Microbial Contaminants of Wooden Toothpicks in Abakaliki Metropolis, Ebonyi State, Nigeria

Elom, Michael Okpara[1], Ugah, Uchenna Iyioku[2] and Omote, Victor[1]

[1]Department of Medical Laboratory Science, Faculty of Health Sciences and Technology Ebonyi State University, Abakaliki
[2]Department of Medical Biochemistry, Faculty of Basic Medical Sciences, Federal University, Ndufu-Alike Ikwo
ugahuchennaiyioku@gmail.com

Abstract: Evaluation of microbial contaminants of wooden toothpicks was carried out in Abakaliki metropolis, of Ebonyi State, Nigeria. A total of one hundred (100) samples comprising of ten (10) toothpicks each from four different sources within six zones were analyzed for microbes, using wet preparation techniques, concentration techniques, culture, and biochemical tests. Out of the 80 (eighty) test samples analyzed, 53 (66%) showed microbial contamination while 27 (34%) were sterile. Of the 53 positive samples, 24 (45%) gave mixed contaminations while 29 (55%) were contaminated by single microbes. Bacteria accounted for the highest contamination (positive for 42 samples out of the 53) while parasites were recovered from 2 samples only. Ogoja Road and Ishieke village showed the highest rates of contamination with 78% and 77% respectively while PRESCO and CAS campuses gave 57% and 54% contaminations respectively. Samples collected from restaurants showed 86% contamination while those collected from eateries gave 54% contamination. Yeast cells and fungal spores contaminated 22 (twenty-two) and 18 (eighteen) of the 53 positive samples respectively while *staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeroginosa* were the most recorded bacteria with prevalence of 8 (eight) each. *Proteus* and *Salmonella* contaminated 2 (two) samples each while *Giardia lambia* and *Ascaris lumbricoides* were isolated from one sample.
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1. Introduction

Microorganisms are ubiquitous in nature. They live in every part of the biosphere including soil, hot springs, on the ocean floor, in the atmosphere and deep inside rocks within the earth crust (Ronniie *et al.,* 2013). Microbes are very important tools that have been exploited by people in biotechnology, both in traditional food and beverages preparation, and in modern technologies based on genetic engineering. However, there are many pathogenic microbes, which are harmful and can even cause death in plants and animals (Talaro and Chess). The pathogenic microbes possess various pathogenic mechanisms that enable them to gain access into the host, attach to receptors on cell surfaces, replicate locally, escape host immune responses, spread to different parts of the body and cause cellular and tissue destructions.

A toothpick is a tiny piece of wood, plastic, bamboo, metal, bone and other substances used to remove debris from the teeth, usually after a meal. Toothpicks usually have one or two sharp ends to insert between teeth. They come in different shapes, sizes, colours, and may also have different flavors. Those made from metals, plastics and wood are the most common types found. Besides being used to remove food from and between teeth, they can be used to perform a number of functions ranging from designing building plans in architecture, to supporting broken stems in floriculture, cleaning and painting hard to reach or awkward places, cake baking and designing, cleaning of nails, applying glue to tight spots and others (Petroski, 2007).

Toothpicks are one of the most poorly handled eating accessories. They are usually placed in bowls or plates and left open in restaurants, bars, eateries and other places where they are used. This unhealthy habit renders them exposed to contaminants from the air, hands, skin and vectors of microbes, such as the houseflies and rodents. The contaminated toothpicks serve as formites, transmitting the microbes, which have the potentials of causing serious public health problems.

The fear that toothpicks could serve as germ spreaders because of how they are usually exposed in bowls or cups where restaurants patrons often recklessly handle them, fingering many and leaving them behind, started gaining attention in the early 20th century and led to their elimination from dinner tables and the ban of open containers of toothpicks by health authorities in Minneapolis (Whitaker, 2012). The propounded inherent danger in the use of toothpicks gained support through the works of Chang and colleagues in which pathogenic bacteria were isolated from hands injured by toothpicks (Chang *et al.,* 2003).

Recovery of microorganisms from toothpicks in restaurants, bars, eateries etc. signifies a looming

public health danger, considering the nutritional, immunological and physiological effects of the microbes on their human hosts. There is little information on the health implications of toothpicks as fomites. This can be largely attributed to the fact that its use has reduced drastically over the years because most elite societies see its use as vulgar and as an offensive and uncivilized practice and most researchers often neglect the pathogenic-bearing potentials of toothpicks. The dearth of information on the pathogenic transmission potentials of toothpicks encouraged the execution of this study, so as to close the information gap.

2.Materials and Methods

2.1 Study area

The study was carried out in Abakaliki metropolis in Ebonyi State, South –eastern Nigeria. Abakaliki is the capital of Ebonyi State, Nigeria. The climate is tropical with two distinct seasons (rainy and dry). The former commences from April and ends in October while the latter starts from November and ends in March. The average rainfall of the city is about 1500 mm while the temperature range is 28-30□C. The city is located very close to the surrounding rural communities where mostly farming activities take place.

2.2 Sampling techniques

A total of one hundred (100) samples (each comprising of ten (10) toothpicks) from four (4) different sources were used in the study. The sources were restaurants, bars, eateries and the market. The study area was divided into six zones namely PRESCO campus and its environs, CAS campus and its environs, Water-works Road and its environs, Ogoja Road, Federal Teaching Hospital Abakaliki (FETHAI) and its environs and Ishieke village. Eighty (80) samples (800 toothpicks) out of the total population were test samples while the remaining twenty samples (200 toothpicks) were bought from the market and used as control samples. The samples were collected from July through September, 2013, using simple random sampling technique. Each of the collected toothpicks were placed in a sterile polythene bag (newly bought drug envelop) sealed and transported to the Research Laboratory in the Department of Medical Laboratory Science, Ebonyi State University, Abakaliki, for analysis.

2.3 Laboratory analysis

The laboratory methods and techniques followed WHO (WHO, 2003). The toothpicks were placed in a sterile universal container containing 10 ml of normal saline solution and the container was shaken vigorously. Thereafter, a sterile forcep was used to remove the toothpicks. The solution was poured into a centrifuge tube and centrifuged at 2,000 g for 5 minutes. The supernatant was discarded leaving, behind the sediment. A part of the sediment from the centrifuged solution was used to asceptically inoculate blood agar and MacConkey agar plates as described by Barker and Silverton (1998) and was incubated for 24 hours at 37^0C.

A small quantity of the sediment was placed on a clean, dry and grease-free glass slide. The preparation was covered with a cover glass and examined under the microscope using x10 and x40 objectives respectively. After 24 hours of incubation, the cultured plates were examined macroscopically for growth of any microbes. The microbial isolates were identified and characterized using standard methods (Chessbrough, 2000).

2.4 Statistical analysis

The analysis of recovered and isolated microbes was carried out using descriptive statistics and inferential statistics of Chi-Square.

3.Results

Out of the 80 test samples, 53 (66%) showed microbial contamination while 27 (34%) were sterile. The 20 control samples showed no microbial contamination of any sort. Forty-five percent (45%) of the contaminated samples gave mixed contamination (contained more than one microbe) while 55% were contaminated by only one microbe. Bacterial contamination was found in 42 of the 53 contaminated samples (79%). Fungi contamination was found in 33 samples (63%) while only 2 samples (3%) were contaminated by parasites. Ogoja Road had the highest contamination while CAS campus had least (Table 1).

Table 1: Prevalence of contamination in relation to zones

Zones	Sample size (n)	Number of contaminated samples	Percentage of contaminated samples
Ogoja Road	14	11	78.57
Ishieke Village	13	10	76.92
Water-works Road	13	9	69.23
FETHA1 and Its environs	13	8	61.54
PRESCO Campus	14	8	57.14
CAS campus	13	7	53.85

Key: FETHA 1: Federal Teaching Hospital, Abakaliki; PRESCO campus: College of Health Sciences, Ebonyi State University; CAS: College of Agricultural Sciences

Of the three sources of sample, restaurants had the highest contamination while eateries had the least contamination. (Table2).

Table 2: Microbial contamination in relation to sources

Sources	Sample size	Number of Contaminated samples	Percentages of Contaminated samples
Restaurants	29	25	86.21
Bar	26	15	57.69
Eatery	25	13	52.00

The prevalence of contamination with respect to microbial species is presented in table 3. Yeast cells had the highest prevalence while the parasites (*Giardia lamblia* and *Ascaris lumbricoides*) had the least.

Table 3: Prevalence of contaminants with respect to microbial species

Microbes	Number of contaminated samples	Percentage of contaminated samples
Yeast cells	22	41.51
Fungal spores	18	33.96
Staphylococcus aureus	8	15.09
Escherichia coli	8	15.09
Pseudomonas aeroginosa	8	15.09
Non-coagulase Staphylococci	5	9.43
Streptococci spp	5	9.43
Klebsiella spp	4	7.55
Salmonella spp	2	3.77
Proteus spp	2	3.77
Giardia lamblia cyst	1	1.89
Ascaris lumbricoides	1	1.89

4. Discussion

Out of the 80 test samples, 53 (66%) showed contamination. This high prevalence of contamination could be attributed to the fact that the toothpicks are made from woods which are naturally porous and have tiny fissures and grooves that can harbor microbes, particularly bacteria (Annett *et al.,* 2005). This fact was supported by the work of Gilbert and Watson (1971) which showed that microbes were more abundant on wooden boards when compared to metal or plastic surfaces after contact with food products. The fact that the study area (Abakaliki) is surrounded by rural communities (Elom *et al.,* 2012) where sanitation and personal hygiene is low might also be a contributing factor to the high prevalence of the microbes recorded. Forty-five percent (45 %) of the contaminated samples showed mixed contamination. This work agrees with the findings of Lam and Choi (2004) in which more than one bacterium species were isolated from the sample of patients injured by toothpicks.

Bacteria had the highest level of contamination, followed by fungi, while the parasites had least. A number of factors might be responsible for this but of paramount importance might be the species of woods involved. Bacteria recovered from birch wood after contamination with *E.coli* for 3 days, indicated that birch wood has reduced antibacterial effect when compared to other species of wood (AK *et al.,* 1994). Moisture content, temperature and humidity are the key factors for fungal growth on different materials (Annett *et al.,* 2005). This may account for the high prevalence of fungi and low prevalence of parasites recovered in the study, as Abakaliki is known for such environmental factors.

Ogoja Road and Ishieke village gave the highest rate of contamination of 78% and 77% respectively while PRESCO and CAS campuses showed contaminations of 57% and 54% respectively. Ogoja Road is the heart of commercial activities in Abakaliki metropolis and the home to most small scale business firms in Ebonyi State. The increased commercial activities as well as the low standard of infrastructure in this area help to promote poor sanitation and poor personal hygiene which may be responsible for the high prevalence of microbial contaminations observed. Ishieke village is inhabited by most natives and few students of the Faculties of Education, Management Sciences, Social Sciences, and Arts and Humanities of Ebonyi State University, Abakaliki. Ignorance, unwholesome practices and lack of basic social amenities in the area might be responsible for poor sanitary conditions which may have accounted for the high prevalence of toothpick contaminations recorded. The reduced prevalence observed in both PRESCO and CAS campuses may be attributed to

health education and enlightenment which are factors that promote personal hygiene and improved sanitation. Most persons residing in those areas were students, lecturers, some members of university staff, a few traders and other individuals with educational exposures and enlightenments.

With respect to sources, the highest rate of microbial contamination was observed in restaurants. This might be due to the fact that most restaurants in Abakaliki are below standard. The restaurants are managed by people with poor personal hygiene and low sanitary values as well as being patronized by individuals with poor knowledge, attitude and practices of unwholesome habits. Most bars in the study area are combined with restaurants. This might be responsible for the prevalence of 58% contamination recorded for bars.

Yeast cells and fungal spores were the most recorded microbes. This work agrees with Valentine (2007) who showed that fungal species contaminated Archives and Museums than bacteria species. This may be attributed to the strong cellulolytic activity exerted by fungi on wood (Anna *et al.,* 2010). *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeroginosa* showed prevalence of 15% contamination each. This study is in consonance with that of Famurewa and David (2009) which revealed that the above-named bacteria showed the highest prevalence among bacteria that were recovered from cell phones. The high prevalence of *Staphylococcus aureus* may be attributed to the fact that it can remain viable for about 9 weeks on dry surfaces (Beard-Peglear *et al.,* 1997). It is also estimated that 20 % of the human population are long-term carriers of *S. aureus* and that it is a normal flora of the skin (Kluytmans *et al.,* 1997). This might also be a contributing factor to the high prevalence recorded. Fecal-oral transmission is the major route through which strains of *E. coli* are transmitted (Feng *et al.,* 2002). Thus, poor personal hygiene such as non-washing of hands after passing out faeces and fecal contamination of water sources may be responsible for the high prevalence of *E.coli* in the study.

P. aeruginosa is a ubiquitous organism present in diverse environmental settings (Lister *et al.,* 2009). Its ability to survive on minimal nutritional requirements and to tolerate a variety of physical conditions (Lister *et al.,* 2009) may be responsible for the high prevalence of *P. aeruginosa* on toothpicks. The survival of parasite (cysts, ova, trophozoites or adult worms) in the environment depends on both biotic and abiotic factors (Alum *et al.,* 2010). The biotic factors include other microbes and their by-products while the abiotic factors include temperature, relative humidity and residence time (Alum *et al.,* 2010). *E. histolytica* can survive for only 18 hours on dry surface where as it can survive for 72 hours and up to 10 days on moist and damp surfaces respectively (Feacham *et al.,* 1981). The poor contamination of the studied toothpicks by parasites may be as a result of increased contamination by other microbes and the dry nature of the toothpicks studied. The only helminth recovered in the study was *Ascaris lumbricoides*. This may be attributed to the fact that Ascaris eggs are more resistant than other intestinal parasite eggs (Alum *et al.,* 2010) and the mucopolysaccharide that coats the eggs serves as an adhesin that aids its attachment to surfaces (Crompton, 1989).

Statistical analysis using Chi-square at a significant level of 95% showed that the prevalence of the recovered microbes is independent of the studied zones and the sources of the sample. The high prevalence of microbial contaminants recorded in the study, indicates a looming public health danger. Most of the microbes recovered were pathogens that could be very destructive on their hosts and are also implicated in the growing antimicrobial resistance phenomenon that is posing a threat to chemoprophylaxis and chemotherapeutics of infectious diseases. Recovered microbes such as yeast and some fungal species that had not been termed pathogens in the past are now threats to the health of immunocompromised individuals. Further research that may lay emphasis on the quantitative analysis of the microbes is recommended. Such a study may find out the least quantities of microbial counts that could establish asymptomatic pathogenic effects on users of toothpicks.

Poor sanitation practices and personal hygiene have been incriminated as the main factors responsible for the high prevalence of microbial contaminants in this study. Therefore, government and relevant authorities should ensure the provision of basic social amenities, as well as the enactment and enforcement of health laws that would reduce microbial contamination of objects and surfaces in restaurants, bars and eateries. Health education and enlightenment programmes on preventive and control measures of infectious diseases and prompt diagnosis and treatment of infected persons should also be emphasized. Regular seminars should always be organized for restaurant managers and other food sellers Toothpicks made of other materials or other wood species with potent antimicrobial activities are recommended in preference to the common ones made from birch wood.

Corresponding to:
Ugah Uchenna Iyioku
Department of Medical Biochemistry
Faculty of Basic Medical Sciences
Federal University Ndufu-Alike Ikwo

PMB 1010, Abakaliki Ebonyi State, Nigeria
e-mail: ugahuchennaiyioku@gmail.com
Mobile: +2347062154353

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