ORIGINAL ARTICLE

A comparative analysis of isolation and antibiotic sensitivity pattern of *Pseudomonas aeruginosa* isolated from pus and urine with special reference to phenotypic and genotypic expression of extended spectrum beta lactamases (ESBLs)

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ABSTRACT

Pseudomonas aeruginosa (*Ps. aeruginosa*) is a classic opportunistic pathogen with innate resistance to many antibiotics and disinfectants. The aim of this study was to find the prevalence and the resistance pattern, phenotypic, and genotypic characterization of *Ps. aeruginosa* from different source of infection. The present study was carried out with a total of 1000 clinical samples including 500 pus samples and 500 urine samples, which were received from patients admitted in the various departments of Rajah Muthiah Medical College and Hospital, Annamalai University, Chidambaram. Of the 500 pus and 500 urine samples screened, the percentage positivity of *Ps. aeruginosa* was 12.8% (64) and 4% (20), respectively. *Pseudomonas aeruginosa* from different samples showed different sensitivity patterns to different antibiotics. In case of isolates from pus, all (100%) were sensitive to Imipenem, while resistance was maximum to Cefotaxime (93.75%). When *Pseudomonas aeruginosa* isolated from urine was tested for the same antibiotics, sensitivity was maximum (90%) to Tobramycin, while resistance was maximum (80%) to Cefotaxime and Aztreonam. ESBL positive *Ps. aeruginosa* isolated from pus and urine was 50% and 40%, respectively. The genotype characterization of 25 of these strains showed 6 with CTX-M and 12 with SHV genes.

Key words: Antibiotic sensitivity, ESBL, *Pseudomonas aeruginosa*, pyogenic infections, urinary tract infection

INTRODUCTION

Pseudomonas aeruginosa is a comm on bacterium that causes infection in man, animals, and plants. *Ps. aeruginosa* is an opportunistic pathogen that typically causes infections in burns and wounds and also infects hospitalized and immunosuppressed patients.[1] *Ps. aeruginosa* can also cause urinary tract infection (UTI) in patient with complex urinary tract abnormalities.[2] *Ps. aeruginosa* infestations are of three types- bacterial attachment and colonization, local infection and disseminated systemic disease. However, the disease process may stop at any stage. Nowadays, *Ps. aeruginosa* is developing multidrug resistance to all antibiotics that have been used for treatment by clinicians.

Hence, this study was conducted to correlate the prevalence rate, sex, age, socioeconomic and domicile status, antibiotic sensitivity, extended spectrum beta lactamase (ESBL), and genotypic characterization of *Ps. aeruginosa* causing pyogenic infection and UTI isolated from patients attending Rajah Muthiah Medical College and Hospital, Chidambaram, Cuddalore district, Tamil Nadu, India.

MATERIALS AND METHODS

A total of 1000 clinical specimens including 500 pus and 500 urine samples were received from patients admitted in Rajah Muthiah Medical College and Hospital. Pus samples were collected from patients with pyogenic infections including ulcers, injury, wounds, and burns admitted in various departments including

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Medicine, Surgery, Paediatrics, and Orthopedics. The specimens collected were pus swabs, aspirates, and drained pus. Urine samples were collected from various departments including Medicine and Urology. These patients presented with various diseases such as UTI, pyelonephritis, cystitis and benign prostate hypertrophy. Specimen collected was early morning midstream urine.

The samples were collected in sterile containers and processed immediately. The Gram stained smear of the specimens were examined and culture for aerobic pathogens was done by standard procedures.[3]

The colonies of *Ps. aeruginosa* were presumptively identified by colony morphology, pigment production, characteristic musty or earthy odor. The identification was confirmed by motility and biochemical tests, which included fermentation of sugars, oxidation fermentation (O/F Test), indole production, nitrate reduction, oxidase, and catalase test.[4] Antimicrobial susceptibility testing was conducted with antibiotics Gentamicin (G), Amikacin (AK), Cefepime (Cpm), Cefotaxime (CE), Aztreonam (Ao), Meropenem (M_1) Imipenem (I), Tobramycin (TB), Ofloxacin (OF), Norfloxacin (Nx), and Ticarcillin (T_1) by Kirby–Bauer's disc diffusion method.[5]

RESULTS

A total of 1000 samples including 500 from pyogenic infections and 500 from UTIs were collected and screened for the isolation of bacteria. Of the 500 pus and 500 urine samples screened, culture positivity was obtained in 410(82%) pus samples and 335(66.4%) urine samples, respectively. Isolation rate of *Ps. aeruginosa* was 12.8% in pus and 4% in urine [Figure 1, Table 1].

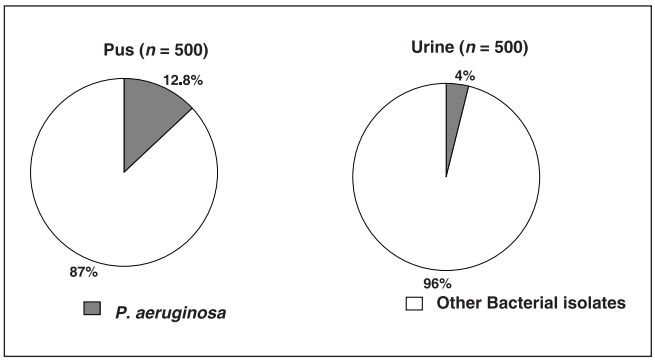

Figure 1: The prevalence of *Ps. aeruginosa* in pyogenic and urinary tract infection.

Demography

Sex wise distribution of *Ps. aeruginosa* showed 59.37% in males and 40.63% in females among pyogenic infection cases. *Ps. aeruginosa* isolated from urine showed higher percentage in males (80%) as against females (20%) [Table 2]. Among pyogenic infection cases males and females showed higher percentage positivity for *Ps. aeruginosa* in the age group of 61-75 years (28.12%).

Of the 64 *Ps. aeruginosa* positive pyogenic infection cases 36 (56.25%) were coolies. *Ps. aeruginosa* positive UTI cases(20) were mainly coolies (30%) and drivers (30%).

80% of *Ps. aeruginosa* positive pyogenic infection cases were from rural areas, whereas *Ps. aeruginosa* positive UTI cases were almost equal in rural (40%), semi urban (30%), and urban areas (30%).

Table 3 shows the results of susceptibility of the *P. aeruginosa* isolates to various antibiotics. All (100%) strains isolated from pus were sensitive to Imipenem, 93.75% strains were resistant to Cefotaxime, and 58% resistant to Ceftazidime.

Of the strains isolated from urine samples, majority (90%) were sensitive to Tobramycin, 80% to Gentamicin, Meropenem & Imipenem, while a maximum of 80% strains were resistant to Cefotaxime and Aztreonam and 60% to Cefepime [Table 3].

Multi drug resistance was seen in 47 (73.43%) of *Ps. aeruginosa* isolated from pus and 17 (26.57%)

Table 1: Isolation of bacteria from pus and urine (*n* = 500 each)

Bacteria isolated	No. of positive cases in pus	No. of positive cases in urine
Pseudomonas aeruginosa	64 (12.8%)	20 (4%)
Staphylococcus aureus	206 (41.2%)	44 (8.7%)
Escherichia coli	84 (16.8%)	149 (29.2%)
Klebsiella pneumoniae	34 (6.8%)	64 (12.8%)
Proteus mirabilis	22 (4.4%)	56 (11.2%)
Enterococci	0	2 (0.4%)
Total	410 (82.0%)	335 (66.4%)

Table 2: Sex wise distribution of *Ps. aeruginosa* positive pyogenic infection and urinary tract infection

Sex	Pus	Urine
Male	38 (59.37%)	16 (80%)
Female	26 (40.63%)	4 (20%)
Total	64 (12.8%)	20 (4%)

Table 3: Antibiogram pattern of *Ps. aeruginosa* isolated from pus and urine samples

Group	Antibiotic tested	Pus (*n* = 64)		Urine (*n* = 20)	
		Sensitive	Resistant	Sensitive	Resistant
Aminoglycosides	Gentamicin [G]	52 (81.25%)	12 (18.75%)	16 (80%)	4 (20%)
	Amikacin [Ak]	56 (87.50%)	8 (12.5%)	14 (70%)	6 (30%)
	Tobramycin [Tb]	52 (81.25%)	12 (18.75%)	18 (90%)	2 (10%)
Fluroquinolones	Ciprofloxacin [Ci]	48 (75%)	16 (25%)	12 (60%)	8 (40%)
	Norfloxacin [Nx]	52 (81.25%)	12 (18.75%)	14 (70%)	6 (30%)
Carbapenems	Meropenem [Mi]	42 (65.62%)	22 (34.38%)	16 (80%)	4 (20%)
	Imipenem [I]	64 (100%)	0	16 (80%)	4 (20%)
Extended spectrum penicillin	Ticarcillin [Ti]	38 (59.38%)	26 (40.62%)	14 (70%)	6 (30%)
Monobactams	Aztreonam [Ao]	42 (65.62%)	22 (34.38%)	4 (20%)	16 (80%)
Cephalosporins	Cefepime [Cpm]	38 (59.37%)	26 (40.63%)	8 (40%)	12 (60%)
	Cefotaxime [Ce]	4 (6.25%)	60 (93.75%)	4 (20%)	16 (80%)
	Ceftazidime [CA]	27 (42%)	37 (58%)	12 (60%)	8 (40%)

Table 4: Expression of antibiotic resistance pattern of *Ps. aeruginosa* isolated from different samples against 13 antibiotics

Type of drug resistance pattern against 13 antibiotics used	Percentage of resistance pattern of *Ps. aeruginosa* from different samples against 13 antibiotics used		
	Pus (*n* = 64)	Urine (*n*=20)	Total
Multidrug resistance	47 (73.43%)	18 (90%)	65 (77.3%)
Single drug resistance	17 (26.57%)	2 (10%)	19 (22.6%)

Table 5: ESBL producing *Ps. aeruginosa* by synergy test

Specimen	ESBL %
Pus (*n* = 64)	32 (50%)
Urine (*n* = 20)	8 (40%)

Table 6: Expression of ESBL Genes by *Pseudomonas aeruginosa* isolated from various sources

Isolates	CTX-M (%)	SHV (%)
25	6 (24%)	12 (36%)

showed resistance to a single drug. Multi drug resistance was seen in 18 (90%) of those isolated from urine and 2 (10%) showed single drug resistance [Table 4].

There were 40 (32 from pus and 8 from urine) strains of *Pseudomonas aeruginosa* that were resistant to 3rd generation cephalosporins and found to be positive by the double disc synergy test for ESBL detection [Table 5]. Genotype characterization of 25 of these strains showed 6 strains with CTX-M and 12 with SHV gene [Table 6].

DISCUSSION

Ps. aeruginosa is widely distributed in nature, existing usually as saprophyte but can give rise to pathological lesions and generalized infections in man.[6] *Ps. aeruginosa* is the most frequently isolated nonfermentative bacilli found in clinical specimens.

Ps. aeruginosa has one of the broad ranges of infectivity among all pathogenic microorganisms. The species can cause disease in plants, insects, fish, amphibians, reptiles, birds, and mammals.[7] *Ps. aeruginosa* causes UTI, respiratory tract infections, dermatitis (hot tub rash), soft tissue infections, bacteremia, bones and joint infection, central nervous system, and gastrointestinal infections in humans. A variety of systemic infections caused by *Ps. aeruginosa* particularly in patients hospitalized with cancer, cystic fibrosis, burns, and fatality rate in these patients is near 50%.[8] *Ps. aeruginosa* produces infection of wounds and burns giving rise to blue green pus. The organism can be acquired by invasive procedures like lumbar puncture, catheterization and instrumentation or from irrigating solutions.[9]

Ps. aeruginosa is notorious for its resistance to antibiotics and is therefore a particularly dangerous and dreaded pathogen. The bacterium is naturally resistant to many antibiotics due to the permeability barrier afforded by its gram negative outer membrane. Also, its tendency to colonize the surface in a biofilm form makes the cells impermeable to therapeutic concentration of the antibiotics. Moreover, *Ps. aeruginosa* maintains antibiotic resistance plasmid, both F factors and resistance transfer factor (RTF) and it is able to transfer the genes by means of the bacterial mechanism of horizontal gene transfer (HGT), mainly transduction and conjugation.[9] The emergence of antibiotic resistance is due to irrational use of antibiotics in treating human infections.[6]

The present study shows that in a total 500 cases of pyogenic infection, 12.8% were positive for *Ps. aeruginosa* as against 4% in 500 UTI cases. In this study *Ps. aeruginosa* isolates from urine were showing higher percentage in males (80%) as against 59.37% positivity in males from pyogenic infections.

Ps. aeruginosa isolated from pus and urine showed higher degree of drug resistance against Cefotaxime, since the clinicians were using Cefotaxime as routine antibiotic therapy against all *Ps. aeruginosa* infections. Although 80% of strains from urine were sensitive to Meropenem, only 63% of strains isolated from pus were sensitive. However, 100% of strains isolated from pus and 80% of strains from urine were sensitive to Imipenem. A higher percentage of ESBL *Ps. aeruginosa* was seen in pus.

The genotypic characterization showed the *Ps. aeruginosa* isolates expressed both the SHV and CTX-M and percentage positivity of SHV was higher than the percentage positivity of CTX-M in the 25 strains tested.

Thus the present study shows that the isolation and testing of *Ps. aeruginosa* for antibiotics is necessary for accurate reporting and to detect chromosomally mediated vertical transmission of drug resistance in *Ps. aeruginosa*.

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