Nig. J. Biotech. Vol. 42(1): 127-133 (June 2025)
ISSN: 0189 1731
Available online at
http://www.ajol.info/index.php/njb/index
and https://bsn.org.ng
DOI: **10.5281/zenodo.18621941**

NIGERIAN JOURNAL OF BIOTECHNOLOGY

Multi-Drug Resistance and Extended-Spectrum Beta-Lactamase Producing Gram-Negative Bacteria from Pig Meat in the Southern Senatorial Zone of Ebonyi State, Nigeria

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Abstract

The emergence of antibiotic-resistant bacteria poses a significant global health threat by undermining the efficacy of antimicrobial drugs and increasing the risk of treatment failure in common infections. This study aimed to determine the prevalence of multidrug-resistant (MDR) and extended-spectrum beta-lactamase (ESBL)-producing Gram-negative bacteria in pig meat obtained from Ebonyi South Senatorial Zone. Samples were systematically collected from five local government areas (Afikpo, Edda, Ohaozara, Onicha, and Ivo), with four samples obtained from each location. Gram-negative bacteria were isolated, subjected to antimicrobial susceptibility testing, and screened for ESBL production using standard methods. The results showed that out of the bacterial isolates identified, Escherichia coli (n = 13) exhibited MDR in 9 (32.1%) cases and ESBL production in 10 (35.7%). Similarly, *Salmonella spp.* (n = 14) had 10 isolates (35.7%) displaying MDR and ESBL traits. *Klebsiella spp*. was isolated once (n = 1), and this isolate was both MDR and an ESBL producer, contributing 3.6% to the total resistant isolates in each category. Overall, 28.5% of *E. coli* isolates, 32.1% of *Salmonella spp.*, and a single *Klebsiella spp*. isolate were identified as MDR ESBL producers. The findings underscore significant contamination of pig meat in the study area with MDR and ESBL-producing Gram-negative bacteria. These resistant pathogens present a serious public health risk due to their potential transmission to humans via consumption or improper handling of contaminated meat.

Keywords: Multidrug resistance (MDR), Extended-spectrum beta-lactamase (ESBL), Gram-negative bacteria, Pig meat contamination, Ebonyi South senatorial Zone

Introduction

The emergence of antibiotic-resistant bacteria has become a significant global health challenge, threatening the efficacy of antibiotic treatments and elevating the risks associated with common infections. Multi-drug resistance (MDR) in bacteria, defined as resistance to three or more classes of antibiotics, complicates infection management, leading to higher morbidity, mortality, and healthcare costs. The situation is particularly concerning for Gram-negative bacteria, which possess structural and genetic mechanisms that allow them to resist multiple antibiotics, making them difficult to treat with standard antimicrobial therapies (Agyepong et al., 2022; Tacconelli et al., 2018). Among these mechanisms, the production of extended-spectrum beta-lactamases (ESBLs) is especially problematic, as ESBLs can hydrolyze a broad spectrum of beta-lactam antibiotics, including penicillin and cephalosporins. These enzymes contribute significantly to the global rise in antibiotic resistance and pose serious implications for public health (Bush and Bradford, 2020).

In recent years, MDR and ESBL-producing bacteria in food sources have raised concerns regarding the foodborne transmission of antibiotic resistance. Animal-derived food products, particularly meat, are recognized as reservoirs for antibiotic-resistant bacteria due to the widespread use of antibiotics in livestock production (Manyi-Loh et al., 2018). Antibiotics are often administered to livestock, including pigs, to promote growth and prevent disease in crowded or unsanitary conditions. However, this practice facilitates the selection and proliferation of resistant bacterial strains within the animal gut microbiome. These resistant strains can then contaminate meat during slaughter and processing, potentially reaching consumers and contributing to the spread of resistant infections in humans (Van Boeckel et al., 2019). Studies have demonstrated that contact with or consumption of contaminated meat, especially when it is undercooked or improperly handled, serves as a direct pathway for MDR bacteria to enter the human population (Schmidt et al., 2021).

Extended-spectrum beta-lactamase-producing bacteria, in particular, have been identified with alarming frequency in food-producing animals. These bacteria produce enzymes that inactivate a wide array of antibiotics, rendering many commonly used treatments ineffective (Bush and Bradford, 2020). The global prevalence of ESBL-producing bacteria has prompted investigations into their occurrence in different types of meat, with studies reporting notable levels of contamination in poultry, beef, and pork (Lynch et al., 2021). Pork, as a major protein source worldwide, has increasingly been studied for bacterial contamination, given that pigs are frequently exposed to antibiotics in both intensive and small-scale farming systems (Kagambega et al., 2018).

Focusing specifically on the Southern Senatorial Zone of Ebonyi State in Nigeria, the presence of MDR and ESBL-producing Gram-negative bacteria in pig meat holds particular relevance. Agriculture, including pig farming, plays a critical role in this region's economy and food supply, but practices such as the use of antibiotics in animal husbandry remain under-regulated. Consequently, this area may represent a unique and understudied environment in which antibiotic resistance can develop and spread (Eze et al., 2020). Previous studies in other regions of Nigeria have reported high levels of MDR pathogens in pig meat, highlighting the need for focused research on antibiotic resistance in this region's food supply to assess and mitigate potential public health risks (Nworie et al., 2020). As there is limited data on the prevalence of ESBL-producing bacteria in pig meat, specifically within Ebonyi State, investigating the presence and spread of these pathogens is critical to understanding local public health risks and informing strategies to control antibiotic resistance.

The aim of this study was to investigate the prevalence and antibiotic resistance patterns of multi-drug resistant (MDR) and extended spectrum beta-lactamase (ESBL)-producing Gram-negative bacteria in pig meat from the Southern Senatorial Zone of Ebonyi State, Nigeria. Given the potential for antibiotic-resistant infections to spread across regions through trade and human movement, understanding and controlling the sources of these bacteria within local food supplies is essential for protecting public health both locally and beyond (Manyi-Loh et al., 2018; Van Boeckel et al., 2019).

Materials and Methods

Materials

Media and Antibiotics

Nutrient Agar (Himedia Laboratories, India), Nutrient Broth (Himedia Laboratories, India), MacConkey Agar (Himedia Laboratories, India), Amoxicillin Clavulanic Acid (GlaxoSmithKline, UK), Cefotaxime (GlaxoSmithKline, UK), Ceftazidime (GlaxoSmithKline, UK), Ciprofloxacin (GlaxoSmithKline, UK), Gentamicin (GlaxoSmithKline, UK), *Salmonella* Shigella Agar (Himedia, India), Simmons Citrate (BD Biosciences, USA), Muller Hinton Agar (Oxoid, UK). METHODS

Sample Collection

Samples were collected from one major market in five different local government areas (LGAs) in Ebonyi State: Afikpo, Edda, Ohaozara, Onicha, and Ivo, to conduct the study. Four distinct samples were obtained at each location following a consistent sampling protocol. First, pig meats were purchased directly from the markets. Next, environmental samples were collected using sterile swab sticks. Specifically, the surfaces of the knife used by the vendor, the table where the meat was processed, and the floor where the pork was occasionally placed were swabbed. This sample collection procedure was repeated across all five LGAs to ensure consistency and allow for comparative analysis.

Isolation of Gram-Negative Bacteria

Selective media played a vital role in this process. This study primarily used MacConkey agar and Xylose Lysine Deoxycholate (XLD) agar as selective media for isolating Gram-negative bacteria. Colonies suspected to be Gram-negative bacteria were characterized using biochemical tests, including indole, methyl red, Voges-Proskauer, and citrate utilization tests.

Antibiotic Susceptibility Testing

Following the isolation of Gram-negative bacteria, antibiotic susceptibility testing was performed to assess the resistance profiles of the isolates. The Kirby- Bauer disk diffusion method was utilized to determine the susceptibility of bacterial isolates to various antibiotics (Hudzicki, 2009). In this method, standardized inoculum suspensions of the isolated bacteria were spread onto Mueller-Hinton agar using a sterile swab to create a uniform lawn of bacterial growth. After the surface of the agar had dried slightly, antibiotic-impregnated discs were aseptically placed on the inoculated plates using sterile forceps. The plates were then incubated for 24 hours at room temperature. Following incubation, the plates were examined for zones of inhibition surrounding the antibiotic discs. The diameters of these zones were measured in millimeters using a ruler, and the results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. The isolates were then classified as susceptible, intermediate, or resistant to each antibiotic tested.

ESBL Detection

The double-disk synergy test (DDST) is a phenotypic method used to detect extended-spectrum β-lactamase (ESBL) production in bacteria. In this test, antibiotic disks containing third-generation cephalosporins (e.g., cefotaxime, ceftazidime) are placed on a Mueller-Hinton agar plate inoculated with the test organism, at a fixed distance from a disk containing clavulanic acid (a β-lactamase inhibitor). If the bacteria produce ESBL, the inhibition zone around the cephalosporin disk expands toward the clavulanic acid disk, forming a

"keyhole" or enhanced zone of inhibition indicating synergy between the two agents and confirming ESBL production (Drieux, 2008).

Data Analysis

Descriptive statistics were employed to summarize the data based on bacterial pathogens contaminating the meat samples, multidrug resistance (MDR) prevalence, and Extended-Spectrum Beta- Lactamase (ESBL) indices. The results were presented as percentages, and bar charts were plotted using OriginLab.

Results and Discussion

The study "Multi-Drug Resistance and Extended Spectrum Beta-Lactamase Producing Gram-Negative Bacteria from Pig Meat in the Southern Senatorial Zone of Ebonyi State" involved identifying bacterial colonies in various pig meat samples, analyzing their biochemical characteristics and examining their resistance patterns, particularly regarding multidrug resistance (MDR) and Extended Spectrum Beta-Lactamase (ESBL) production.

Colony Enumeration

Table 1 presents the colony enumeration of bacterial isolates. This table provides data on the bacterial load found in different pig meat samples across various locations within the Southern Senatorial Zone of Ebonyi State. The results reveal a noticeable difference in colony-forming units (CFU) per gram across locations. For instance, meat samples from Onicha had higher CFU counts (4.8 x 10^6 CFU/g) compared to samples from Edda (2.7 x 10^1 CFU/g) and Ivo (1.2 x 10^1 CFU/g). On the other hand, Afikpo North samples showed moderate CFU counts (1.5 × 10^3 CFU/g for meat samples and 2.2 × 10^3 CFU/g for dropped meat), indicating potential bacterial contamination.

Table 1: Colony enumeration of the bacterial isolates in the different meat samples and locations

S/N	Sample Source	Location	No. of Colonies	CFU/g
1	Meat sample	Afikpo North	75	1.5 x 10^3
2	Dropped meat	Afikpo North	108	2.2 x 10^3
3	Meat sample	Onicha	24	4.8 x 10^6
4	Dropped meat	Onicha	250	5.0 x 10^5
5	Meat sample	Uburu	160	3.2 x 10^5
6	Dropped meat	Uburu	224	4.5 x 10^5
7	Meat sample	Edda	136	2.7 x 10^1
8	Dropped meat	Edda	191	3.8 x 10^1
9	Meat sample	Ivo	62	1.2 x 10^1
10	Dropped meat	Ivo	71	1.4 x 10^1

Frequency of Multidrug Resistant (MDR) and Extended Spectrum Beta-Lactamase (ESBL) producing bacteria

Global public health as well as the efficient prevention and treatment of illnesses have become concerns due to the rise in antimicrobial resistance (Nagvekar et al., 2020; Ejikeugwu et al., 2017). Over the past 20 years, infections caused by these pathogens have become considerably more difficult to manage, especially in poor nations, and are linked to high rates of morbidity and mortality as well as prolonged hospital stays (Agyepong et al., 2018). Figure 1 illustrates the prevalence of multidrug-resistant (MDR) and extended-spectrum beta-lactamase (ESBL) among the Gram-negative isolates from pig meat obtained from the Ebonyi South senatorial zone. The observed prevalence of multidrug- resistant (MDR) and extended- spectrum beta- lactamase (ESBL)-producing bacteria among E. coli, *Salmonella spp.*, and *Klebsiella spp*. isolated from pig meat in the Ebonyi South Senatorial Zone raises significant public health concerns. *Escherichia coli* and *Salmonella spp*. demonstrated high levels of MDR (32.1% and 35.7%, respectively), along with a similarly high proportion of ESBL producers (35.7% each). These findings are consistent with earlier reports suggesting that the gastrointestinal tracts of food-producing animals serve as reservoirs for antimicrobial-resistant pathogens (Tadesse et al., 2012; Nhung et al., 2016). These bacteria pose a substantial risk due to their ability to hydrolyze a broad spectrum of beta-lactam antibiotics. The co-occurrence of MDR and ESBL traits, as seen in most *E. coli* and *Salmonella* isolates in this study, may be due to the presence of complex resistance mechanisms and mobile genetic elements such as plasmids,

integrons, and transposons that facilitate the rapid spread of resistance genes. The detection of a single *Klebsiella* isolate that was both MDR and ESBL-producing, although numerically low (3.6%), is still of clinical concern. The findings of this study

husbandry in the study area. Resistant strains may be transmitted to humans through direct contact with animals or through the consumption of contaminated meat products (Ma et al., 2021).

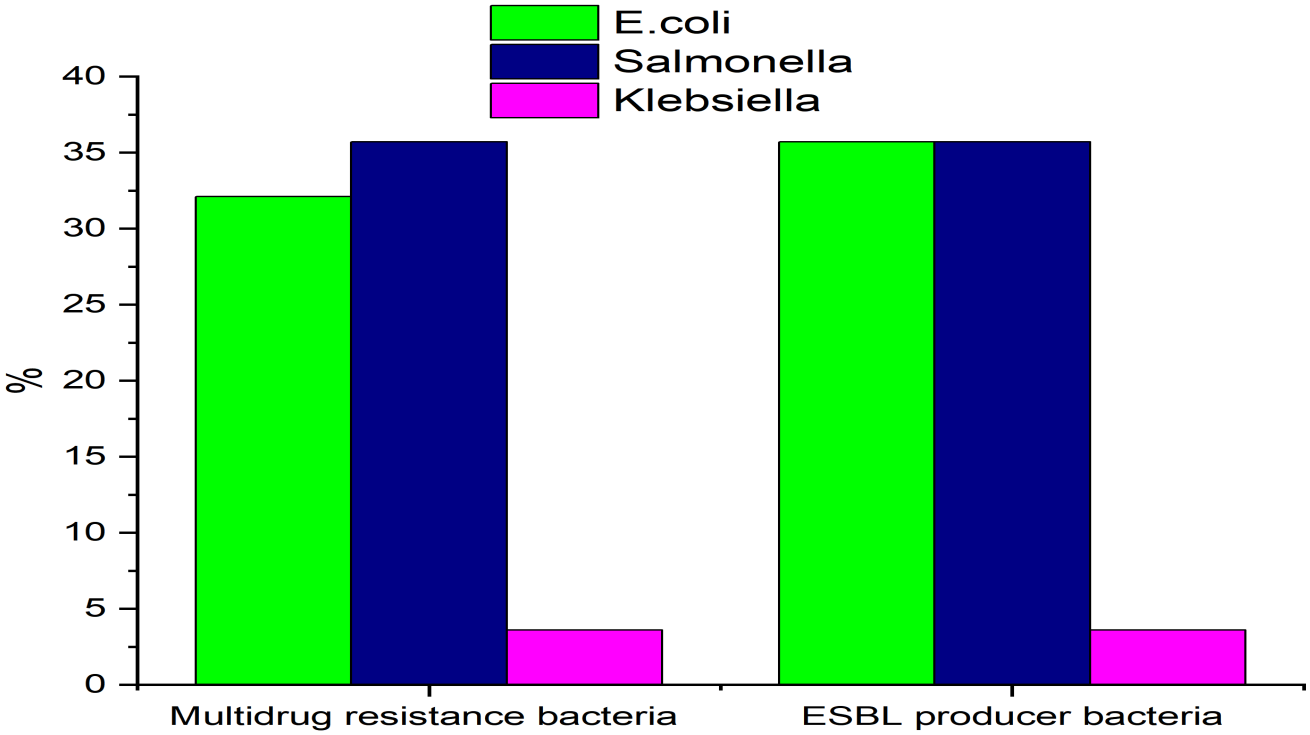

Fig 1: Frequency of multidrug-resistant (MDR) and extended-spectrum beta-lactamase

Frequency of Multidrug-Resistant Bacteria Producing Extended-Spectrum Beta-Lactamase (ESBL)

Figure 2 shows the frequency of multidrug-resistant bacteria producing extended-spectrum beta-lactamase (ESBL). The findings of this study reveal a notable presence of multidrug-resistant (MDR) extended-spectrum beta-lactamase (ESBL)-producing Gram-negative bacteria in pig meat samples collected from the Ebonyi South Senatorial Zone. Specifically, 28.5% of Escherichia coli isolates, 32.1% of *Salmonella spp.*, and one *Klebsiella spp*. isolate (3.6% of the total) were multidrug-resistant bacteria producing extended-spectrum beta-lactamase. The overall detection of multidrug-resistant bacteria producing extended-spectrum beta-lactamase suggests a significant selective pressure likely caused by improper antibiotic use in livestock farming. These results show the need for stringent antimicrobial use in veterinary practice and improved surveillance systems to monitor resistance trends in the food production chain.

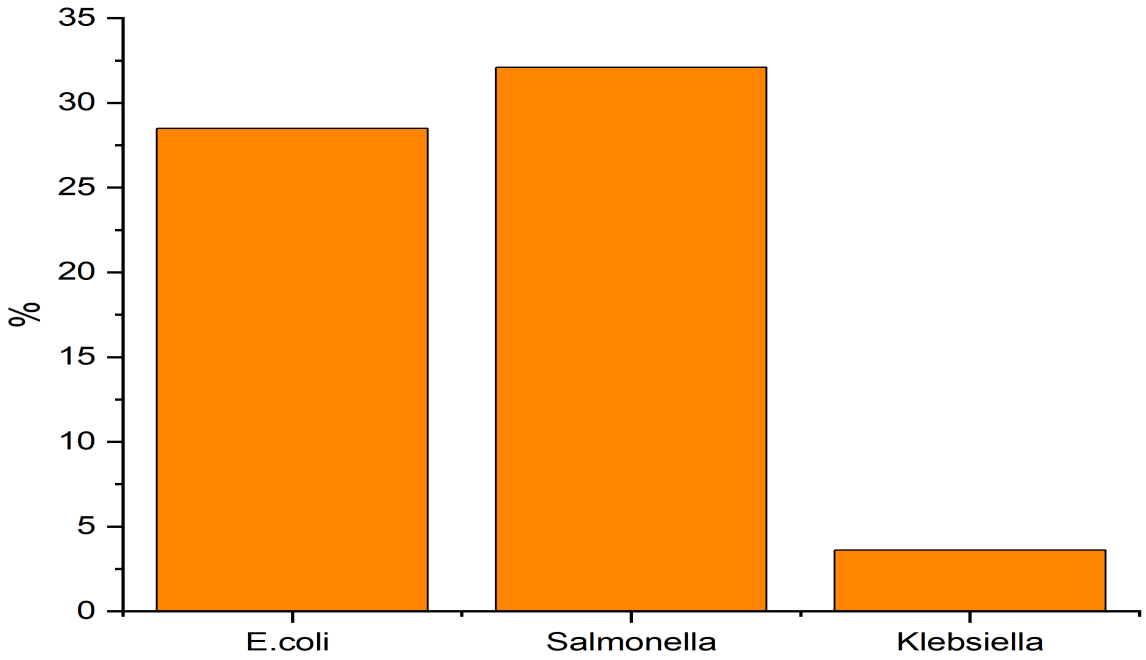

Figure 2: Frequency of multidrug-resistant bacteria producing Extended-Spectrum Beta-Lactamase (ESBL)

Conclusion

This study concludes that pig meat in the Southern Senatorial Zone of Ebonyi State is contaminated with multi-drug-resistant and ESBL-producing Gram-negative bacteria. These resistant bacteria pose a serious public health threat, as they can be transmitted to humans through consumption of contaminated meat or inadequate handling during slaughtering and processing.

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