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**Research Article**

# Isolation, molecular characterization and antimicrobial susceptibility testing of *Pseudomonas aeruginosa* from skin infection of dogs

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**ABSTRACT**

*Pseudomonas aeruginosa* represents one of the most frequently found bacteria on canine skin, and it has been identified as a zoonotic pathogen that spread to human and cause life threatening infections. It is a significant opportunistic pathogen that is challenge to control due to its vast ecological dispersion, inherent resistance to various classes of antimicrobial drugs, high potential of developing novel resistance mechanisms and broad selection of pathogenic causes. The main goal of this research was to detect the frequency of *Pseudomonas aeruginosa* isolated from skin infection of dogs and their susceptibility pattern against antipseudomonal antibiotics. Total of one hundred and twenty skin swabs were collected from infected dogs which were then applied to isolate and identify *Pseudomonas aeruginosa* by applying standard microbiological methods. Molecular characterization of *Pseudomonas aeruginosa* was performed via PCR by utilizing specie specific primers against oprL and Oprl genes of *Pseudomonas aeruginosa*. Antimicrobial susceptibility testing was performed by Disc diffusion assay. Out of 120 skin samples, 24(20%) were identified as *Pseudomonas aeruginosa*. Molecular detection showed 100% positive results for *Pseudomonas aeruginosa*. Antimicrobial resistant pattern showed highest resistance of isolates to Cefepime (70%), while 50% susceptibility of isolates was found to the Colistin. Out of 24 isolates of *Pseudomonas aeruginosa*, 14(58%) were considered as MDR *Pseudomonas aeruginosa*. In conclusion there is an imperative need for ways to regulate and stop the emergence of novel multidrug-resistant microorganisms.

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## Introduction

The skin is a sophisticated ecosystem that supports a wide range of microbiome, including parasites bacteria and yeasts. Animals with a high index of skin humidity provide the ideal habitat for the bacteria to flourish. Bacteria exist in the stratum corneum, the superficial layer of the skin, but not up to the sebaceous gland or the outer region of hair follicles (Tomicic et al. 2018).

*Pseudomonas aeruginosa* is ubiquitous, gram-negative bacilli, considered as a significant pathogen to both animals and humans, it frequently included fundamental infections. *Pseudomonas aeruginosa* is an essential opportunistic as well as nosocomial bacterium in humans primarily affecting immunocompromised patient (Bourely et al. 2019). It has been contemplated that the primary cause of infections in animals, particularly in dogs, including superficial skin infections, otitis externa, wound and urinary tract infections, perianal abscesses and chronic deep pyoderma (Degi et al. 2021). The prevalence of *P. aeruginosa* infections is 11.5% in Europe and 17% in developing countries (Valderrama et al. 2020). As a result of bacterium's persistence in the environment and potential for transmission between animals and humans, these infections are congruent with the "One Health" concept (Chawnan et al. 2021).

It is widely acknowledged that multidrug resistance (MDR) poses a major risk to both human and animal health (Abdullahi et al. 2016). MDR was predominantly associated with nosocomial infections, but as they have spreaded throughout the population (community-acquired MDR), the risk for fatal diseases and their resistance level have increased (Van-Duin and Paterson 2016). Due to the diverse range of antibiotic resistance that MDR bacteria exhibit, treating patients with such infections (whether they are in humans or animals) might be more challenging. Numerous explanations have been proposed for the ongoing increase of MDR in veterinary and human medicine, among which one is directly related to the

improper use of broad-spectrum antibiotics (Kher et al. 2022).

*P. aeruginosa* naturally showed resistance towards numerous kinds of antimicrobial drugs, due to intrinsic mechanism, particularly diminish the permeability of cell wall (Ludwig et al. 2016), constitutive efflux pump systems with a high level of chromosomal AmpC-β-lactamase expression (Pan et al. 2016). As a result, the therapy choices for *P. aeruginosa* are restricted, with relatively few antibiotics helpful as antipseudomonal medicines, the majority of which are not registered for veterinary purpose. Furthermore, *P. aeruginosa* might rapidly gain resistance by overexpressing efflux pumps (Fernandes et al. 2018). Antibiotic target mutations and the production of a broad diversity of β–lactamases (Zhao and Hu 2010). As a result, *P. aeruginosa* may develop resistance to various antibiotic class via a single or many mechanisms (Ijaz et al. 2019).

The primary goal of current investigation was to isolate the *P. aeruginosa* from dogs infected with skin disease and their antimicrobial profile.

## Materials and Methods

### Sample collection

Total of one hundred and twenty samples from dogs with skin infection were collected by swabbing on infected area of skin with the help of sterilized swab from Civil veterinary hospital of District Faisalabad-Pakistan.

### Isolation and purification of *Pseudomonas aeruginosa* on selective media

Samples were streaked via streak plate method on Pseudomonas cetrimide agar, which is selective media for the isolation of *P. aeruginosa.* To obtain pure colonies of *P. aeruginosa* bacterial colony was streaked three dimensionally on different plate. And incubated for 24-48 hours (Hattab et al. 2021).

### Identification of *Pseudomonas aeruginosa*

Identification of *P. aeruginosa* was done by observing colony morphology and pigment production. Furthermore, Gram staining and various biochemical tests including citrate utilization test, indole test, oxidase test, MRVP test and catalase test were performed (Nocera et al. 2021).

### DNA extraction and molecular identification of *Pseudomonas aeruginosa*

DNA was extracted from all isolates by boiling method. The extracted DNA was polymerized by utilizing specific primer for the identification of *P. aeruginosa* Oprl and oprL mentioned in Table 1. The conditions of PCR employed were primary denaturation at 94°C for 4 minutes, followed by 30 cycles of denaturing for 45 seconds, annealing at 57°C for 1 minute, and polymerizing at 72°C for 1 minute, with one final extension cycle at 72°C for 5 minutes. Amplified product of PCR was observed by utilizing the gel doc system on a 1% agarose gel stained with ethidium bromide (Park et al. 2020).

### Antimicrobial susceptibility testing

The Kirby-Bauer disc diffusion technique was applied to assess the antibiotic susceptibility of all isolates in accordance with CLSI 2020 recommendations (de Menezes et al. 2021). Mueller Hinton agar was employed for susceptibility test of different antibiotics, which includes amoxicillin (25µg), Imipenem (10µg), meropenem (10µg), ciprofloxacin (5µg), ceftazidime (30µg), Cefepime (30µg), gentamicin (10µg) and colistin (10µg) (Khalid et al., 2017). By utilizing CLSI guideline results were interpreted.

## Results

### Isolation and identification of *Pseudomonas aeruginosa*

Out of 120 samples, *P. aeruginosa* was isolated from 24(20%) taken from infected skin of dogs by the examination of results of gram staining that showed *P. aeruginosa* is gram negative bacteria and results of different biochemical test were also observed.

### Molecular detection of *Pseudomonas aeruginosa*

Total of 24 isolates were subjected for the detection of polymerase chain reaction. On the basis of molecular characteristics of *P. aeruginosa* out of 24 isolates, 24 (100%) were confirmed positive for *P. aeruginosa*. Prevalence of MDR *P. aeruginosa* was found 58%. Results are given Fig. 1.

### Antimicrobial susceptibility testing

In present study multiple antibiotics including amoxicillin, imipenem, meropenem, ciprofloxacin, ceftazidime, cefepime, gentamicin and colistin were used against all the isolated *P. aeruginosa* shown in Fig. 2. Most of the isolates were found resistant against cefepime (70%) while 50% for all isolates were found susceptible to colistin. Detail susceptibility pattern of isolates against various antibiotics is given in Fig. 3.

## Discussion

Bacterial infection of skin is widespread in canines, and empirical management is a typical therapeutic option to slowing medical progression. Nonetheless, in severe situations, antibiotic susceptibility testing should be regarded mandatory (Gellatly and Hancock 2013). *Pseudomonas aeruginosa* is a significant opportunistic and challenging bacterium because of its distinctive characteristics, which include significant ecological dissemination, inherent resistance too several antibiotic classes, a high potential to develop additional resistance mechanisms, and a diverse set of virulence factors (Hattab et al. 2021).

The primary goal of the current research was to determine the prevalence of *P. aeruginosa* and their antimicrobial susceptibility pattern for different antibiotics in various skin infection of dogs as well as the identification of isolates based on specific primers through PCR. In the present study we employed species-specific primers such as oprL and Oprl to identify *P. aeruginosa* against all isolates, and 24 were verified *P. aeruginosa*. In past, a study was conducted by utilizing oprL and Oprl species specific primer for the identification of *P. aeruginosa* (Plokarz et al. 2022). In present study, significant prevalence (20%) of *P. aeruginosa* was estimated in various skin

infection of dog. In comparison with previously conducted study the prevalence of *P. aeruginosa* is slightly high (29.6%) in dogs infected with skin infection (Noomi 2019). In another study similar results were observed which showed the higher prevalence 40.84% for *P. aeruginosa* from superficial swab samples of infected dogs (Degi et al. 2021).

Current findings suggested that Multi drug resistant *P. aeruginosa* are frequently found 58% (14/24) in dogs infected with skin infections. Similar study was conducted in Brazil showed the highest prevalence (60%) of MDR *P. aeruginosa* in dogs (Menezes et al. 2022). Another study revealed the prevalence of MDR *P. aeruginosa* 39.53% in skin infected dogs (Bicakcioglu et al. 2021).

MDR bacteria are defined as those that exhibit resistance to at least two distinct representatives of at least two classes of antibiotics (Park et al. 2011). *P. aeruginosa* was discovered to be resistant to numerous antibiotics employed in the current study, including amoxicillin, imipenem, meropenem, ciprofloxacin, ceftazidime, cefepime and gentamicin. The susceptibility was observed 50% in colistin. Antimicrobial resistance increases due to improper use of antibiotics, *P. aeruginosa* mutation, the organism's genetic makeup, and the specific area's environmental conditions. In previous study highest resistance was observed against ceftazidime (53.44%) and least resistance was observed against polymyxin B (1.72%) (Degi et al. 2021) while another study showed highest resistance of *P. aeruginosa* against gentamicin (82%) (KuKanich et al. 2022).

**Conclusion**

Our research showed that multidrug-resistant bacteria were involved in the infectious development of dogs at a significant incidence. Given the increased danger of resistant-strains spread among livestock, owners, and healthcare professionals, our results revealed worrying information from the standpoint of the One Health scenario. In veterinary clinics, there is an immediate need for ways to regulate and stop the emergence of novel multidrug-resistant microorganisms. Understanding and emphasizing the responsibility of veterinary specialist in this fight for health care system is equally essential.

**Table 1:** Sequence of specific primer

| Targeted Gene | Sequence of primers | Product size | References |
|---|---|---|---|
| oprL | F-ATG GAAATGCTGAAATTCGGC<br>R-CTTCTTCAGCTCGACGCGACG | 504bp | Abdulhaq et al. (2020) |
| Oprl | F-ATGAACAACGTTCTGAAATTCTCTGCT<br>R-CTTGCGGCTGGCTTTTTCCAG | 249bp | Al-abedi and Abd Al-Mayahi (2019) |

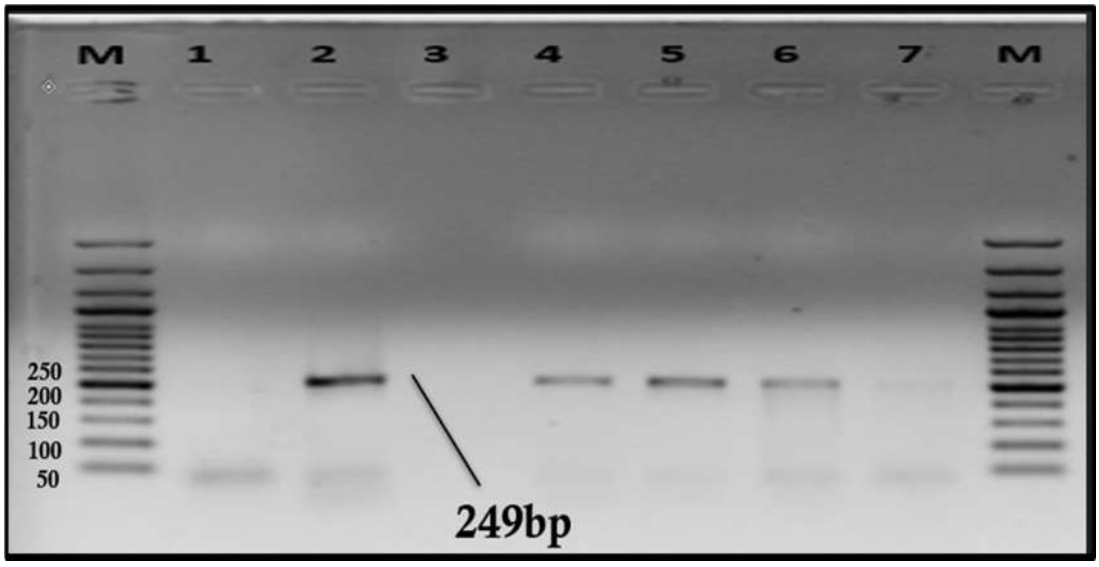



**Fig. 1:** Gel electrophoresis results of Oprl gene

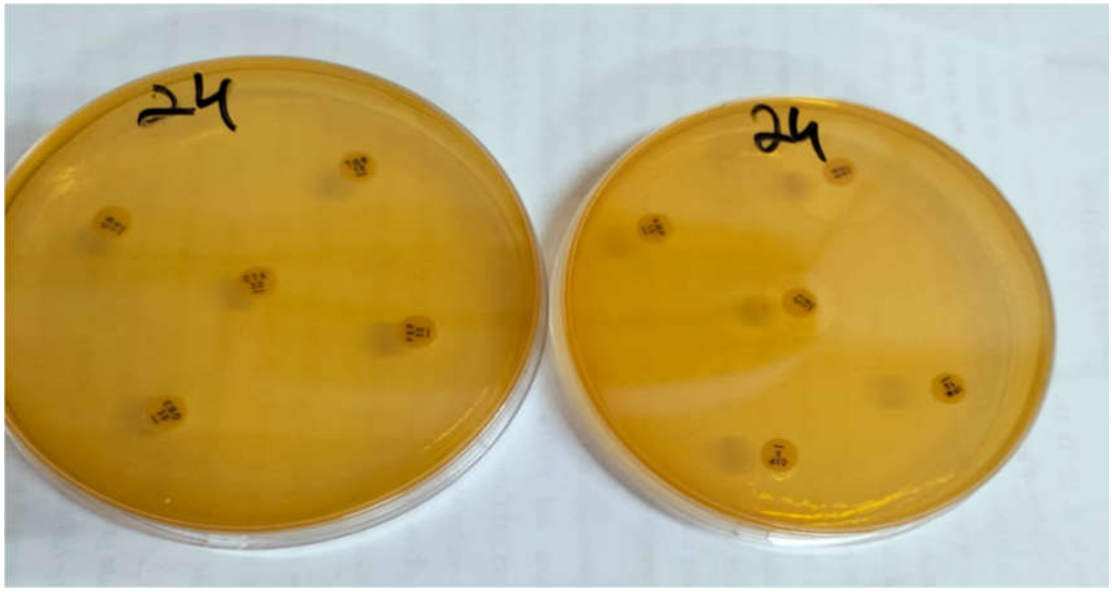
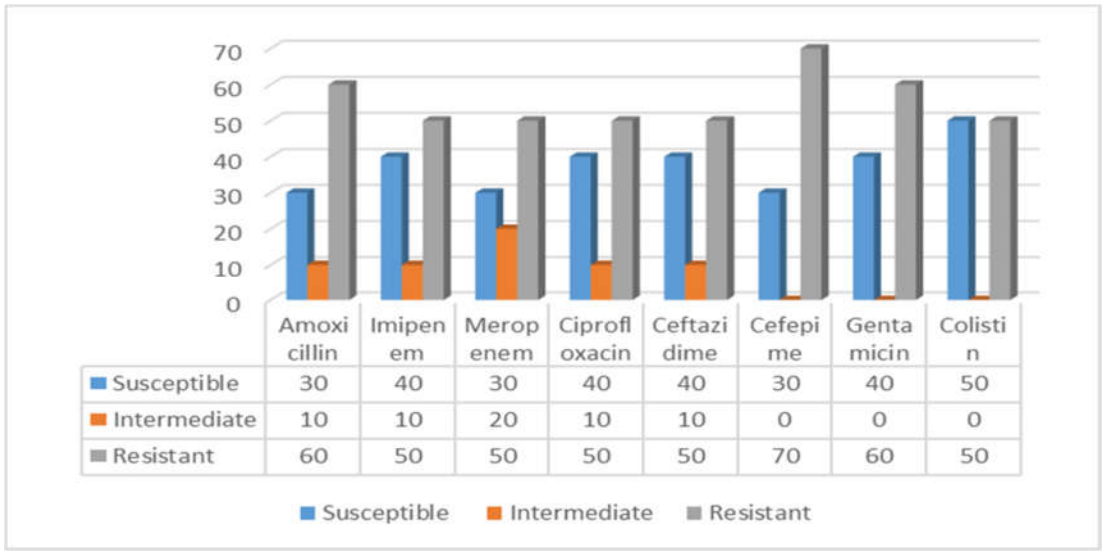



**Fig. 2:** Antimicrobial Susceptibility pattern of *Pseudomonas aeruginosa* against different antibiotics




| | Amoxicillin | Imipenem | Meropenem | Ciprofloxacin | Ceftazidime | Cefepime | Gentamicin | Colistin |
|---|---|---|---|---|---|---|---|---|
| Susceptible | 30 | 40 | 30 | 40 | 40 | 30 | 40 | 50 |
| Intermediate | 10 | 10 | 20 | 10 | 10 | 0 | 0 | 0 |
| Resistant | 60 | 50 | 50 | 50 | 50 | 70 | 60 | 50 |

**Fig. 3:** Percentage prevalence of antimicrobial susceptibility pattern of *Pseudomonas aeruginosa*

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