ORIGINAL ARTICLE

Antibiogram of Pseudomonas Aeruginosa Species Isolated from Various Clinical Samples in a Tertiary Care Hospital

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Abstract:
Background: Pseudomonas aeruginosa a gram-negative microorganism commonly infects hospitalized patients, particularly with burns, respiratory diseases, orthopedics infections, immunosuppression, and catheterization. This infection could be life-threatening and difficult to treat due to resistance to multiple antimicrobials. *Objective*: The present study was done to assess the antibiotic susceptibility patterns of Ps. aeruginosa isolated from various clinical samples in our setup. *Material and Methods*: This study was conducted from Nov 2021 to Oct 2022. Standard microbiological procedures were used for identifying the isolated samples and were tested for antibiotic susceptibility using Mueller-Hinton Agar by Kirby-Bauer disk diffusion method as per Clinical and Laboratory Standards Institute guidelines 2021. *Results*: In this study, a total of 4860 samples were tested, out of which 3888 showed bacterial growth from which 490were isolates of Ps. aeruginosa. Maximum isolates of Ps. aeruginosawere found in pus/wound swabs followed by, sputum and urine. A study showed male predominance. Ps. species demonstrated resistance against monotherapy of Ceftazidime, Gentamicin while combination drugs like Piperacillin + Tazobactam and monotherapy of Amikacin, Cefepime, Norfloxacin, Colistin showed higher sensitivity to Pseudomonas infections; however, the highest sensitivity was shown by Imipenem, Meropenem and Ciprofloxacin. The rate of isolation of ESBL, AmpC, and MBL producers was found to be 72.04%, 15.92%, and 12.04% respectively. Our study showed 28 (5.17%) MDR strains of Ps. aeruginosa. *Conclusion*: From the above results we can conclude that wound infection happened to be the most common among hospital-acquired infections due to Ps. aeruginosa. Alarming resistance to commonly used antibiotics is noted in Ps. aeruginosawhich is rapidly spreading to newer antibiotics as well due to their inappropriate use, lack of awareness amongst physicians, patient non-compliance, etc. Therefore, periodic antimicrobial surveillance should be practiced in hospitals for regular monitoring of susceptibility patterns.

Key words: Pseudomonas aeruginosa, Antimicrobial Susceptibility pattern, ESBL, AmpC, MBL, Multidrug resistance

Introduction:

Pseudomonas aeruginosa is most commonly involved in opportunistic infections mostly in the nosocomial setting[1,2]. It is widely distributed in nature including soil, water, and various types of vegetation throughout the world[3]. It is most harmful to immune-compromised people, such as those suffering from cancer, cystic fibrosis, burns, neutropenia, and AIDS. It may cause many hospital-acquired infections like wound infections, burns, meningitis, urinary tract infections, necrotizing pneumonia, and external ear and eye infections. The bacterium can also cause fatal septicaemia and invasive infections of the blood. [4] P. aeruginosa accounts for 10 - 15% of nosocomial infections worldwide[5]. P. aeruginosa infections are commonly associated with high mortality, attributed to its intrinsic resistance to several categories' classes of antimicrobial agents and ability to acquire resistance by mutation and horizontal transfer of resistance determinants. [6] Pseudomonas aeruginosa develops resistance against almost all antibiotics by several mechanisms like multi-drug resistance efflux pumps, resistance genes, biofilm formation, aminoglycoside modifying enzymes, and mutations in different chromosomal genes. [7] Extended-spectrum beta-lactamases (ESBLs) production in P. aeruginosa has been reported previously; and shows remarkable resistance to different classes of antibiotics, including penicillin's and cephalosporins. ESBLs are newer beta-lactamases that confer resistance to some of the latest beta-lactam antibiotics, especially cephalosporins. ESBL are encoded by genes located on bacterial plasmids which also carry genes responsible for resistance to many other antimicrobials such as aminoglycosides, tetracyclines, and sulphonamides. They are derived from the earlier beta-

lactamases like the TEM enzymes, SHV, and OXA-beta-lactamases with a narrower spectrum of activity in terms of the antibiotics they degrade; and ESBLs are mostly responsible for the multidrug resistance amongst Gram-negative bacteria. [8] All ESBL-type enzymes are classified into two structural ambler classes, viz. A and D. In P. aeruginosa strains, the ESBL enzymes of both these classes are observed, primarily β- lactamases from the PER, GES, VEB, BEL, and PME family (belonging to class A) and from the OXA family (class D), named the extended-spectrum class D β- lactamases (ES-OXAs). [1,8] Additionally, in a few P. aeruginosa isolates, the presence of ESBLs similar to the Enterobacteriaceae family, such as TEM, SHV, and CTX-M-type, was described. [8] Acquired resistance mechanism includes plasmid-mediated AmpC β -lactamase, extended-spectrum β -lactamase, and Metallo β- lactamase (MBL) enzymes. [9] Currently, available drugs against P. aeruginosa include fluoroquinolones (ofloxacin, ciprofloxacin), antipseudomonal Penicillin's (ticarcillin, piperacillin), cephalosporins (ceftazidime, cefepime), aminoglycosides (amikacin, gentamicin) and carbapenems (imipenem, meropenem). Multidrug resistance P. aeruginosa (MDRPA) was defined as "acquired non-susceptibility to at least one agent in three or more antipseudomonal classes (carbapenems, fluoroquinolones, penicillin's, cephalosporins, and aminoglycosides). [10] The incidence of MDRPA was reported to range from 0.6% to 32%.[11] As the antibiotic resistance profiles of P. aeruginosa can change in years, prevalence studies must be carried out regularly. This study aimed to determine the antibiotic susceptibility of P. aeruginosa from various clinical samples and contribute to the application of appropriate empiric therapy in our hospital.

MATERIAL AND METHODS:

The study was carried out in the Department of Microbiology, Swami Ramanand Teerth Rural Government Medical College and Hospital, Ambajogai, Beed. This observational study was of 12 months, conducted from 1 Nov 2021 to 30 Oct 2022. Samples were collected from outdoor patients and indoor patients of all age groups of both genders with all aseptic precautions. Various clinical samples were included such as wound swabs/pus, sputum, urine, blood, etc. All study protocols were approved by the Institutional Review Boards and Ethical Committee. The specimens were inoculated on MacConkey agar, Blood agar, and Nutrient agar and were kept in the incubator at 37℃ for 24-48 hours. MacConkey agar showed lactose non-fermenting pale colonies, blood agar showed greyish irregular beta-hemolytic colonies and Nutrient agar showed various pigments production (pyocyanin, pyoverdine, pyomelanin, and pyorubrum). The isolates were identified by conventional bacteriological methods such as colony morphology, Gram's staining, catalase test, oxidase test, motility test, appropriate biochemical reactions like IMViC (Indole, Methyl Red, Voges-Proskauer, and Citrate) tests, urease test, Triple sugar iron (TSI) test, Oxidative- fermentation (OF) tests, and Phenyl pyruvic acid (PPA) test for the identification of Pseudomonas aeruginosa. [1,10] Pseudomonas aeruginosa strains were subjected to antibiotic susceptibility testing using Mueller-Hinton Agar by Kirby-Bauer disk diffusion method as per Clinical and Laboratory Standards Institute guidelines 2021.[11] The following antibiotics were tested: Ceftazidime (30µg), Cefepime(30µg), Piperacillin-tazobactam(100/10µg),Amikacin(30µg),Gentamicin(10 µg),Imipenem(10µg),Meropenem(10µg),Ciprofloxacin(5µg), Norfloxacin(10µg). For Colistin, Minimum inhibitory concentration (MIC) is determined by broth microdilution and Epsilometer-test.Pseudomonas aeruginosa ATCC 27853 was used as control strain. [11, 12] ESBL was detected by using a Double disc synergy test in which a difference of ≥5 mm between the zone diameters of ceftazidime and ceftazidime/clavulanate disks is seen, as per the CLSI guidelines. AmpC β–lactamase production was tested by Disk Antagonism Test. Ceftriaxone (30µg) and cefoxitin (30 µg) disks were placed 20 mm apart from center to center. Isolates showing blunting of the ceftriaxone zone of inhibition adjacent to the cefoxitin disk were labelled AmpC β-lactamase producers. MBL production was detected by Disk Potentiation Test in which Imipenem (10µg) and Imipenem-EDTA (10/50µg) were placed on the plate and the inhibition zones were compared after 16 to18 hours of incubation at 35 ºC. If the increase in inhibition zone with Imipenem - EDTA disk was ≥7 mm as compared to the Imipenem disk alone was considered

to be the MBL producer.Data was analyzed using Microsoft Excel 2010, Open EPI-Info Version 3.01.

RESULTS:

A total of 4860 samples were tested, out of which 3888 samples showed bacterial growth from which 490 were isolated and identified by standard microbiological procedures as Pseudomonas aeruginosa. Out of total 490 strains, 326 (66.53%) were from males and 164 (33.47%) from females, while 396 were from indoor and 94 from outdoor patients. [Table 1 & Table 2] Pseudomonas aeruginosa infection was seen in all age groups but the rate of isolation was seen more in the age group of 21-40 years (54.49%).[Table 3] Maximum isolates of Pseudomonas aeruginosa, 284 (57.96%) were from pus/wound swabs only, followed by sputum 96(19.59%) and urine 67(13.67%). [Table-4] Out of 490 total samples of Ps. aeruginosa, the maximum number of samples were received from the surgery department 152(31.02%), followed by medicine 98 (20%), ICU 95 (19.39%), burn ward 45 (9.18), ENT 42(8.57%), orthopedics 40 (8.16%), and pediatric 18(3.68%) respectively. [Table-5] Antimicrobial susceptibility patterns of Ps. aeruginosa isolates showed maximum resistance to Ceftazidime (33.06%), Cefepime (25.51%), Gentamicin (25.31%), Amikacin (20.21%), and maximum sensitivity was shown by Imipenem (96.33%), Meropenem (97.96%), Ciprofloxacin (94.90%) and combination drugs like Piperacillin + Tazobactam (93.88%). All isolates were sensitive to the polymyxin group – Colistin (100%). [Table-6] The rate of isolation of ESBL, AmpC, and MBL producerswas found to be 72.04%, 15.92%, and 12.04% respectively.[Table-7] Our study showed 28 (5.17%) MDR strains of Ps. aeruginosa which all are ESBL producers.

Biochemical characteristics of clinical Pseudomonas aeruginosa isolates:

Biochemical test	Result
Oxidase test	Positive*
Oxidation-Fermentation test (O/F)	O^+/F^-**
Gelatin liquefaction	Positive ***
Motility	Motile
Growth at 42°C	+
Growth on triple sugar iron (TSI) agar	K/K****

*Color change to violet within 10 seconds **Only the aerobic tube (O) turned yellow the fermentative tube (F) remains green ***Partial or total liquefaction of the inoculated tube (control tube should be solid) ****K: alkaline slant/ K: alkaline butt reaction

Table 1: Sex-wise distribution of Pseudomonas aeruginosa isolates

Sex	Total No	Percentage (%)
Male	326	66.53
Female	164	33.47
Total	**490**	**100**

Table 2: Indoor & outdoor patients' distribution of Pseudomonas aeruginosa isolates:

	No of isolated Ps. aeruginosa species	Percentage (%)
Indoor patients	396	80.82%
Outdoor patients	94	19.18%
Total	490	100

Table 3: Age-wise distribution of Pseudomonas aeruginosa isolates:

Age (in years)	No of isolated Ps. aeruginosa species	Percentage (%)
0 – 20	97	19.80
21-40	267	54.49
41- 60	71	14.49
61- 80	34	6.94
>80	21	4.29
Total	**490**	**100**

Table 4: Isolation of Ps. aeruginosa from various clinical specimens:

Name of sample	No of isolated Ps. aeruginosa species	Percentage (%)
Pus/ Wound swab	284	57.96
Sputum	96	19.59
Urine	67	13.67
Blood	43	8.78
Total	**490**	**100**

Discussion:

In the present study, 490 Pseudomonas aeruginosawere isolated from 3888 samples i.e., the rate of isolation of Pseudomonas aeruginosa was 12.60%. Being an extremely adaptable organism , it survives and

Table 5: Distribution of P. aeruginosa isolates in different wards/OPDs:

Wards/OPD	Total samples	Percentag (%)
Surgery	152	31.02
Orthopaedics	40	8.16
Burns	45	9.18
Medicine	98	20.00
ENT	42	8.57
Paediatrics	18	3.68
ICU	95	19.39
Total	**490**	**100**

Table 6: Antimicrobial susceptibility pattern of clinical isolates of Ps aeruginosa by disc diffusion method:

Antimicrobial Group	biotic	Sensitive No	(%)	Resist-ance No	(%)
Cephalosporins	Ceftazidime (CAZ)	328	66.94	162	33.06
	Cefepime (CPM)	365	74.49	125	25.51
β-lactam combination	Piperacillin-tazobactam (PIT)	460	93.88	30	6.12
Aminoglycoside	Amikacin (AK)	389	79.39	101	20.61
	Gentamicin (GEN)	366	74.69	124	25.31
Carbapenems	Imipenem (IPM)	472	96.33	18	3.67
	Meropenem (MRP)	480	97.96	10	2.04
Fluoroquinolones	Ciprofloxacin (CIP)	465	94.90	25	5.10
	Norfloxacin (NX)*	62	92.54	05	7.46
Polymyxin	Colistin (CL)** [MIC = 1.5 µg/ml]	490	100	0	0

Norfloxacin (NX)* - used only for urinary isolates. Colistin (CL)** MIC (Minimum inhibitory concentration) is determined by E-test.

Table 7:Rate of isolations among various Beta-lactamase producers:

Total no. of isolates	ESBL	AmpC	MBL
490	353 (72.04%)	78 (15.92%)	59 (12.04%)

Figure 1: Antibiotic sensitivity testing on MHA by Kirby-Bauer disk diffusion method

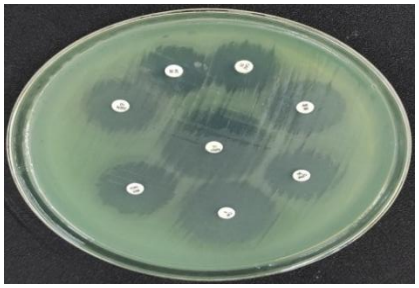
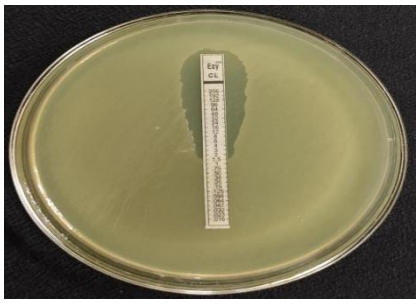


Figure 2 : MIC detection of Colistin detected by E-strip (HiMedia, Mumbai)



multiplies even with minimum nutrients, if moisture is available. The isolation rate of Ps. aeruginosa in our study is comparable with study done by Suprakash Das et al (2020) [13] and Swati Tewari et al (2020) [14] as 10.2% and 18.52% respectively. We can say that duration of stay is directlyproportional to the rate of infection as out of 490 strains of Ps. aeruginosa in our study, 396 (80.82%) were from indoor and 94 (19.18%) were isolated from outdoor patients which are in correlation with Swati Tewari et al (2020) [14]study. In the present study infections caused byPs. aeruginosa were more common in males (66.53%) compared to females (33.47%). Male to female ratio was 1.99: 1. This finding is incomparison with studies done bySwati Tewari et al (2020) [14], P. Jyothi et al (2020) [15]who showed Male to female ratio was 2.25:1and 3:1 respectively. Whereas, Suprakash Das et al (2020) [13] observed infections caused byPs.

aeruginosa were more common in females as compared to males; M: F ratio was 1: 1.4 respectively.

In our study, the maximum isolates of Ps. aeruginosawere from pus/wound swabs (57.96%), followed by sputum (19.59%). These results are in line with 55 of Kokane.V. R. et al (2017)[16]who mentioned that pussamples (36.7%) showed the highest culture positivity followed by sputum (26.06%) and urine (13.8%). In our study, the majority of the isolates weresusceptible to Imipenem, Meropenem,Ciprofloxacin,and combination drugs like Piperacillin + Tazobactam. One striking feature of this study was the restricted use of Imipenem, meropenem, and combination drugsin this hospital. On the other hand, various studies have shown varying degrees of resistance to these drugs in recent years.[13][14][15]

All isolates were sensitive to colistin. Similar findings were reported by, Suprakash Das et al (2020) [13]. In our study, the isolates were least resistant toCeftazidime (33.06%),Cefepime (25.51%), Amikacin (20.61%), and Gentamicin (25.31%). These findings are comparable with the study done by Suprakash Das et al (2020) [13], Swati Tewari et al (2020) [14], P. Jyothi et al (2020) [15] respectively. A total of 28 isolates (5.17%) were found to be MDR, most of which were from ESBL-producing isolates. Pus/wound swabs samples contribute the most of the MDR isolates which are also major contributors to ESBL-producing isolates. The prevalence of MDR isolates in other studies was found as 38.2% and 25.35% respectively. [13][17] This study shows that the clinical isolates ofPs. aeruginosa are Multidrug-resistant (resistant to more than 3 different classes of antibiotics tested) becoming resistant to commonly used antibiotics and infection control procedures need to be implemented. We suggest that there is a need to emphasize the rational use of antimicrobials and strictly adhere to the concept of "reserve drugs" to minimize the misuse of available antimicrobials. Thesolution can be planned by continuous efforts of microbiologists, clinicians, pharmacists, and the community to promote a greater understanding of this problem. gaining maximum resistance to newer antibiotics. Antimicrobial agents are losing their efficacy because of the spread of resistantorganisms due to the indiscriminate use of antibiotics, lack of awareness, patient nonobedience, and unhygienic conditions.

Conclusion:

The results of the present study clearly demonstratedthe occurrence of resistance to various antipseudomonal agents among the Ps. aeruginosaisolates. From our study, we can say that fluoroquinolones can be used for empirical therapy, and amikacin and gentamicin seem to be promising therapy for Pseudomonal infection. Hence, its use should be restricted to severe nosocomial infections to avoid the rapid emergence of resistant strains. Imipenem, Meropenem,and combination drugs like Piperacillin + Tazobactamwerethe only anti-pseudomonal drugs against which all isolates of Ps. aeruginosawere fully sensitive. To prevent the spread of resistant bacteria, it is critically important to have strict antibiotic policies while surveillance programsfor multidrug-resistant organisms onset of diabetes mellitus and it is easily applicable in a primary care setting. Indian diabetes risk score well with body mass index and HbA1c.It can also be usedfor mass screening outreach programs.

Conflict of Interest - Nil

Sources of Support- Nil

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