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Isolation and Characterization of Bacteria Species Related to Food Contact Surfaces in Selected Cafeterias in a Tertiary Institution Campus in ABU, Zaria, Nigeria

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ABSTRACT: The study was carried out to isolate and characterize bacterial species from selected cafeterias in tertiary institution campus in Ahmadu Bello University Zaria, Nigeria. A total of ten samples were selected for analysis which include; spoons, plates, forks, chopping boards, worktops, tables, hands, washing water, plate rinsing water[1] and plate rinsing water[2]. The bacteriological analysis was carried out using the nutrient agar (NA) media, which was prepared by dissolving 28g of the NA powder in 1litre of distilled water, autoclaved and allowed to cool. The growth of the microorganisms were then observed and counted per ml. The identified species include; *Staphylococcus aureus*, *Staphylococcus epidermis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Bacillus anthracis.* The food contact surfaces analyzed revealed that Chopping board has the highest bacterial load with 28 x 10^5, followed by Hand 27 x 10^5, Worktop 20 x 10^5, Table 18.8 x 10^5, Washing water 16 x 10^5, Plate 13.6 x 10^5, Fork 12 x 10^5, Spoon 7.2 x 10^5, plate rinsing water[1] 6 x 10^5, plate rinsing water[2] 5 x 10^5. The frequency of occurrence of the isolated organisms include; *Staphylococcus aureus* 3(10.3), *Staphylococcus epidermis* 9 (31.1), *Escherichia coli* 4(13.8), *Klebsiella pneumonia* 6(20.7), *Pseudomonas aeruginosa* 3(10.3), *Proteus mirabilis* 2(6.9) and *Bacillus anthracis* 2(6.9). It was observed that *Staphylococcus epidermis* have the highest incidence of occurrence, *Bacillus anthracis* and *Proteus mirabilis* have the least incidence of occurrence.

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There are various types of foodborne micro-flora (pathogenic or non-pathogenic), that adheres itself to the food contents and surfaces for a long period of time. These microorganisms play vital role in food degradation, toxification and pathogenicity of consumers. This confers to affect the food quality and safety of food consumers (Bagge-Revn, *et al*., 2004; Vogel *et al*., 2001). Among these microorganisms, some foodborne pathogens cause serious health issues to humans, especially enteropathogenic entrobacteriaceae family i.e. *Escherichia coli*, *Salmonella* and *Shigella* (Lues *et al*., 2007; Borch and Arinder 2002). The causative agents for foodborne diseases are the ingestion of microbial pathogens, chemicals or bio toxins produced by the microorganisms. The degree of disease can be accounted by the rate of mortality and morbidity outbreaks, considering the acute and chronic manifestations or severity that may lead to cause deaths, illnesses, health abnormalities and economic losses due to these foodborne agents (Assefa, *et al*., 2015; WHO 2004). Foodborne illnesses have been a major issue in public health for decades, and food handlers play an important role in its transmission. Although there are various sources by which pathogens can contaminate food, multiply and cause infections in humans, but the persons who handle the food could be the possible cause of transmission. These food handlers contribute in food contamination through many ways i.e. negligence or mishandling of food, incorrect food preparation, personal hygiene, skin, cuts, hair and mouth. Improper sanitation of

surfaces and equipment may influence the burden of foodborne microorganisms to a greater extent (Assefa *et al*., 2015; Campos *et al*., 2009). Several reports have shown that poor personal hygiene and handling of foodstuffs could lead to various illnesses. Centre for Disease Control and Prevention identified over 400 food-related infections, in which 20% are due to food handlers (Michaels *et al*., 2004). Some more studies have reported that improper or poor handling of foods either in the manufacturing sector or homes can cause 97% of foodborne infections (Lambrechts *et al*., 2014). Identification of disease can be made possible by proper laboratory diagnosis for pathogens or toxins, and considering patients recent history for food consumption (Andargie *et al*., 2008; Plaut, 2000). The factors or cautions that could play an important role in foodborne illnesses are the worker training, awareness of handling food and hygiene, correct techniques and implementation of quality standards in food premises (Campos *et al*., 2009). Hence, this research evaluated was carried out to isolate and characterize bacterial species associated with food contact surfaces from selected restaurants in a tertiary institution campus in Ahmadu Bello University Zaria, Nigeria

MATERIALS AND METHODS

Study area: The research was carried out at the Microbiology Laboratory of the Department of Microbiology, Ahmadu Bello University, Zaria. The University began in 1961 with the faculties of Agriculture, Engineering, Law and Sciences, fifteen academic departments and 426 students. The Main Campus of Ahmadu Bello University is located in Samaru, a suburb of Zaria in Kaduna State, Nigeria. Samaru is situated within latitude 11° 15'N to 11°3'N of the equator and longitude 7° 30'E to 7°45'E of Greenwich Meridian , at an altitude of 550-700 meters. It is about 13km from Zaria-city on the Sokoto road, 8km to Shika and 7km from Bassawa. The University covers a land area of about 7,000 hectares and encompasses two campuses, twelve faculties, a Postgraduate School and 82 academic departments. It also has five Institutes, six Specialized centers, a Division of Agricultural Colleges, a School of Basic and Remedial Studies, a Demonstration Secondary School, a Primary School and a Consultancy Outfit which provides a variety of services to the University and the wider society (www.abu.edu.net, 2012).

Fig 1: Map of the study area showing sample collection site within the commercial zone on campus

Sample collection: A total of 100 microbiological swab samples were randomly collected from food contact surfaces from selected cafeterias in a tertiary institution campus in Ahmadu Bello University Zaria, Nigeria between (August to October 2021) Food contact surfaces selected for swab collection were (1) spoons, (2) plates, (3) forks (4) chopping boards, (5) worktops, (6) tables, (7) hands, (8) washing water (9) plate rinsing water[1] and (10) plate rinsing water[2]. Sample collection was performed on working days. Collected swab samples were analyzed for the detection and enumeration of bacterial species associated with food contact surfaces. These samples were treated according to method described in details by ISO 18593 (2004). Swab samples from selected surfaces were collected according to the reference method described by (ISO 18593, 2004). The swab sticks were removed from the peel pouch and inserted into the tube containing the neutralizing buffer. The tip

of the swab was pressed against the wall of the tube to remove any excess liquid.

Swabbing the food preparation surface: The area within each sample was swabbed by rubbing the swab over the surface. The surface was swabbed (whilst rotating the swab between the thumb and forefinger) in two directions at right angles to each other, horizontally and vertically. The area was swabbed for approximately 20 seconds. The swab was inserted more than half way into the neutralizing buffer (10ml) or the recovery diluents (10ml). It was cut aseptically so that the swab remained in the fluid. The swab container was labeled clearly with sample reference number, site, date and time. Collected swabs were aseptically transferred in a cool box to the laboratory within two hours for further analysis.

Sample preparation, inoculation and incubation: Swab samples in tubes were thoroughly mixed for 30 seconds using vortex to make initial dilutions. These dilutions were serially diluted into further decimals. Each suspension was further treated on duplicate plates by pour plate method using 1 ml aliquots for the analysis of desired microorganisms according to the ISO suggested protocols. (ISO, 21528-2, 2004). All media and reagents for analysis were of Oxoid (Hampshire, UK) brand.

Counting and identification of colonies: After completion of specified incubation, counting of colonies was performed as CFU/cm^2 of the surface area according to the ISO protocol (ISO 18593, 2004). Identification and further confirmation of isolated bacterial species was performed through biochemical tests which involve; Citrate utilization test, Sulphate, Indole and Motility test, Methyl-Red Voges-Proskauer (MRVP), Triple Sugar Iron (TSI), and Urease test as mentioned in the respective protocols (ISO 21528-2, 2004).

Preparation of nutrients agar slants: The bacteriological analysis was carried out using the nutrient agar (NA) media, which was prepared by dissolving 28g of the NA powder in 1litre of distilled water, autoclaved and allowed to cool. The NA media was poured into Petri dishes labeled and inoculated for 24hr in an electro-thermal incubator. The growth of the microorganisms were then observed and counted per ml (number of microorganisms per ml of water samples).

Gram staining: Gram staining was carried out to differentiate the bacterial species into two large group based on their cell wall constituents. The Gram stain procedure distinguishes between Gram positive and Gram negative groups by the colour of their cells i.e. red or violet (Gram reaction). About 5 drops of crystal violet stain was added over the fixed culture and allowed to stand for 60 seconds. The stain was poured off and the excess water was gently rinsed with a stream of water from a plastic bottle. The objective of this step is to wash off the stain, not the fixed culture. About five drops of the iodine solution was added on the smear, enough to cover the fixed culture and allowed to stand for 30 seconds. The iodine solution was poured off and the slide was rinsed with running water. Few drops of decolorizer were added so that the solution trickles down the slide. It was rinsed off with water after 5 seconds. It was then counter stain with five drops of Safranin solution for 20 seconds. The Safranin solution was washed off with water and blot with bibulous paper to remove the excess water and air dried. (APHA, 2005).

Biochemical test: All colonies isolated were further screened biochemically after Gram staining using methods described in details by Coghlan *et al*. (1975), which involves the observation of whether or not growth of bacterium in liquid nutrient medium will ferment a particular gas.

RESULT AND DISCUSSION

A total of ten (10) samples were collected from food contact surfaces in selected cafeterias in a tertiary institution campus in Nigeria and isolated and characterized for bacterial species. The results of the morphological characteristics of the probable bacteria isolates and their various shapes, color and pigment formed on the nutrient agar media are presented in Table 1. Table 1 revealed the main bacterial species that were isolated from the food contact surfaces which were *Staphylococcus aureus*, *Staphylococcus epidermis*, *Escherichia coli*, *Klebsiella pneumonia*e, *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Bacillus anthracis*. The bacteria may had found their way onto the surfaces through cross contamination, poor personal hygiene of the users, diseases spreading vectors such as cockroaches and flies (Hood and Zottala, 1997). This variation in the number of positive samples from one place to another is likely to be as a result of the differences in hygiene and sanitary conditions in the environment.

Table 2 shows the characteristics of the isolated bacterial species using biochemical tests such as catalase, coagulase, citrate, indole, urease motility and methyl red /Voges proskauer test. The identified species includes; *Staphylococcus aureus*, *Staphylococcus epidermis*, *Escherichia coli*, *Klebsiella pneumonia*e, *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Bacillus anthracis*. Table 2 shows the identification of the suspected isolated

organisms from different biochemical tests. All the samples were identified to be contaminated with the suspected target organisms. The biochemical test results showed similarities with the standard biochemical test results.

Table 1: Morphological characteristics of the isolated bacterial species

Sr. No	Organism	Morphological characteristics
1	*Staphylococcus epidermis*	Small in size, milky in colour and circular in shape
2	*Staphylococcus aureus*	Large and golden yellow colonies on nutrient agar (NA)
3	*Escherichia coli*	Large yellow and pinkish colonies on nutrient agar (NA)
4	*Klebsiella pneumoniae*	Shows large mucoid and yellow colonies on nutrient agar(NA)
5	*Pseudomonas auroginosa*	Forms a bright green colony on nutrient agar (NA)
6	*Proteus mirabilis*	Large size, irregular in shape and milky in colour
7	*Bacillus anthracis*	Small in size, milky in colour and circular in shape

Table 2: Characterization of the isolated bacterial species

Sample	A	B	C	D	E	F	G	H	I	Organisms
Spoon[1]	+	-	+	-	+	+	-	+	-	*Staphylococcus aureus*
Spoon[2]	+	-	+	-	+	+	-	+	-	*Staphylococcus aureus*
Plate[1]	+	-	-	-	-	+	+	+	-	*Pseudomonas aeruginosa*
Plate[2]	+	-	-	-	-	+	-	+	-	*Klebsiella pneumonia*
Forks[1]	+	+	-	-	-	-	-	+	-	*Klebsiella pneumonia*
Forks[2]	+	+	-	-	+	-	-	+	-	*Staphylococcus epidermis*
Chopping board[1]	+	+	-	-	+	+	+	+	-	*Staphylococcus aureus*
Chopping board[2]	+	-	-	-	+	+	-	+	-	*Staphlococcus aureus*
Worktop[1]	+	-	-	-	+	-	+	+	-	*Stapylococcus epidermis*
Worktop[2]	+	-	+	+	-	-	+	-	-	*Escherichia coli*
Table[1]	+	+	-	-	+	+	-	+	-	*Staphylococcus aureus*
Table[2]	+	-	-	-	+	-	-	-	-	*Bacillus anthracis*
Hand[1]	+	-	-	-	-	+	+	+	-	*Pseudomonas aeruginosa*
Hand[2]	+	-	-	-	-	+	-	+	-	*Klebsiella pneumonia*
Washing water[1]	+	-	-	+	-	+	+	+	+	*Proteus mirabilis*
Washing water[2]	+	-	-	+	-	+	+	+	+	*Proteus mirabilis*
Plate rinsing waterA[1]	+	-	-	-	+	-	-	-	-	*Bacillus anthracis*
Plate rinsing waterA[2]	+	-	-	-	+	+	-	+	-	*Staphylococcus aureus*
Plate rinsing waterB[1]	+	-	+	+	-	-	+	-	-	*Escherichia coli*
Plate rinsing waterB[2]	+	-	+	+	-	-	+	-	-	*Escherichia coli*

A= Catalase; B= Coagulase; C = Indole; D = MR; E = VP; F = Citrate; G = Motility; H = Urease; I = H_2S

Table 3 shows the bacterial load of the isolated bacterial species. The food contact surfaces analyzed revealed that chopping board has the highest bacterial load with 28 x 10^5, followed by hand 27 x 10^5, Worktop 20 x 10^5, Table 18.8 x 10^5, Washing water 16 x 10^5, Plate 13.6 x 10^5, Fork 12 x 10^5, Spoon 7.2 x 10^5, plate rinsing water[1] 5 x10^5 and plate rinsing water[2] 6 x 10^5 respectively.

Table 3: Microbial load of the isolated bacterial species

Sr. No	Sample	TAPC(CFU/ml)
1	Spoon	7.2 x 10^5
2	Plate	13.6 x10^5
3	Fork	12 x 10^5
4	Chopping board	28 x 10^5
5	Worktop	20 x 10^5
6	Table	18.8 x 10^5
7	Hand	27 x 10^5
8	Washing water	16 x 10^5
9	Plate rinsing water[1]	6 x 10^5
10	Plate rinsing water[2]	5 x 10^5

Table 4 shows the frequency of occurrence of the isolated organisms; *Staphylococcus aureus* 3 (10.3), *Staphylococcus epidermis* 9 (31.1), *Escherichia coli* 4(13.8), *Klebsiella pneumonia* 6(20.7), *Pseudomonas aeruginosa* 3(10.3), *Proteus mirabilis* 2(6.9) and *Bacillus anthracis* 2(6.9). It was observed that *Staphylococcus epidermis* have the highest incidence of occurrence, *Bacillus anthracis* and *Proteus mirabilis* have equal and the least incidence of occurrence.

Table 4: Frequency of occurrence of the isolated bacterial species

S/ No	Organism	Occurrence	Frequency (%)
1	*Staphylococcus epidermis*	3	10.3
2	*Staphylococcus aureus*	9	31.1
3	*Escherichia coli*	4	13.8
4	*Klebsiella pneumonia*	2	6.9
5	*Pseudomonas aeruginosa*	3	10.3
6	*Proteus mirabilis*	2	6.9
7	*Bacillus anthracis*	6	20.7
	Total	**29**	**100**

As observed in table 3 the significant rise in bacterial load could be as a result of poor processing method, poor hygiene practice, improper and unhygienic handling of the food product, bad sanitation operations and use of unclean formites and utensils. Baird-Parker (1993) stated that one of the major sources of contamination arises from the handlers during food preparation. However an important factor which

significantly contributes to the great increase in the count is the location of the retail outlet which is basically markets where food can easily be contaminated by aerial spores or bacterial spores carried in the air and several other insects, such as flies, which are uncountable at such sites. Monica and Chessbrough (2000) states that insects such as flies can cause contamination by continuous contact with the product, so also dust particles from heavily contaminated atmospheres around market places and motor parks. Another source of contamination could be the food vendors themselves, as most are dressed in filthy clothes. The high microbial counts recorded for hand swabs and easy contact surfaces could be a reflection of the level of exposure and thus cross contamination. The hand is the main organ used for manipulating the environment and pick microorganism in these diverse environments. Microorganisms are picked by the hands and spread from person to person during exchange of pleasantries or contacted from surfaces and this could be the reason why *Staphylococcus aureus* and *Bacillus anthracis* were isolated from the foods surfaces because these organisms could have been picked up from a place and then spread to another where they were picked up by individuals.

As observed in table 4 most frequently isolated bacterium was *Staphylococcus aureus* 9(31.1%). This is because they are major components of the normal flora of the skin and nose, which probably explains its high prevalence as contaminant as it can easily be discharged by several human activities. This observation is in conformity with the finding of other researchers (Brooks *et al*., 2007). *Staphylococcus aureus* is the most important potential pathogen that cause boils, abscesses, wound infections toxic shock syndrome and pimples. The highest percentage frequency of occurrence of *Staphylococcus aureus* could also be due to the ability of the organism to withstand the effect of heat. Also this could be due to the fact that *Staphylococcus aureus* is abundant in the human body especially skin as a normal flora. A high percentage of *Bacillus spp* isolated could be explained by their spore forming ability which makes them able to resist harsh environmental condition, withstand dry heat and certain chemical disinfectants for a considerable period. This is in accordance with the research carried out by Brooks *et al*., (2007) who reported that *Bacillus spp* was found to be among the predominant organism that was isolated from door handles, Samy *et al.*, (2012) also reported the isolation of *Bacillus spp* from environmental sites in Mecca city. *Bacillus species* are known to bear resistant spores and are common environmental contaminants; it has been shown to be a transient micro flora of the hand and surfaces due to the spore forming ability. *Bacillus cereus* has been implicated in food poisoning (Schmitt *et al*., 1990) and several other *Bacillus species* have been implicated in human pathogenesis and as food spoilage organisms (Collins and Lyne 1986).

Conclusion: The findings of this study have confirmed that pathogenic bacteria can exist in cooked foods even though they may physically appear to be quite wholesome; thus, proper steps should be taken to ensure that the occurrence of such organisms in foods is kept within limits. It can be concluded that pathogenic bacteria capable of causing food poisoning can be isolated from cooked foods using microbial analysis. Also such bacteria can be identified using appropriate biochemical tests and the total microbial load of such foods can as well be ascertained thereby providing information on their microbial quality.

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