RESEARCH ARTICLE

Antibiotic susceptibility pattern of *Escherichia coli* isolated from food, cooking utensils and palms of food handlers in some restaurants in Zaria, Nigeria

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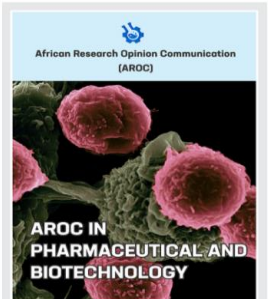

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ABSTRACT

Foodborne disease is a major public health problem causing considerable morbidity and mortality annually. In the present study, the antibiotic susceptibility patterns of *Escherichia coli* isolated from food, cooking utensils and palms of food handlers in some restaurants in Zaria, Nigeria were evaluated**.** A total of 250 samples (220 food samples, 7 hand samples of food handlers, 10 plate samples within restaurants and 13 spoon samples) were collected from five locations in Zaria, Nigeria and analysed for microbial contaminations using standard microbiological techniques. The antibiotic susceptibility pattern of the isolates was determined using Kirby-Bauer modified disc agar diffusion technique. Results revealed that out of 158 acclaimed *Enterobacteriaceae* isolates evaluated, 19 % (30) were confirmed to be *E. coli*, while 81 % were *Klebsiella.* spp, *Citrobacter fruendii, Enterobacter* spp, *Shigella* spp, *Salmonella* spp, *Serratia* spp, and *Cronobacter sakazaki*. The majority of the isolates were resistant to amoxiclav (26.08%), ampicillin (26.08%), tetracycline (26.08%) and metronidazole (13.04%). A 33.3% of the isolates were multidrug-resistant. The *E. coli* isolates were mostly multiple antibiotic resistance with 43.3% having multiple antibiotic resistance index (MARI) ≥ 0.2. In conclusion, *E. coli* evolved resistance to ampicillin, Amoxicillin Clavulanic acid, and Tetracycline and other tested antimicrobial drugs which would make the treatment of *Escherichia coli* infections difficult.

Keywords: *P. aeruginosa*; Modulation factor; *Xylopia aethiopica*; Zones of Inhibition; Minimum Inhibitory Concentration

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1.0 Introduction

Food is an essential ingredient for the good health and sustenance of human life. It's important, therefore cannot be overemphasized. In most African countries especially Nigeria, people depend mostly on vended food and food from public restaurants for consumption [1]. There is a rise in the patients with foodborne diseases resulting from street vended fruits and vegetables. Food purchased from the food vendors such as restaurants or food hunts is also susceptible to microbial contaminations. Improper washing of fruits and vegetables, untreated human faeces and agricultural bio-solid were major predisposing factors [2].

Adequate education on proper hygiene, decontamination approaches towards improving the quality of fruits and vegetables vended in Nigeria were recommended by most authors. These foods are consumed at the point of sale without any further treatment such as heating [1-2]. In the northern part of Nigeria such as Kaduna foods such as cooked rice, cooked beans, moimoi, fried yam, Salad, eba, Egusi soup, cooked semovita, Beans cake, and Pounded yam are mostly purchased at food vendors. Based on the relatively cheap and easily available of these ready-to-eat foods, their consumption has increased over the few years [3].

Despite the excessive patronage of these food vendors /handlers; it is very essential to ensure the safety level of these foods from contaminants and

microorganisms. Over the years Foodborne disease outbreaks linked with foods have been associated with various foodborne pathogens [3, 4]. Foodborne diseases are a major global problem causing considerable morbidity and mortality annually [5].

Increased antimicrobial resistance to most antibiotics prescribed has been reported to be mostly caused by *Escherichia coli* which are one of the most important opportunistic pathogens ever recorded from humans [6]. Antibiotic resistance in *E. coli* has become a serious and growing phenomenon in contemporary medicine, and it's emerging as one of the preeminent public health concerns of the 21st century reported by several studies on bacterial drug resistance especially in *E. coli* [7]. The magnitude of extraintestinal infections by *E. coli* was reported to be in the range of 6 to 8 million with 0.1 million cases per year of sepsis in the United States [8], with an increase in antibiotic resistances every- day [9].

Since resistance properties accrue through different routes, such as natural or intrinsic, mutational changes, and acquisition of plasmid or transposons. This study aims to investigate the antibiotic susceptibility of *E. coli* isolates from some food samples, cooking utensils and palm of food handlers in some restaurants in Zaria metropolis; in order to proffer better treatment option during therapeutics.

2.0 Materials and Method

2.1 Sample Collection

A total of 220 samples of food (cooked rice, cooked beans, moimoi, fried yam, Salad, eba, Egusi soup, cooked semovita, Beans cake, and Pounded yam), seven (7) samples of palms of food handlers was swab including ten (10) plates and thirteen (13) spoons samples were also swab, which was used within the restaurant. All the samples were collected within April to June 2019 from restaurants in five different locations (Samaru, Tudunwada, Sabo Gari, Wusasa, and Kwagila) in Zaria, Kaduna State

2.2 Ethical Clearance

Ethical approval for this work was obtained from the ethical committee of Ahmadu Bello University committee for research in human subject.

2.3 Swab sample collection

The hand-swab, spoon-swab and plate-swab samples were collected by swabbing the palms, spoons, and plates of the food vendors with sterile swab sticks and were transported to the Pharmaceutical Microbiology laboratory for analysis

2.4 Identification of the Isolates

The red colonies were selected from each MacConkey agar plate. Gram staining methods and further biochemical tests were carried out to identify and characterize the organisms that were isolated from the food and cooking utensils samples. Further confirmation was carried out using Microgen test kits [10].

2.5 Antimicrobial Susceptibility testing

The Modified Kirby-Bauer disc diffusion method was used to determine the antibiotic susceptibility of isolates which has been identified and confirmed by biochemical tests. Discrete colonies of isolates on nutrient agar plates were emulsified in 5 ml of sterile physiological saline and the turbidity adjusted to 0.5 McFarland standards (approximately a cell density of 1.5×10^8 CFU/ml). The standardized suspension was inoculated on Mueller Hinton agar using a sterile swab to ensure even distribution and confluent growth. The disc of the various antibiotics was aseptically placed using an antibiotic dispenser, a pre diffusion time of 30 minutes was allowed and the plates were then incubated at 37^{o}C for 18 hours. After incubation, the diameter of the zones of inhibition produced by each antibiotic disc was measured and recorded. The results were interpreted according to the Clinical and Laboratory Standards Institute [11].

The selected antibiotics used for this study are: Ampicillin (AMP, 30µg), Amoxicillin-clavulanic acid (AMC, 30µg), Ceftriaxone (CRO, 30µg), Chloramphenicol (C, 30µg), Ciprofloxacin (CIP, 5µg), Gentamicin (CN, 10µg), Imipenem (IMP, 10µg), Tetracycline (TET, 30µg), and Trimethoprim-sulphamethoxazole (SXT, 25µg) (all from Oxoid Ltd. Basingstoke, London)

2.6 Determination of Multiple Antibiotic Resistance (MAR) Index

The multiple antibiotics resistance (MAR) index was determine for each isolates by dividing the numbers of antibiotics to which the isolates is resistant to by the total number of antibiotics tested Paul *et al.,* [12]

3.0 Results

3.1 Occurrence frequency of *E. coli* from food samples

The incidence of *E. coli* isolates from food, cooking utensils and palms of food handler's samples in Zaria metropolis, Nigeria, after biochemical characterization, using Microbact™ 12E Gram-negative identification kit. Out of 158 acclaimed *Enterobacteriaceae* isolates evaluated, 19 % (30) were confirmed to be *E. coli*, while 81 % were other Enterobacteriaceae organisms such as *Klebsiella.* spp, *Citrobacter fruendii, Enterobacter* spp, *Shigella* spp, *Salmonella* spp, *Serratia* spp, *Cronobacter sakaz aki*

3.2 Antibiotic resistant pattern of *E. coli* from food samples

The number of *E. coli* isolates resistant to each antibiotic and their percentages are shown in table 2 and figure 1. The majority of the isolates were resistant to Amoxylclav (26.08%), Ampicillin (26.08%), and Tetracycline (26.08%) and Metronidazole (13.04%). The isolates were mostly MAR isolates being resistant to at least two of the antibiotics resistance. The multiple antibiotic resistance index of > to 0.2 was shown by 43.3% of *E. coli* (Table 3)

Table 1: Identification of *E. Coli* from food samples in Zaria metropolis, Nigeria.

S/N	Organism	Incidence of Isolates	Percentage (%)
1	*E. coli*	30	18.9
2	*Klebsiella.* spp,	22	14
3	*Citrobacter fruendii,*	10	6.3
4	*Enterobacter* spp,	48	30.4
5	*Shigella* spp,	13	8.2
6	*Serratia* spp	15	9.5
7	*Cronobacter sakazaki*	12	7.6
8	*Salmonella,* spp	8	5.1

Table 2: Percentage of *E. Coli* Resistance to Some Common Antibiotics Mostly used for the Treatment of Diarrhea (food borne infection) in Zaria, Nigeria

S/N	Antibiotics	Category of Antibiotics	NIRA	Percentage Resistant
1	Amoxylclav (AMC)	Penicillin	12	26.08
2	Gentamicin (CN)	Aminoglycoside	0	0
3	Ciprofloxacin (CIP)	Fluoroquinolone	0	0
4	Sulphamethonidazole - Trimethroprim (SXT)	Folate pathway inhibitors	2	4.3
5	Chloramphenicol (C)	Phenicol	0	0
6	Imipinem (IPM)	Carbapenems	2	4.3
7	Ampicillin (AMP)	Penicillin	12	26.08
8	Ceftriaxone (CRO)	Cephalosporins	0	0
9	Metronidazole (MTZ)	Nitriomidazoles	6	13.04
10	Tetracycline (TET)	Tetracycline	12	26.08

Key: NIRA = number of isolates resistant to each antibiotics

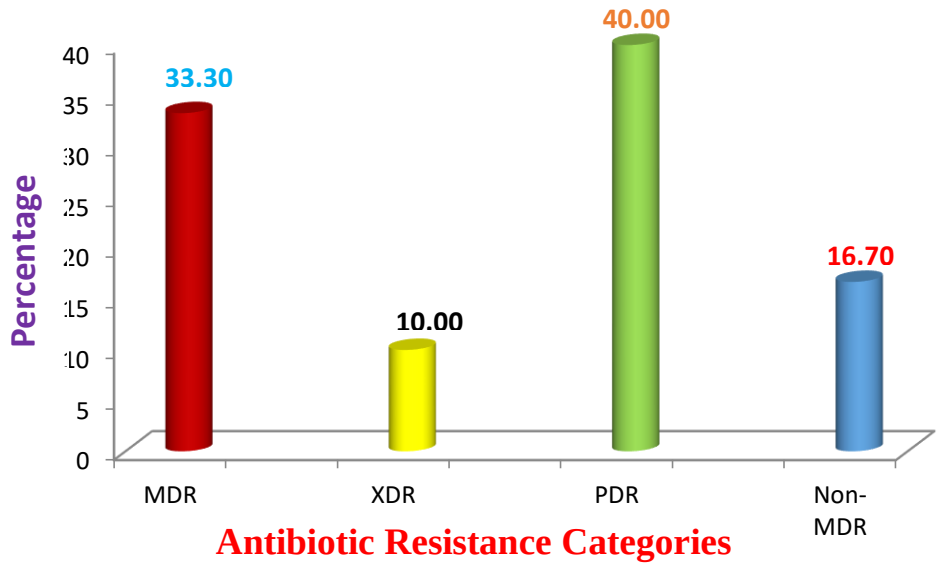

Figure 1: Multidrug resistance pattern of *E. coli* Isolates from Food samples in Zaria, Nigeria. MDR = Multidrug-resistant, XDR = Extensively drug-resistant, PDR = Pandrug-Resistant, Non-MDR = Not Multidrug-resistant

Table 3: Multiple Antibiotic Resistance Index (MARI) of *E. coli* Isolates from Food samples in Zaria, Nigeria

MARI	Number	Percentage
0.2	0	
0.3	4	
0.4	9	
0.5	0	
0.6	0	43.3%
0.7	0	
0.8	0	
0.9	0	
Total	30	100

4.0 Discussion

Foodborne disease is a major public health problem causing considerable morbidity and mortality annually [13]. The isolation of *Escherichia coli, Salmonella,* spp., *Shigella* spp., *Klebsiella* spp., and other *Enterobacteriaceae* as food contaminants in this study is similar to the studies of Idowu *et al.,* [14] which reported that these microorganisms were implicated in contamination of ready-to-eat foods. Similar findings have also been reported by other researchers [15-17].

Out of 158 acclaimed *Enterobacteriaceae* isolates evaluated, 19 % (30) were confirmed to be *E. coli*, while 81 % were other Enterobacteriaceae organisms such as *Klebsiella.* spp, *Citrobacter fruendii, Enterobacter* spp, *Shigella* spp, *Salmonella* spp, *Serratia* spp, *Cronobacter sakaz aki*. The majority of the isolates were resistant to

amoxiclav (26.08%), ampicillin (26.08%), and tetracycline (26.08%) and metronidazole (13.04%). Thirty-three (33.3%) of the isolates were multidrug-resistant. The *E. coli* isolates were mostly multiple antibiotic resistance with 43.3% having multiple antibiotic resistance index (MARI) ≥ 0.2

Ciprofloxacin, Chloramphenicol, Ceftriaxone and Gentamicin susceptibility of *E. coli* isolates (0% overall resistance) in this study proves Ciprofloxacin, Chloramphenicol, Ceftriaxone Gentamicin as one of the possible effective antibiotics. This is corroborated by the findings of Igba et al. [18] in which almost all tested enteric organisms except *E. coli* identified in his study were 100% sensitive to ciprofloxacin, gentamicin, and trimethoprim-sulphamethoxazole. This means that these antimicrobials are still drugs of choice for the management of foodborne illnesses in this locality of Zaria. On the other hand, *E. coli* evolved resistance to ampicillin, Amoxylclav, and Tetracycline and other tested antimicrobial drugs which would make the treatment of *E. coli* infections difficult.

The high percentage (43.4%) of *E. coli* having MAR index ≥ 0.2 in this study, suggests that the isolates originated from a high-risk source of contamination where antibiotics are often used [19]. However, the percentage of MDR (33.3%) obtained in this study (Figure 1) might be an indication that some proportion of the *E. coli* isolates have been pre-exposed to several antibiotics. Furthermore, the combination of microbial characteristics such as selective pressure on antimicrobial usage and technological changes that enhance the transmission of drug-resistant organisms might be the cause of this resistance [20]. Other reasons could be due to the increase in transmission of resistant isolates between vendors from food. Therefore, this attribute has made *E. coli* to be considered an important reservoir of transferable antibiotic resistance [21]. The difficulties in the treatment of food and water associated gastrointestinal diseases due to *E. coli* have been reported [22]. This problem is compounded by the continued emergence of antibiotic resistance to a growing number of antibiotics [23-24].

5.0 Conclusion

This study showed the presence of microbial contamination of food samples. The presence of multidrug resistance E. coli could serve as an indicator for the need to promote awareness about the possible health hazards that could be due to poor handling of these foods. There is, therefore, the need for agencies to ensure that microbiological standards are established and practised by food sellers for the handling distribution of food.

Conflict of Interest: We have no conflict of interests

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Author Contributions: Authors V.E. and G.O.A conceived the idea and designed the study, conducted the study, performed data analysis and interpretation, and wrote the first draft of the manuscript. Authors BAT and P.I. contributed data or analysis tools, correction of draft. All authors read and approved the final version to be published.

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