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RESEARCH ARTICLE **AJHSE040105**

Microbiological Assessment and Antibiotic Susceptibility Pattern of Bacterial Isolates from Exposed Toothpicks in Selected Eateries

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ABSTRACT

Toothpick is mainly used to remove food particles, fishbone and meat stuck between the teeth after a meal. Poor handling and exposure of toothpicks on dinner tables in many eateries could pose a public health risk. In this study, a total of one hundred (100) wooden toothpicks were randomly sampled from twenty (20) eateries in five (5) locations in Benin City. A packet of toothpick purchased from a shop served as control. Microbiological analysis and antibiotic sensitivity tests involved the use of standard methods and disc agar diffusion method, respectively. The percentage occurrence of bacterial isolates from the samples include *Bacillus* sp. (25 %), *Staphylococcus* sp. (20 %), *Streptococcus* sp. (20 %), *Proteus* sp. (15 %), *Salmonella* sp. (10 %), *Escherichia coli* (5 %) and *Klebsiella* sp. (5 %) while the fungal isolates include *Saccharomyces cerevisiae* (80 %), *Penicillium* sp. (10 %) and *Mucor* sp. (10 %). The bacterial isolates were resistant to many antibiotics used in the study with the exception of gentamicin and ofloxacin. In order to prevent microbial contamination of toothpicks in the eateries which could lead to disease transmission, toothpicks should be rinsed with potable water before using it. Toothpicks should not be exposed on dinner tables. Instead, automatic toothpick dispenser should be provided in eateries.

Keywords: *Antibiotic resistance, Dental hygiene, Eating accessories, Fomite, Oral hygiene products*

INTRODUCTION

Poor handling of toothpicks in many eateries in Nigeria could lead to microbiological contamination of toothpicks which poses a health risk to customers who regularly make use of eating accessories especially exposed toothpicks to remove food particles trapped by their teeth after a meal. Toothpick is among the few ancient inventions still in use till date (Lozano *et al*., 2013). As far back as 7000 BC, toothpicks was among the oral hygiene products used by the people. Excavations revealed that Sumerians of 3000 BC used gold extensively to decorate toothpick which they used in maintaining oral hygiene (Gurudath *et al*., 2012). Cast bronze pendants produced by ancient Chinese were used as toothpicks. This practice spread to Europe and became popular between 15th and 19th Century (Fischman, 2000). Today, toothpicks are very common and affordable unlike many centuries ago when it was associated with the elitist class in the society (Fischman, 2000).

Toothpick is a small wooden stick characterized by a sharp end in one or both sides. This object is a description of what is referred as 'modern toothpick'. Although wood is commonly used to produce toothpicks, other materials such as bamboo, bone, ivory plastic, quills and metal could also be used as long as the finished product has a sharp end. Toothpick fashioned from these materials were common in ancient times. Today, toothpick made from wood has become predominant (Christen and Christen, 2003; Lozano *et al*., 2013; Hu and McGwin, 2021). According to Jardim *et al*. (2009), twigs made from wood is known as 'primitive toothpicks'. Mastix tree (*Pistacia lenticus*) commonly known as 'toothpick tree' was commonly used by Greeks and Romans to produce toothpick (Fischman, 2000). Toothpicks are round or triangular in shape (Buunk-Werkhoven, 2017). It is commonly used to remove food particles stuck in the teeth after eating which leaves the individual with a slight feeling of discomfort (Fischman, 2000; Ng and Lim, 2019). The sharp end of toothpick aid in the removal of dental plaques (Zanatta *et al*., 2008; Rudak and Andruškienė, 2019). For many centuries, toothpick has been part of the tools used in maintaining oral hygiene (Mitha *et al*., 2018).

In time past, toothpicks used to be a part of the items found in the personal kit that also contain an ear wax scoop and depilatory tweezers. Archeological studies suggests that golden toothpicks was part of the items found in a famous toilet set used by a Mesoptamian king around 3000 B.C.E (Fischman, 1997; Gurudath *et al*., 2012). It is believed that toothpick evolved into chew stick after many years. Around 1600 B.C., the Chinese started using chew stick which had one end chewed (like a brush) and the other pointed (like a toothpick) (Gurudath *et al*., 2012). The habit of chewing toothpick could promote oral health (Buunk-Werkhoven, 2017). However, dentists do not recommend the use of toothpicks (Lozano *et al*., 2013). According to Christen and Christen (2003), indiscriminate use of toothpicks might result to mouth ulcers, allergic reactions, sensitive teeth, halitosis, gingival abscesses, dental caries, among others. The habit of using toothpick has been associated with the development of Lemierre's syndrome (Wu *et al*., 2013). Although toothpick is mainly used to maintain dental hygiene, it is part of the items used in food service and preparation as well as art projects executed at home (Hu and McGwin, 2021).

In 1869, toothpick was patented and later introduced in restaurants in 1870 by Charles Forster (Buunk-Werkhoven, 2017; Hu and McGwin, 2021). In many restaurants, eateries and bars, it is common to see toothpicks inside saucer or plates on top of dining tables. This practice exposes toothpicks to cross contamination. Exposed toothpicks could be contaminated with dust, sweat, saliva, nasal fluids, houseflies, cockroaches and other vectors. Many customers have formed the habit of using unwashed fingers which they have already used to touch different surfaces inside or outside the eatery to collect toothpick and insert in their mouth (Obi *et al*., 2021). Although toothpick

has not been listed as a leading fomite, there are concerns that exposed toothpicks could be a vehicle for transmitting infectious pathogens to humans (Elom *et al*., 2014). Wooden toothpick harbour bacteria and other microorganisms because wood is a naturally porous material which possess tiny fissures and grooves (Annett *et al*., 2005).

A study carried out by Elom *et al*. (2014) showed that exposed toothpicks were contaminated by *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus* sp., *Salmonella* sp. yeast spores, fungal spores, *Giardia lamblia* cyst and *Ascaris lumbricoides*. The study did not subject the bacterial isolates to antibiotic susceptibility test to ascertain which antibiotics should be recommended for persons infected by pathogens isolated from exposed toothpicks. Therefore, the aim of this study was to carry out microbiological assessment and antimicrobial sensitivity test of bacterial isolates found in exposed toothpicks in selected eateries in Benin City, Nigeria.

MATERIALS AND METHODS

A total of one hundred (100) toothpicks were collected from twenty (20) eateries located at Irhirihi, Siluko, Ugbowo, Airport Road and UBTH environs in Benin City. A brand new toothpick inside a plastic container purchased from Oka market serve as control. Figure 1 shows a Google map specifying the location of the eateries and the market where the control was obtained. Each sample which comprise of five (5) toothpicks was collected aseptically from each of the eateries using sterile transparent zip-lock bags. All the samples were put inside a big sterile cellophane bag and immediately transported to the Microbiology Laboratory, Wellspring University, Benin city, within three (3) hours sampling for analyses.

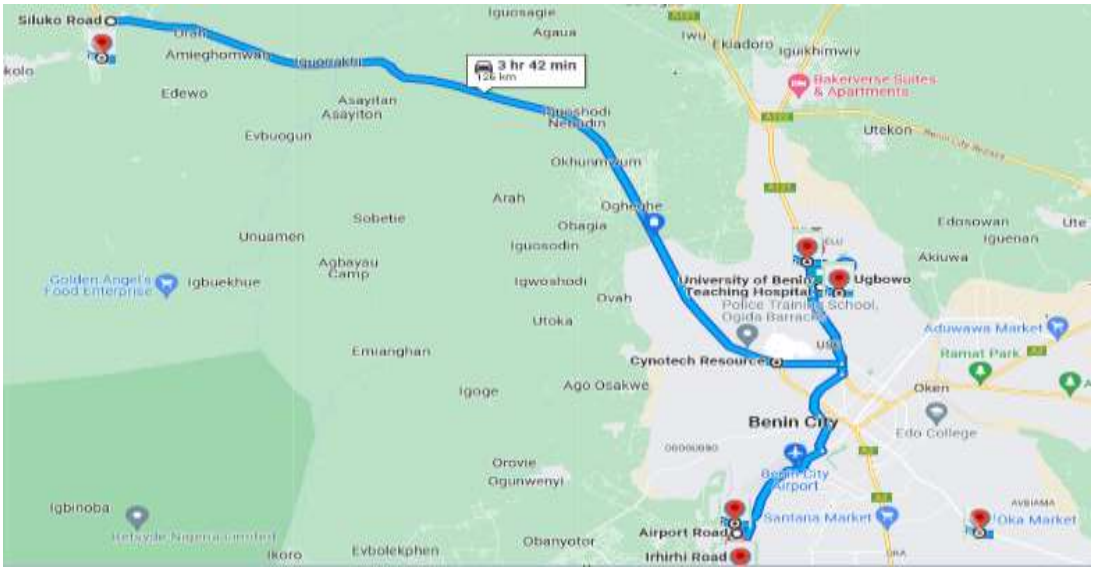

Figure 1: Google map of Benin City specifying the locations of the eateries and the market where the control was obtained

Serial dilution

Five (5) toothpicks from each eatery were soaked in 5 ml sterile peptone water for 30 minutes using a sterile universal container. At intervals, the toothpicks inside the peptone water were agitated vigorously. Sterilized forceps were used to remove the toothpicks from the solution. Nine millilitre (9 ml) of sterile distilled water was dispensed into sterile

test tubes and 1 ml solution of each sample was transferred into the first test tube using a sterile pipette which constitute 10^{-1} homogenate. Stepwise transfer of 1 ml solution from the homogenate into other test tubes containing 9 ml sterile distilled water was done using a sterile pipette for each transfer until 10^{-5} dilution was achieved.

Microbiological analysis

Determination of total heterotrophic bacterial counts

Exactly 0.1 ml dilution 10^{-3} of each sample was used to inoculate into sterile Petri dishes containing molten sterilized nutrient agar (NA) and this was done in duplicates. The inoculated Petri dishes were gently rocked anti-clockwise and allowed to solidify. The inoculated plates were incubated at 37 ^{o}C for 24 h. Upon incubation, the total number of colonies formed were counted manually and the result was recorded. The bacterial population was calculated using the formula below and expressed as colony forming unit per millilitre (CFU/ml).

$$\text{CFU/ml} = \text{no. of colonies} \times \frac{1}{dilution\ factor} \times \frac{1}{volume\ plated} \quad - \quad - \quad - \quad - \quad \text{i}$$

Determination of total coliform counts

Exactly 0.1 ml dilution 10^{-3} of each sample was transferred into sterile Petri dishes containing molten sterilized MacConkey agar and this was done in duplicates. The inoculated plates were gently rocked anti-clockwise and allowed to solidify. The inoculated plates were incubated at 37 ^{o}C for 24 h. Colonies appearing purplish-red surrounded by reddish zone of precipitated bile were counted manually and the result was recorded. The total coliform count of each sample was calculated using the formula below and expressed as colony forming units (CFU) per millilitre.

$$\text{CFU/ml} = \text{no. of colonies} \times \frac{1}{dilution\ factor} \times \frac{1}{volume\ plated} \quad - \quad - \quad - \quad \text{ii}$$

Determination of total fungal counts

Exactly 0.1 ml dilution 10^{-3} of each sample was inoculated into a sterile Petri dish containing molten sterilized potato dextrose agar (PDA) and this was done in duplicates. The inoculated Petri dishes were gently rocked anti-clockwise and allowed to solidify. The inoculated plates were incubated at 28±2 ^{o}C for 5 days. After incubation, the total number of colonies were counted manually and the result was recorded. The fungal population of each sample was calculated using the formula below and expressed as colony forming units (CFU) per millilitre.

$$\text{CFU/ml} = \text{no. of colonies} \times \frac{1}{dilution\ factor} \times \frac{1}{volume\ plated} \quad - \quad - \quad - \quad \text{iii}$$

Determination of pure culture of the isolates

Repeated subculturing of the bacterial and fungal isolates were carried out using freshly prepared NA and PDA, respectively until pure cultures were obtained. Incubation of the culture plates for growth of bacteria and fungi were carried out at 37 ^{o}C and 28±2 ^{o}C for a period of 24 and 120 h, respectively.

Characterization and identification of bacterial isolates

Pure culture of bacterial isolates from the samples were characterized based on their cultural and morphological characteristics followed by Gram staining and biochemical tests. The tests carried out include catalase, coagulase, urease, oxidase, citrate, indole, motility and sugar fermentation (Shoaib *et al*., 2020).

Identification of fungal isolates

The colonial characteristics and morphology of pure culture of fungi in the PDA culture plates were observed and noted. Two (2) drops of lactophenol was placed on a clean glass slide. Mycelia growth on the culture plates were teased out and placed on lactophenol. A coverslip was gently placed on top of the preparation and observed under the microscope using x10 and x40 objective lens. Based on morphological characteristics, the fungal isolates were identified using a guide published by Watanabe (2010).

Antibiotics susceptibility test

The antibiotics susceptibility pattern of the bacterial isolates from the toothpicks was performed in vitro using a modified disk diffusion test of Kirby-Bauer method on Mueller-Hinton agar according to the method described by the Clinical Laboratory Standard Institute (CLSI) (NCCL, 2003). Antibiotic disks (ABTEK) were used in assaying antibiotic resistance of the bacterial isolates. The names of the antibiotics contained in the antibiotic disks are ceftazidime, cefuroxime, gentamicin, cefixime, ofloxacin, augmentin, nitrofurantoin, ciprofloxacin, erythromycin, ceftriaxone and cloxacillin, Forceps were sterilized and used to lift the commercially prepared antibiotic disc and gently placed on top of Mueller-Hinton agar surface of 24 h old culture of the test organisms. The culture plates were placed in inverted position and incubated at 37 ^{0}C for 24 h. The diameters of zones of inhibition after incubation of the plates were measured using a transparent metre ruler. The results were interpreted as resistant (≤19 mm) and sensitive (≥ 23 mm) in accordance with standard specified by CLSI.

RESULTS AND DISCUSSION

Figure 2 shows the total heterotrophic bacterial counts (THBC) of exposed toothpicks obtained from the eateries at different locations. The THBC of toothpicks obtained from Siluko, Ugbowo, Irhirihi, Airport Road and UBTH environs were within the range 4.95-5.48, 4.90-5.61, 4.60-5.48, 4.0-5.18 and 5.04-5.45 $\log_{10}$CFU/ml, respectively.

Depicted in Figure 3 is the total coliform counts (TCC) which ranged between 3.04 and 5.44 $\log_{10}$CFU/ml of exposed toothpicks obtained from eateries in different locations. Although an acceptable limit for THBC and TCC of toothpicks could not be accessed, fairly acceptable bacterial counts of crockery and utensils between 5.0 x 10^4 and 2.5 x 10^5 CFU/ml per container is the standard recommended by Public Health Service, USA (Maori and De, 2010).

Table 1 and Figure 5 shows the characterization and the percentage frequency of occurrence of the isolates. The percentage occurrence of the bacterial isolates include *Bacillus* sp. (25 %), *Streptococcus* sp. (20 %), *Staphylococcus* sp. (20 %), *Proteus* sp. (15 %), *Klebsiella* sp. (5 %), *Salmonella* sp. and *Escherichia* coli (5 %). In a related study, Elom *et al*. (2014) reported the presence of *Staphylococcus aureus* (15.09 %), *Escherichia coli* (15.09 %), *Streptococci* sp. (9.43%), *Klebsiella* sp. (7.55 %) and *Proteus* sp. (3.77 %) in toothpicks. Some of the bacterial genera reported in this study were isolated from patients suffering from periodontal disease (Jabuk *et al*., 2015).

Bacillus sp. (25 %) had the highest percentage occurrence compared with other bacterial isolates encountered in the toothpicks. This could be attributed to wide distribution of *Bacillus* sp. in nature. Air and dust which harbours *Bacillus* sp. spores are possible sources of contamination. The presence of *Streptococcus* sp. in the toothpicks could be from saliva. *Streptococcus* sp. is part of the normal flora of the buccal cavity and throat. Contamination of exposed toothpicks by *Staphylococcus* sp. could be as a result of excessive handling. *Staphylococcus aureus* is part of normal flora of the skin, throat, hairs and palms. A possible source of contamination of toothpicks by *Proteus* sp. in the eateries is from the floor (Ahaotu *et al*., 2019). The presence of *Klebsiella* sp. in exposed toothpicks in the eateries could be attributed to unhygienic handling and dirty environment. In many eateries, frequent cleaning of tables and counters where foods and drinks are served customers especially during business hours is neglected. Some waiters and customers return toothpicks that accidentally fell on the table to its container to avoid wastages. In a related study, Maki (2019) isolated *Klebsiella pneumoniae* and other organisms from dining tables located in home kitchens. Isolation of *Escherichia coli* from exposed toothpicks is a thing of concern because it is an indication of fecal contamination. Dirty toilets and unhygienic practices of some individuals who use unwashed hands to touch toothpicks will contaminate it. The THBC and bacterial genera identified in the control (packaged toothpick) was 2.30 log10CFU/ml and *Bacillus* sp., respectively. However, no culturable fungi was isolated from the control.

Figure 4 shows the total fungal counts (TFC) of exposed toothpicks obtained from different eateries. The result obtained showed that TFC of exposed toothpicks obtained from Siluko, Ugbowo, Irhirihi, Airport Road and UBTH environs were within the range 2.08-3.95, 1.90-3.15, 2.48-3.40, 2.08-3.23 and 1.11-3.04 log_{10}CFU/ml, respectively. Colonial characteristics and morphology of fungi isolated from exposed toothpicks are reported in Table 2. The percentage occurrence of the fungal isolates is depicted in Figure 6. The fungal isolates include *Penicillium* sp. (10 %), *Mucor* sp. (10 %) and *Saccharomyces cerevisiae* (80 %). In a related study, Elom *et al*. (2014) reported that yeast cells (41.51 %) and fungal spores (18 %) contaminated toothpicks obtained from restaurants, eatery and bar. A possible source of fungi isolated from exposed toothpicks in the eateries is from the environment.

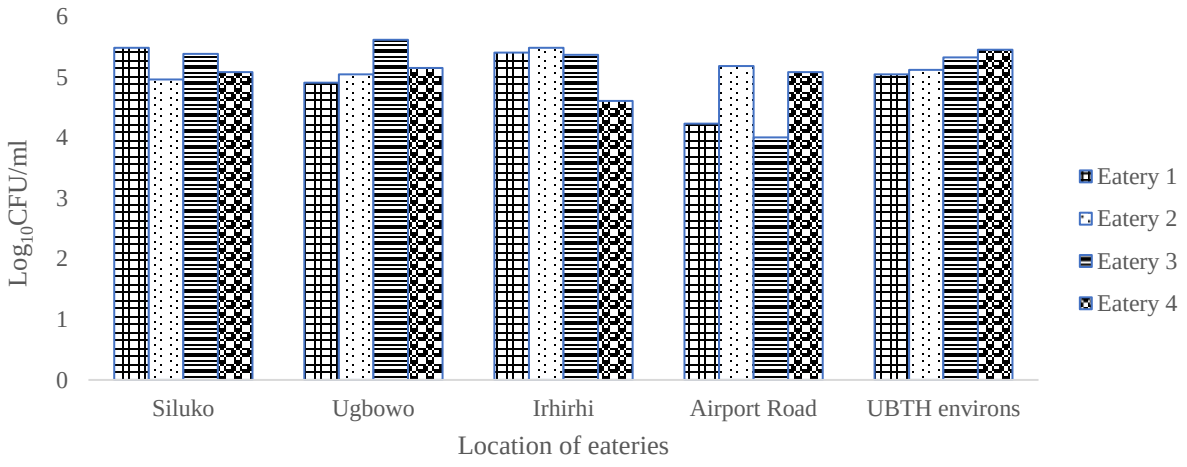

Figure 2: Total heterotrophic bacterial counts of exposed toothpicks obtained from eateries in different locations

Figure 3: Total coliform counts of exposed toothpicks obtained from eateries in different locations

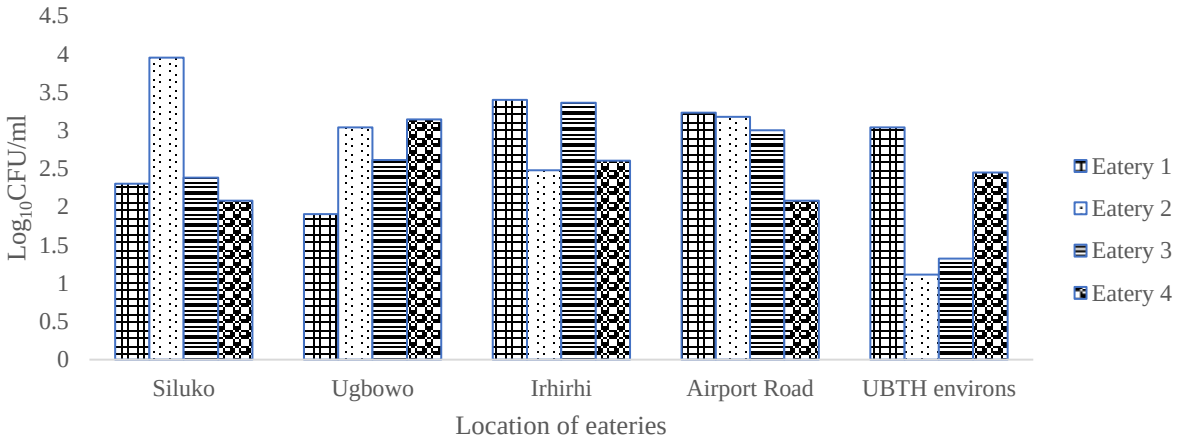

Figure 4: Total fungal counts of exposed toothpicks obtained from eateries in different locations

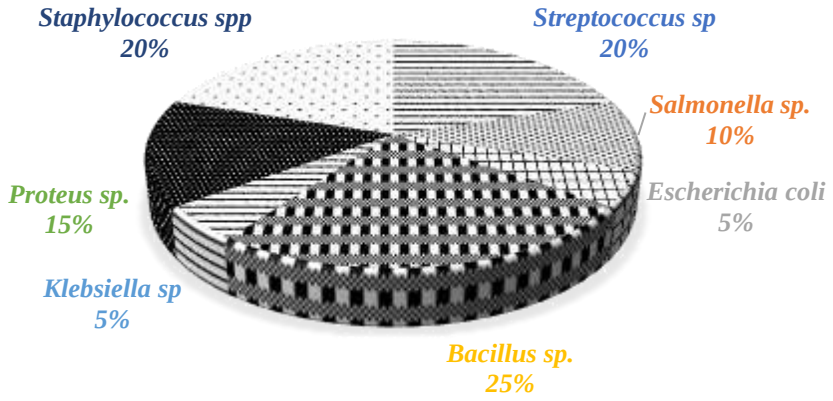

Figure 5: Percentage occurrence of bacterial isolates from exposed toothpicks

Table 1: Biochemical characterization of bacterial isolates from exposed toothpicks

Isolate code	Gram reaction	Cell shape	Cell arrangement	Catalase	Oxidase	Urease	Coagulase	Indole	Citrate	Motility	Sugar fermentation		Probable organism
											Glucose	Lactose	
SU1a	+	Rod	Cluster	+	N/A	N/A	N/A	-	N/A	+	N/A	N/A	*Bacillus* sp.
SU2a	+	Cocci	Cluster	+	-	+	+	-	-	-	+	+	*Staphylococcus* sp.
SU1b	-	Cocci	Chain	+	-	-	+	-	+	+	+	-	*Salmonella* sp.
SU3a	-	Rods	Singly	+	-	-	-	+	+	+	+	+	*Escherichia coli*
SU4a	-	Rod	Cluster	+	-	-	-	-	+	+	+	-	*Proteus* sp.
UW1a	+	Cocci	Cluster	+	-	+	+	-	-	-	+	+	*Staphylococcus* sp.
UW2a	+	Rod	Cluster	+	N/A	N/A	N/A	-	N/A	+	N/A	N/A	*Bacillus* sp.
UW3a	+	Cocci	Chain	-	-	-	-	-	+	-	+	+	*Streptococcus* sp.
UW4a	-	Cocci	Chain	+	-	-	+	-	+	+	+	-	*Salmonella* sp.
IH1a	-	Rod	Cluster	+	-	-	+	-	+	+	+	-	*Proteus* sp.
IH2a	+	Cocci	Cluster	+	-	+	+	-	-	-	+	+	*Staphylococcus* sp.
IH4a	+	Cocci	Chain	-	-	-	-	-	+	-	+	+	*Streptococcus* sp.
IH4b	+	Cocci	Chain	+	N/A	N/A	N/A	-	N/A	+	N/A	N/A	*Bacillus* sp.
AR2a	+	Rod	Cluster	+	+	+	-	-	+	-	+	N/A	*Klebsiella* sp.
AR3a	+	Cocci	Cluster	+	-	+	+	-	-	-	+	+	*Staphylococcus* sp.
AR3b	+	Rod	Cluster	+	N/A	N/A	N/A	-	N/A	+	N/A	N/A	*Bacillus* sp.
AR4a	+	Cocci	Chain	-	-	-	-	-	+	-	+	+	*Streptococcus* sp.
UE1a	+	Cocci	Chain	-	-	-	-	-	+	-	+	+	*Streptococcus* sp.
UE2a	+	Rod	Cluster	+	N/A	N/A	N/A	-	N/A	+	N/A	M/A	*Bacillus* sp.
UE3a	-	Rod	Cluster	+	-	-	-	+	-	+	-	+	*Proteus* sp.

Key: SU –Siluko, UW- Ugbowo, IH-Irhirihi, AR-Airport Road, UE-UBTH environs, Eatery 1, Eatery 2, Eatery 3, Eatery 4, a & b - Isolates, +positive, -negative, N/A-Not applicable

Table 2: Colonial characteristics and morphology of fungi isolated from exposed toothpicks

Sample code	Colonial characteristics	Morphology and cellular structure	Probable organism
H	Smooth colonies, white to cream colour, glistening or dull	Large globose to ellipsoidal budding yeast-like cells or blastoconidia	*Saccharomyces cerevisiae*
I	Colonies are velvety green to yellow	The mycelia were irregularly arranged, asymmetrical with branches of various lengths. Sparse and irregular metulae with phialides on them. Conidia smooth and ellipsoidal	*Penicillium* spp.
J	Colonies are floccose, pale or greyish-brown. They first appear white and fluffy like cotton candy. As the organism ages, it started turning brownish/greyish	Sporangiophores are erect, simple or branched, forming large, terminal, globose to spherical, multi-spored sporangia, without apophyses and with well-developed subtending columellae	*Mucor* spp.

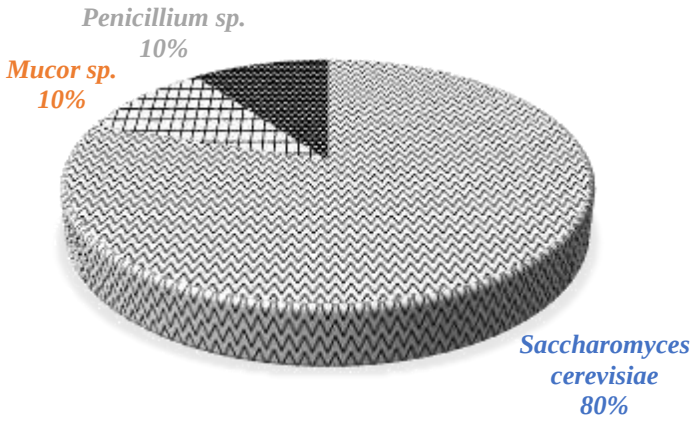

Figure 6: Percentage occurrence of fungal isolates from exposed toothpicks

Table 3 shows the antibiotic susceptibility pattern of bacterial isolates from exposed toothpicks. All the bacterial isolates were sensitive to ofloxacin and gentamicin, but resistant to ceftazidime. Gentamicin is commonly used alongside other antibiotics to treat some severe infections caused by Gram positive and negative bacteria (Tam *et al*., 2006). The bacterial

isolates were resistant to cefixime and augmentin with the exception of *Bacillus* sp. and *E. coli*, respectively. Based on the results, ofloxacin and gentamicin are recommended for treatment of infections associated with the contaminated toothpicks. *Klebsiella* sp., *Proteus* sp., *E. coli* and *Salmonella* sp. were sensitive to both nitrofurantoin and ciprofloxacin. According to Ehiaghe *et al.* (2020), ciprofloxacin and ofloxacin are popular antibiotics capable of inhibiting the growth of many bacterial species.

Table 3: Antibiotic susceptibility pattern of bacteria isolated from exposed toothpicks

	Zones of inhibition (mm) in diameter/status										
Isolates/Antibiotics	CRX	CAZ	GEN	ERY	CTR	CXC	OFL	AUG	NIT	CPR	CXM
Streptococcus sp.	14(R)	12(R)	26(S)	14(R)	16(R)	14(R)	24(S)	14(R)	-	-	-
Bacillus sp.	24(S)	23(R)	24(S)	22(S)	14(R)	12(R)	26(S)	13(R)	-	-	-
Klebsiella sp.	13(R)	14(R)	25(S)	-	-	-	25(S)	12(R)	24(S)	25(S)	15(R)
Proteus spp.	16(R)	12(R)	23(S)	-	-	-	24(S)	15(R)	26(S)	24(S)	13(R)
Escherichia coli	13(R)	14(R)	26(S)	-	-	-	26(S)	25(S)	23(S)	24(S)	14(R)
Salmonella sp.	14(R)	13(R)	25(S)	-	-	-	26(S)	15(R)	24(S)	24(S)	16(R)

Key:
CAZ- ceftazidime (30 µg), CXM – cefuroxime (30 µg), GEN – gentamicin (10 µg), CRX – cefixime (5 µg), OFL- ofloxacin (5 µg), AUG – augmentin (30 µg), NIT – nitrofurantoin (30 µg), CPR- ciprofloxacin (5 µg), ERY – erythromycin (5 µg), CTR- ceftriaxone (30 µg), CXC - cloxacillin **(**5 µg), **S -** Sensitive; **R** - Resistance

CONCLUSION

Wooden toothpicks obtained from the eateries at different locations were contaminated by pathogenic microorganisms. The bacterial and fungal isolates from the exposed toothpicks belong to seven (7) and three (3) genera, respectively. Surprisingly, the control was also contaminated by bacteria. The use of microbial contaminated toothpicks to remove food particles trapped in the teeth might increase the risk of any individual to manifest gastrointestinal illness, gastroenteritis, typhoid fever and diarrhea. The bacterial isolates from the toothpicks were resistant to the antibiotics used in the study with exception of ofloxacin and gentamicin. Going forward, toothpicks should be rinsed with potable water before putting it inside the mouth. The essence of rinsing toothpicks is to reduce its microbial load. Secondly, to reduce the risk of humans manifesting illnesses that will necessitate the intake of antibiotics.

RECOMMENDATIONS

Owners and managers of eateries should provide automatic toothpick dispensers for customers; avoid exposing toothpicks on dinner tables; ensure that staff maintain personal hygiene and sanitation. Regular inspection of eateries should be carried out by the authorities. Health education and enlightenment programmes on transmission of foodborne infections and diseases should be sustained.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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