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Research Paper

Phenotypic Detection & Prevalence of Metallo-β-lactamases in Acinetobacter Spp. in Clinical Isolates at Tertiary Care Hospital

Medical Science

KEYWORDS : Acinetobacter boumanni complex, Metallo–β-lactamase [MBL], Imipenem resistant

Dr Dipal K Jethwa	MD MIcrobiology, Department of Microbiology, BJMC, Ahmedabad
Dr Darshan chauhan	MD Padiatrics, Assistant professor, Government medical college Surat
Dr. M M Vegad	MD Pathology & Microbiology, Professor and Head, Department of Microbiology, BJMC, Ahmedabad

ABSTRACT

Background: Acinetobacter, although saprophytic, the emergence and spread of Acinetobacter in hospital environment is a major area of concern due to multi drug resistant that complicates both therapy and infection control. Carbapenem resistance has been increasing nowadays due to production of a variety of Carbapenemases including Metallo- β -lactamases.

Aims & objectives:

1. To know the prevalence & antimicrobial susceptibility of Acinetobacter spp.isolates from the various clinical samples.

2. To determine the prevalence of Metallo –β-lactamases in Acinetobacter spp. isolates by phenotypic method.

Material & Methods:

854 isolates were obtained from various clinical samples, and subjected to antibiotic susceptibility testing. Imipenem resistant isolates were selected for MBLs detection.

Observation and Result:

A total of 854 isolates of Acinetobacter spp. were found, 79 (9.3%) of these showed resistance to Imipenem, 68 isolates (7.96%) were confirmed MBL producers phenotypically.

Conclusion:

MBL producing Acinetobacter spp .are present in this institute in low prevalence. The Phenotypic detection of MBLs is easy to perform where resources are restricted. To keep it in check, regular detection of this bacteria and judicious use of antibiotics to which it is still susceptible is mandatory.

INTRODUCTION

- *Acinetobacter baumannii*, one of the most important nosocomial pathogen with multiple drug resistance (MDR), is of great concern because of its intrinsic and acquired resistance mechanisms, limiting the treatment options. [1]These bacteria survive for a long time in the hospital environment, with enhanced opportunities for transmission between patients. [2] Infection with the MBLs producing organisms is associated with higher rates of mortality, morbidity, and health care costs.
- Acquired metallo-β-lactamases (MBLs) in *Acinetobacter spp*. have recently emerged as one of the most worrisome resistance mechanism necessitating their detection.
- Metallo β-lactamase (MBL) was first detected in 1960, in bacillus cereus which was chromosomal in location. Then, first plasmid mediated metallo-β-lactamase isolates were found in Pseudomonas aeruginosa in 1991 in Japan.MBLs belongs to Class B Carbapenemases in Ambler Classification requiring Zn2+ for their catalytic activity. (1)MBL hydrolyze all beta-lactam antibiotics including carbapenems, with the exception of aztreonam (monobactam). (2) MBL producing strains are resistant to serine beta lactamase inhibitors, (e.g. clavulanate) but inhibited by Metal chelators, such as EDTA and THIOL compounds. [2,3]Five classes of MBLs: VIM-like, IMP-like, SIM-1, SPM-1, and GIM-1.
- In present study, Imipenem- EDTA combined disc synergy test (cdst) is used for detection of MBLs. [5]

MATERIAL AND METHODS

- During period of July 2010 to June 2011, total of 23973 clinical samples like urine, pus+drain, swab, sputum,blood and body fluids. Samples were cultured on MacConkey agar and Sheep Blood agar. Identification was done by biochemical test [3] and a total of 854 isolates of *Acinetobacter baumannii* group were found and the isolates were stored at -20° C.
- Antibiotic susceptibility was assessed by the Kirby Bauer disc diffusion method following CLSI guidelines. [4] The antibiotics incorporated were: amikacin (30 µg), gentamycin (10 µg), tobramycin (10µg), ceftriaxone (30 µg), ceftazidime (30 µg), cefepime (30 µg), ampicillin-sulbactum(10/10µg), piperacillin/tazobactum (100/10 µg),ciprofloxacin (5 µg) levofloxacin(5 µg), imipenem(10 µg) and trimethoprim-sulfamethoxazole (1.25/23.75 µg). [4]
- Isolates with reduced susceptibility to imipenem were further screened for MBL production by combined disc synergy test (CDST).
- For CDST, the test strain were inoculated on to MHA plates. A 0.5 M EDTA solution was prepared by dissolving 18.61 g. of EDTA in 100 ml of distilled water and adjusting its ph 8.0.The Mixture was sterilized by autoclaving. [5]Two imipenem (10µg) discs were placed on the surface of MHA plate at distance of 25 mm and 4 µl EDTA solution was added to one of them to obtain a desired concentration of 750 µg. [5]
- The inhibition zone of Imipenem and Imipenem- EDTA discs were compared after 16 to18 h of incubation in air at 35^{0}c.
- Enhanced zone around EDTA impregnated Imipenem discs of ≥7 mm were identified as MBL producing Acinetobacter spp.

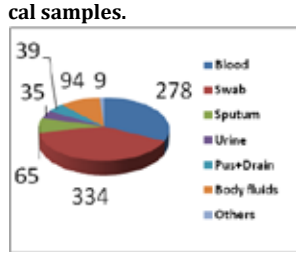
Figure 1. Combined disc synergy test showing MBL producing Acinetobacter spp.



RESULTS

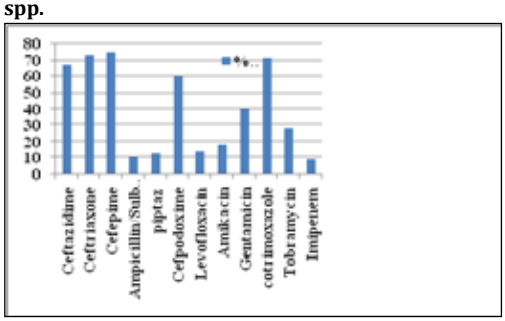
A total of 854 (3.56%) Acinetobacter spp. were isolated in this study from various clinical samples which is shown below in diagram.

Figure 2. Isolation of Acinetobacter spp.from various clinical samples.



Volume : 2 | Issue : 7 | July 2013 • ISSN No 2277 - 8179

Figure 3. Antibioltic susceptibility pattern of Acinetobacter spp.



Out of 854 Acinetobacter spp. isolates, 79 (9.3%) were imipenem resistant and 68 (7.96%) were MBLs Producer detected by cdst method. [5,7]

SUMMARY AND CONCLUSION

- 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, thats why they are used as the last resort for treating MDR Gram negative infections in any noscomial setting..However, there has been an alarming increase in the reports on carbapenem resistant Acinetobacter spp. over the last decade.
- In this study, the prevalence of MBLs producing Acinetobacter spp. was 7.96%.
- Results of our study and those conducted in other two part of India are shown below.

Table:1 Comparison of present study with other studies.

	Our Study	Dibyendu et al [6]	Anil et al [7]
Total Acinetobacter spp.	854 (100%)	66 (100 %)	45(100)
Imepenem resistant isolates	79 (9.3%)	5(7.5%)	32(71.1)
MBL positive isolates	68 (7.96%)	1(1.5%)	7(15.5%)

- Resistance patterns among nosocomial bacterial pathogens may vary widely from country to country at any given point and within the same country over time.
- Apart from being imipenem resistant (9.3%), Acinetobacter isolates were resistant to important groups of antibiotics tested, including ceftriaxone, ciprofloxacin, gentamycin with resistant rate of 72.5%,59.4%,39.4% respectively - a characteristic feature of MBL producers.
- For MBLs, limited treatment options are available and combination therapy is often employed in treatment of multidrug-resistant Acinetobacter spp.
- Our study shows that the Sulbactam was superior to tazobactam which is correlated with study done by Paul GH et al [8], so it represents an alternative treatment option for infections due to multiresistant Acinetobacter spp.
- The Phenotypic detection of MBLs is easy to perform where resources are restricted.
- As phenotypic detection of MBLs is easy to perform, identification and reporting of MBLs producing organisms will aid infection control practitioner in preventing spread of the multi drug resistant isolates and also guides appropriate selection for empiric therapy.
- Carbapenems plays a pivotal role in the management of severe Acinetobacter infections. However, reports of carbapenem resistance have been increasing alarmingly due to production of a variety of carbapenemases including metallo-b-lactamases (MBLs).

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