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Domestic chopping boards represent important vehicles of microbial food contamination and human pathogens

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ABSTRACT

Chopping boards may harbor pathogenic microorganisms that cross contaminate food products leading to food borne illnesses. he present study aimed at comparing the microbial di ersity of plastic, glass and wooden chopping boards. Microorganisms were reco ered from chopping boards by swabbing and enumerated for mesophilic aerobic bacteria, Escherichia coli, Listeria spp., Clostridium perfringens, Salmonella spp. and yeasts and molds. n addition, fungi reco ered were identified by se uencing their ribosomal se uences, and phylogenetic analyses. E. coli was undetectable by the plating method on wooden chopping boards but was isolated from glass and plastic. he mean population density of Salmonella spp., Listeria spp. and C. perfringens reco ered from plastic chopping boards was 2. , 2.4 and 2.5 log cfu ^{2}cm respecti ely. Lastly, the population density of yeasts and molds was found to be higher on wooden boards .0 log cfu ^{2}cm compared to their plastic counterparts 2.2 log cfu^{2} cm he isolated fungi were identified as Penicillium citrinum, Peyronellaea glomerata and Cladosporium halotolerans. o the best of our nowledge, this study is one of the few which has compared the microbiological status and di ersity of different types of chopping boards, highlighting their cross contamination potential.

1. Introduction

Chopping boards are common accessories used in kitchens of domestic households and food catering establishments. Traditionally, the surface used for food preparation was wood but, nowadays, chopping boards are available in different materials such as glass, plastic, stainless steel and wood. However, chopping boards are often perceived as important vehicles harboring foodborne pathogens (1). As a matter of fact, a major concern in households is the transmission of foodborne pathogens by cross-contamination of foods via food contact surfaces, particularly chopping boards. In fact, chopping boards are considered as one of the top five sites most contaminated with microorganisms in domestic kitchens (2).

Numerous studies have evaluated the hygienic potential of chopping boards made of plastic, wood and stainless steel with varying results. For instance, the studies done by Snyder and Worobo (3) and Oliveira *et al.* (4) demonstrated differences in the microbiology of different chopping board materials whereby higher recovery of microorganisms on plastic chopping boards was observed while granite chopping boards were more prone to colonization by *Salmonella* than polyethylene or polypropylene ones.

Thus, microorganisms may attach to chopping boards and remain viable even after cleaning and disinfection and subsequently contaminate food during processing. They pose a potential risk to the consumers,

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particularly if contamination occurs in ready-to-eat foods which have already gone through a heat-killing step. For instance, some fungi have the potential for production of mycotoxins and the latter are heat-resistant. Hence, the presence of fungi on chopping boards can impact on consumers' health directly (3). Therefore, the aim of this study was to compare the hygienic and microbiological status of used domestic chopping boards, collected from different households, made of plastic, glass and wood and to identify the fungal isolates recovered on those surfaces.

2.Materials and methods

2.1 Microbiological survey of chopping boards

Fifteen used chopping boards (5 glass, 5 plastic and 5 wooden) were collected from individual households in Mauritius. They were carefully sealed in plastic bags and brought to the Microbiology laboratory of the University of Mauritius. An area of 25 cm^2 (5 cm x 5 cm) of the chopping boards was swabbed in different orientations using sterile disposable wooden swabs, moistened with 0.1% buffered peptone water. Each swab was then immediately inserted into a test tube containing 10 ml of 0.1% sterile buffered peptone water and vigorously vortexed to form a bacterial suspension. A 5-step decimal dilution series was set up and inoculant from all five tubes was aseptically spread-plated onto petri plates of Plate Count Agar (HiMedia, India) (ISO 4833:2003), Potato Dextrose Agar (PDA, HiMedia, India) (ISO 21527:2008), Iron Sulfite Agar (HiMedia, India) (ISO 15213:2003), Eosin Methylene Blue agar (HiMedia, India) (5), Xylose-Lysine-Tergitol 4 (Oxoid, UK) Agar (6) and PALCAM Agar (Difco, France) (7) to recover mesophilic aerobic bacteria (MAB), yeast and molds (YM), presumptive *Clostridium perfringens*, *Escherichia coli*, presumptive *Salmonella spp* and presumptive *Listeria* spp. respectively. Presumptive *Salmonella* and *Listeria* isolates were confirmed using commercial lateral flow immunoassays (Reveal 2.0, Neogen).

The population densities of the different microorganisms were calculated per cm^2 using the formula:

$$(counts \times dilution\ factor)/(volume\ of\ inocula\ plated \times area\ swabbed)$$

The values obtained were then log transformed by taking their $\log_{10}$ value. A statistical analysis was carried out by using a single factor analysis of variance (ANOVA) and Tukey's one-way multiple comparisons in Minitab® Release 17 software, to compare the log population densities obtained for each type of chopping board. The significant differences were considered at the 95% confidence level ($P < 0.05$).

2.2 Microscopic examination of fungal species

Pure colonies of four fungal isolates, chosen based on their physical characteristics, were subcultured on separate PDA petri plates and allowed to grow for 5 to 7 days at room temperature. After their growth and sporulation, each fungal specie was viewed aseptically under a light microscope after staining with Lacto-phenol cotton blue dye. Briefly, a scalpel was flame-sterilized and used to transfer a small amount of each fungal mycelium onto four different clean glass slides. An aliquot of 1-2 drops of Lacto-phenol cotton blue dye was placed onto the fungal mycelia and a cover slip was placed on top. After 5 min, the specimens were examined under the microscope at low and high magnification.

2.3 Molecular phylogeny

The genomic DNA of the four fungal species was extracted using a modified Cetyl Trimethyl Ammonium Bromide (CTAB) method (8). The extracted DNA samples were used for PCR amplification. ITS was then amplified using primer pairs ITS4 primer and ITS5 primer. PCR amplicons were then purified using Fermantas PCR purification kits following the manufacturers' instructions. DNA sequencing reactions were done using a Big Dye Terminator v. 3.1 Cycle Sequencing Kit (Applied Biosystems) following the protocol outlined by the manufacturers. Forward and reverse sequences were assembled and edited using CLC Main Workbench Version 7.6 (https://www.qiagenbioinformatics.com/). DNA sequences were aligned using the online version of MAFFT 5.66 using the iterative refinement method and the following settings: the Needleman-Wunsch algorithm active, 2 tree rebuilding steps, 1000 iterations and the program's default values for gap opening and gap extension penalties. A number of other fungal species were retrieved from GenBank (Supplementary Table 1) and used in the phylogenetic analysis. Equally, maximum parsimony (MP) and Bayesian inference (BI) methods were used in phylogenetic reconstruction. Finally, the most nucleotide substitution model was determined using Jmodel Test with model selection based on the Akaike information criterion (AIC).

3.Results

3.1 Microbiological survey

The mean population density of microorganisms recovered from the three types of chopping boards is summarized in Table 1.

Table 1. Mean population density (log cfu/cm²) of microorganisms recovered from different types of chopping boards.

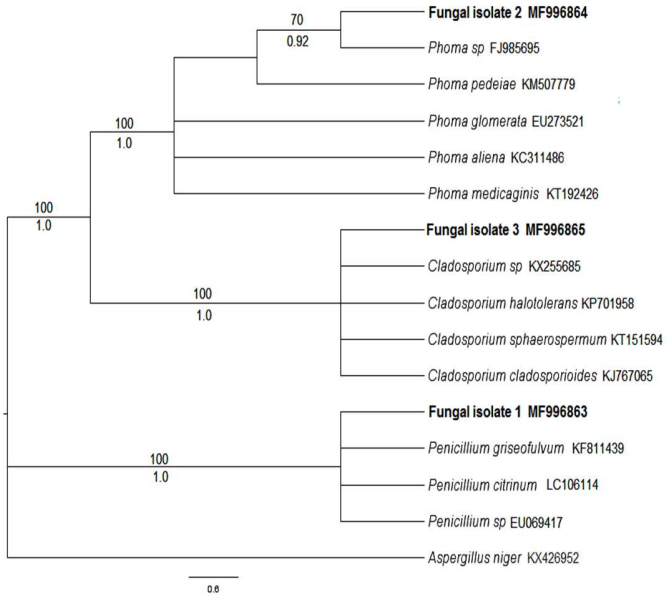
Parameters	Chopping Board Materials		
	Glass	Plastic	Wood
Total Viable Counts	2.6 ± 0.01[a]	3.6 ± 1.13[a]	3.3 ± 0.26[a]
Yeast and Molds	2.3 ± 0.59[a]	2.2 ± 0.69[a]	3.0 ± 0.91[a]
C. perfringens	1.8 ±0.26[a]	2.5 ± 0.80[a]	1.9 ± 0.69[a]
Listeria spp.	1.7 ± 0.31[b]	2.4 ± 0.44[a]	<1.6 ± 0.00
Salmonella spp.	<1.6 ± 0.00	2.3 ± 0.71	<1.6 ± 0.00
E. coli	1.9 ± 0.26[a]	1.8 ± 0.33[a]	<1.6 ± 0.00

*Values with similar superscript letters within the same row are not significantly different (P > 0.05)
*Limit of detection by the plating methodology was <1.6 log cfu/cm² for *E. coli*, presumptive *Salmonella* spp. and presumptive *Listeria* spp.

The population density of mesophilic aerobes on chopping boards varied between 2.6 log cfu/cm² (glass) and 3.6 log cfu/cm² (plastic), although the difference was not statistically significant (P > 0.05). The population density of yeasts and molds was highest on wooden chopping boards (3.0 log cfu/cm²) and lowest on plastic ones (2.2 log cfu/cm²). *E. coli* was undetectable by the plating method on wooden chopping boards (<1.6 log cfu/cm²) but was isolated from the glass (1.9 log cfu/cm²) and plastic (1.8 log cfu/cm²) counterparts. *Salmonella spp* was recovered from plastic chopping boards at levels as high as 2.3 log cfu/cm² but it was undetectable on glass or wooden boards. The population of *Listeria* spp. recovered from plastic, glass and wooden chopping boards were 2.4, 1.7 and < 1.6 log cfu/cm² respectively while the population of *C. perfringens* recovered from plastic, glass and wooden chopping boards were 2.5, 1.8 and 1.9 log cfu/cm² respectively.

3.2 Molecular identification of fungal isolates

Basic Local Alignment Search Tool (BLAST) identified the three fungal species as *Penicillium citrinum*, *Peyronellaea glomerata* and *Cladosporium halotolerans* respectively (Supplementary Figure 1). Sequence analyses of the ITS region revealed maximum identities of 97% with *Penicillium citrinum,* 92% with *Peyronellaea glomerata* and 99% with *Cladosporium halotolerans* (Supplementary Figure 1).



Supplementary Figure 1: One of the 26 most parsimonious trees obtained from the maximum parsimony (MP) using ITS 1 dataset. *Aspergillus niger* was used as "outgroup" to root the tree. Numbers above branches are MP bootstrap values, while numbers below the branches are posterior probability values obtained from a Bayesian analysis. Scale bar: Number of steps.

4.Discussion

Overall, the prevalence of TVC, *C. perfringens*, *Salmonella* and *Listeria* spp. was highest on plastic chopping boards. The results therefore suggest a higher probability of microbial cross-contamination events with plastic chopping boards probably due to the presence of "knife-scars" or scratches observed on plastic chopping boards. This could partly explain the higher persistence of microorganisms on the boards, rendering them difficult to clean and disinfect manually (4). Yeast and mold counts were highest on wooden chopping boards. This is probably due to the

fact that wood retains more water as compared to plastic and glass chopping boards, which is conducive for growth of yeast and molds (3). Aviat *et al.* (1) reported that fungi such as *Aspergillus*, *Penicillium* and *Mucor spp* can even be recovered from wooden food contact surfaces. Also, a lower bacterial population density including that of *E. coli*, was obtained on wooden chopping boards in this study compared to the other surfaces. This could be due to some antimicrobial properties of wood. For instance, Vainio-Kaila (9) observed that wood extracts suppressed the growth of *E. coli*. On the other hand, the highest *E. coli* count was observed on glass chopping boards (1.6 log cfu/cm^2). This may be due to the ability of *E. coli* to form biofilms on glass surfaces (10) owing to extracellular polymeric substances produced by the cells. It is also to be noted that the counts of *C. perfringens* and *Listeria* spp. were lowest on glass chopping boards but highest on plastic surfaces probably due to their ability to form spores and biofilms respectively (4). Overall, the hygienic quality of different chopping board materials ranked in the order of glass ~ wood > plastic. All three fungi (*P. citrinum*, *P. glomerata* and *C. halotolerans*) are quite ubiquitous in the environment and can infect humans and animals with adverse health effects (11,12). Moreover, *P. citrinum* is a well-known producer of mycotoxins (11).

5.Conclusion

This study revealed that chopping boards made of plastic harbored the highest counts of microorganisms, thus presenting the greatest risk of cross-contamination to food. On the other hand, wooden chopping boards presented the lowest risk and thus represent the safest material of choice. Fungal isolates from chopping boards were identified as ubiquitous, spore-forming, airborne mold species that could be directly or indirectly associated with food spoilage or intoxication due to mycotoxin production. This study warrants further research to detect and quantify the level of mycotoxins potentially present on chopping boards.

Conflict of interest

The authors declare no conflicts of interest in this paper.

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