Antibiogram Profile of *Pseudomonas aeruginosa* from a Cattle Farm Located in Epe Lagos State, Nigeria

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Abstract: Antibiotic resistant bacteria (ARB) may be transmitted from livestock to humans through the oral-faecal route. *Pseudomonas aeruginosa* has been reported to have intrinsic resistance to many antibiotics combined with its ability to easily acquire antibiotic resistance determinants, which poses a significant threat to public health. This study investigated the presence of *Pseudomonas aeruginosa*, and also determined the antibiotic resistance profiles of the strains to other classes of antibiotics, in a cattle farm in Epe, Lagos State, Nigeria. Sixteen *P. aeruginosa* strains were isolated after the standard microbiological methods and biochemical testing. The strains were subjected to antimicrobial susceptibility testing, through the disc diffusion method against seven antimicrobial agents, and the minimum inhibitory concentration (MIC) for carbapenem (imipenem) antibiotics. Resistance was found in all antibiotics tested. The highest resistance values were observed for imipenem (68.75%), chloramphenicol (37.50%) and gentamicin (31.25%). The *P. aeruginosa* isolates also exhibited resistance to ciprofloxacin (12.50%), amoxicillin (12.50%), streptomycin (12.50%), and sparfloxacin (6.25%). The MIC values ranged between 8.0 and 32 µg of imipenem, with a resistance prevalence of 68.75% of the isolates. The findings highlight the significance of awareness and proactive actions in agricultural settings to protect public health and maintain the efficacy of current antibiotics. Also, there is an urgent need for increased surveillance and effective control methods to limit the spread of antibiotic-resistant bacteria.

Key word: Antibiotics, multidrug resistance, Cattle farm; *Pseudomonas aeruginosa*

INTRODUCTION

Resistance to antibiotics in the treatment of bacterial infections has remained a prevailing threat in clinical healthcare and veterinary management, making the treatment of some bacterial infections insurmountable. The spread of antibiotic resistance continues to be a public health problem and can be transferred between humans, animals, and the environment (Larsson *et al*., 2022). The main routes of antibiotics in the environment are the discharge of antibiotic residues during production processes and after their usage in human medicine that is, through clinical and urban wastewater (Garcia-Torné *et al*., 2025; Obayiuwana *et al*., 2025), as well as in veterinarian practices, including large-scale farming for food production and aquaculture (Ibekwe *et al*., 2023).

Pseudomonas aeruginosa among other Gram-negative bacteria has been listed as high-burden resistant pathogens in the WHO bacterial priority pathogens list (BPPL) (WHO, 2024). According to the report, the addition of these pathogens to the list emphasizes their global impact in terms of burden, as well as issues related to transmissibility, treatability, and prevention options (WHO 2024). A particular group of relevance amongst the *Pseudomonas aeruginosa* group is the carbapenem resistance *Pseudomonas aeruginosa* (CRPA) (Bakhat *et al*., 2019), that have developed resistance against carbapenem antibiotics (WHO, 2017). The carbapenem group of antibiotics has been reported as the last resort for the treatment of infections of Gram-negative bacteria. This antibiotic has ultimately become less effective due to the rapid rise of resistance strains among these bacteria (Buehrle *et al*., 2017).

The frequent use of antibiotics in animal feed and the treatment of ill animals, tend to presuppose that animal farms are thought to be potential breeding grounds for bacteria resistant to antibiotics (Ibekwe *et al*., 2023). The soil and drinking water of animal farm habitats have been found as a possible reservoir of *Pseudomonas aeruginosa*. A

comprehensive study on the antibiogram profile, virulence, and antibiotic resistance genes of environmental *Pseudomonas aeruginosa* in slaughterhouses, gave useful insights in their development of multidrug resistance and the presence of resistance genes strains (Al-Kadmy, *et al.*, 2024). Similarly, another comprehensive study established the prevalence of multidrug-resistant *Pseudomonas aeruginosa* isolated from dairy cattle, milk, environment, and even farm workers' hands from samples collected from farms and households (Badawy *et al*., 2023). Other studies have also reported the direct isolation of strains of *Pseudomonas aeruginosa* from farm animals with high antimicrobial resistance patterns and virulence gene contents (Morales-Espinosa *et al*., 2024 and Siddique *et al*., 2025).

Considering the high risk of transmission of *Pseudomonas aeruginosa*, particularly the CRPA to both humans and animals when these antibiotic-resistant bacteria are present in animal farm contexts, this study was aimed at evaluating the incidence of *Pseudomonas aeruginosa* in a cattle farm with a large stream of human and animal activities. Overall, this study investigated the prevalence and distribution of *Pseudomonas aeruginosa* in environmental samples, namely soil and water samples from the cattle farm.

MATERIALS AND METHODS

Study site: The sample site was a massive land area comprising several slaughterhouses, which are also referred to as abattoirs, sited at the popular T-junction in Epe, Lagos metropolis, Nigeria. This community houses several activities involved in rearing livestock, mainly beef cattle, reared essentially for their meats. Activities such as stunning, exsanguination, skinning, evisceration, and so on, take place within the facilities, with feaces, wastewaters, litters of hays, and silages were observed all over the vicinity.

Sample collection: A total of sixteen (16) samples were taken from the cattle farm environment (soil, n = 8, and drinking water, n = 8). The samples were collected between October 2022 and February 2023 at the slaughterhouse previously described. The cattle farm environmental samples (soil and their drinking water) were aseptically collected from the herd and dispensed into sterile screw-cap containers in order to form composite samples. The water samples were collected aseptically in sterile 2-l glass bottles. All samples were collected in duplicates and used to form composite samples. Samples were all adequately labelled and transported to the Microbiology laboratory of the Department of Biological Sciences, Augustine University, for bacteriological analysis. Samples were kept at 4˚C in the dark and were processed within 24 hours of collection.

Bacteriological analysis of samples: The water and soil samples were allowed to resume room temperature at 25˚C on the bench before bacterial isolation. The water samples were homogenized before being diluted serially in ten-fold. The soil sample was mixed into 9.0 ml of sterile peptone water and was serially diluted in 10 folds. After serially diluting the samples, 1.0 ml aliquots were taken from pertinent dilutions (10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6}) aseptically introduced into sterile Petri plates using 1000 µl micropipettes. The pour plate method was used to aseptically pour well-prepared, sterile Pseudomonas agar, Mac-Conkey agar, and blood agar into the Petri plates, and incubated for 24 hours at 37˚C in an aerobic atmosphere. Predicted big, flat, blue-green-pigmented colonies on Pseudomonas agar with irregular borders were sub-cultured and purified on nutrient agar after incubation. Accordingly, sixteen (16) assumed colonies from the positive Pseudomonas, blood, and Mac-Conkey agar plates were transferred onto nutrient agar plates for subculturing of the isolates.

Identification of bacterial isolates: The bacterial isolates were identified using standard cultural, morphological and biochemical tests which include Gram staining, motility test, catalase test, citrate

utilization test, triple sugar Iron test, oxidase test, methyl red/Voges-Proskauer (MRVP) test, indole test, urease test, starch hydrolysis test, gelatin hydrolysis test, tryptophan deaminase test, tartrate utilization test, coagulase test, haemolysis test and carbohydrate fermentation tests. The identities of the isolates were confirmed by comparing the results of the standard tests to the taxonomic scheme of the Bergey's Manual of Determinative Bacteriology (Krieg *et al*, 2010) and online identification software (https://www.microrao.com/).

Antibiotic susceptibility test

Screening for antimicrobial susceptibility using the disk diffusion method: The disc diffusion method was used to determine the antibiotic susceptibility of the 16 identified probable *Pseudomonas aeruginosa* isolates to selected antibiotics. The antibiotic sensitivity discs (Maxicare Laboratories) of chloramphenicol (30 µg), sparfloxacin (10 µg), ciprofloxacin (10 µg), amoxicillin (30 µg), gentamycin (10 µg), streptomycin (30 µg) and imipenem (10 µg) were used. The tests were carried out according to the guidelines as provided in the Clinical and Laboratory Standards Institute (CLSI, 2018) with modified Kirby-Bauer disc diffusion technique as recommended by the World Health Organization. An overnight culture of each isolate was prepared in nutrient broth (Biolab, Canada) and incubated at 37°C for 18 hours. The culture was adjusted to a 0.5 McFarland standard. Dry sterile plates of Mueller-Hinton agar (Oxoid, UK) were prepared, and each of the isolates was aseptically and uniformly inoculated onto the Mueller-Hinton agar plates using sterile swab sticks. The sensitivity discs were aseptically and carefully layered on each plate using sterile forceps, and the plates were incubated overnight at 37°C, after which zones of growth inhibition around each disc were measured and interpreted by the zone breakpoint standards of the Clinical and Laboratory Standards Institute (Bauer *et al*., 1966; CLSI, 2018).

Determination of minimum inhibitory concentration (MIC): The CLSI Standards guidelines (CSLI, 2020) were followed in determining the MICs of the carbapenem antibiotic under study, against the 16 probable *Pseudomonas aeruginosa* isolates. This was determined by a standard two-fold serial broth microdilution method using Mueller–Hinton broth according to the CLSI standards guidelines (CLSI, 2018). Sixteen of the isolates were treated with the imipenem antibiotic, with concentrations solution ranging from 2 to 512 g/ml. The prevalence of resistance was determined by dividing the number of resistant bacteria by the total number of strains in the population (Li *et al*., 2010). *Escherichia coli* ATCC 25922 was used as reference strain (CLSI, 2018).

RESULTS

The plate counts of the bacterial colonies were carried out within 24 hours and 48 hours. The different samples collected from the slaughterhouse showed relatively high number of colony forming units per milliliter (cfu/ml) including the drinking water given to the herd. After 48 hours of incubation, the total cell counts of the samples for drinking water, and soil ranged between 9 .0 × 10^4 and 3.3 × 10^7 cfu/ml, and 8.5 × 10^4 - 3.0 x 10^7 respectively (Table 1). The observations are in the average values of triplicate plating for each of the samples.

On *Pseudomonas* agar medium, colonies of irregular borders with big, flat, blue-green-pigmented were selected. After the Grams, biochemical and sugar testing, sixteen (16) most probable phylogenetic identities of *Pseudomonas aeruginosa* were revealed.

The *Pseudomonas aeruginosa* species isolated from the slaughterhouse samples showed relatively high resistance (68.75%) to imipenem, with none of the isolates susceptible to the antibiotics. The *Pseudomonas* spp showed highest susceptibility to streptomycin at 87.5%, followed by amoxicillin with a prevalence of 81.25% (Table 2). However, the least resistance was found in only one of the isolates to sparfloxacin with prevalence at 6.25%. This was followed by resistance to amoxicillin, ciprofloxacin and streptomycin by only 2 of the bacterial isolates at prevalence of

12.5% (Table 2). The *Pseudomonas* isolates showed resistance to imipenem at 68.75% prevalence, the highest rates of resistance in the study, followed by chloramphenicol and gentamycin with resistance prevalence of 37.50% and 31.25% respectively (Figure 1).

The minimum inhibitory concentration (MIC) of the test antibiotics carbapenem (imipenem) shows that the resistance prevalence for the antibiotics was relatively high in all the *Pseudomonas* species at an MIC range of 8.0 to 32 µg of the antibiotics. The findings show that 11(68.75%) of the 16 *Pseudomonas* isolates were resistant to the imipenem antibiotics (Table 3). As shown in Table 3, the antibiotic levels of the bacterial communities in all bacterial isolates were reflected by the MIC50s and MIC90s, which represent MICs required for the inhibition of 50% and 90% of bacterial strains, respectively. The isolates had the MIC50 and MIC90 values of 8.0 and 16 µg/ml respectively.

Table 1: Plate Counts of Bacterial Isolates from Collected Sample (Cattle farm)

Sample	Dilution Factor	Vol. of Sample Plated (ml)	Number of Colonies	cfu/ml
Water 1	10^{-4}	1	75	7.5×10^5
	10^{-6}	1	33	3.3×10^7
Water 2	10^{-3}	1	90	9.0×10^4
	10^{-5}	1	35	3.5×10^6
Soil 1	10^{-4}	1	70	7.5×10^5
	10^{-6}	1	30	3.0×10^7
Soil 2	10^{-3}	1	85	8.5×10^4
	10^{-5}	1	40	4.0×10^6

Table 2: Antimicrobial Susceptibility Showing the Resistant Pattern of the Isolated *Pseudomonas sp*. Expressed in Percentage

Antibiotic	No of Isolates	Sensitive Number (%)	Intermediate Number (%)	Resistance Number (%)
Sparfloxacin (SP)	16	12 (75.00%)	3 (18.75%)	1 (6.25%)
Gentamycin (CN)	16	5 (31.25%)	6 (37.50%)	5 (31.25%)
Chloramphenicol (CH)	16	4 (25.00%)	6 (37.50%)	6 (37.50%)
Amoxicillin (AM)	16	13 (81.25%)	1 (6.25%)	2 (12.50%)
Ciprofloxacin (CPX)	16	11 (68.75%)	3 (18.75%)	2 (12.50%)
Streptomycin (S)	16	14 (87.50%)	0 (0%)	2 (12.50%)
Imipenem (I)	16	0 (0.00%)	5 (31.25%)	11 (68.75%)

Table 3: Minimum Inhibitory Concentration (MIC) of Imipenem Antibiotics against Sixteen *Pseudomonas aeruginosa* Isolates

	Imipenem (Carbapenem Antibiotics)	
Isolates Code	**MIC µg/ml**	**Interpretation**
CFAU01	**08**	**R**
CFAU02	**16**	**R**
CFAU03	**08**	**R**
CFAU04	**06**	**I**
CFAU05	**08**	**R**
CFAU06	**08**	**R**
CFAU07	**08**	**R**
CFAU08	**08**	**R**
CFAU09	**32**	**R**
CFAU10	**06**	**I**
CFAU11	**08**	**R**
CFAU12	**08**	**R**
CFAU13	**06**	**I**
CFAU14	**08**	**R**
CFAU15	**06**	**I**
CFAU16	**04**	**I**

Key: R=Resistance, I = Intermediate

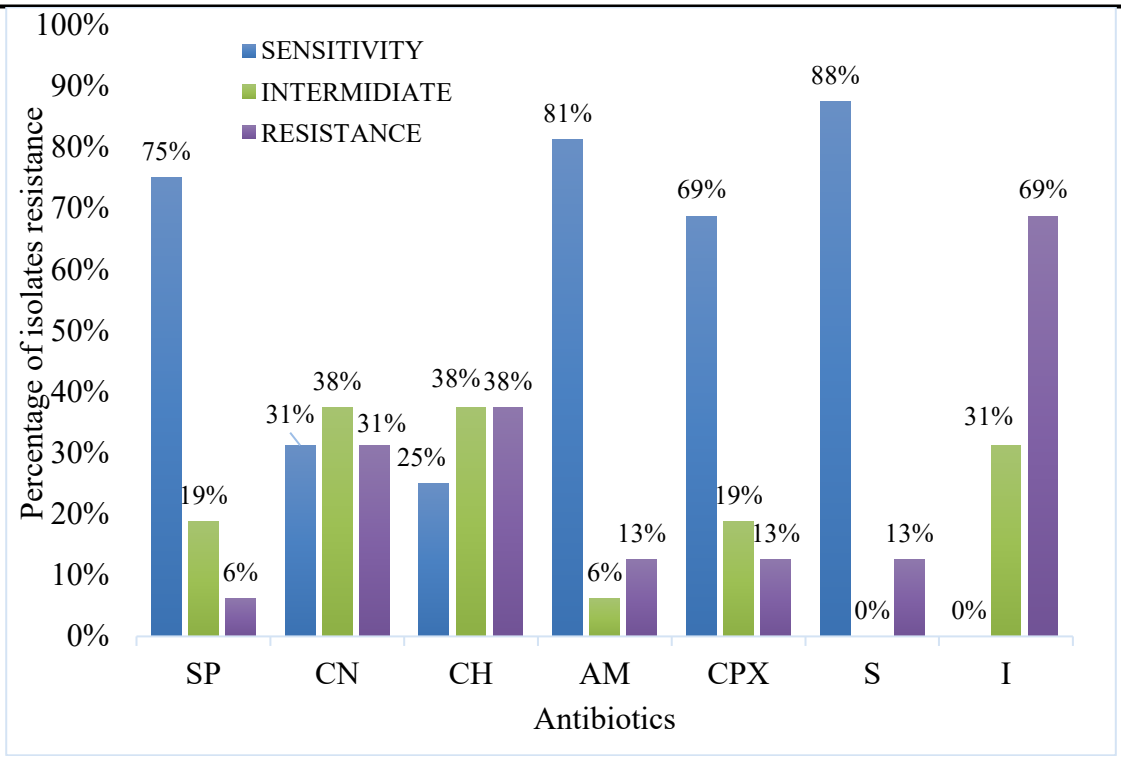

Figure 1: Antimicrobial susceptibility test of the bacterial isolates
Keys: SP = Sparfloxacin, CN = Gentamycin, CH = Chloramphenicol, CPX = Ciprofloxacin, AM = Amoxicillin, S = Streptomycin, I = Imipenem

DISCUSSION

It is becoming extremely difficult to treat infections due to the global problem of antimicrobial agent resistance. This has mostly been linked to the use of antimicrobials in clinical treatments and agricultural practices (Aljeldah, 2022). According to how urgently new antibiotics are needed, the WHO list is classified into three categories to include critical, high, and medium priority. One of the top pathogens on WHO's 2017 list of priority pathogens for research and development of new antibiotics is carbapenem-resistant *Pseudomonas* (WHO, 2017). It is the most dangerous species of the *Pseudomonas*. *Pseudomonas aeruginosa* can cause disease conditions resulting in serious infections that are frequently fatal, like pneumonia and bloodstream infections. This study established the occurrence of carbapenem resistance *Pseudomonas aeruginosa* on a selected cattle farm in Epe, Lagos. An area of the world where little is known about antibiotic use and its contribution to the development and dissemination of resistance to antibiotics in animal husbandry (Adelowo *et al*., 2014). A primary limitation in this study is the difficulty encountered in sample collection, with the non-compliance of most of the farmers which reduced the number of isolates used in the analysis.

The burden of *Pseudomonas aeruginosa* has a substantial effect on cattle production, public health, and the global economy due to the rise in antibiotic resistance. This study shows a high count for the bacterial community in both drinking water and soil samples. *Pseudomonas aeruginosa* isolates were also obtained from both the study farm's drinking water and soil samples drinking water and soil samples from the study farm. These findings become very worrisome when these drinking water sources are given directly to the cattle, and the soil with the feaces are applied directly to farmlands, establishing the horizontal transfer of resistance determinants between humans and animals (Wang *et al*., 2024), such practice is regarded as direct

consumption. An improved hygiene, given the right environmental conditions, could help to have a better understanding of how *Pseudomonas aeruginosa* spreads and can be better controlled. Also, the prevalence of carbapenem resistance varies depending on regional antibiotic policies, the source of the strain, and geographic location.

The findings of this study demonstrated the existence of *Pseudomonas* spp which is known as being resistant to carbapenem antibiotics on the cattle farm. Using the Kirby-Bauer disc diffusion method, the antibiotic susceptibility profiles of the *Pseudomonas* isolates were revealed, with their high resistance to imipenem (68.75%). It also reveals multidrug resistance (MDR) *P. aeruginosa* by use of other antibiotic classes in the disc diffusion approach. The current investigation revealed that 65% of the *P. aeruginosa* from the cattle farm samples (water and soil) were resistant to imipenem (Carbapenem). Similar results were obtained by Rehab *et al.* (2022), who reported that 69% of their isolates of *P. aeruginosa***,** exhibited a 68.75% Imipenem resistance prevalence in their investigation. This study also showed a high percentage (68.75) of *Pseudomonas* species showed resistance to Imipenem in the MIC test. Similarly, the work of Ahmed *et al* (2022) also revealed the same level of resistance (70%) for MIC analysis *of Pseudomonas aeruginosa* strains to Meropenem in their study.

CONCLUSION

According to these findings, there may be a cross-transmission of *Pseudomonas aeruginosa* (CRPA) between cattle and their immediate environment as well as between the cattle and humans, giving rise to possible public health concerns, considering how these slaughterhouses are being operated. The MIC results for the bacterial isolates reveal a 68.75% resistance to carbapenem (Imipenem). This finding is very worrisome considering that carbapenem is a broad-spectrum antibiotic reserved for the treatment of severe bacterial infections, such as from MDR bacteria. The findings from this study give insight on the need to implement targeted strategies to combat antimicrobial resistance and protect human and animal health. To prevent the emergence of resistance, comprehensive antibiotic stewardship procedures, including the prudent use of antibiotics, must be implemented in animal husbandry. Additionally, encouraging excellent cleanliness habits and appropriate waste management on farms can aid in lowering the risk of the spread of bacteria that are resistant to antibiotics.

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