Original Research Article

Antimicrobial Susceptibility Pattern and ESBL Prevalence of Bacteria Isolated from Street Vended Snacks

ABSTRACT

Aims: The objective of the study was to ascertain the antimicrobial susceptibility pattern and ESBL prevalence of bacteria isolated from snacks.
Place and Duration of Study: Department of Microbiology (Laboratory Unit) Michael Okpara University of Agriculture Umudike.
Methodology: The snacks were mashed aseptically, serially diluted and inoculated onto nutrient agar and MacConkey agar. Isolates were identified using standard microbiological procedures. Antimicrobial susceptibility of the isolates and ESBL detection was done using disk diffusion method. ESBL production was confirmed using Double Disc Synergy Test (DDST) method following CLSI recommendations.
Results: *Escherichia coli, Salmonella typhi, Staphylococcus aureus* and *Klebsiella pneumoniae* were the bacteria isolated with *Escherichia coli* as the most prevalent isolate with 42% occurrence in the samples screened. There was significant difference in the sensitivity of the bacteria isolates to the different antibiotics used at *P*=0.05. *Salmonella typhi* isolates exhibited highest resistance to an antibiotic with 86% resistance to ciprofloxacin while *Klebsiella Pneumoniae* isolates exhibited the lowest resistance to an antibiotic with 10% resistance to cefotaxime. Among the Gram-negative bacteria, 36% of suspected ESBL producing *E.coli* isolates were confirmed as ESBL producers indicating the highest occurrence.
Conclusion: The study confirmed the presence of bacteria in street vended snacks which exhibited high resistance to antibiotics that could be attributed to the presence of ESBL producers among the isolates.

Keywords: Antimicrobial, Susceptibility, ESBL, Bacteria (~~*Arial, inclined, 10 font, justified)*~~

1. INTRODUCTION

Food-borne diseases caused by microbes and chemical contamination of food is a major health challenge especially in developing countries [1]. Microbial pathogens cause a wide range of infections or intoxications, such as enteric complications, abdominal pain, fever, haemorrhagic colitis, joint infections, kidney failure and paralysis. The common manifestation of food poisoning occurs by diarrheal diseases, which is usually caused by toxins released from microbes. It is estimated that globally, food-borne and waterborne diseases together kill about 2 million people yearly. Food borne diseases cause illness mainly in developing

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countries due to poor hygiene of handlers during preparation and packaging [2]. The unhygienic practices significantly contributing to the entry of bacterial pathogens to food include contamination from surfaces of cooking utensils, improper storage and unsanitary conditions of surfaces in the operational environment.

Street vended foods are ready to eat (RTE) foods that are prepared and served for immediate consumption with low preparation time and can be consumed at the point of sale without further handling [3]. They are foods consumed in the same state as they are sold and they may be cooked or raw. The major problem associated with RTE's is the frequent incidence of contamination. Due to the nature of these foods and their methods of preparation involving extensive handling, they are usually prone to contamination from water, air, storage equipment and human activities [1]. Street vended foods pose a risk to public health because they are openly displayed and are exposed to dust, insects, and the hands of the food handlers and customers. In developing countries such as Nigeria, clean water is often not available at both preparation and vending sites. Furthermore, sanitary facilities are rarely available for workers. Wastes are disposed of nearby, providing nutrients for flies and rodents and this may harbour food borne pathogens [4]. As a result, ready to eat prepared street foods are commonly exposed to pathogenic microorganisms like *Escherichia coli*, *Salmonella* sp, *Shigella* sp, and *Staphylococcus aureus*.

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Antimicrobial resistance is one of the greatest threat to human health and developing countries including Nigeria are worst hit by this crisis. This increasing resistance can be attributed to various factors such as the abuse of antibiotics. The situation is aggravated by indiscriminate use of antimicrobials especially beta lactams, in therapeutic doses for growth promotion, prophylaxis and treatment of bacterial diseases in animals [5]. Currently, β-lactam drugs are a key factor in the treatment of bacterial infections worldwide and account for over 60% of antibiotics in use [6]. There are six groups of β-lactam drugs based on the chemical structure of the β-lactam ring and they include Penicillins, Cephalosporins, Cephamycins, Carbapenems, Monobactams, and β-lactamase inhibitors. They inhibit bacterial growth by blocking cell wall synthesis. Antibiotic resistance is acquired by bacteria through different mechanisms such as production of efflux pumps, alteration of cell membranes and production of extended spectrum beta-lactamases (ESBLs). ESBLs are β-lactamases enzymes which have the capacity to hydrolyze β-lactam antibiotics containing Penicillins, Aztreonam, as well as the Cephalosporins [7]. They are however inhibited by Clavulanic acid and Tazobactam. ESBLs are a group of enzymes encoded by genes of *Enterobacteriaceae.* There are currently hundreds of ESBL variants, and these have been clustered into nine different structural and evolutionary families based on amino acid sequence.

2. MATERIAL AND METHODS

2.1 Collection of Samples

A total of 30 different snack samples consisting of eggroll, meat pie, and doughnut were purchased from different street food vendors in Umuahia, Abia State, Nigeria. The samples were aseptically collected in sterile polythene bags, and transferred immediately to the microbiology laboratory of Michael Okpara University of Agriculture, Umudike for further analysis.

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2.2 Isolation and purification of bacteria from snacks samples

The snacks samples were mashed in a sterile laboratory type mortar into a paste. Ten percent of the stock solution was prepared by weighing 10g into 100ml of sterile normal saline. The solution was properly shaken and sieved before a ten-fold serial dilution was performed. Using the 10^{-6} dilution, 0.1 ml of the samples was inoculated onto sterile nutrient agar media for total aerobic bacteria count and MacConkey media for coliform count using the spread plate method. All plates were incubated at $37^{o}C$ for 24 hours [8]. For pure colonies, distinct colonies were sub-cultured onto nutrient agar slants and stored in a refrigerator at 4^{o}C.

2.3 Identification of bacteria

The isolated bacteria were identified based on cellular morphology, Gram's staining and biochemical tests [9]. The isolated bacteria was confirmed as using 16S rRNA method.

2.4 Antimicrobial susceptibility testing and ESBLs detection

The antimicrobial susceptibility testing of all identified isolates was done using the Kirby Bauer disk diffusion method on Mueller-Hinton agar according to the guidelines of Clinical and Laboratory Standards Institute [10]. 0.5 McFarland standard isolates were inoculated with sterile cotton swab onto Mueller Hinton agar to make a lawn of bacterial growth after which antibiotic discs were applied. The plates were incubated overnight at 37°C and zones of inhibition measured. The following standard antibiotic discs for the isolates were used; Ceftazidime (30 µg), Ofloxacin (05 µg), Gentamycin (10 µg), Nitrofurantoin (300 µg), Ampicillin (10 µg), Ciprofloxacin(05 µg), Cefotaxime (30 µg), Ceftriaxone (30 µg), Imipenem (10 µg) and Nalidixic Acid(30 µg).

2.4.1 Phenotypic Identification of Esbl Producing Strains (DDST)

Detection of ESBL-producing organisms was performed by Double Disc Synergy Test (DDST) method following CLSI recommendations. A suspension was prepared for each pure bacterial isolate according to the 0.5 McFarland turbidity standard and cultured on Mueller–Hinton agar. After inoculation, antibiotic disks containing Cefazidime (30 µg) with Cefazidime/Clavulanic acid (30/10 µg), and Cefotaxime (30 µg) with Cefotaxime/ Clavulanic acid (30/10 µg) were placed on Mueller–Hinton agar medium at a distance of 20 mm apart from each other. The plates were incubated for 24 h at 37 °C after which the diameter of inhibition zone was measured. According to CLSI guidelines, an increase of ≥5 mm in the zone diameter around the clavulanic acid combination disks versus the same disks alone confrmed the presence of ESBLs producing strains [11].

3. RESULTS AND DISCUSSION

3.1 Identification of Isolates

Morphological and physiological characteristics of the bacterial isolates were investigated according to the method described by [9]. They were identified as *Escherichia coli, Klebsiella pneumonia*, *Salmonella typhi* and *Staphylococcus aureus*. Taxanomic identification using 16S rRNA method was used to confirm the isolates.

3.2 Occurrence of Isolates

E.coli isolates were the most prevalent bacteria isolated from the snacks samples (42%). This was followed by *Klesbsiella pneumoniae,* (24%), *Salmonella typhi* (20%) and *Staphylococcus aureus* (14%). This is shown in figure 1

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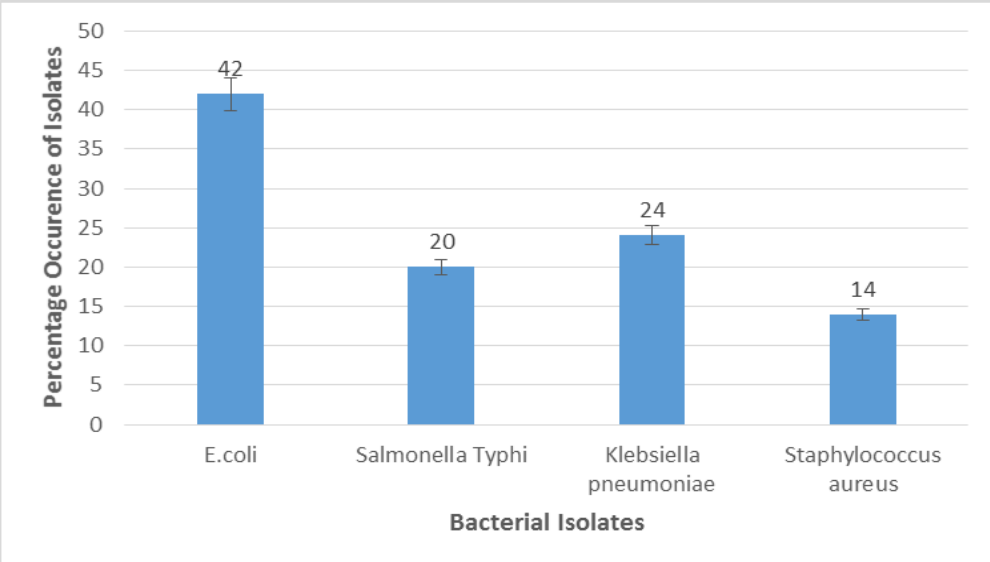

Figure 1. Percentage Occurrence of Bacterial Isolates

3.3 Antibiogram of Bacteria Isolates

Escherichia coli isolates exhibited highest resistance to ceftazidime (76%). High resistance was also exhibited towards cefotaxime, ceftriaxone, gentamycin and ciprofloxacin. Most isolates (60%) exhibited susceptibility to ampicillin, ofloxacin and nitrofurantoin. There was significant difference in the sensitivity of isolates to the different antibiotics (*P*<0.05). This is shown in Table 1.

Table 1. Antibiogram of *E.coli* Isolates

Antibiotic	% Susceptible	% Intermediate	% Resistant
Ceftazidime	24	0	76
Cefotaxime	40	0	60
Ciprofloxacin	35	10	55
Ceftriaxone	40	0	60
Gentamycin	55	0	45
Imipenem	35	15	50
Nitrofurantoin	60	10	30
Ampicillin	60	20	20
Nalidixic Acid	40	20	40
Ofloxacin	60	0	40

Salmonella typhi isolates exhibited highest resistance to ciprofloxacin (86%) followed by ceftazidime (80%). Most *Salmonella typhi* isolates were susceptible to ceftriaxone (70%). They also exhibited high susceptibility to ampicillin, cefotaxime and ofloxacin (60%). There

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was significant difference in the sensitivity of the isolates to the different antibiotics ($P<0.05$). This is shown in Table 2.

Table 2. Antibiogram of *Salmonella typhi* Isolates

Antibiotic	% Susceptible	% Intermediate	% Resistant
Ceftazidime	20	0	80
Cefotaxime	60	20	20
Ciprofloxacin	14	0	86
Ceftriaxone	70	0	30
Gentamycin	30	20	50
Imipenem	30	10	60
Nitrofurantoin	50	20	30
Ampicillin	60	10	30
Nalidixic Acid	50	0	50
Ofloxacin	60	10	30

Klebsiella pneumoniae isolates exhibited most resistance to ciprofloxacin (70%) followed by gentamycin with 60% resistance. Most of the isolates showed sensitivity to cefotaxime (80%) followed by ampicillin (70%). They however exhibited high resistance to ciprofloxacin (70%) and gentamycin (60%). There was statistical difference in the response of the isolates to the different antibiotics. This is displayed in Table 3.

Table 3. Antibiogram of *Klebsiella pneumoniae*

Antibiotic	% Susceptible	% Intermediate	% Resistant
Ceftazidime	50	0	50
Cefotaxime	80	10	10
Ciprofloxacin	30	0	70
Ceftriaxone	55	15	30
Gentamycin	40	0	60
Imipenem	30	30	40
Nitrofurantoin	40	40	20
Ampicillin	70	15	25
Nalidixic Acid	30	20	50
Ofloxacin	50	30	20

There was significant difference in the response of *Staphylococcus aureus* isolates to different antibiotics ($P<0.05$). Most *Staphylococcus aureus* isolates were resistant to ampicillin (84%) and ceftriaxone (70%). Most of the isolates were susceptible to cefotaxime (60%). This is shown in Table 4.

Table 4. Antibiogram of *Staphylococcus aureus* isolates

Antibiotic	% Susceptible	% Intermediate	% Resistant
Ceftazidime	40	0	60
Cefotaxime	60	10	30
Ciprofloxacin	40	20	40
Ceftriaxone	30	0	70
Gentamycin	30	20	50

Imipenem	16	0	50
Nitrofurantoin	50	20	30
Ampicillin	50	0	84
Nalidixic Acid	40	10	50
Ofloxacin	50	10	40

3.4 Comparative Antibiotic Resistance Pattern of Isolates

The four different bacterial species exhibited different resistance patterns to different antibiotics. This is displayed in figure 2. *Salmonella typhi* isolates exhibited highest resistance to an antibiotic with 86% resistance to ciprofloxacin in comparison to the other bacterial species. This was followed by *Staphylococcus aureus with* 84% resistance to ampicillin. Only 10% of *Klebsiella pneumoniae* isolates were resistant to cefotaxime, representing the lowest resistance of the isolates to the antibiotics used. *Salmonella typhi* isolates also exhibited only 20% resistance to ampicillin similar to *Staphylococcus aureus* with 20% to ofloxacin. There was no significant difference in the comparative resistance pattern of the four different bacterial isolates to the different antibiotics tested at *P*=0.05.

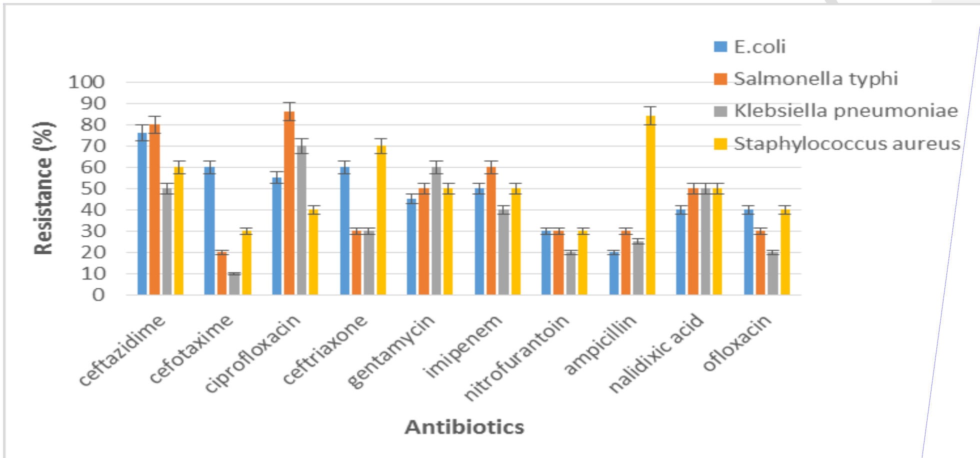

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Figure 2. Comparative resistance pattern of isolates

3.5 Occurrence of ESBL Producers among Gram Negative Isolates

Gram negative bacterial isolates which showed resistance to two of ceftazidime, ceftriaxone and cefotaxime were confirmed for ESBL production using double disc synergy test (DDST) method following CLSI recommendations. A higher percentage of *E.coli* isolates suspected to be ESBL producers were confirmed to be ESBL producers (36%) while *Salmonella typhi* isolates had the least percentage of confirmed ESBL producers (17%).

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Table 5. Percentage occurrence of ESBL producers among Gram negative bacteria

Isolate	ESBL Producers (%)	Non-ESBL Producers (%)
Escherichia coli	36	64
Salmonella typhi	17	83
Klebsiella pneumonia	24	76

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3.6 Discussion

Globalization has made ready to eat foods such as eggroll, meat pie and doughnut popular amongst the urban population in Nigeria. The presence of pathogenic microbes in such foods is a major cause of foodborne diseases. In this study, *Escherichia coli, Salmonella typhi, Klebsiella pneumoniae* and *Staphylococcus aureus* were the bacteria isolated from street vended snacks. *E.coli* was the most prevalent isolate. The presence of *E.coli* and *Salmonella typhi* was similar to findings by [8] and [12]. The presence of enterobacteriaceae such as *E.coli*, *Salmonella typhi* and *Klebsiella pneumoniae* in the samples indicates a lack of good personal hygiene amongst the vendors and poor environmental sanitation. It can also be attributed to the use of contaminated water in food preparation and washing of utensils. The presence of *Staphylococcus aureus* isolates was similar to findings by [13] and could be attributed to contamination from the hands of the vendors and from cooking utensils.

Staphylococcus aureus exhibited high resistance to ampicillin similar to findings by [14]. *E.coli* exhibited relative high resistance to ciprofloxacin which was similar to findings by [15]. *Salmonella typhi* exhibited high resistance to ciprofloxacin similar to findings on *Salmonella* resistance to fluoroquinolones such as ciprofloxacin by [16]. Like other enterobacteriaceae isolated, *Klebsiella pneumoniae* also exhibited high resistance to ciprofloxacin similar to findings by [17]. *E.coli* and *Salmonella typhi* also exhibited high resistance to ceftazidime, indicating the likely presence of ESBL producers amongst the isolates. Among the ESBL producers, the highest rate was observed in *E.coli* followed by *Klebsiella pneumoniae* and was similar to findings by [6]. The least ESBL producers were found among *Salmonella typhi* isolates.

The high resistance shown by the microbes to antibiotics such as ciprofloxacin and ampicillin could be attributed to their abuse of in the Nigerian society. This indiscriminate use of antibiotics has led to increased resistance by pathogenic microorganisms.

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4. CONCLUSION

The study indicated a significant presence of bacteria in snacks sold in Umuahia, Nigeria. Most of the isolates were gram-negative bacteria with *E.coli* the most prevalent. The presence of coliforms in the samples indicates the poor hygienic standard maintained by handlers both at the preparation stage and at points of sale. Equally disturbing is the high resistance exhibited by the isolates to antibiotics and the presence of ESBL producing bacteria. Food safety regulations and policies should be strengthened and enforced to reduce incidences of contamination. Awareness should be created to reduce abuse of antimicrobials. There should be synergy between regulatory bodies to control the spread of pathogens and to combat antibiotic resistance.

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