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Plasmid-Borne Mobile Colistin Resistant Gene (*Mcr-1*) Detection and Multidrug Resistant Bacteria Isolated from Some Abattoir Environments in Benin City, Edo State, Nigeria

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Abstract

Antibiotics resistance is an increasing public health challenge globally, and very recently, global attention has focused on colistin, which is termed "last resort antibiotics". This study was aimed at investigating the prevalence of plasmid-borne mobile colistin resistant and multidrug resistant bacteria from 6 major abattoirs located in Benin City, Edo State, Nigeria. Two hundred and eighty-eight samples from fresh water, wastewater, utensils, and handlers were obtained over a 6-months period. Mean mesophilic aerobic bacteria (MAB) and thermotolerant coliform bacteria (TCB) were determined by pour plate method, while the indoor air of the abattoirs was sampled using passive sedimentation technique. Bacterial isolates were identified by morphological, biochemical and 16S rRNA analysis. Phenotypic detection of colistin-resistance as well as multi-drug resistant profile of all isolates was done by the modified Kirky Bauer method. The presence and/or absence of colistin-resistance gene (*mcr-1* to *mcr-8*) were investigated by polymerase chain reaction. The MAB ranged from 0.3 ± 0.0 cfu/m^3 in indoor air from both Holy Ghost B and Bob Izua abattoir to 2.6 ± 0.3 cfu/ml in wash water from Holy Ghost A abattoir, while the TCB ranged from 0.0 ± 0.0 cfu/ml in wastewater from Lawal and Sons abattoir to 0.6 ± 0.1 cfu/ml in wash water from Osazee abattoir. A total of 149 bacterial isolates, belonging to 6 different species (*Pseudomonas aeruginosa* PA01, *Enterobacter ludwigii* EN-119, *Providencia stuartii* PRV00010, *Klebsiella quasipneumoniae* strain KqPF26, *Enterococcus saccharolyticus* ATCC 43076 and *Provincia rettgeri* strain AR_0082) were obtained with the majority (>90%) being multidrug resistant. Seven (4.7%) of the isolates were phenotypically resistant to colistin, while only 3 harbored the *mcr-1* gene. This result shows that plasmid-borne colistin-resistant and multidrug resistant bacteria are prevalent in abattoir environment located in Benin City, Edo State, Nigeria. This is an indication that abattoir facilities could be a source of human exposure to colistin resistant bacteria, and efforts must be made at reducing the high dependence of antibiotics in farm animals.

Keywords: Abattoir; Beef Processing; Colistin; *mcr-1* gene; Multidrug Resistance

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Introduction

Consumer demand for beef is increasing in Nigeria (Famubo et al., 2020). However, hygiene practices remain poor along the meat production chain (Osemwowa et al. 2021), particularly the abattoirs. Majority of these abattoirs are reported to harbour a number of coliform as well as pathogenic microorganisms (Omoruyi et al., 2011; Uzoigwe et al., 2021), making them unfit for meat production. Meanwhile, standard abattoir with adequate facilities is required for aseptic meat production; otherwise, the abattoir could become a potential source of meat contamination (Uzoigwe et al., 2021). In Nigeria and most developing countries, beef are processed on the floors, often contaminated with blood spills and fecal materials (Omoruyi et al. 2011). These floors are major contributors to the high microbial burden across major abattoirs in Nigeria (Omoruyi et al., 2011; Adegunloye, 2013).

It is generally recognized that the most significant food-borne hazards associated with abattoir are bacteria, such as *Salmonella enterica*, *Listeria monocytogenes*, *Campylobacter jejuni*, *Staphylococcus aureus*, *Escherichia coli* etc, many of which harbor the potential to cause diseases in humans. The main sources of contamination by these pathogens include; floor (Adegunloye, 2013; Uzoigwe et al., 2021) , slaughtering knives (Omoruyi et al., 2011; Uzoigwe et al., 2021), contact surfaces (Adegunloye, 2013; Ayalew et al., 2015; Zailani et al., 2016), workers/butchers (Adegunloye, 2013; Uzoigwe et al., 2021), carcass dressing water (Omoruyi et al., 2011), hide of animals (Uzoigwe et al., 2021), aerosols (Omoruyi et al., 2011) etc. More burdensome is the potential of these pathogenic bacteria to be resistant to the commonly used antibiotics. The indiscriminate use of antibiotics in animals has contributed to the magnitude of global challenge of antibiotics resistance (FAO, 2022). The increased pressure of food security has further intensified antibiotics use as chemotherapeutic, metaphylactic, prophylactic as well as growth promoters, further exacerbating the emergence and spread of antibiotic resistance (Founou et al., 2016).

Colistin is one of the last resort antibiotics, previously banned for use due to its nephrotoxic and neurotoxic effects (European Medicines Agency, 2016). The re-introduction of colistin by the World Health Organization into the group of the "highest priority critically important antimicrobials" was largely based on its high impact (WHO, 2016). Thus, colistin became the antibiotics of choice in clinical cases for which no alternative options are available (Savin et al., 2020).

In 2016, colistin was also re-introduced in agriculture (both as feed additives and in the treatment of animal diseases) by the World Organization for Animal Health (World Organization for Animal Health, 2018), and since its re-introduction, colistin resistant bacteria have been reported extensively in livestock from different parts of the world (Huang et al., 2017; Yamamoto et al., 2019; Anyanwu et al., 2021; Valiakos and Kapna, 2020; Effelsberg et al., 2021; Odoi et al., 2021). Studies on the prevalence of colistin resistant bacteria in abattoir environment are limited, especially in developing countries. The current study was aimed at investigating the prevalence of colistin resistant and multidrug resistant bacteria in major abattoirs located in Benin City, Edo State, South-South Nigeria.

Materials and Methods

Study area

The research was conducted in selected abattoirs in Benin City, Edo State, Nigeria. Samples were obtained weekly, from six (6) abattoirs, for 12 weeks. Four (4) of the abattoirs were located along Akpakpava/Ikpoba Slope. One of the abattoirs was located along the Benin-Sapele expressway and the last, along Ugbor road, GRA, Benin City (Figure 1).

Sample collection and preparation

At each visitation, eight samples from each abattoir were obtained from wastewater, surfaces and air during slaughtering operations, using standard microbiological techniques. Using a sterile universal container, wastewater

emanating from minimally processed bovine carcass was collected at two (2) separate sections within the respective abattoirs. The floors in the slaughtering halls of the visited abattoirs were swabbed with sterile swab sticks, pre-immerged in 2ml of normal saline (Bridson, 2006). The aerobiological flora associated with the circulating indoor air of the slaughtering halls was sampled using passive sedimentation technique as previously described (Augustowska and Dutkiewicz, 2006). All samples were immediately taken to the Microbiology Laboratory of Benson Idahosa University, in cold pack, for immediate analysis.

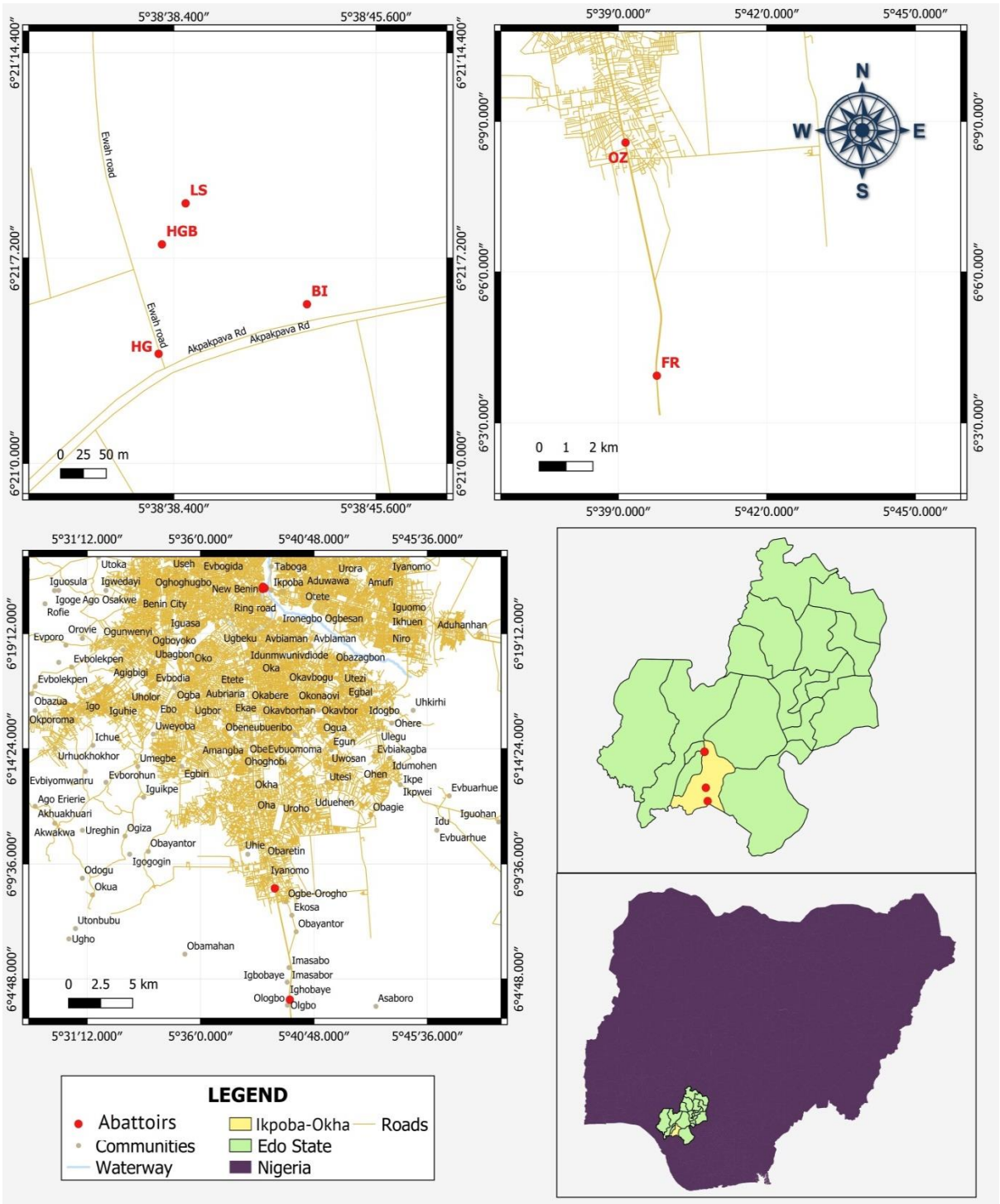

Fig 1: Sampling map revealing the locations of the respective abattoirs visited during the study period.

KEY: **LS**: Lawal and Sons abattoir, **BI**: Bob-Izua abattoir, **HG**: Holy Ghost abattoir, **HGB**: Holy Ghost B abattoir, **OZ**: Osazee abattoir, **FR**: Freedom abattoir

Mean mesophilic aerobic bacterial (MAB) and thermotolerant coliform bacterial (TCB) counts.

MAB and TCB were determined by plate count agar and violet, red bile agar plates respectively (Osemwowa et al. 2021). Briefly, Ten-fold dilutions were plated in triplicate on plate count agar (VWR, Germany) for MAB and violet, red bile agar (Labema, Finland) plates TCB. PCA plates were incubated at 30ºC for 24–48 hrs and TCB plates at 44ºC for 24 hrs. All cultures were done in triplicate and under aseptic conditions (Omoruyi and Ojubiaja, 2022). The plate counts were converted to cfu/m^3 values using an empirical formula described by Stryjakowska-Sekulska et al. (2007).

Isolation, characterization and identification of bacterial isolates

Following incubation, the resultant discrete colonies were culturally and morphologically characterized. One anatomically distinct bacterial colony was sub-cultured from each plate, onto freshly prepared nutrient agar plates, and the isolates were further identified by their biochemical characteristics, and by 16S rRNA analyses.

Phenotypic detection of colistin resistance

All isolates presumptively identified by their biochemical characteristics were further screened for their resistance and/or sensitivity to the antibiotics colistin. Muller Hilton agar was prepared and the isolates in cell suspension were spread on each plate, followed by the introduction of colistin (10µg) onto the agar plates, before being incubated for 24hr at 37ºC. The medium without any zone of inhibition around the bacterial growth indicated colistin-resistance.

Plasmid DNA extraction

Plasmid DNA extraction was carried out using plasmid extraction kit (Zymo Research, USA), according to the manufacturer's instruction. DNA was stored in sterile Eppendorf tube at -20˚C before use.

Detection of colistin resistance gene

Following phenotypic identification of colistin resistance, bacterial cultures with positive outcome were further screened for the presence and/or absence of colistin resistance gene (*mcr-1, mcr-2, mcr-3, mcr-4, mcr-5, mcr-6, mcr-7* and *mcr-8*). Following DNA extraction, the respective DNAs were amplified by PCR, using a Thermocycler (Biometra, Gottingen, Germany) in a 25µl reaction, containing 12.5µl of one Taq quick-load master mix (New England Biolabs, Inc.), 0.5µl of forward primer, 0.5µl of reverse primer, 1.5µl of template DNA and 10µl of nuclease free water. The primer base compositions as well as the annealing temperature are presented by Huang et al. (2017). All PCR products were given a holding temperature of 4˚C. The PCR conditions included 94˚C for 5 min, 94˚C for 1 min (39 cycles), annealing for 1 min, 72˚C for 1 min and 72˚C for 10 min.

Antibiotics susceptibility pattern of bacterial isolates

The antibiotic susceptibility patterns (antibiogram) of the bacterial isolates were evaluated by the disc diffusion technique (Collins et al., 1995). Isolates were inoculated unto freshly prepared nutrient broth and incubated overnight. The turbidity of each culture was adjusted to match the opacity standard ($BaSO_4$ turbidity standard). The standard had a resulting broth culture of 10^8cfu/mL. Freshly prepared Muller Hinton agar plates were seeded on standardized bacterial broth cultures by spread plate techniques (Collins et al., 1995). The inoculated plates were left to dry for 15min, and antibiotic discs (ceftazidine (30ug), cefuroxime (30ug), gentamicin (10ug), cefixime (5ug), augmentin (30ug), ofloxacin (5ug), nitrofurantion (300ug), ciprofloxacin (5ug) and colistin sulphate, (10ug)) were seeded on the agar plates, and incubated for 24hr at 37°C. The resultant zones of inhibition were recorded

according to the Clinical and Laboratory Standards Institute (CLSI) guideline (CLSI, 2020).

Polymerase chain reaction (PCR) and 16S rRNA analysis

PCR sequencing preparation cocktail consisting of 10µl of 5x Go Taq colourless reaction, 3µl of 25mM MgCl2, 1µl of 10mM of dNTPs mix, 1µl of 10pmol each of the desired gene. The PCRs was conducted using universal primers, in a Gene Amp 9700 PCR System Thermal cycler (Applied Bio system Inc., USA) with a PCR protocol consisting of an initial denaturation at 94°C for 5 min; followed by a 30 cycles consisting of 94°C for 30sec, 30sec annealing of primer at 56°C and 72°C for 1min 30sec; and a final termination at 72°C for 10min and hold at 4°C.

Gel integrity

The integrity of the DNA and PCR amplification was checked on 1% and 1.5% agarose gel respectively. The gel was electrophoresed at 120V for 45 min visualized by ultraviolet trans-illumination and photographed.

Purification of amplified product

After gel integrity, the amplified fragments were ethanol purified in order to remove the PCR reagents. The purification of amplified products was done as described by Odeyemi et al. (2018).

Blast analysis.

The blast analysis was done on the National Centre for Biotechnology Information (NCBI) website (http://blast.ncbLnlm.nih.gov/). DNA sequences of each of the test organism was copied in fasta format into the nucleotide sequence search engine and used to query the NCBI data base in search of sequences producing significant alignments with a view to determining the best fit identity for each of the test organisms. The 16S rRNA partial sequence of the test isolates were submitted to GenBank (NCBI) with receipt of corresponding GenBank accession numbers.

Statistical analysis

The mean bacterial counts were subjected to one way analysis of variance (ANOVA) utilizing the software; SPSS version 25. This was done to ascertain if the recorded variations amongst the mean counts derived from the respective abattoir facilities were significantly different (α=0.05).

Results

The results of the current study show that aerobic bacteria, *Pseudomonas* species and coliform are prevalent in Abattoir facilities located in Benin City, Edo State, Nigeria. The mean aerobic bacterial counts ranged from 1.1 ± 0.3 x 10^5 cfu/mL to 2.6 ± 0.3 x 10^5 cfu/mL in wash water; 0.8 ± 0.1 x 10^5 cfu/mL to 1.1 ± 0.1 x 10^5 cfu/mL for wastewater; 0.4 ± 0.1 x 10^5 cfu/m^3 to 2.1 ± 0.5 x 10^5 cfu/m^3 for floor and 0.4 ± 0.3 to 0.6 x 10^5 cfu/m^3 for indoor air (Table 1). The mean aerobic bacteria counts obtained for indoor air and floor was significantly different from those obtained from wash water and wastewater at 95% confidence level. Also, the mean coliform counts ranged from 0.2 ± 0.0 to 0.6 ± 0.1 x 10^3 cfu/mL, 0.0 ± 0.0 to 0.5 ± 0.1 x 10^3 cfu/mL, 0.1 ± 0.0 to 0.3 ± 0.1 x 10^3 cfu/m^3 and 0.1 ± 0.0 to 0.1 ± 0.0 x 10^3 cfu/m^3 for wash water, wastewater, floor and air respectively (Table 1). A total of 149 bacterial isolates, belonging to 6 different genera were isolated from all 288 samples based on their cultural, morphological, biochemical and 16S rRNA analysis. They included *Pseudomonas aeruginosa* (47), *Enterobacter ludwigii* (34), *Providencia stuartii* (31), *Klebsiella quasipneumoniae* (01), *Enterococcus saccharolyticus* (19) and *Providencia rettgeri* (17) (Table 2). The bacterial isolates and their base sequence have been deposited in the gene bank for reference purposes. Of the six bacterial genera, *Pseudomonas aeruginosa* was the most prevalent (47); 28% of which was reported in Osazee Abattoir. *Klebsiella quasipneumoniae* was obtained in only one sample from Bob-Izua Abattoir. *Providencia stuartii* was more prevalent in Holy Ghost A abattoir (43%) and least prevalent in both Osazee and Freedom abattoir (2% each).

The majority of the isolates were observed to be multidrug resistant, showing remarkable resistance against the commonly used antibiotics, especially the β-lactams. All

Pseudomonas aeruginosa and *Enterobacter ludwigii* were 100% resistant to the *β*-lactam antibiotics (ceftazidime, cefuroxime, cefixime and augmentin) tested against, while *Providencia stuartii*, *Enterococcus saccharolyticus* and *Providencia rettgeri* were 100% resistant against 3 (ceftazidime, cefuroxime and augmentin) of the 4 *β*-lactam antibiotics tested against them (Table 3). Meanwhile, gentamicin was 100% active against *Providencia stuartii* and *Enterococcus saccharolyticus*. Three bacterial isolates (*Providencia stuartii*, *Enterococcus saccharolyticus* and *Providencia rettgeri*) were sensitive to ofloxacin.

Table 1: Mean Aerobic Bacterial and Thermotolerant Coliform Bacterial Counts of wash water, wastewater, floor and indoor air obtained from selected abattoirs in Benin City, Edo State, Nigeria.

Abattoir	Sampling	No. of Samples	MAB (x 10^5)	(x 10^5)	TCB (x 10^3)	(x 10^3)
			Mean ± SD	Min – Max	Mean ± SD	Min - Max
Osazee	Wash water	12	1.2 ± 0.3†	1.0 – 1.5	0.6 ± 0.1	0.0 – 1.0
	Wastewater	12	1.1 ± 0.2	0.7 – 1.3	0.5 ± 0.1†	0.3 – 0.6
	Floor	12	0.7 ± 0.2	0.5 – 0.8	0.2 ± 0.0	0.0 – 0.3
	Indoor air	12	0.4 ± 0.0	0.2 – 0.5	0.1 ± 0.0	0.0 – 0.3
Freedom	Wash water	12	1.1 ± 0.3†	0.8 – 1.8	0.4 ± 0.1	0.3 – 0.5
	Wastewater	12	1.0 ± 0.2	0.6 – 1.2	0.2 ± 0.0	0.2 – 0.2
	Floor	12	0.4 ± 0.1	0.4 – 0.5	0.3 ± 0.1	0.1 – 0.4
	Indoor air	12	0.6 ± 0.2	0.4 – 0.8	0.1 ± 0.0	0.1 – 0.1
Bob-Izua	Wash water	12	1.2 ± 0.4†	0.9 – 1.5	0.2 ± 0.1	0.0 – 0.3
	Wastewater	12	0.8 ± 0.1	0.5 – 1.2	0.3 ± 0.1	0.2 – 0.4
	Floor	12	0.7 ± 0.0	0.4 – 0.8	0.2 ± 0.0	0.0 – 0.2
	Indoor air	12	0.3 ± 0.0	0.1 – 0.3	0.1 ± 0.0	0.0 – 0.1
Holy Ghost A	Wash water	12	2.6 ± 0.3¶	0.9 – 3.3	0.2 ± 0.0	0.0 – 0.3
	Wastewater	12	1.1 ± 0.2	0.7 – 1.4	0.3 ± 0.0	0.2 – 0.4
	Floor	12	0.5 ± 0.1	0.4 – 0.7	0.2 ± 0.0	0.0 – 0.2
	Indoor air	12	0.5 ± 0.1	0.1 – 0.7	0.1 ± 0.0	0.0 – 0.1
Holy Ghost B	Wash water	12	1.6 ± 0.5	1.3 – 2.5	0.2 ± 0.0	0.0 – 0.2
	Wastewater	12	0.8 ± 0.1	0.6 – 0.8	0.1 ± 0.0¶	0.0 – 0.3
	Floor	12	1.9 ± 0.3	0.6 – 2.9	0.2 ± 0.0	0.0 – 0.2
	Indoor air	12	0.3 ± 0.0	0.1 – 0.3	0.1 ± 0.0	0.0 – 0.1
Lawal and Sons	Wash water	12	1.1 ± 0.3†	0.8 – 1.8	0.3 ± 0.0	0.3 – 0.5
	Wastewater	12	0.9 ± 0.2	0.9 – 1.1	0.0 ± 0.0¥	0.0 – 0.1
	Floor	12	2.1 ± 0.5	0.4 – 2.7	0.1 ± 0.0	0.0 – 0.3
	Indoor air	12	0.4 ± 0.1	0.1 – 0.5	0.1 ± 0.0	0.0 – 0.1

The different symbol signifies significant difference (α=0.05) at 95% confidence level.

Klebsiella quasipneumoniae showed resistance to ceftazidine, cefuroxime, gentamicin, ofloxacin and nitrofurantion and sensitive to cefixime, augmentin, ciprofloxacin and colistin. Only 12 out of the 149 isolated bacteria (8%) showed phenotypic colistin resistance, and included *Pseudomonas aeruginosa* (33%), *Enterobacter ludwigii* (25%) and *Providencia stuartii* (42%). The isolates were further observed to possess *mcr-1* gene (Table 4).

Discussion

Abattoirs play a major role in the contamination of beef, as the abattoir environments continue to be a source of human exposure to pathogenic microorganisms. These pathogens of public health importance sometimes go through the beef processing chain and become a threat to public health (Barsisa et al., 2019). Abattoir wash water, wastewater, floor and indoor air are some of the popular sources of beef contamination, especially in developing countries. These abattoirs are usually located near water bodies where access to water for beef processing and wastewater discharge is guaranteed (Adelegan, 2004; Dauda et al., 2016).

Abattoir operations in Nigeria are generally unregulated by the relevant Government ministry/agency, thus limiting the management and operations of abattoirs to the patrons/proprietors, who have little or no training/knowledge on infection control practices. Improper management and supervision of abattoir activities is also a major source of risk to public health (World Bank, 1995). Abattoir floor, beef wash water, utensils (e.g., knives), tables and workers have previously been reported as a major source of contamination of beef in slaughter houses (Omoruyi et al., 2011; Uzoigwe et al., 2021). This is in keeping with the report of the current study, were wash water samples, handlers, air flora and waste water were reported to harbor aerobic and coliform bacteria.

Pseudomonas aeruginosa is an opportunistic pathogen in environmental waters, and a common inhabitant in abattoir environment (Igbinosa et al., 2012; Igbinosa and Obuekwe, 2014). This bacterium exhibits high level of resistance to a large number of antibiotics, and are predominantly multidrug resistant. Multidrug resistant *Pseudomonas aeruginosa* is a pervasive and growing environmental problem, making them a threat to public health. *Pseudomonas aeruginosa* have also been reported in an epidemiological study (Mushin and Ziv, 1973), with the gut, wash water, and udder being major reservoirs in the spread of the bacterium.

Providencia species are opportunistic pathogens of clinical significance (Wie, 2015), and are scarcely reported in abattoir facilities globally. Of the nine species of *Providencia*, *P. stuartii* is the most frequently encountered of all *Providencia* species, especially in human pathogen and are mostly found in hospital environment and particularly frequent in the urinary tract of chronically catheterized patients in hospitals and long-term care facilities (Liu et al., 2020). To date, there is only one report on the isolation of *Providencia stuartii* from abattoir effluent in Nigeria (Ogunnusi and Olorunfemi, 2018). Although abattoir effluent is generally reported as a major reservoir of other *Providencia* species such as *P. alcifaciens*, report on the prevalence of *Providencia stuartii* in environmental matrix, remain scarce. One possible reason being that bacterial identification in most studies is limited to morphological and biochemical characteristics, especially in developing nations, where abattoir maintenance remains a public health challenge. *Providencia rettgeri* on the other hand is also of potential public health concern and have been previously reported in sheep abattoir effluent in Sweden (Soderquist et al., 2012). Furthermore, the presence of multidrug resistant *Providencia stuartii* and *Providencia rettgeri* in retail beef (Nossair et al., 2015; Di et al., 2018), is an indication that these bacteria could be present in abattoir and abattoir environment, going through the food chain, to contaminate ready-to-eat animal products, and should be taken seriously, considering their pathogenic potentials. Both species of *Providencia* reported in this study, have also been reported in chicken, beef and pork, as well as stool of patients with diarrhea (Shima et al., 2016). Considering their high prevalence is different meat sources, Shima et al. (2016) concluded that retail meat are the major source of *Providencia* infections in humans.

Table 2: Prevalence (%) of bacteria isolated from selected abattoirs in Benin City, Edo State, Nigeria.

ISOLATE	**SAMPLING SITE**						
	Accession number	**Osazee (N = 48)**	**Freedom (N = 48)**	**Bob- Izua (N = 48)**	**Holy Ghost A (N = 48)**	**Holy Ghost B (N = 48)**	**Lawal & Sons (N = 48)**
Pseudomonas aeruginosa	**ON258647**	28 (47)	16 (47)	12 (47)	22 (47)	11 (47)	11 (47)
Enterobacter ludwigii	**ON258664**	14 (34)	27 (34)	18 (34)	9 (34)	14 (34)	18 (34)
Providencia stuartii		02 (31)	02 (31)	37 (31)	43 (31)	10 (31)	6 (31)
Klebsiella quasipneumoniae	**OM751839**	0 (1)	0 (1)	100 (1)	0 (1)	0 (1)	0 (1)
Enterococcus saccharolyticus		22 (19)	12 (19)	22 (19)	24 (19)	10 (19)	10 (19)
Providencia rettgeri	**ON394533**	10 (17)	38 (17)	7 (17)	14 (17)	27 (17)	4 (17)

Key: N = No. of samples; Total number of isolates in bracket

Table 3: Antibiotic susceptibility profile of bacterial isolated from selected abattoirs in Benin City, Edo State, Nigeria.

Antimicrobial agent	Antimicrobial classes	*Pseudomonas aeruginosa* (N = 47)		*Enterobacter ludwigii* (N = 34)		*Providencia stuartii* (N = 31)		*Klebsiella quasipneumoniae* (N = 01)		*Enterococcus saccharolyticus* (N = 19)		*Providencia rettgeri* (N = 17)	
		S	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**
Ceftazidime	*β*-lactam	0	100	0	100	0	100	0	100	0	100	0	100
Cefuroxime	*β*-lactam	0	100	0	100	0	100	0	100	0	100	0	100
Gentamicin	Aminoglycoside	10	90	17	83	100	0	0	100	100	0	0	100
Cefixime	*β*-lactam	0	100	0	100	100	0	100	0	27	73	0	100
Ofloxacin	Fluoroquinolones	63	37	53	47	100	0	0	100	100	0	100	0
Augmentin	*β*-lactam	0	100	0	100	0	100	100	0	0	100	0	100
Nitrofurantion	Nitrofuran	0	100	9	91	68	32	0	100	0	100	100	0
Ciprofloxacin	Quinolones	100	0	100	0	0	100	100	0	100	0	19	81
Colistin	Polymyxin	91	09	91	09	84	16	100	0	100	0	100	0

KEY: R: Resistant, S: Sensitive, N: Number of isolates

Table 4: Prevalence of colistin resistant gene in bacterial isolated from abattoir environment in Benin City, Edo State, Nigeria.

ISOLATE	**COLISTIN RESISTANT GENES**							
	mcr-1	*mcr-2*	*mcr-3*	*mcr-4*	*mcr-5*	*mcr-6*	*mcr-7*	*mcr-8*
Pseudomonas aeruginosa	+	-	-	-	-	-	-	-
Enterobacter ludwigii	+	-	-	-	-	-	-	-
Providencia stuartii	+	-	-	-	-	-	-	-
Klebsiella quasipneumoniae	-	-	-	-	-	-	-	-
Enterococcus saccharolyticus	-	-	-	-	-	-	-	-
Provincia rettgeri	-	-	-	-	-	-	-	-

Food animals are considered key reservoirs of antibiotics resistant bacteria with increased and indiscriminate use of antibiotics in food production chain reported to have contributed to the global challenges of antibiotics resistance (Founou et al., 2016). As with other food animals, such as poultry, swine, sheep and goat, antibiotics resistant bacteria have also been reported in cattles, abattoir environment (Igbinosa et al., 2012) as well as processed beef (Di et al., 2018). This situation, although high in developing countries, owing to self-medication, abuse and public vending of antibiotics, antibiotics resistant bacteria have now become a global threat, with no geographic boundaries to impede their worldwide spread. In the past, studies on antibiotics resistance were largely phenotypic, but have evolved to antibiotics gene detection, using molecular approach, making it possible to compare and predict the origin as well as evolution of such resistance. The gene for antibiotics resistance is usually transferred from one bacterium to another via direct and indirect contact, and could withstand conventional and advanced treatment techniques (Savin et al., 2020). This outcome of this study is an indication that plasmid-borne mobilizable colistin resistant and multidrug resistant bacteria are present in abattoir facilities in Benin City, Edo State, Nigeria. Effort must therefore be made to limit the usage and abuse of colistin and other antibiotics.

Conclusion/Recommendation

Plasmid-borne mobilizable colistin-resistant bacteria and other multi-drug resistant bacteria are present in abattoir environment in Benin City, Edo State, Nigeria. The presence of these isolates was independent of the location of abattoir, as well as social class for whom beef is processed for. It is therefore recommended as follows;

i. The indiscriminate use of antibiotics in cattle rearing should be discouraged.
ii. The hygienic conditions of abattoirs located in Benin City, Edo State, Nigeria must be closely monitored by the relevant authorities.
iii. Appropriate sanctions must be given to defaulters of relevant safety guidelines, governing the operations of abattoirs in Nigeria.
iv. Proprietors of abattoirs should engage staff knowledgeable in infection control practices, to oversee the safety operations of the abattoirs.

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Conflict of Interest: The Authors declare that there are no potential conflicts of interest.

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