THESE DE DOCTORAT DE

L'ÉCOLE CENTRALE DE NANTES

ECOLE DOCTORALE N° 602

Sciences pour l'Ingénieur

Spécialité : Génie des Matériaux

Libellé : Biologie, médecine et santé

Par

Muhammad Tanveer MUNIR

Wood and hospital hygiene

Investigating the hygienic safety and antimicrobial properties of wood materials

Thèse présentée et soutenue à l'École supérieure du bois (Nantes), le 1er février, 2021
Unité de recherche : Laboratoire Innovation Matériau Bois Habitat Apprentissage (LIMBHA), École Supérieure du Bois (Nantes)

Rapporteurs avant soutenance :

Mélanie Morel-Rouhier	Professeur, Université de Lorraine
Nicole Orange	Professeur, Université de Rouen

Composition du Jury :

Président :	André POTVIN	Professeur, Université Laval, Québec
Examinateurs:	Marie France THEVENON	Chercheuse - HDR, CIRAD
	Luciano VIDAL	Chercheur, Ecole Centrale Nantes
Dir. de thèse:	Mark IRLE	Enseignant-chercheur- HDR, directeur de recherche, École supérieure du bois, Nantes
Co-directeur :	Michel FEDERIGHI	Enseignant-chercheur- HDR, UMR-1014 SECALIM, ONIRIS, Nantes
Co-encadrant :	Christophe BELLONCLE	Enseignant-chercheur, directeur de recherche, École supérieure du bois, Nantes

Abstract

Wood materials provide a nature-based theme to construction because of its natural appearance, ecofriendly nature and biophilic effects on humans. However, its organic and porous nature is questioned when using it in hygienically important places such as hospitals. Studies have shown that wood has antimicrobial properties against some pathogens; work is still needed, however, to demonstrate this antimicrobial action and its relation to wood and microbiological variables.

This research gathers and generates information to guide stakeholders of hospital hygiene on the hygienic safety of wood materials. First, a simple and direct method was developed to study the antibacterial and antifungal activity of solid wood, which also identified the role of wood and microbial variables on this antimicrobial behavior. Further, an elution-based bacterial recovery method was investigated which showed that the most common nosocomial bacteria did not survive as well on wood as compared to smooth surfaces. Meanwhile, an innovative tool was developed, involving the use of fluorescent probes to study the bacterial distribution on and inside wood using confocal spectral laser microscopy.

These experiments produced the information to help the decision-makers regarding the choice of wood material in the healthcare buildings. It not only enhances our understanding of the hygienic safety of wood in healthcare buildings but also provides the basis for future research on the prevalence of pathogens in wooden healthcare buildings and the perception of occupants.

Keywords: wood, indoor, healthcare buildings, antimicrobials, hospital hygiene

Résumé

Les matériaux en bois donnent un thème naturel à la construction car leur apparence naturelle, de caractère écologique et d'effets "biofiliques" sur les humains. Cependant, sa nature organique et poreuse est questionnée lorsqu'il est utilisé dans des lieux importants du point de vue de l'hygiène, comme les hôpitaux. Des études ont montré que le bois possède des propriétés antimicrobiennes contre certains pathogènes ; mais des travaux sont encore nécessaires pour démontrer cette action antimicrobienne et sa relation avec les variables de bois et microorganismes.

Cette recherche rassemble et génère des informations pour guider les acteurs de l'hygiène hospitalière sur la sécurité hygiénique des matériaux en bois. Au début, une méthode simple et directe a été mise au point pour étudier l'activité antibactérienne et antifongique du bois massif, qui a également permis d'identifier le rôle du bois et des variables microbiologiques sur ce comportement antimicrobien. En outre, une méthode de récupération des bactéries par élution a été étudiée, qui a montré que la plupart des bactéries nosocomiales courantes ne survivaient pas aussi bien sur le bois que sur les surfaces lisses. Parallèlement, un outil innovant a été développé, impliquant l'utilisation de sondes fluorescentes pour étudier la distribution bactérienne sur et à l'intérieur du bois en utilisant la microscopie laser spectrale confocale.

Ces expériences ont permis d'obtenir des informations fructueuses qui pourraient améliorer la compréhension du rôle du bois dans la sécurité hygiénique des bâtiments de soins de santé. En outre, les futures recherches et les directives d'application ont été fournies concernant la prévalence des pathogènes dans les bâtiments de soins de santé en bois et la perception des occupants des hôpitaux vis-à-vis de l'environnement intérieur en bois.

Mots clés : bois, intérieur, bâtiments de soins de santé, antimicrobien, hygiène hospitalière.

Résumé exécutif

L'utilisation du bois par l'homme est aussi ancienne que l'histoire de l'humanité. Il a été utilisé pour construire de nombreux objets et surfaces de contact importants du point de vue de l'hygiène, utilisés pour la préparation des aliments, l'emballage des aliments, les ustensiles de cuisine, la construction de bâtiments et l'aménagement intérieur. Le bois est également une composante importante des thèmes liés à la nature et il est de plus en plus évident que l'effet réparateur biophile de ce matériau pourrait être responsable de l'amélioration de la santé psychologique et physiologique des hospitalisés. Les études ont montré que les personnes préfèrent cette ressource naturelle et renouvelable à d'autres matériaux synthétiques comparatifs. Les interventions basées sur la nature deviennent un concept de plus en plus populaire à l'intérieur des bâtiments de soins de santé pour réduire le stress psychologique et améliorer les résultats des traitements. Les études ont montré que les intérieurs en bois améliorent la santé psychologique dans les bâtiments de soins de santé et ne causent pas de problèmes de composés organiques volatils s'ils sont entretenus conformément aux normes recommandées en matière de ventilation et d'émissions de produits du bois. En outre, ce matériau est non seulement écologique, mais il possède également des propriétés antimicrobiennes contre de nombreux agents pathogènes d'origine humaine et animale.

Outre toutes ses propriétés bénéfiques attribuées, le matériau bois est également perçu comme peu hygiénique ou difficile à nettoyer en raison de sa nature organique et poreuse. En outre, il n'existe pas beaucoup d'études décrivant les propriétés hygiéniques ou antimicrobiennes du matériau bois concernant son utilisation éventuelle dans les bâtiments de soins de santé. Par conséquent, de nombreux hygiénistes sont réticents à autoriser l'utilisation de ce matériau dans des lieux importants du point de vue de l'hygiène. Par conséquent, l'objectif de cette recherche est d'étudier largement l'interaction des bactéries et des champignons les plus communs causant des infections nosocomiales avec le matériau en bois et de générer des informations pour que les décideurs tels que les hygiénistes, les microbiologistes, les architectes

et les ingénieurs considèrent le bois comme un matériau d'intérieur pour les bâtiments de soins de santé sans aucun risque hygiénique.

Les objectifs scientifiques spécifiques de ce projet comprenaient l'essai des propriétés antimicrobiennes de différentes espèces de bois contre divers champignons et bactéries nosocomiaux. Il était également prévu d'identifier les variables liées au bois et aux micro-organismes, telles que l'essence de bois, l'emplacement de l'arbre, la direction de la coupe, la méthode de préparation, la souche microbienne et sa résistance aux antibiotiques, qui pourraient influencer l'activité antimicrobienne. L'objectif suivant était de déterminer la survie et la distribution de certaines bactéries sur et à l'intérieur des structures en bois, en utilisant une méthode de récupération basée sur l'élution et la microscopie confocale laser spectrale (CSLM). La revue de la littérature a fourni les lignes directrices pour tester les expériences mentionnées ci-dessus et a élaboré un plan de recherche sur le terrain qui pourrait démontrer les propriétés antimicrobiennes du matériau bois à l'intérieur des bâtiments de soins de santé en évaluant la prévalence des agents infectieux. En outre, la méthodologie préliminaire a été préparée pour étudier la prévalence des agents pathogènes nosocomiaux à l'intérieur des bâtiments de soins de santé et l'impact de notre recherche sur la société, qui pourrait être évalué comme la perception des occupants des bâtiments hospitaliers construits en bois vis-à-vis de leur environnement et de son impact sur leur bien-être.

Les études antibactériennes et antifongiques ont utilisé une méthode modifiée de diffusion directe sur gélose (antiboisgram) pour tester les propriétés antimicrobiennes du bois massif. Pour tester les propriétés antibactériennes du bois, les plaques de gélose Mueller-Hinton ont été inoculées avec une suspension de 0,5 McFarland par stripping sur écouvillon. Les disques ronds en bois (diamètre 9-10 mm et épaisseur 2,5-4 mm) ainsi que les disques en papier filtre inerte et les disques de contrôle (Vancomycine 5 µg pour les bactéries Gram-positives : *Enterococcus* sp. et *Staphylococcus aureus* ; Colistine 25 µg pour les bactéries Gram-négatives : *Pseudomonas aeruginosa, Escherichia coli, Acinetobacter* sp.) ont été placés directement sur la gélose inoculée. Les plaques ont été incubées pendant 18-24 heures à 37 °C, puis les zones d'inhibition (ZOI) ont été mesurées manuellement comme indication de l'activité

antimicrobienne. Comme l'antibiogramme classique est obtenu à l'aide d'extraits chargés sur des disques de papier filtre, directement placés sur gélose ou versés dans les puits créés sur gélose, nous avons utilisé les disques en bois massif, c'est pourquoi nous avons appelé cette méthode innovante "antiboisgram" (elle a été nommée ainsi en référence au mot bios).

Lorsque quatre espèces de bois, Chêne américain (*Quercus* sp.), hêtre européen (*Fagus sylvatica*), sapin européen (*Abies alba*), chêne européen (*Q. robur*) couramment utilisées ont été testées par antiboisgram contre les principales bactéries nosocomiales (*E. faecalis, S. aureus, P. aeruginosa* et *E. coli*), les résultats ont montré que cette méthode permettait de passer au crible de manière fiable les propriétés antimicrobiennes du bois. *S. aureus* était la bactérie la plus sensible et les espèces de chêne étaient les bois les plus actifs. C'est pourquoi des recherches supplémentaires ont été menées en utilisant le chêne sessile (*Q. petraea*) qui est un bois couramment utilisé pour la fabrication de meubles en Europe, et *S. aureus* (la bactérie la plus courante responsable des infections associées aux soins de santé) pour tester les variables d'impact du bois sur l'activité antimicrobienne.

Pour tester l'influence des variables du bois sur l'activité antimicrobienne du bois, le bois de *Q. petraea* sélectionné a été obtenu à partir de différentes régions de France, sur la base de la fertilité du sol. Les résultats de l'antiboisgram ont permis d'identifier l'influence des paramètres expérimentaux, notamment la stérilisation et les directions de coupe, sur l'activité antimicrobienne observée du bois. Les échantillons de bois coupés transversalement ont montré la plus forte activité antimicrobienne basée sur la diffusion, très probablement en raison de l'alignement des fibres qui a permis la plus forte diffusion dans la direction longitudinale du bois. En revanche, les échantillons autoclavés ont montré la plus faible activité antimicrobienne qui pourrait être due à la dégradation ou à la perte de molécules actives (très probablement les composés organiques volatils) par chauffage. En revanche, l'origine de l'arbre, la méthode de coupe des disques, la stérilisation aux rayons gamma et le statut de résistance aux antibiotiques des micro-organismes n'ont pas influencé les propriétés antimicrobiennes du matériau en bois. Par conséquent, les disques découpés transversalement, non stérilisés et fabriqués au laser pouvaient être utilisés avec la plus grande facilité et pour des résultats d'activité maximum.

Les disques en bois de chêne sessile découpés transversalement, non stérilisés et fabriqués au laser ont été utilisés dans des études visant à identifier le rôle des paramètres liés aux bactéries tels que l'espèce (cinq espèces), la souche (10 isolats chacun) et le statut de résistance aux antibiotiques sur la sensibilité de ces cinquante isolats à l'activité antimicrobienne de ces échantillons de bois. Il a été observé que presque toutes les espèces, souches et isolats de bactéries avaient une sensibilité différente au matériau bois. Parmi toutes les bactéries testées, *E. coli* était la plus résistante à l'activité antimicrobienne du bois, et dans une étude complémentaire, il a été constaté que les doses les plus élevées des extractions pouvaient inactiver cette bactérie. Le deuxième genre le plus résistant était *Enterococcus* spp. bien que tous les isolats testés aient montré une légère sensibilité (ZOI 11-12 mm), le chêne a été considéré comme inactif sur la base des points de rupture de l'activité antimicrobienne. Une activité antimicrobienne moyenne a été observée contre les isolats de *P. aeruginosa,* où 6/10 des isolats étaient sensibles. Les *Acinetobacter* spp. et *S. aureus* étaient les isolats bactériens les plus sensibles. Ces résultats ont montré que les propriétés antimicrobiennes du bois pouvaient potentiellement être utilisées pour arrêter la croissance des bactéries nosocomiales les plus courantes dans les bâtiments de soins de santé.

L'antiboisgram a également été utilisé pour étudier le comportement antifongique des matériaux en bois. Deux espèces de bois, dont le chêne sessile et le sapin de Douglas (deux arbres chacun), qui sont des bois couramment utilisés pour les constructions intérieures, ont été utilisées dans cette étude. Un total de 27 isolats fongiques, appartenant aux espèces *Candida* spp. et *Aspergillus* spp., résistantes et sensibles aux antifongiques, ont été testés. La méthode basée sur la diffusion a consisté à tester l'activité antimicrobienne des disques en bois massif qui ont été placés directement sur de l'agar dextrose Sabouraud inoculé, et de la sciure de bois qui a été remplie dans les puits créés dans l'agar inoculé. Les témoins négatifs étaient des disques de papier filtre inerte (épaisseur 0,5 mm, diamètre 8 mm), tandis que les témoins positifs étaient des disques de papier filtre imprégnés de 20 µl de fluconazole (10, 50 et 100 µg/ml pour *Candida* spp.) et d'amphotéricine B (40, 4 et 0,4 µg/ml pour *Aspergillus* spp.) dans une solution stérile de DMSO à 1% dans de l'eau distillée. L'activité antimicrobienne a été observée 48 et 72

heures après l'inoculation pour *Candida* spp. et *Aspergillus* spp. Le bois de sapin de Douglas a montré une activité antifongique significativement plus élevée ($p<0,05$) par rapport au bois de chêne. Seules trois souches fongiques étaient résistantes à l'activité antimicrobienne du sapin de Douglas. Deux arbres de chaque bois présentaient une différence non significative d'activité antifongique, ce qui prouve que la zone d'origine des arbres d'une même espèce n'influence pas l'activité antimicrobienne du bois. Toutes les souches fongiques sauf une, étaient sensibles à l'activité antimicrobienne du bois, indépendamment de leur résistance aux agents antifongiques, ce qui montre que le statut de résistance aux antibiotiques du microbe n'influence pas sa sensibilité au matériau bois. Cependant, la souche et les champignons ont déterminé leur résistance à l'activité antifongique du bois. Aucune activité n'a été observée autour des puits remplis de sciure de bois, très probablement parce que la quantité (30 mg) n'était pas suffisante pour provoquer une activité antifongique sur gélose. C'est la première étude jamais réalisée sur les propriétés antifongiques du bois massif contre un large éventail de champignons nosocomiaux. En outre, des études sont proposées pour identifier les molécules actives responsables de l'activité antifongique unique du sapin de Douglas, en particulier contre *C. auris*, qui était considéré comme une menace biologique émergente avant la crise COVID19.

Les expériences susmentionnées ont étudié les propriétés antimicrobiennes des produits chimiques du bois qui se diffusent du bois massif à l'agar. Cependant, le rôle de la structure physique du bois, en contact avec les microbes, n'a pas été bien démontré. Par conséquent, des recherches supplémentaires ont été prévues pour déterminer l'interaction microbienne avec le matériau en bois qui a été testée par des méthodes basées sur la récupération pour étudier la survie des microbes et par la CSLM pour étudier la distribution microbienne à l'intérieur du bois.

La distribution des bactéries à l'intérieur des structures en bois a été identifiée en les marquant par des sondes fluorescentes et en les observant par CSLM. La contamination des *E. coli* marqués au DRAQ5 a été identifiée par des méthodes directes et indirectes. De plus, l'application directe de DRAQ5 sur le bois a donné des signaux fluorescents, qui ne sont observés que lorsque cette sonde se lie à l'ADN, indiquant ainsi que le bois stérilisé par rayons

gamma peut contenir les traces d'ADN et pourrait interférer avec l'interprétation des études liées aux acides nucléiques sur les surfaces en bois. La bactérie produisant la protéine fluorescente mCherry a été utilisée pour la première fois pour étudier la distribution microbienne sur une surface poreuse. Différentes variables d'espèces de bois, de direction de coupe et de temps d'incubation ont été étudiées. Ces approches innovantes ont produit des résultats inédits montrant que les bactéries descendaient plus profondément dans le bois coupé transversalement en raison des éléments de vaisseaux s'étendant longitudinalement, par rapport aux coupes longitudinales. La distribution microbienne était plus élevée à la surface du peuplier et plus faible sur le sapin de Douglas, ce qui montre indirectement la survie microbienne à la surface du bois. Il a été observé que la fluorescence bactérienne diminuait continuellement sur toutes les surfaces en bois mais persistait même après 7 jours d'étude, ce qui indique que les bactéries pouvaient survivre plus longtemps dans ces conditions. Pour tester si les bactéries pouvaient former un biofilm sur les surfaces en bois et en mélamine, elles ont été complétées avec une solution saline normale comme condition humide et un bouillon comme condition enrichie en nutriments. Des cerises fluorescentes *S. aureus* ont formé un biofilm sur la mélamine dans un environnement enrichi, mais pas sur le bois de chêne, de peuplier ou de sapin de Douglas dans des conditions similaires. En conclusion, la bactérie a survécu et a produit une fluorescence, mais n'a pas pu former de biofilm sur les surfaces de bois.

La survie microbienne sur le bois et d'autres matériaux a été étudiée à l'aide de méthodes basées sur la récupération. L'étude a fourni les premiers résultats concernant la survie de la plupart des pathogènes nosocomiaux courants, dont *Klebsiella pneumoniae, Acinetobacter baumannii, Enterococcus faecalis* et *S. aureus*, sur les matériaux en bois par rapport à d'autres matériaux de construction intérieurs couramment utilisés tels que le plastique, l'acier et l'aluminium. À cette fin, une méthode de récupération microbienne basée sur l'élution a été employée et les bactéries récupérées ont été dénombrées par la méthode de comptage sur plaque. En général, toutes ces bactéries ont montré une survie significativement plus faible sur le bois par rapport à d'autres matériaux. Le temps de survie de *K. pneumoniae* et *E. faecalis* sur le bois était d'un à deux jours, alors qu'il était de plus de 15 jours sur tous les autres matériaux.

A. baumannii n'a survécu que quelques heures sur le bois et a continué à diminuer sur tous les autres matériaux testés jusqu'à une semaine. La bactérie qui a survécu le plus longtemps est *S. aureus* qui a persisté sur le bois de chêne pendant presque une semaine et plus de deux semaines pour tous les autres matériaux. Les résultats ont montré qu'il n'y avait pas de différences significatives ($p<0,05$) entre les bactéries récupérées dans les coupes de bois LT et RT. En attendant, le plan RT est censé avoir une plus grande porosité, on peut donc conclure que cette méthode a donné des résultats de récupération similaires, indépendamment de la porosité des surfaces.

Enfin, l'application de ces résultats aux conditions de terrain a été suggérée sous la forme de méthodologies d'études prospectives sur le terrain. Il s'agissait notamment de déterminer la prévalence des agents pathogènes nosocomiaux et la perception des occupants à l'intérieur des bâtiments de soins de santé. Les informations ont été recueillies dans la littérature et rédigées sous forme de méthodes qui ont été discutées plus en détail avec des experts afin d'être validées pour des applications sur le terrain. Dans l'étude de perception, la première section du questionnaire comprenait les questions relatives aux données démographiques des sujets. La deuxième section était destinée à déterminer la perception des personnes en leur demandant leur avis sur le matériau bois et son rôle dans l'environnement intérieur. La troisième section a été principalement conçue pour les entretiens et comprenait les questions permettant aux participants de diagnostiquer s'ils souffrent du syndrome des bâtiments malsains ou de symptômes de fatigue chronique liés à l'environnement. Les statistiques descriptives sont recommandées pour analyser ce type de données et identifier le rôle du matériau bois sur le bien-être des personnes et le niveau d'acceptation de ce matériau dans l'opinion des occupants de l’hôpital. Un autre objectif basé sur la bibliographie était de concevoir des méthodologies pour déterminer la prévalence des agents infectieux dans l'environnement et l'influence du matériau bois sur leur persistance. Pour ce faire, deux types d'études ont été conçus. D'abord, la collecte des échantillons d'air et de surface dans l'environnement en bois et non en bois des bâtiments de soins de santé, puis la détermination de la présence d'agents infectieux. Deuxièmement, une chambre modèle de démonstration en laboratoire (chambre statique)

pourrait être testée en injectant l'air dans la chambre en présence ou en l’absence de bois, puis en le recueillant à nouveau à la sortie pour étudier la perte de miocrobes. Ce modèle devrait montrer si la présence de bois dans la chambre a influencé la survie des microbes dans l'air circulant.

Dans l'ensemble, cette recherche a permis d'étudier les propriétés antimicrobiennes du bois contre les agents pathogènes nosocomiaux et a fourni une feuille de route pour l'application de ces résultats dans les bâtiments de soins de santé dans un avenir proche.

Acknowledgments

I would like to pay my special regards and appreciation to my thesis supervisors: Dr Christophe Belloncle for managing the project, bringing me into his team, listening to me and my ideas, always being solution-oriented, teaching me methods in chemistry, and guiding me to freedom of research and growth, Prof. Dr. Michel Federighi, a substance of genius, an experienced campaigner, who taught me new approaches in microbiology, research and career, and my thesis director, Dr. Mark Irle, for polishing my scientific writing skills and teaching me research methods of communication. They convincingly guided and encouraged me to be professional and do the right thing even when the road got tough.

I wish to show my gratitude to our project team for their monumental contribution to my study: Dr. Helene Pailhories and Prof. Dr. Mattieu Eveillard for always bringing a solution-oriented answer to my questions and providing me a helping hand in laboratory research and write up, Prof Dr. Didier Lepelletier for furnishing administrative support and ideas for the research, Prof. Dr. Patric LePape, for welcoming me in his world of antifungal research, Dr. Laurence Dubreil, for consistently providing support and help in conducting the microscopy experiments, discussing the innovative methodologies and applying all her knowledge and experience to guide me, and last but not the least, wonderful project manager, Dr. Florence Aviat for keeping the research running smoothly. Without their persistent help, this magnificent project would have not been realized.

I would thank all the people whose assistance was a milestone in the completion of this project and my studies: my colleagues Julia, Abraham, Francois, other staff of ESB, SECALIM, IRS2, and APEX, and the tutored group of students I supervised from engineering (ESB) and Master's (MANIMAL M2) programs. I whole-heartedly appreciate the role of Master's students I co-supervised, including Ismael, Nattar, Marjan and Ju-Chi, for conducting experiments and data collection for my research that also served as their master's thesis. I recognize their invaluable assistance provided during my study.

I wish to express my deepest gratitude for the invaluable support and great love of my family. They kept me going on and this work would not have been possible without their input. I also acknowledge the backing from my friends, for boosting my confidence and always being there in thick and thin.

I recognize the role of funding agency CODIFAB and Region Pays de la Loire for financing our research.

Publications and communications of research

Publications of original results

1. Munir, M.T.; Pailhories, H.; Irle, M.; Aviat, F.; Federighi, M.; Dubreil, L.; LePape, P.; Lepelletier, D.; Belloncle, C. and Eveillard, M. (2020) Wood materials for limiting the bacterial reservoir on surfaces in hospitals: would it be worthwhile to go further? *Future Microbiology* 15(15): 1431-37; doi: 10.2217/fmb-2019-0339
2. Munir, M.T.; Pailhories, H.; Eveillard, M.; Irle, M.; Aviat, F.; Federighi, M.; Belloncle, C. Experimental Parameters Influence the Observed Antimicrobial Response of Oak Wood (*Quercus petraea*). *Antibiotics* 9(9): 535; doi: 10.3390/antibiotics9090535
3. Chen, J-C.; Munir, M.T.; Aviat, F.; Federighi, M.; Dubreil, L.; Irle, M.; LePape, P.; Lepelletier, D.; Belloncle, C.; Eveillard, M. and Pailhories, H. (2020) Survival of bacterial strains on wood (*Quercus petraea*) and other materials. *Antibiotics* 9(11) 804; doi: 10.3390/antibiotics9110804
4. Munir, M.T.; Pailhories, H.; Eveillard, M.; Irle, M.; Aviat, F.; Federighi, M.; Belloncle, C. (2019) Direct screening method to test antimicrobial properties of untreated wood. *European Journal of Wood and Wood Products* 77(2): 319-22; doi: 10.1007/s00107-019-01390-2

Publications of bibliography

On the main subject

5. Munir, M.; Pailhories, H.; Eveillard, M.; Aviat, F.; M.; Dubreil, L.; LePape, P.; Lepelletier, D.; Belloncle, C. and Federighi, M. (2019) Antimicrobial Characteristics of Untreated Wood: Towards a Hygienic Environment. *Health* 11(2): 152-170; doi: 10.4236/health.2019.112014
6. Munir, M.T.; Pailhories, H.; Eveillard, M.; Irle, M.; Aviat, F.; Dubreil, L.; Federighi, M.; Belloncle, C. (2020) Testing the antimicrobial characteristics of wood materials: A review of methods. *Antibiotics*, 9(5), 225; doi : 10.3390/antibiotics9050225

Related to subject

7. Federighi, M.; Munir, M.T.; M.; Irle and Belloncle, C. (2020) Propriétés antimicrobiennes des litières de bois en productions animales. *Le point Vétérinaire Rural* n° 406 du 01/06/2020
8. Munir, M.T.; Federighi, M.; Irle M. and Belloncle, C. (2019) Wood-based litter in poultry production: a review. *The World's Poultry Science Journal* 75 (1): 5-16; doi: 10.1017/S0043933918000909
9. Munir, M.T.; Irle M.; Belloncle, C. and Federighi, M. (2019) Wood Based Bedding Material in Animal Production: A Minireview. *Approaches in Poultry, Dairy and Veterinary Sciences* 6(4): 582-88. doi: 10.31031/APDV.2019.06.000644

Communications in scientific conferences and meetings

10. Munir, M.T. (2020). Antimicrobial properties of wood material: opportunities and challenges for hygienically sensitive places. Antibiotics 2020, Meeting International, December 11, 2020, 2nd Webinar.
11. Munir, M.T.; Belloncle, C.; Aviat, F.; Federighi, M.; Eveillard, M. and Pailhories, H. (2020). Are oak wood surfaces a solution to control *Acinetobacter baumannii* of indoor human and animal environment? 6th World One health Congress. 30 October – 03 November, 2020, Virtual edition.
12. Munir MT, (2020) A one health perspective on the microbial safety of wood material. *Seminar of project Wood and Hospital Hygiene (BoisH2)*, ESB, 14 Jan 2020.
13. Baude, J.; Munir M.T.; Belloncle, C.; Dubreil, L.; Lina, G.; Federighi, M. and Vandenesch, F. (2019) Observation of mCherry labelled *S. aureus* on woods [Titre provisoire : « J'aime *S. aureus* le soir au fond du bois»]. Concours photos MICROBES 30 sep – 2 oct 2019 Paris.
14. Munir, M.T.; Belloncle, C.; Aviat, F.; Federighi, M.; Pailhories, H. and Eveillard, M. (2019). Antimicrobial properties of oak against Acinetobacter: a one health approach. Euro-Medit Conf Environ Integ. 10-13 October 2019: Sousse, Tunisia.
15. Munir M.T. (2019). Antimicrobial properties of wood to control nosocomial infections. These du Bois, University of Laval, 30 September, Quebec Canada.

16. Munir, M.T.; Belloncle, C.; Aviat, F.; Federighi, M.; Pailhories, H. and Eveillard, M. (2019). Oak wood has antimicrobial activity against *Acinetobacter baumanni* isolates of human and animal origin. ICOPHAI, 23-26 sept, 2019: Quebec, Canada
17. Munir, M.T.; Pailhories, H. and Eveillard, M.; Belloncle, C. and Federighi, M. (2019) A new, simple, method to assess antimicrobial properties of untreated wood. 15th conference of French Society of Microbiology 30 sept-2 oct., 2019: Paris, France.
18. Munir, M.T. (2019) Interaction of pathogens with wood material: A One-Health approach in multidisciplinary environment. OHEJP summer school "One health operationalization", 18-31 August, 2019 at University of Surrey and Chatham House, London UK.
19. Munir, M.T.; Maneewan, N.; Federighi, M.; Baude, J.; Aviat, F.; Belloncle, C. and Dubreil, L. (2019). An investigation of hygienic risk of Staphylococcus aureus on wood surfaces via spectral confocal laser scanning microscopy. 16th meeting of French Society of microscopy (SFM), 2-5 July 2019, Poitiers (pp 268-269).
20. Munir, M.T.; Chouet, A. and Dubreil, L. (2019) Imagerie non destructive en microscopie confocale spectrale et microscopie biphotonique : détection de contaminants du bois et analyse structurale d'un produit céréalier de cuisson. Gen2Bio, 14 Mars, 2019 : Terra Botanica, Angers, France.
21. Munir, M.T; Federighi, M.; Aviat, F.; Oumarou-Mahamane, I.; Maneewan, N.; Pailhories, H. and Eveillard, M.; Belloncle, C. and Dubreil, L. (2019) The nondestructive microscopic imaging of hygienically important microbes and contaminants on wood. Poster and presentation in Folle journée de l'imagerie Nantaise, 27 Feb, 2019.
22. Munir, M.T; Oumarou-Mahamane, I.; Federighi, M.; Pailhories, H.; Eveillard, M.; Aviat, F.; Belloncle, C. and Dubreil, L. (2018) Direct DRAQ5 DNA staining on sterilized and contaminated wood surface, investigation by confocal microscopy. 7th GDR Bois, 20-23 November 2018, Cluny, France.
23. Munir, M.T; Belloncle, C.; Pailhories, H.; Eveillard, M.; Aviat, F. and Federighi, M. (2018) Survival of Nosocomial pathogens on Maritime pine and European fir wood. PTFBPI, 19-21 September 2018. TUM, Munich, Germany (pp 213-217).

Table of contents

List of figures

List of tables

Abbreviations

AFM	Atomic force microscopy
AMR	Antimicrobial resistance
ATCC	American Type Culture Collection
AU	Arb units
ATP	Adenosine triphosphate
BLSE	Beta-lactamases spectrum extended
CASFM	Committee of the French Society of Microbiology
CCA	Chromated copper arsenate
CCM	Czech Collection of Microorganisms
CDC	Center of disease control
CFU	Colony forming unit
CI	Confidence interval
COVID19	Coronavirus infectious disease 2019
CPAB	Carbapenemase-producing *Acinetobacter baumannii*
CSLM	Confocal spectral laser microscopy
DGGE	Denaturing gradient gel electrophoresis
DMSO	Dimethyl sulfoxide
DNA	Deoxy ribonucleic acid
DRAQ5	Deep red-fluorescing bisalkylaminoanthraquinone number five
EDTA	Ethylenediaminetetraacetic acid
EPA	Environmental protection agency
Eq.	Equation

GFP	Green fluorescent protein
GISA	Glycopeptide-intermediate *Staphylococcus aureus*
HAI	Healthcare associated infections
ICU	Intensive care unit
KGy	Kilo Greys
LCI	Lowest concentration of interest
LR	Radial cut
LT	Tangential cut
MDL	Minimum detectable limit
MIC	Minimum inhibitory concentration
MLSB	Macrolide-lincosamide-streptogramin B
MPa	Mega pascals
mRNA	Messenger RNA
NCVC	Non cultivable viable counts
NDM	New Delhi metallo-β-lactamase
PBS	Phosphate buffer solution
PCP	Pentachlorophenol
PCR	Polymerase chain reaction
PFA	Paraformaldehyde
PVC	Polyvinyl chloride
qPCR	Quantitative PCR
RFP	Red fluorescent protein
RH	Relative humidity
RNA	Ribonucleic acid

RODAC	Replicate Organism Detection and Counting
rRNA	Ribosomal RNA
RT	Transversal cut
SARM	*Staphylococcus aureus* Multi Resistant
SDA	Sabouraud Dextrose Agar
SEM	Scanning electron microscope
TEM	Transmission electron microscope
TLC	Thin layer chromatography
TVOC	Total volatile organic compounds
US/USA	United states of America
UV	Ultraviolet
VAP	Ventilator associated pneumonia
VOC	Volatile organic compound
WHO	World health organization
2D	Two dimensional
3D	Three dimensional

Chapter 1. Introduction

Wood is an important component of most nature-based building themes. The benefits are not only aesthetic as studies have shown that this material could improve the psychological wellbeing of inhabitants when it is used for indoor constructions. These restorative effects can be extremely useful in highly stressed places such as healthcare buildings, where the stress in patients and staff is often high.

Currently, the use of wood in hygienically important places is often questioned owing to lack of sufficient evidence on its hygienic properties and safety. Moreover, in some instances, it is regarded as a hygienically unacceptable material because of the perception that its porosity and organic nature could harbor or support the growth of harmful pathogens.

Contrarily, the chemical composition and physical structure of wood give it some antimicrobial properties to resist some microorganisms that might attack it. These antimicrobial properties also work against some hygienically important pathogens. However, there are not many studies available to guide decision-makers regarding the microbiological safety of wood material inside healthcare buildings.

Therefore, measuring the antimicrobial potential of wood could support its use in health institutes. With this in mind, a pilot study funded by Comité Professionnel de Développement des Industries Françaises de l'Ameublement et du Bois (CODIFAB) was conducted in 2016, showed that certain wood species have antibacterial properties against different bacteria which are known to cause healthcare-associated infections (HAI).

The positive results from the pilot study provided the incentive for the study presented here. This thesis is part of a broader project called "Bois et Hygiène Hospitalière (BoisH^2)" that is investigating the interactions of wood materials with the bacteria and fungi that are responsible for HAI. In particular, it develops and demonstrates protocols for observing the antimicrobial properties of wood in field studies. The BoisH^2 project is funded by the Region Pays de la Loire and CODIFAB.

The studies for this report were conducted in partnership with Ecole Supérieure du Bois (LIMBHA), ONIRS (UMR INRA/Oniris 1014 Secalim and APEX/Panther), CHU of Angers (laboratory of bacteriology) and CHU of Nantes (laboratory of bacteriology and mycology).

Objectives

The main aim of this research is to provide indoor construction and hygiene maintenance stakeholders the confidence to specify specific woods in certain situations. This aim requires data on the antimicrobial properties of wood, which, in turn, requires protocols that can reliably screen different wood species for their performance against microbes.

The thesis is divided into the following steps that are designed to achieve the main aim of this project.

Specific 0: A literature review on wood and hospital hygiene

Conduct an extensive literature review to identify the available research and the knowledge gaps regarding:

- Enhancing the understanding of wood and its hygienic (antimicrobial) properties
- Exploring the opportunities and challenges related to using wood inside healthcare buildings
- Identifying and compare methodologies that study the hygienic properties of wood

Specific 1: Testing the antibacterial activity of wood materials

Developing a simple, direct and quick screening method to test the antimicrobial properties of wood material. In addition, evaluating the influence of experimental parameters related to wood variables and type of bacteria on the observed antibacterial activity.

Specific 2: Testing antifungal properties of wood material

Conducting laboratory experiments to evaluate the antifungal properties of solid sessile oak and Douglas fir wood against multiple fungal strains. The direct and quick agar-based diffusion methods will be developed to screen the antifungal activity of wood and the

influencing parameters such as type of wood, tree, cutting plane, type of fungi and their resistance status to antifungal agents.

Specific 3: Distribution and survival of bacteria on and inside wood

Developing new, quick and simple methods to elaborate the fate of bacteria on and inside wood. In the first part, studying the survival of nosocomial bacteria on wood and other material used as hospital surfaces by employing an elution-based microbial recovery method. Secondly, evaluating the efficacy of (chemical and genetically modified protein) fluorescent probes to study the bacterial distribution on and inside wood using a confocal spectral laser microscope (CSLM).

Chapter 2. Literature review

This chapter reviews the literature on the interaction of wood with hygienically important microorganisms. Firstly, it introduces wood regarding its structure, chemical profile, environmental credentials and its uses in buildings where it may contribute to healthy living. Then this chapter describes healthcare-associated infections and hygienically important microbes in healthcare buildings. Further, the antimicrobial properties of wood and their possible use against hygienically important microbes are described. Specifically, it elaborates on how the physical and chemical constituents of wood interact with microorganisms to inactivate or kill them. Finally, the chapter describes the possible methodologies which could be used to investigate the antimicrobial nature of wood. The focus of this manuscript is mainly on the untreated wood because the surfactants or polishing of wood may cover the natural behavior of this material.

2.1. Wood from tree: a porous and organic material

Wood is the substance that forms the branches, roots, and stems of shrubs and trees. This chapter, however, specifically considers wood as a material that can be used for indoor constructions. This biologically complex structure is composed of different types of cells fulfilling the structural and functional needs of trees and define the nature and properties of the material. In general, wood is considered as a solid and homogeneous material, however, as it is obtained from the living organism, tree, the structure, and composition may vary depending upon the life experiences of a tree (Dadswell and Hillis 1962).

In industry, wood is termed as ‘hardwood’ when obtained from angiosperms (dicots, often deciduous broad-leaved, including oak, birch, beech, ash), and ‘softwood’ when it comes from gymnosperms (plants producing uncovered seeds such as spruce, pine, fir). This nomenclature, however, does not truly reflect the actual properties of wood, for example, balsa is a hardwood, and it is much softer than a typical softwood (Ramage et al. 2017).

2.1.1. Anatomy and histology of wood

The trunk of tree, from outside to inside, consists of outer and inner bark, vascular cambium, sapwood, heartwood and central pith (Figure 2.1). Outer bark covers the softer inner bark to protect it and minimize evaporation losses. The vascular cambium is the area that contains the xylem and phloem for sugar and water transport (Rowell 2012). The sapwood contains functional parenchyma cells and about 10% of the cells are living (Pallardy 2008). They are involved in the conduction of the sap, and formation and storage of phytochemicals. It is generally lighter in color and almost has similar strength as heartwood that is the darker part of wood (Blankenhorn 2001). Most of the cells in sapwood are already dead. The change to heartwood is still not completely understood, but generally it is thought that parenchyma cells exude extractives into adjacent, recently died cells. Therefore, the heartwood and pith appear darker in color. These parts provide mechanical support but are not involved in physiological processes. This is the reason that even when the inner part of tree is degraded the tree continues living because sapwood can retain all functions (Pallardy 2008).

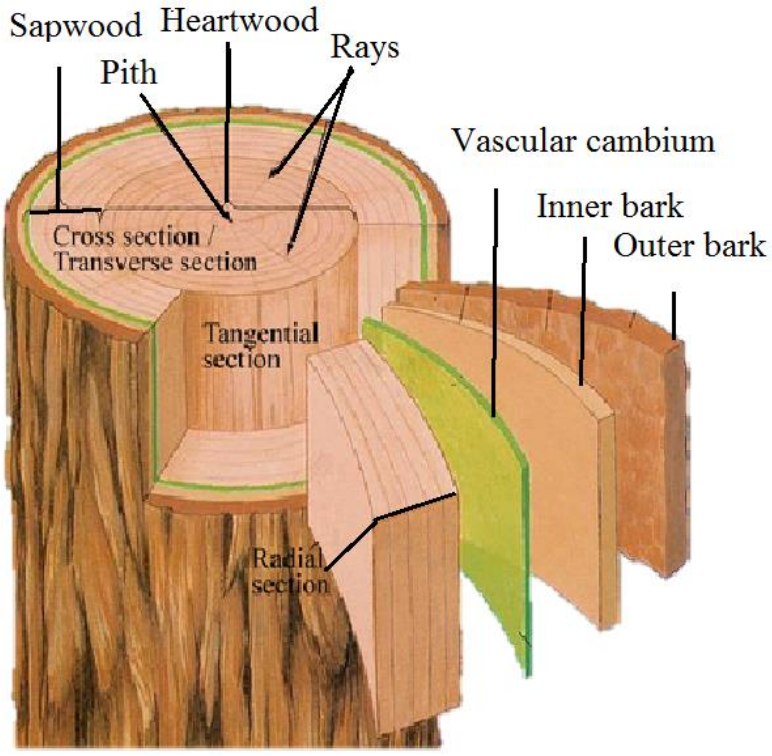


Figure 2. 1: A cut-through of tree stem (University of Cambridge 2020)

When wood is cut in different planes, different cell and fiber directions are exposed (Figure 2.1 and 2.2). The transversal (RT) and longitudinal, are divided into radial (LR) and tangential (LT), sections. These sections are important regarding the utilization of wood in construction because of their different mechanical properties (Holmberg et al. 1999; Franco 2019).

On tissue level, there are two distinct cell types, axial and radial. These cells are elongated in shape and are found interpenetrating and interconnected to each other. The cells of axial system run parallel to long axis of a tree; hence this system is responsible for vertical transmission of nutrients. On the other hand, the radial cells are present perpendicular to long axis and they run from pith to bark direction, performing the function of lateral transport and storage of phytochemicals (Carlquist 2001).

Softwood is composed of simple type of cells where a majority (90%) are elongated cells called tracheids. These cells have perforation, called pits, between them which allow transport of fluids, but their overlapping makes the route zigzag. They are thin-walled in early growth rings and thick-walled in aged rings. Ray parenchyma cells are rectangular prism or brick-shaped and they are involved in the synthesis, storage and transport of phytochemicals. Sometimes, axial parenchyma cells are also present in softwood. These are similar to ray parenchyma cells but are vertically oriented (Rowell 2012).

The structure of hardwood is more complex than softwood (Figure 2.2). It contains specialized water directing cells called vessel elements (50-200 µm). When these vessel elements meet each other, they form a space known as perforation plate. Slicing the hardwood in RT section shows these vessel elements as pores (Rowell 2012). The size, frequency and arrangement of pores vary in different species of wood. Depending on the size, pores are classified into four categories: micropores (80–1.8 nm), mesopores (500–80 nm) and macropores (radius 58–2 µm and 2–0.5 µm) (Plötze and Niemz 2011). Hardwood has specialized thick-walled fibers (200-1200 µm) which provide mechanical support to wood. A wide variety of axial parenchyma cells and more complex rays are also present in

hardwood (Rowell 2012). Overall cellular composition by volume of hardwood is 36%–70% fiber cells, 20%–55% vessel elements, 6%–20% ray cells, and about 2% parenchyma cells (Bajpai 2018).

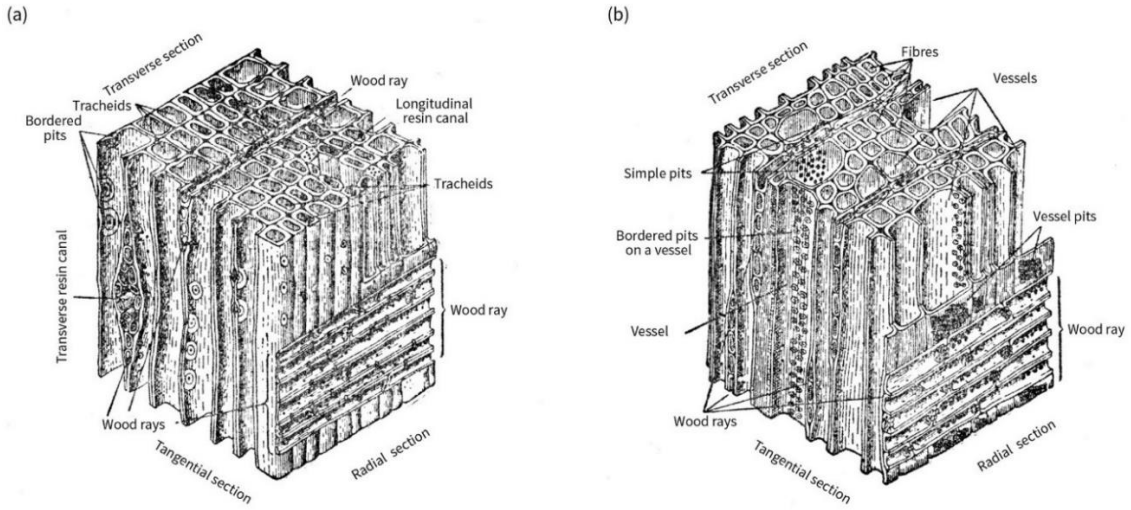


Figure 2. 2: Anatomy of wood in the transverse (RT), radial (LR) and tangential (LT) sections; (a) softwood **(b)** a hardwood (Wadum et al. 1961)

The living plant cells have a 'cell wall' that is the outer covering of cells and the inner content 'protoplast' is enveloped by the cell membrane. The structure of cell wall is very specific based on age, organ, and the part of wood. The cell wall has three layers, middle lamella (outermost), primary wall and a secondary wall (Figure 2.3).

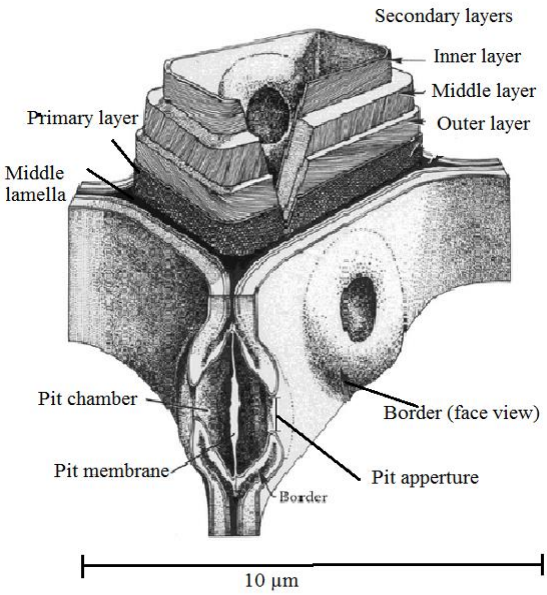


Figure 2. 3: Illustration of the cell wall layers (Rowell 2012)

Cell walls of adjoining cells are modified to allow communication and transport, these modifications give rise to a chamber called ‘pit’ (Conners 2001). Wood cells tend to die after attaining a certain maturity and then they create a void space known as ‘lumen’**,** which is significant regarding the water conduction potential of wood (Rowell 2012).

2.1.2. Chemical composition of wood

The approximate elemental composition of oven-dried wood consists of Carbon (49.5%), Oxygen (44%), Hydrogen (6%), Nitrogen (0.5%), and a trace amount of several metal ions (Fengel and Wegener 1983; Pettersen 1984).

Chemically, wood constituents can be categorized into low molecular weight substances and high molecular weight substances. Organic (extractives) and inorganic (ash) matter are low molecular weight substances. Whereas the high molecular weight substances are polysaccharides (cellulose, hemicellulose) and lignin (polyses) (Figure 2.4).

The extractives are chemicals which are originated from already present precursors, as a result of wounds and as a defense mechanism of the plant. They are relatively smaller molecules and makeup 2-10% of the dry weight of wood (Bajpai 2018). The common extractives are lipids, fatty acids, waxes, terpenes, phenols, steroids, rosin, resin acids, alcohols and many other organic compounds (Quesada-Granados et al. 2016). In general, softwood has higher extractives compared to hardwood, and they are localized mostly in heartwood, imparting color, odor, and, sometimes, durability to wood (Bajpai 2018). Historically, these chemicals have been used for waterproofing, binding, flavoring, perfume production and burning. They also have applications as medicines, cosmetics and preservatives (Beatson 2011). In medicine, they are used as an antifungal (Zare et al. 2019), antibacterial (Moshfeghy et al. 2018) and nutrient supplements (Das et al. 2020). It is believed that extractives are cheaper and safer than synthetic preservatives (Arango et al. 2006). Synthetic preservatives cause harmful effects in woodworkers and they do not degrade easily after disposal of wood, moreover, they are toxic to the environment and may cause pollution (Syofuna et al. 2012).

The cell wall has three major high molecular weight organic components: microfibrils of cellulose (polymers), hemicellulose (smaller and branched molecules), and matrix (pectin in primary and lignin in the secondary cell wall) (Figure 2.4).

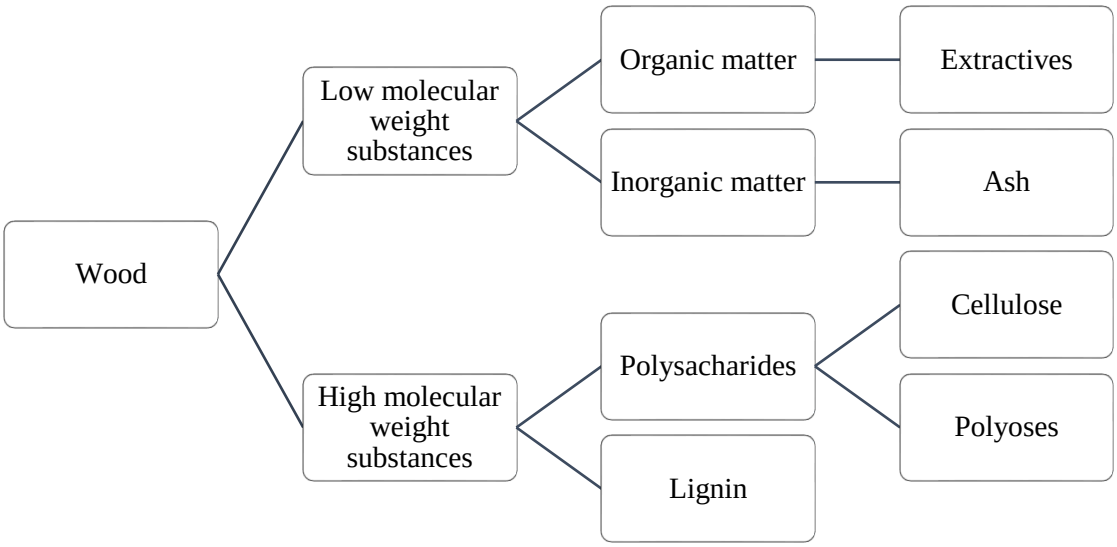


Figure 2. 4: Schematic presentation of wood chemical compounds [adapted from Fengel and Wegener (1983)]

Cellulose and hemicellulose make the cell wall hygroscopic, while lignin is the limiting factor in the uptake of water. The moisture absorption potential of the cell wall greatly affects the behavior of wood in wood products. The water in wood can be available in free form (within lumina) or bound to available hydroxyl groups, the majority of which are in the cellulose and hemicellulose fractions. This moisture level is also important in expressing the density of wood. In North America and Europe, density of wood is considered air-dried at given moisture of 12% while it is 15% in tropics (Rowell 2012). This moisture level is considered equal to its anticipated equilibrium moisture content and for interior uses in dry regions it is 4–9% and 8–13% for coastal areas (Brauns and Rocens 2005). When the fiber wall is completely saturated with bound water and no free water is present in cell cavities; this stage is called fiber saturation point (FSP) which generally ranges from 21-35% (Rowell 2012). FSP determines the physical and chemical properties of wood and it has direct relation to microbial survival. If the moisture content is higher than FSP the wood is

dimensionally stable but if it is lower than this point, wood may shrink. The longitudinal shrinkage is very low as compared to radial shrinkage, which in turn is typically half of the tangential shrinkage (Brauns and Rocens 2005); therefore, wood cut in different directions may exhibit different properties, including the antimicrobial behavior.

2.2. Wood in the healthcare buildings: opportunities and challenges

Healthcare buildings are the workplaces for caregivers that provide health treatments to patients. These facilities range from small doctor clinics to large healthcare centers including hospitals. The occupants of these buildings often have a high level of stress which escalates the risk of mental health problems. For example, medical professionals, including doctors, nurses and veterinarians have 2-5 times higher chances of developing mental health illness due to this stress (Kim et al. 2018). It is well-established fact that the working conditions are responsible for this extreme level of stress and should be managed accordingly (Koinis et al. 2015). Along with the improvement of working conditions, the environment can also be considered as a factor to reduce the level of stress and improve wellbeing markers.

There are examples of nature-based themes being used to provide restorative environments in these facilities. Wood as a natural material maintains its prominent place in such constructive modifications. There is mounting evidence that wood as an indoor material also provides restorative benefits. Therefore, many new healthcare facilities are adapting this material in their buildings. Most of the time, it is used in polished form as furniture, however, the natural material also has the potential to be applied in the buildings. The reason behind using natural material is reducing the use of harmful chemicals as surfactants and reducing the use of cleaning agents that could be toxic to people and the environment. Moreover, wood has natural antimicrobial properties, and thus we assume that its advantages can be best achieved when used without surfactants. Wood material in untreated form can be used as low or no contact surfaces or even the non-contact sides of high contact surfaces, such as bed, table and chair. Studies have shown that such uses can increase the psychological well-being of hospital occupants (Kotradyová and Kaliňáková 2014; Kotradyova et al. 2019); and they

neither increase the indoor emissions (Nyrud et al. 2012) and nor pose a problem in the cleaning process to maintain hygiene (Kotradyova et al. 2019).

The European regulations do not prohibit the use of wood material inside hospital buildings. The requirement is the cleanability of used material. Based on general perceptions, however, the wood material is regarded as unhygienic. Its organic and porous consistency is generally given as a reason.

2.2.1. Wood as a sustainable building material

Wood as a "green" material is used for sustainable and eco-friendly constructions. This renewable resource will be available indefinitely, on the condition that sustainable forest management and harvesting are respected (Falk 2010). Regarding deforestation, many countries are depleting their natural forest resources to clear land for crop operation, this is the case with Brazilian amazon forests (Walker et al. 2020). Meanwhile, the forest cover in the European Union (currently 42%) has increased since world war II and in ast 30 years only it increased by 10% along with a 40% increase in timber stock and 30% more wood production (Europa 2020). However, these claims are debatable as a recent study has shown that 26 European countries have increased deforestation by almost 50 percent in the last few years (Ceccherini et al. 2020).

The ecofriendly title to wood in construction is attributed due to two main reasons, the low embodied energy (the amount of energy required to harvest, mine, manufacture, and transport to the point of use) as compared to other materials (plastic, steel, concrete, etc.) and the carbon-neutral character. Forests play a major role in fixing carbon from the air. The trees use sunlight and carbon dioxide to generate sugar and oxygen (eq. 1).

The usage life of wood, in the form of furniture or construction material, keeps this carbon stored. Furthermore, this material can be recycled and be used to produce secondary products such as particleboards. Finally, the stored carbon is released in the atmosphere when the wood is degraded completely or burned for fuel (eq. 2).

$$(\text{Sunlight}) + 6H_2O + 6CO_2 \rightarrow C_6H_{12}O_6 + 6O_2 \qquad \text{(eq. 1)}$$

$$C_6H_{12}O_6 + 6O_2 \rightarrow (\text{Heat}) + 6H_2O + 6CO_2 \qquad \text{(eq. 2)}$$

On the other hand, the other materials, such as plastics, steel and concrete not only have high embodied energy but also become an environmental hazard during and after their usage life (Table 2.1). The manufacturing of these materials is responsible for 11% of the global CO_2 emissions, and overall, the buildings and construction sector used 36% of final energy and accounted for 39% of energy and process-related CO_2 emissions in 2018 (IEA 2019).

Table 2. 1: Carbon emissions from different materials

Material	**Net carbon emissions (kg C/t)**	**Near-term net carbon emissions including C storage within material (kg C/t)**
Framing lumber	33	–457
Medium-density fiberboard (virgin fiber)	60	–382
Brick	88	88
Glass	154	154
Steel (virgin)	694	694
Recycled steel (100% from scrap)	220	220
Concrete	265	265
Aluminum (virgin)	4,532	4,532
Recycled aluminum (100% recycled content)	309	309
Steel (virgin)	694	694
Plastic	2,502	2,502

Adapted from (Falk 2010)

2.2.2. Wood as an indoor material

It is very difficult to find clues to when humans first started using wood, however, some shreds of evidence suggest that wood has been used for the last 300,000 years, basically for fuel and construction. This versatile material is light and easy to work with, has good insulation properties, and efficiently bears compression and traction forces, therefore it is used as a structural, insulating, and surface material (Alapieti et al. 2020).

Wood, as indoor material, is generally used for two purposes i.e., construction and designing, and mostly both go along together, for example, in making furniture, floors, ceiling and walls (Alapieti et al. 2020). Surface of wood is frequently polished with some treatment to protect it from scratches, stains, weathering and pests. In fact, various chemicals are used during processing of wood surfaces, such as adhesives, pesticides, solvents, resin binders, waterproofing compounds, pigments and paints, and varnishes. Some of these chemicals can be toxic (Wang et al. 2015), for example, creosote, pentachlorophenol (PCP), and chromated copper arsenate (CCA) are carcinogenic (EPA 2015). In addition, various volatile organic compounds (VOC) are emitted during such treatments (wood processing) and cause respiratory or other health-related issues/illnesses. Therefore, less toxic compounds are being researched by scientists for wood treatments (EPA 2015).

In this scenario, the use of untreated wood can be appreciated. Moreover, to retain the natural properties of wood it should be free of any chemical treatments. The untreated wood is already used as an indoor material in form of non-contact furniture surfaces, ceilings, floors and some wall frames. Such untreated materials are more preferred to be recycled as compared to treated wood. Hence, the subject of our study is also describing the hygienic nature of untreated wood.

2.2.3. Wood and psychological wellbeing in the healthcare buildings

The natural environments have restorative effects on humans (Totaforti 2018). Some earlier studies on this subject found that the visual effect of nature leads to beneficial health effects in human subjects (Felippe et al. 2017). For example, it was seen, in hospitalized

patients who had undergone similar surgeries, that the group with a view to park recovered faster than the other having view of a building (Ulrich 1984). In the current scenario of rapidly urbanization world, humans spend more than 90 percent of their time indoors (Fell 2010; Ikei et al. 2018a). Therefore, adapting the indoor hospital environment to make it look more natural could be a significant restorative solution (Ohta et al. 2008; Andrade et al. 2017; Totaforti 2018). However, bringing the nature components indoor needs maintenance, for example, the plants inside the rooms require light and regular watering (Fell 2010), and also the use of natural elements as a decoration or ornamentation is neither cost-effective nor favored by stakeholders (Xue et al. 2019). Thus, the researchers are focusing on the overall architectural implementation of salutogenic and biophilic designs to play a restorative role in mitigating symptoms of psychological and mental discomfort, for both patients and medical staff in healthcare settings (Nyrud et al. 2014; Abdela and Soebarto 2018). The wood being an organic material is an important component of nature-based themes (Zhang et al. 2016), and even there is a widespread belief in the public about its naturalness (Zanetti et al. 2003; Kimura et al. 2011; Cronhjort et al. 2017; Burnard et al. 2017; Nakamura et al. 2019) and superior qualities (Rice 2004; Rice et al. 2007; Jiménez et al. 2016; Høibø et al. 2018; Lee et al. 2018; Larasatie et al. 2018). Moreover, the preference studies in Norwegian hospitals and healthcare buildings showed that the medical professional preferred the patient rooms with moderate level wooden interiors (Bringslimark and Nyrud 2010; Nyrud et al. 2010, 2014). Therefore, the psychological effects of wood inside the health institutes need to be explored more, because many psychological advantages of using wood in interiors have been documented, for example, the researchers have observed that the stress-relieving physiological and psychological indexes including the heart rate and diastolic blood pressure of subjects were better when the rooms were designed, finished or constructed using the wood material (Tsunetsugu et al. 2002, 2007; Sakuragawa et al. 2005; Kelz et al. 2011; Song and Fei 2016; Nakamura et al. 2019). Likewise, the comparative studies consisting of wooden and non-wooden indoor environment showed that the wooden environment inflicted the positive physiological regulation of visual, respiratory and autonomic nervous system, and

the workers in wooden rooms felt less tension and fatigue after a consecutive period of work and enjoyed a more delightful sense of color, odor and light (Zhang et al. 2016, 2017).

Generally, the above-mentioned physio-psychological effects are considered owing to the visual stimulations to moderately complex structures like wood (Fell 2010; Song and Zhao 2012; Watchman et al. 2016; Lipovac and Burnard 2020), but not very complex (Høibø and Nyrud 2010), for instance, the presence of too many knots in wood is neither good for the physical strength of wood and nor their visual appearance reduce the level of stress in subjects as much as plane wood surfaces do (Yoshida et al. 2016). Visually, the wood gives warm, elegant, innovative, energetic, beautiful, clear, bright, soft, natural, homely, inviting and peaceful perceptions to the viewers (Ridoutt et al. 2002; Rice et al. 2007; Song et al. 2017; Watchman et al. 2017; Strobel et al. 2017; Demattè et al. 2018; Poirier et al. 2019) because of its structure, generally having hue coloration, little UV reflection, low contrast and small deviation of distance between annual rings and arrangement of cells which reflect the light like parallel mirrors (Masuda and Nakamura 1990; Masuda 2004; Jafarian et al. 2016, 2018; Poirier et al. 2017; Demattè et al. 2018). Even though, most of these studies compared the wood with visually distinct materials such as plastic, leather, carpets, concrete, glass or stone, the comparison with similar material like laminate also show distinct psychological effects (Jiménez et al. 2015, 2016). A recent study by Burnard and Kutnar (2019) reported that the overall stress levels studied in the subjects were lower in the office-like environment in presence of oak wood than the control room without wood, however, no such effect was evident in the room with walnut wood. They also observed that the stress recovery, however, was not influenced by material type probably due to the short experimental period and lower sample size.

The tactile stimulations with wood have a different effect on subjects as compared to some other materials including steel, laminate, plastics or glass (Yu et al. 2006; Sakuragawa et al. 2008; Ikei et al. 2017a). In a study by Berger et al. (2006), the subjects were blindfolded and were asked to touch or walk on different surfaces and they evaluated wood surfaces as

warmer as and more enjoyable than the laminate version. In another study, when subjects were in physical contact with the materials, it was observed that changes in temperature of acrylic and steel plates, negatively affected the blood pressure, while in case of Japanese cypress, Japanese cedar or oak (*Quercus crispula*), it remained constant (Sakuragawa et al. 2008). Earlier, similar effects have been observed, where contact with a sawn surface of hinoki and sugi wood had a lesser negative effect on the pulse and systolic blood pressure as compared to a vinyl bag filled with cold water and stainless-steel board (Morikawa et al. 1998). Another study examined the physiological effects in subjects after blindly touching the palm on uncoated white oak, stainless steel, marble and tile. The results showed that the tactile stimulation with white oak significantly increased ln(HF)-reflected parasympathetic nervous and decreased the oxygen-hemoglobin concentration in the left/right prefrontal cortex relative to marble, tile, and stainless, concluding that touching the wood induces physiological relaxation (Ikei et al. 2017b). Similar effects were also observed when hinoki cypress (*Chamaecyparis obtusa*) was touched by feet sole and hand palm (Ikei et al. 2018b, a). The tactile sensation of wood changes after processing and finishing with some coatings, which may change the responsiveness towards this material (Ikei et al. 2017c). It has been reported that touching the natural and smooth pine and oak wood surfaces by finger gives a stronger positive emotional perception than coated surfaces (Bhatta et al. 2017). A recent study by (Lipovac et al. 2020) measured cognitive performance and the affective states of 16 subjects before and after spending 15 min at 10 desks with different surfaces including untreated, oiled, or lacquered oak or spruce solid wood, laminated or oak-veneered particleboard, glass, and mineral-filled thermoplastic composite. They observed that the affective states and cognitive performance in participants did not differ according to desk surfaces. Such findings were attributed to the low amount of wood material exposure.

The olfactory sensing of wood can also have a stress-relieving effect on humans (Azuma et al. 2016; Ikei et al. 2017a). It has been reported that the average scores of negative mood items e.g., confusion, fatigue, depression–dejection, tension-anxiety and anger-hostility, significantly decreased after spending half an hour in a wood-finished interior,

while the vinyl-finished interior had no significant effect on studied mood parameters (Saito et al. 2009). In a case-control study by Azuma et al. (2016), it was found that the inhalation of cedar emissions (Cedrol and β-eudesmol) slightly improved the vigor of participants. Previously, cedrol has also been reported to reduce heart rate, systolic and diastolic blood pressure, suppress sympathetic nervous activity, and enhance parasympathetic nervous system activity compared with the control condition (air) (Yada et al. 2007; Matsubara and Ohira 2018). The α-pinene and D-limonene are wood extracts known to induce relaxation effects on inhaling (Joung et al. 2014; Ikei et al. 2016). It is quite clear that the odor from wood depends on the type of chemicals present in it, intriguingly, the type of processing method can influence such emissions, for example, the emissions from air-dried Japanese cypress wood chips have been reported to reduce oxygen-hemoglobin concentrations in the prefrontal cortex of subjects, whereas the high-temperature-dried wood chips do not induce any such effect (Ikei et al. 2015). Moreover, the wood odor is also regarded as a pleasant smell (Schreiner et al. 2018; Demattè et al. 2018), which automatically gives an idea of the likeness of humans towards this material (Matsubara et al. 2017), contrarily, there are many wood chemicals which are regarded as unpleasant but their quantity is low (Liu et al. 2018).

Real healthcare building studies are scarce on this subject. Recently, (Kotradyova et al. 2019) performed a study in the waiting area of a hospital, where the visitors were asked questions to determine their perception regarding the wooden environments. The physio-psychlogical parameters were measured by recording the face expressions, heart rate, respiration rate, electrocardiogram, blood pressure and cortisol levels after exposure to wooden and non-wooden environments. The results showed that the presence of wooden architecture had a regenerative and positive impact on the human nervous system, through appealing aesthetics (color, texture, and structures), high contact comfort, pleasant smell and acoustic well-being in the experimental space.

2.2.4. Wood emissions and indoor hospital environment

As described in the chemical composition section, wood contains multiple VOC. Their emission is an important concern because they may contaminate the indoor air which people inhale in healthcare buildings (Capolongo and Settimo 2017; Hüppe et al. 2017). The most common of these VOC emitted from wood are terpenes, aliphatic aldehydes, and organic acids (Sassoli et al. 2017). These chemicals and their metabolites keep forming and degrading during the life of wood due to processing and weathering (Schreiner et al. 2018); for example, acetic acid originates as a result of the breaking of acetyl groups from hemicelluloses, whereas hexanoic acid is formed by the decay of fatty acids (Sassoli et al. 2017). The emission of VOC from wood depends upon the type, age, product and condition of wood (Sassoli et al. 2017), and on ambient temperature, humidity and ventilation conditions (Nore et al. 2017).

Many of the chemicals identified in wood may belong to the family of hazardous compounds, for example, formaldehyde, acetone and toluene (Jensen et al. 2001; Rahman et al. 2017). Luckily, their quantity and emission from wood are so low that no negative health effects are expected (Kirkeskov et al. 2009; Nyrud et al. 2012; Nore et al. 2017), even, some of them have positive psychological health effects (Joung et al. 2014; Ikei et al. 2016) and antimicrobial activity (Vainio-Kaila et al. 2017a). Although wood pellet storage rooms or wood product factories may pose an occupational hazard to the inhabitants and workers, possibly because of wood dust or synthetic chemicals (Al-Amili 2017), such places are not representative of residential buildings or healthcare institutes. Meanwhile, proper ventilation can eliminate this hazard of chemical gasses from closed environments (Rahman et al. 2017). In a health evaluation study of VOC from interior use wood products, it was observed that the highest emissions were of formaldehyde from rubberwood furniture and they measured 58, 42 and 33 $\mu g/m^3$ at 3, 10 and 28 days (Kirkeskov et al. 2009); these concentrations were lower than the recommended lowest concentration of interest (LCI) of <100 $\mu g/m^3$ at 28 days (Lathauwer 2014). The LCI is defined for each emitted compound based on the available reference values to prevent the health effects during long-term exposure to emissions from

building and decoration materials (ANSES 2016). In a total volatile organic compounds (TVOC) emission study of low energy wooden houses, the emission was recorded to be 2.1 mg/m^3, which falls within the safe limits of LCI, meaning, it does not cause a health risk to the occupants of building (Patkó et al. 2013).

The wood-based panels, such as particleboard and plywood, are generally perceived to have high VOC emissions compared to solid wood because of their manufacture with adhesives, e.g., the urea-formaldehyde resin that is used to bind the wood fibers (Yali et al. 2018). Consequently, most countries have regulations that limit emissions so that VOC concentrations are within safe limits (da Silva et al. 2016; Alapieti et al. 2017). For example, Nyrud et al. (2012) investigated the emissions of VOC from wooden wall panels of birch (*Betula pubescens*) and oak (*Quercus robur*) mounted in the hospital rooms. They reported that the use of wood in the furnishing of hospital rooms has a negligible influence on the TVOC content, therefore, wood is very unlikely to negatively affect the public health in hospital interiors in terms of air quality (Nyrud et al. 2012).

The reason for this low quantity of emissions can be attributed to the drying and processing of wood, which removes a vast number of chemical compounds (Kirkeskov et al. 2009). Moreover, the quantities of VOC can be higher than recommended limits in fresh wood (Salo 2017) and decrease over time (Lee et al. 2018), therefore it is recommended to give some time to new furniture or wood products to release VOC before employing them into use (Kirkeskov et al. 2009). The sinking or diffusion barrier effects of certain wood products are also considered to reduce or time-delay the emission of VOC (Niedermayer et al. 2013; Höllbacher et al. 2016; Nore et al. 2017; Huber et al. 2018; Kotradyova et al. 2019). Another factor, which reduces wood emissions, is the surface treatment that encapsulates the evaporation (Salo 2017). A 10-year follow-up study from Sweden showed that the use of PVC flooring is linked to a higher risk of asthma in children as compared to wooden flooring (Shu et al. 2014). However, a cross-sectional study showed that the use of hardwood wood flooring is positively linked to asthma and related symptoms in children aged 2 to 14 years

in 25 districts of 7 cities of northeast China in 2008–2009 (Dong et al. 2014). These findings were supported by another one-year retrospective cohort study from China, which showed that the use of solid wood flooring in homes could be a risk factor of asthma, wheeze and cough in children, as compared to laminate, tile or stone flooring (Zhang et al. 2018). The authors thought that the adhesives and bonding agents could be the culprits for some unwanted emissions that cause respiratory problems. Moreover, the risk factor of high humidity, due to damping or fungal contamination of wooden floors, cannot be omitted (Wang et al. 2017). Meanwhile, the type of wood and chemical treatments should be studied in similar studies to identify the main cause, because different wood species may have different emissions, as Zylkowski and Frihart, (2018) reported that the VOC from the adhesives used in North American wood products seemed to play a minor role because the Douglas fir plywood, southern pine plywood and oriented strand board, all had different types of VOC emissions even though they had employed similar adhesives and bonding agents (Zylkowski and Frihart 2018). In addition, the surfactants and paints, and even the cleaning agents and disinfectants in hospital conditions could be the core malefactors of hazardous emissions than the wood itself (Du et al. 2015; Bulian and Fragassa 2016; Apanpa-Qasim and Adeyi 2018; Su et al. 2018; Kotradyova et al. 2019) and they can cause the stress of asthma, allergies and sick building-related symptoms in the people staying or working there (Baurès et al. 2018; Rautiainen et al. 2019).

2.3. Healthcare-associated infections

Nosocomial or healthcare-associated infections (HAI) are transmitted to patients when they receive medical care in healthcare facilities. The World Health Organization (WHO) remarks HAI as the most common adverse events among hospitalized patients (WHO 2011).

2.3.1. Epidemiology

In general, the HAI concern 7% of the hospitalized patients in developed and 10% in developing countries (Khan et al. 2017). A study from European Union healthcare facilities

reported that from 2016 to 2017 the point prevalence of HAI was estimated to be 6.5% in acute care hospitals and about 4% in long-term care facilities. In addition, HAI episodes per year were estimated to be almost 9 million patients (Suetens et al. 2018). In Canada, the prevalence of patients with at least one HAI was 7.9% in 2017for 9.9% in 2002 and 11.3% in 2009 (Mitchell et al. 2019). At the moment, the CDC (Centers for Disease Control and Prevention) of the United States reports HAI in 3.1% of hospitalized patients (CDC 2019). The case-fatality rate in the case of HAI ranges from 2.3% to 14.4% depending on the type of infection (Al-Tawfiq and Tambyah 2014). HAI cause approximately 100,000 deaths in the US (CDC 2019), 37,000 direct deaths in Europe and 110,000 indirect deaths due to resulted complications of HAI (ECDC 2020).

2.3.2. Economic burden

In any case, HAI are responsible for increasing the economic burden on the healthcare system by prolonged staying time, disability and treatment cost (Cassini et al. 2016; Benenson et al. 2020). HAI in the US hospitals are associated with direct medical costs of $28.4 billion/year with an additional $12.4 billion coming as a cost from early deaths and lost productivity (CDC 2020a). They do not only cause social disturbance but are also highly important in transmission of antimicrobial resistance (AMR). Among all HAI, a high percentage of AMR marker was observed, for example, 30% of HAI caused by resistant microbes in acute and long-stay healthcare facilities (Suetens et al. 2018), and more than 50% in the intensive care units (Despotovic et al. 2020). The AMR microbes increase the mortality, readmissions, visits to the emergency department, and length of hospital stay compared to susceptible strains (Barrasa-Villar et al. 2017; Jia et al. 2019; Inagaki et al. 2019).

2.3.3. Types and etiologies of HAI

Generally, HAI are characterized as ventilator-associated pneumonia (VAP), central line-associated bloodstream infections, catheter-associated urinary tract infections, and surgical site infections (CDC 2020a). Among these, VAP is responsible for the highest

number of deaths followed by bloodstream infections (Cassini et al. 2016). The severity of these infections can range from mild to fatal depending upon the causative microorganism (e.g., bacteria, fungi or viruses) and the type of infection or system infected. The prevalence of these microbial agents differs depending upon patient populations, available medical facilities and condition of the indoor environment. Most notable etiological agents causing HAI can be listed as: *Staphylococcus aureus*, *Enterococcus* spp. (e.g., *E. faecalis, E. faecium*), *Escherichia coli*, *Candida* spp. (e.g., *C. albicans, C. glabrata*), *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Aspergillus fumigatus* (Haque et al. 2018). The viruses are also very important in the context of HAI, especially, the seasonal influenza virus, and the recent Severe Acute Respiratory Syndrome associated Coronavirus (COVID-19 virus).

2.3.4. Reservoirs and transmission: Role of surfaces

HAI spread through two main routes: contact (direct or indirect) and inhalation. In the contact transmission, patient, caregiver and environmental surfaces (medical instruments, the bed, door, furniture, exercise equipment, sink, etc.) can be considered as risk factors (Khan et al. 2017; Gontjes et al. 2020) and the survival of microbes in the environment depends upon the type of material used (Table 2.2). The aerosols can be responsible for the transmission of pathogens to the patients through the respiratory route or by infecting open wounds or surgical sites (Suleyman et al. 2018). Mirhoseini et al. (2016) reported that the hospital air could be contaminated (prevalence 3-34%) with β-lactam–resistant bacteria, predominantly by *Acinetobacter* spp., *A. baumannii*, and *Staphylococcus* spp. However, the air is not necessarily contaminated by the excretions of patients. A recent study showed higher contamination of outdoor air as compared to indoor air suggesting that the contamination of indoor air was from outdoor sources (Mirhoseini et al. 2020).

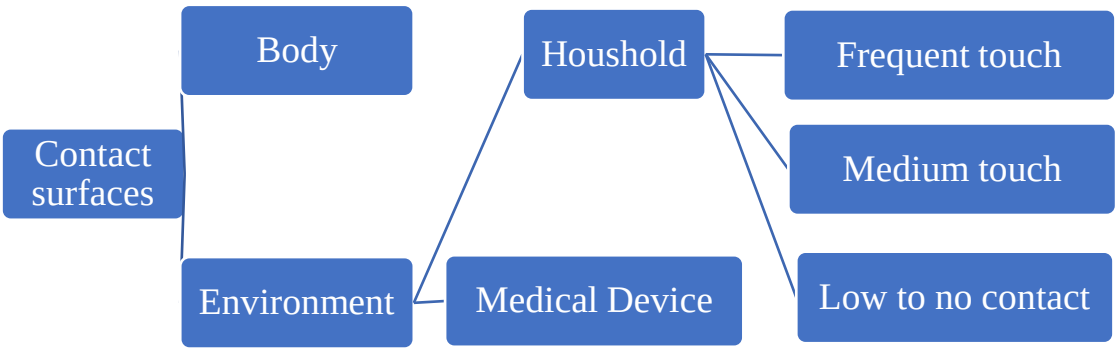


Figure 2.5. Classification of contact surfaces in healthcare buildings

The contact surfaces which may be contaminated with microbial agents causing HAI can be classified into different categories (Figure 2.5). First, the contaminated hands of a healthcare worker or patient's own body can be responsible for the transmission of pathogens. Secondly, cross-contamination *via* the hands of a caregiver, which became contaminated by encountering ill patients or contaminated environmental surfaces; such cross-contamination is responsible for 20 to 40% of HAI (Suleyman et al. 2018). The next factor is linked to surfaces and they can be categorized as surfaces of medical devices (catheters, surgical instruments, stethoscopes, electronic thermometers, blood pressure cuffs, infusion pumps, hemodialysis machines, etc.), and the household surfaces of the patient room, washroom and surrounding objects. The household surfaces can be further classified based on the frequency of touch as the high contact (bed railings, sink, tap, door handle, curtain, etc.) medium contact (furniture, window frame, floor, etc.) and low contact surfaces (ceilings, walls, pillars, etc.) (Suleyman et al. 2018).

The role of non-contact surfaces, such as ceilings, walls, floors, window frames, and some furniture is not documented as an important source of microbial transmission. However, these surfaces are feared to sustain the microbes which can transmit infections to individuals by accidental contacts or by contaminating the air during cleaning and construction works.

Table 2.2: Survival of the most common nosocomial pathogens on different conditions

Pathogen	Survival time [a]	Survival on wood
Acinetobacter spp.	3 days to 1 year (*in-vitro*). 36 days within biofilm vs. 15 days for non-biofilm-forming strains	N/A
Aspergillus spp.	>30 days	> 28 days [b, c]
Candida spp.	>30 days	N/A
Clostridium difficile	5 months for spores. 15 min (dry surface) and 6 h (moist surface) for vegetative form	N/A
Coronavirus SARS 1 and 2	<5 min up to 24 h (on paper), 5–28 days (at room temp.), 28 days (at 4°C)	≤24 h SARS-Cov-2 [d] 8 to ≤96 h SARS-Cov-1 [e]
Enterococcus spp.	5 days to 30 months	>7 days (flood water)[f]
Escherichia coli	1.5 h to 16 months	1h (food contact) [g], >7 days (flood water)[f], 28 days (farm surface) [h]
Influenza virus	1–28 days (strain dependent), 1–3 days (on banknotes), up to 8 days (admixed in mucous)	24-48 h [i], 1-28 days (depending upon environmental conditions) [j]
Klebsiella spp.	2 h to >30 months, 144 h in detergent solution	N/A
Pseudomonas aeruginosa	6 h up to 16 months (5 weeks on dry floor and few hours in aerosol)	N/A
Staphylococcus aureus	7 days up to 1 year (*in-vitro*), 9–12 days (plastic surfaces), 72 h (stainless steel), 6 h (copper), 28 days (dry mops) and 14 days (in water)	> 28 days on hardwood floor [b]

N/A= no data found; [a] (Kramer and Assadian 2014); [b] (Gupta et al. 2017); [c] (Mensah-Attipoe et al. 2016); [d] (van Doremalen et al. 2020; Chin et al. 2020); [e] (Duan et al. 2003); [f] (Taylor et al. 2013); [g] (Aviat et al. 2020) ; [h] (Williams et al. 2005); [i] (Tiwari et al. 2006; Sakaguchi et al. 2010; Oxford et al. 2014); [j] (Guan et al. 2017)

2.4. Up to date knowledge regarding hygienic and antimicrobial behavior of wood

In the last two decades, several studies proved that wood surfaces are better able to inhibit microbial growth and minimize certain microbial transmission (Tiwari et al. 2006; Laireiter et al. 2013; Montibus et al. 2016; Ismail et al. 2017; Pailhories et al. 2017) and aid the psychological welfare of inhabitants when used for indoor construction (Nyrud and Bringslimark 2010; Burnard and Kutnar 2015; Dematte et al. 2018; Kotradyova et al. 2019). In an outdoor environment, wood contains a microbial population according to its moisture content, decay status and duration of storage after cutting the tree (Dutkiewicz et al. 1992). These microbes are not usual human pathogens but the parts of the total flora of microorganisms commonly found in soil and on plants (Cosenza et al. 1970; Munir et al. 2014). Nevertheless, the types of microbe present, determine the interrelated population diversity because of their symbiotic relations (Johnston et al. 2016).

Hygienic characteristics of wood are often misunderstood because of their organic, porous and moisture-absorbing nature. In fact, these properties could be positives because the organic nature of wood makes it environment-friendly, the absorption potential of wood can cause desiccation conditions for microbes, and the presence of extractives can kill harmful microorganisms (Milling et al. 2005b; Alpert 2006). Such implications may allow decreasing the use of chemical agents for cleaning operations, which are a big concern regarding chemical hazards and AMR (Obe et al. 2018; Fahimipour et al. 2018). There is a gap of knowledge to relate the antimicrobial nature of wood to its applications as a suitable hygienic surface for interior constructions.

These data of this section have been published as a review article (Munir et al. 2019f) and it describes the microbe inhibiting physicochemical strategies of untreated wood material. It summarizes the antimicrobial mechanism of wood involving porous structure, moisture content and chemical profile.

2.4.1. Is porosity a shelter for microbes?

The complex porous structure of wood is generally thought to retain microbes and make cleaning difficult because of the probability that bacteria meets disinfectant is very low. Ultimately, the wood surfaces are considered to be more contaminated than other, non-porous surfaces (Gilbert and Watson 1971; Abrishami et al. 1994). Wood surfaces indeed retain bacteria but it does not strictly mean that microorganisms are then, necessarily, transferred to another material in contact with wood (Ismail et al. 2015). For instance, the wood absorbs inoculum more rapidly as compared to other smooth materials (Soares et al. 2012) and the difficulty to recover microbes from wood surfaces implies that these organisms are stuck inside wood structures (Moore et al. 2007). Thus, it can be assumed that these microorganisms do not contaminate the contact objects like food or hands (Carpentier 1997; Montibus et al. 2016; Ismail et al. 2017). This phenomenon was confirmed in a later study investigating *E. coli* and *L. monocytogenes* counts which decreased faster on pine heartwood as compared to a glass surface (Vainio-Kaila et al. 2011). In their study, when wood pieces that had been recovered after a period of inoculation were resuspended in a broth and incubated for a day, and still no colonies were observed in the suspension. However, further comparative survival studies are needed to recover microbes from different cuttings of wood.

The porosity helps in the drying process of wood, contrarily, non-porous materials take a longer time to get dry (Gehrig et al. 2000). Chiu et al. (2006) reported that *Vibrio parahaemolyticus* survived better on smooth surfaces (plastic, stainless steel and glazed ceramic) as compared to porous material (bamboo and wood), probably because smooth surfaces could maintain higher surface moisture conditions for a longer time (Shi et al. 2017).

Porosity of wood material varies in different planes of cutting (Boucher et al. 1998). Furthermore, the presence of more pores could result in more exposure of extractives from cut cells and deeper retention of bacteria inside the wood. Prechter et al. (2002) studied the penetration depth of *E. coli* and spores of *B. subtilis* in wooden cutting boards in LT and RT directions. They observed that bacteria and spores could enter deeper (around 3 mm) in RT cuttings than in longitudinal cut woods; a deeper penetration implies a lower threat of

recontamination. Moreover, the diffusion of antimicrobial extractives is likely to faster in the longitudinal direction, i.e., an RT face (Pailhories et al. 2017). In contrast, boards with LT or LR faces were easier to clean because of shallow and wider openings on the surface (Dubreil et al. 2018). Further microscopic studies are needed to provide information about the microbes stuck inside the structures of wood material with respect to its grain direction.

Contrarily, the porosity not only offers difficulties in microbial recovery but may also provide shelter to some of them. The study of Boucher et al. (1998) reported that the *Campylobacter jejuni* cells, when stressed by aeration of the liquid culture medium, were protected from death when a block of beech wood was present in the broth. They did not observe any protective effect by using wood chemicals (free radical scavengers) or sawdust which means access to the physical structure of wood, to be precise sufficiently small pores (around 16 μm) and at least 4 mm thickness, was necessary for the protection of cells. Interestingly, the deeply scored plastic blocks did not enhance the survival of cells in aerated broths. In this case, wooden pieces were kept in broth, which eliminates the possibility of desiccation effect owing to porosity that may have resulted in survival of bacteria on wood.

2.4.2. Do the hygroscopicity and capillary action dry out the bacteria?

The hygroscopicity of wood is the property of taking moisture from environment. It is influenced by relative humidity and temperature of the environment (Munir et al. 2019d), and the free water, bound water and fiber saturation point of wood, which also determines the shrinkage and swelling of wood (Ramananantoandro et al. 2018). The porous structure and hygroscopic characteristics of wood can lead to desiccation of bacteria (Aviat et al. 2016). Most bacteria are desiccation-sensitive and require a water potential of -2.8 MPa or less for growth in wood (Milling et al. 2005b; Alpert 2006). It is significantly above the moisture content of air-dried wood stored indoors (Thybring et al. 2018), therefore, the properly dried wood does not offer enough water for microbial growth and multiplication (Milling et al. 2005a; Stienen et al. 2014).

The hygroscopicity of wood leads to faster absorption of moisture as compared to other non-porous contact surfaces, therefore, the microbes survive longer on smooth and non-absorptive surfaces such as metal and plastics (Chiu et al. 2006; Xi et al. 2013). Coughenour (2009) observed that Methicillin-Resistant *S. aureus* (MRSA) survived longer on plastic, vinyl, flannel cloth and glass as compared to wood surface. In another study, the turkey coryza agent (*Bordetella spp*.) survived for shortest period on wood as compared to aluminum, glass, dust and feces (Cimiotti et al. 1982).

Once the fiber saturation point is reached the wood does not absorb more moisture, therefore the hygroscopic antimicrobial potential may decrease (Mousavi et al. 1998). Gehrig et al. (2000) studied the survival of *E. coli* on wood and polyethylene by comparing bacterial counts after manual and machine washing of these surfaces and 15 hours of storage at room temperature. It was observed that both wood and polyethylene showed very high counts of bacteria in high moisture conditions. However, bacterial number was lower on wood in a drier environment. This effect was attributed to the faster drying potential of wood, particularly the drainage capacity, as compared to polyethylene surface. If wood surfaces are exposed to external weathering conditions, especially, abundant rain and humidity levels, the passive effect of wood against microbes may decrease. Williams et al. (2005) observed that the *E. coli* O157 persisted greater on wood than on galvanized steel, on the common farmyard surfaces, for a considerable length of time, under high moisture environmental conditions.

2.4.3. Microbial adherence and biofilm formation on wood surface

The adherence of microbes to a substrate is a complex phenomenon and it is the first step to biofilm formation (Tomičić et al. 2017). This bonding is carried out by van der Waals, electrostatic and acid–base interactions, which depend on the physicochemical properties of the microbe and substrate, especially hydrophobicity, surface charge, and electron donor–electron acceptor properties (Soumya et al. 2013). Wood can serve as a support material for biofilm formation of such microbes which use cellulose as nutrition for survival (Asri et al. 2018). However, hygienically important microbes can show different results regarding their adhesion. Vainio-Kaila et al. (2011) observed less CFU of *E. coli* and *L. monocytogenes* on

pine wood surface as compared to glass, and on the next day of study, still wood showed lower microbial counts. The finding suggested that there was no significant adherence of the bacterial cells on the porous surface of pine.

Dantas et al. (2018) experimented with microbial transfer of 10 biofilm-forming *S. enteriditis* strains from chicken meat to cucumber *via* glass, plastic and wood cutting boards. The formation of biofilm was higher on wood (60%), followed by plastic (40%) and glass (10%). Once the biofilm was formed, they are difficult to clean and disinfect on surfaces, and the microbial transfer from cutting boards to cucumbers was also higher in wood. However, in cheese-making process, the presence of lactic acid bacteria counters the adherence of many pathogens including *Listeria* spp., *Salmonella* and other *Enterobacteriaceae* (Galinari et al. 2014; Cruciata et al. 2018).

The presence of biofilms from natural wood flora may stop the growth of some harmful organisms. Therefore, this factor should be considered for microbial safety in hygienic surfaces, such as cheese ripening wooden boards and biocontrol for nosocomial pathogens in hospital environment (Lortal et al. 2009, 2014; Settanni et al. 2012; Didienne et al. 2012; Di Grigoli et al. 2015; Gaglio et al. 2015; Scatassa et al. 2015; Licitra et al. 2017). Mariani et al. (2011) tested the fate of two *L. monocytogenes* strains, over time as a function of the presence of a native biofilm, the farmhouse origin of cheeses, and the wooden shelves properties. In presence of native microbial flora on the shelves, deposited populations of *L. monocytogenes* remained stable or even decreased by up to 2 log10 (CFU/cm2) after 12 days of incubation at 15°C in all tested conditions. By contrast, *L. monocytogenes* populations increased by up to 4 log10 (CFU/cm2) when the resident biofilm was thermally inactivated, suggesting a microbial origin of the observed inhibitory effect. In a similar study, no inhibitory compounds by biofilm microflora were observed. Therefore, this reduction in *L. monocytogenes* counts can be attributed to "Jameson effect" according to the nutrient consumption and exhaustion by competitive microorganisms (Guillier et al. 2008; Ye et al. 2018; Cruciata et al. 2018). This type of effect can be used on wood for treatment with

probiotic type microorganisms and their bio-surfactants, which may antagonize the growth of nosocomial pathogens on inanimate surfaces (Vandini et al. 2014).

In any case, the biofilms models on wood are needed to study the microbial survival and efficacy of cleaning agents. However, there are no standard methods available to form biofilm on wood without changing the humidity levels of untreated wood used in construction.

2.4.4. Hygienic suitability of aged wood surfaces

Wood is an organic material that changes its structure and properties over time under different use and weathering conditions (Buchner et al. 2019). In general, the wood surfaces get rough and cracked with time and thus are feared to entrap bacteria that can cause hygienic risk (Gilbert and Watson 1971; Welker et al. 1997). The studies have shown, however, that the weathering conditions affect other materials too, and scarred wood surfaces have been seen to perform better than others in use surfaces like plastic, regarding the survival of microbes. For example, the electron microscopy revealed that the cuts on wood surfaces open in the drying process and cleaning also becomes easier (Gehrig et al. 2000), at least not more difficult as compared to plastic (Boursillon and Riethmüller 2007; Lucke and Skowyrska 2015). Meanwhile, under similar circumstances, the plastic surface cuts have a closing structure that can provide shelter to microbes (Gehrig et al. 2000). Koch et al. (2002) also reported that artificially aged plastic surfaces supported more bacterial survival as compared to that of wood. Gough and Dodd (1998) assessed the survival of *Salmonella* Typhimurium persistence on food preparation surfaces, wood and plastic chopping boards both new and after heavy scoring. Survival was assessed by counting the CFU of *S.* Typhimurium recovered after rinsing the inoculum off the board surface followed by contact plates. Recovery of the board inoculum from the rinse diluent was significantly greater from plastic than wood, and from untreated than scored boards. However, the disinfection was more readily carried out on plastic than both types of wood boards.

In the case of wood material used inside the healthcare buildings, the wooden pores may be filled with dirt and organic matter which can ultimately lessen the porosity-based antimicrobial advantages of wood material (Kotradyova et al. 2019).

As previously described, wood has extractives that may act as antimicrobial agents. Some of these extractives are VOC with antimicrobial activity (Vainio-Kaila et al. 2017a) and over time, these compounds may diminish, leaving wood less potent against microbes. Even the use of chemicals for surface disinfection (Kotradyova et al. 2019) or heat treatments such as steaming (Imhof et al. 2017) may change the chemical profile of wood. Mariani et al. (2007) studied the wooden shelves used in cheese making and found that the age of materials did not significantly impact the water activity, pH and salt concentration, and neither on the major microflora, such as, *Leuconostoc sp.*, facultative heterofermentative *Lactobacilli*, *Staphylococci*, *Enterococci* and *Pseudomonads*. Consequently, the effect of treatments and aging on antimicrobial behavior of wood needs further investigation.

2.4.5. Contact time and contamination rate from wood surfaces

Wood absorbs moisture and liquid microbial inoculum rapidly compared to non-absorptive materials, leading to lower recovery concentration on contact from wood surface, for example touching wood with hand or to the food which is prepared on the surface. Most of the studies regarding the microbial transfer and contact time with wood material are conducted considering wood as a food contact material. However, these studies provide guidelines regarding the survival of hygienically important pathogens, thus they can be implied to healthcare-associated pathogens too.

Regarding the transfer time of microbes from contaminating material (food or hands) to contaminated surface, Miller et al. (1996) observed that swabbing recovered a non-significant difference in the bacterial numbers after short contact time (0 and 90 min) of placing ground beef onto plastic and hardwood cutting boards at room temperature. On the other hand, the studies considering inoculation of microbes on surfaces could be considered as longer contact time of microbes on wood, thus the results also differ accordingly. Revol-Junelles et al. (2005) inoculated *E. coli* cells and *Bacillus cereus* spores on surfaces and

observed that they became metabolically inactive faster on dry poplar wood as compared to glass surface on room temperature with the prolonged contact time, which made their viable contact recovery very low. Moore et al. (2007) reported that the number of bacteria recovered from Formica and stainless steel was not only higher than polypropylene or wood, but regardless of application medium or holding time, the transfer to the model food was also high.

Similarly, the transfer of microbes from wood surfaces to the contact medium also directly depends upon contact time (Miranda and Schaffner 2016). For example, Dawson et al. (2007) reported that transfer rates of *Salmonella* Typhimurium from carpet, tile and wood to bologna rose with an increase in the brief contact time of 5, 30 and 60 s. However, for longer contact time the transfer rate may be lower because of the antimicrobial activity of wood, for example, Mohammad and Al-Taee (2014) observed that the transferrable quantities of *E. coli* and *Salmonella* spp. after 5 and 15 minutes from the surface to meat and vegetables were higher on glass, plastic and steel compared to wood. Montibus et al. (2016) studied the transfer rate of *Penicillium expansum* from poplar crates and plastic surfaces to apples during one week of trial. They observed that the transfer rate continued to decrease on wooden surfaces during the study period while it was constant or increasing from plastic. Goh et al. (2014) experimented with the contact transfer of *L. monocytogenes* from wooden and plastic cutting boards to the uncooked and cooked meat. For this study, the chicken meat was contaminated with 200 µl solution at 7 log CFU/ml of bacteria and the contacted with test surfaces for 5 s. Later, the cooked and uncooked chicken was contacted on contaminated surfaces to determine the transfer. After 1 h of holding time, the transfer of microbes from meat to wood (6%) and wood to meat (11%) was lower than that of meat to plastic (71.8%) and plastic to meat (25%).

To further investigate the role of antimicrobial properties of wood on contact time transmission, comparative studies are needed to investigate the microbial survival on wood surfaces with respect to time.

2.4.6. Are wood surfaces difficult to clean?

As a misconception, the absorbance potential and porous nature of wood are generally considered as a hindrance in the cleaning process. However, no specific studies are mentioning the washing and cleaning of wood surfaces in healthcare buildings. Therefore, the information is adapted from the food industry.

Many studies have shown that wood surfaces are not more difficult to clean as compared to other non-porous surfaces (Ak et al. 1994a, b; Lucke and Skowyrska 2015). Ordinary washings of wood and plastic preparation surfaces in the kitchen (Ak et al. 1994a, b; Koch et al. 2002) and in industrial settings (Zangerl et al. 2010; Imhof et al. 2017) give satisfactory results regarding the elimination of hygienically important microbes. Moreover, such washing does not decrease the overall hygienic nature of wood material (Cruciata et al. 2018). The cleaning efficiency would generally depend upon the disinfection or cleaning method used. Deza et al. (2007) inoculated the pine and plastic pieces with *E. coli, L. monocytogenes, P. aeruginosa,* and *S. aureus* at 9 to 10 log CFU/ml. They studied the cleaning of these materials with disinfectant solutions (sodium hypochlorite, acidic electrolyzed water and neutral electrolyzed water). It was found that all the solutions decreased the microbial count to a minimum detection limit on plastic after one minute while for wood it took 5 minutes. DeVere and Purchase (2007) reported the survival of *E. coli* and *S. aureus* on four different surfaces cleaned with four types of cleaning agents including wipes and sprays. The microbial solution was inoculated on all surfaces and dried for 30, 60 and 120 min before being cleaned with antibacterial products. The results showed that wood was more efficiently cleaned with all types of products as compared to glass, plastic and antibacterial plastic surfaces. Lucke and Skowyrska (2015) also reported that after proper cleaning, the microbial counts were the same on polyethylene, maple and beech cutting boards, suggesting that the wood material is not worse in cleanability than commonly used plastic.

Some microorganisms could be difficult to clean on wood material, for example, C. *jejuni* has been reported to be protected by the physical structure of wood (Boucher et al.

1998). Acuff et al. (1986) reported the hand washing of cutting boards did not remove bacteria from them, however washing with detergent on the dishwasher removed the *C. jejuni*. Therefore, attention should be given while dealing with *Campylobacter* contaminated food products on wooden surfaces. Thormar and Hilmarsson (2010) observed that the viable *Campylobacter* counts were reduced below the detectable level on plastic and wooden board surfaces after treatment with monocaprin emulsions for 2 min. Al-Qadiri et al. (2016) reported that *C. jejuni*, *Salmonella* Typhimurium, *E. coli* O157:H7, *L. monocytogenes* and *S. aureus* counts were significantly reduced both on wooden and plastic cutting boards after 1 to 5 minutes of treatment with neutral electrolyzed water, quaternary ammonium, and lactic acid-based solutions.

2.4.7. Do the species and part of wood have a role in antimicrobial behavior?

Every wood species has unique anatomy and chemistry which leads to specific action against microbes (Alañón et al. 2015; Munir et al. 2019d). The studies have shown these variations of antimicrobial properties of wood as shown in Table 2.3. Johnston et al. (2001) tested the antimicrobial activities of essential oils extracted from the wood of Alaska cedar (*Chamaecyparis nootkatensis*), western juniper (*Juniperus occidentalis*) and Douglas fir (*Pseudotsuga menziesii*), as well as, methanol extracts of ponderosa pine (*Pinus ponderosa*) and western red cedar (*Thuja plicata*) against *Clostridium perfringens, Fusobacterium necrophorum, Candida albicans and Actinomyces bovis,* which are common causes of multiple infections in farm animals. The extracts of western juniper and Alaska cedar exhibited strong growth inhibitory activities against all tested pathogens while of the Douglas fir showed activity only against *A. bovis*. Regarding the level of beneficial lactic acid bacteria on wooden vats used in dairy processing, Cruciata et al. (2018) reported that the level of these microbes varied depending on the type of wood species being used. For instance, the high levels were registered on the surfaces of cedar, ash, walnut and poplar vats. Within this bacterial group, *Enterococci* were only detected on cedar and cherry woods.

Table 2.3: Grading of wood species depending upon the hygienic suitability

Reference	Bacteria	Ranking
(Laireiter et al. 2013)	*S. aureus*, *Pseudomonas aeruginosa*, *Enterobacter faecium*, and *Bacillus subtilis*	Pine > larch
(Milling et al. 2005a)	*E. coli* and	Pine = oak = larch > maple > spruce > beech > polar
	E. faecium	Pine = oak > larch = maple = spruce = beech = polar
(Koch et al. 2002)	*B. subtilis* and *P. fluorescens*	Oak > spruce
(Milling et al. 2005b)	Poultry manure flora	Pine > larch = maple
(Miller et al. 1996)	*E. coli* 0157:H7	White ash > red oak > black cherry > maple
(Schönwälder et al. 2002)	*E. coli* and *E. faecium*	Pine > polar = beech
(Munir et al. 2017)	*S. aureus*, *P. aeruginosa*, and *Acinetobacter baumannii*	Oak > Douglas fir = pine > poplar
(Vainio-Kaila et al. 2013, 2015, 2017b)	*S. aureus*, *E. coli*, *E. faecalis*, *Streptococcus pneuminae*	Pine > spruce

Wood is a complex material having different structural and chemical composition from different parts of a tree. Therefore, the studies have shown that the extractives from bark, heartwood and sapwood have different effects on microbes (Johnston et al. 2001; Laireiter et al. 2013; Vainio-Kaila et al. 2015, 2017b).

2.4.8. Role of biochemical profile and emissions of wood to the antimicrobial activity

The wood contains many types of extractives that principally protect it against microbial degradation. The antimicrobial chemicals include tannins, phenolic acids, flavenoids and terpenoids etc. (Valette et al. 2017). The mode of action of different wood chemicals can be seen in Table 2.4.

Table 2.4: Antimicrobial actions of wood chemicals against microbes*

Target	Wood chemicals
Cell wall and cell membrane	Flavonoids, tannins, aldehydes, phenolic acids, terpenoids, alkaloids, terpenes
Nucleic acid	Flavonoids, aldehydes, alkaloids
Metals metabolism	Tannins
Protein synthesis	Aldehydes, tannins
Energy metabolism	Flavonoids, phenolic acids
Adhesion and Biofilm formation	Phenolic acids, quinones

*The data were adapted from (Silva and Fernandes Júnior 2010; Teodoro et al. 2015; Ahmad et al. 2015; Valette et al. 2017).

As table 2.4 shows, many wood metabolites affect the microbial cell wall and cell membrane, the difference of membrane structure among different types of microorganisms may give them support or vulnerability to this antimicrobial effect (Silhavy et al. 2010). For example, the Gram-negative bacteria, *E. coli*, survives less on wood comparing to Gram-positive, *E. faecium*, isolates (Schönwälder et al. 2002). However, the extractive action of wood is stronger against multiple Gram-positive microbes *(S. aureus* and *E. faecium*) as compared to Gram-negative bacteria (*E. coli*) (Vainio-Kaila et al. 2013; Laireiter et al. 2013), which shows that probably, the physical microbial effect of wood is stronger against Gram-negative, while the chemical effect is stronger against Gram-positive bacteria. It might be because the Gram-positive bacteria have thicker cell wall which might provide them shield

against the desiccation effect of wood. Unlike Gram negative bacteria, the Gram-positive bacteria lack the outer membrane containing lipopolysaccharide (Silhavy et al. 2010; Riaz et al. 2017), which may render them prone to chemical action of wood metabolites. Further research is needed to justify the difference of survival of different microbes on similar wood types.

The VOCs from wood have antimicrobial properties and their loss may decrease the antimicrobial properties which are linked to the direct effect of these chemicals (Vainio-Kaila et al. 2013). If the other non-volatile extractives are responsible for antimicrobial activity the emissions would not change this behavior; for example, it has been reported that the age and storage time of pinewood did not influence its antimicrobial behavior (Schönwälder et al. 2002). Therefore, studies are needed to investigate the factors which may reduce the VOC linked antimicrobial properties of wood. These factors may include the heat treatment, environmental humidity and the storage time of wood material.

2.5. Methods to test hygienic and antimicrobial properties of wood

This section contains the literature review on the available methods to test the antimicrobial and hygienic properties of wood material and it has been published as a review article (Munir et al. 2020a).

According to the literature findings, it is possible to categorize the methods into two broad groups based on the form of test material used e.g., solid wood or extractives. Furthermore, they were sub-classified into different groups according to the methodology, as shown in Figure 2.6.

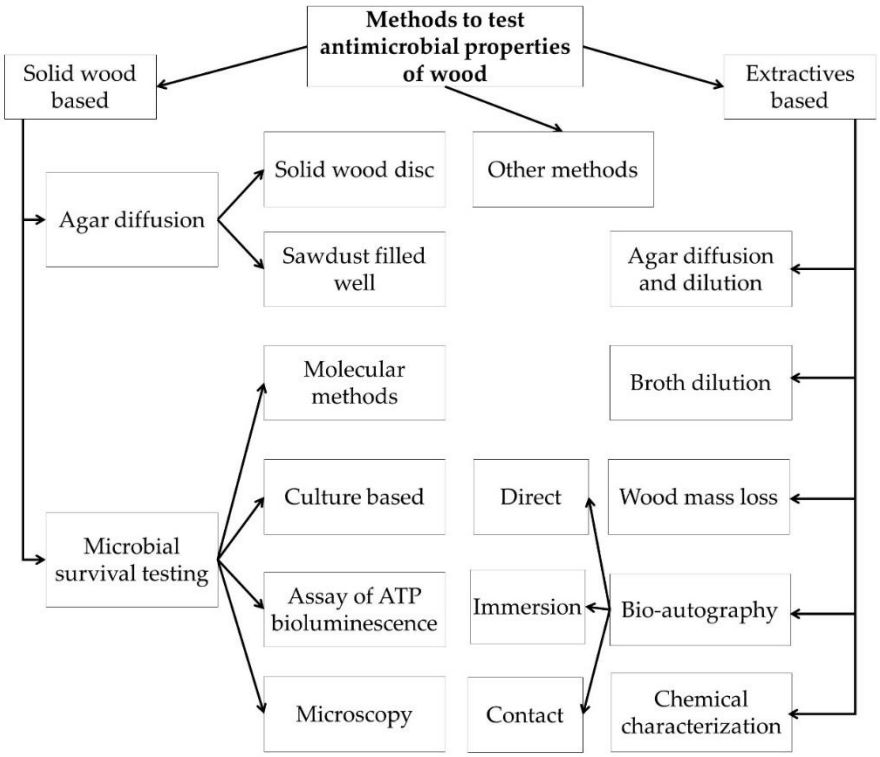


Figure 2. 6: Flow diagram outlining the classification of methods to study the antimicrobial and hygienic properties of wood material.

2.5.1. Direct Methods

In such methods, microbial survival is investigated in direct contact with wood samples. These methods give a better understanding of the role of the physical structure of wood as a microorganism inhibitor. In general, they are easy to implement because usually no chemical handling or complicated preparation steps are required.

For research purposes, the direct methods may require an extra step of sterilization of the test material. Generally, the wood test samples are sterilized by autoclaving, ultraviolet irradiation, gamma radiation, fumigation, or by disinfection with alcohols (Revol-Junelles et al. 2005; Deza et al. 2007; Goh et al. 2014; Gonçalves et al. 2016; Ismail et al. 2017; Pailhories et al. 2017; Nikiforuk et al. 2017; El-Hefny et al. 2020). It would be interesting to know if the sterilization methods interfere with the antimicrobial properties of wood material. For example, heat treatment may alter the chemical composition of the wood surfaces (Vainio-Kaila et al. 2013), and immersing wood pieces in ethanol may extract some compounds from them, thus, influencing the outcomes of the antimicrobial research.

2.5.1.1.Agar diffusion method

The agar diffusion method is commonly used in routine for antibiotic susceptibility testing in clinical microbiology laboratories (Bonnet et al. 2012). In this technique, an agar plate is conventionally used and is inoculated with a standardized bacterial or fungal suspension. The test sample, containing the potential active ingredients (added as a disc or deposited in a well created in the agar or a cylinder (plug)) is placed on the inoculated agar plate (CLSI 2018; EUCAST 2020). When such a system is incubated at a specific temperature, more often 37 °C, for a recommended time, the observation of growth inhibition around the test sample indicates the susceptibility of the incubated microbe (Bonnet et al. 2012). This growth inhibition diameter is dependent on the antimicrobial susceptibility of an organism, the diffusion potential of testing antimicrobial agents in agar medium, and the efficacy of the active compounds (Rios et al. 1988; Jorgensen and Ferraro 2009).

The choice of the agar medium depends upon the type of microorganism being tested in the experiment. For many microbes, the recommendations have been defined by international organizations, such as the European Committee for Antimicrobial Susceptibility Testing (EUCAST 2020) and the Clinical and Laboratory Standards Institute (CLSI 2018). In general, the antimicrobial susceptibility of bacteria is tested on Mueller–Hinton agar (Pailhories et al. 2017). However, plate count agar (PCA) (Montibus et al. 2016), Iso-Sensitest® agar (Khan et al. 2000), tryptone soy agar (Plumed-Ferrer et al. 2013), and other nutrient mediums have also been used (Nakmee et al. 2016). Antimycogram experiments generally involve the use of Sabouraud agar (Khan et al. 2000). However, malt agar (Montibus et al. 2016) and potato dextrose agar (PDA) are also employed for this purpose (Subhashini et al. 2016), depending upon the type of species being tested (Kim et al. 2009).

The incubation period depends on the growth requirement conditions of the tested microorganisms. Generally, most of the bacterial incubations vary from 18 to 24 hours at 37 °C, while in case of fungi, 48 to 72 hours are recommended at room temperature (25-30 °C) (Das et al. 2010; Vek et al. 2013; Fernández-Agulló et al. 2015). Then, the zone of inhibition (diameter) is measured to the nearest mm (Valimaa et al. 2007).

2.5.1.1.1. Direct wood disc agar diffusion method (antiboisgram)

In our previous studies, we reported a direct diffusion method to screen the bacterial growth inhibition potential of multiple wood species (Figure 2.7) (Pailhories et al. 2017; Munir et al. 2019a). In this method, a Mueller–Hinton agar plate was inoculated with a 0.5 McFarland bacterial suspension via swab streaking. Then, wood test samples with a disc form

(2-4 mm thickness and 9-10 mm diameter) were directly placed on it. After an incubation time of 18-24 hours, the diameters of the inhibition zones were manually measured by two different readers (Annex 1.3). (Laireiter et al. 2013; Munir et al. 2019a) used this method as a qualitative screening method and the presence of a zone of inhibition was considered as a positive antimicrobial activity, while Pailhories et al. (2017) further used this method and took into account the variability of the method for the interpretation of the results. This method can also be modified to test the antimicrobial properties and durability of treated solid wood samples (5 mm) against different fungi and bacteria (Mansour and Salem 2015; Salem et al. 2016d, c, 2019). Recently, a similar approach was applied by treating the *Melia azedarach* wood samples with acetone extract of *Withania somnifera* Fruit. Subsequently, the antimicrobial action was investigated against *Agrobacterium tumefaciens, Dickeya solani, Erwinia amylovora, Pseudomonas cichorii, Serratia pylumthica, Fusarium culmorum,* and *Rhizoctonia solani*. The positive antibacterial and antifungal responses were observed in the form of inhibition zones around samples on agar (El-Hefny et al. 2020).

This method has the prospect to test the effect of solid wood variables (part of tree, species of tree, physical treatment of sample, cutting direction, storage time) on the diffusion-based antimicrobial activity (Laireiter et al. 2013; Pailhories et al. 2017). The direct diffusion method can give screening results very quickly and even the results of this technique can be interpreted in the absence of wood sterilization (Munir et al. 2019a). It can also help to determine the influence of antimicrobial potential-affecting variables including the species of tree or part of a tree.

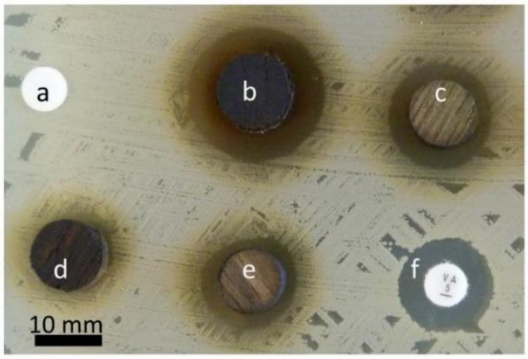


Figure 2. 7 : An antibiogram showing the results of filter paper discs (6 mm) and different oak tree wood discs (10 × 3 mm) tested against *Staphylococcus aureus* ATCC 29213 inoculated on a Mueller–Hinton agar plate: (a) negative control inert filter paper disc; **(b)** oak wood transversal cut; (**c - e**) oak wood longitudinal cut, and **(f)** positive control antibiotic (Vancomycin (Oxoid, Basingstoke, United Kingdom))

2.5.1.1.2. *Sawdust-Filled Well Diffusion Method*

This method was also uniquely developed by our team. If the wood sample is only available in particulate and sawdust form, which is a common case in animal husbandry practices, then it can be placed in a well, created in the agar (Munir et al. 2019d, e). This method is a slight modification of the agar diffusion method, where uniform-sized wells (5 to 10 mm) are punched aseptically with a sterile borer or a tip on agar plate (Fernández-Agulló et al. 2015). Then, the sample particles are filled in these holes, and the system is incubated. The diameters of the zone of inhibition around these wells are measured as an indication of antimicrobial action (Munir et al. 2019a) (Figure 2.8).

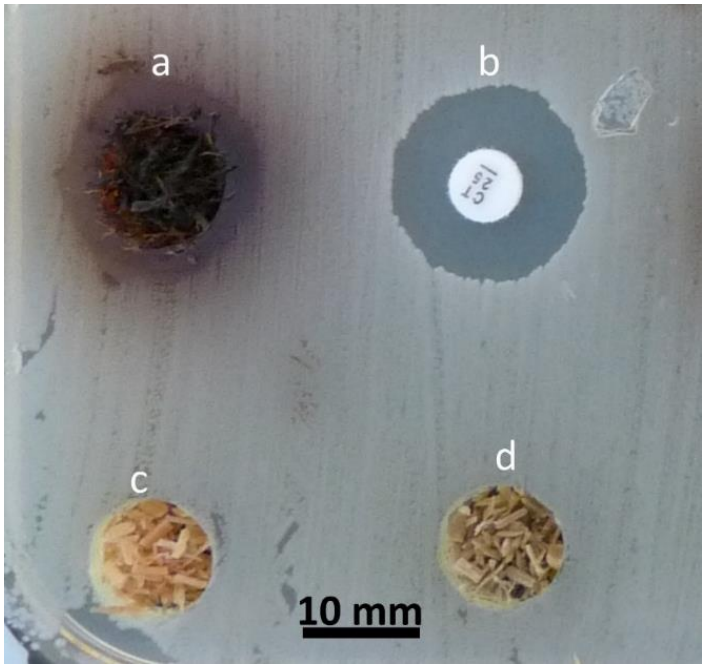


Figure Figure 2. 8: Antibiogram result of the well diffusion method to test the antimicrobial activity of sawdust (1-2 mm particle size), filled in wells (10 mm diameter) created in Mueller–Hinton agar against *Acinetobacter baumannii*: (a) oak wood showing the zone of inhibition around the well as a positive result; **(b)** positive control antibiotic disc (Colistin (Oxoid, Basingstoke, United Kingdom)–6mm diameter disc); **(c)** poplar sawdust with no activity, and **(d)** ash sawdust with no antimicrobial activity.

Although this method gives good results for screening purposes, it is not easy to fill the wells precisely without disrupting or contaminating the inoculated agar surface (Munir et al. 2019a); for example, figure 2.8a shows that the few fibers are spreading out of the well. In addition, the particle size within samples may affect the diffusion and quantity of test material because finer particle sizes have a higher surface area to volume ratio compared to larger particles (Lu et al. 2010). Therefore, granulometric studies are needed to standardize this protocol.

2.5.1.2.Evaluation of microbial survival in contact with solid wood surfaces

The antimicrobial properties of wood can also be studied by observing the viability of microorganisms on wood. Recovery methods and visualization methods alone or in combination are employed to study the role of physical and chemical composition of wood

to counter microbial growth (Figure 2.9). Moreover, such methods also provide good evidence of safety studies of comparative materials such as plastic, glass, and steel.

In previous studies, recovery methods were described as destructive and non-destructive methods (Ismail et al. 2013, 2015); however, this classification may vary depending upon the availability of sample or employment of methodology. For example, planning can be both a destructive and non-destructive method for constructed surfaces. Therefore, Figure 2.9 describes a more complete illustration of methodologies to study microbial survival on wooden surfaces.

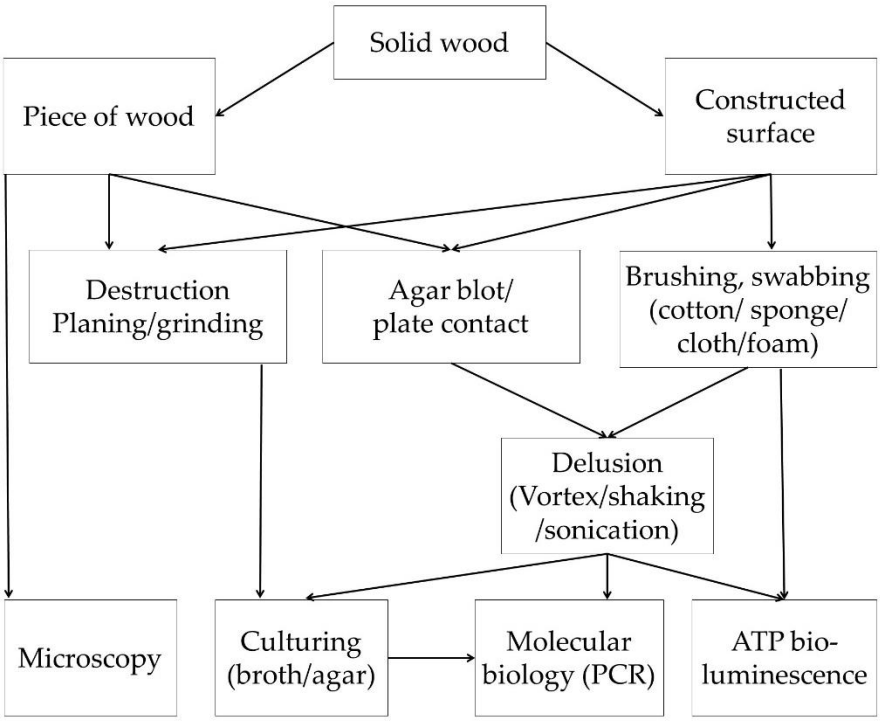


Figure 2. 9: Flow diagram outlining review findings on the methods to study microbial survival on solid wood material.

2.5.1.2.1. Microbial recovery

Here, the recovery is defined as "*the percentage of cells detected from the number of initially inoculated cells on a surface*". The microbial recovery gives information on their survival on different surfaces at different times (Ismail et al. 2013). Such methods are also used to study microbial adhesion and biofilm formation on wood surfaces (Cruciata et al. 2018). In general, the microbial recovery from surfaces depends upon multiple factors, including the type of wood material, surface roughness, size of surface, porosity, moisture content, type of microbes, recovery method, contact time, skills of the handler, and the media

used for collection, transport, and processing of samples (Ismail et al. 2015; Frontino 2019; Rawlinson et al. 2019).

As wood is a porous material with a very complex distribution of porosity (Plötze and Niemz 2011), the recovery of total microbial content is difficult (Cliver 2006; Ismail et al. 2013). Even the transfer of microbes from the wooden contact surface to food is lower as compared to other surfaces (Welker et al. 1997); for example, (Ismail et al. 2017) reported that the transfer rates of *L. monocytogenes* from wood (0.6%) to cheese were lower than perforated plastics (1.1%) and glass (3%).

2.5.1.2.1.1. Culture-based methods

A simple method of microbial recovery is blotting or agar plate contact, which involves directly touching the wood sample to agar to transfer microbes on it (Zangerl et al. 2010; Soares et al. 2012; Buchner et al. 2019; Kotradyova et al. 2019). It involves contacting contaminated pieces of wood on agar at a specific pressure for a known time, e.g., 650 g for 10–20 seconds (Schönwälder et al. 2002; Moore et al. 2007). Kavian-Jaromi et al. (2015) studied the survival of *K. pneumoniae* and MRSA on Larch wood [*Larix decidua* (Mill)]. Both heartwood and sapwood cubes ($10 \times 10 \times 5$ mm^3) were inoculated with about 100 µL of bacterial suspension (10^6 CFU /ml). These samples were blotted onto blood agar plates (Columbia Blood Agar) after 0, 3, and 24 h of inoculation, and subsequently, the developed colonies were counted after 24 h of incubation at 37 °C. Gupta (2017) reported the contact RODAC (Replicate Organism Detection and Counting) plates method for the recovery of fungi and bacteria from different surfaces, including wood. These plates containing sterilized tryptic soy agar (TSA) and potato dextrose agar (PDA) for bacterial and fungal colonies respectively, were impressed upon test surfaces for 20 seconds and incubated directly at 37 °C and 24 °C for TSA and PDA plates, respectively. They also compared this method with a vacuuming and bulk rinsate method. Vacuuming was similar to air sampling for microbes with certain modifications adapted for surfaces. The contact method showed a slightly higher recovery than vacuuming, and the bulk rinsate method gave two times higher recovery compared to the aforementioned methods.

Another direct method has been described in the literature where inoculated food contact surfaces, including wood (DeVere and Purchase 2007), were covered with agar and after incubation, nitroblue tetrazolium solution (pale yellow) was used to stain colonies (purple) at the agar–test surface interface. Stained colonies could be readily detected and counted, and this method gave 5 times higher recovery than the swabbing method (Barnes et al. 1996).

A simple rinsing of a wood surface with normal saline to collect microbes has also been reported (Tang et al. 2011; Goh et al. 2014). However, this method is not very suitable for porous materials such as wood because microbes may descend in the depth of pores and do not come out with a rinsing solution; in addition, even the surface-adhered microbes would not detach. Meanwhile, elution-based methods recover the higher microbial concentrations from such surfaces (Gonçalves et al. 2016; Gupta 2017). They involve the direct immersion of contaminated pieces of test material (e.g., cubes, sawdust, shavings) or a collection device (e.g., swabs, sponges) in an eluent (sterilized phosphate buffer saline or peptone water) and then a physical dissociation method such as shaking, sonication, vortexing, or Stomacher used to recover the microorganisms (Williams et al. 2005; Chiu et al. 2006; Moore et al. 2007; Elom et al. 2014; Kavian-Jahromi et al. 2015; Montibus et al. 2016; Miranda and Schaffner 2016; Chai et al. 2018; Gupta et al. 2019; Ripolles-Avila et al. 2019). Then, this suspension is further vortexed for 5–20 seconds and plated using serial dilution when appropriate (Gupta et al. 2017). Although this method gives higher recovery than the contact and vacuum method (Gupta 2017), the question arises if all the microbes are recovered from wood by this method. Earlier, Vainio-Kaila et al. (2011) used a similar technique to remove all adhered *L. monocytogenes* and *Escherichia coli* cells from the surface of wood and glass samples. Samples were vortexed in 15 mL BHI (brain heart infusion) broth for 5 s. To enumerate the colony-forming units (CFU), the suspension was subjected to a plate count method. Meanwhile, the test samples after microbial recovery were re-incubated in broth to determine the remaining microbial quantity; however, no qualitative growth was observed after 24 h of incubation. This method has also been used to test the survival of microbes on wood shavings (Milling et al. 2005a; Kavian-Jahromi et al. 2015). Ripolles-Avila et al. (2019) used this method with certain modifications for the evaluation of antibacterial activity on grounded high-density polyethylene, expanded polystyrene, pine, and poplar wood. The materials were ground to obtain 0.4 g of each, and they were then suspended in 20 mL of buffered peptone water. The *S. aureus* bacterial suspension was prepared and added in the same, together with test material, to obtain the final bacterial concentrations of around 1 to 3 $\times 10^5$ CFU mL^{-1} adjusted by the McFarland turbidity method. Then, the suspension was vortexed to homogenize and incubated at 37 °C for 24 h. After the incubation time, the decimal dilutions of suspension were made in tryptone saline solution, and then the TEMPO® system was used to quantify the remaining viable bacterial cells.

In addition, microbes are also collected by swabbing (Elserogy et al. 2016) and by destructive methods such as grinding (Montibus et al. 2016) and the planing (Imhof et al. 2017) of wood, and then they were further subjected to vortexing protocol for recovery (Zangerl et al. 2010; Ismail et al. 2015).

Swabbing is also a common method for collecting microorganisms from wooden surfaces (Miller et al. 1996; Deza et al. 2007; Moore et al. 2007; Coughenour 2009; Lortal et al. 2009; Coughenour et al. 2011; Yoon et al. 2012; Lucke and Skowyrska 2015; Elserogy et al. 2016; Exum et al. 2017; Imhof et al. 2017; Kotradyova et al. 2019). The swabs can be wet or dry and could be in form of cotton, foam, cloth, and sponge (Welker et al. 1997; Copes et al. 2000; Buttner et al. 2007; DeVere and Purchase 2007). The microbial collection depends on the type of swabbing approach adapted (Buttner et al. 2007; Frontino 2019). Ahnrud et al. reported that the sonicating swab device that combines swabbing, sonication, and suction can recover a significantly ($p < 0.05$) higher number of *L. monocytogenes* cells from wooden cutting boards as compared to sponge, foam, and cotton swabbing (Ahnrud et al. 2018).

Wood is intrinsically porous, which allows organic debris and bacteria to descend into the pores of wood unless a highly hydrophobic residue covers the surface (Cliver 2006; Kotradyova et al. 2019). It is highly likely that the porous structure of wood provides valleys and holes in which microbes are protected from any swabbing action (Filip et al. 2012). In addition, a higher number of microbes were recovered by swabbing a longitudinally cut wood surface as compared to a transversally cut wooden surface owing to the difference of surface porosity (Boucher et al. 1998; Prechter et al. 2002).

In general, the recovery methods give lower recovery from wood in dry conditions as compared to moist surfaces (Ismail et al. 2015). Welker et al. (1997) reported that the recovery of *E. coli* with the sponge swab method was similar on plastic and moist maple wood, while it was very low on dry wood (0.1%) and plastic (0.25%). Imhof et al. (2017) reported that the recovery of *Listeria* spp. from spruce wood was higher by an abrasive (planing) method as compared to swabbing (cotton rolls) in dry conditions; however, both methods gave similar results when wet with a low detection sensitivity of < 32 CFU/cm^2 (Imhof et al. 2017). The role of surface moisture is also linked to longer survival of microbes, which could lead to higher cultivable microbial recovery. Ismail et al. (2015) reported that the microbial recovery rate from wood was greater at higher moisture contents, regardless of the method of recovery (planing, brushing, or grinding), wood species (pine or poplar), and microorganism (*E. coli*, *L. monocytogenes*, or *P. expansum*). For example, the recovery rates

for *E. coli* at 18% and 37% moisture contents were 19% and 30% from pine and 8% and 27% from poplar wood, respectively. They also reported that the grinding method was found to be the most sensitive, giving the highest recovery rates in all conditions as compared to the planing and brushing method. In another study, Coughenour et al. (2011) reported that the addition of Bovine Serum Albumen to the glass, wood, vinyl, plastic, and cloth surfaces enabled methicillin-resistant *S. aureus* to survive for a significantly longer duration ($p < 0.001$). Interestingly, the recovery of number of CFU was significantly lesser on surfaces stored in 45–55% versus 16% relative humidity.

2.5.1.2.1.2. Molecular biology methods

The specific amplification of nucleic acids, such as in polymerase chain reaction (PCR), can be employed as a culture-independent method to investigate the microbial diversity in different environmental settings with complex mixture communities, non-cultivable viable cells (NCVC), interfering contaminants, and low levels of target DNA (Baymiev et al. 2020). In the first step of the PCR technique, the genetic material is isolated and purified from the target samples (Rozman and Turk 2016). The step can also be a culture-independent method; for example, (Abdul-Mutalib et al. 2015, 2016) used the swabbing of cutting boards for sample collection. Further, they vortexed the samples to obtain microbes and then extracted DNA without culturing these samples. Finally, they used the pyrosequencing technique to identify bacteria.

In PCR, the probes to target various genes can be designed depending upon the objective of the study. The common probes are the phylogenetic probes to get information about the phylogeny of the microorganism, functional gene probes to identify the particular activity of the microbial community, and the species-specific primers to determine the presence of a specific microorganism (Rozman and Turk 2016). These probes can also be used to detect the quantitative growth of microbes in different conditions. Milling et al. (2005a, b) studied the survival of fecal microbes in contact with wood material. For microbial recovery, the contaminated wood particles (3 g) were transferred to sterile plastic bags containing an extraction buffer (1:10 ratio). The samples were mechanically treated in a Stomacher lab blender for 3 min at 260 rpm to dislodge the adhering bacteria. The obtained suspension was used for DNA extraction and culturing for counting bacterial numbers. The decrease in the number of microbes as compared to initial inoculation was regarded as a loss of microbial survival in contact with wood material.

Genetic identification approaches are also important to recover NCVCs that are in a dormant state in the environment but are capable of cell division, metabolism, or gene transcription (mRNA production). Generally, the culture-based methods cannot identify NCVCs. (Buttner et al. 2007) reported that the efficiency of sponge and swab recovery with culture-based methods, to obtain *Erwinia herbicola* from different laminated wood surfaces, was very low (11% and 29%) as compared to qPCR.

As the DNA of dead microbes can persist for an extended period in environments, the molecular assessment (especially for DNA-based methods) can overestimate the viable cell numbers (Lee and Bae 2018). There are other markers proposed to overcome this limitation. Messenger ribonucleic acid (mRNA) is turned over rapidly in living bacterial cells. It has very short half-life inside the cell and can be used as a marker for microbial viability and identification of NCVCs (Rozman and Turk 2016). The nutritional stimulation of bacterial cells immediately produces a significant amount of rRNA precursors (pre-rRNA); these strands are easier to detect than mRNAs (Baymiev et al. 2020). Therefore, they can also be used as a marker for differentiating NCVC from dead cells that have been inactivated by UV irradiation, pasteurization, serum exposure, and chlorine (Lee and Bae 2018). However, these techniques have not been used to study the microbial survival on wood, but the prospect has to be employed.

2.5.1.2.1.3. ATP bioluminescence assay

The ATP bioluminescence assay can rapidly detect adenosine triphosphate (ATP), which is a component of all living cells. This process uses the luciferin enzyme derived from fireflies. When ATP from test samples reacts with luciferin in the presence of oxygen, the bioluminescence is generated as a byproduct, which is measured in relative light units (RLU) (Gibbs et al. 2014; Raia et al. 2018; Nguyen et al. 2018; Lane 2019). This device is generally applied on the surfaces after cleaning to detect the remaining contamination of microbes and organic matter in real-time (Welker et al. 1997). This method uses the swabbing of surfaces to collect organic matter, and results can be understated because of the lower recovery of microbes (Gibbs et al. 2014). Shimoda et al. (2015) used ATP assay to test the contamination of hospital surfaces (melamine, vinyl chloride, stainless steel, wood, and acrylonitrile–butadiene styrene) and found that wood material showed significantly high RLU values with huge variability. The authors cautioned that ATP values on wooden surfaces were likely to be inaccurate because the CFU on all surfaces were similar. Likewise, the sensitivity and specificity of a bioluminescence test as compared to the aerobic colony count method were

reported to be 46% and 71% (Raia et al. 2018). A recent study has also shown that ATP measurement is not an appropriate tool to measure bacterial contamination on wood and bamboo surfaces in hygienically important places (Lane 2019). These variations are linked to the organic nature of wood, and some traces of ATP may be present in this material, which interferes with the results, as Welker et al. (1997) reported a higher level of bioluminescence in new wood samples as compared to plastic. From the results of these studies, it can be concluded that an initial reading before contamination and another after contamination can give clearer information about actual microbial presence. Moreover, ATP bioluminescence assay should be coupled with culture-based methods to determine the microbial survival on wood.

Apart from these methods, some other direct methods can be developed for measuring the metabolites of living bacteria, for example, CO_2.

2.5.1.2.2. Microscopy to study microbial survival and distribution on and inside wood

The microscopic approaches are promising tools to study the morphology and probes as an indication of microbial distribution, survival and viability on different surfaces. Scanning Electron Microscopy (SEM) is widely used to observe the presence of contaminants on wood surfaces. Many articles are found in the literature with biofilm structure analyses by SEM to describe the morphological effects of fungi or bacteria distribution (Welker et al. 1997; Boucher et al. 1998; Mariani et al. 2007; Guillier et al. 2008; Lortal et al. 2009; Didienne et al. 2012; Bang et al. 2014; Gaglio et al. 2015; Scatassa et al. 2015; Imhof et al. 2017; Cruciata et al. 2018). Cruciata et al. (2018) described the formation and characterization of early bacterial biofilms on different wood species (Calabrian chestnut, Sicilian chestnut, cedar, cherry, ash, walnut, black pine, and poplar woods) used in dairy production. By using SEM, they observed a visible exopolysaccharide matrix that is typical of biofilm structures and showed the presence of both rod and coccus bacteria on the wood surfaces.

However, SEM is restricted to 2D exploration, and 3D observation of microbial colonization inside the pores and cracks of wood is very difficult (Schubert et al. 2014). Furthermore, such a method requires a series of highly invasive fixation steps incompatible with live imaging and is unable to provide direct information on the survival status of bacteria on analyzed wood surfaces (Xiao et al. 2000). Moreover, direct microscopy such as environmental SEM can change the morphology of wooden structures and microbial cells during the imaging process (Robson et al. 2018; Dubreil et al. 2018). Therefore, the

application of microscopy to study microbial survival and interaction with wood components is a challenging task.

Confocal scanning laser microscopy (CSLM) in conjunction with digital image processing techniques has been reported as a potent non-invasive optical sectioning tool (Schubert et al. 2014). It allows micro-morphologies of microbe interaction within the wood to be examined at a depth of around 50 µm of a specimen without incision, depending on the density of the wood sample (Table 2.5).

Table 2.5: Confocal microscopy in comparison to other microscopic techniques

	TEM/SEM/AFM	**Wide-field Microscopy**	**Confocal Microscopy**
High resolution	++++ (0.1-100 nm)	+/-	++ (200 nm)
Fixed sample	++++ required	Not required	Not required
Nondestructive sample preparation	- (section)	+/- (section)	++ (thick sample)
Fluorescent multi-labeling	-	++++	++++
3D investigation	++	-	++++
Compatible with live imaging	-	++++	++++

Xiao et al. (2000) reported that after fixation with glutaraldehyde, it was possible to locate fungal hyphae in wood, and counterstaining wood with a fluorescent phospholipid probe enabled the visualization of bacterial colonization and even distinguished Gram types to detect them in wood cell walls. Dubreil et al. (2018) developed an innovative method where they applied CSLM to observe *E. coli* labeled with a DNA probe DRAQ5^{TM} on poplar wood (Figure 2.10).

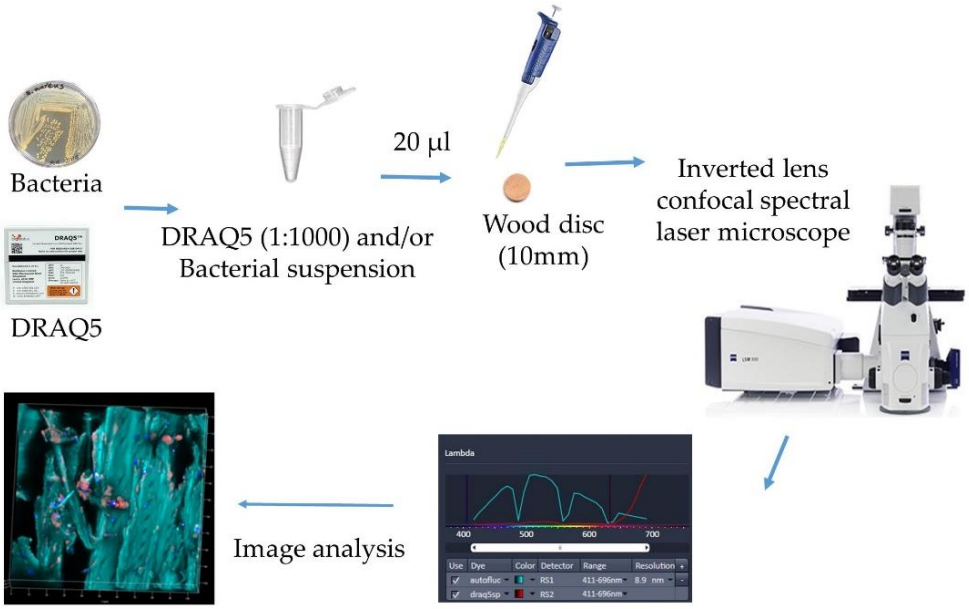


Figure 2. 10: Methodology to observe DRAQ5-labeled bacteria with confocal spectral laser microscopy (CSLM) [adapted from (Dubreil et al. 2018)].

This approach helped to visualize the presence and localization of bacterial cells, and it can be an interesting approach to determine the hygienic risk of microbial presence. However, the application of this method for a wider range of wood and bacterial species remains has not been tested yet. The use of laser scanning confocal microscope has also been reported to track the movements of *E. coli* in a porous matrix (jammed hydrogel media) (Bhattacharjee and Datta 2019).

2.5.2. Extractive-based methods to study the antimicrobial properties of wood

Wood contains biochemical compounds that enhance its resistance to microbial degradation. These special chemicals or extractives are not structural components, so they can be extracted by using different solvents (Rowell 2012; Fernández-Agulló et al. 2020). The quantity and type of extractives vary between wood species even within different parts of the wood in the same tree (Rowell 2012). Moreover, the antimicrobial activities of different extractives in various plants vary according to solvents used (Laireiter et al. 2013). On one side, extraction-based protocols give precise information of antimicrobial activity, and on the other side, the extraction adds an extra step in the antimicrobial test and requires chemical handling.

2.5.2.1. Agar diffusion and dilution methods

The antimicrobial properties of wood extractives can be tested by different agar diffusion-based methods that are classified based on loading the test solution on agar.

In the first method, wooden extractives in viscous form can be directly loaded on inoculated agar as circular points and after the incubation period, zones of inhibitions are observed as indicators of antimicrobial activity (Vainio-Kaila et al. 2015).

In the well diffusion method, extractives (50–100µL) diluted in different concentrations are directly pipetted into 6 mm diameter wells made in the agar (Jain et al. 2010; Hammud et al. 2015; Sarwar et al. 2018).

In the filter paper disc diffusion method, the extractives in different concentrations are impregnated into filter paper discs that are subsequently placed on agar plates. During the test disc preparation, the absorption potential of filter paper discs can vary depending upon the type of paper material being used. There are commercial paper discs available that have a diameter of 6 mm. Their general application is in antimicrobial sensitivity experiments in clinical microbiology laboratories. These discs are impregnated with 15–50 µl of stock solutions (Nostro et al. 2000). However, different sizes of the discs ranging from 5 to 10 mm can be created from blotting paper or simple filter paper (Whatman, no. 1 or 3) (Valgas et al. 2007; Das et al. 2010) and they can be impregnated with 10–200 µL of test solution extracted from wood material (Laireiter et al. 2013; Nakmee et al. 2016; Salem et al. 2016c). However, some studies have reported the soaking method in which the crude extracts were dissolved in TWEEN-20 solvent [to emulsify carrier oil in water (Salem et al. 2016b)] and 10% stock solutions were prepared. The blotting paper discs (6 mm diameter) were soaked in various dilute solvent extracts and dried for 5 minutes to avoid the flow of extracts in the test media (Subhashini et al. 2016; Fentahun et al. 2017). The following step is air drying, maintaining the sterility of the test material. The repetition of impregnation and drying can allow the loading of more liquid on discs. Finally, the sample loaded filter paper discs are subjected to the agar diffusion method to study the antimicrobial properties (Figure 2.11).

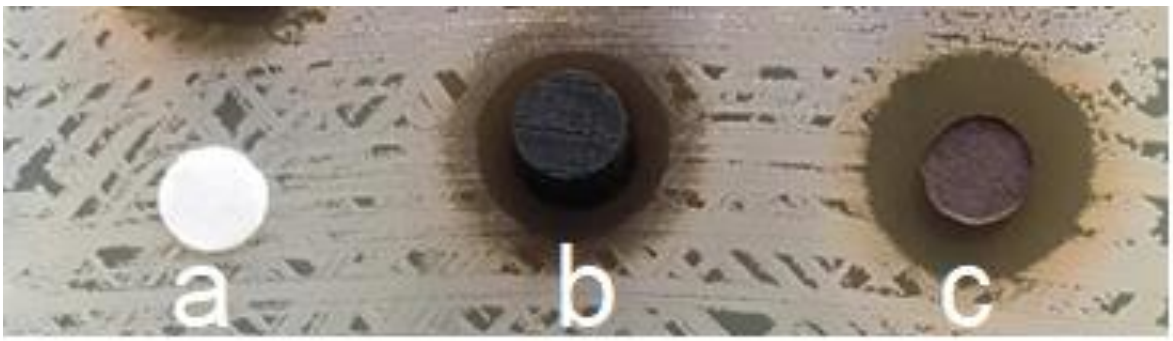


Figure 2. 11: Antibiogram to test the antimicrobial properties of oak wood (*Quercus petraea*) against *S. aureus* with the agar diffusion method*:* **(a)** an inert filter paper disc (negative control); **(b)** a wooden disc showing antimicrobial activity by forming a zone of inhibition and **(c)** a filter paper disc impregnated with wood extractives (10 mg extractive content extracted with methanol) showing antimicrobial activity by forming a zone of inhibition.

Another method of using agar microdilution has been described in the literature, which involves the dispersion of a test compound in molten agar and dispensing the mixture into a 96-well microplate in a small volume of 100 µL per well, which allows a rapid, easy, and economical preparation of samples as well as providing a uniform and stable dispersion without the separation of the oil-water phases, which occurs in methods with liquid medium (Golus et al. 2016).

The extractives in different quantities can also be mixed with agar before pouring into Petri dishes. Later, the bacteria are inoculated by steaking or spreading (Gonçalves et al. 2016). This method is also used for studying the antifungal response of wood extractives, and for this purpose, a piece of agar from a fungi-cultured plate is taken and placed on the extractive-infused petri dish. The size of the circular growth of fungi on agar gives a reading of fungal resistance against extractives (Khan et al. 2000).

2.5.2.2. Broth dilution methods

This method is more common to determine the minimum inhibitory concentration (MIC) (Balouiri et al. 2016), which is the lowest concentration of an antimicrobial product inhibiting the visible growth of a microorganism after overnight incubation (Dasgupta and Krasowski 2020). It requires the homogenous dispersion of a sample agent in a solvent, and dilutions of different concentrations are tested to determine MIC (Rios et al. 1988; Salem et al. 2016a) (Figure 2.12).

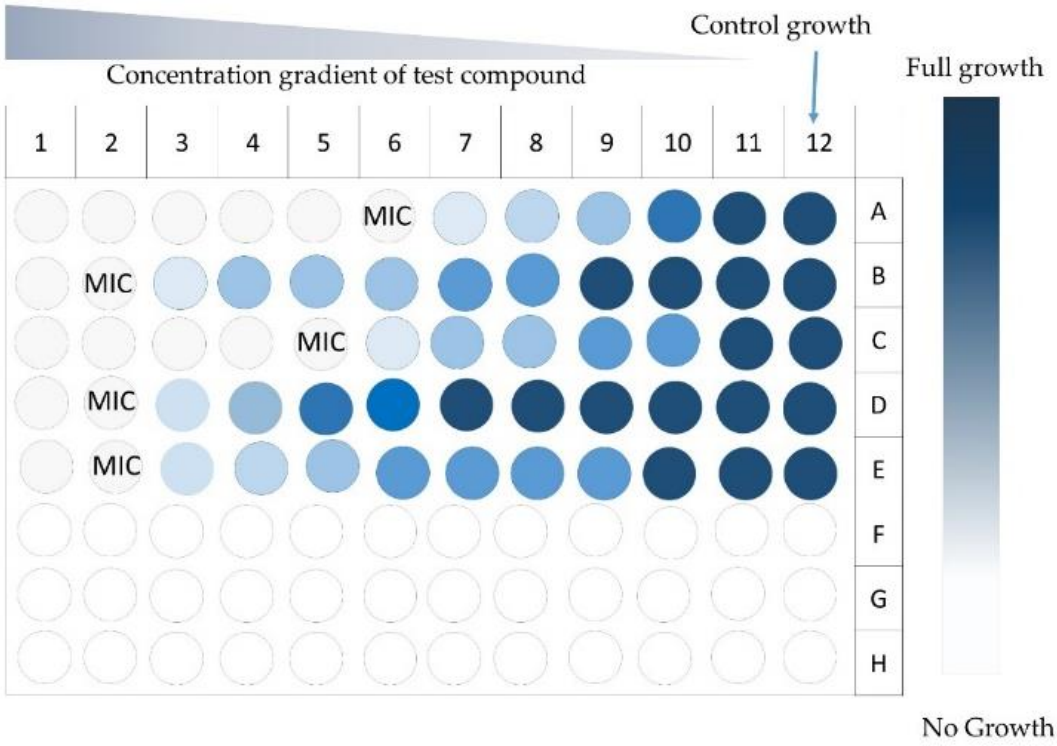


Figure 2. 12: A 96-well plate showing results of the broth microdilution method for an antimicrobial test and minimum inhibitory concentration (MIC).

If the purpose of an experiment is just to test the antimicrobial potential of wood extractives, only one selected dose can be added (Valimaa et al. 2007). The inoculation, incubation, and reading can be performed manually or by an automated system, and the results can be read either by the formation of microbial colonies or the stoppage of growth (Rios et al. 1988; Balouiri et al. 2016). In an automated method, the formation of bacterial colonies gives turbidity to the medium, and it is measured by spectrophotometry (Valimaa et al. 2007; Bonnet et al. 2012).

2.5.2.3. Measurement of wood mass loss to decaying

Wooden surfaces are treated with several synthetic and natural products, including wooden extracts, to increase resistance against microbial biodegradation. Measurement of the loss of wooden mass to degradation over time is used as a parameter to evaluate the protective effect of surface treatment or wood itself. However, this method has not been used to test the antimicrobial properties of wood against hygienically important microbes. Cai et al. (2019) studied the protective effect of *Pterocarpus* spp. extracts on Poplar samples against wood-degrading fungi. The wood was blast dried in an oven at 40 °C until the mass was constant and then immersed in the prepared extract solution for 2 h. The samples were dried again until the mass was constant. The control and treated samples were placed in culture

flasks and incubated at 75% relative humidity and 28 °C for decaying for 3 months. Later, the samples were taken out, hyphae and impurities on the surface were removed, and the samples were oven-dried. The percentage of sample mass loss was used as an indication of the antimicrobial effect.

2.5.2.4. Bioautography

This extractive-dependent method involves the hybridization of planar chromatography (for phytochemical analysis of extracts) with biological detection methods (for antimicrobial potential) (Dewanjee et al. 2015). The technique is similar to the agar diffusion method except that the tested compound diffuses from the chromatographic layer (Valgas et al. 2007).

2.5.2.4.1. Direct bioautography

This is a widely used bioautographic method, which links detection on the adsorbent layer with biological tests performed directly on it (Choma and Jesionek 2015). In this method, extractive is loaded on a thin-layer chromatographic (TLC) plate to obtain a chromatogram. Further, this plate is dipped or sprayed with a suspension of microbes grown on a proper culture, and it is then incubated in a vapor chamber to provide a humid atmosphere (Valgas et al. 2007; Suleimana et al. 2009; Masoko and Masiphephethu 2019). In the case of anaerobic microbes, the scenario is different, the incubation in a sealed jar may result in high humidity potentially, causing a softening and peeling of silica gel layer from the aluminum base; the shorter incubation period and concentrated bacterial suspension are recommended to avoid this problem (Kovács et al. 2016). Finally, the inhibition of microbial growth can be spotted directly (Figure 2.13). To improve this visualization, Suleimana et al. (2009) used p-iodonitrotetrazolium violet, which did not reduce the zone of inhibitions and was visible as white bands. The targeted compounds can also be identified using spectroscopic methods, mostly mass spectrometry, which can be performed directly on a TLC plate (Choma and Jesionek 2015; Moricz et al. 2015). This high-throughput method enables analyses of many samples in parallel and the comparison of their activity, making both the screening and semi-quantitative analysis possible (Suleimana et al. 2009; Choma and Jesionek 2015).

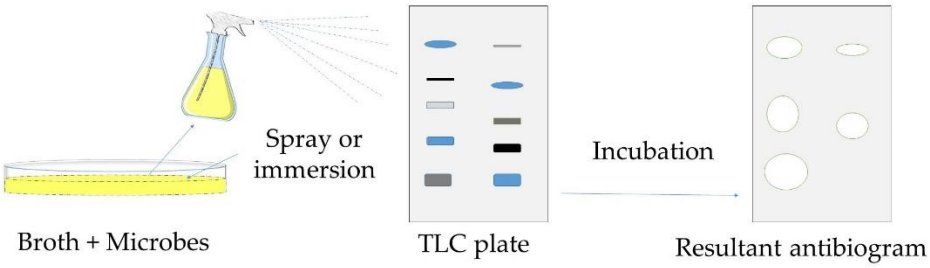


Figure 2. 13: Schematic presentation of direct bioautographic method: (a) a developed chromatographic plate is placed in a dish; **(b)** agar is poured into this dish, and later, microbes are inoculated and **(c)** after the incubation time, the zones of inhibition can be seen on agar around the active antimicrobial compounds [the figure is adapted from (Valgas et al. 2007; Suleimana et al. 2009; Choma and Jesionek 2015; Masoko and Masiphephethu 2019)].

2.5.2.4.2. Contact bioautography

In this method, the TLC plate or paper chromatograms are placed in contact with the inoculated agar surface for some minutes or hours to allow diffusion (Khurram 2011). Next, the plate is removed, and the agar layer is incubated for 1–3 days (Moricz and Ott 2017). The zones of growth inhibition appear in the places where the antimicrobial compounds were in contact with the agar layer (Kovács et al. 2016) (Figure 2.14). The visualization can be enhanced by using vital dyes (Moricz and Ott 2017).

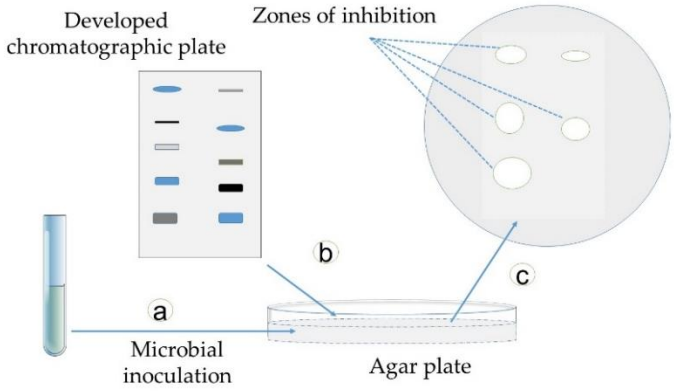


Figure 2. 14 : Schematic presentation of the contact bioautographic method: **(a)** microbes are inoculated on an agar plate; **(b)** a developed chromatographic plate is flipped over an agar plate to create a chromatographic image and transfer the active compounds, and inoculated plates are incubated for 48 hours at 37 °C, and finally, **(c)** the zones of inhibition can be seen on the agar around the active antimicrobial compounds [adapted from (Dewanjee et al. 2015; Choma and Jesionek 2015; Balouiri et al. 2016; Kovács et al. 2016)].

2.5.2.4.3. Immersion (agar-overlay) bioautography

This is the combination of two formerly described methods. In this technique, an extractive inoculated, developed chromatographic plate is immersed in or covered with molten agar (Dewanjee et al. 2015). After the solidification of agar, the plate is seeded with the tested microorganisms and then incubated (Choma and Grzelak 2011) (Figure 2.15).

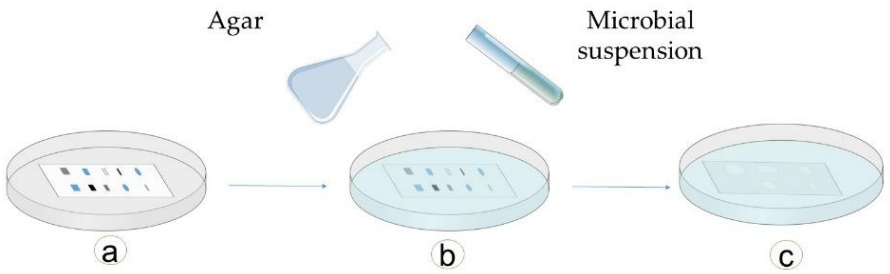


Figure 2. 15: Schematic presentation of the immersion bioautographic method: **(a)** a developed chromatographic plate is placed in a dish; **(b)** agar is poured into this dish, and later, microbes are inoculated; **(c)** after an incubation time, the zones of inhibition can be seen on agar around the active antimicrobial compounds [adapted from (Choma and Grzelak 2011; Dewanjee et al. 2015; Choma and Jesionek 2015; Balouiri et al. 2016)].

2.5.2.5. Active antimicrobial ingredient identification

For the sake of active ingredient or compound identification, the wood extracts are fractioned by chromatographic and spectrophotometric techniques to obtain the pure compounds, which can be further tested for their antimicrobial properties by the conventional methods described above (Graikou et al. 2012; Sarwar et al. 2018; Mukai et al. 2019; Mai et al. 2020). However, the fractioning of compounds to test for antimicrobial activities is a laborious bioactivity-guided isolation procedure that yields an extremely low quantity of active substances after purification (Abedini et al. 2020). In this scenario, the characterized chemical profile can be labeled as antimicrobial compounds according to previous research done on them (Peng et al. 2017).

2.5.3. Non-categorized methods

There are several other ways to detect the antimicrobial properties of natural compounds, and they remain to be tested for their application in wood science. One of such methods is inducing infection in animal models and using the dose of extractives as antimicrobial compounds to treat or eliminate the infection. In more sophisticated studies, the mode of action of different compounds is identified against different microorganisms. Plumed-Ferrer et al. (Plumed-Ferrer et al. 2013) studied the antimicrobial effects of wood-associated

polyphenols on food pathogens and spoilage organisms. They identified the mode of antimicrobial effect of these compounds by studying the microbial membrane permeability and membrane damage.

When it comes to bioaerosol quality of indoor air, the effect of the presence of wooden material on the microbial flora is also an important subject of research. Such studies need to utilize static chambers; however, there is no standard method published for wood material. There is an innovative study conducted by (Vainio-Kaila et al. 2017a) regarding the effect of VOC from *Pinus sylvestris* and *Picea abies* wood on *S. aureus*, *E. coli*, *Streptococcus pneumoniae*, and *S. enterica* Typhimurium. The experiment was carried out in a closed glass container (volume 1.9 L). First, 70 g of sawdust was placed on the bottom. A bacterial solution (20 µL) was inoculated on the glass discs on a rack above the bottom. After the incubation at room temperature for 2, 4, and 24 h, glass discs were dropped in test tubes to recover and enumerate the microbes by the plate count method. This method successfully measured the antimicrobial effect of VOC on microbial survival in different situations of time, air humidity and sample moisture.

2.5.4. Pros and cons of methods used to study antimicrobial behavior of wood

Several factors influence the choice of method selection to study the antimicrobial properties of wood materials. These factors are related to the availability of experimental material, test samples, purpose of study, and skills of handlers. The advantages and disadvantages of the methods discussed in this review are summarized in Table 2.6.

Table 2.6 : Pros and cons of the methods used to study the antimicrobial behavior of wood material

	Method Name	Procedure	Advantage	Disadvantage
Direct methods	Direct diffusion method (Well and disc)	• The wood material is directly placed on microbe-inoculated agar or in a well and incubated for the recommended time • Presence of the zone of inhibition is considered a positive result	• Rapid and time-saving • Applicable for the low amount of material • Adapted for screening	• Disc preparation time • High variability for quantitative applications • Studies only the effect of agar-diffused chemicals • May require the sterilization of wood samples
	Culture-based microbial survival test	• Initial microbial quantity is inoculated on wood samples and after the incubation time, the microbes are recovered, cultured, and viable cells are counted	• Can study the structural and chemical role of wood components • Qualitative and quantitative results • Applicable for low amount of material	• Difficult to recover all microbes present in pores • Microbial quantification is an extra step needed • Only viable cells are identified, while there can be still non-viable infectious cells present
	Microscopy	• The behavior and distribution of inoculated microbes on wooden structures is observed via microscopy	• Rapid and time-saving • Applicable for low amount of material • Adapted for screening	• May require the fixation of samples • Difficult to differentiate microbial structures from wooden structures • May require competencies of image analysis
	ATP luminescence	• The ATP of microbes on wood is measured	• Rapid and easy • Applicable for low amount of material • Adapted for screening	• Difficult to differentiate the microbial ATP from other organic debris • Adapted only for solid surfaces
	Molecular biology methods	• Quantity and viability of microbes is tested via nucleic acid amplification	• Accurately measures the microbial survival	• Expensive • Require sophisticated handling
Extractive based methods	Extractive-based diffusion and dilution method	• Extractives are placed on agar or in agar wells, or in broth, after loading on filter paper discs or directly	• Adapted for qualitative and quantitative antimicrobial studies • Specific chemicals can be extracted depending upon the solvent used	• Involves chemical handling • Extra step of extraction • One solvent cannot extract all active components • Does not study the role of structure of wood
	Bioautography	• Extractives are loaded on a chromatographic layer, and diffusion of active chemicals is studied for antimicrobial activity	• Adapted for qualitative antimicrobial studies • Specific chemicals can be extracted depending upon the solvent used and identified based on their diffusion on the chromatographic layer	• Involves chemical handling and extraction • One solvent cannot extract all active components • Does not study the role of structure of wood • Not a quantitative method
	Mass spectrometry	• The total profile of microbes is measured	• Applicable for a low amount of material • Accurately measure the content of the active ingredient	• For more specific results, the identified compounds are supposed to be tested by other culture-based methods

2.6. Conclusions

The literature review summarizes the research on wood and hospital hygiene. It highlights the possibilities of the application of wood material in healthcare buildings and identifies the advantages and related disadvantages. Further, the hygienic properties of wood material regarding the antimicrobial properties against hygienically important pathogens are explored. This review helps to identify the gaps in knowledge on this subject. It shows that wood material has the potential to be considered as the ecofriendly material of nature and could benefit the inhabitants' psychophysiological health and does not introduce chemical hazards via emissions in residential buildings.

There are not many studies available to provide evidence on the hygienic suitability of wood material in healthcare buildings. The antibacterial studies are limited to a few bacteria. Meanwhile, the antifungal properties of wood compounds have been studied mostly against the wood degrading fungi. Therefore, studying the clinical isolates and standard representative strains of most common nosocomial bacteria and fungi would generate applied research results.

Most of the studies were performed on the extractives to identify the antimicrobial properties of wood material. Meanwhile, it is known that the physical structure of wood also plays a role in countering microbial growth, thus the extractive based studies may neglect the role of physical structure in the determination of antimicrobial activity, and therefore methods should be developed to test the solid wood samples.

The literature shows that the different wood species have different antimicrobial properties, and it is needed to understand the reason for this variability. Moreover, the wood material is used in different forms and undergoes multiple types of treatments during processing and usage life, however, the influence of solid wood variables and treatments on the antimicrobial activity is not known.

The survival of most common nosocomial pathogens including *K. pneumoniae, S. aureus, A. baumannii* and *Pseudomonas aeruginosa* has not been studied on wood material. Therefore, the controlled in vitro studies are needed to elaborate their persistence time on wood in comparison to other inanimate surfaces.

The studies on the distribution of microbes on and inside wood used conventional electron microscopy, which is a time-consuming method and difficult to apply, therefore, innovative techniques are needed to assess the microbial contamination of wood. Moreover, there is no concrete evidence to elaborate on the depth of microbial penetration and distribution inside the wood structures. In addition, the available literature regarding the biofilm formation potential

of different bacteria on wood, discusses the naturally persisting microbes of the food chain, however, there is no evidence on the biofilm formation potential of nosocomial pathogens in experimental or hospital conditions on wood material.

As there is less evidence of hygienic properties of wood material, the perception of people is also based on the knowledge that wood is porous and organic, hence not suitable for application in hygienically important places such as healthcare buildings. However, there is growing application of nature-based themes in the healthcare buildings but there is limited data available to show that applying wood in the healthcare buildings could increase the wellbeing of individuals. Based on this literature review, a retrospective study was prepared in the form of questionnaires and interview questions to determine the perception and wellbeing of wooden hospital building's occupants (Annex 5).

Few studies show the use of wood material in the residential buildings and its relation to asthma, which can be an allergic reaction, however, it is not reported if the use of wood material would influence the persistence of infectious agents in the hospital buildings. Field studies are needed to identify if the healthcare buildings, employing indoor wood material, have any influence on the prevalence of chronic respiratory symptoms in occupants and the prevalence of infectious agents in the environment. The data from this literature review was used to identify and outline the methodology of a study aimed to determine the prevalence of infectious agents in the wooden healthcare buildings and demonstrate the antimicrobial properties of wood by field representative studies (Annex 6).

Chapter 3. Materials and methods

This chapter contains information on general materials and methods employed in this project, including details on the tested wood, bacteria and fungi, and their origin, collection and processing. It also includes information on the sterilization techniques and microscopy. In later sections, the specific methodology of each experiment is provided.

3.1. General materials and methods

3.1.1. Wood material

Different wood species were used in this study and were selected based upon their use in industry, availability and suitability to the investigation.

American oak (*Quercus* sp.), Douglas fir (*Psuedotsuga menziesii*), European Beech (*Fagus sylvatica*), European Fir (*Abies alba*), European oak (*Quercus robur*) and Poplar (*Populus euramericana alba* L.) wood species were the same as used in the pilot study (Munir 2016). All samples were obtained directly from a sawmill and originated from vicinities around Nantes, France except *Quercus* sp. wood that was an imported wood. The logs were naturally dried for 3 months and the samples were cut from heartwood (Tomczak et al. 2020).

Sessile oak wood (*Q. petraea*) was chosen for further investigations based on its known durability (Aloui et al. 2004), use in industry (Sejdiu et al. 2020), and demonstrated antimicrobial behavior of oak wood species (Pailhories et al. 2017). This wood was obtained from six trees growing in Département de la Sarthe in the Région Pays de la Loire of France and having three different soil types (Table 3.1). Logs were cut above 1.30 m. All the cut wood samples went through a natural drying process of 6-8 weeks until moisture content reached almost 12%.

Table 3.1 : Characteristics of oak trees (*Quercus petraea*) from Région Pays de la Loire, France

Sample number	1 and 2	3 and 6	4 and 5
Area	Parcelle 10 A 4ha 48	Parcelle 402 F 6ha 48	Parcelle 21E 4ha 37
Coordinates	47°51'18.4"N 0°08'25.5"W	47°45'35.2"N 0°16'51.3"W	47°50'38.4"N 0°11'28.0"W
Age (years)	90	80	100
Soil fertility	Medium	Good	Medium
Total Height (m)	30	30	33

3.1.1.1. Wood discs preparation

The logs were initially cut into boards of 40 mm thickness with no sapwood nor pith. Then boards were further cut by the electric saw (Altendorf-F45, Minden, Germany) into thinner

(3.5±0.4mm) sheets, with respect to, transversal (RT), tangential (LT) and radial (LR) planes. These veneers were used to prepare uniform-sized circular discs (diameter 9.95±0.1 mm, thickness 3.5±0.4) using a laser cutting machine (Trotec-SP500 C60, Wels, Austria) or a manual punch machine (SyretteSyderic ET23N). The samples were conditioned to ~12% moisture content in a climatic chamber (50% RH, 22 °C).

3.1.1.2. Sawdust preparation

The wood veneers were broken with chisel and hammer to approximately 5 x 10 cm pieces, and ground in a grinder (Retsch ZM200, Haan, Germany) to obtain sawdust that was further separated into three different particle sizes including 500-1000 µm, 250-500 µm and less than 250 µm by mesh packs. Samples were conditioned to ~12% moisture content in a climatic chamber (50% RH, 22 °C) before using in experiments.

3.1.1.3. Wood extracts

The sawdust samples from sessile oak and Douglas fir wood were dried in an oven for 24 h at 103 °C. The dried material was kept in a desiccator for two hours and then 10 g of this sawdust was filled in pure cellulose cartridges (Whatmann or Grosseron, 30 x 3 cm). The material was loaded in the Soxhlet apparatus with 180 ml of solvent (Hexane or Ethanol from Riedel – analytical grade used as received). The extraction was done for six hours. Then the solution was evaporated under reduced pressure in a Buchi rotatory evaporator. The extractive yield (mean ± SD) was 5.56 ± 0.42% for Douglas fir and 9.6 ± 2.3% for sessile oak wood. These extractives were solubilized using DMSO (100%) and stored at 4 °C.

3.1.2. Sterilization of materials

The wood samples were packed in sterile polyethylene bags and divided into three groups. One group was kept unsterilized (N), and the other two were sterilized by either gamma irradiation (G) at 25 kGy (Ionisos, Sablé Sur Sarthe, France) or by autoclaving (A) at 121 °C and 100 kPa for 15 minutes (VAPOUR- Line Lite, VWR). The same autoclaving conditions were used to sterilize the glassware, plastics, metals and culture media used for experiments.

3.1.3. Bacterial and fungal isolates

All reference strains and clinical isolates of bacteria for antimicrobial studies were obtained from the collection of the University Hospital of Angers, France (Table 3.2).

Resistant bacterial isolates were defined with criteria depending upon the bacterial species/genus: carbapenemase-producing *A. baumannii* (CPAB), *E. coli* resistant to third-generation cephalosporins, vancomycin-resistant Enterococci, *P. aeruginosa* resistant to ceftazidime, and MRSA or glycopeptide-intermediate *S. aureus* (GISA).

For CSLM studies, the following two genetically modified fluorescent strains of *Staphylococcus aureus*, containing mCherry (a fluorescent protein) expressing genes in plasmid or chromosome, were obtained from CNR *Staphylococcus aureus* (CNR: Centre National de Référence), Lyon, France, courtesy of Dr. Jessica Baude.

- Chromosomal mCherry label LUG2495: SH100:: PsarA-mCherry
- Plasmidic mCherry label, LUG2929: SH100/pLUG1315: pSK:: PsarA-mCherry

Both these strains were coagulase-positive and had peak growth curve time at 15 to 16 hours (data provided by Dr. Simon O. Khelissa, UMR UMET, CNRS- INRA, France).

Table 3.2: Bacteria used in this project

Bacterial species	Reference number	Susceptibility characteristics
Staphylococcus aureus	ATCC 29213	MSSA/VS
	KSKS7326	MRSA/GISA
	15015019102	MRSA
	16005880401	MRSA
	16009349201	MRSA
	16518728101	MRSA
	16006004401	MSSA/VS
	14528592702	MRSA/GISA
	16518868104	MRSA
	16513145401	MRSA
Acinetobacter baumannii	16521722701	CS
	ATCC 19606	CS
	ATCC 17978	CS
	15519454101	R (Carbapenemase $bla_{\text{NDM-1}}$)
	15015364001	CS/Carbapenem R without carbapenemase
	15517172701	R (Carbapenemase $bla_{\text{OXA-23}}$)
	185 160 30701	R (Carbapenemase $bla_{\text{OXA-23}}$)
	13504498703	R (Carbapenemase $bla_{\text{NDM-1}}$)
	18529482201	CS
	17536228901	CS
	18526909702	CS
Escherichia coli	ATCC 25922	CS
	ATCC 35218	Penicillin R (Tem-1)
	18528747601	R (carbapenemase $bla_{\text{OXA-48}}$)
	18528531001	Cephalosporin R (ESBL+)
	18540723702	CS
	18541756901	Cephalosporin R (ESBL-)
	18541702301	Cephalosporin R (ESBL+)
	18541175601	CS
	18541731901	CS
	18541481601	Cephalosporin R (ESBL-)
	18540722802	Cephalosporin R (ESBL+)

Pseudomonas aeruginosa	ATCC 27853	CS
	18015393101	CS
	18541890201	CS
	18541668202	CS
	18541207902	CS
	18015225701	CS
	18540722802	CS
	18015116701	CS
	18529486401	Carbapenem R
	18010906401	Carbapenem R
Enterococci spp.	ATCC 51299	*E. faecalis* R to glycopeptides (*Van B*)
	ATCC 29212	VS
	18541660506	VS
	18541204901	VS
	18541746201	VS
	18541593801	VS
	18541584501	VS
	18541073802	VS
	18541796701	VS
	18524021401	*E. faecium* R to glycopeptides (*vanA*)
Klebsiella pneumoniae	ATCC 700603	Cephalosporin R (ESBL+)

CS: Colistin sensitive; VS: vancomycin sensitive; R: resistant according to the criteria defined in the study; ESBL+: production of extended-spectrum beta-lactamase; ESBL-: absence of production of extended-spectrum beta-lactamase; MRSA: methicillin-resistant *Staphylococcus aureus*; MSSA: methicillin-susceptible *Staphylococcus aureus;* GISA: Glycopeptide-intermediate *S. aureus.*

Clinical isolates of *Candida* spp. and *Aspergillus* spp. (Table 3.3) were obtained from the Laboratory of Parasitology and Medical Mycology of Centre Hospitalier Universitaire (CHU) of Nantes, France. The *Candida auris* isolates were obtained from Valledupar Medical Center, Colombia.

All fungi were maintained at -192 °C in liquid nitrogen for long term storage and at 4 °C for short term storage. They were sub-cultured on Sabouraud Dextrose Agar (SDA) and kept at 37 °C. The resistant strains of *Candida* spp. are resistant to fluconazole, one of the first-line antifungal drugs for candidiasis. For the diffusion-based tests, square (10x10 cm) Petri dishes were used containing SDA and chloramphenicol (100 μg/ml).

Table 3.3: Fungi used in this project

Species	Isolate/strain*	
	Resistant	**Susceptible**
Candida albicans	CAAL 117	CAAL 16, CAAL 93, CAAL97, CAAL 118
Candida parapsilosis	CAPA 3	CAPA 18
Candida glabrata	---	CAGL 3
Candida krusei	CAKR 1	--
Candida tropicalis	--	CATR 1
Candida auris	CAAU 1, CAAU 6, CAAU 20, CAAU 22	--
Aspergillus fumigatus	--	ATCC 76, ASFU 7, KA 66, WT 104

* Susceptibility/resistance of selected strains was defined in reference to fluconazole and itraconazole for *Candida* sp and *Aspergillus* sp. respectively.

3.1.4. Control samples for antimicrobial studies

For the direct diffusion method, the negative control samples were the inert filter paper discs (6 mm diameter) prepared commercially (Bio-Rad, Marne-la-Coquette France). The positive control discs (diameter 6 mm; Oxoid, Basingstoke UK) for antibacterial studies were Vancomycin (5µg; for Gram-positive bacteria: *Enterococcus sp., S. aureus*) and Colistine (25µg; for Gram-negative bacteria: *P. aeruginosa, E. coli, Acinetobacter sp.*).

Inert filter paper discs (diameter 8 mm) were used as a negative control for the antifungal direct diffusion method. The positive control discs (thickness 0.5 mm, diameter 8 mm) were prepared in the laboratory. For *Candida* spp., fluconazole was used as a positive control, and three concentrations of 100, 50, and 10 µg/ml were prepared in sterile distilled water. For *Aspergillus fumigatus* strains, amphotericin B was used as a positive control, and diluted in three concentrations of 40, 4, and 0.4 µg/ml in sterile 1% DMSO in water solution. The dilutions were protected from light and were stored at -20 °C until use. Finally, 20 µl of each concentration of antibiotics was deposited on inert filter paper discs.

3.1.5. Statistical analysis

Normality tests were done to evaluate the data distribution and then the appropriate statistical analyses were applied to the data. The data in antimicrobial studies were represented in mean ± SD. Student t-test was used to determine the difference between two groups (e.g. LT vs. RT) using MS Excel (Microsoft, Office 2013, 2016). One-way analysis of variance (ANOVA) was performed to evaluate difference among multiple groups and variables (e.g. trees, treatments). In case of significant difference among groups, Tuckey's post hoc test (SAS

Institute Inc., Cary, NC, USA) was used to identify the different groups and level of significance. Kruskal-Wallis tests were used to test the different microbial growth on different surfaces. Mann-Whitney Statistic test was used to compare the values of two groups (e.g. fluorescence at two different times or from two different samples). A p-value of ≤ 0.05 was considered statistically significant in all experiments.

3.2. Methods for antibacterial studies

3.2.1. Screening the antibacterial properties of wood by direct agar diffusion method

This method was employed as described by Pailhories et al. (2017). Briefly, gamma sterilized and non-sterilized round wood discs (diameter 9 mm and thickness 2.5 mm) from 4 different wood species were collected and tested against four bacteria *S. aureus* (ATCC 29213), *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853) and *E. faecalis* (ATCC 29212). The bacterial strains were cultured on blood agar. The colonies were taken *via* a swab and suspended in 2 ml of normal saline to prepare a suspension of 0.5 MacFarland density. The suspension was inoculated by streaking on the square (12 × 12 cm) Mueller-Hinton agar plates (BioRad, Marnes La Coquette, France). The test samples, along with positive and negative control discs, were directly put on the agar plates (16 samples could be placed; arrangement is shown in Figure A1.1), and incubated at 37 °C for 24 h. The inhibition zones, as defined by CASFM and EUCAST (European Committee on Antimicrobial Susceptibility Testing) (CASFM / EUCAST 2019), were manually measured. The tests were carried out in triplicate and all the experiments were conducted in the laboratory of bacteriology of the University Hospital of Angers, France. As this method was mainly used by our research group, we named it “antiboisgram” which is a modified version of antibiogram.

3.2.2. Effect of wood variables on antibacterial activity

Antiboisgram was used, with slight modifications of test samples, to test the hypothesis that wood variables may influence the antimicrobial activity. A total of 648 *Q. petraea* wood discs (diameter 9.95±0.1 mm and weighing 213±15 mg) representing variables of six trees (from 3 locations), three sterilization conditions (non-sterilized, gamma-sterilized and autoclaved), three cutting directions (RT, LT and LR) and two preparation methods (manual punch and laser cutting), were tested against *S. aureus* ATCC 29213 and *A. baumannii* OXA-23 in triplicates (sample arrangement: Figure A1.3).

3.2.3. The bacterial resistance against oak wood

Antiboisgram was used for this experiment as well, with exception of round petri dishes which could test 7 samples. A total of 900, RT plane, non-sterilized and laser-cut, *Q. petraea*

wood discs were tested in triplicates against a set of 8 reference strains (*A. baumannii* ATCC 19606 and ATCC 17978, *S. aureus* ATCC 29213, *E. faecalis* ATCC 29212 and ATCC 51299, *E. coli* ATCC 35218 and *P. aeruginosa* ATCC 27853) and 42 clinical isolates belonging to five bacterial species/genus: *A. calcoaceticus-A.baumannii* complex (Acb), *E. coli*, *Enterococcus (E. faecalis and E. faecium)*, *P. aeruginosa* and *S. aureus*. For each of them, 10 bacterial isolates presenting different patterns of resistance to antibiotics were tested.

3.3. Antifungal activity of wood

3.3.1. Direct disc diffusion method to screen antifungal properties of wood

3.3.1.1. Suspension preparation

The fungal suspensions were prepared as described elsewhere (Stein et al. 2005). Briefly, the *Candida* spp. colonies were taken *via* a swab from freshly grown culture and then suspended in normal saline solution. For *Aspergillus fumigatus* strains, the normal saline solution was supplemented with 0.1% Tween 20 to give better dispersion. In the next step, both fungi were vortexed at 25 Hz for 15 s. The suspension of *Aspergillus fumigatus* was kept still for 10 to 15 minutes, allowing the hyphal fragments to sediment down to bottom, and separating the suspension from top. Finally, the turbidity of both fungi was attained to 0.5 McFarland (containing ~1.5 x 10^8 CFU/ml) measured by Densicheck equipment.

3.3.1.2. Inoculation and incubation

The suspension was streaked *via* a cotton swab in three directions on the SDA (containing chloramphenicol, 100 µg/ml) in the square plates (10x10 cm) according to the arrangement shown in figure 3.1.

D 1 or O3 LR	D1 or O3 LT	D1 or O3 RT
D 2 or O5 LR	D 2 or O5 LT	D2 or O5 RT
Flu: 100 µg/ml Or Amp: 40 µg/ml	Flu: 50 µg/ml Or Amp: 4 µg/ml	Flu: 10 µg/ml Or Amp: 0.4 µg/ml

Figure 3. 1 : Arrangement of samples of different cutting direction (LR: radial, LT: tangential and RT: transversal) of Douglas fir (D1 and D2) and oak (O3 and O5) and Amphotericin B (Amp 0.4, 4 and 40 µg/ml) for *Aspergillus fumigatus* or Fluconazole (Flu: 10, 50 and 100 µg/ml) for *Candida* spp.

The inoculated plates were dried for 15 minutes at 37 °C. Next, the three positive control discs and oak and Douglas fir circular wood discs (diameter 9.95±0.1 mm) of three cuttings (LT, LR, RT) were directly placed on this inoculated agar and incubated at 37 °C for 24 and 48 h for *Candida* spp. And *Aspergillus fumigatus* strains, respectively. The experiment was performed in triplicates and the zones of inhibitions were recorded manually.

3.3.2. Direct diffusion method for wooden sawdust

This experiment was similar to the above-mentioned disc diffusion method, with the difference of using sawdust in the wells rather than solid discs of wood. For this purpose, 8 mm wells were created inside inoculated SDA media with a perforating punch (Jeulin, Evreux, France). These wells were filled with 30 mg of different sizes of sawdust (A: 1000-500 µm, B: 250-500 µm and C: <250 µm) as shown in the figure 3.2. The plates were then incubated at 37 °C for 24 and 48 h for *Candida* spp. and *Aspergillus fumigatus* strains, respectively.

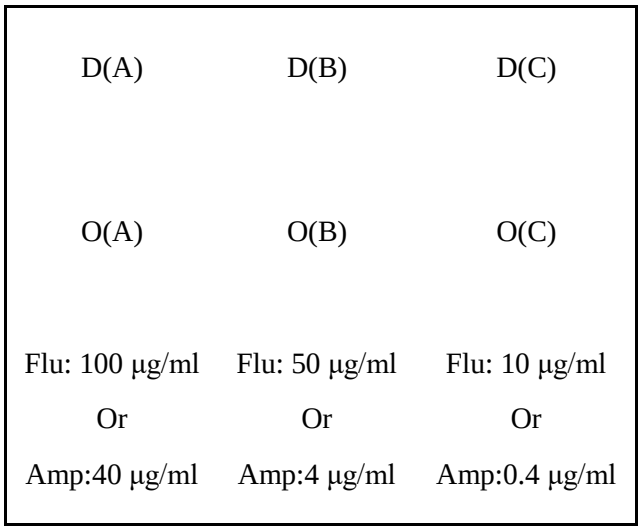


Figure 3. 2 : Arrangement of samples of different granulometry of Douglas fir (D) and oak (O) sawdust (A: 1000-500µm, B: 250-500µm and C: <250µm) and Amphotericin B (for *A. fumigatus* isolates) or Fluconazole (for *Candida* spp.)

3.3.3. Fluorometric screening test for possible antifungal activities of wood

A fluorometric method was also tested to determine the antifungal activity of wood species. In this method, the broth (RPMI-1640, Sigma-Aldrich) inoculated with *C. albicans* and Douglas fir sawdust was incubated for 24 h. Resazurin dye (non-fluorescent blue color) was added to the panel that is expected to be reduced by fungal enzymes and form resorufin (fluorescent pink color) reflecting the fungal cell viability (Pagniez and Le Pape 2001).

3.4. Study the bacterial survival and distribution on and inside wood

3.4.1. Comparative Survival of nosocomial bacteria on wood and other materials

3.4.1.1.Bacterial suspension

All the bacteria (*K. pneumoniae, A. baumannii, S. aureus* and *E. faecalis*) were pre-cultured overnight on blood agar. Then culturing was done in Brain Heart Infusion (BHI) broth by inoculating one colony from each strain and incubating for 12-18 hours at 37 ℃. Three milliliters of this culture were centrifuged for 5 mins at 12000 g. The supernatant was discarded, and the pellet was re-suspended in distilled water and repeated the centrifugation process twice. The final pellet was suspended in 1 ml distilled water to prepare inoculum solution.

3.4.1.2.Enumeration via plate count method

The inoculum solution was diluted 100 times by adding 10 µl of this suspension into 990 µl of distilled water. Then this suspension was used to prepare 10-fold serial dilution by adding 1 ml into 9 ml of distilled water each time. After, 10 µl from each suspension was inoculated on blood agar (BIO-RAD, Columbia + 5% sheep blood #63784) and incubated for 18 h at 37 ℃. The bacterial colonies were counted visually and multiplied by the dilution factor to determine the number of viable bacteria per ml of inoculum suspension.

3.4.1.3.Inoculation and incubation

The test material included sterilized (autoclaved) steel (ST), aluminum (AL), polycarbonate (PC) and stainless steel (SS), and non-sterilized wood discs (diameter 9.95±0.1 mm, thickness 3.5±0.4) from oak (*Q. petraea*) cut into LT and RT *via* a laser machine. 21 pieces from each material placed in a petri dish and 20 µl of bacterial suspension were deposited on them under a safety cabinet. The material was left open for 2 hours to let the inoculum dry, and then covered and kept in the same conditions until tested 3 pieces for bacterial recovery at each time on day 0 (2 h after inoculation or just after drying) 1, 2, 3, 6, 7 and 15.

3.4.1.4.Microbial recovery and enumeration

The individual pieces were held with sterilized forceps and placed in tubes containing 5 beads and 4 ml of distilled water. These tubes were placed in the mixer (Retsch® Mixer Mill MM 400) at 30 Hz to recover the bacteria from the chips. Then serial dilution was conducted for bacterial enumeration in the recovered rinsate. 100µl of the rinsate was diluted with 900µl distilled water then made 10-fold serial dilution to obtain dilutions of 1:10, 1:100, 1:1000 and 1:10000. Later, the 10 µl of each suspension was plated on the blood agar and incubated at 37 ℃ for 18-24 hours. For counting the low bacteria concentrations in the rinsate 10, 100, and 1000 µl of rinsate were directly inoculated on the blood agar. The Petri dishes with 1ml rinsate

were left open for 2 h in the safety cabinet to let the inoculum dry. Then the plates were incubated for 24h to 48h, according to the strain of bacteria, and enumerated the countable colonies visually.

3.4.2. Fluorescent probes to label the bacteria to follow their distribution on and inside tested wood

The autofluorescence spectra of LT and RT of three wood (oak, Douglas fir and poplar) was obtained using CSLM (Zeiss LSM 780, Germany). A stack of at least 30 images (one image every 1 µm) was done for each acquisition Three to ten acquisitions were done per wood specimen placed on a drop of PBS on glass slide. The individual fluorescence signals of different products (wood, bacteria, plastic and steel) were captured using 32 GaAsps detectors capable of detecting fluorescence between 405 nm and 665 nm in spectral mode. The dual or triple sequential laser excitation approach, with 405nm, 488 nm and 561 nm beam laser, was adapted to observe fluorescent signal generating bacteria on the material which were separated by using linear separation technique (Zimmermann et al. 2003).

Different laser excitations to image the auto-fluorescence were used according to the target specimen (Table 3.4). The spectral imaging to separate spectra of different products was done using a highly sensitive online fingerprinting module.

Table 3.4: Laser excitations used for confocal spectral laser microscopy (CSLM)

Specimen	Laser excitation (nm)
Wood	405
DRAQ5™ labelled bacteria	633
Fluorescent mCherry *S. aureus*	561
Melamine	405/488
Steel	405/488
Plastic	405/488

3.4.2.1.DRAQ5™ labelling of bacteria to determine their localization in wood

The DRAQ5™ (deep red-fluorescing bisalkylaminoanthraquinone number five) labeling and confocal spectral laser microscopy (CSLM) developed by (Dubreil et al. 2018) to study the *E. coli* contamination on poplar and pine wood, was adopted for this experiment. *E. coli* ATCC 25922 and *S. aureus* ATCC 29213 bacteria were cultured on plate count agar (PCA). The fresh colonies were used to prepare a suspension of 1.7 McFarland, containing

about $1.5x10^9$ CFU/ml of *E. coli* and $3.5x10^8$ CFU/ml of *S. aureus*, in 10 ml of PBS with 20 mM of EDTA. 1 ml of suspension was taken in an Eppendorf tube and stained with 1 µl of DRAQ5™ [1:1000 dilution of the 0.1% commercial solution (BioStatus Limited, U.K.)]. The tube was incubated for 30 min at 37 °C protected from light by wrapping in aluminum foil. This suspension was centrifuged at 12,000g for 10 min and the pellet was rinsed with 1 ml of PBS. Then again 1 ml PBS was added and vortexed the tube to remake a suspension and repeated the centrifugation process three times. This suspension was used for the following studies.

3.4.2.1.1. DRAQ5™ labeling efficacy for E. coli and S. aureus

Ten microliters of prepared suspension were deposited on a glass slide to observe the labeling efficacy *via* CSLM.

3.4.2.1.2. Influence of oak and poplar wood sterilization on the fluorescence signal of E. coli

A 50 µl of labeled bacterial suspension ($3x10^8$ CFU/ml) was deposited on the center of the longitudinally cut, gamma sterilized, autoclaved or non-sterilized, oak and poplar wood discs (diameter 9.95 ± 0.1 mm; thickness; 3.7 ± 0.4 mm). These discs were incubated for one hour at 37 °C.

Later, 4% paraformaldehyde (PFA) solution was used to fix bacteria on the samples. The bacteria fixation took place for 10 min at room temperature and washed three times (pause of 5 minutes) with PBS in a petri dish and stored at 4 °C, protected from light until CSLM imaging.

3.4.2.1.3. Direct experiment to label bacteria on wood

Ten gamma-sterilized wood discs of oak and poplar each were used in this experiment. One disc of each wood was left as a control to obtain spectra of wood for observation. 20 µl of *E. coli* were directly inoculated on the center of 6 discs and the remaining 3 were left without bacterial inoculation. Then 20 µl of the DRAQ5™ diluted in PBS (1.5: 200), was deposited on all 9 discs and incubated for 10 minutes (3 bacteria + DRAQ5™ inoculated discs) and 120 minutes at 37 °C, protected from light. Samples were fixed by the above-mentioned method and observed *via* CSLM.

3.4.2.2. Observing the distribution of fluorescent mCherry bacteria inside wood

3.4.2.2.1. Culture medium for bacteria

The chromosomal strain was grown on the PCA medium, while the plasmidic strain was cultured on the PCA media containing chloramphenicol (10 µg/ml), with incubation at 37 °C for 24 h.

The chloramphenicol solution was prepared by mixing 4 mg of chloramphenicol powder (Sigma Life Sciences, China) with 5 ml of distilled water (solubility in water 2.5 mg/ml). The solution was sterilized by filtered *via* a 0.22 µm size filter and stored at 4 °C until further use.

The agar was prepared according to the recommendations of the supplier. Briefly, 9.4 g of the PCA powder was suspended in 400 ml distilled water. The mixture was stirred continuously and boiled until the powder was completely dissolved. The medium was sterilized by autoclaving at 121 °C for 15 min. Then the bottle of the medium was placed under the laminar flow safety cabinet until the temperature reached around 55 °C.

The 5 ml of chloramphenicol (800 µg /ml) was added to this medium and gently agitated the solution to dissolve without forming air bubbles.

Finally, 25-30 ml of the prepared molten agar with or without chloramphenicol was poured into Petri dishes (90 mm diameter) and let it solidify in the laminar flow. The agar containing Petri dishes were stored at 4 °C until further use.

3.4.2.2.2. pH of wood samples

Poplar, oak and Douglas fir were chopped into small pieces and 15 g of this material was added to 23.5 ml of distilled water and mixed. Wood pieces immersed in water were left at room temperature for 24 h. Finally, the liquid was separated, and pH was measured using a Mettler Toledo pH-meter (3 points calibration method).

3.4.2.2.3. Bacterial suspension

The bacteria were pre-cultured and cultured on the respective agar. The fresh colonies were harvested and suspended in 5 ml distilled water or PBS (pH 7.4). To attain a homogenous dispersion, the tube was vortexed for one minute and then sonicated at 65 Hz for 30 seconds. The turbidity of suspension was adjusted to 1.7 McFarland.

3.4.2.2.4. Measuring fluorescence of two strains

In the initial step, a drop of each suspension was placed on a glass slide and tested *via* CSLM for their fluorescence intensity.

3.4.2.2.5. Bacterial fluorescence on different pH in solution

Buffer solutions of pH 3.5, 4, 5, 6, 7 and 10 were prepared. A 50 µl of each buffer solution was mixed with 50 µl of distilled water-based bacterial suspension on the Labtech plate and observed the reaction by laser scanning microscope at 0, 1 and 24 h.

3.4.2.2.6. Inoculation on test surfaces

A micropipette was used to inoculate 50 µl of the PBS based bacterial suspension on the center of wooden discs (diameter 9.95±0.1 mm, thickness 3.5±0.4 mm) and incubated at room temperature (20-22 °C).

3.4.2.2.7. Fixation of inoculated samples

After the specific incubation time, the wooden discs were immersed in glutaraldehyde (0.5%) for 10 minutes at room temperature (20 °C). Later the discs were rinsed with distilled water and washed in PBS (3 washes of 10 min), thrice. Finally, the discs were submerged in the PBS in a 12 well container and stored at 20 °C until observation.

Following variables were tested:

- Incubation time: 1h, 1, 4 and 7 days
- Wood species: Oak, polar, Douglas fir
- Cutting directions: Transversal, longitudinal (tangential)
- Observation: Surface and cross-section

3.4.2.2.8. Sectioning of wood discs

After observing bacteria on the wood surfaces, the penetration of the bacteria was also studied in the samples with an incubation time of 1 and 24 hours. For this purpose, the wood discs were sliced perpendicularly to the surface by a microtome (Thermo Scientific™ HM 450 Sliding Microtome) at 50 µm. Sections were mounted on a PBS drop on a slide of glass and recovered by a coverslip. The coverslip was sealed to the main slide by using the nail varnish to prevent a PBS leakage and dryness of the sample (Figure 3.3).

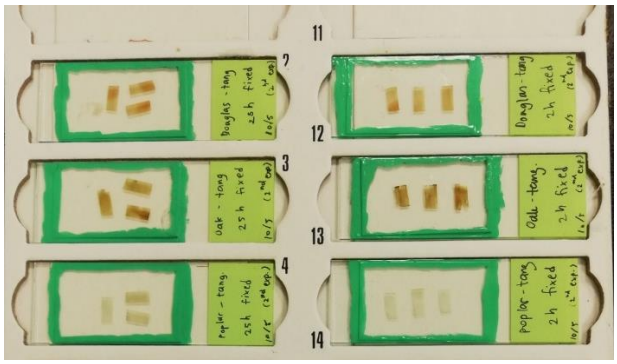


Figure 3. 3: The cross-sections of wood discs mounted on glass slides.

3.4.2.2.9. Biofilm formation potential of fluorescent mCherry on wood and melamine

Oak, poplar, Douglas fir and melamine discs were placed in 12 well cell culture plates (CELLSTAR®, Greiner Bio-one). Nine discs of each material were inoculated with 20 µl of

bacterial (*S. aureus* plasmidic strain) suspension (1.7 McFarland). One disc from each group was kept as negative control and inoculated with PBS only. After one hour, all the samples were rinsed with PBS to remove unattached bacteria. Three discs from each material were fixed after this step, according to the above-mentioned methodology, and observed to study the comparative microbial attachment.

To determine the biofilm formation potential of the bacteria and the role of culture and substrate, three discs from each material were covered with 2 ml of Trypton Soy Broth (TSB) media (Liofilchem, Italy) and the other three with 2 ml of normal saline (Physiodose+, Gilbert). After 24 h of incubation, the liquid medium was gently removed, and the samples were rinsed with PBS and fixed according to the above-mentioned methodology. All this process took place in the laminar flow cabinet at room temperature (~22 °C).

3.4.2.2.10. Image analysis with Fiji

A 2D projection was performed to get Zmax projection from each stack. Max projection image obtained in the green channel was subtracted to Max projection image obtained in the red channel to remove background due to autofluorescence of wood. The area covered by red bacteria was measured (image in red channel) and reported to area covered by wood autofluorescence (image in green channel). At least three distinct experiments were done with five images by the condition.

Chapter 4: Antibacterial properties of wood

4.1. Screening the antibacterial properties of wood

A direct diffusion method (antiboisgram) was used to study the antimicrobial properties of different wood species against selected nosocomial bacteria. This experiment also tested the influence of wood sample cutting direction and sterilization status on the antimicrobial activity. These results were published in a peer-reviewed journal (Munir et al. 2019a).

4.1.1. Results

The results of the different tests with wood discs with different faces (tangential (LT) and transversal (RT) cuts), sterilized or non-sterilized, and sterilized sawdust are presented in Table 4.1.

Table 4.1: Presence (+) or absence (-) of inhibition zone, for 4 bacterial strains, around 9 mm diameter wood discs (LT and RT cuts)

Wood	Test sample	*P. aeruginosa*	*S. aureus*	*E. faecalis*	*E. coli*
European Fir	Disc LT γ	-	+	-	-
	Disc LT	-	+	-	-
	Disc RT γ	-	+	-	-
	Disc RT	-	+	-	-
American Oak	Disc LT γ	-	+	-	-
	Disc LT	-	+	-	-
	Disc RT γ	+	+	-	-
	Disc RT	+	+	-	-
European Oak	Disc LT γ	+	+	-	-
	Disc LT	+	+	-	-
	Disc RT γ	+	+	+	-
	Disc RT	+	+	+	-
European Beech	Disc LT γ	-	+	-	-
	Disc LT	-	+	-	-
	Disc RT γ	-	+	-	-
	Disc RT	-	+	-	-
Vancomycin	Impregnated paper Disc	-	+	+	-
Colistin	Impregnated paper Disc	+	+	-	+

+ (antimicrobial effect) or – (no effect); γ is for the gamma irradiated samples

Gamma-irradiated and non-irradiated samples showed no difference of antimicrobial activity. All the tested wood species showed antimicrobial effects against *S. aureus*, two against *P. aeruginosa* and one against *E. faecalis*. The RT plane showed higher activity as compared to LT plane. This difference can be seen in case of activity of American oak against *P.*

aeruginosa, and European oak against *E. faecalis*, in these two instances, there is no antimicrobial activity evident in LT discs while it can be seen in RT samples.

4.1.2. Discussion

This experiment confirmed the previously performed pilot study regarding the use of direct diffusion method to screen the antimicrobial properties of wood material. *S. aureus* was the most susceptible and *E. coli* was the most resistant species to the diffusion of compounds from wood. It is assumed that the difference of cell wall structures might be the reason for this variation of susceptibility because *S. aureus* is Gram positive (G+) and *E. coli* is a Gram negative (G-) bacterium. Similar results of the higher sensitivity of G+ bacteria to *Q. ilex* and *Q. suber* were reported by other studies (Touati et al. 2015; Merghache et al. 2018). This reason was considered more likely because the mechanism of action of most of the wood antimicrobial compounds, including flavonoids, tannins, aldehydes, phenolic acids, terpenoids, alkaloids and terpenes, is to damage the cell wall of bacteria (Munir et al. 2019d). However, the presence of activity against *E. faecalis* (G+) and *P. aeruginosa* (G-) in the present study contradicts this hypothesis. In addition, Al Hawani et al. (2020) reported that the alcoholic extracts of oak wood showed the greatest effect against *E. coli* as compared to *S. aureus*. Both the G- and G+ bacteria had the minimum inhibition concentration of 100 mg/ml for water and ethanol extracts. Thus, it can be considered that there were some unknown factors involved in the susceptibility of different bacterial species to wood chemicals.

The higher resistance of *E. coli* could be because only heartwood parts of wood were tested in this study. Previous studies have shown that although there is a higher quantity of extractives in the heartwood part of wood, the sapwood extractives were more active against *E. coli* (Vainio-Kaila et al. 2015). It shows that the quantity of total extractives does not matter, it is the type of molecules present in certain parts of the wood which exhibit the antimicrobial activity against various types of microorganisms. Moreover, the survival of *E. coli* on sapwood has also been reported to be lower as compared to heartwood (Vainio-Kaila et al. 2013). In those studies, it was described that the sapwood may contain the live cells and therefore the metabolic activities take place which can produce antimicrobial substances. However, it is only true if the wood has not been dried and not too old; the aged and dried wood will not have such live cells.

This experiment showed that different wood species have different antimicrobial activity, and the oak wood has higher antimicrobial activity as compared to fir and beech wood in the case of *E. faecalis* and *P. aeruginosa*. This difference in activity might be due to higher extractive contents of oak wood. Moreover, the difference of antimicrobial activity of two

Quercus species (European and American oak) against *P. aeruginosa* and *E. faecalis* shows that different wood species of the same genus may have different antimicrobial activity. It is known that different species of wood and specifically oaks have different content and chemistry of extractives as well as different anatomical properties (Rowell 2012).

The two *Quercus* spp. also showed different responses according to the orientation of the cutting. The RT seems to be more active, as it can be seen in the case of American oak against *P. aeruginosa* and European oak against *E. faecalis.* These differences have been reported in our previous study also (Pailhories et al. 2017). The possible explanation of this effect could be that the wood is an orthotropic material with a specific organization of the cells in the three planes and also the antimicrobial chemicals are often localized in the inner part of the lignified cell wall. The diffusion of the extracts into agar is dependent on the direction of cells in contact with agar, ultimately causing the difference in the effect of LT and RT cuts against different bacteria.

The method described here used a quick laser cutting method that facilitated the preparation of uniform discs rapidly. This cutting does not require an intensive manipulation of the samples. In addition, there were some laser made discs showing no antimicrobial activity which shows that there are no new antimicrobial chemicals formed during the laser cutting process which could induce false-positive results in the experiment.

The similar antimicrobial activity of non-sterilized and sterilized samples showed that the sample contamination (presence of microbes in case of non-sterilized samples) did not influence the antimicrobial activity. In general, if there are contaminating microorganisms present on the wooden samples, they may influence the survival of newly contaminating microbes (Cruciata et al. 2018). Moreover, the fungi are the more commonly reported contaminations on wood material (Buchner et al. 2019), therefore it is possible that 24 h incubation time was not sufficient for contaminant fungi to interfere with the measured antimicrobial activity.

This antibiogram tested the diffusion based antimicrobial activity of solid wood samples, thus when the extractives are lost this activity is expected to diminish. This hypothesis was further tested in a complementary experiment (Annex 1.1). The wood discs, which were already tested for their antimicrobial activity *via* antiboisgram, were shifted to new inoculated petri dishes as test samples. The process was repeated for five days. It was observed that the antimicrobial activity was lost gradually with each passage on agar because each time, presumably, extractives were diffused into agar from same discs. It is expected that the

chemical analysis of discs before and after coming in contact with agar would further justify phenomena of extractive loss assumed in this method.

4.1.3. Conclusion

According to the aim of this study, it was found that the antiboisgram with discs having RT faces can be used as a quick screening method for potent antimicrobial woods. Moreover, this method is simple because it does not require any sample sterilization and/or specific chemical handling.

4.2. Effect of wood variables on antibacterial activity

The previous experiment showed that the antiboisgram could be used to screen the antimicrobial properties of solid wood. Moreover, the influence of cutting plane and sterilization status was also estimated. The oak woods had strongest antimicrobial activity while *S. aureus* was most susceptible bacterium. Therefore, this experiment was conducted to further investigate the influence of various wood variables using commonly used sessile oak wood against *S. aureus* (G+) and *A. baumannii* (G-) bacteria.

4.2.1. Results

Antiboisgram results showed that the variation of activity among six trees and three different locations was non-significant ($p < 0.05$) in all the experiments (Figure 4.1 and 4.2). Tree one showed an exception in the case of transversal face (lRT and mRT in figure 4.2) which had slightly lower activity as compared to the other five trees. The mean values (mean±SD) of activity of six trees against *S. aureus* regarding LA, LG, LN, MA, MG and MN were 13.61±1.3, 17.81±1.4, 17.94±1.4, 13.04±0.6, 17.80±1.3 and 16.57±1.03 (Table A1.1).

The results of the effect of different sterilization techniques on the antimicrobial activity of six oak trees are shown in Figure 4.1. Autoclaved samples (LA, MA), irrespective of preparation method, had significantly ($p < 0.05$) lower antimicrobial activity against *S. aureus* as compared to non-sterilized (LN and MN) and gamma sterilized samples (LM and MG). There was no significant difference ($p < 0.05$) between the activities of non-sterilized and gamma sterilized samples.

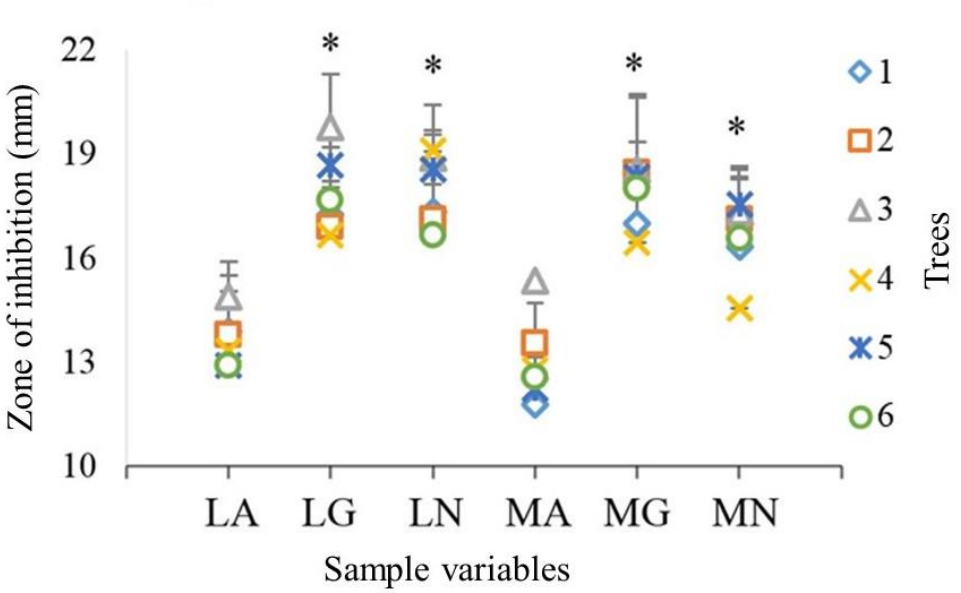


Figure 4. 1: Effect sterilization on the antimicrobial activity of different trees against *Staphylococcus aureus*. Y-axis is the mean ± standard deviation (n=3) of zone of inhibition (mm) including the diameter of wooden discs (9.95±0.1 mm); LA = Laser cut autoclaved samples; LG = Laser-cut gamma-irradiated samples; LN = Laser-cut non-sterilized samples; MA = Manual cut autoclaved samples; MG = Manual cut gamma-irradiated samples; MN = Manual cut non-sterilized samples; 1 - 6 number of trees; * $p < 0.05$

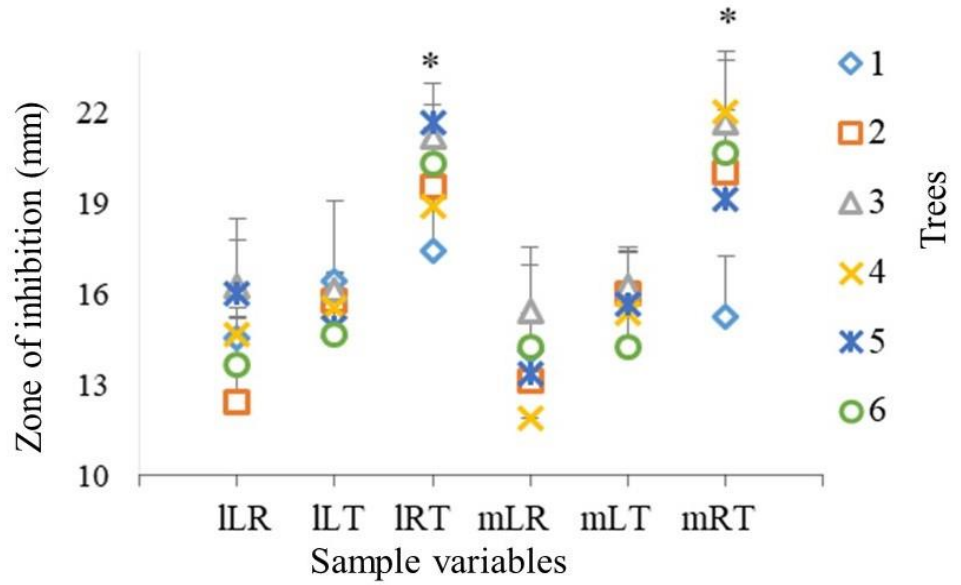


Figure 4. 2: Effect cutting planes on the antimicrobial activity of 6 trees against *S. aureus*. Y-axis is the value of zone of inhibition including the diameter of wooden discs (9.95±0.1 mm); lLR = Laser cut radial plane samples; lLT = Laser cut tangential plane samples; lRT = Laser cut transversal plane samples; mLR = Manual cut radial plane samples; mLT = Manual cut tangential plane samples; mRT = Manual cut transversal plane samples; 1 - 6 number of trees; * $p < 0.05$

Similar trends were observed in the case of *A. baumannii*, but, with higher variation and resistance of bacteria. The gamma irradiated and non-sterilized samples showed higher antimicrobial activity as compared to autoclaved samples. However, results were not significant at p-value <0.05 (Annex 1.2: Figure A1.4 and A1.5).

Data analyses regarding the effect of cutting planes on the antimicrobial activity of oak wood against *S. aureus* showed that the longitudinal and transverse faces had different antimicrobial effect, but none between radial longitudinal and tangential longitudinal (Figure 4.2).

The two discs preparation/cutting methods - manual and laser- had a non-significant ($p < 0.05$) effect on the antimicrobial activity of tested wood (Figures 4.1 and 4.2).

4.2.2. Discussion

The results of this study regarding the antimicrobial activity of wood material are supported by the previous publications reporting that different woods including oak (Pailhories et al. 2017), larch (Laireiter et al. 2013), pine (Vainio-Kaila et al. 2015), spruce (Vainio-Kaila et al. 2017a), beech and fir (Munir et al. 2019a) have antimicrobial properties against various isolates of *S. aureus*. In this study, however, the antimicrobial activity of wood material was very low against *A. baumannii* as compared to *S. aureus*.

No significant difference ($p < 0.05$) of antimicrobial activity among the 6 trees (Figure 4.1 and 4.2) was observed even though they originated from three different locations (Table 1). On the basis of these findings we can hypothesize that all trees had similar levels of anti-microbial compounds. Earlier studies have also reported that ecological zone has negligible effect on chemical composition of different oak species e.g. *Q. robur* and *Q. petraea* (Prida et al. 2006). This uniformity may also be due to the fact that similar parts of wood were collected from different trees (mature heartwood), which reduced the chances of high variations in chemical composition. Additionally, it is possible that the total extractive content varied among different trees, however, the active antimicrobial ingredients were similar in all trees. Another, possibility of lack of variations among groups might be owing to low sensitivity of this method which could not identify these minor variations.

The wood discs with transverse (RT plane) faces showed stronger antimicrobial activity as compared to both longitudinal faces (LT and LR). These findings are in line with the earlier reports (Pailhories et al. 2017; Munir et al. 2018, 2019a). In those publications, this variation of activity was attributed to the arrangement of fibers in wood. The fibers in wood mostly run longitudinally and the diffusion in the longitudinal direction is about 10 to 15 times faster than tangential or radial diffusion (Simpson 1999), therefore, when the RT faced discs were placed

on agar, there is potentially a higher diffusion of active compounds into agar than from the LT and LR samples. Consequently, the wood cut into RT direction would be more efficient to control the microbial growth. The difference among the activity of LT and LR cutting was non-significant which could be due to minor differences of fiber arrangement in both cuttings and the current method did not sensitively measure this difference.

The antimicrobial activities of non-sterilized and gamma sterilized samples were similar as previously described (Munir et al. 2019a). It shows that even if the non-sterilized samples are contaminated with other microbes (Buchner et al. 2019), this method is not influenced by their presence, especially, when used for screening (Munir et al. 2019a). Otherwise, in natural environment, many microbial interactions occur on wooden surfaces, which could be antagonistic or symbiotic, and can interplay with the antimicrobial activity of wood against specific microbes. For example, the studies have shown that wood being an acidic material supports the growth of lactic acid bacteria which can counter the growth of some bacterial pathogens e.g. *Listeria, Salmonella* and *Campylobacter* (Guillier et al. 2008; Di Grigoli et al. 2015; Gaglio et al. 2019). In addition, researchers have used certain wood microbes e.g. *Bacillus subtilis,* to treat surfaces and stop the growth of undesired wood degrading fungi (Borges et al. 2018; Sajitha et al. 2018; Fantinel et al. 2018).

The lower antimicrobial activity of autoclaved wood samples, as compared to non-sterilized and gamma sterilized wood, shows the loss of some active compounds. As many of the VOC are responsible for the antimicrobial action of wood (Vainio-Kaila et al. 2017a), their loss by high-temperature treatment (Vainio-Kaila et al. 2013; Pohleven et al. 2019) might have reduced this ability. This finding is important because the loss of VOC from wood continues throughout the usage life until reaching a minimum level. In such conditions, the antimicrobial activity is also likely to reduce over time. In an earlier study, it was observed that the two-year storage of wooden discs in polythene bags did not reduce the antimicrobial activity of samples (Munir et al. 2019a), thus we can assume that the lost molecules degraded faster at the higher temperature (of autoclaving) than at room conditions. It is not clear, however, if the autoclaving changed the physical structures of wood, leading to reduced diffusion and ultimately, antimicrobial activity.

The samples prepared by two-disc preparation methods, manual punch or laser cutting, did not show any significant difference ($p < 0.05$) of antimicrobial activity. In earlier studies the question was raised, the laser cutting uses very high temperatures to cut the samples, so are there any chances that the degradation of wood chemicals at very high temperatures could increase or decrease? The results answered the question by showing similar activities

irrespective of the preparation method. In addition, to explain the point that why chemicals were not degraded at so high temperatures, it can be stated that the laser beam cuts the wood samples only on the sides, and not the contact surface which is supposed to be tested for antimicrobial action. Therefore, it can be assumed that there are no new chemicals formed or reduced to interfere with the antimicrobial activity. In earlier studies, laser cutting has been described as an efficient and quicker method for uniformly sized sample preparation as compared to manual sawing (Pailhories et al. 2017; Munir et al. 2019a). Further research should be targeted to study the influence of surface burning techniques on antimicrobial activity also because this technique is becoming very common in the furniture industry.

The method used in this study has a limitation of variability when the antimicrobial activity is low. For example, the minimum detection limit of the direct diffusion method was considered as 1 mm (10 + 1 mm) of the zone of inhibition and in the case of *A. baumannii*, the activity was very low as compared to *S. aureus* (variability of method tested for these two bacteria in Annex 1.4: Table A1.4 and A1.5). Consequently, the influence of wood variables in the case of *A. baumannii* was non-significant when considering $p < 0.05$. Therefore, future studies should consider this point when interpreting the results.

4.2.3. Conclusion

The findings show that the direct diffusion method can be used to determine the antimicrobial properties of wood material. In addition, some influencing variables such as the effect of sterilization and cutting planes should be taken into consideration when testing the antimicrobial properties of wood material. Based on the sampling set, antibacterial properties of oak (*Q. petraea),* tree activity was found to be neither origin nor tree dependent. Future studies should apply this methodology with uniform-sized and weighed discs to confirm these variables in multiples wood species and different microbes.

4.3. Susceptibility of different bacteria to oak wood

The previous experiment confirmed that wood variables influenced the observed antimicrobial response against tested bacteria. Hence, optimum conditions, of non-sterilized, laser made, RT cut wood samples could be further used to test the variables related to bacterium itself. Therefore, this study was planned to test the antimicrobial activity of selected wood samples against a wide range of bacteria and their isolates.

4.3.1. Results

The global results of wood activity against 50 isolates are given in table 4.2. The main findings are that none of the *E. coli* isolates were susceptible to the antimicrobial activity of oak wood, and only one *Enterococcus* isolate was susceptible. While, oak presented activity

against six *P. aeruginosa* isolates, nine of Acb and eight of *S. aureus*. The antibacterial activity of oak was irrespective of the antibiotic resistance status of bacteria. The activities (mean ±SD) of six trees were 15.25±2.85, 11.72±1.32, 18.90±1.05 and 20.26±5.93 for *Acinetobacter* spp., *Enterococci* spp., *P. aeruginosa* and *S. aureus* respectively. Meanwhile, the variation of activity among 6 trees were non-significant (p-values are given in Annex: Table A1.2).

Table 4.2: Results of the assessment of activity of *Q. petraea*.

Bacterial Species Cut-off	**Isolate**	**Resistance**	Means of diameters	Interpretation
Acinetobacter calcoaceticus-Acinetobacter baumannii complex SD = 4 mm Cut-off 14 mm	ATCC 19606		14	Active
	15519454101	R (Carbapenemase *bla*$_{\text{NDM-1}}$)	15	Active
	15015364001		14	Active
	15517172701	R (Carbapenemase *bla*$_{\text{OXA-23}}$)	19	Active
	18516030701	R (Carbapenemase *bla*$_{\text{OXA-23}}$)	14	Active
	ATCC 17978		11	Inactive
	13504498703	R (Carbapenemase *bla*$_{\text{NDM-1}}$)	15	Active
	18529482201		20	Active
	17536228901		17	Active
	18526909702		14	Active
Escherichia coli SD = 1 mm Cut-off = 11 mm	ATCC 35218		10	Inactive
	18528747601	R (carbapenemase *bla*$_{\text{OXA-48}}$)	10	Inactive
	18528531001	R (ESBL+)	10	Inactive
	18540723702		10	Inactive
	18541756901	R (ESBL-)	10	Inactive
	18541702301	R (ESBL+)	10	Inactive
	18541175601		10	Inactive
	18541731901		10	Inactive
	18541481601	R (ESBL-)	10	Inactive
	18540722802	R (ESBL+)	10	Inactive
Enterococci SD = 2 mm Cut-off 12 mm	18524021401	*E. faecium* R (*vanA*)	11	Inactive
	ATCC 29212		11	Inactive
	ATCC 51299	*E. faecalis* R (*Van B*)	11	Inactive
	18541660506		12	Active
	18541204901		11	Inactive
	18541746201		11	Inactive
	18541593801		11	Inactive
	18541584501		11	Inactive
	18541073802		11	Inactive
	18541796701		11	Inactive
Pseudomonas aeruginosa SD = 2 mm Cut-off = 12 mm	ATCC 27853		10	Inactive
	18529486401	R	11	Inactive
	18010906401	R	11	Inactive
	18015393101		14	Active
	18541890201		11	Inactive
	18541668202		12	Active

	18541207902		14	Active
	18015225701		12	Active
	18540722802		12	Active
	18015116701		14	Active
Staphylococcus aureus SD = 7 mm Cut-off 17 mm	15015019102	MRSA	24	Active
	16005880401	MRSA	17	Active
	16009349201	MRSA	18	Active
	16518728101	MRSA	35	Active
	16006004401		16	Inactive
	14528592702	MRSA/GISA	20	Active
	16518868104	MRSA	16	Inactive
	16513145401	MRSA	18	Active
	ATCC 29213		21	Active
	KSKS7326	MRSA/GISA	18	Active

R: resistant according to the criteria defined in the study
ESBL+: production of extended-spectrum beta-lactamase
ESBL-: absence of production of extended-spectrum beta-lactamase
MRSA: methicillin-resistant *Staphylococcus aureus*
GISA: Glycopeptide-intermediate *Staphylococcus aureus*

4.3.2. Discussion

The wood material presents antimicrobial properties to a wide range of microorganisms. This character is a plus point as compared to other inert surfaces. This study widened the testing to 50 bacterial isolates to evaluate the antimicrobial activity of oak wood. The general results showed that wood had antimicrobial activity against most of these isolates. The active extractive components are considered as the cause of this activity.

Different bacteria showed a different level of susceptibility to the oak wood. First of all, the antimicrobial activity was dependent upon the bacterial species.

The *E. coli* species was the most resistant and tested wood samples did not show any activity against any of 10 tested isolates. Similar results were found in previous studies (Basri and Fan 2005; Safary et al. 2009; Touati et al. 2015; Munir et al. 2019a). The second most resistant bacteria were *Enterococcus* spp.*;* although all the tested isolates showed mild susceptibility (ZOI 11-12 mm), oak was considered inactive based on the breakpoints of antimicrobial activity. Previous studies have shown that extracts from various wood species show equally stronger antimicrobial activities against these two bacteria (Sati et al. 2011; Nagesh et al. 2012; Kaynar 2017; Bahar et al. 2017; Gul et al. 2017; Tayel et al. 2018; Elansary et al. 2019; Smailagić et al. 2020). These differences could be attributed to the lack of specific antimicrobial chemicals in heartwood and the low dose of active molecules. To support the first possibility, it has been postulated that there could be a lesser amount of active

antimicrobial chemicals in heartwood than in sapwood, which acts against *E. coli* (Vainio-Kaila et al. 2013, 2015). As there were only heartwood parts of tree used in the present experiment, the antimicrobial activity against *E. coli* and *Enterococcus* spp. were not evident. According to the second possibility, higher doses of extractives are needed to achieve bactericidal activity. This hypothesis was tested in a separate experiment (Annex 1.5) by extracting the wood chemicals and then loading their concentrated doses to filter paper discs and testing them against *E. coli* and *S. aureus via* an agar diffusion method. The results showed that *S. aureus* is susceptible at a minimum dose of 2 mg per disc, while concentrated doses of extractives (5-20 mg/disc) were needed to stop the *E. coli* growth in a dose-dependent manner. Besides, double zones of inhibition were observed around discs tested against *E. coli* indicating bacteriostatic effects at outer circles of diffused chemicals; whereas clear zones of inhibition were formed closer to discs which were considered as a real bacteriostatic effect.

An average antimicrobial activity was observed against the *P. aeruginosa* isolates where 6/10 isolates were susceptible. This study was the first investigation testing the antimicrobial properties of *Q. petraea* against *P. aeruginosa.* (Mohabi et al. 2017) reported that *Q. infectoria* galls extracts at a concentration of 200 µg/ml inhibited the growth of five *P. aeruginosa* strains tested; meanwhile, at a dose of 100 µg/ml, the extract had only a mild effect on the colony size and did not inhibit the bacterial growth. Likewise, in other studies, this bacterium has shown an average susceptibility to extracts of *Q. incana* (Gul et al. 2017), *Q. suber* (Touati et al. 2015), *Q. brantii* (Safary et al. 2009; Bahar et al. 2017; Zare et al. 2019), *Q. virginiana* (Adonizio et al. 2008), *Q. leucotrichophora* (Sati et al. 2011), *Q. infectoria* (Basri and Fan 2005; Zachariah et al. 2014; Tayel et al. 2018; Ahmed and Salih 2019), *Q. robur* (Kaynar 2017), *Q. macrocarpa* and *Q. acutissima* (Elansary et al. 2019), and resistance to *Q. rubra* (Omar et al. 2000) and *Q. brantii* at given concentrations (Shahi et al. 2017). *P. aeruginosa* is ubiquitous in the environment and forms biofilms for survival. It is known to cause HAI of the blood, lungs (pneumonia), or other parts of the body after surgery (Adonizio et al. 2008). Therefore, identifying the antimicrobial properties of oak would help to prevent the persistence of this bacteria in the environment (Adonizio et al. 2008) because it has been reported that oak wood (*Q. infectoria*) extracts show high anti-quorum sensing activity and reduce the pyocyanin, protease, and elastase production and thus stop biofilm formation (Karbasizade et al. 2017). These extracts can also be used as a component of some anti-infectious medications or inanimate surface treatments (Basri and Fan 2005; Zare et al. 2019).

In this study, *Acinetobacter* spp. and *S. aureus* were the most susceptible bacterial isolates. These findings are in line with previous studies showing a wide range of antimicrobial properties of solid oak wood against these two bacteria (Pailhories et al. 2017; Munir et al. 2019b, c, 2020b). *S. aureus* is the most commonly studied bacteria regarding the antimicrobial properties of wood and in general, most susceptible to wood chemicals (Basri and Fan 2005). Various studies reported the susceptibility of different isolates of *S. aureus*, ranging from susceptible test strains to multi-resistant isolates, against the oak wood extractives including *Q. petraea* (Smailagić et al. 2020), *Q. alba* (Dettweiler et al. 2019), *Q. incana* (Gul et al. 2017), *Q. leucotrichophora* (Sati et al. 2011), *Q. brantii* (Bahar et al. 2017), *Q. rubra* (Omar et al. 2000), *Q. suber* (Touati et al. 2015), *Q. robur* (Kaynar 2017), *Q. infectoria* (Basri and Fan 2005; Zachariah et al. 2014; Tayel et al. 2018; Ahmed and Salih 2019), *Q. acutissima*, *Q. macrocarpa*, *Q. robur* (Elansary et al. 2019). These studies did not necessarily use the wood parts of oak, rather fruits, leaves, roots, and bark were employed for extraction and further antimicrobial testing. In addition, the type of solvent (Kaynar 2017) used, and method of testing is also known to influence the observed antimicrobial response (Munir et al. 2020a).

On the other hand, there is not much research available on the interaction of *Acinetobacter* spp. with solid wood or its extractives. (Dettweiler et al. 2019) reported that *Q. alba* extracts (branch galls and bark) attained 50% growth inhibition against multiple *A. baumannii* strains at 32–256 µg/ml. In their study, the susceptibility of *S. aureus* (susceptible and multiresistant) and *A. baumannii* strains were almost similar. This bacterium can maintain its virulence properties for a long-time after desiccation or starvation (Chapartegui-González et al. 2018). For instance, despite long-time desiccation, some *A. baumannii* strains maintained their ability to form biofilms after rehydration, the addition of nutrients, and changing temperature, conditions that they can easily find when they are transmitted from a hospital surface to hospitalized patients (Chapartegui-González et al. 2018). The observed weak antimicrobial properties of wood could prove mild stress to these bacteria, and we do not know if it is linked to the survival of this bacterium on wooden surfaces. Moreover, one of the antimicrobial actions of wood is by desiccating the microbes, therefore, it would be interesting to see if *Acinetobacter* spp. and other bacteria could maintain or revive their virulence on wood surfaces.

In the current study, the antimicrobial activity of wood does not seem to be influenced by the resistance of all these tested isolates to antibiotics. The reason could be that the bacteria become resistant to antibiotics because each antibiotic has a specific mode of action to kill

bacteria or stop its growth and over time if the bacteria adapt itself, they become resistant (Reygaert 2018). If the bacteria must fight two stressors at the same time, they are less likely to develop resistance (Hiltunen et al. 2018). Likewise, the extractives of wood are a combination of various antimicrobial compounds and they affect (probably synergistically) different parts of bacteria for growth inhibition (Munir et al. 2019f), consequently, even if the microorganism is resistant to a target-specific antibiotic, it can still remain prone to wood chemicals. Such results, at the time of ever-increasing antimicrobial resistance threat, could retain the attention of infection control teams and hospital administrators, and in the perspective of using wood as a material in the hospital setting.

In general, all these tested common nosocomial pathogens, and their survival time, on hospital surfaces, ranges from few hours to several months depending upon the environmental conditions (Kramer and Assadian 2014). Therefore, current findings are very important regarding the control of these two pathogens on wooden surfaces in hygienically important places.

It is recommended in the antimicrobial sensitivity testing methods that two independent readers should manually take the measurements of zone of inhibitions to reduce the chances of error. The zones of inhibition in above-mentioned methods were also measured by at least two readers and there was only a minor difference in measurements. The data analysis regarding the difference in readings of two independent readers has been presented in Annex 1.3 (Table A1.3).

4.3.3. Conclusion

This experiment investigated the susceptibility of different bacteria against the antimicrobial activity of oak wood tested *via* a direct diffusion method. It was found that the susceptibility of most bacteria to oak wood depended mostly upon the species of wood rather than strains or isolates. Moreover, the resistance status of bacteria to antibiotics was also not an influencing factor. On the side of wood variables, the six trees tested showed similar activity against all the tested isolates. Further studies are needed to understand the mechanism of action of wood chemicals and how the antimicrobial properties can be employed in healthcare settings to utilize their antimicrobial potential.

Chapter 5. Antifungal activity of wood

There are many fungal species responsible for infections in humans and animals. The most common of them are *Candida* and *Aspergillus* genera, therefore, they were selected for this study. The persistence of these pathogens in healthcare buildings can be deadly, especially for immunocompromised individuals (Talento et al. 2019). However, it is not known if the wood material is a risk factor for fungal infections in healthcare or residential buildings. The reason for this lack of information can be due to the lack of fungal infections research because most of the research and related funding goes for bacteria or virus research. Even the fungal infections are equally devastating, for example, *Cryptococcus neoformans* and *C. gattii*, cause meningitis resulting in 20 times more deaths as compared to bacterial meningitis caused by *Neisseria meningitidis*, however, fungal research funding in this domain is only 25% of that allocated for bacterial research (BSAC 2020). There are millions of fungal infections reported around the globe with 1.5 million deaths and only two percent of the total infection research funding is allocated to the medical fungal research (Rodrigues and Nosanchuk 2020). This might be the possible reason that there is not enough evidence on understanding the role of wooden surfaces in fungal infections. Some epidemiological studies exist regarding the sick building syndrome in different residential buildings (Zhang et al. 2018; Smajlović et al. 2019; Babaoglu et al. 2020) but it is not evident that fungi are responsible for that. In fact, most of the research is published regarding the fungal deterioration of wooden buildings (Haas et al. 2019; Reinprecht et al. 2020). Meanwhile, some studies have shown that wood extracts could be used to stop the growth of various wood degrading fungi (Mansour et al. 2015; Mansour and Salem 2015; Salem et al. 2016d, c, 2019; El-Hefny et al. 2020) and against some strains which could cause infections in humans and animals (Johnston et al. 2001; Khurram 2011; Tomičić et al. 2017; Valette et al. 2017). Therefore, these experiments were conducted to enhance the understanding of antimicrobial properties of solid wood against pathogenic fungi responsible for HAI.

5.1. Direct disc diffusion method to screen antifungal properties of wood

The previous studies showed that a direct diffusion method can be used to test the antibacterial properties of wood material against different bacteria. However, this method has not been tested against fungi. Therefore, this study was planned to test the antifungal properties of two solid wood species against some common susceptible and resistant fungal isolates responsible for HAI.

5.1.1. Results

The antifungigram results are shown in Table 5.1. Douglas fir wood had higher antimicrobial activity against all the fungi as compared to oak. The fungal strains CAPA 18, CAPA3, CAAU6 and CAAU22 were susceptible to all the tested wood samples. The RT faced samples showed the strongest antifungal activity followed by LR and then LR.

The quantitative values and the statistical analysis results are given in Annex 2 (Table A2.1 and A2.2). There was no significant ($p<0.05$) difference among the antimicrobial activities of two longitudinal cuts (LT and LR) and neither between two trees for each wood. However, RT cut samples in all trees showed significantly ($p<0.05$) stronger antifungal activity as compared to LT and LR. In addition, the Douglas fir showed significantly ($p<0.05$) higher antifungal activity against most of the tested fungi as compared to that of oak wood. It is interesting to note that the samples exhibited high activity against fluconazole-resistant isolates such as the new emerging *C. auris*.

Table 5.1: Zones of inhibition (diameter in millimeter mean±SD) around the different cuts of sessile oak and Douglas fir wood discs in against various fungal isolates

Fungi		Wood												Control
		Douglas (D)						Oak (O)						
		1			2			3			5			
		RT	LT	LR	RT	LT	LR	RT	LT	LR	RT	LT	LR	
Susceptible *Candida* spp.	CAAL 16	14±1.41	0	0	14.5±0.7	0	0	0	0	0	0	0	0	44
	CAAL 93	11.5±0.7	0	0	11.5±0.7	0	0	0	0	0	0	0	0	44
	CAAL 97	11.5±0.7	0	0	11.5±0.7	0	0	0	0	0	0	0	0	44
	CAAL 118	13.5±0.7	0	0	14.5±0.7	0	0	0	0	0	0	0	0	36
	CAPA 18	14±1.41	11.5±0.7	0	14.5±0.7	11.5±0.7	0	12.5±0.7	10.5±0.7	0	12±0.0	11±0.0	0	22
	CAGL 3	0	0	0	0	0	0	15±1.41	11.5±0.7	0	15.5±0.7	11.5±0.7	0	18
	CATR 1	0	0	0	0	0	0	0	0	0	0	0	0	32
Resistant *Candida* spp.	CAAL 117	17.5±0.7	11.5±0.7	0	16±1.41	12.5±0.7	0	0	0	0	0	0	0	0
	CAPA 3	14±1.41	12.5±0.7	0	13.5±0.7	11.5±0.7	0	12.5±0.7	11±0.0	0	12±0.0	10.5±0.7	0	28
	CAKR 1	0	0	0	0	0	0	0	0	0	0	0	0	22
	CAAU1	16±1.41	0	0	17±1.41	0	0	0	0	0	0	0	0	15
	CAAU 6	23.5±0.7	15±1.41	13±1.41	22±1.41	16±1.41	12.5±0.7	14±1.41	12.5±0.7	11.5±0.7	13.5±0.7	13.5±0.7	11±0.0	14
	CAAU 20	14.5±0.7	0	0	14±0.0	0	0	12.5±0.7	0	0	11.5±0.7	0	0	14
	CAAU 22	21±1.41	13±0.0	11.5±0.7	16.5±0.7	12±1.41	10.5±0.7	16.5±0.7	12.5±0.7	10±0.0	15.5±0.7	11.5±0.7	10.5±0.7	0
Aspergillus spp.	ATCC 76	11.5±0.7	0	0	11.5±0.7	0	0	0	0	0	0	0	0	22
	ASFU 7	15.5±0.7	0	0	15±0.0	0	0	0	0	0	0	0	0	22
	WT 104	11±0.0	0	0	12±0.0	0	0	0	0	0	0	0	0	22
	KA 66	14±1.41	0	0	14.5±0.7	0	0	0	0	0	0	0	0	22

Values are given in mean ±SD. Control 50µg/ml fluconazole (*Candida* spp.) and 40µg/ml amphotericin B (*Aspergillus* spp.)

5.1.2. Discussion

The sessile oak and Douglas fir showed antimicrobial activity against a wide range of fungal isolates tested. Previous studies have also reported antifungal properties of these wood species against various fungi including *Candida* spp. and *Aspergillus* spp. (Johnston et al. 2001; Valimaa et al. 2007; Tešević et al. 2009; Smailagić et al. 2020). However, the antifungal activities in those studies were based on extracts or essential oils of trees and not solid wood.

This study shows that the direct diffusion method could screen the antimicrobial properties of two wood species when tested against a wide range of susceptible and resistant fungi. These findings were also reported in our previous studies in the case of different bacterial strains. The antimicrobial activities in such methods are attributed to the chemicals diffused from the wood into the agar. However, in this study, it is very likely that low amounts of the active chemicals (present in wood discs) diffused into the agar and it could have led to an underestimation of results.

There was a significant difference (p-value <0.001) between the antifungal activities (mean±SD) of Douglas fir and oak wood which were 13.6±2.9 and 11.3±1.7 respectively. In general, the Douglas fir wood showed higher antimicrobial activity, except for the positive activity of oak against CAGL 3. The results can be explained by the fact that these woods belonged to different genera, oak is a hardwood (angiosperm) and Douglas fir is a softwood (gymnosperms), and their chemical composition was different; likewise, their antifungal activities also varied. One of the major roles in this difference might of flavonoid compounds, which are known to have antifungal activities (Valimaa et al. 2007; Al Aboody and Mickymaray 2020). It has been reported that the heartwood of Douglas fir contained 3.6% extractives and among them, 75-80% were flavonoids (Nisula 2018). While sessile oak wood has been reported to contain about 4% extractives (Smailagić et al. 2020) [some other oaks may have 12-19% (Doussot et al. 2002; Miranda et al. 2017)] and flavonoids constituted only about 5-12% of them (Kim et al. 2012; Touati et al. 2015). In such cases, if a similar weight of wooden discs is placed on agar, we can expect that more flavonoids will transfer to medium from Douglas fir as compared to those from oak. Therefore, in the present case possibly the difference of flavonoid content was the reason for, in general, the higher antifungal activity of Douglas fir. However, the oak wood is denser as compared to Douglas fir, so the hypothesis of more flavonoid content in similar sized (volume) of discs may not be true, therefore, further chemical characterization of test samples would give helpful information in future studies.

The antifungal activities of all the tested wood samples were independent of the fungal resistance against fluconazole or amphotericin B. As it is evident from results (Table 5.1) that the activity of wood samples was almost the same against both the fluconazole susceptible and the resistant isolates. This effect might be linked to a wide range of chemicals present in wood, which can stop fungal growth in different ways. For example, fluconazole and itraconazole interact with a cytochrome P-450 enzyme called 14- lanosterol demethylase that is is implicated in ergosterol biosynthesis which is important for the integrity of the fungal cell membrane, thus they disrupt the cellular permeability (Spampinato and Leonardi 2013). Amphotericin B (the positive control used for *Aspergillus* spp.) also works similarly by directly binding to ergosterol and disrupting the cell membrane functioning (Spampinato and Leonardi 2013). On the other hand, the flavonoids have antifungal properties, which are not limited to only membrane disruption but also inhibit or affect the efflux pumps, cell division, cell wall formation, nucleic acid and protein synthesis, and mitochondrial functioning (Al Aboody and Mickymaray 2020).

None of the *Aspergillus* spp. strains were susceptible to the antimicrobial activity of oak wood. An earlier study has also shown that the oak (*Q. ilex*) extracts did not have any antifungal activities against filamentous fungi *A. flavus*, whereas the inhibition effects were seen against the growth of all *C. albicans* isolates tested (Güllüce et al. 2004). However, studies have shown the anti-aspergillus activities of other oak species, such as *Q. incana* (Sarwar et al. 2015, 2018; Gul et al. 2017), *Q. dilatata* (Ahmed 2018), *Q. robur, Q. macrocarpa, Q. acutissima* (Elansary et al. 2019) and *Q. infectoria* (Abdulla 2018) against *A. niger* and *A. flavus*. (Jamil et al. 2012) reported that the *Q. dilatata* extracts showed strong antifungal activity against wide range of filamentous fungi including *A. niger, A. flavus, Fusarium moniliformes, F. solani* and *Alternaria* sp., however, the weakest activity was observed against *A. fumigatus*. These studies show that the oak species have generally mild to weak antifungal activity against *A. fumigatus*. The absence of antimicrobial activity of *Q. eubra* against *A. fumigatus* was also reported by (Omar et al. 2000), however, their study also did not observe any activity against *C. albicans* that might be because there was a fungistatic effect not fungicidal as observed in a later study (Andrensek et al. 2004). Some other studies have also shown antifungal activity against *C. albicans* by *Q. paterae* (Smailagić et al. 2020), *Q. infectoria* (Tayel et al. 2018), *Q. robur* (Kaynar 2017), *Q. brantii* (Moshfeghy et al. 2018), *Q. castaneifolia* (Sefidgar et al. 2015), *Q. ilex* (Merghache et al. 2018), *Q. macranthera* subsp. *syspirensis, Q. cerris, Q. pubescens* and *Q. coccifera.* (Söhretoglu et al. 2007). (Söhretoglu et al. 2007) also observed antifungal properties of different extracts from the mentioned oak

species against *C. krusei* and *C. parapsilosis*. Omar et al. (2000) observed that, in general, the hardwood extracts were more active against filamentous fungi (*Trichophyton mentagrophytes* and *Microsporum gypseum*) than yeast-like fungi (*Cryptococcus neoformans* and *Sacchromyces cervisiae*), which is not the case, however, with *Candida* spp. and *Aspergillus* spp. fungi or at least for sessile oak wood. Therefore, assumptions can be made that the anti-aspergillus compounds in sessile oak are either absent or are present in so low quantities that the current diffusion-based method could not measure their activity. The second assumption is more appreciable because the anti-aspergillus activity was also absent in the longitudinal cut Douglas fir wood and this difference is linked to the diffusion of chemicals into the agar.

The difference of antimicrobial activity within the species of tested wood was non-significant ($p<0.05$). Similar findings were observed in our previous studies where the trees originated from different locations had similar antibacterial activity. This similarity of activity can be explained by the fact that the main factor influencing extractives level in wood is the species (Guchu et al. 2006), not the trees (Prida et al. 2007). The effect of the local environment, such as geographical location (Guchu et al. 2006) and soil type, is very low (Smailagić et al. 2020) and could be considered negligible (Prida and Puech 2006).

The effect of cutting planes on the antifungal activity of wood discs in all the tested samples was significant ($p<0.05$). The RT showed a significantly ($p<0.05$) higher antimicrobial activity as compared to LT and LR. However, the difference of activities between the LR and LT was non-significant ($p<0.05$), even though it appears that LT had slightly higher activity as compared to LR, for example, LT showed a positive antimicrobial activity against 4 of the tested strains while in case of LR it was only against 2 in all the tested trees. Our previous results on the influence of cutting planes on the antibacterial activity of wood showed similar findings.

5.2. The study of antifungal properties of wood via the direct well diffusion method

This study was planned to develop a direct antifungigram method that could test the antifungal properties of sawdust of different sizes. The antifungigram results were manually observed as shown in Figure 5.1 (according to the arrangement of Figure 3.2). The zones of inhibition were mostly absent or very mild and inconsistent. Therefore, the results were considered negative in all cases.

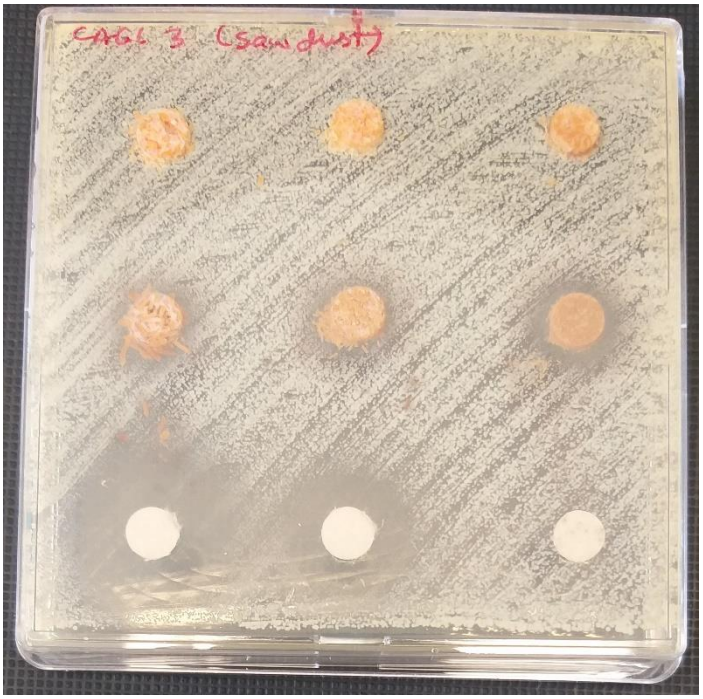


Figure 5. 1: Antifungigram result using different granulometric sizes of sawdust of sessile oak and Douglas fir in the wells created on *Candida glabrata* inoculated Sabouraud Dextrose Agar, 24 h after incubation

This very low antifungal activity of sawdust as compared to the wooden discs was possibly because a very low quantity of sawdust (30 mg) material was used as compared to discs which weighed on an average from 130.7±13 mg for Douglas fir and 190.2±17 mg for oak wood. Contrarily, our previous study has shown that the sawdust well diffusion method could be used to screen the antimicrobial properties of wood against susceptible bacterial strains (Munir 2016; Munir et al. 2019a).

5.3. Fluorometric method

The results showed that both the control and wood samples showed pink color formation. It was assumed that the components of wood material contain some compounds which can reduce the resazurin. Therefore, it was not possible to determine the inhibition of fungal growth by sawdust using spectrofluorimetry.

5.4. Conclusions

The broad objective of this chapter was to test the antifungal properties of wood material. The results showed that oak and Douglas fir exhibited antifungal behavior. The antifungal action was similar in both susceptible and resistant strains of fungi. The Douglas fir wood showed a wider range of antifungal action compared to oak wood. However, the

influence of tree type (based on origin) was not significant. The discs cut in transversal directions showed better antifungal activity as compared to two longitudinal cuttings.

The two other methods used in this study including the well diffusion method and fluorometric method were not compatible to study the antifungal properties of wooden sawdust. The reasons being the low quantity of sawdust for the first and the reduction of fluorochrome indicator by wood component of wood components in the second method.

Chapter 6. Distribution and survival of bacteria on and inside wood

In previous experiments, it was observed that the many wood species showed antimicrobial properties against a wide range of fungi and bacteria. As the objective of this thesis is to really concentrate on the antimicrobial properties of solid wood material, the earlier experiments provided the opportunity for antimicrobial compounds to diffuse from wood and observed their impacts. Clearly, the chemical profile of wood will have a great influence on the observed effects. In the real world, however, it is the survival and distribution of microbes on and in wood products that is of greater interest. Two main approaches were used in this section: first was to recover the microbes from wood samples after inoculation to determine their survival and second to observe the microbes on and inside wood via innovative tools of CSLM and fluorescent probes.

6.1. Comparative Survival of nosocomial bacteria on wood and other materials

This study investigated the survival of different nosocomial bacteria (*K. pneumoniae, A. baumannii, S. aureus* and *E. faecalis*) on Sessile oak wood and other commonly used indoor hospital surfaces (steel, aluminum, polycarbonate). The number of inoculated bacteria, 1 - 24 x 10^6, were high enough to reliably show the reduction of microorganisms on all the tested materials. All the experiments were done in triplicate, increasing the reliability of the study.

6.1.1. Results

The global results show that the tested bacteria survived for the least time on wood material as compared to other materials. The mean values and statistical analysis results are given in the Annex 4.

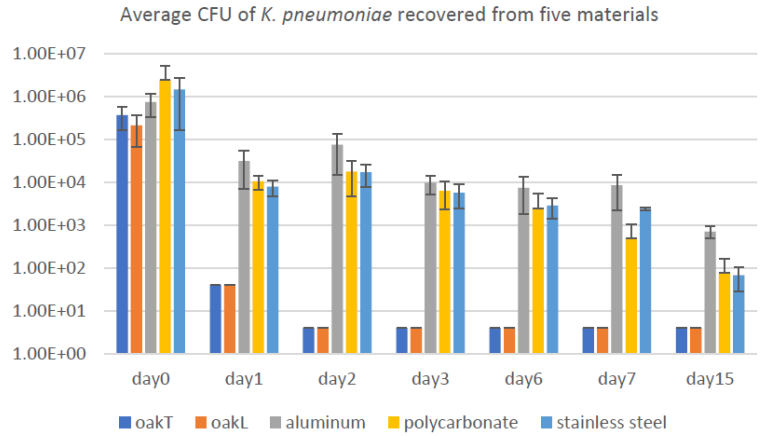


Figure 6.1: Average colony forming units (CFU) of *Klebsiella pneumoniae* recovered from five surfaces. oakT: transversal-cut sessile oak; oakL: tangential cut of sessile oak.

A total of 1.59 x 10^7 viable cells of *K. pneumoniae* were inoculated on all the tested surfaces. The recovery results throughout the experimental period are given in figure 6.1. On day 0, the recovered bacterial number (mean±SD x10^6) were: OT = 0.38±0.2, OL = 0.22±0.15, AL = 0.75±0.4, PC = 2.5±2.7 and SS = 1.48±1.3 (Annex 3: Table A3.1). Statistically, there was no significant ($p<0.05$) difference among the number of microbes recovered from each surface on day 0 (Annex 3: Figure A3.1). The bacterial number on both wood surfaces significantly decreased on day one as compared to day 0. Also, the global decrease of *K. pneumoniae* viable count on wood was significantly greater than for other materials ($p < 0.05$). Minimum detectable limit (MDL, 4 CFU) was attained on wood between 1 and 2 days after inoculation, and henceforth, stayed low throughout the study period. Meanwhile, the bacterial survival gradually decreased on all other surfaces also without any significant difference among those surfaces (Annex 3).

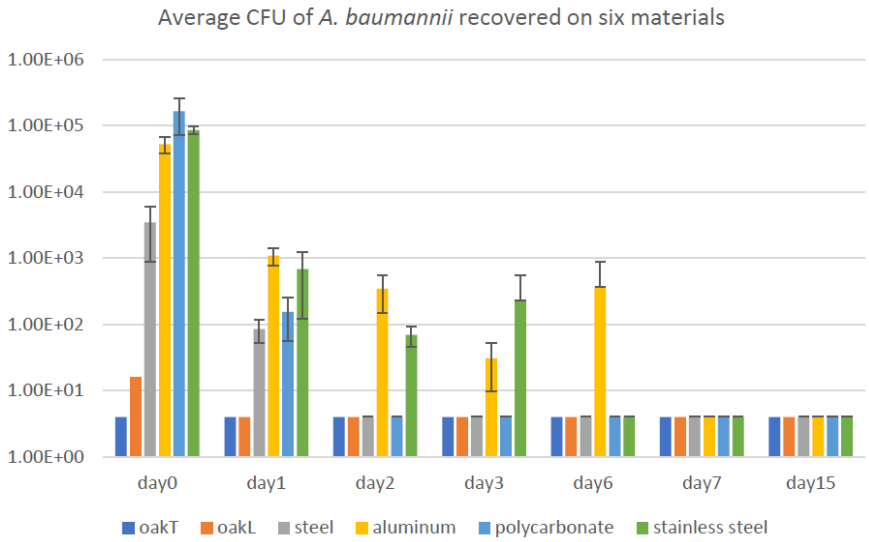


Figure 6.2 : Average CFU of *Acinetobacter baumannii* recovered from six surfaces. oakT: transversal-cut sessile oak; oakL: tangential cut of sessile oak.

A total of 1.4 x 10^6 viable cells of *A. baumannii* were inoculated on each test sample. The recovery results throughout the experimental period are given in figure 6.2 (mean values are given in Annex 3: Table A3.2). The bacterial CFUs decreased significantly ($p<0.05$) faster on both wood surfaces from day 0 as compared to all other test surfaces. The MDL on wood was reached within a few hours after inoculation. Meanwhile, bacteria were detected on all other surfaces after 24 hours of inoculation with no significant difference ($p<0.05$) among them (Annex 3: Figure A3.2). On day two, bacteria were detectable only on AL and SS, and still persisted on AL at day six; finally, MDL was attained by all surfaces one week after inoculation.

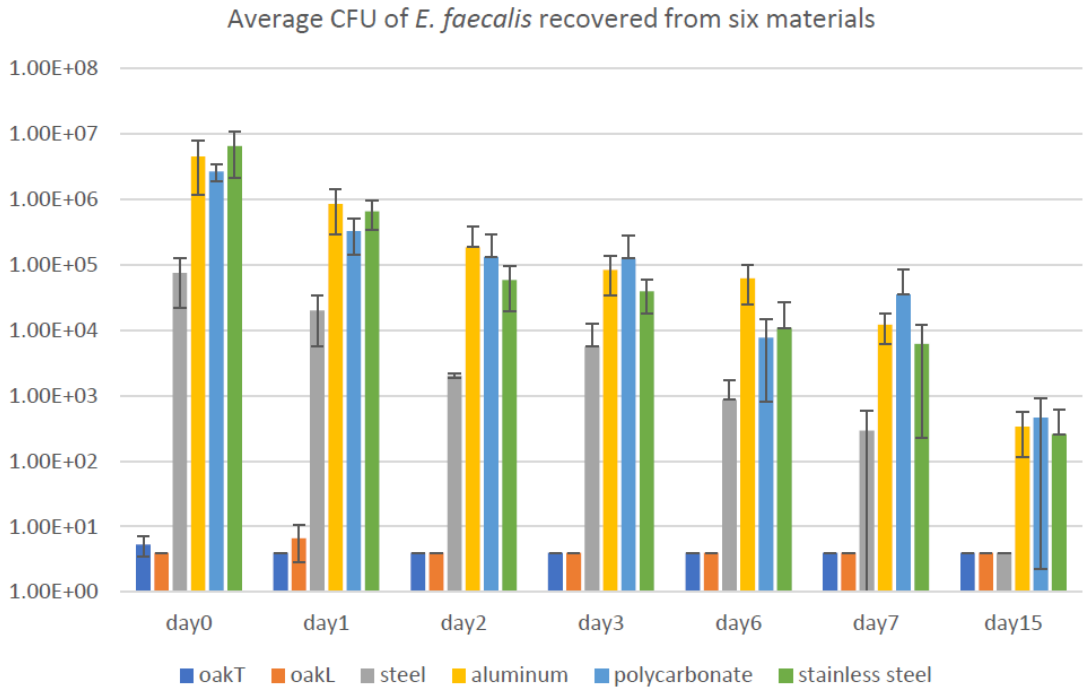


Figure 6.3 : Average CFU of *Enterococcus faecalis* recovered from six surfaces. oakT: transversal-cut sessile oak; oakL: tangential cut of sessile oak.

Approximately 20 x 10^6 CFU of *E. faecalis* were inoculated on six test surfaces. Figure 6.3 shows that similar trends were observed to those mentioned above for other bacteria, i.e. the bacterial numbers fell rapidly and significantly (p<0.01) on both wood surfaces compared to all other test materials (Annex 3: Table A3.3 and Figure A3.3). The MDL was reached on the wooden surfaces within a few hours of inoculation and remained at this level throughout the study period (Figure 6.3). Meanwhile, the viable cells slowly decreased on all other surfaces and there was a non-significant difference in CFU recovered from these four materials. The 2nd fastest decrease after wood was on ST, however, CFUs could still be detected at one week of inoculation, and even on day 15 except on ST.

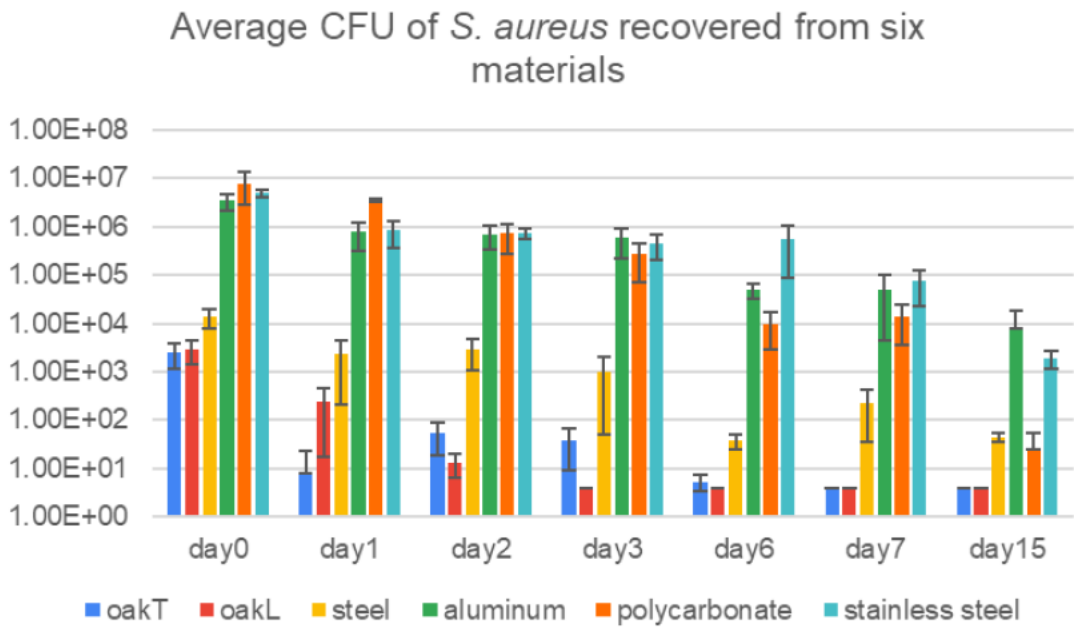


Figure 6.4: Average CFU of *S. aureus* recovered from six surfaces. oakT: transversal cut sessile oak; oakL: tangential cut of sessile oak.

Almost 24 x 10^6 CFU of *S. aureus* were inoculated on the surfaces. Alike above-mentioned results, the bacteria decreased fastest on oak and ST surfaces (Figure 6.4). At time 0 the recovered bacterial number was similar (p<0.05) on wood and ST. The number of recovered CFUs continuously decreased on OL and finally reaching to MDL between days two and three. However, in the case of OT, there were few variations; the number of viable cells decreased very much on day one and then slightly increased on day two where it maintained the level on day three and even few cells were detectable day six, and finally reaching to MDL at day seven. On all other surfaces, CFUs were still detectable at the end of the study period. The comparative recovery analysis showed that there was no significant difference (p<0.05) among OL, OT and ST (Annex 3: Table A3.4 and Figure A3.4). However, these three samples showed significantly (p<0.05) lower CFU as compared to other tested surfaces.

6.1.2. Discussion

The results show that all the tested bacteria had the lowest survival time on wood surfaces as compared to other materials. The most probable reason behind this is the antimicrobial activity of wood material (Munir et al. 2019f). The literature shows that different oak wood species exhibit antimicrobial properties against *S. aureus, K. pneumoniae, A. baumannii* and *E. faecalis* (Omar et al. 2000; Basri and Fan 2005; Safary et al. 2009; Sati et al. 2011; Zachariah et al. 2014; Touati et al. 2015; Kaynar 2017; Bahar et al. 2017; Pailhories et al. 2017; Gul et al. 2017; Tayel et al. 2018; Ahmed and Salih 2019; Elansary et al. 2019; Munir

et al. 2019a, 2020b; Dettweiler et al. 2019; Smailagić et al. 2020). Almost all of the mentioned studies tested the extractive content of oak wood, meaning that only chemical activity was documented (Munir et al. 2020a). However, the antimicrobial properties of wood are a combination of physicochemical parameters which create unsuitable growth conditions for many pathogenic bacteria (Aviat et al. 2016). These antimicrobial properties of wood against bacteria depend upon multiple factors including, the type of bacteria and wood, and environmental conditions (Munir et al. 2019e). Likewise, the microbial recovery rates are also linked to these parameters.

The lower microbial recovery from the porous wood material could have mildly impacted the results. However, it is not a significant factor because previous studies have shown that the elusion-based recovery method used here is more reliable than surface contact or swabbing methods (Ismail et al. 2015; Gupta 2017). In this study, it can be seen that *K. pneumoniae* recovery on day 0 was the same on all the tested surfaces and then the antimicrobial properties of wood lead to a decrease in CFU throughout the study period while it did not happen on other surfaces. A previous study by Vainio-Kaila et al. (2011) tested the hypothesis that live bacteria were left inside the pores after using a vortex-based recovery method. They used a similar vortex-based technique, to the one used here, to remove all adhered bacterial cells from the surface of wood and glass samples. They recovered a low number of CFU from the wood sample. The wood samples were then re-incubated in broth to allow any "hidden" bacteria to multiply; however, no qualitative growth was observed after 24 h of incubation. Which proved that the actual antimicrobial behavior of wood was responsible for lower microbial survival. Another evidence of efficient microbial recovery is the non-significant ($p<0.05$) difference between bacterial recovery from two cutting directions of wood (OT and OL). Theoretically, if the porosity is a major factor in the recovery of microbes from the surfaces, then two cutting directions should have had a difference in recovered CFUs because the RT face is very much porous as compared to the LT face. It has been shown in previous studies that the bacteria can penetrate RT faces more readily due to the depth of pores. However, the non-significant differences of this study prove that even if the bacteria are hidden or penetrated the wooden structure, the elusion-based recovery methods can still recover them.

In previous studies, *A. baumannii* and *E. faecalis* showed mild susceptibility to the diffusion-based antimicrobial properties of solid oak wood (Munir et al. 2019a, 2020b). However, in the current investigation, these two bacteria survived less than 24 hours on wood.

It proves that microbial survival is not only influenced by the extractives of wood but also physical properties. The same is true for other bacteria also, for example, *Escherichia coli* is the most resistant bacteria to the agar diffused antimicrobial chemicals (Munir et al. 2019a), however, (Milling et al. 2005a) reported that *E. coli* and *E. faecium* significantly decreased in contact with pine and oak as compared to poplar, maple, spruce, beech and plastic.

The antimicrobial properties of oak wood against multiple *S. aureus* strains have been well documented. However, in the current case, it survived longer as compared to other microbes. The reason behind this survival could be the strain of bacteria tested (Wendt et al. 1997) because in some studies the antimicrobial properties of oak wood (*Q. alba*) against *S. aureus* have been reported to be less potent compared to other bacteria such as *A. baumannii* and *K. pneumoniae* (Dettweiler et al. 2019). However, it has been reported the difference of resistance of strains against wood is independent of their specific antibiotic resistance status. Apart from these results, the previous studies have also shown that the survival and recovery of *S. aureus* on wood being lower as compared to other materials such as plastic, steel and glass, etc. Ripolles-Avila et al. (2019) reported that due to antimicrobial properties of wood material, *S. aureus* (ATCC 6538) number on poplar and pine wood reduced by 0.1 and 2.8 Log CFU/cm^2 as compared to initial bacterial numbers (5.7 Log CFU), while they increased on polyethylene and polystyrene by 0.7 and 1.2 Log CFU/cm^2, respectively. Kotradyova et al. (2019) reported that the *S. aureus* (CCM 3953) was undetectable on wood (oak and pine) as compared to laminated particle boards at 20 hours after inoculation when tested with blotting, swabbing and girding methods. Coughenour et al. (2011) inoculated 10^7 CFU of MRSA (ATCC 43300) on five different surfaces, they observed that bacteria persisted on vinyl and plastic surfaces even five days after inoculation; however, no bacterial counts were detectable on cloth at day four and on wood even at day two. Gupta et al. (2017) inoculated almost 10^7 *S. aureus* (ATCC 6538) cells on 5 different floor materials (vinyl, porcelain, hardwood and carpets) and recovered them at different times by the elusion-based method. They observed that the bacterial number continuously decreased on all materials and were undetectable on the carpet at the end of the study (week 4), while there were still 2-3 logs of bacteria detectable on all other materials. In their experiment, the information regarding the surface treatment of wood was not provided which most probably was a varnish treated material and may have masked the antimicrobial potential of wood.

A. baumannii reached MDL within a week after inoculation on all surfaces. However, previous studies have shown a longer survival on different surfaces in diverse conditions of

incubation. For example, Bravo et al. (2019) reported that after inoculating 10^8 cells of *A. baumannii* ATCC 19606 in saline suspension, on different surfaces, it survived on SS for more than 90 days and there was only a 1-2 log reduction in viable and cultivable cells, in dry and wet conditions, at 20 °C and 37 °C. It is assumed that these differences could have been due to initial inoculum dose used or the type of SS employed. In a previous study, it was reported that a difference of less than 2 log10 (10^7 vs. 10^5) affected the survival of bacteria in the same environment and on the same surface (Otter and French 2009). This might be the reason another study showed lower survival of *A. baumannii* on SS surface (41 days) with an initial inoculum of 10^6 cells/ml of saline or per test surface (Lodi et al. 2019). In addition, these studies showed that the survival of *A. baumannii* was surface dependent. However, Wendt et al. (1997) studying the survival of *Acinetobacter* sp. on different surfaces by inoculating 100 µl of bacterial suspension prepared in distilled water with 10^8 CFU/ml, observed that the long-time persistence was not influenced by type of substrate surfaces or the species, rather it depended upon the strain. As our study showed that the *A. baumannii* survival was dependent somewhat on the porosity of the surface, this bacterium survived longer on nonporous surfaces and there was no significant difference among them, except with steel. These variations show that further studies are needed to better understand the mechanisms of survival of this pathogen on different surfaces. Besides, there are not any studies providing information on the survival of *A. baumannii* on wooden surfaces.

The antimicrobial properties of wood which lead to lower survival of bacteria on its surface may be a combination of bacteriostatic and bactericidal interactions. During this study, both *S. aureus* and *A. baumannii* showed a prolonged growth rate after recovering from oak compared to those recovered from other material. For example, after 24 hours of incubation, the colonies were either mildly visible or absent. Whereas after a prolonged incubation time (42 h), more colonies were present. Therefore, it can be said that the wood also caused a bacteriostatic effect on the growth of tested bacteria. Meanwhile, it is not evident if the bacteria went into dormant form and can pose risk later.

In this study, the water-based bacterial suspension was prepared to avoid the effects of nutrients (Wendt et al. 1997; Coughenour et al. 2011). Havill et al. (2014) reported that *K. pneumoniae* dried in TSB were recovered on day 40, while those dried in water were not; suggesting that the suspension medium influences the microbial survival. Still, when interpreting the results of this study, it must be noted that not only water but also numerous

liquids (urine, saliva, nasal discharge, aerosol, etc.) may act as a medium for HAI transmission, hence affecting the survival of bacteria.

Untreated wood material was used in this investigation to avoid the interference of surfactants and finishes, like paints and varnishes. Thus, the wood behavior described here are for natural wood that is ready to be used for indoor construction, however, after using the wood material as the surface may cause a change in surface properties, for example, the pores may be filled with dirt and interfere with antimicrobial properties (Kotradyova et al. 2019). Likewise, the persistence of nosocomial bacteria would change. Saka et al. (2017) studied the prevalence of different HAI agents on hospital surfaces, they observed that *S. aureus* was the predominant contaminants on AL surfaces, *A. baumannii* and *K. pneumoniae* of ceramic, while *A. baumannii* was the major contaminant of wooden surfaces. However, their study did not provide the condition of wood samples or surfaces and recovery was done based on swabbing.

Regarding the application of this study, the results are not limited to these four isolates of bacteria but it will also serve as a guideline for testing various other microorganisms in all hygienically important places where wood is or could be employed, such as food contact surfaces (Aviat et al. 2016) and healthcare buildings (Kotradyova et al. 2019). Therefore, future studies are needed to provide a wider perspective of the survival of various pathogens on wooden surfaces inside the healthcare buildings.

6.1.3. Conclusions

This study found that the survival of four bacteria, known to cause healthcare-associated infections, is lowest on oak wood (*Quercus petraea*) as compared to other commonly used hospital surfaces (polycarbonate, steel, stainless steel and aluminum). Although the two cuttings of wood had different porosity the survival of microbes was similar on both which showed that the porosity did not influence the recovery of bacteria from samples. Based on these findings, it can be expected that wood surfaces can be used to counter the growth of some common bacteria. However, there are some limitations of this study such as the number of replicates and control of humidity. Further studies are needed to test the survival of various other microorganisms and their isolates to apply this research on a broader scale.

6.2. Fluorescent probes to label the bacteria for following their distribution on and inside wood material

The fluorescence microscopy techniques allow the identification of targets with specific fluorescence emissions in complex samples. Therefore, the CSLM was used in these experiments to identify the microbial contamination and distribution on and wooden surfaces. Firstly, we characterized endogenous fluorescence of different wood species i) to choose the fluorescent probe which will be used for bacteria tracking on wood in the next experiment ii) to investigate endogenous fluorescence of wood in order to localize fluorescent bacteria on wood without previous staining of the material. Measurements were done on the wood disc by using 405 nm excitation and fluorescence collection between 405 to 690 nm. It can be observed that the autofluorescence spectra of wood material are quite large with emission wavelengths ranging from 435 to 670 nm (Figure 6.5).

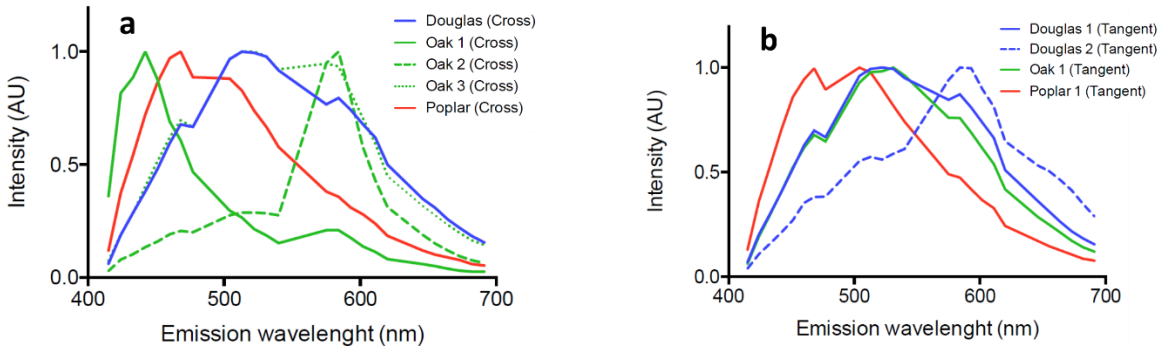


Figure 6.5: Autofluorescence spectra of different wood samples measured by a confocal spectral laser microscope (CSLM). (a) Transversal section; **(b)** Tangential section; **AU** = arb units

These measurements were done both for RT and LT sections and for the three species of wood (sessile oak, Douglas fir and poplar). Two distinct spectra were obtained from the oak cross-section (green curve), first spectra with a maximum value at 415 nm and second spectra with a peak at 590 nm. These two spectra are probably due to the presence of components with different fluorescence properties which were more visible on transversal (RT) compared to the tangential section (LT). Autofluorescence spectra of poplar (red curve) was very close for cross and tangential section whereas autofluorescence of Douglas fir (blue curve) was represented by one spectra on cross section and two spectra on tangential section. These spectra will be used as reference spectra in further measurements to separate wood fluorescence from bacteria fluorescence.

Meanwhile, the autofluorescence of most microorganisms, including *S. aureus* and *E. coli*, which are important common nosocomial and foodborne pathogens, falls in the same range, making it difficult to differentiate microbes from wood. The use of fluorescent probes can help overcome this difficulty by labeling the microbes causing them to fluoresce at a different wavelength to wood. As the autofluorescence of wood is mainly in the green color, the red fluorescent markers (DRAQ5™, mCherry) appears to be the most suitable fluorescent probes to track bacteria on wood by using fluorescence microscopy. The results were compiled based on the visual analysis of images to localize bacteria on wood and measurement of area recovered by total red fluorescence attributed to bacteria reported to total area recovered by green fluorescent attributed to wood surface.

6.2.1. DRAQ5™ labeling to study microbial contamination and distribution

The DRAQ5™ is a proven far-red fluorescent DNA dye for live or fixed cell analysis. Previously, it has been described to successfully label *E. coli* (G-) and some other gram-positive bacteria (Herrero et al. 2006; Quirós et al. 2007; Silva et al. 2010; Dubreil et al. 2018). Therefore, it was employed to label *S. aureus* (G+) and *E. coli* bacteria in this study, for optimizing the methodology and expand the application of the method to different wood and bacteria.

6.2.1.1. E. coli and S. aureus labeling efficacy with DRAQ5™

The images obtained via CSLM were visually analyzed for evaluating the difference of labeling efficacy of DRAQ5™ on *S. aureus* and *E. coli*. Figure 6.6 shows that almost 90 percent of *E. coli* were fluorescent (Figure 6.6a), whereas only half of the *S. aureus* cells showed red fluorescent after labeling with DRAQ5™ probe (Figure 6.6b).

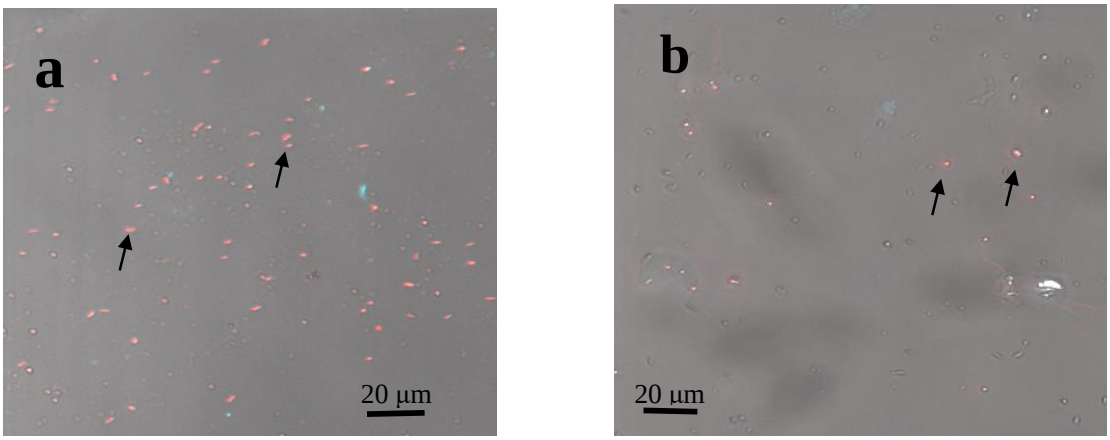


Figure 6. 6: CSLM imaging of DRAQ5™ (7.5mM) labeled suspension of (a) *Escherichia coli* and (b) *S. aureus* bacteria. Arrows are pointing the fluorescent bacterial cells.

6.2.1.2. Influence of sterilization on the bacterial presence on wood

Based on previous results, only *E. coli* was selected for further DRAQ5™ labeling experiments. A total of $1.5x10^7$ *E. coli* cells were inoculated on each test sample on an area of 78.5 mm^2. The CSLM images were qualitatively analyzed to see if the bacteria maintained their fluorescence on oak and poplar wood. Another objective was to determine whether or not the sterilization status of wood samples influenced the presence of bacteria on tested samples.

Figure 6.7 shows that the DRAQ5™ labeled *E. coli* was present on all the samples irrespective of sterilization status. However, a visual qualitative observation showed a higher number of cells on poplar wood as compared to oak (data not quantitatively analyzed).

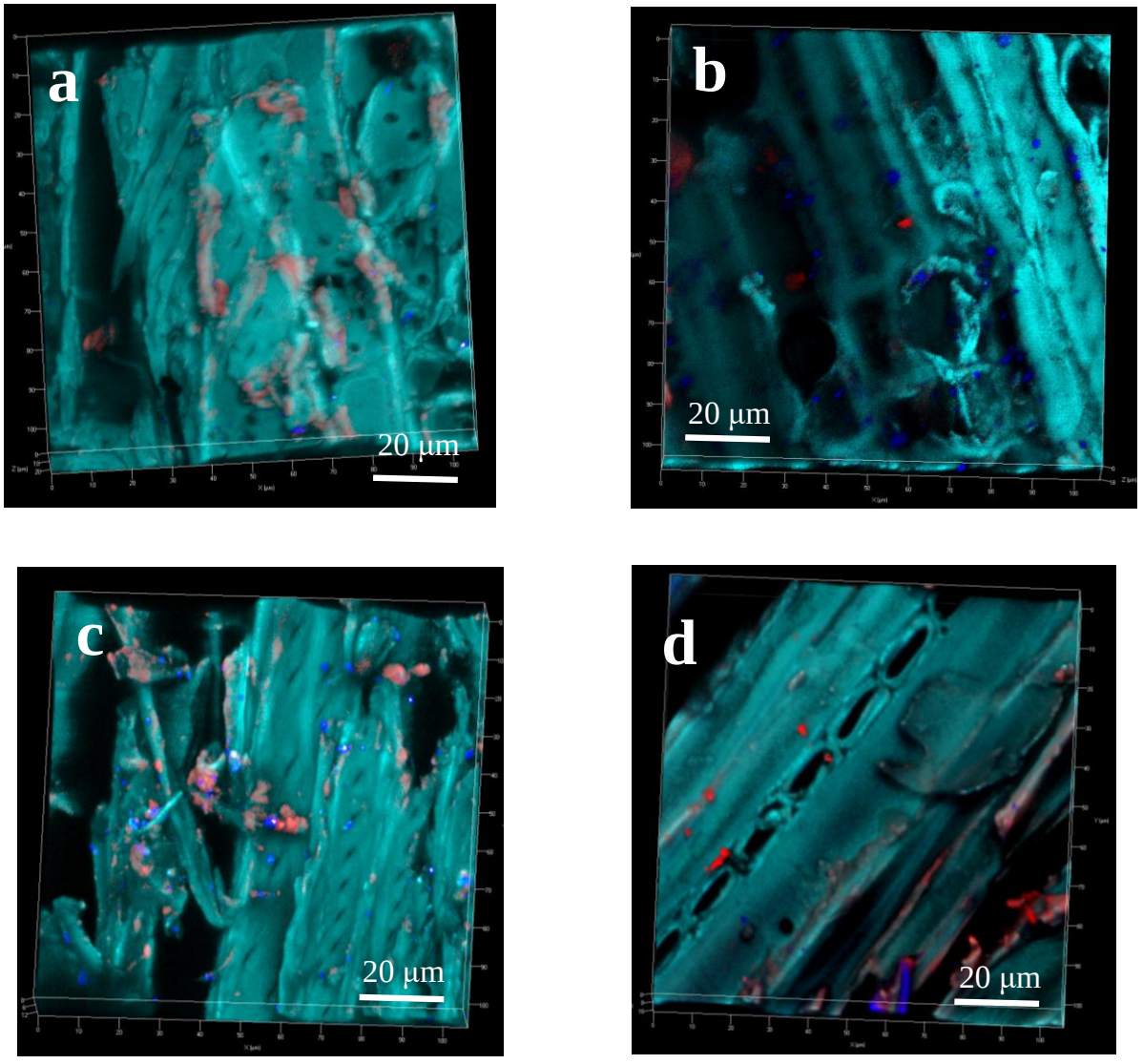


Figure 6. 7: CSLM image of DRAQ5™ labeled *E. coli* on wood. (a) Autoclaved oak; **(b)** Gamma irradiated oak **(c);** Non-sterilized oak; **(d)** Gamma irradiated poplar. Red = DRAQ5™ fluorescence; Greenish blue = autofluorescence of wood; Blue = contaminants/extractives

6.2.1.3. Direct labeling of E. coli on wood

The previous studies and above-mentioned experiment showed that the presence of DRAQ5™ labeled *E. coli* cells could be identified by CSLM. Further, it is not known if directly applying DRAQ5™ on wood samples can label the bacterial cells which could be used as a direct method to detect microbial contamination on wood. Therefore DRAQ5™ was directly applied on gamma sterilized oak wood with or without pre inoculation of *E. coli*. The visual observation of CSLM obtained images showed that the DRAQ5™ probe, surprisingly, showed the red signals on both types of samples irrespective of *E. coli* inoculation (Figure 6.8).

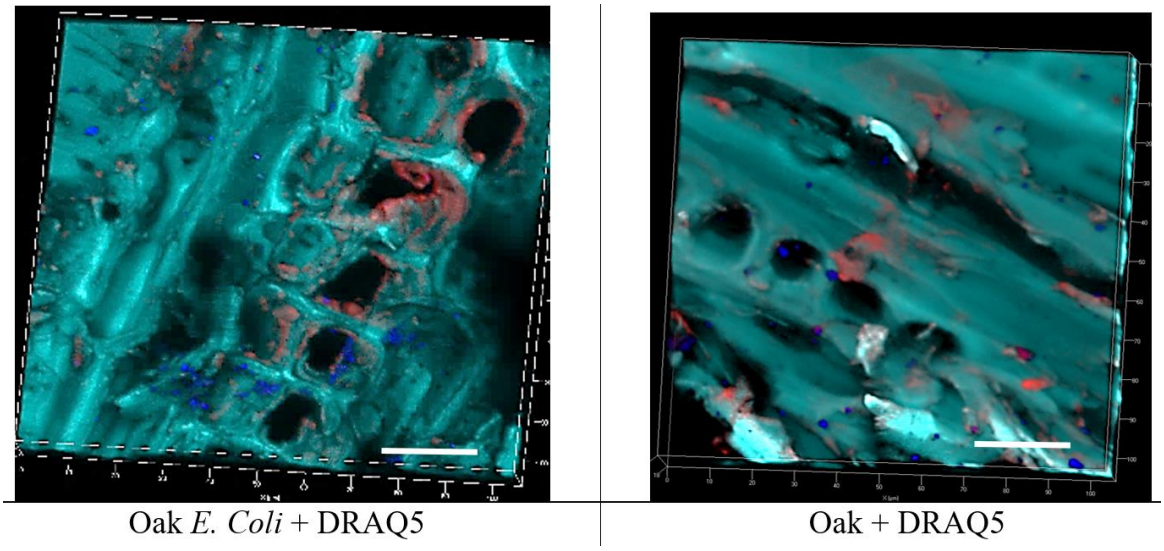


Figure 6.8: Direct inoculation of DRAQ5™ on wood in presence and absence of *E.coli*. Scale bar indicates 20 µm.

6.2.1.4. Discussion

First of all, the experiment analyzed the comparative DRAQ5™ labeling efficacy for *E. coli* and *S. aureus* bacteria. The *E. coli* cells showed a strong red fluorescence signal after this labeling. Previous studies have also reported the use of DRAQ5™ for labeling the DNA of *E. coli* (Silva et al. 2010; Dubreil et al. 2018). However, only half of the *S. aureus* cells showed red fluorescence after labeling. This difference might have been due to G- (*E. coli*) and G+ (*S. aureus*) cell wall characteristics of these bacteria. Generally, DRAQ5™ is a synthetic anthracycline dye and rapidly crosses cell membranes and binds to nucleic acids (Sánchez-Pérez et al. 2013). However, the cell wall may have posed some hindrance, as G- bacteria have a thin (<10 nm) and porous cell wall (Mai-Prochnow et al. 2016), and the DRAQ5™ supposedly passed through it easily and bonded with the nucleic acid. In contrast, G+ bacteria are enclosed in a thicker cell wall (20-80 nm) (Mai-Prochnow et al. 2016) that could have been a hindrance for DRAQ5™ molecules to pass through. Contrary to this hypothesis, previous

studies have shown the labeling of *Lactobacillus hilgardii* (G+), by DRAQ5™ for flow cytometry analysis (Herrero et al. 2006; Quirós et al. 2007). Apart from *E.coli*, DRAQ5™ has also been reported to label *Mycoplasma haemofelis* and *M. haemominutum* (Sánchez-Pérez et al. 2013), which are G- bacteria and lack a cell wall around their cell membranes. Therefore, the current experiment does not provide enough information on why *S. aureus* could not be efficiently labeled as compared to *E. coli*.

The higher microbial presence on poplar as compared to oak can be justified by the fact that the oak wood is acidic and has shown antimicrobial properties against many bacteria (Andrensek et al. 2004; Subhashini et al. 2016; Pailhories et al. 2017). Therefore, lower red fluorescence from this surface might have been an indication of lower microbial content on this surface. However, as this probe is not recommended for cell viability studies and it is difficult to interpret that the higher fluorescence signal or labeled bacteria on two wood surfaces are due to bacterial survival differences, therefore further studies are needed to confirm this hypothesis. Another possible reason for their higher number of microbes on poplar wood could be that it readily absorbed the inoculum while oak wood took longer time for absorbing. Thus, the bacteria on poplar had more time to adhere to surface and on oak wood they stayed suspended in a drop for a longer time, hence lesser time for coming in contact with wood and adhere. Consequently, a higher number of unattached bacteria might have lost on oak surface during rinsing and fixation of samples. However, the rinsate was not collected after washing the samples, thus it can be assumed that the future study of unattached bacteria in the washed solution would give more insights to this hypothesis.

The direct application of DRAQ5™ on wood labeled the *E. coli* cells. This is an interesting finding and could be used to label this bacterium on wood to determine the sterilization status of the surface, on the condition that there is no contaminating nucleic acid present on the wood surface. Theoretically, it was expected that gamma sterilization degrades the DNA (Munir and Federighi 2020) present on wood and the addition of DRAQ5™ would not give any red signal. However, there was a red signal present negating the hypothesis of the absence of DNA on sterilized wood material. Therefore, it can be expected that even the sterilized wood has traces of DNA that can influence the genetic studies of microbes on wood. However, it will be interesting to see if these sterilizations are influencing the genetic identification of wood.

The chemical probe (DRAQ5™) labeling gave good qualitative results; however, the quantitative analysis is a challenge. It had certain limitations, such as requiring the step of bacterial labeling, and the chances of interfering with the natural behavior of bacteria since it binds with DNA. Moreover, the studies regarding the fate of the most common nosocomial bacterium (*S. aureus*) inside wood could not be studied. Consequently, avoiding chemical labeling, obtaining quantitative results and studying *S. aureus* on wood requires some innovative tool application that could minimize the effect of the mentioned limitations. Therefore, the genetically modified *S. aureus* was selected for further studies.

6.2.2. mCherry protein to study *S. aureus* contamination and distribution on wood

Two genetically modified strains of S. *aureus* were selected for studying their distribution inside wood. These strains had red fluorescent protein mCherry expressing genes inserted in their chromosome or plasmid, thus not requiring chemical labeling. Previously, it has been reported that mCherry gene does not interfere with microbial growth (van Zyl et al. 2015). This probe was selected instead of conventional green fluorescent protein (GFP) because of its higher stability in an acidic environment (Doherty et al. 2010) since most of wood species have acidic pH (Geffert et al. 2019). Another reason was that the fluorescence emission of wood was mainly in the green channel which would be very difficult to differentiate from that of GFP.

6.2.2.1. Selection of fluorescent strains

The first step was to compare the fluorescence of two mCherry strains, so further studies could evaluate their comparative distribution on wood. However, a higher fluorescent intensity was exhibited by plasmid strain as compared to chromosomal strain (Figure 6.9). Fluorescence intensity is a function of mCherry protein expression level (Doherty et al. 2010; Kim et al. 2020). This means that less expression of mCherry protein was obtained from chromosomal construction compared to plasmidic construction. Consequently, the plasmid strain was selected for further experiments due to better fluorescence signal and simplicity of the method with a single type of bacterium.

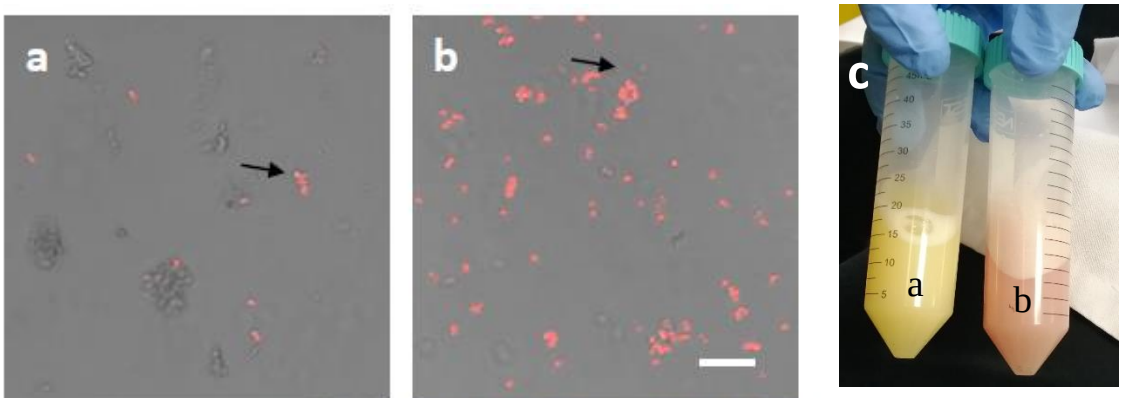


Figure 6. 9: mCherry fluorescence expression of *S. aureus*. a) Chromosomal strain; **b)** plasmidic strain; **c)** suspension of bacteria. Scale bar indicates 20 µm.

6.2.2.2. pH of wood and its effect on mCherry fluorescence

The pH of poplar, Douglas fir and oak wood samples were 6.40, 4.35, and 3.75 respectively. Similar ranges of pH have been described previously for these wood species (Geffert et al. 2019).

In a previous study, it has been shown that the mCherry fluorescence slightly decreased with an increase in acidity until pH 4 (Doherty et al. 2010). As the above-mentioned results show that the pH of wood is acidic and for oak, it is even around 3.50-3.75, a confirmatory experiment was conducted to measure the stability of plasmidic mCherry strain in suspensions with different pH and on oak wood samples with and without buffering.

The results of mCherry spectra at different pH in solution are given in figure 6.10. According to the literature, fluorescence maximum emission peak of mCherry was obtained at 610 nm between pH 4 and pH 10 (Doherty et al. 2010), whereas a dramatic shift of mCherry spectra was observed at pH 3.5 with a peak at 500 nm.

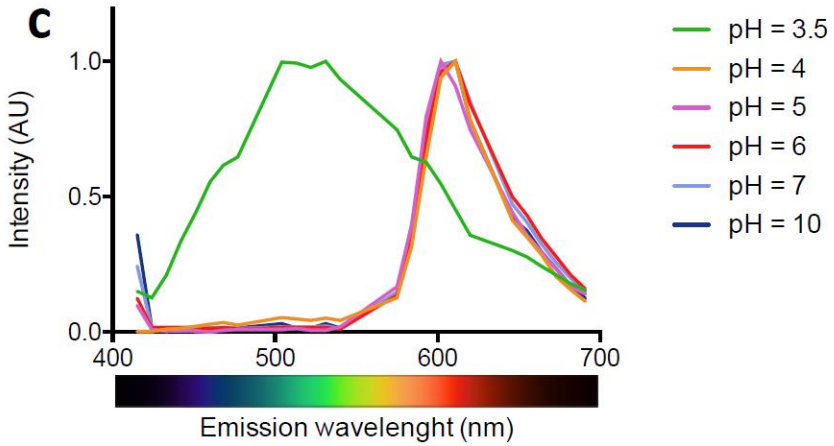


Figure 6.10: Spectra evolution of plasmidic mCherry *S. aureus* at different pH

The fluorescence of inoculated oak at different pH was also measured as shown in figure 6.11. Bacteria appeared bright red at neutral pH. On the other hand, at the 3.5 pH, the bacterial fluorescence was greatly modified both in solution and inoculated on oak wood (Figure 6.11) in fact, the bright red color of this bacteria was modified to a greenish color. Therefore, in further experiments, the phosphate buffer saline (PBS) was used to prepare bacterial suspension and disc storage after fixation, to minimize the influence of wood pH on mCherry fluorescence.

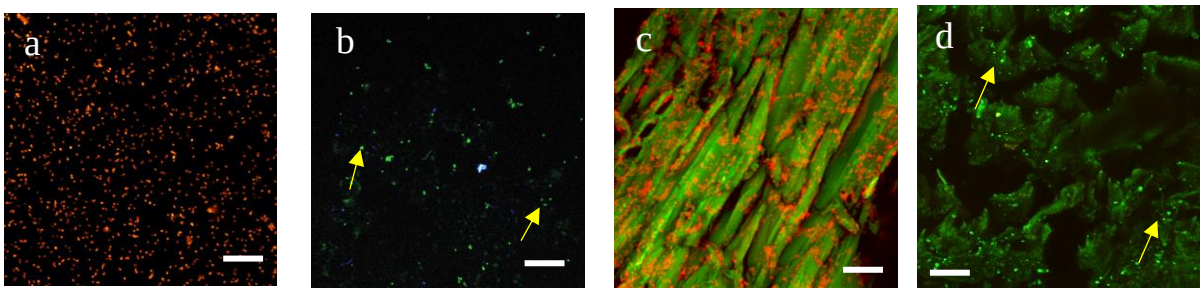


Figure 6.11: Fluorescence of mCherry *S. aureus* on wood and in suspension at different pH. a) mCherry *S. aureus* in PBS pH 7.4; **b)** mCherry *S. aureus* in acidic solution pH 3.5; **c)** mCherry *S. aureus* on wood store in PBS with pH 7.4 until observation; **d)** mCherry *S.aureus* on wood store without control of pH until observation; arrows are pointing the bacteria and scale bar represents 20 µm.

6.2.2.3. Bacterial distribution on and inside wood surfaces

The LT and RT faces of wood have very different porosities. In general, the transversal surface is more porous compared to longitudinal sections. Therefore, the distribution of microbes is also expected to vary depending upon the porosity of the surface. This hypothesis was tested by inoculating bacteria on LT and RT sections of oak, Douglas fir and poplar wood and then observing the bacterial distribution after one hour of incubation. First, the visual qualitative observations were done on the CSLM collected images (Figure 6.12). Only a few bacteria were present on all the RT surfaces one hour after inoculation. Meanwhile, the bacterial surface cover was very much higher on LT sections for all wood samples. A similar trend was observed in samples after 24 h of incubation (Figure Annex 4.1).

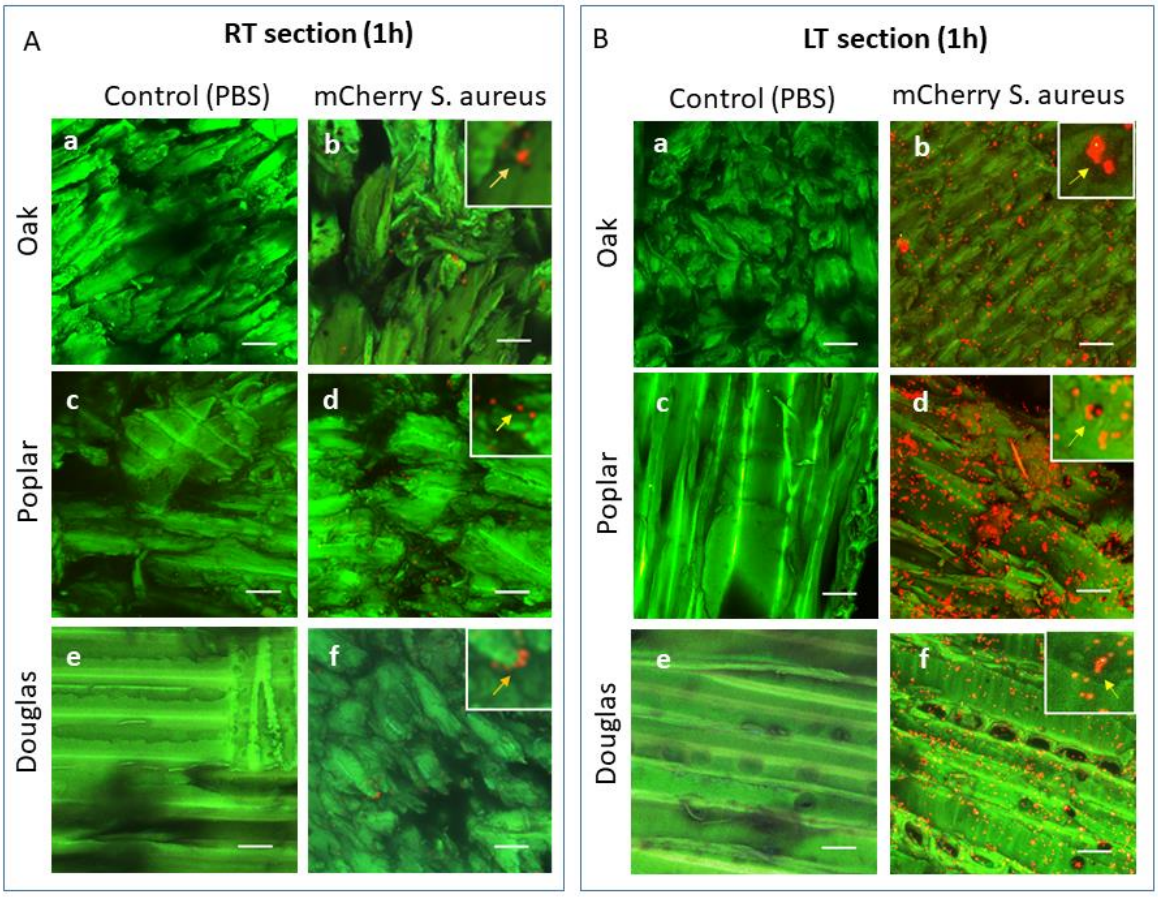


Figure 6. 12 : CSLM obtained images (z projection) showing mCherry *S. aureus* (red fluorescence) distribution on wood (green fluorescence). (A) RT and **(B)** LT sections, 1 hour post-inoculation**; a, c, e** control wood disc without mCherry *S. aureus* inoculation. **b, d, e** mCherry *S. aureus* inoculated wood disc; Scale bar indicates 20 µm.

Further analysis was done on the collected images by image analysis software (ImageJ, NIH, US). The results were obtained based on the fluorescence cover of red mCherry spectra as compared to green wood spectra. It was observed that the bacterial fluorescence cover was higher on LT cutting as compared to RT cutting on all three kinds of wood after inoculation (Figure 6.13). Similar results were shown by the samples incubated for 24 hours (Figure Annex 4.2 and 4.3).

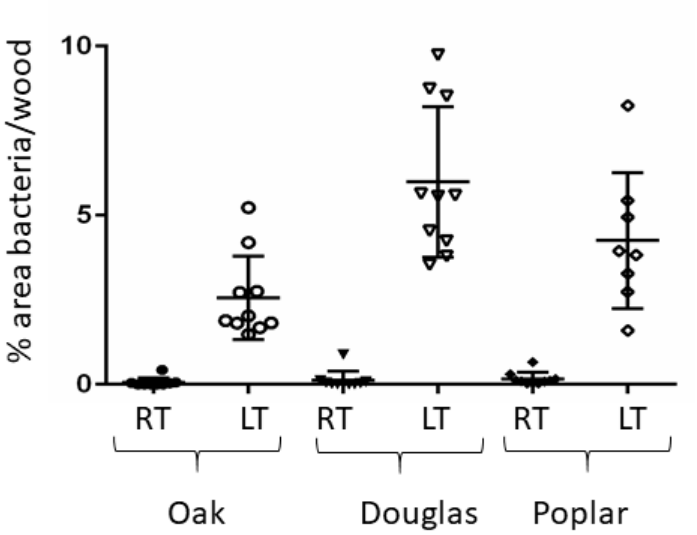


Figure 6. 13: The area covered by red fluorescence (mCherry *S. aureus)* compared to the area recovered by wood autofluorescence at 1 h after bacteria inoculation, for the RT and LT sections.

As a higher red fluorescent signal was observed on LT surfaces as compared to RT, it is hypothesized that bacteria penetrate the interior of wood more easily on RT surfaces and are, as a consequence, hidden. To confirm this assumption, the inoculated wooden discs were cut in cross-sections and mounted on slides to observe the bacteria inside wood elements.

The images obtained from the cross-sections show that the bacteria remained closer to the surfaces of LT samples as compared to the RT samples (Figure 6.14 and 6.15). For the LT section, bacterial penetration was 35-70 µm and their concentration seems higher as compared to RT, which indicates penetration of up to 95 µm (data for all tested wood are presented in the Annex: Figure A4.4). Therefore, a survey of bacterial presence was conducted on the cross-sections of RT planes through the whole thickness (3.7 ± 0.4 mm) of the wood disc. An example of poplar wood can be seen in Figure 6.15 (data for oak and Douglas fir are presented in Annex4: Figure A4.5), where some bacteria could be seen even at the bottom of the disc both at 1 h and 24 h after inoculation This implies that the bacteria penetrated throughout the length of the pores. The area covered by red fluorescence to green fluorescence was also estimated on top, middle and bottom of cross-sectional cut discs (Annex 4: Table A4.1).

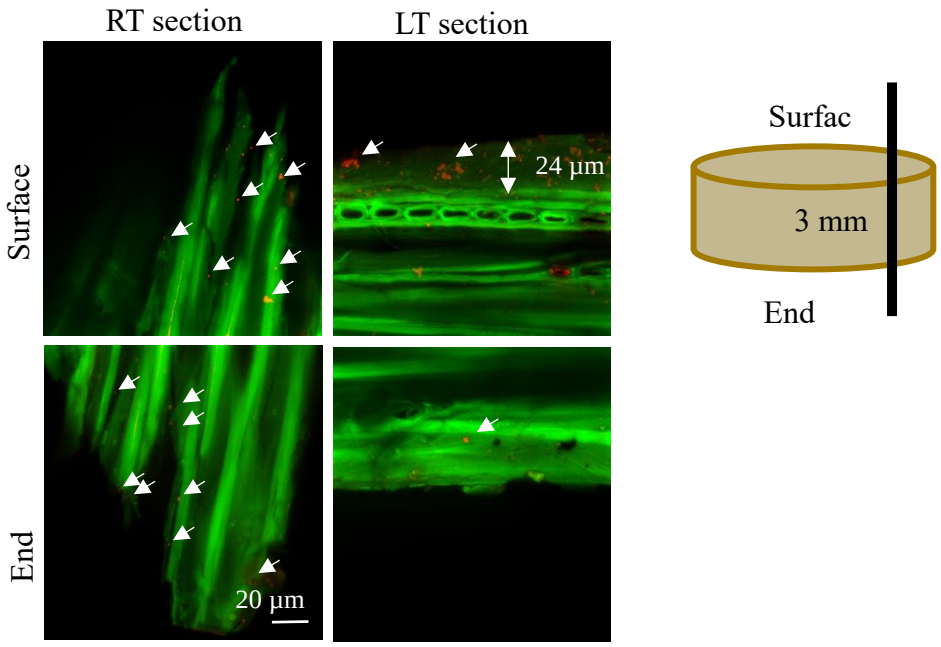


Figure 6. 14: CSLM image of the cross-section of wood samples (Poplar from top to 3mm depth) in RT and LT plane, 24 hours after inoculation. Arrows are pointing the bacteria; scale bar represents 20 µm.

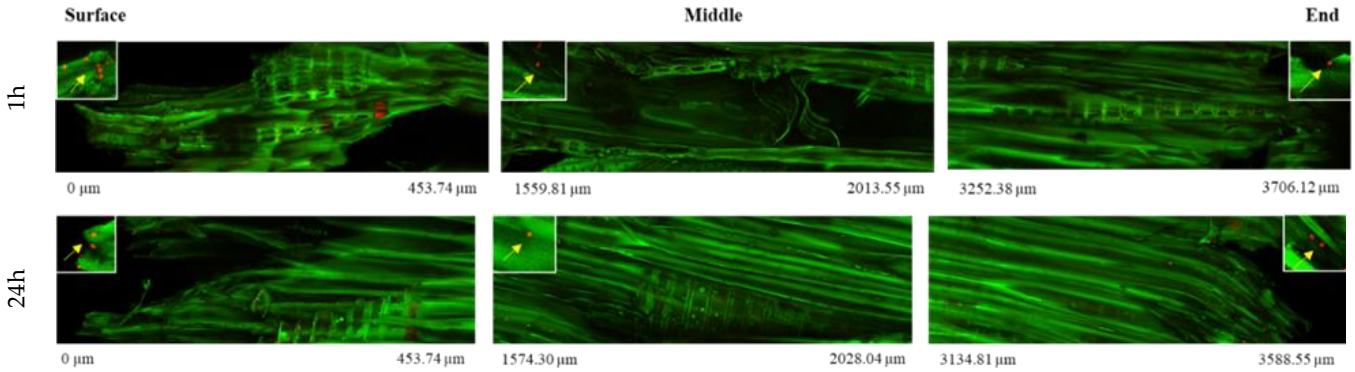


Figure 6. 15: CSLM image of the cross-section of poplar wood samples in RT plane, at 1 and 24 h after inoculation. Arrows pointing the bacteria.

After discovering that bacteria tend to remain near the surfaces of LT faces, it was decided to employ this plane for further investigations. It was assumed that the unfixed mCherry would lose the fluorescence after death; as a previous study has reported an excellent correlation between mCherry fluorescence and viable cell numbers suggesting that the photons emitted by the mCherry protein are a good reflection of the metabolic activity (van Zyl et al. 2015).

The objective of the further experiment was to test the bacterial survival on LT wood surfaces for 7 days. The image collected via CSLM were tested for bacterial quantity based on the red fluorescent area covered by wood compared to the green area of wood (Figure 6.16).

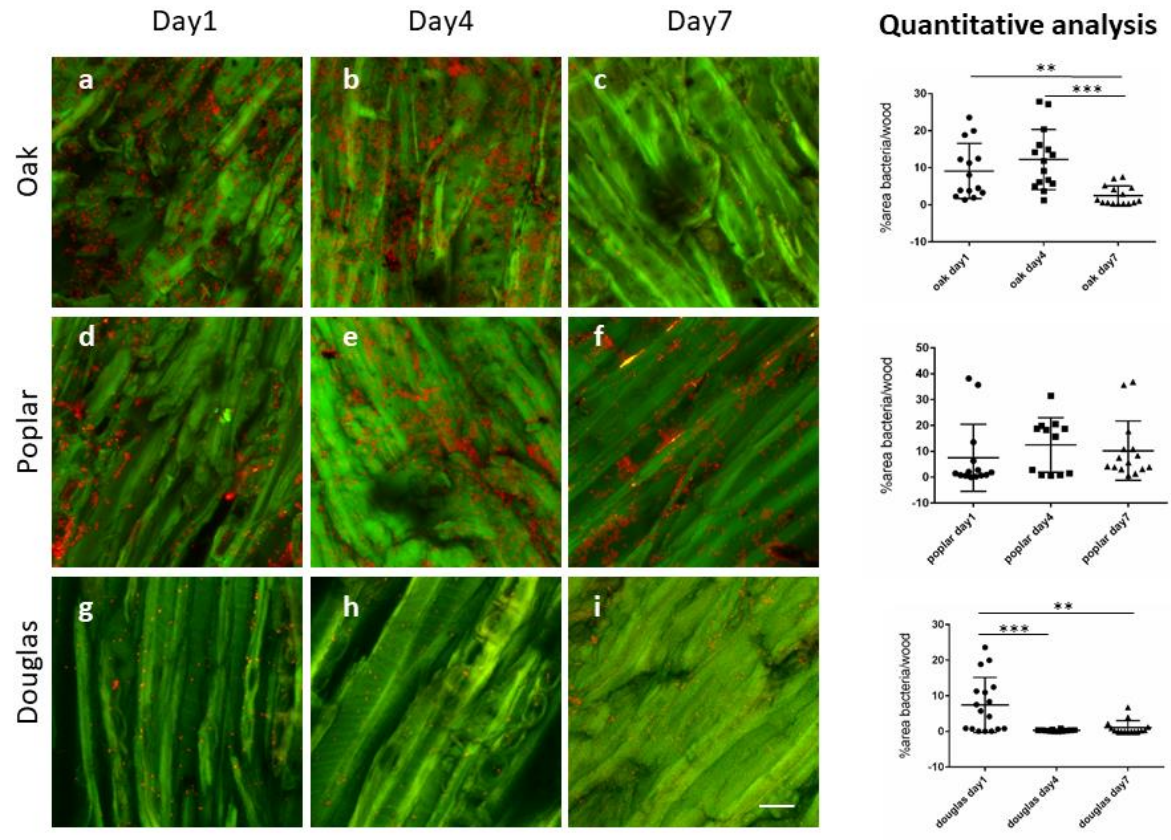


Figure 6.16: mCherry *S. aureus* on LT cut wood surfaces at different times after inoculation. Sessile oak at **(a)** 1 day, **(b)** 4 days and **(c)** 7 days after inoculation; poplar at **(d)** 1 day, **(e)** 4 days **(f)** 7 days after inoculation; Douglas fir at **(g)** 1 day), **(h)** 4 days, **(i)** 7 day after inoculation; z-projection; scale bar represents 20 µm; n=3 experiments; Mann-Whitney test, error bars represent the SEM of the mean. *p < 0.05, **p < 0.01 and ***p < 0.001.

The presence of bacteria on wood surface was analyzed three-time day1, day 4 and day7. It can be seen that bacterial fluorescence (numbers) declined with time depending upon the type of wood material. In general, the percentage of area recovered by red fluorescence was close to three wood species at day one (8-10%). Bacterial content decreased significantly (p<0.05) on Douglas fir wood at day 4 and day 7 as compared to day 1. Whereas the bacterial content on oak wood was highly variable on day 4 and decreased significantly (p<0.05) only on day 7 compared to day 1. On the other hand, bacterial fluorescence was unchanged on the poplar surface throughout the study period (p<0.05). These experiments have shown that one week was a sufficient duration to compare the antibacterial properties of wood. This new

method represents an innovative tool to assess antimicrobial properties of wood and could be used to assess bacterial contamination on numerous other materials at the same time.

6.2.2.4. Bacterial biofilm formation on wood and melamine

The mCherry *S. aureus* gave good results regarding distribution on wood and it survived for several days on wood. In this scenario, there is always a question of whether the bacteria can form biofilm in given and enriched conditions. Moreover, understanding biofilm formation in a given situation is necessary concerning surface cleaning studies, especially those with a hygienic perspective. Therefore, a pilot study was conducted where the bacteria were supplemented with PBS or diluted agar after one hour of incubation (surface attachment time) on poplar, Douglas fir and oak wood. After one day of incubation, the results showed that the bacteria were present on all wood surfaces, but they did not form a biofilm (data shown in Figure A4.6). The use of diluted agar might have been the limitation of this study because the other studies have used broth rather than agar (Khelissa et al. 2017).

The previous experiment was improvised by using Tryptic soy broth (TSP) instead of diluted agar. However, only oak wood was selected this time because it showed good bacterial cover in the previous experiment when a nutrient medium was added (Figure A4.6). Meanwhile, a non-porous material (melamine) was also included in the investigation. Melamine is a synthetic material that is extensively used as a contact surface in healthcare buildings (Shimoda et al. 2015). The CLSM taken images show that almost equal fluorescence of mCherry could be seen on both surfaces after 1h of incubation (Figure 6.17).

After supplementation with PBS and 24 hours of incubation, the quantities of bacteria were similar on both the oak wood and melamine surfaces. However, the interesting finding was that the bacteria formed biofilm and their number increased exponentially on the melamine surface in presence of broth (Figure 6.17). Meanwhile, no such changes were evident on oak wood.

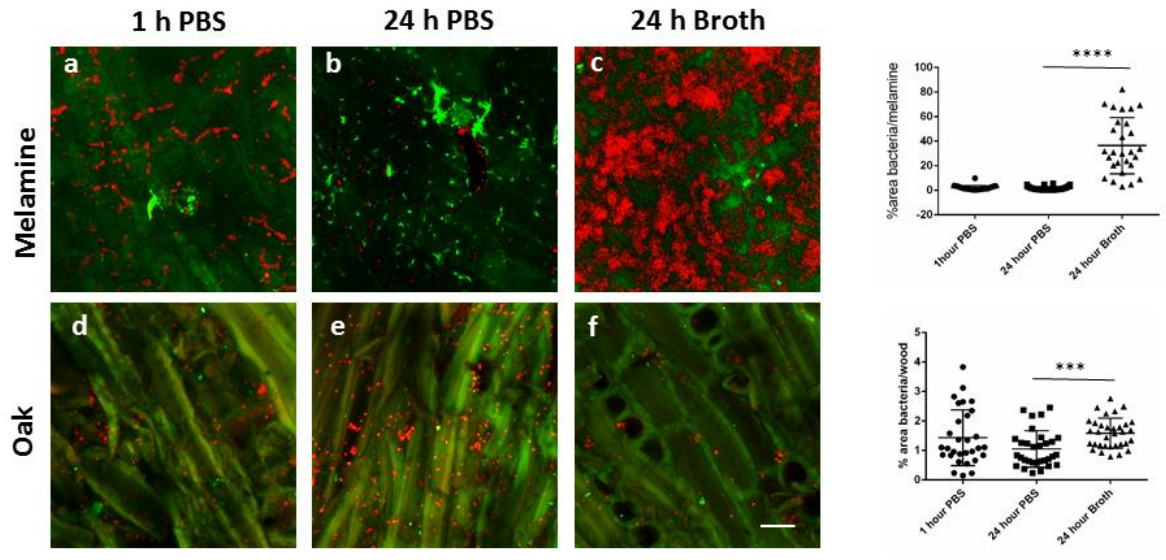


Figure 6.17: CSLM obtained image (z projection) of mCherry *S. aureus* (red) on surfaces (green) of oak wood and melamine. Scale bar represents 20 µm; n=3 experiments; Mann-Whitney Mann-Whitney test, error bars represent the SEM of the mean. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$.

6.2.2.5. Discussion

This experiment studied the distribution of genetically modified mCherry *S. aureus* on different wood surfaces at different times. In general, it was observed that the fluorescence of mCherry protein-containing bacteria was very stable to pH changes from four to 10 as it has been reported previously (Doherty et al. 2010). However, our study was the first investigation to find that the fluorescence of mCherry *S. aureus* changes at pH 3.5 or lower. In fact, the emission becomes like GFP and it becomes difficult to differentiate the spectra of bacteria from that of wood because both appear in the green channel. Most of the wood species are acidic (Geffert et al. 2019), and mCherry could be used if the pH remains above 4.5, however, in case of very acidic materials, buffer solutions should be used to manage the pH and thus avoid different fluorescence behavior. In our study, the pH of wood material was found to be acidic which was due to water-soluble acidic components extracted to be tested *via* pH meter. However, being more precise, the pH might slightly vary because this water technique does not measure the influence of non-soluble acid functional groups in the cell walls.

Qualitatively, it was very obvious that there was higher red fluorescence on inoculated LT planes as compared to RT. The first assumption was that the transverse face may have permitted higher diffusion of antimicrobial compounds, as observed in previous experiments

(Pailhories et al. 2017; Munir et al. 2019a). However, the more likely reason was the penetration of bacteria into the pores which are more prevalent in the RT plane (Figure 2.2). Because a previous study also reported that a higher number of *E. coli* cells and *B. subtilis* spores were seen on the LT faced wooden cutting boards compared to RT (Prechter et al. 2002). They observed that bacteria and spores could penetrate further around 3 mm into transverse faces compared to only 1 mm in longitudinal planes of wood.

Based on these findings and literature reports, the next objective of experiments was to find bacteria inside LT and RT faced samples of wood. The inoculated and control discs were cut cross-sectional to take interior images by CSLM. It was observed that higher bacteria concentrations were present on surfaces of LT surfaces compared to RT, presumably because they had been transported by the flow of the liquid into the sample. Observations of the full cross-section of the discs showed that the bacteria were distributed throughout the length and present even at the bottom of the wood material. However, contamination of the bottom side of disc caused by a rinsing operation during sample preparation, cannot be excluded. It is an important consideration if the sectioning knife moved the microbes throughout the section. This point can be explained by the fact that similar conditions were used for cutting the sections of discs in RT and LT planes, the difference of microbial descending in these two planes justified that the knife did not move the fixed bacteria along the cut. The cross-contamination risk of the bacteria deeply descended inside pores was not studied in this experiment, however, it is expected to be negligible. However, it is up to hygienists if they consider the persistence of dormant or live bacteria inside the porous surface as a hygienic risk because if the bacteria are stuck inside the complex structures they are not expected to cross-contaminate, as shown in previous studies (Cliver 2006; Moore et al. 2007; Tang et al. 2011; Soares et al. 2012; Ismail et al. 2015). Regarding the existence of porosity on wood material, it is important to mention that during the usage life of wood inside healthcare buildings, the pores are covered by dust and bacterial descending deep into wood may diminish (Kotradyova et al. 2019). If the porosity and descending of bacteria is considered as a problem the LT cuttings would be preferable to avoid such descending of microbes.

When observing the cross-section of the LT plane of wood discs, the mCherry bacteria were mainly localized on the first 30 µm into wood structure (6.14). These results are in accordance with those obtained from the surface of un-sectioned wood discs (Figure 6.12B), where CSLM could create spectral images up to 60 µm depth into wood. These findings show that the CSLM can be used to study the bacterial contamination on wood surfaces without

sample destruction (sectioning) and the porosity on LT sections would not much influence the results.

The long-term persistence of *S. aureus* on indoor hospital surfaces has been reported (Kramer and Assadian 2014; Saka et al. 2017). Likewise, the current strain of bacterium showed that it could persist on wood surfaces for more than one week. The number of bacteria decreased throughout the study period on all the wood surfaces. Longer study periods are needed to identify the survival length of these microbes on wood. In addition, the microscopy coupled with microbial recovery method are also expected to provide better risk assessment, however, no such examples were found in the literature.

In all the cases, lower bacteria to wood fluorescence percentages were observed on Douglas fir compared to oak and poplar. Meanwhile, the poplar always showed higher bacterial concentrations as compared to other woods. Both the anatomy and chemical composition of the wood could be influencing factors as they are known to interfere with the microbial survival on wood material (Munir et al. 2019f). Moreover, same microbes are known to have different survival time on different wood species (Table 2.3).

The bacteria survived on wood and melamine material in presence of PBS after 24 hours of observation. Meanwhile, almost similar findings were observed with slight changes in bacterial numbers on wood when the broth was added as the nutrient support for bacteria. However, nutrient enrichment allowed the bacteria to form biofilm only on the melamine surface. A similar broth enrichment method has been used to successfully form *S. aureus* biofilms on plastic and steel surfaces (Khelissa et al. 2017). However, this is the first study that compares wood and melamine surfaces in the same experiment. Another study showed that MRSA and vancomycin-intermediate *S. aureus* could form biofilm and survive for more than six weeks on Formica® (trademark of condensed formaldehyde melamine paper sheet) and other hospital surfaces (latex, cotton fabric, vinyl flooring) (Zarpellon et al. 2015).

Although bacterial cells could be seen on the wood samples dosed with broth or PBS, in neither case did a biofilm form. So, although the wood did not prevent bacterial growth or persistence for 24 hours, the biofilm did not form. Similar findings were reported in a previous study by Hobby et al. (2012) who used static crystal violet staining methods and CSLM, and observed that the leaf and stem butanol fraction extracts from *Q. cerris* at the dose of 200 μg/ml reduced the *S. aureus* biofilm formation (in solution) by 63±10% and 74±4% respectively. They also reported that, at this dose, no bactericidal effects were observed in the stem extracts,

therefore, some other mechanism is involved in the stoppage of biofilm formation. Another study reported that the 50% *S. aureus* biofilm (in solution) inhibition was attained minimum at 1 µg/ml (1 – 32 µg/ml range) of *Q. alba* extracts (Dettweiler et al. 2019).

The CSLM is generally used for observing biofilms in solution and on smooth solid surfaces. On wood, CSLM is generally used to study the topography and surface roughness (Van den Bulcke et al. 2007). However, it can give promising results when used to observe bacterial colonization on wood surfaces. Lortal et al. (2009) used CSLM to observe yeast and bacterial biofilms on Douglas fir wood used in the cheese industry. They used syto9 and concanavalin staining to differentiate microbes and the polysaccharide covering of biofilms from wood material. Another study used CSLM with fluorescence in situ hybridization (FISH) probe to observe the microorganisms present on kitchen sponge surfaces (Cardinale et al. 2017). They also demonstrated the 3D images showing the colonization to a depth of 50 µm. This type of demonstration can be a very promising tools to demonstrate the real distribution of bacteria inside the porous materials, especially for hygiene education. In another study, the use of a laser-scanning confocal microscope has also been reported to track the movements of *E. coli* in a porous matrix (jammed hydrogel media) (Bhattacharjee and Datta 2019).

The limitation of the study is that the bacteria detached from surfaces were not collected for further investigation. The studies have shown that the detached bacteria can give information on the influence of surface on the microbial behavior and physiology (Khelissa et al. 2017, 2019; Abdallah et al. 2019).

6.2.3. Conclusions

These experiments were conducted using the fluorescent probes as a direct approach to study the microbial distribution on and inside different wood material. The DRAQ5™ fluorescent probe was used based on that used in an earlier publication (Dubreil et al. 2018) to qualitatively test the *E. coli* and *S. aureus* presence on pine and poplar wood surfaces. The results showed that DRAQ5™ was not very efficient in labeling the *S. aureus*, however, the distribution of *E. coli* could be studied with this marker. The direct DRAQ5™ application on wood gave signals irrespective of *E. coli* presence, implying the presence of nucleic acids on wood; hence it can interfere with the result analysis to study microbial distribution on wood. Further quantitative studies should be conducted using similar methodology and involving wide range of hygienically important microbes.

The second fluorescent probe used was the mCherry protein produced by genetically modified *S. aureus.* This study investigated the use of CSLM to study mCherry *S. aureus* in

terms of microbial colonization, survival, distribution and biofilm formation on wood surfaces over different times. The plasmidic mCherry strain showed excellent results regarding the study of microbial distribution. This probe showed good stability over a wide range of pH variations (4.5-10), however, at a very acidic pH of 3.5, same as oak wood, the fluorescent emission changed extensively, therefore the fixed samples were stored in PBS to maintain the red fluorescence. The CSLM observations showed that the bacteria were present mostly on the tangential surface and they descended down into pores more inside the transversally cut samples. The qualitative and quantitative results showed that the Douglas fir was the least colonized, while poplar was most colonized wood. In addition, bacterial numbers decreased gradually on wood from day 4 to 7 but were still present one week after inoculation. Finally, the experiment showed that the plasmidic mCherry *S. aureus* strain formed a biofilm on a melamine surface in the presence of broth, but, not on oak wood.

General conclusions

The principal aim of the investigation was to provide information regarding the hygienic properties of wood material. Thus, the antibacterial and antifungal properties of wood against nosocomial pathogens were studied. This information is intended to provide information on the survival of microorganisms on and inside of this material when used in healthcare settings. Overall, the antimicrobial properties against a wide range of pathogens were observed and these results are expected to provide the basis for benefitting from the antimicrobial properties of wood in healthcare buildings.

Major findings

This study shows that the direct diffusion method (antiboisgram), developed during this project, could be used to screen the antimicrobial properties of solid wood material against a wide range of bacteria and fungi that are responsible for healthcare-associated infections. More than ten wood species were used in a pilot project and of the five most common wood species tested in this research, the oak species showed the highest antibacterial activity.

The application of antiboisgram identified the influence of experimental parameters, notably sterilization and cutting directions, on the observed antimicrobial activity of wood. The origin of tree, disc cutting method, gamma sterilization, and antibiotic resistance status of microorganisms had no influence or very low influence on the antimicrobial properties of wood material. Meanwhile, the microbial susceptibility to antimicrobial properties of wood is was also dependent upon the type of bacteria and fungi. The *E. coli* was most resistant to the antimicrobial activity of all the tested wood and it was found that the higher doses of extractives could inactivate this bacterium (Annex 1.5).

Douglas fir wood exhibited better antifungal activity as compared to oak wood, especially against *Candida auris* that was considered as an emerging threat before the current COVID-19 crisis (Jeffery-Smith et al. 2018; CDC 2020b). Douglas fir is an important species used in construction, while sessile oak is a local species with high volume that could be used in healthcare buildings for indoor construction and furniture.

This study also provided the first results regarding the survival of most common pathogens including *K. pneumoniae*, *S. aureus*, *A. baumannii* and *Pseudomonas aeruginosa* on wood material compared to other commonly used indoor construction materials including plastic, steel and aluminum. In general, all the bacteria showed lower survival on wood as compared

to other materials. The survival time of *K. pneumoniae* and *E. faecalis* on wood material was one to two days and more than 15 days on all other materials. *A. baumannii* survived on wood only for a few hours and survived up to a week on all other tested materials. The longest surviving bacteria were *S. aureus* which persisted on oak wood for almost a week and more than two weeks on all other materials. This study also shows that the elution-based recovery method isolated almost similar amounts from longitudinal and transversal cut wooden discs, which shows that substrate porosity did not influence the recovery.

The distribution of bacteria was identified by a confocal spectral laser microscope (CSLM). An innovative approach of using fluorescent probes was used to facilitate the differentiation of bacteria from wood. The first probe used in this case was DRAQ5 which is a DNA probe and gives a signal when it binds with nucleic acid. When labeled *E. coli* cells were inoculated on wood surfaces, they could be seen via CSLM, thus providing qualitative results regarding the microbial contamination of wood material. When this probe was directly applied to the sterilized wood surfaces, it still gave a red signal, implying that the wood surfaces have sufficient amounts of DNA that could interfere with the nucleic acid-based hygienic risk assessment studies of wood. The second method developed involved the use of genetically modified bacteria (*S. aureus*) containing a gene in plasmid or chromosome, which codes for a fluorescent protein called mCherry. The results showed that the bacteria could be seen on the surfaces and also almost 60 μm below the surface. In addition, it was found that the bacteria penetrated deeper into transversally cut wood because of the presence of longitudinally running vessel elements, as compared to longitudinal faced samples. These findings were also confirmed by cutting the cross-sections of inoculated discs. The tested fluorescent signals of mCherry containing *S. aureus* could be detected even at seven days after inoculation on oak, Douglas fir and poplar wood samples, however, the quantities fell with time. When the culture conditions were enriched by adding normal saline or broth on inoculated discs of wood and melamine, the bacterium formed a biofilm in presence of broth on melamine surface but not on wood. Consequently, even if a bacterial colony survived for a long time on wood, it would have limited ability to form a biofilm even in enriched conditions.

Limitations and future research directions

The first limitation regarding the antimicrobial testing of wood against fungi and bacteria was that the method was not compared with other available methods. The reason being that the conventional methods do not test the antimicrobial behavior of solid wood rather than extractives. The antiboisgram could also test the effect of physical modifications made in wood

samples; for example, a study collaborated with Dr Báder Mátyás, University of Sopron, Faculty of Wood Sciences, Hungary, showed an effect of thermos-hydro-compression method on the antimicrobial activity of oak and beech wood (unpublished results; Annex 1.6). On the other hand, extractive based methods should be employed to identify the active molecules responsible for antimicrobial activity.

In addition, the methods were not applied to a wide range of wood species, therefore, future studies should apply this methodology to an even wider range of wood types and microorganisms to have a more complete picture of the antimicrobial properties of wood material in healthcare settings. Moreover, based on screening results, further research should also be conducted to identify the active molecules responsible for antimicrobial activity of wood material.

The microbial survival was tested by a single elution-based recovery method. The comparative methods can be used, however, to maintain the sensitivity of methods, all the trials should be conducted at the same time. Moreover, experiments were conducted in triplicate owing to time constraints, further studies are recommended to adapt five replicate methodology. Similar methodologies should be applied to other bacteria and hygienically important fungi to determine their survival and distribution on wood material. Such results will help in the development of methodologies that can also test the efficacy of different disinfectants and cleaning methods used for wood material that could be untreated or treated via physical modification or by applying the surfactants, varnishes, etc. Such surface modifications may act as a barrier to the antimicrobial activity of wood and may increase the contact transfer due to increased surface smoothness. The wooden discs after recovering the bacteria were not studied microscopically, such investigation would give an idea to see if there were still microbes left hidden after elution-based recovery method.

Regarding the microscopy studies, the initial objective was to develop methods for quick and easy detection of nosocomial pathogens on wood surfaces. However, there were no reliable methods available literature, therefore, a methodology to follow the bacteria on wood surfaces was developed, which would be applied in future investigations to study the hygienic risk of nosocomial pathogens on wood surfaces. The first specific limitation of this research was that the results of the DRAQ5^{TM} experiments were analyzed qualitatively. The images, however, are archived for future analyses that might provide quantitative data. In addition, the viability of cells could not be tested using this probe. In case of studying the fluorescent *S. aureus,* the data were collected during two years of study and the environmental condition may have slightly varied each time. Another limitation of this method was image collection and

processing time, which spanned days for a single experiment. However, this time was still shorter than other microscopic approaches such as scanning electron microscopy. In addition, the resolution of images obtained by CSLM was not very high, and it does not provide sufficiently clear images to study any changes in the structure of individual bacterial cells. The development of biofilm on melamine as compared to oak surface was an interesting result, however, further studies are still required to develop an *in vitro* biofilm model on wood. In these investigations, the bacteria detached from the wooden surfaces during processing were not studied, studying their behavior would give further information on their attachment and survival in contact with wood material.

Overall, this research provided information on the antimicrobial properties of solid untreated wood material tested in laboratory settings. The data will be used to further help implement in-field studies. The root maps of indoor hospital studies were identified in the literature and used to outline the methodologies for future research (Annex 5 and 6). The epidemiological aspect of the prevalence of infectious agents in wooden healthcare buildings will be addressed as discussed in Annex 6. As the antimicrobial activities of wood were observed in laboratory studies, it is expected that there will be a lesser or equal prevalence of some specific pathogens in the wood containing the hospital environment. This effect could also be demonstrated in a laboratory-scale static chamber method and field-scale collection of the air and surface samples inside the hospital buildings (Figure A6.1). Meanwhile, social science and psychological themes would evaluate the perception of the occupants of healthcare buildings towards the indoor uses of wood material and its influence on their wellbeing (Annex 5). This study will measure the real social impact of our research and should be repeated for 5-10 years.

References

Abdallah M, Mourad R, Khelissa SO, et al (2019) Impact of growth temperature on the adhesion of colistin-resistant *Escherichia coli* strains isolated from pigs to food-contact-surfaces. Arch Microbiol. https://doi.org/10.1007/s00203-019-01632-0

Abdela MS, Soebarto V (2018) The death of modern hospitals: Towards a comprehensive approach for restorative healthcare environmental design. In: Proceedings of the 1st Annual Design Research Conference. Sydney, Australia, pp 139–153

Abdulla NQF (2018) The effect of aqueous and alcoholic extracts of galls of *Quercus infectoria* on the growth of some pathogenic fungi. Zanco Journal of Pure and Applied Sciences 30:46–57. https://doi.org/10.21271/ZJPAS.30.6.4

Abdul-Mutalib N-A, Amin Nordin S, Osman M, et al (2015) Pyrosequencing analysis of microbial community and food-borne bacteria on restaurant cutting boards collected in Seri Kembangan, Malaysia, and their correlation with grades of food premises. International Journal of Food Microbiology 200:57–65. https://doi.org/10.1016/j.ijfoodmicro.2015.01.022

Abdul-Mutalib N-A, Nordin SA, Osman M, et al (2016) The prevalence of foodborne pathogenic bacteria on cutting boards and their ecological correlation with background biota. AIMS Microbiology 2:138–151. https://doi.org/10.3934/microbiol.2016.2.138

Abedini A, Colin M, Hubert J, et al (2020) Abundant extractable metabolites from temperate tree barks: the specific antimicrobial activity of *Prunus Avium* extracts. Antibiotics 9:111. https://doi.org/10.3390/antibiotics9030111

Abrishami SH, Tall BD, Bruursema TJ, et al (1994) Bacterial Adherence and Viability on Cutting Board Surfaces. Journal of Food Safety 14:153–172. https://doi.org/10.1111/j.1745-4565.1994.tb00591.x

Acuff GR, Vanderzant C, Hanna MO, et al (1986) Effects of handling and preparation of Turkey products on the survival of *Campylobacter jejuni*. Journal of Food Protection 49:627–631. https://doi.org/10.4315/0362-028X-49.8.627

Adonizio A, Kong K-F, Mathee K (2008) Inhibition of quorum sensing-controlled virulence factor production in *Pseudomonas aeruginosa* by South Florida plant extracts. Antimicrobial Agents and Chemotherapy 52:198–203. https://doi.org/10.1128/AAC.00612-07

Ahmad A, Kaleem M, Ahmed Z, Shafiq H (2015) Therapeutic potential of flavonoids and their mechanism of action against microbial and viral infections—A review. Food Research International 77:221–235. https://doi.org/10.1016/j.foodres.2015.06.021

Ahmed AA, Salih FA (2019) *Quercus infectoria* gall extracts reduce quorum sensing-controlled virulence factors production and biofilm formation in *Pseudomonas aeruginosa* recovered from burn wounds. BMC Complementary and Alternative Medicine 19:177. https://doi.org/10.1186/s12906-019-2594-5

Ahmed M (2018) Evaluation of biological potential of *Quercus dilatata* L. and isolation of active compounds. PhD Thesis, Quaid-i-Azam University

Ahnrud GP, Mendoza AJ, Hurley MJ, Marek PJ (2018) Efficacy of a sonicating swab for removal and capture of microorganisms from experimental and natural contaminated surfaces. Appl Environ Microbiol 84:e00208-18. https://doi.org/10.1128/AEM.00208-18

Ak NO, Cliver DO, Kaspar CW (1994a) Cutting boards of plastic and wood contaminated experimentally with bacteria. Journal of Food Protection 57:16–22. https://doi.org/10.4315/0362-028X-57.1.16

Ak NO, Cliver DO, Kaspar CW (1994b) Decontamination of plastic and wooden cutting boards for kitchen use. Journal of Food Protection 57:23–30. https://doi.org/10.4315/0362-028X-57.1.23

Al Aboody MS, Mickymaray S (2020) Anti-fungal efficacy and mechanisms of Flavonoids. Antibiotics 9:45. https://doi.org/10.3390/antibiotics9020045

Al Hawani IAA, Aldhaher AHS, Abdalzahra IM (2020) A biological study on *Quercus* bark as antimicrobial agent. Annals of Tropical Medicine and Public Health 23:SP23119. https://doi.org/10.36295/ASRO.2020.231109

Al-Amili WA (2017) Evaluation of DNA damage for some Iraqi workers employed in wooden furniture factories. Journal of Kerbala University Proceedings of the Third Scientific Conference of the Faculty of Veterinary Medicine / University of Kerbala on 10th April 2017:221–228

Alañón ME, García-Ruíz A, Díaz-Maroto MC, et al (2015) Antimicrobial and antioxidant activity of pressurized liquid extracts from oenological woods. Food Control 50:581–588. https://doi.org/10.1016/j.foodcont.2014.09.043

Alapieti T, Mikkola R, Pasanen P, Salonen H (2020) The influence of wooden interior materials on indoor environment: a review. Eur J Wood Prod 78:617–634. https://doi.org/10.1007/s00107-020-01532-x

Alapieti T, Vornanen-Winqvist C, Mikkola R, et al (2017) The effect of wooden building materials on measured and perceived indoor environment quality: A review. International Society of Indoor Air Quality and Climate, Lublin University of Technology CampusLublin; Poland, p Code 138756

Aloui F, Ayadi N, Charrier F, Charrier B (2004) Durability of European oak (Quercus petraea and Quercus robur) against white rot fungi (Coriolus versicolor): relations with phenol extractives. Holz Roh Werkst 62:286–290. https://doi.org/10.1007/s00107-004-0489-7

Alpert P (2006) Constraints of tolerance: why are desiccation-tolerant organisms so small or rare? Journal of Experimental Biology 209:1575–1584. https://doi.org/10.1242/jeb.02179

Al-Qadiri HM, Ovissipour M, Al-Alami N, et al (2016) Efficacy of neutral electrolyzed water, quaternary ammonium and lactic acid-based solutions in controlling microbial

contamination of food cutting boards using a manual spraying technique. Journal of Food Science 81:M1177–M1183. https://doi.org/10.1111/1750-3841.13275

Al-Tawfiq JA, Tambyah PA (2014) Healthcare associated infections (HAI) perspectives. Journal of Infection and Public Health 7:339–344. https://doi.org/10.1016/j.jiph.2014.04.003

Andrade CC, Devlin AS, Pereira CR, Lima ML (2017) Do the hospital rooms make a difference for patients' stress? A multilevel analysis of the role of perceived control, positive distraction, and social support. Journal of Environmental Psychology 53:63–72. https://doi.org/10.1016/j.jenvp.2017.06.008

Andrensek S, Simonovska B, Vovk I, et al (2004) Antimicrobial and antioxidative enrichment of oak (Quercus robur) bark by rotation planar extraction using ExtraChrom. Int J Food Microbiol 92:181–187. https://doi.org/10.1016/j.ijfoodmicro.2003.09.009

ANSES (2016) Labelling of building and decoration products with respect to VOC emissions. In: Anses - Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail. https://www.anses.fr/en/content/labelling-building-and-decoration-products-respect-voc-emissions. Accessed 20 Sep 2020

Apanpa-Qasim AFI, Adeyi AA (2018) Oxygenated volatile organic compounds (anti-freezing agents) in decorative water-based paints marketed in Nigeria. Journal of Health and Pollution 8:. https://doi.org/10.5696/2156-9614-8.18.180606

Arango RA, Green F, Hintz K, et al (2006) Natural durability of tropical and native woods against termite damage by Reticulitermes flavipes (Kollar). International Biodeterioration & Biodegradation 57:146–150. https://doi.org/10.1016/j.ibiod.2006.01.007

Asri M, El Ghachtouli N, Elabed S, et al (2018) Wicherhamomyces anomalus biofilm supported on wood husk for chromium wastewater treatment. Journal of Hazardous Materials 359:554–562. https://doi.org/10.1016/j.jhazmat.2018.05.050

Aviat F, Gerhards C, Rodriguez-Jerez J, et al (2016) Microbial safety of wood in contact with food: a review. Comprehensive Reviews in Food Science and Food Safety 15:491–505. https://doi.org/10.1111/1541-4337.12199

Aviat F, Le Bayon I, Federighi M, Montibus M (2020) Comparative study of microbiological transfer from four materials used in direct contact with apples. International Journal of Food Microbiology 333:108780. https://doi.org/10.1016/j.ijfoodmicro.2020.108780

Azuma K, Kouda K, Nakamura M, et al (2016) Effects of inhalation of emissions from cedar timber on psychological and physiological factors in an indoor environment. Environments 3:37. https://doi.org/10.3390/environments3040037

Babaoglu UT, Milletli Sezgin F, Yag F (2020) Sick building symptoms among hospital workers associated with indoor air quality and personal factors. Indoor and Built Environment 29:645–655. https://doi.org/10.1177/1420326X19855117

Báder M, Németh R (2017) Hygroscopicity of longitudinally compressed wood. Acta Silvatica et Lignaria Hungarica 13:135–144. https://doi.org/10.1515/aslh-2017-0010

Báder M, Németh R (2018a) The effect of the relaxation time on the mechanical properties of the longitudinally compressed wood. Wood Research 63:383–398

Báder M, Németh R (2018b) The effect of the rate of longitudinal compression on selected wood properties. Acta Silvatica et Lignaria Hungarica 14:83–92. https://doi.org/10.2478/aslh-2018-0005

Báder M, Németh R (2019) Moisture-dependent mechanical properties of longitudinally compressed wood. Eur J Wood Prod 77:1009–1019. https://doi.org/10.1007/s00107-019-01448-1

Báder M, Németh R, Konnerth J (2019) Micromechanical properties of longitudinally compressed wood. Eur J Wood Prod 77:341–351. https://doi.org/10.1007/s00107-019-01392-0

Bahar Z, Ghotaslou R, Taheri S (2017) In vitro anti-biofilm activity of *Quercus brantii* subsp. persica on human pathogenic bacteria. Research Journal of Pharmacognosy 4:67–73

Bajpai P (2018) Wood and Fiber Fundamentals (Chapter 2). In: Bajpai P (ed) Biermann's Handbook of Pulp and Paper (Third Edition). Elsevier, pp 19–74

Balouiri M, Sadiki M, Ibnsouda SK (2016) Methods for in vitro evaluating antimicrobial activity: A review. Journal of Pharmaceutical Analysis 6:71–79. https://doi.org/10.1016/j.jpha.2015.11.005

Bang J, Hong A, Kim H, et al (2014) Inactivation of *Escherichia coli* O157:H7 in biofilm on food-contact surfaces by sequential treatments of aqueous chlorine dioxide and drying. International Journal of Food Microbiology 191:129–134. https://doi.org/10.1016/j.ijfoodmicro.2014.09.014

Barnes B i., Cassar C a., Halablab M a., et al (1996) An in situ method for determining bacterial survival on food preparation surfaces using a redox dye. Letters in Applied Microbiology 23:325–328. https://doi.org/10.1111/j.1472-765X.1996.tb00200.x

Barrasa-Villar JI, Aibar-Remón C, Prieto-Andrés P, et al (2017) Impact on morbidity, mortality, and length of stay of hospital-acquired infections by resistant microorganisms. Clin Infect Dis 65:644–652. https://doi.org/10.1093/cid/cix411

Basri DF, Fan SH (2005) The potential of aqueous and acetone extracts of galls of *Quercus infectori* a as antibacterial agents. Indian J Pharmacol 37:26–29. https://doi.org/10.4103/0253-7613.13851

Baurès E, Blanchard O, Mercier F, et al (2018) Indoor air quality in two French hospitals: Measurement of chemical and microbiological contaminants. Science of The Total Environment 642:168–179. https://doi.org/10.1016/j.scitotenv.2018.06.047

Baymiev AnKh, Baymiev AlKh, Kuluev BR, et al (2020) Modern approaches to differentiation of live and dead bacteria using selective amplification of nucleic acids. Microbiology 89:13–27. https://doi.org/10.1134/S0026261720010038

Beatson RP (2011) Chemicals from Extractives. In: Zhu J (J. Y), Zhang X, Pan X (Jun) (eds) Sustainable Production of Fuels, Chemicals, and Fibers from Forest Biomass. American Chemical Society, Washington, DC, pp 279–297

Benenson S, Cohen MJ, Schwartz C, et al (2020) Is it financially beneficial for hospitals to prevent nosocomial infections? BMC Health Services Research 20:653. https://doi.org/10.1186/s12913-020-05428-7

Berger G, Katz H, Petutschnigg AJ (2006) What consumers feel and prefer: Haptic perception of various wood flooring surfaces. Forest products journal 56:42–47

Bhatta SR, Tiippana K, Vahtikari K, et al (2017) Sensory and emotional perception of wooden surfaces through fingertip touch. Front Psychol 8:367. https://doi.org/10.3389/fpsyg.2017.00367

Bhattacharjee T, Datta SS (2019) Bacterial hopping and trapping in porous media. Nature Communications 10:2075. https://doi.org/10.1038/s41467-019-10115-1

Blankenhorn PR (2001) Wood: Sawn Materials. In: Buschow KHJ, Cahn RW, Flemings MC, et al. (eds) Encyclopedia of Materials: Science and Technology. Elsevier, Oxford, pp 9722–9732

Bonnet R, Kahlmeter G, Leclercq R (2012) Antimicrobial susceptibility testing. In: Cornaglia G, Courcol R, Herrmann J-L (eds) European manual of clinical microbiology, 1st edn. European Society for Clinical Microbiology and Infections Diseases : Société française de microbiologie, Basel, pp 77–85

Borges RCF, Marques E, Macedo MA, et al (2018) Biocontrol ofteak canker caused by Lasiodiplodia theobromae. Revista Árvore 42:e420304. https://doi.org/10.1590/1806-90882018000300004

Boucher SN, Chamberlain AHL, Adams MR (1998) Enhanced survival of *Campylobacter jejuni* in association with wood. Journal of Food Protection 61:26–30. https://doi.org/10.4315/0362-028x-61.1.26

Boursillon D, Riethmüller V (2007) The safety of wooden cutting boards: Remobilization of bacteria from pine, beech, and polyethylene. British Food Journal 109:315–322. https://doi.org/10.1108/00070700710736561

Brauns J, Rocens K (2005) Elasticity in wood and concrete: hygromechanical effects. In: Buschow KHJ, Cahn RW, Flemings MC, et al. (eds) Encyclopedia of Materials: Science and Technology. Elsevier, Oxford, pp 1–5

Bravo Z, Orruño M, Navascues T, et al (2019) Analysis of *Acinetobacter baumannii* survival in liquid media and on solid matrices as well as effect of disinfectants. Journal of Hospital Infection 103:e42–e52. https://doi.org/10.1016/j.jhin.2019.04.009

Bringslimark T, Nyrud AQ (2010) Patient rooms with different degrees of wood: A preference study conducted among hospital staff. In: Proceedings of a meeting held 20-24 June 2010. Trees and Timber Institute, National Research Council, Trentino, Italy, pp 2645–2648

BSAC (2020) The forgotten superbug: Is it time to bring fungal infections out of the dark? In: British Society for Antimicrobial Chemotherapy. http://bsac.org.uk/the-forgotten-superbug-is-it-time-to-bring-fungal-infections-out-of-the-dark/. Accessed 6 Aug 2020

Buchner J, Irle M, Belloncle C, et al (2019) Fungal and bacterial colonies growing on weathered wood surfaces. Wood Material Science & Engineering 14:33–41. https://doi.org/10.1080/17480272.2018.1443975

Bulian F, Fragassa C (2016) VOC emissions from wood products and furniture: a survey about legislation, standards and measures referred to different materials. FME Transactions 44:358–364. https://doi.org/doi: 10.5937/fmet1604358B

Burnard MD, Kutnar A (2015) Wood and human stress in the built indoor environment: a review. Wood Sci Technol 49:969–986. https://doi.org/10.1007/s00226-015-0747-3

Burnard MD, Kutnar A (2019) Human stress responses in office-like environments with wood furniture. Building Research & Information 48:316–330. https://doi.org/10.1080/09613218.2019.1660609

Burnard MD, Nyrud AQ, Bysheim K, et al (2017) Building material naturalness: Perceptions from Finland, Norway and Slovenia. Indoor and Built Environment 26:92–107. https://doi.org/10.1177/1420326X15605162

Buttner MP, Cruz P, Stetzenbach LD, Cronin T (2007) Evaluation of two surface sampling methods for detection of *Erwinia herbicola* on a variety of materials by culture and quantitative PCR. Appl Environ Microbiol 73:3505–3510. https://doi.org/10.1128/AEM.01825-06

Cai M, Lv H, Cao C, et al (2019) Evaluation of antimicrobial activity of *Pterocarpus* extracts. Industrial Crops and Products 140:111668. https://doi.org/10.1016/j.indcrop.2019.111668

Capolongo S, Settimo G (2017) Indoor Air Quality in Healing Environments: Impacts of Physical, Chemical, and Biological Environmental Factors on Users. In: Capolongo S, Settimo G, Gola M (eds) Indoor Air Quality in Healthcare Facilities. Springer International Publishing, Cham, pp 1–11

Cardinale M, Kaiser D, Lueders T, et al (2017) Microbiome analysis and confocal microscopy of used kitchen sponges reveal massive colonization by Acinetobacter, Moraxella and Chryseobacterium species. Scientific Reports 7:5791. https://doi.org/10.1038/s41598-017-06055-9

Carlquist S (2001) Evolution in Wood: An Ecological/Functional Synthesis. In: Carlquist S (ed) Comparative Wood Anatomy: Systematic, Ecological, and Evolutionary Aspects of Dicotyledon Wood. Springer, Berlin, Heidelberg, pp 335–379

Carpentier B (1997) Sanitary quality of meat chopping board surfaces: a bibliographical study. Food Microbiology 14:31–37. https://doi.org/10.1006/fmic.1996.0061

CASFM / EUCAST (2019) Comité de l'Antibiogramme (CA-SFM) CASFM / EUCAST V2.0 Mai 2019. Société Française de Microbiologie, Paris, France

Cassini A, Plachouras D, Eckmanns T, et al (2016) Burden of six Healthcare-Associated Infections on European population health: estimating incidence-based disability-adjusted life years through a population prevalence-based modelling study. PLoS Med 13:e1002150. https://doi.org/10.1371/journal.pmed.1002150

CDC (2019) HAI Data | CDC. https://www.cdc.gov/hai/data/index.html. Accessed 28 Jul 2020

CDC (2020a) Health Topics - HAI - POLARIS. https://www.cdc.gov/policy/polaris/healthtopics/hai.html. Accessed 28 Jul 2020

CDC (2020b) Candida auris | Fungal Diseases. In: Centers for Disease Control and Prevention. https://www.cdc.gov/fungal/candida-auris/index.html. Accessed 5 Oct 2020

Ceccherini G, Duveiller G, Grassi G, et al (2020) Abrupt increase in harvested forest area over Europe after 2015. Nature 583:72–77. https://doi.org/10.1038/s41586-020-2438-y

Chai J, Donnelly T, Wong T, Bryce E (2018) Environmental sampling of hospital surfaces: Assessing methodological quality. Canadian Journal of Infection Control 33:138–145

Chang C-J, Yang H-H, Wang Y-F, Li M-S (2015) Prevalence of sick building syndrome-related symptoms among hospital workers in confined and open working spaces. Aerosol and Air Quality Research 15:2378–2384. https://doi.org/10.4209/aaqr.2015.01.0040

Chapartegui-González I, Lázaro-Díez M, Bravo Z, et al (2018) *Acinetobacter baumannii* maintains its virulence after long-time starvation. PLoS One 13:e0201961. https://doi.org/10.1371/journal.pone.0201961

Chin AWH, Chu JTS, Perera MRA, et al (2020) Stability of SARS-CoV-2 in different environmental conditions. The Lancet Microbe 1:e10. https://doi.org/10.1016/S2666-5247(20)30003-3

Chiu T-H, Duan J, Liu C, Su Y-C (2006) Efficacy of electrolysed oxidizing water in inactivating Vibrio parahaemolyticus on kitchen cutting boards and food contact surfaces. Letters in Applied Microbiology 43:666–672. https://doi.org/10.1111/j.1472-765X.2006.02006.x

Choma I, Jesionek W (2015) TLC-direct bioautography as a high throughput method for detection of antimicrobials in plants. Chromatography 2:225–238. https://doi.org/10.3390/chromatography2020225

Choma IM, Grzelak EM (2011) Bioautography detection in thin-layer chromatography. Journal of Chromatography A 1218:2684–2691. https://doi.org/10.1016/j.chroma.2010.12.069

Cimiotti W, Glunder G, Hinz KH (1982) Survival of the bacterial turkey coryza agent. Veterinary Record 110:304–306. https://doi.org/10.1136/vr.110.13.304

Cliver DO (2006) Cutting boards in Salmonella cross-contamination. J AOAC Int 89:538–542. https://doi.org/10.1093/jaoac/89.2.538

CLSI (2018) Performance Standards for Antimicrobial Disk Susceptibility Tests, 13th edn. Clinical and Laboratory Standard Institute, Pennsylvania, USA

Conners TE (2001) Wood: Ultrastructure. In: Buschow KHJ, Cahn RW, Flemings MC, et al. (eds) Encyclopedia of Materials: Science and Technology. Elsevier, Oxford, pp 9751–9759

Copes J, Pellicer K, Malvestiti L, Stanchi NO (2000) Sobrevivencia en tablas de cocina de madera y plástico inoculadas experimentalmente con *Listeria monocytogenes* [Survival of Listeria monocytogenes in cutting boards of plastic and wood experimentally contaminated]. Analecta Veterinaria 20:47–50

Cosenza BJ, McCreary M, Buck JD, Shigo AL (1970) Bacteria Associated with Discolored and Decayed Tissues in Beech, Birch, and Maple. Phytopathology 60:1547. https://doi.org/10.1094/Phyto-60-1547

Coughenour C (2009) An evaluation of Methicillin Resistant *Staphylococcus aureus* survival on five environmental surfaces under two different humidities, with and without the addition of bovine serum albumin. Master Thesis, University of Nevada, Las Vegas

Coughenour C, Stevens V, Stetzenbach LD (2011) An evaluation of Methicillin-Resistant *Staphylococcus aureus* survival on five environmental surfaces. Microbial Drug Resistance 17:457–461. https://doi.org/10.1089/mdr.2011.0007

Cronhjort Y, Tulamo T, Verma I, Zubillaga L (2017) Interior design and care environments: End –user perceptions of wood material. Aalto University School of Arts, Design and Architecture, Finland

Cruciata M, Gaglio R, Scatassa ML, et al (2018) Formation and characterization of early bacterial biofilms on different wood typologies applied in dairy production. Appl Environ Microbiol 84:e02107-17. https://doi.org/10.1128/AEM.02107-17

da Silva CFFP, Rana C, Maskell D, et al (2016) Influence of eco-materials on indoor air quality. Green Materials 4:72–80. https://doi.org/10.1680/jgrma.16.00002

Dadswell HE, Hillis WE (1962) CHAPTER 1 - Wood. In: Hillis WE (ed) Wood Extractives and their Significance to the Pulp and Paper Industries. Academic Press, pp 3–55

Dantas STA, Rossi BF, Bonsaglia ECR, et al (2018) Cross-contamination and biofilm formation by *Salmonella enterica* serovar Enteritidis on various cutting boards. Foodborne Pathog Dis 15:81–85. https://doi.org/10.1089/fpd.2017.2341

Das K, Tiwari RKS, Shrivastava DK (2010) Techniques for evaluation of medicinal plant products as antimicrobial agents: Current methods and future trends. J Med Plants Res 4:104–111. https://doi.org/10.5897/JMPR09.030

Das Q, Islam MR, Lepp D, et al (2020) Gut microbiota, blood metabolites, and spleen immunity in broiler chickens fed berry pomaces and phenolic-enriched extractives. Front Vet Sci 7:150. https://doi.org/10.3389/fvets.2020.00150

Dasgupta A, Krasowski MD (2020) Chapter 10 - Therapeutic drug monitoring of antimicrobial, antifungal and antiviral agents. In: Dasgupta A, Krasowski MD (eds) Therapeutic Drug Monitoring Data (Fourth Edition). Academic Press, pp 159–197

Dawson P, Han I, Cox M, et al (2007) Residence time and food contact time effects on transfer of *Salmonella* Typhimurium from tile, wood and carpet: testing the five-second rule. Journal of Applied Microbiology 102:945–953. https://doi.org/10.1111/j.1365-2672.2006.03171.x

Demattè ML, Zucco GM, Roncato S, et al (2018) New insights into the psychological dimension of wood–human interaction. European Journal of Wood and Wood Products 76:1093–1100. https://doi.org/10.1007/s00107-018-1315-y

Despotovic A, Milosevic B, Milosevic I, et al (2020) Hospital-acquired infections in the adult intensive care unit—Epidemiology, antimicrobial resistance patterns, and risk factors for acquisition and mortality. American Journal of Infection Control. https://doi.org/10.1016/j.ajic.2020.01.009

Dettweiler M, Lyles JT, Nelson K, et al (2019) American Civil War plant medicines inhibit growth, biofilm formation, and quorum sensing by multidrug-resistant bacteria. Scientific Reports 9:7692. https://doi.org/10.1038/s41598-019-44242-y

DeVere E, Purchase D (2007) Effectiveness of domestic antibacterial products in decontaminating food contact surfaces. Food Microbiology 24:425–430. https://doi.org/10.1016/j.fm.2006.07.013

Dewanjee S, Gangopadhyay M, Bhattacharya N, et al (2015) Bioautography and its scope in the field of natural product chemistry. Journal of Pharmaceutical Analysis 5:75–84. https://doi.org/10.1016/j.jpha.2014.06.002

Deza MA, Araujo M, Garrido MJ (2007) Efficacy of neutral electrolyzed water to inactivate *Escherichia coli*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* on plastic and wooden kitchen cutting boards. Journal of Food Protection 70:102–108. https://doi.org/10.4315/0362-028X-70.1.102

Di Grigoli A, Francesca N, Gaglio R, et al (2015) The influence of the wooden equipment employed for cheese manufacture on the characteristics of a traditional stretched cheese during ripening. Food Microbiology 46:81–91. https://doi.org/10.1016/j.fm.2014.07.008

Didienne R, Defargues C, Callon C, et al (2012) Characteristics of microbial biofilm on wooden vats (‘gerles’) in PDO Salers cheese. International Journal of Food Microbiology 156:91–101. https://doi.org/10.1016/j.ijfoodmicro.2012.03.007

Doherty GP, Bailey K, Lewis PJ (2010) Stage-specific fluorescence intensity of GFP and mCherry during sporulation In Bacillus Subtilis. BMC Research Notes 3:303. https://doi.org/10.1186/1756-0500-3-303

Dong G-H, Qian Z (Min), Wang J, et al (2014) Home renovation, family history of atopy, and respiratory symptoms and asthma among children living in China. Am J Public Health 104:1920–1927. https://doi.org/10.2105/AJPH.2013.301438

Doussot F, De Jéso B, Quideau S, Pardon P (2002) Extractives content in cooperage oak wood during natural seasoning and toasting; influence of tree species, geographic location, and single-tree effects. J Agric Food Chem 50:5955–5961. https://doi.org/10.1021/jf020494e

Du L, Batterman S, Godwin C, et al (2015) Air exchange rates and migration of VOCs in basements and residences. Indoor Air 25:598–609. https://doi.org/10.1111/ina.12178

Duan S-M, Zhao X-S, Wen R-F, et al (2003) Stability of SARS coronavirus in human specimens and environment and its sensitivity to heating and UV irradiation. Biomed Environ Sci 16:246–255

Dubreil L, Aviat F, Anthoine V, et al (2018) Confocal spectral microscopy—an innovative tool for tracking of pathogen agents on contaminated wooden surfaces. European Journal of Wood and Wood Products 76:1083–1085. https://doi.org/10.1007/s00107-018-1294-z

Dutkiewicz J, Sorenson WG, Lewis DM, Olenchock SA (1992) Levels of bacteria, fungi and endotoxin in stored timber. International Biodeterioration & Biodegradation 30:29–46. https://doi.org/10.1016/0964-8305(92)90022-G

ECDC (2020) Healthcare-associated infections. In: European Centre for Disease Prevention and Control. https://www.ecdc.europa.eu/en/healthcare-associated-infections. Accessed 28 Jul 2020

Elansary HO, Szopa A, Kubica P, et al (2019) Polyphenol profile and pharmaceutical potential of quercus spp. Bark extracts. Plants 8:486. https://doi.org/10.3390/plants8110486

El-Hefny M, Salem MZM, Behiry SI, Ali HM (2020) The potential antibacterial and antifungal activities of wood treated with *Withania somnifera* Fruit Extract, and the Phenolic, Caffeine, and Flavonoid composition of the extract according to HPLC. Processes 8:113. https://doi.org/10.3390/pr8010113

Elom MO, Ugah UI, Omote V (2014) Microbial Contaminants of Wooden Toothpicks in Abakaliki Metropolis, Ebonyi State, Nigeria. World Journal of Life Sciences and Medical Research 3:101–106

Elserogy A, Kanan G, Hussein E, Khreis SA (2016) Isolation, characterization and treatment of microbial agents responsible for the deterioration of archaeological objects in three jordanian museums. Mediterranean Archa eology and Archaeometry 16:117–126. https://doi.org/DOI: 10.5281/zenodo.35525

EPA (2015) Overview of Wood Preservative Chemicals. In: US EPA. https://www.epa.gov/ingredients-used-pesticide-products/overview-wood-preservative-chemicals. Accessed 29 Jul 2020

EUCAST (2020) EUCAST Disk Diffusion Method for Antimicrobial Susceptibility Testing, Version 8.0. European Society of Clinical Microbiology and Infectious Diseases

Europa (2020) The European Union and forests | Fact Sheets on the European Union | European Parliament. https://www.europarl.europa.eu/factsheets/en/sheet/105/the-european-union-and-forests. Accessed 29 Jul 2020

Exum NG, Kosek MN, Davis MF, Schwab KJ (2017) Surface sampling collection and culture methods for *Escherichia coli* in household environments with high fecal contamination. Int J Environ Res Public Health 14:947. https://doi.org/10.3390/ijerph14080947

Fahimipour AK, Mamaar SB, McFarland AG, et al (2018) Antimicrobial Chemicals Associate with Microbial Function and Antibiotic Resistance Indoors. mSystems 3:e00200-18. https://doi.org/10.1128/mSystems.00200-18

Falk RH (2010) Wood as a sustainable building material (Chapter 1). In: Wood Handbook: wood as an engineering material, Centennial Edition. Forest Products Laboratory • United States Department of Agriculture Forest Service, Madison, Wisconsin, pp 1–3

Fantinel VS, Muniz MFB, Poletto T, et al (2018) Biocontrole in vitro de Colletotrichum siamense utilizando Trichoderma spp. e Bacillus thuringiensis var. kurstaki. Revista Ciência Agrícola 16:43–50. https://doi.org/10.28998/rca.v16i3.4818

Felippe ML, Kuhnen A, Silveira BB da, Lelli G (2017) What is a restorative hospital environment? Environmental meaning, affective stress restoration and physical attributes in pediatric inpatient rooms. Children, Youth and Environments 27:17–46. https://doi.org/10.7721/chilyoutenvi.27.1.0017

Fell DR (2010) Wood in the human environment : restorative properties of wood in the built indoor environment. PhD Thesis, University of British Columbia

Fengel D, Wegener G (1983) Wood: chemistry, ultrastructure, reactions. Walter de Gruyter

Fentahun M, Yilkal B A, Amsalu N, et al (2017) Antibacterial evaluation and phytochemical analysis of selected medicinal plants against some pathogenic enteric bacteria in Gozamin district, Ethiopia. Journal of Pharmacovigilance 5:244. https://doi.org/10.4172/2329-6887.1000244

Fernández-Agulló A, Freire MS, González-Álvarez J (2015) Effect of the extraction technique on the recovery of bioactive compounds from eucalyptus (*Eucalyptus globulus*) wood industrial wastes. Industrial Crops and Products 64:105–113. https://doi.org/10.1016/j.indcrop.2014.11.031

Fernández-Agulló A, Freire MS, Ramírez-López C, et al (2020) Valorization of residual walnut biomass from forest management and wood processing for the production of bioactive compounds. Biomass Conv Bioref. https://doi.org/10.1007/s13399-019-00598-9

Filip S, Fink R, Oder M, Jevšnik M (2012) Hygienic acceptance of wood in food industry. Wood Science and Technology 46:657–665. https://doi.org/10.1007/s00226-011-0440-0

Franco JT (2019) How tree trunks are cut to produce wood with different appearances and uses. In: ArchDaily. https://www.archdaily.com/894449/how-tree-trunks-are-cut-to-produce-wood-with-different-appearances-and-uses. Accessed 5 Oct 2020

Frontino G (2019) Comparison of methods for detection of *Listeria* on wooden shelves used for cheese aging: challenges associated with sampling porous surfaces. Master Thesis, University of Vermont

Gaglio R, Cruciata M, Gerlando RD, et al (2015) Microbial activation of wooden vats used for traditional cheese production and evolution of the neo-formed biofilms. Appl Environ Microbiol AEM.02868-15. https://doi.org/10.1128/AEM.02868-15

Gaglio R, Cruciata M, Scatassa ML, et al (2019) Influence of the early bacterial biofilms developed on vats made with seven wood types on PDO Vastedda della valle del Belìce cheese characteristics. International Journal of Food Microbiology 291:91–103. https://doi.org/10.1016/j.ijfoodmicro.2018.11.017

Galinari É, da Nóbrega JE, de Andrade NJ, de Luces Fortes Ferreira CL (2014) Microbiological aspects of the biofilm on wooden utensils used to make a Brazilian artisanal cheese. Braz J Microbiol 45:713–720. https://doi.org/10.1590/s1517-83822014000200047

Gawande S, Tiwari R, Narayanan P, Bhadri A (2020) Indoor air quality and sick building syndrome: Are green buildings better than conventional buildings. Indian Journal of Occupational and Environmental Medicine 24:30–32. https://doi.org/10.4103/ijoem.IJOEM_148_19

Geffert A, Geffertova J, Dudiak M (2019) Direct method of measuring the pH value of wood. Forests 10:852. https://doi.org/10.3390/f10100852

Gehrig M, Schnell G, Zürcher E, Kucera LJ (2000) Hygienische Eigenschaften von Holz- und Kunststoffbrettern in der Nahrungsmittelverarbeitung und -präsentation: Ein Vergleich. Holz als Roh- und Werkstoff 58:265–269. https://doi.org/10.1007/s001070050423

Gibbs SG, Sayles H, Colbert EM, et al (2014) Evaluation of the relationship between the Adenosine Triphosphate (ATP) Bioluminescence Assay and the presence of *Bacillus anthracis* spores and vegetative cells. Int J Environ Res Public Health 11:5708–5719. https://doi.org/10.3390/ijerph110605708

Gilbert RJ, Watson HM (1971) Some laboratory experiments on various meat preparation surfaces with regard to surface contamination and cleaning. International Journal of Food Science & Technology 6:163–170. https://doi.org/10.1111/j.1365-2621.1971.tb01604.x

Goh SG, Leili A-H, Kuan CH, et al (2014) Transmission of *Listeria monocytogenes* from raw chicken meat to cooked chicken meat through cutting boards. Food Control 37:51–55. https://doi.org/10.1016/j.foodcont.2013.08.030

Golus J, Sawicki R, Widelski J, Ginalska G (2016) The agar microdilution method - a new method for antimicrobial susceptibility testing for essential oils and plant extracts. J Appl Microbiol 121:1291–1299. https://doi.org/10.1111/jam.13253

Gómez-Acebo I, Dierssen-Sotos T, Pérez-Belmonte E, Llorca J (2013) Investigating an outbreak of non-specific building-related symptoms in workers of a general hospital. International Journal of Occupational Medicine and Environmental Health 26:563–571. https://doi.org/10.2478/s13382-013-0130-2

Gómez-Acebo I, Llorca J, Ortiz-Revuelta C, et al (2011) Sick building syndrome in a general hospital and the risks for pregnant workers. International Journal of Gynecology and Obstetrics 113:241–242. https://doi.org/10.1016/j.ijgo.2011.01.008

Gonçalves F, Correia P, Silva SP, Almeida-Aguiar C (2016) Evaluation of antimicrobial properties of cork. FEMS Microbiol Lett 363:fnv231. https://doi.org/10.1093/femsle/fnv231

Gontjes KJ, Gibson KE, Lansing B, et al (2020) Contamination of common area and rehabilitation gym environment with Multidrug-Resistant Organisms. Journal of the American Geriatrics Society 68:478–485. https://doi.org/10.1111/jgs.16284

Gough NL, Dodd CER (1998) The survival and disinfection of *Salmonella* Typhimurium on chopping board surfaces of wood and plastic. Food Control 9:363–368. https://doi.org/10.1016/S0956-7135(98)00127-3

Graikou K, Gortzi O, Mantanis G, Chinou I (2012) Chemical composition and biological activity of the essential oil from the wood of *Pinus heldreichii* Christ. var. leucodermis. Eur J Wood Prod 70:615–620. https://doi.org/10.1007/s00107-012-0596-9

Guan J, Chan M, VanderZaag A (2017) Inactivation of Avian Influenza Viruses on porous and non-porous surfaces is enhanced by elevating absolute humidity. Transboundary and Emerging Diseases 64:1254–1261. https://doi.org/10.1111/tbed.12499

Guchu E, Díaz-Maroto MC, Díaz-Maroto IJ, et al (2006) Influence of the Species and Geographical Location on Volatile Composition of Spanish Oak Wood (Quercus petraea Liebl. and Quercus robur L.). J Agric Food Chem 54:3062–3066. https://doi.org/10.1021/jf053055z

Guillier L, Stahl V, Hezard B, et al (2008) Modelling the competitive growth between *Listeria monocytogenes* and biofilm microflora of smear cheese wooden shelves. International Journal of Food Microbiology 128:51–57. https://doi.org/10.1016/j.ijfoodmicro.2008.06.028

Gul F, Khan KM, Adhikari A, et al (2017) Antimicrobial and antioxidant activities of a new metabolite from *Quercus incana*. Natural Product Research 31:1901–1909. https://doi.org/10.1080/14786419.2016.1266347

Güllüce M, Adigüzel A, Oğütçü H, et al (2004) Antimicrobial effects of *Quercus ilex* L. extract. Phytother Res 18:208–211. https://doi.org/10.1002/ptr.1419

Gupta M (2017) Characterization of microbial contaminants associated with floor material types. PhD Thesis, The Ohio State University

Gupta M, Bisesi M, Lee J (2017) Comparison of survivability of *Staphylococcus aureus* and spores of *Aspergillus niger* on commonly used floor materials. American Journal of Infection Control 45:717–722. https://doi.org/10.1016/j.ajic.2017.02.014

Gupta M, Lee S, Bisesi M, Lee J (2019) Indoor Microbiome and Antibiotic Resistance on Floor Surfaces: An Exploratory Study in Three Different Building Types. International Journal of Environmental Research and Public Health 16:4160. https://doi.org/10.3390/ijerph16214160

Haas D, Mayrhofer H, Habib J, et al (2019) Distribution of building-associated wood-destroying fungi in the federal state of Styria, Austria. Eur J Wood Prod 77:527–537. https://doi.org/10.1007/s00107-019-01407-w

Hammud KK, Neema RR, Ali SG, Hamza IS (2015) Direct solid disc as a novel antibacterial testing method. Int J Adv Pharm Biol Chem 4:844–851

Haque M, Sartelli M, McKimm J, Abu Bakar M (2018) Health care-associated infections – an overview. Infect Drug Resist 11:2321–2333. https://doi.org/10.2147/IDR.S177247

Havill NL, Boyce JM, Otter JA (2014) Extended survival of Carbapenem-resistant *Enterobacteriaceae* on dry surfaces. Infection Control & Hospital Epidemiology 35:445–447. https://doi.org/10.1086/675606

Herrero M, Quirós C, García LA, Díaz M (2006) Use of flow cytometry to follow the physiological states of microorganisms in cider fermentation processes. Appl Environ Microbiol 72:6725–6733. https://doi.org/10.1128/AEM.01183-06

Hiltunen T, Cairns J, Frickel J, et al (2018) Dual-stressor selection alters eco-evolutionary dynamics in experimental communities. Nature Ecology & Evolution 2:1974–1981. https://doi.org/10.1038/s41559-018-0701-5

Hobby GH, Quave CL, Nelson K, et al (2012) *Quercus cerris* extracts limit *Staphylococcus aureus* biofilm formation. Journal of Ethnopharmacology 144:812–815. https://doi.org/10.1016/j.jep.2012.10.042

Høibø O, Hansen E, Nybakk E, Nygaard M (2018) Preferences for urban building materials: Does building culture background matter? Forests 9:504. https://doi.org/10.3390/f9080504

Høibø O, Nyrud AQ (2010) Consumer perception of wood surfaces: The relationship between stated preferences and visual homogeneity. Journal of Wood Science 56:276–283. https://doi.org/10.1007/s10086-009-1104-7

Höllbacher E, Ters T, Rieder-Gradinger C, Srebotnik E (2016) Influences of interior fitting processes on the indoor air quality of two model rooms. Eur J Wood Prod 74:693–701. https://doi.org/10.1007/s00107-016-1051-0

Holmberg S, Persson K, Petersson H (1999) Nonlinear mechanical behaviour and analysis of wood and fibre materials. Computers & Structures 72:459–480. https://doi.org/10.1016/S0045-7949(98)00331-9

Huber D-J, Huber J, Hesser F, et al (2018) Two experts, three opinions: volatile organic compounds' testing methods and regulative systems. Eur J Wood Prod 76:5–12. https://doi.org/10.1007/s00107-017-1240-5

Hüppe T, Lorenz D, Wachowiak M, et al (2017) Volatile organic compounds in ventilated critical care patients: a systematic evaluation of cofactors. BMC Pulmonary Medicine 17:116. https://doi.org/10.1186/s12890-017-0460-0

IEA (2019) Global Status Report for Buildings and Construction 2019. International Energy Agency, Paris, France

Ikei H, Song C, Lee J, Miyazaki Y (2015) Comparison of the effects of olfactory stimulation by air-dried and high-temperature-dried wood chips of hinoki cypress (*Chamaecyparis obtusa*) on prefrontal cortex activity. J Wood Sci 61:537–540. https://doi.org/10.1007/s10086-015-1495-6

Ikei H, Song C, Miyazaki Y, et al (2018a) Physiological effects of touching the wood of Hinoki Cypress (*Chamaecyparis obtusa*) with the soles of the Feet. International Journal of Environmental Research and Public Health 15:2135. https://doi.org/10.3390/ijerph15102135

Ikei H, Song C, Miyazaki Y (2017a) Physiological effects of wood on humans: a review. Journal of Wood Science 63:1–23. https://doi.org/10.1007/s10086-016-1597-9

Ikei H, Song C, Miyazaki Y (2017b) Physiological Effects of Touching Wood. Int J Environ Res Public Health 14:. https://doi.org/10.3390/ijerph14070801

Ikei H, Song C, Miyazaki Y (2018b) Physiological effects of touching hinoki cypress (*Chamaecyparis obtusa*). J Wood Sci 64:226–236. https://doi.org/10.1007/s10086-017-1691-7

Ikei H, Song C, Miyazaki Y, et al (2017c) Physiological effects of touching coated wood. International Journal of Environmental Research and Public Health 14:773. https://doi.org/10.3390/ijerph14070773

Ikei H, Song C, Miyazaki Y (2016) Effects of olfactory stimulation by α-pinene on autonomic nervous activity. J Wood Sci 62:568–572. https://doi.org/10.1007/s10086-016-1576-1

Imhof R, Schwendimann L, Scettrini PR (2017) Sanitising wooden boards used for cheese maturation by means of a steam-mediated heating process. J Consum Prot Food Saf 12:255–263. https://doi.org/10.1007/s00003-017-1114-0

Inagaki K, Lucar J, Blackshear C, Hobbs CV (2019) Methicillin-susceptible and Methicillin-resistant *Staphylococcus aureus* bacteremia: nationwide estimates of 30-day readmission, in-hospital mortality, length of stay, and cost in the United States. Clin Infect Dis 69:2112–2118. https://doi.org/10.1093/cid/ciz123

Ismail R, Aviat F, Gay-Perret P, et al (2017) An assessment of *L. monocytogenes* transfer from wooden ripening shelves to cheeses: Comparison with glass and plastic surfaces. Food Control 73:273–280. https://doi.org/10.1016/j.foodcont.2016.08.014

Ismail R, Aviat F, Michel V, et al (2013) Methods for recovering microorganisms from solid surfaces used in the food industry: A review of the literature. International Journal of Environmental Research and Public Health 10:6169–6183. https://doi.org/10.3390/ijerph10116169

Ismail R, Bayon IL, Michel V, et al (2015) Comparative study of three methods for recovering microorganisms from wooden surfaces in the food industry. Food Anal Methods 8:1238–1247. https://doi.org/10.1007/s12161-014-0008-3

Jafarian H, Demers CMH, Blanchet P, Laundry V (2016) Impact of indoor use of wood on the quality of interior ambiances under overcast and clear skies: case study of the Eugene H. Kruger building, Québec City. BioResources 11:1647-1663–1663. https://doi.org/10.15376/biores.11.1.1647-1663

Jafarian H, Demers CMH, Blanchet P, Laundry V (2018) Effects of interior wood finishes on the lighting ambiance and materiality of architectural spaces. Indoor and Built Environment 27:786–804. https://doi.org/10.1177/1420326X17690911

Jain P, Shekhar N, Gaurav K (2010) Antimicrobial activity and phytochemical analysis of *Eucalyptus tereticornis* bark and leaf methanolic extracts. International Journal of Pharmaceutical Sciences Review and Research 4:126–128

Jamil M, ul Haq I, Mirza B, Qayyum M (2012) Isolation of antibacterial compounds from *Quercus dilatata* L. through bioassay guided fractionation. Annals of Clinical Microbiology and Antimicrobials 11:11. https://doi.org/10.1186/1476-0711-11-11

Jeffery-Smith A, Taori SK, Schelenz S, et al (2018) Candida auris: a Review of the Literature. Clinical Microbiology Reviews 31:e00029-17. https://doi.org/10.1128/CMR.00029-17

Jensen LK, Larsen A, M⊘lhave L, et al (2001) Health Evaluation of Volatile Organic Compound (VOC) Emissions from Wood and Wood-Based Materials. Archives of Environmental Health: An International Journal 56:419–432. https://doi.org/10.1080/00039890109604477

Jia H, Li L, Li W, et al (2019) Impact of Healthcare-Associated Infections on length of stay: A study in 68 hospitals in China. BioMed Research International 2019:Article ID 2590563. https://doi.org/10.1155/2019/2590563

Jiménez P, Dunkl A, Eibel K, et al (2016) Wood or laminate?—Psychological research of customer expectations. Forests 7:275. https://doi.org/10.3390/f7110275

Jiménez P, Dunkl A, Eibel K, et al (2015) Evaluating psychological aspects of wood and laminate products in indoor settings with pictures. Forest Products Journal 65:263–271. https://doi.org/10.13073/FPJ-D-14-00003

Johnston SR, Boddy L, Weightman AJ (2016) Bacteria in decomposing wood and their interactions with wood-decay fungi. FEMS Microbiol Ecol 92:fiw179. https://doi.org/10.1093/femsec/fiw179

Johnston WH, Karchesy JJ, Constantine GH, Craig AM (2001) Antimicrobial activity of some Pacific Northwest woods against anaerobic bacteria and yeast. Phytother Res 15:586–588. https://doi.org/10.1002/ptr.765

Jorgensen JH, Ferraro MJ (2009) Antimicrobial Susceptibility Testing: A Review of General Principles and Contemporary Practices. Clinical Infectious Diseases 49:1749–1755. https://doi.org/10.1086/647952

Joung D, Song C, Ikei H, et al (2014) Physiological and psychological effects of olfactory stimulation with D-limonene. Advances in Horticultural Science 28:90-94–94. https://doi.org/10.13128/ahs-22808

Karbasizade V, Dehghan P, Sichani MM, et al (2017) Evaluation of three plant extracts against biofilm formation and expression of quorum sensing regulated virulence factors in *Pseudomonas aeruginosa*. Pak J Pharm Sci 30:585–589

Kavian-Jahromi N, Schagerl L, Dürschmied B, et al (2015) Comparison of the antibacterial effects of sapwood and heartwood of the larch tree focusing on the use in hygiene sensitive areas. Eur J Wood Prod 73:841–844. https://doi.org/10.1007/s00107-015-0935-8

Kaynar P (2017) Antimicrobial activity of *Quercus robur* L. (Acorn). Fresenius Environmental Bulletin 26:6992–6995

Kelz C, Grote V, Moser M (2011) Interior wood use in classrooms reduces pupils' stress levels. In: Proceedings of the 9th Biennial Conference on Environmental Psychology. Eindhoven University of Technology, Eindhoven,The Netherlands

Khan HA, Baig FK, Mehboob R (2017) Nosocomial infections: Epidemiology, prevention, control and surveillance. Asian Pacific Journal of Tropical Biomedicine 7:478–482. https://doi.org/10.1016/j.apjtb.2017.01.019

Khan MN, Ngassapa O, Matee MIN (2000) Antimicrobial Activity of Tanzanian Chewing Sticks Against Oral Pathogenic Microbes. Pharmaceutical Biology 38:235–240. https://doi.org/10.1076/1388-0209(200007)3831-SFT235

Khelissa SO, Abdallah M, Jama C, Chihib N-E (2019) Actively detached *Pseudomonas aeruginosa* biofilm cell susceptibility to benzalkonium chloride and associated resistance mechanism. Arch Microbiol. https://doi.org/10.1007/s00203-019-01643-x

Khelissa SO, Jama C, Abdallah M, et al (2017) Effect of incubation duration, growth temperature, and abiotic surface type on cell surface properties, adhesion and pathogenicity of biofilm-detached *Staphylococcus aureus* cells. AMB Express 7:191. https://doi.org/10.1186/s13568-017-0492-0

Khurram M (2011) Evaluation of anticandidal potential of *Quercus baloot* Griff. using contact bioautography technique. African Journal of Pharmacy and Pharmacology 5:1538–1542. https://doi.org/10.5897/AJPP11.386

Kim JJ, Bimal KG, Hyeun CS, et al (2012) Comparison of phenolic compounds content in indeciduous *Quercus* species. Journal of Medicinal Plants Research 6:5228–5239. https://doi.org/10.5897/JMPR12.135

Kim M-S, Kim T, Lee D, et al (2018) Mental disorders among workers in the healthcare industry: 2014 national health insurance data. Ann Occup Environ Med 30:31. https://doi.org/10.1186/s40557-018-0244-x

Kim SW, Kim KS, Lamsal K, et al (2009) An in vitro study of the antifungal effect of silver nanoparticles on oak wilt pathogen Raffaelea sp. J Microbiol Biotechnol 19:760–764

Kim SY, Kim KW, Kwon YM, Kim JYH (2020) mCherry protein as an *in vivo* quantitative reporter of gene expression in the chloroplast of *Chlamydomonas reinhardtii*. Mol Biotechnol 62:297–305. https://doi.org/10.1007/s12033-020-00249-9

Kimura A, Sasaki S, Yatagai M, et al (2011) Psychological and physiological effects in humans induced by the visual and olfactory stimulations of an interior environment made of Hiba (*Thujopsis dolabrata*) wood. Mokuzai Gakkaishi/Journal of the Japan Wood Research Society 57:150–159. https://doi.org/10.2488/jwrs.57.150

Kirkeskov L, Witterseh T, Funch LW, et al (2009) Health evaluation of volatile organic compound (VOC) emission from exotic wood products. Indoor Air 19:45–57. https://doi.org/10.1111/j.1600-0668.2008.00560.x

Koch AP, Kofod CJ, Konova D, et al (2002) Wood, plastic and steel- comparason of hygenic properties. The Danish Technological Institute

Koinis A, Giannou V, Drantaki V, et al (2015) The impact of healthcare workers' job environment on their mental-emotional health, coping strategies: the case of a local general hospital. Health Psychol Res 3:1984. https://doi.org/10.4081/hpr.2015.1984

Kotradyová V, Kaliňáková B (2014) Wood as material suitable for healthcare and therapeutic facilities. Advanced Materials Research 1041:362–366. https://doi.org/10.4028/www.scientific.net/AMR.1041.362

Kotradyova V, Vavrinsky E, Kalinakova B, et al (2019) Wood and its impact on humans and environment quality in healthcare facilities. International Journal of Environmental Research and Public Health 16:3496. https://doi.org/10.3390/ijerph16183496

Kovács JK, Horváth G, Kerényi M, et al (2016) A modified bioautographic method for antibacterial component screening against anaerobic and microaerophilic bacteria. Journal of Microbiological Methods 123:13–17. https://doi.org/10.1016/j.mimet.2016.02.006

Kramer A, Assadian O (2014) Survival of microorganisms on inanimate surfaces. In: Borkow G (ed) Use of Biocidal Surfaces for Reduction of Healthcare Acquired Infections. Springer International Publishing, Cham, Switzerland, pp 7–26

Laireiter CM, Schnabel T, Köck A, et al (2013) Active anti-microbial effects of Larch and Pine wood on four bacterial strains. BioResources 9:273–281. https://doi.org/10.15376/biores.9.1.273-281

Lane K (2019) The efficacy of ATP monitoring devices at measuring organic matter on postharvest surfaces. Master Thesis, University of Massachusetts Amherst

Larasatie P, Guerrero JE, Conroy K, et al (2018) What does the public believe about tall wood buildings? an exploratory study in the US Pacific Northwest. Journal of Forestry 116:429–436. https://doi.org/10.1093/jofore/fvy025

Lathauwer DD (2014) List of LCI values for the Belgian Royal Decree of 8/5/2014 regarding threshold values for emissions towards the indoor environment of construction products for certain intended uses. Royal Decree and the JRC ECA 29 report

Lee K, Choi J-H, Lee S, et al (2018) Indoor levels of volatile organic compounds and formaldehyde from emission sources at elderly care centers in Korea. PLoS One 13:e0197495. https://doi.org/10.1371/journal.pone.0197495

Lee S, Bae S (2018) Molecular viability testing of viable but non-culturable bacteria induced by antibiotic exposure. Microbial Biotechnology 11:1008–1016. https://doi.org/10.1111/1751-7915.13039

Licitra G, Caccamo M, Valence F, Lortal S (2017) Traditional wooden equipment used for cheesemaking and their effect on quality. In: Global Cheesemaking Technology. Wiley-Blackwell, pp 157–172

Ling S, Hui L (2019) Evaluation of the complexity of indoor air in hospital wards based on PM2.5, real-time PCR, adenosine triphosphate bioluminescence assay, microbial culture and mass spectrometry. BMC Infect Dis 19:646. https://doi.org/10.1186/s12879-019-4249-z

Lipovac D, Burnard MD (2020) Effects of visual exposure to wood on human affective states, physiological arousal and cognitive performance: A systematic review of randomized trials. Indoor and Built Environment 1420326X20927437. https://doi.org/10.1177/1420326X20927437

Lipovac D, Podrekar N, Burnard MD, Šarabon N (2020) Effect of desk materials on affective states and cognitive performance. Journal of Wood Science 66:43. https://doi.org/10.1186/s10086-020-01890-3

Liu R, Wang C, Huang A, Lv B (2018) Characterization of odors of wood by gas chromatography-olfactometry with removal of extractives as attempt to control indoor air quality. Molecules 23:203. https://doi.org/10.3390/molecules23010203

Lodi FG, Viana GF, Uber AP, et al (2019) Can the resistance profile affect the survival of *Acinetobacter baumannii* on hospital surfaces? Journal of Hospital Infection 103:e120-122. https://doi.org/10.1016/j.jhin.2019.03.005

Lortal S, Di Blasi A, Madec M-N, et al (2009) Tina wooden vat biofilm: A safe and highly efficient lactic acid bacteria delivering system in PDO Ragusano cheese making. International Journal of Food Microbiology 132:1–8. https://doi.org/10.1016/j.ijfoodmicro.2009.02.026

Lortal S, Licitra G, Valence F (2014) Wooden Tools: Reservoirs of Microbial Biodiversity in Traditional Cheesemaking. Microbiology Spectrum 2:CM-0008–2012. https://doi.org/10.1128/microbiolspec.CM-0008-2012

Lu H, Ip E, Scott J, et al (2010) Effects of particle shape and size on devolatilization of biomass particle. Fuel 89:1156–1168. https://doi.org/10.1016/j.fuel.2008.10.023

Lucke F-K, Skowyrska A (2015) Hygienic aspects of using wooden and plastic cutting boards, assessed in laboratory and small gastronomy units. J Verbr Lebensm 10:317–322. https://doi.org/10.1007/s00003-015-0949-5

Mai P-Y, Levasseur M, Buisson D, et al (2020) Identification of antimicrobial compounds from *Sandwithia guyanensis*-associated endophyte using molecular network approach. Plants 9:47. https://doi.org/10.3390/plants9010047

Mai-Prochnow A, Clauson M, Hong J, Murphy AB (2016) Gram positive and Gram negative bacteria differ in their sensitivity to cold plasma. Scientific Reports 6:38610. https://doi.org/10.1038/srep38610

Mansour MMA, Abdel-Megeed A, Nasser RA, Salem MZM (2015) Comparative evaluation of some woody tree methanolic extracts and Paraloid B-72 against phytopathogenic mold fungi *Alternaria tenuissima* and *Fusarium culmorum*. BioResources 10:2570–2584. https://doi.org/10.15376/biores.10.2.2570-2584

Mansour MMA, Salem MZM (2015) Evaluation of wood treated with some natural extracts and Paraloid B-72 against the fungus *Trichoderma harzianum*: wood elemental composition, in-vitro and application evidence. International Biodeterioration & Biodegradation 100:62–69. https://doi.org/10.1016/j.ibiod.2015.02.009

Mariani C, Briandet R, Chamba J-F, et al (2007) Biofilm ecology of wooden shelves used in ripening the French raw milk smear cheese Reblochon de Savoie. Journal of Dairy Science 90:1653–1661. https://doi.org/10.3168/jds.2006-190

Mariani C, Oulahal N, Chamba J-F, et al (2011) Inhibition of *Listeria monocytogenes* by resident biofilms present on wooden shelves used for cheese ripening. Food Control 22:1357–1362. https://doi.org/10.1016/j.foodcont.2011.02.012

Masoko P, Masiphephethu MV (2019) Phytochemical investigation, antioxidant and antimycobacterial activities of *Schkuhria pinnata* (Lam) Thell extracts against *Mycobacterium smegmatis*. Journal of Evidence-Based Integrative Medicine 24:1–8. https://doi.org/10.1177/2515690X19866104

Masuda M (2004) Why wood is excellent for interior design? From vision physical point of view. Proceedings of the 8th World Conference on Timber Engineering, Lahti, Finland, 2004 101–106

Masuda M, Nakamura M (1990) The wood ratio in interior space and the psychological images. Bulletin of the Kyoto University Forests 62:297–303

Matsubara E, Ohira T (2018) Inhalation of Japanese cedar (*Cryptomeria japonica*) wood odor causes psychological relaxation after monotonous work among female participants. Biomedical Research (Tokyo, Japan) 39:241–249. https://doi.org/10.2220/biomedres.39.241

Matsubara E, Tsunetsugu Y, Ohira T, Sugiyama M (2017) Essential oil of japanese cedar (*Cryptomeria japonica*) wood increases salivary dehydroepiandrosterone sulfate levels after monotonous work. International Journal of Environmental Research and Public Health 14:97. https://doi.org/10.3390/ijerph14010097

Mensah-Attipoe J, Reponen T, Veijalainen A-M, et al (2016) Comparison of methods for assessing temporal variation of growth of fungi on building materials. Microbiology, 162:1895–1903. https://doi.org/10.1099/mic.0.000372

Merghache D, Boucherit-Otmani Z, Haci IAE, et al (2018) Inhibitory effect of *Quercus ilex* wood ash on the growth of pathogenic microorganisms. Phytothérapie 16:S269–S272. https://doi.org/10.3166/phyto-2018-0071

Miller AJ, Brown T, Call JE (1996) Comparison of wooden and polyethylene cutting boards: Potential for the attachment and removal of bacteria from ground beef. Journal of Food Protection 59:854–858

Milling A, Kehr R, Wulf A, Smalla K (2005a) Survival of bacteria on wood and plastic particles: Dependence on wood species and environmental conditions. Holzforschung 59:72–81. https://doi.org/10.1515/HF.2005.012

Milling A, Smalla K, A. Wulf (2005b) The use of wood in practice – a hygienic risk? Holz Roh Werkst 63:463–472. https://doi.org/10.1007/s00107-005-0064-x

Miranda I, Sousa V, Ferreira J, Pereira H (2017) Chemical characterization and extractives composition of heartwood and sapwood from *Quercus faginea*. PLOS ONE 12:e0179268. https://doi.org/10.1371/journal.pone.0179268

Miranda RC, Schaffner DW (2016) Longer contact times increase cross-contamination of *Enterobacter aerogenes* from surfaces to food. Appl Environ Microbiol 82:6490–6496. https://doi.org/10.1128/AEM.01838-16

Mirhoseini SH, Didehdar M, Akbari M, et al (2020) Indoor exposure to airborne bacteria and fungi in sensitive wards of an academic pediatric hospital. Aerobiologia 36:225–232. https://doi.org/10.1007/s10453-020-09624-0

Mirhoseini SH, Nikaeen M, Shamsizadeh Z, Khanahmad H (2016) Hospital air: A potential route for transmission of infections caused by β-lactam-resistant bacteria. Am J Infect Control 44:898–904. https://doi.org/10.1016/j.ajic.2016.01.041

Mitchell R, Taylor G, Rudnick W, et al (2019) Trends in health care–associated infections in acute care hospitals in Canada: an analysis of repeated point-prevalence surveys. CMAJ : Canadian Medical Association Journal 191:E981–E988. https://doi.org/10.1503/cmaj.190361

Mohabi S, Kalantar-Neyestanaki D, Mansouri S (2017) Inhibition of quorum sensing-controlled virulence factor production in *Pseudomonas aeruginosa* by *Quercus infectoria* gall extracts. Iranian Journal of Microbiology 9:26–32

Mohammad GA, Al-Taee SM (2014) Comparison of various types of Cutting boards in bacterial contamination. Journal of College of Education for Pure Sciences 4:301–308

Mohan GBM, Parker CW, Urbaniak C, et al (2020) Microbiome and metagenome analyses of a closed habitat during human occupation. mSystems 5:e00367-20. https://doi.org/10.1128/mSystems.00367-20

Montibus M, Ismaïl R, Michel V, et al (2016) Assessment of *Penicillium expansum* and *Escherichia coli* transfer from poplar crates to apples. Food Control 60:95–102. https://doi.org/10.1016/j.foodcont.2015.07.025

Moore G, Blair IS, McDowell DA (2007) Recovery and transfer of *Salmonella* Typhimurium from four different domestic food contact surfaces. J Food Prot 70:2273–2280. https://doi.org/10.4315/0362-028X-70.10.2273

Moricz ÁM, Häbe TT, Böszörményi A, et al (2015) Tracking and identification of antibacterial components in the essential oil of Tanacetum vulgare L. by the combination of high-performance thin-layer chromatography with direct bioautography and mass spectrometry. Journal of Chromatography A 1422:310–317. https://doi.org/10.1016/j.chroma.2015.10.010

Moricz AM, Ott PG (2017) Screening and characterization of antimicrobial components of natural products using planar chromatography coupled with direct bioautography,

spectroscopy and mass spectrometry: a review. Current Organic Chemistry 21:1861–1874. https://doi.org/10.2174/1385272821666170127154900

Morikawa T, Miyazaki Y, Kobayashi S (1998) Time-series variations of blood pressure due to contact with wood. J Wood Sci 44:495–497. https://doi.org/10.1007/BF00833417

Moshfeghy Z, Asadi K, Akbarzadeh M, et al (2018) *Quercus Brantii* Lindl. Vaginal douche versus clotrimazole on vaginal candidiasis, a randomized clinical trial. Journal of Pharmacopuncture 21:185–194. https://doi.org/10.3831/KPI.2018.21.022

Mousavi SM, Desobry S, Hardy J (1998) Mathematical modelling of migration of volatile compounds into packaged food via package free space. Part II: Spherical shaped food. Journal of Food Engineering 36:473–484. https://doi.org/10.1016/S0260-8774(98)00059-4

Mukai A, Takahashi K, Kofujita H, Ashitani T (2019) Antitermite and antifungal activities of thujopsene natural autoxidation products. Eur J Wood Prod 77:311–317. https://doi.org/10.1007/s00107-018-1370-4

Munir MT (2016) Antimicrobial potential of wood used in hygienically sensitive places. Master Thesis, ONIRIS

Munir MT, Aviat F, Pailhories H, et al (2019a) Direct screening method to assess antimicrobial behavior of untreated wood. European Journal of Wood and Wood Products 77:319–322. https://doi.org/10.1007/s00107-019-01390-2

Munir MT, Belloncle C, Aviat F, et al (2019b) Oak wood has antimicrobial activity against *Acinetobacter baumanni* isolates of human and animal origin. Quebec, Canada

Munir MT, Belloncle C, Aviat F, et al (2019c) Antimicrobial properties of oak against Acinetobacter: a one health approach. Springer, Sousse, Tunisia

Munir MT, Belloncle C, Irle M, Federighi M (2019d) Wood-based bedding in poultry production: a review. World's Poultry Science Journal 75:5–16. https://doi.org/10.1017/S0043933918000909

Munir MT, Belloncle C, Pailhories H, et al (2017) Wood as a safe material for indoor surface construction of hygienically sensitive places. Northwestern Polytechnical University Xi'an, China, pp 79–80

Munir MT, Belloncle C, Pailhoriès H, et al (2018) Survival of nosocomial pathogens on Maritime pine and European fir wood. In: Proceedings of the 5th International Conference on Processing Technologies for the Forest and Bio-based Products Industries. Freising/Munich, Germany, pp 213–217

Munir MT, Federighi M (2020) Control of foodborne biological hazards by ionizing radiations. Foods 9:878. https://doi.org/10.3390/foods9070878

Munir MT, Irle M, Belloncle C, Federighi M (2019e) Wood based bedding material in animal production: a minireview. Approaches in Poultry, Dairy & Veterinary Sciences 6:582–88

Munir MT, Munir AR, ul Hasan M, Abubakar M (2014) Epidemiology and management strategies of Johne's disease in endemic situations. Research Journal for Veterinary Practitioners 2:84–90. http://dx.doi.org/10.14737/journal.rjvp/2014/2.5.84.90

Munir MT, Pailhories H, Eveillard M, et al (2019f) Antimicrobial characteristics of untreated wood: towards a hygienic environment. Health 11:152–170. https://doi.org/10.4236/health.2019.112014

Munir MT, Pailhories H, Eveillard M, et al (2020a) Testing the antimicrobial characteristics of wood materials: a review of methods. Antibiotics 9:225. https://doi.org/10.3390/antibiotics9050225

Munir MT, Pailhories H, Eveillard M, et al (2020b) Experimental parameters influence the observed antimicrobial response of oak wood (*Quercus petraea*). Antibiotics 9:535. https://doi.org/10.3390/antibiotics9090535

Nagesh L, Sivasamy S, Muralikrishna KS, Bhat KG (2012) Antibacterial potential of gall extract of *Quercus infectoria* against *Enterococcus faecalis* -an in vitro study. Pharmacognosy Journal 4:47–50. https://doi.org/10.5530/pj.2012.30.9

Nakamura M, Ikei H, Miyazaki Y (2019) Physiological effects of visual stimulation with full-scale wall images composed of vertically and horizontally arranged wooden elements. Journal of Wood Science 65:55. https://doi.org/10.1186/s10086-019-1834-0

Nakmee PS, Khuntong S, Nuengchamnong N (2016) Phytochemical constituents with antimicrobial and antioxidant activities from *Xylia xylocarpa* (Roxb.) Taub. sawdust extracts. Chiang Mai Journal of Science 43:11–21

Nguyen DT, Kim HR, Jung JH, et al (2018) The development of paper discs immobilized with luciferase/D-luciferin for the detection of ATP from airborne bacteria. Sensors and Actuators B: Chemical 260:274–281. https://doi.org/10.1016/j.snb.2018.01.009

Niedermayer S, Fürhapper C, Nagl S, et al (2013) VOC sorption and diffusion behavior of building materials. Eur J Wood Prod 71:563–571. https://doi.org/10.1007/s00107-013-0713-4

Nikiforuk AM, Cutts TA, Theriault SS, Cook BWM (2017) Challenge of liquid stressed protective materials and environmental persistence of Ebola Virus. Scientific Reports 7:4388. https://doi.org/10.1038/s41598-017-04137-2

Nisula L (2018) Wood extractives in conifers: a study of stemwood and knots of industrially important species. PhD Thesis, Åbo Akademi University

Nore K, Nyrud AQ, Kraniotis D, et al (2017) Moisture buffering, energy potential, and volatile organic compound emissions of wood exposed to indoor environments. Science and Technology for the Built Environment 23:512–521. https://doi.org/10.1080/23744731.2017.1288503

Nostro A, Germanò M p., D'Angelo V, et al (2000) Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. Letters in Applied Microbiology 30:379–384. https://doi.org/10.1046/j.1472-765x.2000.00731.x

Nyrud AQ, Bringslimark T (2010) Is interior wood use psychologically beneficial? A review of psychological responses toward wood. Wood and Fiber Science 42:202–218

Nyrud AQ, Bringslimark T, Bysheim K (2014) Benefits from wood interior in a hospital room: A preference study. Architectural Science Review 57:125–131. https://doi.org/10.1080/00038628.2013.816933

Nyrud AQ, Bringslimark T, Englund F (2012) Wood use in a hospital environment: VOC emissions and air quality. Eur J Wood Prod 70:541–543. https://doi.org/10.1007/s00107-011-0578-3

Nyrud AQ, Bysheim K, Bringslimark T (2010) Health benefits from wood interior in a hospital room. In: Proceedings of the International Convention of Society of Wood Science and Technology and United Nations Economic Commission for Europe – Timber Committee. Geneva, Switzerland, p Paper WS-56

Obe T, Nannapaneni R, Sharma CS, Kiess A (2018) Homologous stress adaptation, antibiotic resistance, and biofilm forming ability of *Salmonella enterica* serovar Heidelberg ATCC8326 on different food-contact surfaces following exposure to sublethal chlorine concentrations1. Poult Sci 97:951–961. https://doi.org/10.3382/ps/pex346

Ohta H, Maruyama M, Tanabe Y, et al (2008) Effects of redecoration of a hospital isolation room with natural materials on stress levels of denizens in cold season. International Journal of Biometeorology 52:331–340. https://doi.org/10.1007/s00484-007-0125-4

Omar S, Lemonnier B, Jones N, et al (2000) Antimicrobial activity of extracts of eastern North American hardwood trees and relation to traditional medicine. J Ethnopharmacol 73:161–170. https://doi.org/10.1016/S0378-8741(00)00294-4

Otter JA, French GL (2009) Survival of Nosocomial Bacteria and Spores on Surfaces and Inactivation by Hydrogen Peroxide Vapor. Journal of Clinical Microbiology 47:205–207. https://doi.org/10.1128/JCM.02004-08

Oxford J, Berezin EN, Courvalin P, et al (2014) The survival of influenza A(H1N1)pdm09 virus on 4 household surfaces. American Journal of Infection Control 42:423–425. https://doi.org/10.1016/j.ajic.2013.10.016

Pagniez F, Le Pape P (2001) New fluorometric screening test for possible antifungal drugs. Journal de mycologie médicale (Paris) 11:73–78

Pailhories H, Munir MT, Aviat F, et al (2017) Oak in hospitals, the worst enemy of *Staphylococcus aureus*? Infection Control and Hospital Epidemiology 38:382–384. https://doi.org/10.1017/ice.2016.304

Pallardy SG (2008) The Woody Plant Body. In: Pallardy SG (ed) Physiology of Woody Plants, 3rd edn. Academic Press, San Diego, pp 9–38

Patkó C, Patkó I, Pásztory Z (2013) Indoor air quality testing in low-energy wooden houses: Measurement of formaldehyde and VOC-s. Acta Polytechnica Hungarica 10:105–116

Peng W, Li D, Zhang M, et al (2017) Characteristics of antibacterial molecular activities in poplar wood extractives. Saudi Journal of Biological Sciences 24:399–404. https://doi.org/10.1016/j.sjbs.2015.10.026

Pettersen RC (1984) The Chemical Composition of Wood. In: The Chemistry of Solid Wood. American Chemical Society, Washington, DC, pp 57–126

Plötze M, Niemz P (2011) Porosity and pore size distribution of different wood types as determined by mercury intrusion porosimetry. Eur J Wood Prod 69:649–657. https://doi.org/10.1007/s00107-010-0504-0

Plumed-Ferrer C, Väkeväinen K, Komulainen H, et al (2013) The antimicrobial effects of wood-associated polyphenols on food pathogens and spoilage organisms. International Journal of Food Microbiology 164:99–107. https://doi.org/10.1016/j.ijfoodmicro.2013.04.001

Pohleven J, Burnard MD, Kutnar A (2019) Volatile organic compounds emitted from untreated and thermally modified wood - a review. Wood and Fiber Science 51:231–254. https://doi.org/10.22382/wfs-2019-023

Poirier G, Demers CM, Potvin A (2019) Wood perception in daylit interior spaces: an experimental study using scale models and questionnaires. BioResources 14:1941–1968. https://doi.org/Wood perception in daylit interior spaces: An experimental study using scale models and questionnaire

Poirier G, Demers CMH, Potvin A (2017) Experiencing wooden ambiences with Nordic light: scale model comparative studies under real skies. BioResources 12:1924-1942–1942. https://doi.org/10.15376/biores.12.1.1924-1942

Prechter S, Betz M, Cerny G, et al (2002) Hygienische Aspekte von Schneidebrettern aus Holz bzw. Kunststoff [Hygienic aspects of wooden or plastic cutting boards]. Holz als Roh- und Werkstoff [European Journal of Wood and Wood Products] 60:239–248. https://doi.org/10.1007/s00107-002-0301-5

Prida A, Boulet J-C, Ducousso A, et al (2006) Effect of species and ecological conditions on ellagitannin content in oak wood from an even-aged and mixed stand of *Quercus robur* L. and *Quercus petraea* Liebl. Annals of Forest Science 63:415–424. https://doi.org/10.1051/forest:2006021

Prida A, Ducousso A, Petit RJ, et al (2007) Variation in wood volatile compounds in a mixed oak stand: strong species and spatial differentiation in whisky-lactone content. Ann For Sci 64:313–320. https://doi.org/10.1051/forest:2007008

Prida A, Puech J-L (2006) Influence of geographical origin and botanical species on the content of extractives in American, French, and East European oak woods. Journal of Agricultural and Food Chemistry 54:8115–8126. https://doi.org/10.1021/jf0616098

Qayyum S, Tariq S, Younas F (2020) Sick building syndrome and job performance in women factory workers. Journal of Postgraduate Medical Institute 34:22–28

Quesada-Granados JJ, Samaniego-Sánchez C, Blanca-Herrera RM (2016) Rhum–Ron–Rum: Technology and Tradition. In: Caballero B, Finglas PM, Toldrá F (eds) Encyclopedia of Food and Health. Academic Press, Oxford, pp 618–627

Quirós C, Herrero M, García LA, Díaz M (2007) Application of flow cytometry to segregated kinetic modeling based on the physiological states of microorganisms. Appl Environ Microbiol 73:3993–4000. https://doi.org/10.1128/AEM.00171-07

Quoc CH, Huong GV, Duc HN (2020) Working conditions and sick building syndrome among health care workers in vietnam. International Journal of Environmental Research and Public Health 17:. https://doi.org/10.3390/ijerph17103635

Rahman MA, Rossner A, Hopke PK (2017) Occupational exposure of aldehydes resulting from the storage of wood pellets. J Occup Environ Hyg 14:417–426. https://doi.org/10.1080/15459624.2017.1285491

Raia DD, Cannova L, Provenzano S, et al (2018) Comparison between adenosine triphosphate bioluminescence and aerobic colony count to assess surface sanitation in the hospital environment. Epidemiology, Biostatistics and Public Health 15:e12710. https://doi.org/10.2427/12710

Ramage MH, Burridge H, Busse-Wicher M, et al (2017) The wood from the trees: The use of timber in construction. Renewable and Sustainable Energy Reviews 68:333–359. https://doi.org/10.1016/j.rser.2016.09.107

Ramananantoandro T, Eyma F, Belloncle C, et al (2018) Effects of machining parameters on raised grain occurring after the application of water-based finishes. European Journal of Wood and Wood Products 76:1323–1333. https://doi.org/10.1007/s00107-017-1250-3

Rautiainen P, Hyttinen M, Ruokolainen J, et al (2019) Indoor air-related symptoms and volatile organic compounds in materials and air in the hospital environment. International Journal of Environmental Health Research 29:479–488. https://doi.org/10.1080/09603123.2018.1550194

Rawlinson S, Ciric L, Cloutman-Green E (2019) How to carry out microbiological sampling of healthcare environment surfaces? A review of current evidence. Journal of Hospital Infection 103:363–74. https://doi.org/10.1016/j.jhin.2019.07.015

Reinprecht L, Vidholdová Z, Iždinský J (2020) Bacterial and Mold Resistance of Selected Tropical Wood Species. BioResources 15:5198–5209

Revol-Junelles A-M, Miguindou-Mabiala R, Roger-Maigné D, Millière J-B (2005) Behavior of *Escherichia coli* cells and *Bacillus cereus* spores on poplar wood crates by impedance measurements. J Food Prot 68:80–84. https://doi.org/10.4315/0362-028X-68.1.80

Reygaert WC (2018) An overview of the antimicrobial resistance mechanisms of bacteria. AIMS Microbiol 4:482–501. https://doi.org/10.3934/microbiol.2018.3.482

Riaz A, Umar S, Munir MT, Tariq M (2017) Replacements of antibiotics in the control of Necrotic Enteritis: a review. Science Letters 5:208–216

Rice J (2004) An analysis of interior wood products and their psychological impact. Master Thesis, University of British Columbia

Rice J, Kozak RA, Meitner MJ, Cohen DH (2007) Appearance wood products and psychological well-being. Wood and Fiber Science 38:644–659

Ridoutt BG, Ball RD, Killerby SK (2002) First impressions of organizations and the qualities connoted by wood in interior design. Forest Products Journal 52:30. https://doi.org/10.1016/S0378-1127(01)00766-6

Rios JL, Recio MC, Villar A (1988) Screening methods for natural products with antimicrobial activity: A review of the literature. Journal of Ethnopharmacology 23:127–149. https://doi.org/10.1016/0378-8741(88)90001-3

Ripolles-Avila C, Hascoët AS, Ríos-Castillo AG, Rodríguez-Jerez JJ (2019) Hygienic properties exhibited by single-use wood and plastic packaging on the microbial stability for fish. LWT 113:108309. https://doi.org/10.1016/j.lwt.2019.108309

Robson A-L, Dastoor PC, Flynn J, et al (2018) Advantages and Limitations of Current Imaging Techniques for Characterizing Liposome Morphology. Front Pharmacol 9:80. https://doi.org/10.3389/fphar.2018.00080

Rodrigues ML, Nosanchuk JD (2020) Fungal diseases as neglected pathogens: A wake-up call to public health officials. PLOS Neglected Tropical Diseases 14:e0007964. https://doi.org/10.1371/journal.pntd.0007964

Rowell RM (2012) Handbook of wood chemistry and wood composites. CRC press, Boca Raton

Rozman U, Turk SŠ (2016) PCR Technique for the Microbial Analysis of Inanimate Hospital Environment. Polymerase Chain Reaction for Biomedical Applications 119–134. https://doi.org/10.5772/65742

Safary A, Motamedi H, Maleki S, Seyyedneja SM (2009) A preliminary study on the antibacterial activity of *Quercus brantii* against bacterial pathogens, particularly enteric pathogens. International Journal of Botany 5:176–180. https://doi.org/10.3923/ijb.2009.176.180

Saito Y, Nishimaki M, Tatsumi Y, et al (2009) Effects of wooden and vinyl interior finishes on stress reduction estimate by biological and psychological parameters. Journal of the Japan Wood Research Society (Japan)

Sajitha KL, Dev SA, Maria Florence EJ (2018) Biocontrol potential of *Bacillus subtilis* B1 against sapstain fungus in rubber wood. Eur J Plant Pathol 150:237–244. https://doi.org/10.1007/s10658-017-1272-z

Saka K, II AA, Obasa T, et al (2017) Bacterial contamination of hospital surfaces according to material make, last time of contact and last time of cleaning/disinfection. Journal of Bacteriology & Parasitology 08:312. https://doi.org/10.4172/2155-9597.1000312

Sakaguchi H, Wada K, Kajioka J, et al (2010) Maintenance of influenza virus infectivity on the surfaces of personal protective equipment and clothing used in healthcare settings. Environ Health Prev Med 15:344–349. https://doi.org/10.1007/s12199-010-0149-y

Sakuragawa S, Kaneko T, Miyazaki Y (2008) Effects of contact with wood on blood pressure and subjective evaluation. J Wood Sci 54:107–113. https://doi.org/10.1007/s10086-007-0915-7

Sakuragawa S, Miyazaki Y, Kaneko T, Makita T (2005) Influence of wood wall panels on physiological and psychological responses. J Wood Sci 51:136–140. https://doi.org/10.1007/s10086-004-0643-1

Salem MZM, Elansary HO, Elkelish AA, et al (2016a) In vitro bioactivity and antimicrobial Activity of *Picea abies* and *Larix decidua* wood and bark extracts. BioResources 11:9421–9437. https://doi.org/10.15376/biores.11.4.9421-9437

Salem MZM, Mansour MMA, Elansary HO (2019) Evaluation of the effect of inner and outer bark extracts of sugar maple (*Acer saccharum* var. Saccharum) in combination with citric acid against the growth of three common molds. Journal of Wood Chemistry and Technology 39:136–147. https://doi.org/10.1080/02773813.2018.1547763

Salem MZM, Zayed MZ, Ali HM, El-Kareem MSMA (2016b) Chemical composition, antioxidant and antibacterial activities of extracts from *Schinus molle* wood branch growing in Egypt. J Wood Sci 62:548–561. https://doi.org/10.1007/s10086-016-1583-2

Salem MZM, Zidan YE, El Hadidi NMN, et al (2016c) Evaluation of usage three natural extracts applied to three commercial wood species against five common molds. International Biodeterioration & Biodegradation 110:206–226. https://doi.org/10.1016/j.ibiod.2016.03.028

Salem MZM, Zidan YE, Mansour MMA, et al (2016d) Antifungal activities of two essential oils used in the treatment of three commercial woods deteriorated by five common mold fungi. International Biodeterioration & Biodegradation 106:88–96. https://doi.org/10.1016/j.ibiod.2015.10.010

Salo L (2017) The effects of coatings on the indoor air emissions of wood board. Master Thesis, Aalto University

Sánchez-Pérez A, Brown G, Malik R, et al (2013) Rapid detection of haemotropic mycoplasma infection of feline erythrocytes using a novel flow cytometric approach. Parasites and Vectors 6:158. https://doi.org/10.1186/1756-3305-6-158

Sarwar R, Farooq U, Khan A, et al (2015) Evaluation of antioxidant, free radical scavenging, and antimicrobial activity of *Quercus incana* Roxb. Frontiers in Pharmacology 6:277. https://doi.org/10.3389/fphar.2015.00277

Sarwar R, Farooq U, Naz S, et al (2018) Isolation and characterization of two new antimicrobial acids from *Quercus incana* (Bluejack Oak). BioMed Research International Article ID 3798105. https://doi.org/10.1155/2018/3798105

Sassoli M, Taiti C, Guidi Nissim W, et al (2017) Characterization of VOC emission profile of different wood species during moisture cycles. iForest - Biogeosciences and Forestry 10:576. https://doi.org/10.3832/ifor2259-010

Sati SC, Sati N, Sati OP (2011) Chemical investigation and screening of antimicrobial activity of bark of *Quercus leucotrichophora*. International Journal of Pharmacy and Pharmaceutical Sciences 3:89–91

Scatassa ML, Gaglio R, Macaluso G, et al (2015) Transfer, composition and technological characterization of the lactic acid bacterial populations of the wooden vats used to produce traditional stretched cheeses. Food Microbiology 52:31–41. https://doi.org/10.1016/j.fm.2015.06.008

Schönwälder A, Kehr R, Wulf A, Smalla K (2002) Wooden boards affecting the survival of bacteria? Holz als Roh- und Werkstoff 60:249–257. https://doi.org/10.1007/s00107-002-0300-6

Schreiner L, Bauer P, Buettner A (2018) Resolving the smell of wood - identification of odour-active compounds in Scots pine (Pinus sylvestris L.). Scientific Reports 8:8294. https://doi.org/10.1038/s41598-018-26626-8

Schubert M, Stührk C, Fuhr MJ, Schwarze FWMR (2014) Imaging hyphal growth of Physisporinus vitreus in Norway spruce wood by means of confocal laser scanning microscopy (CLSM). Holzforschung 68:727–730. https://doi.org/10.1515/hf-2013-0183

Sefidgar SAA, Taghizadeh Armaki M, Pournajaf A, et al (2015) Evaluation of antimicrobial activity of alcoholic and aqueous extracts from common hop (*Humulus lupulus*) and Oak (*Quercus castaneifolia*). Arak Medical University Journal 17:39–46

Sejdiu M, Marku P, Sejdiu R (2020) The Use of Sawn Timber for Barrel Production from Oak Wood (Quercus Petraea L.). European Journal of Engineering and Formal Sciences 4:17–23

Settanni L, Di Grigoli A, Tornambé G, et al (2012) Persistence of wild *Streptococcus thermophilus* strains on wooden vat and during the manufacture of a traditional Caciocavallo type cheese. International Journal of Food Microbiology 155:73–81. https://doi.org/10.1016/j.ijfoodmicro.2012.01.022

Shahi F, Hashemi A, Abdolmaleki K, et al (2017) Antibacterial effects of *Quercus brantii* fruits and *Stachys lavandulifolia* methanol extracts on imipenemase-type metallo-beta lactamase-producing *Pseudomonas aeruginosa*. Research Journal of Pharmacognosy 4:59–66

Shi Y, Sun R, An D, et al (2017) Mathematical quantification of inactivation of *Vibrio parahaemolyticus* on two types of surface soiled with different substrates. Food Control 74:1–8. https://doi.org/10.1016/j.foodcont.2016.11.025

Shimoda T, Yano R, Nakamura S, et al (2015) ATP bioluminescence values are significantly different depending upon material surface properties of the sampling location in hospitals. BMC Research Notes 8:807. https://doi.org/10.1186/s13104-015-1757-9

Shu H, Jönsson BA, Larsson M, et al (2014) PVC flooring at home and development of asthma among young children in Sweden, a 10-year follow-up. Indoor Air 24:227–235. https://doi.org/10.1111/ina.12074

Silhavy TJ, Kahne D, Walker S (2010) The bacterial cell envelope. Cold Spring Harbor Perspectives in Biology 2:414. https://doi.org/10.1101/cshperspect.a000414

Silva F, Lourenço O, Pina-Vaz C, et al (2010) The use of DRAQ5 to monitor intracellular DNA in *Escherichia coli* by Flow Cytometry. J Fluoresc 20:907–914. https://doi.org/10.1007/s10895-010-0636-y

Silva NCC, Fernandes Júnior A (2010) Biological properties of medicinal plants: a review of their antimicrobial activity. Journal of Venomous Animals and Toxins including Tropical Diseases 16:402–413. https://doi.org/10.1590/S1678-91992010000300006

Simpson WT (1999) Drying and control of moisture content and dimensional changes. In: Wood handbook: wood as an engineering material. USDA Forest Service, Forest Products Laboratory, Madison, WI, p 12.1-12.20

Smailagić A, Ristivojević P, Dimkić I, et al (2020) Radical scavenging and antimicrobial properties of polyphenol rich waste wood extracts. Foods 9:319. https://doi.org/10.3390/foods9030319

Smajlović SK, Kukec A, Dovjak M (2019) Association between sick building syndrome and indoor environmental quality in slovenian hospitals: A cross-sectional study. International Journal of Environmental Research and Public Health 16:17. https://doi.org/10.3390/ijerph16173224

Soares VM, Pereira JG, Viana C, et al (2012) Transfer of *Salmonella* Enteritidis to four types of surfaces after cleaning procedures and cross-contamination to tomatoes. Food Microbiology 30:453–456. https://doi.org/10.1016/j.fm.2011.12.028

Söhretoglu D, Ekizo¤lu M, K›l›ç E, Sakar MK (2007) Antibacterial and antifungal ctivities of some *Quercus* species growing in Turkey. Fabad Journal of Pharmaceutical Sciences 32:127–130

Song SS, Fei B (2016) The psychological effects of different types of housing environment under different weather conditions. Wood Research 61:105–120

Song SS, Fei B, Wang XH, et al (2017) Effects of different types of housing environments on the physical index and physiological index. Wood Research 62:515–516

Song SS, Zhao GJ (2012) Expression of physiological sensation of anatomical patterns in wood: An event-related brain potential study. BioResources 7:5593–5610. https://doi.org/10.15376/biores.7.4.5593-5610

Soumya E, Saad IK, Abdellah H, Hassan L (2013) Experimental and theoretical investigations of the adhesion time of Penicillium spores to cedar wood surface. Materials Science and Engineering: C 33:1276–1281. https://doi.org/10.1016/j.msec.2012.12.026

Spampinato C, Leonardi D (2013) Candida infections, causes, targets, and resistance mechanisms: traditional and alternative antifungal agents. Biomed Res Int Article ID 204237: https://doi.org/10.1155/2013/204237

Stein AC, Sortino M, Avancini C, et al (2005) Ethnoveterinary medicine in the search for antimicrobial agents: Antifungal activity of some species of Pterocaulon (Asteraceae). Journal of Ethnopharmacology 99:211–214. https://doi.org/10.1016/j.jep.2005.02.011

Stienen T, Schmidt O, Huckfeldt T (2014) Wood decay by indoor basidiomycetes at different moisture and temperature. Holzforschung 68:9–15. https://doi.org/10.1515/hf-2013-0065

Strobel K, Nyrud AQ, Bysheim K (2017) Interior wood use: linking user perceptions to physical properties. Scandinavian Journal of Forest Research 32:798–806. https://doi.org/10.1080/02827581.2017.1287299

Su F-C, Friesen MC, Stefaniak AB, et al (2018) Exposures to Volatile Organic Compounds among Healthcare Workers: Modeling the Effects of Cleaning Tasks and Product Use. Ann Work Expo Health 62:852–870. https://doi.org/10.1093/annweh/wxy055

Subhashini S, Begum SM, Rajesh G (2016) Antimicrobial characterisation combining spectrophotometric analysis of different oak species. International Journal of Herbal Medicine 4:32–35

Suetens C, Latour K, Kärki T, et al (2018) Prevalence of healthcare-associated infections, estimated incidence and composite antimicrobial resistance index in acute care hospitals and long-term care facilities: results from two European point prevalence surveys, 2016 to 2017. Eurosurveillance 23:1800516. https://doi.org/10.2807/1560-7917.ES.2018.23.46.1800516

Suleimana M, McGaw L, Naidoo V, Eloff J (2009) Detection of antimicrobial compounds by Bioautography of different extracts of leaves of selected South African tree species. Afr J Tradit Complement Altern Med 7:64–78

Suleyman G, Alangaden G, Bardossy AC (2018) The role of environmental contamination in the transmission of nosocomial pathogens and Healthcare-Associated Infections. Curr Infect Dis Rep 20:12. https://doi.org/10.1007/s11908-018-0620-2

Syofuna A, Banana AY, Nakabonge G (2012) Efficiency of natural wood extractives as wood preservatives against termite attack. Maderas Ciencia y tecnología 14:155–163. https://doi.org/10.4067/S0718-221X2012000200003

Talento AF, Fitzgerald M, Redington B, et al (2019) Prevention of healthcare-associated invasive aspergillosis during hospital construction/renovation works. Journal of Hospital Infection 103:1–12. https://doi.org/10.1016/j.jhin.2018.12.020

Tang JYH, Nishibuchi M, Nakaguchi Y, et al (2011) Transfer of *Campylobacter jejuni* from raw to cooked chicken via wood and plastic cutting boards. Letters in Applied Microbiology 52:581–588. https://doi.org/10.1111/j.1472-765X.2011.03039.x

Tayel AA, El-Sedfy MA, Ibrahim AI, Moussa SH (2018) Application of *Quercus infectoria* extract as a natural antimicrobial agent for chicken egg decontamination. Revista Argentina de Microbiología 50:391–397. https://doi.org/10.1016/j.ram.2017.12.003

Taylor J, Davies M, Canales M, Lai K man (2013) The persistence of flood-borne pathogens on building surfaces under drying conditions. International Journal of Hygiene and Environmental Health 216:91–99. https://doi.org/10.1016/j.ijheh.2012.03.010

Teodoro GR, Ellepola K, Seneviratne CJ, Koga-Ito CY (2015) Potential Use of Phenolic Acids as Anti-Candida Agents: A Review. Front Microbiol 6:1420. https://doi.org/10.3389/fmicb.2015.01420

Tešević V, Milosavljević S, Vajs V, et al (2009) Chemical composition and antifungal activity of the essential oil of Douglas fir (*Pseudosuga menziesii* mirb. Franco) from Serbia. Journal of the Serbian Chemical Society 74:1035–1040. https://doi.org/10.2298/JSC0910035T

Thormar H, Hilmarsson H (2010) Killing of Campylobacter on contaminated plastic and wooden cutting boards by glycerol monocaprate (monocaprin). Letters in Applied Microbiology 51:319–324. https://doi.org/10.1111/j.1472-765X.2010.02898.x

Thybring E, Kymäläinen M, Rautkari L (2018) Moisture in modified wood and its relevance for fungal decay. iForest - Biogeosciences and Forestry 11:418–422. https://doi.org/10.3832/ifor2406-011

Tiwari A, Patnayak DP, Chander Y, et al (2006) Survival of two avian respiratory viruses on porous and nonporous surfaces. Avian Diseases 50:284–287. https://doi.org/10.1637/7453-101205R.1

Tomczak K, Tomczak A, Jelonek T (2020) Effect of natural drying methods on moisture content and mass change of Scots Pine roundwood. Forests 11:668. https://doi.org/10.3390/f11060668

Tomičić R, Tomičić Z, Raspor P (2017) Adhesion of Candida spp. and Pichia spp. to wooden surfaces. Food Technology and Biotechnology 55:138–142. https://doi.org/10.17113/ftb.55.01.17.4514

Totaforti S (2018) Applying the benefits of biophilic theory to hospital design. City, Territory and Architecture 5:Article 1. https://doi.org/10.1186/s40410-018-0077-5

Touati R, Santos SAO, Rocha SM, et al (2015) The potential of cork from Quercus suber L. grown in Algeria as a source of bioactive lipophilic and phenolic compounds. Industrial Crops and Products 76:936–945. https://doi.org/10.1016/j.indcrop.2015.07.074

Tsunetsugu Y, Miyazaki Y, Sato H (2002) The visual effects of wooden interiors in actual-size living rooms on the Autonomic Nervous activities. Journal of Physiological Anthropology and Applied Human Science 21:297–300. https://doi.org/10.2114/jpa.21.297

Tsunetsugu Y, Miyazaki Y, Sato H (2007) Physiological effects in humans induced by the visual stimulation of room interiors with different wood quantities. J Wood Sci 53:11–16. https://doi.org/10.1007/s10086-006-0812-5

Ulrich RS (1984) View through a window may influence recovery from surgery. Science 224:420–421. https://doi.org/10.1126/science.6143402

University of Cambridge (2020) DoITPoMS - TLP Library The structure and mechanical behaviour of wood - The structure of wood (II). In: DoITPoMS. https://www.doitpoms.ac.uk/tlplib/wood/structure_wood_pt2.php. Accessed 14 Sep 2020

Vafaeenasab MR, Morowatisharifabad MA, Taghi Ghaneian M, et al (2015) Assessment of sick building syndrome and its associating factors among nurses in the educational hospitals of Shahid Sadoughi University of Medical Sciences, Yazd, Iran. Global Journal of Health Science 7:247–253. https://doi.org/10.5539/gjhs.v7n2p247

Vainio-Kaila T, Hänninen T, Kyyhkynen A, et al (2017a) Effect of volatile organic compounds from *Pinus sylvestris* and *Picea abies* on *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus pneumoniae* and *Salmonella enterica* serovar Typhimurium. Holzforschung 71:905–912. https://doi.org/10.1515/hf-2017-0007

Vainio-Kaila T, Kyyhkynen A, Rautkari L, Siitonen A (2015) Antibacterial effects of extracts of *Pinus sylvestris* and *Picea abies* against *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, and *Streptococcus pneumoniae*. BioResources 10:7763–7771. https://doi.org/10.15376/biores.10.4.7763-7771

Vainio-Kaila T, Kyyhkynen A, Viitaniemi P, Siitonen A (2011) Pine heartwood and glass surfaces: easy method to test the fate of bacterial contamination. European Journal of Wood and Wood Products 69:391–395. https://doi.org/10.1007/s00107-010-0453-7

Vainio-Kaila T, Rautkari L, Nordström K, et al (2013) Effect of extractives and thermal modification on antibacterial properties of Scots pine and Norway spruce. International Wood Products Journal 4:248–252. https://doi.org/10.1179/2042645313Y.0000000038

Vainio-Kaila T, Zhang X, Hänninen T, et al (2017b) Antibacterial effects of wood structural components and extractives from *Pinus sylvestris* and *Picea abies* on Methicillin-Resistant *Staphylococcus aureus* and *Escherichia coli* O157:H7. BioResources 12:7601–7614. https://doi.org/10.15376/biores.12.4.7601-7614

Valette N, Perrot T, Sormani R, et al (2017) Antifungal activities of wood extractives. Fungal Biology Reviews 31:113–123. https://doi.org/10.1016/j.fbr.2017.01.002

Valgas C, Souza SM de, Smânia EFA, Smânia Jr. A (2007) Screening methods to determine antibacterial activity of natural products. Brazilian Journal of Microbiology 38:369–380. https://doi.org/10.1590/S1517-83822007000200034

Valimaa A-L, Honkalampi-Hämäläinen U, Pietarinen S, et al (2007) Antimicrobial and cytotoxic knotwood extracts and related pure compounds and their effects on food-associated microorganisms. International Journal of Food Microbiology 115:235–243. https://doi.org/10.1016/j.ijfoodmicro.2006.10.031

Van den Bulcke J, Van Acker J, Saveyn H, Stevens M (2007) Modelling film formation and degradation of semi-transparent exterior wood coatings. Progress in Organic Coatings 58:1–12. https://doi.org/10.1016/j.porgcoat.2006.10.003

van Doremalen N, Bushmaker T, Morris D, et al (2020) Aerosol and surface stability of HCoV-19 (SARS-CoV-2) compared to SARS-CoV-1. New England Journal of Medicine 382:1564–1567. https://doi.org/DOI: 10.1056/NEJMc2004973

van Zyl WF, Deane SM, Dicks LMT (2015) Use of the mCherry Fluorescent Protein To Study Intestinal Colonization by Enterococcus mundtii ST4SA and Lactobacillus plantarum 423 in Mice. Applied and Environmental Microbiology 81:5993–6002. https://doi.org/10.1128/AEM.01247-15

Vandini A, Temmerman R, Frabetti A, et al (2014) Hard surface biocontrol in hospitals using microbial-based cleaning products. PLOS ONE 9:e108598. https://doi.org/10.1371/journal.pone.0108598

Vek V, Oven P, Humar M (2013) Phenolic extractives of wound-associated wood of beech and their fungicidal effect. International Biodeterioration and Biodegradation 77:91–97. https://doi.org/10.1016/j.ibiod.2012.10.013

Wadum J, Currie C, Streeton N, et al (eds) (1961) Wooden Supports. Archetype Pubications and CATS, London

Walker WS, Gorelik SR, Baccini A, et al (2020) The role of forest conversion, degradation, and disturbance in the carbon dynamics of Amazon indigenous territories and protected areas. Proceedings of the National Academy of Sciences 117:3015–3025. https://doi.org/10.1073/pnas.1913321117

Wang J, Engvall K, Smedje G, et al (2017) Current wheeze, asthma, respiratory infections, and rhinitis among adults in relation to inspection data and indoor measurements in single-family houses in Sweden—The BETSI study. Indoor Air 27:725–736. https://doi.org/10.1111/ina.12363

Wang Y, Zhang Y, Li X, et al (2015) Exploring the effects of different types of surfactants on Zebrafish embryos and larvae. Scientific Reports 5:10107. https://doi.org/10.1038/srep10107

Watchman M, Potvin A, Demers CMH (2016) Wood and comfort: a comparative case study of two multifunctional rooms. BioResources 12:168-182–182. https://doi.org/10.15376/biores.12.1.168-182

Watchman M, Potvin A, Demers CMH (2017) A post-occupancy evaluation of the influence of wood on environmental comfort. BioResources 12:8704-8724–8724. https://doi.org/10.15376/biores.12.4.8704-8724

Welker C, Faiola N, Davis S, et al (1997) Bacterial retention and cleanability of plastic and wood cutting boards with commercial food service maintenance practices. Journal of Food Protection 60:407–413. https://doi.org/doi: 10.4315/0362-028X-60.4.407.

Wendt C, Dietze B, Dietz E, Rüden H (1997) Survival of *Acinetobacter baumannii* on dry surfaces. Journal of Clinical Microbiology 35:1394–1397

WHO (2011) Report on the burden of endemic health care-associated infection worldwide

Williams AP, Avery LM, Killham K, Jones DL (2005) Persistence of *Escherichia coli* O157 on farm surfaces under different environmental conditions. Journal of Applied Microbiology 98:1075–1083. https://doi.org/10.1111/j.1365-2672.2004.02530.x

Xi L, Qin D, An X, Wang G (2013) Resistance of natural bamboo fiber to microorganisms and factors that may affect such resistance. BioResources 8:6501–6509. https://doi.org/10.15376/biores.8.4.6501-6509

Xiao Y, Wakeling RN, Singh AP (2000) Use of confocal microscopy in examining fungi and bacteria in wood. Biofouling 15:231–239. https://doi.org/10.1080/08927010009386313

Xue F, Gou Z, Lau SS-Y, et al (2019) From biophilic design to biophilic urbanism: Stakeholders' perspectives. Journal of Cleaner Production 211:1444–1452. https://doi.org/10.1016/j.jclepro.2018.11.277

Yada Y, Sadachi H, Nagashima Y, Suzuki T (2007) Overseas survey of the effect of Cedrol on the Autonomic Nervous System in three countries. J Physiol Anthropol 26:349–354. https://doi.org/10.2114/jpa2.26.349

Yali S, Jun S, Shen X, Jiankun Q (2018) Effect of panel area-volume ratio on tvoc released from decorative particleboards. Wood and Fiber Science 50:132–142. https://doi.org/10.22382/wfs-2018-015

Ye K, Liu J, Liu M, et al (2018) Effects of two *Weissella viridescens* strains on *Listeria monocytogenes* growth at different initial inoculum proportions. CyTA - Journal of Food 16:299–305. https://doi.org/10.1080/19476337.2017.1401667

Yoon H, Lee J-Y, Suk H-J, et al (2012) Modeling to predict growth/no growth boundaries and kinetic behavior of Salmonella on cutting board surfaces. Journal of Food Protection 75:2116–2121. https://doi.org/10.4315/0362-028X.JFP-12-094

Yoshida M, Nakamura M, Kikuchi Y (2016) Effect of observation of wooden wall panels on eye fixation related potentials, eye movement and sensory evaluation. Mokuzai Gakkaishi 62:275–283. https://doi.org/10.2488/jwrs.62.275

Yu H, Liu Y, Xiao X (2006) Preliminary study on the characteristics of tactility of wood by physiological index HRV. Frontiers of Forestry in China 1:361–366. https://doi.org/10.1007/s11461-006-0009-5

Zachariah SM, Kumar NM, Darsana K, et al (2014) Phytochemical screening, formulation and evaluation of dried galls of *Quercus Infectoria* Oliv. Int J Pharm Sci Revi Res 26:125–130

Zanetti M, Mothe F, Merlin A, et al (2003) Conséquences du vieillissement du bois de chêne sessile (Quercus petraea (Matt.) Liebl.) sur sa perception esthétique par les utilisateurs. Ann For Sci 60:61–68. https://doi.org/10.1051/forest:2002074

Zangerl P, Matlschweiger C, Dillinger K, Eliskases-Lechner F (2010) Survival of *Listeria monocytogenes* after cleaning and sanitation of wooden shelves used for cheese ripening. European Journal of Wood and Wood Products 68:415–419. https://doi.org/10.1007/s00107-009-0381-6

Zare A, Moshfeghy Z, Zarshenas MM, et al (2019) Quercus brantii Lindl. Vaginal cream versus placebo on Bacterial Vaginosis: A randomized clinical trial. Journal of Herbal Medicine 16:100247. https://doi.org/10.1016/j.hermed.2018.11.003

Zarpellon MN, Gales AC, Sasaki AL, et al (2015) Survival of vancomycin-intermediate *Staphylococcus aureus* on hospital surfaces. Journal of Hospital Infection 90:347–350. https://doi.org/10.1016/j.jhin.2015.04.005

Zhang J, Sun C, Liu W, et al (2018) Associations of household renovation materials and periods with childhood asthma, in China: A retrospective cohort study. Environment International 113:240–248. https://doi.org/10.1016/j.envint.2018.02.001

Zhang X, Lian Z, Ding Q (2016) Investigation variance in human psychological responses to wooden indoor environments. Building and Environment 109:58–67. https://doi.org/10.1016/j.buildenv.2016.09.014

Zhang X, Lian Z, Wu Y (2017) Human physiological responses to wooden indoor environment. Physiology & Behavior 174:27–34. https://doi.org/10.1016/j.physbeh.2017.02.043

Zimmermann T, Rietdorf J, Pepperkok R (2003) Spectral imaging and its applications in live cell microscopy. FEBS Letters 546:87–92. https://doi.org/10.1016/S0014-5793(03)00521-0

Zylkowski S, Frihart C (2018) Volatile organic compounds emissions from North American engineered wood products. Forestry Research and Engineering: International Journal 2:204–207. https://doi.org/10.15406/freij.2018.02.00049

Annexes

Annex 1: Antibacterial activity of wood

In this annex the additional or supporting data of antibacterial properties of wood.

ALG	BRN	DLG	IRN
	DLN	ELN	GLN
ARG	ELG	FRN	
DRN	HLN	ERN	GRN

ILG		JLN	GLG
BLG	ARN	GRG	ILN
IRG	ALN	ERG	
DRG	CRG	JLG	HRN

BLN	CLN	FLN	BRG
	FLG	HLG	HRG
CRN	FRG	CLG	

Figure A1.1: Sample arrangement on square (12x12 cm) Mueller-Hinton agar plates (screening experiment: sections 3.2.1 and 4.1). The three alphabets/digit in each block represents: 1 = species of tree (A-J); 2 = cutting plane (L: longitudinal, R: transversal); 3 = sterilization (G: gamma-irradiated, N: non-sterilized)

Annex 1.1: Antibacterial activity of oak wood on different days

The diffusion-based antimicrobial activity is tested via antiboisgram. In this method, the diffused chemicals are emitted from the solid wood discs, hence the activity of the remaining sample would decrease. The experiment was conducted to follow the decrease in antimicrobial activity during successive testing. Each time when the zone of inhibition reading was taken, the wood samples were transferred to a new bacterium inoculated petri agar plate and incubated in similar conditions of antiboisgram. Around 10 wood species were tested in this protocol, and four of them showed positive results, and only oak showed activity on the third day and successively (Figure A 1.2).

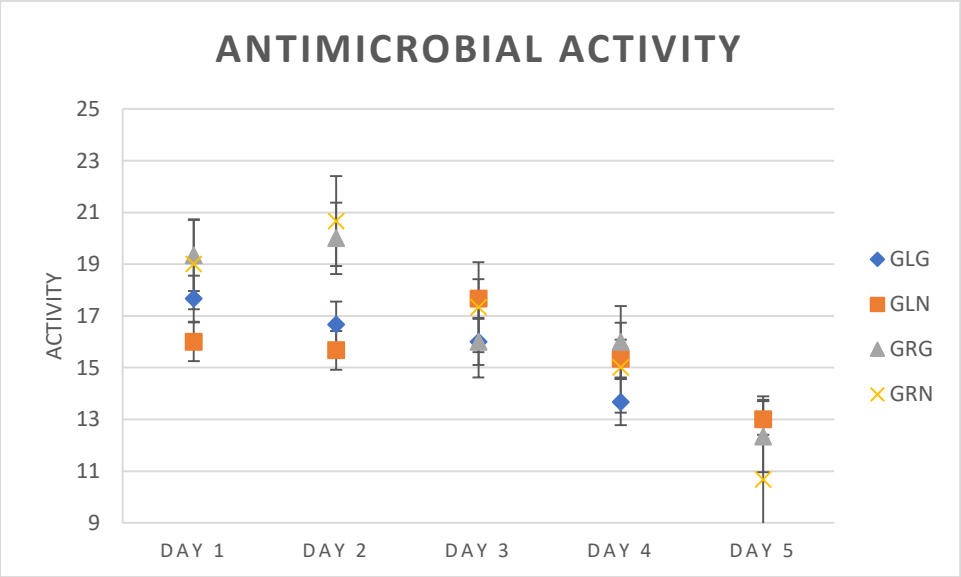


Figure A1.2: Antimicrobial activity of oak wood against Staphylococcus aureus at different days. GLG: longitudinal- gamma-sterilized; GLN: longitudinal- non-sterilized; GRG: transversal- gamma-sterilized; GRN: transversal- non-sterilized. Activity is mentioned as zone of inhibition including the diameter of disc (9 mm) mean ±SD (n=3)

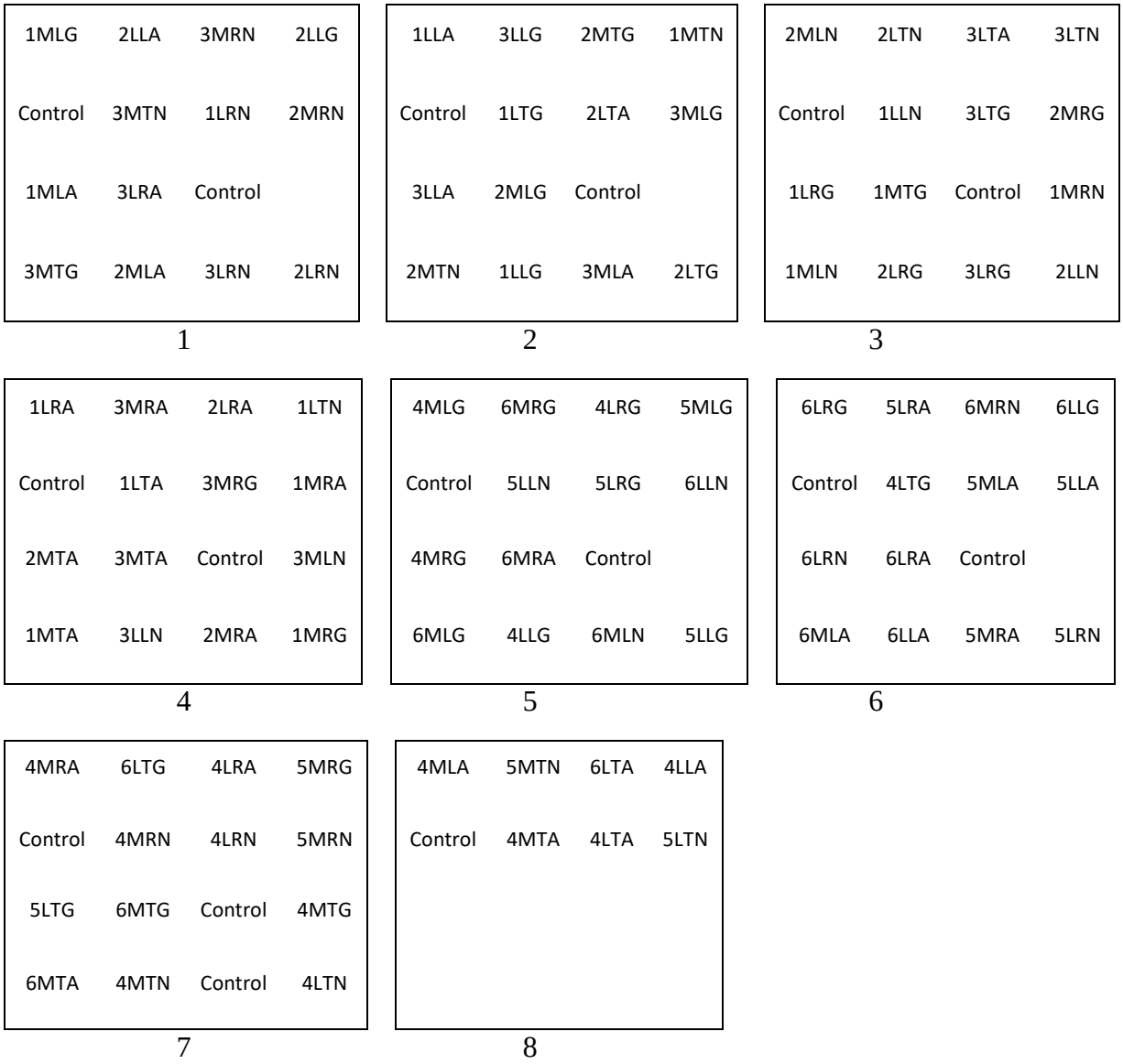


Figure A1.3: Test sample arrangement of antiboisgram on square (12x12 cm) Mueller-Hinton agar plates (effect of wood variables: sections 3.2.2 and 4.2). The four alphabets/digit in each block represents: 1 = tree number (1-6); 2 = preparation (L: laser made M: manually cut); 3 = cutting plane (L: radial (LR), R: tangential (LT), T: transversal (RT)); 4 = sterilizations (G: gamma-irradiated, A: autoclaved, N: non-sterilized)

Table A1.1: Effect sterilization and cutting planes on the antimicrobial activity of six trees against *Staphylococcus aureus*.

Variables	Trees					
	1	2	3	4	5	6
LA	13.9±1.15	13.78±1.7	14.89±1	13.3±0.6	12.8±1.2	12.9±0.6
LG	17.22±1.0	16.9±1.15	19.78±1.5	16.67±0.6	18.7±2.5	17.7±1.5
LN	17.33±1.7	17.11±1.0	18.8±1.5	19.1±0.6	18.6±2.1	16.6±1.7
MA	11.78±0.6	13.56±1.2	15.3±0	12.7±0.6	12.2±0.6	12.6±0.6
MG	17±1.0	18.44±0	18.6±2.1	16.4±0.0	18.3±2	18.0±2.7
MN	16.33±2.3	17.1±1.2	17.33±1	14.5±0.0	17.6±1.2	16.6±0.6
lLR	14.6±1.0	12.4±1.20	16.2±1.5	14.7±0.6	16±2.5	13.7±1.5
lLT	16.4±2.6	15.78±0.0	16.1±0.0	15.56±1.2	14.89±0	14.67±2
lRT	17.4±2.5	19.5±0.60	21.2±1.7	18.8±1.5	21.67±0.6	20.3±1.2
mLR	14.1±1.0	13.11±0.0	15.4±0.1	11.89±0	13.33±2	14.2±2.7
mLT	15.78±0	16.0±1.50	16.2±1.15	15.3±2.1	15.67±0	14.2±2.1
mRT	15.2±2.0	20.0±2.10	21.67±2.1	22.0±2.0	19.1±1.7	20.7±0.6

Values are in mean ± standard deviation (n=3) of zone of inhibition (mm) including the diameter of wooden discs (9.95±0.1 mm); LA = Laser cut autoclaved samples; LG = Laser-cut gamma-irradiated samples; LN = Laser-cut non-sterilized samples; MA = Manual cut autoclaved samples; MG = Manual cut gamma-irradiated samples; MN = Manual cut non-sterilized samples; lLR = Laser cut radial plane samples; lLT = Laser cut tangential plane samples; lRT = Laser cut transversal plane samples; mLR = Manual cut radial plane samples; mLT = Manual cut tangential plane samples; mRT = Manual cut transversal plane samples1 - 6 number of trees

Annex 1.2: Effect sterilization nd cutting planes on the antimicrobial activity of six trees against *Acinetobacter baumannii.*

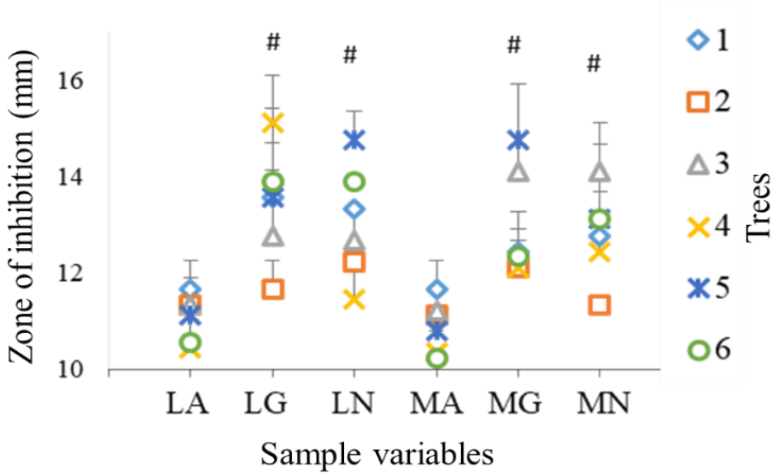


Figure A1.4: Effect sterilization on the antimicrobial activity of different trees against *Acinetobacter baumannii*. Y axis is the mean ± standard deviation (n=3) of zone of inhibition (mm) including the diameter of wooden discs (10 mm); LA = Laser cut autoclaved; LG = Laser cut gamma irradiated; LN = Laser cut non-sterilized; MA = Manual cut autoclaved; MG = Manual cut gamma irradiated; MN = Manual cut non-sterilized; 1 - 6 number of trees; # $p < 0.1$.

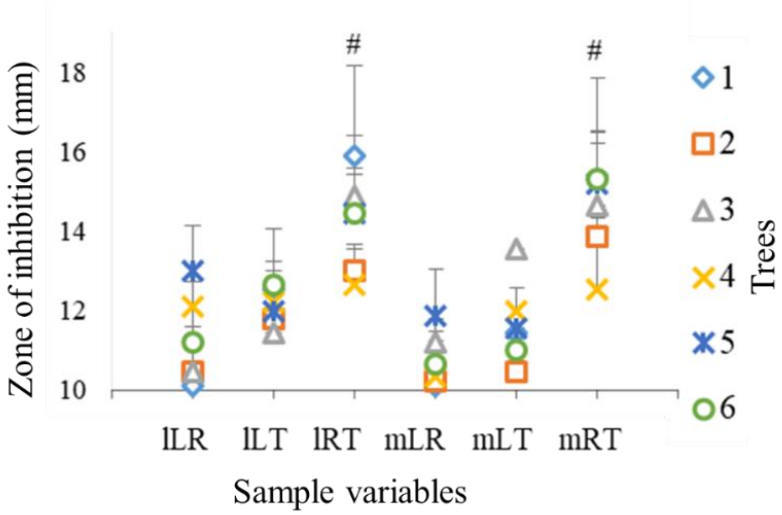


Figure A1.5: Effect cutting planes on the antimicrobial activity of six trees against *A. baumannii*. Y axis is the value of zone of inhibition including the diameter of wooden discs (9.95±0.1 mm). lLR = Laser cut radial plane samples; lLT = Laser cut tangential plane samples; lRT = Laser cut transversal plane samples; mLR = Manual cut radial plane samples; mLT = Manual cut tangential plane samples; mRT = Manual cut transversal plane samples; 1 - 6 number of trees; # $p < 0.1$.

Table A1.2: Difference among trees when tested against 10 isolates of 5 bacteria

Bacteria	Activity	p-value	
	(mean ±SD)	Trees	Isolates
Acinetobacter spp.	15.25±2.85	0.71	<0.001
E. coli	N/A	N/A	N/A
Enterococci spp.	11.72±1.32	0.27*	<0.051
P. aeruginosa	18.90±1.05	0.57	<0.001
S. aureus	20.26±5.93	0.08	<0.001

*The p-value was taken excluding the activity of Tree 5, otherwise it was <0.001. N/A data not available due to lack of antimicrobial activity

Annex 1.3: Influence of data collected by two independent readers on the observed antimicrobial response of wood against bacteria

According to the recommendation of antimicrobial sensitivity test reading, two independent readers collected data for all the experiments. To identify the difference if the two readers got different results, the antimicrobial activity of six trees of oak against *E. faecalis* and *P. aeruginosa,* were compared for identifying the effect of the personal error on data validity. Table A1.3 shows that there was no significant difference ($p<0.05$) of the values (mean±SD) measured by two different readers (13.58±2.3 vs. 12.6±2.3).

Table A1.3: t-Test: Paired two sample for means

	Tanveer	*Helene*
Mean	13,58163	12,59913
Variance	5,437139	5,501832
Observations	98	98
Pearson Correlation	0,970024	
Hypothesized Mean Difference	1	
df	97	
t Stat	-0,30232	
P(T<=t) one-tail	0,381526	
t Critical one-tail	1,660715	
P(T<=t) two-tail	0,763052	
t Critical two-tail	1,984723	

Annex 1.4: The results of variability of antibiogram method

All the antibiogram results were conducted in triplicates, however, there was high variability sometimes reaching to a standard deviation of ±3. Therefore, a complementary experiment was performed to test the variability of this method by increasing the number of replicates to ten. In this experiment, the mass of discs was made uniform by rubbing the non-contact surface *via* sandpaper. The antibiogram results are presented in the following table. The variations of activity with ten samples were very low with a maximum standard deviation of 1.93. Thus it can be concluded that the antimicrobial activity tested by antibiogram is reliable when the disc size is uniform.

Meanwhile these results also showed that the samples belonging to three regions showed similar antimicrobial activity.

Table A1.4: The antimicrobial acitivty (mean ± SD) of six sessile oak trees against *A. baumannii* and *S. aureus*

Tree number	***A. baumannii***	***S. aureus***
1	12.45±2.18	29.47±1.53
2	10.75±0.70	26.44±1.86
3	10.95±0.80	30.50±1.02
4	10.50±0.67	30.25±0.90
5	10.55±0.92	25.70±1.93
6	10.45±0.67	25.78±1.93
Mean	10.94	28.02

Table A1.5: Statistical analysis (ANOVA) to identify the difference of activity among different groups based on replicates of experiment and area of origin of the tested trees

ANOVA	**Three locations**					
Source of Variation	*SS*	*df*	*MS*	*F*	*p*	*F crit*
Between Groups	9.5789	2	4.7895	0.6103	0.5469	3.16825
Within Groups	423.79	54	7.848			
Total	433.37	56				

ANOVA	**Ten replicates**					
Source of Variation	*SS*	*df*	*MS*	*F*	*p*	*F crit*
Between Groups	14.8167	9	1.6463	0.1887	0.9944	2.0734
Within Groups	436.167	50	8.7233			
Total	450.983	59				

Annex 1.5: Antibacterial activity of oak (*Quercus petraea*) wood extract against *E. coli*

The extractives were extracted from oak wood discs using ethanol solvent. The obtained solution was loaded on inert filter paper discs (diameter 10 mm) at the rate of 5 mg, 10 mg and 20 mg on each disc. Agar diffusion method was employed to test the antimicrobial activities of extracted material (Munir et al. 2020a). The Muller Hinton agar plates were inoculated with *E. coli* by swab streaking and then the filter paper discs and extracted and non-extracted wooden discs were directly placed on agar. After incubation at 37°C for 24 hours, the zone of inhibition (clear areas with no bacterial growth) were recorded manually according to the joint recommendations of the Antibiogram Committee of the French Society of Microbiology (CASFM) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST).

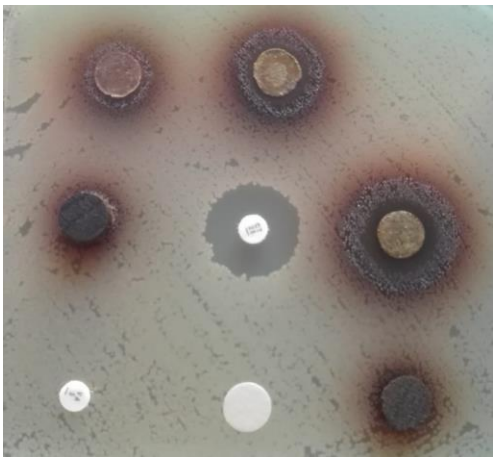


Figure A1. 6: Antibiogram of oak wood extractives tested against *E. coli.*

The extractive content of different oak wood trees was 2.1 ± 0.2 %. The antibiogram results showed that there were no zone of inhibitions around solid wood discs figure A1.6. The extractive loaded filter paper discs had mild activity against *E.coli* at 5mg dose and it increased in a dose-dependent manner reaching a maximum at 20 mg. Interestingly, there were two zones observed around the paper discs, the outer, low diffusion zone, is evidence of bacteriostatic action. Therefore, it can be concluded that wood extractives have bacteriostatic activity against *E.coli* at low doses and bactericidal activity at higher doses.

Annex 1.6: Effect of thermo-hydro-mechanical treatment on the diffusion-based antimicrobial behavior of wood

Thermo-hydro-mechanical treatment is used to increase the bendability of wood material. The effect of such treatments on the natural antimicrobial behavior of wood is unknown. This study investigates the use of a direct diffusion-based method to test the antimicrobial activity of two wood species undergone thermo-hydro-mechanical treatment. The beech (*Fagus sylvatica* L.) and oak wood (*Quercus petraea* (Matt.) Liebl.) were steamed, longitudinally compressed and expanded according to a method described earlier (Báder and Németh 2017, 2018a, b, 2019; Báder et al. 2019). The earlier described method antibiogram was employed to test the antimicrobial properties of different solid samples (Pailhories et al. 2017; Munir et al. 2019a). All these three treated materials with 4th untreated control were cut into 3 mm veneers in transversal (RT) and longitudinal (LT) planes. The round discs (diameter 10 mm) were prepared from these planes *via* a laser machine or a manual punch machine. The antimicrobial activity of wood was tested using a direct diffusion method against *Staphylococcus aureus* and *E. coli*. The zones of inhibition around the wooden discs were manually recorded according to recommendations (CASFM / EUCAST 2019). Only *S. aureus* showed susceptibility to diffused chemicals from wood.

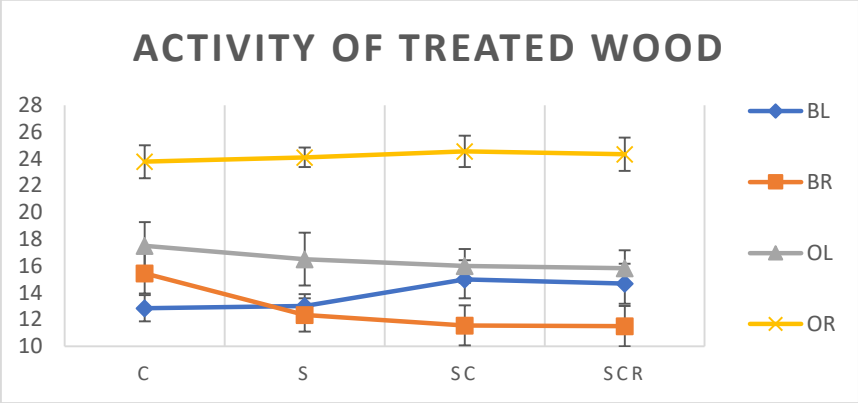


Figure A1. 7: Antimicrobial activity of thermo-hydro-mechanically treated oak and beech wood against *S. aures* tested via antiboisgram. Y axis: Zone of inhibition including the diameter of disc (10 mm); C: control (untreated); S: steamed; SC: steamed compressed; SCR: steamed compressed relaxed; BL: beech longitudinal cut; BR: beech transversal cut; OL: Oak longitudinal cut; OR: Oak transversal cut.

The thermo-hydro-mechanical treatments did not influence the antimicrobial activity of oak wood but the beech samples showed variations of activity under the influence of these treatments. The new samples with better traceability and uniformity were ordered and would be tested in future studies.

Annex 2: Antifungal properties of wood material

This annex includes the complementary data for antifungal studies.

Table A2.1: Statistical analysis results to comparing differences between groups as mean differences (p-values)

	D1	D2	O3	O5
RT vs. LT	3.97 (0.000)*	3.67 (0.000)*	2.42 (0.007)*	1.92 (0.049)*
RT vs. LR	4.57 (0.000)*	4.33 (0.000)*	3.58 (0.000)*	3.00 (0.003)*
LT vs. LR	0.60 (0.752)	0.67 (0.599)	1.17 (0.226)	0.67 (0.332)
Between trees [b]	D1 vs. D2 = 0.09 (0.194)		O3 vs. O5 = 0.04 (0.219)	
Between wood [b]	D vs. O = 2.33 (0.000)*			

* Significant at $p<0.05$

[a] Multiple comparisons obtained via Tuckey's post hoc test used after ANOVA

[b] One tailed t-Test: Paired Two Sample for Means

Annex 3: Microbial survival on wood and other materials

Table A3.1: Average and standard deviation of recovered *K. pseumoniae* CFU on each time point

	Material	day0	day1	day2	day3	day6	day7	day15
oakT	average	376133	40	4	4	4	4	4
	SD	205718.3	0	0	0	0	0	0
oakL	average	217933	40	4	4	4	4	4
	SD	148441.7	0	0	0	0	0	0
aluminum	average	754000	31866	76800	9840	7546	8580	715
	SD	421448.2	24942.2	61477.8	4640.8	5744.5	6342.8	220.9
polycarbonate	average	2544000	10666	18062	6400	2516	485	76
	SD	2727777.6	3771.2	13283.1	4092.2	2986.5	578.2	91.6
Stainless steel	average	1476000	8000	17286.6	5746.6	2910	2406.6	68
	SD	1312699.0	3265.9	9276.4	3262.7	1448.7	209.9	39.5

oakT: transversal-cut sessile oak; oakL: tangential cut of sessile oak.

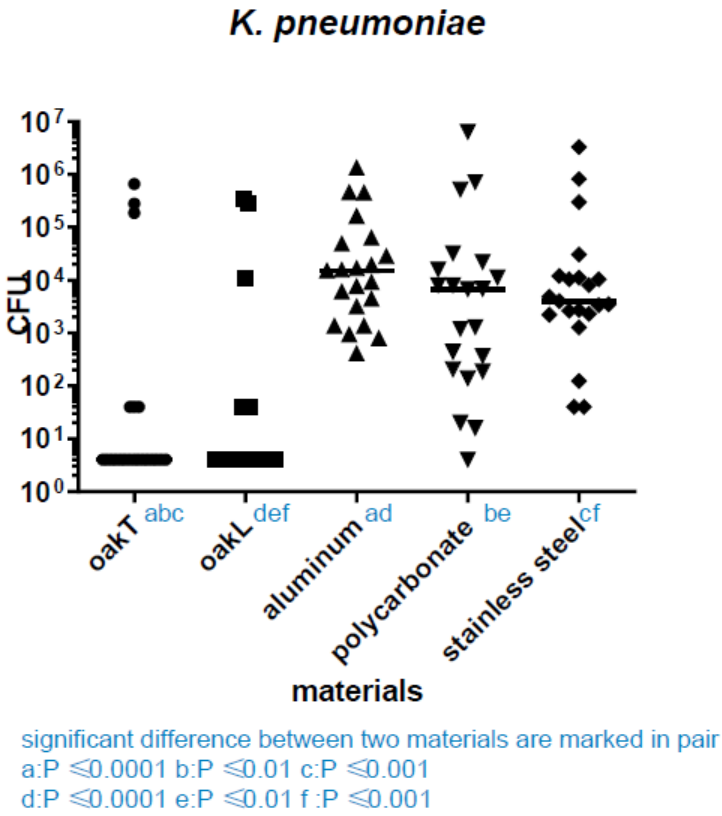


Figure A3. 1: A scatter graph showing the recovered *K. pseumoniae* CFU during the total experimental time from different surfaces. oakT: transversal-cut sessile oak; oakL: tangential cut of sessile oak.

Table A3.2: Average and standard deviation of recovered *A. baumannii* CFU on each time point

		day0	day1	day2	day3	day6	day7	day15
oakT	average	4	4	4	4	4	4	4
	SD	0	0	0	0	0	0	0
oakL	average	16	4	4	4	4	4	4
	SD	16.9	0	0	0	0	0	0
steal	average	3476	84	4	4	4	4	4
	SD	3187.8	39.3	0	0	0	0	0
aluminum	average	52933	1096	344	30	372	4	4
	SD	17907.9	402.2	243.0	25.7	637.3	0	0
polycarbonate	average	165066	153	4	4	4	4	4
	SD	114831.5	119.2	0	0	0	0	0
stainless steel	average	78000	906	60	347	4	4	4
	SD	14589.4	685.5	28.9	396.0	0	0	0

oakT: transversal-cut sessile oak; oakL: tangential cut of sessile oak.

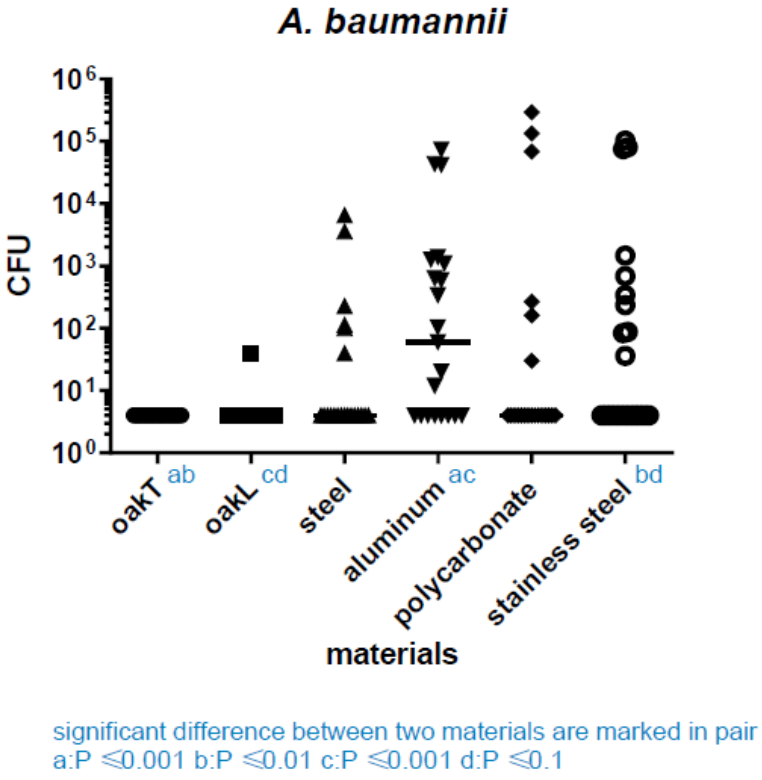


Figure A3. 2: A scatter graph showing the recovered *A. baumannii* CFU during the total experimental time from different surfaces. oakT: transversal-cut sessile oak; oakL: tangential cut of sessile oak.

Table A3.3: Average and standard deviation of recovered *E. faecalis* CFU on each time point

		day0	day1	day2	day3	day6	day7	day15
oakT	average	5	4	4	4	4	4	4
	SD	1.8	0	0	0	0	0	0
oakL	average	4	6	4	4	4	4	4
	SD	0	3.7	0	0	0	0	0
steel	average	75000	20046	2026	5613	867	294	4
	SD	53000.3	14341.0	191.3	6809.9	868.1	293.9	0
aluminum	average	4545333	855333	185800	84466	62200	12186	336
	SD	3373829.2	566830.5	203176.8	50616.5	37770.1	5950.5	222.5
polycarbonate	average	2680000	328000	133333	124326	7773	35251	462
	SD	797997.4	183244.8	160639.49	155771.3	6958.64	49744.0	459.7
stainless steel	average	6532666	657333	58666	39220	10985	6214	259
	SD	4412745.2	308568.0	38883.5	21415.4	15425.4	5983.8	346.5

oakT: transversal-cut sessile oak; oakL: tangential cut of sessile oak.

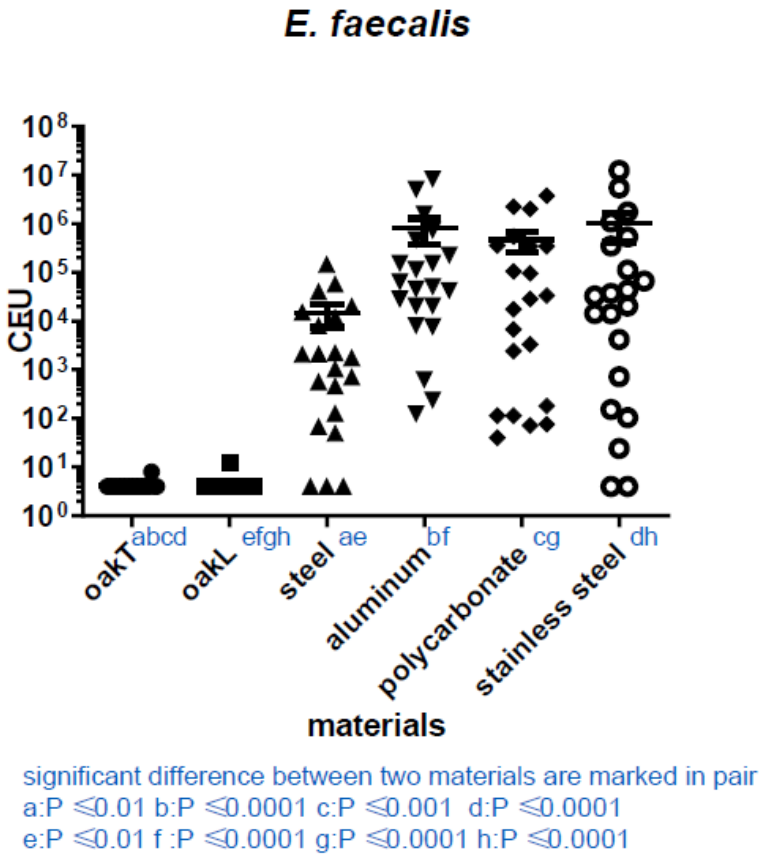


Figure A3. 3: A scatter graph showing the recovered *E. faecalis* CFU during the total experimental time from different surfaces. oakT: transversal-cut sessile oak; oakL: tangential cut of sessile oak.

Table A3.4: Average and standard deviation of recovered *S. aureus* CFU on each time point

		day0	day1	day2	day3	day6	day7	day15
oakT	average	2520	8	54	38	5	4	4
	SD	1405.5	15.0	35.8	29.4	1.8	0	0
oakL	average	2893	232	13	4	4	4	4
	SD	1439.0	214.9	6.7	0	0	0	0
steal	average	13866	2311	2986	1026	38	230	44
	SD	6025.1	2106.0	1904.5	974.9	13.1	195.9	9.8
aluminum	average	3393333	772000	674333	579333	48600	50900	7610
	SD	1224236.7	451684.2	348440.6	357182.8	15295.3	46344.5	10458.1
polycarbonate	average	7926666	3506666	715000	266666	9893	13542	24
	SD	5145362.5	187853.3	447138.3	196783.0	7031.8	9944.0	28.2
stainless steel	average	4913333	852000	743000	458666	558420	73853	1910
	SD	980657.3	491872.6	192843.6	247576.6	470100.0	51260.5	736.1

oakT: transversal-cut sessile oak; oakL: tangential cut of sessile oak.

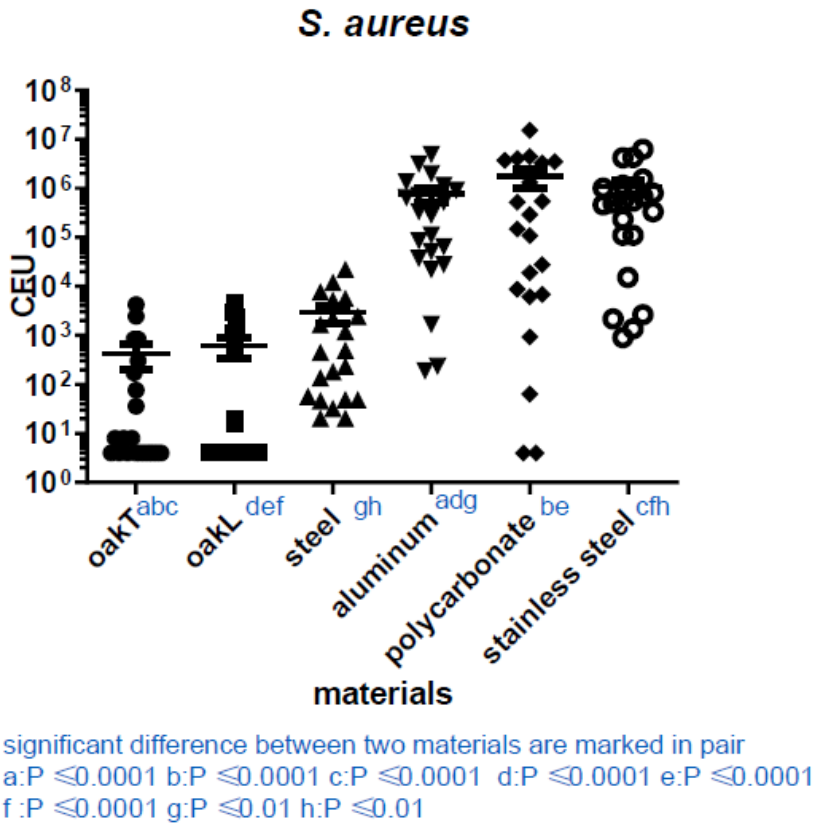


Figure A3. 4: A scatter graph showing the recovered *S. aureus* CFU during the total experimental time from different surfaces. oakT: transversal-cut sessile oak; oakL: tangential cut of sessile oak.

Annex 4: Microbial distribution on and inside wood

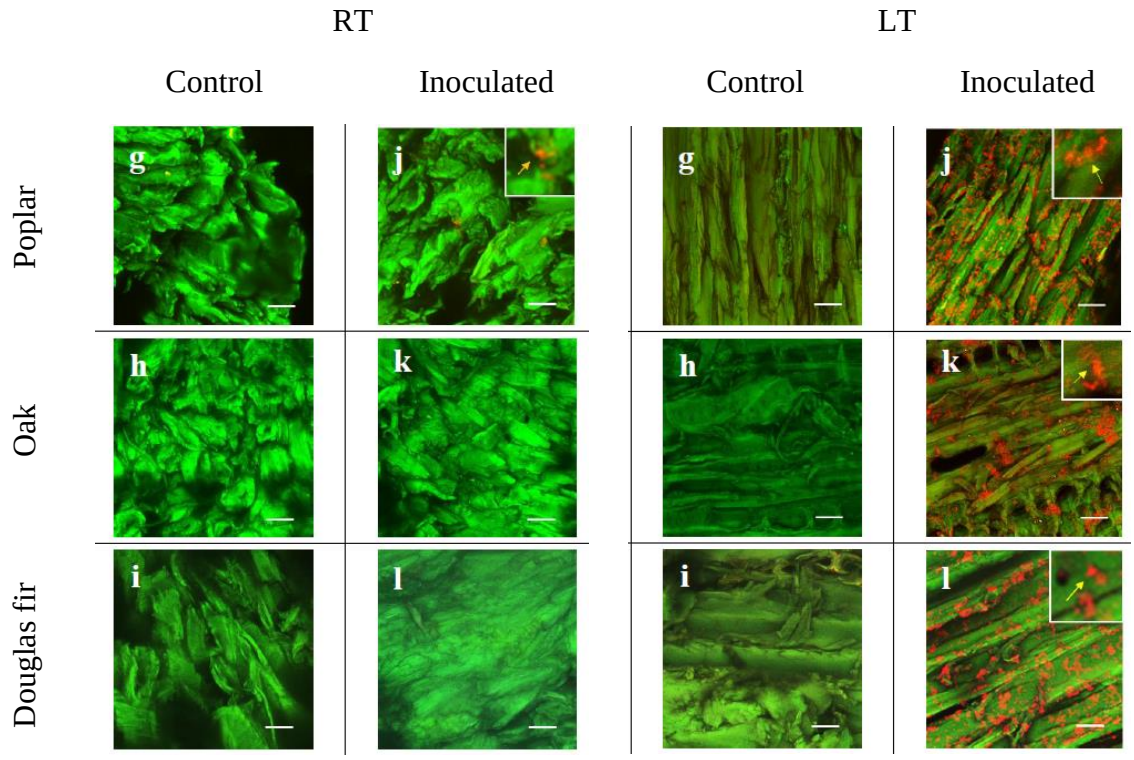


Figure A4. 1: mCherry *S. aureus* distribution on the wood surface of RT and LT sections at 24 h after inoculation. Bar = 20 μm**.**

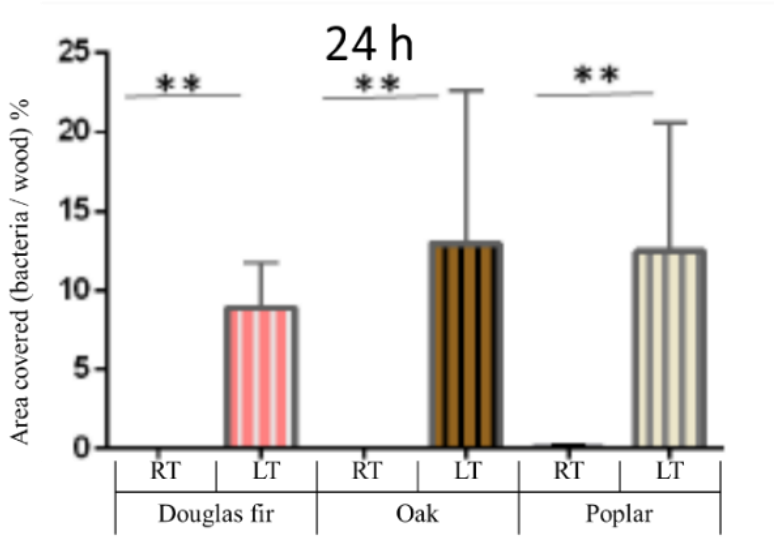


Figure A4. 2: The area covered by red fluorescence (mCherry *S. aureus*) compared to the area recovered by green fluorescence (wood) at 24 h after bacteria inoculation, for the transverse (RT) and tangential (LT) section. Mann-Whitney Statistic test, **p<0.001.

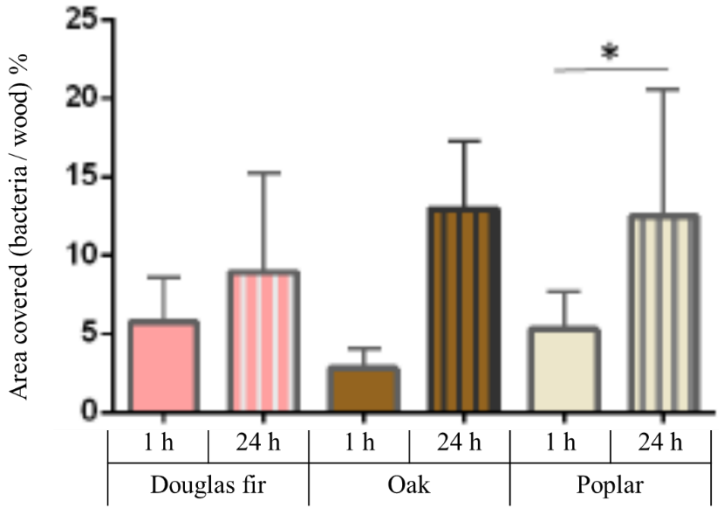


Figure A4. 3: The area covered by red fluorescence (mCherry *S. aureus*) compared to the area recovered by green fluorescence (wood) at 1 and 24 hour after inoculation on LT section. Mann-Whitney Statistic test, *p<0.05.

The presence of bacteria on LT cutting of different wood is shown in the figure 6.9. At 1 h after inoculation, bacteria could be seen on all surfaces. The poplar showed the highest number of bacteria based on visual observations at 1h. At this time, the bacterial fluorescence was equal on oak and Douglas fir. The bacterial fluorescence was equally prevalent on all three LT wood surfaces one day after inoculation.

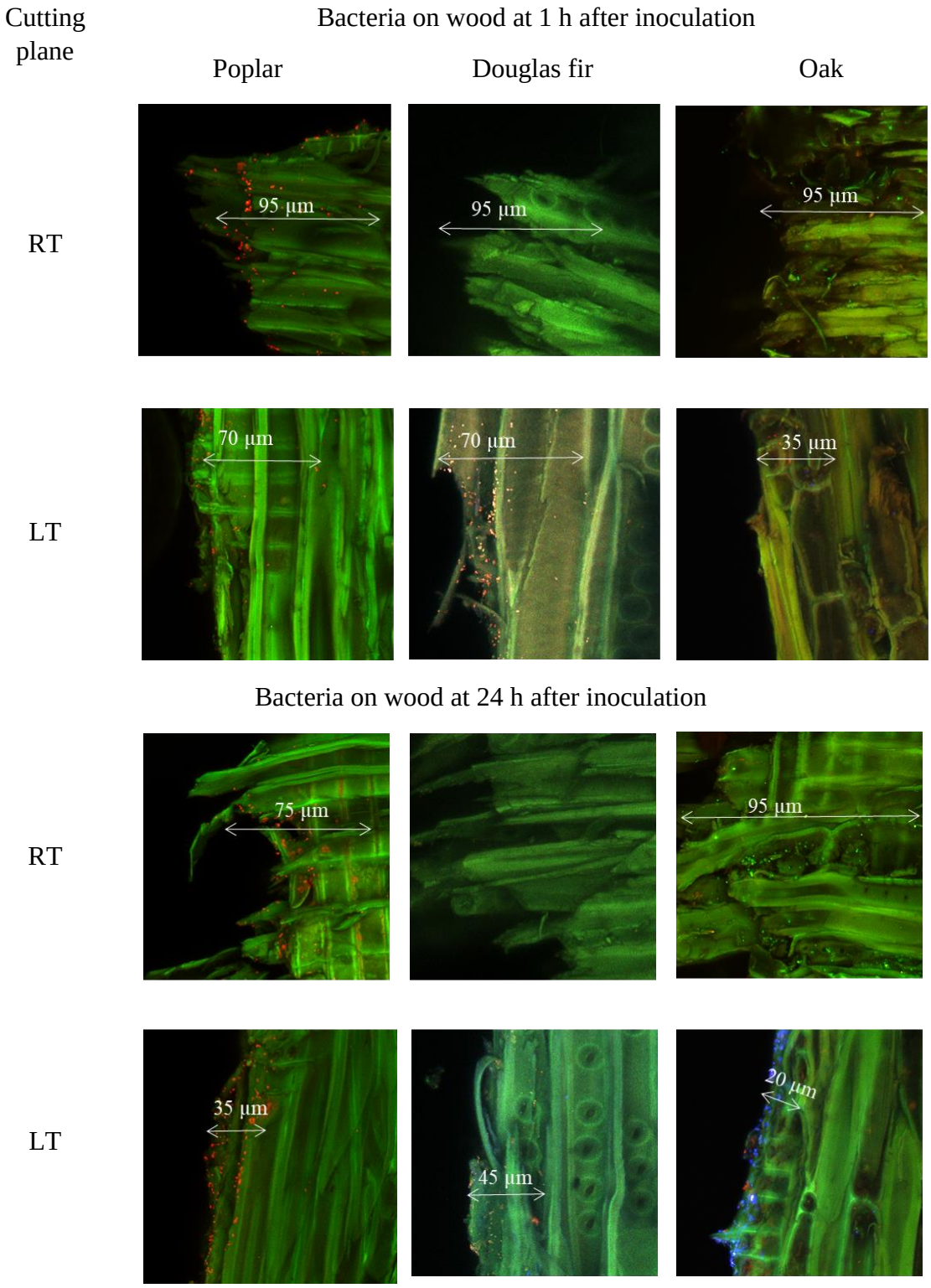
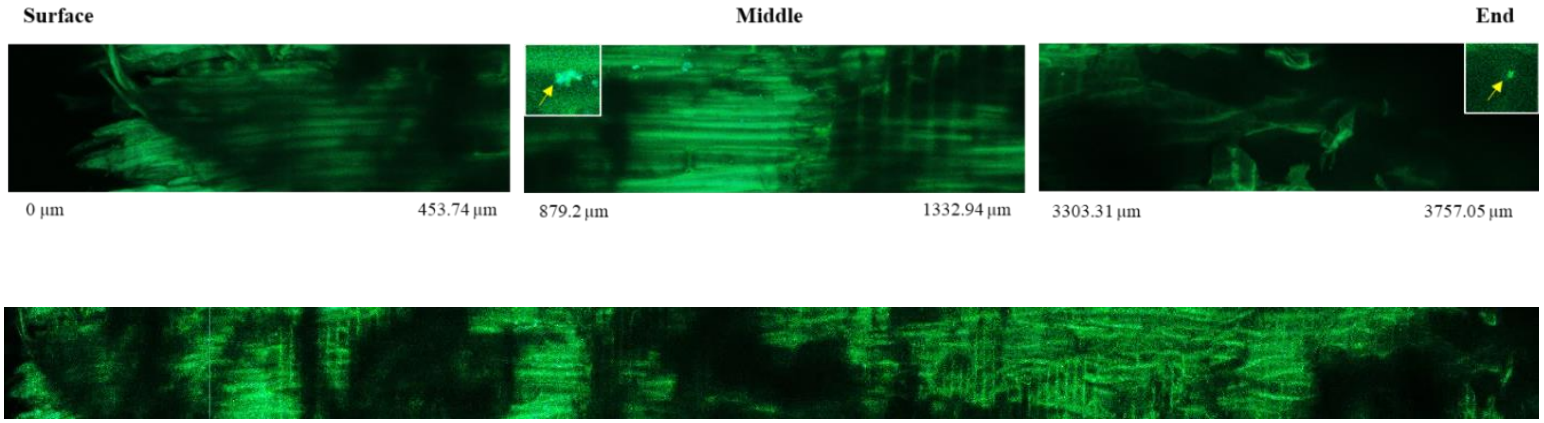
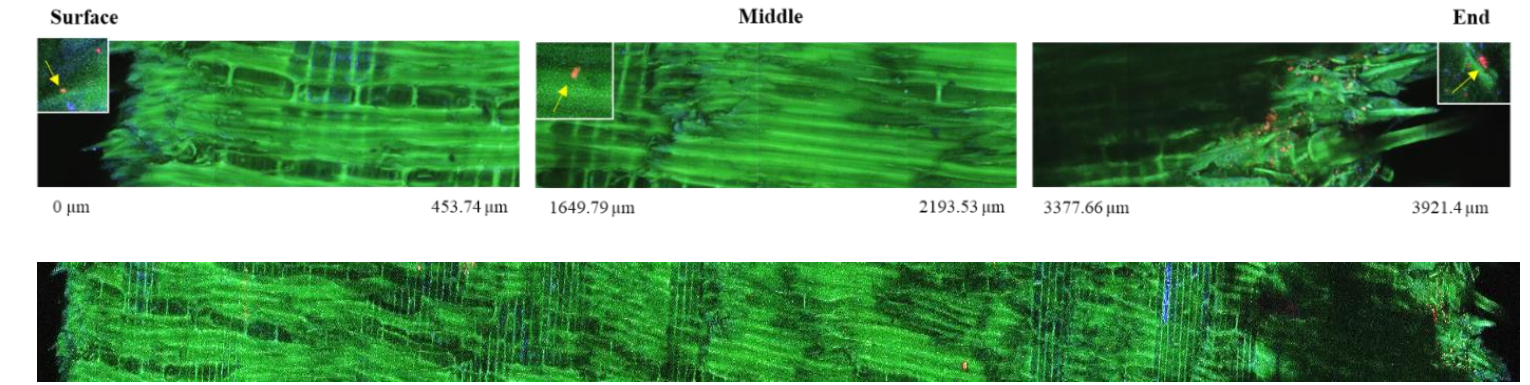


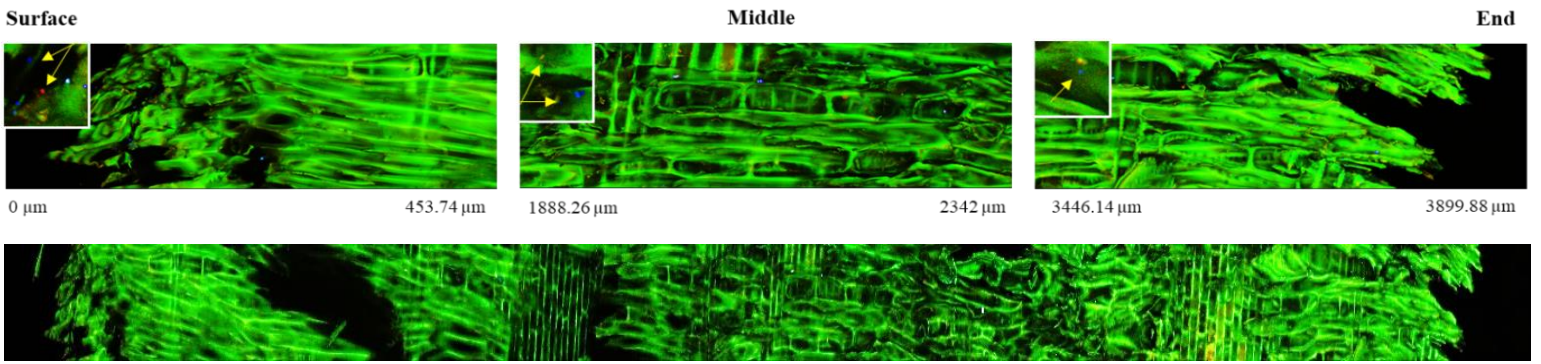
Figure A4. 4: CSLM image of cross section of wood samples (from top to ~ 100 µm depth) in RT and LT plane, at different times after inoculation.

Oak, 24-hours RT

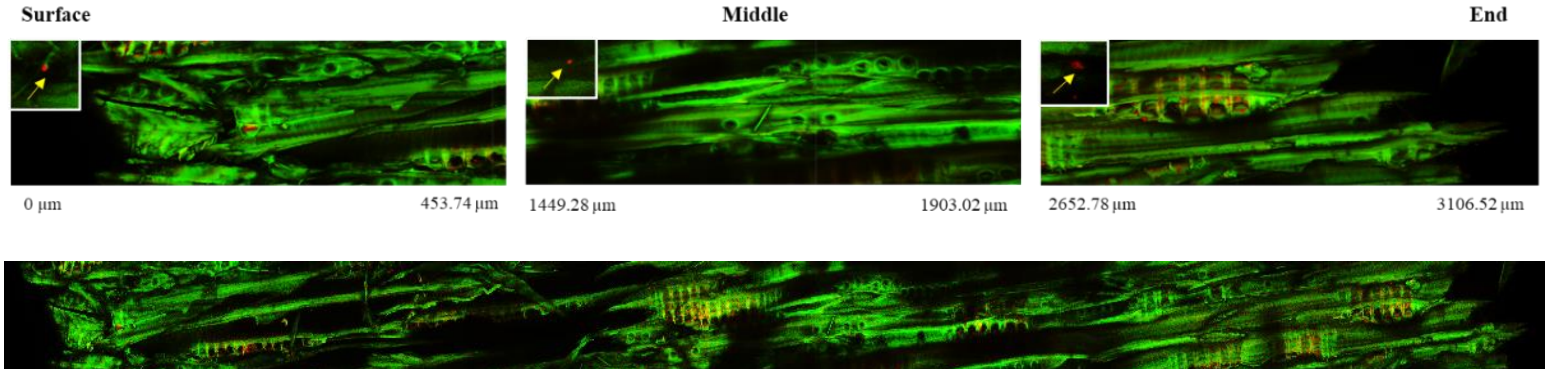




Oak, 24-hours, LT



Douglas fir, 1h, RT



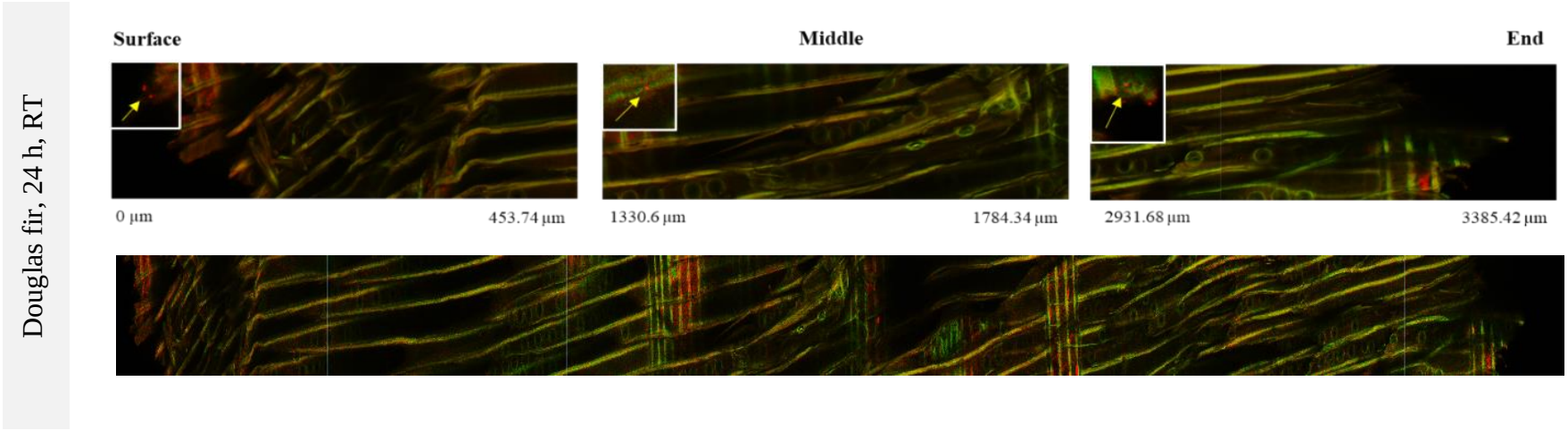


Figure A4. 5: CSLM image of cross section of oak and Douglas fir wood in LT and RT plane, at 1 and 24 h after inoculation. The mCherry fluorescence of bacteria is shown in red pointed with arrows.

Table A4.1: Estimated* number of bacteria inside wood

Wood spp.	Section	Incubation time (hour)	The estimated number of bacteria inside wood per area (cells/ µm²)		
			Surface of wood	Middle of wood	End of wood
Poplar	Transverse	1	+	48 / 395,476	+
		24	+	65 / 378,862	+
	Tangential	1	++	-	-
		24	+++	-	-
Oak	Transverse	1	-	-	-
		24	+++	42 / 413,179	+
	Tangential	1	++	-	-
		24	+++	65 / 424,502	++
Douglas	Transverse	1	rare	13 / 310,746	rare
		24	rare	28 / 350,158	rare
	Tangential	1	++	-	-
		24	++	-	-
Comments:	The estimated number of bacteria; rare, low (+), moderate (++), high (+++) No data / Cannot observe (-)				

*The number of bacteria from top and bottom images were manually counted per slide and for the middle of wood estimation were made by ratio of red fluorescence to green fluorescence in the image.

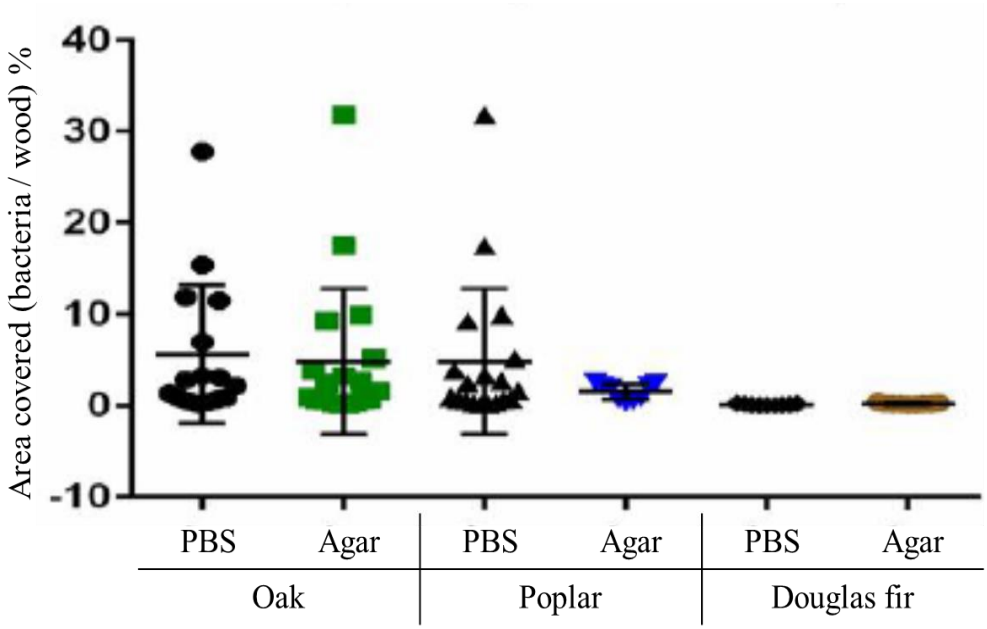


Figure A4. 6: Estimated bacterial quantity on different wood species incubated with phosphate buffer saline (PBS) and diluted agar for 24 hours

Annex 5: Perception of hospital occupants regarding the benefits of a wooden environment

The objective of this investigation was the preparation of a questionnaire and interview questions to evaluate the perception of people in wooden healthcare buildings. This study will identify the current perception of people regarding the use of wood in healthcare buildings regarding its hygienic status and restorative properties. This investigation will measure the social impact of our research starting from the perception of occupants of healthcare buildings

Annex 5.1: Literature search methodology

The literature research was conducted to create a questionnaire. The articles were identified from the previously collected literature used in the bibliography section. The keyword of "perception" was searched to specify the studies and then read them to identify the questions for questionnaires used to collect data on the perception of people regarding their indoor environment. Preference was given to healthcare-associated studies.

For specific questions to identify the sick building syndrome a study the Scopus search was conducted with keyword "sick building syndrome" (n=2078). The period was selected from 2011 to the present (02/09/2020) and the search was limited to medicine, environmental health, social science and psychology (n=392). Questionnaires were further limited to specific to hospital environment (n= 125). Finally, the titles and abstracts were read to identify the most relevant studies (n=9) (Gómez-Acebo et al. 2011, 2013; Chang et al. 2015; Vafaeenasab et al. 2015; Smajlović et al. 2019; Babaoglu et al. 2020; Gawande et al. 2020; Qayyum et al. 2020; Quoc et al. 2020). The methodology of these studies was read to finally prepare the interview questions.

Annex 5.2: Results

Annex 5.2.1. Study population

The study population (n=135) was identified as the hospital staff, visitors and patients of wood constructed healthcare building ESEAN, Nantes (47°11'08.4"N 1°31'28.2"W).

The resultant questionnaire and interview questions were also evaluated by a psychologist (Mr. Dian Lipovac, InnoRenew CoE, Slovenia) and a public health researcher (Prof Dr Didier Lepelletier, University of Nantes).

Annex 5.2.2. Questionnaire – Environmental perception study in ESEAN

- Researching the perception of indoor environments, including the perception of wood material by occupants of ESAN hospital.
- The results of this study will enable us to assess the acceptance of wood as natural indoor material and its effects on the wellbeing of inhabitants.
- The responses and identity of participants of this questionnaire will be kept safe and will be published only in anonymized form.
- There are no right or wrong answers in this questionnaire, we are just interested in the opinion of participants.

Annex 5.2.2.1. Demographic data

1. **Age**:

☐ Under 15 ☐ 16-20 ☐ 21-30 ☐ 30-40 ☐ 41 or more

2. **Gender:** ☐ Female ☐ Male
3. **Last completed educational qualification:**

☐ Primary ☐ Secondary ☐ High ☐ College ☐ University

4. **Field of study:**

☐ Formal science (mathematics, logics)

☐ Natural science (biology, chemistry, physics, earth science)

☐ Engineering and technology

☐ Medical and health

☐ Agricultural science

☐ Social science

☐ Humanities

If you are patient:

5. *How often you visit this hospital?*

☐ 2-3 times per week ☐ Once a week ☐ Once a month ☐ Rarely

6. *What is your current hospital stay time?*

☐ 1-3 days ☐ 4-7 days ☐ 8-15 days ☐ 16-30 days ☐ more than a month

If you are a relative of patient:

7. *How often you visit your relative?*

☐ Everyday ☐ Twice a week ☐ Once a week ☐ Twice a month ☐ Only single visit

If you are member of staff:

8. *What is your position in staff of hospital?*

☐ Admin

☐ Medical assistant (nurse)

☐ Medical position (pharmacist, physician, physiotherapist)

9. *Job type*

☐ Full time ☐ Part time

10. *Number of weekly hours?*

☐ 1-10 ☐ 11-20 ☐ 20-30 ☐ 31 or more

11. *Do you work somewhere else as well?*

☐ Yes ☐ No

12. *Years of experience at this hospital?*

☐ less than 1 years ☐ 1-3 years ☐ 4 -10 years

13. *Years of experience in this occupation?*

☐ less than 1 years ☐ 1-3 years ☐ 4 -10 years ☐ more than 10 years

If you are related to hospital hygiene:

14. Job type

☐ Full time ☐ Part time

15. Years of experience in this occupation?

☐ less than 1 years ☐ 1-3 years ☐ 4 -10 years ☐ more than 10 years

16. Any allergy developed during this work?

☐ Yes ☐ No

Annex 5.2.2.2.: Perception of wood in environment

1. **On a scale 1 to 7, what you think about the following characteristics of this hospital indoor environment due to wood material as compared to some other artificial material (glass, plastic, concrete and steel)?**

	1	2	3	4	5	6	7	
Warm								Cold
Modern								Old
Productive								Boring
Pleasing to eyes								Ugly
Durable								Nondurable
Clean								Dirty
Bright								Dark
Natural								Artificial
Sustainable								Not eco friendly
Easy to clean								Difficult to clean
Pleasant smell								Unpleasant
Homely feeling								Uncomfortable
Peaceful								Noisy

2. **Keeping in view the current use of wood in this hospital, on scale of 1 to 7, what do you say about following statements? [1 strongly disagree, 2 disagree, 3 somewhat disagree, 4 not disagree or agree, 5 somewhat agree, 6 agree and 7 strongly agree]**

	1	**2**	**3**	**4**	**5**	**6**	**7**
Wooden surfaces are physically injurious							
Wooden environment has harmful chemical emissions							
Wooden surfaces transmit infections							
Wooden buildings have highest risk of fire							

3. **To what extent do you like wood being in each of the following furnishings? [1 strongly dislike, 2 moderately dislike, 3 slightly like, 4 not like or dislike, 5 slightly like, 6 moderately like and 7 strongly like]**

	1	**2**	**3**	**4**	**5**	**6**	**7**
Doors							
Beds							
Toys							
Kitchen utensils							
Stair railings							
Window frames							
Walls							
Floors							
Ceilings							
Pillars							
Exercising equipment							

4. **To what extent do you like wood being in each of the following places?**

[1 strongly dislike, 2 moderately dislike, 3 slightly like, 4 not like or dislike, 5 slightly like, 6 moderately like and 7 strongly like]

	1	**2**	**3**	**4**	**5**	**6**	**7**
Reception							
Corridors							
Waiting room							
Washrooms							
Offices							
Cafeteria							
Patient room							
Operating room (operation theatre)							
Break room							
Storage room							
Charging room							
Common living room							

5. **How does including wood influences certain qualities of the indoor environment?**

[1 considerably deteriorates, 2 moderately deteriorates, 3 slightly deteriorates, 4 does not influence, 5 slightly improves, 6 moderately improves, 7 considerably improves]

	1	**2**	**3**	**4**	**5**	**6**	**7**
Indoor air quality							
Acoustic quality							
Visual appearance							
Thermal insulation / regulation							

6. **On the extent of wood material usage in this hospital, what is your say?**

Should be more	Slightly more can be added	It is enough	It is too much	Should be removed

7. **Do you believe that wooden surfaces have antimicrobial properties against infectious pathogens?**

   ☐ Yes ☐ No

Annex 5.2.3. Interview questions to identify the signs of sick building syndrome
In the past one year have you had more than two episodes of:

1. **Dry throat** ☐ Yes ☐ No

Was this better away from this hospital environment? ☐ Yes ☐ No

How frequent were the symptoms in this hospital? (Please check the box)

Everyday	More than 2 times in a week	Once a week	Once or twice a month	Less often

2. **Lethargy and/or tiredness** ☐ Yes ☐ No

Was this better away from this hospital environment? ☐ Yes ☐ No

How frequent were the symptoms in this hospital? (Please check the box)

Everyday	More than 2 times in a week	Once a week	Once or twice a month	Less often

3. Headache ☐ Yes ☐ No

Do you have migraine? ☐ Yes ☐ No

Was this better away from this hospital environment? ☐ Yes ☐ No

How frequent were the symptoms in this hospital? (Please check the box)

Everyday	More than 2 times in a week	Once a week	Once or twice a month	Less often

4. Dry, itching or irritated skin ☐ Yes ☐ No

Was this better away from this hospital environment? ☐ Yes ☐ No

How frequent were the symptoms in this hospital? (Please check the box)

Everyday	More than 2 times in a week	Once a week	Once or twice a month	Less often

Annex 6: Prevalence of nosocomial pathogens in the hospitals

The objective of this data collection was to outlay a study plan of a future experiments for evaluation of prevalence of nosocomial pathogens inside the wooden and non-wooden healthcare buildings. It will give an outline of a static chamber method, and the sample collection from the healthcare buildings. Future study will also aim to evaluate the influence of wood material utilization in the hospitals on the point prevalence of pathogenic fungi and bacteria on the surfaces and in the indoor air.

Annex 6.1. Methodology of literature search

The literature research was conducted to identify the methodologies and modify them according to the wooden healthcare buildings. The articles were scrutinized from the previously collected literature used in the bibliography section. The keywords of "prevalence" was searched to specify the studies and then read them to identify methodologies to collect data on the healthcare associated studies infectious agents. The hospital buildings containing wooden and non-wooden indoors will be selected.

Annex 6.2. Results

The results obtained from literature study were used to prepare the following experimental methodologies.

Annex 6.2.1. Air sampling

Active sampling is done to collect air from different parts of hospital at different times with respect to activity in the building and cleaning. The air is collected via an air aspirator with a flow rate of 180 L/min for 3 minutes. The air is passed through 50 ml of liquid PBS leaving the biological material suspended.

Annex 6.2.2. Surface sampling

Three types of surface sampling were planned:

1. Swabbing the constructed surfaces
2. Pieces of constructed surfaces
3. Test surface pieces installed in hospital buildings

For swabbing, the surface area of 25 cm^2 is selected and a boundary is made with a scotch tape. After wetting the surface with PBS, the swabbing is performed in three directions with 60° angle of test surface each time and swab is also rotated each time at 120° angle. The used

swab is placed in a swab collection container. All the swabs are aseptically transported to the laboratory at 4°C.

Annex 6.2.3. Static chamber method

In this method a plastic or glass box representing the hospital room is taken with inlets and outlets for air flow (Figure 7.1).

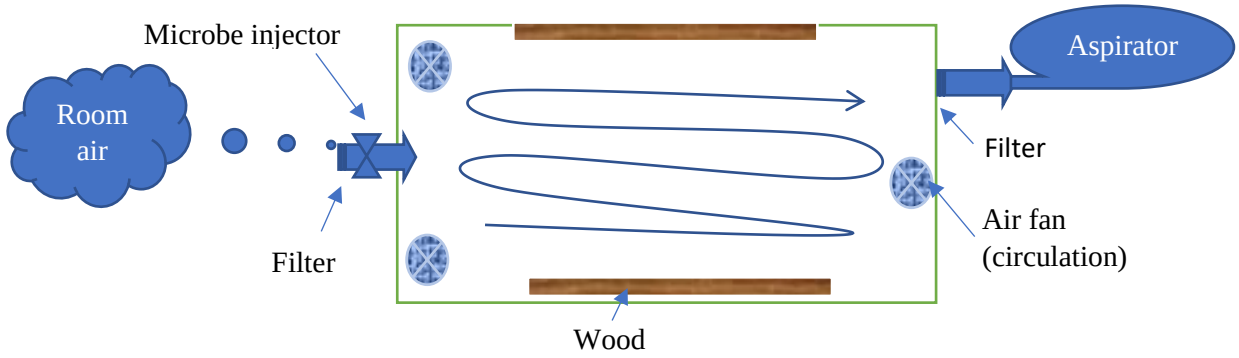


Figure A6. 1: A hypothetical room model (static chamber) showing influence of wood material on microbial quantities in room air and on surfaces. Wood planks are fitted on ceiling, floor and wall of chamber made up of an innert material (glass or plastic). Continuous room air is filtered and passed to this system. The known quantities of microbes are injected via a nebulizer in form of aerosol with more than 75% particles being the size of <3 µm. Fans homogenize the air circulation. Hygrometer and thermometer show the temperature and humidity variations. An aspirator ejects the air from chamber and the filter fitted on face of this machine will collect the microbes or pass the air through a liquid medium for further quantification.

Annex 6.2.4. Sample processing

The tips of control unused and microbial sample containing swabs are broken and placed in 5 ml of sterile PBS (pH 7.4) (Saka et al. 2017). The pieces of wood (1 cm^3) collected by cutting the pieces out of constructed surfaces are also placed in tubes in similar fashion. Then the tubes are vigorously shaken for 2 minutes to detach the microbes (Gupta 2017).

The microbial suspension collected from air or surface sampling is transported to lab in sterile conditions maintaining the temperature at 4°C, and further used to determine the total microbial counts and determine the microbiome.

Annex 6.2.5. Total viable microbial count

For total bacterial and fungal counts, 0.5 ml of suspension is diluted in 10 time serial dilution as mentioned above. Finally, 100 µl from each dilution is spread on PDA (chloramphenicol at 100 µg/ml) for total fungi counts and blood agar media for total bacterial counts. After

incubating, the petri dishes are inoculated at 35°C for 48 h for bacteria and 25°C for one week for fungi (Mohan et al. 2020).

Annex 6.2.6. Identification of microbes

During the incubation time, the plates were observed regularly and the colonies with distinct morphological features were picked and plated. Once the culture was identified as pure it is further analyzed by matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) for microbial identification (Ling and Hui 2019).

Titre : Le bois et l'hygiène hospitalière : Enquête sur la sécurité hygiénique et les propriétés antimicrobiennes du bois
Mots clés : bois, intérieur, bâtiments de soins de santé, antimicrobien, hygiène hospitalière.

Résumé : Le matériau bois est un composant bien connu des thèmes de construction basés sur la nature en raison de son aspect naturel, de sa nature écologique et de ses effets biophiles chez l'homme. L'utilisation de ce matériau dans des endroits sensibles sur le plan de l'hygiène est toutefois remise en question en raison de sa nature organique et poreuse. Des études antérieures ont montré que le bois possède des propriétés antimicrobiennes contre de nombreux microorganismes importants du point de vue de l'hygiène. Les travaux sont encore nécessaires pour démontrer cette action antimicrobienne et sa relation avec le bois et les variables microbiologiques.

Cette recherche visait à rassembler et à générer des informations pour guider les acteurs de l'hygiène hospitalière en ce qui concerne la sécurité hygiénique du matériau bois. Les méthodes ont été développées pour étudier l'action antimicrobienne du bois et identifier les variables qui influencent ce comportement. La première méthode développée dans ce contexte a été une méthode de diffusion directe sur gélose (appelée antiboisgram) qui a donné de bons résultats en ce qui concerne le - dépistage de plusieurs espèces de bois pour leur action antibactérienne et antifongique. Elle a également permis d'identifier le rôle du bois et des variables microbiennes dans la détermination des propriétés antimicrobiennes du matériau en bois. En outre, une méthode de récupération bactérienne basée sur l'élution a été étudiée, qui a montré que la plupart des bactéries nosocomiales courantes survivent moins bien sur le bois que sur les surfaces lisses. Parallèlement, un outil innovant a été mis au point, impliquant l'utilisation de sondes fluorescentes pour étudier la distribution des bactéries sur et à l'intérieur des matériaux en bois à l'aide de la microscopie laser spectrale confocale.

Ces expériences ont permis d'obtenir des informations fructueuses qui pourraient améliorer la compréhension du rôle du bois dans la sécurité hygiénique des bâtiments de soins de santé. En outre, les futures recherches et les directives d'application ont été fournies concernant la prévalence des pathogènes dans les bâtiments de soins de santé en bois et la perception des occupants des hôpitaux vis-à-vis de l'environnement intérieur en bois.

Title : Wood and hospital hygiene: Investigating the hygienic safety and antimicrobial properties of wood materials
Keywords : Wood, indoor, healthcare buildings, antimicrobials, hospital hygiene

Abstract: The wood material provides a nature-based theme to construction because of its natural appearance, ecofriendly nature and biophilic effects on humans. However, its organic and porous nature is questioned when using it in hygienically important places such as hospitals. Studies have shown that wood has antimicrobial properties against some pathogens; work is still needed, however, to demonstrate this antimicrobial action and its relation to wood and microbiological variables.

This research gathers and generates information to guide stakeholders of hospital hygiene on the hygienic safety of wood materials. First, a simple and direct method was developed to study the antibacterial and antifungal activity of solid wood, which also identified the role of wood and microbial variables on antimicrobial behavior. Further, an elution based bacterial recovery method was investigated which showed that the most common nosocomial bacteria did not survive as well on wood as compared to smooth surfaces such as aluminum, steel and polycarbonate. Meanwhile, an innovative tool was developed, involving the use of fluorescent probes to study the bacterial distribution on and inside wood using confocal spectral laser microscopy.

These experiments produced the information that will help the decision makers regarding the choice of wood material in the healthcare buildings. It not only enhances our understanding of hygienic safety of wood in healthcare buildings but also provides the basis for future research on the prevalence of pathogens in the wooden healthcare institutes and the perception of the occupants those buildings.