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Prevalance of Pseudomonas Aeruginosa Isolated From Various Clinical Sample with Antibiotic Resistance Pattern at Our Tertiary Care Hospital

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Abstract: Pseudomonas aeruginosa a gram negative, non-lactose fermenting, motile, non- sporing, pigment producing nosocomial pathogen, found in normal microbial flora of humans like skin, nasal mucosa, throat, is now emerging as a multidrug resistant organism in various clinical samples. 1. To isolate and obtain the prevalence of Pseudomonas aeruginosa from various clinical samples. 2. To assess the rate of antibiotic resistance among pseudomonas aeruginosa. The study was carried out in Department of Microbiology, Sree Balaji Medical College and Hospital, Chrompet, Chennai, for a period of four months. A total of 780 samples were processed in central laboratory, among which 127 were isolated and identified as Pseudomonas aeruginosa by direct microscopy, colony morphology, pigment production, oxidase test, motility, catalase test, triple sugar iron test, indole test, urease test, citrate utilization test. Antibiotic susceptibility was determined for Amikacin (30mcg), Gentamycin (10mcg), Tobramycin (10mcg), Ceftazidime (30mcg), Piperacillin-Tazobactam (100mcg/10mcg), Aztreonam (30mcg), Imipenem (10mcg), Cefepime (30 mcg), Levofloxacin (5 mcg), Ciprofloxacin (5mcg). Antibiotic susceptibility testing showed high level resistance to cefepime (26%) followed by ceftazidime (25.2%), gentamycin and levofloxacin (18.1%), amikacin (15%), ciprofloxacin (13.4%), aztreonam (12.6%), tobramycin (10.2%), piperacillin – Tazobactam (5.5%), and least resistance were imipenem (4.7%). Colistin is 100% sensitive to all strains of pseudomonas aeruginosa.
Keywords: Various clinical samples, pseudomonas aeruginosa, antibiotic resistance pattern, MDR.

INTRODUCTION

Pseudomonas aeruginosa a gram negative, non-fermenting, aerobic, non-spore forming bacilli that either do not use carbohydrates as a source of energy or degrade them through metabolic pathways other than fermentations. Pseudomonas a ubiquitous microorganism, that continues to be the major pathogen in patients with immune suppressions, cystic fibrosis, and malignancy. It is responsible for about 10-20 % of nosocomial infections which are seen as septicemia in burn and wound infections, cystic fibrosis in intensive care units, *et al*. [1]. Pseudomonas aeruginosa is responsible for 16% of nosocomial pneumonia, 12% of hospital acquired infections, 10% blood stream infections, 8 % surgical wound infections. The pathogenic Pseudomonas aeruginosa is most often isolated by culturing the samples of infected body parts such as ear, eye, burns, skin, and from the surfaces of medical devices and facilities [12, 13]. MDR pseudomonas is defined as bacterium which is resistant to antimicrobial agents which are included in three or more anti-pseudomonal anti-microbial classes (carbapenems, fluoroquinolones, penicillins/ cephalosporins and aminoglycosides. Multidrug resistance pseudomonas is an emerging cause of mortality and morbidity in burn patients, which cause 4-60% nosocomial infection in different part of the world [2]. These strains are frequently isolated among patients suffering from nosocomial infections, primarily those in intensive care units. The morbidity and mortality associated with pseudomonas are mainly attributed to inadequate empirical therapy and or delay in the initiation of appropriate therapy [3]. Mechanism that causes antimicrobial drug resistance and multidrug resistance in pseudomonas aeruginosa is due to the acquisition of resistance genes (those encoding β-lactamase and aminoglycosides modifying enzymes through horizontal gene transfer and mutation of chromosomal genes) are the target of the fluoroquinolones particularly ciprofloxacin's [4].

Resistance in P. aeruginosa to β-lactams is more complex and involves the production of β-lactamases and poor membrane permeability (e.g., efflux pumps and porins). Pseudomonas aeruginosa are intrinsically resistance to a panel of drug that includes, ampicillin, Amoxicillin-Clavulanic acid, ampicillin-sulbactam comibations, cefotaxime, ceftriaxone, tetracycline, trimethoprim, cotrimoxazole. Penicillin, linezolid and chloramphenicol. In this study, our aim is to establish the prevalence of pseudomonas aeruginosa and its antimicrobial susceptibility and resistance pattern that leading to MDR strains.

MATERIALS AND METHODS

The present study was conducted over a period of four months at Sree Balaji medical college and hospital, Chennai. A total of 780 samples were obtained from inpatients of various department to central laboratory out of which 127 were pseudomonas aeruginosa. pseudomonas aeruginosa isolates that are considered clinically relevant of all age group and both sex was included, from various clinical samples like urine, pus/wound swab, sputum, ET secretion, ear swabs, conjunctival swab, other body fluids etc.

Collection and processing

All clinical samples are collected in a sterile containers or tubes and were processed for the isolation of the bacterial pathogen in the central laboratory of Microbiology. The processing involved gram staining and culturing onto nutrient agar, mac conkey agar, blood agar plates by streaking. The inoculated plates were incubated at 37°C for 24hours.colonies grown on different media were further subjected to morphological and biochemical identifications. Suspected pseudomonas aeruginosa colonies were further identified according to standard microbiological procedures.

Colony characteristics

On nutrient agar: The colonies are 1-2 mm diameter, smooth, circular, large, low convex, opaque colonies with diffusible blue-green pigment.

On mac conkey agar: The colonies are 2-3 mm circular, smooth, low convex, non- lactose fermenting, pale colonies. On blood agar: They exhibit 1-2 mm diameter, opaque, circular, smooth hemolytic colonies.

From colony, gram staining showed gram negative bacilli, 1.5-3µm x 0.5 µm and hanging drop preparation were actively motile which possess a polar flagellum.

Biochemical reactions

Table-1

S.NO	TEST	CHARACTERS
1.	Catalase	Positive
2.	Oxidase	Positive
3.	Motility	Motile
4.	gram staining	Gram negative bacilli
5.	Indole production	Negative
6.	Citrate utilization	Positive
7.	Urea hydrolysis	Negative
8.	TSI agar	Alkali/alkali with no gas and hydrogen sulphide production
9.	Mannitol motility test	Mannitol non fermenting and motile

Antimicrobial susceptibility testing

A total of 127 samples of pseudomonas were obtained from various clinical samples, for which antibiotic susceptibility testing was performed by Kirby-Bauer disc diffusion method following guidelines from the CLSI (Clinical and Laboratory Standards Institute). A battery of pseudomonas aeruginosa panel drugs were used. Amikacin (30mcg), Gentamycin (10mcg), Tobramycin (10mcg), Ceftazidime (30mcg), Piperacillin-Tazobactam (100mcg/10mcg), Aztreonam (30mcg), Imipenem (10mcg), Cefepime (30mcg), Levofloxacin (5mcg), Ciprofloxacin (5mcg), colistin(10mcg).

Sex wise distribution of patients suggests that out of 127 patients 77(60.6%) were males and 50(39.4%) were females.

Table-2: Sex wise distribution

Gender	Frequency	Percent(%)
Female	50	39.4
Male	77	60.6
Total	127	100.0

Table-3: Age wise distribution

Age group		Frequency	Percent
	<20	6	4.7
	21-40	28	22.0
	41-60	50	39.4
	>61	43	33.9
	Total	127	100.0

Age wise distribution of patients suggest that highest cases were found in age group between41-60(39.4%) followed by age group above 61(33.9%), between 21-40(22%) and least patients belong to age-group 20(4.7%) respectively.

Table-4

Sample	Frequency	Percent
BLOOD	13	10.2
OTHERS	12	9.4
SPUTUM	16	12.6
TISSUE	3	2.4
URINE	29	22.8
PUS/WOUND	54	42.5
Total	127	100.0

Out of 127 pseudomonas aeruginosa isolates, the highest samples isolated were wound (42.5%), 22.8% were isolated from urine sample, 12.6% were from sputum sample and lowest isolate were from tissue sample 2.4%

Table-4: Antimicrobial Sensitivity and resistance pattern of pseudomonas aeruginosa

ANTIBIOTIC	SENSITIVE	INTERMEDIATE	RESISTANT
Amikacin	107(84.3%)	1(0.8%)	19(15%)
Aztreonem	107(84.3%)	4(3.1%)	16(12.6%)
Ciprofloxacin	108(85%)	2(1.6%)	17(13.4%)
Ceftazidime	92(72.4%)	3(2.4%)	32(25.2%)
Cefepime	91(71.7%)	3(2.4%)	33(26%)
Imipenem	116(91.3%)	5(3.9%)	6(4.7%)
Tobramycin	110(86.6%)	4(3.1%)	13(10.2)
Piperacillin- Tazobactam	116(91.3%)	4(3.1%)	7(5.5%)
Gentamycin	104(81.9%)	-	23(18.1%)
Levofloxacin	103(81.1%)	1(0.8%)	23(18.1%)
Colistin	127(100%)	-	-

RESULTS AND DISCUSSION

In our study, 127 strains of pseudomonas aeruginosa were isolated from 780 various clinical samples like pus/wound, sputum, blood, urine, tissue, eye and ear swab. The rate of isolation of Pseudomonas aeruginosa was 16.2%. Out of these, Sex wise distribution of patients suggests that 77(60.6%) were males and 50(39.4%) were females (table 1).

Age wise distribution of patients suggest that highest cases were found in age group between 41-60 (39.4%) followed by age group of above 61(33.9%), between 21-40(22%) and less infected patients belong to age-group of less than 20(4.7%) respectively (table 2).

Among the samples processed, Pus/wound (42.5%) were the predominant source of specimens of pseudomonas aeruginosa followed by urine sample (22.8%), sputum (12.6%), blood (10.2%), others (9.4%) and tissue (2.4%) (table 3).

Antibiotic susceptibility testing was done and they demonstrated highest resistance towards cefepime (26%) followed by ceftazidime (25.2%), gentamycin and levofloxacin (18.1%), amikacin (15%), ciprofloxacin (13.4%), aztreonam (12.6%), tobramycin (10.2%), piperacillin – Tazobactam (5.5%), and least resistance were imipenem (4.7%). Colistin is 100% sensitive to all strains of pseudomonas aeruginosa (table 4).

A study done at K.G. Halli, Bengaluru, india, saroj golia *et al*. [5] showed (24%) pseudomonas aeruginosa isolated, which was higher than our study (16.2%) and (33.3%) of patients infected were around 41-60 years of age group which is similar to our study (39.4%). Sex wise, male patients were more affected than female patients constituting about 60.6% in our study, similarly gagan priya *et al*.[6], reported an increased incidence in male sex for about (70%). The distribution of specimens for P. aeruginosa various in each hospital. In our study (42.5%) of P. aeruginosa were obtained from pus/wound samples compared to other samples, which was similar to other studies in India (55.83%) saroj golia *et al*. [5] and (30%) gagan priya *et al*. [6].

Pseudomonas aeruginosa an emerging pathogen for multidrug resistance has showed highest resistance pattern to cefepime (26%) in our study, which was similar to basanthi pathi *et al*. [10] (76.6%). The carbapenems, imipenem and meropenem are usually active against multidrug resistant isolates of P. aeruginosa; however, resistance to these compounds has also become a growing therapeutic problem [9]. In our study imipenem showed (4.7%) resistant to pseudomonas aeruginosa which was similar to pooja kamaria *et al*. [7] 14.8%. Among 127 isolates, 16/127 (13%) showed multidrug resistance to more than three antipseudomonal antibiotics (i.e., gentamycin, Piperacillin+Tazobactam, ceftazidime, levofloxacin). Colistin showed 100% sensitive to all isolates which is similar to saroj *et al*. [5] Combination treatments are generally recommended for suspected pseudomonal infections. It has been reported that the choice of carbapenems, cefepime or piperacillin-Tazobactam, ciprofloxacin and gentamycin combination with amikacin or tobramycin appears to provide widest potential antimicrobial activity against MDR P. aeruginosa [5].

CONCLUSION

Pseudomonas aeruginosa an emerging nosocomial pathogen, posing a multidrug resistance (MDR) world-wide. In our study we concluded with high percentage of sensitive drugs and lower percentage of drug resistant strains. Though low MDR strains were isolated, rigorous monitoring for MDR among pseudomonas aeruginosa is crucial due to lack of newer antimicrobial agents with activities against P. aeruginosa. Rapid and accurate detection of resistant antibiotic is essential for appropriate treatment in clinical practices. Prevention and control of spread in nosocomial infection should be followed to lessen the burden of multidrug resistant organism.

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