



Prevalence and Antibigram of Bacteria Associated with Food Vending in Awka-South LGA, Anambra State, Nigeria

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ABSTRACT

Food vending is recently a booming part of the food industry and millions of people depend on it for their daily nutritional needs especially because of its easy accessibility. It has become an important public health issue due to widespread food-borne diseases which are leading cause of morbidity and mortality. This study is aimed at assessing the prevalence and antibiogram assay of bacteria associated with food vending in Awka-South LGA, Anambra State. The randomly selected towns for this study were three towns in Awka- South LGA in Anambra state namely Awka (State capital), Mbaukwu and Nise. Descriptive cross-sectional study and multistage sampling technique was adopted and sample size determined using a standard formula. Total of 65 food vendors were sampled. Sterile swab sticks were used to swab the palms, aprons, plates and spoons of the food vendors and the rinse method was used for bacteria isolation. Antibiotics sensitivity test of isolates was carried out using Kirby-Bauer disk diffusion method. The bacteria isolates from this study were *E. coli* (24.41%), *S. aureus* (21.60%), *B. cereus* (9.39%), *Pseudomonas aeruginosa* (15.96%), *Serratia marcescens* (4.70%), *Klebsiella pneumonia* (10.56%) and *Salmonella enterica* (13.38%). The bacterial isolates during the course of this study showed wide resistance to conventional antibiotics especially beta-lactams. The findings of this study therefore necessitate the need for sensitization of food vendors and appropriate measures should be established to monitor susceptibility patterns of microorganisms involved as this will help to avoid possible outbreaks.

Key words: *Antibiogram, Food vendors, Bacteria, Antibiotic sensitivity*

1. INTRODUCTION:

In developing countries like Nigeria in Western Africa, street food vending is very common because of their easy accessibility, low cost and convenient availability. The consumption of such food products have increased tremendously, becoming daily source of diet to millions of people in the country. Food is one of the most basic human needs and it is essential to sustain life (Agu, 2014). In as much as food is eaten primarily to sustain life, food should be nourishing, attractive and free from noxious substances such as poisonous chemicals, toxins and pathogenic microorganisms (McLauchlin and Little 2007). Food if not carefully guarded against contaminations by the food vendors can do much harm to the consumer than the intended good.

The safety of street food has become one of the major concerns of public health and a focus for governments and scientists to raise public awareness (FAO, 2007; Mukhola, 2007). Food contamination is caused by many factors including traditional food processing methods, inappropriate holding temperatures, and poor personal hygiene of food handlers (Feglo and Sakyi, 2012). According to Monney *et al.* (2013), food vendors contaminate food by poor personal hygiene, cross-contaminating raw and processed food, as well as inadequate cooking and improper storage of food. Food is not supposed to cause harm to the consumer, but when it causes harm, a reverse of its intended good leads to food-borne diseases.

The world Health Organisation (WHO) (2010) stated that millions of people fall sick or die as a consequence of eating unsafe food. WHO (2015) estimates that as many as 600 million, or about one in every 10 people around the world is sickened by food-

borne disease each year and 420, 000 people die as its result, including 125, 000 children under the age of 5 years. Although epidemiological data on the incidence of food borne diseases are inadequate, and the outbreak often not investigated; the recurrent incidences of food borne illnesses with symptoms of gastro intestinal distress like diarrhea, vomiting, abdominal cramp and nausea has remained a major cause of mortality and morbidity in Nigeria (Nweze, 2010). According to Ihenkuronye (2012), more than 200, 000 persons die of food contamination annually in Nigeria, and these deaths are caused by contaminated foods through improper processing, preservation and service.

Assefa *et al.* (2015) reported that food handlers are carriers of potential food pathogens like *Staphylococcus aureus*, *Klebsiella* spp, *E. coli*, *Enterobacter* spp, *Citrobacter* spp, *Serratia marcescens*, *Pseudomonas aeruginosa*, *Proteus* spp, *Providencia rettgeri* and *salmonella* spp. Sharmila (2011) also reported that food vendors are carriers of food-borne pathogens like *Escherichia coli*, *Salmonella*, *Shigella*, *Campylobacter* and *Staphylococcus aureus* which they eventually transfer as food-borne hazards to the consumers.

Several food-borne diseases caused by bacterial pathogens are cured by using different class of conventional antibiotics already available but current development of resistance in almost all bacterial species to different classes of antibiotics poses a major threat to global healthcare. Several mechanisms are known to induce antibiotic resistance in bacteria, but the most common type of resistance develops and transmits horizontally via conjugation of a plasmid (Farzana *et al.* 2011). Likewise, evolution of multidrug-resistant (MDR) in bacterial strains, known as “superbugs” may create serious threat which results in resistance to several antibiotics (Alanis, 2005). With antibiotics resistance in microorganisms, the treatment of food-borne illnesses becomes difficult and if not handled with care leads to mortality. According to Farzana *et al.* (2011), the knowledge of susceptibility or resistance of bacterial species to various antibiotics helps in the effective treatment.

This study is aimed to reveal the prevalence of bacterial strains and their antibiotics susceptibility pattern in Awka-south LGA, Anambra State, Nigeria.

2. MATERIALS AND METHODS

Study Area

The study areas for this research were three selected towns in Awka- South LGA in Anambra state which includes Awka (State capital), Mbaukwu and Nise.

Study Design

In this study, Cross-sectional design was used.

Study Population

The target population was all food vendors who cooked and sold ready to eat foods within the selected study area and a total of 65 vendors were sampled.

a. Inclusion Criteria

The inclusion criteria were food vendors who sell cooked/prepared food on streets, markets, restaurants, fast-food joints and hawkers.

b. Exclusion Criteria

The exclusion criteria were food vendors who were not selling cooked foods, but snacks, fruits and vegetables.

Ethical Approval and Consent to Participate

Prior to data collection, permission was sought from the Ethical and Ethics Committee of Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra State, Nigeria. Permission to conduct study in Awka-South LGA was equally sought from Federal Ministry of Health, Anambra State Directorate. At the individual level, Letter of Introduction and written informed consent was approved and used before food vendors were involved in the study.

Sampling Technique

This study employed multi-stage sampling technique which includes random, stratified and cluster techniques. The selected towns used in this study were randomly selected from the nine towns in Awka-South LGA, Anambra state, Nigeria. Each of the selected towns was considered a stratum, and the strata were clustered into groups pending on the availability and location of the food vendors. Proportionate sampling based on the number of food vendors in each cluster was done to get the number of respondents.

Swab Collection

A total of two hundred and twenty seven (227) swabs were aseptically collected from palms, plates, spoons and aprons of the food vendors (65 vendors) from the

three selected towns. A sterile swab stick was dipped into normal saline and aseptically used to swab each of the surfaces of their palms, plates, spoons and aprons. After collection, the swab sticks were placed in sterile bags and conveyed to the Microbiology laboratory of Department of Applied Microbiology and Brewing, Nnamdi Azikiwe University, Awka for analysis.

Source of Reagents and Materials

Culture media used (Nutrient Agar, Blood Agar, MacConkey Agar, Mannitol Salt Agar, Salmonella-Shigella Agar, Ceftrimide Agar, Mueller-Hinton agar) were manufactured by Oxoid Ltd., UK. All reagents and materials used were of analytical standard.

Preparation of Culture Media

All culture media used were prepared according to the manufacturer's instructions. They were sterilized by autoclaving at 121°C, 15psi and for 15 minutes while *Salmonella-Shigella* agar was prepared by boiling in a water bath at temperature of 100°C.

Bacterial Isolation

The rinse method as described by Lambrechts *et al.* (2014) was adopted. Each swab stick was aseptically rinsed into freshly prepared Nutrient Broth in test tubes (5ml per test tube and plugged); the test tubes were incubated at 37°C for 24 hours for growth which is detected through turbidity. After incubation, a loop full of each broth was streaked progressively to obtain discrete colonies on appropriate culture media. The plates were incubated at 37°C for 24 hours and then observed at the end of the incubation for the kind of growth present on each agar. Discrete colonies were

sub-cultured and pure cultures stored in slants of their respective media. The pure cultures were preserved at 4°C in a refrigerator.

Identification and characterization of the Isolates

The isolates were identified by Gram-staining, characterized biochemically and identified up to species level by performing standard tests.

Antibiotics Susceptibility Testing and Analysis

The susceptibility tests were performed using Kirby-Bauer disk diffusion method as recommended by Clinical Laboratory Standards Institute (CLSI, 2012) and National Committee for Clinical Laboratory Standards (NCCLS, 1997) using Mueller Hinton Agar. The bacterial strains were tested against the following Oxoid antibiotic sensitivity discs: Ciprofloxacin (CPR, 5µg); Ceftazidime (CAZ, 30µg); Cefuroxime (CRX, 30µg); Gentamicin (GEN, 10µg); Cefixime (CXM, 5µg); Ofloxacin (OFX, 5µg); Amoxicillin/Clavulanic acid (AUG, 30µg); Nitrofurantoin (NIT, 5µg). Inhibition Zone Diameter (IZD) was interpreted using standard charts, CLSI (2012) and Oxoid-CLSIFDA guidelines (2013).

3. RESULT

Potential Bacteria Isolated and Prevalence (%)

Out of the 65 food vendors sampled and 227 samples collected, a total of 426 bacteria were isolated. Table 1 shows the morphological characteristics and biochemical identification of the bacteria isolates while the frequency of the isolates and their percentage prevalence are shown in Table 2. The isolates are potential bacteria implicated in food-borne disease outbreaks.

Table 1: Morphological and Biochemical Characteristics of Bacteria Isolates

Sl. No.	Colony Morphology	Gram Stain/ Shape	Catalase Test	Coagulase Test	Motility Test	Indole Test	Urease Test	V-P Test	Methyl Red Test	Citrate Test	Oxidase Test	Starch Hydrolysis	Spore Test	Glucose utili.	Lactose Utili.	Maltose Utili.	Mannitol Utili.	Sucrose Utili.	Organism
1	Circular, raised, smooth and cream Coloured.	+ Cocci in Clusters	+	+	-	-	V	+	+	+	-	-	-	+	+	+	+	+	<i>Staphylococcus aureus</i>
2	Oval, Opaque and Pinkish	- Rods	+	-	+	+	-	-	+	-	-	-	-	+	+	-	+	V	<i>Escherichia coli</i>
3	Black centered on SS Agar	- Rods	+	-	+	-	-	-	+	-	-	-	-	+	-	+	+	-	<i>Salmonella enterica</i>
4	Bluish-Green on Cetrimide Agar	- Rods	+	-	+	-	-	-	-	+	+	-	-	-	-	-	+	-	<i>Pseudomonas aeruginosa</i>
5	Creamy, Flat	+ Rods	+	-	+	-	-	+	-	V	-	+	+	+	-	+	-	+	<i>Bacillus cereus</i>
6	Pinkish-red, mucoid	- Rods	+	-	-	-	+	+	-	+	-	-	-	+	+	+	+	+	<i>Klebsiella pneumonia</i>
7	Red coloured on Nutrient Agar	- Rods	+	-	+	-	-	+	-	+	-	-	-	+	-	+	+	+	<i>Serratia marcescens</i>

**Key: + = Positive, V-P = Voges-Proskauer, V = varied
- = Negative, Utili. = Utilization**

Table 2: Frequency and Percentage Prevalence of the Bacteria Isolates

Isolates	Frequency	Percentage prevalence (%)
<i>Escherichia coli</i>	104	24.41
<i>Staphylococcus aureus</i>	92	21.60
<i>Bacillus cereus</i>	40	9.39
<i>Pseudomonas aeruginosa</i>	68	15.96
<i>Serratia marcescens</i>	20	4.70
<i>Klebsiella pneumonia</i>	45	10.56
<i>Salmonella enterica</i>	57	13.38

Total number of isolates = 426

Antibiogram Assay of the Isolates

The bacteria isolates from this study showed a wide range of resistance to conventional antibiotics especially the beta-lactams. The results of their percentage (%) antibiogram assay were presented in figures 1–7. The susceptibility profile of the isolates was graded as susceptible, intermediate and resistant.

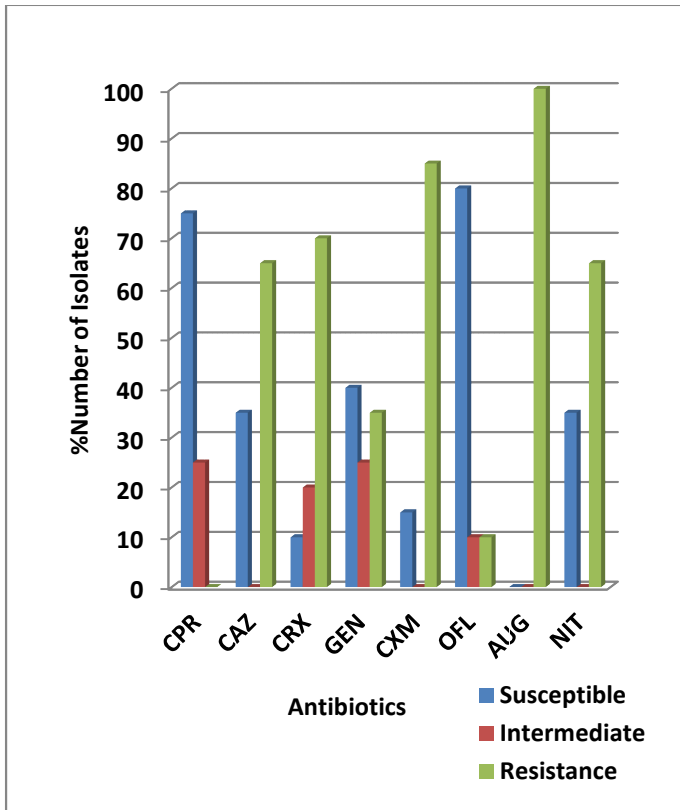


Fig 1: Percentage (%) Antibiogram Assay on *Escherichia coli* isolates.

Fig 2: Percentage (%) Antibiogram Assay on *Staphylococcus aureus* Isolates.

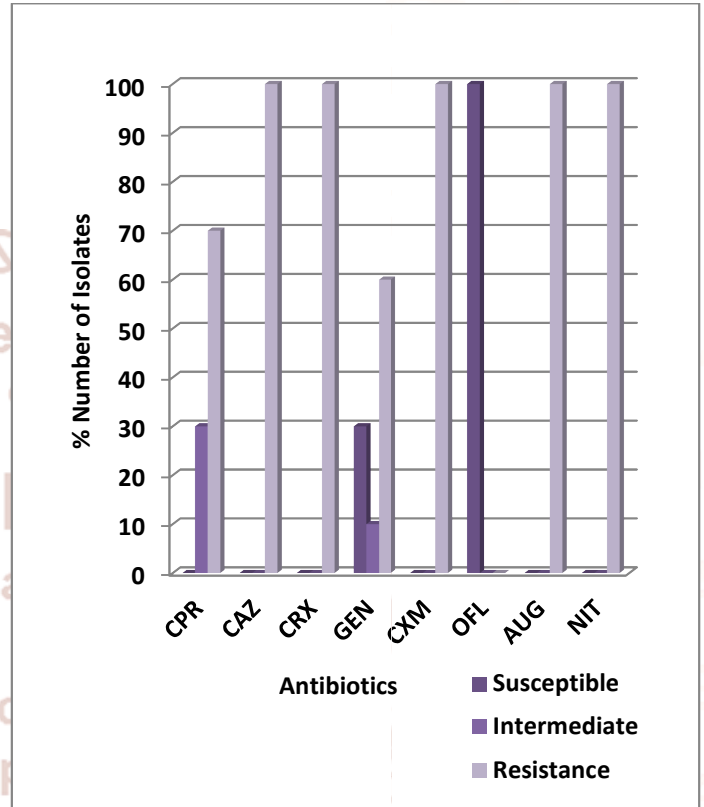
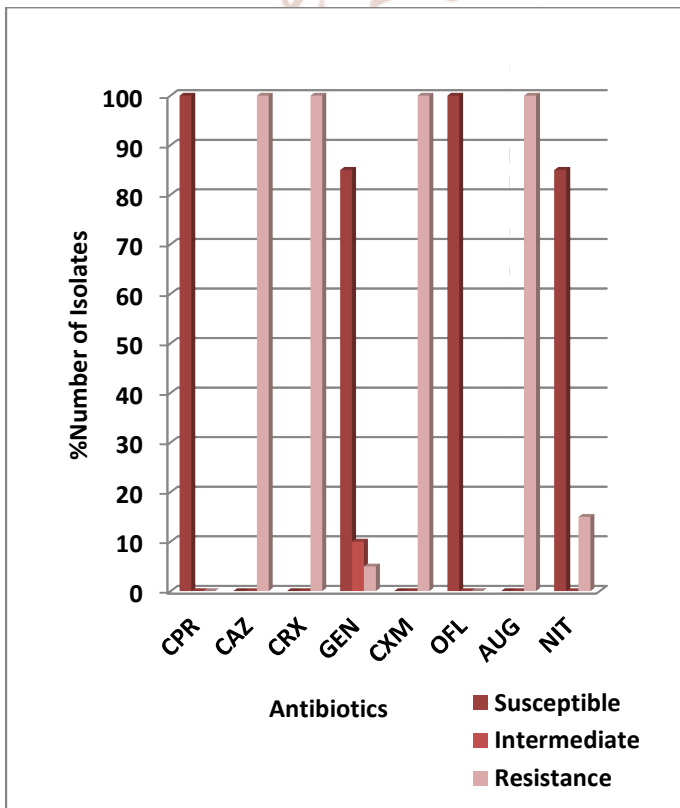


Fig 3: Percentage (%) Antibiogram Assay on *Bacillus cereus* Isolates.



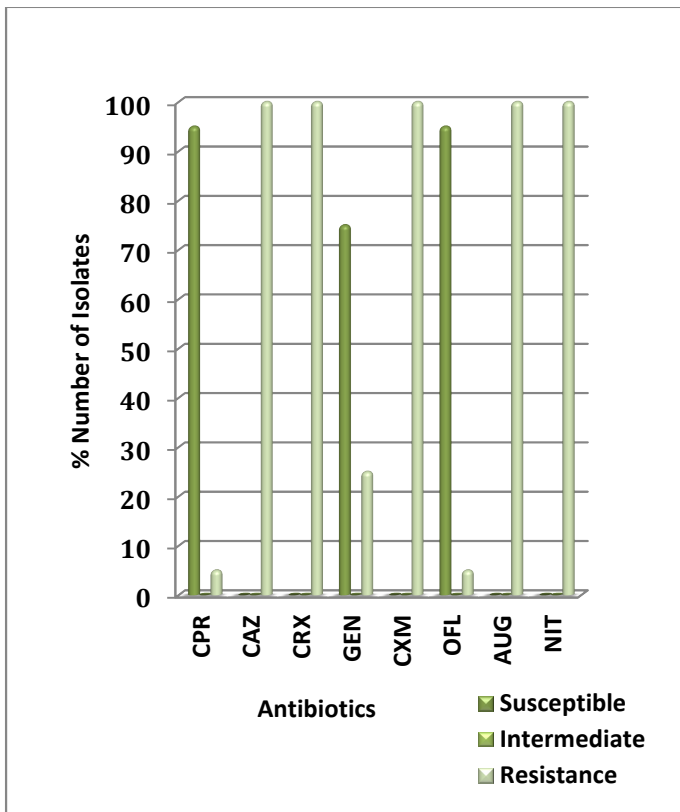


Fig 4: Percentage (%) Antibigram Assay on *Pseudomonas aeruginosa* Isolates.

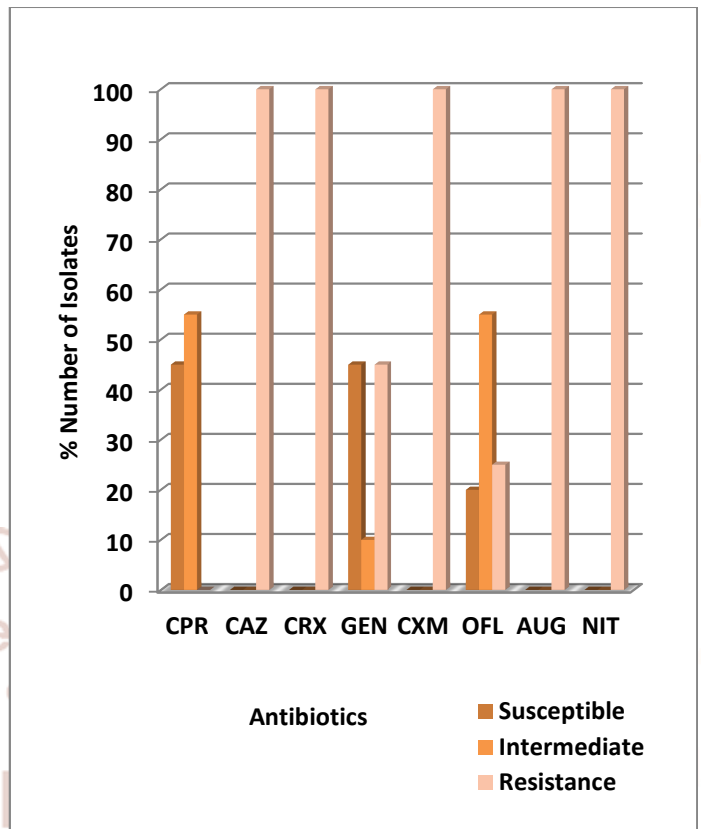


Fig 6: Percentage (%) Antibigram Assay on *Klebsiella pneumoniae* Isolates.

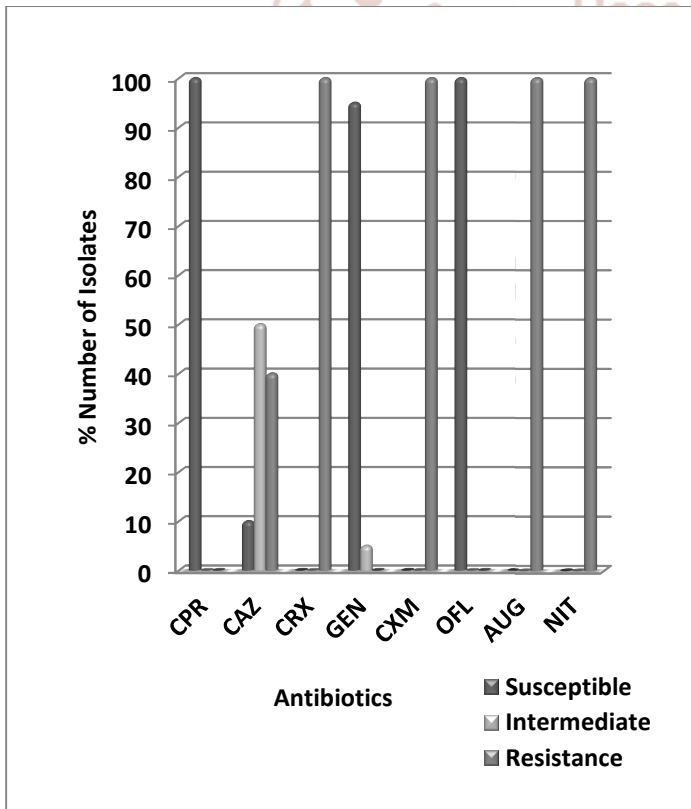


Fig 5: Percentage (%) Antibigram Assay on *Serratia marcescens* Isolates.

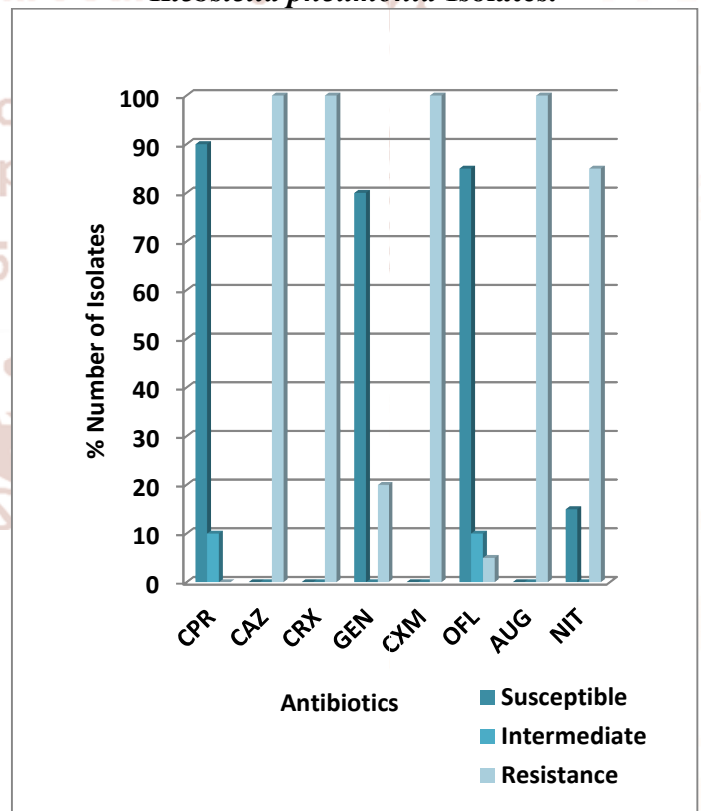


Fig 7: Percentage (%) Antibigram Assay on *Salmonella enterica* Isolates.

4. DISCUSSION

Food vending practices over the years has been a major cause of food-borne diseases globally especially in developing countries, where high incidence of food-borne diseases are recorded annually. The results of the study clearly indicates high level of unhygienic and sanitary practices among food vendors which concurs with Assefa *et al.* (2015) that food handlers are important vehicle for microorganisms, and their improper handling practices may cause food contamination and consequently food-borne diseases which pose a potential risk to public health.

The bacteria isolated in this study and their percentage prevalence had previously been implicated in food-borne disease outbreaks and they include; *Escherichia coli* (24.41%), *Staphylococcus aureus* (21.60%), *Bacillus cereus* (9.39%), *Pseudomonas aeruginosa* (15.96%), *Serratia marcescens* (4.70%), *Klebsiella pneumonia* (10.56%) and *Salmonella enterica* (13.03%). Similar types of microbial contaminants had been identified in previous studies in Benin City, Ogun State and Ondo State, Nigeria (Okareh and Erhahon, 2015; Bankole *et al.*, 2009; Ibrahim *et al.*, 2013). Furthermore, the isolates from this study also agrees with isolates from previous researches carried out in Gondar and Jimma, Ethiopia (Andargie, 2008; Assefa, *et al.*, 2015); Saudi Arabia (Zaglool *et al.*, 2011); Egypt (Fadel and Ismail, 2009); Puerto Rico (Dharod *et al.*, 2009) and Brazil (Souza and Santos, 2009).

The microbial isolates from the study were accessed using antibiotics susceptibility assay (antibiogram) which was carried out to evaluate the activity of conventional antibiotics against the isolated bacteria; this is mainly to ascertain the nature of the microbes. The *Escherichia coli* isolates showed wide resistance to beta-lactam antibiotics i.e. 100% resistance to augmentin, 85% resistance to cefixime, 70% resistance to cefuroxime and 65% resistance ceftazidime. There was 75% and 80% susceptibility to ciprofloxacin and ofloxacin (fluoroquinolones) respectively. The antibiotics susceptibility profile of *Escherichia coli* isolates from this research agrees with similar findings by Abuchi *et al.* (2016) which recorded 100% resistance to beta-lactams (Ampicillin, ceftriaxone and cefuroxime) and 100% susceptibility to fluoroquinolones.

The *Staphylococcus aureus* isolates showed 100% resistance to beta-lactam antibiotics especially penicillin and cephalosporins (augmentin, ceftazidime, cefuroxime and cefixime). There was 100% susceptibility to fluoroquinolones (ciprofloxacin and ofloxacin) and 85% susceptibility to nitrofurantoin and gentamicin respectively. Consistent with the findings of this study, a similar study in India by Kuljinder and Kahlon (2017) reported 70% and 80% susceptibility to ciprofloxacin and ofloxacin respectively. The study also reported 80% susceptibility to gentamicin which is consistent with 85% susceptibility reported in this study. The *Pseudomonas aeruginosa* isolates showed 100% resistance to beta-lactam antibiotics and nitrofurantoin, they were susceptible to fluoroquinolones and gentamicin (75%). *Salmonella enterica* isolates from this study were mostly resistant to beta-lactams and nitrofurantoin, but were susceptible to fluoroquinolones and gentamicin. A similar study in Kenya by Sang *et al.* (2016) also reported high susceptibility to fluoroquinolones but in contrast to this study recorded susceptibility to the beta-lactam augmentin. *Klebsiella pneumonia* isolates were 100% resistant to beta-lactams and nitrofurantoin, they were mainly intermediate at 55% to fluoroquinolones, and 45% susceptible to gentamicin. The *Bacillus cereus* isolates showed 100% resistance to beta-lactams and nitrofurantoin. They showed 100% susceptibility to ofloxacin but 70% and 60% resistance to ciprofloxacin and gentamicin respectively. *Serratia marcescens* isolates showed wide resistance to betalactams and nitrofurantoin, but they were highly susceptible to fluoroquinolones and gentamicin. The bacteria isolated during the course of this study showed wide resistance to beta-lactam antibiotics and this calls for public health attention.

5. CONCLUSION

Considering the high rate of resistance to antibiotics shown by the strains isolated from the food vendors, it can be concluded that there is wide spread of antibiotics resistance among microorganisms from different sources. This study emphasizes the need for intensive surveillance on isolates throughout the food production continuum to prevent food-borne diseases and also to detect emerging antimicrobial resistant microorganisms especially in developing countries like Nigeria.

6. RECOMMENDATION

Since microorganisms are ubiquitous and mostly opportunistic pathogens, this study recommends that proper hygiene and sanitary practices should be adopted by these vendors. Such hygiene practices can include sterilizing their wares (utensils, aprons) after daily vending activities. Also, use of antiseptic soaps to wash their hands often and regular use of hand sanitizers are recommended. Active antibiotics resistance surveillance system has to be developed in the country by the government as seen in developed countries. This will easily detect the presence of multidrug resistant contaminants in ready to eat foods and their selling environments. In cases of outbreaks and food-borne diseases, effective antibiotics should be used to treat diseases as this will help to avoid induced resistance in causative microbes.

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