Journal of Community Medicine and Primary Health Care. 32 (2) 80-94
https://dx.doi.org/10.4314/jcmphc.v32i2.7

JOURNAL OF COMMUNITY MEDICINE AND PRIMARY HEALTH CARE

ORIGINAL ARTICLE

Distribution of Enterobacteria in Ready-to-Eat Food in Cafeterias and Retail Food Outlets in Benin City: Public Health Implications

Igbinosa IH[1,2], Beshiru A[1,3], Egharevba NE[1,4], Igbinosa EO[1,4]

[1]Applied Microbial Processes & Environmental Health Research Group, Faculty of Life Sciences, University of Benin, Private Mail Bag 1154 300283, Nigeria
[2] Department of Environmental Management & Toxicology, Faculty of Life Sciences, University of Benin, Private Mail Bag 1154 Benin City 300283, Nigeria
[3] Department of Microbiology, College of Natural and Applied Sciences, Western Delta University, Private Mail Bag 10 Oghara, 331101 Delta State, Nigeria
[4]Department of Microbiology, Faculty of Life Sciences, University of Benin, Private Mail Bag 1154 Benin City 300283, Nigeria

Keywords

Antibiotic-resistant; Enterobacteria; Foodborne pathogens; Microbial quality; Public health; Street foods

ABSTRACT

Background: The aim of this study was to determine the occurrence and antibiotic susceptibility profile of enterobacteria isolated from ready-to-eat foods within Benin metropolis, Nigeria.

Methods: This was a descriptive study of 210 ready-to-eat food samples comprising fried rice, jollof rice, moi-moi, salad, oil beans, non-oil beans, and African salad obtained from roadside food vendors between January and June 2017. Isolation and identification of enterobacteria isolates were carried out using standard bacteriological and molecular methods. Antibiotic susceptibility profile was carried out using the disc diffusion method.

Results: The mean mesophilic bacterial count expressed in $\log_{10}$ CFU/g from the ready-to-eat foods ranged from oil beans (4.3±0.52) to African salad (7.2±1.38). *Escherichia coli* count ranged between oil beans (1.8±0.16) and African salad (4.1±0.10). *Salmonella* species count ranged from non-oil beans (2.3±0.17) to African salad (5.2±0.09). Significant differences were observed from the population count of the ready-to-eat foods ($p < 0.05$). Bacterial isolates recovered from the ready-to-eat foods include *Pseudomonas aeruginosa*, *Citrobacter freundii*, *Enterobacter cloacae*, *Salmonella enterica*, *Escherichia coli*, *Klebsiella oxytoca*. The highest occurrence of the bacterial isolates was *Escherichia coli* 23(41.07%) while the least was *Citrobacter freundii* 3(5.36%) and *Enterobacter cloacae* 3(5.36%). The antimicrobial susceptibility profile of the bacterial isolates revealed that all bacterial isolates were 100% resistant to cefepime, ceftazidime, cefuroxime, ertapenem, and meropenem; with considerable sensitivity to kanamycin and gentamycin.

Conclusion: The occurrence of these bacterial isolates in the foods constitutes public health risk to consumers as these pathogens have been associated with foodborne infections.

Correspondence to:
Dr E. O. Igbinosa
Department of Microbiology,
Faculty of Life Sciences,
University of Benin, Benin City, Nigeria.
Email: etinosa.igbinosa@uniben.edu
Telephone: +234 (0) 811 4744 531

INTRODUCTION

Foods contain some essential nutrients for the prevention of disease and the advancement of human health and one of the primary needs of human. As a result of the important role of food in the existence of

human, it is important to sustain and improve the level of food safety connected with food.[1,2] Food safety conditions include efforts to circumvent contamination from chemical, biological agents and other particles and/or substances that can threaten human health.[1] As a consequence of socio-economic variations categorized by amplified mobility, resulting in increased ready-to-eat foods obtained outside the home, services of food vendors are on the rise and obligation for the safety of food have been reassigned from families/individuals to the food hawkers who infrequently apply good manufacturing practices. [2] Street foods are often connected with gastrointestinal ailments such as typhoid fever and diarrhoea as a result of serving practices and improper handling.[1,3,4] Poor environmental sanitation and poor personal hygiene among the food handlers are to a large extent accountable for a considerable proportion of the contamination.[3] These bacteria can emanate in connection with the ready-to-eat foods when prepared particularly in contaminated cooking utensils and unhygienic environments.[5, 6]

Food contaminated with antibiotic-resistant pathogenic bacteria is an important threat to public health.[7] Aside from infecting humans, they act as potential reservoirs of antimicrobial resistance and the pathogens easily convey antibiotic-resistant elements to unrelated and related bacterial species.[8] Globally, there is an increase in the occurrence of antimicrobial resistance among foodborne bacterial pathogens in recent year.[9,10] In Nigeria, ready-to-eat food in markets and streets as well as laterally within the road for travellers is a norm. Unfortunately, none of these food vendors is monitored or licensed by appropriate agencies or organizations to certify the safety of foods.[11] As a result of the conditions and manner, these food hawkers displaced their foods, there are possibilities that several ready-to-eat foods are contaminated with bacteria pathogens. Food hygiene has been largely neglected in the study area despite the fact that it is the most important component, essential to health and productivity of individuals and the community at large. Therefore, this study was carried out to determine the bacteriological quality and antimicrobial susceptibility of retiled ready-to-eat foods within Benin metropolis, Nigeria.

METHODOLOGY

Study Design: This descriptive study was carried out in Benin City which is the capital of Edo State in southern Nigeria. Benin City has an estimated human population of over 1 million and a lot of people usually patronize roadside food vendors which makes roadside food of different varieties very popular to residents in the city. Only ready-to-eat food samples available from roadside food vendors were considered in this study.

Ethical Consideration: Ethical consideration was not considered in this study as samples from human subjects were not included. However, informed consent

was obtained from individual food vendor owners prior to sample collection.

Sample Size Determination: The sample size used in this study was determined using the sample size determination formula as stated below:

$$\text{Sample (N)} = \frac{(Z_{1-\alpha/2})^2 \, P(1-P)}{d^2}$$

$Z_{1-\alpha/2}$ = Standard normal variant at 5% type I error ($p < 0.05$)

P = Expected prevalence based on previous study

d = Absolute error or precision (which is 5%)

Prevalence of 14.58% from a previous study [4] was used in this study.

$$\text{Sample (N)} = 191.59$$

Sample Collection: Ready-to-eat food samples were obtained from non-hygienic sources within Benin City, Nigeria. Non-hygienic sources are characterized by vending sites without running water, toilets, unclean food preparation surfaces, fridges to store food and untidy environment. A total of 210 ready-to-eat food samples comprising fried rice (*n*=30), jollof rice (*n*=30), moi-moi (*n*=30), salad (*n*=30), oil beans (*n*=30), non-oil beans (*n*=30), and African salad (*n*=30) were obtained from roadside food vendors within Benin City, Nigeria between January and June 2017. Samples were obtained using sterile plastic pets and separately parked and conveyed to the Applied Microbial Processes & Environmental Health Research Laboratory in ice pack within 4 hours after collection for microbiological analysis.

Bacteriological Assessment of the Samples: Ten grams (10g) of the samples were homogenized in 90 mL of sterile distilled water which makes up the stock solution. A 1.0 mL of the stock solution was then serially diluted from 10^1 to 10^9. The diluents (100 µL) were spread on Nutrient agar (NA) plates (Lab M, Lancashire, United Kingdom), Xylose Lysine Deoxycholate (XLD) agar plates (Lab M, Lancashire, United Kingdom) and Chromocult Coliform agar (CCA) plates (Lab M, Lancashire, United Kingdom) and incubated at 35±2°C for 18-24 hours. After incubation, colonies on NA were enumerated for mesophilic bacterial density; colonies with black centres on XLD agar were enumerated for *Salmonella* species; while purple to violet colonies on CCA was enumerated for *Escherichia coli* isolates. All bacterial cell count was expressed in colony-forming units per grams (CFU/g). Distinct colonies from respective agar were repeatedly sub-cultured on nutrient agar and incubated at 35±2°C for 18-24 hours. After incubation, purified isolates were stored on agar slants and maintained at 4°C until ready for further characterization.

Identification of Isolates: Colonies were presumptively identified by colony pigmentation and Gram reactions using 3% potassium hydroxide (3% KOH) (Gram-positive or Gram-negative) characteristics. Pure cultures were obtained by streaking a

portion of an isolated colony on NA and incubated aerobically at 37°C for 18-24 hours. The isolates were confirmed by catalase, oxidase, and indole activity. Isolates were further characterized biochemically using Analytical Profile Index (API) 20E. The tests were performed according to the manufacturer's instruction for use. Data interpretation was performed using the Analytical profile index (API) database (V4.1) with the apiweb™ identification software. Pure isolates where DNA was extracted were resuscitated on tryptone soy broth (Lab M, Lancashire, United Kingdom) and incubated for 18 hours at 37°C. DNA isolation was carried out using PeqGold Bacterial DNA kit (Peqlab Biotechnologie GmbH, Germany) following the manufacturers' instruction. For the amplification of the genomic DNA primers 27-F with the sequence 5'-AGAGTTTGATCMTGGCTCAG-3' and primer 1540-R the sequence 5'-TACGGYTACCTTGTTAC GACT-3' was used for identification of the bacteria. The 50 µL PCR approaches for the 16S rRNA gene sequencing the gDNA included 10 µL of gDNA, 5 µL PCR buffer with $MgCl_2$, 2.5µL 27-F primer, 2.5 µL 1540-R primer, 6 µL dNTP mix, 0.3 µL Taq polymerase and 23.7 µL nuclease-free water, were introduced into the appropriate PCR-tubes. The samples were commercially sequenced in the laboratory according to Sanger. The individual sequences of the forward and reverse primers were combined to form an entire 16S rRNA sequence and compared with the database of the National Center for Biotechnology Information (NCBI). By "Basic Local Alignment Search Tool" (BLAST) the inserted 16S rRNA sequences were assigned to those bacterial strains that had an identical or very similar 16S rRNA sequence.

Antimicrobial Susceptibility Profile: Antimicrobial susceptibility profile of the bacterial isolates was carried out using Kirby-Bauer disc diffusion method and readings interpreted by adopting the breakpoints of Clinical and Laboratory Standard Institute. [12] Briefly, purified isolates were inoculated on 5 mL TSB and incubated overnight. The optical density (OD) of the turbidity of the broth was determined to conform with the OD of the McFarland standard where the cells are equivalent to $\times 10^6$ CFU/mL. Using a sterile swab stick, respective standards were aseptically swabbed on Mueller Hinton agar (Lab M, Lancashire, United Kingdom). A total of 14 antibiotic discs (Mast Diagnostics, Merseyside, United Kingdom) which includes kanamycin (30 µg), gentamycin (10 µg), piperacillin (100 µg), imipenem (10 µg), ertapenem (10 µg), meropenem (10µg), cefotaxime (30 µg), cefepime (30 µg), tetracycline (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg) ciprofloxacin (10µg), cefuroxime (30 µg), and aztreonam (30µg) were employed. The respective discs were also aseptically impregnated on the agar plates using a sterile forceps equidistant apart. An average of 5 antibiotic discs was impregnated per plate. Plates were allowed to stand at room temperature for 5 min to allow the media to absorb effectively and incubated at 37 °C for

18-24 hours. Characterization of the resistance, intermediate or susceptibility profile of the isolates was determined by measuring inhibitory zones and then compared with the interpretative chart to determine the sensitivity, intermediate and resistant nature of the isolates to the antibiotics used using the CLSI (2017) interpretative chart.

Statistical Analysis: Data from this study were analyzed using the statistical package SPSS and Microsoft Excel 2013. Descriptive statistics were used to express values in mean and standard deviations. One Way Analysis of Variance was also used to compare multiple variables. P-values less than 0.05 were considered statistically significant.

RESULTS

The mean mesophilic bacterial counts from the ready-to-eat foods are expressed in $\log_{10}$ CFU/g as follows: fried rice (5.7±0.13), jollof rice (6.0±0.33), moi-moi (6.2±0.07), salad (6.6±0.03), oil beans (4.3±0.52), non-oil beans (5.1±1.13), and African salad (7.2±1.38). Significant differences were observed where *p*-values were < 0.05 (Table 1). The mean *E. coli* counts from the ready-to-eat foods are expressed in $\log_{10}$ CFU/g as follows: fried rice (2.1±0.04), jollof rice (2.7±0.11), moi-moi (2.9±0.07), salad (3.5±0.02), oil beans (1.8±0.16), non-oil beans (2.0±1.14), and African salad (4.1±0.10). Significant differences were observed where *p*-values were < 0.05 (Table 2).

The mean *Salmonella* species counts from the ready-to-eat foods are expressed in $\log_{10}$ CFU/g as follows: fried rice (3.0±0.11), jollof rice (3.2±0.05), moi-moi (3.9±0.12), salad (4.3±0.24), oil beans (2.8±0.46), non-oil beans (2.3±0.17), and African salad (5.2±0.09). Significant differences were observed where *p*-values were < 0.05 (Table 3). Bacterial isolates recovered from the ready-to-eat foods and their percentage (%) homology to bacterial isolates in the National Centre for Biotechnology Information (NCBI) database after they were sequenced and profiled with the Basic Local Alignment Search Tool (BLAST) include *Pseudomonas aeruginosa* (100%), *Citrobacter freundii* (99.5%), *Enterobacter cloacae* (99-100%), *Salmonella enterica* (99%), *Escherichia coli* (98-100%), *Klebsiella oxytoca* (99-100%).

The distribution of the bacterial isolates from the different food samples as shown in Table 4. are as follows: *Pseudomonas aeruginosa* [fried rice (1), jollof rice (1), moi moi (2), salad (2), African salad (4) and none from oil beans and non-oil beans]; *Citrobacter freundii* [moi moi (1), salad (1), African salad (1) and none from other food sources]; *Enterobacter cloacae* [jollof rice (1), moi moi (1), African salad (1) and none from others]; *Salmonella enterica* [fried rice (1), jollof rice (2), moi moi (2), salad (2), oil beans (1), non-oil beans (1) and African salad (3)]; *Escherichia coli* [fried rice (2), jollof rice (3), moi moi (3), salad (6), oil beans (2), non-oil beans (2) and African salad (5)]; *Klebsiella oxytoca* [fried rice (1), moi moi (2), salad (1), African salad (1) and none from other food

sources]. The frequency of occurrence of the bacterial isolates as shown in Figure 1 includes *Pseudomonas aeruginosa* 10(17.86%), *Citrobacter freundii* 3(5.36%), *Enterobacter cloacae* 3(5.36%), *Salmonella enterica* 12(21.43%), *Escherichia coli* 23(41.07%), and *Klebsiella oxytoca* 5(8.93%).

The antimicrobial susceptibility profile of the bacterial isolates revealed that all bacterial isolates in Table 5 were 100% resistant to cefepime, ceftazidime, cefuroxime, ertapenem, and meropenem. Antimicrobial sensitivity to gentamycin is as follows: *P. aeruginosa* 7(70%), *C. freundii* 2(66.7%), *E. cloacae* 3(100%), *S. enterica* 9(75%), and *E. coli* 21(91.3%). In addition, all isolates of *P. aeruginosa* were resistant to cefotaxime, aztreonam, and tetracycline. All isolates of *C. freundii* were also resistant to cefotaxime. All isolates of *E. cloacae* were also resistant to ceftriaxone and aztreonam. *S. enterica* 9(75%) were also resistant to ceftriaxone and tetracycline. All *E. coli* isolates were also resistant to ceftriaxone. All isolates of *K. oxytoca* were also resistant to cefotaxime, ceftriaxone, and aztreonam (Table 5). The multiple antibiotic-resistant characterizations of the bacterial isolates revealed for *P. aeruginosa*, 3(30%) of the isolates were resistant to 11 antibiotics (CTX^R, CPM^R, CRO^R, CAZ^R, CXM^R, PTZ^R, ATM^R, TET^R, IMI^R, ETP^R, MEM^R) which belong to 5 antimicrobial class with a multiple antibiotic resistant (MAR) index of 0.79. For *C. freundii*, 2(66.7%) of the isolates were resistant to 9 antibiotics (CTX^R, CPM^R, CRO^R, CAZ^R, CXM^R, ATM^R, TET^R, ETP^R, MEM^R) which belong to 4 antimicrobial class with a multiple antibiotic resistant (MAR) index of 0.64. For *E. cloacae*, 2(66.7%) of the isolates were resistant to 9 antibiotics (CTX^R, CPM^R, CRO^R, CAZ^R, CXM^R, ATM^R, TET^R, ETP^R, MEM^R) which belong to 4 antimicrobial class with a multiple antibiotic resistant (MAR) index of 0.64. For *S. enterica*, 4(33.3%) of the isolates were resistant to 10 antibiotics (CTX^R, CPM^R, CRO^R, CAZ^R, CXM^R, PTZ^R, ATM^R, TET^R, ETP^R, MEM^R) which belong to 5 antimicrobial class with a multiple antibiotic resistant (MAR) index of 0.71. For *E. coli*, 5(21.7%) of the isolates were resistant to 11 antibiotics (CTX^R, CPM^R, CRO^R, CAZ^R, CXM^R, PTZ^R, ATM^R, TET^R, IMI^R, ETP^R, MEM^R) which belong to 5 antimicrobial class with a multiple antibiotic resistant (MAR) index of 0.79. For *K. oxytoca*, 2(40%) of the isolates were resistant to 12 antibiotics (CTX^R, CPM^R, CRO^R, CAZ^R, CXM^R, PTZ^R, ATM^R, TET^R, IMI^R, ETP^R, MEM^R, CIP^R) which belong to 6 antimicrobial class with a multiple antibiotic resistant (MAR) index of 0.86.

DISCUSSION

Microbial contamination is an indicator of the degree of safe handling of food which is a globally recognized vehicle for transmission of pathogens.[13,14] In this study, bacterial load was adopted as a measure of the microbial quality of food served to the general populace. All the food items examined in this study were contaminated in varying degrees and can be

Table 1: Mesophilic bacteria counts from ready-to-eat foods

Food types	Bacterial count range (log_{10} CFU/g)	Mean bacterial count (log_{10} CFU/g) ±SD	*p*-value						
			F	**J**	**M**	**S**	**O**	**D**	**A**
Fried rice (*n* = 30)	4.1-5.8	5.7±0.13	-	0.923	0.713	0.705	**0.032**	**0.041**	**0.033**
Jollof rice (*n* = 30)	4.5-6.3	6.0±0.33	0.923	-	0.052	**0.041**	**0.004**	**0.024**	**0.038**
Moi-moi (*n* = 30)	5.8-6.3	6.2±0.07	0.713	0.052	-	0.060	**0.033**	**0.047**	**0.040**
Salad (*n* = 30)	6.1-6.6	6.6±0.03	0.705	**0.041**	0.060	-	**<0.001**	**<0.001**	**0.048**
Oil beans (*n* = 30)	3.6-4.8	4.3±0.52	**0.032**	**0.004**	**0.033**	**<0.001**	-	0.086	**<0.001**
Non-oil beans (*n* = 30)	3.9-5.4	5.1±1.13	**0.041**	**0.024**	**0.047**	**<0.001**	0.086	-	**<0.001**
African salad (*n* = 30)	6.9-7.8	7.2±1.38	**0.033**	**0.038**	**0.040**	**0.048**	**<0.001**	**<0.001**	-

Legend: F: Fried rice; **J:** Jollof rice; **M:** moi-moi; **S:** salad; **O:** oil beans; **D:** non-oil beans; **A:** African salad, -: no comparison is done. The mean difference was considered statistically significant at $p < 0.05$.

Table 2: *Escherichia coli* count from ready-to-eat foods

Food types	Population of *E. coli* range (log_{10} CFU/g)	Mean *E. coli* count (log_{10} CFU/g) ±SD	*p*-value						
			F	**J**	**M**	**S**	**O**	**D**	**A**
Fried rice (*n* = 30)	0.0-2.6	2.1±0.04	-	**0.036**	**0.014**	**0.010**	**0.019**	0.073	**<0.001**
Jollof rice (*n* = 30)	0.0-2.9	2.7±0.11	**0.036**	**-**	**0.046**	**0.001**	**<0.001**	**0.002**	**<0.001**
Moi-moi (*n* = 30)	0.0-3.2	2.9±0.07	**0.014**	**0.046**	**-**	**<0.001**	**<0.001**	**0.022**	**<0.001**
Salad (*n* = 30)	0.0-3.7	3.5±0.02	**0.010**	**0.001**	**<0.001**	**-**	**<0.001**	**0.004**	0.052
Oil beans (*n* = 30)	0.0-2.1	1.8±0.16	**0.019**	**<0.001**	**<0.001**	**<0.001**	-	**0.060**	**<0.001**
Non-oil beans (*n* = 30)	0.0-2.3	2.0±1.14	0.073	**0.002**	**0.022**	**0.004**	0.060	-	**<0.001**
African salad (*n* = 30)	0.0-4.6	4.1±0.10	**<0.001**	**<0.001**	**<0.001**	0.052	**<0.001**	**<0.001**	-

Legend: **F:** Fried rice; **J:** Jollof rice; **M:** moi-moi; **S:** salad; **O:** oil beans; **D:** non-oil beans; **A:** African salad, -: no comparison is done. The mean difference was considered statistically significant at $p < 0.05$.

Table 3: *Salmonella* species count from ready-to-eat foods

Food types	Population of *Salmonella* species range (log_{10} CFU/g)	Mean *Salmonella* species count (log_{10} CFU/g) ±SD	*p*-value						
			F	J	M	S	O	D	A
Fried rice (n = 30)	0.0-3.1	3.0±0.11	-	0.120	**0.016**	**<0.001**	0.201	**0.046**	**<0.001**
Jollof rice (n = 30)	0.0-3.4	3.2±0.05	0.120	-	0.050	**<0.001**	**0.034**	**<0.001**	**<0.001**
Moi-moi (n = 30)	0.0-4.0	3.9±0.12	**0.016**	0.050	-	**0.004**	**<0.001**	**<0.001**	**<0.001**
Salad (n = 30)	0.0-4.6	4.3±0.24	**<0.001**	**<0.001**	**0.004**	-	**<0.001**	**<0.001**	**0.007**
Oil beans (n = 30)	0.0-3.0	2.8±0.46	0.201	**0.034**	**<0.001**	**<0.001**	-	**<0.001**	**<0.001**
Non-oil beans (n = 30)	0.0-2.4	2.3±0.17	**0.046**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	-	**<0.001**
African salad (n = 30)	0.0-5.5	5.2±0.09	**<0.001**	**<0.001**	**<0.001**	**0.007**	**<0.001**	**<0.001**	-

Legend: F: Fried rice; J: Jollof rice, M: moi-moi, S: salad, O: oil beans, D: non-oil beans, A: African salad, -: no comparison is done. The mean difference was considered statistically significant at $p < 0.05$

Table 4: Distribution of the bacterial isolates in the different food samples

Bacterial isolates	Fried rice	Jollof rice	Moi-moi	Salad	Oil beans	Non-oil beans	African salad
Pseudomonas aeruginosa	1	1	2	2	0	0	4
Citrobacter freundii	0	0	1	1	0	0	1
Enterobacter cloacae	0	1	1	0	0	0	1
Salmonella enterica	1	2	2	2	1	1	3
Escherichia coli	2	3	3	6	2	2	5
Klebsiella oxytoca	1	0	2	1	0	0	1

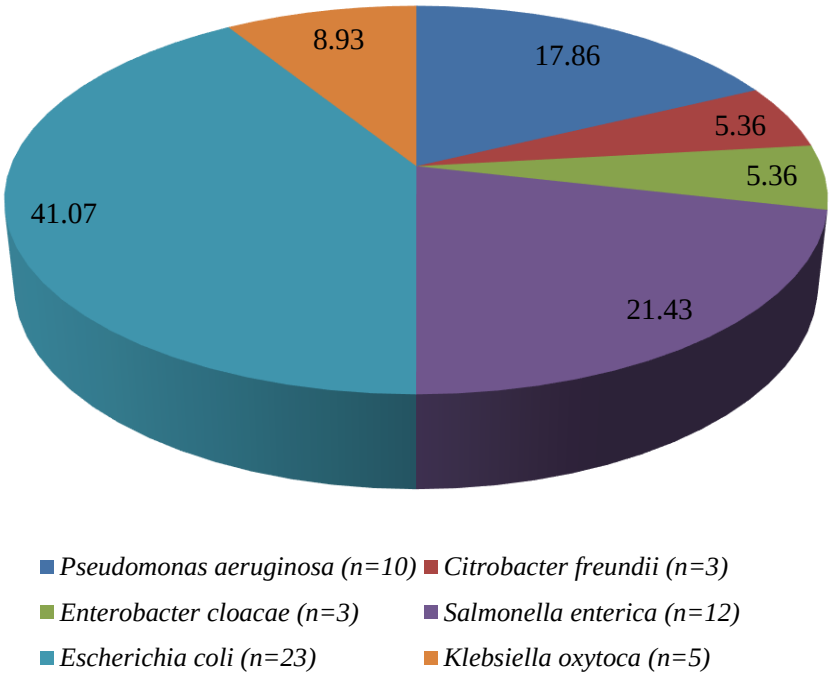

Figure 1: Frequency of occurrence of the bacterial isolates

categorized as not satisfactory in terms of microbial quality. This agrees with reports of several investigators that street foods in some African and low resources countries contained enteric pathogens of insignificant proportions.[10,14-17] The high bacteria counts observed in the foods sold generally and other various food centres examined in this study is of public health concern. The presence of coliform bacteria in almost all food samples may be an indication of unhygienic handling of the food after cooking and or lack of good manufacturing practices which may account for post-processing contamination recorded in this study. It is also an indication of post-processing faecal contamination due to poor handling. Food handlers are normally directly responsible for contamination of foods with enteric pathogens in particular, during preparation, post-processing handling and cross-contamination also due to attitude of the food handler/vendors.[15,18] This highlights the epidemiological significance of the role of food handlers or vendors in foodborne illnesses worldwide.

The findings in this study are a likely reflection of the risk of exposure to bacterial pathogens that can be transmitted through ready-to-eat foods sold in eating centres in Benin City, Nigeria. Eating out is gradually becoming more popular in this fast-emerging metropolis; probably out of necessity and the category of eating places reflect the socioeconomic status or social stratification of consumers.[16] The results showed that *Escherichia coli* (*E. coli)* was the most common coliform recovered in the food items. *E. coli* were isolated from all cooked food samples.

Table 5: Antimicrobial susceptibility profile of the bacterial isolates

Antimicrobial class	Antibiotics	*P. aeruginosa* (*n*=10)			*C. freundii* (*n*=3)			*E. cloacae* (*n*=3)			*S. enterica* (*n*=12)			*E. coli* (*n*=23)			*K. oxytoca* (*n*=5)		
		R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S
Cephems	CTX	10(100)	0(0)	0(0)	3(100)	0(0)	0(0)	2(66.7)	1(033.3)	0(0)	8(66.7)	4(33.3)	0(0)	20(86.9)	3(13.0)	0(0)	5(100)	0(0)	0(0)
	CPM	10(100)	0(0)	0(0)	3(100)	0(0)	0(0)	3(100)	0(0)	0(0)	12(100)	0(0)	0(0)	23(100)	0(0)	0(0)	5(100)	0(0)	0(0)
	CRO	9(90)	1(0)	0(0)	2(66.7)	1(33.3)	0(0)	3(100)	0(0)	0(0)	9(75)	3(25)	0(0)	23(100)	0(0)	0(0)	5(100)	0(0)	0(0)
	CAZ	10(100)	0(0)	0(0)	3(100)	0(0)	0(0)	3(100)	0(0)	0(0)	12(100)	0(0)	0(0)	23(100)	0(0)	0(0)	5(100)	0(0)	0(0)
	CXM	10(100)	0(0)	0(0)	3(100)	0(0)	0(0)	3(100)	0(0)	0(0)	12(100)	0(0)	0(0)	23(100)	0(0)	0(0)	5(100)	0(0)	0(0)
Penicillins	PTZ	6(60)	2(20)	2(20)	0(0)	2(66.7)	1(33.3)	0(0)	0(0)	3(100)	4(33.3)	2(16.7)	6(50)	11(47.8)	6(26.1)	6(26.1)	3(60)	2(40)	0(0)
Monobactams	ATM	10(100)	0(0)	0(0)	2(66.7)	1(33.3)	0(0)	3(100)	0(0)	0(0)	8(66.7)	4(33.3)	0(0)	10(43.5)	13(56.5)	0(0)	5(100)	0(0)	0(0)
Tetracyclines	TET	10(100)	0(0)	0(0)	2(66.7)	1(33.3)	0(0)	2(66.7)	0(0)	1(33.3)	9(75)	3(25)	0(0)	11(47.8)	10(43.5)	2(8.7)	3(60)	2(40)	0(0)
Carbapenems	IMI	4(40)	6(60)	0(0)	0(0)	1(33.3)	2(66.7)	0(0)	2(66.7)	1(33.3)	3(75)	5(41.7)	4(33.3)	6(26.1)	9(39.1)	8(34.8)	3(60)	0(0)	2(40)
	ETP	10(100)	0(0)	0(0)	3(100)	0(0)	0(0)	3(100)	0(0)	0(0)	12(100)	0(0)	0(0)	23(100)	0(0)	0(0)	5(100)	0(0)	0(0)
	MEM	10(100)	0(0)	0(0)	3(100)	0(0)	0(0)	3(100)	0(0)	0(0)	12(100)	0(0)	0(0)	23(100)	0(0)	0(0)	5(100)	0(0)	0(0)
Aminoglycosides	KAN	1(10)	3(30)	6(60)	0(0)	0(0)	3(100)	0(0)	0(0)	3(100)	1(8.3)	3(25)	8(66.7)	4(17.4)	2(8.7)	17(73.9)	0(0)	1(20)	4(80)
	GEN	2(20)	1(10)	7(70)	0(0)	1(33.3)	2(66.7)	0(0)	0(0)	3(100)	1(8.3)	2(16.7)	9(75)	2(8.7)	0(0)	21(91.3)	0(0)	4(80)	1(20)
Fluoroquinolones	CIP	1(10)	6(60)	3(30)	0(0)	3(100)	0(0)	0(0)	2(66.7)	1(33.3)	3(25)	3(25)	6(50)	4(17.4)	12(52.2)	7(30.4)	2(40)	3(60)	0(0)

Legend: ***P. aeruginosa***: *Pseudomonas aeruginosa*, ***C. freundii***: *Citrobacter freundii*, ***E. cloacae***: *Enterobacter cloacae*, ***E. coli***: *Escherichia coli*, ***K. oxytoca***: *Klebsiella oxytoca*, **PTZ**: Piperacillin, **CPM**: Cefepime, **CTX**: Cefotaxime, **CRO**: Ceftriaxone, **CXM**: Cefuroxime, **CAZ**: Ceftazidime, **ATM**: Aztreonam, **ETP**: Ertapenem, **IMI**: Imipenem, **MEM**: Meropenem, **GEN**: Gentamycin, **KAN**: Kanamycin, **TET**: Tetracycline, **CIP**: Ciprofloxacin, **R**: Resistant, **I**: Intermediate, **S**: Sensitive. Values in parenthesis represent percentage (%)

Escherichia coli is an ecologically versatile bacterium, known to adapt to a variety of environmental conditions encountered in both animal hosts and the external environments.[19] This attribute may, therefore, have been responsible for the presence of the organism in the ready-to-eat foods examined in this study. Other bacteria detected include *Citrobacter freundii*, *Pseudomonas aeruginosa*, *Salmonella*, *Enterobacter cloacae* and *Klebsiella* spp. However, pathogens have been recovered from foods, or have been known to survive and grow in such foods.[20] Reports from both developing and the developed nations suggest that the majority of food-borne gastrointestinal illnesses occur as a result of unhygienic handling and or the unsanitary environment during and after the food preparation. Humans are primarily exposed to gastrointestinal pathogens through direct or indirect contact with human and animal faecal wastes, or contamination of food. Evidence indicates that the incidence of diarrhoeal diseases can be reduced by preventing or controlling exposure to enteropathogens that are frequently present in foods.[21] In order to control enteric infections and gastrointestinal diseases, particularly in low resources nations, an understanding of the incidence of the organisms responsible for these illnesses and their survival in food is very crucial.

Antibiotic resistance among the enteric bacteria recovered from cooked food revealed that the antibiotics are commonly used to treat and cure infections both in human and veterinary medicine. Other reports have highlighted the existence of unacceptable high antibiotic residues in meat in Kenya and Nigeria[22,23] indicating that the use of antimicrobials for animal husbandry in Africa is not a rare occurrence and may suggest misuse of antimicrobial agents. The high antibiotic resistance and multiple antibiotic resistances (MAR) observed among the bacteria recovered from food samples in the study area is, therefore, a cause for concern to public health. However, Teuber[24], Teuber et al.[25] and Verraes et al.[26] reviewed the significant role of food and food-borne pathogens in food chain in the epidemiology of antibiotic-resistant bacteria, thus highlighting the need for urgent attention, particularly in low resources countries that may not possess the means to curtail any major outbreak by such MAR organisms. The occurrence of antibiotic-resistant commensals and antibiotic resistance gene in ready-to-eat retailed foods has been reported.[26,27] In their study, Van et al.[27] reported that more than 90% of foods sampled were contaminated with *E. coli* and 83.3% of the *E. coli* was resistant to at least one antibiotic. Furthermore, the role of commensals, especially food-borne microbes in transmitting antibiotic resistance genes through horizontal transfer has been highlighted.[26] The present study emphasizes the importance of surveillance of bacterial isolates throughout the food production continuum to detect emerging antimicrobial resistance phenotypes in Nigeria. These may include but not limited to an increased number of hospitalization,

increased risk of invasive infections, and failures in medical treatment, increased health costs and mortality.

Food safety in the food market is one of the key areas of focus in public health because it affects people of every age, race, gender, and income level around the world. The local and international food marketing continues to have significant impacts on food safety and the health of the public. Food supply chains cross multiple national borders which increase the internationalization of health risks. Overall, this study suggests that food safety-related public health risks are more common in developing countries than in developed countries. This can be justified that foods get easily contaminated with microbes due to poor hygiene and sanitation in developing countries.[28,29] Transmission of antimicrobial resistant bacteria to humans and the human gastrointestinal tract is of concern due to either direct infections or the possibility of horizontal gene exchange with other potentially pathogenic members of the gut microbiota favoured by the high cell densities found in the gut.[30,31] In recent years, several global and national public health organizations have highlighted the growing number of multidrug-resistant microbes as a major public health priority. Food plays an important role in the transmission of foodborne pathogens. Transmission of microorganisms between food and humans occurs during the handling of raw materials as well as cross- and re-contamination between different food products at production, distribution, and household levels.[32] Approximately 15% of emerging infectious disease events have been associated with foodborne transmission.[33] This is of concern, given that the interconnectedness of global food systems is resulting in increased antibiotic-resistant foodborne disease transmission.

Antimicrobial resistance is now widely acknowledged as a major global public health challenge.[34,36] Food contamination with antibiotic-resistant bacteria can be a major threat to public health, as the antibiotic resistance determinants can be transferred to other bacteria of human clinical significance. In low- and middle-income countries, antibiotic resistance remains largely unaddressed. The World Health Organization states that "large gaps in knowledge exist about the status of antibiotic resistance surveillance capacities worldwide, particularly in resource-limited settings."[37] Inappropriate antibiotic prescriptions, over-the-counter availability, poor patient adherence to prescribed medications, use of substandard medications, and self-medication with previously unused antibiotics all increase the development of antibiotic resistance.[38] The use and misuse of antibiotics, resistance can develop in bacteria in human beings and animals. Hence, infections that normally respond to antibiotic treatment can become difficult and sometimes impossible to cure. The resulting treatment failures lead to increased disease cases and deaths, a growing challenge to develop new antibiotics and consequently higher costs to society. The prevention and containment of

antibiotic resistance, therefore require addressing all risk factors for the development and spread of antibiotic resistance across the full spectrum of conditions, sectors, settings (from health care to use in food-animal production) and countries. Collection of antibiotic usage data supports planning and implementation of evidence-based public health policies and strategies and provides data for interventions and their evaluation. The Centers for Disease Control and Prevention emphasizes four priority action areas: stewardship, infection control, resistance tracking, and novel antibiotic and diagnostic test development. Measures aimed at reducing or eliminating pathogens and indicator organisms from food, i.e. good agricultural practices (GAP), good hygiene practices (GHP) and Hazard Analysis and Critical Control Point (HACCP)-based procedures should substantially reduce the risk of transmission of viable antimicrobial-resistant bacteria through the food chain.

Conclusion: The occurrence of these bacterial isolates in the foods could result in a public health threat to consumers as all these bacterial pathogens have been associated with diarrheal illness and other foodborne infection. The identification of these bacteria in the foods samples could be attributed to poor personal hygiene, non-compliance to hazard analysis and critical control point's scheme. To minimize this unwholesome trend of ready-to-eat food contamination, it is important for appropriate agencies in food safety and public health to organize training on hygiene and food safety for food vendors.

Conflict of Interest: None

REFERENCES

1. Adolf JNP, Azis BS. Microbiological status of various foods served in elementary school based on social-economic status differences in Karawachi Region, Tangerang District-Indonesia. International Food Research Journal. 2012; 19(1): 65-70.

2. Musa OL, Akande TM. Effect of health education intervention on food safety practices among food vendors in Ilorin. Sahel Medical Journal, 2002; 5: 120-124.

3. Nkere CK, Ibe NI, Iroegbu CU. Bacteriological quality of foods and water sold by vendors and in restaurants in Nsukka, Enugu State, Nigeria: A comparative study of three microbiological methods. Journal of Health Population and Nutrition, 2011; 29(6): 560-566.

4. Beshiru A, Igbinosa IH, Igbinosa EO. Prevalence of antimicrobial resistance and virulence gene elements of *Salmonella* serovars from ready-to-eat (RTE) shrimps. Frontiers Microbiology, 2019; 10: 1613.

5. Shamsuddeen U, Ameh JB. Survey of the possible critical control points during the production of Balangu in Kano. Bayero Journal of Pure and Applied Sciences, 2008; 1(1): 76-79.

6. Kawo AH, Abdulmumin FN. Microbiological quality of pre-packaged sweets sold in metropolitan Kano, Nigeria. Bayero Journal of Pure Applied Sciences, 2009; 2(1): 154-159.

7. Gajraj R, Pooransingh S, Hawker J, Olowokure B. Multiple outbreaks of *Salmonella* Braenderup associated with consumption of iceberg lettuce. International Journal of Environmental Health Research, 2012; 22(2): 150-155.

8. Odu NN, Akano UM. The microbiological assessment of ready-to-eat food (shawarma) in Port Harcourt City, Nigeria. Nature and Science, 2012; 10(8): 1-8.

9. David OM, Oluyege AO. Antibiotic resistance and plasmid carriage among *Salmonella* typhi isolated from food and hands of food handlers in a Nigeria University.

International Journal of Current Microbiology and Applied Sciences, 2015; 4(3): 906-914.

10. Beshiru A, Okareh OT, Okoh AI, Igbinosa EO. Detection of antibiotic resistance and virulence genes of Vibrio strains isolated from ready-to-eat shrimps in Delta and Edo States, Nigeria. Journal of Applied Microbiology, 2020; 129(1): 17-36. doi.org/10.1111/jam.14590

11. Ire FS, Imuh VT. Bacteriological quality evaluation and safety of randomly selected ready-to-eat foods sold in Port Harcourt City, Nigeria. Journal of Applied Life Sciences International, 2016; 7(1): 1-10.

12. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing M02-A12, M07-A10, and M11-A8. 27th Edition; 282pp. 2017.

13. Chukwu OOC, Chukwuedo AA, Otalike P, Chukwu ID, Echeonwu NOG, Bitrus JG, Akubo SI. Studies of foodborne bacteria in commercially hawked ready-to-eat fish in Jos and its environments. African Journal of Food Science, 2013; **7**(4): 71-75.

14. Igbinosa EO, Beshiru A. Antimicrobial resistance, virulence determinants, and biofilm Formation of *Enterococcus* species from ready-to-eat seafood. Frontiers Microbiology, 2019; 10:728. DOI: 10.3389/fmicb.2019.00728.

15. Haileselassie M, Taddele H, Adhana K. Source (s) of contamination of 'raw' and 'ready-to-eat' foods and their public health risks in Mekelle City, Ethiopia. ISABB Journal of Food Agricultural Sciences, 2012; 2(2): 20-29.

16. Famurewa O, Oyagade JO, Femi-Ola TO, Laleye SA. Assessment of microbial quality of ready-to-eat food retailed in AdoEkiti. Nigerian Society for Experimental Biology Journal, 2003; 3: 71-77.

17. Mensah P, Yeboah-Manu D, Owusu-Darko K, Ablordey A. Street foods in Accra, Ghana: how safe are they? Bulletin of the World Health Organization, 2002; 80(7): 546-554.

18. Baro N, Bello AR, Savadogo A, Quattara CAT, Ibuodo AJ, Traore AS. Hygienic status assessment of dishwashing waters, utensils, hands and pieces of money from street food processing sites in Ouagadougou (Burkina Faso). African Journal of Biotechnology, 2006; 5(11): 1107-1112.

19. Large TM, Walk ST, Whittam TS. Variation in acid resistance among shiga toxin-producing clones of pathogenic *Escherichia coli*. Applied and Environmental Microbiology, 2005; 71(4): 2493-2500.

20. Olasupo NA, Smith SI, Akinside KA. Examination of the microbial status of selected indigenous fermented foods in Nigeria. Food Safety, 2002; 22: 85-94.

21. Madic J, Vingadassalon N, de Garam CP, Marault M, Scheutz F, Brugère H, Jamet E, Auvray F. Detection of shiga toxin-producing *Escherichia coli* serotypes O26:H11, O103:H2, O111:H8, O145:H28, and O157:H7 in raw-milk cheeses by using multiplex real-time PCR. Applied Environmental Microbiology, 2011; 77(6): 2035-2041.

22. Muriuk FK, Ogara WO, Njeruh FM, Mitema ES. Tetracycline residue levels in cattle meat from Nairobi slaughter house in Kenya. Journal of Veterinary Science, 2001; 2(2): 97-101

23. Dipeolu M, Alonge D. Residues of streptomycin antibiotic in meat sold for human consumption in some states in Nigeria. Archives of Zootechnology, 2002; 51: 477-480.

24. Teuber M. Spread of antibiotic resistance with food-borne pathogens. Cellular and Molecular Life Science, 1999; 56: 755-763.

25. Teuber M, Meile L, Schwarz F. Acquired antibiotic resistance in lactic acid bacteria from food. Antonie van Leeuwenhoek, 1999; 76: 115-137.

26. Verraes C, Van Boxstael S, Van Meervenne E, Van Coillie E, Butaye P, Catry B, et al. Antimicrobial resistance in the food chain: A Review. International Journal of Environmental Research and Public Health, 2013; 10: 2643-2669.

27. Van TH, Moutafis G, Tran LT, Coloe PJ. Antibiotic resistance in food-borne bacterial contaminants in Vietnam. Applied and Environmental Microbiology, 2007; 73(24): 7906-7911.

28. Baluka SA, Miller R, Kaneene JB. Hygiene practices and food contamination in managed food service facilities in Uganda. African Journal of Food Science, 2015; 9(1): 31-42.

29. Gizaw Z. Public health risks related to food safety issues in the food market: a systematic literature review. Environmental Health and Preventive Medicine, 2019; 24: 68.

30. Haug MC, Tanner SA, Lacroix C, Stevens MJ, Meile L. Monitoring horizontal antibiotic resistance gene transfer in a colonic fermentation model. FEMS Microbiology Ecology, 2011; 78: 210-219.

31. Collignon PC, Conly JM, Andremont A, Mcewen SA, Aidara-Kane A, Agerso Y. World Health Organization ranking of antimicrobials according to their importance in human medicine: a critical step for developing risk management strategies to control antimicrobial resistance from food animal production. Clinical Infectious Diseases, 2016; 63: 1087-1093.

32. Macdonald E, White R, Mexia R, Bruun T, Kapperud G, Lange H. Risk factors for sporadic domestically acquired Campylobacter infections in Norway 2010-2011: a national prospective case-control study. PLoS ONE, 2015; 10: e0139636.

33. Parmley J, Leung Z, Leger D, Finley R, Irwin R. One Health and food safety-the Canadian experience: a holistic approach toward enteric bacterial pathogens and antimicrobial resistance surveillance. In: Improving Food Safety through a One Health Approach, ed. Choffnes ER, Relman DA, Olsen L, Hutton R, Mack A. Washington, DC: National Academic Press. 2012. pp. 176-188.

34. FAO/WHO/OIE. Joint Expert Meeting on Critically Important Antimicrobials. 2007.

35. WHO. Tackling antibiotic resistance from a food safety perspective in Europe. 2011.

36. WHO. Antimicrobial resistance global report on surveillance: 2014 summary WHO/HSE/PED/AIP/2014.2

37. WHO. Worldwide Country Situation Analysis: Response to Antimicrobial Resistance. Geneva: World Health Organization 2015.

38. Center for Disease Control and Prevention). Antibiotic Resistance Threats in the United States. Atlanta, GA: US Department Health Humanitarian Services 2013. http://www.cdc.gov/drugresistance/pdf/ar-threats-2013-508.pdf