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Molecular detection of carbapenem-resistant *Pseudomonas aeruginosa* from canine skin infections

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ABSTRACT

Pseudomonas aeruginosa (*P. aeruginosa*) is a common bacterium identified in canine skin and has been recognized as a zoonotic bacterium. The treatment of *P. aeruginosa* infections is becoming increasingly challenging due to the rapid development of antimicrobial resistance during treatment. Carbapenems are used as a last-resort treatment option. *P. aeruginosa* is resistant to carbapenems due to the presence of carbapenemase enzymes. This study aimed to identify carbapenemase-encoding genes in *P. aeruginosa* associated with skin infections in dogs. Three hundred skin swabs were collected from dogs with skin infections. Standard microbiological methods were used to isolate and identify *P. aeruginosa*. A polymerase chain reaction was employed for the molecular characterization of *P. aeruginosa* and carbapenemase-producing genes. A disc diffusion assay was used to determine antimicrobial susceptibility. Among 300 skin samples, 78 (26%) were positive for *P. aeruginosa*. Molecular characterization revealed that 78/78 (100%) were positive for *P. aeruginosa*. Antimicrobial resistance patterns exhibited the highest resistance of isolates against ciprofloxacin (82%), meropenem (76%), and imipenem (73%), while 65% susceptibility of isolates was found to colistin. Among 78 isolates of *P. aeruginosa*, 42 (53.8%) were considered as multi-drug-resistant *P. aeruginosa*. Among the 78 isolates, 11 (14.1%) harbored carbapenemase genes: 3 (27.3%) *blaVIM*, 4 (36.3%) *blaNDM*, 3 (27.3%) *blaKPC*, and 1 (9.1%) *blaIMP*. This research discovered the prevalence of carbapenemase-producing *P. aeruginosa* in companion animals, such as dogs, which could be a source of transmission of these resistant bacteria or their genomes to humans.

Key words: carbapenemase; dogs; multidrug resistance; *Pseudomonas aeruginosa*; skin infection

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Introduction

The skin is the largest organ in humans and animals. It is the first line of defense against infectious agents, the most vulnerable organ to physical and chemical harm, and the site of numerous pathogens that can cause wounds and infections (NOCERA et al., 2021). The skin is a complex ecosystem with many different habitats, folds, invaginations, and specialized niches that are home to various microorganisms. The skin microbiota varies depending on the location of the body and includes bacteria (*Pseudomonas aeruginosa* [*P. aeruginosa*], *Proteobacteria spp.*, *Corynebacterium spp.*, *Staphylococcus* spp., and others), fungi, and viruses (SHAHID et al., 2023; ZAFAR et al., 2025). Animals with a high skin humidity index offer optimal conditions for the growth of microorganisms (HATTAB et al., 2021).

P. aeruginosa is a widespread Gram-nega- tive bacillus usually associated with basic infections, and causes severe diseases in humans and animals. It causes urinary tract infections, wounds, chronic deep pyoderma, and otitis externa in dogs (CURRAN et al., 2021). The persistence of the bacteria in the environment supports the "One Health" concept, highlighting the potential for transmission between humans and animals.

Carbapenemase-producing bacteria have been found in companion animals, food-producing animals, and the environment (ASLAM et al., 2022; MWAFY et al., 2023). Although the Food and Drug Administration has not approved the carbapenem class, including imipenem and meropenem, for use in animals, veterinarians have prescribed these antimicrobials as extra-label medications for animals with chronic infections resistant to treatment (BASIT et al., 2021). Long-term antibiotic treatment is frequently necessary because *P. aeruginosa* infections in dogs are persistent and resistant to treatment (SANKAR et al., 2022).

P. aeruginosa is resistant to several antimicrobial agents due to its intrinsic mechanisms, including reduced cell permeability, high basal production of AmpC β-lactamase, and efflux pump systems (IJAZ et al., 2019; ABBAS et al., 2022). Therefore, there are few effective treatments for *P. aeruginosa*, and most require veterinary licenses. Additionally, *P. aeruginosa* can quickly develop resistance by overexpressing efflux pumps, losing the ability to function, mutating antibiotic targets, and expressing various β-lactamases (GHAFOUR et al., 2021). The global spread of resistant strains poses a substantial risk to human and animal health, with the human-pet bond being a major factor in the spread of clinically relevant multidrug-resistant (MDR) bacteria (KIM et al., 2022).

Carbapenemases, which may be chromosomally or plasmid-encoded or linked to other mobile genetic structures (insertion sequences, integrons, or transposons), are the most common resistance mechanisms. These include Verona integron-encoded metallo-beta-lactamase (VIM), oxacillinase (OXA), imipenemase (IMP), New Delhi metallo -beta-lactase-1(NDM-1),and *Klebsiella pnuemoniae* carbapenemase (KPC). The rise of carbapenem -resistant *Enterobacteriaceae* is a rapidly developing global public health issue that necessitates an immediate response from the scientific community worldwide. Numerous studies have confirmed that carbapenemase is becoming increasingly common in animals (HOLMES et al., 2021).

Carbapenem-resistant *P. aeruginosa* is increasingly known to be found in companion animals, particularly dogs that suffer from persistent skin infections. Their exposure to extra-label carbapenem use and intimate contact with humans both aid in the spread of resistance. Since resistant bacteria can spread from animals to people and the environment, this presents a zoonotic and threat to the environment. Misuse of vital antibiotics and the human-animal link increase the establishment and spread of strains that are resistant to multiple drugs. Therefore, in relation to antimicrobial resistance worldwide, companion animals offer an important yet unappreciated reservoir. This emphasizes how urgently the One Health framework's surveillance and intervention are needed (IBRAHIM et al., 2024).

This investigation primarily aimed to determine the prevalence of carbapenemase-producing *P. aeruginosa* and the antimicrobial susceptibility pattern of various antibiotics against *P. aeruginosa* collected from different skin infections in dogs.

Materials and methods

Sample collection. Three hundred samples were collected from dogs with skin infections at veterinary clinics in Faisalabad District, Pakistan. Samples were collected by a trained veterinarian using swabs on the infected skin area (PERRY et al., 2022). Written informed consent was obtained from each dog owner before sampling.

Isolation of P. aeruginosa. The specimens were streaked onto Pseudomonas cetrimide agar, a differential medium for isolating *P. aeruginosa*. Bacterial colonies were streaked in three directions on multiple plates and incubated at 37°C for 24– 48 h to obtain pure colonies (PŁÓKARZ et al., 2022).

Preliminary identification of P. aeruginosa. Pigment synthesis and colony morphology were used to identify *P. aeruginosa*. Additionally, Gram staining and biochemical tests, including indole, oxidase, methyl red–Voges-Proskauer, and catalase tests, were performed (HATTAB et al., 2021; ABED et al., 2024).

Molecular characterization of P. aeruginosa. DNA was extracted from all isolates using a commercial kit (Thermo Scientific, United States). Specific primers were used to identify the genes (*Oprl* and *oprL*) of *P. aeruginosa* (Table 1). The polymerase chain reaction (PCR) conditions were as follows: initial denaturation at 94°C for 4 min, 30 cycles of denaturation at 94°C for 45 s, annealing at 57°C for 1 min, and polymerization at 72°C for 1 min, followed by one final extension cycle at 72°C for 5 min. PCR products were analyzed by electrophoretic migration on a 1.5% agarose gel stained with ethidium bromide and viewed using a gel doc system (PARK et al., 2020).

Antimicrobial susceptibility testing. Kirby -Bauer disc diffusion was used to evaluate the antibiotic susceptibility of all isolates, according to the Clinical and Laboratory Standards Institute (CLSI 2020) recommendations (WEINSTEIN et al., 2020). Susceptibility testing for antibiotics included imipenem, meropenem, ciprofloxacin, ceftazidime, cefepime, gentamicin, and colistin. The results were interpreted using CLSI guidelines (WEINSTEIN et al., 2020; BUI et al., 2024).

Detection of carbapenemase genes using PCR. All positive *P. aeruginosa* isolates were con- firmed to contain carbapenemase genes (*oprL*, *Oprl*, *blaKPC*, *blaNDM*, *blaIMP*, and *blaVIM*) using PCR. The conditions for PCR were set as follows: initial denaturation for 3 min at 95°C, followed by 30 cycles of denaturation for 30 s at 95°C, annealing at 65°C for *NDM*, 52°C for *VIM*, 60°C for *IMP*, 61°C for *KPC*, extension for 45 s at 72°C, and final extension for 5 min at 72°C (SANKAR et al., 2022). The primer sequences of the carbapenemase-producing genes are listed in Table 1. The PCR amplicons were subjected to agarose

Table 1. Primer sequences of species-specific and carbapenemase genes

TARGETED GENES	FORWARD PRIMERS	REVERSE PRIMERS	PRODUCT SIZE (BP)	REFERENCES
OPRL	5'-ATG GAA ATG CTG AAA TTC GGC-3'	5'-CTT CTT CAG CTC GAC GCG ACG-3'	504	(ABDULHAQ et al., 2020)
OPRL	5'-ATG AAC AAC GTT CTG AAA TTC TCT GCT-3'	5'-CTT GCG GCT GGC TTT TTC CAG-3'	249	(AL-ABEDI et al., 2019)
BLAKPC	5'-CGT CTA GTT CTG CTG TCT TG-3'	5'-CTT GTC ATC CTT GTT AGG CG-3'	538	(MOHAN et al., 2015)
BLANDM	5'-GGT TTG GCG ATC TGG TTT TC-3'	5'-CGG AAT GGC TCA TCA CGA TC-3'	561	(MOHAN et al., 2015)
BLAIMP	5'-GAA GGA GTT TAT GTT CAT AC-3'	5'-GTA CGT TTC AAG AGT GAT GC-3'	432	(AL-OUQAILI et al., 2018)
BLAVIM	5'-GTT TGG TCG CAT ATC GCA AC-3'	5'-AAT GCG CAG CAC CAG GAT AG-3'	382	(DE SOUSA et al., 2023)

gel electrophoresis to confirm the size of the specific products

Results

Isolation and identification of P. aeruginosa. Among 300 samples from infected dogs, *P. aeruginosa* was isolated from 78 (26%) samples. *P. aeruginosa* was identified by observing the Gram's staining results and the results of various biochemical tests, including methyl red, indole, catalase, and oxidase tests.

Molecular characterization of P. aeruginosa. All isolates were confirmed to be *P. aeruginosa* on the basis of their molecular features (Fig. 1).

Antimicrobial susceptibility testing. In this investigation, ciprofloxacin resistance was reported in most isolates (82%), while colistin sensitivity was observed in 65% of all isolates. The prevalence of MDR *P. aeruginosa* was 53.8%. Table 2 illustrates the detailed susceptibility patterns of the isolates to different antibiotics.

Table 2. Antimicrobial susceptibility pattern of *P. aeruginosa* isolated from canine skin infections (n = 78)

Antibiotics	Antibiotic susceptibility test	*Pseudomonas aeruginosa* strains isolated from canine skin infections	
		N	*%*
Imipenem (IPM/10 µg)	R	57	73
	S	21	27
Meropenem (MEM/10 µg)	R	59	76
	S	19	24
Ceftazidime (CAZ/30 µg)	R	46	59
	S	32	41
Cefepime (FEP/30 µg)	R	52	67
	S	26	33
Gentamicin (GM/10 µg)	R	48	62
	S	30	38
Ciprofloxacin (CIP/5 µg)	R	64	82
	S	14	18
Colistin (CT/10 µg)	R	27	35
	S	51	65

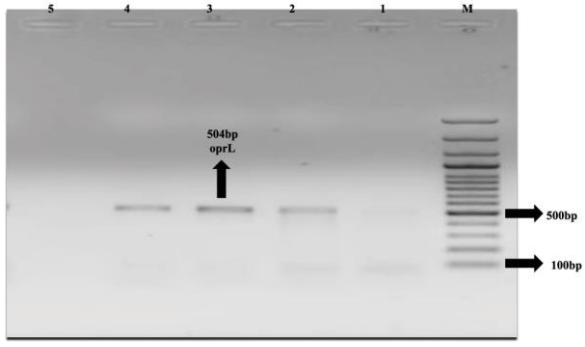

Fig. 1. Molecular detection of Pseudomonas aeruginosa with 50 bp ladder and lanes 3, 5, 6, and 7 presenting band size 504 bp confirming *P. aeruginosa*

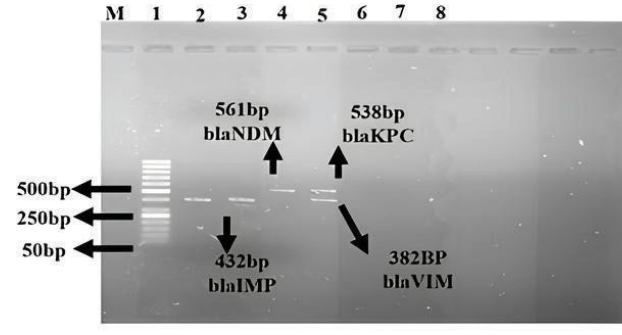

Fig. 2. Agarose gel electrophoresis of PCR products for carbapenemase genes in *Pseudomonas aeruginosa* isolates from canine skin infections by using a 50bp ladder

Lanes 2, 3 showing the band size 432bp for blaIMP, Lane 4 showing the band size 561bp for blaNDM, lane 5 showing the band size of 382bp for blaVIM and 538bp for blaKPC

Molecular characterization of carbapenemase. All 78 *P. aeruginosa* isolates were examined for the molecular detection of carbapenemase genes. Among the 78 isolates, carbapenemase-encoding genes were detected in 11 (14%) isolates: *blaVIM* in 3 (27.27%) isolates, *blaNDM* in 4 (36%) isolates, *blaKPC* in 3 (27%) isolates, and *blaIMP* in 1 (9%) isolate (Fig. 2).

Discussion

Skin infections caused by bacteria are common in dogs, and empirical treatment is a common therapeuticchoice for halting thedisease progression. However, antibiotic susceptibility testing should be considered obligatory in emergencies (AHSAN et al., 2022; ALI et al., 2023). *P. aeruginosa* is an important opportunistic pathogen that is challenging to treat due to its unique traits, including widespread environmental diffusion, inherent resistance to many classes of antibiotics, a high propensity to acquire new resistance mechanisms, and a diverse range of virulence factors (MOSER et al., 2021).

This study aimed to identify the prevalence of carbapenemase-producing *P. aeruginosa* and the antimicrobial susceptibility patterns of vari- ous antibiotics against these isolates collected from different skin infections in dogs. In this rese- arch, the prevalence of *P. aeruginosa* in canine skin infections was 78 (26%), which is higher than the previously prevalence (10.6%) reported by CHAUDHARY et al. (2019).

This research indicated that 54% (42/78) of the dogs with skin infections were infected with MDR *P. aeruginosa*. Similarly, a study conducted in Brazil revealed that dogs had the highest prevalence (60%) of MDR *P. aeruginosa* (MENEZES et al., 2022). In contrast, BICAKCIOGLU et al. (2021) reported a lower prevalence of MDR *P. aeruginosa* (39.53%) in dogs with cutaneous infections. It is especially important to take into account that variations in reported prevalence and resistance patterns between studies may be caused by variations in study design, geographic location, clinical settings, and local antibiotic usage practices.

MDR bacteria are resistant to two or more antibiotics from different classes (EXNER et al., 2017). In this investigation, various antibiotics were found to be resistant to *P. aeruginosa*, as follows: ciprofloxacin (82%), meropenem (76), imipenem(73%),cefepime(67%),gentamicin(62%), and ceftazidime (59%). However, *P. aeruginosa* was susceptible to colistin (65%). A similar study in Romania revealed the highest resistance against polymaxicin B (98%), ciprofloxacin (82%), meropenem (74%), and imipenem (77.58%), while ceftazidime (53%) was the most susceptible, as explained by DÉGI et al. (2021). Antibiotic misuse, *P. aeruginosa* mutation, the organism's genetic makeup, and the local environment contribute to increased antimicrobial resistance.

Due to the increased frequency of extended -spectrum β-lactamases and other MDR organisms, carbapenems are the last line of defense against MDR *P. aeruginosa* infections (JAMIL et al., 2022). The evolution of carbapenem resistance in pet animals is a public health concern due to the close contact between humans and their pets, and the possibility of cross-species transmission (WOODFORD et al., 2014). The extensive use of carbapenems in humans, or environmental factors may be responsible for developing carbapenem resistance in companion animals. Since resistance to carbapenems in animal isolates is not regularly assessed because these medications are not currently permitted in the veterinary industry, its incidence has been underestimated (MELETIS, 2016). The transmission of *P. aeruginosa* from pet animals to humans can cause a significant increase in the number of carbapenemase-producing bacteria in humans. This reflects an emerging problem in treating diseases (PAULSON et al., 2019).

Standardized PCR was developed to identify the presence of common carbapenemases in dogs with skin infections. Among the 78 isolates, carbapenemase-encoding genes were detected in 11 (14%) isolates. The highest prevalence was observed for *blaNDM* in 4 (36%) isolates, followed by *blaKPC* in 3 (27%) and *blaVIM* in 3 (27.27%) isolates. This is consistent with prior research by GONZÁLEZ-TORRALBA et al. (2016) on companion animals, where *NDM* and *OXA* were the carbapenemases most frequently found. These results revealed a latent hazard in

treating pseudomonal infections in dogs. Therefore, controlling the irrational, excessive, and underuse of antibiotics in animals is important. Current national policies and standards could be the starting point for addressing this issue. The transfer of resistance between animals and humans should be monitored to ensure the effectiveness of antibiotic resistance (AHSAN et al., 2024).

Conclusions

In conclusion, this study found a high prevalence of carbapenem resistance in *P. aeruginosa*, indicating an imminent crisis in treating clinical infections. To stop the rise of carbapenem resistance, it is crucial to implement strict infection control, surveillance techniques, and sensible antibiotic use in human and veterinary medicine. Since humans and companion animals have frequent interaction, the discovery of carbapenem-resistant *P. aeruginosa* in dogs raises concerns about possible zoonotic transmission, and emphasizes the significance of One Health strategies to antimicrobial resistance surveillance and management.

Ethics statement

This study was approved by the Ethical Review Committee (ERC), Government College University, Faisalabad, vide Ref no. GCUF/ERC/23/113, dated 24/10/2023.

Authorship contribution statement

HA and RN: Samples collection and Research Methodology, RH and BA: Research methodology and Manuscript preparation, ZN and MZN: Manuscript Preparation and Review, ABS: Research Idea and Supervision

Declaration of competing interests

The authors declare no conflict of interest.

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SAŽETAK

Pseudomonas aeruginosa (*P. aeruginosa*) česta je bakterija izolirana iz kože pasa i prepoznata kao zoonotski uzročnik. Liječenje infekcija uzrokovanih ovom bakterijom postaje sve zahtjevnije zbog brze pojave antimikrobne rezistencije tijekom liječenja. Karbapenemi se upotrebljavaju kao lijekovi posljednjeg izbora. Rezistencija bakterije *P. aeruginosa* na karbapeneme posljedica je prisutnosti enzima karbapenemaza. Cilj istraživanja bio je identificirati gene koji kodiraju karbapenemaze u bakteriji *P. aeruginosa* povezanoj s kožnim infekcijama pasa. Ukupno je prikupljeno 300 brisova kože pasa s kožnim infekcijama. Za izolaciju i identifikaciju bakterije *P. aeruginosa* primijenjene su standardne mikrobiološke metode. Lančana reakcija polimerazom (PCR) primijenjena je za molekularnu karakterizaciju bakterije *P. aeruginosa* i gena koji kodiraju karbapenemaze. Osjetljivost na antimikrobne lijekove određena je disk-difuzijskom metodom. Od 300 uzoraka kože, 78 uzoraka (26%) bilo je pozitivno na *P. aeruginosa*. Molekularna karakterizacija pokazala je da je svih 78 uzoraka (100%) bilo pozitivno na bakteriju *P. aeruginosa*. Uzorci su pokazali najveću rezistenciju na ciprofloksacin (82%), meropenem (76%) i imipenem (73%), dok je najveća osjetljivost (65%) utvrđena na kolistin. Među 78 izolata ove bakterije, njih 42 (53,8%) klasificirano je kao višestruko rezistentni sojevi *P. aeruginosa*. Od 78 izolata, 11 izolata (14,1%) sadržavalo je gene za karbapenemaze: 3 izolata (27,3%) sadržavala su gen *blaVIM*, 4 izolata (36,3%) sadržavala su gen *blaNDM*, 3 izolata (27,3%) sadržavala su gen *blaKPC* i 1 izolat (9,1%) gen *blaIMP*. Ovim je istraživanjem utvrđena prisutnost sojeva bakterije *P. aeruginosa* koji proizvode karbapenemaze u kućnih ljubimaca poput pasa, koji mogu biti izvor prijenosa ovih rezistentnih bakterija ili njihovih genoma na ljude.

Ključne riječi: karbapenemaza; psi; višestruka rezistencija na lijekove; *Pseudomonas aeruginosa;* kožna infekcija