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Plasmid Profile and Antibiotic Resistance Pattern of Bacteria from Abattoirs in Port Harcourt City, Nigeria

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Authors' contributions

This work was done in collaboration between both authors. Author DNO designed the study, performed the statistical analysis, wrote the protocol and manuscript. Author TCA managed the analyses of the study and literature searches under the strict supervision of author DNO. Both authors read and approved the final manuscript.

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ABSTRACT

Several activities occur in abattoirs including receiving, slaughtering and sale of cattle for meat protein. In rearing of these animals, antibiotics and vaccines are incorporated as part of their meals as well as in the treatment of their infections. The regular use of antibiotics leading to the increased occurrence of antibiotic resistant organisms worldwide and also from abattoir samples, has prompted the determination of plasmid profile in these microorganisms as the plasmids act as a faster means of transmission of resistance genes. This study was thus aimed at determining the plasmid profile of multi-resistant microorganisms isolated from abattoirs. Several samples including swabs from the tables, cow blood, faecal matter and service water were collected from the Iwofe, Rumuodumaya and Trans-Amadi abattoirs located within Port Harcourt City. Antibiotics including Gentamicin (10 µg), Ofloxacin (5 µg), Augmentin (30 µg), Ceftazidime (30 µg), Cefuroxime (30 µg), Nitrofurantoin (300 µg), Cefixime (5 µg) and Ciprofloxacin (5 µg) were used to determine the sensitivity pattern of the isolated microorganims.The plasmid profile of the multiple antibiotic

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resistant microorganisms was determined using standard microbiological procedures. From the results, gram-positive isolates of the genera *Bacillus* and *Staphylococcus* exhibited 100% resistance to Cefuroxime, Ceftazidime and Augmentin while they exhibited 100% susceptibility to Ofloxacin. The gram negative isolates including those of the genus *Escherichia, Pseudomonas* and *Proteus* exhibited 100% resistance to Cefuroxime as well as 100% susceptibility to Ofloxacin. Strains of *Escherichia coli*, *Bacillus amyloliquefaciens*, *Bacillus flexus* and *Klebsiella pneumoniae* lacked plasmids while strains of *Pseudomonas*, *Klebsiella, Escherichia, Staphylococcus aureus* and *Proteus mirabilis* had at least one plasmid each. The absence of plasmids in some of these isolates, indicate that their resistance may be chromosome-mediated and not plasmid-mediated. The occurrence of plasmids in multi-resistant microorganisms, poses a serious public health threat as other susceptible organisms may become resistant to the regularly used antibiotics over time.

Keywords: Plasmid profile; bacteria; antibiotic resistance; abattoir.

1. INTRODUCTION

The increased demand for cow meat and other by-products from cow has led to the establishment of abattoirs in almost every locality within Port Harcourt. As an advantage, this has not only provided ease of meat purchase but also reduced cost of the meat. Also, it serves as source of employment for the increasing population [1]. However, with the increase in the establishment of abattoirs, there is proportional increase in the generation of wastes resulting from the rearing and slaughter of the animals. In most cases, these wastes are channeled through tributaries into major streams or rivers such as that of the Trans-Amadi abattoir in Port Harcourt [2]. These wastes which are either in solid or liquid form often contain microorganisms which may be pathogenic and in some cases resistant to regularly prescribed antibiotics. These microorganisms include rotaviruses, *Cryptosporidium parvum*, *Giardia lamblia,* hepatitis E virus, *Yersinia enterocolitica*, *Campylobacter* sp., *Vibrio* sp., *Salmonella* sp. and *E. coli* 0157:H7 [3,2]. Conversely, the butchers, meat sellers and buyers or the consumers are exposed to these microorganisms. In some instances, the water used in washing the slaughtered animals may not be portable and could as well be a source of microbial contamination of the meat and possibly the handlers. The faecal material from the animals, which often contain pathogenic organisms, may act as a vehicle for the transmission of zoonotic infections [4].

Zoonotic infections can be spread through hand contact and have over time been a public health concern [5]. The reduction of these diseases in animal farms has been effective using antibiotics; however, awareness on the use of antibiotics has been on the increase [6]. In the US, it was reported that about 40% of the produced antibiotics were added to livestock feeds. According to studies, penicillins and cephalosporins which are β-lactam inhibitors are among the antibiotics regularly prescribed, worldwide [7]. This scenario has led to increased resistance of bacteria to these antibiotics. This rapid growth in the occurrence of antibiotic resistance has been made possible through different means including the acquisition of plasmids. These plasmids are extra-chromosomal materials which are able to effect the production of β-lactamases. Some scholars have suggested that these β-lactamases have not only been active against β-lactam drugs but also on aminoglycosides and quinolones which are non-β-lactam drugs; this have more destroying effect on patients who are immuno-compromised and makes treatment of illnesses difficult [8].

Plasmids are often implicated in increasing drug resistance as they are able to transfer the genes both within species and between different species [9,10]. Mechanisms of antibiotic resistance include structural modification of the target, degradation of the drug by enzymes and efflux of antibiotics [11]. Conversely, the genes responsible for resistance are either located on the chromosome or on the plasmid. This provides a medium for the quick spread of resistance genes than mutation and vertical evolution [12]. Plasmid profiling has proved to be relevant in the epidemiologic study of drug resistance as this explains the pattern, occurrence and likely future picture of the resistance when linked with some parameters [13]. It also aids in surveillance in relating strains with outbreaks and their spread [14]. The occurrence of Multiple Drug Resistance in some microorganisms isolated from abattoirs has also been reported [15]. This study was therefore

aimed at determining the antibiotic susceptibility pattern and plasmid profile of isolated microorganisms from some abattoirs in Port Harcourt as this provides information on the potential health and environmental effects of the presence of the microorganisms.

2. MATERIALS AND METHODS

2.1 Sampling Locations

The locations where the samples were collected included Iwofe, Rumuodumaya and Trans-Amadi abattoirs. All three abattoirs are located within Port Harcourt City in Rivers State, Nigeria (Fig. 1). The Trans-Amadi and Rumuodumaya abattoirs are however usually busy as it is located within popular markets. Trans-Amadi abattoir is located at longitude 04 48.442 N and latitude 007 2.303E; Rumuodumaya abattoir at longitude 04 '52' 48.0 N and latitude 7'58'20.0 E while Iwofe abattoir is located at latitude 4 59'14.0N and longitude 7 16' 12.0 E (Table 1).

2.2 Sample Collection

Samples used in this study were cow blood, table swabs, service water and faecal matter from the abattoirs. All samples were collected under aseptic conditions using sterile syringes and swab sticks while service water was collected by allowing the tap flow for 1 minute into sterile bottles and faecal samples were collected by scooping surface faeces of the cow using sterile sample bottles. All collected samples were put in ice packed coolers and transported to the Microbiology laboratory immediately for analyses.

2.3 Isolation of Microorganisms

Microbiological analyses were done according to the method of [16]. The swabs were dipped in 5 ml peptone water and allowed to stand for 30 minutes to resuscitate the microorganisms; after which 1 ml was transferred to 9 ml normal saline (diluent) for serial dilution. One milligram of faecal material from the slaughtered animal was added to sterile 9 ml peptone water from where serial dilution was done. One millilitre of the blood and water samples each were transferred to 9 ml of normal saline (diluent) and a 10-fold serial dilution was carried out up to 10^6 dilution. Aliquots (0.1 ml) of the samples were plated on sterilized and cooled Nutrient agar plates, spread using a sterile bent glass rod and incubated at 37°C for 24 - 48 hours. Biochemical tests including gram's stain, catalase, indole, coagulase, spore test and sugar fermentation tests were performed to identify the isolates. Molecular characterization of the isolates was carried out to further characterize the isolates. The identified microorganisms were preserved in Nutrient agar slants in a refrigerator and used for subsequent tests [16].

2.4 Antibiotic Susceptibility Testing

Antibiotic sensitivity testing was carried out using standard gram negative and gram positive discs (Abtek Biologicals, Liverpool, UK). Each ring was embedded with 8 antibiotics. Antibiotics used include Gentamicin (10 µg), Ceftriaxone (30 µg), Erythromycin(5 µg), Cloxacillin (5 µg), Ofloxacin (5 µg), Augmentin (30 µg), Ceftazidime (30 µg), Cefuroxime (30 µg), Nitrofurantoin (300 µg), Cefixime (5 µg) and Ciprofloxacin (5 µg).

Table 1. Sampling points, GPS coordinates and sample types

Sampling stations	Sampling points	Sampling coordinates		Type of samples
		Northing	Easting	
Iwofe	i	004° 48.598′	006° 58.517′	Blood
	ii	004° 48.592′	006° 58.501′	Swab
	iii	004° 48.601′	006° 58. 525′	Water
	iv	004° 48.594′	006° 58.518′	Faecal matter
Rumuodumaya	i	004° 52.118′	006° 59.580′	Blood
	ii	004° 52.102′	006° 59.571′	Swab
	iii	004° 52.124′	006° 59. 602′	Water
	iv	004° 52.120′	006° 59.582′	Faecal matter
Trans-Amadi	i	004° 48.442′	007° 02.303′	Blood
	ii	004° 48.434′	007° 02.293′	Swab
	iii	004° 48.456′	007° 02.319′	Water
	iv	004° 48.444′	007° 02.301′	Faecal matter

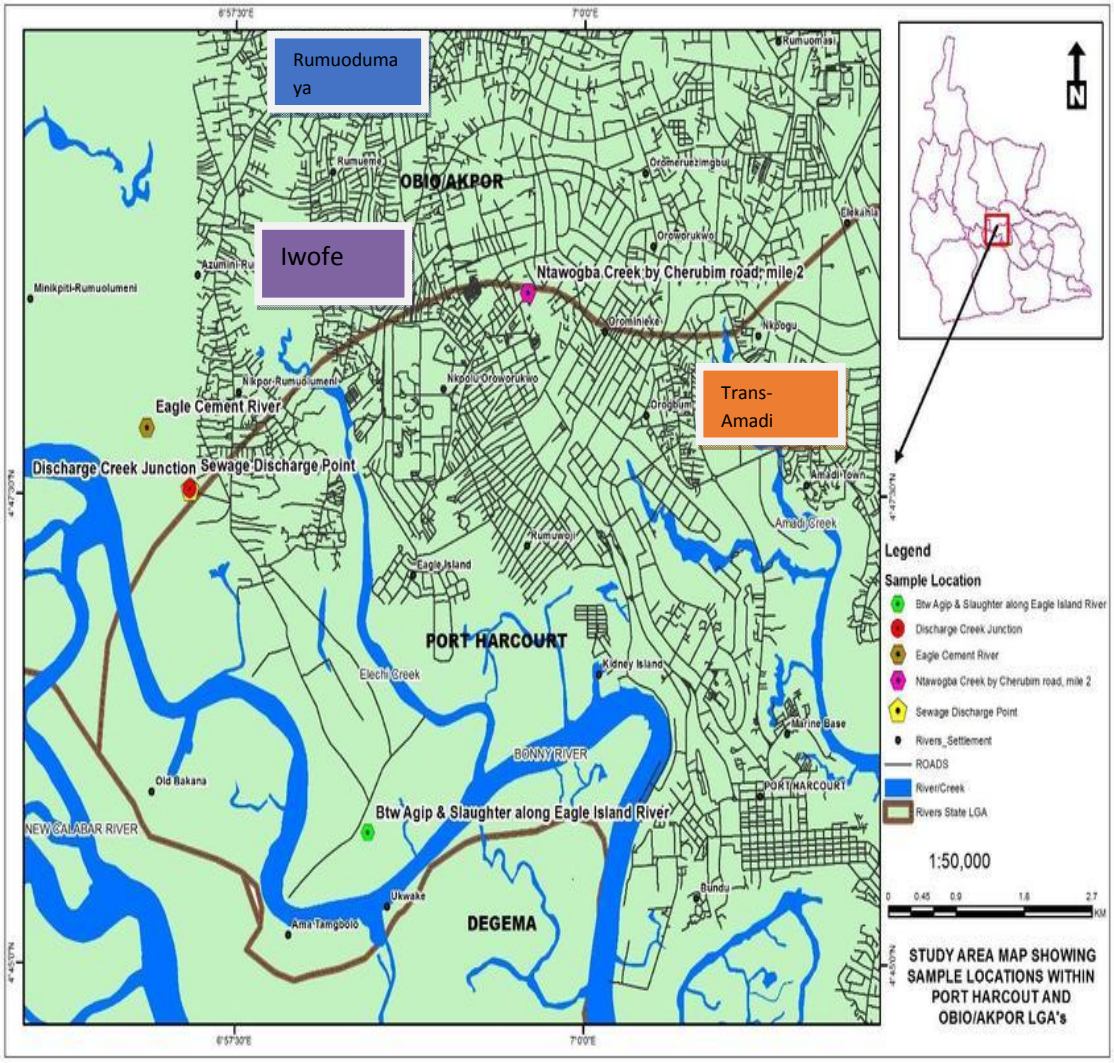

Fig. 1. Map of Port Harcourt Metropolis showing the sampling points
(Source: Rivers State Ministry of Lands and Survey, Port Harcourt)

Mueller-Hinton (MH) agar was prepared accrording to manufactrurers instructions, allowed to cool and solidify. Cultures of the organims were prepared by diluting the isolate in 5 ml of sterile distilled water to 0.5 Macfarland's standard. Sterile swab stick was used to pick the culture and stricked on MH agar medium ensuring even distribution of the culture, allowed to stay at room temperature for 30 miniutes and then the antibiotic ring containing the dics were aspetically placed on the plate using sterilized forcep. The plates were incubated for 18 - 24 hours at 37°C. The zones of inhition of the antibiotic visible as a clear halo around the discs were measured and recorded in millimeters. The percentage sensitivity of the isolates was determined per antibiotic using Microsoft Excel [17].

2.5 Extraction of Plasmid DNA

Isolates that exhibited Multiple Drug Resistance (MDR) to at least three antibiotics were used for plasmid analyses. The plasmids were extracted using the Zyppy™ Plasmid Miniprep Kit (Zymo Research, Irvine, California). Pure isolates were inoculated on Luria-Bertani broth and incubated at 37°C overnight. Sixty microliter (60 µl) of the bacterial culture from Luria-Bertani broth (LB) was transferred to a 1.5 ml micro-centrifuge tube; 100 µl of 7X lysis buffer was added to the above tube and mixed by inverting the tube 6 times.

The setup was incubated for 1-2 minutes. Complete cell lysis was evident by the change of colour from opaque to blue; 350 µl of cold neutralization buffer was added and mixed thoroughly. Complete neutralization was evident by the change of colour to yellow; The mixture was centrifuged for 2-4 minutes at 14000 rpm; The supernatant was transferred to a Zymo-Spin™ IIN column placed in a Collection Tube; and spun for 15 seconds at 14000 rpm; The flow-through was discarded and column returned to the Collection tube; 200 µl of Endo-Wash buffer was added to the column and spun for 30 seconds at 14000 rpm; 400 µl of Zyppy™ Wash buffer was added to the column and spun for 1 minute at 14000 rpm; The column was transferred to a clean sterile 1.5 ml micro-centrifuge tube and then 35 µl of Zyppy™ Elution buffer was added directly to the column matrix and incubated for 1 minute at room temperature; The setup was spun for 30 seconds at 14000 rpm to extract the plasmid DNA. The quantity of the extracted plasmid DNA was determined using a Nanodrop 1000 spectrophotometer and after which 1% agarose gel electrophoresis was used to verify the integrity of the extracted plasmids.

2.6 Gel Electrophoresis

Plasmids were separated by electrophoresis in 1% agarose (Sigma Aldrich, USA) at a voltage of 4.5 V/cm; buffer used was TAE (Tris-Acetate-EDTA) for 3 hours. Following electrophoresis, the gels were stained for 15 minute with ethidium bromide solution (1.0 µg/ml EtBr in 0.5 x Tris-Acetate-EDTA (TAE)), and then observed under UV light. The image was registered and analyzed using Quantity One software, version 4.1 [18].

3. RESULTS

Biochemical and Genomic characterization of the isolates revealed that 10 distinct microorganisms including strains of *Escherichia coli* (2017C-4109), *Pseudomonas* sp (6174), *Bacillus amyloliquefaciens* (WU-12), *Klebsiella pneumoniae* (K20), *Bacillus flexus* isolate Murraya, *Escherichia coli* (SAMA_EC), *Staphylococcus aureus* (NCIM2654), *Klebsiella* sp (EIKU11) and *Proteus mirabilis* (46X4) were isolated from the samples (blood, swab, water and faecal matter). Table 2 shows the weight of the isolated plasmid DNA of each of the organisms in ng/µl. Fig. 2 shows the sensitivity test for *Escherichia coli*. The clear zones show the antibiotics which were active against the isolates. Fig. 3 shows that the gram-positive isolates (*Bacillus* sp. and *Staphylococcus* sp.) exhibited 100% resistance to Cefuroxime (30 µg), Ceftazidime (30 µg) and Augmentin (30 µg) while they exhibited 100% susceptibility to Ofloxacin (5 µg). Cloxacillin (5 µg) and Gentamicin (10 µg) were however effective against the test isolates with 78% and 52% respectively. The gram negative isolates (*E. coli, Pseudomonas* sp. and *Proteus* sp.) were resistant to Cefuroxime with 100%, Augmentin with 97.1 while they were susceptible to Ofloxacin with 100% (Fig. 4). The gram-negative bacteria were susceptible to Nitrofurantoin and Ceftazidime with 25.2% each, while they were susceptible to Ciprofloxacin with 69.8%. Fig. 5 shows the plasmid profile of the 10 isolates with the lines showing the isolates with plasmids. The figure shows that isolates 2 (*Pseudomonas* sp. strain 6174), 7 (*Escherichia coli* strain SAMA_EC), 8 (*Staphylococcus aureus* strain NCIM2654), 9 (*Klebsiella* sp. strain EIKU11) and 10 (*Proteus mirabilis* strain 46X4) all had plasmids. Whereas isolates *Klebsiella* sp. strain EIKU11 and *Proteus mirabilis* strain 46X4 had multiple plasmids, isolate *Pseudomonas* sp. strain 6174 had only 1 plasmid.

4. DISCUSSION

Majority of the isolates such as *E. coli, Pseudomonas* sp and *Proteus* sp were coliforms and according to Okonko et al. [19], these organisms are commonly encountered in aquatic environments and soil; these are possible sources in the meat products or abattoir environments. Plasmids have been known to be extra-chromosomal elements that can replicate on their own. They are distinct from chromosomal DNA in that they can exist independent of the host [20]. In most cases, the plasmids are not required for the day to day surivival of the bacteria; but confer addittional features for survival especially in harsh conditions such as in the case of antibiotic resistance. The link between Multiple Drug Resistance (MDR) and plasmid profile of bacteria gives a hint on the important role played by plasmids in the spread of MDR among bacterial species [21]. Over the years, plamid profile has been used to determine the presence, size, type and number of plasmids in a bacteria [22]. Multidrug-reistanace mediated by plasmids has also, been a constraint in the treatment of infectious diseases [23].

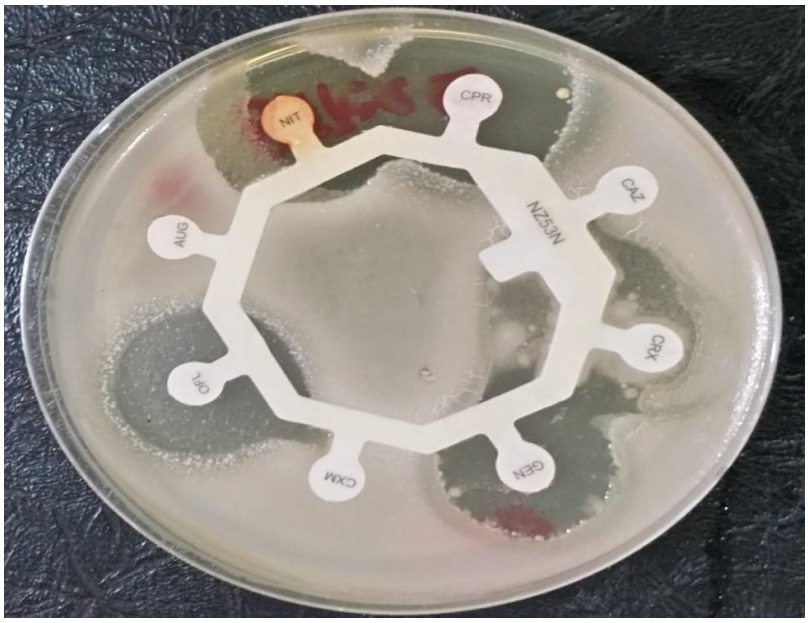

Fig. 2. Multiple antibiotic sensitivity test for *Escherichia coli*

Key: GEN- Gentamicin (10µg), OFL- Ofloxacin (5 µg), AUG- Augmentin (30 µg), CAZ- Ceftazidime (30 µg), CRX- Cefuroxime (30 µg), NIT- Nitrofurantoin (300 µg), CXM- Cefixime (5 µg) and CPR-Ciprofloxacin (5 µg)

The antibiotics sensitivity pattern of the isolates showed that all the gram negative isolates were resistant to Cefuroxime while all the Gram positive isolates exhibited high level of resistance to Augmentin, Cefuroxime and Ceftazidime. The high rate of resistance of the isolates to Cefuroxime, Ceftazidime and Augmentin is of concern as most physicians are quick to presecribe these drugs especially Augmentin in the treatement of bacterial infections. Resistance of bacteria to Cefuroxime has been reported by Harrison and Bratcher [24] who investigated the susceptibility of some microorganims to antibiotics such as cefuroxime. Augmentin resistance by microorganisms of the genera *Klebsiella, Escherichia, Proteus* and *Pseudomonas* from abattoir sources has been reported by Adesoji et al. [25]. This resistance may be caused by mechanisms such as the synthesis of low-affrinity β-lactams binding proteins and the production of penicillinase [26]. Although gentamicin resistance of 63.3% and 52% resistance has been reported among the gram negative and postive isolates respectively from abattoirs [27]. The occureence of Gentamicin reistance has been linked to transferable genetic elements which included plasmids [28]. The high percentage susceptibility of the test isolates to Ofloxacin is in agreement with reports by other authors on multiple-drug resistance bacteria [29]. In this study, it was observed that drugs with higher MIC values such as Augmentin were not as effective against the isolates as those with lower MIC such as Ofloxacin (5 µg). This may be due to the frequency of usage of the drug.Multiple antibiotic resistances among bacterial isolates from abattoir sources have been attributed to the increased use of antibiotics in cattle farms in Nigeria for prophylaxis and treatment infections in cows. Antibiotics are also used in producing animal food [30]. Excretion of quantities of administered drugs from the animals to the environment either as metabolized or non-metabolized compounds undergo transformation in the environment into their active forms [31]. Also, the inappropriate use of antimicrobials by individuals has promoted the presence of strains with resistance plasmids [32]. According to Nsofor and Iroegbu [33], resistant *E. coli* strains can be transmitted to humans from animals through food and can act as an in vivo source of transmission of resistance plasmids to strains that are drug sensitive in the intestine of the animal through conjugation.

The plasmid profile of 10 multi-resistant isolates were determined. Gel electrophoresis of the plasmid DNA showed that all the isolates except *Escherichia coli* strain 2017C-4109, *Bacillus amyloliquefaciens* strain WU-12, *Klebsiella pneumoniae* strain K20, *Klebsiella pneumoniae*

strain K20 and *Bacillus flexus* isolate *Murraya* had at least one plasmid. The two strains of Bacillus isolated lacked plasmid DNA while one strain of *E. coli* (*Escherichia coli* strain 2017C-4109) lacked plasmid DNA but the other (*Escherichia coli* strain SAMA_EC) possessed

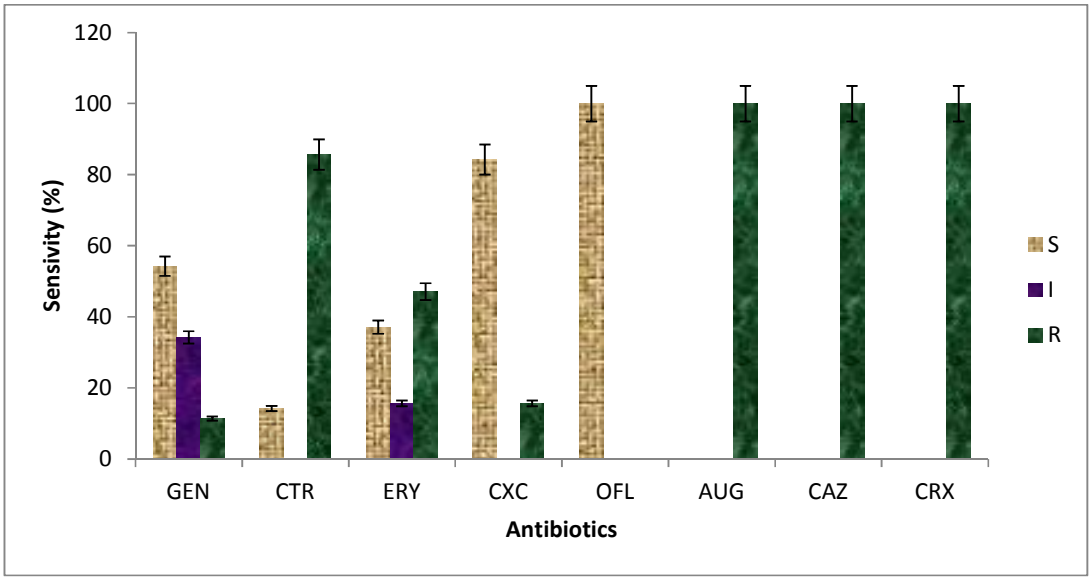

Fig. 3. Percentage sensitivity of gram-positive isolates to tested antibiotics

Key: S - Sensitive, I - Intermediate, R- Resistant, % - Percentage, GEN- Gentamicin (10 µg), OFL- Ofloxacin (5 µg), AUG- Augmentin (30 µg), CAZ- Ceftazidime (30 µg), CRX-Cefuroxime (30 µg), NIT- Nitrofurantoin (300 µg), CXM- Cefixime (5 µg) and CPR-Ciprofloxacin (5 µg)

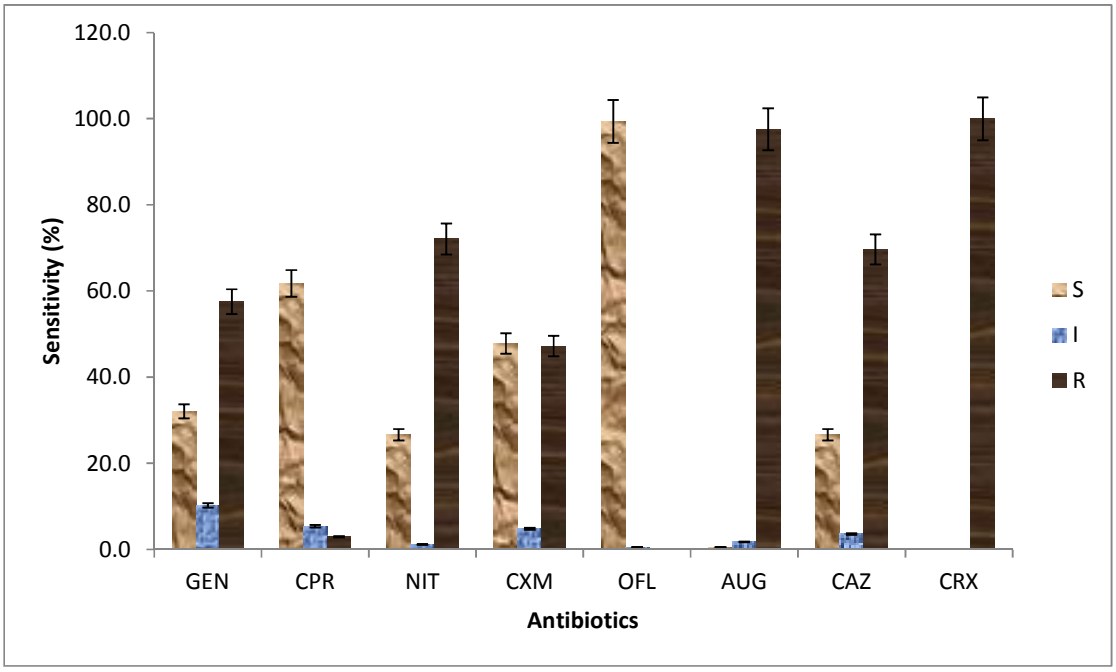

Fig. 4. Percentage sensitivity of gram-negative isolates to tested antibiotics

Key: S - Sensitive, I - Intermediate, R- Resistant, % - Percentage, GEN- Gentamicin (10 µg), OFL- Ofloxacin (5 µg), AUG- Augmentin (30 µg), CAZ- Ceftazidime (30 µg), CRX-Cefuroxime (30 µg), NIT- Nitrofurantoin (300 µg), CXM- Cefixime (5 µg) and CPR-Ciprofloxacin (5 µg)

Table 2. Nanodrop quantification of plasmid DNA

S/N	Microorganism	Quantity (ng/µl)
1	*Escherichia coli* strain 2017C-4109	25.64
2	*Pseudomonas* sp. strain 6174	27.24
3	*Bacillus amyloliquefaciens* strain WU-12	11.70
4	*Klebsiella pneumoniae* strain K20	6.41
5	*Klebsiella pneumoniae* strain K20	14.25
6	*Bacillus flexus* isolate *Murraya*	14.04
7	*Escherichia coli* strain SAMA_EC	11.19
8	*Staphylococcus aureus* strain NCIM2654	18.27
9	*Klebsiella* sp. strain EIKU11	15.44
10	*Proteus mirabilis* strain 46X4	17.14

Table 3. Resistance pattern of the selected isolates

Bacteria	Antibiotics
Escherichia coli strain 2017C-4109	CAZ
Klebsiella pneumoniae strain K20	CAZ
Pseudomonas sp. strain 6174	CAZ
Escherichia coli strain SAMA_EC	CAZ
Klebsiella sp. strain EIKU11	CAZ
Proteus mirabilis strain 46X4	CAZ
Klebsiella pneumoniae strain K20	CAZ
Bacillus amyloliquefaciens strain WU-12	CAZ, CRX, AUG
Staphylococcus aureus strain NCIM2654	CAZ, CRX, AUG
Bacillus flexus isolate Murraya	CAZ, CRX, AUG

Key: CAZ- Ceftazidime (30 µg), CRX-Cefuroxime (30 µg), AUG- Augmentin (30 µg)

Fig. 5. Agarose gel electrophoresis showing plasmids profile of the bacterial isolates. M represents the 1kb ladder

plasmid. The presence of plasmid in *Pseudomonas* sp. especially *Pseudomonas aeruginosa* has been reported by Akingbade et al. [34] who studied the plasmid profile of *P. aeruginosa* isolated from wound infections in South West, Nigeria. The absence of plasmids in *Escherichia coli* strain 2017C-4109, *Bacillus amyloliquefaciens* strain WU-12, *Klebsiella pneumoniae* strain K20 and *Bacillus flexus* isolate *Murraya* implies that the resistance may not be plasmid-mediated and may invariably be chromosome-mediated and as such may be caused by mechanisms other that plasmid-mediation [35,36]. This study recorded the

presence of *Klebsiella pneumoniae* strain K20 with a weight of 6.41 kbp. Agbagwa et al. [37] reported that strains containing larger plasmids were mainly from non-human sources while the smaller ones of about 4.7 to 10.8 kbp were mostly from humans. Also, this difference in plasmid sizes may be attributed to the presence of different resistance plasmids (R plasmids) responsible for multiple antibiotic resistances [38]. A study carried out by Nsofor and Iroegbu [32], also reported the presence of plasmids in strains of *E. coli* isolated from cattle. The sizes of the plasmids isolated were between 1-20 kbp. These small-sized plasmids are transmissible and may be linked with resistance genes belonging to the class 1 integrons [39]. These integrons further help in the quick transmission of resistance genes between environmental bacteria and human pathogens [40].

5. CONCLUSION

The location of two of the abattoirs within markets not only exposes the meat handlers to these pathogenic resistant organims but also other traders who may come in contact with the meat handlers or the runoffs from the slaughterslab. The findings of this study points to the presence of plasmid-containing strains which serve as reservoirs for resistance plasmids that may be transferred to otherwise susceptible bacteria making them resistant, thus increasing the ocurrence of antibiotic resistance among these pathogenic organims. This causes difficulty in the treatment of infections caused by them. It is strongly recommended that the frequent and indescrimate use of antibiotics be discouraged by both humans and animals. The service water from the abattoirs be treated regularly to reduce the transfer of resistant microorganims from the water source to the animals as they consume these abatoir waters too. The abattoir wastes should be properly disposed to reduce the persistence of these resistance microorganims in the environment.

CONSENT AND ETHICAL APPROVAL

Approval for this research was obtained from the Department of Microbiology of the Rivers State University as well as a consent from the abattoir authorities.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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