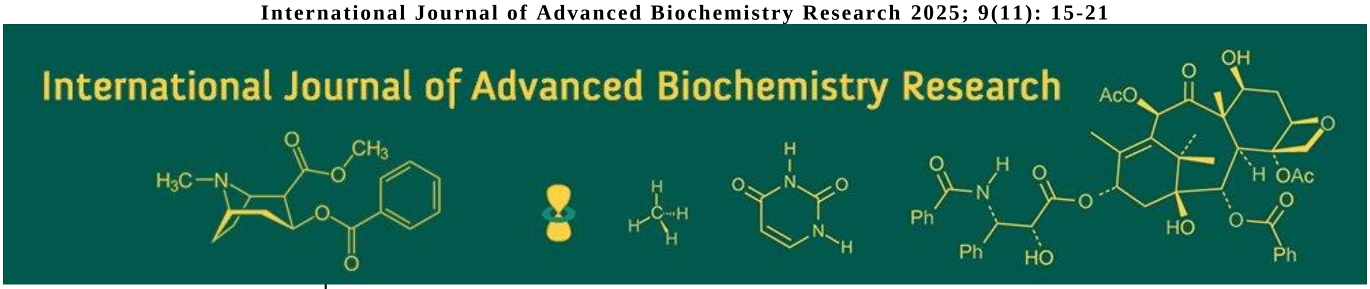

ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2025): 5.29
IJABR 2025; 9(11): 15-21
www.biochemjournal.com
Received: 13-08-2025
Accepted: 16-09-2025

B Srilatha
Department of Veterinary Public Health and Epidemiology, NTR College of Veterinary Science, Sri Venkateswara Veterinary University, Gannavaram, Andhra Pradesh, India

R Annie Supriya
Department of Veterinary Public Health and Epidemiology, College of Veterinary Science, Sri Venkateswara Veterinary University, Garividi, Andhra Pradesh, India

CH Bindu Kiranmayi
Department of Veterinary Public Health and Epidemiology, NTR College of Veterinary Science, Sri Venkateswara Veterinary University, Gannavaram, Andhra Pradesh, India

V Samatha
Animal Husbandry Polytechnic, Sri Venkateswara Veterinary University, Venkataramannagudem, Andhra Pradesh, India

T Srinivasa Rao
Department of Veterinary Public Health and Epidemiology, NTR College of Veterinary Science, Sri Venkateswara Veterinary University, Gannavaram, Andhra Pradesh, India

Corresponding Author:
R Annie Supriya
Department of Veterinary Public Health and Epidemiology, College of Veterinary Science, Sri Venkateswara Veterinary University, Garividi, Andhra Pradesh, India

Comparative evaluation of biofilm detection methods and quorum sensing gene prevalence and antimicrobial resistance in *Pseudomonas aeruginosa* isolates from chicken meat and meat contact surfaces

B Srilatha, R Annie Supriya, CH Bindu Kiranmayi, V Samatha and T Srinivasa Rao

DOI: https://www.doi.org/10.33545/26174693.2025.v9.i11a.6167

Abstract
Biofilm formation plays a crucial role in protecting microorganisms from the host immune system, facilitating their persistence and contributing to chronic infections with high resistance to antimicrobials. This study evaluated biofilm formation in 20 *Pseudomonas aeruginosa* isolates using three methods: Tube method, Congo red agar method, and Microtitre plate method. The Tube method identified biofilm formation in 18 (90%) isolates, categorizing 3 as strong, 9 as moderate, and 6 as weak biofilm producers, while 2 isolates were non-producers. The Congo red method detected biofilm formation in all 20 (100%) isolates, with 4 strong, 7 moderate, and 9 weak biofilm producers. Similarly, the Microtitre plate method also confirmed biofilm formation in all isolates, identifying 3 strong, 6 moderate, and 11 weak biofilm producers. Additionally, quorum sensing, a key regulatory mechanism for virulence, biofilm formation, and antibiotic resistance in *P. aeruginosa*, was investigated by screening for *lasI* and *lasR* genes using Polymerase Chain Reaction. Both genes, with amplicon sizes of 295 bp and 130 bp, were detected in 14 isolates. The highest occurrence of Quorum sensing genes was observed in isolates from cutting board swabs, followed by chicken meat (64.28%) and knife swabs (50%). Antimicrobial resistance profiling of all isolates against 12 antibiotics revealed the highest resistance to tetracycline (95%), followed by cefotaxime (85%). Conversely, imipenem demonstrated the highest sensitivity (75%).
These findings underscore the high biofilm-forming capacity, prevalence of quorum sensing genes, and substantial antimicrobial resistance among *P. aeruginosa* isolates, highlighting their potential to contribute to persistent infections and treatment challenges.

Keywords: Biofilm, cutting board, chicken meat, knives, *P. aeruginosa*, quorum sensing genes

Introduction
Biofilms are bacterial communities enclosed within a self-produced extracellular polymeric substance (EPS) matrix, enabling survival in fluctuating environments (Rollet *et al.*, 2009) [1]. The biofilm matrix formed by *P. aeruginosa* comprises over 90% of its biomass, serves as a scaffold for attachment to various surfaces such as biotic and abiotic surfaces and provides a protective barrier in hostile conditions (Jackson *et al.*, 2004; Ryder *et al.*, 2007; Strempel *et al.*, 2013) [2, 3, 4]. Bacteria in biofilms evade immune defenses and show up to 1000-fold greater resistance to antimicrobials (Lewis, 2001) [5]. Biofilms contribute to over 65% of noso-comial infections (Romling and Balsalobre, 2012) [6] and 60% of infections in humans (Boyle *et al.*, 2013) [7], prompting extensive research into the mechanisms of their formation.
Biofilm formation in *P. aeruginosa* is regulated by quorum-sensing (QS) systems, which enable cell-to-cell communication. These systems coordinate various processes, such as bioluminescence, plasmid transfer, and the synthesis of various virulence factors (El-Khashaab *et al.*, 2016) [8]. Quorum sensing (QS) in *P. aeruginosa* enables cells to communicate and coordinate behavior, especially at high cell densities, using signaling molecules called autoinducers (Rutherford and Bassler, 2012) [9]. *P. aeruginosa* has four QS systems: las, rhl, PQS, and IQS (Sarabhai *et al.*, 2016) [10]. The las and rhl systems consist of autoinducer synthases (*lasI*, *rhlI*) and transcriptional regulators (*lasR*, *rhlR*), which together control the production of various virulence factors essential for host tissue colonization (Luo *et al.*, 2016) [11].

The poultry industry utilizes antibiotics to enhance meat production by improving feed efficiency, accelerating growth rates and preventing diseases. When administered at sub-therapeutic doses, antibiotics have been shown to effectively promote growth (Emami *et al.*, 2012) [11] and support bird health by influencing the immune system of broiler chickens (Lee *et al.*, 2012) [13]. The overuse of antibiotics in chicken promotes antibiotic-resistant bacteria, which can spread to humans through food. Consuming small antibiotic doses via chicken may also contribute to resistance in humans (Sahu and Saxena, 2014) [14]. This surge in antibiotic resistance has significantly increased mortality rates from bacterial infections, presenting a major threat to human health (Xu *et al.*, 2009; Kumar *et al.*, 2020) [15, 16]. These resistant strains result in more complex and prolonged illnesses, which in turn lead to substantial economic losses due to increased treatment and control costs.

Materials and Methods

Isolation and Identification of *Pseudomonas aeruginosa*

A total of 20 *Pseudomonas aeruginosa* isolates were obtained and subjected to identification using standard microbiological procedures. The isolates were first examined using Gram staining to determine their morphological characteristics. Biochemical tests, were performed to confirm their identity. Further molecular confirmation was carried out using polymerase chain reaction (PCR) with specific primers targeting the *P. aeruginosa*-specific gene.

Assessment of Biofilm Formation

The biofilm-forming ability of the isolates was evaluated using the Tube Method, Microtiter Plate (MTP) Method, and Congo Red Agar (CRA) Method

Tube method

A qualitative evaluation of biofilm formation was conducted by inoculating 10 mL of BHI broth with a loopful of microorganisms from overnight culture plates and incubating them for 24 h at 37 °C. After incubation, the broth was discarded and the tubes were washed with phosphate buffer saline (PBS) at pH 7.3 and allowed to dry. The dried tubes were then stained with 0.1% crystal violet for one minute. Excess stain was removed and the tubes were rinsed with deionized water before being dried in an inverted position for observation of biofilm formation.

Biofilm formation was considered positive when a visible violet film was observed lining the tube's wall and bottom. The biofilm presence was assessed using a scoring system: 0 for absent, 1 for weak, 2 for moderate, and 3 for strong biofilm formation (Christensen *et al.*, 1985) [17].

Microtiter plate (MTP) method

Colonies of *P. aeruginosa* isolates from *Pseudomonas* isolation agar plates were transferred into TSB and incubated overnight at 37 °C. The turbidity of the broth was then adjusted to match a 0.5 Mc Farland standard using a nephelometer, which corresponds to 1.5×10^8 colony-forming units (CFU) per ml, or 3×10^6 CFU per well.

For MTP assay 180 µL of TSB was added to each well in the first row of the microtiter plate (MTP), followed by the addition of 20 µL of bacterial inoculum, adjusted to 0.5 McFarland standard, into each well. Three wells containing only sterile TSB without inoculum served as negative controls. The plate was then incubated at 37 °C for 24 h. After incubation, the plate contents were gently discarded and the wells were washed three times with 250 µL of PBS (pH 7.2). The plates were inverted and heat-fixed at 60 °C for 60 min in an incubator. To fix the attached bacteria, 200 µL of methanol was added to each well for 15 min at room temperature. Subsequently, 150 µL of 0.1% crystal violet was used to stain the wells for 15 min at room temperature. After staining, the wells were emptied and rinsed with sterile distilled water. The plates were inverted and dried for 15-30 min at room temperature. Next, 150 µL of 95% ethanol was added to each well to elute the biofilm bound to the walls. The plates were covered and incubated for 30 min at room temperature. The optical density (OD) of each well was then measured at 570 nm using ELISA plate reader (Robinson *et al.*, 2019) [18].

The quantification of biofilm and non-biofilm producing colonies was determined using the cut-off OD (ODc). The ODc is defined as the mean OD of the negative control plus three standard deviations. Based on this, strains were categorized as follows:

Non-biofilm producers (OD≤ ODc)

Weak biofilm producers (ODc < OD ≤ 2 x ODc)

Moderate biofilm producers (2 x ODc < OD ≤ 4 x ODc)

Strong biofilm producers (OD > 4 x ODc).

Congo red Agar (CRA) method

For the CRA method, a specially prepared solid medium was used, consisting of BHI broth supplemented with 5% sucrose and Congo red **(**Freeman *et al.*, 1989) [19]. Congo red was prepared as a concentrated aqueous solution and autoclaved separately from the other medium components at 121 ℃ for 15 minutes, then added to the BHI medium once it had cooled to 55 ℃. The plates were then streaked with a *P. aeruginosa* culture and incubated at 37 ℃ for 24 to 48 h.

Positive results were indicated by the formation of black colonies with a dry, crystalline appearance. Weak slime producers were usually pink, with occasional dark centers in the colonies. Intermediate results showed colony darkening without the dry crystalline morphology.

Molecular confirmation of Quorum Sensing genes in *P. aeruginosa* isolates

All the confirmed *P. aeruginosa* isolates from chicken meat and the meat contact surfaces were subjected to m-PCR for the detection of quorum sensing genes. A m-PCR assay capable of detecting the *lasI* and *lasR* genes, which produce amplification products of 295 bp and 130 bp, respectively (Table 1) was used. PCR amplification was optimized in 25 µL PCR reaction mixture consisting of PCR master mix 12.50 µL, Forward & Reverse primers (10 pmol/µL) each 1.25 µL, Template DNA (50 ng/µL) 2.5 µL and Nuclease free water 7.50 µL was subjected to standardized PCR conditions of initial denaturation at 94 ℃ for 2 min, followed by 30 cycles each of denaturation at 9 5 ℃ for 40sec, annealing at 59.4 ℃ for 1min, extension at 72 ℃ for 2 min, final extension at 72 ℃ for 10 min for detection of *Pseudomonas aeruginosa* according to (da Costa Lima *et al.*, 2018) [20].

Table 1: Nucleotide sequences for quorum sensing genes of *P. aeruginosa*

Species	Target gene	Nucleotide sequence (5'-3')	Amplicon size (bp)
P. aeruginosa	*las I*	CGTGCTCAAGTGTTCAAGG TACAGTCGGAAAAGCCCAG	295
	las R	AAGTGGAAAATTGGAGTGGAG GTAGTTGCCGACGACGATGAAG	130

Antimicrobial Susceptibility testing

P. aeruginosa isolates were initially cultured in BHI broth and incubated for at 37 °C for 24hr. The turbidity of the culture was adjusted to match 0.5 McFarland standards, equivalent to a cell density of approximately 1.5×10^8 CFU/mL which corresponds to an absorbance of 0.132 at 600 nm. To prepare a lawn culture, 200 µL of each bacterial suspension was spread evenly on Muller Hinton agar using a sterile cotton swab. After allowing the surface to dry, antibiotic discs were positioned with sterile forceps, with a minimum of 15mm distance. The antibiotic resistance pattern of *P. aeruginosa* was determined using the Kirby-Bauer disc diffusion method (Bauer *et al.*, 1966) [21] against 12 different antibiotics used in veterinary medicine. The antimicrobial agents tested and their corresponding concentrations were as follows Amoxicillin/Clavulanic acid (30µg), Ampicillin (10 µg), Aztreonam (30 µg), Ceftriaxone (30 µg), Co-trimoxazole (25 µg), Ciprofloxacin (5 µg), Amikacin (30µg), Imipenem (10 µg), Tetracycline (30µg), Cefotaxime (30 µg), Chloramphenicol (30 µg), Ceftazidime (30 µg). The plates were then incubated at 37 °C for 18 h, and the zones of inhibition were measured. Interpretation of susceptibility and resistance was performed according to (CLSI, 2021) [22].

Results

In this study, biofilm formation by *P. aeruginosa* isolates was detected using both qualitative and quantitative methods. The qualitative evaluation involved Christensen's tube method and Congo red agar (CRA) while the quantitative measurement was carried out by microtiter plate assay (MTP).

The biofilm formation ability of *P. aeruginosa* was detected after 24-48 h of incubation by test tube method. The adherence capacity was measured after staining with 0.1% crystal violet solution and were classified as strong, moderate, weak and non-biofilm former. Out of the 20 isolates, four isolates showed dark colour after crystal violet staining which indicated them as strong biofilm formers, eight isolates exhibited moderate adherence indicated as moderate biofilm formers, six isolates as weak biofilm formers with low adherence and two isolates did not exhibit any adherence indicated as non-biofilm formers (Fig.1).

The isolates were also tested by using CRA method for biofilm formation. Out of the 20 isolates, 4 isolates showed black colonies with dry crystalline consistency indicating them as strong biofilm formers (Fig. 2), 7 isolates showed dark colonies without dry crystalline colonial morphology indicating intermediate (Fig. 3), 9 were weak slime producers as they remained pink though darkening of the centers were observed but rarely (Fig.4). None of the *P. aeruginosa* isolates were negative for biofilm formation by CRA.

The quantification of biofilm was done for the 20 *P. aeruginosa* isolates using the microtiter plate assay. The isolates were classified as strong, moderate, weak and non-biofilm producer based on the OD values obtained (Fig. 5 and Table. 2).

All the 20 isolates (100%) were found to be biofilm producers on MTP. Out of 20, 3 isolates were found to be strong biofilm producers as their OD values were ≥ (i.e., 4x OD_c), 6 isolates were moderate biofilm formers with OD values ranging from (i.e., 2x OD_c < OD ≤ 4x OD_c) and 11 isolates were weak biofilm formers with OD values between (i.e., OD_c < OD ≤ 2x OD_c). None of the isolates were non-biofilm producers on MTP as their OD values were less than 0.312 (i.e., OD_c of negative controls). The comparative analysis of the three methods is shown in (Table. 3).

Table 2: Biofilm formation ability by *P. aeruginosa* isolates using tube method, CRA and MTP assay

S. No	Sample No.	Tube method	CRA	MTP	
				Adherence	OD values
1	CM5	Moderate	Moderate	Moderate	1.007
2	CM14	Weak	Weak	Weak	0.521
3	CM25	Weak	Weak	Weak	0.546
4	CM38	Moderate	Weak	Moderate	0.657
5	CM72	Weak	Moderate	Weak	0.573
6	CM78	Strong	Strong	Strong	2.028
7	CM89	Absent	Weak	Weak	0.368
8	CM100	Absent	Weak	Weak	0.394
9	CM113	Weak	Weak	Weak	0.488
10	CM146	Strong	Strong	Strong	2.081
11	CM163	Moderate	Weak	Weak	0.563
12	CM171	Moderate	Moderate	Weak	0.627
13	CM175	Weak	Weak	Weak	0.508
14	CM185	Moderate	Moderate	Moderate	1.155
15	CB6	Moderate	Moderate	Weak	0.588
16	CB8	Moderate	Strong	Moderate	0.647
17	CB11	Strong	Moderate	Moderate	0.744
18	CB12	Strong	Strong	Strong	2.090
19	KS7	Weak	Weak	Weak	0.486
20	KS12	Moderate	Moderate	Moderate	0.702

CM-Chicken meat, CB-Cutting board, KS-Knife swabs

Table 3: Comparative analysis of biofilm formation ability by *P. aeruginosa* isolatesusing tube, CRA and MTP method

Biofilm formation assay	Absent (%)	Weak (%)	Moderate (%)	Strong (%)
Test tube method	2 (10)	6 (30)	8 (40)	4 (20)
Congo red agar method	0 (0)	9 (45)	7 (35)	4 (20)
Microtiter plate method	0 (0)	11(55)	6 (30)	3 (15)

In the current study, all the 20 *P. aeruginosa* isolates were further assessed for the presence of quorum sensing genes (*lasI* and *lasR*). Out of the 20 isolates, both the genes *lasI* and *lasR* with amplicon size of 295 and 130bp were noticed in 14 isolates. The highest occurrence of *lasI* and *lasR* genes were noticed in all the cutting board swabs followed by chicken meat (64.28%), knife swabs (50%). (Fig. 6 and Table 4).

Table 4: Distribution of quorum sensing genes in *P. aeruginosa* isolates

S. No	Type of sample	No. of isolates analysed	Quorum sensing genes	
			***lasI* (%)**	***lasR* (%)**
1	Chicken meat	14	9(64.28)	9(64.28)
2	Cutting board swab	4	4(100)	4(100)
3	Knife swab	2	1(50)	1(50)
4	Hand swab	0	0/0(0)	0/0(0)
	Total	20	14(70)	14(70)

All the 20 *P. aeruginosa* isolates were subjected to an *in vitro* antibiotic sensitivity test, carried out by disc diffusion method to asses the resistance/sensitivity patterns against 12 antimicrobial drugs. The antimicrobial drugs belonging to different classes cephalosporins, phenolics, fluoroquinolones, aminoglycosides, carbapenems, penicillins, sulphonamides, tetracyclines and monobactams were used to detect the multidrug resistance in *P. aeruginosa* isolates.

Highest resistance was observed against tetracycline (90%) followed by cefotaxime (85%), ampicillin (75%), ceftriaxone (60%), ceftazidime (55%), amikacin (50%), chloramphenicol (35%), co-trimoxazole and amoxicillin and clavulonic acid (30% each), aztreonam (25%), imipenem (20%) and ciprofloxacin (10%). The *P. aeruginosa* isolates showed highest sensitivity against imipenem (75%) followed by co-trimoxazole (60%), ciprofloxacin (50%), chloramphenicol and amoxicillin/clavulonic acid (45%), amikacin (40%), ceftazidime and aztreonam (30% each), ampicillin (15%), ceftriaxone and tetracycline (5% each).

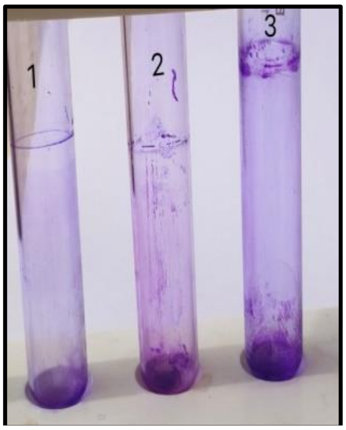

Fig 1: Detection of Biofilm formation by tube method
1-Weak biofilm
2-Moderate biofilm
3-Strong biofilm

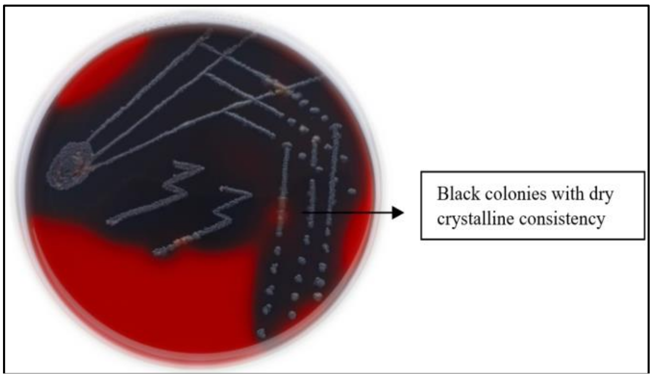

Fig 2: Strong biofilm formation by *P. aeruginosa* on congored agar.

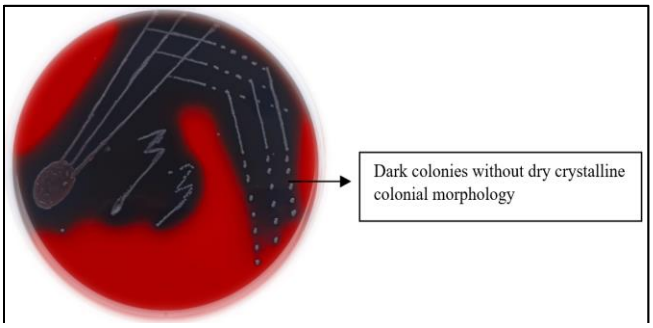

Fig 3: Intermediate biofilm formation by *P. aeruginosa* on congo red agar.

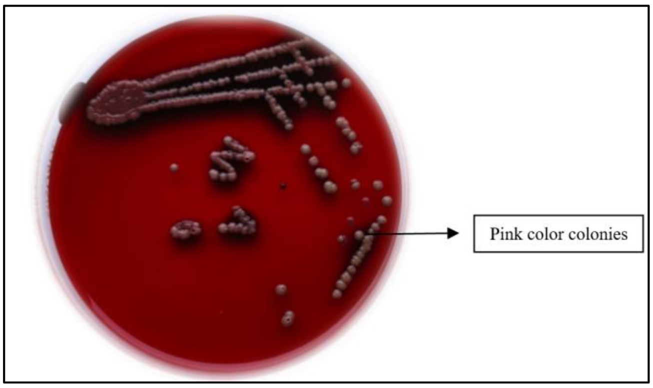

Fig 4: Weak biofilm formation by *P. aeruginosa* on congo red agar.

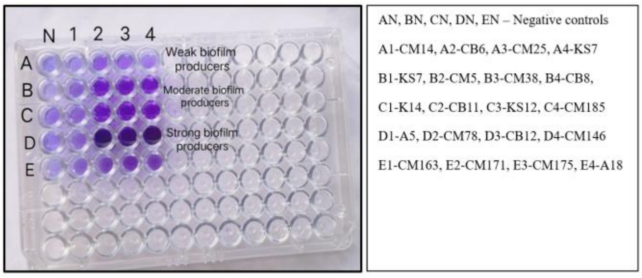

Fig 5: Detection of Biofilm formation of *P. aeruginosa* by MTP

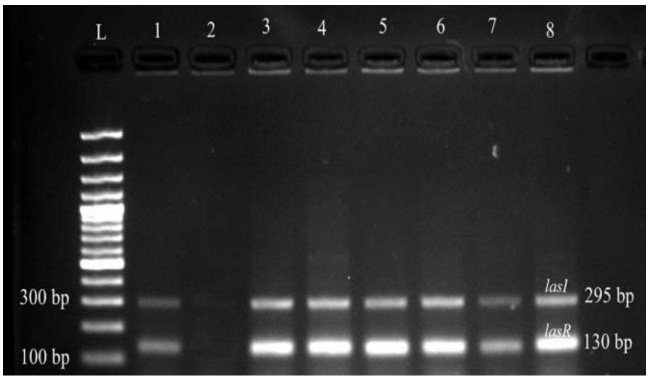

Fig 6: Gel photograph of quorum sensing genes in *P. aeruginosa* isolates
{Lane L: DNA ladder (100bp), Lane 1: *P. aeruginosa* isolate showing *lasI* (295bp)
and *lasR* (130 bp), Negative control (Lane2), Different *P. aeruginosa* isolates showing
sensing gene *lasI* (295 bp) and *lasR* (130 bp) (Lane 3-8)}

Discussion

In the current study, evaluation of biofilm production was done by tube method, CRA and MTP assay which demonstrated slight differences in their efficiency to detect the biofilm formation phenotypically. In a study conducted by (Salem *et al.*, (2024) [23] to detect biofilm producing ability of *P. aeruginosa* using tube method revealed that 27.3% of the isolates were non-adherent, 18.1% were weak biofilm formers, 27.3% were moderate biofilm producers and 27.3% were strong biofilm formers. In the current study tube method results indicated that 10% of the isolates were non-adherent, 30% were weak biofilm formers, 40% were moderate and 20% were strong biofilm formers. The difference in the reports might be due to the interpretation of results by different observers as it was hard to visually differentiate the non-biofilm formers, weak, moderate and strong biofilm formers in crystal violet assay.

Locatelli *et al.*, (2004) [24] and (Freitas and Barth, 2002) [25] analyzed the biofilm formation of *P. aeruginosa* using CRA technique and observed that all (100%) *P. aeruginosa* strains analyzed were considered to be biofilm producers. Similar results were observed in this study where all the 20 isolates were detected as biofilm formers using CRA technique, of which 45% were weak, 35% were moderate and 20% were strong biofilm producers. In contrary (Inat *et al.*, 2021) [26] reported 80% of the *P. aeruginosa* isolates as biofilm producers. The reason behind the differences between present study and previous studies may be due to inconsistent congo red staining which can lead to over estimation or under estimation of biofilm formation.

In the current study, the quantitative assessment of biofilm formation was performed using MTP assay. All the 20 (100%) *P. aeruginosa* isolates were found to be biofilm formers, of which 55% were weak, 30% were moderate and 15% were strong biofilm formers. The current study reported 100% of the isolates as biofilm formers using CRA method and MTP assay. This result was strongly in accordance with (Ramanarayana, 2022) [27] who reported that all (100%) of the positive isolates had ability to produce biofilm. In contrary (Abbas *et al.*, 2022) [28] reported 22.7% as strong biofilm producers, 45.7% as moderate, 18.1% as weak and 13.6% as non biofilm formers. The variations in results with crystal violet staining in microtiter plates may arise from inconsistent biofilm adherence to the plate surface. Additionally, optical density measurements of stained biofilms can be influenced by factors such as washing steps, dye retention, and the calibration of the plate reader.

In the current study, all the 20 *P. aeruginosa* isolates were tested for the presence of quorum sensing genes *lasI and lasR* which play a major role in the production of virulence factors and formation of biofilms. The results demonstrated that 14 (14/20 each) isolates were positive for both *lasI* and *lasR* genes. In the present study, the prevalence of *lasI* gene in chicken meat was 64.28% (9/14) which is in tune with the findings of (Inat *et al.*, 2021) [26] who also reported the prevalence of *lasI* gene from chicken meat as 72%. In contrary (Bakheet and Torra, 2020) [29] reported the high prevalence of *lasI* gene (100%) in chicken meat.

In the present study, the prevalence of *lasI* and *lasR* gene in cutting board swabs is 100%, where as 50% in knife swabs and none of the isolates from hand swabs were found to be positive for *lasI* and *lasR* genes.

The production of extracellular enzymes that degrade quorum-sensing (QS) coding genes could lead to QS gene deficiencies. Additionally, mutations in QS coding genes may result in variations in gene expression. Differences in bacterial origins, genetic traits, isolation sources and environmental conditions may further account for the discrepancies observed between the present study and previous investigations.

Antimicrobial resistance (AMR) poses an intensifying global health threat with substantial implications for public health, healthcare systems, economies and the environment. The rise in antimicrobial resistance is particularly alarming as resistant *P. aeruginosa* strains may transfer their resistance genes to other bacteria. Consequently, research on the antibiotic susceptibility of *P. aeruginosa* isolates is essential to prevent the spread of multidrug-resistant strains. In this context, the antimicrobial susceptibility profiles of *P. aeruginosa* isolates were evaluated against 12 antibiotics using the Kirby-Bauer disc diffusion method.

The antibiotic sensitive patterns of *P. aeruginosa* vary depending on the dosage and prolific usage of antimicrobials, the inherent resistance capacity and biofilm formation ability of the bacteria. In the present study, the results of the antibiotic sensitivity test which was conducted with a panel of 12 different antibiotics revealed highest sensitivity to imipenem (75%) and highest resistance to tetracycline (90%). Results also indicated that *P. aeruginosa* isolates showed 85% resistance to cefotaxime, 75% resistance to ampicillin, 60% to ceftriaxone, 55% to ceftazidime, 50% to amikacin, 35% to chloramphenicol, 30% each to amoxicillin/clavulanic acid and co-trimoxazole, 25% to aztreonam, 20% to imipenem and 10% resistance to ciprofloxacin.

In the present study, the resistance towards tetracycline (90%) and ceftaxime (85%) were in slight agreement with the findings of (Algammal *et al.*, 2023) [30] who reported the resistance 100% towards tetracycline and 92.6% to cefotaxime. The highest resistance noticed might be due to indiscriminate use of antibiotics by veterinarians in farm animals as treatment or as growth promoters.

Resistance rates of ceftriaxone (60%) and cotrimoxazole (30%) were in line with the observations of (Elbehiry *et al.*, 2022) [31] who reported the resistance of 66.6% to ceftriaxone and 33.33% to co-trimoxazole, respectively.

In the present study, sensitivity patterns of imipenem (75%) and ciprofloxacin (50%) were similar to the findings of (Salem *et al.*, 2024) [32] who also reported the sensitivity patterns of 78.2% to imipenem and 52.7% to ciprofloxacin. On contrary (Abbas *et al.*, 2022) [33] detected 33.3% sensitivity to imipenem and 4% sensitivity to ciprofloxacin. This may be due to the regional variations in the usage of antibiotics, leading to the differences in antibiotic resistance.

Conclusion

In conclusion, the detection of biofilm formation in *Pseudomonas aeruginosa* isolates from chicken meat highlights the potential risk posed by these bacteria in food contamination and public health. The ability of *P. aeruginosa* to form biofilms enhances its resistance to environmental stress, disinfectants, and antibiotics, making it a significant concern in food safety. Furthermore, the presence of quorum sensing genes plays a crucial role in controlling virulence factors, biofilm maturation, and bacterial communication, contributing to the persistence of *P. aeruginosa* in food processing environments. Understanding these mechanisms is essential for developing effective control strategies to minimize bacterial contamination in poultry products. In this regard, future studies should focus on exploring alternative antimicrobial approaches, such as quorum sensing inhibitors, to mitigate the risks associated with *P. aeruginosa* in the food industry. Additionally, restricting antimicrobials to therapeutic use under veterinary supervision, promoting natural alternatives, strengthening regulatory enforcement, and raising awareness about antimicrobial resistance are crucial steps. These measures will protect public health, combat AMR and support sustainable poultry farming.

Acknowledgement
The authors express gratitude to Sri Venkateswara Veterinary University, Tirupati, Andhra Pradesh, for providing valuable support throughout the course of this research.

References

1. Rollet C, Gal L, Guzzo J. Biofilm-detached cells, a transition from a sessile to a planktonic phenotype: a comparative study of adhesion and physiological characteristics in *Pseudomonas aeruginosa*. FEMS Microbiol Lett. 2009 Jan 1;290(2):135-142.
2. Jackson KD, Starkey M, Kremer S, Parsek MR, Wozniak DJ. Identification of *psl*, a locus encoding a potential exopolysaccharide that is essential for *Pseudomonas aeruginosa* PAO1 biofilm formation. J Bacteriol. 2004 Jul;186(14):4466-4475.
3. Ryder C, Byrd M, Wozniak DJ. Role of polysaccharides in *Pseudomonas aeruginosa* biofilm development. Curr Opin Microbiol. 2007 Dec 1;10(6):644-648.
4. Strempel N, Neidig A, Nusser M, Geffers R, Vieillard J, Lesouhaitier O, *et al.* Human host defense peptide LL-37 stimulates virulence factor production and adaptive resistance in *Pseudomonas aeruginosa*. PLoS One. 2013 Dec 13;8(12):e82240.
5. Lewis KI. Riddle of biofilm resistance. Antimicrob Agents Chemother. 2001 Apr 1;45(4):999-1007.
6. Romling U, Balsalobre C. Biofilm infections, their resilience to therapy and innovative treatment strategies. J Intern Med. 2012 Dec;272(6):541-561.
7. Boyle KE, Heilmann S, van Ditmarsch D, Xavier JB. Exploiting social evolution in biofilms. Curr Opin Microbiol. 2013 Apr 1;16(2):207-212.
8. El-Khashaab TH, Erfan DM, Kamal A, El-Moussely LM, Ismail DK. *Pseudomonas aeruginosa* biofilm formation and quorum sensing *lasR* gene in patients with wound infection. Egypt J Med Microbiol. 2016 Jan;38(87):1-8.
9. Rutherford ST, Bassler BL. Bacterial quorum sensing: its role in virulence and possibilities for its control. Cold Spring Harb Perspect Med. 2012 Nov 1;2(11):a012427.
10. Sarabhai S, Kaur A, Capalash N, Sharma P. Quorum sensing in *Pseudomonas aeruginosa*: mechanism and regulation of virulence. In: *Pseudomonas*: Molecular and Applied Biology. Cham: Springer; 2016. p. 231-256.
11. Luo J, Kong JL, Dong BY, Huang H, Wang K, Wu LH, *et al.* Baicalein attenuates the quorum sensing-controlled virulence factors of *Pseudomonas aeruginosa* and relieves the inflammatory response in *P. aeruginosa*-infected macrophages by downregulating the MAPK and NFκB signal-transduction pathways. Drug Des Devel Ther. 2016 Jan 7;10:183-203.
12. Emami NK, Samie A, Rahmani HR, Ruiz-Feria CA. The effect of peppermint essential oil and fructooligosaccharides, as alternatives to virginiamycin, on growth performance, digestibility, gut morphology and immune response of male broilers. Anim Feed Sci Technol. 2012 Jul 20;175(1-2):57-64.
13. Lee KW, Hong YH, Lee SH, Jang SI, Park MS, Bautista DA, *et al.* Effects of anticoccidial and antibiotic growth promoter programs on broiler performance and immune status. Res Vet Sci. 2012 Oct 1;93(2):721-728.
14. Sahu R, Saxena P. Antibiotics in chicken meat. Centre for Science and Environment. 2014 Jul;41.
15. Xu Z, Li L, Shirtliff ME, Alam MJ, Yamasaki S, Shi L. Occurrence and characteristics of class 1 and 2 integrons in *Pseudomonas aeruginosa* isolates from patients in southern China. J Clin Microbiol. 2009 Jan;47(1):230-234.
16. Kumar SB, Arnipalli SR, Ziouzenkova O. Antibiotics in food chain: The consequences for antibiotic resistance. Antibiotics (Basel). 2020 Oct 13;9(10):688.
17. Christensen GD, Simpson WA, Younger JJ, Baddour LM, Barrett FF, Melton DM, *et al.* Adherence of coagulase-negative staphylococci to plastic tissue culture plates: a quantitative model for the adherence of staphylococci to medical devices. J Clin Microbiol. 1985 Dec;22(6):996-1006.
18. Robinson VH, Paterson S, Bennett C, Steen SI. Biofilm production of *Pseudomonas* spp. isolates from canine otitis in three different enrichment broths. Vet Dermatol. 2019 Jun;30(3):218-e67.
19. Freeman DJ, Falkiner FR, Keane CT. New method for detecting slime production by coagulase negative staphylococci. J Clin Pathol. 1989 Aug 1;42(8):872-874.
20. da Costa Lima JL, Alves LR, de Araújo Jacomé PR, Neto JP, Maciel MA, *et al.* Biofilm production by clinical isolates of *Pseudomonas aeruginosa* and structural changes in LasR protein of isolates non biofilm-producing. Braz J Infect Dis. 2018 Mar 1;22(2):129-136.
21. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol. 1966 Apr;45(4):493-496.
22. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. CLSI supplement M100. 31st ed. Wayne (PA): CLSI; 2021.
23. Salem M, Younis G, Sadat A, Nouh NA, Binjawhar DN, Abdel-Daim MM, *et al.* Dissemination of *mcr-1* and β-lactamase genes among *Pseudomonas aeruginosa*: Molecular characterization of MDR strains in broiler chicks and dead-in-shell chicks infections. Ann Clin Microbiol Antimicrob. 2024 Jan 28;23(1):9.
24. Locatelli CI, Englert GE, Kwitko S, Simonetti AB. *in vitro* bacterial adherence to silicone and polymetylmethacrylate intraocular lenses. Arq Bras Oftalmol. 2004;67:241-248.
25. Freitas AL, Barth AL. Antibiotic resistance and molecular typing of *Pseudomonas aeruginosa*: focus on imipenem. Braz J Infect Dis. 2002;6:01-06.

26. İnat G, Sırıken B, Başkan C, Erol İ, Yıldırım T, Çiftci A. Quorum sensing systems and related virulence factors in *Pseudomonas aeruginosa* isolated from chicken meat and ground beef. Sci Rep. 2021 Aug 2;11(1):15639.
27. Ramanarayana P. Assessment of antibiotic resistance pattern and biofilm formation in *Pseudomonas aeruginosa* isolates from buffaloes and dogs [dissertation]. Tirupati (A.P) India: Sri Venkateswara Veterinary University; 2022.
28. Abbas R, Nawaz Z, Siddique AB, Aslam R, Rafique A, Zahoor MA, *et al.* Molecular Detection of Biofilm Production among Multidrug Resistant Isolates of *Pseudomonas aeruginosa* from Meat Samples. Pak Vet J. 2022 Oct 1;42(4).
29. Bakheet AA, Torra DE. Detection of *Pseudomonas aeruginosa* in Dead Chicken Embryo with Reference to Pathological Changes and Virulence Genes. Alex J Vet Sci. 2020 Jun 1;65(1).
30. Algammal AM, Eidaroos NH, Alfifi KJ, Alatawy M, Al-Harbi AI, Alanazi YF, *et al. Opr l* gene sequencing, resistance patterns, virulence genes, quorum sensing and antibiotic resistance genes of xdr *Pseudomonas aeruginosa* isolated from broiler chickens. Infect Drug Resist. 2023 Dec 31:853-867.
31. Elbehiry A, Marzouk E, Aldubaib M, Moussa I, Abalkhail A, Ibrahem M, *et al. Pseudomonas* species prevalence, protein analysis, and antibiotic resistance: an evolving public health challenge. Amb Express. 2022 May 9;12(1):53.
32. Salem M, Younis G, Sadat A, Nouh NA, Binjawhar DN, Abdel-Daim MM, *et al.* Dissemination of *mcr-1* and β-lactamase genes among *Pseudomonas aeruginosa*: Molecular characterization of MDR strains in broiler chicks and dead-in-shell chicks infections. Ann Clin Microbiol Antimicrob. 2024 Jan 28;23(1):9.