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Multiple Antibiotic-resistant *Escherichia coli* in Ready-to-eat Foods from Food Outlets in Ekiti State University and Its Environ

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Authors' contributions

This work was carried out in collaboration between all authors. Authors OJO, OMD and OF designed the study, performed the statistical analysis. Authors OJO, OMA and AAA wrote the protocol and authors OJO and AAA wrote the first draft of the manuscript and authors OJO, OMD and OF managed literature searches. Authors OJO, OMD, OMA and OF managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The potential health risk associated with consumption of ready-to-eat (RTE) food sold in food outlets in and around Ekiti State University was investigated using standard techniques. The microbiological analysis of 96 food samples obtained randomly from 6 food outlets for a period of 12 months carried out. Antibiotic resistance of 379 *Escherichia coli* recovered from the samples was also determined using disk diffusion method. Results indicated that the total bacterial counts (TBC), total coliform counts (TCC) and total *E. coli* counts recorded for the food samples ranged between a mean value of 2.450±0.834 and 65.550±27.430x10^5 cfu/g; 0.050±0.022 and 12.250±6.735x10^5 cfu/g; and 0.033±0.021 and 5.333±2.809x10^5 cfu/g respectively. The values obtained for the TBC for rice, scotch egg, doughnut, semo and beans from all outlets were not significantly different ($p<0.05$). RTE Foods obtained from ST carried the highest number of *E. coli*

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with 21.1% of the total isolates, followed by food samples obtained from DKA with 20.3%. RTE foods from CJ had 18.5% of the total *E. coli* isolates, while 13.5% and 13.7% of the total *E. coli* isolates were recovered from foods from TTA and CCA respectively. Generally, the highest number of *E. coli* (61) was isolated from pounded yam while the least occurrence (26) was recovered from doughnut. Ofloxacin was the most effective antibiotic as only 2 (0.6%) of the *E. coli* isolates were resistant, while all the 379 isolates (100.0%) were resistant to augmentin and 80.2% and 65.7% of the isolates were resistant to ceftazidime and cefuroxime, respectively. Of the total *E. coli* recovered from this study, 73.1% were multiple antibiotic-resistant (MAR) with varying resistotypes. The highest percentage of MAR with 8 resistotypes was 0.3% of the *E. coli* isolates, while highest number (28%) had 4 resistotype with CXM-CRX-CAZ-AUG as the most common resistotype. The results of this study imply that there is need to improve on hygienic and good manufacturing practices in public food outlets in order to attain a relatively safe level in RTE foods for human consumption.

Keywords: Ready-to-eat foods; Bukateria; antibiotic resistance; Resistotype; Ekiti State University.

1. INTRODUCTION

Ensuring that food for human consumption is wholesome, is a necessity for human well-being, though many foods are frequently contaminated with naturally occurring microorganisms including pathogenic ones, which unfortunately cannot be detected organoleptically and therefore cause diseases of varying severity including death [1]. The severity of these diseases depends specifically on preparation and exposure during sales, storage processes and or mode of handling of the foods. All these play a significant role in the proliferation of the pathogenic microorganisms to certain level of contamination. Thus, food safety issues are of major importance to human health [2]. Moreover illness resulting from the consumption of contaminated foods has become one of the most widespread public health challenges in contemporary society [3]. In Nigeria, like many other developing countries, increased population, much of itinerary workers, less home-centered activities coupled with laziness and daily work load especially among the youth including students, have really hiked the reason for alternatives. The situation however resulted in many going for ready-to-eat (RTE) foods which are taken outside the home. Thus, food vending services are on the increase and also, the responsibility for good manufacturing practices of food such as good sanitary measures and proper food handling have been transferred from individuals/families to the food vendors who rarely observe such practices [4]. Majority of these RTE foods are prepared and/or sold at public places such as schools, market places, in canteens and cafeterias, on street corners and at easily accessible places by street vendors at relatively cheaper rate [5,6].

Furthermore, the food vendor services offer varieties of meals including traditional meals of which preparations are quite laborious and time consuming. Thus, with the increase in the number of hours spent at work places and schools, the importance of these food vending services in human daily survival is increasingly unavoidable among all socio-economic groups [7]. Several studies have shown that foods sold in these centers; cafeterias, canteens among others, are sometimes made and held at improper temperatures, excessively handled by food vendors while some are sold in very dirty environment [8-11]. In addition, vendors practice poor personal hygiene and reports of these food vendors serving as carriers of potential pathogens have been documented, therefore serving as a major potential source of transmission of enteric fevers [5]. Hence, the microbiological quality of foods served in these vending centers should be more paramount as equally as the aroma, attractive look and other attributes of good foods are considered. [12,13] emphasized the role of food vendors in the epidemiology of food-borne infections and diseases.

However, most people that use the services of these food vendors are more interested in the conveniences and taste of the foods rather than the quality and hygiene. Thus, concerns have been raised by the Food and Agricultural Organization (FAO) and other quality assurance agencies on these types of foods serving as a potential source of food poisoning outbreaks [14]. Moreover, majority of students do not prepare food themselves, neither take food along with them to lecture classes. The demand for food therefore gives opportunity to the cafeterias and canteens to serve as the major vending sites

where students purchase food daily. Hence, the present study was aimed at assessing the microbiological quality as regard the incidence of multiple antibiotic resistant (MAR) *E. coli* in RTE foods sold in some *bukateria*, cafeteria and canteens in and around the Ekiti State University campus.

2. MATERIALS AND METHODS

2.1 Sample Collection

A total of 96 food samples comprising of different numbers of each of eight (8) ready-to-eat foods including rice, beans, semo, pounded yam, and snacks such as scotch-eggs, meat-pies, doughnut and fish-pies. The foods were obtained from six (6) food-vending sites in Ado-Ekiti, three (3) of which were canteens and *bukateria* (ST, SM and CJ) in the Ekiti State University campus while other three (3) were eateries (TTA, CCA and DKA) in the town of Ado-Ekiti. The names of the food-vending sites are coded for obvious reasons. The samples were collected over a period of 12 months (January to December, 2013). Fresh food samples were purchased and collected in sterile specimen containers and transported on ice to the Laboratory for microbiological analysis which was performed within 2 hours of collection.

2.2 Microbiological Analysis

A 10 g amount of each food sample was homogenized into 90 ml of sterile physiological saline solution using a sterile warring blender. Ten-fold serial dilution of the sample homogenate was made and 1 ml aliquot of appropriate dilutions of the homogenate was plated in duplicates on Nutrient agar (Lab M, UK), MacConkey agar (Oxoid Thermo Fisher, UK) and Eosin Methyleneblue agar (Lab M, UK), using pourplate method. The plates were then incubated at 37℃ for 24 hrs. Nutrient agar was used to determine total bacterial count, MacConkey agar was used for coliform enumeration while Eosine methylene blue agar was used for the enumeration of *E*. *coli*. At end of the incubation periods, culture plates were examined for microbial growth and colonies were counted using the illuminated colony counter (Gallenkamp, England).The counts for each plate were expressed as colony forming unit (cfu/g) of the sample homogenate [15].

2.3 Identification of *Escherichia coli*

The green metallic sheen colouration and morphological attributes of *E. coli* on Eosine methylene blue agar were closely examined, observed and recorded. Discrete colonies were purified by repeated sub-culturing. For confirmation, the isolates were further subjected to Gram staining and other typical *E. coli* characteristics according to Cheesbrough [16] and the pure cultures were kept as stock on slants at 4℃ until needed for use.

2.4 Antibiotic Susceptibility Testing

The antibiotic susceptibility of the isolates was determined using the disk Kirby-Bauer disk diffusion method [17] on Müller-Hinton agar according to CLSI [18]. The bacterial isolates were tested against eight Gram-negative antibiotic disc (ABTEK) with their concentrations (in µg). They included; ceftazidime (30), cefuroxime (30), gentamicin (10), cefixime (5), ofloxacin (5), augmentin (30), nitrofurantoin (300) and ciprofloxacin (5).

The inocula were standardized in Müller-Hinton broth by adjusting its density to equal the turbidity of a Barium sulphate ($BaSO_4$) (0.5 McFarland turbidity standards). The standardized inocula were ceded on Müller-Hinton agar plates using a swab-stick and left for 5 minutes. With a sterile forceps, the appropriate antibiotic disc was placed on the inoculated plates and left for about 30mins after which the plates were incubated aerobically at 37℃ for 18 hrs in inverted position. The diameter of inhibition zone (including the diameter of the disk) was measured and interpreted using the standard guidelines of the Clinical Laboratory Standards Institute [18].

2.5 Statistical Analysis

All data obtained from bacterial count and sensitivity test were subjected to analysis of variance (ANOVA) using the Statistical Package for the Social Sciences (SPSS), version 20.0 at 95% confidence level and 5% significance level.

3. RESULTS

The microbial composition of all RTE food samples obtained from the six different outlets; three of which are within the Ekiti State University campus and the other three outside the campus premises, were enumerated. The distribution of RTE foods based on food-vending site and food types are respectively presented on Tables 1 and 2. Furthermore, the visible and noticeable environmental and hygienic conditions relating to the outlets were noted as presented on Table 3.

Table 1. Distribution of ready-to-eat food samples based on food vending sites

S/N	Vending sites	Number of samples
Within the campus		
1	ST	17
2	SM	6
3	CJ	25
Outside the campus		
4	CCA	12
5	DKA	15
6	TTA	21
Total		**96**

Table 2. Distribution of ready-to-eat food samples based on food types

Food samples	Number of samples
Rice	15
Beans	13
Semo	14
Pounded yam	10
Scotch egg	11
Meat pie	10
Doughnut	12
Fish pie	11
Total	**96**

The microbial counts of RTE food samples obtained from food outlets in the Ekiti State University campus and environ are depicted in Tables 4a, 4b and 4c. The total bacterial count (TBC) for the food samples showed a range between a mean value of 2.450±0.834 and 65.550±27.430 x 10^5 cfu/g, the total coliform count (TCC) ranged between mean a value of 0.050±0.022 and 12.250±6.735 x10^5 cfu/g, the total *E*. *coli* count (TEC) ranged between a mean value of 0.033±0.021 and 5.333±2.809 x10^5 cfu/g. The mean total bacteria count, coliform count, and total *E. coli* count presented in Tables 4a, 4b, and 4c indicated that there was no significant difference ($p<0.05$) in values obtained for Scotch Egg in all the sales outlets. The mean of total bacterial counts presented on Table 4a shows that there was no significant difference ($p<0.05$) in the value obtained for scotch egg and doughnut from all the outlets, but there was significant difference ($p>0.05$) in the value obtained for pounded yam from ST and CJ. The mean of the total coliform counts depicted in Table 4b showed that there was no significant difference ($p<0.05$) in the value obtained for semo and Scotch egg from all the outlets, but there was significant difference ($p>0.05$) in the value obtained for beans from CCA and DKA. While the mean of total *E*. *coli* counts as portrayed in Table 4c showed that there was no significant difference at $p>0.05$ in the value obtained for scotch egg in all the outlets, however there was significant difference in the values obtained for beans, meat pie and doughnut from CCA and DKA compared to other outlets such as ST, CJ and SM.

Table 5 depicts the distribution of *E. coli* in the food samples. The result shows that the foods obtained from ST had the highest number of *E. coli* with 21.1% of the total isolates, followed by food samples obtained from DKA with 20.3%. *Bukateria* in the University Campus had 18.5% of the total *E. coli* isolates, while 13.5% and 13.7% of the total *E. coli* isolates were recovered from foods from TTA and CCA respectively. The least occurrence of *E. coli* (12.9%) was recorded in the food samples obtained from SM. Generally, the highest number of *E. coli* (61) was isolated from pounded yam while the least occurrence (26) was observed in doughnut. Number of *E. coli* isolated from other food samples included; beans (56), meat pie (53), semo (48), fish pie (48) and scotch egg (44).

Overall, a total of 379 *E. coli* isolates were obtained from the RTE food samples collected from six food outlets in the Ekiti State University, Ado-Ekiti and its eniviron. Table 6 shows the antimicrobial susceptibility pattern of the 379 *E. coli* isolates against eight conventional antibiotics, revealing that ofloxacin was the most active antibiotic as only 2 (0.6%) of the isolates were resistant, while all the 379 isolates (100.0%) were resistant to augmentin and 80.2% and 65.7% of the isolates were resistant to ceftazidime and cefuroxime, respectively.

Escherichia coli isolated from DKA food samples had the highest resistance of 26.0% to nitroflorantoin, while the least *E. coli* (9.6%) resistant to nitroflorantoin recovered were from CCA. Except the *E*. *coli* recovered from food samples from SM and the University *bukateria* that had 2.0% and 1.4% resistance respectively, the isolates from DKA, TTA, ST and CCA food samples were not resistant to ofloxacin. Furthermore, 18.4% and 10% of *E. coli* isolated from SM and ST food samples were respectively resistant to gentamicin. The percentage resistance of *E*. *coli* with respect to each food outlet is depicted in Fig. 1 while that of the isolates from all the RTE food samples is presented in Fig. 2 showing that the isolates from all food samples were generally resistant to augmentine. However, the isolated from rice,

beans, semo, scotch egg and doughnut were not resistant to ofloxacin.

Multiple antibiotic resistance (MAR) with varying resistotypes was observed in 277/379 (73.1%) (Table 7). The highest levels of MAR with resistance to all the eight antibiotics tested was observed in 1 (0.3%) of the isolates; resistance to seven antibiotics was in 6 (1.6%), resistance to six in 26 (6.9%) of the isolates. Resistance was highest against four (106; 28.0%) and three (85, 22.4%) respectively, while these were followed by resistance to five antibiotics in 53 (14.0%) of the isolates (Table 7).

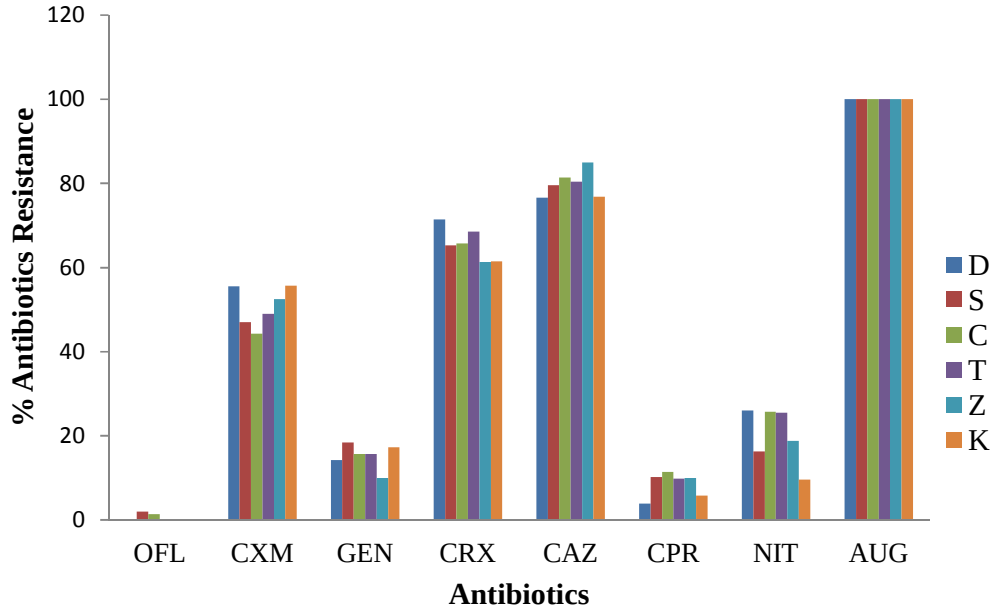

Fig. 1. Antibiotic resistance of the *Escherichia coli* isolated from food outlet in Ekiti State University an d Environs

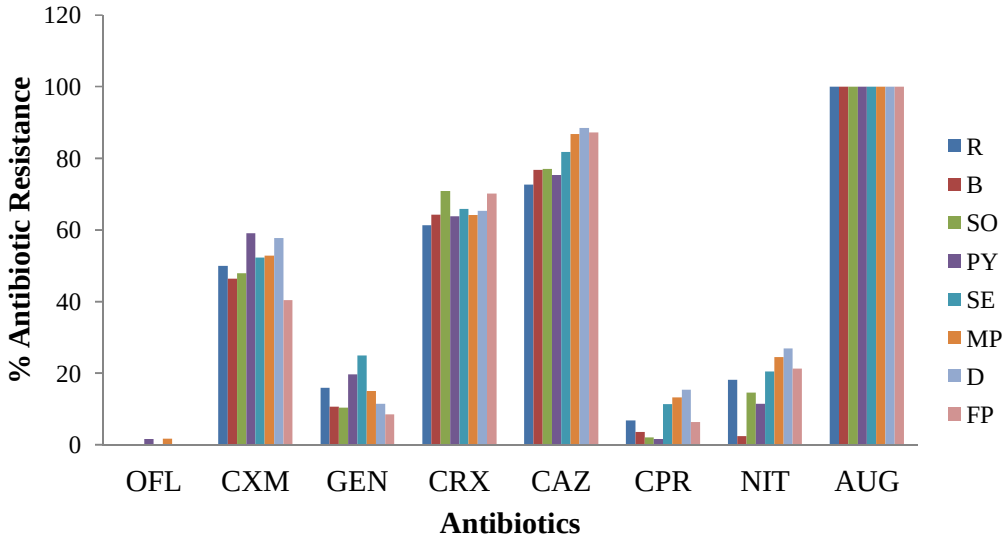

Fig. 2. Antibiotic resistance of *Escherichia coli* isolated from food samples obtained from food outlets in Ekiti State University and Environs

Keys: *D- DKA; S- SM; C- C.J; T- TTA; Z- ST; K- CCA; R- Rice; B- Beans; SO- Semo; PY- Pounded Yam; SE- Scotch egg; MP- Meat pie; D- Doughnut; FP- Fish Pie; OFL- Ofloxacin; CXM- Cefixime; GEN- Gentamicin; CRX- Cefuroxime; CAZ- Ceftazidime; CPR- Ciprofloxacin; NIT- Nitrofurantoin; AUG- Augmentin*

Table 3. Notable conditions of targeted food outlets

Parameters	Condition					
	DKA	SM	CJ	TTA	ST	CCA
Water supplies	Well	Well	Pipe-borne	Bore-hole	Pipe-borne	Well
Environment	Clean	Clean	Not clean	Clean	Clean	Very clean
Service	Crowded	Not crowded	Crowded	Crowded	Crowded	Not crowded
Fast selling foods	Snacks	Rice	Rice, Semo, Pounded yam, Beans	Rice, Snacks, Semo	Rice, Snacks	Rice, Semo, Pounded yam, Beans
Medium of service	Serving tray	Serving tray	Cooking pot	Serving tray	Cooler	Serving tray
Serving means	Washable plates	Washable plates	Washable plates	Disposable plates	Washable plates	Disposable plates

Table 4a. Mean bacterial counts of RTE food samples from outlets in Ekiti State University and environ

Food outlets	Mean bacterial count ($x10^5$cfu/g) of food samples							
	Rice	Beans	Semo	Pounded yam	Scotch egg	Meat pie	Doughnut	Fish pie
ST	4.950±1.834^{a}	8.433±2.804^{ab}	14.400±5.406^{a}	18.217±7.654^{a}	6.567±2.036^{a}	20.600±5.993^{ab}	6.000±2.544^{a}	18.350±5.657^{b}
C.J	17.783±6.073^{a}	21.550±7.908^{b}	22.083±10.991^{ab}	43.867±16.473^{b}	18.850±5.881^{a}	22.089±6.832^{ab}	10.917±3.382^{a}	8.750±3.252^{ab}
SM	16.283±5.328^{a}	0.000±0.000^{a}	33.000±10.733^{ab}	0.000±0.000^{a}	12.033±5.574^{a}	0.000±0.000^{a}	16.100±5.322^{a}	0.000±0.000^{a}
CCA	34.150±11.302^{ab}	0.650±0.191^{a}	64.033±20.919^{b}	0.000±0.000^{a}	11.550±3.779^{a}	2.450±0.834^{a}	10.250±3.466^{a}	2.717±0.783^{a}
DKA	25.117±8.925^{a}	14.783±4.907^{b}	48.267±15.255^{ab}	12.183±3.724^{a}	20.717±6.135^{a}	38.550±13.480^{b}	17.650±5.076^{a}	11.535±4.714^{ab}
TTA	65.550±27.430^{b}	17.017±5.583^{b}	39.961±17.553^{ab}	22.800±7.424^{ab}	13.883±4.625^{a}	14.450±5.462^{a}	11.283±3.658^{a}	10.817±3.662^{ab}

Keys: *Values with similar superscript(s) along column are not significantly different from each one*

Table 4b. Mean coliform counts of RTE food samples from outlets in Ekiti State University and environ

Food outlets	Mean coliform count ($x10^5$cfu/g) of food samples							
	Rice	Beans	Semo	Pounded yam	Scotch egg	Meat pie	Doughnut	Fish pie
ST	1.267±0.557^{a}	1.883±0.697^{ab}	2.950±0.917^{a}	9.067±4.040^{b}	1.700±0.460^{a}	8.300±2.549^{b}	1.967±1.217^{a}	7.550±2.437^{b}
C.J	5.483±1.719^{ab}	3.250±1.308^{b}	5.533±2.844^{a}	10.117±3.959^{b}	5.300±1.655^{a}	8.450±3.113^{b}	2.633±1.010^{a}	2.267±0.834^{a}
SM	2.700±0.957^{a}	0.000±0.000^{a}	6.750±2.348^{a}	0.000±0.000^{a}	4.783±2.884^{a}	0.000±0.000^{a}	3.050±0.872^{a}	0.000±0.000^{a}
CCA	2.167±0.603^{a}	0.083±0.040^{a}	7.517±2.186^{a}	0.000±0.000^{a}	2.900±0.939^{a}	0.500±0.169^{a}	1.300±0.313^{a}	0.050±0.022^{a}
DKA	5.500±1.741^{ab}	3.833±1.068^{b}	11.267±3.459^{a}	3.767±1.294^{ab}	6.017±2.022^{a}	9.183±2.567^{b}	9.100±2.639^{b}	3.617±2.108^{ab}
TTA	12.250±6.735^{b}	4.717±1.650^{b}	10.000±3.263^{a}	4.900±1.386^{ab}	2.550±0.812^{a}	4.700±2.019^{ab}	2.117±0.678^{a}	2.567±0.642^{a}

Keys: *Values with similar superscript(s) along column are not significantly different from each one*

Table 4c. Mean *Escherichia coli* counts of RTE food samples from outlets in Ekiti State University and environ

Food outlets	Mean *Escherichia coli* count (x10^5CFU/g) of food Samples							
	Rice	**Beans**	**Semo**	**Pounded yam**	**Scotch egg**	**Meat pie**	**Doughnut**	**Fish pie**
ST	0.283±0.151^{a}	0.250±0.157^{ab}	1.200±0.573^{abc}	2.733±1.176^{b}	0.450±0.182^{a}	1.900±0.419^{c}	0.433±0.328^{ab}	1.850±0.514^{b}
C.J	0.683±0.299^{a}	0.667±0.303^{abc}	0.650±0.483^{ab}	2.767±1.164^{b}	1.350±0.291^{a}	1.117±0.294^{bc}	0.633±0.167^{ab}	0.833±0.272^{ab}
SM	0.817±0.380^{a}	0.000±0.000^{a}	0.100±0.045^{a}	0.000±0.000^{a}	0.400±0.327^{a}	0.000±0.000^{a}	1.000±0.447^{b}	0.000±0.000^{a}
CCA	0.133±0.061^{a}	0.033±0.052^{a}	1.133±0.285^{abc}	0.000±0.000^{a}	0.650±0.157^{a}	0.000±0.000^{a}	0.700±0.134^{ab}	0.000±0.000^{a}
DKA	0.950±0.228^{a}	1.500±1.178^{c}	2.333±0.616^{c}	0.117±0.054^{a}	0.600±0.300^{a}	1.750±0.437^{c}	0.800±0.089^{ab}	1.233±1.381^{ab}
TTA	5.333±2.809^{b}	1.317±1.840^{bc}	1.717±0.587^{bc}	0.733±0.464^{ab}	1.450±0.629^{a}	0.533±0.173^{ab}	0.067±0.033^{a}	1.367±0.543^{b}

Keys: *Values with similar superscript(s) along column are not significantly different from each one*

Table 5. Distribution of *Escherichia coli* isolates in RTE food samples from Ekiti State University and environ

Food outlets	Number of occurrence in food samples								Total number (%)
	R	**B**	**S**	**PY**	**SE**	**MP**	**D**	**FP**	
ST	5	14	6	15	4	9	11	16	80(21.1)
C.J	3	13	5	17	10	10	6	6	70(18.5)
SM	6	4	6	9	12	6	1	5	49(12.9)
CCA	7	9	8	10	4	2	5	7	52(13.7)
DKA	10	9	15	7	14	15	NA	7	77(20.3)
TTA	13	7	8	3	NA	11	3	6	51(13.5)
Total	44	56	48	61	44	53	26	48	**379(100.0)**

Keys: *R- Rice, B- Beans, SO- Semo, PY- Pounded yam, SE- Scotch egg, MP- Meat pie, D- Doughnut, FP- Fish pie*

Table 6. Antibiotic susceptibility pattern of *E. coli* isolated from RTE food samples

S/N	Antibiotics	Disc content (µg)	Susceptibility (n=379)		
			Sensitive n (%)	**Intermediate n (%)**	**Resistance n (%)**
1	OFL	5	367 (96.8)	10 (2.6)	2 (0.6)
2	CXM	5	90 (23.7)	97 (25.6)	192 (50.7)
3	GEN	10	283 (74.7)	40 (10.6)	56 (14.7)
4	CRX	30	44 (11.6)	86 (22.7)	249 (65.7)
5	CAZ	30	31 (8.2)	44 (11.6)	304 (80.2)
6	CPR	5	307 (81.0)	40 (10.6)	32 (8.4)
7	NIT	300	252 (66.5)	48 (12.7)	79 (20.8)
8	AUG	30	0 (0.0)	0 (0.0)	379 (100.0)

Keys: *OFL- Ofloxacin; CXM- Cefixime; GEN- Gentamicin; CRX- Cefuroxime; CAZ- Ceftazidime; CPR- Ciprofloxacin; NIT- Nitrofurantoin; AUG- Augmentin*

Table 7. Phenotypic Pattern of multiple antibiotics resistance *E. coli* isolated from RTE food samples

Number of antibiotics	Resistotypes	Number of occurrence no (%)
3	CRX,CAZ,AUG	47
	CRX,NIT,AUG	7
	CXM,CAZ,AUG	25
	CXM,CPR,AUG	1
	GEN,CRX,AUG	1
	CXM,CRX,AUG	4
	Sub-Total	**85(22.4)**
4	CXM,CRX,CAZ,AUG	70
	CRX,CAZ,NIT,AUG	23
	CXM,GEN,CRX,AUG	1
	CXM,CAZ,CPR,AUG	2
	Sub-Total	**106 (28.0)**
5	CXM,CRX,CAZ,NIT,AUG	25
	CXM,GEN,CRX,CAZ,AUG	22
	CXM,CRX,CAZ,CPR,AUG	5
	CXM,GEN,CRX,NIT,AUG	1
	Sub-Total	**53 (14.0)**
6	CXM,GEN,CRX,CAZ,NIT,AUG	8
	CXM,GEN,CRX,CAZ,CPR,AUG	16
	CXM,CRX,CAZ,CPR,NIT,AUG	2
	Sub-Total	**26 (6.9)**
7	CXM,GEN,CRX,CAZ,CPR,NIT,AUG	5
	OFL,CXM,GEN,CRX,CAZ,CPR,AUG	1
	Sub-Total	**6(1.6)**
8	OFL,CXM,GEN,CRX,CAZ,CPR,NIT,AUG	1
	Sub-Total	**1 (0.3)**
Total		**277(73.1)**

Keys: *OFL- Ofloxacin; CXM- Cefixime; GEN- Gentamicin; CRX- Cefuroxime; CAZ- Ceftazidime; CPR-Cefuroxime; NIT- Nitrofurantoin; AUG- Augmentin*

4. DISCUSSION

Good Hygienic Practices (GHP) otherwise known as Good Manufacturing Practices (GMP) are essential to guarantee food safety. They are required by law under national and international food hygiene regulations and are frequently considered as pre-requisites to food safety systems based on Hazard Analysis and Critical Control Point (HACCP) [19]. Compromising good hygiene practices almost always results in establishment and proliferation of pathogenic as well as spoilage microorganisms on the processing and storage food contact surfaces. This has led to contamination of food with hazardous microorganisms making them unsafe for consumption [20].

From this present study, most of the outlets lack access to quality water supply; the major reliance is on well and bore-hole water (underground water) which can be easily contaminated and can hamper the possibility of providing safe food [21]. More so, defective personal hygiene could also facilitate the transmission of contaminants found in the environment via the mode of handling. Odu and Akano [22] reported that food handling by personnel plays important role in ensuring food safety throughout the chain of food production, processing, storage and vending. Mishandling and disregard for hygienic measures on the part of the food vendors have been reported as a major source of contaminants and pathogens that survive and multiply in sufficient numbers to cause illness in the consumers [1].

The 96 RTE food samples collected from six outlets in this study revealed that most of the food samples from the outlets were contaminated with coliform bacteria; which was an indication of poor microbiological quality. Several authors have observed that bacteria from unclean (impure) water obtained from dish washing and other sources can adhere to the surfaces of utensils and hence, constitute a risk of contamination during food vending [23-25]. Generally, there was higher microbial load in food samples obtained from outlets outside the University campus than those within the campus; this agrees with the findings of [26]. The high microbial load recorded for semo obtained from all the outlets both within and outside the campus

could be attributed to the mode of preparation and packaging. Semo shortened for semovita, a cereal-base powdered food type, is prepared by stirring the powder for some time on hot plates or fire and packaged in nylons of unknown microbial quality and hence could not be guaranteed and might probably serve as source of the contaminants. The microbial load recorded for rice obtained from food outlets outside the campus was equally higher than that obtained from samples from within the campus; with rice from TTA having the highest level of contamination followed by the sample from DKA and CCA. This could be as a result of the rich medium of coleslaw (salad cabbage, shredded carrots, dressed with mayonnaise) added to the rice sold in these outlets. This agrees with the report of Oranusi et al. [7] that noted that preparation, exposure and handling of RTE foods at temperature conducive for microbial proliferation, coupled with rich coleslaw, could be factors that increase microbial load in food.

The results obtained from this study show that most food samples collected from different outlets from Ekiti State University and environs harbored high number of *E*. *coli* which was among the microorganisms reported by Okwonko et al. [27] encountered in food samples examined. The presence of *E. coli* and other coliforms is an indication of possible faecal contamination of food through food handlers/vendors and poor hygienic practices, and possible improper storage conditions of foods [7,21,28]. This supports the report of Moyo and Baudi [29] that attributed the presence of indicator organisms, pathogens or high bacterial counts in food stuffs, food contact surfaces, equipment and utensils, to a direct and relevant measure of cleaning efficiency and hygiene. Considering the report of The International Commission for Microbiological Specification for Foods (ICMSF) [30] which stated that; ready-to-eat foods with total *E. coli* counts between 0 – 10^3 is acceptable, betwee 10^4 - ≤ 10^5 is tolerable while 10^6 and above is unacceptable, the result of the present study (as regards the *E. coli* load from all the outlets) signified unacceptable situation that is potentially hazardous to health as there is high tendencies of contracting food-borne illnesses caused by *E. coli*. Although *E. coli* is majorly considered part of the normal flora of human and some animals and hence harmless, other strains of *E. coli* exist that are pathogenic and may not easily be differentiated or distinguished [31].

The antibiotics susceptibility testing of *E*. *coli* isolated from food samples shows that there is high resistance to cefixime (50.7%), cefuroxime (65.7%), ceftazidime (80.2%), augmentin (100%). Likewise, 14.7% and 20.8% of the *E*. *coli* isolates were resistant to gentamicin and nitroflorantoin respectively. Only 0.6% and 8.4% of the isolates were respectively resistant to ofloxacin and ciprofloxacin, therefore ofloxacin and ciprofloxacin that is the second generation fluoroquinolone antibiotics can be used in the treatment of diseases caused by *E. coli.* These antibiotics are valued for their broad-spectrum activity, tissue penetration and availability in both oral and intravenous formulations. This is in agreement with the findings of Okereh and Erhahon [32]. However, twenty different types of MAR patterns (resistotypes) were observed from the 379 *E. coli* (Table 7). A high percentage (73.1%) of the *E. coli* isolates were MAR and the resistance pattern was such that the *E. coli* isolates were resistant to augmentin consisting of amoxillin (penicillin antibiotics) and clavulanic acid (β-lactamase inhibitor), cefuroxime (second generation cephalosporin), cefixime, ceftazidime (third generation cephalosoporin), gentamycin (broad-spectrum aminoglycoside), nitrofurantoin (nitrofurans), lesser resistotypes with ciprofloxacin, ofloxacin (flouroquinolones). The observation of MAR isolates was similar to the findings of Oluyege et al. [33]. The high level of antibiotic resistance recorded in this study may be ascribed to the possible previous and continuous of the bacterial contaminants to various antimicrobial agents such as used in the agricultural sector and with the same or similar modes of action. Many of these agents incidentally get into the food chain and eventually get to human beings through consumption. The consequences of these resistance patterns would be grave in terms of treatment of food-borne diseases with possibility of transfer of antibiotic resistance gene to humans.

5. CONCLUSION

These findings demonstrated that RTE foods sold in outlets in and outside the Ekiti State University are microbiologically contaminated and constitute likely potential hazard to human health. The isolation of *E*. *coli* in cooked RTE foods is an indicator of post-processing contamination or inadequate cooking. This study thus emphasized the need for intensive surveillance of this isolate in RTE foods continuum, to prevent food-borne infections and detect emerging antimicrobial resistant

phenotypes. Good hygienic practices are primary preventive measures hence the monitoring of their effectiveness will not only provide an early warning of potential problems but also evidence of due diligence.

Relevant agencies such as Consumer Protection Rights, NAFDAC and SON should therefore orientate and ensure strict compliance of these vendors to improved personal hygiene, food safety and proper waste disposal so as to enhance the quality of RTE food sold in the university and its environ and the general public otherwise the already poor public health system may be further burdened.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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