RESEARCH ARTICLE

Molecular Characterization and Antibiogram of *Pseudomonas aeruginosa* Isolated from Various Meat Samples in and around Tirupati, Andhra Pradesh

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Abstract

Pseudomonas aeruginosa is an emerging opportunistic pathogen and is one of the pathogens in the acronym ESKAPE highlighting a global threat to human health due to antimicrobial resistance. This study aimed to molecularly characterize *P. aeruginosa*, and to assess its antimicrobial resistance in different meat samples, including chicken, beef, pork, frozen chicken, frozen beef and frozen pork, collected from various meat markets in Tirupati, Andhra Pradesh, India. A total of 18 (16.07%) *P. aeruginosa* isolates were confirmed from 112 meat samples, *viz.,* chicken (18.75 %), beef (16.6%), pork (20 %), and frozen pork (20%) by PCR targeting the 16S rRNA and *rpo*B genes, respectively. None of the isolates were identified in frozen chicken and frozen beef samples. Further, all the 18 *P. aeruginosa* isolates were phenotypically characterized for antimicrobial resistance patterns using the disc diffusion method and found 100% resistance to erythromycin, 17 isolates were resistance against amoxycillin (94%), 15 isolates to tertracycline and vancomycin (83%), 14 isolates to cotrimoxazole (77.7%), 8 isolates against ciprofloxacin (44%) and 4 isolates were resistance against gentamicin (22 %). The presence of multiple antibiotic-resistant *P. aeruginosa*, which carry virulence genes, in various meat samples, poses a significant public health risk from consuming raw or undercooked meat. Consequently, the detection of these resistant strains in meat highlights the urgent need for improved food safety measures and monitoring to protect public health.

Key words: Antimicrobial resistance, ESKAPE, PCR, *Pseudomonas aeruginosa, rpoB,* 16S rRNA.

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Introduction

Pseudomonas aeruginosa is a Gram-negative, aerobic, non-fermenting bacterium known for its metabolic versatility and environmental adaptability. It thrives in nutrient-limited conditions, allowing it to persist in various environments, including soil, water, plants, animals, animal faeces, food, and clinical settings (Balasubramanian *et al*., 2013; Da Silva Luz *et al*., 2015). Its high genetic plasticity enables rapid adaptation and colonization of diverse hosts, contributing to its wide distribution (Benie *et al*., 2017). It is recognized globally as a multidrug-resistant "superbug" and an ESKAPE (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* spp.) pathogen and poses significant challenges in clinical settings (Breidenstein *et al*., 2011). As a major opportunistic pathogen, *P.aeruginosa* causes a range of infections, especially in immunocompromised individuals, from mild conditions like swimmer's ear to severe nosocomial infections, often resulting in high morbidity and mortality (Streeter *et al*., 2016). Its pathogenicity is supported by metabolic flexibility, biofilm formation, antimicrobial resistance, and virulence factors (Balasubramanian *et al*., 2013).

In the food industry, *P.aeruginosa* is a common spoilage organism found in poultry, fruits, dairy, and meat, with spoilage characterized by off-odours, slime, and reduced shelf life (Bellanger *et al*., 2013; Stellato *et al*., 2017). Raw meat can harbour this bacterium, leading to spoilage and potential foodborne exposure, particularly in environments with inadequate hygiene (Rouger *et al*., 2017; Bantawa *et al*., 2018). Water is also a significant contamination source (Kim and Wei, 2007). While *P. aeruginosa* infections rarely lead to severe foodborne mortality, they can cause gastrointestinal

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symptoms such as vomiting and diarrhoea (Huang *et al*., 2024). Considering its public health significance, the present study was aimed to isolate and confirm *P. aeruginosa* from various meat samples and analyse the antibiotic resistance patterns of these isolates.

Materials and Methods

Sample Collection

The present study was conducted at the Department of Veterinary Public Health and Epidemiology, College of Veterinary Science, SVVU, Tirupati (India) during the period from June 2025 to December 2025. A total of 112 various meat samples, *viz.,* chicken meat (n=64), beef (n=12), pork (n=15), frozen chicken meat (n=11), frozen pork (n=5), and frozen beef (n=5), were randomly collected from local markets, slaughterhouses, and retail outlets in Tirupati (Andhra Pradesh, India). Each sample was collected under aseptic conditions in a sterile zip-lock bag. The cold chain was maintained until the samples were transported to the College laboratory, and were processed within 2-3 h after collection.

Isolation and Identification of *P. aeruginosa*

Approximately 10-20 g of each sample was homogenized using a mortar and pestle by adding 90 mL of peptone water. A 3 mL aliquot of this mixture was pre-enriched in Tryptic Soy Broth (TSB) and incubated at 37°C for 24 h under aerobic conditions. The pre-enriched sample was then streaked onto Cetrimide agar (HiMedia Laboratories) and incubated at 37°C for 24-48 h for the isolation of *Pseudomonas aeruginosa*. The presumptive colonies were collected and screened by Gram staining and biochemical tests, *viz.,* catalase test, oxidase test, IMViC tests, urease test, Triple Sugar Iron (TSI) agar test, gelatin liquefaction test, and esculin hydrolysis test, using standard microbiological techniques for confirmation of the isolates (Austin and Austin, 2016).

Molecular Confirmation of *P. Aeruginosa* Isolates by PCR

The DNA was extracted from all biochemically confirmed *P. aeruginosa* isolates by boiling and snap-chilling methods as described by Benie *et al*. (2017). These DNA templates were subjected to PCR targeting the 16S rRNA and *rpoB* genes for molecular confirmation. The primers used in the present study are listed in Table 1. For standardisation of both genes, a 25 µL of PCR reaction mixture was used for amplification which consists of 12.5 µL of 2 X PCR master mixture, 1 µL (10 pmol/µL) of each primer, 3 µL of DNA template and the remaining volume of Nuclease Free Water (NFW) to make a volume up to 25 µL and kept under the suitable cycling conditions (Table 2**)**.

Phenotypic Characterization of Antimicrobial Resistance

All the *P. aeruginosa* isolates obtained in the present study were subjected to antimicrobial susceptibility testing against commonly used antibiotics in human and veterinary therapy by the disk diffusion method (Kirby-Bauer method) for phenotypic characterization. Each isolate was cultured in Tryptone Soya Broth (TSB) at 37°C for 18-24 h, and the concentration of the culture was adjusted to 0.5 McFarland standard. A sterile swab was soaked in the bacterial suspension and spread onto Mueller-Hinton agar plates. Selected 8 antibiotic discs (erythromycin, amoxicillin, tetracycline, vancomycin, cotrimoxazole, ciprofloxacin, cefepime, and gentamicin) were gently placed onto the inoculated Mueller-Hinton agar plates and incubated at 37°C for 18-24 h. 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, 2018). 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).

Results and Discussion

Out of the 112 samples analysed, 21 samples were identified as *P. aeruginosa* by conventional methods, *viz.,* cultural and biochemical tests. Further, the presumptive isolates were confirmed by PCR targeting the 16S rRNA and *rpoB* genes

Table 1: Primers used for molecular characterization of *P. Aeruginosa*

Target Gene	Primer sequence (5^{l}–3^{l})	Amplicon size	Reference
16s rRNA	F: AGAGTTTGATCCTGGCTCAG R: CTACGGCTACCTTGTTACGA	1500 bp	Benie *et al.* (2017)
rpoB	F: CAGTTCATGGACCAGAACAACCCG R: ACGCTGGTTGATGCAGGTGTTC	759 bp	

Table 2: Cycling conditions for the *16S rRNA* and *rpoB* genes of *P. aeruginosa* **(**Benie *et al*., 2017)

Genes targeted	Cycling conditions				
	Initial denaturation	Final denaturation	Annealing	Extension	Final extension
16S rRNA	94° C 5 min	94° C 60 s	58° C 60 s Repeat for 30 cycles	72°C 120 s	72°C 10 min
rpoB	94° C 60 s	94° C 3 min	58° C 60 s Repeat for 30 cycles	72°C 120 s	72°C 10 min

for genus and species confirmation, respectively (Fig. 1, 2). It was observed that only 18 (16.07%) isolates were confirmed as *P. aeruginosa* by PCR from the screening of 112 various meat samples, *viz.,* chicken meat (18.75%), beef (16.6%), pork (20%), frozen pork (20%), which were randomly collected from different local markets, slaughterhouses, and retail outlets in Tirupati (Table 3). None of the isolates were identified in frozen chicken and frozen beef samples.

Table 3: Number of positives of *P. aeruginosa* in various meat samples (n=112)

Type of sample	No. of samples screened	Number positive for *P. aeruginosa* (%)
Chicken	64	12 (18.75)
Beef	12	02 (16.60)
Pork	15	03 (20.00)
Frozen chicken	11	0
Frozen pork	05	1 (20.00)
Frozen beef	05	0

The overall prevalence rate of *P. aeruginosa* recorded in the present study (16.07%) was comparable to the findings of Srilatha *et al*. (2025), who reported a prevalence of 12% in chicken meat and contaminated surfaces such as chopping boards, knives, and handlers. Swetcha *et al*. (2023) also reported a similar prevalence of *P. aeruginosa* (10.47%) in various fresh and chilled meat samples, including chicken, mutton, and pork, in their study. In contrast, Bantawa *et al*. (2018) from Nepal, and Abbas *et al*. (2022) from Pakistan observed a higher prevalence of *P. aeruginosa* from meat samples, *viz.,* 40% and 24%, respectively, whereas Chakraborty *et al*. (2020) from India recorded a lower prevalence (5.5%) in faeces of pigs, oropharyngeal swabs of poultry and cloacal swabs of chicken.

The prevalence of *P. aeruginosa* varied in meat samples of different species. Out of 64 chicken samples screened in this study, 12 (18.75%) were confirmed as *P. aeruginosa* which is contrary to findings of Bantawa *et al*. (2018) from Nepal (46.6%), Abbas *et al*. (2022) from Pakistan (28.0%), Swetcha *et al*. (2023) from Tamil Nadu (8.57%), and Srilatha *et al*. (2025) from Andhra Pradesh (7.32%). In the present study, two of 12 beef samples showed presence of *P. aeruginosa* isolates (16.6%) which is similar to the finding (14.9%) of Benie *et al*. (2017).

Out of 15 pork samples screened, three samples (20.0%) were found positive for *P. aeruginosa,* which was similar to the finding (12.8%) of Swetcha *et al*. (2023). In contrast 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.

Among the fresh meat samples screened, chicken samples showed higher prevalence than the beef and pork samples, which might due to easily availability of chicken as it is cheap source of protein and may widely sold at roadside stalls which are dirty/polluted places leading to its contamination (Abbas *et al*., 2022). The variations in prevalence of *P. aeruginosa*in fresh meats worldwide could be related to geographical distribution, environmental factors, host susceptibility and the season of sample collection (Sowmya *et al*., 2024).

Simultaneously, a total of 21 frozen samples, *viz.,* chicken (n=11), pork (n=5) and beef (n=5) screened for the presence of *P. aeruginosa* isolates revealed only one pork sample positive (20.0%). None of the frozen chicken and beef samples yielded *P. aeruginosa* isolate. In this study, frozen meat samples showed lower prevalence (4.76%) of *P. aeruginosa* than the fresh meat samples 16.07%), which is similar to the findings of Abbas *et al.* (2022) from Pakistan, who has recorded 16% and 32% prevalence in frozen and fresh meats, respectively. In contrary to this, Swetcha *et al*. (2023) found higher positive isolates of *P. aeruginosa* in frozen samples than fresh meat samples. In general, fresh meats will provide high water content, oxygen availability and nutrients for the growth and spoilage activities of *P. aeruginosa.* This organism is only a psychrotolerant organism but not psychrophilic. While at freezing temperatures, the metabolic activity is arrested, low

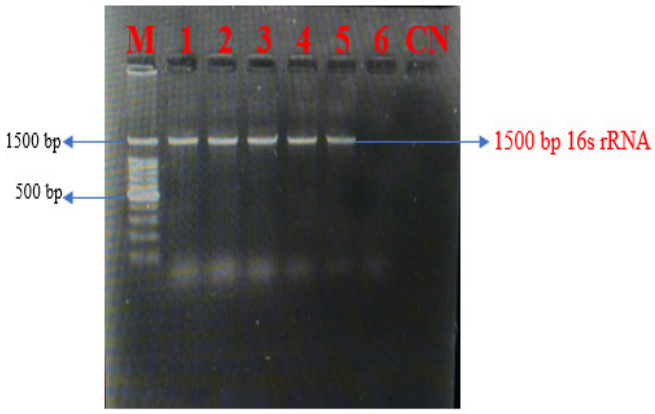

Fig. 1: 16s rRNA profiles of *P. aeruginosa* isolates. Lanes 1-5: Presence of *P. aeruginosa* in analyzed products; CN: Negative control; M: Marker gene ruler, 100 bp

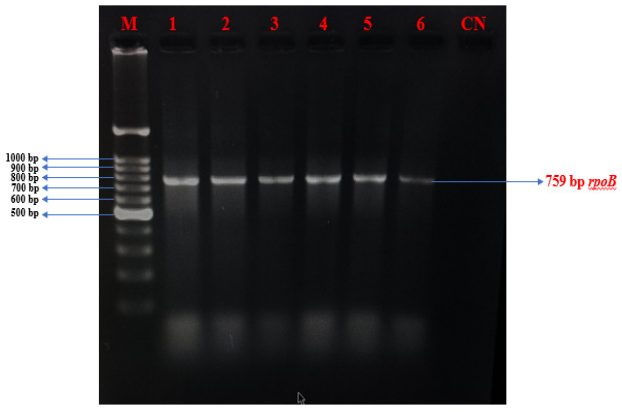

Fig. 2: *rpoB* profiles of *P. aeruginosa* isolates. Lanes 1-6: Presence of *P. aeruginosa* in analyzed products; CN: Negative control; M: Marker gene ruler, 100 bp

oxygen supply and further cells cannot multiply, that may lead to lower prevalence in frozen meat than in fresh meat (Da Silva *et al*., 2026).

The 18 *P. aeruginosa* isolates recovered from fresh and frozen meat samples in this study, were subjected to phenotypic characterization of antimicrobial resistance patterns by disc diffusion method. Out of 18 isolates, all the isolates were shown resistance to erythromycin (100%), while 17 isolates were resistance against amoxycillin (94.0%), 15 isolates to tertracycline and vancomycin (83.0%), 14 isolates to cotrimoxazole (77.7%), 8 isolates against ciprofloxacin (44.0%) and 4 isolates were resistance against gentamicin (22.0%). Many of the researchers from the previous studies also found different antimicrobial resistance patterns against *P. aeruginosa* and also observed MDR isolates from various types of samples, *viz.,* chicken, fish, mutton, pork and beef (Chakraborty *et al*., 2020; Abbas *et al*., 2022; Swetcha *et al*., 2023; Sowmya *et al*., 2024). These discrepancies in the antimicrobial resistance patterns might due to variations in drugs usage and several drug resistance mechanisms (Zeng *et al*., 2014).

The findings of the present study on antimicrobial resistance patterns underscore the critical importance of continuous monitoring and systematic surveillance of antimicrobial resistance (AMR) in *Pseudomonas aeruginosa* at both local and regional levels. Such surveillance is essential for early detection of emerging resistance trends and for guiding evidence-based therapeutic strategies. Furthermore, the results highlight the need to strengthen the implementation of the One Health approach, integrating human, animal, and environmental health sectors to effectively address the growing threat of AMR. The study also emphasizes the necessity for judicious, rational, and restricted use of antibiotics across human and veterinary practices. Prudent antimicrobial stewardship at all levels is imperative to prevent the further emergence and spread of resistance to commonly used antibiotics, thereby preserving their efficacy for future therapeutic interventions.

Conclusion

The present study revealed the occurrence of *Pseudomonas aeruginosa* in various fresh and frozen meat samples collected from retail markets, slaughterhouses, and outlets in and around Tirupati. The antimicrobial susceptibility testing revealed a high level of resistance to commonly used antibiotics, with several isolates exhibiting multidrug resistance. These findings emphasize the growing concern of antimicrobial resistance in foodborne pathogens and its potential public health implications. The presence of multidrug-resistant *P. aeruginosa* in retail meat samples suggests possible contamination during slaughtering, processing, handling, and marketing practices. Therefore, continuous monitoring and systematic surveillance of *P. aeruginosa* and other foodborne pathogens in meat and meat products are essential to ensure food safety and public health. Strengthening of the One Health approach through coordinated efforts among animal, human, and environmental health sectors is necessary to effectively control and prevent the dissemination of antimicrobial-resistant pathogens.

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ANNOUNCEMENT - I

XIII Annual Convention and National Symposium of SVSBT-2026

XIII Annual Convention of the Society for Veterinary Sciences & Biotechnology ***(SVSBT)*** and ***National Symposium*** will be **organized** by the Bihar Veterinary College (BVC), Bihar Animal Sciences University (BASU), **Patna-800014, Bihar, India**, **during October 6-7, 2026**. The detailed Brochure cum Announcement showing Theme Areas / Sessions, Registration Fee, Bank Details for online payment and deadlines for abstract submission/registration, etc. will be floated on the Whatsapp group and e-mails soon.

Dr. Dushyan Yadav, Assistant Professor, LFC-VGO (Mob No. 82993 08955) will act as Organizing Secretary, while **Dr. Amrita Behera,** Assistant Professor, VBC (Mob No. 70080 86783) and **Dr. Manmohan Kumar**, Assistant Professor, LFC (Mob No. 82988 87456) will act as Co-organizing Secretaries. For further details, please contact any one of them.