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Original Research Article

Prevalence of Metallo-β-Lactamase producing *Pseudomonas aeruginosa* in tertiary care center (BPKIHS)

Gurung R[*1], Shrestha S[2], Baral R[1], Poudyal N[1] and Khanal B[1]

[1]*Department of Microbiology, BPKIHS, Dharan, Nepal*
[2]*Department of Microbiology, Nepal Medical College, Kathmandu, Nepal*

QR Code

***Correspondence Info:**
Dr. Gurung R
Department of Microbiology,
BPKIHS, Dharan, Nepal

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Abstract

Background: Carbapenem resistance in Pseudomonas aeruginosa is an emerging threat and matter of concern, therefore detection of metallo-β-Lactamase (MBL) - producing *P. aeruginosa* is crucial in preventing its spread to other Gram negative bacteria.
Aims and Objective: To determine the prevalence of MBL production and antimicrobial resistance in *P. aeruginosa* isolated from in-patients and out-patients of a tertiary care center in Eastern Nepal.
Methodology: Identification and antimicrobial susceptibility pattern of isolated *P. aeruginosa* from different clinical samples received in Microbiology laboratory was performed following CLSI guidelines and MBL production was determined by IPM-EDTA - disk synergy test.
Results: A total of 137 *P. aeruginosa* strains were isolated from different clinical samples, out of which 79.6% isolates were resistant to Carbapenem, and among them 35.78% isolates were MBL producer. Overall prevalence of MBL production among *P. aeruginosa* was found to be 31.4%.
Conclusion: This study reveal high level of resistance being exhibited by a notorious organism like *P. aeruginosa* to commonly used antimicrobials for treatment of infection, and an alarming threat of MBL production necessitating a routine screening for MBL production. Also a more effective, less toxic and cheap antimicrobial for the future is imperative.
Keywords: Antimicrobial resistance, Ethylenediamine-tetraacetic acid, *Pseudomonas aeruginosa*.

1. Introduction

Infection caused by *Pseudomonas aeruginosa* (*P. aeruginosa*) is common, with the burden of infection mostly among hospitalized patients. *It is reported* to be the second most common organism isolated in nosocomial pneumonia, the third most common organism isolated in both urinary tract infections (UTIs) and surgical site infections (SSIs), and the fifth most common organism isolated from all sites of nosocomial infection.[1] The problem of antibiotic resistance in *P. aeruginosa* is on the increase.[2] Frequently encountered multidrug- resistant (MDR) bacterial isolates like Ceftazidime- resistant *P. aeruginosa* is common and prevalent in a hospital environment.[3]

MDR *P. aeruginosa* is inherently resistant to many drug classes and is able to acquire resistance to all effective antimicrobial drugs[4], it elaborates inactivating enzymes that make beta-lactams and carbapenems ineffective, such as Extended Spectrum Beta Lactamases (ESBLs) and Metallo- β-lactamases (MBLs).[5] Consequently, treatment options are narrowed down to a few antibiotics. Carbapenems are the antibiotics of choice for severe pseudomonas infections.[6] However, there is paucity data from Nepal, which have systematically studied the underlying mechanisms for MBL resistance among *P. aeruginosa* isolates. Therefore, this prospective study was carried out to determine the prevalence of MBL production and antimicrobial resistance in *P. aeruginosa* isolated from different clinical samples received from in-patients and the out-patients of a tertiary care center in Eastern Nepal.

2. Materials and Methods

A cross sectional study conducted over a period of one year (2011) at Microbiology laboratory, BP Koirala Institute of Health Sciences (BPKIHS). All clinical specimens received for culture and Antimicrobial

Susceptibility Testing (AST) from In-patient and Out-patient were included. Isolation of *P. aeruginosa* was done on Blood agar and MacConkey agar plates, further identification and antimicrobial susceptibility pattern against Piperacillin(100µg), Carbenicillin(100µg), Ceftazidime(30µg), Ceftriaxone(30µg), Tobramycin(10µg), Amikacin(10µg) and Ciprofloxacin(5µg) according was determined using Clinical and Laboratory Standards Institute (CLSI) guidelines on Muller Hinton Agar (MHA) by disc diffusion method.[7] Standard antibiotics discs used in this study were purchased from HiMedia India.MBL production was determined using standard Ethylenediamine-tetraacetic acid (EDTA) disc synergy test method.[8]

3. Results

One hundred thirty seven strains were confirmed as *P. aeruginosa* by specific biochemical test. Out of which 64.2% was isolated from exudates (Figure 1). Overall MBL production in *Pseudomonas* was found to be 31.4%, amongst which MBL production detected in clinical sample was 69.8%, 20.9% and 9.3% from exudates, blood and urine samples respectively (Figure-2). High degree of resistance was exhibited by *P. aeruginosa* against commonly used antimicrobial agents with highest being seen against Ceftazidime (89.83%) followed by Amikacin (86.1%), Piperacillin (81%) (Figure-3).Almost all antibiotics showed equal number of resistance to MBL producing *P. aeruginosa* (Table 1).

Figure 1: Distribution of Pseudomonas isolates in different samples

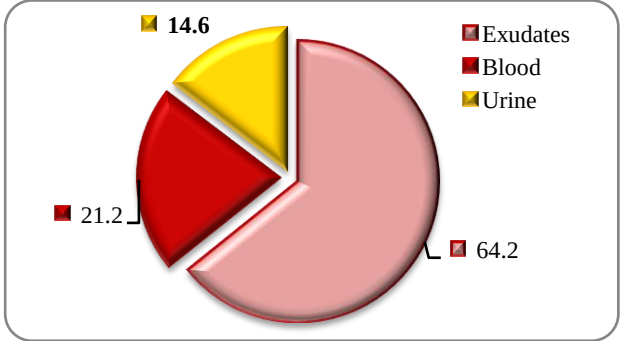

Figure 2: Distribution of MBL positive Pseudomonas aeruginosa in different samples

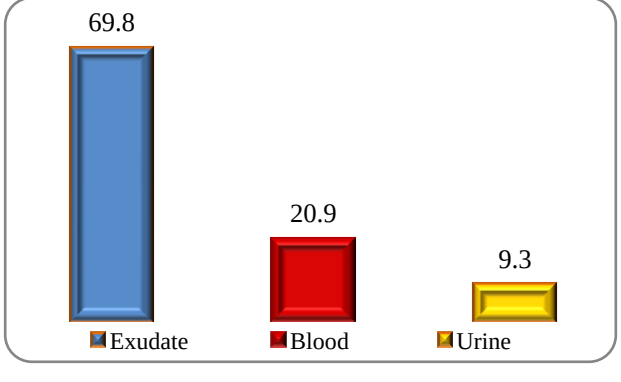

Figure 3: Antibiotic resistance pattern of *Pseudomonas aeruginosa* isolates

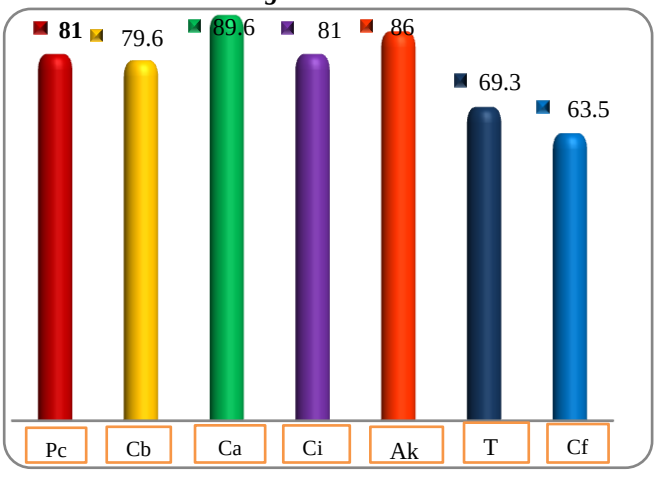

Table I: Antibiotic resistance pattern of MBL producing and non MBL producing *Pseudomonas aeruginosa*

Antimicrobial agents	MBL Producer (%)	MBL Non-producer (%)
Piperacillin (n=111)	35.13	64.87
Carbenicillin (n=109)	35.78	64.22
Ceftazidime (n=123)	33.33	66.67
Ceftriaxone (n=111)	35.13	64.87
Amikacin (n=118)	33.05	66.95
Tobramycin (n=95)	32.63	67.37
Ciprofloxacin (n=87)	34.48	65.52

4. Discussion

Spread of MBLs capable of hydrolyzing carbapenems in *P. aeruginosa* and other major gram-negative pathogens has been a worrying problem in Japanese hospitals since early 1990, and recent reports suggest that a similar phenomenon is now also emerging in Europe and other parts of the world.[9] The occurrence of an MBL-positive isolate in a hospital setting poses a therapeutic problem, as well as a serious concern for infection control management. The accurate identification and reporting of MBL-producing *P. aeruginosa* will aid infection control practitioners in preventing the spread of this multidrug-resistant isolates.[10]

Over a period of one year total, 137 isolates were processed for identification of MBL production. Among these majorities of isolates were from exudates followed by blood and urine. According to various studies, MBL production ranged from 7-65%.[11] In our context, overall MBL production was 31.4 %, where the percentage of MBL production among the carbapenem resistant isolates was found to be 35.78%. Our prevalence is correlating well with other studies (30.3 – 36%).[10-14]

Our Prevalence of MBL in *P. aeruginosa* does not correlate with other studies across the country. In a study by Baniya *et al*[15], 16.4% of MBL production *P. aeruginosa* was reported and in another study performed in Kathmandu by Thapa P *et al* reported 14.29% of *P. aeruginosa*.[16] This variation seems to reflect the different diagnostic

methods and the different rates of antibiotics used in different places.

Apart from being imipenem resistant, MBLs were resistant to important groups of antibiotics tested, including the third-generation cephalosporins, aminoglycosides, and quinolones a characteristic feature of MBL producers.[10,13] For MBLs, limited treatment options are available and the only therapeutic option may be polymyxins, but it should not be used as monotherapy. It can be combined with an appropriate aminoglycoside.[13,17]

5. Conclusion

This study revealed a high level of resistance to commonly used antimicrobials for treatment of infection with a notorious organism like *P. aeruginosa*, in addition to the alarming threat of MBL production necessitates a routine screening for MBL production. Also, a more effective, less toxic and cheap antimicrobial for the future is imperative.

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