IJMAAS International Journal of Microbiology and Applied Sciences
ISSN 1115-4004 Volume 4 issue 2 May, 2025 Research Article

Microbial Flora and Antibiogram of Bacteria Associated With Wooden Toothpicks Used In Restaurants in Port Harcourt Metropolis

Ogbonna, S. I*., Chuku, W., Ogbuleka, N. A. C. and Elechi, D. N.
Department of Microbiology, Rivers State University,
P.M.B. 5080, Port Harcourt, Nigeria
***Corresponding Author:** *solomon.ogbonna@ust.edu.ng*

ABSTRACT

Wooden toothpicks are small thin sticks with pointy ends used in dislodging food particles or debris from between the teeth. It is a commonly used disposable kitchen utensil in Nigeria and other parts of the world. This study aimed to determine the microbial flora associated with wooden toothpicks used in restaurants in Port Harcourt Metropolis. A total of forty (40) samples of wooden toothpicks from eight (8) restaurants within Port Harcourt metropolis were aseptically collected and cultured on appropriate Agar medium such as nutrient Agar, Mannitol salt Agar, Sabouraud dextrose Agar using standard microbiological techniques for colony count and isolation of bacteria and fungi. Results showed that the mean total heterotrophic bacteria count ranged from 1.3 x 10^6 to 21.0 x 10^6CFU/g, total Staphylococcal count ranged from 0.0 x 10^4 to 7.0 x 10^4CFU/g while total heterotrophic fungi count ranged from 1.5 x 10^4 to 216.5 x 10^4CFU/g respectively. Fourteen (14) bacterial species belonging to four (4) genera were identified. Their occurrence (%) were; *Bacillus* sp. (57%), *Pseudomonas* sp. *(*14.29%), *Staphylococcus* sp. (7.14%) and *Escherichia* sp. (21.43%). While a total of 10 fungal species belonging to three (3) fungal genera were isolated. They include *Saccharomyces* sp. (66.7%), *Penicillium* sp. (16.7%) and *Mucor* sp. (8.3%). *Bacillus* sp. isolates were highly susceptible to most antibiotics like gentamicin and levofloxacin with 100% susceptibility but resistant to ciprofloxacin. *Escherichia coli* isolates were also susceptible to ofloxacin and gentamicin with 100% susceptibility but also showed resistance to few antibiotics like streptomycin and aureomycin at (33.3%) respectively. This study shows that there is a high rate of contamination of these wooden toothpicks which may represent a unique route in the spread of bacterial and fungal pathogens to the public. This is of public health concern, therefore compliance to proper hygiene standards and usage of alternative materials with low susceptibility to microbial contamination should be encouraged.

Keywords: Restaurants, Toothpick, Oral Hygiene, Bacteria, Antibiotic Resistance, Antibiogram.

Introduction

The use of toothpicks, especially wooden ones, as a means of dental hygiene has been a regular practice across cultures worldwide. However, concerns have been raised regarding the potential risk of microbial infection associated with their use. While toothpicks are commonly utilized for dislodging food particles and debris from between teeth, their porous nature and the potential presence of microorganisms on the surface pose a risk of introducing pathogens into the oral cavity. Other studies have also emphasized and identified wooden toothpicks as potential vectors for microbial contamination due to their porous nature, which allows microorganisms to adhere and multiply on their surfaces (Tsugita *et al.,* 2017). Wooden toothpicks, typically made from materials such as birchwood or bamboo, have been shown to harbour bacteria and fungi due to their organic composition and rough texture (Johnson and Guthmiller, 2007).

Research indicates that wooden toothpicks can serve as reservoirs for various microorganisms, including potentially pathogenic species such as *Enterococcus faecalis* and *Staphylococcus aureus* (Sedgley *et al*., 2005). Moreover, studies have demonstrated that wooden toothpicks can retain these microorganisms even after rinsing with water or disinfectants, which lays emphasis on the challenge of effectively sterilizing them for reuse (Ng *et al*., 2008).

The potential for microbial transmission through the use of wooden toothpicks raises concerns, particularly among individuals with compromised immune systems or pre-existing oral health conditions. Furthermore, inadequate oral hygiene practices, such as using contaminated toothpicks, may contribute to the development of dental infections and other oral health issues (Stuart *et al.,* 2006).

Citation: Ogbonna *et al*. (2025). Microbial flora and antibiogram of bacteria associated with wooden toothpicks used in restaurants in Port Harcourt Metropolis. *International Journal of Microbiology and Applied Sciences*. *4(2)*: 51 – 59.

Wooden toothpicks are frequently utilized for various purposes, including dental hygiene, food handling, and even craft projects, owing to their widespread availability, convenience, and cost effectiveness (Jones *et al.,* 2019). The use of contaminated toothpicks can lead to the transmission of various pathogens and potentially compromise oral hygiene and overall well-being (Nascimento *et al.,* 2020).

Environmental sources, such as air, water, soil, and human contact, can introduce microbes onto the toothpicks' surfaces (Jumpponen, 2019). Bacterial, fungal, and viral pathogens are among the microorganisms that have been identified on wooden toothpicks (Huang *et al.,* 2018). These contaminants can pose potential health implications, ranging from oral infections to more systemic illnesses (Farhadian *et al.,* 2021).

To ensure public health and safety, it is necessary to examine the factors that contribute to microbial growth on wooden toothpicks. Moisture content is a critical factor, as wooden toothpicks can absorb moisture from the environment or come into contact with moisture-rich substances, creating an ideal breeding ground for microorganisms (Nascimento *et al.,* 2020). Temperature fluctuations can also influence microbial proliferation on toothpicks, as different temperature ranges can favour the growth of specific pathogens (Kirschner and Melzner, 2017). Additionally, handling and storage practices, both during manufacturing and daily use, can impact the level of microbial contamination on wooden toothpicks (Tsugita *et al.,* 2017).

The potential for microbial contamination in wooden toothpicks poses a significant concern that must be addressed to safeguard public health and ensure the safety of individuals who use them. Despite these concerns, the use of wooden toothpicks remains prevalent due to their affordability and convenience. Therefore, it is imperative to educate users about the potential risks associated with their use and promote alternative methods of interdental cleaning that prioritize hygiene and safety. Understanding the sources and types of microorganisms that may be present on wooden toothpicks are significant in developing appropriate prevention strategies.

While wooden toothpicks are widely used for various purposes due to their convenience and affordability, the potential for microbial contamination raises concerns regarding public health and safety.

Addressing these concerns requires a detailed examination of the sources and types of microorganisms, as well as the factors influencing their growth on wooden toothpicks.

Despite their widespread use, there is a lack of detailed and specific research on the extent and diversity of microbial contamination on wooden toothpicks and the potential health risks it poses on individuals who makes use of it. Thus, it is important to assess the risks associated with microbial contamination in wooden toothpicks and implement preventive measures to mitigate these risks effectively. This study was aimed to determine the microbial contamination associated with wooden toothpicks used in restaurants in Port Harcourt metropolis

Materials and Methods

Study Area

The study was carried out amongst eight (8) restaurants located in four (4) different areas of Port Harcourt Metropolis of Rivers State. The restaurant locations and their sample codes were Agip (AG), Iwofe (IW), Staff Club (SC), and University Back Gate (BG). Samples were collected from two (2) restaurants in each location and designated 1 and 2 making a total of eight restaurants studied.

Sample Collection

A total of forty toothpick (40) samples were collected from the eight (8) different restaurants. Five (5) toothpick samples were collected from each restaurant. The samples were collected aseptically using sterile forceps, hand gloves and placed into a sterile sample bottle. The samples collected were exposed but assumed unused toothpicks and it was then placed inside the sample bottles. All the samples collected were immediately transported in an ice pack, to the microbiology laboratory in Rivers State University for Microbiological analysis.

Sample Preparation

Ten (10g) grams of sample was weighed and aseptically immersed into 9ml sterile normal saline and soaked for 24 hours (Obire and Ogbonna, 2017). The samples were agitated and 1ml was transferred into 9ml sterile normal saline in test tube given 10^{-1} dilution. 1ml was transferred from initial test tube into 9ml sterile normal saline in series to the sixth test tube as 10^{-6} dilution (Taylor, 2008).

Citation: Ogbonna *et al*. (2025). Microbial flora and antibiogram of bacteria associated with wooden toothpicks used in restaurants in Port Harcourt Metropolis. *International Journal of Microbiology and Applied Sciences*. *4(2)*: 51 – 59.

Enumeration of Bacteria and Fungi

The total heterotrophic bacterial and fungal load from the different samples was enumerated using standard plate count (Prescott *et al.,* 2011). After the serial dilutions, aliquot of 10^{-4} and 10^{-6} dilutions were transferred in duplicates into prepared Nutrient agar and Mannitol Salt agar, aliquot of 10^{-2} and 10^{-4} were transferred in duplicate on prepared Sabouraud Dextrose agar which had been fortified with tetracycline antibiotics for the inhibition of bacterial growth (Ogbonna *et al*., 2022). Plates were incubated at 37°C for 24 – 48 hours for the Nutrient Agar and Mannitol Salt Agar. The Sabouraud Dextrose Agar was incubated 37°C for 5 days. After incubation, plates were observed for microbial growth and ensuing colonies on Nutrient Agar, Mannitol Salt Agar and Sabouraud Dextrose Agar plate were counted and discrete colonies were sub-cultured onto sterile nutrient agar for colonies on Nutrient Agar, Mannitol Salt Agar and Sabouraud Dextrose Agar plates to obtain pure isolates (Cheesebrough, 2000).

Identification of Bacterial and Fungal Isolates

The pure bacteria isolates were characterized by Gram's staining and Biochemical tests such as catalase test, indole test, methyl red test, citrate test, coagulase test, Voges Proskauer test and sugar fermentation tests. Identity of the isolates was matched with the Bergey's Manual of Determinative Bacteriology and ABIS online for confirmation. (Buchanan & Gibbons, 1974; Costin, 2024). The Fungal Isolates were identified using their morphological features such as colony color, shape, texture and size of colony followed by microscopic examination (conidial shape, arrangement of hyphae and type of spore) of their wet mounts prepared with lactophenol cotton blue. Characterization of fungal isolates was drawn from matching results with those reported by Kidd *et al.* (2023) and Elis *et al.* (2007).

Antibiotics Sensitivity Test

The disk diffusion method of antibiotics testing according to the Clinical laboratory standard institute was adopted. Twenty-four (24) hours old cultures was standardized using the 0.5 McFarland standard (CLSI, 2020). This was done by matching the turbidity of the isolates in sterile 4mL normal saline to the 0.5McFarland standard. Sterile swab sticks were then dipped into the standardized isolates and swabbed uniformly on the surface of the dried Mueller-Hinton agar plates. The bacterial isolates were tested against already prepared commercial antibiotics: Ciproflox (10µg), Augmentin (30µg), Tarivid (10µg), Streptomycin (30µg), Reflacine (10µg) Nalididixic Acid (30µg), Ceporex (10µg), Septrin (30µg), Norfloxacin (10µg), Levofloxacin (20µg), Ampiclox (20µg) Chloramphenicol (30µg), Amoxil (20µg), Rifampicin (20µg), Erythromycin (30µg) and Ampicilin (30µg). The plates were held at room temperature for 3-5mins to allow drying. The antibiotics discs were placed on the plates and the plates were incubated for 18-24 hours at 37°C. The diameters of zone of inhibition were recorded to millimeter and classified as resistant (R), intermediate (I) and susceptible (S) according to published interpretive chart (CLSI, 2020).

Results

The result of mean microbial counts on the wooden toothpicks is presented in Table 1. Total heterotrophic bacterial count ranged from 1.3 x 10^6 to 21.0 x 10^6CFU/g. Total *Staphylococcal* count, ranged from 0.0 x 10^4 to 7.0x10^4CFU/g and total heterotrophic fungi count, ranged from 1.5 x 10^4 to 216.5 x 10^4CFU/g.

Table 2 shows the Physiological and biochemical characteristics and probable identification of the bacteria isolated from wooden toothpick samples.

Table 1: Microbial Population (CFU/g) of Toothpick Samples in Restaurants

Toothpick Samples	Total Heterotrophic Bacteria (x 10^6 CFU/g)	Total *Staphylococcus* Count (x 10^4CFU/g)	Total Heterotrophic Fungi (x 10^4 CFU/g)
Agip (AG 1)	6.6 ± 0.1 ^{b}	3.5 ± 0.7 ^{b}	9.5 ± 07^{a}
Agip (AG 2)	4.4 ± 1.6 ^{ab}	0.0 ± 0.0 ^{a}	39.0 ± 1.4^{b}
Back-Gate (BG 1)	1.3 ± 0.1^{a}	2.5 ± 0.7^{ab}	2.5 ± 07^{a}
Back-Gate BG 2)	13.7 ± 0.3^{c}	0.0 ± 0.0^{a}	1.5 ± 0.7^{a}
Iwofe (IW 1)	16.1 ± 0.1^{c}	1.5 ± 0.7^{ab}	2.5 ± 0.7^{a}
Iwofe (IW 2)	4.5 ± 2.1^{ab}	7.0 ± 1.4^{c}	2.0 ± 0.0^{a}
Staff Club (SC 1)	21.0 ± 0.2^{d}	4.0 ± 1.4^{b}	207.0 ± 5.7^{c}
Staff Club (SC 2)	14.3 ± 0.2^{c}	0.0 ± 0.0	216.5 ± 2.1^{d}
P-value	***0.001***	***0.00***	***0.00***

Key: Data in Mean ± Standard Deviation *Means with similar superscript down the group have no significant difference (P> 0.05).

Citation: Ogbonna *et al*. (2025). Microbial flora and antibiogram of bacteria associated with wooden toothpicks used in restaurants in Port Harcourt Metropolis. *International Journal of Microbiology and Applied Sciences*. *4(2)*: 51 – 59.

Table 2: Physiological and biochemical characteristics and probable identification of the bacteria isolated from wooden toothpicks

Toothpick Isolate Code	Gram's Reaction	Catalase	Oxidase	Motility	Citrate	Indole	Coagulase	Methyl Red	Voges Proskauer	Glucose	Sucrose	Lactose	Maltose	Identified Bacterium
AG 1	Gram Positive Rod	+	+	-	-	-	-	-	+	A	A	N	A	*Bacillus* sp
AG 2	Gram Positive Cocci	+	-	-	+	-	+	-	+	AG	A	AG	AG	*Staphylococcus aureus*
IW 1	Gram Positive Rod	+	+	+	+	-	-	-	-	A	N	N	A	*Bacillus* sp
SC 1	Gram Negative Rod	+	-	+	-	+	-	+	-	A	AG	A	A	*Escherichia coli*
SC 2	Gram Negative Rod	+	+	+	-	+	-	-	-	A	A	N	A	*Escherichia coli*
AG 2b	Gram Positive Rod	+	+	+	-	-	-	-	-	A	A	N	A	*Bacillus* sp
IW 1b	Gram Positive Rod	+	+	+	+	-	-	-	-	A	A	N	A	*Bacillus* sp
IW 1c	Gram Negative Rod	+	+	+	+	-	-	-	-	N	N	N	N	*Pseudomonas* sp
IW 2b	Gram Negative Rod	+	+	+	+	+	-	+	-	A	A	A	A	*Escherichia coli*
BG 2	Gram Positive Rod	+	+	+	+	-	-	-	-	N	N	N	N	*Bacillus* sp
AG 1b	Gram Positive Rod	+	+	+	-	+	-	-	-	N	A	N	N	*Bacillus* sp
SC 2b	Gram Negative Rod	+	+	+	+	+	-	-	-	N	N	N	N	*Pseudomonas* sp
BG 1	Gram Positive Rod	+	-	+	+	-	-	+	-	AG	AG	A	AG	*Bacillus* sp
BG 1	Gram Positive Rod	+	-	+	+	-	-	-	-	AG	AG	N	AG	*Bacillus* sp.

Key: Restaurant location - AG = Agip; IW = Iwofe; SC = Staff Club; BG = Back-Gate

Citation: Ogbonna *et al*. (2025). Microbial flora and antibiogram of bacteria associated with wooden toothpicks used in restaurants in Port Harcourt Metropolis. *International Journal of Microbiology and Applied Sciences*. *4(2)*: 51 – 59.

The cultural characteristics, morphology of fungi isolated from toothpick in restaurants is presented in Table 3. Fourteen (14) bacterial species belonging to four (4) genera were identified with the bacteria isolated from wooden toothpicks from the different restaurants. While a total of 10 fungal species belonging to three (3) fungal genera were isolated. The occurrence (%) of bacteria presented in Figure 1 were; *Bacillus* sp. (57%), *Pseudomonas* sp. (14.29%), *Staphylococcus* sp. (7.14%) and *Escherichia* sp. (21.43%). While the occurrence (%) of fungi as presented in Figure 2 were *Saccharomyces* sp. (66.7%), *Penicillium* sp. (16.7%) and *Mucor* sp. (8.3%). *Bacillus* sp and *Saccharomyces* sp recorded the highest occurrence for bacteria and fungi respectively.

Table 3: Cultural characteristics, morphology of fungi isolated from toothpick in restaurants

S/N	Macroscopic/Colony Appearance	Microscopic/Morphology	Fungal Isolate
1	Milky, mucoid, convex, 2mm growth/cream colour, mucoid growth.	Oval shaped cells.	*Saccharomyces* sp. (2)
2	Green growth with white radial periphery and brown reverse.	Branching septate hyphae with chain like conidia head.	*Penicillium* sp. (6)
3	Initial white, then brown colour, fluffy growth with yellow reverse.	Aseptate hyphae with rod conidia head.	*Mucor* sp. (2)

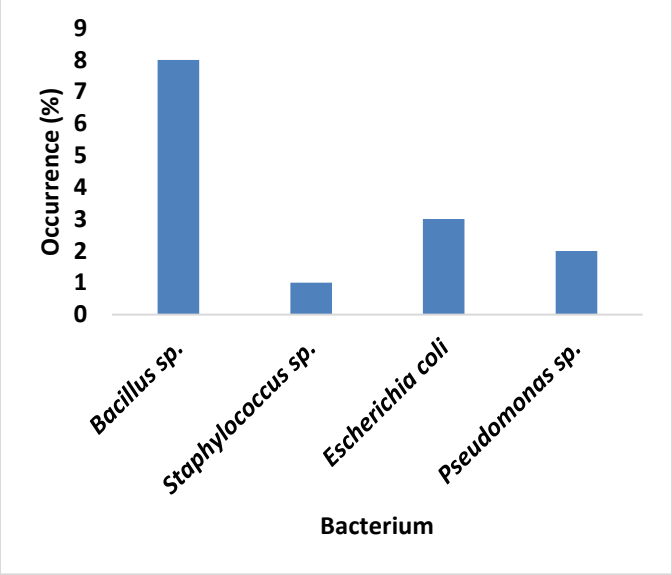

Fig. 1: Percentage Occurrence of Bacteria isolated from toothpick samples

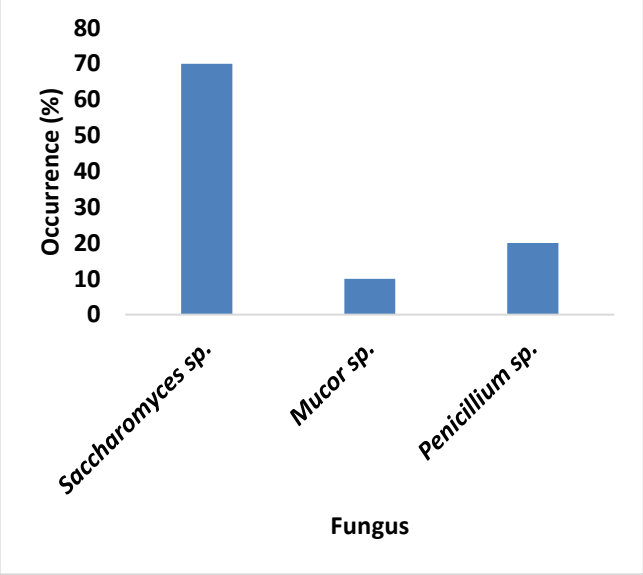

Fig. 2: Percentage Occurrence of fungi isolated from toothpick samples

Tables 4 and 5 shows the susceptibility pattern of the bacteria isolates. The results showed that *Bacillus* sp. isolates were highly susceptible to antibiotics like gentamicin and levofloxacin with 100% susceptibility but resistant to ciprofloxacin.

Escherichia coli isolates were also susceptible to ofloxacin and gentamicin with 100% susceptibility but also showed resistance to antibiotics like streptomycin and aureomycin at (33.3%) respectively.

Citation: Ogbonna *et al*. (2025). Microbial flora and antibiogram of bacteria associated with wooden toothpicks used in restaurants in Port Harcourt Metropolis. *International Journal of Microbiology and Applied Sciences*. *4(2)*: 51 – 59.

Table 4: Antibiotics Susceptibility Pattern of Gram-Negative Bacteria

Antibiotics	*Escherichia coli* (n = 3)			*Pseudomonas* sp (n =2)		
	R n (%)	I n (%)	S n (%)	R n (%)	I n (%)	S n (%)
Cefadorxil	0	0	3 (100)	1 (50)	1 (50)	0
Ofloxacin	0	0	3 (100)	0	0	2 (100)
Aureomycin	1(33.3)	0	2 (66.7)	1 (50)	0	1 (50)
Pefloxacin	1(33.3)	0	2 (66.7)	0	0	2 (100)
Ceftazidime	0	0	3 (100)	1 (50)	0	1 (50)
Gentamicin	0	0	3 (100)	1 (50)	0	1 (50)
Ciprofloxacin	0	0	3 (100)	0	0	2 (100)
Cephalexin	0	1 (33.3)	2 (66.7)	0	0	2 (100)
Trimethoprim	0	1 (33.3)	2 (66.7)	1 (50)	0	1 (50)
Streptomycin	1(33.3)	0	2 (66.7)	1 (50)	0	1 (50)

Keys: R = Resistant, I =Intermediate, S = Susceptible.

Table 5: Antibiotics Susceptibility Pattern of Gram-Positive Bacteria

Antibiotics	*Bacillus* sp (n = 8)			*Staphylococcus* sp (n =1)		
	R n (%)	I n (%)	S n (%)	R n (%)	I n (%)	S n (%)
Levofloxacin	0	0	8 (100)	0	0	1 (100)
Gentamicin	0	0	8 (100)	0	0	1 (100)
Cephalexin	0	1 (12.5)	7 (87.5)	0	1 (100)	0
Rifampin	0	0	8 (100)	1 (100)	0	0
Ceftazidime	1 (12.5)	0	7 (87.5)	1 (100)	0	0
Streptomycin	0	0	8 (100)	0	0	1 (100)
Azithromycin	0	0	8 (100)	0	0	1 (100)
Amoxicillin	0	0	8 (100)	1 (100)	0	0
Ciprofloxacin	1 (12.5)	0	7 (87.5)	0	1 (100)	0
Erythromycin	0	0	8 (100)	0	0	1 (100)

Keys: R = Resistant, I =Intermediate, S = Susceptible.

Discussion

This study showed a significant microbial load on wooden toothpicks used in restaurants in Port Harcourt metropolis. The total heterotrophic bacterial count (THBC) ranged from 1.3 x 10^6 to 21.0 x 10^6 CFU/g, which is a substantial level of microbial contamination compared to the study made by Farhadian *et* al., 2021, which had a maximum total heterotrophic bacterial count of 1.5 x10^5 CFU/toothpick. The significant difference in the total heterotrophic bacterial count (THBC) may be as a result of variations in restaurant hygiene practices or the possible difference in sampling and culturing methods.

The total heterotrophic fungal count (THFC) ranged from 1.5 x 10^4 to 216.5 x 10^4 CFU/g. These counts indicate a high level of microbial contamination, which raises concerns about the hygiene practices and safety of wooden toothpicks used in restaurants in Port Harcourt metropolis. Several factors such as the porous nature of wooden toothpicks and the organic composition of the wood provides an ideal environment for the colonization of microorganisms and also serves as a nutrient source for their growth (Johnson and Guthmiller, 2007; Tsugita *et al.,* 2017).

Citation: Ogbonna *et al*. (2025). Microbial flora and antibiogram of bacteria associated with wooden toothpicks used in restaurants in Port Harcourt Metropolis. *International Journal of Microbiology and Applied Sciences*. *4(2)*: 51 – 59.

The bacterial population varied significantly among samples from different locations (p = 0.001). Samples from Iwofe (IW 1) and Staff Club (SC 1 and SC 2) showed the highest bacterial counts, suggesting that the restaurants in these locations may have poorer hygiene practices or storage conditions for toothpicks.

The fungal population also showed significant variation among samples (p = 0.00). Notably, samples from Staff Club (SC 1 and SC 2) exhibited extremely high fungal counts (207.0 x 10^4 and 216.5 x 10^4 CFU/g, respectively), which were substantially higher than those from other locations. This indicates a potential fungal contamination issue specific to the restaurant in the Staff Club location.

This study identified fourteen (14) different bacteria species belonging to four (4) main bacterial genera. They include: *Bacillus* sp. (57%), *Escherichia coli* (21.43%), *Pseudomonas* sp. (14.29%), *Staphylococcus aureus* (7.14%).

The predominance of *Bacillus* species is not surprising, as it is a common environmental bacterium. However, the presence of *Escherichia coli*, a fecal indicator organism, and *Staphylococcus aureus,* a potential pathogen, is concerning and suggests possible contamination from human handling or unsanitary conditions. The presence of these organisms are in alignment with the study made by Jones *et al.,* (2021) who also isolated these organisms during their research.

This study also identified ten (10) different fungal species belonging to three (3) main fungal genera. They include: *Saccharomyces* sp. (66.7%), *Penicillium* sp. (16.7%), *Mucor* sp (8.3%). The high prevalence of *Saccharomyces,* a yeast genus, could be attributed to environmental factors or cross-contamination during the manufacturing processes of these wooden toothpicks and packaging and storing of these wooden toothpicks in restaurants. Huang *et al.* (2018) had reported that, bacterial, fungal, as reported in this present study; and viral pathogens are among the microorganisms that have been identified on wooden toothpicks. These contaminants can pose potential health implications, ranging from oral infections to more systemic illnesses (Farhadian *et al.,* 2021).

The high prevalence of microorganisms in wooden toothpicks indicates the lack of proper hygienic practices from their production and distribution, down to restaurant owners in the storage of these toothpicks for customer's usage. The variation in microbial load and species diversity across different samples indicates that factors such as storage conditions, handling practices, and environmental exposure play crucial roles in determining the level of microbial contamination on wooden toothpicks (Jumpponen, 2019).

Antibiotic susceptibility for Gram-negative bacteria revealed that, *Escherichia coli* isolates showed 100% susceptibility to several antibiotics, including cefadorxil, ofloxacin, ceftazidime, gentamicin, and ciprofloxacin. This suggests that these antibiotics could be effective in treating infections caused by strains of *Escherichia coli* if necessary. However, some resistance was observed against aureomycin, pefloxacin, and streptomycin (33.3% resistance each). This result was in agreement with the research done by Rasheed *et al.,* 2014, who also had *Escherichia coli* isolates that showed slight resistance to these same antibiotics. *Pseudomonas* sp showed 100% susceptibility to ofloxacin, pefloxacin, and ciprofloxacin, but 50% resistance to cefadorxil, aureomycin, ceftazidime, gentamicin, trimethoprim, and streptomycin. The variation in the resistance profile highlights the importance of proper antibiotic selection in case of infections gotten from the usage of wooden toothpicks. Antibiotic susceptibility for Gram-positive bacteria revealed that, *Bacillus* sp isolates demonstrated high susceptibility to most antibiotics tested, with 100% susceptibility to levofloxacin, gentamicin, rifampin, streptomycin, azithromycin, amoxicillin, and erythromycin. This result was in agreement with the research done by Maduka & Olie (2023), who also had *Bacillus* species that were susceptible to these same antibiotics. Minor resistance was observed for ceftazidime and ciprofloxacin (12.5% each). The single *Staphylococcus aureus* isolate showed complete resistance to rifampin, ceftazidime, and amoxicillin, which is concerning given the pathogenic potential of this species. This may be as a result of the acquisition of resistance genes. However, it was susceptible to several other antibiotics, including levofloxacin, gentamicin, streptomycin, azithromycin, and erythromycin.

Citation: Ogbonna *et al*. (2025). Microbial flora and antibiogram of bacteria associated with wooden toothpicks used in restaurants in Port Harcourt Metropolis. *International Journal of Microbiology and Applied Sciences*. *4(2)*: 51 – 59.

The presence of antibiotic-resistant strains among the isolates from wooden toothpicks is particularly worrisome, as it suggests that wooden toothpicks could potentially serve as vectors for the transmission of drug-resistant pathogens. The high prevalence of microorganisms of wooden toothpicks underscores the need for appropriate measures should be taken to reduce the risk of infection gotten from the usage of wooden toothpicks.

Conclusion

This study has showed a substantial level of microbial contamination of wooden toothpicks in restaurants. This is an indication that wooden toothpicks can serve as vehicles for various microbial contaminations which poses potential risk to oral and systemic health. The presence of potentially pathogenic organisms such as *Escherichia coli*, *Pseudomonas* sp. and *Staphylococcus aureus,* on wooden toothpicks raises the risk of systemic infections if these organisms enter the bloodstream through micro-injuries in the oral cavity.

In light of the findings from this study, appropriate measures should be taken to reduce the risk of infection gotten from the usage of wooden toothpicks. it is important to adopt and promote the usage of hygienic alternatives such as interdental brushes, floss picks and other tools which are made from materials that have low affinity for microbial contamination. There should also be improved manufacturing and storage practices which includes minimizing exposure to contaminants during manufacturing and proper packaging with moisture-proof containers during storage which minimizes exposure to air, moisture and human contact to reduce the risk of microbial contamination.

References

Buchanan, R. E., & Gibbons, N. E. (Eds.). (1974). *Bergey's manual of determinative bacteriology* (8th ed.). Baltimore, MD: Williams & Wilkins

Cheesborough, M. (2006). *Medical laboratory manual for tropical Countries.* Butterworth Heinemarm Ltd., Oxford. United Kingdom. *pp* 200-234.

CLSI, (2020). *Performance standards for antimicrobial susceptibility testing. CLSI approved standard M100-S23. Clinical and Laboratory Standard Institute, Wayne*, *pp* 72-76.

Costin, D. (2024). *ABIS online – Advanced Bacterial Identification Software*. Retrieved June 30, 2025, from https://www.tgw1916.net/bacteria_logare.html

Ellis, D., Davis, S., Handke, R. and Barkely, R. (2007). *Description of Medical Fungi, Second Edition*, Nexus Print Solutions, Adelaide, South Australia. *pp* 61-67

Farhadian, A. (2021). Microbial quality of wooden toothpicks in restaurants and fast-food outlets: A cross-sectional study. *Journal of Food Protection, 84*(1), 85-91.

Garcia, D., Sanchez, M., & Perez, A. (2021). Molecular characterization of bacteria isolated from wooden toothpicks. *Microbial Ecology, 82*(2), 422-431.

Garcia, E., Perez, M., & Martinez, A. (2022). Meta-analysis of microbial contamination on wooden toothpicks. *Environmental Microbiology Reports, 14*(3), 378-385.

Huang, J. (2018). Microbiological quality and safety analysis of wooden toothpicks obtained from hotels in Wuhan city, central China. *Journal of Hygiene Research, 47*(2), 243-249.

Johnson, G. K., & Guthmiller, J. M. (2007). The impact of cigarette smoking on periodontal disease and treatment. *Periodontology 2000, 44*, 178-194.

Johnson, K., Williams, R., & Brown, C. (2024). Whole-genome sequencing of *Enterococcus faecalis* strains isolated from wooden toothpicks in a healthcare setting. *Infection Control & Hospital Epidemiology, 45*(1), 10-17.

Jones, T. S. (2019). Risk behavior and pathogens associated with wooden toothpick use. *Journal of Dental Hygiene, 93*(5), 21-27.

Jumpponen, A. (2019). Wooden toothpicks: To eat or not to eat. *Manufacturing Engineering, 164*(4), 89-93.

Karaca, O. B., Akkus, S., & Ozdemir, T. (2022). Identification of bacteria in toothpicks produced by using different packaging materials. *Food Science and Technology, 45*(1), 10-17.

Citation: Ogbonna *et al*. (2025). Microbial flora and antibiogram of bacteria associated with wooden toothpicks used in restaurants in Port Harcourt Metropolis. *International Journal of Microbiology and Applied Sciences*. *4(2)*: 51 – 59.

Kidd, S., Halliday, C. & Ellis, D. (2023). *Descriptions of Medical Fungi (4th Edition). CABI Publishing,* UK.
Kirschner, A. K. T., & Melzner, D. (2017). Influence of temperature and mouse strain on the composition of wild-type microbiota. *FEMS Microbiology Ecology, 93*(11), 1-11.

Lee, C., & Zhang, S. (2018). Microstructure and mechanical properties of bamboo. In *Handbook of Bamboo Utilization*. *Springer*, Singapore, pp 95-128.

Maduka, N. & Olie, R. (2023). Microbiological Assessment and antibiotic susceptibility pattern of bacterial isolates from exposed toothpicks in selected eateries. *African Journal of Health, Safety and Environment*, *41*(1), 45-46.

Martinez, D., Rodriguez, J., & Garcia, E. (2023). Isolation and characterization of *Staphylococcus aureus* from wooden toothpicks. *Journal of Clinical Microbiology, 61*(2), e02543-22.

Nascimento, M. M. (2020). Wooden toothpicks contaminated by bacteria: Prevalence, profile of pathogens and antimicrobial susceptibility. *Brazilian Dental Journal, 31*(2), 170-179.

Ng, Y. L., Mann, V., Rahbaran, S., Lewsey, J., & Gulabivala, K. (2008). Outcome of primary root canal treatment: systematic review of the literature - Part 2. Influence of clinical factors. *International Endodontic Journal, 41*(1), 6-31.

Obire, O. and Ogbonna, S.I. (2017). Antimicrobial Activity of Some Seed Extracts on Bacteria Isolated from Maize Slurry (Akamu) in Port Har court Metropolis. *Current Studies in Comparative Education, Science and Technology*, *4*(1), 188-202

Ogbonna, S.I., Akani, N.P., Williams, J. O. & Peekate, L. P. (2022). Prevalence and Antibiogram of Bacteria Isolated from Saloon Equipment in Rivers State, Nigeria. *International Journal of Health and Pharmaceutical Research*, *7* (1), 2695-2165.

Rasheed, M. U., Thajuddin, N., Ahamed, P., Teklemariam, Z., & Jamil, K. (2014). Antimicrobial drug resistance in strains of *Escherichia coli* isolated from food sources. *Revista do Instituto de Medicina Tropical de São Paulo*, *56*(4), 341-346.

Sedgley, C. M., Lennan, S. L., & Appelbe, O. K. (2005). Survival of *Enterococcus faecalis* in root canals ex vivo. *International Endodontic Journal*, 38(10), 735-742.

Smith, J., Brown, A., & Patel, K. (2021a). Microbial composition of wooden toothpicks: Implications for hygiene. *Journal of Applied Microbiology, 131*(5), 2231-2240.

Smith, J., Brown, A., & Patel, K. (2021b). Microscopic analysis of wooden toothpick surfaces: Implications for oral hygiene. *Journal of Microscopy, 279*(3), 236-245.

Stuart, C. H., Schwartz, S. A., Beeson, T. J., & Owatz, C. B. (2006). *Enterococcus faecalis*: Its role in root canal treatment failure and current concepts in retreatment. *Journal of Endodontics, 32*(2), 93-98.

Taylor, D. J., Green, N. P. O. & Stout, G. W. (2008). *Biological Science (6th ed.). Cambridge University Press*, England, pp 35-37.

Tsugita, T. (2017). Characterization and comparison of the oral microbiome of edentulous infants and their mothers using high-throughput sequencing. *Scientific Reports,* 7, 44285.

Citation: Ogbonna *et al*. (2025). Microbial flora and antibiogram of bacteria associated with wooden toothpicks used in restaurants in Port Harcourt Metropolis. *International Journal of Microbiology and Applied Sciences*. *4(2)*: 51 – 59.