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Prevalence of Carbapenem Resistance Among *Pseudomonas Aeruginosa* Isolates Obtained From Various Clinical Samples

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ABSTRACT

Background: The most powerful drug considered for treating multi-drug resistant Gram-negative bacilli infections is beta lactam ring containing antibiotics, Carbapenems. Carbapenems which are available in India for use are meropenem and imipenem. Few bacteria especially *Pseudomonas* species has been reported to show resistance against these antibiotics. *Pseudomonas aeruginosa*, Gram-negative bacilli from last decades are emerging as prevalent and potent causative agents of nosocomial infection because of emerging antimicrobial resistance due among these microorganisms.

Aim & Objectives: The goal of this study is to ascertain the prevalence of Carbapenem resistance in *P. aeruginosa*.

Method: All clinical samples were processed by standard microbiological procedures and isolates so obtained were identified according to the CLSI guidelines. The isolates of *P. aeruginosa* obtained were processed for antimicrobial susceptibility pattern by Kirby Bauer disk diffusion method. Carbapenem resistant isolates were further studied for carbapenemase production by Modified Hodge test.

Result: Out of total 956 clinical samples the number of *Pseudomonas* spp. isolated were 45. Among these 45 isolates resistance to meropenem was 20 (44.44%), which was more than the resistance to imipenem 17 (37.78%). Resistance to other antibiotics was also high which includes aminoglycosides (44-66%), and β-lactams (50-86%). Only 27(60%) were sensitive to poly-myxin B and 24(55.56%) to Tobramycin. Maximum resistance bacteria were isolated from intensive care units (ICU). Finally, by Modified Hodge test 7 (15.55%) isolates were obtained which produced Carbapenemase.

Conclusion: High rates of *P. aeruginosa* resistant to carbapenems is increasing overall burden of infection in the hospitals by reducing the availability of effective agents. There is a need for initiating

ISSN: 0971-1260
Vol-22-Issue-17-September-2019

some policy changes to prevent such resistance against highly effective drugs like meropenem and other carbapenems.

Keywords: Antimicrobial resistance, Carbapenem, Kirby Bauer Test, Modified Hodge's Test, *Pseudomonas aeruginosa*.

INTRODUCTION

Nosocomial infection also known as hospital acquired infection is commonly caused by several infectious organisms which are present in the hospital environment [1]. One of the most potent etiological agents of nosocomial infections is *Pseudomonas aeruginosa*. [2]. It has the ability to adapt itself in different environmental conditions [3]. *P. aeruginosa* is actively motile by a polar flagellum & is non-capsulated. However, many strains of this spp. have a mucoid cover. It has a characteristic feature of producing number of pigments, the best known being pyocyanin & fluorescein [4].Various types of infections are caused by these bacteria, for example, urinary tract infection (UTI) and respiratory tract infection (RTI) in cystic fibrosis patients and behave as a potent opportunistic pathogen incase of immunocompromised patients [5].

Carbapenem is the class of β-lactam antibiotic having a broad spectrum of antibacterial activity. They have been the drug of choice incase of multidrug resistant bacteria [6, 7]. It is one of the most active groups of β-lactam antibiotics against *P. aeruginosa* . *Pseudomonas* spp. has been increasingly becoming resistant to cephalosporin drug used in the health care settings that leads to increased use of stronger β-lactam antibiotics. Misuse has resulted in carbapenem resistance in a gram-negative organism such as *P. aeruginosa* [8, 9].

Carbapenems, including meropenem and imipenem, are the last-line antibiotics used extensively to treat the infections caused by MDR-*Pseudomonas aeruginosa* [7, 10]. The multifactorial carbapenem resistnace mechanism includes over-expression of efflux-pumps, carbapenemase production, mutation in OprD gene and AmpC overproduction [7, 11, 12]. Out of theses resistance mechanisms, carbapenemase production is one of the most important resistant mechanism. So, due to development of drug resistance, *P. aeruginosa* related infection remains to be disturbing, with 18% to 61% mortality rate [13].

ISSN: 0971-1260
Vol-22-Issue-17-September-2019

MATERIALS AND METHODS

In the course of 4 months study (January 2018 to April 2018) at a tertiary care hospital in Jalandhar, from various clinical samples including urine, pus, sputum, tissue, high vaginal swab samples collected from In Patient Department (IPD), Carbapenem resistant *P. aeruginosa* was isolated on different agar medium including chocolate agar, blood agar and MacConkey agar. These isolated strains were further distinguished through colony morphology, Gram staining, oxidase test and glucose fermentation test.

The antibiogram report was constructed for the clinical isolates obtained by Kirby Baeur disc diffusion method [14]. Carbapenemase production activity of resistant strains so obtained was detected by Modofied Hodge test[15].

RESULTS

In this study from 956 clinical samples collected and processed, a total of 45 *P. aeruginosa* isolates were obtained over an interval of 4 months (January 2018 to April 2018)

Table No. 1 Age and sex wise distribution of *P. aeruginosa* isolates

Age group(years)	Female(no.)	Male(no.)	Total (no.)%
<20	2	3	5(11.11%)
21-40	3	7	10(22.22%)
41-60	8	9	17(37.78%)
>60	7	6	13(28.89%)
Total	20	25	45(100%)

From 45 isolates of *P. aeruginosa*, 25 (55.56) isolates were obtained from males and 20(44.44%) from females. Maximum number of isolates obtained belong to 41-60 years age group.

Table No. 2 Clinical sample wise distribution of *P. aeruginosa*

Samples	No. of Isolates	%
Urine	7	15.56
Pus	17	37.78
Sputum	9	20
Tissue	2	4.44
ET Secretion	8	17.78
HighVaginal Sample	2	4.44
Total	45	100

ISSN: 0971-1260
Vol-22-Issue-17-September-2019

Maximum isolates of *P. aeruginosa* were yielded from pus 17(37.78%), followed by sputum 9(20%), ET secretion 8(17.78%), urine 7(15.56%), tissue 2(4.44%) and high vaginal sample 2(4.44%).

Table No. 3 Antibiogram of *P. aeruginosa* isolates

Antibiotic	Resistance no. (%)	Sensitive no. (%)
Aminoglycosides		
Ofoxacin	30(66.67%)	10(22.22%)
Gentamycin	12(26.67%)	29(64.44%)
Amikacin	40(88.89%)	2(4.44%)
Tobramycin	11(24.44%)	27(60%)
Netilmycin	20(44.44%)	17(37.78%)
Fluroquinolones		
Ciprofloxacin	19(42.22%)	25(55.56%)
B-lactams		
Ceftriaxone	34(75.55%)	7(15.56%)
Cefoperazone	22(48.89%)	15(33.33%)
Ceftazidime	25(55.56%)	12(26.67%)
Cefotaxime	27(60%)	8(17.77%)
Cefoxitin	41(91.11%)	1(2.22%)
Cefepime	35(77.77%)	4(8.89%)
Pipracilin/tazobactam	27(60%)	9(20%)
Cefperazone/sublactam	37(82.22%)	4(8.89%)
Amoxycilin/clavulanate	40(88.80%)	2(4.44%)
Meropenem	20(44.44%)	18(40%)
Imipenem	17(37.78%)	16(35.56%)
Polymyxin B	15(33.33%)	24(53.33%)

Among 45 isolates of *P. aeruginosa* from various clinical specimen, 20(44.44%) isolates were resistant to meropenem and 17(37.78) to imipenem. Resistance to other antibiotics was also high which includes aminoglycosides (44-66%) and fluoroquinoles (42.22%). Around (50-86%) were resistant to β lactams. Only 24(53.33%) were sensitive to poly-myxin B and 27(60%) to Tobramycin.

ISSN: 0971-1260
Vol-22-Issue-17-September-2019

Table No. 4 Ward wise distribution of Carbapenem Resistant *P. aeruginosa*

Ward	No. of Resistant isolates	%
Paediatric	1	5.88
OBG	1	5.88
ENT	3	17.65
Medicine	2	11.77
Surgery	1	5.88
Orthopaedics	1	5.88
ICU	5	29.41
HDU	1	5.88
Emergency	2	11.77
Total	17	100

Among 45 isolates of *P. aeruginosa*, the no. of carbapenem-resistant *Pseudomonas* in different wards was 17. The maximum no. of resistance was found in ICU (29.41%), followed by ENT (17.65%), Medicine & Emergency (11.77%) and resistance in other wards was 1 (5.88%)

Table No. 5 Modified Hodge Test positive for *P. aeruginosa*

Total no. of *Pseudomonas*	MHT positive	Percentage (%)
45	7	15.55

From 17 Carbapenem resistant isolates, 7 (15.55%) isolates were able to produce carbapenemase enzyme.

DISCUSSION

P. aeruginosa, gram negative bacteria is a ubiquitous microorganism that can act as an opportunistic pathogen and is responsible for hospital acquired infection. Carbapenem

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resistant *Pseudomonas* spp. is a major concern, as many resistant isolates *Pseudomonas* spp. are detected in nosocomial infection. Among the various carbapenem resistance mechanisms described for *P. aeruginosa*, efflux pump, β-lactamases production and loss of porins can be highlighted.

Age and gender wise distribution

In our study the maximum number of *P. aeruginosa* were isolated from male patients 25(%) and minimum were isolated from female patients 20(44.44%). Most of the isolates were procured from 41-60 years age group (37.78%) and minimum from >20 years age group (11.11%)

Current study is similar to the study of Saroj Golia *et.al*, 2016**,** the rate of isolation of *P. aeruginosa* was 24 %. Maximum isolates (66.6%) were from males and mimimum (33.3%) from females. Most of them were from 41-60 years age group (40, 33.3%) [16].
Anotherr study by Viren A. Javiya *et.al*, reported maximum isolates from males (62.5%) than from females (37.5%) [17].

Sample wise distribution

From the total 45 isolates of *P. aeruginosa* the maximum strains were derived from pus (n=17) 37.78%, followed by sputum (n=9) 20%, ET secretion (n=8)17.78%, urine (n=7)15.56%, Tissue & High vaginal sample (n=2)4.44% as shown in Table 3. The present study is similar to the study of Saroj Golia *et.al*,2016, where 120 strains of *P. aeruginosa* obtained were identified and the maximum strains of *P. aeruginosa* were derived from wound, accounting for 55.83% of the total number, followed by sputum(20%), Ear discharge, Tracheal aspirate (8.33%), urine(5%), High vaginal sample(1.66%) [18]

Another study by Anita Nandi *et.al* maximum number of isolates were procured from pus sample (n=58) and minimum from other body secretions (n=3) [19].

Antibiotic resistance pattern of Pseudomonas aeruginosa

In this study from 45 isolates of *P. aeruginosa*, 20(44.44%) were meropenem resistant and 17(37.78%) were imipenem resistant. Around (44-66%) resistance was found in aminoglycosides, and fluoroquinoles were (42.22%) resistant. Around (50-86%) were resistant to β lactams. Only 24(53.33%) were sensitive to polymyxin B and 27(60%) to

ISSN: 0971-1260
Vol-22-Issue-17-September-2019

Tobramycin shown in Table 4. Prakash *et al*. (2014) showed 22% imipenem resistant isolates that is lower than the one reported in current study (33%) [20]

Ward wise distribution of Carbapenem resistant *P. aeruginosa*

In present study, maximum number of carbapenem-resistant isolates were found in ICU (29.41%), then in ENT (17.65%), followed by Medicine and Emergency ward (11.77%), the minimum was isolated in Pediatric, surgery, OBG and HDU ward (5.88%) shown in Table 5. In the study of Iara Rossi *et.al*, 2017, maximum number of Carbapenem-resistant *P. aeruginosa* was isolated from ICU (55.07 %%) ward then from Surgery (53.07%), this study is similar to current study[21].

Modified Hodge test

In this study 7(15.55%) isolates were given MHT positive result. Whereas the study by A Amjad et.al, 2011 reported that out of a total 200 isolates showing intermediate or susceptible zone i.e16mm-21mm for imipenem, 138(69%) were positive for production of carbapenemase by Modified Hodge test [15].

Conclusion:

Treating infectious diseases is becoming a more challenging task with each passing year. The opportunistic pathogen *P. aeruginosa* being one of the most important example because of its potential of developing a multidrug-resistant phenotype. Carbapenem resistance is newly emerging in isolates of *P. aeruginosa.* High rates of Carbapenem resistant *P. aeruginosa*, reduces the availability of effective agents. Only few antibiotics like polymyxin B and Tobramycin are known to be effective against these carbapenem-resistant *Pseudomonas,* but these drugs have low safety profile and associated with attributable mortality in the patient treated by them. So the lack of new antibiotics effective against multidrug-resistant gram-negative bacilli necessitates need for initiating some policy changes to prevent such resistance against highly effective drugs like meropenem and other carbapenems.

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