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A Study on Isolation and Antibiotic Sensitivity Testing of *Pseudomonas aeruginosa* Isolated from Patients with Respiratory Tract Infection with Special Reference to Phenotypic and Genotypic Characterization of Extended Spectrum Beta Lactamases (ESBL)

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Abstract

Backgrounds: *Pseudomonas aeruginosa* is a classic opportunistic pathogen with innate resistance to many antibiotics and disinfectants. The lung is a main target for colonization and infection by the bacteria either in the context of a chronic, progressively deteriorating infectious and inflammatory pulmonary disease such as cystic fibrosis (CF) or in a more acute setting such as severe pneumonia in immunocompromised patients [1]. Aim and Objectives: To study the prevalence, virulence and the resistance pattern, phenotypic and genotypic characterization of *P. aeruginosa* from sputum samples. *Materials and Methods*: The present study was carried out with a total of 500 clinical sputum samples, which were received from patients, admitted to the various departments of Rajah Muthiah Medical College & Hospital, Annamalai University, Chidambaram. *Result*: Of the 500 samples subjected for isolation and identification of *P. aeruginosa*, 116 (23.20%) were positive. The isolated strains were tested for antibiotic sensitivity patterns. 93.10% of *P. aeruginosa* showed a maximum sensitivity to Ofloxacin, Norfloxacin and 86.20% of strains were highly resistant to Cefotaxime. The same isolates were also tested for phenotypic characterization of Extended Spectrum of Beta Lactamases by double disc synergy method against Cefotaxime and Clavulanic acid, according to the criteria of Hi-Media [2]. Of the resistant strains of *P. aeruginosa* iso-

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lated from sputum, 59% were positive for ESBL. The genotype characterization of ESBL *P. aeruginosa* showed 40% of CTX-M and 46.66% SHV gene. *Conclusion*: The present study strongly recommends for further checking of the antibiotic resistant strains of *P. aeruginosa* for phenotypic characterization of ESBL for effective treatment.

Keywords

***Pseudomonas aeruginosa*, Prevalence in Patient with Respiratory Tract Infection (RTI), Socioeconomic Status, ESBL**

1. Introduction

P. aeruginosa is an aerobic gram-negative, motile, non-spore forming rods that are oxidase positive and lactose nonfermenters. *P. aeruginosa* is a member of the genus Pseudomonas. It is hydrophilic and widely distributed in nature. It forms smooth fluorescent green colonies at 42˚C, with a characteristic sweet (grape-like) odor, making it easy to recognize on solid media in the laboratory. It is a common bacterium which causes respiratory tract infection in humans [3]. Nowadays, *P. aeruginosa* is producing resistance to all antibiotic by the production of Extended Spectrum Beta Lactamase due to hyper production of Broad Spectrum B-lactamases, decreased outer membrane permeability and active efflux [4]. It is found that high prevalence of multidrug resistance and extended spectrum Beta lactamase strains in hospital [5].

This study was conducted to correlate the prevalence, sex, age, socio-economic, domicile status, fluorescence and non fluorescence strain, virulence, antibiotic sensitivity, phenotypic and genotypic characterization of Extended Spectrum Beta Lactmase (ESBL) of *P. aeruginosa* causing respiratory tract infection among the patient attending Rajah Muthiah Medical Collage and Hospital, Chidambaram, Cuddalore District, Tamil Nadu, India.

2. Materials and Methods

A total of 500 Sputum samples were collected from patients admitted to the Rajah Muthiah Medical College and Hospital, Chidambaram, Cuddalore District, Tamil Nadu. The sputum samples were collected from the department of Chest medicine, and Pediatrics. Patients presented with various lung diseases such as chronic bronchitis, bronchiectasis, cystic fibrosis, bronchial asthma, fibrosing alveolitis, pulmonary tuberculosis and healed tuberculosis with fungal super infections.

The specimens collected were expectorated sputum and bronchial washings. The sputum samples were collected on the three consecutive days in a sterile wide mouthed container and processed immediately.

The specimen were examined microscopically in gram's stained smear and cultured for aerobic pathogens by standard procedures. Presumptively identified by colony morphology, pigment production and the characteristic musty or earthy odor and confirmed by motility and by other biochemical tests. Antimicrobial susceptibility pattern was tested for the following antibiotics namely, Gentamicin (G), Amikacin (AK), Cefepime (Cpm), Cephotaxime (CE), Aztreonam(Ao), Meropenem (M1), Imipenem (I) Tobramycin (TB), Ofloxacin (OF), Norfloxacin (NX) Ticarcillin (Ti) by Kirby-Bauer's disc-diffusion technique [6]. The pattern was graded into sensitive, intermediate and resistant, according to standard sensitivity charts from Hi-media. The fluorescence and non fluorescence strain of *P. Aeruginosa* were studied. The extracellular virulence factors such as Haemolysin, Protease, DNAse, Gelatinase, Lipase and Amylase were also studied. The *P. aeruginosa* was also tested for phenotypic characterization of extended spectrum Beta lactamase by double disc synergy method against Ceftazidime and Clavulanic acid, according to criteria of Hi-media [2]. The genotypic characterization of extended spectrum Beta lactamase was done by polymerized chain reactions.

3. Results

A total of 500 sputum samples were screened for aerobic bacteria. Of the 500 sputum samples screened, 72% were positive for bacterial isolates. Among the bacterial isolates, the *P. aeruginosa* (23.20%) *Streptococcus*

(2.2%) *Staphylococcus aureus* (4%), *Escherichia coli* (13.66%), *Klebsiella pneumoniae* (27%), *Proteus mirabilis (0.6%*), *Enterococci* (0.3%) (**Figure 1** and **Table 1**).

The sex wise distribution for *P. aeruginosa* positive cases showed, 57.75% of infected males and 42.25 % of infected females (**Figure 2**).

Both males and females, showed a higher percentage positivity for *P. aeruginosa* in the age groups of 61-75 (39.28%) years and 76 - 90 (33.34%) years. The percentage positivity for *P. aeruginosa* patients belonging to the age group of 61 - 75 years, was also found to be statistically significant ($P = 0.005$) (**Table 2**).

Of the 116 *P. aeruginosa* positive cases, 57 cases (49.13%) were coolies, among which, 47 cases were males. Most of the infected females, 39 cases (33.62%), were housewives (**Table 3**).

The percentage of P. aeruginosa positive respiratory tract infection cases, according to the domicile status of the patients, were as follows, with 53% seen in rural areas, 34% in urban areas and 13% of cases seen in semi urban areas (**Figure 3**).

In sputum, 93.10% of strains were sensitive to ofloxacin and norfloxacin, 79.32% to tobramycin, 73% to ciprofloxacin, 63.80% to gentamicin, amikacin, meropenem and imipenem, 57.75% to ticarcillin and 56.90% to cefepime. The 86.20% of cases were resistant to cefotaxime, 59% to cefatazidime and 57.75% to aztreonam (**Table 4**).

93.11% of the *P. aeruginosa*, showed multi drug resistance and 6.89% showed single drug resistance (**Table 5**).

The percentage of Extended Spectrum Beta Lactamase (ESBL) positive *P. aeruginosa* was 59% (**Table 6**).

Of the 116 strains isolated, 15 strains were randomly chosen and tested for fluorescence and non fluorescence producing strains (**Table 7**), for the production of extracellular virulence factors and also, for the expression of ESBL gene (bla CTX-M, bLa SHV).

93.33% of the strains produced haemolysin, protease and lipase whereas only 73.33% of the strains produced DNAse and gelatinase (**Table 8**).

The genotype characterization showed 40% of CTX-M and 46.66% SHV gene in *Ps. aeruginosa*, isolated from sputum ($n = 15$) (**Table 9**).

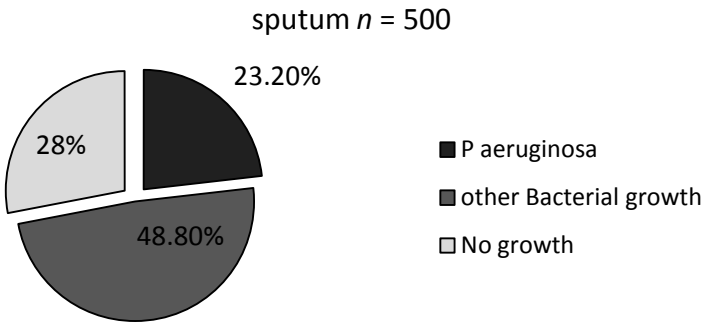

Figure 1. The prevalence of *Pseudomonas aeruginosa* and other bacterial isolates in respiratory tract infection (n = 500).

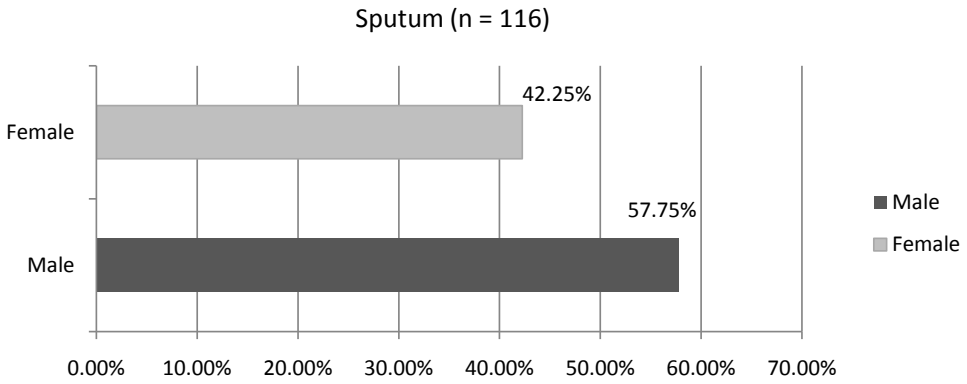

Figure 2. Sex wise distribution of *Pseudomonas aeruginosa* positive cases (n = 116).

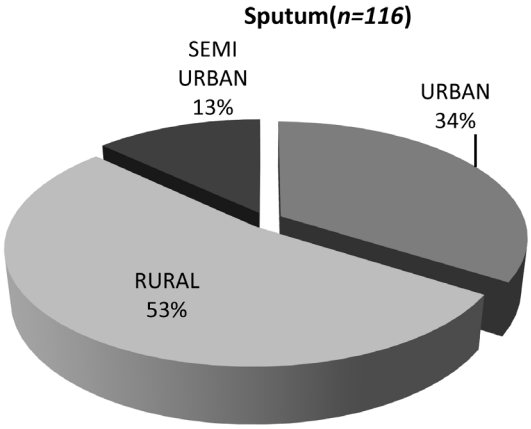

Figure 3. Distribution of *Pseudomonas aeruginosa* positive cases according to domicile status.

Table 1. Isolation of bacteria from sputum (n = 500).

S. No	Bacteria isolated	No. of isolates	%
1.	*Pseudomonas aeruginosa*	116	23.20%
2.	*Klebsiella pneumoniae*	135	27%
3.	*Escherichia coli*	68	13.66%
4.	*Staphylococcus aureus*	20	4%
5.	*Streptococcus*	11	2.2%
6.	*Proteus mirabilis*	3	0.6%
7.	*Enterococci*	2	0.4%
Total No. of bacterial isolates		217	72.33%
No growth		140	28%

Table 2. Age wise distribution of *Pseudomonas aeruginosa* positive respiratory tract infection cases [sputum n = 116].

Age group	No. of cases screened	No. of positive cases	No. of negative cases
0 - 15	10 (2%)	-	10 (100%)
16 - 30	124 (24.8%)	**17 (13.71%)**	107 (86.29%)
31 - 45	134 (26.8%)	**33 (24.62%)**	101 (75.38%)
46 - 60	124 (24.8%)	**25 (20.16%)**	99 (79.84%)
61 - 75	84 (16.8%)	**33 (39.28%)***	51 (60.72%)
76 - 90	24 (4.8%)	**8 (33.34%)**	16 (66.66%)
Total	500	**116 (23.2%)**	384 (76.8%)

*(S)—significant.

Table 3. Profession wise distribution of *P. aeruginosa* positive cases (n = 116).

S. No	Occupation	Male	Female	Total No. of *P. aeruginosa* positive cases
1	Coolie	47	10	57 (49.13%)
2	Driver	20	0	20 (17.25%)
3	Housewife	0	39	39 (33.62%)
Total No. of *P. aeruginosa* positive cases		67 (57.75%)	49 (42.25%)	116

Table 4. Antibiogram pattern of 116 strains isolated from sputum (n = 116).

Groups	Antibiotic Tested	Sputum (n = 116)	
		Sensitive	Resistance
Aminoglycosides	Gentamicin [G]	74 (63.80%)	42 (36.20%)
	Amikacin [Ak]	74 (63.80%)	42 (36.20%)
	Tobramycin [Tb]	92 (79.32%)	24 (20.68%)
Fluroquinolones	Ciprofloxacin[Ci]	85 (73%)	31 (27%)
	Ofloxacin [Of]	108 (93.10%)	8 (6.90%)
	Norfloxacin [Nx]	108 (93.10%)	8 (6.90%)
Carbapenems	Meropenem [Mi]	74 (63.80%)	42 (36.20%)
	Imipenem [I]	74 (63.80%)	42 (36.20%)
Extended Spectrum Penicillin	Ticarcillin [Ti]	67 (57.75%)	49 (42.25%)
Monobactams	Aztreonam [Ao]	49 (42.25%)	67 (57.75%)
Cephalosporins	Cefepime [Cpm]	66 (56.90%)	50 (43.10%)
	Cephotaxime [Ce]	16 (13.80%)	100 (86.20%)
	Cefatazidime [Ca]	47 (41%)	69 (59%)

Table 5. Expression of antibiotic resistance pattern of *Pseudomonas aeruginosa* isolated from sputum against 13 antibiotics.

Type of drug resistance pattern against 13 antibiotics used	Percentage of resistance pattern of *Pseudomonas aeruginosa* from different samples against 13 antibiotics used
	Sputum (n = 116)
Multi drug resistance	108 (93.11%)
Single drug resistance	8 (6.89%)

Table 6. Extended spectrum beta lactamase producing *Pseudomonas aeruginosa* by synergy test.

Specimen	ESBL %
Sputum (n = 116)	69 (59%)

Table 7. Fluorescence and non fluorescence producing *Pseudomonas aeruginosa* from sputum (n = 15).

Category according to fluorescence	Sputum (n = 15)
Fluorescence	11 (73.34%)
Non fluorescence	4 (26.66%)
Total	15

Table 8. Production of extracellular virulence factors by *Pseudomonas aeruginosa* isolated from sputum (n = 15).

No. of *Pseudomonas aeruginosa*	Haemolysin	Protease	DNAse	Gelatinase	Lipase	Amylase
Sputum n = 15	14 (93.33%)	14 (93.33%)	11 (73.33%)	11 (73.33%)	14 (93.33%)	0 (0%)

Table 9. Expression of ESBL genes by *Pseudomonas aeruginosa* strains isolated from sputum (n = 15).

ESBL genes expressed	Strains expressing ESBL genes	Percentage
bla CTX-M	6 strains (S_1, S_5, S_8, S_{10}, S_{11}, S_{15})	40%
bLa SHV	7 strains (S_1, S_5, S_7, S_8, S_{10}, S_{11}, S_{15})	46.66%

4. Discussion

Pseudomonas aeruginosa infection occurs all over human and animal body. *P. aeruginosa* is the predominant respiratory tract pathogen in patients with cystic fibrosis and other chronic infections [3]. *P. aeruginosa* develops acquired resistance easily, either by mutation in chromosomally-encoded gene transfer or by the horizontal gene transfer of antibiotic resistance determinants. One of the most worrisome characteristics of *Ps. aeruginosa* is its low antibiotic susceptibility. The phenotypic resistance is associated to emergence of small colony variant and biofilm formation in *P. aeruginosa*. [7]

The present study showed that in 500 sputum samples, 23.20% were positive for *P. aeruginosa* and other Bacterial isolates were 72%. *P. aeruginosa* is a common contaminant in the environment and showed higher percentage positivity in sputum. The sex wise distribution of *P. aeruginosa* positive cases was males (57.75%) and females (42.25%). When compared to females, males were more prone to pseudomonas infection because of their increased exposure to various adverse environmental factors.

The higher percentage positivity of *P. aeruginosa* was seen in the age group 61 - 75 years (39.28%). *P. aeruginosa* a major opportunistic pathogen in humans and is capable of infecting various tissues and organs and causing severe, damaging, and often fatal disease [8].

The study also indicated that a prevalence of *P. aeruginosa* in rural (53%) and urban (34%) areas. This is because in urban areas, people are more affected due to pollution, industrialization, precipitating factors and hospital acquired infection.

Among the antibiotics tested, the isolates were highly sensitive to Ofloxacin and Norfloxacin (86.20%) and highly resistant to Cephotaxime (86.20%). Since the antibiotic Cephotaxime was used commonly by the clinician, *P. aeruginosa* developed resistance [9].

In the present study, the *P. aeruginosa* isolated from respiratory tract infection was showing 73.34% fluorescence positive *P. aeruginosa* and 26.66% nonfluorescence strains. The present study also revealed that the percentage of fluorescence strains were predominantly isolated from respiratory tract infection like other studies [10]. Among the *P. aeruginosa* isolated from sputum, the production of haemolysin, protease and lipase was 93.33%, DNAse and gelatinase was 73.33%. Also the study revealed that there was correlation between the production of extracellular virulence and multi drug resistance [11].

A study was also conducted for Extended Spectrum Beta Lactamase producing *P. aeruginosa* among the drug Cephotaxime with Clavulanic drug and 59% Cephotaxime resistant strain showed ESBL positive [12]. In our study the genotype characterization showed 40% of CTX-M and 46.66% showed SHV expressing gene of *P. aeruginosa*.

5. Conclusion

Pseudomonas aeruginosa is the second most common cause of respiratory tract infections. It is one of the most common opportunistic pathogens with multidrug resistant. Moreover, the present study strongly recommends for further checking of the antibiotic resistant strains of *P. aeruginosa* for phenotypic characterization of ESBL for effective treatment.

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