

A Study on *Pseudomonas aeruginosa* in Chicken Meat: Isolation, Molecular Characterization, and Virulence Genes Detection

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ABSTRACT

Foodborne diseases caused by contaminated foods pose significant economic and public health risks worldwide. This study focused on the isolation and molecular characterization of *Pseudomonas aeruginosa* from chicken meat and contaminated surfaces, including cutting boards, knives, and workers hands in retail chicken meat outlets. A total of 250 samples were analyzed, comprising 191 chicken meat samples, 20 cutting board swabs, 20 knife swabs, and 19 hand swabs. Conventional methods identified *P. aeruginosa* in 30 (12.0%) samples, of which 20 (8.0%) were confirmed via species-specific PCR. The highest occurrence was in cutting board swabs (20.0%), followed by knife swabs (10.0%) and chicken meat (7.32%), while no isolates were found in hand swabs. Virulence gene analysis revealed that *P. aeruginosa* harbored multiple pathogenic factors. Among 20 isolates, 12 (60.0%) carried the *lasB* gene, 6 (30.0%) carried *exoS*, 11 (55.0%) carried *plcH*, and none harbored *algD*. The *lasB* gene was most prevalent in cutting board swabs (75.0%), *exoS* was equally present in cutting board and knife swabs (50.0%), while *plcH* was most frequent in chicken meat (57.14%). These findings highlight the potential risk of *P. aeruginosa* contamination in poultry retail environments.

Key words: Chicken meat, Cutting boards, Knives, *Pseudomonas aeruginosa*, Virulence genes, Workers hands.

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INTRODUCTION

India is one of the world's leading producers of poultry meat, with consumption surpassing 4 million metric tons in 2023 (Minhas, 2024). The rapid growth of the poultry sector has increased concerns about food safety, particularly regarding microbial contamination. Chicken meat, a vital protein source, is highly perishable due to its rich nutritional profile and high water content, making it susceptible to microbial growth (Alexa *et al.*, 2024). Foodborne illnesses linked to contaminated poultry underscore the importance of stringent hygiene practices in processing and retail environments (Momtaz *et al.*, 2013; Rahimi *et al.*, 2014). Among the pathogens of concern, *Pseudomonas aeruginosa* is an emerging cause of foodborne diseases and spoilage (Gao *et al.*, 2023). This motile, Gram-negative bacterium thrives in diverse environments, including poultry processing plants and contaminated water sources (Li *et al.*, 2023). *P. aeruginosa* is an opportunistic pathogen that can cause serious infections in immunocompromised individuals, affecting the bloodstream, respiratory tract, and soft tissues (Klockgether and Tummler, 2017). Its transmission through undercooked poultry and improper handling presents a significant public health risk (Alimi *et al.*, 2022).

Additionally, *P. aeruginosa* possesses multiple virulence factors, including elastase B (*lasB*), exoenzyme S (*exoS*), phospholipase C (*plcH*), and alginate (*algD*), which contribute to its pathogenicity (Benie *et al.*, 2017). Previous research has indicated that the presence of *exoS* is associated with heightened virulence, particularly in cases of burn wound

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infections and lung infections (Fazeli *et al.*, 2017). In mouse models, the secretion of *lasB* by *P. aeruginosa* isolates has been linked to haemorrhagic alveolar damage (Zhu *et al.*, 2021). Additionally, phospholipase C negatively impacts lung tissue integrity, leading to impaired lung function (Wargo *et al.*, 2009). Alginate, a polymer composed of α -L-guluronic acid and β -D-mannuronic acid, is crucial for biofilm protection and structural stability (Wei and Ma, 2013). Therefore keeping in view of its potential threat to food safety, this study was

designed to investigate the prevalence of *P. aeruginosa* in chicken meat, highlighting the need for improved hygiene practices in poultry processing to mitigate contamination risks.

MATERIALS AND METHODS

Sample Collection

A total of 191 chicken meat samples were collected from various chicken retail outlets in Vijayawada (Andhra Pradesh, India). Additionally, 59 samples were collected from chicken retail shops, including contaminated surfaces such as hand swabs (19) from retailers, swabs from knives (20), and cutting board (20). About 25 g of chicken meat samples were collected in sterile polythene zip lock packs under aseptic conditions. Swabs from hands, cutting boards, and knives were collected by moistening them with physiological saline. These samples were immediately placed on ice and transported to the food safety laboratory, Department of Veterinary Public Health and Epidemiology of the College and examined within 24-48 h of collection.

Cultural Isolation and Identification of *P. aeruginosa*

Selective enrichment of samples was done in Brain Heart Infusion broth at 37°C for 24 h aerobically. Later, enriched samples were streaked on cetrinide agar and incubated at 37°C for 24 h aerobically. After 24 h of incubation, cetrinide agar plates showing characteristic bluish green colonies or yellowish green colonies were identified as presumptive *P. aeruginosa* colonies. These colonies were further examined using Gram staining, which revealed Gram-negative rods. Biochemical tests confirmed the isolates as catalase-positive, oxidase-positive, indole-negative, methyl red-negative, Voges-Proskauer-negative, and citrate-positive.

Molecular Characterization of *P. aeruginosa*

An overnight *P. aeruginosa* culture (2 mL) was placed in a microcentrifuge tube and spun at 10,956 g for 5 min. The resulting pellet was resuspended in 400 µL of nuclease-free water and heated for 10 min in a boiling water bath. The tube was then immediately transferred to ice. After 20 min, it was centrifuged at 7,025 g for 5 min at 4°C, and the supernatant was collected to serve as the template for PCR reactions. The concentration of DNA was measured with nanodrop and adjusted to 50 ng/µL for further studies. Pure DNA samples (with an optical density ratio of 1.8-2.0 at 260/280 nm) were stored at -20°C, for further use.

P. aeruginosa species specific PCR employing a primer combination that targeted a portion of the 16S *rRNA* gene was used to molecular confirmation of presumptive colonies of *P. aeruginosa* (Table 1). 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 95°C for 5 min, followed by 30 cycles each of denaturation at 95°C for 45 sec, annealing at 55°C for 45 sec, extension at 72°C for 1 min, final extension at 72°C for 5 min for detection of *Pseudomonas aeruginosa* according to Spilker *et al.* (2004).

After the reaction, Agarose gel electrophoresis was performed to analyze PCR amplicons following Sambrook, (2001). A 1.5% agarose gel containing ethidium bromide (0.5 µg/mL) was prepared in 1X TAE buffer and cast in a tray with a comb. After solidification, the gel was placed in a horizontal electrophoresis unit with 1X TAE buffer, and the comb was removed. PCR products (10 µL) were mixed with 2 µL of 6X loading dye (Fermentas, USA) and loaded into the wells. A 100 bp DNA ladder (Genei™, Bangalore) was loaded as a molecular weight marker. Electrophoresis was run at 70 volts

Table 1: Nucleotide sequences and amplicon sizes of *P. aeruginosa* species specific PCR primers

Species	Target gene	Nucleotide sequence (5'-3')	Amplicon size	Reference
<i>P. aeruginosa</i>	16S <i>rRNA</i>	GGGGGATCTTCGGACCTCA TCCTTAGAGTGCCCCACCG	956 bp	Spilker <i>et al.</i> (2004)

Table 2: Primers used for the detection of virulence genes in *P. aeruginosa*

PCR	Target gene	Primer	Nucleotide Sequence(5'-3')	Amplicon Size (bp)	Reference
m-PCR-I	<i>lasB</i>	<i>lasB</i> -F	GGAATGAACGAGGCGTTCTC	300	Benie <i>et al.</i> (2017)
		<i>lasB</i> -R	GGTCCAGTAGTAGCGGTTGG		
	<i>exoS</i>	<i>exoS</i> -F	CTTGAAGGGACTCGACAAGG	504	
		<i>exoS</i> -R	TTCAGGTCCGCGTAGTGAAT		
	<i>algD</i>	<i>algD</i> -F	ATGCGAATCAGCATCTTTGGT	1310	
		<i>algD</i> -R	CTACCAGCAGATGCCCTCGGC		
m-PCR-II	<i>plcH</i>	<i>plcH</i> -F	GAAGCCATGGGCTACTCAA	307	Benie <i>et al.</i> (2017)
		<i>plcH</i> -R	AGAGTGACGAGGAGCGGTAG		

for 1 h until the dye migrated 75–80% of the gel. Bands were visualized under UV light (300 nm) using a Gel Documentation System, and their sizes were determined by comparison with the DNA ladder (Fig. 1).

Molecular Detection of Virulence Genes of *P. aeruginosa* by m-PCR-I and m-PCR-II

All the confirmed *P. aeruginosa* isolates from various sources were subjected to m-PCR for the detection of virulence genes. Two sets of m-PCR assays which detect the presence of *lasB*, *exoS*, *algD* and *plcH* generating amplification products of 300, 504, 1310 and 307 bp, respectively (Table 2) were used (Benie *et al.*, 2017). Optimized PCR reaction mixture consisting of PCR master mix 12.50 µL, Forward & Reverse primers (10 pmol/µL) each 0.375 µL ×4, Template DNA (50 ng/µL) 3.00 µL and Nuclease free water 6.50 µL, was subjected to standardized PCR conditions of initial denaturation at 94°C for 5 min, followed by 35 cycles each of denaturation at 94°C for 35 sec, annealing at 60°C for 1 min, extension at 72°C for 1 min, final extension at 72°C for 10 min for detection of virulence genes in *P. aeruginosa* according to Benie *et al.* (2017) (Fig. 2).

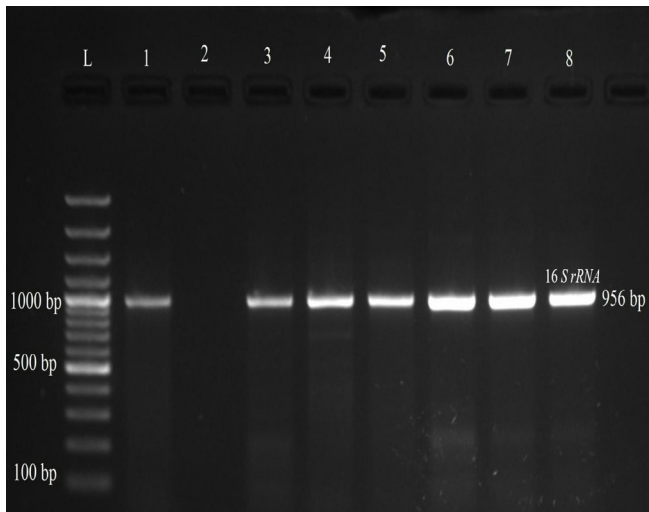


Fig. 1: Gel photograph of *P. aeruginosa* species specific 16S rRNA gene (956 bp). Lane L: DNA ladder, Lane 1: Positive control of *P. aeruginosa* (ATCC 27853), Lane 2: Negative control, Lane 3: *P. aeruginosa* isolate from chicken meat (CM78), Lane 4: *P. aeruginosa* isolate from cutting board swab (CB12), Lane 5: *P. aeruginosa* isolate from knife swab (KS7), Lane 6: *P. aeruginosa* isolate from chicken meat (CM113), Lane 7: *P. aeruginosa* isolate from chicken meat (CM146) Lane 8: *P. aeruginosa* isolate from chicken meat (CM163).

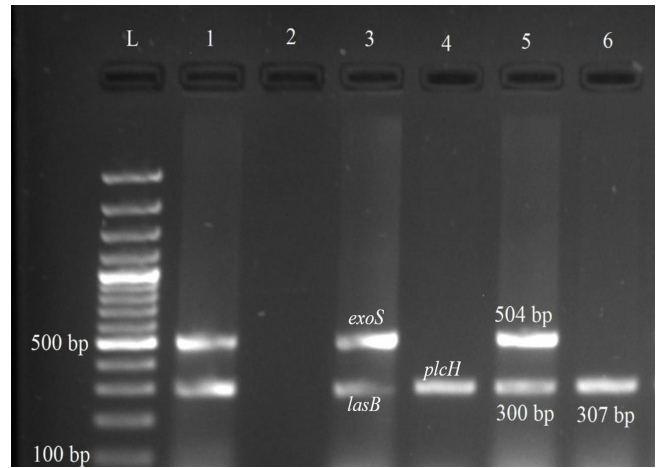


Fig. 2: Gel photograph of *P. aeruginosa* virulence genes using m-PCR-I and m-PCR-II. Lane L: DNA ladder, Lane 1: *P. aeruginosa* isolate carrying *lasB* (300 bp), *exoS* (504 bp) gene, Lane 2: Negative control, Lane 3: *P. aeruginosa* isolate (CM 163) carrying *lasB* (300 bp) and *exoS* (504 bp) gene, Lane 4: *P. aeruginosa* isolate (CM 163) showing *plcH* (307 bp) gene, Lane 5: *P. aeruginosa* isolate (CB 6) showing *lasB* (300) and *exoS* (504 bp) gene, Lane 6: *P. aeruginosa* (CB6) isolate showing *plcH* (307 bp) gene.

RESULTS AND DISCUSSION

In the present study, BHI broth was used as the enrichment medium, followed by selective plating on cetrimide agar plates. *P. aeruginosa* was identified by its characteristic yellowish green colonies. This observation was in line with previous study conducted for isolation of *P. aeruginosa* by Saha *et al.* (2022). After isolation of *P. aeruginosa* from different sources, they were further confirmed by biochemical tests (Oxidase-positive, Catalase-positive, Indole-negative, Methyl red-positive, Voges-proskauer-negative, citrate-positive) as reported earlier by Quinn *et al.* (2011).

In the present study, overall occurrence of *P. aeruginosa* isolated from various sources such as chicken meat, cutting board and knife swabs was 12.0% (30/250) by cultural methods with high prevalence in cutting board swabs (25.0%) followed by chicken meat (12.0%), knife swabs (10.0%)(Table 3). The above results of cutting board swabs aligned with Touimi *et al.* (2019), who reported the phenotypic prevalence of *P. aeruginosa* from cutting board swabs as 30.0%. In the present study the prevalence of *P. aeruginosa* from chicken meat was 12.04% (23/191) which is in tune with the findings of Thanigavel and Anandhan (2015), who also reported the

Table 3: Occurrence of *P. aeruginosa* in chicken retail shops

S.No	Type of sample	No. of samples tested	No. positive for <i>P. aeruginosa</i> by cultural methods	No. positive for <i>P. aeruginosa</i> by PCR
1	Chicken meat	191	23/191 (12.04%)	14/191 (7.32%)
2	Cutting board swab	20	5/20 (25.0%)	4/20 (20.0%)
3	Knife swab	20	2/20(10.0%)	2/20 (10.0%)
4	Hand swab	19	0/19(0.0%)	0/19 (0.0%)
	Total	250	30/250 (12.0%)	20/250 (8.0%)

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

S.No	Type of sample	Isolates analysed	<i>lasB</i> (%)	<i>exoS</i> (%)	<i>algD</i> (%)	<i>plcH</i> (%)
1	Chicken meat	14	9 (64.2)	3 (21.4)	0	8 (57.14)
2	Cutting board swab	4	3 (75.0)	2 (50.0)	0	2 (50.0)
3	Knife swab	2	0	1 (50.0)	0	1 (50.0)
4	Hand swab	0	0	0	0	0
	Total	20	12 (60.0)	6 (30.0)	0 (0)	11 (55.0)

phenotypic prevalence of *P. aeruginosa* from chicken meat as 8.5%. In contrary, Jawher and Hassan (2022) reported the high prevalence of *P. aeruginosa* (38.0%) in chicken meat. In this study the prevalence of *P. aeruginosa* in knife swabs was 10.0% which coincided with Elkammoshi *et al.* (2024), who reported the prevalence rate of 4.3%. In contrast, Vaidya *et al.* (2007) reported high prevalence of *P. aeruginosa* as 88.8% from cutting board and 33.33% from knife swabs.

In the present study the overall prevalence of *P. aeruginosa* from chicken meat, cutting board, knife swabs and hand swabs was 8.0% (20/250) using PCR. The highest occurrence of *P. aeruginosa* was noticed in cutting board swabs (20.0%), followed by knife swabs (10.0%) and chicken meat (7.32%)(Table 3). This result of chicken meat aligned with Swetcha *et al.* (2023), who also reported the prevalence of *P. aeruginosa* as 8.57% by PCR. In contrast, El-Aziz (2015) reported the high prevalence of *P. aeruginosa* (80.0%) from chicken meat. The variation in prevalence might be due to variation in sample number. Poultry is widely sold at retail outlets which are commonly located at roadside, often in unhygienic and polluted places leading to contamination. Also raw meat being kept in open instead of chilling, lack of personal hygiene and improper handling by the meat handlers also plays a major role in contamination of raw meat.

In the current study, all the 20 confirmed *P. aeruginosa* isolates were subjected for m-PCR to detect the virulence genes *lasB*, *exoS*, *algD*, *plcH* (Benie *et al.*, 2017). Out of 14 *P. aeruginosa* isolates from chicken meat, 9 (64.2%) carried *lasB* gene (Table 4), which was in accordance with Shahat *et al.* (2019), who reported the prevalence of *lasB* gene as 71.4%. In contrary, Benie *et al.* (2017) reported the prevalence of *lasB* gene as 89.2%. Three chicken meat isolates (21.4%) carried *exoS* gene which aligned with 25.9% prevalence by Algammal *et al.* (2023), while Shahat *et al.* (2019) reported the prevalence of *exoS* gene as 71.4%. Eight (57.14%) isolates of the current study carried *plcH* gene which was in accordance with Radwan *et al.* (2018), who reported the prevalence of *plcH* gene as 60.0%, while Benie *et al.* (2017) reported the prevalence of *plcH* gene as 72.1%. None of the isolates carried *algD* gene which was in accordance with Chakraborty *et al.* (2020), whereas Abdullah *et al.* (2024) reported the prevalence of *algD* gene as 80.0%.

In the present study the prevalence of *lasB* gene in cutting board swabs was 75.0%, whereas *exoS* and *plcH* 50.0% each. In knife swabs the prevalence of *exoS* and *plcH*

was 50.0% each, whereas none of the isolates carried *algD* gene (Table 4).

CONCLUSION

The rapid growth in meat production and consumption in India has raised significant hygiene concerns, particularly in poultry meat shops, where substandard facilities and unhygienic practices pose risks to public health. Key concerns include the absence of separate docks for edible and inedible products, poor waste disposal systems, and inadequate cleaning of equipment, such as wooden cutting blocks that absorb moisture and promote microbial growth. Essential measures like proper cleaning with disinfectants, the use of food-grade synthetic chopping blocks and hot water ($\geq 82^{\circ}\text{C}$) for sterilizing equipments are often neglected. Improving hygiene standards, formal education and training programs for meat handlers, along with strict regulatory vigilance, are essential to ensure the availability of fresh and safe poultry meat while safeguarding public health and environmental sustainability.

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