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Isolation and Characterization of Various *Pseudomonas* species from Distinct Clinical Specimens

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Abstract:Pseudomonas aeruginosa *causes infections more common in patients with neutropenia, cystic fibrosis, burns and those on ventilators. Not only the* Pseudomonas aeruginosa *but also other members of genus Pseudomonas have also been implicated in hospital-acquired infections. In the light of this it was decided to identify and characterize the various* Pseudomonas *species from distinct clinical samples along with their antibiotic sensitivity pattern. A total of 12,591 samples were received, from these samples 5,369 bacterial isolates were obtained. Out of 5,369 isolates 400 were identified as* Pseudomonas *species.Out of thispredominant numbers were that of* P. aeruginosa *(84.25%), followed by other* Pseudomonad's*(15.75%). Majority of the isolates were sensitive to imipenem and amikacin. Marked resistance was observed to commonly used drugs like ampicillin, netilmycin and gentamicin.*P. aeruginosa *and other* Pseudomonas *species are associated with human disease, therefore, they should not be discarded as contaminants or non-pathogens.*

Keywords:*Cystic fibrosis, hospital-acquired infections, imipenem,* Pseudomonas aeruginosa.

I. Introduction

The non-fermentative gram-negative bacilli are a group of aerobic, non-spore forming bacilli that either do not use carbohydrates as a source of energy or degrade them through metabolic pathways other than fermentation. Non-fermenters (NF) organisms constitute a heterogeneous group of bacteria because they do not fall into a well-defined taxonomic group, thus several different genera have been included in the group of NF. The predominant species among those strains are *Pseudomonas aeruginosa* followed by *Acinetobacter* species and *Alcaligenes* species [1].

Pseudomonas aeruginosa has one of the broadest ranges of infectivity among all pathogenic microorganisms. It is a significant cause of burn wound infections, nosocomial infections, fulminant infections of bones and joints, endocarditis, meningitis and pneumonia in cystic fibrosis patients. It is isolated from various clinical samples like pus, sputum, respiratory tract secretions, blood, urine, wound swabs, vaginal swab etc. [2].

Other members of genus Pseudomonas have also been implicated in hospital-acquired infections such as bacteremia, otitis media, conjunctivitis and septic arthritis [1]. *P. fluorescence*, *P. putida* and *P. stutzeri* have been reported to cause bacteremia, urinary tract infections, wound infection, respiratory tract infections [1]. *Burkholderia cepacia* can cause pneumonia in cystic fibrosis patients, urinary tract infections and respiratory tract infections. *Brevundimonas diminuta* can cause bacteremia. *Stenotrophomonas maltophilia* has been reported with catheter related infections, bacteremia, wound infections, pneumonia and urinary tract infections [3].

Very few laboratories in India identify different species of *Pseudomonas* as a routine because it requires the use of special culture media and biochemical tests for their identification.Hence, in the light of above, the present study was aimed to find out incidence, identification, characterization and antibiogram of *Pseudomonas* species from various clinical samples in a rural tertiary care hospital.

II. Material And Methods

This prospective study was carried out from January 2013 to December 2013 in a tertiary care hospital. During the study, 12,591 clinical samples received in the department of Microbiology for culture and sensitivity.All the samples were inoculated on MacConkey agar and Blood agar medium by standard methods and incubated overnight at 37°C. All the *Pseudomonas* species were identified by colony morphology and standard biochemical methods [4]. All the non-lactose fermenting colonies, grown on MacConkey agar, were subjected to oxidase test, Gram's staining and carbohydrate utilization (triple sugar iron). The organisms were subjected to various tests depending upon the results of Hugh and Leifson test, growth on MacConkey agar and

oxidase reaction.The organisms which were gram negative, glucose oxidizers, grown on MacConkey agar and having positive oxidase reaction were processed further using various tests as described in table 1 [3]. The organisms which were gram negative, glucose oxidizers, growing on MacConkey agar and having negative oxidase reaction were processed further using various tests as mentioned in table 2 [3].

Sensitivity to relevant antibiotics was determined by the Kirby-Bauer disk diffusion method as per the Clinical and Laboratory Standards Institute (CLSI) guidelines using the commercially available antibiotic disks from Hi-Media (Mumbai, India). NCCLS reference strain **(***Pseudomonas aeruginsa* ATCC 27853) was included as control strain.Two grades were recognized sensitive and resistant by comparing the diameters of inhibition zones with critical zone diameters [5,6].

Table 1; various tests for identification of Gram negative, glucose oxidizers, MacConkey positive and oxidase positive organisms

Organisms	Indole Test	Gas from Nitrate	Growth at 42°C	OF Maltose	Arginine Dihydrolase	Motility Test	Pigments Production	OF Mannitol	Gelatin Liquefaction
Pseudomonas aeruginosa	–	+	+	Alk	+	+	+	V	V
Pseudomonas fluorescens	–	V	–	V	+	+	+	V	+
Pseudomonas putida	–	–	–	V	+	+	–	V	–
Pseudomonas stutzeri	–	+	+	V	V	+	–	A	–
Burkholderia cepacia complex	–	+	V	A	–	+	+	A	V

Note:+ (Positive), **-**(Negative), **A**(Acid), **ALK**(Alkaline), **V** (Variable)

Table 2;various tests for identification of Gram negative, glucose oxidizers, MacConkey positive and oxidase negative organisms

Organisms	Motility Test	OF Mannitol	OF Lactose	Arginine Dehyrolase
Burkholderia cepacia complex	+	A	A	–
Stenotrophomonas maltophilia	+	ALK	ALK	–

Note:+(Positive), **-**(Negative), **A**(Acid), **ALK**(Alkaline)

III. Results

During the study period 12,591 samples were received, from these samples 5,369 bacterial isolates were obtained. Out of 5,369 isolates400 were identified as *Pseudomonas* species.Out of thispredominant numbers were that of *P. aeruginosa* 337 (84.25%), followed by other Pseudomonad's 63 (15.75%). The incidence of other Pseudomonad's had shown in table 3. Majority of the isolates of *P. aeruginosa, P. stutzeri, P. fluorescence* were isolated from pus samples. *P. putida* was mainly isolated from blood culture (Table 4). In antibiotic susceptibility testing, among 337 isolates of *P. aeruginosa*, imipenem was sensitive against majority of isolates (95.86%) followed by amikacin (73.59%) (Table 5). Other Pseudomonad's also showed good sensitivity against imipenem and amikacin. Marked resistance was observed to commonly used drugs like ampicillin, netilmycin and gentamicin. Resistance was also observed to some of the drugs like ciprofloxacin and carbenicillin (Table 6).

Table 3;incidence of other Pseudomonad's

Organisms (n=63)	No. of strains (%)
Pseudomonas stutzeri	28 (44.4%)
Pseudomonas fluorescence	17 (26.9%)
Pseudomonas putida	05 (7.9%)
Pseudomonas pickettii	05 (7.9%)
Stenotrophomonas maltophilia	07 (11.1%)
Burkholderia cepacia complex	01 (1.6%)

Table 4;incidence of *Pseudomonas* speciesin relation to clinical specimens

Organism	(n=400)	Pus	Urine	Aural Swab	Blood Culture	Sputum	EC Swab	Burn Swab	Others
Pseudomonas aeruginosa	337	186	52	46	19	17	2	5	10
Pseudomonas stutzeri	28	17	4	3	4	0	0	0	0
Pseudomonas fluorescence	17	6	3	3	1	2	0	1	1
Stenotrophomonas maltophilia	07	2	1	0	0	1	0	0	3
Pseudomonas putida	05	2	0	0	3	0	0	0	0
Burkholderia cepacia complex	01	0	1	0	0	0	0	0	0
Pseudomonas pickettii	05	3	0	0	2	0	0	0	0

Table 5; antibiotic susceptibility pattern of *Pseudomonas aeruginosa*

Drugs	Total Strains of *Pseudomonas aeruginosa* (n=337)			
	Sensitive		Resistant	
	No.	Percentage	No.	Percentage
Ampicillin	8	2.37	329	97.62
Amoxy + Clav	52	15.41	285	84.56
Gentamicin	39	11.51	298	88.42
Carbenicillin	156	46.29	181	53.70
Ciprofloxacin	209	62.01	128	37.92
Co-trimoxazole	46	13.64	291	86.35
Tetracycline	133	39.46	204	60.53
Netilmycin	176	52.22	161	47.77
Piperacillin	184	54.59	153	45.40
Nitrofurantoin	42	12.46	295	87.63
Amikacin	248	73.59	89	26.40
Imipenem	323	95.86	14	4.24

Table 6; antibiotic susceptibility pattern of Pseudomonad's

Drugs	Total Strains of Pseudomonad's (n=63)					
	P. stutzeri(n=28)	*P. fluorescence* (n=17)	*P. putida* (n=5)	*P. pickettii* (n=5)	*S.maltophilia* (n=7)	*B.c complex*(n=1)
Ampicillin	0	0	0	0	0	0
Amoxy + Clav	6(21.4%)	2(11.7%)	1(20.0%)	0	0	0
Gentamicin	0	4(23.5%)	0	0	0	0
Carbenicillin	1(3.57%)	0	0	0	1(14.2%)	0
Ciprofloxacin	5(17.8%)	2(11.7%)	0	0	1(14.2%)	1(100%)
Co-trimoxazole	7(25.0%)	3(17.6%)	0	0	5(71.4%)	0
Tetracycline	4(14.2%)	1(5.8%)	0	0	0	0
Netilmycin	0	0	0	0	0	0
Piperacillin	17(60.0%)	2(11.7%)	1(20.0%)	0	0	1(100%)
Nitrofurantoin	1(3.57%)	0	1(20.0%)	0	0	0
Amikacin	25(89.2%)	9(52.9%)	3(60.0%)	2(50.00%)	3(42.8%)	0
Imipenem	25(89.2%)	16(94.1%)	5(100%)	4(100%)	7(100%)	1(100%)

IV. Discussion

Most laboratories in India include tests that detect *P. aeruginosa*, but miss other species, as these organisms require special biochemical tests for their identification. Few studies are available from India and abroad on the identification of these organisms and their role in disease [3,7,8].

In the present study,*P. aeruginosa*337 (84.2%) emerged out as the commonest species among all the isolates. The next common isolate was *P. stutzeri* 28 (7%) followed by *P. fluorescens* 17 (4.2%), *Stenotrophomonas maltophilia* 7 (4.2%), *P. putida* 5 (1.2%),*P. pickettii*5 (1.2%) and*B. cepacia complex* 1 (0.2%)(Table 3).Pickett and Pedersen in a similar study on 486 cases reported the highest incidence of *P. aeruginosa* (75%)[9]. In another study,*P. aeruginosa* was also reported 75% [3]. The incidence of *P. aeruginosa* was slightly lesser thanthe present study (84.2%). In another study, the incidence of *P. aeruginosa* was reported 57.4% that is lower as compared to the present study (84.2%) but for *S. maltophilia* incidence was reported as (6.5%), which is higher than that of present study [7]. However, similar findings were obtained by Pickett and Pedersen [9].Veenu et al. studied 300 cases and the incidence of *P. aeruginosa* was reported 72.6% whereas in the present study it was 84.2%. The incidence of *P. fluorescence*, *P. putida* and *P. pickettii* was almost similar to present study [10].

In this study, out of 337 isolates of*P. aeruginosa*, 186were isolated from pus samples, followed by urine samples(52), aural swabs(46), blood cultures(19), sputum samples(17), burn swabs(5), endocervical swabs(2), and others (10) that included pleural fluid, endotrachial tube secretion, throat swabs, tissue and bronchial washing (Table 4). Paramasivan et al. in their study on 108 cases and Veenu et al. on 300 cases also reported the highest incidence of *P. aeruginosa* in pus samples that were 32 and 116 respectively [7,10].

In the current study, out of 28 strains of *P. stutzeri*, 17 were isolated from pus samples, 4 from blood culture, 4 from urine and 3 from aural swabs (Table 4). Yasodhara and Shyamala isolated 2 strains of *P. stutzeri* from blood culture [8]. Veenu et al. in their study isolated only 1 strain of *P. stutzeri* from blood culture [10].

In the present study, out of 17 strains of *P. fluorescence*, 6 were from pus sample, 3 each from urine culture and aural swab, 2 from sputum followed by one each from blood culture, burn swab and endotracheal tube secretions (Table 4).Yasodhara and Shyamala in their study on 100 cases isolated 11 strains of *P. fluorescence* from pus sample (6), urine and catheter tip (2), sputum sample (1), blood culture (1) and high vaginal swab (1)[8]. Veenu et al. in their study on 300 cases isolated 8 strains of *P. fluorescence* from pus sample (4), blood culture (2), urine sample (1) and sputum sample (1) [10]. Thus the incidence of *P. fluorescence* in the present study was higher as compared to the studies done by Yasodhara and Shyamala and Veenu et al.

In this study, out of 7 strains of *S. maltophilia*, 2 each were from pus sample and cerebrospinal fluids, one each from urine sample, sputum sample and endotrachial tube secretions(Table 4). Paramasivan et al. isolated 7 strains of *S. maltophilia*. Out of these,3 were from cerebrospinal fluid, 1 from respiratory tract, 1 from gastrointestinal tract and 2 from others specimens [7]. Veenu et al. isolated only one strain of *S. maltophilia* from sputum sample [14-10].

In the present study,one strain of *B. c complex* was isolated from urine sample (Table 4). Similar results were obtained by Yasodhara and Shyamala [8]. In their study, out of 100 cases, they isolated 2 strains of *B. c complex*, one from sputum sample and another from urine sample.

In the current study,*P. putida* was isolated from 5 cases.Out of five, 2 strains were from pus and 3 from blood culture specimens (Table 4). Yasodhara and Shyamala isolated seven cases of *P. putida*.Out of 7 cases, 3 were from urine sample, one each from pus, sputum and blood culture [8].In this study, 5 strains of *P. pickettii* were isolated from pus (3)and blood culture (2)sample, while Veenu et al. isolated 6 strains of *P. pickettii* from blood (4), pus (1) and stool (1) specimens[10].

In antibiogram, out of 400*Pseudomonas* species, 337 were *P. aeruginosa* and majority of these isolates were sensitive to imipenem 323 (95.86%) followed by amikacin 248 (73.59%). Marked resistance was observed to ampicillin 329 (97.62%), gentamicin 298 (88.42%) and nitrofurantoin 295 (87.53%)(Table 5). Similar results were obtained by Yasodhara and Shyamala [8]. In their study amikacin showed sensitivity against 77.19% isolates. Veenu et al. in another study found amikacin to be the most active drug against *P. aeruginosa* and gentamicin to be the least active drug [10].

In the present study, the antibiotic sensitivity pattern of other pseudomonad's showed that majority of the isolates were sensitive to imipenem followed by amikacin and piperacillin. Marked resistance was observed to commonly used drugs like ampicillin, nitrofurantoin, gentamicin and even some drugs like ciprofloxacin and netilmycin. Veenu et al. found out amikacin to be the most active drug against other pseudomonads [10].

The antibiogram pattern of the *Pseudomonas* species isolated in the present study showed multidrug resistant pattern.The marked resistance was observed to commonly used drugs like ampicillin, gentamicin, amoxy-clav co-trimoxazole and nitrofurantoin. None of the isolates were sensitive to all the drugs. Majority of the isolates were resistant to two or more drugs. On the whole drugs that have shown good *in-vitro* efficacy were imipenem and amikacin respectively.

V. Conclusion

Thus with the available evidences from current literature, there is enough reason to believe that*P. aeruginosa* and other*Pseudomonas* species are associated with human disease. Therefore, careful attempts must be made for their isolation and identification from various clinical samples and they should not be discarded as contaminants or non-pathogens.

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