Health Leadership and Quality of Life. 2025; 4:590
doi: 10.56294/hl2025590

ORIGINAL

Assessment of Virulence Genes and Antibiotic Resistance in *Pseudomonas aeruginosa* Isolates from Poultry Carcasses

Evaluación de genes de virulencia y resistencia a antibióticos en aislados de *Pseudomonas aeruginosa* de canales de aves de corral

Anuradha N[1], Komal Patel[2], Mohan Garg[3], Prabhjot Kaur[4], Nyalam Ramu[5], Subhashree Ray[6]

[1]Department of General Medicine, Tagore medical college and Hospital, Chennai, Tamil nadu, India.
[2]Department of Gynaecology, Parul University, PO Limda, Tal. Waghodia, District Vadodara, Gujarat, India.
[3]Chitkara Centre for Research and Development, Chitkara University, Himachal Pradesh-174103 India.
[4]Centre of Research Impact and Outcome, Chitkara University, Rajpura- 140417, Punjab, India.
[5]Centre for Multidisciplinary Research, Anurag University, Hyderabad, Telangana, India.
[6]Department of Biochemistry, IMS and SUM Hospital, Siksha 'O' Anusandhan (Deemed to be University), Bhubaneswar, Odisha, India.

Cite as: N A, Patel K, Garg M, Kaur P, Ramu N, Ray S. Assessment of Virulence Genes and Antibiotic Resistance in Pseudomonas aeruginosa Isolates from Poultry Carcasses. Health Leadership and Quality of Life. 2025; 4:590. https://doi.org/10.56294/hl2025590

Submitted: 25-05-2024 **Revised:** 10-12-2024 **Accepted:** 18-05-2025 **Published:** 19-05-2025

Editor: PhD. Neela Satheesh

Corresponding author: Anuradha N

ABSTRACT

Pseudomonas aeruginosa, a major human pathogen, poses a significant threat to food, particularly meat products, due to its aggressiveness and drug resistance. Research aims to identify and analyze P. aeruginosa samples from poultry carcasses, examining their antimicrobial resistance and potential pathogenicity patterns. A total of 670 samples of poultry carcasses were gathered from slaughterhouses and shopping centers. Biochemical testing, Polymerase Chain Reaction (PCR) targeted the oprL gene, and culture-based techniques were used to identify the isolates. The virulence factors were identified both genotypically (by PCR amplification of *exoS, toxA,* and *lasB*) and phenotypically (by detection of hemolysin, protease, elastase, and biofilm formation). Data analysis was performed using SPSS software (version 26) to evaluate the results and determine associations between antibiotic resistance profiles and phenotypic virulence features. *P. aeruginosa* was identified from 102 samples (15,2 %) out of 670 samples. Protease activity was identified in 69,6 % of these isolates, hemolysin synthesis in 76,5 %, and biofilm formation in 83,3 %. According to PCR data, 58,8 % of the isolates had *exoS,* 51,9 % had *toxA*, and 64,7 % had *lasB*. Ceftazidime (61,7 %) and ciprofloxacin (52,9 %) showed high resistance, with 48,0 % of cases being categorized as multidrug-resistant (MDR). Public health is likely to be at risk due to the presence of virulent, multidrug-resistant Pseudomonas aeruginosa in poultry carcasses, which emphasizes better hygiene management and antimicrobial surveillance in poultry processing.

Keywords: Antibiotic Resistance; Hemolysin; Protease; Pseudomonas Aeruginosa; Carcass Surface Swab Prevalence; Virulence Factors.

RESUMEN

Pseudomonas aeruginosa, un importante patógeno humano, representa una amenaza significativa para los alimentos, en particular los productos cárnicos, debido a su agresividad y resistencia a fármacos. La investigación busca identificar y analizar muestras de P. aeruginosa de canales de aves de corral, examinando su resistencia a los antimicrobianos y sus posibles patrones de patogenicidad. Se obtuvieron 670 muestras de canales de aves de corral de mataderos y centros comerciales. Se utilizaron pruebas bioquímicas, la reacción en cadena de la polimerasa (PCR) dirigida al gen oprL, y técnicas basadas en cultivo para identificar los aislados.

Los factores de virulencia se identificaron tanto genotípicamente (mediante la amplificación por PCR de exoS, toxA y lasB) como fenotípicamente (mediante la detección de hemolisina, proteasa, elastasa y formación de biopelículas). El análisis de datos se realizó con el programa SPSS (versión 26) para evaluar los resultados y determinar las asociaciones entre los perfiles de resistencia a los antibióticos y las características fenotípicas de virulencia. Se identificó P. aeruginosa en 102 muestras (15,2 %) de un total de 670. Se identificó actividad de proteasa en el 69,6 % de estos aislados, síntesis de hemolisina en el 76,5 % y formación de biopelícula en el 83,3 %. Según los datos de PCR, el 58,8 % de los aislados presentó exoS, el 51,9 % toxA y el 64,7 % lasB. La ceftazidima (61,7 %) y la ciprofloxacina (52,9 %) mostraron alta resistencia, con un 48,0 % de casos clasificados como multirresistentes (MDR). Es probable que la salud pública esté en riesgo debido a la presencia de Pseudomonas aeruginosa virulenta y multirresistente en canales de aves de corral, lo que hace hincapié en una mejor gestión de la higiene y la vigilancia antimicrobiana en el procesamiento avícola.

Palabras clave: Resistencia a los Antibióticos; Hemolisina; Proteasa; Pseudomonas Aeruginosa; Prevalencia en Hisopados de la Superficie de la Canal; Factores de Virulencia.

INTRODUCTION

Animal raw meat can serve as a reservoir for a variety of food-borne viruses, which can cause food to decay and lead to food-borne illnesses. Food microbe *Pseudomonas aeruginosa*, sometimes known as food and the environment, is a common source of *P. aeruginosa*, an opportunistic human disease.[1] Soil and water are among the environmental sources where *P. aeruginosa* is typically found. It can proliferate and develop in meat stored under aerobic conditions and temperature fluctuations.[2] During various phases in the slaughterhouse, raw meat from various animal species could get infected with various types of bacteria. The primary phases are beheading, drawing blood, slaughtering, clarification, dismembering the corpse, examination, and cleaning the corpse.[3] Animal components deemed inappropriate for human consumption are known as animal byproducts. Certain economic value-added by-products make it possible for the meat industry to compete financially with sources of vegetable protein.[4] When meat is stored in more hygienic conditions, its shelf life is increased. *Pseudomonas*, which predominates in chilled meat held under aerobic circumstances, is the most prevalent psychrotroph that can emerge after chilling. Before consumption, the growth of *pseudomonas* alters the organoleptic characteristics of meat, resulting in product loss.[5] Certain spoilage bacteria species, such as *Pseudomonas* strains, are present in meat, poultry, and fish. Among the several varieties of *Pseudomonas*, the most prevalent kind infects humans is *P. aeruginosa*.[6] The research aims to evaluate the resistance to antibiotics rate, frequency, virulence marker allocation, and sensitivity gene expression of the isolated *P. Aeruginosa* variety from surface swabbing samples of cattle along with ovine animals containing raw meat and corpses.

In the research [7] the antibiotic susceptibility of the isolated strains of Y. enterocolitica was determined, and the prevalence of the bacterium in cattle was evaluated. Research [8] developed resistance by *P. aeruginosa*, commonly referred to as the Multidrug resistance (MDR) infection. *P. aeruginosa* has heightened resistance to drug resistance, a significant public health concern with its inclination to form biofilms in proteins such as meat and other dietary items. The investigation [9] presented an inquiry to identify Helicobacter species in chicken carcasses and evaluate the virulence genes and anti-bio-gram of the Helicobacter isolates. Research [10] examined the colistin-resistant Enterobacteriaceae isolates found in poultry products, the traits of antibiotic resistance, and the coexistence of resistance genes. Research [11] discussed collected information about the genetic composition, prevalence, and resistance to antibiotics of the isolated strain of *S. aureus* from the corpses of pigs, chickens, and people who came into contact with Nigerian slaughterhouses. The research [12] focused on measuring the formation of biofilms coupled with antibiotic resistance in *Pseudomonas* species isolated from clinical and dietary materials. The investigation [13] determined the genotypic and phenotypic characteristics of *S. aureus* bacteria resistant to antibiotics were collected from retail meat. Research [14] examined the resistance of 88 *Escherichia coli* strains separated from the factory during one year to eighteen antibacterial drugs, including *colistin, fluoroquinolones, β-lactams*, and virulence factors. Research [15] determined the recovered *isolates*' susceptibility to antibiotics and the incidence of Campylobacter on chicken carcasses was were examined in Poland. The common food-borne pathogens along with the bacteria resistant to antibiotics in retail ground beef and pig, including processed items, were investigated.[16]

METHOD

Prevalent Virulence and Antimicrobial Resistance of P. aeruginosa Isolated from Poultry Carcasses: A Cross-Sectional Research Consisting of Six Months of Sampling. A collection of samples for testing and evaluation from different areas can be done without any contamination, such as abattoirs and retail outlet units, and sample processing could be done much earlier in controlled settings. Isolation can involve selective enrichment and

culture on Cetrimide Agar, subsequently subjected to biochemical, molecular positive confirmation targeting *the oprL* gene. The virulence factors are phenotypically and genotypically assessed, and antimicrobial susceptibility is tested by the Kirby-Bauer disk diffusion method and statistical analysis by using SPSS version 26. Figure 1 shows the experimental design and analytical steps in P. aeruginosa surveillance.

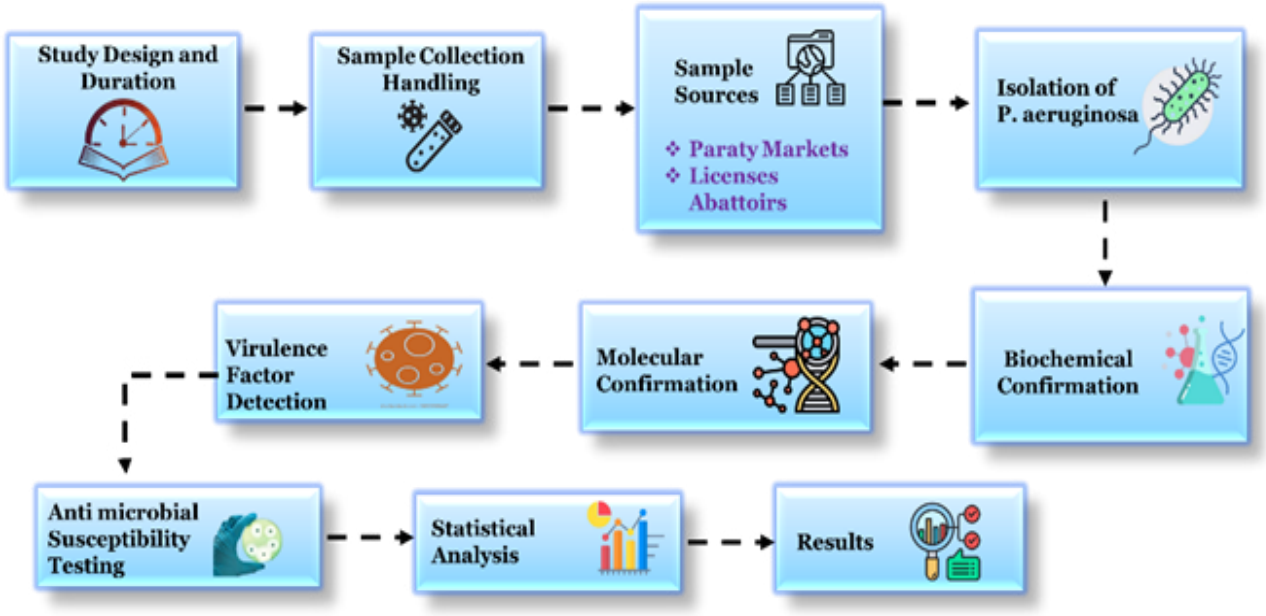

Figure 1. Experimental Design and Analytical Steps in P. aeruginosa Surveillance

Research Design and Duration

A cross-sectional investigation was conducted to determine the prevalence, virulence features, and antibiotic resistance profile characteristics of *P. aeruginosa* isolated from poultry carcass samples over six months. Several slaughterhouses and retail establishments were included in the research to enable representative sampling from a range of poultry meat sources. Actually, identifying the microbiological hazards in poultry items the general public consumes was the research's main goal.

Sample Collection and Handling

The poultry carcass samples were collected through strict aseptic techniques using sterile instruments to prevent contamination during the collection process. Each sample weighed around 25 grams to provide a representative sample for analysis. Immediately after sampling, the samples were packed in sterile labeled containers to avoid mix-ups and transferred to the laboratory under controlled temperature conditions maintained at 4°C. Low temperatures help preserve the integrity of bacterial samples, inhibiting unwanted bacterial growth and degradation before processing. All samples were processed within six hours of collection to allow bacterial viability and accurate testing results.

Sample Sources

Samples were collected from different sources around the country to represent a wide spectrum of possible contamination risks, including poultry markets and licensed slaughterhouses, which served as high points of sale and processing. The diversity of sources meant these could capture the full spectrum of potential contamination patterns and even give indications of potential hot spots for bacterial contamination. It also sought to dye in the wide range of conditions that could influence microbial load on poultry carcasses, from handling practices to hygienic standards, at different points along the poultry supply chain.

Isolation of *P.aeruginosa*

Enrichment of the samples in BPW from which *P. aeruginosa* have been isolated, was initiated. BPW is a selective broth that enhances the organism and many others while suppressing others. For multiplication, the sample was cultured for 24 hours at 37°C. From a loop full of the enriched sample, the sample was streaked onto Cetrimide Agar, a selective medium for Pseudomonas species. Cetrimide promotes *P. aeruginosa* growth while suppressing other microorganisms. After incubation for 24-48 hours, bluish-green colonies showed the isolate was likely *P. aeruginosa*, as pyocyanin is produced as a pigment by the organism.

Biochemical Confirmation

Presumptive *P. aeruginosa* colonies were subjected to many biochemical tests intended to confirm organism identification. Gram-negative rod bacteria were identified by a Gram stain as a characteristic of *P. aeruginosa*. The oxidase test was positive, indicating cytochrome c oxidase is present, which is a key aerobic respiratory enzyme of the electron transport chain of *P. aeruginosa*. Positive results from catalase tests further supported the bacteria's conversion of hydrogen peroxide into water and oxygen. A positive citrate usage test showed *P. aeruginosa* was present because it used citrate as its sole carbon source. *P. aeruginosa* is known for its flagellated motility, which was validated by the final test, motility.

Molecular Confirmation of isolates

Extraction of DNA

Using the boiling approach, which is easy and less expensive for separating Deoxyribonucleic Acid (DNA) from bacterial cells, genomic DNA extraction was carried out. First, bacterial colonies are suspended in sterile distilled water to form a bacterial suspension. The suspension is then subjected to thermal treatment, which lyses the cells and brings about the release of genomic DNA into the solution. The suspension is then centrifuged to separate cellular debris from the supernatant containing the DNA. The supernatant is finally collected and used as a DNA template for subsequent PCR analysis.

PCR Targeting oprL Gene

The PCR was used to amplify a particular gene, *oprL*, to validate the presence of *P. aeruginosa*. One reliable molecular indicator of *P. aeruginosa* is the *oprL* gene, which codes for a lipoprotein found in the bacterium's outer membrane. To guarantee amplification of high specificity and sensitivity, the gene was first amplified using certain primers. Using gel electrophoresis on a 1,5 % agarose gel stained with ethidium bromide for DNA band visualization under Ultra Violet (UV) light, the PCR result was subsequently examined to confirm the presence of *P. aeruginosa* based on the anticipated band size.

Virulence factor detection

Phenotypic Assays

Numerous methods have been employed to characterize Pseudomonas aeruginosa virulence to evaluate its virulence components, including hemolysin, protease, elastase production, and biofilm development. By cultivating the bacteria on blood agar plates, hemolysin activity was assessed; the presence of ß-hemolysin was determined by the development of clear zones surrounding colonies as a result of red blood cell lysis. Protease production was evaluated on skim milk agar, where the clearing of casein due to protease activity gave clear zones around the bacterial growth, which confirmed the enzyme activity. For elastase production, elastin Congo Red agar was used; the clear zones formed were indicative of elastase activity, which degrades elastin and liberates the red dye from agar. Finally, the 96-well microtiter plate method was used to quantify the production of biofilms. Biofilms were dyed with crystal violet, and the amount of dye retained was measured using a spectrophotometer set to 570 nm. The ability of the bacteria to build robust biofilms and the amount of biofilm development were directly correlated with the absorbance.

Genotypic Detection of Virulence Genes

PCR assays were run to identify virulence genes specifically: *exoS, toxA*, and *lasB*. The *exoS*gene encodes an exoenzyme S, which is linked with cytotoxicity, whereas the *toxA*gene codes for an exotoxin A virulence factor. The *lasB* gene codes for the elastase B, which takes part in tissue destruction. PCR amplification conditions were optimized for each gene, and visualization of the amplification products was conducted on agarose gel to reveal the presence of the specific genes. The establishment of these important virulence factors at the molecular level is currently possible.

Testing for antimicrobial susceptibility (AST)

Disk Diffusion Method

The Kirby-Bauer disk diffusion methodology is a traditional technique for assessing bacteria's susceptibility to antibiotics. Paper discs coated with antibiotics are put on a Mueller-Hinton agar plate that has been infected with the bacterial isolate using techniques. Imipenem, Ciprofloxacin, Gentamicin, Amikacin, Ceftazidime, and Cefepime were among the antibiotics that were examined. A distinct area known as the zone of inhibition can appear as a result of the bacterium either preventing the organism's growth or having no effect. To determine resistance or susceptibility, the size of the zone is measured and compared with standard charts.

Statistical Analysis

Data analysis was done with the use of SPSS version 26. Using the Chi-square test, the resistance profiles of

the *P. aeruginosa* isolates were compared to determine whether certain virulence features were present. Any association between the variables under investigation was unlikely to be the product of chance if the p-value was less than 0,05, which was regarded as statistically significant.

RESULTS

Pseudomonas aeruginosa turns out to be a common contaminant of poultry carcasses and potentially poses a food safety threat. The isolates exhibit several important phenotypic virulence attributes, such as biofilm formation and enzyme production, which strengthen pathogenicity. The existence of key virulence genes associated with tissue damage and immune system evasion has been confirmed genotypically. In addition, the situation has been further exacerbated by widespread antimicrobial resistance and a considerable number of isolates being multi-drug resistant, which poses challenges to treatment and control.

Prevalence of *P. aeruginosa*

The word "prevalence" refers to the detection frequency of *P.aeruginosa* in a given population or environment. The presence of organisms in poultry carcasses means contamination with an ensuing hazard to food safety. Maintaining vigilance concerning its prevalence remains crucial in understanding the extent of bacterial spread to enable consideration of control measures.

Table 1. Prevalence of *P. aeruginosa*in Poultry Carcass Samples.

Sample Type	Total Samples	Positive for *P. aeruginosa*	Prevalence (%)
Poultry carcasses	670	102	15,2 %

Table 1 illustrates that the 670 poultry carcass samples were collected from slaughterhouses and retail marketplaces, and 102 had a 15,2 % incidence of *P. aeruginosa*. The incidence indicates *P. aeruginosa*, which can cause human contamination and spread during foodborne illnesses, is quite common in poultry carcasses.

Chi-square test

The chi-square test is a statistical method for figuring out whether category variables significantly correlate with one another. The technique determines how well the data fit a particular hypothesis by comparing observed and expected frequency counts shown in equation (1):

$$x^2 = \sum \frac{(O_i - E_i)^{\ 2}}{E_i} \qquad (1)$$

There is the observed frequency and is the expected frequency.

Table 2. Chi-Square Test Results for Associations between Virulence Traits and Antimicrobial Resistance Profiles

Virulence Trait	Chi-Square Value (x^2)	p-value
LasA	4,50	0,034
ExoS	7,20	0,007
ToxA	1,20	0,275
OprD	5,80	0,016

Table 2 showed LasA (p = 0,034), ExoS (p = 0,007), and OprD (p = 0,016) had significant associations with antibiotic resistance, confirming contributions to *P. aeruginosa* resistance. ToxA had an insignificant association with resistance, with a p-value of 0,275, p-value clearly indicates the supportive role of LasA, ExoS, and OprD concerning the resistance while no role by ToxA. <0,05 is accepted for statistically significant associations. With the findings, the varying influence of virulence factors on antimicrobial resistance is visible.

Phenotypic Virulence Traits in Isolates

The phenotypic virulence traits are the observable characteristics that make a microorganism a pathogen. These include motility, biofilm formation, and the enzymes proteases or hemolysins. Assessing these characteristics could help in determining the pathogenic potential of the isolates.

Table 3. Number of Phenotypic Virulence Factors in Poultry Carcass Isolates of Pseudomonas *aeruginosa*

Virulence Factor	Number of Positive Isolates	Percentage (%)
Biofilm	85	83,3
Hemolysin	78	76,5
Protease	71	69,6
Elastase	66	64,7

Table 3 shows the total 102 isolates of *P. Aeruginosa*; the observed frequencies of phenotypic virulence factors were as follows: An enormous 83,3 % of isolates were biofilm producers, which enhance bacterial survival, resistance to antimicrobial treatment, and persistence in infections. Hemolysin was produced by 76,5 % of the isolate's virulence factor, leading to the damage of red blood cells and tissues during infections. Protease activity was present in 69,6 % of the isolates, with these enzymes helping in the breakdown of host proteins and aiding tissue invasion. Lastly, 64,7 % of the isolates exhibited elastase activity, which further increases virulence in *P. aeruginosa* as a protease helping in immune evasion and tissue destruction.

Genotypic Detection of Virulence Genes

The genotypic detection of virulence genes means the identification of specific DNA sequences associated with pathogenic characteristics in microorganisms. Usually, genotypic detection involves molecular methods such as PCR to identify these genes, which can indicate the virulence potential of bacterial isolates.

Table 4. Virulence-Associated Gene Prevalence in *P. aeruginosa* Isolates

Gene Detected	Positive Isolates	Percentage (%)
exoS	60	58,8
toxA	53	51,9
lasB	66	64,7

Table 4 shows that several virulence genes were found in the isolated strains of *P. aeruginosa*, according to genotypic testing. Thus, exoS was detected in 58,8 % of the isolates, which is a toxin that disrupts the cellular signaling in the host causes tissue damage, and increases virulence. toxA, which is responsible for encoding toxA, was identified to be present in 51,9 % of the isolates. toxA causes cell death and consequent tissue damage by preventing the host cells from synthesizing proteins. Additionally, 64,7 % of the isolates had lasB, which codes for elastase. Elastase is a key player in the breakdown of elastin in host tissue, which facilitates tissue invasion and immune suppression and increases the pathogen's pathogenicity.

Antimicrobial Resistance Profile

The Antimicrobial Resistance Profile outlines the resistance pattern exhibited by bacterial isolates to a variety of antibiotics under investigation. It enables the identification of multiresistant bacteria and the assessment of ineffective antibiotics for treatment. The resistance profile is very important in recommending appropriate therapeutic measures with resistance trend patterns.

Table 5. Antimicrobial Resistance Profile of *P. aeruginosa* Isolates from Poultry Carcasses

Antibiotic	Resistant Isolates (n = 102)	Resistance (%)
Ceftazidime	63	61,7
Ciprofloxacin	54	52,9
Imipenem	17	16,7
Gentamicin	29	28,4
Amikacin	21	20,6
Cefepime	42	41,1
MDR	49	48,0

Table 5 and figure 2 show that the *P. aeruginosa* isolates' antimicrobial resistance profile revealed significant resistance to several widely used medications. 61,7 % exhibited ceftazidime resistance, suggesting the emergence of resistance to the third-generation cephalosporin and, consequently, to ß-lactams. With the fluoroquinolone, ciprofloxacin resistance was notably significant at 52,9 %. Due to imipenem, a carbapenem family member is

often used as a last resort to treat *P. aeruginosa* infections; resistance to antibiotics was lower at 16,7 %. There is some resistance in a family of antibacterials, as seen by the moderate resistance rate to the aminoglycosides gentamicin (28,4 %) and amikacin (20,6 %). Once more, 41,1 % of isolates exhibited resistance to the antibiotic or a combination of both. The MDR of these isolates was thus thoroughly demonstrated. Nearly half of the isolates (48,0 %) were found to be MDR bacteria, which are resistant to at least three different classes of antibiotics. It is a major hazard to public health because these strains are thought to be the hardest to treat and provide fewer therapeutic alternatives.

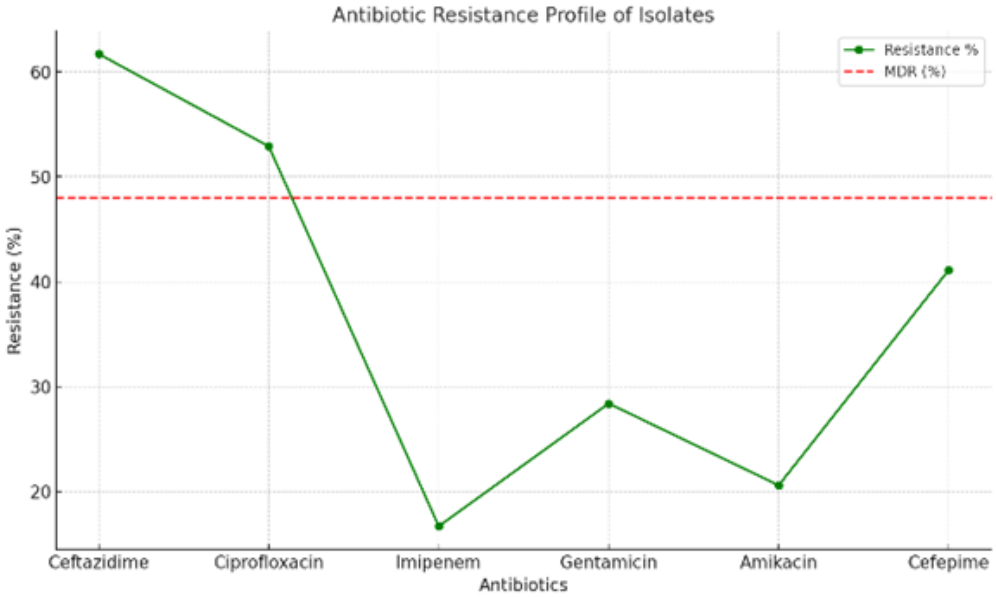

Figure 2. Antibiotic Resistance and MDR Threshold in *P. aeruginosa*Isolates from Poultry Carcasses

Virulence and Resistance Profiles

Understanding *P. aeruginosa* isolates' phenotypic virulence characteristics and antibiotic resistance is essential for focused interventions and risk assessments about poultry illnesses to improve monitoring and treatment strategies (figure 3).

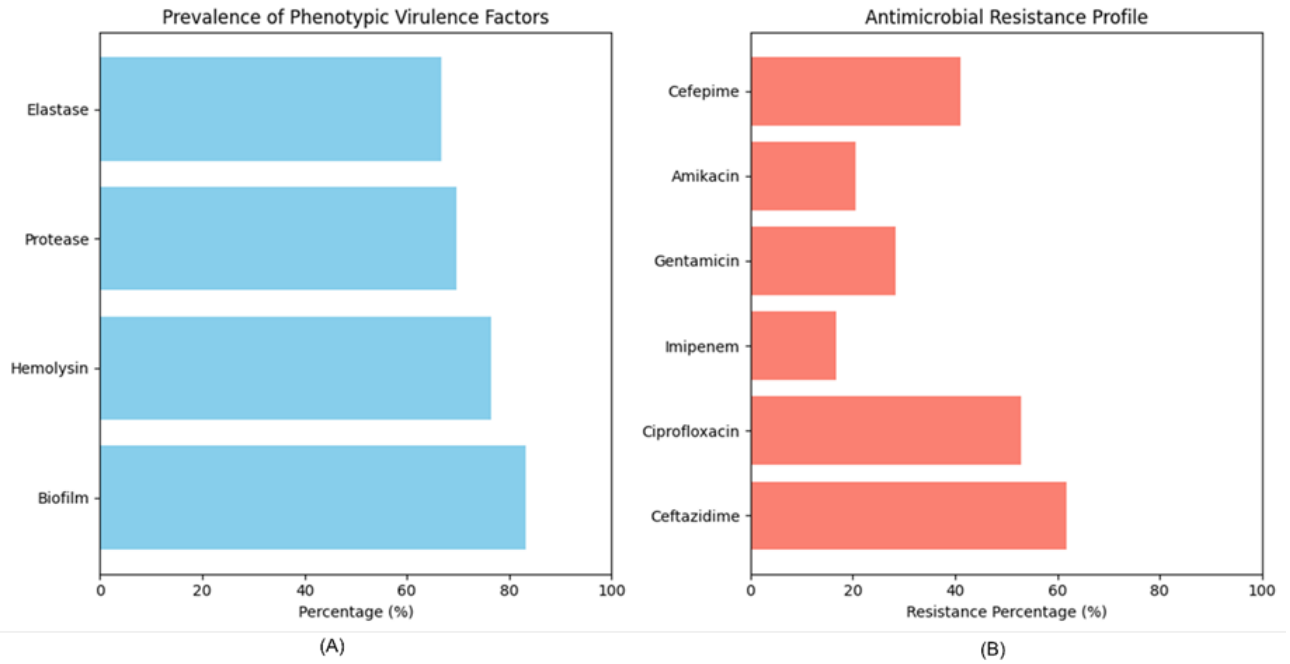

Figure 3. (A) Phenotypic Virulence Factors, and (B) Antimicrobial Resistance Patterns in *P. aeruginosa* Isolates from Poultry Carcasses

DISCUSSION

According to the research, animal rendering plants include multidrug-resistant Escherichia coli that is resistant to fluoroquinolones and ß-lactam antibiotics. The health hazards are highlighted by the discovery of virulence genes and mobile genetic elements, such as ST131 clones. Antibiotic resistance genes can be stored in rendering plants and spread through food chains and environmental exposure. Rendering factories continue to be important control points for the environmental propagation of these infections despite rigorous thermal processing, which presents problems for food safety and public health.[14] Shiga toxin genes were detected in 32,6 % of fecal samples from 470 healthy reindeer, whereas Listeria monocytogenes and Yersinia spp. were discovered in 3,2 % and 9,8 % of the samples, respectively. The high frequency of Shiga toxin genes indicates a possible zoonotic danger even in the presence of poor antibiotic resistance. A thorough evaluation of the risks to human health is hampered by the absence of strain-level identification and virulence profile, underscoring the necessity of more stringent hygienic procedures before slaughter.

The parasitic diseases' method of action and environmental resilience, and the occurrence of *P. aeruginosa* in poultry carcasses present a significant public health risk. The occurrence of Pseudomonas in food products like poultry could provide a reservoir for human exposure, necessitating improved hygiene measures during processing and handling. The phenotypically confirmed virulence traits include biofilm formation, protease, hemolysin, and elastase activity, which confirm the opportunistic pathogenicity of these isolates. Such traits are necessary for invading tissue and evading immune response while aiding in the bacteria's persistence under harsh conditions, such as exposure to antibiotics. Genotypic detection of virulence genes such as exoS, toxA, and lasB supports the aforementioned phenotypic determinations towards the aggressive virulence mechanism employed by *P. aeruginosa*. The presence of genes gives an indication of the higher potential of the isolates causing infection, especially in immunocompromised hosts. The Chi-square test shows that there exist significant statistical associations between a few virulence factors and antibiotic resistance, especially LasA, ExoS, ToxA, and OprD. Antibiotic resistance suggests that these virulence traits can confer a survival advantage for resistant strains. Nonetheless, the antimicrobial resistance profiles indicate high resistance levels to some commonly used antibiotics, presenting the risk of therapeutic failure in clinical settings due to widespread multidrug resistance among the isolates. To prevent the spread of virulent and drug-resistant *P. aeruginosa* across the poultry production chain, all of these findings support integrated surveillance, strict antibiotic stewardship, and control measures.

CONCLUSION

P. aeruginosa, the invasive pathogen, is often isolated from animal-derived food products, posing considerable public health problems. The virulence attributes and multiple antibiotic resistance offered by the organism make control of infection and treatment difficult. Therefore, investigating these attributes in meat and carcass samples is very relevant for food safety and antimicrobial resistance monitoring. Out of 670 samples, 102 samples (15,2 %) included *P. aeruginosa*. In these isolates, 69,6 % showed protease activity, 76,5 % showed hemolysin production, and 83,3 % showed biofilm development. The PCR results showed 64,7 % of the isolates had lasB, 51,9 % had toxA, and 58,8 % had exoS. High resistance was seen to ceftazidime (61,7 %) and ciprofloxacin (52,9 %), with 48,0 % of patients classified as MDR. The limitation of the research concerns the small sample size and its geographic restrictions. These conditions render results not necessarily generalizable to broader distribution patterns. The molecular mechanisms underlying resistance were not investigated sufficiently. Further analysis involving whole-genome sequencing should bring a better understanding of transmission dynamics and the evolution of resistance.

REFERENCES

1. Poursina S, Ahmadi M, Fazeli F, Ariaii P. Assessment of virulence factors and antimicrobial resistance among the Pseudomonas aeruginosa strains isolated from animal meat and carcass samples. Veterinary medicine and science. 2023 Jan; 9(1):315-25. https://doi.org/10.1002/vms3.1007

2. Heredia N, García S. Animals as sources of food-borne pathogens: A review. Animal nutrition. 2018 Sep 1; 4(3):250-5. https://doi.org/10.1016/j.aninu.2018.04.006

3. Vijayalakshmi P, RajaniChowdary A, Vidyullatha P, Sushma M. Identification of bacteriological quality and antimicrobial resistance of microorganisms isolated from animal foods collected from the abattoir, butcher shops and local seafood market. Food Research. 2021 Jun;5(3):144-51. https://doi.org/10.26656/fr.2017.5(3).570

4. 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 Dec; 12(1):1-4. https://doi.org/10.1186/s13568-022-01390-1

5. Jawher IM, Hasan MG. Molecular identification of Pseudomonas aeruginosa in meat at Mosul city retails using PCR technique. Iraqi Journal of Veterinary Sciences. 2022 Oct 1; 36(4):1083-7. https://doi.org/10.33899/ijvs.2022.133086.2173

6. Circella E, Schiavone A, Barrasso R, Camarda A, Pugliese N, Bozzo G. Pseudomonas azotoformans belonging to Pseudomonas fluorescens group as the causative agent of blue coloration in carcasses of slaughterhouse rabbits. Animals. 2020 Feb 6;10(2):256. https://doi.org/10.1002/vms3.1007

7. Łada P, Kończyk-Kmiecik K, Bancerz-Kisiel A. Isolation, characterization and antimicrobial resistance of Yersinia enterocolitica from Polish cattle and their carcasses. BMC veterinary research. 2023 Sep 5; 19(1):143. https://doi.org/10.1186/s12917-023-03700-6

8. Mehmood S, Ali K, Bashir A, Farid N, Fatima K, Naz S.Biorisk. Management and Antibiotic Susceptibility Pattern of Biofilm producing Pseudomonas aeruginosa Isolated from Broiler Chicken: A Public Health Concern: Biorisk Management of Biofilm producing Pseudomonas aeruginosa from Broiler Chicken. Proceedings of the Pakistan Academy of Sciences: B. Life and Environmental Sciences. 2023 Sep 5;60(3):497-503. https://doi.org/10.53560/PPASB(60-3)866

9. Elrais AM, Arab WS, Sallam KI, Elmegid WA, Elgendy F, Elmonir W, et al. Prevalence, Virulence Genes, Phylogenetic Analysis, and Antimicrobial Resistance Profile of Helicobacter Species in Chicken Meat and Their Associated Environment at Retail Shops in Egypt. Foods. 2022 Jun 26;11(13):1890. https://doi.org/10.3390/foods11131890

10. Karim MR, Zakaria Z, Hassan L, MohdFaiz N, Ahmad NI. Antimicrobial Resistance Profiles and Co-Existence of Multiple Antimicrobial Resistance Genes in mcr-HarbouringColistin-Resistant Enterobacteriaceae Isolates Recovered from Poultry and Poultry Meats in Malaysia. Antibiotics. 2023 Jun 15;12(6):1060. https://doi.org/10.3390/antibiotics12061060

11. Okorie-Kanu OJ, Anyanwu MU, Ezenduka EV, Mgbeahuruike AC, Thapaliya D, Gerbig G, et al. Molecular epidemiology, genetic diversity and antimicrobial resistance of Staphylococcus aureus isolated from chicken and pig carcasses, and carcass handlers. PLoS One. 2020 May 14;15(5):e0232913. https://doi.org/10.1371/journal.pone.0232913

12. Radovanovic RS, Savic NR, Ranin L, Smitran A, OpavskiNV,Tepavcevic AM, et al. Biofilm production and antimicrobial resistance of clinical and food isolates of Pseudomonas spp. Current Microbiology. 2020 Dec; 77:4045-52. https://doi.org/10.1007/s00284-020-02236-4

13. TorkiBaghbaderani Z, Shakerian A, Rahimi E. Phenotypic and genotypic assessment of antibiotic resistance of Staphylococcus aureus bacteria isolated from retail meat. Infection and drug resistance. 2020 May 7:1339-49. https://doi.org/10.2147/IDR.S241189

14. Gregova G, Kmet V. Antibiotic resistance and virulence of Escherichia coli strains isolated from animal rendering plant. Scientific reports. 2020 Oct 13;10(1): 17108. https://doi.org/10.1038/s41598-020-72851-5

15. Wieczorek K, Bocian Ł, Osek J. Prevalence and antimicrobial resistance of Campylobacter isolated from carcasses of chickens slaughtered in Poland–a retrospective research. Food control. 2020 Jun 1; 112:107159. https://doi.org/10.1016/j.foodcont.2020.107159

16. Laaksonen S, Oksanen A, Julmi J, Zweifel C, Fredriksson-Ahomaa M, Stephan R. Presence of foodborne pathogens, extended-spectrum ß-lactamase-producing Enterobacteriaceae, and methicillin-resistant Staphylococcus aureus in slaughtered reindeer in northern Finland and Norway. ActaVeterinariaScandinavica. 2017 Dec;59(1):1-8. https://doi.org/10.1186/s13028-016-0272-x

FINANCING

The authors did not receive financing for the development of this research.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORSHIP CONTRIBUTION

Conceptualization: Anuradha N, Komal Patel, Mohan Garg, Prabhjot Kaur, Nyalam Ramu, Subhashree Ray.

Drafting - original draft: Anuradha N, Komal Patel, Mohan Garg, Prabhjot Kaur, Nyalam Ramu, Subhashree Ray.

Writing-Proofreading and Editing: Anuradha N, Komal Patel, Mohan Garg, Prabhjot Kaur, Nyalam Ramu, Subhashree Ray.