Original Research Article

Metallo-beta-lactamase mediated resistance in clinical isolates of Acinetobacter spp

Ivy Viswamohanan¹, S A Lakshminarayana²*, Ashish Jitendranath¹, L Bhargavi³

¹Dept. of Microbiology, Sree Gokulam Medical College & Research Foundation, Trivandrum, Kerala, India
²Dept. of Microbiology, Rajarajeswari Medical College and Hospital, Bangalore, Karnataka, India
³Dept. of Microbiology, GG Hospital, Thiruvananthapuram, Kerala, India

A R T I C L E I N F O

Article history:
Received 18-09-2019
Accepted 24-09-2019
Available online 21-11-2019

Keywords:
Metallo-beta-lactamase
Acinetobacter
Combined dist test
Double disc synergy test
E-test

A B S T R A C T

Introduction: Acinetobacter spp. is one of the most common opportunistic bacterial pathogen causing hospital acquired infections. Its extensive resistance spectrum has earned it a “red alert” label among human pathogens. The emergence of MBL production among Acinetobacter spp has proved a global threat due to its broad spectrum of activity and limited treatment options.

Aims: To determine the prevalence of MBL among clinical isolates of Acinetobacter spp in a tertiary care hospital and to determine their antibiotic susceptibility pattern.

Materials and Methods: 57 clinical isolates of Acinetobacter spp obtained over a period of 1 year was subjected to antibiotic susceptibility testing by Kirby Bauer disc diffusion method as per CLSI guidelines. The carbapenem resistant strains were screened for MBL production by Imipenem-EDTA Combined disc test and Double disc synergy test. MBL E-test was used for confirmation of MBL production.

Results: Of the 57 isolates, carbapenem resistance was recorded in 39 isolates. Screening tests for MBL showed 24 to be positive by CDT and 16 to be positive by DDST. All 24 of the Acinetobacter spp isolates that tested MBL positive by the screening test showed positive result by E-test. The prevalence of confirmed MBL by E-test among Acinetobacter spp was found to be 42.2%. The MBL isolates, in addition to resistance to cephalosporins and carbapenems, showed statistically significant resistance to fluoroquinolones and aminoglycosides. Colistin was found to be 100% sensitive and hence may prove a possible therapeutic option.

© 2019 Published by Innovative Publication. This is an open access article under the CC BY-NC-ND license (https://creativecommons.org/licenses/by/4.0/)

1. Introduction

Acinetobacter spp plays a significant role in the colonization and infection of patients admitted to hospitals.¹ It typically colonizes skin, the respiratory, urinary, gastrointestinal tract, wounds and indwelling plastic devices of the hospitalized patients and can cause infections in burn, trauma, mechanically ventilated and immunocompromised patients. It shows a special predilection for the ICU.²

Multidrug resistant A. baumannii (MDR A. baumannii), defined as an A. baumannii strains resistant to at least three different antibiotic groups; penicillins and cephalosporins (including inhibitor combinations), fluoroquinolones, and aminoglycosides, has emerged and has been reported worldwide to significantly increase the morbidity, mortality, and cost of treatment. Acinetobacter spp. exhibit multidrug resistance through production of beta lactamases, alterations in outer membrane proteins (OMPs) and penicillin-binding proteins (PBPs), and increased activity of efflux pumps. Resistance to β-lactams appears to be primarily caused by production of β-lactamases which include extended-spectrum-β-lactamases (ESBLs), metallo-β-lactamases (MBLs), and oxacillins.³

Metallo-beta-lactamases (MBL) are carbapenemases, belonging to Ambler class B that confers resistance to all beta lactam antibiotics except monobactams.⁴ They are

https://doi.org/10.18231/j.ijmr.2019.071
2394-546X/© 2019 Innovative Publication, All rights reserved.
not inhibited by clavulanic acid, tazobactam, or sulbactam; hence beta-lactam combinations with the currently available beta-lactamase inhibitors are not useful. Apart from their broad spectrum of activity, another factor causing concern is that many of the MBL genes may be located on plasmids with genes encoding other antibiotic resistance determinants, i.e., aminoglycoside resistance genes. These MBL-positive strains are usually resistant to beta-lactams, aminoglycosides, and fluoroquinolones. There are no clinically approved MBL inhibitors, making these enzymes a serious threat to human health.

MBL prevalence among Acinetobacter spp ranged from 14 to 83% in various studies worldwide. The foremost implication of infection with carbapenem resistant A. baumannii is the need to use “last-line” antibiotics such as colistin, polymyxin B, or Tigecycline.

With global increase in the occurrence and types of MBLs early detection is essential for implementing appropriate antibiotic therapy as well as infection control practices. Keeping this in view, the present study was undertaken to determine the prevalence of metallo-beta-lactamase producing strains of Acinetobacter spp in our hospital, and their antibiotic sensitivity pattern, so that appropriate infection control strategy and antibiotic policy can be formulated to prevent their spread.

2. Materials and Methods

57 non repetitive, clinically significant isolates of Acinetobacter spp. obtained during a one year period were subjected to antibiotic susceptibility testing by employing Kirby Bauer disc diffusion techniques according to CLSI guidelines. The isolates showing resistance to carbapenems (imipenem or meropenem) were subjected to screening test for MBL production: Screening tests for MBL:

2.1. Imipenem and Imipenem-EDTA combined disc test (CDT)

The IMP-EDTA combined disc test was performed as described by Yong et al. Test organisms were inoculated as lawn culture on to plates with Mueller Hinton agar. Two 10 μg imipenem disks (Himedia) were placed on the plate, and appropriate amounts of 10 μL of 0.5M EDTA solution were added to one of them to obtain the desired concentration (750 μg). The inhibition zones of the Imipenem and Imipenem-EDTA discs were compared after 16 to 18 hours of incubation in air at 35°C. If the increase in inhibition zone with the Imipenem and EDTA disc was ≥ 7 mm than the Imipenem disc alone, it was considered as MBL positive. (Figure 1)

2.2. Double disc synergy test (DDST)

The test organisms were inoculated as lawn culture on to plates with Mueller Hinton agar. An imipenem (10 μg) disc was placed 20 mm centre to centre from a blank disc containing 10 μL of 0.5 M EDTA (750 μg). Enhancement of the zone of inhibition in the area between imipenem and the EDTA disc in comparison with the zone of inhibition on the far side of the drug was interpreted as a positive result. (Figure 1)

2.2.1. Confirmatory test for MBL: E-test (Figure 2)

Isolates positive for MBL by either or both of the screening tests were confirmed by MBL E-test. The E Test MBL strip (Biomérieux) contains a double sided seven-dilution range of imipenem (4 to 256 μg/mL) and imipenem (1 to 64 μg/mL) in combination with a fixed concentration of EDTA. The E-test was done according to manufacturer’s instructions. The test was considered positive if it satisfied any of the following criteria

1. MIC ratio of IP (Imipenem)/IPI (Imipenem-EDTA) of >8 or >3 log2
2. Appearance of phantom zone
3. Deformation of the elliptical zone of inhibition

3. Results and Discussion

Of the 57 isolates of Acinetobacter spp., 35% were from pus samples, 35% from respiratory specimen (sputum and suction tip), 14% from urine samples, 12.3% from blood and 1.8% from ear discharge and pleural fluid, each. Majority of the isolates were obtained from the wards (49%) and ICUs (44%), the rest being from the OPDs (7%).

Of the 57 isolates, carbapenem resistance was recorded in 39 isolates. Screening tests for MBL showed 24 to be positive by CDT and 16 to be positive by DDST. All the 16 isolates which tested MBL positive by DDST were also positive by CDT. However CDT showed an additional 8 isolates to be MBL positive, as compared to DDST.

All 24 of the Acinetobacter spp isolates that tested MBL positive by the screening test showed positive result by E-test as well. Hence, the prevalence of confirmed MBL by E-test among Acinetobacter spp was found to be 42.2%.

50% of the pus samples (10 out of 20), suction tip samples (10 out of 20) and catheter urine samples (5 out of 10) tested positive for MBL. Sputum samples tested 40% (4 of 10) positive, urine 33.3% (2 of 6), and blood 28.6% (2 of 7).

Comparison of the sensitivity pattern of the MBL positive isolates with that of the MBL negative isolates (Table 1) showed statistically significant difference (P<0.05) for Cephalosporins, piperacillin, piperacillin tazobactam, carbapenems, aztreonam, fluoroquinolones and gentamicin. Colistin was found to be sensitive among all the MBL positive strains.

No statistically significant relation was found between the prevalence of MBL and age group, sex or history of antibiotic intake.
Table 1: Comparison of antibiotic sensitivity patterns of MBLS positive isolates with MBL negative isolates

| Antibiotic           | MBL positive (n=24) | MBL negative (n=33) |
|----------------------|---------------------|---------------------|
| Piperacillin*        | 0                   | 6.1% (2)            |
| Piperacillin-Tazobactam* | 0                   | 54.5% (18)         |
| Ceftazidime*         | 0                   | 15.2% (5)          |
| Cefotaxime*          | 0                   | 9.1% (3)           |
| Cefepime*            | 0                   | 42.4% (14)         |
| Gentamicin*          | 12.5% (3)           | 27.3% (9)          |
| Amikacin             | 29.2% (7)           | 51.55% (17)        |
| Tetracycline         | 4.2% (1)            | 33.3% (11)         |
| Co-trimoxazole       | 37.5% (9)           | 45.5% (15)         |
| Ciprofloxacin*       | 8.3% (2)            | 24.2% (8)          |
| Cefoperazone-Sulbactam | 16.7% (4)          | 72.7% (24)         |
| Imipenem*            | 0                   | 90.9% (30)         |
| Meropenem*           | 0                   | 54.5% (18)         |
| Aztreonam*           | 8.3% (2)            | 60.6% (20)         |
| Colistin             | 100% (24)           | 100% (33)          |
| Tigecycline*         | 33.3% (8)           | 78.8% (26)         |

*P value <0.05

Table 2: Comparison of MBL prevalence among acinetobacter spp with other studies

| S. No. | Year | Study | Location | % Prevalence |
|--------|------|-------|----------|--------------|
| 1      | 2009 | R. Uma Karthika et al(24) | Puducherry | 70.9         |
| 2      | 2011 | John et al(8)                 | Bangalore   | 14.8         |
| 3      | 2013 | Goel V et al(10)              | New Delhi   | 48.72        |
| 4      | 2014 | Kaur et al(13)                | New Delhi   | 30.78        |
| 5      | 2016 | Harekrishna et al (17)        | Assam       | 38.7%        |
| 6      | 2018 | Amandeep Kaur(23)             | Bathinda    | 44.8%        |

**Fig. 1:** Screening tests for MBL

**Fig. 2:** MBL E–test

4. Discussion

Clinical isolates of *Acinetobacter* species initially retained at least partial susceptibility against the 3rd and 4th generations viz cephalosporins, fluoroquinolones, semisynthetic aminoglycosides, carbapenems and 100% susceptibility to imipenem but the clinical utility of this class of antimicrobial is increasingly being jeopardized by the emergence of both enzymatic and membrane-based mechanisms of resistance. The increase in the number of MBLs in *A. baumannii* is an ominous development in the global emergence of resistance to β-lactams.17

Carbapenem resistance among *Acinetobacter* spp was found to be 68.4% (39/57). Other published data indicate a widely varying range of carbapenem resistance among *Acinetobacter* spp, ranging from 23% to 99%.8–13,19

Of the 39 isolates showing carbapenem resistance, 24 were found to be by MBL production. For the remaining 15 isolates, resistance to carbapenems in the absence of
class B metallo β lactamase enzyme (MBL) production can be attributed to the production of other enzymes like Carbapenemase, Oxacillinase or to nonenzymatic mechanisms, including changes in outer membrane proteins (OMPs), multidrug efflux pumps and alterations in the affinity or expression of penicillin-binding proteins.20–22

The prevalence of MBL among Acinetobacter spp was found to be 42.2% in the present study which is comparable to the reports of Goel V et al10 and Amandeep Kaur et al23 (Table 2)

The MBL isolates, in addition to resistance to cephalosporins and carbapenems, showed statistically significant resistance to fluoroquinolones and aminoglycosides. This could be explained by the concurrent carriage of resistance genes to aminoglycosides and fluoroquinolones on the same mobile genetic elements carrying the MBL gene.6

Colistin showed 100% susceptibility among the MBL positive isolates in this study, and hence can be considered to be a treatment option. However this drug has to be used judiciously as various reports of colistin resistant MBL Acinetobacter isolates have emerged.7,17,23 The emergence of colistin resistance can be attributed to selection caused by routine use of colistin as a first-line drug for severe nosocomial infections.24

5. Conclusion

In conclusion, this study has documented the prevalence of MBL production among Acinetobacter spp as 42.2%, in our hospital. The early detection of MBL-producing isolates is important for the reduction of mortality rates of infected patients and also to avoid the intra hospital dissemination of such strains. CDT using imipenem can be used as a convenient screening method for detection of MBL production in gram negative bacilli in routine Microbiology laboratory where molecular methods are not feasible. At present, Colistin appears to be a suitable treatment option for MBL positive Acinetobacter spp, however, it should be used judiciously to prevent emergence of resistance. Continuous monitoring of MBL prevalence and formulation of appropriate antibiotic policy is the need of the hour for surveillance and control of MBL in the hospital.

6. Source of funding

None.

7. Conflict of interest

None.

References

1. Bergogne-Brzin E, Towner KJ. Acinetobacter spp. as nosocomial pathogens: microbiological, clinical, and epidemiological features. Clin Microbiol Rev. 1996;9(2):148–165.

2. Patwardhan RB, Dhakephalkar PK, Niphadkar KB, Chopade BA. A study on nosocomial pathogens in ICU with special reference to multiresistant Acinetobacter baumannii harbouring multiple plasmids. Indian J Med Res. 2008;128(2):178–187.

3. Molecular Study of Acinetobacter baumannii isolates for Metallo-Lactamases and Extended-Spectrum-Lactamases Genes in Intensive Care Unit, Mansoura University Hospital, Egypt. Int J Microbiol. 2017:p. 3925868–3925868. Available from: 10.1155/2017/3925868.

4. Mandell GL, Bennett JE, Dolin R. Drugs and Bennett’s principles and practice of infectious diseases. Philadelphia: Churchill Livingstone Elsevier ; 2010., 7th ed.

5. Bonomo RA, Szabo D. Mechanisms of Multidrug Resistance in Acinetobacter species and Pseudomonas aeruginosa. Clin Infectious Diseases. 2006;43:49–56. Supplement 2.

6. Walsh TR, Toleman MA, Poirel L, Nordmann P. Metallo-Lactamases: the Quiet before the Storm? Clin Microbiol Rev. 2005;18(2):306–325.

7. Anwar H, Ejaz A, Zafar H, Hamid H. Phenotypic Detection of Metallo-Beta-Lactamases in Carabapenem Resistant Acinetobacter baumannii Isolated from Pediatric Patients in Pakistan. J Pathogens. 2016;Available from: 10.1155/2016/8603964.

8. John S, Balagurunathan R. Metallo beta lactamase producing Pseudomonas aeruginosa and Acinetobacter baumannii. Ind J of Med Microbiol. 2011;29(3):302–304.

9. Rit K, Chakraborty B, Dey R, Chakrabarty P, Naha A, Saha R. Prevalence of Pseudomonas aeruginosa and Acinetobacter spp producing metallo–lactamase in a tertiary care hospital. J Dr NTR Univ Health Sci. 2013;2(1):18–21.

10. Goel V, Hogade SA, Karadesai SG. Prevalence of extended spectrum beta lactamases, AmpC beta lactamase, and metallo beta lactamase producing Pseudomonas aeruginosa and Acinetobacter baumannii in an intensive care unit in a tertiary care hospital. J Sci Soc. 2013;40:31–31.

11. Irfan S, Zafar A, Guhar D, Ahsan T, Hasan R. Metallo-Lactamase-Producing Clinical Isolates of Acinetobacter species and Pseudomonas aeruginosa from Intensive Care Unit patients of Tertiary Care Hospital. Ind J Med Microbiol. 2008;26(3):243–245.

12. Kabbaj H, Seffar M, Belefquih B, Akka D, Hador N, et al. Prevalence of Metallo-beta-lactamase producing Acinetobacter baumannii in a Moroccan Hospital. ISRN Infectious Diseases. 2013:p. 1–3.

13. Kaur A, Gupta V, Chhina D. Prevalence of Metallo-beta-lactamase producing (MBL) Acinetobacter species in a tertiary care hospital. Iranian J Microbiol. 2014;6(1):22–25.

14. Noori M, Karimi A, Fallah F, Hashemi A, Alimehr S, Goudarzi H. High Prevalence of Metallo-beta-lactamase producing Acinetobacter baumannii isolated from two hospitals of Tehran. Arch Pediatr Infect Dis. 2014;2(1):15439.

15. Amudhan MS, Sekar U, Kamalanathan A, Balaraman S. bla (IMP) and bla (VIM) mediated carbapenem resistance in Pseudomonas and Acinetobacter species in India. J Infect Dev Cities. 2012;6(11):757–762.

16. Kumar S, De AS, Baveja SM, Gore MA. Prevalence and risk factors of metallo -lactamase producing Pseudomonas aeruginos and Acinetobacter species in burns and surgical wards in a tertiary care hospital. J Lab Physicians. 2012;4(1):39–42.

17. Harekrishna N, Barkataki D. Study of Acinetobacter Isolates from Clinical Specimens in Tertiary Care Hospital and their Antimicrobial Susceptibility Pattern. Int J Curr Microbiol App Sci. 2016;5(10):842–848.

18. Yong D, Lee K, Yum JH, Shin HB, Rossolini GM, Chong Y. Imipenem-EDTA disk method for differentiation of metallo–lactamases producing clinical isolates of Pseudomonas spp and Acinetobacter spp. J Clin Microbiol. 2002;40(10):3798–3801.

19. Hodiwala A, Dhoke R, Urhekar AD. Incidence of metallo-beta-lactamase producing Pseudomonas, Acinetobacter & Enterobacterial isolates in hospitalised patients. Int J Pharm Sci Biol Sci.2013(3):79–83.

20. Sinha M, Srinivasas H. Mechanisms of resistance to carbapenems in meropenem- resistant Acinetobacter isolates from clinical samples. Indian J Med Microbiol. 2007;25(2):121–125.
21. Kempf M, Rolain JM. Emergence of resistance to carbapenems in Acinetobacter baumannii in Europe: clinical impact and therapeutic options. *Int J Antimicrob Agents*. 2012;39(2):105–114.

22. Anandhalakshmi S, Shashikala P, Sheela D, Noyal J, Prashanth K, Reba K. Detection of various resistance mechanisms associated with Acinetobacter infections in hospitalised patients. 2016;5:58–65.

23. Kaur A, Singh S. Prevalence of Extended Spectrum Beta-lactamase (ESBL) and Metallo-beta-lactamase (MBL) Producing Pseudomonas aeruginosa and Acinetobacter baumannii Isolated from Various Clinical Samples. *J Pathol*. 2018:p. 6845985. Published. Available from: 10.1155/2018/6845985.

24. Franco MRG, Caiaffa-Filho HH, Burattini MN, Rossi F. Metallo-beta-lactamases among imipenem-resistant Pseudomonas aeruginosa in a Brazilian University hospital. *Clin*. 2010;65(9):825–829.

Author biography

Ivy Viswamohan *Assistant Professor*

S A Lakshminarayana *Associate Professor*

Ashish Jitendranath *Associate Professor*

L Bhargavi *Consultant Microbiologist*

Cite this article: Viswamohan I, Lakshminarayana SA, Jitendranath A, Bhargavi L. Metallo-beta-lactamase mediated resistance in clinical isolates of Acinetobacter spp. *Indian J Microbiol Res* 2019;6(4):336-340.