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ORIGINAL ARTICLE

Incidence of Metallo Beta-Lactamase Producing Pseudomonas Aeruginosa in Diabetes and Cancer Patients

HENNA KHALID[1], MARIAM RIAZ[2], SARA NAJEEB[3], NAZMA KIRAN[4]
[1]*Associate Professor, Pathology, Women Medical and Dental College, Abbottabad, Pakistan*
[2]*Associate Professor, Pathology, Women Medical and Dental College, Abbottabad, Pakistan*
[3]*Associate Professor Pathology (Microbiology), Mohi-ud-Din Islamic Medical College, Mirpur Azad Kashmir*
[4]*Associate Professor Department of Patholoy, Rai Medical College, Sargodha*
Corresponding author: Mariam Riaz, Email: drmriaz23@gmail.com

ABSTRACT

Background and Aim: Nosocomial infections could be caused by strains of metallo-beta-lactamase (MBL)-producing Pseudomonas aeruginosa. Public health issues are associated with Pseudomonas aeruginosa, a major healthcare associated pathogen. However, the prevalence of Pseudomonas aeruginosa in diabetes and cancer patients are yet to be determined. The present study aimed to determine the prevalence of metallo-beta-lactamase (MBL)-producing Pseudomonas aeruginosa in diabetes and cancer patients.

Methodology: This cross-sectional study was conducted on 186 P. aeruginosa isolates taken from diabetes and cancer patient's samples in the Department of Pathology, Ayub Teaching Hospital Abbottabad from March 2017 to February 2021. Clinical outcome and incidence of P. aeruginosa in diabetes and cancer patients have been investigated. A CLSI-guideline-based susceptibility test was performed on these isolates to assess their susceptibility to anti-pseudomonal drugs. A disc potentiation test using imipenem and meropenem discs impregnated with EDTA was performed on them to screen for MBL production. SPSS version 26 was used for data analysis.

Results: Out of 186 P. aeruginosa isolates, about 54 (29%) had shown resistance to carbapenems (imipenem and meropenem) and MBL producers were found in 42 (22.6%) isolates. Out of 42 MBL producer isolates, the prevalence of diabetes, cancer, and both diabetes and cancer were found in 30 (71.4%), 8 (19%), and 4 (9.5%) respectively. The combine therapy of amikacin, piperacillin with tazobactam, and colistin were responded by 7 (16.7%) whereas 35 (83.3%) patients responded to the combination therapy of gatifloxacin, amikacin, and piperacillin with tazobactam.

Conclusion: The present study found that the prevalence of MBLs was 22.6%. Consequently, It has been reported that the rapid dissemination of MBL producers makes surveillance studies a priority, as well as the proper selection of antibiotics, especially carbapenems. MDR P. aeruginosa infections may be treated with polymyxins, aaminoglycoside or fluoroquinolone molecules without therapeutic MBL inhibitors.

Keywords: Metallo-beta-lactamase-producing, Pseudomonas aeruginosa, diabetes, Cancer patients

INTRODUCTION

Pseudomonas aeruginosa is amongst the utmost prevalent causes of healthcare related nosocomial infection such as septicemia and pneumonia worldwide [1]. When isolates expressing ESBL remain susceptible to carbapenemase and are resistant to all antibiotics except tigecycline, colistin, and polymyxin B it presages an era of untreatable conditions to come [2]. MBL, Ambler molecular type B beta lactamases, also known as Ambler-class B beta lactamases, are predominantly responsible for carbapenem resistance. They recognize bivalent metal ions and have the ability to hydrolyze all lactams, including carbapenems [3]. Carbacenems, which are largely active against Enterobacteriaceae and non-fermenters, are recommended for treating infections caused by ESBL-producing Enterobacteriaceae, particularly Escherichia coli and Klebsiella pneumonia. Acinetobacter spp and.Pseudomonas spp. are the most common non-fermenters [4].

Australian Imipenem (AIM), IMP (Imipenamse), Meyrol, SPM1 (São Paulo Metallo-Beta-lacamase), GIM (German imipenem), and VIM (Verona Integron-encoded metallo-beta lactamase are five different kinds of MBLs whose incidence is increasing [5]. VIM and IMP are the most major MBLs. With the worldwide increase in prevalence and types of MBLs, early detection is critical, the advantages of that embrace timely implementation of strict infection management practices and treatment with different antimicrobials. Of the MBL production available testing methods, the imipenem (IMP)-EDTA combined disc test is the most suitable method in terms of sensitivity and specificity. Acinetobacter spp and Pseudomonas spp. could be detected by (IMP) 10 µg-EDTA 750 µg combined disc test with sensitivity 95.7% and specificity 91% [6, 7].

Acquired metallo beta lactamases have been considered as emerging resistance mechanisms. Because of their ability to hydrolyze all beta-lactams, including carbapenems, these strains are not prone to inhibitors like sulfones and clavulanate of therapeutic serine β-lactamase. Additionally, high mobile elements carry their genes consenting easy diffusion. The higher rate of morbidity and mortality are associated with MBL-producing isolates and invasive infections [8]. The MBL-isolate positivity occurrences are not only causing therapeutic issues due to the hospital environment but severely affect the infection control management. Healthcare related infections are mostly caused by Acinetobacter baumannii and Pseudomonas aeruginosa [9]. Therefore, the detection of such organisms are difficult and pose substantial challenges, especially their unnoticed spreading and participating ability in gene transformation with other pathogens in horizontal MBL. The present study aimed to determine the prevalence of metallo-beta-lactamase (MBL)-producing Pseudomonas aeruginosa in diabetes and cancer patients.

METHODOLOGY

This cross-sectional study was conducted on 186 P. aeruginosa isolates took from diabetes and cancer patient's samples in the Department of Pathology, Ayub Teaching Hospital Abbottabad from March 2017 to February 2021. Clinical outcome and incidence of P. aeruginosa in diabetes and cancer patients have been investigated. A CLSI-guideline-based susceptibility test was performed on these isolates to assess their susceptibility to anti-pseudomonal drugs. A disc potentiation test using imipenem and meropenem discs impregnated with EDTA was performed on them to screen for MBL production. Standard microbiological methods were used for identification and differentiation of Gram-negative bacilli that appears microscopically and as non-lactose fermenting colonies on MacConkey's agar plates. The inoculated blood is incubated on MacConkey's agar properly and their isolates appeared as opaque, flat pigmented colonies, and grayish medium size producing a grape like odour either hemolytic or non-hemolytic on blood agar plates.

According to the CLSI guidelines, Antimicrobial susceptibility testing (AST) was used for all the confirmed isolates of P. aeruginosa using a single disc diffusion method. Selected antibiotic

discs were used for testing on Mueller Hinton (MH) agar plates. Each plate was inspected after aerobic incubation for 24 hours at 37°C. Based on CLSI interpretive criteria, the inhibited zones were recorded and inferred as susceptible (S), intermediate (I) or resistant (R). P. aeruginosa isolates were confirmed and their antimicrobial susceptibility patterns were tested and sub-cultured by aerobic incubation for 24 hours at 37 °C on blood agar plates.

SPSS version 26 was used for data analysis. Numerical variables were expressed as mean and standard deviation. Continuous variables were described as frequency and percentages. Chi-square test was used for comparing different groups. All the descriptive statistics were carried out using 95% confidence interval and 5% level of significance.

RESULTS

Out of 186 P. aeruginosa isolates, resistance to carbapenems (imipenem and meropenem) were found in about 54 (29%) and MBL producers in 42 (22.6%) isolates. Out of 42 MBL producer isolates, the prevalence of diabetes, cancer, and both diabetes and cancer were found in 30 (71.4%), 8 (19%), and 4 (9.5%) respectively as shown in Figure-1. The combine therapy of amikacin, piperacillin with tazobactam, and colistin were responded by 7 (16.7%) whereas 35 (83.3%) patients responded to the combination therapy of gatifloxacin, amikacin, and piperacillin with tazobactam. Gender's distribution is shown in Table-1. Patients were distributed based on their age groups as follows; 15-30 years, 31-45 years, and >45 years as shown in Figure-2. P. aeruginosa isolates producing MBL had higher percentage from respiratory sample 18/42 (42.8%) followed by urine 12/42 (28.6%) and pus and exudate sample 11/42 (26.2%). The remaining one isolate had an ear sample 1/42 (2.4%) as shown in Figure-3. Isolates of positive MBL producing P. aeruginosa distributed based on risk factors are shown in Table-II. Antimicrobial susceptibility patterns of 30 P. aeruginosa isolates producing MBL is represented in Table-III.

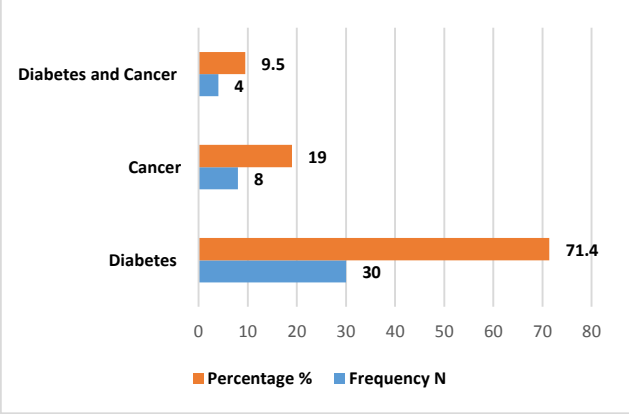

Figure-1: MBL isolates comorbidities conditions (n=42)

Table-1: Gender's distribution of P. aeruginosa isolates producing MBL

Gender	Frequency N	Percentage %
Male	17	40.5
Female	25	59.5
Total N (%)	42	100

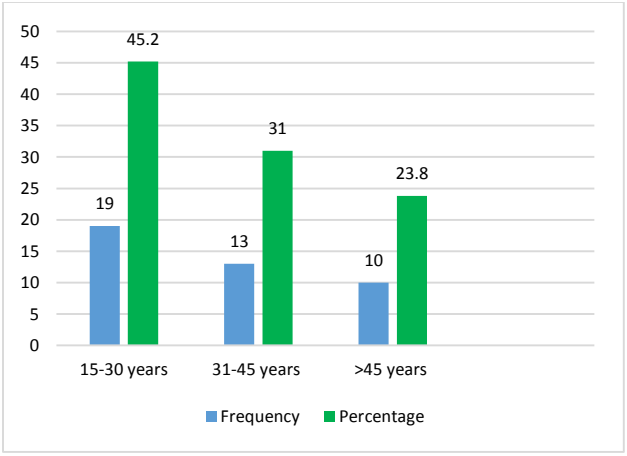

Figure-2: Age-wise distribution of P. aeruginosa isolates producing MBL (n=42)

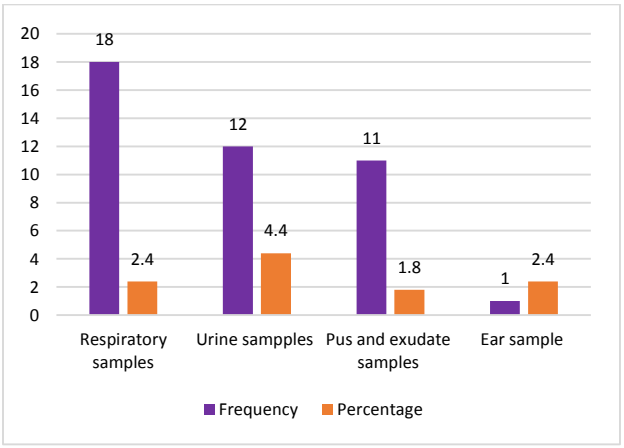

Figure-3: Prevalence of different samples in P. aeruginosa isolates producing MBL (n=42)

Table-2: Isolates of positive MBL producing P. aeruginosa distributed based on risk factors

Risk Factors	MBL producing P. aeruginosa isolates N (%)	P-value
Age (years)	36.4±6.8	0.873
Hospital duration (days) <7 >7	 12 (28.6) 30 (71.4)	0.426
Comorbidities conditions Diabetes Cancer Diabetes and cancer	 30 (71.5) 8 (19) 4 (9.5)	 <0.001 <0.002 <0.003
Devices Mechanical Urinary Catheter	 11 (26.2) 11 (26.2)	0.428

Table-3: Antimicrobial susceptibility patterns of 30 P. aeruginosa isolates producing MBL (n=42)

Combine therapy	Susceptible N (%)	Intermediate N (%)	Resistance N (%)	Total N (%)
Amikacin, piperacillin with tazobactam, and colistin	1 (2.4)	2 (4.8)	4 (9.5)	7 (16.7)
Gatifloxacin, amikacin, and piperacillin with tazobactam.	6 (14.3)	9 (21.4)	20 (47.6)	35 (83.3)

DISCUSSION

The present study mainly focused on the incidence of metallo-beta-lactamase (MBL)-producing Pseudomonas aeruginosa in diabetes and cancer patients and found that Pseudomonas aeruginosa and Acinetobacter species like bacteria are involved in MBLs. Simple detection techniques such as combined disc test are not recommended methods as per CLSI guidelines. The MBLs most prevalent gene alleles could be determined with genotypic

detection or molecular typing [10]. A standardized phenotypic test must be used for determining the multi-drug resistance and straining with increasing prevalence in diagnostic laboratories and hospital settings. Globally, nosocomial infection are caused by isolates of MBL producing Pseudomonas aeruginosa [11].

In the current study, 186 isolates of P. aeruginosa were investigated and prevalence of MBLs was 22.6%. Behbahani et al. reported a lower prevalence of P. aeruginosa isolates that produced 15.2% MBLs in P. aeruginosa isolates [12]. The development of rapid and remarkable resistance offered to antibiotics are the main concerns about P. aeruginosa isolates. Mechanism of antimicrobial resistance wide array described that P. aeruginosa isolates are contender to Gram-negative pathogens of non-fermentative and represents their swifty response to environmental pressure [13].

Despites the availability of progressive antimicrobial agents for infected patients treatment, Pseudomonas aeruginosa isolates causing nosocomial infections and their complications are a major contributing factor for morbidity and mortality. Though numerous studies reported various epidemiological aspects of nosocomial infections caused by isolates of Pseudomonas aeruginosa 14, 15]. Limited susceptibility of Pseudomonas aeruginosa isolates to antimicrobials have been reported in various studies [16, 17].

The positive isolates of MBLs is not only limited to therapeutic issues but severe apprehension for infection control management. A significant risk has been imposed in terms of unnoticed spreading of these infections with other pathogens due to the difficulties of detection [18]. Various methods are used for MBLs detection produced by P. aeruginosa, out of which three key methods were used in the present study; i) imipenem plus EDTA combination and E-test, ii) combined disk test, and iii) reduction in MIC. A previous study reported that the incidence of isolates of P. aeruginosa provided resistance to imipenem was 16% whereas the positive isolates of P. aeruginosa was 17.6% [19]. Mohammad et al [20] reported that resistance of P. aeruginosa isolates was 14.5% and MBLs producer were 4.4% positive among P. aeruginosa isolates.

Of the positive and negative isolates of MBLs, piperacillin/tazobactam combination had maximum sensitivity was 53% as compared to other studies according to which piperacillin / tazobactum had maximum susceptibility among drugs [21, 22]. In fact, combining antibiotics may lead to overuse and the emergence of drug resistance. Combination therapy should adequately cover relevant pathogens for maximum effect while minimizing the risk of drug resistance. Yet, in recent decades, increasing carbapenem resistance was reported in Pseudomonas aeruginosa isolates. The occurrence of positive strains MBLs varies with location and other parameters such as age, gender, multidrug resistance, and length of hospitalization.

CONCLUSION

The present study found that the prevalence of MBLs was 22.6%. Consequently, It has been reported that the rapid dissemination of MBL producers makes surveillance studies a priority, as well as the proper selection of antibiotics, especially carbapenems. MDR P. aeruginosa infections may be treated with polymyxins, aminoglycosides or fluoroquinolone molecules without therapeutic MBL inhibitors.

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