Indian Journal of Basic & Applied Medical Research; December 2011: Issue-1, Vol.-1, P. 38-46

"THE STUDY OF MBL PRODUCERS IN GRAM NEGATIVE ISOLETS FROM ICUs AND WARDS"

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ABSTRACT:

MBL producing gram negative bacteria have been recognized to be among the most important nosocomial pathogens. Identification and reporting of MBL producing organisms will aid in preventing the spread of multi drug resistant isolates.

The prospective study was conducted in the Sassoon General Hospital, Pune.

Total number of 1546 Gram negative bacteria, were isolated from various clinical samples like pus, sputum, blood, urine, CSF and other fluids. Clinical isolates were tested for resistance to carbapenem class of drug with Imipenem by modified Kirby-Bauer disc diffusion technique (CLSI guidelines).

300 (19.04%) serial isolates showed resistance to Imipenem. These samples were screened by Imipenem-EDTA disc method, out of these 59 (19.67%) were found to be MBL producers. The MBL producers consisted of P.aeruginosa (57.63%), Acinetobacter.spp (38.98%) and 1.69% each of Ecsh.coli and K.pneumoniae. The study showed that the ICUs (most from medical ICU) formed a major share in harboring MBL producers (57.63%) as compared to wards. P.aeruginosa and Acinetobacter.spp were the major MBL producing organism from the ICUs as well as wards.

INTRODUCTION:

MBL producing gram negative bacteria have been recognized to be among the most important nosocomial pathogens. In clinical settings proliferation of MBL producing gram negative bacteria will pose a serious global problem in future. MBL's have been identified from clinical isolates worldwide with increasing frequency for over the past few years. Strains producing those enzymes have been responsible for prolonged nosocomial outbreaks that were accompanied by serious infections. Surveillance of MBL producer's identification and reporting will aid infection control practitioners in preventing the spread of these multi drug resistant isolates. It will also help in therapeutic guidance for

confirmed infections. MBL can hydrolyze beta lactams from all classes except the Mono bactams. Higher mortality has been reported in patients infected with the IMP-1 producing strains. The need for early recognition of MBL producing strains, rigorous infection control measures and restricted clinical use of broad spectrum of beta lactams, including carbapenems is significant.

The present study was conducted to evaluate, prevalence of gram negative isolates for the resistance to Imipenem and to confirm production of MBL in the Imipenem resistant isolates.

MATERIAL AND METHODS: This prospective Study was conducted in Sassoon General Hospital, Pune from June 2007 to June 2008. In this study, total of 1546 gram negative clinical isolates were screened for presence of resistance to IMIPENEM according to CLSI guidelines (2007). Indoor patient's samples were from, pus, sputum, blood, urine, CSF, other fluids and secretions like pleural fluid, ascitic fluid, tracheal secretion. All the samples were collected with strict aseptic precautions and were immediately processed without any delay. A total of 300 isolates were found to be resistant to IMEPENAM by the Modified Kirby-Bauer Disc diffusion technique (MKBDDT) The detection of MBL production was performed by phenotypic test IMIPENAM-EDTA disc method.

RESULTS AND OBSERVATIONS:

Table 1: Total samples resistant to Carbapenems by Modified Kirby-Bauer's disc diffusion method

ORGANISM	TOTAL NO. OF ISOLATES TESTED	NO. OF ISOLATES RESISITANT TO CARBAPENEM	% RESISTANT
P.aeruginosa	265	143	53.96
Acinetobacter.spp	313	125	39.94
Esch. Coli	400	20	5
K. pneumonia	248	12	4.84
Citrobacter.spp	100	0	0
Proteus.spp	220	0	0
Total	**1546**	**300**	**19.40**

Table 2: Detection of MBL by Imipenem-Imipenem EDTA disc method

Organism	**No. of isolates resistant by MKBDD method**	**Isolates positive by Imp-EDTA disc method**
P.aeruginosa	143	34
Acinetobacter	125	23
Esch.coli	20	1
K.pneumoniae	12	1
Total	**300**	**59**

All the carbapenem resistant isolates were tested by Imipenem-EDTA disc method showed 59 (19.67%) Carbapenem resistant isolates as MBL producers.

Table 3: Distribution of MBL producers amongst various organisms

Organism	**Isolates producing MBL**	**% of Isolates**
P.aeruginosa	34	57.63
Acinetobacter.spp	23	38.98
Esch.coli	1	1.69
K.pneumoniae	1	1.69
Total	**59**	**100**

Amongst the MBL producing isolates, P.aeruginosa was the most prevalent one (57.63%)

Table 4: Percentage of Carbapenem resistant isolates producing MBL by phenotypic methods.

Organism	**No. of isolates resistant to carbapenem**	**No. of isolates producing MBL**	**% Of isolates producing MBL**
P.aeruginosa	143	34	23.78
Acinetobacter.spp	125	23	18.4
Esch.coli	20	1	5
K.pneumoniae	12	1	8.33
Total	**300**	**59**	**19.67**

Thus out of 143 P.aeruginosa isolates cultured during the study, 32(23.78%) were MBL producers. Similarly, 23(18.40%) of the Acinetobacter.spp out of 125 were MBL producers.

Table 5: Distribution of MBL producing isolates among different samples.

Specimen	**No. of Organism**				
	P.aeruginosa	Acinetobacter.spp	Esch.coli	K.pneumoniae	Total no (%)
Blood	2	5	0	1	8(13.56)
Pus	12	10	0	0	22(37.29)
Urine	15	5	1	0	21(35.59)
Fluid	2	1	0	0	3(5.08)
CSF	2	0	0	0	2(3.39)
Tracheal sec”n	1	2	0	0	3(5.08)
Total	**34 (57.63%)**	**23 (38.98%)**	**1(1.69%)**	**1(1.69%)**	**59 (100%)**

Table 6: Distribution of MBL producing strains in the hospital.

Discipline	**No of cases**		**Total No (%)**
	Ward	ICU	
Medicine	8	23	31(52.54)
Obs/Gyn	6	7	13(22.03)
Surgery	5	3	8(13.55)
Cardiac surgery	1	0	1(1.69)
Pediatrics	0	1	1(1.69)
Orthopedics	4	0	4(6.78)
ENT	1	0	1(1.69)
Total	**25(42.37)**	**34(57.63)**	**59(100)**

Table 7: Distribution of Pseudomonas aeruginosa producing MBL in Ward/ICU

Discipline	**No of cases**		**Total No (%)**
	Ward	ICU	
Medicine	6	12	18 (52.94)
Surgery	3	1	4 (11.76)
Obs/Gyn	3	4	7 (20.59)
Cardiac surgery	1	0	1(2.94)
Pediatrics	0	1	1(2.94)
Orthopedics	3	0	3(8.82)
ENT	1	0	1(2.94)
Total	**17(50%)**	**17(50%)**	**32(100)**

MBL producing P.aeruginosa were equally distributed amongst the wards and ICUs. Amongst the ICUs, it was found to be most prevalent in the Medicine ICU 12/17(70.59%)

Table 8: Distribution of MBL producing isolates in the Ward and ICU

Organism	**No of cases**		**Total No (%)**
	Ward	ICU	
P.aeruginosa	17	17	34(57.63)
Acinetobacter.spp	9	14	23(38.98)
Esch.coli	0	1	1(1.69)
K.pneumonia	0	1	1(1.69)
Total	**26(44.97%)**	**33(55.93%)**	**59(100)**

Most commonly isolated MBL producer was P. aeruginosa (57.63%)

Table 9: Organisms resistant to Carbapenems and producing MBL

Organism	**Total no. of isolates tested**	**No. of isolates positive for MBL**	**%Of total isolates producing MBL**
P.aeruginosa	265	34	12.83
Acinetobacter.spp	313	23	7.35
Esch.coli	400	1	0.25
K.pneumoniae	248	1	0.4
Citrobacter.spp	100	0	0
Proteus.spp	220	0	0
Total	**1546**	**59**	**3.82**

Thus 3.82% of the total isolates tested produced MBL. The organism wise comparison revealed that, out of the total P.aeruginosa tested, 12.83% were MBL producers.

DISCUSSION:

In the present study 19.4% of isolates obtained were Carbapenem resistant, which is in accordance with Jesudasan et al i.e. 18.4% (1). A study by Taneja et al (2004) reported a higher incidence (36.4%) of Carbapenem resistant strain in nosocomial UTI (2). This might be due to selection of patients suffering from nosocomial UTI who were treated with broad spectrum anti-biotics.

All the 300 isolates showing Carbapenem resistance were screened for presence of MBL by using IMIPENEM-EDTA Disc method 59 (19.67%) strains were detected producing MBL amongst the 300 Carbapenem resistant isolates.

Among the 59 MBL producing isolates P.aeruginosa comprised of 34/59 (57.63%) Acinetobacter.spp 23/59 (38.98%) and Esch.coli and K. pneumoniae one each (1.69% each). The result also revealed that 34/143(23.78%) of all carbapenem resistant P.aeruginosa isolates produced MBL.

The results revealed that P.aeruginosa isolates were 34/143(23.78%), Acinetobacter.spp (18.4%), K.pneumoniae 8.33% and Esch.coli 5%. P.aeruginosa was the commonest MBL producer amongst the isolates in this study. Pitout et.al Canada (2005) showed a prevalence of 46% MBL producing P.aeruginosa amongst all Pseudomonas which were resistant to Carbapenem (3)

In the present study the figures are almost half that of Pitout study in Canada, this could be be use of large number of broad spectrum of antibiotics in the patients.

The percentage is less in the present study, signifies the controlled use of broad spectrum antibiotics with limited resources. Stunt et.al. (1998) from Scotland demonstrated a 13% prevalence of MBL producing Pseudomonas amongst all Carbapenem resistant P.aeruginosa strains (4). These figures are slightly lower than the present study, might be due to gradual increase in the acquisition of plasmid bearing the blaIMP gene from the hospital environment over a period (3). Magalhaes et.al Brazil (2005) showed 62.5% P.aeruginosa produced MBL (5).

Patients selected by Pitout et al and Magalhaes et al were critically ill having multiple infections and receiving large numbers of antibiotics leading to selection of MBL producing P.aeruginosa.

Navneeth et al Bangalore (2002) found 12% and Mendiratta et al Nagpur (2005) showed prevalence of 8.62% (6) MBL producing Carbapenem resistant P.aeruginosa isolates whereas, Hemlata et al (2005) reported higher rate i.e. 87.5% because isolation was from critically ill patients from the ICUs only.

In the present study, Esch.coli (5%) and K. Pneumoniae (8.33%) MBL producers were detected. In India presence of MBL producers in other gram negative bacteria are not reported much. This has to be reviewed carefully as the MBLs have spread from the non fermenters to

other gram negative enteric bacilli. This will make the resistance scenario more critical as time passes.

In the present study, highest number of MBL producers i.e. 22 (37.29%) were noted from pus specimens followed by urine specimens 21(35.59%) whereas Lee et al (Korea) and Butt et al (Pakistan) demonstrated a high number of MBL producers in urine (7)

The high numbers of MBL producers in the present study are isolated from pus and urine reveals that such organisms might have been acquired by the patients from the hospital environment. This signifies that the transmission could have been person to person, so the necessity of proper hand washing by the health personnel and the visitors, while attending the patients is necessary.

There is a significant presence of MBL producers, P.aeruginosa and Acinetobacter.spp are noted in pus and urine specimen, in the present study.

Blood specimen showed a high prevalence of MBL producing Acinetobacter.spp as compare to other such isolates. Other body fluids like CSF, Tracheal fluid, harbored very few MBL producing organisms (CSF2, tracheal secrition3, fluids3)

The present study revealed the higher number of MBL producers from the ICUs (57.63%) as compared to wards (42.37%) proving ICU as the epicenter for multi-drug resistant organisms. Lee et al also reported an isolation of 31.7% of MBL producers from ICUs in Korean hospitals (7).

Amongst the ICUs maximum number of MBL producers were isolated from medical ICUs followed by surgical ones. This shows importance of hospital environment as source of MBL producing organisms the environment in the ICUs is more vicious due to their co-morbid conditions along with more invasive procedures super added with irrational and extensive use of antibiotics.

It was found that, the ICUs are harbored by the MBL producing isolates - P.aeruginosa (57.63%) to the highest followed by Acinetobacter.spp (38.98%) and Esch.coli (1.69%), K.pneumoniae (1.69%). Thus it was evident that MBL genes from P. aeruginosa and Acinetobacter.spp are spreading to organisms from Enterobacteriaceae family. Peleg et al. (2004) reported same findings from clinical settings in A Australia (8)

Thus the MBLs have recently emerged as one of the most worrisome resistant organism, owing to the capacity of these bacteria to hydrolyze almost all known beta lactam agents. Also the concerned genes are carried on highly mobile elements which allow their easy dissemination of such organisms among other gram negative bacteria (9). Treatment of this multi drug resistant organism is difficult as very limited options are available.

Extended surveys of Human infections with MBL producers have not been done. Hence the suitable treatment options remain unknown. In vivo studies have shown that Aztreonam in high doses reduces the bacterial load and may be a useful drug (9). The other alternative is the use of Polymixin, which has

a promising outcome so far against gram negative bacilli. Advancement of modern medicine may have prolonged the life of man, but it has also brought problems like “drug resistance'!

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This original research work was conducted in Sassoon General Hospital, Pune from June 2007 to June 2008 by Dr. Wankhede Sachin Vasantrao with Dr. Iyer V.S., Dr. Bharadwaj R.S.

Conflict of Interest: Nil, Source of support: Nil

Date of Initial Acceptance: 7 October 2011

Date of Peer Review Approval: 21 November 2011

Date of Final Approval: 26 November 2011

Date of Publication: 1 December 2011