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ORIGINAL ARTICLE

Evaluation of the relationship between quorum sensing system genes and antibiotic resistance in isolated *Pseudomonas aeruginosa* from cystic fibrosis patients

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ABSTRACT

Background and Objectives: *Pseudomonas aeruginosa* is a Gram-negative bacterium that causes respiratory infections in individuals with cystic fibrosis. Its level of virulence is primarily controlled through Quorum Sensing (QS), a communication mechanism that utilizes small signaling molecules. This study investigates *P. aeruginosa* antibiotic resistance in CF patients in Imam Khomeini Hospital and examines the presence of QS genes in resistant strains.

Materials and Methods: Sixty-five *P. aeruginosa* samples were identified in CF patients in Imam Khomeini Hospital in Tehran. Antibiotic resistance was assessed using the disk diffusion method, and QS genes (*rhlI, rhlR, lasI, lasR*) were evaluated by applying PCR.

Results: Approximately 61.5 % of *P. aeruginosa* strains were multiple-drug-resistant (MDR), with 30.7% classified as extensively drug-resistant (XDR). The highest resistance was observed against amoxicillin, amikacin, and cefepime. The most common QS gene in MDR and XDR strains was *rhlR*. Additionally, 78.9% of XDR isolates carried *rhlI, rhlR, lasI,* and *lasR* genes.

Conclusion: The study specified that more than half of the *P. aeruginosa* strains exhibited resistance to five antibiotic classes, and effective antibiotics against *P. aeruginosa* were colistin, meropenem, ciprofloxacin, piperacillin/tazobactam, and cefotaxime. A noteworthy correlation was identified between MDR and XDR strains and the existence of QS genes in the strains.

Keywords: *Pseudomonas aeruginosa*; Cystic fibrosis; Drug resistance; Quorum sensing

INTRODUCTION

Pseudomonas aeruginosa (P. aeruginosa) is a prevalent hospital-acquired bacterium that represents a major challenge to healthcare systems. It causes death in immunocompromised patients and it is associated with burns, and CF patients infections (1). A key factor exacerbating chronic infections within the cystic fibrosis (CF) community is the alarming prevalence of extensively drug-resistant (XDR) and multiple-drug-resistant (MDR) strains of *P. aeruginosa*. These formidable resistant strains not only elevate morbidity and mortality rates but also create daunting challenges in identifying effective

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treatment options (2, 3).

These bacteria have the ability to develop MDR and XDR patterns due to their capacity to produce biofilms, disperse transmissible resistance elements, and transmit resistant strains in hospitalized patients. *P. aeruginosa* produces a biofilm structure that adheres to biotic and abiotic surfaces. This biofilm can cause persistent and complicated infections. These bacteria produce extracellular polymeric substances (EPS), which form this structure and help the bacteria to be resistant against bacteriostatic and bactericidal agents (2, 4).

The aggregation of the bacterial community causes *P. aeruginosa* to adhere to various surfaces, resulting in what is known as biofilm formation. Infections caused by *P. aeruginosa* that are associated with biofilms exhibit a remarkable 20-30% higher resistance to antibiotics compared to those arising from planktonic cells (4, 5).

The matrix structure created by the EPS undoubtedly impedes antibiotic penetration and also serves as a barrier (6). *P. aeruginosa* masterfully employs cell-to-cell signaling to forge intricate bacterial communities and create resilient biofilms. Quorum sensing (QS) is a fascinating biological process that elegantly controls expression of various genes dependent on the cell population. This sophisticated mechanism allows cells to communicate and coordinate their behavior, showcasing the remarkable intricacies of microbial life (1, 7).

The QS system is an essential regulatory mechanism used by *P. aeruginosa* to coordinate various cellular processes, such as biofilm formation. This enables bacterial communities to successfully withstand host immune responses and antimicrobial agents.

The QS system comprises small signaling molecules called autoinducers, which are produced and sensed. These molecules help bacteria communicate and coordinate their activities according to population density (8, 9). In *P. aeruginosa*, two major quorum sensing systems are Rhl and Las. Both of these systems employ autoinducers specifically, N-butanoyl-L-homoserine lactone (C4-HSL) and N-(3-oxododecanoyl)-L-homoserine lactone (3O-C12-HSL) to facilitate cell-to-cell communication (7, 10). These molecules that function as signals are prepared by *rhlI* and *lasI*. They bind to their respective receptors, RhlR and LasR, when they reach a certain concentration threshold. The Rhl system plays a vital role in the intricate process of biofilm development. It orchestrates the formation of microcolonies, preserves the integrity of open-channel structures, and facilitates the release of embedded bacterial cells. Each of these stages highlights the remarkable complexity and adaptability of microbial life, showcasing the essential functions of the Rhl system in shaping dynamic biofilm ecosystems. Rhl plays a pivotal role in regulating the virulence factors of *P. aeruginosa*, orchestrating the production of powerful agents such as hydrogen cyanide, rhamnolipid, pyocyanin and elastase (7). The Las system is responsible for controlling the expression of genes that encode alkaline proteases, elastase, and exotoxin A. It also plays a role in biofilm maturation and other phases of the biofilm formation process (11, 12). In addition , QS is responsible for regulating drug efflux pumps and enzymes that modify antibiotics, which could result in the evolution of antibiotic resistance in *P. aeruginosa* (13, 14).

Understanding the relationship between the QS system, the ability to form biofilms, and drug resistance could transform the treatment of *P. aeruginosa* infections. This understanding opens up new possibilities for addressing the increasing challenges posed by this pathogen (15). Given the profound challenges posed by MDR and XDR strains, coupled with the limited treatment options available for infections caused by these formidable pathogens, this study embarks on a critical exploration. The goal is to reveal the molecular detection of quorum sensing (QS) genes and examine their important function in producing biofilm and antibiotic sensitivity in the isolated *P. aeruginosa* strains in CF patients. Through this research, we seek to shed light on the intricate mechanisms at play, paving the way for innovative strategies in combating these resilient infections.

The objective of this project was to investigate whether the genetic patterns of *P. aeruginosa* in CF patients are correlated with antibiotic resistance. To develop this idea, MDR and XDR strains of *P. aeruginosa* isolated from CF patients were identified. Subsequently, the genetic patterns of the *P. aeruginosa* strains, including the presence of quorum sensing (QS) genes, were evaluated.

MATERIALS AND METHODS

Bacterial isolates. This research involved gathering 65 isolates of *P. aeruginosa* from the sputum samples of cystic fibrosis patients admitted to the

Children's Medical Center at Imam Khomeini Hospital in Tehran, Iran. Sputum samples were cultured on blood agar plates. The morphology of bacterial colonies was evaluated based on whether they were non-mucoid or mucoid.

Bacterial recognition. Standard and common bacteriological techniques were used to identify the *p. aeruginosa* isolates. To achieve this goal, the identified isolates in the initial step were examined using morphology of colonies , hemolysis, Gram staining, and other routine biochemical tests (16). All isolated strains of *P. aeruginosa* were carefully preserved in TSB medium infused with 15% glycerol and stored at -20°C, ensuring their viability for future analysis (17). It should be noted that all of the reagents and consumed media were supplied by Hi Media, India.

Antimicrobial susceptibility testing. The antibiotic susceptibility profile of *P. aeruginosa* isolates was determined using the Kirby-Bauer disk diffusion technique on Mueller-Hinton agar (HiMedia, India), according to guidelines which were suggested by the Clinical and Laboratory Standards Institute (CLSI 2023) (18).

Furthermore, *P. aeruginosa* ATCC 27853 was used as the quality control strain, and different antibacterial classes were applied to evaluate antimicrobial susceptibility, including, cephalosporins (cefotaxime, ceftazidime, cefepime), fluoroquinolones (ciprofloxacin), carbapenems (meropenem, imipenem), β-lactams (amoxicillin, ticarcillin, piperacillin), penicillins (piperacillin/ tazobactam), polymyxins (colistin), aminoglycosides (gentamycin, amikacin).

Additionally, *P. aeruginosa* isolates that were non-susceptible to at least one agent in three or more different antimicrobial categories were defined as MDR, while those that were non-susceptible to at least one agent in six or more categories were defined as XDR (19, 20).

Detection of QS genes using PCR

DNA extraction. The extraction of DNA was elegantly achieved through the boiling technique. A 1.5 mL sample of *P. aeruginosa* culture grown in TSB medium was combined with 500 µL of distilled water. Then, the *P. aeruginosa* isolate was decomposed by boiling for about 10 minutes. Next, all samples were centrifuged. Subsequently, the PCR procedure was used to detect QS genes in the samples, which were stored at -20°C (6).

PCR assay for QS genes. The presence of the QS genes was evaluated in isolated *P. aeruginosa* by PCR assay. To identify specific genes, we utilized four pairs of primers, meticulously assessing the specificity of each primer for its corresponding gene through the powerful tool, NCBI Primer-BLAST (13). DNA was quantified using spectrophotometry with an Ultraspec 3000 over a wavelength range of 260 to 280 nm.

The following method was applied to detect QS genes:

The PCR mixture was assembled using the following components: 12.5 µL of 2× Taq Master Mix (Vazyme, China), 1 µL of primers at 10 µM concentration, 50 ng of DNA template, and sterile distilled water to bring the total volume to 25 µL. The PCR amplification was carried out on a PTC-100 programmable thermal cycler (MJ Research, Inc). The procedure started with an initial denaturation at 96°C for 3 minutes. This was then followed by 35 cycles consisting of denaturation at 95°C for 45 seconds, annealing at 56.0°C for 45 seconds, and extension at 72°C for 45 seconds. The process ended with a final extension step at 72°C for 10 minutes. The primer sequences used for QS genes are provided in Table 1. The PCR products were subsequently analyzed through electrophoresis on a 1% agarose gel (conducted at 100 V for 1 hour), stained with ethidium bromide at a concentration of 0.5 µg/mL. The sizes of the alleles were determined using a 100-1000 bp DNA ladder (SinaClon, Iran). The validity of our study was confirmed by the positive control *P. aeruginosa* ATCC 27853 (21).

Data analysis using statistical methods. Statistical analyses were conducted with SPSS version 16 to categorize the frequencies of various parameters, such as QS genes and MDR/XDR patterns. The relationships between these parameters were assessed using Fisher's exact test and the chi-squared test. A P-value of 0.05 or lower was deemed indicative of statistical significance.

Ethics approval. This research has been reviewed under a thesis title in Shahid Beheshti University and has been approved with the ethics code IR.SBU.REC.1404.004.

Table 1. The primers utilized to detect QS genes

Genes	Primer sequence (5′ 3′)	Size of amplicon (bp)	Ref
rhlI	F- TTC, ATC, CTC, CTT, TAG, TTC, TTC	155	(10)
	R- TTC, CAG, CGA, TTC, AGA, GAG, C		
rhlR	F- TGC, ATT, TTA, TCG, ATC, AGG, GC	133	(10)
	R- CAC, TTC, CTT, TTC, CAG, GAC, G		
lasI	F- CGT, GCT, CAA, GTG, TTC, AAG, G	295	(10)
	R- TAC, AGT, CGG, AAA, AGC, CCA, G		
lasR	F- AAG, TGG, AAA, ATT, GGA, GTG, GAG	130	(10)
	R- GTA, GTT, GCC, GAC, GAC, GAT, GAA, G		

RESULTS

Antibiotic susceptibility pattern results. Overall, 61.5% of the isolated *P. aeruginosa* strains were identified as MDR, while 31% were classified as XDR. The greatest resistance rates were noted against amoxicillin (96.92%), amikacin (95.38%), and cefepime (93.84%), respectively. Moreover, the highest susceptibility rates were found with colistin (87.69%), meropenem (84.61%), and ciprofloxacin (81.53%), respectively. Additionally, 85% of the isolates represented resistance to more than five antibiotics.

The antibiotic susceptibility pattern of *P. aeruginosa* isolated from CF patients is depicted in a column chart (Fig. 1).

PCR reaction results. The most abundant QS genes among the isolated strains were *rhlI* (66.08%), *rhlR* (83.75%), *lasI* (68.52%), and *lasR* (77.55%). The most prevalent QS gene among MDR and XDR strains was the *rhlR* gene, with rates of 92.6% and 97.3%, respectively. Additionally, it was revealed that 78.9% of the XDR isolates harbored the *rhlI*, *rhlR*, *lasR* and *lasI* genes.

The analysis clearly shows that among the QS genes, *lasI* has the lowest prevalence in XDR isolates (78.9%). In contrast, among MDR isolates, *rhlI* has the lowest prevalence (70.1%).

Table 2 shows the frequencies of QS genes in MDR and XDR strains, mucoid strains and non-mucoid strains.

Furthermore, 85% of the XDR isolates were found to carry the *rhlI*, *rhlR*, and *lasR* genes. The prevalence of the *lasI* gene in these isolates was slightly lower at 78.9%. Additionally, a significant correlation was identified between MDR and XDR strains and the presence of quorum sensing (QS) genes, with a P-value of 0.001 or less.

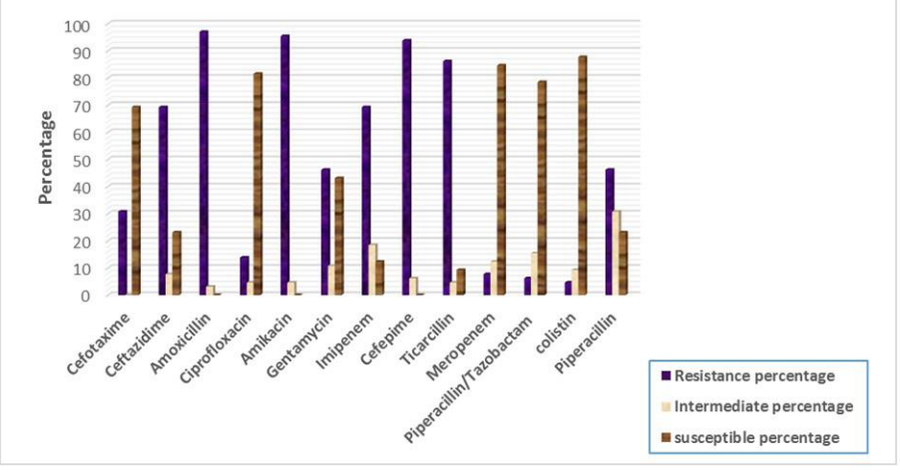

Fig. 1. The graph illustrates the antibiotic susceptibility patterns of the isolated strains.
The horizontal axis displays the types of antibiotics, while the vertical axis indicates the rates of susceptibility and resistance in percentages.

Table 2. Distribution of QS genes in isolated *P. aeruginosa* strains

Tested genes		Mucoid Strains	Non-Mucoid Strains	MDR strains	XDR strains
QS genes	*rhlI*	65.3%	43.65%	70.1%	85.3%
	rhlR	92.5%	52.6%	92.6%	97.3%
	lasI	70.1%	37.9%	87.2%	78.9%
	lasR	80.2%	62.7%	79.67%	87.64%

Figs. 2-5 illustrate the PCR results and band patterns of the QS genes in *P. aeruginosa* isolates obtained from CF patients.

Correlation of MDR, XDR, mucoid, and non-mucoid strains with patient age. The study found that 35.6% of children aged four to six years were infected with MDR strains, 23.2% with XDR strains, 25.4% with mucoid strains, and 74.6% with non-mucoid strains.

According to what was found in this study, children were less likely to be infected with highly resistant and mucoid strains. In addition, 42.1% of patients aged 13-18 were infected with MDR strains, 63.7% with XDR strains, 65.32% with mucoid strains, and 15.6% with non-mucoid strains. Our results revealed that older patients had a greater chance of being infected with XDR and mucoid strains, which was a significant finding. There are two possibilities. One is that non-mucoid strains with lower antibiotic resistance could transform into mucoid and highly resistant strains in the lungs of older individuals. Additionally, there is a risk that these individuals could be infected with mucoid and XDR strains from the environment at the beginning of the disease. Statistical analysis revealed a strong correlation between mucoid strains and the MDR and XDR patterns (P-value < 0.001).

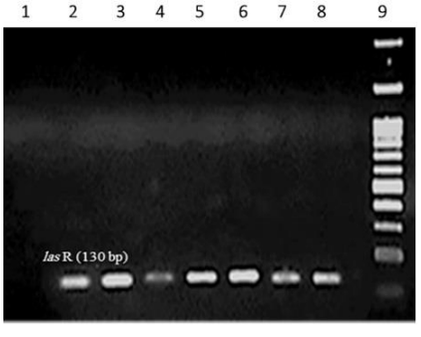

Fig. 2. PCR products for *lasR* (130 bp). 1: Negative control; 2: Positive control strain; 3–8: Isolated *P. aeruginosa* strains; 9: DNA ladder.

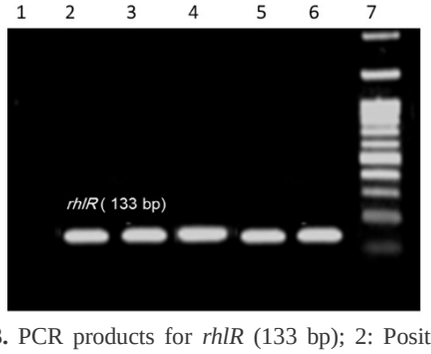

Fig. 3. PCR products for *rhlR* (133 bp); 2: Positive control strain; 1: Negative control; 3-6: Isolated *P. aeruginosa* strains; 7: DNA ladder.

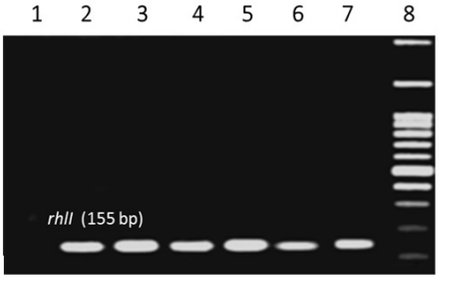

Fig. 4. PCR products for *rhlI* (155 bp); 2: positive control strain; 1: Negative control; 3-7: Isolated *P. aeruginosa* strains; 8: DNA ladder.

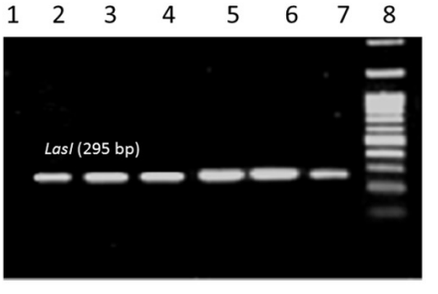

Fig. 5. PCR products for *lasI* (295 bp); 2: positive control strain; 1: Negative control; 3-7: Isolated *P. aeruginosa* strains; 8: DNA ladder.

DISCUSSION

P. aeruginosa is a prevalent Gram-negative opportunistic pathogen that presents considerable difficulties for healthcare systems, particularly affecting individuals with burn injuries, those who are ventilator-dependent, neutropenic patients, individuals with chronic illnesses, or CF patients (7).

Antibiotic resistance poses a major challenge for healthcare systems, making disease treatment more difficult and leading to increased medical expenses from longer hospital stays, ultimately raising patient mortality rates. The rise of MDR and XDR *P. aeruginosa* strains in patients presents a significant public health concern.

Antibiotic resistance trends have been observed in frequently utilized antimicrobial agents, including β-lactams, aminoglycosides, and fluoroquinolones, in *P. aeruginosa* strains isolated from CF patients. The QS system in *P. aeruginosa* represents a sophisticated mechanism that promotes biofilm development, the production of virulence factors, and the emergence of drug resistance, which consequently leads to extended infections in CF patients (21).

Additionally, QS is a complex mechanism in bacteria that controls the expression of genes depending on the population in the community and density. Two significant QS systems, LasI-LasR and RhlI-RhlR, are employed by *P. aeruginosa*, which have vital roles in a variety of cellular processes. These systems greatly enhance the bacteria's virulence, thereby limiting the therapeutic options available for treating *P. aeruginosa* infections (22, 23).

Earlier research has highlighted the crucial role of the quorum sensing (QS) system in the development of MDR and XDR in *P. aeruginosa*. This complex facilitates swarming movement, the production of pathogenicity factors, biofilm production, and enhances the bacterium's overall pathogenic potential. Additionally, another study suggested that strains lacking a functional QS system tend to be more resistant to antimicrobial agents (23, 24).

In the present study, we examined the antibiotic susceptibility patterns and the presence of QS genes in *P. aeruginosa* strains obtained from cystic fibrosis patients at Imam Khomeini Hospital. A significant prevalence of MDR and XDR strains was observed. Furthermore, a significant association was identified between QS systems and mucoid MDR and XDR strains, highlighting the crucial role of quorum sensing in complicating treatment strategies against these resistant bacterial pathogens. This research revealed that although over half of the *P. aeruginosa* strains exhibited high resistance to five different antibiotic classes, they remained susceptible to specific antibiotics such as colistin, meropenem, ciprofloxacin, piperacillin/tazobactam, and cefotaxime. Additionally, the study found that *P. aeruginosa* strains showed significant resistance to certain antibiotics, including amoxicillin, amikacin, cefepime, and ticarcillin.

P. aeruginosa can gradually adjust to the environment of the body and form biofilms in CF patients. Therefore, this study's results on the patterns of antibiotic susceptibility of various strains can be used to determine which antibiotics are best for treating CF patients.

The results of antibiogram examinations and tests in Iran differ slightly from one region to another, which could be due to variations in the application of different classes of antibiotics, as well as differences in the ecosystem, climate, and physical conditions.

Rajabi et al. reported that 75.9% of the *P. aeruginosa* strains were capable of biofilm formation. Furthermore, the antibiotic susceptibility patterns showed that both biofilm-positive and biofilm-negative had high resistance to amoxicillin, with rates of 95.7% and 92.3%, respectively. The resistance rate observed in this study (96.92%) is consistent with the results reported in Rajabi et al. research (25).

In a review of prior research, Bonyadi et al. found that *P. aeruginosa* isolated from CF patients showed the highest resistance to cefotaxime (67%) and the lowest resistance to colistin (5%) among the antibiotics they tested. In current study, 9.44% of the strains were observed to be resistant to colistin, a figure that closely matches Bonyadi's findings. However, resistance to cefotaxime was seen in 26.6% of the strains, which differs from the resistance level reported by Bonyadi. These variations can be attributed to factors such as differences in sample size, the type of infections involved, and the specific antibiotics used in treatment (26).

In a study conducted over a period of time on CF patients, Lucca et al. reported that *P. aeruginosa* susceptibility to fluoroquinolones significantly declined, particularly among patients under 20 years old. Conversely, there was a rise in the susceptibility for antibiotics such as amikacin and colistin. Resistance was more prevalent in younger patients who had received more than three courses of antibiotics annually, with

amikacin and colistin being exceptions. These findings align with the low proportion of mucoid and resistant strains observed in children, as well as the presence of MDR and XDR strains in older patients in the current study. The current study's results regarding colistin susceptibility closely match Lucca et al.'s findings. However, the results concerning amikacin differ markedly, with a high level of resistance to amikacin observed in strains isolated in this study. This discrepancy may be attributed to the widespread and possibly indiscriminate use of amikacin (27).

Emaneini et al. carried out a study examining the molecular characteristics and resistance profiles of *P. aeruginosa* in CF patients. They concluded that piperacillin/tazobactam and meropenem were the most powerful antibiotics against *P. aeruginosa*. Consequently, the findings of present assessment aligned with those of Emaneini et al. (28).

Additionally, in a study on *P. aeruginosa* strains from CF patients, Jarzynka et al. found that 39.4% of isolates exhibited multiple resistance, aligning with the findings of this study (29).

Fig. 6 shows that the probability of encountering mucoid and XDR strains in patients increases as age advances.

There are two potential explanations for this: first, older patients might have been initially infected with mucoid or XDR strains at the beginning of their illness. Second, the non-mucoid and MDR strains already present in their bodies could have developed into mucoid and XDR strains over time. If resistant strains are found in younger patients, it suggests that the hospital environment might be the source of infection, or there may be cross-infection occurring among CF patients.

The high prevalence of multiple antibiotic resistance and the variety of resistance patterns to different classes of antibiotics in *P. aeruginosa* strains isolated from CF patients pose a significant risk to the health and lives of these individuals.

Therefore, antibiotic resistance in the community is important. Moreover, this research can help identify effective antibiotics for treatment and develop appropriate strategies to manage chronic *P. aeruginosa* infections in CF patients. This finding highlights a significant trend which deserves further investigation.

These findings reveal a concerning prevalence of MDR/XDR isolates in this area, emphasizing the critical necessity for comprehensive microbiological surveillance, responsible antibiotic prescribing practices, and unwavering commitment to antimicrobial policies within our healthcare systems. It is imperative that we act decisively to address this urgent challenge and protect public health. Promptly administering the appropriate antibiotic is essential to prevente chronic infections in CF patients. This situation should be considered as a medical emergency. The differences in antibiotic susceptibility highlight the global variations in antibiotic resistance patterns. This highlights the critical importance of strengthening local antimicrobial stewardship programs and strictly adhering to CLSI guidelines. These proactive steps are essential to prevent the development of antimicrobial resistance in *P. aeruginosa* strains and to improve health outcomes for everyone. *P. aeruginosa* strains are renowned for their remarkable capability to form resilient biofilms on various surfaces, a phenomenon that significantly heightens the risk of mortality among CF patients. The QS system plays a crucial role in the regulation of production biofilm

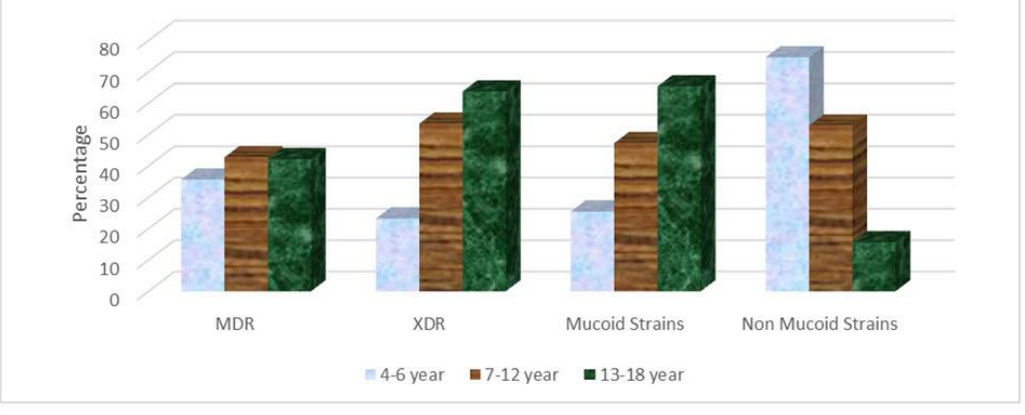

Fig. 6. The prevalence of MDR, XDR, mucoid and non-mucoid isolates among the patients

in *P. aeruginosa*. a critical player in the initial adhesion of cells. Moreover, the las-rhl system comes into play through the unalterable adherence phase, playing a vital role in the maturation of the biofilm and underscoring the complex interplay of mechanisms that enhance the bacteria's survival and persistence (23, 24).

Previous studies (30) demonstrated that the incidence of QS genes in strong biofilm producer isolates is significantly high, establishing a significant connection between QS genes and the production of biofilm.For example, Lima et al. (9) demonstrated that QS genes existed in 97% of the biofilm-producing strains they investigated. Furthermore, a subsequent study (10) revealed that all biofilm-forming *P. aeruginosa* strains capable of biofilm formation harbored the QS genes. Additionally, Alayande et al. (12) discovered that QS signaling elements are crucial in the formation of biofilms.The development of biofilms is a key virulence factor in *P. aeruginosa*, playing an important role in enhancing its ability to cause disease. Research has shown that bacteria within a biofilm structure exhibit resistance level that are 100 to 1,000 times higher than those of individual planktonic cells (31).

In the current study, it was found that among the MDR strains, the *rhlI*, *rhlR*, and *lasR* genes were the most frequently observed, accounting for 70% of the total. In addition, 85% of the XDR isolates tested positive for the *rhlI*, *rhlR*, and *lasR* genes. Additionally, a strong association was discovered between MDR and XDR strains and the existence of QS genes (P-value $\leq$ 0.001).

In this study, the most commonly identified QS genes in MDR strains were *rhlR* and *lasI*, with occurrence rates of 92.6% and 87%, respectively. Furthermore, *lasI* had the lowest prevalence in XDR isolates (78.9%), while *rhlI* had the lowest prevalence in MDR isolates (70.1%).

Our results revealed that 82% of the tested isolates possessed three of the four QS genes. Conversely, Perez et al. (32) discovered that 90.1% of the isolates they examined harbored all the QS genes. Likewise, Lima et al. (9) observed a notably high prevalence of QS genes in Brazil, with 97.5% of the isolates testing positive for these genes. The variation in the prevalence suggests that *P. aeruginosa* may possess multiple mutations within its quorum sensing system.

A key finding in this study was the striking correlation between the existence of QS genes and the pattern of antibiotic resistance. Genetic investigation revealed that an impressive 70% of MDR isolates and a remarkable 78.9% of XDR isolates harbored all QS genes. The critical role of QS genes in the context of antibiotic resistance is underlined by this finding, highlighting their potential as key players in microbial resilience.

As shown in Table 2, the prevalence of QS genes is significantly lower in non-mucoid strains than in mucoid ones. This difference may explain why non-mucoid strains are unable to produce biofilms.

The differences in the percentages and presence of QS genes among various strains in different studies are attributed to the mutations that can occur within these genes. The primary issue is the correlation between QS genes and antibiotic resistance, a connection that has been established in numerous studies.

CONCLUSION

There is a potential increase in drug resistance patterns in the studied region. This finding underscores the urgent need for continuous monitoring and a reassessment of current treatment strategies for *P. aeruginosa* infections. It is necessary to conduct more research to comprehend the factors contributing to the rise in drug resistance and its implications for public health and treatment protocols. Our results suggest that upcoming studies should investigate the molecular mechanisms governing the interactions among transcription factors, signaling molecules, and receptors that control quorum sensing gene regulation. Gaining insight into the environmental factors that shape the QS system holds the promise of unveiling innovative strategies to modify bacterial behavior. This knowledge could pave the way for effectively disrupting QS-mediated processes, such as the formation of biofilms, the emergence of drug resistance, and the production of virulence factors. The potential to transform our approach to these challenges is both exciting and vital.

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