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Phenotypic Characterization & Determination of the Antibiotic Susceptibility Pattern of Clinical Isolates of *Pseudomonas* spp. Collected from Various Diagnostic Centers of Dhaka city, Bangladesh

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Abstract: *Pseudomonas* spp. is one of the foremost etiological agent causing severe infections of hospital admitted patients. This study was carried out to monitor the drug sensitivity patterns of the phenotypically identified *Pseudomonas* spp. collected from a nmber of admired diagnostic centers of Dhaka city, Bangladesh. A total of 50 non-duplicate isolates were characterized from various patients having diverse age groups and sex. The microorganisms found from varied specimens (i.e. urine, pus, sputum, ear secretions, wound swabs) were identified by a number of phenotypic and biochemical tests. Antibiotic susceptibility patterns against 20 different antibiotics were evaluated by the Agar-disc-diffusion method to comprehend antibiotic resistance profile of the isolates. All the isolates were presumptively detected as *Pseudomonas* spp. The female patients (68%) were likely to be more susceptible to the infections caused by *Pseudomonas* spp. than their male counterparts (32%). Most of isoltaes showed multi-drug resistant (MDR) property. The highest resistance was recorded against amoxyclav 100%, nitrofurantion 100%, cotrimoxazole 100%, cefuroxime 98%, azithromycin 92 %, ceftriaxone 86%, tygecycline 78% and nalidixic acid 70%. Thee findings of this study showed that colistin was the most effective drug for the inhibition of *Pseudomonas* spp. followed by piperacillin, meropenem and amikacin.

Keywords: *Pseudomonas* spp., multi-drug resistant, effective drug.

I. INTRODUCTION

Pseudomonas is an aerobic, motile, Gram-negative bacterium and has caused trouble in diverse hospital acquired infections such as pneumonia, infection of urinary tract and skin and in severe burns. They are also one of the major causative agent for producing infections among immunosuppressed persons [1-3]. *Pseudomonas* spp. having multidrug resistant properties is particularly connected with augmented mortality because of the lack of options in healing choices [4].

Some of the mechanisms of drug resistance by *Pseudomonas* are considered to be the expression of aminoglycoside modifying enzymes, syntheis of biofilm and extensive range of β lactamases along with mutations in DNA gyrase and topoisomerase [5]. It is considered to be the fifth frequent pathogen among nosocomial infection causing microorganisms and responsible for 10% of all hospital acquired infections [6]. In the context of Bangladesh, it is third in position and produces a number of infections [7].

An immense challenge stay alive if there is an appearance of Multi Drug Resistant (MDR) *Pseudomonas* as medical practitioners are left with restricted therapeutic options. In any health care setup, preceding information about the antibiotic susceptibility profile against frequently suggested drugs would facilitate the clinicians to choose suitable antimicrobial agents against these resistant strains. The current study therefore was performed to spot the antibiogram profile of the clinical isolates of *Pseudomonas* spp. along with their phenotypic characterization at Department of Microbiology, Stamford University Bangladesh.

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II. MATERIALS AND METHODS

Collection of Samples

The samples were collected between the periods of October 2020 to November 2020. The samples were collected from two diagnostic centers of Mirpur and Dhanmondi of Dhaka city, Bangladesh. A total of 50 clinical isolates of *Pseudomonas* spp. were collected from different types of clinical samples from suspected individuals of various age and gender groups. Sample processing and transportation were maintained as per WHO guidelines [8]. All experiment was performed in the laboratory of Department of Microbiology, Stamford University Bangladesh.

Isolation and confirmation of *Pseudomonas* spp.

Pure culture of *Pseudomonas* spp. was isolated and preserved using Cetrimide aga (CA). Collection of all the strains was done aseptically and transferred into Cetrimide aga (CA). The collected samples were incubated overnight at 37°C for 24 hours and were subjected for additional investigation.

Microscopic analysis

Bacterial size, shape, and staining characteristics were performed through microscopic analysis of the isolates [9]. Gram staining procedure was done for the primary identification of the isolates, followed by different biochemical tests. Cultural and morphological characteristics of the bacterial isolates were presumptively confirmed in accorance to standard microbiological protocols [10].

Biochemical test for the confirmative identification

All isolated bacteria were identified by standard laboratory biochemical tests according to the methods described by Collee et al., 1996 (11). The biochemical tests for *Pseudomonas* spp. were indole test, MR-VP test, coagulase test, catalase test, citrate test, motility test and urease test.

Determination of antimicrobial susceptibility by disk diffusion method

Pure culture of *Pseudomonas* spp. collected from different clinical samples was chosen for determining the antimicrobial susceptibility pattern against a different group of antibiotics such as Amikacin (30μg), Amoxyclav (30μg), Azithromycin (30μg). Cefixime (5μg), Ceftazidime (30μg), Ceftriaxone (30μg), Cefuroxime (30μg), Ciprofloxacin (5μg), Colistin (10μg), Gentamycin (30μg), Imipenem (10μg), Meropenem (30μg), Nalidixic acid (30μg), Nitrofurantoin (300μg), Cotrimoxazole (30μg), Piperacillin (110μg), Doripinum (), Tigecycline (30μg), Levofloxacin (30μg) and Cepepime () by Kirby Bauer disc diffusion method [12] as recommended by clinical and Laboratory Standards Institute (CLSI) guideline 2013.

III. RESULTS

In this study, all suspected plates were screened for the existence of yellow-green colors colonies of *Pseudomonas* spp. on CA media. All assumed *Pseudomonas* spp. isolates were confirmed by Gram staining, microscopic analysis and biochemical tests. Table 1 demonstrates the biochemical test results of all presumptive *Pseudomonas* spp. isolates.

Table 1: Biochemical tests for the bacterial isolates

Assumed Organism	Coagulase Test	Indole Test	MR Test	VP Test	Citrate Test	Oxidase Test	Catalase Test	Urease Test	Motility	Nitrate Reduction Test
Pseudomonas spp.	-	-	-	-	+	–	+	+	+	+

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MR = Methyl red, VP =Voges-Proskauer,
Control organism: *Pseudomonas aeruginosa* ATCC® 27853

Age & sex distribution among study population

A total of 50 patients of suffering from suspected pseudomonad infections were included in the study during October 2020 to November 2020. 16 (32%) respondents were males while the remaining 34 (68%) were females between age group of 1-90 years.

Table 2: Distribution of age and sex among respondents

SL	Age	Total	Male	%	Female	%
1	1 to 10	3	2	67	1	33
2	11 to 20	2	1	50	1	50
3	21 to 30	5	2	40	3	60
4	31 to 40	7	4	57	3	43
5	41 to 50	7	2	28	5	72
6	51 to 60	9	2	22	7	78
7	61 to 70	9	1	11	8	89
8	71 to 80	4	0	0	4	100
9	81 to 90	4	2	50	2	50
7	Total	50	16	32	34	68

A total 50 patients either sex (male-16 and female-34) with the respective ratio of 32:68 between age group of 1-90 years were included in this study (Table 2). Here minimum age was 2 months and maximum age was 85 years old. Table 2 shows that the incidence of disease is higher in females than male and incidence of infection was much higher in the patients having age group 51 years to 70 years age groups when compared to other age groups. The rate of infection seems to vary randomly according to age without any selective pattern, although, the tendency of getting affected by different pseudomonad infections seem to be higher in between the ages from 51-70 years old whereas the lowest prevalence rate was observed between the age group from 1-20 years old according to the present study findings.
50 clinical isolates of *Pseudomonas* spp. were subjected to antimicrobial resistance test against different commercial antibiotics. With the 100% resistance against amoxyclav, nitrofurantion and cotrimoxazole demonstrated as the most ineffective antimicrobial followed by cefuroxime 98%, azithromycin 92 %, ceftriaxone 86%, tygecycline 78% and nalidixic acid 70%. However, colistin was the most effective drug against *Pseudomonas* spp. followed by piperacillin, meropenem and amikacin. The overall result of antibiotic se profile has been summarized in Table 3.

Table 3: Overall antibiobic susceptibity pattern of *Pseudomonas* spp.

SL	Antipsudomonal antibiotics tested	% No. of sensitive isolates (n=50)
1	Amikacin (30μg	86
2	Amoxyclav (30μg),	0
3	Azithromycin (30μg).	8
4	Cefixime (5μg)	8
5	Ceftazidime (30μg)	88

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6	Ceftriaxone (30μg)	14
7	Cefuroxime (30μg)	2
8	Ciprofloxacin (5μg)	80
9	Colistin (10μg)	96
10	Gentamycin (30μg)	74
11	Imipenem (10μg)	84
12	Meropenem (30μg)	86
13	Nalidixic acid (30μg)	30
14	Nitrofurantoin (300μg)	0
15	Cotrimoxazole (30μg)	0
16	Piperacillin (110μg)	92
17	Doripinum ()	82
18	Tigecycline (30μg	22
19	Levofloxacin (30μg)	58
20	Cepepime ()	66

IV. DISCUSSION

In our present study, female patients were more affected (68%) than male patients (32%). Although infection from *Pseudomonas* can occur at any age, in our present study, the patient's age ranged from 51-70 years were more exaggerated in terms of male and female patients whereas the lowest incidence rate of infection was in between 1-20 years of age. All the other age groups had almost similar types of infection rate.

Pseudomonas associated infections having multidrug-resistant feature create an economic load as these are associated with high treatment costs and longer duration of hospital stay when compared to those associated with their drug-susceptible counterparts along with their substantial morbidity and mortality [13]. There is an utmost need for the proper detection and choice of an proper antibiotic to commence therapy for an optimized the clinical outcome [14]. In the current study, the *Pseudomonas* isolates were mainly collected from pus/wound samples followed by urine, sputum and ear infected area swabs which were had some form of similarity in relation to some previous studies [15, 16]. The maximum resistance was observed in amoxyclav, nitrofurantion and cotrimoxazole, and the minimum resistance was observed against colistin. The experiential rate of antibiotic resistance in our observation against aminoglycosides such as amikacin and gentamicin showed a same pattern when compared to some with previous studies [17,18]. Drug resistance against quinolones such as ciprofloxacin showed high sensitivity in the present study which was almost similar to the findings conducted by Fadeyi et al., 2005 [19]. Cephalosporins are regarded as anti-pseudomonal drugs, particularly ceftazidime which is a third generation cephalosporin and shows efficacy in such infections [14]. However, this drug also encountered some degree of resistance (12%) in our study. The chief constraint of our study was that molecular depiction of resistant isolates particularly in case of identifying genes conferring resistant properties to the clinical isolates.

The susceptibility blueprint of antimicrobials against *Pseudomonas* has given us a guideline of the unrestrained use of antibiotics through this study. The sensible use of antibiotics by the concerned personnels such as doctors or health care professionals and attempt to control mishandling of antibiotics and procurement will aid in limiting the mounting rate of antibiotic resistance in the pathogenic microorganisms. Improper prescription of antibiotics for viral infections, against which they have no outcome or antibiotic employ for these conditions are pointless. Coherent drug policy should be in

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exercise before prescribing the effective antibiotics to the country [20]. Antibiotic management should track certain nominal rations [21].

V. CONCLUSION

Pseudomonas spp. remains a worldwide cause of nosocomial infections as well as causing life-threatening diseases such as pneumonia, meningitis, endocarditis, endophthalmitis, external otitis and septicemia [22]. In conclusion, our study emphasizes the development of public awareness regarding the prevention and spread of pseudomoad infection. Constant monitoring of the antimicrobial susceptibility pattern of *Pseudomonas* isolates for the selection of right therapy is also very much mandatory. The at hand circumstances of antimicrobial resistance in Bangladesh should be monitored critically, otherwise, it will become an irrepressible trouble not only in Bangladesh but also global in the days to come.

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