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PHENOTYPIC DETECTION OF METALLO BETA LACTAMASE IN CARBAPENEM RESISTANT ACINETOBACTER SPECIES IN INTENSIVE CARE UNIT IN A TERTIARY CARE HOSPITAL

Microbiology

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ABSTRACT

Introduction: Acinetobacter causes a wide range of illness in debilitated and hospitalized patients. Carbapenem resistance in Acinetobacter species is an emerging problem and is a cause of concern as many nosocomial infections with Acinetobacter species are resistant to most other antibiotics. The present study was aimed to study metallo-β-lactamase (MBL) production in Acinetobacter species. **Material and Methods:** Data was collected retrospectively from October 2021 to March 2022. Out of all the clinical samples obtained (respiratory secretions, pus, blood & urine), all Carbapenem resistant isolates of Acinetobacter species were included. Antimicrobial susceptibility testing was done by standard Kirby Bauer disk diffusion method. MBL detection was done by imipenem-EDTA combined disk method and Modified Hodge test. **Results:** Out of total 325 Acinobacter isolates isolated from intensive care unit (ICU), 228 were found to be carbapenem (Imipenem & Meropenem) resistant and these were further processed for MBL production. Out of 228 Carbapenem resistant Acinetobacter isolates 198 (86.84%) and 170 (74.56%) were found MBL producer by Combined Disc test and Modified Hodge Test respectively. **Conclusion:** This study demonstrated that multidrug resistant strains of Acinetobacter are common in ICU of tertiary care hospitals. Unwarranted and unrestricted usage of antibiotics and production of MBL is associated with emergence of resistance in nosocomial pathogens. Regular monitoring and documentation of carbapenem resistant is crucial in developing strategies to control infection due to these bacteria.

KEYWORDS

Acinetobacter species, Acinetobacter baumannii, Metallo-beta-lactamase, Carbapenem resistance, Clinical and Laboratory Standard Institute

INTRODUCTION

Acinetobacter species causes a wide variety of illness in immunocompromised and hospitalized patients.[1] They are intrinsically more resistant to antibiotics than Enterobacteriaceae. Because of frequent resistance to aminoglycosides, fluoroquinolones, ureidopenicillins and third generation cephalosporins, carbapenems, these bacteria survive for long period in hospital environment[2] and therefore the opportunities for cross infection between patients are enhanced.[1] The resistance of Acinetobacter species to carbapenem is now a major worldwide issue .[3-6]

Metallo-beta-lactamase (MBL) producing Acinetobacter baumannii has become a growing therapeutic concern worldwide.[7] The rapid detection of MBL positive isolates is necessary to control infection and to prevent their dissemination. The aim of this study was to determine the prevalence of MBL among carbapenem resistant strains of *Acinetobacter isolates* in our hospital.

MATERIALS AND METHODS

This retrospective study was conducted in the Department of Microbiology at Shri Ram Murti Smarak Institute of Medical Sciences, Bareilly over a period of 6 months from October 2021 to March 2022. Clinical specimens were obtained from ICU patients aged 15 years and above. The samples were processed following standards laboratory protocols. Samples were inoculated on blood agar, MacConkey agar and CLED agar. After overnight incubation growth was further identified by gram stain and standard biochemical methods. Finally antibiotic susceptibility testing (AST) of the isolates was performed using Kirby-Bauer disc diffusion method as per Clinical and Laboratory Standard Institute (CLSI)[8] guidelines and Carbapenem (Imipenem & Meropenem) resistant *Acinetobacter isolates* were detected. Other antibiotics which were tested: Piperacillin + Tazobactum, Cefaperazone + Sulbactum, Ceftriaxone, Cephalothin, Tigecycline, Fluoroquinolones, Amikacin, Colistin and Polymyxin B. The isolated *Acinetobacter species* were then processed for production of MBL enzyme by using Imipenem-EDTA combined disc test (Figure 1) and Modified Hodge test (Figure 2). *Acinetobacter baumannii* species ATCC 19606 was used as quality control strains. Data analysis was done by graphs, charts and *p* value was calculated with less than 0.05 considered as significant.

Imipenem - EDTA combined disc test (Method 1)

Mueller Hinton agar (MHA) plate is prepared by inoculating with standard inoculum (0.5 McFarland) of the test organism to form a lawn culture. Two 10µg Imipenem discs are placed on the plate. 10µl of 0.5M EDTA solution is added to one of the Imipenem discs to obtain a concentration of 750 mcg and was incubated overnight at 37ºC. If increase in inhibition zone with IMP + EDTA disc was ≥7mm than IMP disc alone, it is considered as MBL producer.[9,10]

Modified Hodge test (Method 2)

The surface of MHA plate was lawned evenly with an overnight culture suspension of Escherichia coli ATCC 25922, which was adjusted to one tenth turbidity of the 0.5 McFarland standards. After allowing the lawn to dry, an imipenem disc was placed in the Centre of the plate and imipenem-resistant isolates from the overnight culture plates were streaked heavily from the edge of the disc to the periphery of the plate. The presence of a distorted inhibition zone (Clover leaf shape) after overnight incubation was interpreted as Modified Hodge test positive.[9]

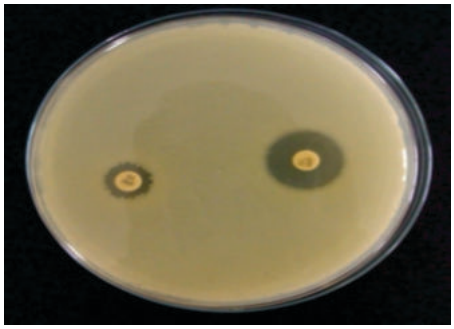

Figure1: Plate showing positive Imipenem- EDTA combined disc test.

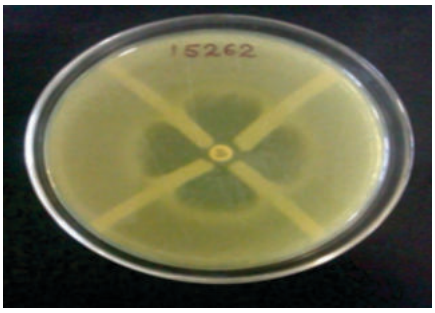

Figure 2: Plate showing positive Modified Hodge test by Modified Hodge test

RESULTS

Total 325 samples were received from ICU and 228 were found to be Carbapenem resistant (Imipenem & Meropenem). Out of 228, 153 (67.1%) were isolated from male patients and 75 (32.9%) were isolated from female patients. Furthermore 182 (79.8%) Acinetobacter isolates are isolated from samples of medicine ICU patients and 46 (20.2%) from the samples of surgery ICU patients.

The most common source of these Acinetobacter isolates (Table 1) were respiratory samples 205 (89.9%) followed by pus 12(5.3%), blood 6 (2.6%) and urine 5 (2.2%).

All the isolated Carbapenem resistant Acinetobacter isolates had a multidrug resistant profile and were resistant to all commonly used antibiotics included in the study except Colistin and Polymyxin B. All isolates were further processed for MBL production by Combined disc test and Modified Hodge test. These phenotypic methods showed 198/228 (86.84%) and 170/228 (74.56%) MBL production by Combined disc test and Modified Hodge test respectively (Table 2).

Table 1. Distribution of Isolates according to types of samples taken

Sample	Total samples (n=325)	Carbapenem Resistance (n=228)
Respiratory sample	250	205
Pus	40	12
Blood	20	6
Urine	15	5

Table 2. Detection of Metallo-β lactamases in Imipenem Resistant isolates of Acinetobacter species

Methods	Carbapenem Resistant	MBL Positive	Percentage	P value
Imipenem-EDTA Combined Disc Test	228	198	86.84%	0.036
Modified Hodge Test	228	170	74.56%	0.045

DISCUSSION

Antimicrobial resistance is an emerging problem worldwide, especially in the ICUs. Identifying the resistance pattern of microorganisms in every hospital is the key to success for the appropriate management. Recent clinical attention has been focused on the increasing frequency of non–lactose-fermenting gram-negative pathogens responsible for hospital-acquired infections.[11] In this group, *Acinetobacter* species are emerging as an important nosocomial pathogen that is associated with life-threatening infections.[12] A study done by Sofianou et.al[13] also found *Acinetobacter species as* predominant (35%) organism in ICU. In an Indian study by Kaur T et al[14], Acinobacter infection in ICU showed 42% mainly from respiratory sample, another study by Khanal et.al.[13] from Nepal also found among Gram-negatives bacteria, Acinetobacter spp. were the most frequently (32.9%) isolated species from ICU. In our study also there were 35% Acinobacter species along with majority respiratory samples. This could be attributed to the fact that majority ICU patients are intubated, so it's the most common source of infection.

Carbapenems are used as the last resort for treating multi drug resistant (MDR) Gram negative bacterial infections in any nosocomial setting. Carbapenems have a broad-spectrum activity and they are stable to hydrolysis by most of the β-lactamases, including the extended spectrum β lactamases (ESBLs) and the AmpC β-lactamases. However, there has been an alarming increase in the reports on carbapenem resistant Acinetobacter spp. over few years.[9]

The wide use of Carbapenems for empirical therapy of nosocomial infections caused by Acinetobacter isolates has resulted in emergence of an additional type of resistance which is called carbapenemase; one of them, MBL. It is important in drug resistance against Carbapenems.[7,16-17]

Numerous Indian studies from different regions have documented low (9%) to very high (90%) carbapenem resistance in Acinetobacter isolates.[18,19,20]

In the present study, Acinetobacter isolates were isolated most commonly from respiratory tract specimens, followed by pus, blood and urine. Many studies also reported that respiratory specimens are the major source of Acinetobacter isolates.[21,22]

In CLSI guidelines, no phenotypic method has been given for screening & confirmatory detection of MBLs in Acinetobacter species therefore different phenotypic methods have been used by different authors to detect Metallo-β-Lactamase in Acinetobacter species.

Different studies carried out in different parts of country and abroad showed different prevalence of Metallo-β-lactamase in Acinetobacter species. The current study showed 86.84% and 74.56% isolates are carbapenemase (MBLs) producer by CDT and by MHT respectively. Noori et al (2014) also had similar results with 86.8% MBL production by CDT method. A study done by Mohamed NM et al (2011) found 48% MBL by CDT. Another study done by Irfan et al (2008) and Pandya et al (2011) shown 96.6% and 96.30% MBL production respectively by Combined disc test. [17,23,24,25]

The results from the MHT in current study showed that 74.56% of Acinetobacter isolates were MBL producers. These results were supported by several other studies such as Lee et al. (2003) and Kumar et al. (2011) found 73% and 71%, Metallo--lactamases producer Acinetobacter isolates respectively by MHT.[26,27]

A study done by Hussein et al. (2013) and Moulana Z. et al (2020) 83.3% and 84% Metallo--lactamases producing Acinetobacter isolates respectively by MHT, these results are similar to current study.[28,29]

Although as per the study of Behra B et all [30] Imipenem-EDTA Combined disc Test and Epsilometer test was found equally sensitive. However, given the cost constraints of Epsilometer test, a simple screening method like Imipenem-EDTA Combined disc technique and modified Hodge test were used in present study because as they were very economical, the materials used were cheap, non-toxic, easily accessible and can be incorporated into the routine testing of MBL in any Microbiology laboratory.[8]

CONCLUSION

The prevalence of drug resistance among Acinetobacter species against most of the antibiotics is very high. Hence, early detection and optimization of infection control practices are the best defences against these organisms. Therefore, systematic surveillance to detect MBL producing bacteria and rational prescription and use of carbapenems could be helpful to prevent the spread of carbapenem resistance.

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