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Prevalence and Antibiogram Pattern of *Pseudomonas Aeruginosa* Isolates in a Tertiary Care Hospital of West Bengal

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Abstract
Background: *Pseudomonas aeruginosa is one of the most common gram-negative microorganisms that continue to be a major cause of opportunistic nosocomial infections, causing around 10% of hospital infections. This lethal microorganism presents a serious therapeutic challenge for treatment due to its intrinsic ability to resist many classes of antibiotics as well as its ability to acquire resistance.*
Aim: *This study was undertaken to determine the prevalence and susceptibility pattern of Pseudomonas aeruginosa isolates among patients in a tertiary care hospital in West Bengal.*
Methodology: *Pseudomonas aeruginosa was identified using standard methods from various clinical samples collected over a period of seven months. Antimicrobial susceptibility test of these isolates were determined by disk diffusion method following CLSI guideline.*
Result: *Our study showed that the prevalence of P.aeruginosa in our institution is 3.73% and most of these bugs were obtained from middle-aged adult male patients particularly from outdoor (22%) and ICU(20%). This organism was maximally isolated from wound infections (45%). Overall, the highest susceptibility was shown to polymyxins categories i.e. polymyxin B (96.8%) and colistin (91.7%) and the lowest to ceftazidime (21.2%) and gentamicin (49%).*
Conclusion: *The high prevalence of P. aeruginosa as an opportunistic nosocomial pathogen and high frequency of antimicrobial resistance among the clinical isolates demand regular monitoring of antibiogram of P. aeruginosa isolates with proper implementation of antimicrobial policy and infection control measures.*
Key words: *Pseudomonas aeruginosa, prevalence, infection, , antibiogram, polymyxins*

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I. Introduction

Pseudomonas aeruginosa is an opportunistic pathogen associated with a range of nosocomial infections like, pneumonia, bacteremia, wound infection and urinary tract infection [1,2]. There are number of factors contributing its high prevalence and propensity of causing infections. Apart from its own gamut of virulence factors; immunocompromised state, misuse of antibiotics, ever-growing figure of invasive procedures also have prompted its increased involvement in health care associated infections and emergence and dissemination of drug resistant bugs [3].Moreover, high risk patients located in intensive care units, oncology departments, burn units and surgery wards are frequently infected with multidrug resistant *P. aeruginosa* isolates leading to high morbidity and mortality[4,5].

Pseudomonas species are ubiquitous gram-negative, motile bacteria that inhabit diverse environments, including soil, water, plants, insects, and animals [1]. They are the most common non-fermenter isolated from clinical specimens. Biochemically, they are oxidase, catalase positive and oxidizer of carbohydrates [5].

It colonizes in natural and artificial surfaces, therefore found on medical equipments including invasive catheters causing cross infections in hospitals and clinics. This bacteria is notorious for its low antibiotic susceptibility which is not only due to its intrinsic resistance but also *P. aeruginosa* can acquire resistance by mutation either in chromosomally encoded genes or by the horizontal gene transfer of antibiotic resistance determinants[6,7]. Low permeability of the bacterial cellular envelopes and action of multidrug efflux pumps contribute significantly in drug resistance. This efflux pump is associated with elevated MICs with penicillins, cephalosporins, quinolones, tetracyclines, chloramphenicol, metallo-b-lactamases and later carbapenems[8-12].

Moreover, literature review showed that the resistance of *P. aeruginosa* to β-lactams, quinolones, aminoglycosides and carbapenems, especially imipenem has steadily increased[8, 11-13]. In this study we investigated in detail about the various sensitivity pattern shown by this virulent organism.

II. Material & Methods

The present study was conducted in Microbiology department, Burdwan Medical College, Purba Bardhaman, India, over 7 months from January 2019 to July 2019. After collection of various samples, first, direct smear was prepared and stained with gram stain and then the samples were cultured on various culture media, like Blood agar and MacConkey's agar. After 24hrs of incubation, colony morphology and pigmentation were noted and from the colony; gram stain, motility and oxidase test were performed. Then, only samples showing oxidase positive gram negative, motile bacilli were taken into account. After that biochemical parameters like oxidative carbohydrate utilization, ability to grow at 42 ˚C and on Cetrimide agar were considered to identify as *Pseudomonas aeruginosa*[14].

Antimicrobial susceptibility test

The antibiotic susceptibility pattern of all the *Pseudomonas aeruginosa* isolates were assessed by modified Kirby–Bauer disc diffusion method on Mueller– Hinton agar against the following antibiotics: amikacin (30µg), gentamicin (10µg), ciprofloxacin (5µg), levofloxacin (5µg), piperacillin-tazobactam (110µg), ceftazidime (30µg), aztreonam (30µg), imipenem (10µg), meropenem (10µg), polymyxin B (300U) and colistin (10µg). After incubation of 24 h at 37°C, the zone diameters measured around each disc were interpreted on the basis of guidelines published by the Clinical and Laboratory Standards Institute (CLSI)[15].

III. Results

In this study, a total of 2439 samples were collected in the Microbiology department, Burdwan Medical College, over a period of seven months from January 2019 to July 2019. Of these, *Pseudomonas aeruginosa* was isolated in ninety-one (91) samples. It was observed that, 68% of the isolates were obtained from male patients while females contribute only 32% of the isolates.(Figure 1)

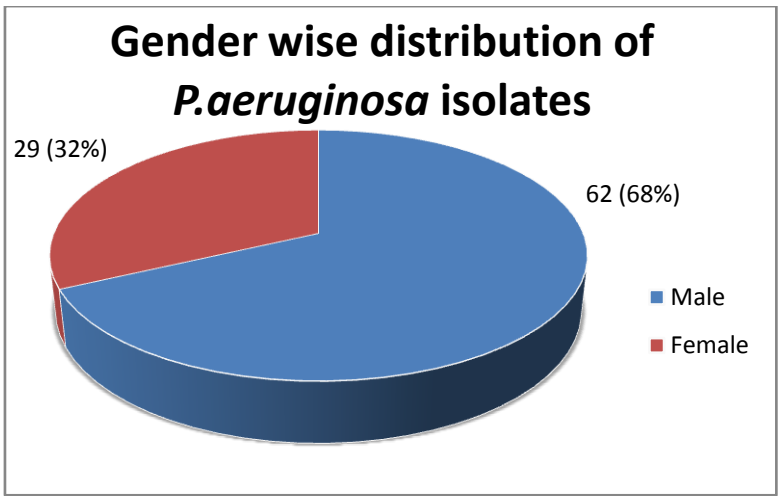

Figure 1: Gender wise distribution of *P. aeruginosa* isolates. (n=91)

This study also revealed that *P. aeruginosa* isolates were mostly retrieved from middle aged adult patient in the age group 45-54 years followed by patients of 15-24 years. (Figure 2, Table 1)

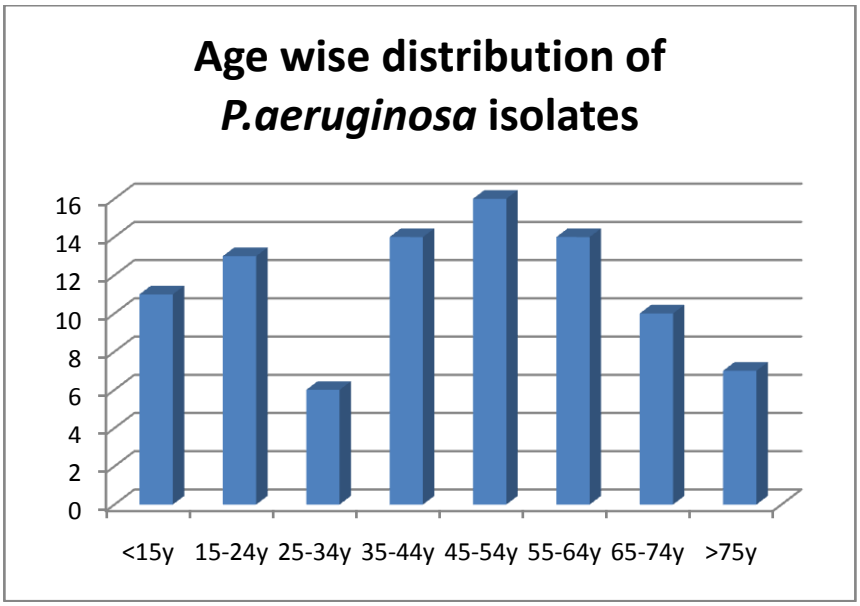

Figure 2:- Age wise (in years) distribution of *P.aeruginosa* isolates. (n=91)

Table 1: Age wise distribution of *P.aeruginosa* isolates. (n=91)

Age groups (in years)	No. of isolates (n)	Percentage (%)
<15	11	12
15-24	13	14
25-34	6	7
35-44	14	15
45-54	16	18
55-64	14	15
65-74	10	11
>75	7	8

In our study, we noticed that 22% of the isolates were from the patients attended OPD, while ICU (20%) and medicine department (20%) contributed significantly. But, comparatively less number of isolates were seen from paediatrics (7%) and gynaecology (5%) departments. (Figure 3, Table 2)

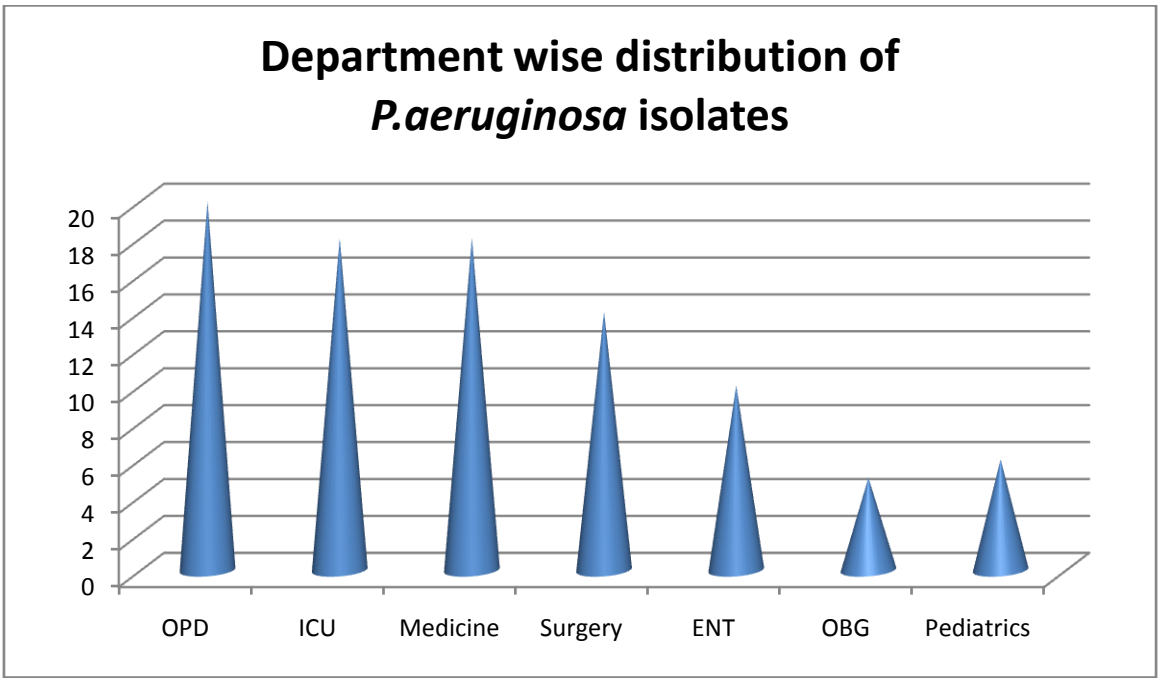

Figure 3: Department wise distribution of *P.aeruginosa* isolates. (n=91)

Table 2: Department wise distribution of *P.aeruginosa* isolates. (n=91)

Department	No. of isolates (n)	Percentage (%)
OPD	20	22
ICU	18	20
Medicine	18	20
Surgery	14	15
ENT	10	11
OBG	5	5
Pediatrics	6	7

Our study also showed that most of the *P. aeruginosa* clinical isolates were obtained from pus (45%) followed by urine (21%), sputum (19%), blood (7%), BAL (4%) and tracheal aspirate (4%). (Figure 3)

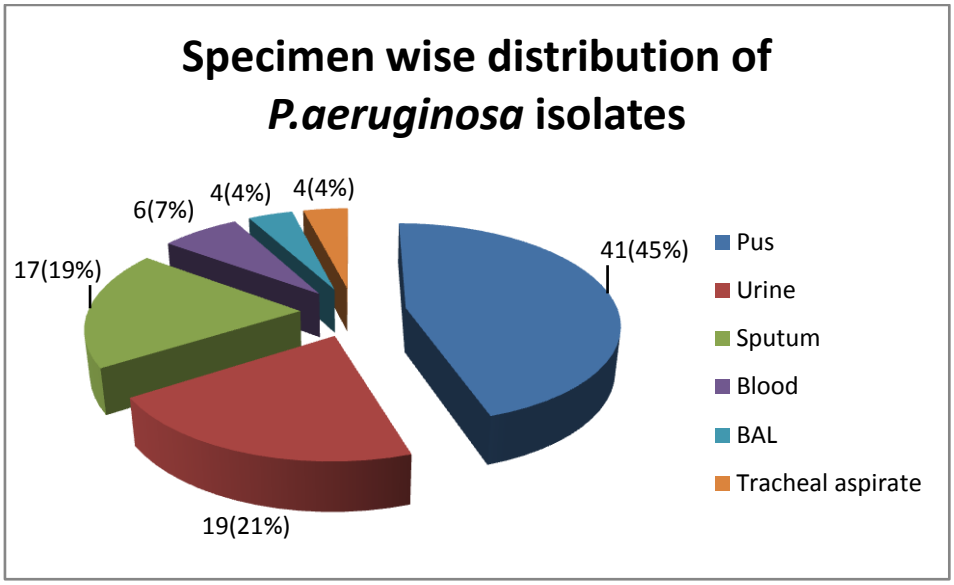

Figure 4: Specimen wise distribution of *P.aeruginosa* isolates. (n=91)

Antimicrobial susceptibility of these 91 *P. aeruginosa* isolates against 11 antimicrobial agents was shown in Table 3. We observed that the highest susceptibility was shown to polymyxins group i.e. polymyxin B (96.8%) and colistin (91.7%) followed by piperacillin-tazobactam (72.1%) and amikacin (71.6%) and the lowest to ceftazidime (21.2%) and gentamicin (49%). (Table 3)

Table 3: Antimicrobial susceptibility patterns of *P. aeruginosa* clinical isolates. (n=91)

Antimicrobial agents	Number of isolates (%)		
	Resistant		Susceptible
Amikacin	26(28.4)		65(71.6)
Gentamicin	46(51)		45(49)
Ciprofloxacin	39(43.1)		52(56.9)
Levofloxacin	37(41.2)		54(58.8)
Piperacillin-tazobactam	25(27.9)		66(72.1)
Ceftazidime	72(78.8)		19(21.2)
Aztreonam	43(46.9)		48(53.1)
Imipenem	36(39.3)		55(60.7)
Meropenem	42(46.5)		49(53.5)
Polymyxin B	3(3.2)		88(96.8)
Colistin	8(8.3)		83(91.7)

IV. Discussion

In this present study, 91 isolates of *P. aeruginosa* were obtained from 2439 samples. This showed prevalence rate of 3.73% which is comparable to the study by Tadvi *et al.* (4.15%)[16]. However, Pathi *et al.* noticed 8.43% and similar studies in Afghanistan and Greece showed the prevalence rate of 6.67% and 16.6% respectively[5, 17-18]. It was observed that, 68% of the isolates were from male patients and middle aged adult patients in the age group 45-54 years were suffered the most. Although, Pathi *et al.* reported that male patients

between 61-80 years of age had highest percentage of Pseudomonas sp. isolates which mimics other studies, where elderly males contributed 62.5- 71% of the study sample[5,19-20].

In this study, we observed that 22% of the isolates were from outdoor patients which closely matches with the findings of Rashid *et al.*(30%)[21]. Moreover, ICU (20%) and medicine department (20%) contributed significantly in our study mimicking observations of Sharma *et al.*(22.8% and 12.3% respectively)[22].

Furthermore, our study showed that most of the *P. aeruginosa* clinical isolates were obtained from pus (45%) followed by urine (21%), sputum (19%), blood (7%), BAL (4%) and tracheal aspirate (4%). Pathi *et al.*found almost comparable figures for pus (29%) ,urine(23%), sputum (18.8%) and blood (11%)[5].

In this present study, we observed that these 91 *Pseudomonas aeruginosa* isolates demonstrated highest susceptibility to polymyxins category i.e. (96.8%) and (91.7%) which is at par with the observation of Saderi *et al.* (polymyxin B and colistin 95.5% and 90.9% respectively)[23]. Colistin which was considered as last resort to treat these isolates but still there have been reports of colistin resistant *P. aeruginosa*[11,24-27].

Among the aminoglycosides, amikacin was found to be superior than the gentamicin and susceptibility to amikacin (71.6%) and gentamicin (49%) were much better than the findings of Saderi *et al.* (amikacin and gentamicin 55% and 27.3% respectively)[23] and Tadvi *et al.* (amikacin and gentamicin 56% and 55% respectively)[16]. Fluoroquinolones, monobactams and carbapenems were fairly active against these isolates (56-59%, 53%, 53-60% respectively) which also mimics results obtained by Iranian researchers[23]. However the Gujarat group noticed much higher percentage of susceptibility for fluoroquinolones (Levofloxacin: 92.66%) and carbapenems (Meropenem: 93.33%)[16].

In our study, we noticed that piperacillin-tazobactam, in the penicillins/ß-lactamase inhibitors category, was relatively better in killing these isolates (72.1%) as compared to other groups and this observation was also comparable to that of Saderi *et al.* (63.6%)[23] and Tadvi *et al.*(80.66%)[16]. But susceptibility to ceftazidime in the cephalosporin category was worse in our study (21.2%) than Iranian group (63.6%)[23] and Gujarat group (80.66%)[16].

V. Conclusion

Our study revealed that the prevalence of *P.aeruginosa* is substantial and most of these bugs were obtained from middle-aged adult male patients particularly from outdoor and ICU. Isolation of this organism was maximum from wound infections and polymyxins, undoubtedly, work best to kill it. Dissemination of this organism within an institution is a threat to infection control efforts. Active screening and compliance with recommended infection control practices play an important role in the control of health-care associated infection. Moreover, monitoring of antibiotic sensitivity pattern of *P. aeruginosa* and strict antibiotic policy with stringent implementation of antimicrobial stewardship programme are mandatory to control the situation.

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