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Isolation and Determination of Multidrug-resistant *Pseudomonas aeruginosa* from Clinical Samples

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A B S T R A C T

As *Pseudomonas aeruginosa* continues to acquire antibiotic resistance, it has emerged as a significant human opportunistic pathogen. It also existed as one of the most prevalent and deadly organisms that cause ventilator-associated pneumonia in incubated patients, with directly attributable mortality reaching 38%. Multidrug-resistant *P. aeruginosa* infections are becoming increasingly common, and conventional cystic fibrosis antimicrobial therapies are inadequate to eliminate *P. aeruginosa* infections. As a result, different strategies for combating *P. aeruginosa* have gained a lot of attention. Thus, in this current work, *P. aeruginosa* isolates from clinical samples were characterized by using standard methods. Out of 52 isolated strains, 15 Multidrug-resistant strains were screened by assessing their antibiogram pattern. *P. aeruginosa* employs a cell-cell signaling system referred to as quorum sensing (QS) to control the expression of several virulent genes. As *P. aeruginosa* develops antibiotic resistance, the quorum sensing enhanced transcriptional regulator LasR is considered a viable alternative target. Thus, the present study was carried out to explore the prevalence of MDR strains of *P. aeruginosa.*

***Key words*:** *Pseudomonas aeruginosa,* Multidrug resistance, Antibiotics, Quorum sensing, Antibiogram

Pseudomonas aeruginosa is a common human pathogen that accounts for 12 percent of hospital-acquired urinary tract infections, ten percent of bloodstream infections, and eight percent of surgical site infections [1]. It's also one of the most prevalent and deadly diseases in incubated patients, with directly related fatalities reaching 38% [2]. Patients with cystic fibrosis are particularly vulnerable to *Pseudomonas aeruginosa* infection, which is responsible for a high rate of sickness and mortality in this population. *Pseudomonas aeruginosa* capacity to infect a prospective host is primarily dependent on its ability to manage several of its virulence characteristics through a process that monitors cell density and allows bacteria to communicate [3].

Multidrug-resistant (MDR) *Pseudomonas aeruginosa* infections are becoming increasingly common and conventional cystic fibrosis antimicrobial therapies are inadequate to eliminate *P. aeruginosa* infections. As a result, different strategies for combating *P. aeruginosa* have a lot of attention [4]. Therapeutics that target and inhibit QS in *P. aeruginosa* might reduce the bacterium's pathogenicity and hence aid the human immune response in clearing the infection. LasR and RhlR, two QS networks, are considered good targets for future treatments [5]. The LasR transcriptional regulator and the LasI synthase protein make up the Las system. The AHL signal molecule N-(3-oxododecanoyl)-l-homoserine lactone requires LasI to be produced (3O-C12-HSL) [6]. To become an active transcription factor, LasR requires 3O-C12-HSL. LasR produces multimers in the presence of 3O-C12-HSL, and only the multimeric form of this protein can bind DNA and control the transcription of numerous genes, according to new research. The RhlI and RhlR proteins in *Pseudomonas aeruginosa* form a second QS system [7-8].

N-butyryl-l-homoserine lactone (C4-HSL) is produced by RhlI synthase, and RhlR is the transcriptional regulator. RhlR regulates the expression of many genes only when it is complex with C4-HSL. Both 3O-C12-HSL and C4-HSL have been found to readily migrate out of bacterial cells, however, 3O-C12-HSL diffusion is slower than C4-HSL diffusion. In addition to LasR and RhlR, QscR is an orphan LasR-RhlR homolog for which no comparable acyl-HSL synthase gene exists [9-10]. A qscR mutant is extremely infectious. QscR's effect on the expression of a few genes regulated by the LasR-I and RhlR-I systems has been investigated. Genes in the phenazine synthesis operons phz1 and phz2; hcnAB, the hydrogen cyanide synthesis operon; lasB, which codes for elastase; rhlI; and lasI are all prematurely activated in a qscR mutant. The mechanism by which QscR suppresses these genes

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transiently is unknown. QscR can form heterodimers with LasR and RhlR at low acyl-HSL concentrations. LasR and RhlR may be inactivated as a result of this [11-12].

The rise and spread of multidrug-resistant bacteria have heightened public health concerns, resulting in an unsettling scenario. In light of available therapeutic options, treating MDR strains appears to have severe limits, necessitates the exploration of novel targets, and the development of novel antimicrobials [13-17]. Many gram-negative bacterial pathogens are known to interact with one another via tiny chemical signal molecules that adjust the expression of numerous genes and coordinate virulence factors that lead to disease in the host [18]. Quorum sensing, a chemical communication system among bacteria, is a new and novel prospective therapeutic target for inhibiting the virulence system as well as the expression of several genes that enhance pathogen-host interactions. It's also plausible that QscR traps acyl-HSL signals, delaying the expression of genes regulated by LasR and RhlR [19]. We concentrated on QscR to gain a better understanding of its involvement in *P. aeruginosa* gene regulation. Given the foregoing, we hypothesize that QscR can directly impact certain genes in response to the 3OC12-HSL signal created by LasI. *P. aeruginosa* was isolated from clinical samples in this investigation, and multidrug-resistant bacteria were tested using an antibiogram pattern.

MATERIALS AND METHODS

Collection and processing of samples

Pus samples were collected from medical laboratories and transferred to the lab [20]. The collected samples were streaked on the nutrient agar plates, which were then incubated at 37 degrees Celsius for 24 hours. Gram's staining was utilized to identify the alleged single colonies, which were then subcultured in Mac Conkey agar [21-23]. *P. aeruginosa* isolates were transferred to a 1 percent nutritional agar slant and kept in the refrigerator at 4°C for further use.

Morphological and biochemical identification

Bergey's manual of determinative bacteriology was used to identify the sub cultured isolates in the selective and differential medium [24], morphological characterization, viz., Gram staining, motility, catalase, and oxidase. Biochemical tests including glucose, lactose, maltose, sucrose, and mannitol fermentation test, Indole production test, Triple Sugar Iron agar test, methyl red test, Voges-Proskauer test, citrate utilization test, urease test, and Nitrate reduction test were used to confirm the identification [25].

Antibiotic sensitivity

Antibiotic sensitivity testing (AST) is performed to assess the susceptibility of *P. aeruginosa* isolates to a variety of prospective treatment drugs. The disc diffusion or Kirby-Bauer test method was used to conduct the experiment [26]. To assess the Antibiotics sensitivity pattern, commercially available antibiotic discs (Himedia) were employed. Around 4-5 bacterial colonies were chosen and injected into the Nutrient broth, which was then incubated at 35°C for 2-5 hours and adjusted to the 0.5 McFarland turbidity standard to yield 1X108CFU/mL as the total count. While sterilized Muller Hinton agar was prepared and dispensed into sterile Petridishes and was allowed to solidify. The plates were infected by swabbing after being dried in an incubator for 30 minutes to eliminate excess moisture from the surface.

The sensitivity of each isolate to antimicrobial drugs such as Ampicillin (10µg), Chloramphenicol (30µg), Streptomycin (10µg), Tetracycline (30 µg), Cefuroxime (30µg), Ceftriaxone (30 µg), Ofloxacin (10µg) and Ciprofloxacin (10µg) was determined using the disc diffusion technique. Under sterile circumstances, the antibiotic discs were carefully deposited on the plates and left to stand for 30 minutes (pre-diffusion period) before being incubated at 37°C for 24 hours. At the end of the incubation time, the diameter of the zones of inhibition was measured using a zone measurement ruler (Hi-Media) without opening the lid [27].

Identification of multi-drug resistant strains

By measuring the zone of inhibition in line with the CLSI standard chart for Enterobacteriaceae, the multidrug-resistant *Pseudomonas aeruginosa* was detected [28]. MDR *Pseudomonas aeruginosa* isolates were defined as those that were resistant to three or more drugs [29-30].

RESULTS AND DISCUSSION

Pseudomonas aeruginosa is an important opportunistic human pathogen that infects immune-compromised individuals and people with cystic fibrosis. The increase in multidrug-resistant strains of *Pseudomonas aeruginosa* attributes to the significant increase in severity of the disease and also difficulty in treating the infection that probably leads to death [31]. Thus, the prevalence study and inhibition of multidrug resistance of *P. aeruginosa* by inhibiting the QS enhanced transcriptional regulator is necessary to control *P. aeruginosa* infection in the public health sector.

Collection, processing of samples, and identification of Pseudomonas aeruginosa

A total of 189 samples were collected from medical laboratories. Based on the growth characteristics of MacConkey Agar enrichment media, 116 samples (61.3%) were observed for turbid growth. Based on the growth characteristics and biochemical tests, a total of 52 samples (45.21%) from 116 samples, were identified as *Pseudomonas aeruginosa* (Table 1).

Table 1 Biochemical tests for the identification of *Pseudomonas aeruginosa*

Name of the test	Observations
Gram's staining	G-ve rod
Motility test	Positive
Catalase test	Positive
Oxidase test	Positive
Glucose fermentation	Negative
Sucrose fermentation	Negative
Lactose fermentation	Negative
Maltose fermentation	Negative
Mannitol fermentation	Positive
Indole production test	Negative
Methyl red test	Negative
Voges-Proskauer test	Negative
Citrate utilization test	Positive
Urea hydrolysis test	Negative
Nitrate reduction test	Positive

In 2007, Gad *et al*. [32], reported 19.2% of *P. aeruginosa* from 445 clinical samples from Minia, Egypt. Similarly, 1548 clinical samples, 495 *P. aeruginosa* isolates were reported by Shenoy et al. [33] from Mangalore. In 2012 Ramana *et al*. [34] isolated 290 *P. aeruginosa* from healthcare-associated infections at a tertiary care hospital from Andhra Pradesh. The studies of Swetha *et al.* [35] reported 39 *P. aeruginosa* isolates

from human samples collected at Uttar Pradesh, India. Rakesh *et al*. [36] reported a significant number of *P. aeruginosa* isolates in Bangalore. The studies of Wattal *et al.* [37] reported *P. aeruginosa* among 85 patients in New Delhi, India.

Antibiotic susceptibility and multi-drug resistance determination of Pseudomonas aeruginosa

Antimicrobial susceptibility testing was performed for all 52 *Pseudomonas aeruginosa* isolates with eight different classes of antibiotics including Ampicillin (10µg), Chloramphenicol (30µg), Streptomycin (10µg), Tetracycline (30 µg), Cefuroxime (30µg), Ceftriaxone (30 µg), Ofloxacin (10µg) and Ciprofloxacin (10µg). The zone of inhibition against each antibiotic in accordance with NCCL standard chart for enterobacteriaceae was considered to determine the isolate as resistant or intermediate or sensitive towards each antibiotics. The zone of inhibition diameter of ≤ 13mm for Ampicillin, ≤ 12mm for Chloramphenicol, ≤ 11mm for Streptomycin, ≤ 11 mm for Tetracycline, ≤ 14mm for Cefuroxime, ≤ 13mm for Ceftriaxome, ≤ 12mm for Oflaxacin and ≤ 15mm for Ciprofloxacin, then the isolate was considered to be resistant (Table 2).

Table 2 NCCL Guidelines to classify *Pseudomonas aeruginosa* as resistant/ intermediate / sensitive to antibiotics

Type	Antibiotics	Zone of inhibition in mm		
		Resistant	Intermediate	Sensitive
Pencillin	Ampicillin	≤ 13	14 – 16	≥ 17
Phenicols	Chlorempheniсol	≤ 12	13 – 17	≥ 18
Aminoglucosides	Streptomycin	≤ 11	12 – 14	≥ 15
Tetracyclins	Tetracycline	≤ 11	12 – 14	≥ 15
CEPHEMS	Cefuroxime	≤ 14	15 – 22	≥ 23
	Ceftriaxone	≤ 13	14 – 20	≥ 21
Fluoroquinolones	Oflaxacin	≤ 12	13 – 15	≥ 16
	Ciprofloxacin	≤ 15	16 – 20	≥ 21

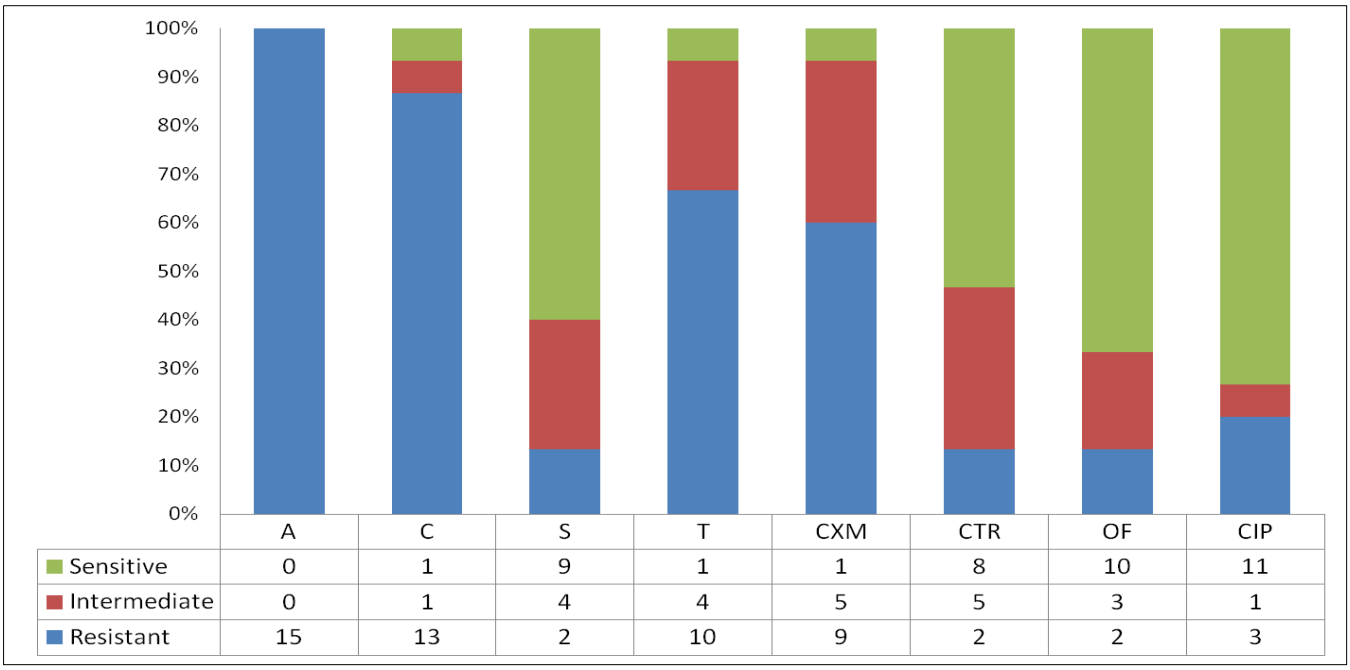
In the present study, isolates that showed resistance towards each antibiotic were recorded. Higher resistance was observed against Ampicillin (87.3%) and the lower resistance against Cefuroxime (3.15%). The number of isolates that were resistant, intermediate, and sensitive towards each antibiotic was given in (Table 3, Fig 1). The isolates that exhibited resistance against three or more antibiotics were considered multidrug-resistant strains. Among these MDR isolates, the resistance of 100% was observed against Ampicillin (Fig 2).

The emergence of drug-resistant isolates is a greater public health concern. In this study, the occurrence of 15 isolates as multidrug-resistant indicates the high level of *Pseudomonas aeruginosa*. The widespread usage of antibiotics has resulted in a surge in multidrug-resistant bacterial strains during the last few decades. Among the determined MDR isolates of the present study, the majority of strains exhibited resistance towards Ampicillin, Chloramphenicol, and tetracycline. Thus, the antibiotics sensitivity pattern of the isolates was studied and the MDR strain of *Pseudomonas aeruginosa* was identified for further studies.

Table 3 Antibiogram pattern of 15 MDR *P. aeruginosa* isolates against eight antibiotics, with zone of inhibition in mm

Isolate	Antibiotics - Zone of inhibition (mm)								
	Ampicillin (13)	Chloramphenicol (12)	Streptomycin (11)	Tetracycline (11)	Cefuroxime (14)	Ceftriaxone (13)	Ofloxacin (12)	Ciprofloxacin (15)	No. of resistance
Pae 1	10 (R)	10 (R)	12 (I)	10 (R)	25 (S)	14 (I)	14 (I)	13 (R)	4
Pae 3	11 (R)	11 (R)	11 (R)	14 (I)	14 (R)	20 (I)	19 (S)	24 (S)	4
Pae 4	10 (R)	10 (R)	16 (S)	10 (R)	12 (R)	22 (S)	18 (S)	22 (S)	4
Pae 5	10 (R)	11 (R)	17 (S)	11 (R)	14 (R)	13 (R)	19 (S)	24 (S)	5
Pae 7	10 (R)	12 (R)	15 (S)	11 (R)	20 (I)	30 (S)	18 (S)	26 (S)	3
Pae 11	12 (R)	12 (R)	18 (S)	14 (I)	13 (R)	18 (I)	19 (S)	25 (S)	3
Pae 13	11 (R)	12 (R)	18 (S)	11 (R)	15 (I)	25 (S)	16 (S)	21 (S)	3
Pae 19	12 (R)	10 (R)	14 (I)	11 (R)	19 (I)	22 (S)	21 (S)	20 (I)	3
Pae 22	10 (R)	11 (R)	12 (I)	14 (I)	14 (R)	22 (S)	17 (S)	24 (S)	3
Pae 27	11 (R)	15 (I)	11 (R)	13 (I)	14 (R)	23 (S)	10 (R)	15 (R)	5
Pae 28	11 (R)	22 (S)	19 (S)	11 (R)	21 (I)	22 (S)	12 (R)	15 (R)	4
Pae 34	10 (R)	12 (R)	18 (S)	15 (S)	13 (R)	12 (R)	21 (S)	24 (S)	4
Pae 36	11 (R)	11 (R)	18 (S)	11 (R)	15 (I)	14 (I)	14(I)	21 (S)	3
Pae 42	10 (R)	12 (R)	18 (S)	11 (R)	13 (R)	23 (S)	16 (S)	29 (S)	4
Pae 46	11 (R)	11 (R)	13 (I)	11 (R)	13 (R)	17 (I)	15(I)	27 (S)	4

R: Resistance; I: Intermediate; S: Sensitive



	A	C	S	T	CXM	CTR	OF	CIP
Sensitive	0	1	9	1	1	8	10	11
Intermediate	0	1	4	4	5	5	3	1
Resistant	15	13	2	10	9	2	2	3

Fig 1 The sensitivity pattern of 15 *P. aeruginosa* isolates against eight antibiotics

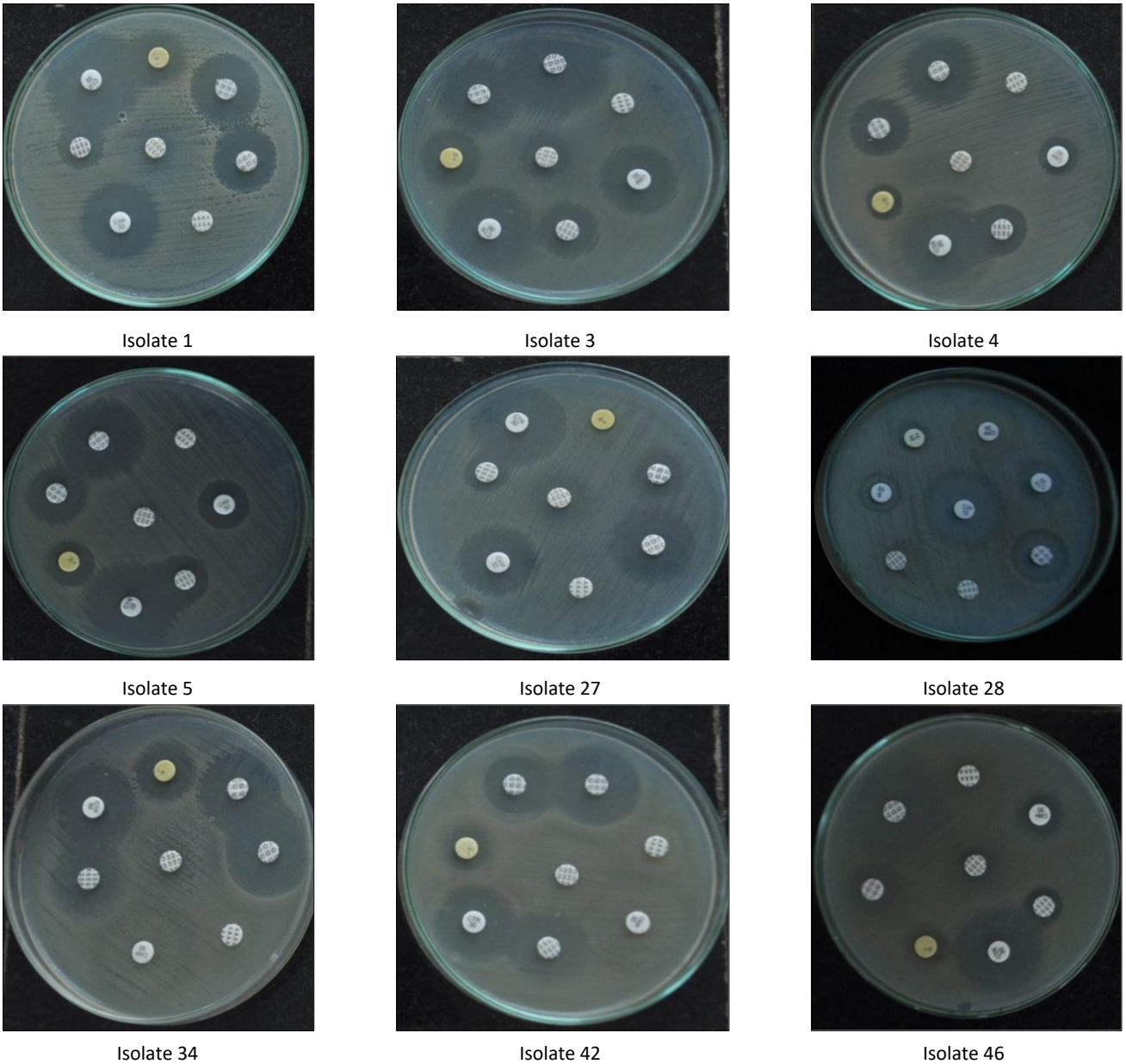


Fig 2 Antibiogram pattern of nine *P. aeruginosa* isolates that showed resistance to four or more antibiotics

CONCLUSION

As it continues to acquire antibiotic resistance, *Pseudomonas aeruginosa* has emerged as a significant human opportunistic pathogen. *Pseudomonas aeruginosa* isolates were identified using pus samples obtained in the Salem region, as well as growth characteristics and biochemical tests. These isolates' antibiotic sensitivity patterns were investigated, and the MDR strain of *P. aeruginosa* was discovered. *P. aeruginosa* surveillance is investigated by extracting 52 strains from samples and characterizing them using conventional morphological, physiological, and biochemical techniques. The antibiogram pattern indicated 15 *P. aeruginosa* MDR strains. The bulk of the strains were resistant to ampicillin, chloramphenicol, and tetracycline.

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