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EVALUATION OF MODIFIED CARBAPENEM INACTIVATION METHOD (MCIM) FOR DETECTION OF MULTIDRUG RESISTANCE IN ACINETOBACTER SPECIES

Microbiology

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ABSTRACT

Background: *Acinetobacter* is a Gram negative bacteria, which causes Healthcare Associated Infections (HAI) like bacteremia, urinary tract infections, pneumonia. Carbapenem Resistant *Acinetobacter baumannii* (CRAB) has been a major concern in critically ill patients.
Aim of the Study: This study was done to isolate *Acinetobacter* from various clinical samples, and also to evaluate Modified Carbapenem Inactivation Method (mCIM) for detection of multidrug resistance in *Acinetobacter* species in a Tertiary Healthcare unit.
Materials & Methods: This cross-sectional study was conducted in 443 samples collected from adult patients admitted with infections in various sites. using sterile swabs. Carbapenemase production in *Acinetobacter* species detection by Modified Carbapenem Inactivation Method (mCIM) and to confirm by EDTA-Modified Carbapenem Inactivation Method (e- mCIM).
Conclusion: *Acinetobacter* was most commonly isolated from urine samples mostly from OG wards and Neonatal units. Modified Carbapenem Inactivation Method (mCIM) was found to be simple, easy to perform and an useful test to detect Carbapenamase production among the isolates. HAIs can be identified at the earliest using this method, so that appropriate control measures can be implemented in the hospitals.

KEYWORDS

Acinetobacter, Carbapenem Resistance, HAI

INTRODUCTION

Acinetobacter baumannii(AB), has emerged as one of the most troublesome pathogens for health care institutions globally as agents of a variety of healthcare associated infections including bacteremia, urinary tract infections and secondary meningitis and particularly in ventilator associated pneumonia.[9]

EMERGENCE OF MULTIDRUG RESISTANT ACINETOBACTER

Acinetobacter spp. are glucose-non-fermentative,non-fastidious, non-motile, catalase-positive, oxidase-negative, aerobic Gram-negative coccobacilli. [12]Carbapenems are powerful broad-spectrum β-lactam antibiotics that are widely regarded by clinicians as "last-line" antibiotics, particularly for the management of critically ill patients and/or those with antimicrobial-resistant Gram-negative infections.[8] Carbapenem resistance rates are high among Gram-negative bacteria in the hospitals of South and Southeast Asia,[1,5] especially in *Acinetobacter baumannii* complex (AB) isolates.[7]

RISK FACTORS FOR INFECTION DUE TO MULTIDRUG-RESISTANT *A.baumannii (*MDRAB)

The only significant independent risk factor for the appearance of imipenem resistant MDRAB in patients formerly infected with imipenem susceptible MDRAB is imipenem or meropenem exposure.[13] The independent risk factors for the acquisition of imipenem resistant *A. baumannii* (IRAB) include a hospital size of > 500 beds, previous antimicrobial treatment, a urinary catheter, surgery,[2] previous intensive care unit (ICU) stay, and prior exposure to imipenem or third-generation cephalosporins.[

AIMS AND OBJECTIVES:

1. To identify *Acinetobacter* species in various clinical samples.
2. To perform anti-microbial susceptibility testing for the *Acinetobacter* isolates.
3. To detect Carbapenemase production in *Acinetobacter* species by Modified Carbapenem Inactivation Method (mCIM) and to confirm by EDTA-Modified Carbapenem Inactivation Method (e-mCIM).

MATERIALS AND METHODS:

This cross-sectional study was conducted in 443 non-repetitive consecutive samples collected from adult patients admitted with infections in various sites during the 2 months study. Samples received in leaky containers with contamination were excluded.

Sample Collection & Processing

The clinical samples were collected using sterile swabs from pus discharges or secretions from patients with infections or wounds in various sites and transported in sterile, leak proof properly labelled containers to the Microbiology Laboratory and processed as per standard operating procedures.

Detection of Carbapenem Resistance in *Acinetobacter*

As per the CLSI guidelines (2019), to detect carbapenemase enzyme production leading to multi-drug resistance among the *Acinetobacter* isolates, Modified Carbapenam Inactivation method (mCIM) and EDTA- Modified Carbapenam Inactivation method (eCIM) were carried out.[3]

Modified Carbapenam Inactivation method (mCIM) for Suspected Carbapenemase Production in *Acinetobacter* species

The above test was done:

- For epidemiological or infection control purposes.
- mCIM was used for detecting carbapenemases in *Acinetobacter* species whereas EDTA- Modified Carbapenam Inactivation method (eCIM) was used together with mCIM to differentiate metallo-β-lactamases from serine carbapenemases in *Acinetobacter* species.
- mCIM can be performed alone; however, eCIM must be performed together with mCIM.
- eCIM is only valid if mCIM is positive.

Test method:

Meropenem disk inactivation

Test reagents and materials:

- TSB (2 mL aliquots)
- Meropenem disks (10 µg)
- 1-µL and 10 -µL inoculation loops
- Nutrient broth (eg, Mueller-Hinton(MHA), Trypticase Soy Broth(TSB) or normal saline (3.0-5.0 mL aliquots)
- MHA plates (100 mm or 150 mm)
- Meropenem-susceptibile indicator strain - *E. coli* (ATCC® 25922)
- 0.5 M EDTA (only for eCIM)

Test procedure for mCIM[3]

1. For each isolate , emulsify a 1- µL loopful of bacteria for *Enterobacteriaceae* or 10 - µL loopful of bacteria for *Acinetobacter* species from an overnight blood agar plate in 2ml TSB.
2. Vortex for 10-15 seconds.

3. Add a 10 -µg Meropenem disk to each tube using sterile forceps or a single disk dispenser. Ensure the entire disk is immersed in suspension.
4. Incubate at 35ºC ± 2ºC in ambient air for 4 hours ± 15 minutes.
5. Just before completion of TSB- meropenem disk suspension incubation, prepare a 0.5 McFarland suspension of *E.coli* ATCC® 25922 in nutrient broth.
6. Inoculate an MHA plate with *E.coli* ATCC® 25922 as for the routine disk diffusion making sure the inoculum suspension preparation and MHA plate inoculation steps are each completed within 15 minutes. Allow the plates to dry for 3-10 minutes before adding the meropenem disks.
7. Remove the meropenem disk from each TSB-meropenem disk suspension using a 10 - µL loop Carefully drag and press the loop along the inside edge of the tube to expel excess liquid from the disk and then place it on the MHA plate previously inoculated with the meropenem-susceptible *E.coli* ATCC® 25922 indicator strain. Disk capacity: 4 disks on a 100 mm Muller Hinton Agar (MHA) plate ; 8 disks on a 150 mm MHA plate.
8. Invert and incubate the MHA plates at 35ºC ± 2ºC in ambient air for 18-24 hours.
9. Following incubation, measure the zones of inhibition as for the routine disk diffusion method.

Test procedure: eCIM[3]

1. For each isolate, label a second 2-mL TSB tube for the eCIM test.
2. Add 20 µL of the 0.5M EDTA to the 2-mL TSB tube to obtain a final concentration of 5 mM EDTA.
3. Follow steps 1 through 9 above as for mCIM procedure. Process the mCIM and eCIM tubes in parallel.
4. Place the meropenem disks from the mCIM and eCIM tubes on the same MHA plate inoculated with the meropenem-susceptible *E.coli* ATCC® 25922 indicator strain.

Test interpretation for mCIM[3]

- **Carbapenemase positive :**
- Zone diameter of 6-15 mm or presence of pinpoint colonies within a 16-18 mm zone.
- If test isolate produces a carbapenemase , the meropenem in the disk will be hydrolyzed and there will be no inhibition or limited growth inhibition of the merponem-susceptible *E.coli* ATCC® 25922.

Carbapenemase negative :

- Zone diameter of ≥ 19 mm (clear zone)
- If test isolate does not produce carbapenemase, the meropenem in the disk will not be hydrolyzed and will inhibit growth of the meropenem-susceptible *E.coli* ATCC® 25922.

Carbapenemase indeterminate :

- Zone diameter of 16-18 mm
- Zone diameter of ≥ 19 mm and the presence of pinpoint colonies within the zone

Test Interpretation for eCIM[3]

Metallo-β-lactamase positive :

A ≥ 5 –mm increase in zone diameter for eCIM vs zone diameter for mCIM (eg. mCIM=6mm; eCIM=15mm; zone diameter difference = 9mm). For only the eCIM test, ignore pinpoint colonies within any zone of inhibition.

If the test isolates produces a metallo- β- lactamase, the activity of the carbapenemase will be inhibited in the presence of EDTA such that the meropenem in the disc will be not be hydrolysed as efficiently as in the tube without EDTA. The result is inhibition of the meropenem–susceptibile *E. coli* and an increase in the zone diameter for eCIM zone diameter when compared to the mCIM zone diameter.

Metallo-β-lactamase negative :

A≤ 4 –mm increase in zone diameter for eCIM vs zone diameter for mCIM (eg. mCIM=6mm; eCIM= 8mm; zone diameter difference = 2mm). For only the eCIM test, ignore pinpoint colonies within any zone of inhibition.

If the test isolates produces a serine carbapenemase, the activity of the carbapenemase will not be affected by the presence of EDTA and there will be no or marginal (≤ 4mm) increase in zone diameter in the presence of EDTA compared to the mCIM zone diameter.[39]

Ethical Clearance

This study was conducted after getting Institutional Ethics Committee Clearance.

STATISTICAL ANALYSIS

This study was statistically analysed using Epi-info software.

OBSERVATIONS AND RESULTS:

Table 1: Sample type-wise distribution of *Acinetobacter* isolates (n=20)

Type of Sample	Isolation of *Acinetobacter*	Total Samples
Urine	10 (50%)	294 (66.4%)
Blood	04 (20%)	46 (10.4%)
Pus	02 (10%)	29 (6.5%)
Umbilical venous catheter (UVC)	01 (5%)	22 (5.0%)
Sputum	01 (5%)	11 (2.4%)
Endo-tracheal (ET) tube	01 (5%)	17 (3.8%)
Pleural fluid	01 (5%)	24 (5.4%)
Total	**20 (100%)**	**443 (100%)**

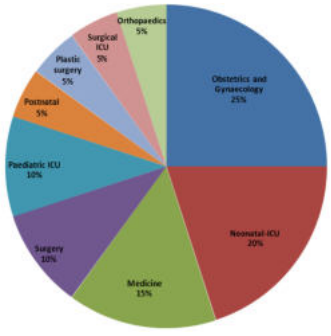

Figure 1: Ward wise distribution of *Acinetobacter* isolates

Table 2: Analysis of Carbapenemase production among Acinetobacter isolates using mCIM and eCIM methods (n=20)

	Zone of inhibition		
S.No.	mCIM	eCIM	Difference in zone of inhibition diameter between mCIM and eCIM
1	23 mm	24 mm	1mm
2	23 mm	23 mm	Nil
3	22 mm	23 mm	1mm
4	22 mm	22 mm	Nil
5	25 mm	25 mm	Nil
6	24 mm	25 mm	1 mm
7	25 mm	25 mm	Nil
8	22 mm	24 mm	2 mm
9	23 mm	25 mm	2 mm
10	22 mm	23 mm	1 mm
11	24 mm	24 mm	Nil
12	22 mm	22 mm	Nil
13	22 mm	23 mm	1 mm
14	25 mm	25 mm	Nil
15	23 mm	24 mm	1 mm
16	23 mm	23 mm	Nil
17	23 mm	25 mm	2 mm
18	24 mm	25 mm	1 mm
19	25 mm	25 mm	Nil
20	22 mm	24 mm	2 mm

Figure 2: Zone of inhibition of an Acinetobacter isolate for mCIM & eCIM methods

DISCUSSION:

In this study,out of 443 samples collected from wound infections, the culture positivity was found in 291 (65.7%) samples. Out of 443 samples 294 were urine samples, 46 blood samples, 29 pus samples, 24 pleural fluid samples, 22 umbilical venous catheter (UVC) samples, 17 endo-tracheal (ET) tube cultures, 11 sputum samples **[Table 1].**

Acinetobacter species were isolated in 20 (6.8%) out of 291 culture positive samples. Among the 20 *Acinetobacter* isolates, 10 (50%) were isolated from urine samples followed by 4 (20%) from blood samples **[Table 1]**. Studies conducted by Rachna T *et al*.,[10] and KK Lahiri *et al*.,[4] in India also suggest that *Acinetobacter* is predominantly isolated from urine samples.

Maximum number of *Acinetobacter* (35%) were isolated from samples of patients belonging to the age group of 0-10 years. Also, *Acinetobacter* species were isolated more from samples of females (60%) than from that of males (40%). This is similar to the study conducted by Rachna T *et al*.,[10] in which they reported the frequency of *Acinetobacter* in male and female patients to be 40.3% and 59.7%.

Ward wise distribution of the isolates has been illustrated in **[Figure1].** 25% of the isolates were from samples received from Obstetrics and Gynaecology (OG) wards, followed by neonatal-ICU (20%), medicine wards (15%), surgery wards (10%) and paediatric-ICU (10%). Increased incidence of infections in OG wards and neonatal ICU may be due to the fact that pregnant women and neonates are comparatively more susceptible to infections, due to their altered and under-developed immune status respectively.

All the 20 (6.8%) *Acinetobacter* isolates from various samples were subjected to anti- microbial susceptibility testing. *Acinetobacter* isolates from urine samples (n=10) were found to be sensitive to Piperacillin / Tazobactam (100%), Gentamicin (90%) and Amikacin (90%) whereas 90% of these isolates were resistant to Amoxicillin / Clavulanic acid. Isolates from blood samples (n=4) were sensitive to Piperacillin / Tazobactam (100%) and Amikacin (100%). Ampicillin was found to be the least effective antibiotic against these isolates.

Piperacillin / Tazobactam (83%) were found to be the most effective antibiotics against the isolates from miscellaneous samples (viz., pus, sputum, pleural fluid, endo-tracheal tube, umbilical venous catheter). Similar results were obtained by Purti C Tripathi *et al.*,[11] Cefotaxime and Piperacillin were the most resistant.

In this study, the mCIM and eCIM methods carried out to detect carbapenemase enzyme production among the 20 *Acinetobacter* isolates, it was found that there was no significant increase in the zone diameter for eCIM, as compared to the zone diameter for mCIM method, **[Table 2]** which helps to differentiate metallo-β-lactamases from serine Carbapenemases. Therefore, none of the *Acinetobacter* isolates were found to produce Carbapenemase of either type. **[Figure 2]**

CONCLUSION:

Acinetobacter spp. is a surrogate marker for Healthcare Associated Infections (HAI). In this study, Modified Carbapenam Inactivation method (mCIM) was found to be a simple,efficient and useful method to detect Carbapenemase production in the *Acinetobacter* isolates. Hence, this method greatly helps to monitor HAIs and help to take appropriate control measures to prevent HAIs.

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