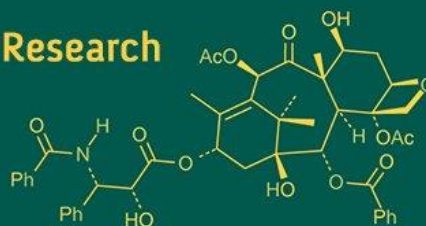
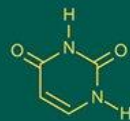


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Prevalence, phenotypic characterization and AntibioGram of *Pseudomonas aeruginosa* isolates recovered from oropharyngeal swabs of Pigs in and around Tirupati

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Abstract

Pseudomonas aeruginosa is an opportunistic Gram-negative bacterium that is widely distributed in the environment and is capable of colonizing the respiratory tract of animals. The organism is having public health concern because of owing to its intrinsic resistance to several antimicrobial agents and its ability to acquire multidrug resistance. The information regarding the prevalence of *P. aeruginosa* in pigs in Tirupati, is limited. Therefore, the present study was undertaken to determine the prevalence and phenotypically characterize *P. aeruginosa* isolated from oropharyngeal swabs of pigs in and around Tirupati. Of the 80 oropharyngeal swabs screened, eleven (13.75%) isolates were confirmed as *P. aeruginosa* by conventional methods. Those isolates were subjected for antimicrobial susceptibility tested against various antibiotics commonly used human and veterinary medicine. Antimicrobial susceptibility testing revealed varying resistance patterns among the isolates, with multidrug-resistant strains being detected. The MAR index values indicated that several isolates originated from environments with frequent antimicrobial exposure. The study demonstrated the presence of *P. aeruginosa* in the oropharyngeal cavity of pigs in the Tirupati region and highlighted the occurrence of antimicrobial resistant isolates. These findings emphasize the need for continuous surveillance, prudent antimicrobial use and improved biosecurity practices in pig farms to minimize the emergence and dissemination of antimicrobial-resistant *P. aeruginosa* and to reduce the associated animal and public health risks.

Keywords: *P. aeruginosa*, oropharyngeal swabs, pigs, Tirupati, MAR index

Introduction

Pig farming is an important component of the livestock sector in India, contributing significantly to rural livelihoods and food security (DAHD, 2023). Respiratory diseases remain one of the major constraints affecting pig production by causing reduced growth, increased mortality, and economic losses (Manasa *et al.*, 2025) [13].

Among bacterial pathogens, *Pseudomonas aeruginosa* is a Gram-negative, aerobic, opportunistic bacterium capable of causing respiratory infections, septicemia, wound infections, and otitis in animals. Although *P. aeruginosa* is not considered a primary respiratory pathogen in pigs, it frequently acts as a secondary invader in animals affected by stress, viral infections, or poor management practices (Wood *et al.*, 2023) [20].

The bodies of animals and humans host a variety of ecological niches that support diverse populations of microorganisms. While most studies on microbiota have concentrated on the intestinal tract or faeces, other body areas, such as the nasal passages, also contain significant microbial populations. However, research on the nasal microbiota in pigs has been limited, with culture-based studies primarily targeting specific pathogens (Weese, 2013; Holman *et al.*, 2017; Correa-Fiz *et al.*, 2016) [5, 9, 19].

The emergence and spread of multiple drug-resistant pathogenic bacteria is a major global health concern. Moreover, *P. aeruginosa* exhibits intrinsic resistance to many antimicrobials and readily acquires additional resistance mechanisms, making infections difficult to treat. The emergence of multidrug-resistant (MDR) *Pseudomonas* strains in food-producing animals

has become a global public health concern due to the potential transmission of resistant bacteria or resistance genes to humans through direct contact or the food chain (WHO, 2023; FAO, 2023; Schwarz *et al.*, 2010) [15].

Despite its importance, limited information is available regarding the prevalence and antimicrobial resistance patterns of *Pseudomonas* spp. in pigs in Andhra Pradesh. Therefore, this study is proposed to investigate the occurrence of *Pseudomonas aeruginosa* in the nasal swabs of pigs collected from different farms in and around Tirupati, Andhra Pradesh and characterization of the isolates by phenotypic methods.

Materials and methods

Study location

A cross-sectional study was designed to study the antimicrobial resistance patterns of the *Pseudomonas aeruginosa* isolates recovered from pigs of various farms located in and around Tirupati. The present study was carried out in the Department of Veterinary Public Health and Epidemiology, College of Veterinary Science, Tirupati during the period January 2026 to June 2026.

Sampling details

A total of 100 oropharyngeal swabs were collected randomly and aseptically from pigs of various farms located in and around Tirupati, Andhra Pradesh. The samples were collected using sterile swabs and transported to the department under chilled conditions for further processing and for bacteriological analysis.

Isolation and Identification of *Pseudomonas aeruginosa* by conventional method

Initially, the oropharyngeal swabs were inoculated in the Tryptone Soya Broth (TSB) and incubated at 37°C for 24 hrs under aerobic conditions for pre-enrichment. Following incubation, the enriched cultures were streaked on to Cetrimide agar and incubated at 37°C for 24 hrs. Cetrimide agar is a selective and differential medium for the identification of *Pseudomonas aeruginosa* in which Cetrimide acts as detergent which inhibits most bacteria and enhances the production of two pigments pyocyanin and pyoverdine.

Biochemical characterization of presumptive *P.aeruginosa* isolates

After incubation, the plates were examined for the presence of typical *P.aeruginosa* colonies, characterized by flat, spreading colonies with irregular margins, blue-green or yellow-green pigmentation, characteristic fruity or grape like odour. Presumptive colonies were subjected to Grams staining, which revealed gram negative slender rods. These presumptive isolates were further characterized using biochemical tests, including the IMViC tests. The isolates showing negative for Indole, Methyl Red and Voges-Proskauer test, along with positive Citrate test, in combination with the characteristic colony morphology and gram staining reaction.

Phenotypic characterization of antimicrobial resistant *P.aeruginosa* isolates

All the confirmed *P.aeruginosa* isolates were tested for antibiotic susceptibility against commonly used antibiotics in human and veterinary therapy by the Kirby Bauer disc

diffusion method on Mueller Hinton agar using commercial discs (Himedia Pvt. Ltd, India). Initially, each confirmed isolates were inoculated into Tryptone Soya Broth (TSB) and incubated at 37°C for 24 hrs. Further, the concentration of turbidity was adjusted to 0.5 McFarland Standard. A sterile swab was dipped in to the culture and pressed against the walls of the test tube to remove excess culture. Later, the swab was spreaded on to Mueller Hinton agar and the selected antibiotic discs (Ceftriaxone, Ciprofloxacin, Chloramphenicol, Gentamicin, Erythromycin, Tetracycline, Ampicillin and Amikacin) were gently pressed against the culture spreaded Mueller-Hinton agar plates and incubated at 37°C for 18-24 hours. The diameters of inhibition zones were measured to the nearest millimeter, and the results were interpreted as resistant (R), intermediate (I), or susceptible (S) according to CLSI guidelines (CLSI, 2014). Isolates showing resistance to three or more antibiotics were considered multidrug resistant (MDR), and the resistance rates were classified as highly resistant (>50%), moderately resistant (10-50%), and low resistance (<10%) (Schwarz *et al.*, 2010) [15].

Evaluation of Multiple Antibiotic Resistance Phenotypes (MARPS) and Multiple Antibiotic Resistance (MAR) Index for *P.aeruginosa* isolates

The multiple antibiotic-resistant phenotypes (MARPs) of *P.aeruginosa* were evaluated for isolates that exhibited resistance to three or more antibiotics according to the method adapted from Iwu and Okoh, 2020. MAR index was calculated for all isolates as per procedure given by Krumpman (1983) by the formula a/b , where 'a' represents the number of antibiotics to which the isolate is resistant and 'b' the total number of antibiotics to which the isolate is exposed [10, 12].

Results and discussion

Out of the 80 oropharyngeal samples screened, only 11 samples (13.75%) were confirmed as *P. aeruginosa* by conventional methods, viz., cultural and biochemical tests. These findings almost similar to Donnik *et al.*, 2020 who recorded 16% prevalence of *P.aeruginosa* from various sample types viz., wash offs from pigs and surfaces, skin of pigs, feedstuff and premises, water samples of different pig farms. Although the sample types differed between the two studies, the prevalence observed in the present investigation was comparable to that reported by Donnik *et al.*, 2020, suggesting the widespread occurrence of *P.aeruginosa* in pig farm environments. In contrast to this, Chakraborty *et al.*, 2020 from India recorded zero and 6% prevalence from oropharyngeal swabs and faecal samples from pigs respectively [7].

Similarly, the present study prevalence is almost similar to the findings of Karthik *et al.*, 2026 (20%) and Swetcha *et al.*, 2023 (12.8%) from pork samples. In contrary, Chakraborty *et al.* (2020) from India and Bantawa *et al.* (2018) from Nepal recorded a lower (3.0%) and higher prevalence (40.0%) from faeces of pigs and pork samples, respectively [2, 3, 11, 17].

The information regarding the prevalence of *P.aeruginosa* in oropharyngeal swabs of pigs is published scarce. Most available studies have focused on experimental respiratory infection models, airway colonization, or isolation from nasal swabs, lungs and other anatomical sites rather than natural colonization of the oropharynx. Consequently, the data related to the prevalence of *P.aeruginosa* in oropharyngeal

swabs of pigs under field conditions remain limited, highlighting the need for epidemiological investigations in pig populations.

The eleven *P.aeruginosa* isolates recovered from oropharyngeal swabs of pigs were studied for phenotypic characterization of antimicrobial resistance patterns by disc diffusion method. Out of these 11 isolates, ten isolates (91%) were resistance to ampicillin and erythromycin, while nine isolates (82%) were resistance to ceftriaxone and tetracycline, six isolates (55%) resistance to ciprofloxacin (Figure 1), four isolates (36%) to chloramphenicol, and three isolates (27%) were resistance against gentamicin and amikacin respectively (Table 1).

Many researchers have reported varying antimicrobial resistance patterns in *Pseudomonas aeruginosa* and have identified multidrug-resistant (MDR) isolates from different food animal sources, including chicken, fish, mutton, pork, and beef (Chakraborty *et al.*, 2020; Abbas *et al.*, 2022; Swetcha *et al.*, 2023; Sowmya *et al.*, 2024). The differences observed in antimicrobial resistance profiles among studies may be attributed to variations in antimicrobial usage practices, geographical locations, management systems, and the presence of diverse resistance mechanisms in *P. aeruginosa* (Zeng *et al.*, 2014) [1, 3, 17, 18].

Table 1: Antimicrobial resistant profile of *P.aeruginosa* isolates

Antibiotic class	Antibiotics tested	No. of <i>P.aeruginosa</i> isolates (%)		
		Susceptible (mm)	Intermediate (mm)	Resistant (mm)
Cephalosporins	Ceftriaxone	18	0	82
Quinolones	Ciprofloxacin	45	0	55
Phenicol	Chloramphenicol	64	0	36
Aminoglycoside	Gentamicin	55	18	27
Macrolide	Erythromycin	9	0	91
Tetracyclines	Tetracycline	18	0	82
Betalactams (aminopenicillins)	Ampicillin	9	0	91
Aminoglycoside	Amikacin	64	9	27

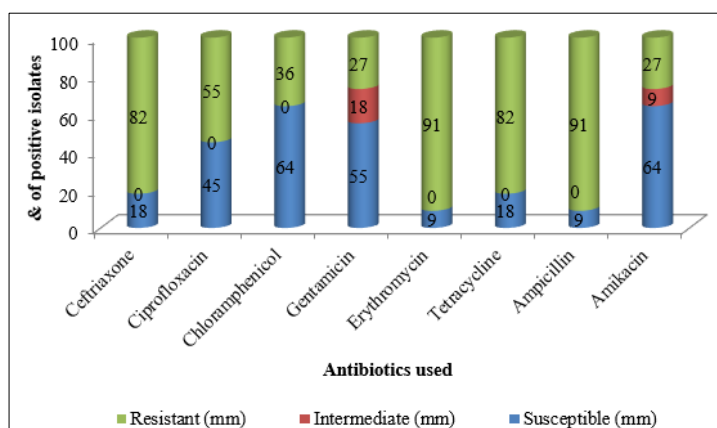


Fig 1: Antimicrobial resistant profile of *P.aeruginosa* isolates

The MARPs and MAR indices patterns of *P.aeruginosa* isolates recovered from oropharyngeal swab samples were given in the Table 2. The eleven *P.aeruginosa* isolates exhibited nine patterns of MARPs to antibiotics used ranging from 4 to 8 and the MAR indices ranges from 0.38 to 1.0 in this present study. The isolates with intermediate resistance were considered as resistant while calculating MAR index. It

was found that only one isolate was resistant to eight different antibiotics, two isolates were resistant to six different antibiotics with varying combination of antibiotics patterns; six isolates were resistant against five different antibiotics patterns and two isolates were resistant to four antibiotics tested (Figure 2).

Isolate No.	CEF	CIP	C	GEN	E	TE	AMP	AK
1	R	R	R	R	R	R	R	R
2	R	S	S	S	R	R	R	R
3	S	R	R	R	R	R	R	S
4	R	S	R	S	R	R	R	S
5	R	R	S	I	R	S	R	S
6	R	S	R	S	R	R	S	R
7	R	R	S	S	R	R	R	R
8	R	R	S	I	S	R	R	S
9	S	R	R	R	R	S	R	S
10	R	S	S	R	R	R	R	S
11	R	S	S	S	R	R	R	I

Sensitive

Intermediate Resistance

Resistance

Fig 2: Heat map of antibiotic resistance detected in *P.aeruginosa* isolates

A Multiple Antibiotic Resistance (MAR) index greater than 0.2 indicates that the isolates may have originated from high-risk sources where antibiotics are frequently used, reflecting a greater risk of exposure to antimicrobial agents (Maurice *et al.*, 2018). In contrast, a MAR index of less than 0.2 suggests that the isolates originated from sources where antibiotics are seldom or never used and therefore represent a lower risk of antimicrobial exposure [14].

In the present study, all the eleven *P.aeruginosa* isolates recovered had the MAR index of greater than 0.2, ranged from 0.5-1.0, which indicates that these isolates falls under high risk category with high risk potential to favour the spread of antimicrobial resistance pathogens in both animals and humans.

Table 2: Patterns of MAR phenotypes and MAR index of *P.aeruginosa* isolates obtained from oropharyngeal samples of pigs

Sample No.	Multiple Antibiotic Resistance Phenotypes (MARPs)	MAR index
1	CEF, CIP,C,GEN,E, TE, AMP, AK	1.00
s2	CEF,E, TE, AMP, GEN	0.63
3	CIP, C, GEN,E, TE, AMP	0.75
4	CEF, C,E, TE, AMP	0.63
5	CEF, CIP, E, AMP	0.50
6	CEF, C,E, TE, AK	0.63
7	CEF,CIP,E, TE, AMP, AK	0.75
8	CEF,CIP, TE, AMP	0.50
9	CIP, C,GEN,E, AMP	0.63
10	CEF,E, TE, AMP, GEN	0.63
11	CEF,E, TE, AMP,AK	0.63

The antimicrobial resistance patterns observed in the present study highlight the need for the judicious prudent use of antibiotics at both veterinary and public health levels. Regular monitoring and continuous surveillance of the antimicrobial susceptibility profiles of *P.aeruginosa* are essential for the early detection of emerging resistance trends, guiding appropriate antimicrobial therapy, preventing treatment failures and minimizing the development and spread of resistance to commonly used antibiotics for the treatment of pseudomoniasis.

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