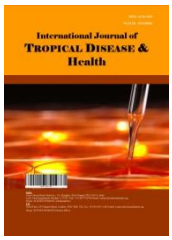

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Quantitative Measurement of Enteric Bacteria Load From Public Surfaces in Restaurants in Nnewi-Town

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: Bio-contamination of surfaces of various items and equipment used by the public is the main cause of disease epidemic. Most outbreaks of infection associated with inanimate objects are caused by items that should be sterile but have been contaminated and are generally known as fomites.
Objective: This study is to evaluate the presence of enteric bacteria from different public surfaces in restaurants in Nnewi town.
Methodology: Selected public surfaces in eight (8) randomly selected restaurants were analyzed for the presence of enteric bacteria via culture and biochemical tests following a standard criterion. The sample sites included; tables, chairs, counters, sinks and walls. Also, Disc susceptibility test were carried out on the isolates using conventional antibiotics.
Results: Eighty-eight (88) bacteria isolates were isolated and identified, and *S. aureus* showed the highest prevlence 29(33%), followed by *E. coli* 15(17%), *Proteus* spp 13(15%), *Klebsiella* spp 12(14%), *Pseudomonas* spp 10(11%), and Coagulase-negative *Staphylococci* 9(10%). Tables and sinks were the most contaminated fomites each constituting of 19(22%) of the total bacteria isolated in this study. *Escherichia coli*, *Pseudomonas* spp, *Proteus* spp and *Staphylococcus*

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aureus, were the most resistant isolates to the antibiotics, Coagulase-negative *Staphylococci* was the most sensitive isolate. Out of the ten antibiotics tested ceporex was found to be the least effective with about 100% resistance by the isolates while ciprofloxacin, ceftriaxone, and levofloxacin were the most effective antibiotics with 0% resistance by the bacteria isolates.
Conclusion: the study has shown that public surfaces in restaurant can easily be contaminated with a variety of bacterial contaminants that may be multi-drug resistant bacteria strains posing as a possible public health issue.

Keywords: Immunology; bacteria; infectious diseases.

1. INTRODUCTION

One of the main causes of epidemics obtained from the environment and nosocomial infections is the bio-contamination of surfaces of various items and equipment used by the public [1]. The increasing incidence of epidemic outbreaks of certain diseases and its rate of spread from one community to the other has become a major public concern [2]. Inanimate objects which become contaminated with pathogenic bacteria and then spread infection to others are often referred to as fomites and in the infectious chain serve as the reservoir for pathogens, from which they spread further through transfer via hands [3].

Most outbreaks of infection associated with inanimate objects are caused by items that should be sterile but have been contaminated [4]. Fomites include door handle of conveniences, showers, toilet, hand lockers especially those found in public offices, hospitals, hotels, restaurants and restrooms [5]. The hypothesis that environmental microorganism cause human diseases arises from two facts, firstly, our interaction with the inanimate environment is constant and close, secondly environmental objects are usually contaminated often with important human pathogens [6]. Unfortunately, though it is fairly easy to assess the prevalence of microorganism in the environment, it is relatively difficult to establish the role the organisms in the environment play in causing human disease [6].

Several factors have been identified to affect the transfer rate of bacteria from surface to another surface. These include bacteria type, source and destination surfaces, time post inoculation, and moisture level [7,8] investigated bacterial transfer rates from food to hands and from hands to food with and without a glove barrier and found that a glove barrier can decelerate the transfer rate of microorganisms from food to hands.

Germs can survive in the microscopic grooves and cracks on surfaces and will go unnoticed. Oils in the skin, dust, grime, moisture and warmth from central heating systems provide an ideal environment for these germs to accumulate [9]. Cold and flu viruses can survive on dry surfaces such as door knob for more than 48 hours [10], while some bacteria, such as *E. coli,* can survive on dry surfaces for months on end [11]. Soft, wet surfaces (particularly those with plenty of food) are perfect for bacteria. Cloth, sponges and carpets that have gotten wet are excellent living places for bacteria because it protects them from exposure to the environment, dry air or sunlight [12].

There are a myriad of surfaces that could potentially become contaminated with enteric microorganisms during normal use in restaurants and include door handles, sink taps and faucets, floor, toilet paper and paper towel dispensers [5]. Such locations are all touched continually by people and may become contaminated by enteric organisms [13]. Frequent touching of these areas can result in the transfer of potentially pathogenic microorganisms from the hand to the nose and mouth resulting in self-inoculation [14]. In addition, the pathogens may be transferred to other vehicles involved in transmission such as foods within food preparation area or via contaminated hands of food handlers [15]. Restaurants have several surfaces that can serve as reservoir of bacterial infection such as door handles, toilet surfaces, kitchen utensils, eating tables and chairs etc. They are also public places where people of different calibre like; occupation, exposure, economic status, health status, age, gender often come to and as such can serve as source of fomites. This forms the basis of this study; to evaluate the presence of enteric bacteria from different public surfaces in restaurants in Nnewi town.

2. MATERIALS AND METHODS

2.1 Study Area

Nnewi town is the only town in Nnewi North LGA. It has four villages (sub-towns) that make up the one-town local government, which includes; Otolo, Uruagu, Umudim and Nnewi-ichi.

2.2 Study Design

This study was a descriptive cross-sectional study in which stratified random selection of restaurants was employed for isolation of enteric bacteria from public surfaces in restaurants in Nnewi town and prevalence recorded. The duration for this study spanned for three months.

2.3 Sampling Technique

Stratified random technique was used in which five restaurants were selected within Nnewi town. From each restaurant, five randomly selected public surfaces were swabbed such that two swab sticks per surface to give a total of ten swab samples per restaurant. Hence, the total swab samples to be collected from the five restaurants that were selected were 80 swab sticks.

2.4 Sample Collection

Modified criteria of [6] was adopted in which sterile swab stick was pre-moistened with sterile normal saline by adding 2-3mls of normal saline to the swab stick vials. The moistened swab sticks was used to swab each surface back and forth in zigzag manner and the swab stick was immediately returned to the vial. The swab samples will then be transported immediately to the Laboratory in the Department of Medical Laboratory Science, Medical microbiology Specialty, College of Health Sciences and Technology Nnamdi Azikiwe University, Nnewi Campus within 2hrs after collection.

2.5 Microbiological Analysis of Samples

Modified cretiria of Kim et al., [16] was employed. For every two swab samples per surface, one was enriched in Nutrient broth and the other in Selenite-F broth and then both were incubated for 18-24hrs at 35-37^{o}C. From the enrichment media, the samples were inoculated onto several selective media (using a flame sterilized wire loop) for preliminary identification. From Selenite-F broth to *Salmonella Shigella* Agar (SSA) for *Shalmonella* spp and *Shigella* spp; from Nutrient broth to Mannitol-Salt Agar (MSA) for *Staphylococcus aureus* and to MacConkey agar for other enteric bacteria such as coliforms, *proteus* spp, *pseudomonas* spp, etc. The inoculated selective media were incubated for 18-24hrs at 35-37^{o}C. In case of mixed growth, the colonies was separately sub-cultured onto new sterile media for 18-24hrs at 35-37^{o}C. Further identification was carried out via Gram staining and Indole test, Oxidase test, Urase test, Citrate test, Catalase test and also Coagulase test in accordance with [17]. Identified pure isolates was stored in glycerol Nutrient broth at 4^{o}C until further test.

2.6 Sterility Test for the Commercially Prepared Antibiotic Discs

One from each antibiotic disc container was used as representative for the test of sterility by incubating for 18-24hrs at 35-37^{o}C in a sterile nutrient agar media. Any visible growth around the antibiotic discs and not all over the media surface shows unsterile contaminated batch of antibiotic discs and need to be discarded and new ones obtained and re-tested.

2.7 Preparation of 0.5 McFarland Turbidity Standards [18]

A 0.5 ml aliquot of 0.048 mol/L Barium chloride (1.175% w/v $BaCl_2.2H_2O$) was added to 99.5 ml of 0.18 mol/L Hydrogen sulphate (1% v/v H_2SO_4) with constant stirring to maintain a suspension. The correct density of the turbidity standard was verified by using a Visible Spectrophotometer (Bioevopeak), SP-LV22 with a wavelength Range: 340-1000nm, and matched cuvette to determine the absorbance. The absorbance at 625 nm should be 0.08 to 0.10 for the 0.5 McFarland standards. The turbidity standard was transferred in 4 to 6 ml aliquots into screw-cap tube of the same size as those to be used in growing or diluting the bacterial inoculum. These tubes was tightly sealed and stored in the dark at room temperature. The $BaSO_4$ turbidity standard was vigorously agitated before each use for a uniformly turbid appearance. If large particles appear, the standard was replaced by preparing a new one.

2.8 Standardization of Isolates

Inocula were obtained from an overnight agar culture of the test organisms. Inocula for the susceptibility test were prepared by taking at least 3-5 well-isolated colonies of the same morphology from an agar plate culture. The top of each colony was touched with a sterile wire loop and the growth transferred into a tube containing 3 ml of sterile normal saline and thoroughly mixed to obtain smooth suspension. The density of the inoculum were adjusted with sterile saline to 0.5 McFarland standards by comparing the inoculums suspension tube and

the 0.5 McFarland standard tubes by viewing against a white background with contrasting black lines. This results in a suspension containing approximately 1-2×10^8 cfu/ml of the isolates. The standardized isolate suspensions were used within 15 minutes because the isolates can multiply and increase in density which will affect the susceptibility result.

2.9 Susceptibility Test

Disc diffusion susceptibility test was carried out using ten (10) different commercially prepared antibiotic discs via modified Kirby-Bauer disc diffusion technique aseptically [19]. Two standardized inoculum suspensions of each isolates (labelled; A = first representative and B = second representative) was prepared. The Nutrient Agar and antibiotic containers were brought to the room temperature by standing for 1-2 hours prior to standardization of inocula to minimize the amount of condensation that occurs when warm air contacts cold surface.

A sterile cotton swab was dipped into the standardized inoculum suspension, rotated several times and pressed firmly on the inside wall of the tube above the suspension level to remove excess inoculum from the swab. The dried surface of a sterile Nutrient agar plate was inoculated by swab streaking over the entire sterile agar surface. This procedure was repeated by streaking two more times, rotating the plate approximately 60° each time to ensure an even distribution of inoculum. As a final step, the rim of the agar was swabbed. The lid was left ajar for 3 to 5 minutes, but not more than 15 minutes, to allow for any excess surface moisture to be absorbed before applying the commercial antibiotic discs.

The commercially prepared antibiotic discs were dispensed onto the surface of the inoculated agar plate. Each disc was placed individually with flame-sterilized forceps and then gently, pressed down onto the agar to make firm contact with the agar surface. The discs were distributed evenly so that they were not closer than 24 mm from centre to centre. The plates were inverted, and placed in an incubator for 18-24hrs at 35-37°C within 1hour after the discs were applied.
After an overnight incubation, the bacterial growth around each disc was observed. If the isolate is susceptible to a particular antibiotic, a clear area of “no growth” was observed around that particular disc and was referred to as the zone of inhibition. The diameter of the observed zone of inhibition was measured using ruler to the nearest whole millimetre (mm) and compared to a standard interpretation chart used to categorize the isolate as super-sensitive (25 mm and above), sensitive (20 mm-24 mm), intermediately sensitive (15 mm-19 mm) or resistant (0-14 mm). If the zones of adjacent discs overlap, the zone diameter was determined by measuring the radius of the zone, measured from the centre of the disc to a point on the circumference of the zone where a distinct edge is present and then multiplied by 2 to determine the diameter of the zone of inhibition.

2.10 Statistical Analysis

The experimental data was analysed using Chi-square to ascertain the prevalence of enteric bacteria from public surfaces in restaurants in Nnewi-town and the significant relationship between variables determined at ≤0.05.

3. RESULTS AND DISCUSSION

Table 1 Shows the overall prevalence of the bacterial isolates in this study. A total of 88 bacterial isolates were obtained. *Staphylococcus aureus* were the most frequently isolated bacterial isolates with a prevalence of 33%, followed by *E. coil* (17%), *Proteus* spp (15%), *Klebsiella* spp (14%), *Pseudomonas* spp (11%), while coagulase-negative *Staphylococcus* (10%) was the least frequently isolated bacterial isolates with a prevalence of 10%.

In this study, it was found that non-food contact surfaces in restaurants post as potential sources of pathogens implicated in food borne illness, as each sample sites which included; tables, sinks, chairs, counters, and walls yielded bacteria growths. This is consistence with the study of [20], who also reported that household and restaurants utensils (air, knife, spoon and cutting board) in Dhaka, Bangladesh, are contaminated with bacteria. A total of about 61 bacterial isolates were identified where *Bacillus* spp. showed the highest prevalence 18 (29.51%) followed by *Vibrio* spp. 17 (27.87%), *Staphylococcus* spp. 13 (21.31%), *Klebsiella* spp. 6 (9.84%), *Shigella* spp. 4 (6.56%), *Salmonella* spp. 2 (3.28%), *and E. coli* 1 (1.63%). The differences in the bacteria isolated and their prevalence may be due to the difference in sample sites, restaurants employed and their hygiene practices, and geographical area where the study was conducted.

Table 2 Shows occurrence/prevalence of bacteria isolates on the sample sites (fomites). Tables and sinks were the most contaminated fomites each constituting of 22% of the total bacteria isolated in this study, followed by chairs - constituting of 20% of the total bacteria isolated, counter - constituting of 19% of the total bacteria isolated, while walls was the least contaminated fomites constituting of 17% of the total bacteria isolated. This study also showed that tables and sinks in the restaurants employed in this study were the most contaminated sample sites, followed by chairs, counters and walls. This is supported by [19], who carried out a study to measure microbial contamination on non-food-contact surfaces in restaurants which included, tables, chairs, highchairs and booster seats, and they were found to have high levels of microbial contamination most especially *staphylococci* on booth seats, table, and chairs with total microbial counts of 151 and 184 cfu/100 cm^2. However, there is lack of enough substantial evidence in literature to back up this finding in this study.

S. aureus in this study was the most prevalent isolate maybe because *S. aureus* is a major component of the normal flora of the skin and nostrils and it can be easily discharged by several human activities. This particular species of bacteria does not form spores but can cause contamination of food products during food preparation and processing. They can grow in a wide range of temperatures (7° to 48.5° C; optimum 30 to 37°C), pH (4.2 to 9.3; optimum 7 to 7.5), and sodium chloride concentration up to 15% NaCl [21]. It is a desiccation tolerant organism with the ability to survive in potentially dry and stressful environments, such as the human nose and on skin and inanimate surfaces such as clothing and kitchen surfaces [20]. Although *E. coli* (second most prevalent) itself is naturally found in the human intestine and although most strains are harmless, but its presence in any numbers can be regarded as evidence that food contact surfaces were contaminated with faecal discharges, if not of human origin then of animal origin from carcass, and at least is an important cause of food intoxication [20].

Table 1. Overall prevalence of the bacterial isolates in this study

Bacterial isolates	Frequency
E. coil	15(17%)
Pseudomonas spp	10(11%)
Klebsiella spp	12(14%)
Proteus spp	13(15%)
Staphylococcus aureus	29(33%)
Coagulase-negative *Staphylococcus*	9(10%)
Total	88(100%)

Table 2. Occurrence/prevalence of bacteria isolates on the sample sites (fomites)

Bacteria isolates	Sample sites					χ^2	P-value
	Tables n(%)	Sinks n(%)	Chairs n(%)	Counters n(%)	Walls n(%)		
Escherichia coli	3(16%)	4(21%)	3(17%)	2(12%)	3(20%)	0.95	0.92
Pseudomonas spp.	2(11%)	2(11%)	2(11%)	3(18%)	1(7%)	1.25	0.87
Klebsiella spp.	4(21%)	1(5%)	3(17%)	2(12%)	2(13%)	2.85	0.58
Proteus spp.	3(16%)	4(21%)	2(11%)	2(12%)	2(13%)	1.66	0.80
Staphylococcus aureus	7(37%)	5(26%)	6(33%)	6(35%)	5(33%)	1.15	0.89
Coagulase-negative *Staphylococcus.*	0(0%)	3(16%)	2(11%)	2(12%)	2(13%)	3.25	0.52
Total (%)	19(22%)	19(22%)	18(20%)	17(19%)	15(17%)		

Key: χ^2= Pearson chi-square values;
P-value = statistical significance level

Table 3. Antibiotic susceptibility profiles of some of the bacteria isolates

	Average diameters of zone of inhibitions (mm) (interpretation/category)									
Bacterial isolates	**PEF**	**GEN**	**AU**	**CPX**	**SXT**	**SM**	**AMP**	**CEP**	**CTO**	**LEV**
Escherichia coli	**15**	0	12	20	0	0	11	6	20	17
	(I)	(R)	(R)	(S)	(R)	(R)	(R)	(R)	(S)	(I)
Pseudomonas spp	**0**	0	0	20	0	21	0	10	22	21
	(R)	(R)	(R)	(S)	(R)	(S)	(R)	(R)	(S)	(S)
Klebsiella spp	**24**	14	0	21	22	14	19	0	25	16
	(S)	(R)	(R)	(S)	(S)	(R)	(I)	(R)	(SS)	(I)
Proteus spp	**0**	0	10	24	5	21	12	0	24	18
	(R)	(R)	(R)	(S)	(R)	(S)	(R)	(R)	(S)	(I)
S.aureus	**17**	0	0	21	0	0	0	11	25	21
	(I)	(R)	(R)	(S)	(R)	(R)	(R)	(R)	(SS)	(S)
Coagulase-negative *Staphylococcus*	**23**	16	18	20	17	22	0	0	23	26
	(S)	(I)	(I)	(S)	(I)	(S)	(R)	(R)	(S)[a]	(ss)
%R	**33%**	83%	83%	0%	67%	50%	83%	100%	0%	0%
%S	**67%**	17%	17%	100%	33%	50%	17%	0%	100%	100%

Key: PEF = Pefloxacin; GEN = Gentamycin; AU = Augmentin; CPX = Ciprofloxacin; SXT =Sulfamethoxazole-trimethoprim; SM = Streptomycin; AMP = Ampicillin; CEP = Ceporex; CTO = Ceftriaxone; LEV = Levofloxacin; "R" = Resistance (0-14mm); "I" = Intermediate (15-19mm); "S" = Sensitive (20-24mm); "SS" = Super- Sensitive (≥ 25mm); "%R" = Percentage resistance; %S = Sensitivity percentage

Table 3 Shows the average diameter of the zone of inhibitions obtained from the duplicate susceptibility test carried out using ten (10) conventional antibiotics on some of the bacterial isolates. The average diameter of the observed zone of inhibitions were compared to a standard interpretation chart used to categorize the isolates as super-sensitive – SS (25 mm and above), sensitive – S (20 mm-24 mm), intermediately sensitive – I (15 mm-19 mm) or resistant – R (0-14 mm). Ciprofloxacin, ceftriaxone, and levofloxacin exhibited the highest activity against the bacterial isolates and then ceporex exhibited the least activity against the bacterial isolates. *Escherichia coli*, *Pseudomonas* spp, *Proteus* spp and *Staphylococcus aureus*, all showed 40% sensitivity and 60% resistance to the antibiotics, *Klebsiella* spp showed 60% sensitivity and 40% resistance to the antibiotics, and then coagulase-negative *Staphylococcus* showed 80% sensitivity and 20% resistance to the antibiotics. Hence, *Escherichia coli*, *Pseudomonas* spp, *Proteus* spp and *Staphylococcus aureus* are the most resistant bacteria, followed by *Klebsiella* spp, and then coagulase-negative *Staphylococcus* which is the least resistant but sensitive bacterial isolate.

Determination of antibiotic susceptibility pattern revealed that all bacteria isolates tested were resistant to at least two antibiotics. Among the bacteria isolated, *Escherichia coli*, *Pseudomonas* spp, *Proteus* spp and *Staphylococcus aureus*, were the most resistant isolates to the antibiotics, Coagulase-negative *Staphylococci* was the most sensitive isolate. Out of the ten antibiotics tested ceporex was found to be the least effective with 100% resistance by the isolates while ciprofloxacin, ceftriaxone and levofloxacin were the most effective antibiotics with 0% resistance by the bacteria isolates. This correlates with the finding of [22], who reported that the bacteria contaminants of household and restaurants utensils show resistant strains with 72.13% found to be resistant to more than two antibiotics and 27.87% resistant to two antibiotics. However, the study recorded gentamycin as the most probable antibiotic of choice as against this present study but also recorded ciprofloxacin as second antibiotic of choice which is in the favour of this study.

The resistance phenomenon of the bacteria to antibiotics may be due to the inappropriate use of the antibiotics which accelerates the evolution of resistant strains of bacteria [23]. The improper use of antibiotics in human and livestock, wrong and substandard prescriptions by unqualified medical personnel along with poor diagnosis or lack of diagnosis, have been reported to be among the main factors contributing to the development of resistant microbes [24].

4. CONCLUSION

The results of this study indicate that, surfaces in restaurant can easily be contaminated therefore, can serve as potential source of food-borne diseases. These bacterial contaminants may be multi-drug resistant bacteria strains thereby posing as a public health issue. Regular cleaning of kitchen utensils, use of dish washing liquid and public awareness on personal hygiene can help minimize the spread of food-borne diseases from kitchen utensils. A comprehensive risk analysis and risk management approach of food safety from production to consumption is recommended to protect public health from such hazards.

CONSENT

Informed consent was verbally obtained from the management of the restaurants before participating in the study.

ETHICAL APPROVAL

Ethical approval was sought from the ethics committee of Faculty of Health Sciences and Technology, Nnamdi Azikiwe University, Nnewi Campus, before the commencement of this study.

DISCLAIMER

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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