Genetic and Molecular Characterization of Biofilm Formation in Multidrug-resistant strains *Pseudomonas aeruginosa* and *Staphylococcus aureus*

Abstract

The virulence and antimicrobial resistance (AMR) of biofilm-forming bacteria pose a growing threat to public health. The aim of this study was to identify molecular determinants of resistance and biofilm formation in *P. aeruginosa* and *S. aureus*. A total of 120 strains consisting of *P. aeruginosa* (60) and *S. aureus* (60) were isolated from animal products. These strains underwent phenotypic, biochemical, and molecular identification. The molecular determinants of resistance (***bla*$_{SHV}$**, ***bla*$_{IMP}$**, and ***bla*$_{TEM}$**) and biofilm formation (*PpyR*, *PslA*, and *PelA*) were detected using the PCR method. In descending order of importance, the prevalence of resistance genes detected in *P. aeruginosa* was 63.3% (***bla*$_{SHV}$**), 53.3% (***bla*$_{IMP}$**), and 35.0% (***bla*$_{TEM}$**). *S. aureus* strains producing coagulase (83.33%) and DNase (75%) harbored ***bla*$_{IMP}$** (48.3%) and ***bla*$_{SHV}$** (36.7%) resistance genes with varying prevalences. The *PpyR* gene (93.3%) was predominantly detected in *P. aeruginosa*, followed by the *PslA* (80.0%) and *PelA* (71.7%) genes. Only the *PslA* (48.3%) and *PelA* (38.3%) genes were detected in *S. aureus*, with prevalences below 50.0%. Detecting and controlling the determinants of resistance and biofilm formation can guide improved diagnosis and contribute to the fight against bacterial biofilm infections.

Keywords: Biofilms, resistance, gene, *P. aeruginosa*, *S. aureus*

1. Introduction

"In nature, microorganisms exist either as planktonic cells or enclosed within an exopolysaccharide matrix called biofilms" (Grari *et al*., 2025). The matrix of these biofilms varies among species, but it is primarily composed of proteins, polysaccharides, and nucleic acids (Mlugu *et al*., 2023). "Biofilms are considered communities of homogeneous or heterogeneous microorganisms bound together and adhering to a biotic or abiotic surface. These surfaces are often composed of living tissues, supports in the food industry, wastewater channels, bathrooms, medical devices, and frequently surfaces immersed or exposed to an aqueous solution" (Grari *et al*., 2025).

When microorganisms adhere to these surfaces, biofilm formation occurs in a structured manner (Mlugu *et al*., 2023). Initially, "free-floating bacteria adhere reversibly to a conditioned surface, and adherent bacteria are irreversibly fixed by surface adhesins" (Grari *et al*., 2025). Subsequently, "an extracellular matrix forms to produce a fully mature biofilm. Finally, bacteria disperse from this matrix to colonize other surfaces before expressing their resistance, virulence, and potential to form new biofilms" (Costa & Carvalho, 2025).

These microorganisms in biofilms develop various molecular mechanisms and phenotypes necessary to express their pathogenicity and increased resistance to antimicrobial treatments (Garcia-Reyes *et al*., 2025). "Approximately 80% of all microbial infections are associated with biofilms, of which 60–70% are nosocomial infections" (Garcia-Reyes *et al*., 2025). However, "in 2017, bloodstream infections associated with biofilms ranked 12th among the leading causes of death, with an overall mortality rate between 15 and 30%" (Shehab *et al*., 2025).

"Several microbial species, such as *Pseudomonas aeruginosa*, *Burkholderia cepacia*, *Pseudomonas pseudomallei*, Haemophilus influenzae, *Escherichia coli*, *Candida albicans*, *Streptococcus pneumoniae*, *Staphylococcus epidermidis*, and *Staphylococcus aureus*, are involved in biofilm formation" (Grari *et al*., 2025; Muhammad *et al*., 2025). Among these, *P. aeruginosa* is a suitable model organism for studying biofilm formation in vitro (Zhou *et al*., 2018). *P. aeruginosa* strains are considered potent biofilm-forming organisms and are often resistant to antimicrobial treatments (Grari *et al*., 2025). In addition to *P. aeruginosa*, *S. aureus* strains contribute to approximately 87% of bloodstream infections and 50-70% of catheter infections (Mlugu *et al*., 2023).

A major problem in infections caused by *P. aeruginosa* and *S. aureus* is their relatively high resistance to most antimicrobials, particularly the antibiotics commonly used) (Di *et al*., 2025). These bacterial strains often cause infections following the acquisition of resistance genes such as Vietnamese Extended Spectrum β-lactamase (VEB), Sulfhydryl Variable (SHV), and Oxacillinases (OXA). Furthermore, various types of Metallo-β-lactamases (MBLs), such as imipenemase (IMP) and Verona imipenemase (VIM), have been identified among these bacterial strains (Di *et al*., 2025).

In addition to resistance genes, certain genetic markers involved in biofilm formation promote this multi-resistance and the pathogenicity of these bacterial strains (Traoré *et al*., 2024). These genetic determinants include the Pel gene encoding dandruff; the Psl gene encoding polysaccharide synthesis; and *PpyR*-type genes that regulate psl operons and pyoverdin biosynthesis (Traoré *et al*., 2024).

In Côte d'Ivoire, some studies have been conducted on biofilms from clinical and animal-derived strains (Traoré *et al*., 2024; Benie *et al*., 2021). Despite this limited research in Côte d'Ivoire, data are scarce, if not nonexistent, regarding the genetic determinants of resistance and biofilm formation in foodborne bacterial strains. This study aims to identify the genetic basis of resistance and biofilm formation in *P. aeruginosa* and *S. aureus* isolated from animal products.

2. Material and methods

2.1. Samples and Bacterial isolates

A total of one hundred and twenty (120) strains composed of *P. aeruginosa* (60) and *S. aureus* (60) were isolated from animal products (beef, fresh fish, and smoked fish). These *P. aeruginosa* and *S. aureus* strains were isolated on Cetrimide and Baird Parker agar, respectively. These bacterial strains isolated on these specific media were identified using standard bacteriological and biochemical techniques and confirmed by PCR.

2.2. Detection of coagulase

"Different colonies of *S. aureus* isolated on Baird Parker agar were transferred to brain-heart broth (BHC) and incubated at 37°C for 24 h. After 24 h, 0.2 mL of each culture was

transferred to sterile hemolysis tubes containing 0.5 mL of rabbit plasma. The culture was incubated again at 37°C and observed after 1 h and 4 h. A firm clot indicates the presence of coagulase-positive *S. aureus"* (Thirunavukkarasu *et al*., 2014).

2.3. Detection of DNA-ase Production

"Thermonuclease, or DNA-ase, was detected in suspected *S. aureus* colonies isolated on Baird-Parker agar. These suspected colonies were then streaked onto Columbia agar (Bio-Rad), and the plates were incubated at 37°C for 24 hours. After 24 hours, the colonies isolated on Columbia agar were removed and inoculated into 1 cm diameter patches on DNA agar (OXOIOD). Finally, the plates containing the inoculated DNA agar were flooded with a 1 mol/L hydrochloric acid solution, and the supernatant was removed after 5 minutes. The presence of a clear zone around the patch indicates that the strain produces DNase (the strain is said to be DNase-positive). In the absence of a clear zone, it does not produce one (the strain is said to be DNase negative)" (Kateete *et al*., 2010).

2.4. Molecular characterization of resistance and biofilm determinants

2.4.1. Extraction and purification of genomic DNA

Strains of *P. aeruginosa* and *S. aureus* were harvested from an overnight broth culture. The genomic DNA of *P. aeruginosa* and *S. aureus* was extracted by thermal lysis and purified according to the method described by Kateete et al.. After extraction, DNA was diluted and stored at -20°C to serve as a DNA template for polymerase chain reactions (PCR).

2.4.2. Reaction mixture

The reaction mixture was prepared according to the method described by Benie et al. This 25 µL reaction mixture consisted of 16 µL of sterile Milli-Q water (milli-Q™, Millipore Corporation, USA), 5 µL of 5X concentration loading buffer, 1.5 µL of MgCl2, 2 mM (Promega Corporation, Madison, WI 53711-5399, USA), 0.2 µL of 10 mM dNTPs, 0.1 µL of each primer, 10 mM (Integral DNA Technology, California, USA), 0.1 µL of Go Taq® G2 Flexi DNA polymerase of final concentration 1.5U (Promega Corporation, Madison, WI 53711-5399, USA) and 2 µL of the DNA template. Sterile Milli-Q water and reference strains were used as negative control and positive control, respectively, for each PCR reaction run.

2.4.3. Gene Amplification

The 16S rDNA gene was amplified according to the method described by Benie *et al*., 2021) using primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (3'TACGGYTACCTTGTTACGACTT-5'). The amplification program consisted of an initial 5-min denaturation at 94°C followed by 35 cycles of denaturation (94°C for 30 s), hybridization (55°C for 40 s), and extension (72°C for 30 s), with a single final 10-min extension at 72°C. Samples were stored at +4°C until the thermocycler was shut down.

2.4.4. Amplification of resistance genes and biofilm formation

The amplification of resistance genes (blaSHV, blaIMP, and blaTEM) and biofilm formation genes (pslA, pelA, and PpyR) was carried out following the procedure outlined by Benie et al.. The amplification procedure included a preliminary 5-minute denaturation at 95°C, succeeded by a cyclic phase that was repeated. Every 33-cycle phase included an amplification cycle featuring a 30-second denaturation phase at 95°C, a 60-second primer attachment (hybridization) phase at 65°C, and a 90-second elongation phase at 72°C. The amplification concluded with a final 5-minute elongation at 72°C. Samples were kept at +4°C until the T9700 Thermocycler, produced by AB (Applied Biosystems) in Singapore, China, was turned off. Table 1 outlines the amplification programs and the nucleotide sequences of the primers employed

2.4.5. Electrophoresis of amplification products

Gene amplification products were detected on a 1% agarose gel after migration at 120 V for 30 min and visualized by illumination on a UV plate of a lighting device and photographed (Molecular Imager Gel DocTM EZ, Bio-Rad, USA) as described previously.

Table 1. Primers for detecting resistance genes and biofilm formation

Gene	Primer sequence (5′→3′)	Amplification program	Size
SHV	F : 5’ TGGTTATGCGTTATATTCGCC-3’	95°C, 5 min	867 pb

Resistance	*TEM*	R : 5’ GCTTAGCGTTGCCAGTGCT-3’ F: 5’ ATAAAATTCTTGAAGAC -3’ R: 5’ TTACCAATGCTTAATCA-3’	33 x [95°C, 30 s; 65°C, 60s; 72°C, 90s] 72°C, 5 min;	1075 pb
	IMP	F : 5’TGAGCAAGTTATCTGTATTC -3’ R : 5’ TTAGTTGCTTGGTTTTGATG-3’		740 pb
Biofilms	*PelA*	F : 5’-CATACCTTCAGCCATCCGTTCTTC-3’ R : 5’-CGCATTCGCCGCACTCAG-3’	95°C, 5 min	656 pb
	PslA	F : 5’-TCCCTACCTCAGCAGCAAGC-3’ R : 5’-TGTTGTAGCCGTAGCGTTTCTG-3’	33 x [95°C, 30 s; 65°C, 60s ; 72°C, 90s]	786 pb
	PpyR	F : 5’-CGTGATCGCCGCCTATTTCC-3’ R : 5’-ACAGCAGACCTCCCAACCG-3’	72°C, 5 min; 4°C.	160 pb

Pel: pellicle (Pel gene encoding dandruff); Psl: polysaccharide synthesis locus; ppyR: psl and pyoverdine operon regulator, PA2663; TEM: Temoneira-Patient Name; SHV: Sulfhydryl Variable; IMP: MβL Imipenemas

2.5. Statistical Analysis

The data underwent computer processing. SPSS 20.0 software was used to analyze the data, while Excel was used to plot the curves. A principal component analysis (PCA) was performed on the distribution of biofilm-forming genes in the studied strains.

3. Results

3.1. Differential Characteristics in *P. aeruginosa* and *S. aureus*

Some phenotypic and biochemical characteristics studied showed that *P. aeruginosa* strains are Gram-negative, oxidase-positive, non-spore-forming bacteria, generally motile by one or more polar flagella. Some produce pigments including pyocyanin (blue-green) and pyoverdine or fluorescein (yellow-green) (Fig. 1). *S. aureus* strains, on the other hand, are Gram-positive, catalase- and coagulase-positive bacteria (Fig. 1). Electrophoretic profiling showed that all the strains studied belonged to the *P. aeruginosa* and *S. aureus* species (Fig. 2).

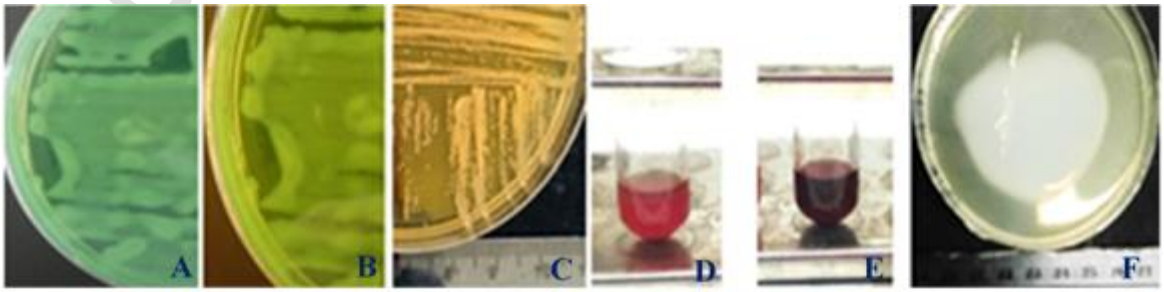

Fig. 1. Differential characteristics in *P. aeruginosa* and *S. aureus*

A: Pyocyanin production on King A; B: Pyoverdine production on King B; C: Appearance of S. aureus; D: Coagulase (-), E: Coagulase (+) and F: DNase production (DNase +)

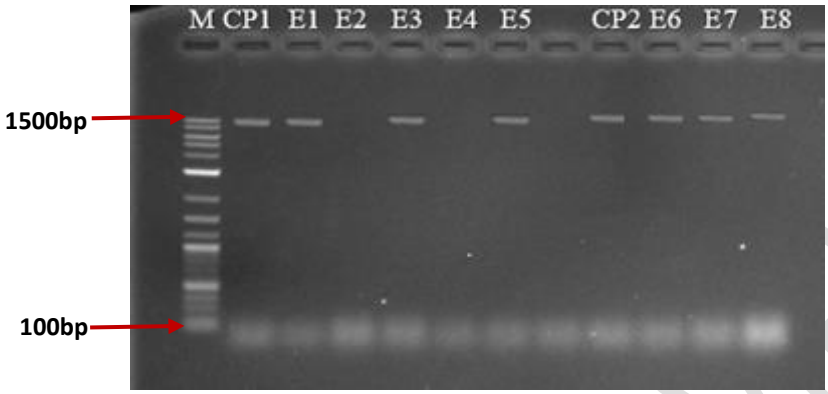

Fig. 2. Electrophoretic profile of the 16S gene with primers 27-F and 1492-R

E1, E3, and E5: Presence of Pseudomonas in the analyzed products. CP1: positive control (P. aeruginosa ATCC 27853); E6-8: Presence of Staphylococcus aureus in the analyzed products. CP2: positive control (S. aureus ATCC29213); CN: negative control; M: Molecular weight marker

3.2. Coagulase and DNase produced in *S. aureus*

Out of a total of sixty (60) strains of *S. aureus*, 83.33% and 75% were producers of coagulase and DNase respectively (Table 2).

Table 2. Proportion of *S. aureus* strains producing coagulase and DNase

Total number of *S. aureus* strains N (%)	Proportion of enzymes produced in *S. aureus* N (%)	
	Coagulase	DNase
60 (100 %)	50 (83,33 %)	45 (75 %)

3.3. Molecular resistance determinants in *P. aeruginosa* and *S. aureus*

The figure shows the electrophoretic profile of resistance genes in *P. aeruginosa* and *S. aureus* strains (Fig. 3). In descending order of importance, the prevalence of resistance genes detected in *P. aeruginosa* was 63.3% (***bla$_{SHV}$***), 53.3% (***bla$_{IMP}$***), and 35.0% (***bla$_{TEM}$***). In *S. aureus*, the prevalence of ***bla$_{IMP}$*** (48.3%) and ***bla$_{SHV}$*** (36.7%) was less than 50.0% (Table 3).

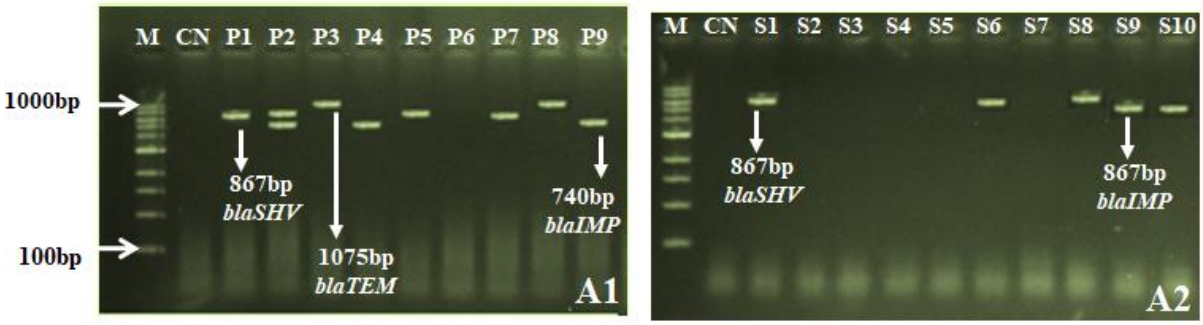

Fig. 3. Electrophoretic profile of resistance determinants

A1: In P. aeruginosa: Lines 1, 2, 5, and 7: Presence of the ***bla$_{SHV}$*** *resistance gene; Lines 2, 4, and 9: Presence of the* ***bla$_{IMP}$*** *resistance gene; Lines 3 and 8: Presence of the* ***bla$_{TEM}$*** *resistance gene. A2: In S. aureus: Lines 1, 6, and 8: Presence of the* ***bla$_{SHV}$*** *resistance gene; Lines 9 and 10: Presence of the* ***bla$_{IMP}$*** *resistance gene. CP: negative control; M: molecular weight marker.*

Table 3. Prevalence of resistance genes in *P. aeruginosa* and *S. aureus*

Bacterial species	Prevalence of resistance genes (%)		
	bla$_{SHV}$	***bla$_{TEM}$***	***bla$_{IMP}$***
P. aeruginosa n=60	38 (63,3)	21 (35,0)	32 (53,3)
S. aureus n=60	22 (36,7)	0 (0,0)	29 (48,3)

SHV: Sulfhydryl Variable; IMP: MβL Imipenemase, TEM: TEMONEIRA-Name of patient

3.4. Molecular determinants of biofilms in *P. aeruginosa* and *S. aureus*

Figure 4 presents the electrophoretic profile of biofilm formation determinants in *P. aeruginosa* and *S. aureus*. The *PpyR* gene (93.3%) was predominantly detected in *P. aeruginosa*, followed by the *PslA* (80.0%) and *PelA* (71.7%) genes (Table 4). Only the *PslA* (48.3%) and *PelA* (38.3%) genes were detected in *S.* aureus, with prevalences below 50.0% (Table 4).

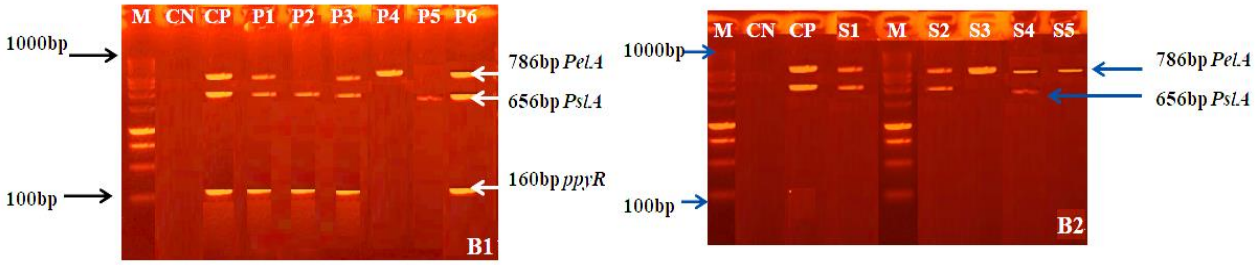

Fig. 4. Electrophoretic profile of genes involved in biofilm formation

B1: In P. aeruginosa: Lines P1, P3, and P6: Presence of biofilm formation genes pelA, pslA, and PpyR; Line P2: Presence of biofilm formation genes pslA and PpyR; Line P4: Presence of biofilm formation genes pelA; Line P5: Presence of biofilm formation genes pslA. B1: In S. aureus: Lines S1, S2, and S4: Presence of biofilm formation genes pelA and pslA; Lines S3 and S5: Presence of biofilm formation genes pelA. CP: positive control; CN: negative control; M: molecular weight marker. PelA: pellicle (gene coding for pellicles); PslA: polysaccharide synthesis locus.

Table 4. Prevalence of biofilm genes in *P. aeruginosa* and *S. aureus*

Genes of Biofilms	*P. aeruginosa* (n=60)		*S. aureus* (n=60)		Total (n=120)	
	Number (N)	Prevalence (%)	Number (N)	Prevalence (%)	Number (N)	Prevalence (%)
PslA	48	80,0	29	48,3	47	39,2
PelA	43	71,7	23	38,3	66	55,0
PpyR	56	93,3	0	0,0	56	46,7

PelA: pellicle (gene encoding dandruff); PslA: polysaccharide synthesis locus; ppyR: psl and pyoverdine operon regulator, PA2663

3.5. Distribution of Biofilm-Forming Genes in *P. aeruginosa* and *S. aureus*

Axis 1 and 2 of the principal component analysis (PCA) account for 100% of the total inertia. Axis 1 of the PCA individual plot shows that the biofilm-forming genes *PslA* and *PelA* are more strongly associated with *S. aureus* strains. However, this PCA indicates that only *P. aeruginosa* strains harbor the *PpyR* gene (Fig. 5). Axis 2 shows that *P. aeruginosa* strains are more strongly associated with the *PpyR* and *PslA* genes (Fig. 5).

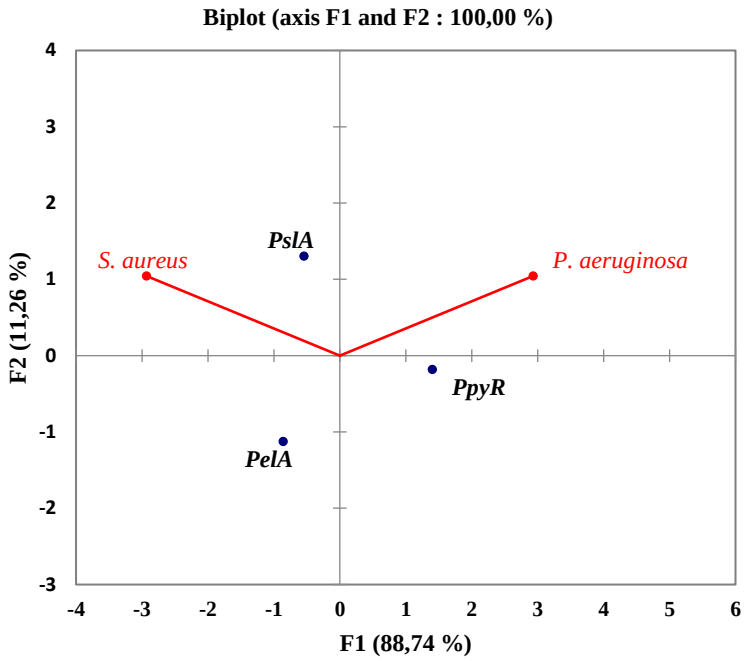

Fig. 5. Distribution of biofilm formation genes in *P. aeruginosa* and *S. aureus*

4. DISCUSSION

"Biofilm formation is a cycle in which organized communities of microbes become embedded in an extracellular polymeric matrix (ESM), promoting 65–80% of microbial infections" (Grari *et al*., 2025). "These biofilms are important virulence determinants responsible for various infectious diseases. In particular, microbial interactions are known to play a significant role in nosocomial and community-acquired infections" (Mlugu *et al*., 2023). "Although some antimicrobials are used to treat bacterial diseases associated with biofilms,

they often do not target the molecular determinants" (Mlugu *et al*., 2023). "Research into alternatives that specifically target these molecular and phenotypic interaction mechanisms by which microorganisms express their pathogenicity and virulence represents an important advance" (Singh *et al*., 2021; Chen *et al*., 2013).

This study highlighted the molecular determinants involved in biofilm formation in multidrug-resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa* isolated from animal products. In descending order of importance, the prevalence of resistance genes detected in *P. aeruginosa* was 63.3% (***bla*$_{SHV}$**), 53.3% (***bla*$_{IMP}$**), and 35.0% (***bla*$_{TEM}$**). "The coagulase- and DNase-producing *S. aureus* strains isolated harbored ***bla*$_{IMP}$** (48.3%) and ***bla*$_{SHV}$** (36.7%) resistance genes with varying prevalences. The presence of these enzymes and resistance genes detected in bacterial strains could explain colonization dynamics and virulence mechanisms as well as those of antibiotic resistance and biofilm formation" (Amer *et al*., 2025; Jarzynka *et al*., 2025; Rasamiravaka *et al*., 2015).

"Studies have shown that the agr and Rhl quorum sensing (QS) communication system in these strains plays a crucial role in regulating virulence determinants by modulating gene expression according to bacterial density" (Amer et al., 2025). "This system exerts both positive (hemolysins and exoenzymes) and negative (coagulase and adhesion proteins) regulation on various determinants of virulence and resistance" (Risser et al., 2022). "It influences the bacterium's ability to colonize and evade host defenses" (Fathi et al., 2022). "The presence of biofilm-forming genes detected in this study also indicated that these potential communication systems promote biofilm formation in *S. aureus* and *P. aeruginosa"* (Mlugu *et al*., 2023).

"Indeed, the *PpyR* gene (93.3%) was predominantly detected in *P. aeruginosa*, followed by the *PslA* (80.0%) and *PelA* (71.7%) genes. The detection of these *PpyR*, *PslA*, and *PelA* genes could indicate the potential for pyoverdin, polysaccharide, and pellicle biosynthesis in *P. aeruginosa*, respectively. Furthermore, this high pyoverdin production in *P. aeruginosa* would explain its ability to spoil food products, particularly those of animal origin" (Mohsenzadeh *et al*., 2025). "These high prevalences of determinants indicating overproduction of pyoverdine, polysaccharide and pellicle could translate into different mechanisms involved in the virulence and pathogenicity of *P. aeruginosa"* (Mohsenzadeh *et al*., 2025; Maisuria *et al*., 2016).

"In *S. aureus*, the same *PslA* and *PelA* genes promoting polysaccharide and pellicle biosynthesis were detected, but no gene encoding pyoverdine production was observed" (Jarzynka *et al*., 2025; Benie *et al*., 2021). "The presence of these genes involved in biofilm formation could explain the level of biofilm formation and the involvement of *P. aeruginosa* and *S. aureus* in the animal production chain" (Mlugu *et al*., 2023).

In this work, principal component analysis (PCA) was used to understand the similarity of these biofilm formation determinants in the genetic makeup of *S. aureus* and *P. aeruginosa*. This PCA analysis indicates the specificity or close link of the *PpyR* gene to *P. aeruginosa* strains (Chimi *et al*., 2021). This affinity of the gene involved in biofilm formation in *P. aeruginosa* could be explained by the diversity of pyocyanic pigments produced by this strain. This study demonstrated the presence and involvement of biofilm formation determinants in *S. aureus* and *P. aeruginosa* of animal origin. The presence of these numerous resistance and biofilm formation determinants indicates the need to improve the production and processing chain for foodstuffs of animal origin.

5.1. Conclusion

This study identified molecular mechanisms for resistance and biofilm formation in *P. aeruginosa* and *S. aureus* isolated from animal products. The study revealed high prevalence of $\boldsymbol{bla_{SHV}}$, $\boldsymbol{bla_{IMP}}$, and $\boldsymbol{bla_{TEM}}$ resistance genes, primarily detected in *P. aeruginosa* strains. Similarly, *PpyR*-type molecular determinants responsible for pyoverdine production, which are involved in biofilm formation, were predominantly detected in *P. aeruginosa*, followed by the *PslA* and *PelA* genes. Only the *PslA* and *PelA* genes were detected in *S. aureus* strains. The presence of these resistance and biofilm formation genes in animal-derived strains indicates a potential risk of their expression in animal products.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

All authors hereby declare that no generative AI technology or image-to-text generators were used in the writing or revision of this manuscript.

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