



Research Article

STUDY OF ANTIMICROBIAL RESISTANCE PATTERN OF NON-FERMENTERS IN A MEDICAL COLLEGE HOSPITAL OF WESTERN INDIA

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Abstract- Background & Objective: The antimicrobial resistance to most of the antimicrobial agent is very commonly seen among non-fermenters. Recently, New Delhi metallo beta-lactamase (NDM) has emerged as a novel carbapenemase. Carbapenem resistance is a major public health concern. Emergence of MBL producing Non-fermenters is alarming and reflects excessive use of carbapenams. **Results:** Out of 1932 non-fermenter isolates from various clinical specimens. 1393 (72%) were *Pseudomonas* spp. and 539 (28%) were *Acinetobacter* spp. **Interpretation & Conclusion:** Antimicrobial resistance to most of the antimicrobial agent which includes MBL in non-fermenter is becoming a therapeutic challenge. Only few drugs such as Polymyxin-B, Colistin and Tigecycline are suggested as possible effective treatment choices which are expensive and most of the times unavailable. Therefore, rapid detection of mechanism of resistance necessary to modify therapy & to initiate effective infection control to prevent their dissemination.

Keywords- MBL, non-fermenter, *Pseudomonas* spp., *Acinetobacter* spp

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Introduction

The most frequent isolates among non fermenters are *Pseudomonas* spp. and *Acinetobacter* spp and antimicrobial resistance multiple antibiotic is observed [1]. Beta lactamase are functionally classified as Class A, B, C, D and the group 3 are the zinc-based metallo beta-lactamases corresponding to the molecular class B [2], which are the only enzymes acting by the metal ion zinc. Metallo B-lactamases are able to hydrolyze penicillin, cephalosporin and carbapenams. Other mechanisms are decreased outer membrane permeability, increased efflux systems etc [3,4]. Metallo beta lactamase (MBL) are Class B is a group of beta-lactamases use divalent cations of zinc as cofactors for enzyme activity. Its hydrolyzing activity is potent against carbapenem and other b-lactam antibiotics. The resistance is transmitted by the plasmids. The IMP and VIM genes responsible for MBL production have been reported as important causes of nosocomial infections [5, 6] associated with clonal spread. The detection of the mechanism of drug resistance is important for the treatment of patients particularly in critically ill and hospitalized patients, and to control the spread of resistance. We conducted the study carried in Microbiology Department, B. J. Medical College, Ahmedabad. During July-December 2017 among 7983 clinical isolates, non-fermenter isolates were 1932. All the non-fermenters were subjected to antibiotic susceptibility testing by Kirby-Bauer disc diffusion test as per the Clinical and Laboratory Standards Institute (CLSI) guidelines. Their antimicrobial resistance pattern is being analysed

Detection of carbapenem resistance

For *Pseudomonas* spp. zone diameter of imipenem less than or equal to 15mm considered as resistance. For *Acinetobacter* spp. zone diameter of imipenem less than or equal to 18mm considered as resistance. MIC (minimum inhibitory concentration) testing of imipenem performed on these isolates, which were further confirmed by imipenem-EDTA combined disc method and imipenem-EDTA double disc synergy test for the mechanism of resistance[7].

MIC (minimum inhibitory concentration) testing of imipenem

MIC (minimum inhibitory concentration) testing of imipenem were performed on these isolates, MIC interpretive criteria for *Pseudomonas* spp. and *Acinetobacter* spp. resistance is a value greater than or equal to 8µg/ml as per current CLSI guidelines.

Imipenem-EDTA combined disc method (CDT)

Imipenem-EDTA combined disc method (CDT) was performed as described by Yong *et al.* A lawn culture of test isolates was prepared. After allowing it to dry for five minutes, two imipenem discs, one with 0.5 M EDTA and the other a plain imipenem disc, were placed on the surface of agar plates approximately 30mm apart. Incubated at 37°C for overnight. An increase in zone diameter of ≥ 7mm around imipenem + EDTA disc in comparison to imipenem disc alone indicated production of MBL.

Imipenem-EDTA double disc synergy test (DDST)

- Imipenem-EDTA double disc synergy test (DDST) was performed as described by Lee *et al.*[9] Test organisms were inoculated on to plates with Mueller Hinton agar as recommended by CLSI.
- An imipenem (10µg) disc was placed 20mm Centre to Centre from a blank disc containing 10µL of 0.5 M EDTA (750µg) [9].
- Enhancement of the zone of inhibition in the area between imipenem and EDTA disc in comparison with the zone of inhibition on the far side of the drug was interpreted as a positive result for MBL production.

Result

Out of 1932 non-fermenter isolates from various clinical specimens. 1393 (72%) were *Pseudomonas* spp. and 539 (28%) were *Acinetobacter* spp. They were identified by using standard microbiological techniques.

Table-1 Showing total non-fermenters from various clinical isolates and imipenem resistant isolates among them

Month	Total Non Fermenter Isolates
July	354
August	371
September	352
October	286
November	267
December	302
Total	1932

Out of 1932 isolates 745 were resistant to carbapenams (imipenem) which is 38.5 % resistance

Table-2 Showing total imipenem resistant non-fermenters isolates and MBL producing strains among them.

Month	Imipenem Resistant Non Fermenter Isolates	MBL Producing Strain
July	117	112
August	146	143
September	136	129
October	102	97
November	106	105
December	138	131
Total	745	717

Table-3 Distribution among *Acinetobacter* Spp.

<i>Acinetobacter</i>	Percentage
<i>Acinetobacter baumannii</i>	71%
<i>Acinetobacter</i> spp.	29%

Table-4 Distribution among *Pseudomonas* Spp.

<i>Acinetobacter</i>	Percentage
<i>Pseudomonas aeruginosa</i>	82%
<i>Pseudomonas</i> Spp.*	18%

**Pseudomonas* Spp. Include *Pseudomonas stutzeri*, *Pseudomonas mendocina*, *Pseudomonas alcaligenes*

Discussion

Antimicrobial resistance to most of the antimicrobial agent which includes MBL in non-fermenter is becoming a therapeutic challenge. Only few drugs such as Polymyxin-B, Colistin and Tigecycline are suggested as possible effective treatment choices which are expensive and most of the times unavailable. Even these drugs have started showing resistance in few cases which makes the problem bigger. Therefore, rapid detection of mechanism of resistance necessary to modify therapy & to initiate effective infection control to prevent their dissemination. Study conducted at Microbiology Department, B. J. Medical College, Ahmedabad during JULY-DECEMBER 2017. Methods used for detecting MBL were Double disc synergy test (DDST), Imipenem-EDTA combined disc Method (CDT), MIC of imipenem. In the present study, the use of EDTA impregnated imipenem disc resulted in a significant increase in the zone size for the MBL producers when compared to the non-producer. In our study, out of 1932 non-fermenter isolates from various clinical specimens, 1393(72%) were *Pseudomonas* spp. and 539(28%) were *Acinetobacter* spp. They were identified by using standard microbiological techniques. Out of 1393 *Pseudomonas* spp., Imipenem resistance was found in 534(38.33%) isolates. Out of 539 *Acinetobacter* spp., Imipenem resistance was found in 211(39.14%) isolates. MBL producing strains out of total non-fermenters is 717(37.11%). Out of total MBL producing strain isolated from non-fermenters 466(65%)MDRO. There are several other mechanisms also for imipenem resistance because of that all imipenem resistant isolates were not MBL positive. Other studies conducted Nandy *et al* shows the percentage of MBL production in *Pseudomonas aeruginosa* to be 19.76%. and conducted in north India by Singla *et al* shows the percentage of MBL production in *Acinetobacter* spp. to be 39%. The emergence of New Delhi metallo beta-lactamase(NDM) a novel carbapenemase has made the scenario uncontrollable. The alarming rise in the antimicrobial resistance indicates the implementation of strict infection control measures and judicious use of antibiotics.

Conclusion

Antimicrobial Resistance including the Carbapenem resistance among nonfermenters is a major public health concern due to excessive use of carbapenams. There is a need to develop new antibiotic to combat antibiotic resistance as per the World Health Organization. Strict guidelines to limit inappropriate uses of antibiotics is also needed at the hospital setting, state and the national level. Only few drugs such as Polymyxin-B, Colistin and Tigecycline are suggested as possible effective treatment choices which are expensive and most of the times unavailable.

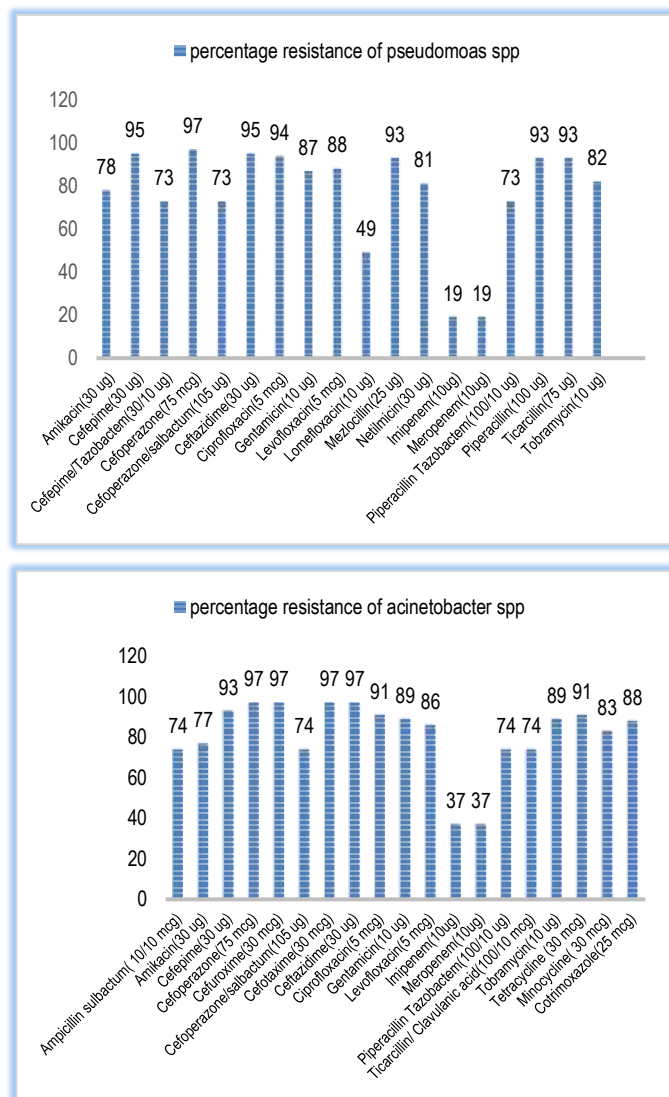


Fig- Antibiotic resistance pattern among MBL producing strains of Non fermenter.

Application of research

Therapeutic options for such isolates are colistin (an old and rather toxic antibiotic) and Polymyxin B for *Pseudomonas* spp. and colistin, Polymyxin B and Tigecycline (a newer antibiotic than can only be used in some, not all types of infection) for *Acinetobacter* as a last resort for the treatment of multi-resistant bacterial infection.

Research Category: Medical microbiology

Abbreviations:

DDST: Double disc synergy test

MBL: Metallo beta lactamase

NDM: New Delhi metallo beta-lactamase

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