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Bacteriological Examination of the Cafeteria Equipments in Karu L.G.A Cafeterias, Nasarawa State, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author NCJA designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors NCJA and WCJ managed the literature searches and analyses of the study performed the spectroscopy analysis. Author NCJA managed the experimental process. Author MOI identified the species of microbes, managed statistical analysis and data presentation. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This study was carried out to determine the rate of bacterial contamination of cafeteria tables, countertops and available equipments used for cooking in Karu Local Government Area cafeterias as a key to determining the hygienic and sanitary conditions of the cafeteria environment.
Study Design: This research study was done using random sampling technique.
Place and Duration of Study: Karu Local Government Area; Department of Biological Sciences, Bingham University, between April 2015 and September 2015.
Materials and Methods: Five cafeterias were used for this study. Swab samples from spoons, mortars, pestles, pots, knifes, chopping boards, tables, countertops from the different cafeterias in Karu L.G.A, Nasarawa state of Nigeria were analyzed for the presence of microorganisms using standard microbiological methods. A total of 200 samples were analyzed, 20 samples were

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collected per visit, using creation of awareness as a parameter (i.e. before and after) making a total of 40 samples from each cafeteria used for the study. Pour plate technique was used for enumeration of microbes after 10 fold serial dilution while streak plate method was used for isolation. Nutrient agar, Mannitol Salt agar, Salmonella-Shigella agar, Eosin Methylene Blue agar and McConkey agar (Oxoid, Cambridge UK) were used for isolation and enumeration.

Results: 73 (36.5%) samples out of the 200 swab samples were positive with bacterial contamination. The contamination rates of the samples obtained from each cafeteria were however, statistically insignificant (P>0.05). The bacteria species isolated were *Escherichia coli*, *Staphylococcus aureus*, *Enterobacter aerogenes*, and *Salmonella typhimirium*. *Escherichia coli* showed the highest frequency of occurrence 72 (57.14%), followed by *Staphylococcus aureus* 24 (19.05%), *Salmonella* 16 (12.70%), *Enterobacter aerogenes* 14 (11.11%). The mean colony count showed *E. coli* to have the highest count (4.84±0.12) in cafeteria A while *S. typhimurium* had the lowest count (1.79±0.07) also in cafeteria A. No *Enterobacter* was however, isolated from cafeteria C. Total aerobic plate count carried out on the equipments from each cafeteria revealed that counters sampled in cafeteria C had the highest count of 5.89±0.43 while pestles from the same cafeteria had the lowest aerobic count of 2.42±0.00 (P>0.05).

Conclusion: This study obtained a considerably high rate of bacterial contamination from cafeteria equipments. The mean counts (level of occurrence) however, were generally low, indicating the non-severity of the microbial occurrence. Although statistically insignificant, it is indicative of poor personal hygiene, uncleanliness of the environment and general neglect of food safety which can pose a health hazard to consumers. Cafeterias should therefore, be thoroughly supervised and mandated by government agencies to comply with a standard hygienic preparation and presentation of not only the food, but also the cafeteria and equipments used in food preparation.

Keywords: Bacteria; food; cross-contamination; cafeteria; equipments; food poisoning.

1. INTRODUCTION

Cross-contamination occurs when bacteria and viruses are transferred from a contaminated food or surface such as a chopping board and utensils to other food. Bacterial contamination usually occurs when bacteria end up in a location where they are not supposed to be. Bacteria readily colonize table surfaces, counters and equipments found in restaurants or cafeterias where food is eaten or served which are likely where we are exposed to the broadest diversity of microbes, and the exchange of microbes between humans and the cafeteria environment can have impact on human health [1,2]. Microbial exposures arise both directly, from contact with surfaces that harbour microbes derived from a range of potential sources, including humans, food and wet clothes used in cleaning the table surfaces, as well as sponges for washing the cooking equipments. Humans are potential sources that serve as a medium for transfer of microbes when their contaminated hands come in contact with the table surfaces or contaminated cafeteria equipments. Hands are among the obvious culprits in transferring bacteria and viruses from raw to ready-to-eat food, but direct contact with soiled raw foods, dirty chopping boards, knives and other food preparation implements and containers can also spread the contamination. Chopping boards, plates and knives, blenders, mixers, bowls, or any other surface that has been in contact with raw meats, seafood and soiled vegetables and herbs needs to be carefully washed with warm water and detergent, then rinsed and thoroughly dried before being used for ready-to-eat foods [3]. The remnants of food left on the table surface can also serve as a medium for transfer of microbes. When contaminated clothes come in contact with surfaces and equipments, microorganisms are readily transferred as well. This may present a risk if there is subsequent contact with food. Kitchen items that often become contaminated include: Can openers, Cutting boards, Countertops (Most people use their countertops not only for food preparation, but also for possibly contaminated items like grocery bags, mail, or household objects), Dishrags, towels, sponges, and scrubbers, Garbage disposals, Sink drains and P-trap (this is the J-shaped pipe under the sink that retains a quantity of water to block sewer gas from seeping back up through the sink), Refrigerators, Complex appliances like food processors, blenders, and eggbeaters [4].

Food borne illness outbreaks can certainly create a bad reputation for the restaurant. Issues of food safety are also, especially critical for restaurant managers or owners; perceptions of poor sanitation might lead to consumers

choosing a safer restaurant, hence, resulting in loss of revenue to the managers or owners [5]. In some of the cafeterias, the table surfaces where food is served or consumed are mostly touched by staff or consumers and their hands can be the medium for bacterial transfer to the table surfaces. Furthermore some of the equipments used to prepare the food may not be clean or cleaning may not be properly done which can also serve as medium for bacterial transfer. Lastly, the cleaning might be done properly, but the environmental condition can also serve as a medium for bacteria transfer as they are ubiquitous. Hence, bacteriological examination is important in determining the sanitary condition of environment, food and hand contact surfaces in order to enlighten the immediate community on the hygienic standard of cafeteria environments (especially the tables, counters and equipments used), the risk associated with possible infections, as well as the role of hygiene in curbing incidence of food-borne infections in Karu L.G.A, Nigeria and the world at large. The aim of this research was to determine the rate of bacterial contamination of cafeteria tables and equipments in Karu L.G.A cafeterias as a key to determining the hygienic and sanitary conditions of the cafeteria environment.

2. MATERIALS AND METHODS

2.1 Study Area and Duration

Five cafeterias in Karu L.G.A were sampled for this study between April 2015 and September 2015. Karu is a Local Government Area in Nasarawa State, North-Central geo-political zone in Nigeria. It is close in proximity to the Federal Capital Territory of Nigeria. It has an area of 2,640 km². Karu local government has its headquarters in New Karu town. From west to east, the urban area includes towns like Kurunduma, New Nyanya, Mararaba, New Karu, Ado, Masaka and newer, fast-growing towns such as One Man Village (which contains over 1 million people) and Gidan Zakara. Apart from the farmers in the LGA, the abattoirs also serve butchers and traders in the some of the satellite towns of the F.C.T such as Kurudu, Jikwoyi and Karu [6]. The cafeterias were selected based on availability, affordable cost of food, and the most frequently visited by consumers.

2.2 Sample Size

The sample size was determined using the following equation as described by Akilu et al. [7].

$$N= \frac{Z^2P(1-P)}{D^2}$$

Where

p (prevalence rate) = 0.15
N= Sample size
D= Precision =5%= 0.05
Z= Statistics for a level of 95% confidence interval = 1.96

$$\frac{1.96^2 \times 0.15(1-015)}{0.05^2}$$

$$\frac{0.57624(0.85)}{0.0025}$$

$$\frac{0.489804}{0.0025}$$

=195.92

Therefore, a total of 200 samples were used for this study.

2.3 Media Preparation

The media used for the isolation of the various microorganisms were prepared following the manufacturers' instruction (Oxoid, Cambridge UK), hence, Salmonella-Shigella agar was not sterilized. Sterilization of the other media was done using an autoclave operated at a temperature of 121℃, for 15 minutes.

2.4 Inclusion Criteria, Sampling and Inoculation

All the clean equipments available and frequently used in the cafeteria were included in this study. All the dirty (unwashed/unclean) equipments, unavailable ones, as well as those that haven't been in use for a while were excluded. The equipments sampled were mortar, pestle, countertops, chopping board, tables (4), spoons (8), knifes (2), and pots (2). Samples were collected aseptically, using sterile swab sticks. The swab sticks were moistened prior to the collection of the samples using 1 ml of sterile maximum dilution media (normal saline). The moist swabs were then rubbed over the surface area of the equipments with firm pressure and rotation of the swab shaft using finger and thumb. The sample area was swabbed horizontally and vertically approximately 10 times in each direction. After swabbing, the swabs were placed in sterile container with 40 ml maximum dilution medium (normal saline). Each

sample was labelled with the sample site, date and time of collection. The swab sticks were then immediately transferred to the laboratory and inoculated on Nutrient agar, Mannitol Salt agar, Salmonella-Shigella agar, Eosin Methylene Blue agar and McConkey agar (Oxoid, Cambridge UK) at 37℃ for 18-24 hours. These samples were then examined using specific parameter (before awareness- day 1 and after awareness-day 2). Biochemical tests were carried out using conventional biochemical reagents for confirmation of the presumptive isolates [8].

2.5 Serial Dilution and Enumeration of Bacteria

Serial dilution was done for each sample to obtain 10 dilutions (10^{-1} to 10^{-10}) by diluting 1 in 9 mls of sterile peptone water, first from stock culture, then from subsequent dilutions. 0.1 ml each of 10^{-5} and 10^{-6} dilution was then inoculated using spread plate technique for total aerobic plate count (TAPC) on Nutrient agar and mean colony count on McConkey agar, Eosin methylene blue agar, Mannitol Salt agar and Salmonella-Shigella agar (at 35-37℃ for 20-24 hours). All culture media used were prepared according to manufacturer's instruction (Oxoid, UK). Plates showing between 30 and 300 colonies were counted using the digital illuminated colony counter (Gallenkamp). Colony counts were expressed as colony forming units per ml (cfu/ml) of sample. All counts were done in triplicate and average values were reported [9].

2.6 Identification of Bacterial Isolates

After incubation at 37℃ for 18-24 hours, the morphological and cultural characteristics of the bacteria were used to identify them. The isolates were further subjected to Gram staining and other conventional biochemical tests (catalase, coagulase, DNA-ase, indole, motility, Citrate utilisation, Voges Proskauer, Lysine decarboxylase, and sugar fermentation as described by Cheesbrough [8] for proper isolation and identification.

2.7 Statistical Analysis of Results

Student's T test was used to determine if there was a difference in bacterial load between the cafeteria equipments sampled before and after creation of awareness. Two-way analysis of variance (ANOVA) was also used to investigate the significant difference in the bacterial load in the equipments from the cafeterias sampled. Each test was conducted at 95% confidence interval, $P<0.05$ at the appropriate degrees of freedom (d.f.). A P-value of $P<0.05$ was considered significant. The data were analysed using the programme IBM SPSS Version 22.

3. RESULTS

A total of 200 samples were collected and microbiologically examined. From the samples examined, a total of 126 isolates were obtained from 73 positive samples. The isolates obtained include *Escherichia coli*, *Staphylococcus aureus, Salmonella typhimurium* and *Enterobacter aerogenes*. *E. coli* had the highest percentage occurrence on cafeteria equipments while the lowest percentage was seen in *E. aerogenes* (Fig. 1). The percentage occurrence of bacteria in the cafeteria sampled was generally higher on second visit (i.e, after creation of awareness on food pathogens and enlightenment on food safety and cafeteria hygiene) (Table 1). From the statistical analysis carried out using SPSS at 0.05 level of significance $_{DF=19,}$ the paired samples test significance for each cafeteria were higher than the 0.05 level of significance, which indicates that the level of microbial contamination in all the tables, counters and equipments used in food preparation was not statistically significant. The mean colony count showed *E. coli* to have the highest count (4.84±0.12) in cafeteria A while *S. typhimurium* had the lowest count (1.79±0.07) also in cafeteria A. No *Enterobacter* was however, isolated from cafeteria C (Table 2). Total aerobic plate count carried out on the equipments from each cafeteria revealed that counters sampled in cafeteria C had the highest count of 5.89±0.43 while pestles from the same cafeteria had the lowest aerobic count of 2.42±0.00 ($P>0.05$) (Table 3).

4. DISCUSSION

The first and foremost suspect "gadget" in the kitchen is the human hand. Too often, people don't wash their hands before preparing food. More often, people don't wash their hands between handling possibly contaminated foods like meat and other foods that are less likely to be contaminated like vegetables. This "cross-contamination" is a leading cause of food borne disease. This study recorded 36.5% occurrence of bacteria in cafeteria equipments, with *E. coli* as the highest occurring organism. Although the level of contamination from this study is not significant ($P>0.05$), it should not be overlooked as food borne pathogens are a menace to the

health of the general population. Many factors may play a role in the incidence and reporting of food borne illness outbreaks that implicate fresh produce, such as an aging population that is susceptible to food borne illness, an increase in global trade, a more complex supply chain, improved surveillance and detection of food borne illness, improvements in epidemiological investigation, and increasingly better methods to identify pathogens [10].

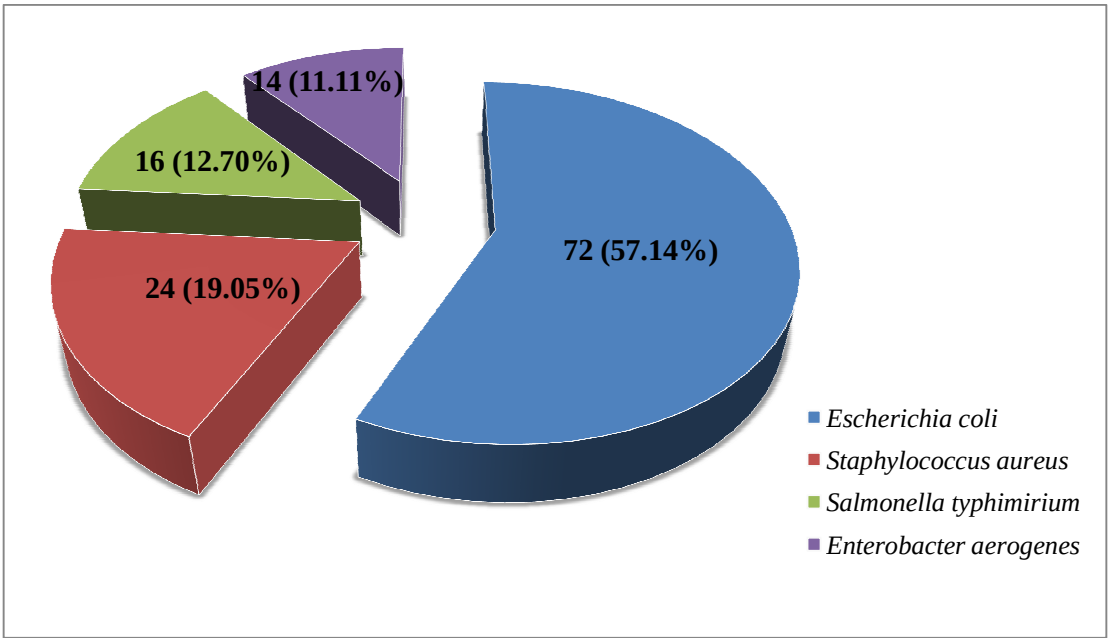

Fig. 1. Percentage occurrence of bacterial isolates on cafeteria equipments

Table 1. Percentage occurrence of bacterial isolates from each cafeteria

Cafeteria	Days of visit	*Escherichia coli* (%)	*Staphylococcus aureus* (%)	*Enterobacter aerogenes* (%)	*Salmonella typhimirium* (%)	Total (%)
A	One	4 (3.17)	2 (1.59)	3 (2.38)	1 (0.79)	10 (7.94)
	Two	2 (1.59)	3 (2.38)	2 (1.59)	1 (0.79)	8 (6.35)
B	One	2 (1.59)	4 (3.17)	1 (0.79)	1 (0.79)	8 (6.35)
	Two	6 (4.76)	1 (0.79)	1 (0.79)	1 (0.79)	9 (7.14)
C	One	14 (11.11)	1 (0.79)	0 (0.00)	1 (0.79)	16 (12.70)
	Two	12 (9.52)	2 (1.59)	0 (0.00)	1 (0.79)	15 (11.90)
D	One	10 (7.94)	3 (2.38)	2 (1.59)	1 (0.79)	16 (12.70)
	Two	8 (6.35)	5 (3.97)	1 (0.79)	3 (2.38)	17 (13.49)
E	One	4 (3.17)	0 (0.00)	3 (2.38)	4 (3.17)	11 (8.73)
	Two	10 (7.94)	3 (2.38)	1 (0.79)	2 (1.59)	16 (12.70)
Total		72 (57.14)	24 (19.05)	14 (11.11)	16 (12.70)	**126 (100)**

Table 2. The mean colony count of bacterial isolates from cafeteria

Cafeteria	Log10 CFU/ml			
	S. aureus	***Salmonella***	***E. coli***	***Enterobacter***
A	3.72±0.11	1.79±0.07	4.84±0.12	3.78±0.08
B	2.57±0.08	1.86±0.07	3.95±0.10	1.93±0.07
C	3.07±0.11	2.82±0.07	4.16±0.12	NG
D	4.46±0.12	4.13±0.88	4.79±0.13	4.67±0.08
E	4.28±0.06	4.36±0.10	3.94±0.12	4.75±0.10

Key: NG – No growth

Table 3. The mean total aerobic plate count from cafeteria

Cafeteria	Pots	Spoons	Log10	CFU/ml	Counter	Knifes	Tables	Chopping board
			Mortar	Pestle				
A	3.32±0.75	4.22±0.42	4.45±0.32	3.64±0.33	2.67±1.10	4.06±0.17	NG	3.06±0.41
B	3.81±0.80	3.59±0.73	2.98±0.51	4.22±0.34	3.60±0.34	NG	3.53±0.43	2.52±0.30
C	4.27±0.65	3.69±0.24	3.54±0.93	2.42±0.00	5.89±0.43	3.78±0.00	3.14±0.80	NG
D	4.33±1.35	5.04±0.35	4.58±0.83	5.18±1.01	NG	4.09±0.82	3.95±0.99	NG
E	3.67±1.34	5.06±0.14	3.58±0.51	4.02±0.85	NG	4.70±0.74	4.56±0.89	NG

Key: NG – No growth

Escherichia coli was seen to have the highest occurrence (57.14%) in this study. *E. coli* is an opportunistic organism which is not harmful in its natural habitat. Most *E. coli* strains are non-pathogenic, found in the intestines of all animals, including humans, and function by suppressing harmful bacterial growth. However, there are minorities of strains such as serotype O157:H7 (enterovirulent strains of *E. coli*) that may cause human illness. *E. coli* O157:H7 is a life-threatening bacterium that produces large quantities of potent toxins that can cause severe damage to the lining of the intestines. Human illness associated with *E. coli* O157:H7 infection may include non-bloody diarrhoea, haemorrhagic colitis, haemolytic uremic syndrome (HUS), or thrombotic thrombocytopenic purpura (TTP). Haemorrhagic colitis progresses from abdominal cramps to non-bloody diarrhoea to bloody diarrhea. HUS largely affects young children and is the leading cause of acute renal failure in children. TTP is a rare syndrome of *E. coli* O157:H7 infection, which largely affects adults and resembles HUS histology. *E. coli* O157:H7 outbreaks have been associated with meat (especially undercooked or raw hamburger), fresh produce, raw milk, unpasteurized apple juice, coleslaw, and contaminated water [4]. *E. coli* isolated from this study could have been transferred by pre-processed food handlers (employees) in the course of preparing ready-made food from the raw produce without observing proper environmental sanitation and personal hygiene. Although this study didn't go further to identify the strains of *E. coli* isolated, Its presence in any number can be regarded as evidence that the cooking equipments, tables and counters were contaminated either by the food handlers or from fresh produce obtained from the open air market prior to preparation, as *E. coli*, if not of human origin, is an important cause of food intoxication [11].

Salmonella causes several diseases such as gastroenteritis typhoid (enteric fever) etc. which is transmitted via food or water. The isolation of *Salmonella* in the cafeteria is of great concern as it is one of the most common cause of food borne illness (salmonellosis) and is responsible for millions of cases of illness each year [10]. The presence of *Staphylococcus aureus* in tables, counter, equipments should also be worrisome as the organism is pathogenic and survives for longer period in water than the coliforms. *Staphylococcus aureus* can cause food poisoning when a food handler contaminates food and then the food is not properly refrigerated. Other sources of food contamination include the equipments and surfaces on which food is prepared. These bacteria multiply quickly at room temperature to produce a toxin that causes illness. *Staphylococcus aureus* is killed by cooking and pasteurization [12]. The presence of *Enterobacter aerogenes* in this study is not a threat as it has hitherto not been associated with foodborne illness or food poisoning. It is an aetiological agent of hospital (nosocomial) infection which has become a cause of concern in community infections [13] as it resides in soil water. Cross contamination seen in this study could have occurred via hands of restaurant workers and/or food handlers as well as consumers who have recently visited hospitals or tended to ill patients. The species of *Enterobacter* associated with food poisoning however, is *Enterobacter sakazakii* (now known as *Cronobacter sakazakii*).

The mean colony count showed *E. coli* to have the highest count in cafeteria A while *S. typhimurium* had the lowest count also in cafeteria A. No *Enterobacter* was however, isolated from cafeteria C. Total aerobic plate count (TAPC) carried out on the equipments from each cafeteria revealed that countertops sampled in cafeteria C had the highest count of 5.89±0.43 microbes while pestles from the same cafeteria had the lowest aerobic count of 2.42±0.00 microbes ($P>0.05$). Countertops in most restaurants in developing countries are not always properly cleaned as most restaurant

managers are business inclined; hence, believe that appropriate cleaning of countertops is done by using supposedly clean dry clothes since there is rarely direct contact with processed food. Pestles, however, are given more sanitary attention since they are used frequently in processing of local food like pounded yam, flour meals, cooking condiments (e.g. cocoyam, ginger, nutmeg, e.t.c) and the tiniest particles left on the pestle surface could go a long way to alter the taste of the subsequent food prepared using same pestle. This could explain why the TAPC was higher in countertops than pestles. The mean counts were generally low, indicating the non-severity of the microbial occurrence. The incidence of the bacteria should however not be overlooked, as they could pose a threat on the health of patronizing consumers in the nearest future. Therefore it is essential that cafeteria managers take urgent intervention and increase the standards of cleanliness of the cafeteria and environment to prevent higher contamination even as the contamination rate is presently low.

5. CONCLUSION

This study obtained a considerably high rate (36.5%) of bacterial contamination from cafeteria equipments. Although statistically insignificant, it is indicative of poor personal hygiene, uncleanliness of the environment and general neglect of food safety which can pose a health hazard to consumers. The creation of awareness on food pathogens and enlightenment on food safety and cafeteria hygiene did not yield any improvement as the percentage occurrence of bacteria in the cafeterias sampled was generally higher on second visit. The mean counts (level of occurrence) were generally low, indicating the non-severity of the microbial occurrence. However, their presence should not be overlooked by the cafeteria personnel as they could pose a threat of possible foodborne infections in the future. Personal hygiene and cleanliness of the environment should not be overlooked as it is important in ensuring food safety.

6. RECOMMENDATION

1. Standards must be set by the appropriate government agencies to ensure a clean and healthy public eating environment.
2. Proper or thorough supervision should be carried out by restaurant managers so as to ensure total compliance with standard hygienic practice.
3. Good hand washing technique should be adopted by: Using soap and warm running water or alcohol-based hand sanitizer. If soap is being used, hands should be well-lathered while washing all surfaces (including between the fingers, the backs of the hands, wrists, and under the fingernails) thoroughly for 20 seconds and then rinsed well.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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