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Original Research Article

A Study of Metallo Beta Lactamase Production in Clinical Isolates of Imipenem Resistant Gram Negative Bacilli

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ABSTRACT

In the clinical isolates of Gram negative Bacilli, Metallo Beta lactamases (MBL) production had been seen. Isolates resistant to Imipenem were screened for MBL production by EDS (EDTA Disc Synergy) test, Modified Hodge test and MIC Reduction test. There was comparison among these methods. Rapid detection of MBL producing Gram negative bacilli is necessary to prevent their dissemination and also it provides therapeutic guidance.

Key words: MBL (Metallo Beta Lactamase), MIC (Minimal Inhibitory Concentration), EDS (EDTA Disc Synergy).

INTRODUCTION

Carbapenem hydrolysing Metallo Beta lactamases, (MBL) effectively hydrolyse Betalactam antibiotics and confer resistance to penicillins, cephalosporins and carbapenems. [6] The emergence of carbapenem hydrolysing enzyme had been reported first in Japan. [1] There may be several other mechanisms for carbapenem resistance such as minimal drug penetration due to mutation in porins, loss of certain outer membrane proteins and efflux mechanisms [19] etc.

The resistance may spread rapidly among various gram negative bacilli, as the MBL genes placed in mobile gene cassettes of integrons. [3,8] Infections due to this resistant gram negative Bacilli are often difficult to treat because of its pathogenic nature and the limited drug of choice. [7,12,17] Hence rapid detection of MBL producing gram negative Bacilli is essential to control infections and dissemination [3]

The aim of this study is to collect Imipenem resistant strains by Kirby Bauer disc diffusion method, performing MIC and to detect the production of MBL by EDTA Disc Synergy (EDS) test, Modified Hodge test and MIC reduction test. Comparison among these methods aid to find the simplest and accurate method for the detection of MBL.

MATERIALS AND METHODS

This study was conducted from Jan 2013 - Jan 2016 at Christian Mission Hospital, Madurai. A total of 76 E. coli, 60 Pseudomonas sp., 9 Klebsiella sp., 3 Proteus sp., 1 Citrobacter sp., 2 Salmonella typhi and 1 Shigella sp., These organisms were isolated from various samples like Urine, Pus, Blood, Stool and Sputum of both out patients and in patients admitted to different wards, were sent to the laboratory for diagnosis and antibiogram.

All these isolates were undergone antibiogram for Imipenem resistance by the routine Kirby - Bauer Disc diffusion using CLSI manual. [2,9,15] A Total of 25 E.coli, 42 Pseudomonas sp., 2 Klebsiella sp., 1 Proteus sp., and 1 Citrobacter sp., were resistant to Imipenem. Their MIC values for Imipenem were also found. The imipenem resistant strains were subjected to EDS test, Modified Hodge test, and MIC reduction test.

Modified Hodge test: The test strains resistant to imipenem were undergone to Modified Hodge test for detection of Carbapenemases. A 24 hour liquid culture of ATCC 25922 strain Escherichia coli adjusted to 0.5 McFarland standard was lawn cultured on the surface of a Mueller-Hinton agar .After drying, 10 ug imipenem disc was placed at the centre of the plate and the test strain was streaked in four different directions from the edge of the disc to the periphery of the plate .The plate was incubated overnight at 37^{o} C. The presence of a `clover shaped' zone of inhibition due to by the test strain was considered as positive.

EDTA disk synergy (EDS) test: It was done with imipenem and EDTA discs for detection of Metallo Beta Lactamases in the imipenem resistant isolates.

A 0.5 M EDTA solution was prepared by dissolving 1.86g of disodium EDTA.2H_2O (NICE chemicals, India) in 10ml of distilled water. The pH was adjusted to 8.0 by adding either Hcl or NaOH and was sterilized using autoclave. 18-24 hours culture in peptone water of the test isolate was adjusted 0.5 McFarland standard and spread on the surface of a MHA plate. A 10ug imipenem disc (HI-MEDIA, Mumbai, India) was placed on the agar. An empty disc prepared from Whatmann filter paper no. 1 had incorporated with 10ul of 0.5 M EDTA was kept 10mm edge-to-edge apart from the imipenem disc. After incubating overnight at 37^{o} C, the appearance of zone between the two discs was interpreted as positive for MBL production.

MIC Reduction test:

MIC reduction test of imipenem was done by agar dilution method. [10] EDTA (1 ml solution of 0.5M) was added to 1 ml of the imipenem. Mix 2 ml of EDTA and imipenem with 18 ml of molten Mueller Hinton agar and poured on plates that were allowed to set. A loopful of test inoculums was spot inoculated on these plates. The reading was taken after overnight incubation. The highest dilution of imipenem that inhibits the growth of the organism was taken as MIC. The four fold reduction from the previous MIC of these strains without EDTA confirmed that the strains were MBL producer.

RESULTS

A total of 154 isolates of 5 various gram negative Bacilli were included in the study. A total of 25 E. coli, 42 Pseudomonas, 2 Klebsiella, 1 Proteus and 1 Citrobacter were found to be Imipenem resistant by the routine antibiogram methods. Minimal inhibitory concentration of these strains for Imipenem is shown in Table.

Table 1

	MIC For Imipenem												
	>256 mg/l	256 mg/l	128 mg/l	64 mg/l	32mg/l	16 mg/l	8 mg/l	4 mg/l	2 mg/l	1 mg/l	0.5 mg/l	0.25 mg/l	0.125 mg/l
E.coli (25)	1	1	6	8	5	1			1		1	1	
Pseudomonas (42)	8	4	13	4	5	1	1	1	1		1	2	1
Proteus (1)									1				
Klebsiella (2)				2									
Citrobacter (1)				1									

All the 71 strains were screened for MBL production by EDS test, Modified Hodge Test, MIC reduction test. 12/25 E.coli, 19/42 Pseudomonas was found to be positive in EDS method. 6/25 E.coli, 9/42 Pseudomonas and 1/1 Citrobacter were

found to be positive by Modified Hodge test. 15/25 E.coli and 23/42 Pseudomonas was found to be positive in MIC reduction Test.

DISCUSSION

This study was undertaken to detect the presence of MBL among Imipenem resistant gram negative Bacilli. This study also reveals the best and the simple method of detection of MBL.

Antibiotic resistance has evolved since fifty years and leads to serious medical problems in hospitals all over the world. [13] One of the most important mechanisms of microbial resistance to Beta lactum antibiotics is hydrolysis by production of Beta lactamases. Among the Beta lactamases, MBL s is most important because it hydrolyse majority of drugs including carbapenem. [19]

Imipenem is the carbapenem antibiotic which is used for various bacterial infections especially caused by Gram negative Bacilli. Though various mechanisms of Imipenem resistance like lack of drug penetration, Mutation, efflux mechanisms, loss of certain outer member proteins, the production of MBL is of great concern and its detection is helpful to prevent their dissemination.

In our study 46% of Imipenem resistance was documented. Sarkar [18] et al., in his study had reported 36.36% of resistance to imipenem. In contrast, G. Agarwal [5] et al., could be detected only 8.05% of imipenem resistance. Minimal Inhibitory Concentration for Imipenem were performed and found the resistance concentration. E.coli (76%) showed their MIC for imipenem ranges between 128 mg/l and 32 mg/ and Pseudomonas (81%) showed their MIC for imipenem ranges from 256 mg/l and 32 mg/l.

In this study, among 25 Imipenem resistant E.Coli 18 were found to produce MBL and among 42 Imipenem resistant Pseudomonas, 29 were found to produce MBL. 1 Imipenem resistant citrobacter were found to produce MBL.

Of these three methods, EDS method and MIC reduction test together detected MBL production in 50% of E.Coli and 48.27% of Pseudomonas. Enwuru [4] et al., documented 50% of E.coli was producing MBL. Johann D.D Pitout [11] et al., reported that 46 % of pseudomonas were producing MBL. M.J.C Noyal [13] et al., could detect MBL production 50% of Pseudomonas. In contrast, MBL Pseudomonas isolates in the study of G. Agarwal [5] et al., Navneeth [16] et al., Hemalatha [7] et al., Mandiratta [14] et al., were 8.05% 12%, 14%, 8.62 % respectively.

MIC Reduction method found additional 11.11% of E.Coli and 13.79% of Pseudomonas which were missed by other methods. Modified Hodge test missed 66.66% of MBL producing E.Coli and 76.86% of MBL producing Pseudomonas. In contrast to other two methods, Modified Hodge test detected MBL producing strains with <2 mg/l MIC. Repeatation of this test also revealed the same result.

Table 2

	MBL Production		
	E.coli (25)	**Pseudomonas (42)**	**Citrobacter (1)**
EDS Method	12	19	
Modified Hodge Test	6	9	1
MIC Reduction Test	15	21	

EDS method also detected as equal to MIC Reduction method. Considering on tedious procedure and time consumption, we consider EDS as the simple and effective method in the detection of MBL. Minimal inhibtory concentration ranges from >256mg/l to 32mg/l were found to produce MBL. Enwuru et al., reported that isolates with MIC >4ug/ml were found to produce MBL. The detection of MBL determines the appropriate therapy for Imipenem resistant gram negative bacilli. The combination of colistin and rifampicin was recommended for the treatment of MBL producing Imipenem resistant gram negative bacilli.

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