Study of metallo-β-lactamase production in nosocomial nil fermenter gram-negative bacterial isolates from clinical samples in a tertiary hospital

Sir,

In the past, carbapenems have been the main stays of the infectious disease community for serious infections because of their broad-spectrum activity and stability to hydrolysis by most of the β-lactamases, including extended spectrum β-lactamases (ESBLs).[1] However, in the last decade, outbreak of carbapenem-resistant *Pseudomonas aeruginosa* and *Acinetobacter* spp. due to metallo-β-lactamases (MBLs) have been reported from different regions. MBLs have spread to other species of gram-negative bacilli.[2] These MBLs belong to Ambler's class B and Bush group 3 classification of ESBL. They can hydrolyze all classes of β-lactams except aztreonam and their activity cannot be inhibited by β-lactam inhibitors.[3] The present study was undertaken considering the paucity of data on MBL producing nosocomial nil fermenter gram-negative isolates from Kumaun region.

A total of 44 nil fermenters consisting of 32 *Pseudomonas* spp. and 12 *Acinetobacter* spp. were screened for MBL production by double disk synergy test (DDST) as described by Lee *et al.*[4] and disk combination test (DCT) as described by Young *et al.*,[5] using ceftazidime (CAZ) and imipenem (IMP) with 0.5 M ethylenediaminetetraacetic acid (EDTA; 750 µg/disk).

CAZ-EDTA combined disk test and DDST detected 36.36% and 20.45% MBL producers, respectively. IMP-EDTA DCT detected 22.7%, while IMP-DDST detected 13.8% MBL producers [Table 1].

Of the 32 *Pseudomonas* spp., 22 were CAZ resistant and 8 were IMP resistant. Five isolates detected by DCT were negative by DDST. Of the 12 *Acinetobacter* spp., 6 were resistant to CAZ and 4 to IMP. Two isolates detected by CAZ-EDTA combination test were negative by DDST. Three *Pseudomonas* spp. and one *Acinetobacter* spp. sensitive to IMP were found to be MBL producer by CAZ-EDTA DCT [Table 2].

The clinical utility of carbapenem is under threat with emergence of acquired carbapenemases, particularly Ambler's class B MBLs.[1] The occurrence of an MBL-positive isolate in a hospital environment not only poses a therapeutic problem, but also is a serious concern for infection control management.[3]

In this study, 29.5% isolates were found to be MBL producers which is in agreement with the study by Hemlata *et al.*[6] CAZ-EDTA gave better results than IMP-EDTA similar to that reported in other studies from India.[6,7]

In this study, we also found three *Pseudomonas* spp. and one *Acinetobacter* spp., sensitive to IMP, to be MBL producers as detected by DCT. The correlation between carriage of MBL genes and carbapenem resistance is often imperfect. It is quite possible that the gene pool is more extensive than what we can detect and is either in a quiescent state or is not being detected, or both.[2] With the emergence of carbapenem-sensitive MBLs, screening only carbapenem-resistant isolates would miss MBL production in carbapenem-sensitive strains.

Table 1: Comparison of methods of MBL production using CAZ and IMP disks

Bacterial species (*n*)	Resistant to		MBL detection by CAZ-EDTA disk		Detected by both methods	MBL detection by IMP-EDTA disk		Detected by both methods
	CAZ	IMP	DCT	DDST		DCT	DDST	
Pseudomonas spp. (32)	22	8	11	06	05	08	04	04
Acinetobacter spp. (12)	6	4	05	03	02	02	02	02
Total (44)	28	12	16	09	07	10	06	06

DCT, disk combination test; DDST, double disk synergy test; CAZ, ceftazidime; IMP, imipenem; MBL, metallo-β-lactamase; EDTA, ethylenediaminetetraacetic acid

Table 2: MBL production by IMP-sensitive and IMP-resistant isolates by using CAZ-EDTA and IMP-EDTA disk combination test

Bacterial species	IMP-resistant MBL +ve		IMP-sensitive MBL +ve	
	CAZ-EDTA	IMP-EDTA	CAZ-EDTA	IMP-EDTA
Pseudomonas spp.	08	06	03	0
Acinetobacter spp.	04	02	01	0
Total	12	08	04	0

CAZ, ceftazidime; IMP, imipenem; MBL, metallo-β-lactamase; EDTA, ethylenediaminetetraacetic acid

Behra *et al*.[3] have recommended MBL screening for all CAZ-resistant isolates. Based on the findings of our study, we conclude that combination disk method is better than DDST. CAZ-EDTA combination disk could pick additional isolates of MBL producers as compared to IMP-EDTA disk. Hence, it could be used as a convenient method in the clinical microbiology laboratories. Though our sample size is low, microbiology laboratories must evaluate the various screening methods for detection of MBL in order to correctly report this important mechanism of antimicrobial resistance.

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