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Carbapenemases Mediated Resistance Among the Isolates of Neonatal Septicemia

VinodKumar C.S[1], Prasad B.S[2], Kalapannavar N.K[2], RaghuKumar K.G[1], Yogeesha Babu K.V[1], Jayasimha V.L[1], Basavarajappa K.G[1]

[1] Department of Microbiology, [2] Department of Pediatrics,
S. S. Institute of Medical Sciences and Research Centre, Davangere, India
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Abstract

Materials & Methods: This study was carried out in Microbiology Department of Gulbarga University, Gulbarga to find out the frequency of metallo beta lactamase producing gram-negative bacilli isolated from neonatal septicemic cases. Methods: This study was conducted for the period of 5 years. A total of 471 consecutive Gram-negative bacilli were recovered during the study period from blood samples. Metallo beta lactamase detection in these isolates was carried out by modified Hodge method and EDTA disk synergy test on Mueller Hinton agar. Escherichia coli ATCC 25922 and K. pneumoniae ATCC 700603 were used as control strains.

Results: The frequency of MβL producing gram-negative bacilli among the neonatal septicemic cases was 68.4%.

Keywords: MβL, Enterobacteriaceae, Neonatal septicemia, modified Hodge test

Introduction:

Neonatal bacterial sepsis is one of the major cause of morbidity and mortality in neonates. It continues to be one of the four leading causes of neonatal mortality in our country[1,2].

In recent years, the metallo-b-lactamases (MBLs) have emerged as one of the most feared resistance mechanisms because of their ability to hydrolyse virtually all β-lactam agents, including the carbapenems, and because their genes are carried on highly mobile elements [3]. So far, five major clinically important groups of MBLs have been identified: IMP, VIM, SPM, GIM and the recently described SIM2,3Initially limited to south-east Asia, MBLs have rapidly spread through Europe, followed by Latin America, especially after 2000 [3]. Recently, MBLs have been described in North America and Oceania, while their prevalence has continued to increase in many countries from the other continents, giving the problem a worldwide dimension [3-5].

The spread of these enzymes in nosocomial Gram-negative rods, particularly among *P. aeruginosa* isolates, severely limits therapeutic options for infections by these pathogens [5]. Although the potential threat of acquired MBLs is no longer questioned, the clinical impact of and the optimal therapy for infections caused by organisms producing these enzymes remain unknown [6,7]. The aim of the present study was to assess the incidence of MβL mediated resistance among the gram negative bacterial isolates in neonatal sepsis.

Material And Methods

A total of 471 consecutive non-duplicate gram-negative bacilli recovered from 1647 suspected neonatal septicemic cases during the five years study period. Two samples of blood were collected aseptically from each patient. About 2ml of blood was added immediately into 20ml of Brain-heart infusion broth with 0.025% sodium polyethol sulphonate as anticoagulant (HI-Media Laboratories, Mumbai). The bottles were incubated for 7 days and subcultures were done

Address Correspondence to:
Dr. VinodKumar C.S, Department of Microbiology,
S. S. Institute of Medical Sciences and Research Centre,
NH-4, Bypass, Davangere-577005, Karnataka, India
E-mail; vinodmicro@yahoo.com, Contact no; 9964402525

approximately. Aerobic bacteria were isolated and identified by standard microbiological methods [1,2].

Modified Hodge test

The Imipenem resistant strains were subjected to modified Hodge test for detection of carbapenemases. An overnight culture suspension of Escherichia coli ATCC 25922 adjusted to 0.5 McFarland standard was inoculated using a sterile cotton swab on the surface of a Mueller-Hinton agar (MHA) (HI-MEDIA, Mumbai, India). After drying, 10 µg imipenem / meropenem disk (HI-MEDIA, Mumbai, India) was placed at the centre of the plate and the test strain was streaked from the edge of the disk to the periphery of the plate in four different directions. The plate was incubated overnight at 37^0C. The presence of a 'cloverleaf shaped' zone of inhibition due to carbapenemase production by the test strain was considered as positive [7].

EDTA disk synergy (EDS) test

EDTA disk synergy (EDS) test was done with simultaneous testing of two different ß-lactams (meropenem and ceftazidime), for detection of metallo-β-lactamases in the meropenem resistant isolates. A 0.5 M EDTA solution was prepared by dissolving 186.1 g of disodium EDTA. H2O (REACHEM, Chennai, India) in 1,000 ml of distilled water. The pH was adjusted to 8.0 by using NaOH (HI-MEDIA, Mumbai, India) and was sterilized by autoclaving. An overnight liquid culture of the test isolate was adjusted to a turbidity of 0.5 McFarland standard and spread on the surface of a MHA plate. A 10 µg meropenem disk (HI-MEDIA, Mumbai, India) was placed on the agar. A blank disk (6 mm in diameter, Whatmann filter paper no. 1) was kept on the inner surface of the lid of the MHA plate and 10 µl of 0.5 M EDTA is added to it. This EDTA disk was then transferred to the surface of the agar and was kept 10 mm edge-to-edge apart from the meropenem disk. After incubating overnight at 37^0C, the presence of an expanded growth inhibition zone between the two disks was interpreted as positive for MBL production [7,8,9].

Results:

Out of 1647 suspected septicemic cases, 877 were culture positive. Four hundred seventy one gram negative bacilli (54.0%), 307(35.1%) Gram positive cocci and 96(10.9%) Candida species were isolated from neonatal scepticemia. *Klebsiella pneumoniae* was the predominant bacteria isolated (40.6%) followed by *Escherichia coli* (19.5%), *Acinetobacter baumannii* (9.6%), *Pseudomonas aeruginosa* (8.9%) and *Enterobacter cloacae* (6.4%). 114(24.2%) isolates were resistant to imipenem and or meropenem and 78(16.6%) were metallo beta lactamases producers. *Klebsiella pneumoniae* (41.0%) was the most frequent MβL producer followed by, *Escherichia coli* 17(21.8%), *Acinetobacter baumannii* 8(10.3%), P*seudomonas aeruginosa* 7(9.0%), and *Enterobacter cloacae* 3(3.8%), (Table-1).

EDTA disk synergy test detected MBL production in additional 3 *Pseudomonas* spp and 1 *Acinetobacter* spp

Discussion

The increase in antibiotic resistance among gram-negative bacteria is a notable example of how bacteria can procure, maintain, and express new genetic information that can confer resistance to one or several antibiotics. This genetic plasticity can occur both inter-and intragenerically. Gram negative bacterial resistance possibly now equals or usurps that of gram positive bacterial resistance and has prompted calls for similar infection control measures to curb their dissemination. Reports of resistance vary, but general consensus appears to prevail that quinolone and broad spectrum β lactam resistance is increasing in members of the family Enterobacteriaceae and Acinetobacter spp. and that treatment regimes for the eradication of Pseudomonas aeruginosa infections are becoming increasingly limited. Gram-negative bacteria have at their disposal a plethora of resistance mechanisms that they can sequester and/or evince, eluding the actions of carbapenems and other β-lactams. The common form of resistance is either through lack of drug penetration (i.e., outer membrane protein [OMP] mutations and efflux pumps), hyperproduction of an AmpC-type β-lactamase, and/or carbapenem-hydrolyzing β-lactamases. Based on molecular studies, two types of

carbapenem-hydrolyzing enzymes have been described: serine enzymes possessing a serine moiety at the active site, and metallo-β-lactamases (MBLs), requiring divalent cations, usually zinc, as metal cofactors for enzyme activity [6,8,9].

Over the last decade there have been several articles summarizing the levels of MBLs in the bacterial community [11-13]. However, in the past 3 to 4 years many new transferable types of MBLs have been studied and appear to have rapidly spread. In some countries, P. aeruginosa possessing MBLs constitute nearly 20% of all nosocomial isolates, whereas in other countries the number is still comparatively small [12]. In recent years MBL genes have spread from P. aeruginosa to Enterobacteriaceae, and a clinical scenario appears to be developing that could simulate the global spread of extended-spectrum β-lactamases. Moreover, given that MBLs will hydrolyze virtually all classes of β-lactams and that we are several years away from the implementation of a therapeutic inhibitor, their continued spread would be a clinical catastrophe. We found that 16.6% of the gram negative bacteria that were resistant to carbapenems were attributable to the MBL production. This is surprisingly higher than documented report in Korea, where 11.4% of imipenem resistant gram negative bacterial isolates produced MBLs and Wattal et al., reported prevalence of resistance to carbapenems ranging 13 to 15% in E.coli and Klebsiella spp from ICUs and wards from a tertiary care hospital in Delhi [14]. In the present study *Klebsiella pneumoniae, E. coli* and *Acinetobacter baumannii* were the predominant MBL producers.

EDTA disk synergy test detected MBL production in additional 3 Pseudomonas species and 1 *Acinetobacter* species, which were missed by modified Hodge test. Based on these findings, EDTA disk synergy test seems to be a better method for MBL detection then modified Hodge test. Though the reason for the difference in the performance of these two tests is not clear, similar results have been observed in other studies [13]. In the EDS test, EDTA-ceftazidime combination detected additional MBL producers which were not identified by EDTA-meropenem combination. The reason for the increased sensitivity of EDTA-ceftazidime combination is the ability of ceftazidime to produce a marked inhibitory effect with EDTA. Therefore, ceftazidime appears to be the better substrate for EDS. Similar results were observed in a study done at the National Institute of Infectious Diseases, Tokyo, Japan.

Two MBL producing *P. aeruginosa* were detected only by EDTA-meropenem combination, but not by EDTA-ceftazidime combination. A synergistic zone of inhibition which must have been normally present between EDTA and ceftazidime due to MBL production was not observed with these two isolates. These two MBL producers were also simultaneously producing AmpC β-lactamase. Hence it could be proposed that the synergistic zone of inhibition was masked by the resistance to ceftazidime conferred by the AmpC β-lactamase, which is independent of zinc ions for its action. Based on this study it is clear that both EDTA-meropenem and EDTA-ceftazidime combination must be used simultaneously to detect all the MBL producers, which may otherwise be missed by using either of this combination alone.

In the absence of specific surveillance, the prevalence of MβL in a country of region may be under recognized, as any routine susceptibility testing methods employed in clinical laboratories may not detect the production of MβL.

Metallo beta lactamases producing gram-negative bacteria has emerged in neonatal septicemic cases. Laboratories can detect MβL production by simple technique of Modified Hodge test or EDTA synergy test. Bacterial strains resistant to most classes of antibiotics will continue to emerge unless the inappropriate use of these drugs is curtailed. Pediatrician should consider MβL production as a possibility in case of treatment failure with carbapenem antibiotics.

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Table-1: Distribution of MβL producing strains among different organisms isolated

Organism	No. of isolates	% of organism	Imipenem resistant	MβL producers	% of ESBL Producers
K. pneumoniae	191	40.6	38	32	41.0
K. oxytoca	41	8.7	07	05	6.4
E. coli	92	19.5	24	17	21.8
Acinetobacter baumanii	45	9.6	16	08	10.3
Acinetobacter lwofii	09	1.9	04	02	2.6
Pseudomonas aeruginosa	42	8.9	12	07	9.0
Enterobacter cloacae	30	6.4	07	03	3.8
Citrobacter sps	21	4.6	06	04	5.1
TOTAL	471		114	78	68.4

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