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Environmental occurrence of carbapenem-resistant *Pseudomonas aeruginosa* harbouring clinically relevant carbapenemase genes in poultry farm soils in Lagos, Nigeria

Abstract

Carbapenem-resistant *Pseudomonas aeruginosa* (CRPA) represents a major global public health threat, yet data on its occurrence in non-clinical environments remain limited, particularly in low- and middle-income countries. Agricultural settings, especially poultry farms, may serve as underrecognized reservoirs for carbapenem-resistant bacteria. Thirty poultry litter–contaminated soil samples were collected from three poultry farms in Lagos State, Nigeria. Isolation of presumptive CRPA was performed using cetrimide agar supplemented with meropenem. Phenotypic carbapenem resistance was assessed by Kirby–Bauer disk diffusion using meropenem and imipenem in accordance with CLSI guidelines. Molecular identification was confirmed by 16S rRNA gene sequencing. Polymerase chain reaction was used to detect carbapenemase-encoding genes (bla_{IMP}, bla_{VIM}, bla_{KPC}, bla_{OXA-48}, *and* bla_{OXA-1}). A total of 41 carbapenem-resistant *Pseudomonas* isolates were recovered, of which 38 were confirmed as *P. aeruginosa*. Molecular screening revealed the presence of bla_{IMP} in 31.57% of isolates, bla_{KPC} in 18.42%, and bla_{VIM} and bla_{OXA-48} each in 7.89%, while bla_{OXA-1} was not detected. All positive isolates exhibited phenotypic resistance to both meropenem and imipenem. This study demonstrates that poultry farm soils harboured carbapenem-resistant *P. aeruginosa* carrying clinically significant carbapenemase genes. These findings highlight agricultural environments as important reservoirs for carbapenem resistance and underscore the need to expand antimicrobial resistance surveillance beyond clinical settings. Strengthened antimicrobial stewardship in poultry production and inclusion of environmental monitoring are essential components of effective AMR containment strategies in Nigeria and similar settings.

Keywords: Carbapenem-resistant, *Pseudomonas aeruginosa*, poultry, 16S rRNA, carbapenemase

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Eddison I Oghonyon,[1] Nzube F Ekpunobi,[2] Sandra C Okoye,[3] Theresa Ariri,[4] Michelle C Okoye[5]

[1]Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, David Umahi Federal University of Health Sciences, Nigeria
[2]Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Nigeria
[3]Department of Biological Sciences, College of Liberal Arts and Sciences, Eastern Illinois University, USA
[4]Department of Biology, College of Arts and Sciences, Texas A and M University, USA
[5]Department of Mid-wifery, School of Mid-wifery, Alex Ekwueme federal university teaching hospital, Nigeria

Correspondence: Nzube F Ekpunobi, Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Nigeria

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Introduction

The increasing resistance of potentially pathogenic bacteria to multiple conventional antibiotics is an urgent problem in global public health.[1] *Pseudomonas aeruginosa* is one of the major causes of diseases such as otitis, mastitis, endometritis, hemorrhagic pneumonia and urinary tract infections in both livestock and companion animals.[2] The multiple-drug-resistant (MDR) *Pseudomonas* can be transmitted from different sources to humans and also to the environment through horizontal gene transfer. The emergence and occurrence of MDR *P. aeruginosa* strains are growing in the world, leading to limited therapeutic options.[3]

In recent years, enough evidence highlighting a link between excessive use of antimicrobial agents and antimicrobial resistance from animals as a contributing factor to the overall burden of antimicrobial resistance has emerged.[4] The extent of usage is expected to increase markedly over the coming years due to the intensification of farming practices in most of the developing countries.[5] The main reasons for the use of antibiotics in food-producing animals include prevention of infections, treatment of infections, promotion of growth and improvement in production in the farm animals. There are also human health concerns about the presence of antimicrobial residues in meat, eggs and other farm products.[6,7] Generally, when an antibiotic is used in any setting, it eliminates the susceptible bacterial strains, leaving behind those with traits that can resist the drug. These resistant bacteria then multiply and become the dominating population and, as such, can transfer (both horizontally and vertically) the genes responsible for their resistance to other bacteria.[8]

As critical broad-spectrum agents of last resort, resistance to carbapenems correlates directly with rising infection rates, increased mortality, prolonged hospitalisations, and escalating healthcare expenditures.[9] A recent meta-analysis by Ramatla *et al.*[10] identified a global pooled prevalence of 34.7% for carbapenem-resistant *Pseudomonas aeruginosa* (CRPA), with regional estimates for Asia, Africa, Europe, South America, and North America reaching 32.8%, 38.5%, 47.6%, 40.9%, 33.3%, and 47.8%, respectively. These figures represent a significant surge compared to the 8% reported for sub-Saharan Africa in 2022 and the 21.36% continental prevalence observed previously.[11] Such variations in resistance rates are likely driven by disparities in national antibiotic consumption patterns. Subgroup analysis at the continental level revealed that the current burden exceeds the 15% CRPA prevalence reported in Ethiopia by Gobezie *et al.*[12] Furthermore, Africa experienced a sharp rise in CRPA prevalence, jumping from 16.7% during the 2018–2021 period to 38.5% between 2022 and 2024.[10] These consistent periodic increases underscore a burgeoning global crisis in CRPA-related infections.

Despite increasing reports of CRPA in clinical settings, data on carbapenem-resistant P. aeruginosa in poultry-associated soils in Nigeria remain scarce. Thus, this study aims to evaluate the prevalence

of CRPA within farm soil samples and to characterise the genetic determinants responsible for the carbapenem resistance observed in these isolates.

Methodology

Sample collection

This study was carried out on 30 soil samples. These samples were collected from three selected farm locations in Lagos State. 10 soil samples were collected from each of the poultry farms and placed in sterile containers, and immediately sent to the laboratory for further processing.

Isolation and identification

The isolation was done as described by Abdulrahman and Saleh[13] with some modifications. From each soil sample, 1g of the poultry faecal littered soil was suspended in 9mL of sterile distilled water, followed by successive serial dilutions by transferring 1mL of an aliquot into another 9mL of sterile distilled water and continuing in this manner till a 10^{-5} dilution was reached. An aliquot of 0.1mL of 10^{-3} and 10^{-5}each was plated by the spread plate method on cetrimide agar and in the presence of meropenem antibiotics, allowing only those that are resistant to meropenem to grow, and it was immediately incubated at 37 °C for 24-48hours. The *Pseudomonas* spp. was identified to be *Pseudomonas aeruginosa* because it produced yellow-green/ yellow-brown colonies (pyoverdin). Colonies of the presumptive *Pseudomonas aeruginosa* were isolated, obtained as pure cultures and stored.

Molecular identification/characterization of bacterial isolates

Genomic DNA was extracted using the Quick-DNA™ Fungal/ Bacterial Miniprep Kit according to the manufacturer's instructions. The 16S rRNA gene was amplified using universal primers 27F and 1492R in a 25µl reaction volume containing PCR Master Mix. Thermal cycling (Eppendorf Nexus Gradient) involved initial denaturation at 94°C for 30s, followed by 35cycles of 94°C (20s), 54°C (45s), and 72°C (1min), with a final 5-minute extension. Amplicons were resolved on a 1.5% agarose gel and visualised via UV transillumination. Purified products were sequenced and identified by comparing results against the NCBI GenBank database using the BLASTN program.

Carbapenem susceptibilty screening tests

To screen for carbapenem resistance, bacterial suspensions from 18–24h cultures were standardized to 0.5 McFarland units and streaked onto Mueller-Hinton agar. Meropenem (10µg) and Imipenem (10µg) discs were applied using the Kirby-Bauer method, followed by incubation at 37°C for 24h. Following CLSI[14] guidelines, isolates were classified as resistant, intermediate, or susceptible based on measured zones of inhibition. Isolates displaying simultaneous resistance to both meropenem and imipenem were defined as carbapenem-resistant.

Molecular detection of carbapenem-resistant encoding genes

PCRs for detection of the $bla_{IMP,}$ $bla_{VIM,}$ $bla_{KPC,}$ $bla_{OXA-48,}$ *and* bla_{OXA-1} genes. Specific primers for amplifying the selected genes by PCR. The reaction mixture consisted of 25µl Platinum™ HotStart PCR Master Mix (Invitrogen™), 1µl DNA extract, 0.5µl of each primer in a concentration of 20pmol, and nuclease-free water up to 50µl. The cycling conditions included denaturation for 10min at 95°C, amplification for 30 cycles of 30s at 95°C, 1min at 55°C, and 1 min at 72°C, and extension for 10min at 72°C.

Results

Thirty (30) soil samples from the locations were examined for the presence of carbapenem-resistant *P. aeruginosa,* and 41 isolates of carbapenem-resistant *P. aeruginosa* were presumptively identified from the 30 samples.

Molecular identification confirmed that only 38 of the presumptively identified carbapenem-resistant isolates were *P. aeruginosa.*

The isolates were further characterised for the presence of the carbapenemase encoding resistance genes; $bla_{IMP,}$ $bla_{VIM,}$ $bla_{KPC,}$ $bla_{OXA-48,\ and}$ bla_{OXA-1} (Table 2).

Table 1 List of Primers used for this study

Gene	Sequence	Annealing temp	Amplicon size
blaIMP	F:TGAGCAAGTTATCTGTATTC	52	740
	R:TTAGTTGCTTGGTTTTGATG		
blaVIM	F:TTGGTCTACATGACCGCGTCT	55	747
	R:TTTGACAACGTTCGCTGTGT		
blaKPC	F:ATGTCACTGTATCGCCGTCTA	55	821
	R:TCGCTGTGCTTGTCATCCT		
blaOXA-48	F:TTGGTGGCATCGATTATCGG	55	744
	R: GAGCACTTCTTTTGTGATGGC		
blaOXA-1	F:ACACAATACATATCAACTTCGC	50	814
	R:AGTGTGTTTAGAATGGTGATC		

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Table 2 Occurrence of carbapenem-resistant encoding genes in *P. aeruginosa* isolates

GENE	Number of samples positive	Occurrence (%)
bla_{IMP}	12	31.57
bla_{OXA-48}	3	7.89
bla_{VIM}	3	7.89
bla_{KPC}	7	18.42
bla_{OXA-1}	0	0

From the study, 12 out of 38 isolates were positive for bla_{IMP}, 7 of the isolates were positive for $bla_{KPC,}$ and 3 were positive for bla_{OXA-48} *and* $bla_{VIM,}$ respectively

Discussion

The global emergence of carbapenem-resistant bacteria (CRB) poses a critical threat to public health.[15-17] Despite their clinical importance, the prevalence and specific characteristics of CRB in non-clinical environments remain insufficiently understood.[10,18] Beyond traditional healthcare settings, agricultural operations are increasingly recognised as contributors to the environmental dissemination of these pathogens.[6]

Recent studies, particularly in Europe, have documented the presence of CRB in both agricultural and non-agricultural soil samples, suggesting that soil serves as a significant, yet underrecognized, reservoir for resistance.[19,20]

This study demonstrates the presence of carbapenem-resistant *Pseudomonas aeruginosa* (CRPA) in poultry farm soils in Lagos State, Nigeria, providing further evidence that non-clinical environments contribute meaningfully to the global epidemiology of antimicrobial resistance (AMR). The recovery of 38 molecularly confirmed CRPA isolates from poultry litter–contaminated soils highlight the role of agricultural ecosystems as reservoirs for clinically relevant resistance determinants.

The selective isolation of *P. aeruginosa* on meropenem-supplemented media suggests sustained antimicrobial selective pressure within poultry farm environments. In Nigeria and other low- and middle-income countries, antimicrobials are widely used in poultry production for disease prevention and growth promotion, often in the absence of regulatory oversight.[21,22] Such practices facilitate the persistence and amplification of resistant bacteria in soil matrices enriched with animal waste, creating opportunities for environmental maintenance and dissemination of resistance genes.[23]

Phenotypic susceptibility testing confirmed resistance to both meropenem and imipenem among the isolates, consistent with CLSI-defined carbapenem resistance. The detection of CRPA outside healthcare facilities is of particular concern, given the critical role of carbapenems as last-line agents for severe *P. aeruginosa* infections. Increasing reports of environmental and community-associated CRPA suggest that resistance is no longer restricted to hospital settings, but rather circulates across interconnected ecological compartments.[24,25] This environmental circulation may facilitate reintroduction of resistant strains into clinical settings, complicating infection control efforts.[7,26]

Molecular characterisation revealed the presence of multiple carbapenemase-encoding genes, with bla_{IMP} detected most frequently (31.57%), followed by bla_{KPC} (18.42%), while bla_{VIM} and bla_{OXA-48} were identified at lower frequencies. The predominance of bla_{IMP} aligns with previous studies reporting metallo-β-lactamases as major contributors to carbapenem resistance in *P. aeruginosa*, particularly in environmental and non-clinical isolates.[8] These enzymes are often associated with integrons and plasmids, enhancing their potential for horizontal gene transfer and global dissemination.[27]

The detection of bla_{KPC} in poultry farm soil is noteworthy, as KPC enzymes are primarily associated with clinical Enterobacterales and healthcare-associated outbreaks. Its presence in environmental *P. aeruginosa* suggests gene spillover from human or animal waste streams and highlights the increasing convergence between environmental and clinical resistomes.[28,29] This finding is epidemiologically significant, as environmental persistence of KPC-producing organisms may undermine efforts to control carbapenem resistance in healthcare settings.

The relatively lower prevalence of bla_{VIM} and bla_{OXA-48} observed in this study may reflect regional differences in carbapenemase gene distribution or variations in antimicrobial selective pressure within poultry production systems. The absence of bla_{OXA-1} indicates that resistance in the studied isolates is predominantly mediated by carbapenemase production rather than extended-spectrum β-lactamase–associated mechanisms, further underscoring the clinical relevance of the resistance determinants detected.

Beyond microbiological considerations, these findings reflect broader structural challenges related to antimicrobial governance in the veterinary and agricultural sectors. Despite existing national and international guidelines discouraging the use of critically important antimicrobials in food-producing animals, enforcement remains weak in many LMICs, including Nigeria.[22,30] Antimicrobials are frequently administered for prophylaxis and growth promotion without veterinary prescription or laboratory diagnosis, generating sustained selective pressure within farm environments. The detection of carbapenemase-producing *P. aeruginosa* in poultry farm soils illustrates the downstream consequences of such regulatory gaps and highlights the urgent need for strengthened antimicrobial stewardship, improved regulatory enforcement, and routine monitoring of antimicrobial use in animal production systems.

From a global AMR perspective, poultry farms represent critical interfaces where antimicrobial use, animal waste, and environmental contamination intersect. Occupational exposure of farm workers, environmental runoff, and contamination of surrounding ecosystems may facilitate transmission of CRPA and resistance genes beyond farm boundaries. These pathways reinforce the need for surveillance strategies that extend beyond hospitals to include agricultural and environmental reservoirs, particularly in regions with high antimicrobial consumption and limited regulatory control.

In Nigeria, these findings are directly relevant to national AMR containment efforts. The National Action Plan on Antimicrobial Resistance emphasises the expansion of surveillance and the optimisation of antimicrobial use across human and animal sectors. The detection of carbapenemase-producing *P. aeruginosa* in poultry farm soils highlights a critical surveillance gap and supports the inclusion of environmental sampling in routine AMR monitoring frameworks.

Conclusion

This study provides evidence that poultry farm soils in Lagos State harbour carbapenem-resistant *P. aeruginosa* carrying clinically

important carbapenemase genes. These findings underscore the importance of including agricultural and environmental reservoirs in global AMR surveillance and highlight the need for improved antimicrobial stewardship in poultry production to mitigate the environmental spread of high-risk resistance determinants.

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None.

Conflicts of interest

We declare that there is no conflict of interest of any kind.

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