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Original Article

Microbiology Section

Prevalence and Antibiogram of *Pseudomonas Aeruginosa* Isolated from Various Clinical Specimens: A Cross-sectional Study from a Rural Teaching Tertiary Care Hospital in Southern Haryana, India

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ABSTRACT

Introduction: *Pseudomonas aeruginosa* is an opportunistic pathogen because of its adaptive nature and a well-known cause of both community and hospital acquired infections. Varied prevalence and antimicrobial susceptibility pattern has been observed due to several reasons. Multidrug Resistant (MDR) *P.aeruginosa* is a global concern.

Aim: To find out the prevalence and antimicrobial susceptibility pattern of *P.aeruginosa* isolates obtained from various clinical samples from a rural teaching tertiary care hospital in Nalhar (Nuh), Haryana, India.

Materials and Methods: This was a one year cross-sectional study done in Department of Microbiology, SHKM GMC, Nalhar, Haryana, India from February 2019 to January 2020. On the total 6306 samples were collected and processed. The isolates were processed and identified by standard microbiological techniques. Antimicrobial Susceptibility Testing (AST) was done by Kirby-Bauer disc diffusion method as per Clinical and Laboratory Standards Institute (CLSI) guidelines. Metallo-Beta-Lactamase (MBL) detection in *Pseudomonas aeruginosa* was done by epsilometer-test. Bivariate analysis was done using Chi-square statistics. Statistically significant association was set with p-value <0.05.

Results: Total of 170 *P.aeruginosa* isolates were received,among which 100 (58.82%) isolates were from male patients and remaining 70 (41.18%) from female patients. The age of patients infected with *P.aeruginosa* ranges from ≤20 years to ≤60 years with the mean age 33.6 years. *P.aeruginosa* was isolated in 170 (2.7%) out of total 6306 samples received in the bacteriology laboratory during the study period. It was significantly observed among indoor patients, elderly (>60 years), and had undergone any invasive procedure. Antibiotic sensitivity patterns of *P.aeruginosa* isolates were colistin and polymixin B (98.82%), imipenem, meropenem and piperacillin-tazobactum (70%), amikacin (64.12%), gentamycin (48.82%), ciprofloxacin (54.12%) cefepime (54.71%), ceftazidime (38.24%). The most common specimen source for both MBL P.aeruginosa (PA) and non MBL PA was pus (75.61% and 53.49%). MDR was shown by (42.35%) isolates. All the MBL producers (100%) were MDR in comparison to of non MBL producers (24%).

Conclusion: This study would help to formulate the antibiotic guidelines and guide the physician in patient management which in turn has a great impact in preventing the mortality and morbidity associated with *Pseudomonas aeruginosa* infections.

Keywords: Metallo-beta lactamase, Multidrug Resistant, Gram negative bacteria, Hospital acquired infections

INTRODUCTION

Pseudomonas aeruginosa is a non fermentative gram negative bacteria. It is a well-known cause of both community and hospital acquired infections [1]. It has broad ecological adaptability, ubiquitous in nature and ability to acquire and disseminate drug resistance. It remains viable on inanimate and animate objects. It also survives in antiseptic solutions. It can survive in wide range of temperature. The adaptive nature makes the bacilli an excellent opportunistic pathogen [2].

The prevalence rate of *P.aeruginosa* infection varies from one region to other. In a multicentric study conducted by Ling JM and Cheng AF, it was 3%-16%. In India, prevalence rate of *P.aeruginosa* infection varies from 10.5%-30% [3,4]. Nosocomial infections caused by *P.aeruginosa* are very common, worldwide it ranges from 11-13.8% but in Intensive Care Unit (ICU) patients the rate is higher that is 13.2-22.6% [5]. In India, it accounts for 9-15% of all nosocomial infections and 15-31% in critical care settings [6].

Drug resistance in *Pseudomonas aeruginosa* can be intrinsic as well as extrinsic. Drug resistance to different classes of antibiotics such as beta-lactams, carbapenems, aminoglycosides, fluoroquinilones and polymixins has been reported [7]. Phenotypes of MDR, Extensive Drug Resistant (XDR) and Pan Drug Resistant (PDR) are frequently encountered in *P.aeruginosa* causing nosocomial infections are associated with higher rates of mortality, morbidity, and overall healthcare costs [7].

Therefore, area-wise studies on antimicrobial susceptibility profiles are essential to guide policy on the appropriate use of antibiotics. The present study was conducted to find out the prevalence and antimicrobial susceptibility pattern of *P.aeruginosa* isolates obtained from various clinical samples in tertiary care hospital from Nalhar, Haryana, India.

MATERIALS AND METHODS

This was a cross-sectional study done in Department of Microbiology, SHKM GMC, Nalhar, Haryana, India from February, 2019 to January, 2020. Ethical approval was taken from Institutional Ethics Committee (EC/0A-34/2018).

Sample size calculation: $n=Z^2\ p\ q/e^2$

Prevalence (p) =12 [3-6]

q=100-p=88, e=5%, z=1.96 n=168.9 (170)

Inclusion criteria: Total of 170 isolates of *Pseudomonas aeuruginosa* from clinical samples of patients attending different outdoor departments and admitted in this hospital was included.

Exclusion criteria: Repeat isolates from the same patient were excluded.

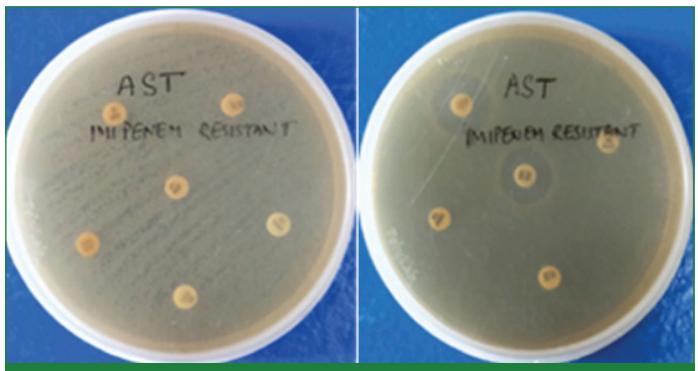
Procedure

P.aeruginosa isolates were identified by colony characteristics, fruity smell, pigment production and biochemical tests [Table/Fig-1]. AST of these isolates to various classes of antibiotics was done by Kirby-Bauer disc diffusion method on Mueller-Hinton agar (MHA) plate as per CLSI guidelines [8].

[Table/Fig-1]: Growth on Blood agar-metallic sheen, MacConkey agar-Non lactose fermenting colonies, Nutrient agar-Bluish green pigmented colonies.

In this method, the inoculum was prepared by touching the tops of each of 3-5 identical colonies from the primary culture plate of the organism to be tested, with the help of sterile loop and transferred into nutrient broth. It was incubated at 37ºC for 2-3 hours. The turbidity of the broth was adjusted to 0.5 McFarland's standard using normal saline to give almost confluent growth. Within 15 minutes of preparation of the suspension, a sterile cotton-wool swab was dipped into the suspension and the surplus was removed by rotating the swab against the side of the test tube. With this swab, the MHA plate was inoculated by even streaking of the swab over the entire surface of the plate in three directions so as to obtain a lawn culture. The plates were allowed to dry for five minutes [9,10].

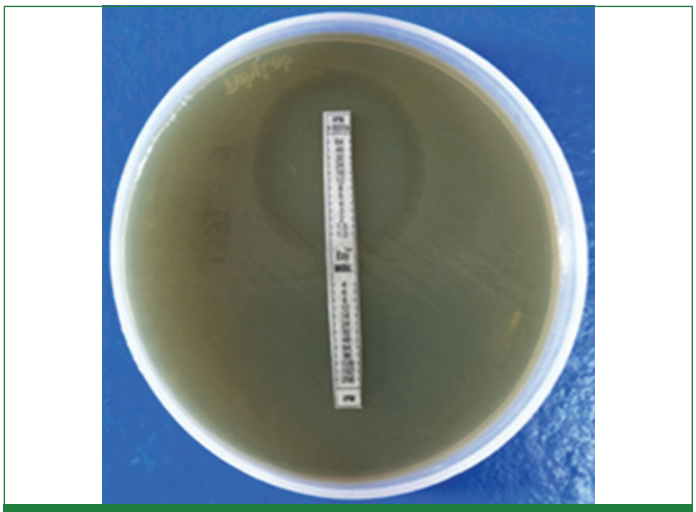
The commercially available antibiotic discs (HI-MEDIA) were taken out from the refrigerator and allowed to attain room temperature. With the help of sterile forceps, these antibiotic discs were placed on the inoculated plates at a distance not less than 24mm apart, center to center and were gently pressed down to ensure even contact with the medium. The American Type Culture Collection (ATCC) *P.aeruginosa* 27853 strain was used for quality control. The drugs used for antimicrobial susceptibility were piperacillin-tazobactum (100/10 µg) ceftazidime (30 µg), cefepime (30 µg), gentamycin (10 µg), amikacin (30 µg), ciprofloxacin (5 µg), aztreonam (30 µg), imipenem (10 µg), meropenem (10 µg), colistin (10 µg), polymixin B (300 U) [Table/Fig-2].



[Table/Fig-2]: AST of the *P.aeruginosa* isolate.
Resistant=PIT, CAZ, CPM, GEN, AK, CIP, AT, IPM, MRP. Sensitive=CL, PB

The plates were incubated overnight at 37°C. The diameter of zone of inhibition was recorded and interpreted as sensitive, intermediate and resistant as per CLSI Guidelines except for colistin and polymixin B [8,11].

MBL production in *P.aeruginosa* was detected by epsilometer test after doing lawn culture on MHA agar and overnight incubation at 37°C. Minimum Inhibitory Concentration (MIC) ratio of imipenem: imipenem+Ethylenediaminetetraacetic acid (EDTA) ≥8 was considered positive for MBL production [2]. The presence of phantom zone or deformation of imipenem eclipse or if zone is observed on the side coated with imipenem+EDTA and no zone is observed on the opposite side coated with imipenem was also considered positive [Table/Fig-3].



[Table/Fig-3]: Positive for MBL production by E-test.

STATISTICAL ANALYSIS

The data was entered in spread sheet and analysed using Epi-info (version 7.2.3.1) Centers for Disease Control (CDC) Atlanta, Georgia. Univariate analysis were summarised using frequencies and percentages. Bivariate analysis was done using Chi-square statistics. Statistically significant association was set with p-value <0.05.

RESULTS

Total of 170 *P.aeruginosa* isolates were received, among which 100 (58.82%) isolates were from male patients and remaining 70 (41.18%) from female patients. The age of patients infected with *P.aeruginosa* ranges from ≤20 years to ≤60 years with the mean age 33.6 years. A total of 6306 samples received in the laboratory in the bacteriology laboratory during the study period. *P.aeruginosa* was isolated in 170 (2.7%) out of total 6306 samples. The samples cultured were pus, blood, urine sputum, catheter tip, throat swab, body fluids, respiratory secretions, CSF and others. Out of 170 isolated *P.aeruginosa*, the most common samples which gave positive growth were pus 100 (58.82%) followed by blood 33 (19.41%), urine 19 (11.18%), as shown in [Table/Fig-4].

Sample	Total (n)	Percentage (%)
Pus	100	58.82%
Blood	33	19.41%
Urine	19	11.18%
Sputum	9	5.29%
Catheter tip	3	1.76%
Throat swab	2	1.18
Body fluids	2	1.18%
Tracheal aspirate	1	0.59%
Cerebrospinal fluid	1	0.59%
Total	170	100%

[Table/Fig-4]: Distribution of P.aeruginosa isolates from various clinical specimens.

The organism was most commonly isolated from the age group ≤20 (n=71, 41.76%), followed by 21-40 age group (n=38, 22.35%), 41-60 age group (n=37, 21.76%) and >60 years age group (n=24, 14.12%), respectively. *P.aeruginosa* infection was significantly observed among indoor patients, elderly (>60 years), and patients who had undergone any invasive procedure (p-value <0.05). There was no significant association of gender with infection (p-value >0.05) [Table/Fig-5].

Demographic profiles	Total no. of patients (n=6306)	No. of *P.aeruginosa* isolated (n=170)	Chi-square with Yates correction	p-value
Age (years)				
>60	1505	24	8.190	0.0042
≤60	4801	146		
Gender				
Male	3527	100	0.4509	0.501
Female	2779	70		
Attended hospital				
Indoor patient	3450	116	11.6984	0.00062
Outdoor patient	2856	54		
Invasive procedure				
Conducted (catheterisation, intubation, ventilation)	756	57	68.019	0.00001
No invasive procedure	5550	113		

[Table/Fig-5]: Demographic profiles for *P.aeruginosa* infection among patients.

The sensitivity patterns of *P.aeruginosa* isolates in current study are shown in [Table/Fig-6]. Almost all the isolates were sensitive to colistin and polymixin B (98.82%). Carbapenems (meropenem and imipenem) and β-lactam/β-lactam inhibitor (piperacillin-tazobactum) were sensitive in 70% of isolates. The *Pseudomonas aeruginosa* isolates were least sensitive to ceftazidime and gentamycin.

Antibiotics	Sensitive n (%)	Resistant n (%)
Piperacillin -Tazobactum (PIT)	119 (70.00%)	51 (30%)
Ceftazidime (CAZ)	65 (38.24%)	105 (61.76%)
Cefepime (CPM)	93 (54.71%)	77 (45.29%)
Gentamycin (GEN)	83 (48.82%)	87 (51.18%)
Amikacin (AK)	109 (64.12%)	61 (35.88%)
Ciprofloxacin (CIP)	92 (54.12%)	78 ((45.88%)
Aztreonam (AT)	105 (61.76%)	65 (38.24%)
Meropenam (MRP)	119 (70.00%)	51 (30.00%)
Imipenam (IPM)	119 (70.00%)	51 (30.00%)
Colistin (C)	168 (98.82%)	2 (1.18%)
Polymixin B (PB)	168 (98.82%)	2 (1.18%)

[Table/Fig-6]: Antibiotic susceptibility pattern of P.aeruginosa isolates from different specimens.

The most common specimen source for both MBL producing and non MBL-PA was pus (75.61% and 53.49%, respectively) [Table/Fig-7]. MBL producing *P.aeruginosa* were 41 (24.11%) and 129 (75.88%) were non MBL producers. Among MBL positive isolates, 34 (82.92%) were from indoor patient and 7 (17.07%) were from outdoor patient. Non MBL producing *P.aeruginosa* were 82 (63.56%) from indoor patients and 47 (36.43%) were from outpatients, respectively. MDR was shown by 72/170 (42.35%) isolates. All the MBL producers, 41/41(100%) were MDR in comparison to 31/129(24%) of non MBL producers. Pan drug resistance was seen in two MBL-PA.

Specimen	MBL-PA (n,%)	Non MBL-PA (n,%)	Total (n,%)
Pus	31 (75.61%)	69 (53.49%)	100 (58.82%)
Blood	2 (4.87%)	31 (24.03%)	33 (19.41%)
Urine	1 (2.43%)	18 (13.95%)	19 (11.7%)
Sputum	3 (7.31%)	6 (4.65%)	9 (5.29%)
Catheter tip	2(4.87%)	1 (0.77%)	3(1.76%)
Throat swab	1 (2.43%)	1(0.77%)	2 (1.17%)
Body fluids	-	2 (1.55 %)	2 (1.17%)
Tracheal aspirate	1 (2.43%)	-	1 (0.588%)
Cerebrospinal fluid	-	1 (0.77 %)	1 (0.588%)
Total	41 (24.11%)	129 (75.89%)	170 (100%)

[Table/Fig-7]: Distribution of Metallo-B-Lactamase and Non metallo-B-Lactamase producing *P.aeruginosa* isolates among various specimens.

DISCUSSION

P.aeruginosa is a common opportunistic pathogen causing both community and hospital-acquired infections across the globe. Identification and selection of appropriate antibiotic to initiate therapy is essential to optimising the clinical outcome.

The present study includes 170 clinically significant, consecutive, non duplicate *P.aeruginosa* isolates. The overall isolation rate among all samples processed was 2.7%. Various previous studies have documented prevalence rate of *P.aeruginosa* isolates between 2.76%-16.9% [Table/Fig-8] [3,12-15].

Authors, References	Place of study	Year of publication	*P.aeruginosa* prevalence
Senthamarai S et al., [3]	Kanchipuram, TN	2014	2.76%
Harshada V et al., [12]	Goa	2019	3.1%
Tadavi J et al., [13]	Baroda, Gujarat	2015	4.15%
Pathi B et al., [14]	Bhubaneswar, Odisha	2013	8.43%
Bhargava D et al., [15]	Birgunj, Nepal	2015	16.9%
Present Study	Nalhar, Haryana	2023	2.7%

[Table/Fig-8]: Prevalence of *P.aeruginosa* in various studies [3,12-15].

In the present study, *P.aeruginosa* was most commonly isolated from pus 100 (58.82%), blood 33 (19.41%) and urine 19 (11.18%). Harshada V et al., reported 40.2% *P.aeruginosa* isolates from pus [12]. Golia S et al., has documented 55.83% *P.aeruginosa* isolates from pus or wound in his study [16]. Similarly Pathi B et al., had highest recovery rate from pus or wound swabs followed by urine [14]. Similar result in Nepal had been reported by Chander A and Shahid RM [17]. Ramana BV and Chaudhury A, has documented higher isolation rate from urinary catheters (52%) [18]. In another study from Gujarat done by Javiya VA et al., reported higher isolation rates from urine, pus and sputum which accounts to 27% each, followed by Endotracheal (ET) secretion 14%. This variation among these studies could be due to the difference in study period and inclusion criteria of the patient population [19].

The *P.aeruginosa* isolates recovered from indoor patients was 68.23% and from outdoor patients 31.8%. This shows that it is an important pathogen for nosocomial infections but can also cause community acquired infections.

Out of the 170 isolates,71 (41.76%) isolates were from the patients of age group ≤20 followed by 38 (22.35%) isolates in 21-40 age group, 37 (21.76%) isolates in 41-60 age group and 24(14.12%) in age group >60. In study done by Harshada V et al., elderly age group >61 was most commonly affected age group (31.5%). Gupta R et al., reported a higher prevalence among elderly patients of age group 61-80 (43.92%) [20]. Golia S et al., and Yadav VC et al., has reported highest prevalence (33.3%) and (41%) in the age group 41-60. According to Bennett, very young and very old patients had overall higher rates of infection than did other age groups; however, the risk of infection in different age groups differed between sites [16,21].

In the present study, among the 170 *P.aeruginosa* isolates, 100 (58.82%) isolates were from male patients and remaining 70 (41.18%) isolates were from female patients. Male female ratio was 1.42:1 (p-value=0.501) in this study which is similar to the finding of study done by Harshada V et al., that is 1.2:1 [12]. The prevalence of *P.aeruginosa* isolated from different samples, except urine was higher in males as has been documented previously by other authors [12,21].

P.aeruginosa is rarely seen as a member of human normal flora. However, colonisation rates may exceed 50% during hospitalisation, especially among impaired immunity patients. The first line of

Study	Cephalosporins		Aminoglycosides		Carbapenems		BL/BLI	FQ	Monobactam	Polymixn B
	CAZ	CEP	GEN	AMK	MER	IMP	PIT	CIP	AZT	CL
Present study	38.2%	54.7%	48.8%	64.1%	70%	70%	70%	54.1%	61.76%	98.82%
AMR Surveillance Network. ICMR 2021 [22]	62.7%	64.7%	63.5%	69.6%	67.2%	65%	69.7%	60.3%	-	96.9%
Harshada V et al., 2019 [12]	44.9%	24.9%	47.1%	48.4%	59.3%	59.3%	59.3%	48.4%	57.7%	83.4%
Pragasam AK et al., and Ellappan K et al., 2018 [23,24]	0%	-	21%	3%	0%	0%	-	5%	-	88%
Pragasam AK et al., and Dhaneria M et al., 2018 [23,25]	17%	-	33%	67%	-	-	33%		-	-
Pragasam AK et al., 2018, Agarwal R and Sankar J, 2016 [23,26]	53%	53%	3%	21%	69%	69%	-	5%	-	88%
Pragasam AK et al., 2018, Kotwal et al., 2016 [23,27]	60%	26%	23%	40%	-	72%	93%	18%	-	-
Pragasam AK et al., 2018, Gandra et al., 2016 [23,28]	32%	32%	43%	43%	53%	53%	38%	-	-	100%
Pragasam AK et al., Gupta R et al., 2016 [23,20]	32%	26%	29%	37%	-	-	37%	-	-	-
Yadav VC et al., 2016 [21]	51.5%	47.5%	53%	78%	88.5%	91.4%	93%	51%	63.7%	-
Golia S et al., 2016 [16]	91%	95.5%	82.1%	86.6%	-	100%	91%	-	-	100%
CMC year: 2014-2016 [22]	76%	76%	85%	77%	74%	73%	76%	-	68%	99%
Pragasam AK et al., 2018 Wattal C et al., 2014 [23,29]	42%	40%	27%	32%	33%	33%	45%	29%	-	100%
Senthamarai S et al., 2014, Pragasam AK et al., [3,23]	34%	-	48%	70%	-	80%	60%		-	-

[Table/Fig-9]: Antimicrobial susceptibility pattern of *P.aeruginosa* in various studies [3,12,16,20-29].
BL/BLI: β-lactam/β-lactamase inhibitor; FQ: Fluoroquinolone

defense like normal skin and mucosal barrier is breached by various invasive procedures and increases the chances of infection.

Our study revealed *P.aeruginosa* infection was significantly associated among hospitalised, elderly (>60 years) and had undergone any type of invasive procedure such as catheterisation, intubation or ventilation (p-value <0.05).

The antimicrobial sensitivity pattern of the *P.aeruginosa* isolates was studied. Colistin and polymixin B were sensitive to (98.82%) isolates. Meropenam and imipenam sensitivity to the isolates was (70%). The sensitivity of the isolates to pipercillin-tazobactum was (70%), azetreonam (61.76%), cefepime (54.71%), ceftazidime (38.24%). The isolates were more sensitive to amikacin (64.12%) than gentamycin (48.82%). Ciprofloxacin showed (54.12%) resistance in these isolates. The annual report, AMR surveillance network Indian Council of Medical Research 2021 mentioned low susceptible rates for fluoroquinolones (57.9%-60.3%) followed by cephalosprorins (62.7%-64.7%), carbapenems (65%-67.2%), aminoglycosides (63.5%-69.6%) and colistin (96.9%) [22]. [Table/Fig-9] gives a comparison of the different antimicrobial susceptibility rates reported from other studies [3,12,16,20-29].

Detection of MBL was done using epsilometer test as standard. In this study the prevalence of MBL producing *P.aeruginosa* was 41/170(24.11%). This lies in the range of 10-30% which has been reported in India from different regions. However, some studies like the one done by Behera B et al., Manoharan A et al., have reported very high incidence rates of 39.56% and 42.6% MBL positive *P.aeruginosa* [1,30]. The rate of MBL detection varies from region to region and also with time at the same place. The factors determining the MBL positive isolates are the type of sample, infection control practices, antibiotic prescription and methods to detect MBL production [1,30].

In the present study, MBL positive *P.aeruginosa* was most commonly isolated from pus samples (75.61%) similar to the findings of Khakhkhar VM et al., (66.67%) and Mitra AN et al., (62.75%), whereas Choudhary V et al., reported blood as most common source of MBL positive *P.aeruginosa* [31-33]. Sood MS and Sangeetha KT et al., has reported maximum MBL producing *P.aeruginosa* from respiratory secretions (39.13% and 35.29%), respectively. Urine was the most common source of MBL positive *P.aeruginosa* (44.12%) in a study done by Wankhede SV et al., [34-36].

In this study, MDR in MBL isolates was 100% and 24% in non MBL isolates. Choudhary V et al., and Ranjan S reported 44.4% and 55.17% [33,37]. MDR in MBL positive isolates whereas in non MBL isolates it was 5.55% and 8.62%, respectively [Table/Fig-7].

Combination treatments of (carbapenem, cefepime, or piperacillin+ tazobactam, in combination with amikacin or tobramycin), are generally recommended for suspected *Pseudomonas aeruginosa* infections. Outbreaks caused by strains resistant to multiple classes of antibiotics, including carbapenems in different parts of the world demands rigorous monitoring for such isolates.

Limitation(s)

We could not do molecular study to know the genes prevalent in our region responsible for antibiotic resistance due to lack of facilities and finance.

CONCLUSION(S)

Emergence of MDR *P.aeruginosa* especially MBL-PA is a matter of concern. Frequent monitoring, appropriate antibiotic usage and infection control practices should be implemented to avoid emergence of MDR-PA. Due to the availability of few studies in our hospital, studies like this would help to formulate the antibiotic guidelines to the physician in treatment part which in turn has a great impact in preventing the mortality and morbidity associated with *Pseudomonas aeruginosa*, infection.

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