



## Prevalence of Carbapenem Resistant Enterobacteriaceae across One Health Interface from selected Abattoirs in Abeokuta, Ogun State

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### Abstract

Carbapenem Resistance Enterobacteriaceae (CRE) are commonly detected infectious bacteria in livestock. These pathogens have emerged a global health concern due to their vital roles as vectors for the transfer of resistance genes. This study investigated the prevalence of CRE from selected abattoirs across one health interface. Fifty (50) samples which include; cow beef, soil, water and hand swabs were purposively collected from three major slaughterhouses in Abeokuta Ogun State and assessed phenotypically for CRE after cultural characterization, biochemical and antibiotic susceptibility test. Isolates were confirmed for carbapenemase production using Modified Hodges test (MHT). Four members of Enterobacteriaceae were identified from 56 isolates namely; *Escherichia coli* (10.7 %), *Klebsiella* spp. (59 %), *Proteus* spp. (19.6 %) and *Enterobacter* spp. (10.7 %). Antibiotic susceptibility test showed that all Bacteria except *Enterobacter* spp. showed resistance with highest prevalence of 59 % from *Klebsiella* spp. Further screening showed that 33 out of 46 MDR isolates were carbapenem resistant but confirmatory analysis with MHT proved all CRE negative for carbapenemase production. The study revealed that CRE was significantly high in samples from the abattoir with cattle beef having the highest proportion of Enterobacteriaceae.

**Keywords:** Carbapenem Resistance Enterobacteriaceae (CRE), Abattoirs, Enterobacteriaceae, One health approach, Multidrug-resistance, Modified Hodges test (MHT).

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### Introduction

Enterobacteriaceae, one of the largest family of gram-negative Bacteria have changed over time due to high target mechanism in resisting antimicrobial agents. Thus making present antibiotic lose their efficiency especially carbapenem, a last-resort antibiotic (Malley, 2000).

This diverse collection of bacteria is extensively distributed in various settings, including water, soil, and plants, in addition to being a regular component of the human flora and other animals (Odeyemi, 2012). In a public health fight against Bacterial infections, Carbapenem Resistance

Enterobacteriaceae (CRE)-caused infections, have appeared to be a global health concern (Barisics *et al.*, 2017) and the spread of carbapenem-resistant Bacteria jeopardizes the efficiency to these antibiotics (Kohlenberg *et al.*, 2010). In other to combat this threat, medical personnel turned to carbapenems and some other broad-spectrum antibiotics, which has contributed to selection-driven resistance accelerated by the development of antibiotic resistant genes (D'Angelo *et al.*, 2016). The main mechanism by which resistance in Enterobacteriaceae are expressed is the production of

carbapenemase enzyme, by varying species via the transfer of mobile genetic elements.

An abattoir is where animals are slaughtered for consumable meat production. As important as the activity and its operations are in providing a needed source of protein for humans, the way it is handled, and its by-products or wastes can pose a health risk if proper precautions are not met (Weobong and Adinyira, 2011). Without adequate sanitary control, the environment for ablation could significantly be a source of meat contamination by these Bacteria (Bersisa *et al.*, 2019). The causes of food-borne infections in most underdeveloped nations include poor environmental sanitations and unhygienic practices, lack of adequate food safety regulations, poor regulatory structures, insufficient financial resources to invest in more secure food manufacturing equipment, as well as a lack of hygiene orientation by food handler (Oluwafemi *et al.*, 2013).

Studies conducted across Nigeria have revealed that most meat is of poor grade (Chuku *et al.*, 2016). Most of the time, animal health before butchering and processing, in addition to environmental hygiene, processing, and marketing, significantly impacts the microbiological state of meat.

As a result of abattoir operations, such as animal slaughter and butchery on floors contaminated with blood, splatters and feces (Nwanta *et al.*, 2010). Wheelbarrows, meat conveyors, slaughterhouses, wooden tables, hands, weighing scales, and water containers such as metal buckets and plastic containers could be potential sources of Meat contamination (Faleke *et al.*, 2017). Therefore, evaluating the total Bacterial load in these food products can aid in assessing the risk of Bacterial infection to man's health (Bersisa *et al.*, 2019).

To prevent meat contamination in slaughterhouses, quality hygiene practices must be curtailed by handlers. A good indicator for proper meat hygiene is by employing microbiological quality assay which can minimize rate of contamination. Without sufficient sanitary practices, slaughterhouses could be a major avenue for microbial accumulation which can in turn

result to meat contamination. This research aimed to determine the Prevalence of Carbapenem

Resistance Enterobacteriaceae in Abattoirs across one health interface.

### Materials and Methods

#### Area of study

The study location were abattoirs from community markets at Eleweran Abeokuta, Ogun State, South-West Nigeria. The location has a population of 449,088 according to the population census of 2006 and a density of 510 persons per square kilometer. The city is situated at longitude 709'39N and 3020'54E. Ogun State as a whole is made up of 20 Local Government Areas.

#### Sample Collection

A cross sectional study was conducted at three selected abattoirs from study location. A total of 50 samples (10grams of cattle beef-20, water-10, soil- 10, hand swabs-10) were randomly collected. All samples were transported, under controlled conditions using transport medium to the Microbiology Laboratory of the Federal University of Agriculture Abeokuta for further analysis.

#### Isolation and Identification of Bacteria

The collected Samples were cultured aseptically on MacConkey agar plates which allows the growth of gram-negative Bacteria and incubated aerobically at 37°C for 24 hours. Phenotypic characterization and identification of isolates were based on colony morphology, cultural appearance and series of biochemical tests (Cheesebrough, 2006).

#### Antibiotic Susceptibility Test

The sensitivity of the organisms to conventional antibiotics was determined using the Kirby Bauer's disk diffusion method and in accordance to clinical and laboratory institute (CLSI) Guidelines, 2021. Muller Hinton agar (MHA) was prepared according to manufacturer's specification. Few colonies were resuscitated in a broth medium and inoculated via streaking throughout the surface of MHA plate. Sterile forceps were used to pick the gram-negative antibiotics

disc namely; Chloramphenicol (CH), Septrin (SXT), Amoxicillin (AM), Augmentin (AU), Tarivid (OFX), Gentamycin (CN), Sporfloxacin (SP), Ciprofloxacin (CPX), Pefloxacin (PEF), Streptomycin (S). They were aseptically impregnated on the agar plate and incubated at 37°C for 24 hours. Zones of inhibition were measured (mm) and classified as susceptible or resistance following the CLSI standard and guidelines.

#### Carbapenem Resistance Enterobacteriaceae Test

Multi drug resistant isolates were subjected to carbapenem using imipenem. Disc was placed at the center of the inoculated MHA plate using Kirby Bauer's disk diffusion method. Diameter of inhibition zones  $\leq 19$  were measured as resistant based on CLSI breakpoints for imipenem against gram negative Bacteria. Imipenem resistant Bacteria were further tested for carbapenemase production using Modified Hodges Test (MHT) as recommended by CLSI (2021).

#### Results

Out of fifty (50) samples, fifty-six (56) isolates were observed from cultured plates, and four (4) members of enteric Bacteria

were identified, as shown in **Table 1**. The *Enterobacteriaceae* species detected were *Escherichia coli* (10.7 %) and *Klebsiella spp.* (59 %), followed by *Proteus spp.* (19.6 %) and *Enterobacter spp.* (10.7%). High prevalence was recorded for *Klebsiella spp.* from all samples isolated. **Figure 1** depicts the results of antibiotic resistance pattern of the different isolates of Enteric Bacteria. All the organisms were resistant to all antibiotics used except *Enterobacter spp.* which showed susceptibility to almost all antibiotics.

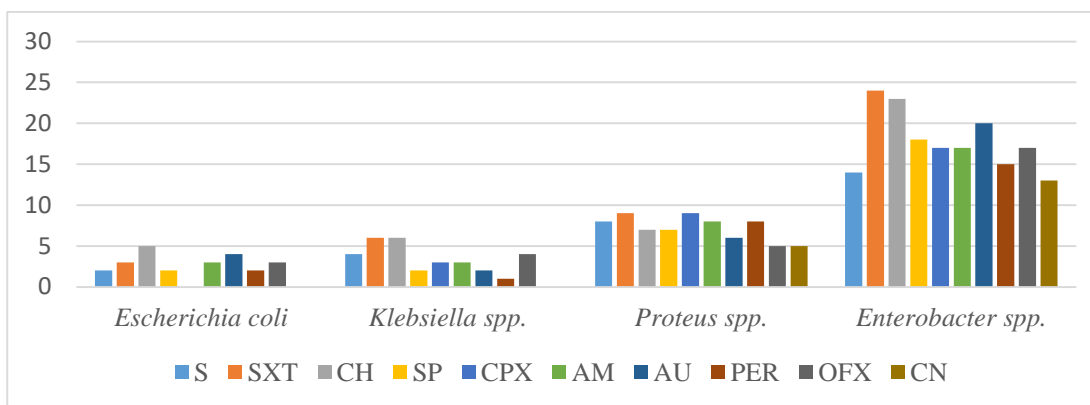
**Figure 2** shows the resistance pattern of Enteric pathogens to carbapenem using imipenem disc. *Klebsiella spp.* were the most resistant isolates to imipenem with highest recorded prevalence of 59 %, subsequently *Proteus spp.* (24 %) and *Escherichia coli* (15 %) but lowest prevalence was observed in *Enterobacter spp.* due to the high proportion of antibiotics susceptibility.

Out of forty-six (46) Multi drug resistant (MDR) isolates subjected to carbapenem using imipenem disk, thirty-three were resistant with zero percentage recorded for carbapenemase production as confirmed with Modified Hodges Test (MHT) for all 33 isolates (**Table 2**).

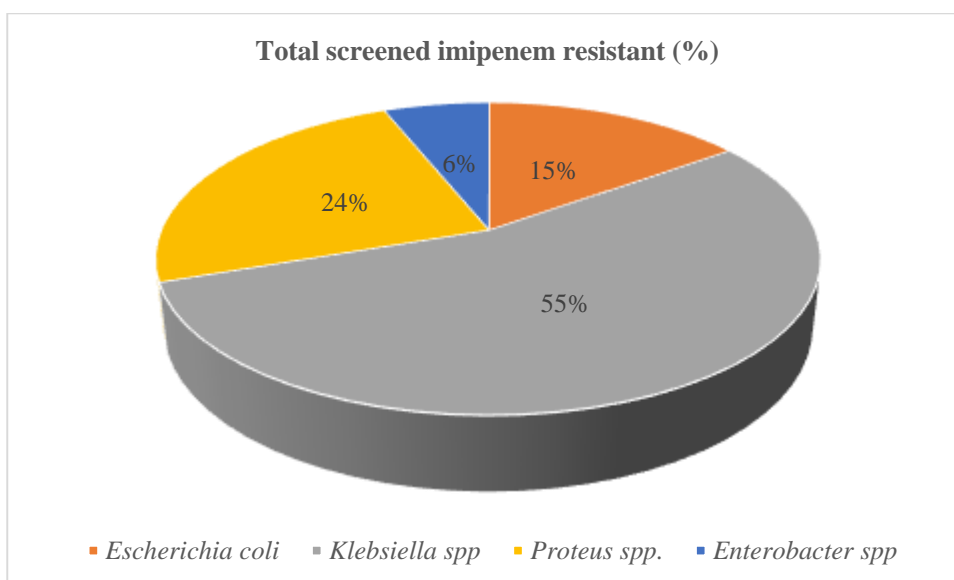
**Table1:** Frequency of Enteric Bacteria from samples in selected slaughterhouses across one health interface.

Total number of isolates (Percentage) from samples					
Enteric Bacteria	Isolates n (%)	Cow beef n (%)	Soil n (%)	Water n (%)	Hand swab n (%)
<i>Escherichia coli</i>	6 (10.7)	1 (16.67)	2 (33.33)	1 (16.67)	2 (33.33)
<i>Klebsiella spp.</i>	33 (59)	18 (54.55)	7 (21.21)	7 (21.21)	1(3.03)
<i>Proteus spp.</i>	11 (19.6)	4 (36.36)	2 (18.18)	3 (27.27)	2(18.18)
<i>Enterobacter spp.</i>	6 (10.7)	4 (66.67)	0 (0)	0 (0)	2(33.33)
<b>Total</b>	<b>56</b>	<b>27</b>	<b>11</b>	<b>11</b>	<b>7</b>

## Prevalence of Carbapenem Resistant Enterobacteriaceae across One.....



**Figure 1:** Multidrug-resistant pattern of enteric Bacteria from slaughterhouses in Abeokuta, Ogun State. **KEYS:** S- Streptomycin AM - Amoxicillin SXT- Septrin AU – Augmentin CH Chloramphenicol PEF - Pefloxacin SP - Sparfloxacin OFX - Tarivid CPX - Ciprofloxacin CN - Gentamycin



**Figure 2:** Prevalence of Carbapenem resistant Enterobacteriaceae across one health interface from selected Abattoirs in Abeokuta.

**Table 2:** Percentage frequency of phenotypic detection of CRE across one health interface

Gram negative bacteria	Frequency of isolates n (%)	Total Screened MDR	Total screened imipenem resistant (%)	Total screened MHT positive (%)
<i>Escherichia coli</i>	6 (10.7)	5 (8.9)	5 (8.9)	0 (0)
<i>Klebsiella spp.</i>	33 (59)	27 (48.2)	18 (32.1)	0 (0)
<i>Proteus spp.</i>	11 (19.6)	11 (19.6)	8 (14.2)	0 (0)
<i>Enterobacter spp.</i>	6 (10.7)	3 (5.4)	2 (3.6)	0 (0)
Total	56	46 (82.1)	33 (59)	0 (0)

## Discussion

This study shows that Enteric Bacteria species circulates among all samples collected from all Slaughterhouses. The isolated organisms include: *Klebsiella* spp., *Proteus* spp., *Enterobacter* spp., and *Escherichia coli*. Bacteria with highest prevalence was *Klebsiella* spp. (59 %). This result corresponds with studies of Putri and Kiran (2014), Ahmed *et al.* (2013), Ramana *et al.* (2013), and Egbe *et al.* (2011) but contradicts the work of Atia *et al.*, (2018) that had *S. pneumoniae* as the most frequently recovered pathogen while *Klebsiella* spp. was observed to be the least isolated. The study also shows that the antibiotic susceptibility test of the four Enteric Bacteria isolated from the Sampling Area had varying multidrug-resistant patterns to various classes of antibiotics. Isolated Bacteria were resistant to antibiotics with few exceptions, although the order of resistance differed among the organisms. This correlates with the studies of Ilusanya *et al.* (2010), where resistance occurred in identical Bacteria isolates. The overall prevalence of multidrug-resistant Enteric Bacteria in the study were high, which supports the studies of Odoki *et al.* (2015) and Kabugbo *et al.* (2016), where a high prevalence of multidrug resistance was recorded among the same enteric Bacteria isolated. The result does not correspond with the research of Odongo *et al.* (2013), with a lower prevalence of the same enteric Bacteria to multidrug resistance. The resistance result of all the isolates to Streptomycin corresponds with the works of Chat *et al.* (2018), Fashe *et al.* (2018), and Ojo *et al.* (2016), who reported that *Enterobacteriaceae* isolated from beef (cattle) are all resistance to Streptomycin although it contradicts the work of Adesokan *et al.*, (2015) where more of enteric isolates observed from their studies were not resistant to Streptomycin and this is quite unusual as Streptomycin is frequently used to treat most Bacterial infections in cattle thereby predisposing the animal to transfer of resistance strains.

Rearing of animals in unhygienic kens as well as exposure of animals to several antibiotic treatments by herders who are certainly not veterinary doctors can be a significant contributing factor to the circulation and prevalence of CRE among these settings, as they are ignorant of the dangers in the persistent use of antibiotics when not prescribed. Additionally, as observed among the inhabitants of the study location there are no efficient waste disposal of cattle droppings. The waste is being discharged in a close stream besides the market from which they also use in washing of equipment used for slaughtering. This can result to cross contamination and transfer of resistant genes among organisms.

## Conclusion and Recommendations

The prevalence of Carbapenem Resistance Enterobacteriaceae was significantly high in abattoir in which the cow beef had the highest proportion. The present study indicates that the isolate from cattle beef spread the highest Enterobacteriaceae resistant organisms. Isolates showed more resistance to various antibiotics and few susceptibility patterns. Due to high resistance of antibiotics among Bacteria isolates, it is impossible to consider antibiotics to be a true effective treatment for Bacterial infection, caused by these organisms. This study also revealed that the few Bacteria that showed resistance to some antibiotics were however not carbapenem resistance. The family Enterobacteriaceae have certain traits which aids their transmission and pathogenicity. This includes resistance mechanisms to many antimicrobial agents. Their ability to produce, carbapenemase enzyme thus enables their survival and capability to invade man's immune system. Strict implementation of standard hygiene and sanitation should be ensured in abattoirs specifically in the study location in other to prevent the increasing prevalence of CRE infections. Health history of cattle handlers should be properly supervised to prevent cross contamination and wide spread of the Bacteria under study.



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