

# EVALUATION OF FOOD CONTACT SURFACES IN SELECTED RESTAURANTS OF KADUNA STATE UNIVERSITY FOR THE PRESENCE OF *Escherichia coli* AND *Staphylococcus aureus*

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## ABSTRACT

The evaluation of food contact surfaces in Kaduna State University restaurants for the presence of *Escherichia coli* and *Staphylococcus aureus* were investigated. Five (5) selected restaurants in Kaduna State University were evaluated to assess the cleanliness of the surfaces that come into contact with food. Fifty (50) swab samples were collected from five (5) restaurants with three (3) samples each from plates, spoons and two (2) from chopping boards and tables. The samples were analysed using streak plate technique and biochemical tests to identify the bacterial isolates. Out of the 50 samples analysed, 13 (26 %) were positive for *Escherichia coli* and 0% was positive for *Staphylococcus aureus*. Out of the 13 that were positive for *Escherichia coli*, 8 (61.5 %) were from plates, 3 (23.1 %) were from chopping boards and 1 (7.7 %) was from table and spoon each. Eleven (11) (84.6 %) isolates of *E. coli* were susceptible to pefloxacin and tarivid each and 3 (23.1 %) isolates of *E. coli* were intermediate to septrin and ciprofloxacin each. While 6 (46.2 %) isolates of *E. coli* were resistant to amoxicillin and 7 (53.8 %) isolates of *E. coli* were resistant to sparfloxacin. This suggests that there is need for enlightenment of food handlers on the importance of good hygienic practices. Like introducing Hazard Analysis and Critical Control Point (HACCP) systems in food production and practices.

**Keywords:** Contact surfaces, restaurants, swab, streak plate, hygienic practices

## INTRODUCTION

Food is defined as any edible or nourishing substance which when eaten sustains life, promotes growth and provides energy. Foods eaten are rarely sterile as they carry microbial associations. Since before the beginning of recorded history, food and microorganisms have interesting associations. Nutrition of food is not just limited to consumers; they are also a good source of nutrition for growth of microorganisms (Dilbaghi and Sharma, 2007). Microorganisms in food can be from the natural micro-flora of the raw material and can be introduced during slaughter or harvesting, processing, storage and distribution (Adams and Moss, 2008).

Surfaces that food comes in contact with are known as food contact surfaces which include; utensils, worker's hands, worker's clothing, all equipment, facilities and packaging materials. These surfaces and food handler's hands are important means of pathogen transmission (Blackburn, 2003). These surfaces have been found to constitute a constant risk to transfer of microbes

and also contribute to cross infection. Many cases of foodborne illnesses are linked to contaminated raw ingredients usage, improper cooking and control of temperature but also, cross contamination of food through food contact surfaces is also a risk factor (DeCasare *et al.*, 2003).

Food contact surfaces are defined as surfaces that come in contact with human food during food processing. They include utensils, equipment, packaging material, hands and worker clothing. Food needs to be safe, wholesome and acceptable as it is important for human survival (Begani *et al.*, 2012).

In food service establishments, the main sources of microbial contamination has been discussed by several studies which include dirty food contact surfaces, poor personal hygiene practices, and inappropriate storage temperatures. After these studies, it has been revealed that the main sources of microbial contamination are food contact surfaces (Griffith and Clayton, 2005).

Occurrence of food borne diseases and improper sanitary practices in food production causes risk in food safety. As a result of the nature of interpersonal interactions, school environments are prone to epidemiological outbreaks (Lee and Greiga, 2010). Introduction of an additional variable that supports microbial growth, such as food increases the risk. During food preparation, food contact surfaces may easily be contaminated with significant amount of pathogenic microbes thereby, compromising food quality (Begani *et al.*, 2012). Upon consumption of contaminated foods, students can be infected with pathogenic bacteria such as *Escherichia coli*, *Salmonella sp*, *Staphylococcus aureus* and are at risk of developing pathological conditions (Lee and Greiga, 2010).

*Escherichia coli* popularly abbreviated as *E. coli* is a member of the bacterial family *Enterobacteriaceae* (Meng and Schroeder, 2007). *Escherichia coli* is a gram negative bacillus and is found in the intestines of mammals. They are often the most abundant facultative anaerobes in the environment. Due to its occurrence in faeces, it is used as an indicator of faecal contamination (Adams and Moss, 2008). Contaminated food and water are the sources of exposure to *Escherichia coli*. There are strains of *Escherichia coli* that are harmless and there are also strains that are pathogenic or harmful. Some harmful strains of *Escherichia coli* include: Enteropathogenic *Escherichia coli* (EPEC), Enterohemorrhagic *Escherichia coli* (EHEC), Enteroinvasive *Escherichia coli* (EIEC), Enterotoxigenic *Escherichia coli* (ETEC) (Gerba, 2009).

*Staphylococcus aureus* on the other hand is a gram positive coccus. It is a member of the bacterial family *Staphylococcaceae* and is part of the genus *Staphylococci*. The genus consists of

many species such as *Staphylococcus epidermidis*, *Staphylococcus saprophyticus* and *Staphylococcus haemolyticus* (Washington, 2006). It was discovered in 1880 by a surgeon, Sir Alexander Ogston and in 1884, it was named by Rosenbach as *Staphylococcus aureus* (aureus; golden) due to the colonial pigmentation which is golden. It is usually found as a commensal which is associated with skin, skin glands and mucous membranes particularly in the nose of healthy individuals. As estimated, approximately 20-30% of the population are *Staphylococcus aureus* carriers (Heyman, 2004). However, it can become pathogenic when it enters into host tissue through inoculation by needles or trauma of the cutaneous barrier or through ingestion of contaminated food (Murray *et al.*, 2003). The aim of this research was to evaluate food contact surfaces in Kaduna State University restaurants for the presence of *Escherichia coli* and *Staphylococcus aureus*.

## MATERIALS AND METHODS

### Collection of Samples

Fifty (50) swab samples were collected from five (5) restaurants with three (3) samples each from plates, spoons and two (2) chopping boards and tables. The swab sticks were removed from the sterile wrappings and the tip were moisturized by immersing it in nutrient broth for each sampling respectively. For each swab, the tip was pressed against the inside of the tube containing the moisturizer to remove excess fluid. The area to be swabbed were selected and the tips of the swabs were pressed onto the surface and streaked in two directions at right angles whilst rotating the swab stick tween the fore finger and the thumb. Then the swabs were deposited back in the sterile wrapping, screwed tight and labelled separately as described by United States Department of Agriculture, USDA (2012).

### Media Preparation

All media used were prepared according to the manufacturer's instruction. After preparation, it was allowed to cool to about 45°C. The working area was then disinfected using cotton wool and disinfectant. The prepared media was then poured into sterile petri dishes, bijour bottles or test tubes as the case may be and were allowed to solidify.

### Isolation of Bacteria from Contact Surfaces

The swab sticks were streaked on the surface of Eosin Methylene blue agar and Mannitol Salt agar and were incubated at 37°C for 48 h. The suspected colonies were subcultured to obtain pure isolates (Oyeleke and Manga, 2008).

### Characterization and Identification of Bacterial Isolates

#### Morphological and Cultural Characteristics

The following characteristics were observed; colour, shape, elevation, margin, surface and texture.

#### Gram Staining

Each isolate was picked and emulsified in a drop of saline on a slide to make a smear. The smeared slides were then allowed to air dry. After air drying, they were heat fixed in the flame of a Bunsen burner. After that, the slides were covered with crystal violet stain as the primary stain for a minute. It was rinsed with distilled water and then Gram's iodine (mordant) was applied for a

minute also. It was then rinsed with distilled water and acetone (decolouriser) was applied and rinsed immediately with distilled water. Lastly, safranin was applied as counterstain and rinsed with distilled water after 30 seconds. The slides were then left to air dry and after drying they were heat fixed by passing through the flame of a Bunsen burner; it was then viewed under the microscope using oil immersion objective to see the gram reaction (Oyeleke and Manga, 2008).

### Biochemical Tests

#### Indole Test

This was performed on tryptophan broth by inoculating the media with the isolates. The test tubes were incubated for 24 h at 37°C. Result was read after adding Kovac's reagent (Acharya, 2013).

#### Methyl Red Test and Voges-Proskauer Test

These was done in the prepared methyl red-voges-proskauer (MR-VP) broth. The isolates were inoculated in the media and incubated for 24 h at 37°C. Reagents added vary according to the test. For methyl red test, methyl red reagent was added and for the voges-proskauer test Barritt's A and Barritt's B reagents were added (Acharya, 2013).

#### Citrate Utilization Test

This was performed on Simmon's citrate agar. The medium was prepared according to the manufacturer's instruction and it was poured into test tubes and kept in a slanted position to make an agar slants. The isolates were inoculated on the media and incubated for 24 hours at 37°C (Acharya, 2013).

#### Sugar Fermentation Test

Peptone water with yeast extract was prepared and then about 8 drops of methyl red was added as an indicator. Exactly 1 % of each sugar (glucose, lactose, sucrose and maltose) was then added to the media in test tubes containing Durham tubes. Wire loop was then used to inoculate the media with the isolates and incubated for 24 h at 37°C (Kar, 2008).

#### Antibiogram of Bacterial Isolates against Selected Antibiotics

The Kirby-Bauer disc diffusion method was used. Using an inoculating loop, test tubes of nutrient broth were inoculated with the test organisms and incubated at 37°C for 24 h to obtain a 24 h broth culture of the isolates. Then, the turbidity of the 24 h broth cultures were adjusted to 0.5 McFarland standard. McFarland standard was prepared by mixing 99.5ml of 1 % sulphuric acid and 0.5ml of 1 % barium chloride. After that, Mueller Hinton agar was prepared according to the manufacturer's instruction and then, it was poured into sterile petri dishes and allowed to solidify. A sterile swab was then used to inoculate the plates by dipping it in the suspension of the isolates and streaking on the surface of the agar. The plates were then allowed to stand for about 3 minutes. Using a sterile forceps, the gram negative multiple discs were then placed on the corresponding inoculated plates. The discs were pressed slightly to ensure contact with the agar. They were then incubated at 37°C for 18 h (Adetunji and Odetokun, 2012). The zones of inhibition were then measured and interpreted according to the recommendations of National Committee for Clinical Laboratory Standards, NCCLS (2004).

**RESULTS**

Table 1 showed the result of isolation of bacteria from food contact surfaces. Thirteen (13) bacteria isolates from the contact surfaces were suspected to be *Escherichia coli* due to production of green metallic sheen colonies on Eosin methylene blue agar (EMB). Table 2 showed the result of isolation of bacteria from food contact surfaces in which none of the bacteria had the characteristics of *Staphylococcus aureus* on mannitol salt Agar (MSA) which appears as yellow zones in the medium. Table 3 showed the morphological and the biochemical characteristics of the bacteria isolates. All the isolates were found positive for indole and methyl red test and were negative for voges-proskauer and citrate utilization test. They were also able to ferment the four sugars used (lactose, glucose, maltose and sucrose) by producing acid and gas. Acid production was indicated by colour change from red to yellow and gas production was indicated by appearance of bubbles in the Durham tubes. Table 4 showed the result of the sensitivity test of the bacteria isolates to 10 different antibiotics. Eleven (11) (84.6 %) isolates of *E. coli* were susceptible to pefloxacin and tarivid each and 3 (23.1 %) isolates of *E. coli* were intermediate to septrin and ciprofloxacin each. While 6 (46.2 %) isolates of *E. coli* were resistant to amoxicillin and 7 (53.8 %) isolates of *E. coli* were resistant to sparfloxacin.

**Table 1:** Isolation of *Escherichia coli* from Food Contact Surfaces

Restaurants	Surface type				Number of positive
	Plates	Spoons	Tables	Chopping boards	
A	3	3	2	2	2/10
B	3	3	2	2	4/10
C	3	3	2	2	0/10
D	3	3	2	2	3/10
E	3	3	2	2	4/10
Total	15	15	10	10	13/50

**KEY:**

- A – First restaurant
- B – Second restaurant
- C – Third restaurant
- D – Fourth restaurant
- E – Fifth restaurant

**Table 2:** Isolation of *Staphylococcus aureus* from Food Contact Surfaces

Restaurants	Surface type				Number of positive
	Plates	Spoons	Tables	Chopping boards	
A	3	3	2	2	0/10
B	3	3	2	2	0/10
C	3	3	2	2	0/10
D	3	3	2	2	0/10
E	3	3	2	2	0/10
Total	15	15	10	10	0/50

**KEY:**

- A – First restaurant
- B – Second restaurant
- C – Third restaurant
- D – Fourth restaurant

**E – Fifth restaurant**

**Table 3:** Morphology and Biochemical Characteristics of the Bacterial Isolates from Food Contact Surfaces

Isolates	Colonial morphology	Gram Reaction	Indole	Methyl Red	Voges Proskauer	Citrate	Lactose	Glucose	Sucrose	Maltose	Probable Organisms
PA1	Bacilli	-	+	+	-	-	+	+	+	+	<i>E. coli</i>
PA2	Bacilli	-	+	+	-	-	+	+	+	+	<i>E. coli</i>
PB	Bacilli	-	+	+	-	-	+	+	+	+	<i>E. coli</i>
TB	Bacilli	-	+	+	-	-	+	+	+	+	<i>E. coli</i>
CB1	Bacilli	-	+	+	-	-	+	+	+	+	<i>E. coli</i>
CB2	Bacilli	-	+	+	-	-	+	+	+	+	<i>E. coli</i>
PD1	Bacilli	-	+	+	-	-	+	+	+	+	<i>E. coli</i>
PD2	Bacilli	-	+	+	-	-	+	+	+	+	<i>E. coli</i>
CD	Bacilli	-	+	+	-	-	+	+	+	+	<i>E. coli</i>
PE1	Bacilli	-	+	+	-	-	+	+	+	+	<i>E. coli</i>
PE2	Bacilli	-	+	+	-	-	+	+	+	+	<i>E. coli</i>
PE3	Bacilli	-	+	+	-	-	+	+	+	+	<i>E. coli</i>
SE	Bacilli	-	+	+	-	-	+	+	+	+	<i>E. coli</i>

PA1: first plate from the first restaurant, PA2; second plate from the first restaurant, PB; plate from the second restaurant, TB; table from the second restaurant, CB1; first chopping board from the second restaurant, CB2; second chopping board from the second restaurant, PD1; first plate from the fourth restaurant, PD2; second plate from the fourth restaurant, CD; chopping board from the fourth restaurant, PE1; first plate from the fifth restaurant, PE2; second plate from the fifth restaurant, PE3; third plate from the third restaurant, SE; spoon from the fifth restaurant.

**Table 4:** Antibiogram of Bacteria Isolates from Food Contact Surfaces to Commercially Available Antibiotics

Antibiotics	Concentration (ug)	PA1	PA2	PB	TB	CB1	CB2	PD1	PD2	CD	PE1	PE2	PE3	SE
		2												
Seprtrin	30	12	-	10	-	12	-	-	15	21	18	24	-	26
Chloramphenicol	10	-	20	25	-	19	26	13	16	26	23	-	-	20
Sparfloxacin	30	23	-	22	-	13	-	25	-	26	16	13	12	26
Ciprofloxacin	10	18	13	26	22	23	18	-	25	20	24	25	24	26
Amoxicillin	30	-	-	-	18	-	16	-	26	14	26	-	18	18
Augmentin	30	-	24	26	20	24	18	13	22	-	21	26	-	21
Gentamycin	30	26	-	24	-	26	20	16	18	16	26	19	21	-
Pefloxacin	10	26	22	25	-	23	26	21	24	19	26	16	22	24
Tarivid	10	22	24	16	26	14	26	-	26	16	19	22	26	20
Streptomycin	30	14	20	26	26	21	23	13	26	14	26	-	15	26

**KEY:**

PA1: first plate from the first restaurant, PA2; second plate from the first restaurant, PB; plate from the second restaurant, TB; table from the second restaurant, CB1; first chopping board from the second restaurant, CB2; second chopping board from the second restaurant, PD1; first plate from the fourth restaurant, PD2; second plate from the fourth restaurant, CD; chopping board from the fourth restaurant, PE1; first plate from the fifth restaurant, PE2; second plate from the fifth restaurant, PE3; third plate from the third restaurant, SE; spoon from the fifth restaurant.

**DISCUSSION**

Result of this study has shown that there were contaminations of food contact surfaces in Kaduna State University with *Escherichia coli*. This might be as a result of the type of water used to wash and clean the surfaces which may be feacally contaminated. Absence of *Staphylococcus aureus* might be that the organism is transmitted through the food handlers or those washing the surfaces by activities such as sneezing or coughing which may not necessarily occur. This agrees with the report of the findings of

Sudheesh *et al.* (2013) who reported that out of the five restaurants sampled, one was negative for *Escherichia coli* and all were negative for *Staphylococcus aureus*. Out of the 50 samples collected in this study, 13 (26 %) were positive for *Escherichia coli*. This is slightly lower than the findings of Groenewald *et al.* (2014) where they reported 30% for *Escherichia coli* and Begani *et al.* (2012) reported 0% for *Staphylococcus aureus*. Among the 13 (26 %) isolates that were positive for *Escherichia coli*, plates were the highest contaminated surfaces with 61.5% followed by chopping boards with 23.1% then tables and spoons with 7.7% each. This could be as a result of using dirty water for washing the dishes and utensils because some restaurants use the same water to wash the plates throughout the day. Source of the water is also important because some are initially faecally contaminated. And after washing these dishes and utensils, warm water is not usually used to sanitize those. Though the presence of *Escherichia coli* on tables from the result of this study was very low, it might be attributed to the fact that the tables are not cleaned with clean towels and sanitizers or were not cleaned frequently during service hours. Contamination of these surfaces might result possibly from inadequate cleaning and sanitisation of these surfaces as well as inadequate personal hygiene of the food handlers which can lead to contamination of the surfaces and can serve as a vehicle for *Escherichia coli* foodborne infection. This agrees with a similar report by Cobsy *et al.* (2008). The antimicrobial sensitivity test against the bacteria isolates showed that out of the thirteen (13) positive samples, 11 (84.6 %) *E. coli* were susceptible to pefloxacin and tarivid each, 3 (23.1%) were intermediate to septrin and ciprofloxacin each while 6 (46.2 %) were resistant to amoxicillin and 7 (53.8 %) were resistant to sparfloxacin. These findings are in partial agreement with Al-Ferdous *et al.* (2012).

### Conclusion

Contamination of food contact surfaces by only *Escherichia coli* was revealed in this study. Thirteen (13) *Escherichia coli* isolates were isolated and identified while no *Staphylococcus aureus* was isolated from the contact surfaces sampled. Antibioqram of the isolates showed that they were more susceptible to pefloxacin and tarivid and were more resistant to sparfloxacin. Therefore there is need for food handlers to be educated on the importance of sanitation and safe practices because when food is served with microbiologically contaminated utensils. The organisms present on such surfaces can pose a great risk to the health of consumers. Obtainment of zero contamination of microbes is not possible, as such, good hygiene, cleaning and sanitation is important to achieve low levels of microorganisms on food contact surfaces and final product.

### Recommendations

1. Water used for washing and rinsing dishes and utensils should be from a good source and should be changed at regular intervals before it is too dirty.
2. Food handlers should improve their personal hygiene especially of their hands by washing thoroughly after using the toilet or after handling raw foods.
3. Food handlers should clean their food contact surfaces with warm soapy water and sanitize with sanitizers such as chlorine especially after these surfaces have come in contact with raw foods.

Washed dishes and utensils should be kept covered to prevent contamination by dust, droplets from speaking and sneezing and

flies that carry infectious agents on their body surfaces.

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