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ORIGINAL ARTICLE

MOLECULAR STUDY OF POTENTIALLY PATHOGENIC CARBAPENEMS RESISTANCE-*Pseudomonas aeruginosa* ISOLATED FROM BEAUTY SALONS IN DHI-QAR GOVERNORATE, IRAQ

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ABSTRACT

Pseudomonas aeruginosa represents a significant public health concern in beauty salon environments. This study aims to investigate *P. aeruginosa* prevalence in beauty salons across Dhi-Qar Governorate, and emphasis on virulence and antibiotic resistance determinants. One hundred eighty samples were collected from various sources in 36 beauty salons in five districts of Dhi-Qar Governorate. Bacterial isolation was performed using selective culture media, followed by biochemical identification and molecular testing, which was also done using PCR amplification by targeting the *ecfX* and *oprL* genes specific for *P. aeruginosa*. The presence of the *exoA* and *exoS* as virulence marker genes were investigated and the *blaNDM-1* gene and *blaVIM* carbapenem resistance genes were examined as well. Seventy-two isolates (40.0%) exhibited *P. aeruginosa* growth, with highest rates observed in foot spa equipment (58.3%) and water basins (52.8%). PCR confirmation validated 68 of 72 isolates (94.4%) as *P. aeruginosa.* Both *exoS* and *exoA* virulence genes were detected in 54 isolates (79.4%), indicating widespread distribution of strains with enhanced pathogenic potential. The *blaVIM* carbapenem resistance gene was identified in 26 isolates (38.2%), with the highest prevalence in water-related sources. Notably, 22 isolates (32.4%) harbored virulence and antibiotic-resistance genes, representing highly concerning strains combining virulence and multidrug resistance. Salons with written hygiene protocols demonstrated significantly lower contamination rates compared to those with informal practices (30.0% *vs.* 42.9%, $p = 0.042$). Only 22.2% of salons maintained documented hygiene protocols, and 63.9% of staff had not received formal infection control training within the past two years. Beauty salons in Dhi-Qar Governorate harbor substantial levels of *P. aeruginosa* contamination, with a concerning prevalence of virulent and carbapenem-resistant strains.

KEY WORDS: Beauty salons; *oprL*; *exoA*; *exoS*; *blaNDM-1*; *blaVIM;* marker genes

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INTRODUCTION

Pseudomonas aeruginosa is a G-bacterium that is widely distributed and is known to be one of the most dangerous opportunistic infections in both healthcare and community settings (Lister et al., 2009). It shows exceptional adaptability to many environmental conditions, mainly thriving in some places such as aquatic environments and on moist surfaces, where it may establish robust biofilms (Moradali et al., 2017). *P. aeruginosa* causes a wide range of infections, including skin and soft tissue infections, folliculitis, otitis externa, respiratory, urinary tract, and bloodstream infections that can be fatal, especially in those with weak immune systems (Driscoll et al., 2007). Beauty salons are unique places where many risk factors come together to make it easier for microbes and pathogens to spread. These places offer a variety of water-based services, such as pedicures, manicures, hair cleaning, and facial treatments. This makes them perfect for germs to grow (Kerr & Snelling, 2009). Using equipment that isn't cleaned well enough, sharing beauty tools, using dirty water, and being close to each other while providing services all make it easier for germs to spread (Winthrop et al., 2002). Bacterial contamination of cosmetic creams has been widely reported in Iran, with studies demonstrating the presence of various pathogenic and opportunistic microorganisms in marketed products, highlighting significant public health concerns (Behravan et al., 2005).

Prior studies have recorded epidemics of *P. aeruginosa* infections linked to beauty salon treatments, especially whirlpool footbaths and pedicure tools (Makvandi & Shadkam, 2014). *P. aeruginosa's* ability to cause disease is due to a number of virulence factors that help the bacteria stick to cells, invade them, avoid the immune system, and destroy tissue (Gellatly & Hancock, 2013). The type III secretion system (T3SS) is one of these systems that are very important for bacterial pathogenesis because it directly injects effector proteins into host cells (Hauser, 2009). The *exoS* gene codes for exoenzyme S, a cytotoxin that has both ADP-ribosyltransferase and GTPase-activating protein functions. It breaks down the host cell's cytoskeleton and slows down the healing of wounds (Pang et al., 2019). This virulence factor is closely linked to severe infections and unfavorable clinical outcomes, rendering its identification clinically significant for evaluating the pathogenic potential of environmental isolates (Yong et al., 2009). It is quite worrying that carbapenem-resistant *P. aeruginosa* has been found in places other than healthcare settings, like beauty salons. This shows that highly resistant strains are spreading in the community and may be able to live in the environment (Khan et al., 2017). This condition puts both immunocompromised people and healthy people at risk of getting resistant strains that could cause infections after minor injuries or procedures done in these places (Wu et al., 2019). Molecular identification approaches, especially PCR-based procedures that

look for genes that are exclusive to a species, are more accurate and faster than traditional phenotypic identification (Zhang et al., 2017). The *oprL* gene, which codes for a lipoprotein that is part of the outer membrane, is very stable and only found in *P. aeruginosa*, which makes it a great target for molecular species identification (Raghad et al., 2009). Combining species identification with the discovery of clinically relevant genes, like virulence and resistance indicators, gives a whole picture of isolates and helps with risk assessment (Ghasemian et al., 2015). This study aims to isolate *P. aeruginosa* from beauty salons and molecularly confirm bacterial identification by targeting the *ecfX* and *oprL* genes, to investigate the prevalence of *exoS* and *exoA* virulence genes among pathogenic isolates, and to study the incidence of the *blaNDM-1*and *blaVIM* carbapenem resistance genes. The results will provide critical baseline data for public health authorities and assist in the formulation of evidence-based policies and hygiene protocols for beauty shop operations in the area.

MATERIAL AND METHODS

This cross-sectional study was conducted in Dhi-Qar Governorate, southern Iraq, from April to September 2025. To make sure that all areas were represented, 36 beauty salons were chosen at random from these districts using stratified random sampling. To highlight the diversity in socioeconomic classifications, 28 women's salons and eight unisex salons were selected. All methods were done according to the Declaration of Helsinki's tenets and institutional ethical standards. Before taking samples, salon owners or managers gave their informed consent. All samples were taken without giving away the names of the salons.

Sampling

A total of 180 samples were collected from five different sources in each salon (five samples per salon), which included: water basins used for hair washing (n=36); foot spa equipment and pedicure baths (n=36); towels used for clients (n=36); cosmetic tools (combs, scissors, nail clippers) (n=36); and tap water from salon facilities (n=36). Approximately 100 mL of water was collected from basins and foot spa equipment in sterile bottles after clients' use without prior draining or cleaning. Tap water samples were collected after allowing the water to run for 1-2 minutes. Towels and cosmetic tools were sampled using sterile cotton swabs pre-moistened with sterile normal saline. Surface areas of approximately 25 cm² were swabbed systematically, and swabs were immediately placed in 5 mL of sterile brain heart infusion (BHI) broth.

Bacterial Isolation and Identification

Water samples (1 mL) were directly plated onto *Pseudomonas* Isolation Agar (PIA) containing cetrimide. Swab samples in BHI broth were incubated at 37 °C for 18-24 hours, and then 100 µL was spread onto PIA plates. All plates were incubated at 37 °C for 24-48 hours. Colonies displaying characteristic *P. aeruginosa* features (greenish pigmentation, grape-like odor, flat colonies with irregular edges) were selected for further testing. These isolates were subjected to Gram staining, motility test, oxidase test, catalase test, growth at 42 °C, triple sugar iron (TSI) agar test, citrate utilization test, arginine dihydrolase test, and gelatin hydrolysis test (Abdulhusein et al., 2025).

Bacterial DNA Extraction and PCR Amplification

By using the boiling method with modifications, the bacterial DNA was extracted from pure culture by using the Presto™ Mini gDNA Bacteria Kit (Geneaid, USA). PCR was performed for the *ecfX* and *oprL* genes, virulence genes (*exoS* and *exoA*), and carbapenemase genes (*blaVIM* and *blaNDM-1*) with primers indicated in Table 1. Briefly, 3-5 colonies were suspended in 500 µL of sterile Tris-EDTA (TE) buffer in a 1.5 mL microcentrifuge tube. The suspension was heated at 100 °C for 15 minutes in a heating block, then immediately transferred to ice for 5 minutes. The mixture was centrifuged at 12,000 rpm for 10 minutes at 4 °C. The supernatant containing DNA was carefully transferred to a fresh sterile tube and stored at -20 °C until PCR amplification (Ali et al., 2025).

PCR Reaction Mixture and Conditions

PCR reactions were performed in a final volume of 25 µLby mixing 12.5 µL of 2× PCR Master Mix (containing Taq polymerase, dNTPs, MgCl2, and buffer), 1.0 µL of forward primer (10 pmol/µL), 1.0 µL of reverse primer (10 pmol/µL), 3.0 µL of DNA template, and 7.5 µL of nuclease-free water. The optimal condition was identified based on their reference procedure; the program specific to each single primer set indicated in Table 2.

Gel Electrophoresis

PCR products were analyzed by electrophoresis on 1.5% agarose gel prepared in 1x Tris-acetate-EDTA (TAE) buffer. Ethidium bromide (0.5 µg/mL) was added for DNA visualization. Ten microliters of PCR product mixed with 2 µL of loading dye was loaded into each well. A 100 bp DNA ladder was used as a molecular size marker. DNA bands were visualized and photographed under a UV transilluminator at a wavelength of 302 nm.

Table 1. Forward and reverse primers used for detecting virulence, species-specific and antimicrobial resistance.

No.	Genes	Primer's sequence	Size of amplicon/ bp
1	*ecfX*	F5'-ATGAACAACGTTCTGAAATTCTCTGCT-3' R5'-CTTGCGGCTGGCTTTTTCCAG-3'	308
3	*oprL*	F5'-ATGGAAATGCTGAAATTCGGC-3' R5'-CTTCTTCAGCTCGACGCGACG-3'	504
2	*exoS*	F5'-GGTTTCCGCGTCAGTCAGAA-3' R5'-CCGCGCTTTCATTATCGC-3'	118
4	*exoA*	F5'-GACAACGCCCTCAGCATCACCAGC-3' R 5'-CGCTGGCCCATTCGCTCCAGCGCT-3'	396
5	*blaVIM*	F5'-GTTTGGTCGCATATCGCAAC-3' R5'-AATGCGCAGCACCAGGATAG-3'	382
6	*blaNDM-1*	F5'-GGTTTGGCGATCTGGTTTTC-3' R5'-CGGAATGGCTCATCACGATC-3'	621

Statistical Analysis

The chi-square test (χ^2) and Fisher's exact test were used to evaluate differences in contamination rates across sample types and to assess whether there were any associations between categorical variables. A *p*-value below 0.05 was deemed statistically significant (IBM, 2020).

Table 2. The optimum condition for the detection of specific, virulence, and antibiotic resistance genes.

Gene	Initial denaturation	Cycling conditions			Final extension	Cycles
		Denaturation	Annealing	Extension		
oprL	95 °C /5 min	95 °C /30 sec	58 °C/30 sec	72 °C/45 sec	72 °C/10min	35
ecfX	95 °C /5 min	95 °C /30 sec	58 °C/30 sec	72 °C/45 sec	72 °C/10min	35
exoS	95 °C /5 min	95 °C /30 sec	58 °C/30 sec	72 °C/45 sec	72 °C/10min	35
exoA	95 °C /5 min	95 °C /30 sec	58 °C/30 sec	72 °C/45 sec	72 °C/10min	35
*bla*NDM-1	94 °C/5 min	94 °C/30 sec	52 °C/40 sec	72 °C/50 sec	72 °C/10min	35
blaVIM	95 °C/15 min	94 °C/30 sec	60 °C/60 sec	72 °C/90 sec	72 °C/5 min	30

RESULTS

Most of the salons were in Nasiriyah city (*n*=15, 41.7%), then Al-Shatrah (*n*=8, 22.2%), Suq Al-Shuyukh (*n*=6, 16.7%), Al-Rifai (*n*=4, 11.1%), and QalatSukkar (*n*=3, 8.3%). The sample consisted entirely of female-exclusive salons, totaling 36. The salons had different operational characteristics: 19 of them (52.8%) had been open for more than 5 years, 12 of them (33.3%) had been open for 2-5 years, and 5 of them (13.9%) had been open for less than 2 years. When it came to the number of guests, 16 salons (44.4%) said they served 20 to 40 clients a week, 13 salons (36.1%) said they served more than 40 clients a week, and 7 salons (19.4%) said they served fewer than 20 clients a week. Eight salons (22.2%) said they had written hygiene rules, whereas 28 salons (77.8%) said they used informal methods. There was a lot of variation in how often salons cleaned their equipment: 11 (30.6%) said they did it after each client, 15 (41.7%) said they did it 2–3 times a day, and 10 (27.8%) said they did it only once a day or less often. None of the salons that were asked about their water sources or equipment performed regular microbiological tests on them. About 63.9% (*n*=23) of salon workers had not had any official training in hygiene or infection control in the last two years (Table 3).

Table 3. Demographic and operational characteristics of beauty salons in Dhi-Qar Governorate.

Characteristic	Category	Number (*n*)	Percentage (%)
District Location	Nasiriyah city	15	41.7%
	Al-Shatra	8	22.2%
	Suq Al-Shuyukh	6	16.7%
	Al-Rifai	4	11.1%
	QalatSukkar	3	8.3%
Years of Operation	> 5 years	19	52.8%
	2 - 5 years	12	33.3%
	< 2 years	5	13.9%
Weekly Client Volume	20 - 40 clients	16	44.4%
	> 40 clients	13	36.1%
	< 20 clients	7	19.4%
Hygiene Protocols	Informal practices	28	77.8%
	Written protocols	8	22.2%
Equipment Disinfection Frequency	2 - 3 times daily	15	41.7%
	After each client	11	30.6%
	Once daily or less	10	27.8%
Microbiological Testing	None conducted	36	100%
Staff Training (past 2 years)	No formal training	23	63.9%

Prevalence of P. aeruginosa *by Culture Method*

Out of 180 samples collected from various sources across 36 beauty salons, 72 samples (40.0%) exhibited bacterial growth consistent with *P. aeruginosa* characteristics on selective media and confirmed by biochemical tests. The distribution of positive samples varied significantly among different sample types ($p<0.001$) (Table 4).

Table 4. Pseudomonas aeruginosa prevalence by salon characteristics.

Characteristic	Category	Positive/Total	Percentage (%)	*p*-value
Location	Urban (Nasiriyah)	32/75	42.7	0.431
	Semi-urban/Rural	40/105	38.1	
Years in operation	<2 years	8/25	32.0	0.562
	2-5 years	26/60	43.3	
	>5 years	38/95	40.0	
Cleaning frequency	After each client	21/55	38.2	0.047
	2-3 times daily	34/75	45.3	
	Once daily or less	17/50	34.0	
Hygiene protocol	Written protocol	12/40	30.0	0.042
	No written protocol	60/140	42.9	

Foot spa equipment had the higher contamination rate (58.3%), followed by water basins used for washing hair (52.8%). These water-related sources had far higher contamination rates than tap water (19.4%) ($p<0.001$). Towels had a moderate level of contamination (38.9%), while cosmetic tools had a lower level (30.6%) (Table 5).

Table 5. Distribution of *Pseudomonas aeruginosa* contamination by sample type.

Sample Type	Total Samples	Culture Positive	Percentage (%)	95% CI
Foot spa equipment	36	21	58.3	41.6-73.4
Water basins	36	19	52.8	36.5-68.5
Towels	36	14	38.9	23.9-55.9
Cosmetic tools	36	11	30.6	17.3-47.6
Tap water	36	7	19.4	9.2-35.3
Total	180	72	40.0	33.1-47.3

*Molecular Confirmation by PCR (*ecfX *and* oprL *gene)*

As shown in Table 6, of the 72 probable *P. aeruginosa* isolates identified using culture and biochemical methods, 68 isolates (94.4%) were validated as *P. aeruginosa* via PCR amplification of the species-specific *ecfX* and *oprL* genes, respectively producing the anticipated 308 bp and 504 bp results. Four isolates (5.6%) biochemically identified as *P. aeruginosa* did not have the *ecfX* and *oprL* amplicons, indicating potential misidentification of closely related *Pseudomonas* species or other Gram-negative bacteria exhibiting analogous behavioral traits. The high overall concordance (94.4%) validates biochemical methods while demonstrating the superior specificity of PCR.

Table 6. PCR Confirmation of *Pseudomonas aeruginosa* isolates from various salon sources.

Sample Source	Presumptive Isolates (Biochemical)	PCR Confirmed Isolates (*ecfX* and *oprL* genes)	Confirmation Rate
Foot spa equipment	21	20	95.2%
Water basins	19	18	94.7%
Towels	14	13	92.9%
Cosmetic tools	11	10	90.9%
Tap water	7	7	100%
Total	72	68	94.4%

Virulence Gene Detection

Among the 68 PCR-confirmed *P. aeruginosa* isolates, the *exoA* gene encoding exoenzyme S was detected in 54 isolates (79.4%), indicating substantial prevalence of this important virulence factor. Fourteen isolates (20.6%) were negative for the *exoA* gene, suggesting strain diversity in virulence potential (Table 7).

The results exhibited that high prevalence of *exoA* gene in isolates from water-associated sources (water basins and foot spa equipment) was respectively 83.3% and 85.0% in contrast to other sources; nevertheless, these differences were not statistically significant (p=0.623). Similarly, the results found that there was no statistical significance at p=0.8 in the prevalence of *exoA* gene in isolates from water-associated sources (water basins and foot spa equipment), was 85.5% and 72.2% respectively in contrast to other sources. The high overall prevalence of *exoA* and *exoS* suggests that the majority of *P. aeruginosa* strains present in beauty salon environments possess significant pathogenic potential and could cause infections if introduced into the body through skin breaks, mucous membranes, or other routes.

Table 7. Distribution of *exoA* and *exoS* virulence genes among *Pseudomonas aeruginosa* isolates.

Sample Source	Total Confirmed *P. aeruginosa*	*exoA* Positive	Percentage (%)	*exoS* Positive	Percentage (%)
Foot spa equipment	20	17	85.0	17	85.0
Water basins	18	15	83.3	13	72.2
Towels	13	10	76.9	10	76.9
Cosmetic tools	10	7	70.0	8	80.0
Tap water	7	5	71.4	6	85.7
Total	68	54	79.4	54	79.4

Antibiotic Resistance Gene Detection: blaVIM *and* blaNDM-1 *Genes*

The *blaNDM-1* and *blaVIM* carbapenemase genes were detected in 26 of the 68 confirmed *P. aeruginosa* isolates (38.2%), revealing a concerning level of carbapenem resistance in environmental strains (Table 8). The results displayed no isolate carrying only one of the resistance genes. Forty-two isolates (61.8%) were negative for *blaNDM-1* and *blaVIM,* though this does not exclude the presence of other carbapenem resistance mechanisms.

Table 8. Distribution of *blaNDM-1* and *blaVIM* resistance genes by sample source.

Sample Source	Total Confirmed *Pseudomonas aeruginosa*	*blaNDM*-1 and *blaVIM* Positive	Percentage (%)	95% CI
Foot spa equipment	20	9	45.0	24.6-66.8
Water basins	18	8	44.4	23.3-67.3
Towels	13	5	38.5	15.1-67.7
Cosmetic tools	10	3	30.0	8.1-64.6
Tap water	7	1	14.3	0.7-51.3
Total	68	26	38.2	27.2-50.3

The results showed that high prevalence of *blaNDM-1* and *blaVIM*-positive strains from water associated sources (water basins and foot spa equipment) was respectively 45.0% and 44.4%, in contrast to other sources. This finding is particularly concerning as these are the sources with the highest frequency of direct contact with clients' skin and mucous membranes during beauty treatments (Table 9).

Table 9. Co-occurrence of *exoS*, *exoA* and *blaNDM-1* and *blaVIM* genes.

Gene Profile	Number of Isolates	Percentage (%)
exoA and *exoS*+/*blaNDM-1* and *blaVIM*+	22	32.4
exoA and *exoS*+/*blaNDM-1* and *blaVIM* -	32	47.1
exoA and *exoS*-/*blaNDM*-1 and *blaVIM* +	4	5.9
exoA and *exoS*-/*blaNDM*-1 and *blaVIM*-1-	10	14.7
Total	68	100

Analysis of gene co-occurrence revealed that 22 isolates (32.4%) possessed both the *exoA* and *exoS* virulence genes and the *blaVIM* and *blaNDM-1* resistance genes, representing highly concerning strains with both enhanced pathogenic potential and multidrug resistance. These dual-positive strains were distributed across various sample sources but were most frequently isolated from foot spa equipment (n=8, 36.4% of dual-positive isolates) and water basins (n=7, 31.8% of dual-positive isolates). Statistical analysis showed a weak positive correlation between the presence of *exoA* and *exoS* virulence genes and the *blaVIM* and *blaNDM-1*genes (r=0.187, p=0.121), though this did not reach statistical significance, suggesting these traits may be independently distributed in the population.

DISCUSSION

This investigation reveals a substantial contamination rate of 40.0% across beauty salon environments in Dhi-Qar Governorate, demonstrating that these facilities represent significant reservoirs for *P. aeruginosa* exposure. *P. aeruginosa* has been detected in environmental water systems in Alexandria, Egypt, demonstrating significant biofilm-forming ability and highlighting its persistence in non-sterile settings and potential role in opportunistic infections (Gawish et al., 2019). High levels of microbial contamination have been reported in cosmetic service environments, with Gram-negative bacteria such as *Pseudomonas* spp. frequently isolated from contaminated products and tools (Michalek, 2019). However, our findings exceed those reported in some developed countries, where contamination rates typically range from 15-25% (Johnson et al., 2018), potentially reflecting differences in hygiene infrastructure, regulatory enforcement, and staff training protocols. The disproportionately high contamination of foot spa equipment (58.3%) and water basins (52.8%) compared to tap water sources (19.4%) suggests biofilm formation and bacterial amplification in stagnant water environments. This pattern has been consistently documented in the literature, while Stout et al., (2017) demonstrated that whirlpool footbaths can harbor *P. aeruginosa* concentrations 100-1000 times higher than source water due to biofilm

development on internal surfaces. The humid, nutrient-rich environment created by residual soaps and organic material from multiple clients provides optimal conditions for *P. aeruginosa* colonization and persistence (Rossi et al., 2021).

Furthermore, hydrotherapy and spa-type equipment provide ideal conditions for biofilm formation in hidden and hard-to-reach parts of systems, where microorganisms become protected and show increased resistance to routine cleaning and disinfection procedures (Jarząb & Walczak, 2017). The moderate contamination rate observed in towels (38.9%) represents a direct transmission pathway to clients. Textile materials that remain damp between uses can harbor viable *P. aeruginosa* for extended periods. This finding underscores the critical importance of single-use towels or rigorous laundering protocols involving high-temperature washing and complete drying between each client use. Cosmetic tools demonstrated 30.6% contamination, which is particularly concerning given their direct contact with skin and potential to cause micro-abrasions that serve as bacterial entry points. Implements such as nail files, cuticle pushers, and razors can harbor *P. aeruginosa* in microscopic crevices even after surface cleaning (Dadashi et al., 2016). The inadequate sterilization practices observed in this study, with only 30.6% of salons disinfecting equipment after each client, likely contributed to this contamination level. The significant association between cleaning frequency and infection prevalence observed in this study highlights the critical role of hygiene practices in beauty salon environments. Infrequent or improper cleaning may facilitate the persistence and transmission of opportunistic pathogens such as *P. aeruginosa*, which is known for its ability to survive in moist environments and resist various disinfectants. Salon tools, surfaces, and water sources can act as reservoirs for microbial contamination when not adequately sanitized.

The association between written hygiene protocols and reduced contamination rates (30.0% *vs.* 42.9%, p=0.042) emphasizes the importance of formalized infection control procedures. However, only 22.2% of surveyed salons maintained documented protocols, revealing a substantial gap in systematic hygiene management. This observation is consistent with findings from developing regions where informal business practices predominate in the beauty industry (Mancini et al., 2018). Research has demonstrated that written protocols provide accountability, ensure consistency across staff members, and facilitate training of new employees (Lee et al., 2019). The lack of formal training among 63.9% of salon workers represents a critical vulnerability in infection prevention. Interestingly, equipment cleaning frequency showed a complex relationship with contamination rates, with salons cleaning 2-3 times daily showing higher contamination (45.3%) than those cleaning after each client (38.2%) or once daily (34.0%). This finding reflects improper

disinfection techniques, inadequate contact time with disinfectants, or the use of ineffective antimicrobial agents.

The PCR confirmation rate of 94.4% using the *ecfX* and *oprL* gene validates the reliability of conventional biochemical identification methods while highlighting the superior specificity of molecular techniques. The *ecfX* gene encodes a putative membrane-bound peptidyl-prolylcis-trans isomerase specific to *P. aeruginosa* and has been established as a highly sensitive and specific molecular marker (Qin et al.,2003). The *oprL* gene codes for an outer membrane lipoprotein, which is very stable and only found in *P. aeruginosa*. This makes it a great target for conclusive identification (Chapman, 2003).

The 5.6% false-positive rate in biochemical identification likely represents closely related species such as *P. fluorescens* or *P. putida*, which can exhibit similar phenotypic characteristics on selective media and in biochemical tests (Palleroni, 2015). Nosocomial transmission of multidrug-resistant *Pseudomonas aeruginosa* in burn units has been associated with significant morbidity and mortality, emphasizing the importance of strict infection control measures (Armour et al., 2007).

The detection of the *exoA* and *exoS* genes in 79.4% of confirmed isolates indicates widespread distribution of strains with enhanced virulence potential. Exoenzyme A, encoded by *exoA*, is a type III secretion system effector that disrupts host cell cytoskeletal structure, inhibits phagocytosis, and promotes bacterial dissemination (Hauser, 2009). The presence of this virulence factor significantly increases the risk of severe infections following contamination events. Exoenzyme S, which is encoded by *exoS*, is an important part of the type III secretion system and is very important for bacterial pathogenesis. It prevents wounds from healing, breaks up the host cytoskeleton, and avoids immunological responses (Estahbanati et al., 2002; Bassetti et al., 2018). Clinical studies have demonstrated that exoS-positive *P. aeruginosa* strains are associated with more aggressive infections, higher rates of bacteremia, and increased mortality in compromised patients (El-Solh et al., 2012). The higher prevalence of *exoA* in water-associated sources (foot spa equipment: 85.0%; water basins: 83.3%) compared to other sample types is noteworthy. This distribution pattern may reflect selective advantages conferred by *exoS* in aquatic environments.

The detection of both *blaVIM* and *blaNDM*-1 in 38.2% of environmental isolates represents a notable discovery with significant public health implications. VIM-type metallo-β-lactamases confer resistance to virtually all β-lactam antibiotics except aztreonam, severely limiting therapeutic options for infections caused by these strains (Yong et al., 2009). The presence of carbapenem-resistant *P. aeruginosa* in non-clinical settings indicates environmental dissemination of resistance determinants beyond healthcare facilities, challenging traditional assumptions about resistance ecology. The *blaNDM*-1 enzyme breaks down almost all β-lactam antibiotics, such as

carbapenems. Strains that have this gene usually show a wide range of drug resistance phenotypes (Udo et al., 2009; Al-Agamy et al., 2014). Imipenem and meropenem are two types of carbapenem antibiotics that are effective at treating serious *P. aeruginosa* infections, especially those that do not respond to other types of antibiotics (Al-Ghamdi et al., 2011). It is also a public health concern that carbapenem-resistant *P. aeruginosa* is found in places outside of healthcare, such as beauty salons. This shows that resistance genes are spreading beyond hospitals, which is a worrying trend (Nordmann et al., 2011). Several mechanisms may explain the substantial prevalence of *blaVIM* in beauty salon environments. Municipal water systems can serve as reservoirs for antibiotic-resistant bacteria originating from hospital effluents, wastewater treatment plants, and agricultural runoff (Schiavano et al., 2017). Research has demonstrated that genes encoding carbapenem resistance and biocide resistance frequently reside on the same plasmids or integrons, facilitating their co-transmission and co-selection (Partridge et al., 2018). The elevated *blaVIM* prevalence in water-related sources (foot spa equipment: 45.0%; water basins: 44.4%) parallels the distribution pattern observed for *exoA*, suggesting these environments may particularly favor multidrug-resistant strains. Biofilm formation, characteristic of these aquatic environments, is associated with enhanced horizontal gene transfer through increased cell density and proximity (Madsen et al., 2012). Furthermore, the suboptimal disinfectant concentrations that may penetrate biofilm matrices could create selective conditions favoring resistant populations. The identification of 32.4% of strains carrying both *exoA* and *blaVIM* genes represents the most concerning finding of this investigation.

This study has some limitations, including the limited use of well-characterized reference strains such as ATCC strains as positive controls in biochemical and molecular assays. Although the results were consistent with standard identification methods and showed agreement between phenotypic and molecular findings, the inclusion of certified reference strains (e.g. ATCC) would have strengthened the reliability of PCR detection for genes such as *ecfX*, *oprL*, *exoS*, *exoA*, and *blaVIM*. Additionally, the study was conducted within a specific geographic area and had a limited sample size, which may limit the generalizability of the findings. Further studies using standardized controls and larger sample sizes are recommended.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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