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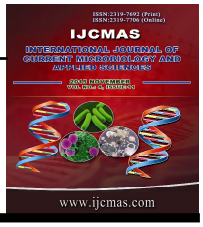

Original Research Article

The Occurrence of (MDR/MDS) *Pseudomonas aeruginosa* among Nosocomial and Community Acquired Infections in and around Coimbatore, India

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A B S T R A C T

Pseudomonas aeruginosa is a ubiquitous organism present in many diverse environmental settings, and it can be isolated from various living sources, including plants, animals, and humans. The ability of *P. aeruginosa* to survive on minimal nutritional requirements and to tolerate a variety of physical conditions has allowed this organism to persist in both community and hospital settings. Despite the wide distribution of *P. aeruginosa* in nature and the potential for community- acquired infections, serious infection with. *P. aeruginosa* are predominantly nosocomial acquired. In this study, out of 9860 clinical isolates, 499 positive strains (*P. aeruginosa*) were taken for the study. The samples taken were divided based on type of infection *i.e.* Hospital acquired and Community acquired, gender wise distribution of *P. aeruginosa*, distribution of organism in diabetic and non diabetic patients, type of infection, age wise distribution, distribution based on economic status of patients is also carried out in the study. This study reveals the prevalence of infection is more with community acquired male patients with diabetic and high in age group above 50 years of economically poor class people.

K e y w o r d s

Pseudomonas aeruginosa, Infection, Nosocomial, Community

Introduction

Pseudomonas aeruginosa is seldom a member of the normal microbial flora in humans. *P. aeruginosa* is a ubiquitous organism present in many diverse environmental settings, and it can be isolated from various living sources, including plants, animals, and humans. The ability of *P. aeruginosa* to survive on minimal nutritional requirements and to tolerate a variety of physical conditions has allowed this organism to persist in both community and nosocomial settings. Despite the wide distribution of *P. aeruginosa* in nature and the potential for community-acquired infections, serious infection with. *P. aeruginosa* are predominantly nosocomial acquired. In the nosocomial, *P. aeruginosa* can be isolated from a variety of sources, including respiratory therapy equipment, antiseptics, soap, sinks, mops,

medicines, and physiotherapy and hydrotherapy pools. Community reservoir of this organism includes swimming pools, whirlpools, hot tubs, contact lens solution, home humidifiers, soil and rhizosphere and vegetables (Pollack, 1995). *P. aeruginosa* is identified as the second leading cause of nosocomial pneumonia (14 to 16%), third most common cause of urinary tract infections (7 to 11%), fourth most frequently isolated pathogen in surgical site infections (8%), and seventh leading contributor to blood stream infections (2 to 6%). Data from more recent studies continue to show *P. aeruginosa as* the second most common cause of nosocomial pneumonia, health-care associated pneumonia, and ventilator associated pneumonia (Kollef *et al.,* 2005) and the leading cause of pneumonia among pediatric patients in the intensive care (Richards *et al.,* 1999). *P. aeruginosa* presents a serious therapeutic challenge for treatment of both community- acquired and nosocomial infections and selection of the appropriate antibiotic to initiate therapy is essential to optimizing the clinical outcome.

The present study focuses on the occurrence of *P. aeruginosa* among the patients of community and nosocomial acquired infections and discussed in terms of gender, age, clinical condition, etc. Our previous study reports the infection of *P. aeruginosa* among diabetic and non-diabetic patients and effective antibiotics (Mohan raj *et al.,* 2015).

Materials and Methods

Collection of Specimen

Specimens were collected from November 2012 to September 2014 from different tertiary care centre in and around Coimbatore. Urine, pus, sputum and ear swab samples were collected in and around Coimbatore, Tamilnadu, India. The specimens collected were urine, pus, sputum and ear swab. The samples were collected periodically and immediately transfer to microbiology lab for further studies.

Isolation and Identification of *Pseudomonas aeruginosa*

Primary identification was carried with Gram's staining. Further analysis done with colony morphology, motility tests, biochemical tests such as sugar fermentation, oxidase, urease, indole, methyl red, Voges-Proskauer and citrate to conform the isolates as *P. aeruginosa* and were inoculate on routine culture media like nutrient agar, Mac-Conkey agar, blood agar and cetrimide agar (Collee *et al.,* 1996).

Antibiotic Susceptibility Test (Kirby Bauer-Disk Diffusion Method)

The Kirby Bauer test is a qualitative assay whereby disks of filter paper are impregnated with a single concentration of different antibiotics or any chemicals that will diffuse from the disk into the agar. The selected antibiotic disks are placed on the surface of Mueller Hinton agar plate which has already been inoculated with test bacteria. During the incubation period, the antibiotics/chemicals diffuse outward from the disks into the agar. This will create a concentration gradient in the agar which depends on the solubility of the chemical and its molecular size. The absence of growth of the organism around the antibiotic disks indicates that, the respected organism is susceptible to that antibiotic and the presence of growth around the antibiotic disk indicates the organism is resistant to that particular antibiotic. This area of no growth around the disk is known as a zone of inhibition, which is uniformly circular with a confluent lawn of growth in the media.

Antibiogram was performed using commercially available antibiotic discs with a Kirby-Bauer method. The identified *Pseudomonas aeruginosa* strains were tested against the following antibiotic discs. Amikacin (AK) 30µg, Amoxycillin (AMX)10µg, Augmentin (AMC) 30µg, Cefoperazone/Sulbactam 50/50 mcg/disc, Cefotaxime (CTX) 30µg, Ceftazidime (CAZ) 30µg, Ceftriaxone (CTR) 30µg, Ciprofloxacin (CIP) 5µg, Co-Trimoxazole (COT) 25µg, Gentamicin (GEN) 10µg, Imipenem (IPM) 10µg, Levofloxacin (LE) 5µg, Norfloxacin (NX) 10µg, Ofloxacin (OF) 5µg, Piperacillin / Tazobactam (PIT) 100/10µg, Netillin (NET) 30µg, Tobramycin (TOB) 10µg.

Results and Discussion

The clinical samples from different centers collected and processed systematically as per the microbiological procedure. The *Pseudomonas aeruginosa* from samples was identified by its biochemical characteristics and was shown in table 1. The Gram stain showing Gram negative bacilli, biochemical characters like oxidase, catalase were positive and growth on nutrient agar at 42°C and the production of pyocyanin and fluorescein were observed, cetrimide agar, β haemolysis on blood agar and non lactose fermenting colonies of *Pseudomonas aeruginosa* were observed visually. The quantum of samples collected was shown in table 2. Out of 9860 various microbial isolates, 499 *Pseudomonas aeruginosa* isolates alone were taken for the study.

Nosocomial acquired *Pseudomonas aeruginosa infections*

Table 3 shows, 104 positive strains isolated from nosocomial acquired patients, of which 60 are male and 44 are female.

In male (non diabetic- 27 and diabetic- 33) 7 isolates are from age group below 15 and are non diabetic from different clinical specimens (pus- 1, urine- 3, sputum- 2 and ear swab - 1). From age group between 16-50, 11 are non diabetic (pus- 7, urine- 3 and sputum- 1), 15 are from diabetics (pus- 9, urine- 3, sputum- 1 and ear swab 2). From age group above 50, 9 are from non diabetics (pus- 6, urine- 2, sputum- 1) and 18 are from diabetics (pus- 11, urine- 5 and sputum- 2).

In female (non diabetic- 18 and diabetic-26), from age group below 15, 6 are non diabetic (pus- 3, urine -2 and swab- 1) 1 isolates from diabetics (urine- 1). From age group between 16- 50, 9 are non diabetic (pus- 6, urine -3), 10 are from diabetics (pus- 4, urine- 2, sputum- 3 and ear swab - 1). From age group above 50, 3 are from non diabetics (pus- 2, urine- 1) and 15 are from diabetics (pus- 9, urine- 4, sputum- 2).

Community Acquired *Pseudomonas aeruginosa Infections*

Table 4 shows 395 positive strains isolated from community acquired patients, of which 247 are male and 148 are female.

In male (non diabetic- 99 and diabetic-148) 42 isolates are from age group below 15 and are non diabetic from different clinical specimens (pus-26, urine- 11, sputum-5). From age group between 16- 50, 42 are non diabetic (pus-27, urine -12 and sputum -1 and ear swab-1), 64 are from diabetics (pus-39, urine- 18, sputum- 3 and ear swab-4). From age group above 50, 15 are from non diabetics (pus- 8, urine- 3, sputum- 4) and 84 are from diabetics (pus- 48, urine- 28, sputum- 4 and ear swab- 4).

In female (non diabetic- 60 and diabetic-88), from age group below 15, 22 are non diabetic (pus- 13, urine -7, sputum- 2) 1

isolates from diabetics (urine- 1). From age group between 16- 50, 19 are non diabetic (pus- 11 urine -7 and sputum 1), 37 are from diabetics (pus- 27, urine- 7, sputum- 2 and ear swab 1). From age group above 50, 19 are from non diabetics (pus- 9, urine- 5, sputum- 4 and ear swab 1) and 50 are from diabetics (pus- 30, urine- 13, sputum- 5 and ear swab- 2). In both the cases, the most affected patients are diabetic in the age group of 16–50. In the collected samples the highest number of samples and *Pseudomonas aeruginosa* is with pus sample followed by others (Fig. 3).

In the present study, *P. aeruginosa* is less obtained from nosocomial acquired compared to community acquired (Fig. 1). It is alarming that resistant bacteria are emerging from both groups of patients. The pre-eminent of *Pseudomonas aeruginosa* in nosocomial infections is due to its resistance to common antibiotics and antiseptics, and its ability to establish itself widely in nosocomials. Being an extremely adaptable organism, it can survive and multiply even with minimum nutrients, if moisture is available.

Pseudomonas aeruginosa Infections among male and Female

In the present study, uropathogenic *P. aeruginosa* was found higher in females than males in case of diabetic. The one reason may be that *P. aeruginosa* may be a common inhabitant of lower intestinal tract and in female the distance between anal and vaginal opening is small, thus *P. aeruginosa* through fecal contamination invade and colonized urinary tracts causing infection (Mohan *et al.,* 2013). But in comparing nosocomial and community acquired infections the male were dominating ratio with respect to their clinical condition (Fig. 2).

Occurrence of *Pseudomonas aeruginosa* infections among various age groups

Age wise it was high in age group above 50 followed by 16- 50 and below 15 years. Economic status of the patient was also included in the study, in which it is high in poor. The prevalence is seen high in age group above 50 years and poor class people. Adults with chronic illnesses, who reside in nursing homes, who have recently been treated with antibiotics, or who are alcoholics are at risk for unique infections. Individuals with low hygienic practices and lives in low hygienic conditions indulge to have infections. In both nosocomial and community acquired infections, the most affected patients are diabetic in the age group 16–50.

Antibiotic sensitive / resistance pattern

In the present study, the sensitivity pattern of clinical isolates of *P. aeruginosa*, showed (Table 5 and 6) a antibiotic sensitivity pattern (S/R %) for the following antibiotics cotriaxone (71.5/ 28.5), cotrimaxazole (70.5/29.5), gentamycin (74.5/25.5), amikacin (86.5/13.5), norfloxacin (63.5/33.5), cephotoxime (52.5/47.7), ciprofloxacin (72.5/27.5), netillin (54.5/46.5), oflaxacin (66/34), imipenem (93/7), levofloxacin (82/16), nitrofurantoin (58.5/48.5), ceftazidime (81.5/18.5), tobramycin (78.5/21.5), polymyxin B (68.5/31.5), cefoperazone (88/12), pipercillin/tazobactum (88.5/11.5). The detailed antibiotic sensitive / resistance pattern was discussed in our previous study (Mohan Raj *et ai*., 2015). Nosocomially acquired *Pseudomonas aeruginosa* isolates tend to be more resistant than community acquired strains to antimicrobial agent frequently displaying resistance and cross resistance to many antibiotics (Pascale *et al.,* 1994).

Table.1 Biochemical characteristics of *Pseudomonas aeruginosa*

S.No	Biochemicals	Result
1	Gram staining	G-ve
2	Motility	+
3	Catalase	+
4	Oxidase	+
5	Indole	-
6	Methyl red	-
7	Voges Proskauer	-
8	Citrate	+
9	Urease	+
10	Triple Sugar Iron	k/k Alkaline slant/ alkaline butt
11	Nitrate reduction	+
12	Mannitol	-
13	Pigment production	+ (pyocyanin)

(+ indicates Positive result and - indicates negative result)

Table.2 Number of samples collected and *Pseudomonas aeruginosa* positive isolates

Sl.No	Sample	Total no. of sample	Positive sample
1.	Pus	5706	296
2.	Urine	3455	141
3.	Sputum	453	44
4.	Ear Swab	246	18
5.	Fluid	-	-
6.	Other	-	-
Total		9860	499

Table.3 Distribution of nosocomial acquired *Pseudomonas aeruginosa* among different samples

NOSOCOMIAL ACQUIRED (104) ND: 45 D:59												
Male ND:27 D:33						Female ND:18 D:26					
	<15		16 – 50		Above 50		< 15		16-50		Above 50	
	ND	D	ND	D	ND	D	ND	D	ND	D	ND	D
Pus	1	-	7	9	6	11	3	-	6	4	2	9
Urine	3	-	3	3	2	5	2	1	3	2	1	4
Sputum	2	-	1	1	1	2	-	-	-	3	-	2
Ear Swab	1		-	2	-	-	1	-	-	1	-	-

Table.4 Distribution of community acquired *Pseudomonas aeruginosa* among different samples

COMMUNITY ACQUIRED (395) ND: 159 D:236												
Male () ND: 99 D: 148							Female () ND: 60 D:88					
	<15		16-50		Above 50		<15		16-50		Above 50	
	ND	D	ND	D	ND	D	ND	D	ND	D	ND	D
Pus	26	-	27	39	8	48	13	-	11	27	9	30
Urine	11	-	12	18	3	28	7	1	7	7	5	13
Sputum	5	-	2	3	4	4	2	-	1	2	4	5
Ear Swab	-		1	4	-	4	-	-	-	1	1	2

Table.5 Number of *Pseudomonas aeruginosa* among different samples sensitive to antibiotics (MDS)

Antibiotics	**Symbol**	**Disc Content**	**Out of 491**	**Pus**	**Urine**	**Sputum**	**Ear Swab**
Amikacin	AK	30mcg	491	288	141	44	18
Amoxycillin	AMX	10mcg	48	20	12	9	7
Amoxyclav	AMC	30mcg	154	86	38	21	9
Cefoperazone/Sulbactam	CFS	50/50 mcg/disc	491	288	141	44	18
Cefotaxime	CTX	30mcg	415	247	133	19	16
Ceftazidime	CAZ	30mcg	491	288	141	44	18
Ceftriaxone	CTR	30mcg	413	246	132	21	14
Ciprofloxacin	CIP	5mcg	396	276	87	21	12
Co-Trimoxazole	COT	25mcg	102	53	32	11	6
Gentamicin	GEN	10mcg	491	288	141	44	18
Imipenem	IPM	10mcg	491	288	141	44	18
Levofloxacin	LE	5mcg	491	288	141	44	18
Norfloxacin	NX	10mcg	398	274	91	20	13
Ofloxacin	OF	5mcg	399	267	93	27	12
Piperacillin/Tazobactam	PIT	100/10mcg	491	288	141	44	18
Netillin	NET	30mcg	491	288	141	44	18
Tobramycin	TOB	10mcg	491	288	141	44	18

Table.6 Number of *Pseudomonas aeruginosa* among different samples resistant to antibiotics (MDR)

Antibiotics	**Symbol**	**Disc Content**	**Out of 4**	**Pus**	**Urine**	**Sputum**	**Ear Swab**
Amikacin	AK	30mcg	4	1	0	0	0
Amoxycillin	AMX	10mcg	4	0	0	0	0
Amoxyclav	AMC	30mcg	4	0	0	0	0
Cefoperazone / Sulbactam	CFS	50/50 mcg/disc	4	2	0	0	0
Cefotaxime	CTX	30mcg	4	0	0	0	0
Ceftazidime	CAZ	30mcg	4	1	0	0	0
Ceftriaxone	CTR	30mcg	4	0	0	0	0
Ciprofloxacin	CIP	5mcg	4	0	0	0	0
Co-Trimoxazole	COT	25mcg	4	0	0	0	0
Gentamicin	GEN	10mcg	4	1	0	0	0
Imipenem	IPM	10mcg	4	4	0	0	0
Levofloxacin	LE	5mcg	4	1	0	0	0
Norfloxacin	NX	10mcg	4	0	0	0	0
Ofloxacin	OF	5mcg	4	0	0	0	0
Piperacillin/Tazobactam	PIT	100/10mcg	4	3	0	0	0
Netillin	NET	30mcg	4	1	0	0	0
Tobramycin	TOB	10mcg	4	1	0	0	0

Fig.1 Distribution of *P. aeruginosa* among type of infection

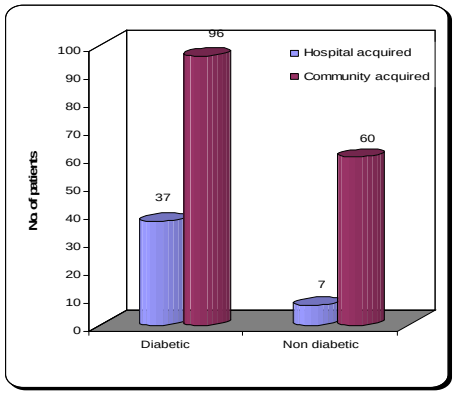


Fig.2 Distribution of *Pseudomonas aeruginosa* among gender

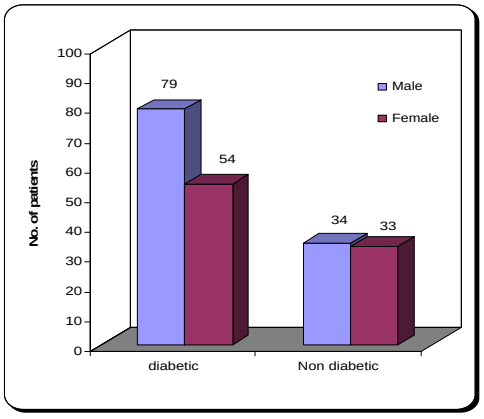
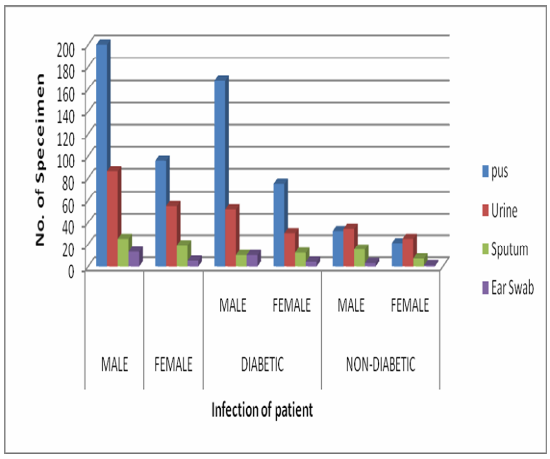


Fig.3 Distribution of *P. aeruginosa* among different specimen



On comparing the sensitivity patterns observed in present study and previous studies, it was found that most of the isolates of *P. aeruginosa* isolated from various samples shows resistance to most of the antibiotics commonly used. This may be due to excessive β-lactamase production and/or active efflux mechanism (Carmeli *et al.,* 1999). Multi drug efflux pumps in the inner and outer membrane of *P. aeruginosa* may protect the bacterium from β-lactam agents (Srikumar *et al.,* 1997).

In conclusion, the present study reveals that, *Pseudomonas aeruginosa* is one of the most important bacterial pathogen seriously contributing the problem of nosocomial infection and drug resistance to *Pseudomonas aeruginosa* is rapidly increasing. The prevalence of *P. aeruginosa* is more common in males than in females and also high in diabetics than in non diabetics. The age wise distribution of *P. aeruginosa* from the present study shows that, it is seen common in age group above 50 followed by between16-50 and below 15 in diabetics. The antibiotic susceptibility pattern of *P. aeruginosa* to different commonly used antibiotics showed low resistance to imipenem followed by pipercillin/ tazobactum, cefoperazone/ sulbactum, amikacin, levofloxacin, ceftazidime, tobramycin and gentamycin and shown high resistace to cefotaxime followed by netillin, nitrofurantoin, norfloxacin, oflaxacin, cotrimaxazole, ceftriaxone and ciprofloxacin.

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