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Evaluation of Antibiotics Pattern of Extended Spectrum Beta-Lactamase Producing Multi-Drug Resistant *Pseudomonas aeruginosa*

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Abstract

Background: *Pseudomonas aeruginosa* (*Ps. aeruginosa*) is considered as an opportunistic pathogen and the leading cause of morbidity and mortality in immunocompromised individuals. Globally, approximately 10-15% of the nosocomial infections are caused by *Ps. aeruginosa*. The *Ps. aeruginosa* can acquire resistance against broad-spectrum antibiotics. According to recent studies increased mortality has been observed due to infection with extended-spectrum-beta-lactamase (ESBL) producing *Ps. aeruginosa* strains. This study was designed to determined antibiogram of ESBL producing multi-drug resistant *Ps. aeruginosa* in Khyber Pakhtunkhwa.

Methods: The clinical confirmed *Ps. aeruginosa* samples were collected according to the standard protocol, at Khyber Teaching Hospital (KTH), Peshawar. All collected samples were sub-cultured on appropriate culture media. After isolation and identification, the antibiotics susceptibility testing was performed. The detection of ESBL was carried out by the double-disc diffusion method. Carbapenemase-producing bacteria was confirmed by the modified Hodge test. Descriptive analysis was performed for statistical analysis of collected data.

Results: A total of one hundred and sixty-two (n=162) *Ps. aeruginosa* confirmed isolates were collected, in which 59.3% were male and 40.7% were from female patients. The percentages of ESBL and carbapenemase producing *Ps. aeruginosa* isolates were 5.5% and 23.5%, respectively. The multidrug resistance was observed against 27.2% isolates. Among tested antibiotics highest percentages of resistance was observed against ciprofloxacin (43%) and ceftazidime (39.5%).

Conclusion: We observed highest level of drug resistance in *Ps. aeruginosa* clinical isolates against tested antibiotics and majority of the isolates were Multi-drug resistant (MDR).



Introduction

Pseudomonas aeruginosa (*Ps. aeruginosa*) is a gram-negative, rod shaped, non-lactose fermenter and opportunistic pathogen. As an opportunistic pathogen it is considered as a leading cause of morbidity and mortality [1]. It causes both hospital and community-acquired infections [2]. Worldwide approximately 10-15% of the nosocomial infections are caused by *Ps. aeruginosa* [3]. Various virulence factors that contribute in its pathogenesis are endotoxins, exotoxins, ability to produce biofilm and different enzymes [4]. Usually, for the treatment of *Ps. aeruginosa* infections broad spectrum antibiotics such as carbapenems, extended-spectrum cephalosporin, anti-pseudomonal penicillin, and polymyxin B/colistin are used [5]. With the passage of time these antibiotics acquired resistance due to over use or misuse of these antimicrobial agents [3]. Other factors that contribute in its increase resistance are production of beta-lactamase enzymes such as extended spectrum beta lactamases (ESBLs) and metallo-beta-lactamases (MBLs), target site modification, efflux pump and biofilm formation [6]. The *Ps. aeruginosa* acquire resistance by intrinsic as well as extrinsic mechanism [7]. As a result increase in morbidity and mortality have been observed from the infections of multi drug resistant (MDR) *Ps. aeruginosa* [8].

Globally, the prevalence of ESBL and carbapenemase producing *Ps. aeruginosa* have been reported [9]. The infections caused by MDR *Ps. aeruginosa* are difficult to treat. It is important to periodically check the antibiotic susceptibility pattern of important clinical pathogens that will improve empirical treatment of clinical infections. Therefore, this study was conducted to evaluate the antibiotic susceptibility profile and phenotypic detection of ESBL and carbapenemase enzyme among clinical isolates of *Ps. aeruginosa*.

Methods

This study was carried out in Khyber Teaching Hospital, Peshawar from February to September 2019. A total of one hundred and sixty-two (n=162) *Ps. aeruginosa* confirmed samples were collected. The *Ps. aeruginosa* strains were isolated from blood, bone marrow, fluids, pus, sputum, tissue, urine, and wound etc. The collected strains were sub-cultured on Blood agar and MacConkey agar (OXIDE England) and incubated overnight. The isolated strains were confirmed by standard microbiological procedures such as colony morphology, Gram staining, and biochemical testing.

The antibiotic susceptibility testing was carried out by Kirby-Bauer disc diffusion method as per CLSI, 2018 recommended guidelines [10]. The antibiotic discs and concentrations used were as follows; ciprofloxacin (5µg), gentamicin (10µg), imipenem (10µg), sulzone (100µg), meropenem (10µg), ceftazidime (30µg), tazocin (100µg), amikacin (30µg) and polymyxin B/colistin (10µg). The bacterial suspension was prepared in a sterile normal saline solution and compared with 0.5 McFarland's turbidity standard. The tested antibiotic discs were placed on Muller Hinton agar (OXIDE England) plate and overnight incubated at 37C. The zone size interpretation

was carried out as per CLSI guidelines 2018 [10]. The *Ps. aeruginosa* ATCC 27853 reference strain was used as quality control. The imipenem resistant strains were tested for carbapenemase production by modified Hodge test [11]. ESBL detection was carried out by double disc synergy method. The disc used were amoxicillin clavulanic acid, cefotaxime and ceftazidime [12].

All the data were analyzed through statistical package for social science software version 21. The descriptive analysis (percentage and number) were performed for collected data.

Results

A total of one hundred and sixty-two (n=162) confirmed isolates of *Ps. aeruginosa* were collected in which 59.3% were from male and 40.7% were from female patients. In this study, age wise the patients were categorized into eight groups (Table no.1). Majority of the *Ps. aeruginosa* were recovered from the age grouped between 21-30 years.

Age (Years)	Male % (n)	Female % (n)	Total (Male + Female) % (n)
0-10	10.4 (10)	9.1 (6)	9.9 (16)
11-20	9.4 (9)	7.6 (5)	8.6 (14)
21-30	14.6 (14)	25.7 (17)	19.1 (31)
31-40	11.5 (11)	9.1 (6)	10.5 (17)
41-50	11.5 (11)	13.6 (09)	12.4 (20)
51-60	17.7 (17)	10.6 (07)	14.8 (24)
61-70	18.7 (18)	16.7 (11)	17.9 (29)
≥70	6.2 (6)	7.6 (5)	6.8 (11)
Total	59.3 (96)	40.7 (66)	100 (162)

Table 1: Age & gender wise distribution of *Ps. aeruginosa* clinical isolates in patients attending KTH.

The strains of *Ps. aeruginosa* were collected from nine different clinical samples (Shown in table no. 2), which includes; Urine 34.0% (n=55), Pus 30.2% (n=49), Sputum 24.1% (n=39), Wound 04.3% (n=07), Tissue 1.2% (n=2), Blood 2.5% (n=4), Bone marrow 1.9% (n=3), Fluid 1.2% (n=2) and CSF 0.6% (n=1). The highest number of specimens were collected from urine samples. All the isolates (n=162) were tested for the antibiogram against commonly prescribed antibiotics used in our locality and overall MDR has been observed as shown in table no 3.

Among the tested isolates 27.16% isolates were MDR (The isolates showed resistance to at least one antibiotic in three or more antimicrobial classes were classified as Multidrug resistant *Ps. aeruginosa*). Overall, 23.4% and 5.5% of the isolates were carbapenemase and ESBL producers respectively.

Discussion

The *Ps. aeruginosa* is a non-fermenting multidrug resistant pathogen and the main cause of hospital and

Sample	Male %(n)	Female%(n)	Total (Male + Female) %(n)	ESBL%(n)	Carbapenemase %(n)	MDR%(n)
Blood	3.1 (3)	1.5 (1)	2.5 (4)	00 (00)	5.2 (2)	(00)
Bone	2.1 (2)	1.5 (1)	1.9 (3)	11 (1)	5.2 (2)	4.5 (2)
CSF	1.0 (1)	00 (00)	0.6 (1)	00 (00)	00 (00)	00 (00)
Fluid	2.1 (2)	00 (00)	1.2 (2)	00 (00)	00 (00)	00 (00)
Pus	32.3 (31)	27.3 (18)	30.2 (49)	44.5 (04)	15.9 (6)	20.5 (09)
Sputum	21.9 (21)	27.3 (18)	24.1 (39)	00 (00)	34.2 (13)	31.8 (14)
Tissue	2.1 (2)	00 (00)	1.2 (2)	00 (00)	00 (00)	00 (00)
Urine	31.2 (30)	37.9 (25)	34 (55)	44.5 (04)	34.2 (13)	40.9 (18)
Wound	4.2 (4)	4.5 (3)	4.3 (7)	00 (00)	5.2 (2)	2.3 (1)
Total	59.3 (96)	40.7 (66)	100 (162)	5.5 (9)	23.5 (38)	27.2 (44)

Note: n: Sample number, %: percentage, MDR: Multi-Drug Resistant

Table 2: Specimen wise distribution of *Ps. aeruginosa* strains isolated from patients attending KTH.

Variants	Male n=96 %(n)	Female n=66 %(n)	Total n=162 %(n)
ESBL	5.2 (05)	6.0 (04)	5.6 (09)
Carbapenemase	25 (24)	21.2 (14)	24.5 (38)
MDR	32.3 (31)	19.7 (13)	27.2 (44)
Ciprofloxacin	50 (47)	33.3 (22)	43 (69)
Gentamicin	39.6 (38)	28.8 (19)	35.2 (57)
Suzlon	36.4 (35)	27.3 (18)	32.7 (53)
Imipenem	37.5 (36)	25.8 (17)	32.7 (53)
Meropenem	37.5 (36)	27.3 (18)	33.3 (54)
Ceftazidime	41.7 (40)	36.4 (24)	39.5 (64)
Tazocin	37.5 (36)	31.8 (21)	35.2 (57)
Amikacin	38.5 (37)	27.3 (18)	34 (55)
Colistin/polymyxin	16.7 (16)	15.2 (10)	16 (26)

Note: ESBL: Extended Spectrum Beta lactamases, MDR: Multi-Drug Resistant, n: sample number, %: percentage.

Table 3: Resistance determinants of *Ps. aeruginosa* clinical isolates among patients attending KTH.

No of resistant antibiotics	Male (n=96) %(n)	Female (n=66) %(n)	Total (male and female) n=162) %(n)
R-0	45.8 (44)	56.1 (37)	50 (81)
R-1	6.3 (6)	4.5 (3)	5.5 (09)
R-2	5.2 (5)	7.6 (5)	6.2 (10)
R-3	7.3 (7)	4.5 (3)	6.2 (10)
R-4	9.4 (9)	3 (2)	6.8 (11)
R-5	4.2 (4)	1.5 (1)	3.1 (05)
R-6	4.2 (4)	9.1 (6)	6.2 (10)
R-7	5.2 (5)	3.0 (2)	4.3 (07)
R-8	11.5 (11)	9.1 (6)	10.5 (17)
R-9	00 (00)	3 (2)	1.2 (2)

Note: R-0: No resistance, R-1: Resistant to single antibiotic, R-2: Resistant to two antibiotics, R-3: Resistant to three antibiotics, R-4: Resistant to four antibiotics, R-5: Resistant to five antibiotic, R-6: Resistant to six antibiotics, R-7: Resistant to seven antibiotics, R-8: Resistant to eight antibiotics, R-9: Resistant to nine antibiotics

Table 4: Antibiotic resistance combination among *Ps. aeruginosa* isolated from patients.

community-acquired infections [13]. The prevalence of resistant strains varies in different regions. In this study, the increase prevalence of *Ps. aeruginosa* infection was recorded in patients with age group of 21-30 years (19.1%), followed by 61-70 years (17.9%). A study

conducted at Peshawar in 2017, revealed that age group 41-61 years have a high prevalence (36.6%) of *Ps. aeruginosa* infection, which are not in consistence with the current study [14]. The possible reason that caused difference could be origin of bacterial isolation.

A study conducted at Karachi, Pakistan, showed that infection caused by *Ps. aeruginosa* is more prevalent in females (64.71%) than in men (35.29%) [15]. Comparatively we observed low prevalence among females. In our study, the resistance against Ciprofloxacin, Gentamicin, Imipenem, Suzlon, Meropenem, Ceftazidime, Tazocin, Amikacin and Colistin were 43%, 35.2%, 32.7%, 32.7%, 33.3%, 39.5%, 35.2%, 34%, and 16%, respectively. A study conducted in 2009 at North West region of Pakistan, showed resistance against Amikacin (70%), Gentamicin (25%), and Ciprofloxacin (49%) [16]. In correlation to this study, the resistance against Amikacin is less. Another study carried out at Burn Center Islamabad in 2015, reported resistance to Tazocin (Piperacillin and Tazobactam) 80.55%, Imipenem (63.88%), Ciprofloxacin (44.44%), Polymyxin/colistin (36.11%), Suzlon (Cefoperazone and Sulbactam) 30.55%, ceftazidime (11.11%) and Amikacin (8.33%) [17]. We observed a low level of resistance against Amikacin and Ceftazidime. The reported resistance in another study were as follows; Ciprofloxacin (60%), Cefepime (57%), Levofloxacin (56%), Ceftazidime (53.9%), Amikacin (53%), Gentamicin (51%) and Tazobactam/Piperacillin 81(37.9%) [16].

The reported frequency of carbapenemase and ESBL producer from Lahore was 3.4% and 12.5% respectively, and all the strains were multidrug-resistant [18]. Our results of ESBL are in consistence with the previous report whereas a slight increase have been observed in case of carbapenemase. The frequency of MDR-ESBL and MDR-carbapenemase were almost similar with the results of the previous study. Ullah *et al.* at Peshawar in 2014 reported the frequency of MDR 2.75% (n=102/3700) [19].

Conclusively, we identified *Ps. aeruginosa* clinical isolates from a tertiary care hospital. Majority of the studied isolates were ESBL producers and MDR. This increase prevalence of MDR will lead to treatment failure of infection caused these strains. This study recommends development of alternative treatment regimen. Furthermore, periodical surveillance and observation of resistance to antimicrobial agents at local and national level will be better for management of bacterial infections and for effective empirical therapy.

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Competing interest

All the authors declare that they have no competing interest that can affect the current study.

Authors' Contribution

Conceived and designed the experiments: Anees Muhammad, Ihsan Ali, Muhammad Owais
 Performed the experiments: Anees Muhammad, Sadiq Noor Khan, Irfan Qadir Afridi
 Analyzed the data: Anees Muhammad, Muhammad Owais and Nasir Ali
 Contributed materials/analysis/tools: Ihsan Ali, Sadiq Noor Khan and Nasir Ali
 Wrote the paper: Ihsan Ali, Muhammad Owais, Irfan Qadir Afridi and Nasir Ali
 Critical Review: Ihsan Ali, Sadiq Noor Khan, Irfan Qadir Afridi and Nasir Ali

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