

# Bacteriological Quality of Some Ready-to-Eat Foods Served in some Food Centres in Zaria, Kaduna State, Nigeria

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**Abstract** The aim of this research was to examine bacteriological quality of some ready-to-eat foods that were served in some food centres in Zaria. Samples of jollof rice, pounded yam and melon seed soup were collected from three categories of food service centres and enumerated on selective media for the isolation of *Bacillus cereus*, *Escherichia coli* and *Staphylococcus aureus*. Analysis of variance of the mean counts of the organisms from the three categories of food service centres were not significantly different ( $p > 0.05$ ). However, mean counts ranging from 5.76 to 7.91  $\log_{10}$  cfu/g for *B. cereus* and 5.99 to 7.91  $\log_{10}$  cfu/g for *S. aureus* recovered from the foods were above the standard acceptable limits of less than 5.0  $\log_{10}$  cfu/g for *B. cereus* and less than 4.0  $\log_{10}$  cfu/g for *S. aureus*. Out of the 108 food and swab samples examined, 82 (75.9%) were contaminated with *S. aureus*, 68 (62.9%) with coliforms and 57 (52.7%) with *B. cereus*. *E. coli* was isolated from pounded yam and melon seed soup in bukateria and cafeteria. The antibiotic-sensitivity test of the bacteria revealed that most of the bacteria were resistant to the common antibiotics used in the treatment of infections.

**Keywords:** Jollof rice, pounded yam, melon seed soup, *Bacillus cereus*, *Escherichia coli*, *Staphylococcus aureus*

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## 1. Introduction

Ready-to-eat foods sold in food service centres are conveniently consumed by diverse group of people including students, workers and other professions. This is mainly due to lack of time for home cooking of meals. However, when food poisoning outbreaks are investigated, it has been shown that small and medium food service centres are often important locations in the transmission of food-borne illness [1,2]. In fact, food-borne diseases are the most widespread health problems in the contemporary world and an important cause of reduced economic productivity [3]. More than two hundred known diseases are transmitted through food [4]. The causes of food-borne illnesses include bacteria, fungi, viruses, parasites and toxins. Symptoms range from mild gastroenteritis to life threatening neurological, hepatic, and renal syndromes [5]. Research studies have clearly indicated that biological contaminants are the major cause of food-borne diseases [3]. In the developing world including Nigeria, health risks are associated with initial contamination of raw foods with pathogenic bacteria and subsequent contamination by food handlers during preparation from

cross-contamination, survival of pathogens during preparation and microbial proliferation after cooking of food, as well as during holding of cooked foods [4]. The use of bare hands to feel the adequacy of cooking increases level of contamination as enteropathogens can survive on the hands for three hours or longer [6]. The container in which foods are served is an important risk factor, similarly, use of polythene bags, papers and leaves in wrapping ready-to-eat foods also increase risk of contamination. The papers used for packaging some ready to eat foods are usually newsprint of questionable origin. Leaves are usually wiped with a piece of cloth without washing or disinfecting. Moreover, micro-flora on leaves and microbes acquired through poor handling of foods could be transferred to ready-to-eat foods [6]. Handling of food at ground level also increases risks of contamination because dust could easily get into cooked foods. There is also a risk of contamination associated with exposure of food to flies [2]. The preparation of pounded yam involves pounding the staple after cooking by means of a mortar and pestle and turning the stiff dough with bare hands [6]. The fact cannot be overemphasized that ready-to-eat foods sold in food service centres pose a direct health hazard to consumers if they contain an infective dose of pathogens or toxic levels of their toxins [7].

## 2. Materials and Methods

### 2.1. Selection of samples

Three food centres from the three categories of food service providers (restaurant, cafeteria and bukateria) were chosen for this research. The selection of food-service centres for this study was based on consent given by the proprietors of the food-service centres. The foods that were examined were; jollof rice, pounded yam and melon seed soup.

### 2.2. Description of Preparation Methods of Selected Samples

**Jollof rice:** This dish is the most frequently consumed food in all the categories of the food service centres studied. Preparation involved, cooking parboiled rice with other ingredients such as tomatoes, red bell peppers, chillies, onions, beef, groundnut oil and seasoning. The method of cooking in all the food centres was the same with a slight variation, with the addition of diced carrots and peas in restaurant and cafeteria. Carrots and peas were not added in the jollof rice of bukateria (buka).

**Pounded yam:** The preparation of pounded yam involved pounding yam after cooking by means of a mortar and pestle. The stiffed dough is scooped in small proportions and wrapped in polythene bags. The general method of cooking and handling pounded yam was the same in all the categories of food service centres.

**Melon seed soup:** This soup is made from a mixture of ground tomatoes, red bell peppers, chillies, onions, beef or fish, ground melon seeds, spinach, palm oil and seasoning. It is usually eaten with pounded yam.

### 2.3. Sampling of Food Samples for Enumeration

Samples were taken at sequential stages of preparation and handling using sterile wide-mouth bottles with tight-fitting lids. Hot samples were cooled immediately in an insulated plastic box containing ice blocks. Samples were subsequently examined for total aerobic plate count, *S. aureus*, *B. cereus* and *E. coli*. Water samples, swab of working surfaces and worker's hands were collected. These were placed on ice-packed insulated container and transported to the laboratory for analysis. All media and diluent were prepared according to manufacturer's instructions and sterilized by autoclaving for 15 minutes at 121 °C.

### 2.4. Enumeration of Micro-organisms

Enumeration of micro-organisms from food samples was conducted by the methods described by [8]. Ten grams of each sample was aseptically transferred into 90 milliliters of 0.1% sterile peptone water and homogenized. Each sample was ten-fold diluted in 0.1% sterile peptone water and 0.1 ml of dilutions  $10^{-2}$  to  $10^{-4}$  were spread onto nutrient agar, Baird Parker agar, Vasconcellos Rabinovitch medium [9] and Eosin methylene blue agar. After inoculating, the surface of the medium was allowed to dry.

The plates were inverted and incubated at 37 °C for 24 hours. Colonies were counted, calculated and reported as cfu/g. Creamy or pale-yellow colonies on Vasconcellos Rabinovitch medium (VRM) were counted as presumptive *Bacillus cereus*, greenish metallic sheen on EMB was counted as presumptive *E. coli* and black shiny colonies on BPA were counted as presumptive *S. aureus*.

### 2.5. Purification and Storage of Isolates

Colonies of *S. aureus*, *E. coli* and *B. cereus* from BPA, EMB and VRM plates respectively were purified by repeated streaking on nutrient agar plates, Gram stained and stored on nutrient agar slants at 4°C.

### 2.6. Biochemical Characterization of Isolates

The biochemical characterization of isolates stored on nutrient agar slants was carried out as described by [10,11,12]. Briefly, the tests were carried out as follows;

**Methyl- red (MR)/ Voges-Proskauer (VP) test:**

All Gram positive and Gram negative organisms on slants were tested for acid production. Four millilitre of MR-VP broth in tubes were inoculated with isolates and incubated at 37 °C for 48 to 72 hours. One millilitre of the incubated broth was transferred into a tube and 2-3 drops of methyl red added to the tube. The appearance of a red colour on the addition of the indicator indicated a positive methyl red test. To the rest of the broth in the original tube, 0.2 ml of 40% potassium hydroxide solution was added followed by 0.6 ml of an ethanolic solution of 5% alpha-naphthol. The tube was vigorously shaken and placed in a sloping position. The tubes were examined after 30 and 60 minutes. The development of red colour from the top of the broth indicated a positive VP test.

**Indole test:**

Presumptive *E. coli* and *S. aureus* isolates were grown in 5 ml of peptone water for 48 hours, to the culture was then added Kovac's indole reagent (0.5 ml per 5 ml culture) and shaken gently. In the positive test, indole (present in the culture) dissolves in the reagent which then becomes red and forms a layer at the surface of the medium.

**Urease test:**

Isolates were inoculated onto slants of Christensen's urea agar and incubated at 32 °C for one week. The slants were observed daily within the incubation period for colour change from yellow to red which is an indication of a positive result.

**Oxidase test**

Both Gram positive and negative isolates on slants were smeared on a filter paper soaked with a few drops of 1% freshly prepared oxidase reagent (Tetramethyl-p-phenylene diamine dihydrochloride), using a sterile toothpick stick. The development of an intense purple colour within 30 seconds indicated a positive test; failure of colour development (purple) within 30 seconds indicated a negative result.

**Catalase test:**

All Gram positive cocci and rods stored on nutrient agar slants were tested for catalase production. Tubes containing 2-3 ml of 3% hydrogen peroxide solution were inoculated with 24 hours culture using a sterile glass rod. A positive test was indicated by active bubbling while the absence of bubble indicated a negative result.

**Citrate utilization:**

Isolates were inoculated onto Simmon's citrate agar slants in bijou bottles and incubated at 32 °C for 24 to 72 hours. A positive result was indicated by development of a deep blue colour on the slants.

**Motility test:**

All Gram positive and Gram negative isolates on nutrient agar slants were tested for motility. Tubes of motility indole urea (MIU) agar were stab-inoculated with test organisms and incubated at 37 °C for 24 to 48 hours. Development of growth from the line of stabbing towards the rest of the entire medium and cloudiness of the medium was recorded as positive for motility, while restriction of growth to the line of stabbing indicate a negative result.

**Spore stain:**

A smear of the presumptive *Bacillus* was made on a slide, dried and heat fixed. The entire slide was flooded with 5% aqueous malachite green and steamed for 3-6 minutes, rinsed under running water and was counter stained with 0.5% aqueous safranin. Spores stain green while vegetative cells are pink or red.

**Nitrate reduction:**

About 0.5 ml of sterile nitrate broth was inoculated with isolates and incubated at 37 °C for 4 hours. One drop of sulphanic acid reagent and one drop of alpha-naphthylamine reagent were added and shaken. Development of a red colour in the tubes indicated a positive result (nitrate reduced to nitrite).

**Gelatin liquefaction test:**

Presumptive *Bacillus* isolates were inoculated onto tubes of plain gelatin, incubated at 37 °C and observed for gelatin liquefaction for a period of 3-7 days. Before observation, the tubes were refrigerated at 4°C for 2 hours to enable solidification of gelatin. Tubes that solidified during refrigeration period were indication of negative results while those that did not solidify indicated positive results.

**Growth in 7% NaCl**

Presumptive *Staphylococcus* and *Bacillus* isolates were inoculated into tubes of nutrient broth containing 7% NaCl and incubated at 37 °C. The tubes were then observed for growth for a period of 3 to 7 days.

**Starch Hydrolysis:**

Gram positive isolates on slants were inoculated onto plates of nutrient agar incorporated with 10% potato starch suspension. The plates were then incubated at 37°C for 48 hours. After the incubation period, the plates were flooded

with iodine solution. Starch hydrolysis was indicated by the appearance of clear zones around colonies.

**Phenylalanine deaminase test and litmus milk reaction:**

The Gram positive isolates were grown overnight on phenylalanine agar, 0.2 ml of a 10% solution of ferric chloride was added over the growth on the culture slants. A positive enzyme production was indicated by the formation of a green colour on the surface of the culture slant. The isolates were also inoculated into tubes of litmus milk and incubated at 37 °C for 3 to 7 days. The tubes were observed within this period for colour change from mauve to white or pale yellow and curd formation.

**Coagulase test:**

The tube coagulase test was adopted in testing for the production of coagulase from presumptive *S. aureus* isolates using freshly prepared human and sheep plasma. One in ten dilutions (0.2 ml of plasma and 1.8 ml of physiological saline) were made and inoculated with test organisms, which were then incubated at 37 °C for 6 hours. The tubes were checked every 30 minutes for 6 hours for evidence of production of clot or formation of a loose web of fibrin indicated a positive coagulase test.

Haemolysis of human and sheep erythrocytes (red blood cells):

All cocci isolates were inoculated on blood agar containing 10% human and sheep blood respectively. The plates were incubated at 37 °C for 24 hours. Clear haemolysis and discolouration of the blood medium to pale yellow indicated a positive haemolysis test.

**Utilization of sugar:**

Screw capped test tubes containing 5 ml aliquots of basal medium made up of 0.5% (w/v) yeast extract, 0.75% (w/v) peptone and 0.1% (w/v) bromothymol blue were incorporated with either 6% (w/v) glucose, mannitol and lactose. The sugar solutions were added at a rate of 2 ml to the 5 ml basal medium and sterilized. Each tube was inoculated with 0.1 ml of 24 hour cultures of the isolates, incubated at 37°C and observed daily for 2 weeks for fermentation. Fermentation was positive with change in colour from green to yellow.

## 2.7. Antibiotic Sensitivity Test

The biochemically confirmed *E. coli*, *B. cereus* and *S. aureus* isolates were examined for antibiotic sensitivity. Sensitivity of isolates to antibiotics was determined by the disc-diffusion technique (Kirby-Bauer) as recommended by [13] on Mueller Hinton agar and Multo-disc (HABdiscs ABTEK) containing ampicillin (10µg), chloramphenicol (10µg), cloxacillin (5µg), erythromycin (5µg), gentamicin (10µg), penicillin (1i.u), streptomycin (10µg) and tetracycline (10µg), for gram-positive microorganisms and ampicillin (25µg), colistin (25µg), gentamicin (10µg), nalidixic acid (30µg), nitrofurantoin (200µg), co-trimoxazole (25µg), streptomycin (25µg) and tetracycline (25µg) for gram-negative organisms. A 24 hour culture of the isolates was emulsified in 3-4 ml of 0.85% physiological saline and standardized by visual comparison with McFarland

0.5 turbidity standard (0.6 ml of 1% w/v of barium chloride to 99.4 ml of 1%v/v of sulphuric acid) as described by [10]. Using a sterile swab, the plates were streaked evenly on the surface and allowed to dry for about 3-5 minutes, antibiotic paper discs were placed in the centre of the plates using sterile forcep. All plates were incubated at 35°C for 24 hours. Using a ruler on the underside of the plates, the diameter of each zone of inhibition in millimeter was measured. The zones sizes of each antimicrobial were interpreted using the interpretative chart and reporting the organisms as 'Resistant', 'Intermediate' and 'Susceptible'. The standard positive and negative control strains used to test the performance of the method were:

- i. Staphylococcus aureus ATCC021001
- ii. Escherichia coli ATCC11775.

## 2.8. Statistical Analysis

The Tukey's test, a multiple comparison test obtained under the ONE-WAY Analysis of Variance menu of the SPSS statistical package was used for analysing the data at the 0.05 level of significance.

## 3. Results

### 3.1. Bacteriological Analysis

A total of 108 samples were collected during food preparation and handling from three food centres (restaurant, cafeteria and bukateria) as well as water, hand and working surface swabs. Eighty-two (75.9%) were contaminated with *S.aureus*, 68(62.9%) were contaminated with coliforms and 57(52.7%) were contaminated with *B. cereus*. Six *E. coli* were isolated from pounded yam and melon seed soup in bukateria and cafeteria. The microbiological analyses revealed that aerobic count for foods from the three restaurants were not significantly different ( $P>0.05$ ). Similarly, staphylococcal, *Bacillus* and coliform counts were not different ( $P > 0.05$ ). However most of the counts were above the permissible level of ( $>5.0 \log_{10}/g$  and  $>4.0 \log_{10}/g$ ) for *Bacillus* and Staphylococcal counts respectively [14]. The mean counts of the organisms isolated from the restaurant, cafeteria and bukateria are shown in Table 1, Table 2 and Table 3 respectively.

Table 1. Bacteriological quality of foods at different stages of preparation and handling in the restaurant

Parameter	Counts Log <sub>10</sub> cfu/g*			
	APC	SC	BC	CC
<b>Jollof rice</b>				
RF	6.73±1.09	5.95±0.30	5.70±0.15	<1x10
IAC	<1x10	<1x10	<1x10	<1x10
H	6.12±0.14	6.69±1.13	5.80±6.20	5.90±0.11
<b>Melon seed soup</b>				
RF	7.38±0.39	6.61±0.92	6.30±0.75	6.1±0.25
IAC	<1x10	<1x10	<1x10	<1x10
H	6.03±4.88	6.66±1.08	5.58±0.48	5.76±2.11
<b>Pounded yam</b>				
RF	7.59±0.45	6.72±0.88	7.15±0.98	5.89±1.28
IAC	<1x10	<1x10	<1x10	<1x10
IAP	5.77±0.54	5.55±0.45	<1x10	5.58±0.47
H	6.74±1.07	6.80±0.87	6.64±1.08	6.57±1.08
Water	<1x10	<1x10	<1x10	<1x10
Hand swab	6.07±0.19	7.56±0.40	<1x10	<1x10
Working surface swab	5.93±3.85	6.57±1.19	<1x10	5.54±0.0

APC = Aerobic Plate Count; SC= Staphylococcal Count; BC = *Bacillus* Count; CC= Coliform Count; RF= Raw Food; H= Holding for an average of six hours; IAC= Immediately after Cooking; IAP = Immediately after Pounding; \* Counts are means of triplicate experiments; ± = Standard deviation; <1x10 = Organisms not isolated

Table 2. Bacteriological quality of foods at different stages of preparation and handling in the cafeteria

Parameter	Counts Log <sub>10</sub> cfu/g*			
	APC	SC	BC	CC
<b>Jollof rice</b>				
RF	6.44±0.38	6.33±0.57	6.33±0.62	6.84±0.10
IAC	<1x10	<1x10	<1x10	<1x10
H	6.14±0.48	6.41±0.44	5.96±0.84	6.86±0.10
<b>Melon seed soup</b>				
RF	5.56±0.45	6.39±0.51	6.33±0.51	6.11±0.61
IAC	<1x10	<1x10	<1x10	<1x10
H	6.23±0.4	6.48±1.50	6.09±0.55	5.88±6.27 <sup>(+)</sup>
<b>Pounded yam</b>				
RF	6.05±0.12	5.86±0.12	0.46±0.55	5.92±6.27
IAC	<1x10	<1x10	<1x10	<1x10
IAP	6.36±0.46	6.18±0.54	5.60±0.45	6.38±0.46 <sup>(+)</sup>
H	6.97±0.27	6.77±0.20	6.87±0.32	6.66±0.11 <sup>(+)</sup>
Water	5.94±0.15	6.04±0.55	5.91±0.10	<1x10
Hand swab	6.95±0.21	6.91±2.9	5.78±0.12	6.85±0.20
Working surface swab	6.23±0.0	5.63±0.0	<1x10	6.28±0.82

APC = Aerobic Plate Count; SC= Staphylococcal Count; BC = *Bacillus* Count; CC= Coliform Count; RF= Raw Food; H = Holding for an average of six hours; IAC= Immediately after Cooking; IAP = Immediately after Pounding; \* Counts are means of triplicate experiments; ± = Standard deviation; <1x10 = Organisms not isolated; <sup>(+)</sup> = *E. coli* isolated.

Table 3. Bacteriological quality of foods at different stages of preparation and handling in the bukateria

Parameter	Counts Log <sub>10</sub> cfu/g*			
	APC	SC	BC	CC
<b>Jollof Rice</b>				
RF	5.72±0.63	7.69±0.63	5.91±3.70	<1x10
IAC	<1x10	<1x10	<1x10	<1x10
H	7.25±1.03	6.54±0.93	7.83±0.20	5.87±6.41
<b>Melon seed soup</b>				
RF	7.28±0.92	6.27±1.33	6.62±1.17	6.29±1.35
IAC	<1x10	<1x10	<1x10	<1x10
H	6.89±1.52	7.03±0.98	6.07±1.00	6.28±1.17 <sup>(+)</sup>
<b>Pounded yam</b>				
RF	6.33±0.64	6.36±1.38	6.33±1.20	5.37±0.47
IAC	<1x10	<1x10	<1x10	<1x10
IAP	5.63±0.47	5.72±0.17	5.98±2.60	5.93±4.60 <sup>(+)</sup>
H	7.76±0.12	6.67±0.76	6.22±0.18	5.92±5.72 <sup>(+)</sup>
Water	5.94±0.0	5.60±0.4	<1x10	5.76±0.25
Hand swab	5.41±0.43	7.34±1.17	<1x10	<1x10
Working surface swab	7.04±1.02	5.66±0.53	5.78±0.14	<1x10

APC = Aerobic Plate Count; SC= Staphylococcal Count; BC = *Bacillus* Count; CC= Coliform Count; RF= Raw Food; H= Holding for an average of six hours; IAC= Immediately after Cooking; IAP = Immediately after Pounding; \* Counts are means of triplicate experiments; ± = Standard deviation; <1x10 = Organisms not isolated; <sup>(+)</sup> = *E. coli* isolated.

Table 4. Biochemical characterisation of presumptive *E. coli*, *B. cereus* and *S. aureus* from food and swab samples

Test	No. (%) of isolates positive		
	<i>E. coli</i> n=6	<i>B. cereus</i> n=57	<i>S. aureus</i> n=82
Gram reaction	6(100)	57(100)	82(100)
Methyl red	6(100)	-	-
V-P reaction	0(0.0)	57(100)	-
Indole	6(100)	0(0.0)	82(100)
Urease	0(0.0)	0(0.0)	0(0.0)
Oxidase	0(0.0)	0(0.0)	51(62)
Catalase	6(100)	57(100)	82(100)
Citrate	0(0.0)	55(96)	73(89)
Motility	6(100)	57(100)	0(0.0)
Anaerobic growth	6(100)	54(94)	51(62)
Spore formation	-	53(92)	-
Nitrate reduction	-	57(100)	82(100)
Gelatin liquefaction	-	57(100)	-
Growth in 7% NaCl	-	53(92)	82(100)
Reaction with litmus milk	-	57(100)	-
Starch hydrolysis	-	57(100)	-
Phenylalanine deaminase	-	0(0.0)	-
Coagulase	-	-	82(100)
Haemolysis	-	-	82(100)
<b>Utilization of:</b>			
Mannitol	6(100)	-	-
Glucose	6(100)	57(100)	82(100)
Lactose	6(100)	-	-

- Not tested.

### 3.2. Antibiotic-Sensitivity Test

Table 5 shows the results of the antimicrobial susceptibility test. Most of the 92 isolates tested were resistant to the commonly used antibiotics. All the six *E. coli* tested, were resistant to ampicillin, four (66.6%) were resistant to tetracycline and two (33.3%) were resistant to co-trimoxazole. All the *E. coli* were sensitive to nitrofurantoin and gentamycin while five (83.3%) were

sensitive to nalidixic acid, and streptomycin. Similarly, most of the 54 *S. aureus* isolates tested (92%) were resistant to ampicillin, 59.2% to tetracycline, 83.3% penicillin. However, 90.7% were sensitive to gentamicin and streptomycin, and 44.4% to cloxacillin. Additionally, out of the 32 *B. cereus* isolates tested, 81.2 % were resistant to ampicillin, 90.6% to cloxacillin and 100% to penicillin. However, 90.6% were sensitive to streptomycin, 78.1% to tetracycline and 62.5% to chloramphenicol.

Table 5. Antibiogram of *B. cereus*, *E. coli*, and *S. aureus* isolated from food and swab samples.

Antimicrobial agents (Concentration)/Test organism	No. (%) Susceptible	No. (%) Intermediate	No. (%) Resistant
<b><i>B. cereus</i> (n = 32)</b>			
Ampicillin (10µg)	3(9.3)	3(9.3)	26(81.2)
Chloramphenicol(10µg)	20(62.5)	11(34.3)	1(3.1)
Gentamicin(10µg)	28(87.5)	4(12.5)	0(0.0)
Streptomycin(10µg)	29(90.6)	2(6.2)	1(3.1)
Tetracycline(10µg)	25(78.1)	5(15.8)	2(6.2)
Cloxacillin(5µg)	2(6.2)	1(3.1)	29(90.6)
Erythromycin(5µg)	17(53.1)	14(43.7)	1(3.1)
Penicillin(1 i.u)	0(0.0)	0(0.0)	32(100)
<b><i>E. coli</i> (n = 6)</b>			
Nitrofurantoin(200µg)	6(100)	0(0.0)	0(0.0)
Nalidixic Acid (30µg)	5(83.3)	1(16.6)	0(0.0)
Tetracycline (25µg)	2(33.3)	0(0.0)	4(66.0)
Ampicillin (25µg)	0(0.0)	0(0.0)	6(100)
Colistin(25µg)	0(0.0)	5(83.3)	1(16.6)
Streptomycin(25µg)	5(83.3)	0(0.0)	1(16.6)
Co-trimoxazole(25µg)	3(50.0)	1(16.6)	2(33.3)
Gentamicin(10µg)	6(100)	0(0.0)	0(0.0)
<b><i>S. aureus</i> (n = 54)</b>			
Ampicillin (10µg)	0(0.0)	4(7.4)	50(92.5)
Chloramphenicol(10µg)	18(33.3)	16(29.6)	20(37.0)
Gentamicin(10µg)	49(90.7)	4(7.4)	1(1.8)
Streptomycin(10µg)	49(90.7)	2(3.7)	3(5.5)
Tetracycline(10µg)	11(20.3)	10(18.5)	32(59.2)
Cloxacillin(5µg)	24(44.4)	8(14.8)	22(40.7)
Erythromycin(5µg)	7(12.96)	27(50.0)	20(37.0)
Penicillin(1 i.u)	8(14.8)	1(1.8)	45(83.3)
<b><i>Staphylococcus aureus</i> ATCC021001 (n=1)</b>			
Ampicillin (10µg)	Resistant		
Chloramphenicol (10µg)	Intermediate		
Gentamicin (10µg)	Susceptible		
Streptomycin (10µg)	Susceptible		
Tetracycline (10µg)	Intermediate		
Cloxacillin (5µg)	Resistant		
Erythromycin (5µg)	Susceptible		
Penicillin (1 i.u)	Resistant		
<b><i>Escherichiacoli</i> ATCC11775 (n = 1)</b>			
Nitrofurantion (200µg)	Susceptible		
Nalidixic Acid (30µg)	Susceptible		
Tetracycline (25µg)	Resistant		
Ampicillin (25µg)	Resistant		
Colistin (25µg)	Intermediate		
Streptomycin (25µg)	Susceptible		
Co-trimoxazole (25µg)	Resistant		
Gentamicin (10µg)	Susceptible		

## 4. Discussion

High prevalence rate of *B. cereus*, and *S. aureus* in all the raw food samples was observed in this study. This is not surprising because most of the food commodities were bought from an open market where they were displayed in open bowls or sacks and sometimes on the ground. Since micro-organisms are widely distributed in the soil and the atmosphere, contamination of these products during processing and handling is expected. Similar high counts of  $2.4 \times 10^6$  to  $1.7 \times 10^7$  cfu/g were observed in dry food condiments sold in a local market [15]. The high prevalence rate of *S. aureus* could also be attributed to its ubiquity. The clinical significance of this organism is

largely due to the ubiquity of this species, being found in a wide variety of habitats and frequently as part of the normal human micro flora [16,17].

The isolation of *E. coli* from raw yam suggests contamination from food handlers, water or the source of the raw yam. Moreover, faecal contamination of water supplies and contamination from food handlers have been frequently implicated in outbreaks caused by *E. coli* [18]. Furthermore, detection of coliforms in food is an indication of contamination from human source [19]. Coliforms are of intestinal origin and they get into foods because of poor hygienic conditions of handlers [20]. The preparation of raw food ingredients at ground level and on inadequately cleaned working surfaces as observed in this study could obviously increase the initial microbial load

of these foods. Moreover, subsequent heat treatment might not kill all the organisms especially *B. cereus* that produces spore. Heat treatment of food (e.g. cooking) not only improves the taste, smell, appearance and digestibility, it also reduces the number of micro-organisms, improves keeping quality by inhibiting moulds, yeast, and bacteria that promote decay and infection. Thus, heat treatment is a practice aimed at improving the overall safety of food. The contamination of pounded yam with *E. coli* could be attributed to post cooking contamination by food handlers, utensils, mortar and pestle and water used for dipping pestle at intervals of pounding and moulding into shapes as well as wrapping in polythene bags. Time-temperature exposure during cooking was adequate to kill vegetative microbes and not the spores, but potential for recontamination existed from the hands of food workers, utensils, and cloths and sponges used in wiping as reported by [21]. In a similar study by [6], fufu was heavily contaminated because of excessive handling after cooking [6]. The pounding process, moulding and wrapping of pounded yam as observed in this study entails profuse sweating of which some of the sweats could gain access into the pounded yam resulting in post cooking contamination. Most of these foods were generally prepared in bulk and displayed for an average of six hours in pots and food warmers without reheating. Therefore, potential for bacterial growth and proliferation exist. Although the temperature used in cooking and frying is always high enough to kill most vegetative cells, resistant spores may survive [8]. Hazard analysis and epidemiological studies have revealed that foods prepared in advance and kept at room temperature for a long time (4-6 hrs or more) showed a massive increase in bacterial counts, reaching a critical level that may cause food-borne illness [2,21]. In a similar situation, when rice and related foods were held at room temperature for 4 hrs or longer particularly overnight, high number of bacteria were observed [8]. In this study most of the counts 5.76 to 7.91  $\log_{10}$ cfu/g for *B. cereus* and 5.99 to 7.91  $\log_{10}$ cfu/g for *S. aureus* were higher than the permissible levels, of less than 5.0  $\log_{10}$ cfu/g for *B. cereus* and less than 4.0  $\log_{10}$ cfu/g for *S. aureus* [14]. Susceptibility of individuals to pathogens varies greatly with age, acquired immunity and state of health. 'Special risk' groups, in which the risk is greater and disease may be far more serious, include children, the very old, pregnant women and immuno-compromised. The mean count of *B. cereus* from jollof rice in this study was less than that reported by [22] which ranged from  $10^6$  to  $10^8$  cfu/g. Similar counts were also recorded for eba ( $3.0 \times 10^5$ ) and tuwo ( $4.6 \times 10^4$ ) by [23] and from street foods in Accra, Ghana [5].

The wrapping of pounded yam in polythene bags could be a source of contamination by *B. cereus*, so also the mortar and pestle. Strains of *B. cereus* were isolated from paper board used for packaging foods [24]. The high counts of *S. aureus* obtained in this study could be attributed to the regular opening of the food warmers at intervals of serving, talking or un-stifled sneezing while serving and regular touching of nostrils. *S. aureus* inhabits the nostrils and skins of humans, each nostril could contain about  $10^8$  cells. The organism is disseminated to other parts of the body and to the environment by hands

[25,26,27]. Sweating during pounding yam and the use of inadequately cleaned hands during wrapping obviously increased the contamination of this food. Among the various metabolites produced by *S. aureus*, enterotoxins pose the greatest risk to consumer's health [16]. The presence of *S. aureus* in food indicates a high potential for the production of enterotoxin that causes food poisoning [25]. *E. coli* was isolated from pounded yam and melon seed soup during holding. The detection of *E. coli* in these foods is an indication of contamination most likely from human source. In a similar study, *E. coli* was isolated from tomato stew and shito [6]. Therefore, use of bare hands during wrapping pounded yam, opening and closing of warmers containing soups at intervals of serving could lead to cross-contamination. These observations corroborate with the findings of other researchers [8,28]. The antibiotic-sensitivity test of some of the isolates to antimicrobial agents revealed that the isolates were resistant to the common drugs of choice used in the treatment of infections. The high resistance of *E. coli*, *S. aureus* and *B. cereus* to the beta-lactam agents (ampicillin, penicillin and cloxacillin) and tetracycline recorded in this study were similar to that studied by [22]. Many studies have attributed resistance of *B. cereus* to penicillins as a result of production of beta-lactamase enzyme. [22,29]. Additionally, *S. aureus* strains carry a wide variety of multi-drug resistant genes on plasmids, which could spread among different species of Staphylococci [30]. The resistance of *B. cereus* and *S. aureus* to  $\beta$ -lactam antibiotics as observed in this study could be attributed to frequent misuse of the antibiotics in humans and animal feeds. This finding corroborates with studies of other researchers on bacterial resistance to antimicrobial agents [22,30,31,32]. Bacteria are increasingly developing resistance to many antibiotics and strains that were previously considered to be harmless are posing significant health threats. The situation is made more difficult in developing countries especially Nigeria where antimicrobial drugs are readily available over the counter with or without prescription from a medical practitioner. Such practice has led to misuse of antimicrobial drugs and emergence of multi-drug resistance strains of *B. cereus*, *E. coli* and *S. aureus*. The isolation of antimicrobial resistant bacteria in ready-to-eat food products reveals potential dissemination of antimicrobial resistant bacteria from food to humans [32].

## 5. Conclusion

This study has revealed high level of bacteriological contaminants in some ready-to-eat foods sold in some food service centres in Zaria. Food borne pathogens pose potential health hazards to consumers and therefore, the need for educating food service providers and consumers on food safety and hygiene. Public health authorities should also monitor conditions of hygiene, food safety and sanitation in food service centres. The high resistance of some of the bacteria to commonly used antibiotics in this study indicates frequent misuse and abuse of these drugs. Public awareness on dangers of indiscriminate purchase and use of antibiotics is important in the control of antibiotic resistance.

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