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MODIFIED CARBAPENEM INACTIVATION METHOD FOR PHENOTYPIC DETECTION OF CARBAPENEMASE PRODUCTION AMONG ENTEROBACTERIACEAE

Microbiology

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ABSTRACT

Introduction: Carbapenemase-producing enterobacteriaceae (CPE) are increasing worldwide. Infections due to carbapenem-resistant organism have become a great concern for the clinicians due to the limited therapeutic options & so rapid and accurate detection of CPE is necessary for appropriate antimicrobial treatment and hospital infection control. The aim of this study was phenotypic detection of carbapenemase production among enterobacteriaceae by modified carbapeneme inactivation method recommended by CLSI 2017.

Material and Methods: In this test colonies were transferred to tryptic soya broth meropenem (10µg) disk was added & incubated for 4 hours. Muller Hinton Agar plate was lawn cultured with E. coli ATCC 25922 as for Disc Diffusion test. Meropenem disk was removed & placed on E. coli lawn & incubated overnight.

Results:

1) Out of 180 isolates 51 (28.33%) were carbepenemase producing.
2) Incidence of carbepenemase producing organism was more in isolates from IPD(85%) as compared to isolates from OPD(15%) patients.
3) Incidence of carbapenem resistance organism is more in isolates from ICU (41.1%).
4) Among carbapenem resistant isolates, 15.51% was carbapenem resistant E.coli and 56.5% was carbapenem resistant Klebsiella.

Discussion & Conclusion: The mCIM is a simple, inexpensive, accurate, and reproducible method for the identification of carbapenemase production among Enterobacteriaceae where automated system are not available.

KEYWORDS

Enterobacteriaceae; mCIM; carbapenemase;

INTRODUCTION:

Carbapenemase-producing Enterobacteriaceae (CPE) are increasing worldwide. Infections due to carbapenem-resistant organism have become a great concern for the clinicians due to the limited therapeutic options & so rapid and accurate detection of CPE is necessary for appropriate antimicrobial treatment and hospital infection control. Carbapenem resistance is mostly mediated by the production of carbapenemase enzymes that are present on the mobile genetic elements, followed by chromosomal-mediated porin loss and efflux pumps overexpression. In these circumstances, detection of carbapenemase producers is of significant importance for guiding effective antimicrobial therapy and for infection control. Several tests are described for the screening and detection of carbapenemases, but it requires more time, expertise and a well-established laboratory to perform these assays.[1],[2],[3],[4],[5] For these reasons, to have a point of care test for the detection of carbapenemase producers; Nordmann et al.[6] have developed a biochemical-based assay Carba NP, which can detect the presence of carbapenemase in <2 h of time. Carba NP has been extensively evaluated and proved to be an excellent rapid test.[6]

Clinical and Laboratory Standards Institute (CLSI), 2015 included Carba NP as a screening test for the carbapenemase detection (CLSI M100-S25).[7] Recently, CLSI 2017 (CLSI M100-S27) included "modified CIM (mCIM)" as a carbapenemase screening test for Enterobacteriaceae (CSLI M100-S27).[10] Compared to Carba NP, which was a rapid method with a turnaround time of 2 h, mCIM requires an overnight incubation for the detection of carbapenemases. Although mCIM is time consuming, it is relatively simple with the sensitivity and specificity of >99%.[8],[10]

A reliable and robust test for detecting carbapenemase producers is the need of the time. In view of this present study was carried out to the carbapenemese producing Enterobacteriaceae by recently recommended mCIM method by CLSI in tertiary care hospital.

MATERIAL AND METHOD:

mCIM testing:

Using a sterile inoculating loop, 1 µl of test organism was added into a tube containing 2 ml of tryptic soy broth (TSB) the bacterial suspension was vortexed for 10 to 15 s. Next, a 10-µg Meropenem (MEM) disk (Hi-media susceptibility test disc) was aseptically added into the bacterial suspension. The tube was then incubated for 4 h ± 15 min at 35°C ± 2°C in ambient air. Just prior to completion of the 4-h carbapenem inactivation step, a suspension of the mCIM indicator organism (Escherichia coli ATCC 25922, a carbapenem-susceptible strain) with turbidity equivalent to a 0.5 McFarland standard was prepared, and the surface of a Hi-media Mueller-Hinton agar plate (MHA) was lawn cultured using the procedure for standard disk diffusion susceptibility testing [13]. The MEM disk was then removed from the TSB bacterial suspension using a 10-µl inoculating loop; the loop was dragged along the edge of the tube during removal to remove excess liquid, and the disk was placed on the MHA plate, which was then incubated in an inverted position for 18 to 24 h at 35°C ± 2°C in ambient air.

mCIM result interpretation:

The diameter of the zone of inhibition around each MEM disk was measure. A zone diameter of 6-15 mm or presence of colonies within a 16-18 was considered a positive result (i.e., carbapenemase production detected), a zone diameter of 16-18 mm was considered an indeterminate result, and a zone diameter of ≥19 mm was considered a negative result (i.e., no carbapenemase production detected)[8,9] .

RESULTS:

Bacterial isolate:

All the isolates were from different clinical specimens obtained from hospitalized patients in various medical wards and OPD (n = 180). The source of isolates was as follows: intensive care units (n = 34), medicine (n = 46), surgery (n = 41), Pediatric (n = 13), Obstetric and gynecology(n = 09), orthopedics (n = 06), oncology (n = 8) and OPD(n = 30). (table.1)

Table 1. Percentage of isolates in IPD and OPD patients

Sr no	Ward	Carbapenem Sensitive	Carbapenem Resistant	Total	Percentage (%)
1	MEDICINE	35	11	46	25.5
2	SURGERY	32	09	41	22.7
3	ICU	20	14	34	18.8
4	PAEDIATERIC	07	04	11	07.2
5	OBGY	06	03	09	05.0
6	ORTHOPEDIC	06	00	06	03.3
7	ONCOLOGY	02	01	03	01.6
8	OPD	25	05	30	16.6

Incidence of carbepenemase producing organism:
Among 180 isoates, 51 (28.33%) were identified as carbapenem-resistant. Incidence of carbepenemase producing organism was more in isolates from IPD(83.4%) as compared to isolates from OPD(16.6%) patients. Incidence of carbapenem-resistant (CR) organism was as follows: ICU (41.1%), oncology ward(33.3%), obstetrics & gynecology ward (33.3%), pediatrics ward (30%), medicine ward (23.9%), surgery ward (21.9%), isolates from OPD patients which is 16.6%.(table.2)

Table 2. Percentage of carbapenem-resistant isolates in IPD and OPD patients

Sr no	Ward	Total	Carbapenem Resistant	Percentage (%)
1	ICU	34	14	41.1
2	OBGY	09	03	33.3
3	ONCOLOGY	03	01	33.3
4	PAEDIATERIC	13	04	30.0
5	MEDICINE	46	11	23.9
6	SURGERY	41	09	21.9
7	ORTHO	06	00	00.0
8	OPD	30	05	16.6

Out of 116 strains of E.coli 18 were CR, 26 of 46 Klebsiella pneumonia were CR, 1 of Citrobacter koseri was CR, all 4 isolates of Citrobacter freundii were CR, 1 of 4 Proteus mirabilis & 1 of 2 Enterobacter cloacae were CR. None isolates of K.oxytoca & S,marcescens were CR. (table.3)

Table 3: showing number of carbapenem-resistant and sensitive organisms

Name of organism	Total	Carbapenem-resistant	Carbapenem-sensitive
E. coli	116	18	98
K. pneumonia	46	26	20
K. oxytoca	04	00	02
C. koseri	05	01	04
C. freundii	04	04	00
P. mirabilis	02	01	03
Ent. cloaecae	02	01	01
S. marcescens	01	00	01
	180	51 (28.33%)	129 (71.67%)

Antimicrobial susceptibility testing:
All mCIM results of resistant to carbapenem production are comparable to VITEK. Carbapenemase-resistant strains of E.coli, Klebsiella and Citrobacter were resistant to maximum drugs except Colistin and Tigecycline (Only four isolates Klebsiella were resistant to Tigecycline). Trimethoprime sulfamethoxazole was resistant to 32 isolates i.e. all 18 isolates of E.coli, 12 of Klebsiella, 1 of both Citrobacter and Enterobacter. Proteus was resistant only to Meropenem.

Isolates not resistant to carbapenem were sensitive to more number of drugs as compared to carbapenem resistant isolates.

DISCUSSION:
Carba NP test was developed by Nordmann & Poirel in 2012; however, this method was subsequently modified.[10] This method was approved by CLSI in 2015.[7] Carba NP, with high sensitivity and rapid detection (≤2 h), can detect not only all the known carbapenemases, but also it identifies newly emerging carbapenemase, compared to molecular methods.[6] This method has some disadvantages such as high cost in contrast to other phenotypic methods, home-made cell lysis reagent (PER II, Bacterial Protein Extraction Reagent), short shelf life of reagent, false negative results with some isolates that produce OXA-type carbapenemase and report of results requiring an experts.[6,7]

A phenotypic test, referred to as CIM, was introduced to detect carbapenemase activity in gram-negative rods within 8 hours[8] CIM has yielded very promising results considering its sensitivity, specificity, very low-cost and easy interpretation.[10,12] In our setting, incubation period after putting the disc on MHA was overnight. If results are required within the same day, they can be read after six hours. CIM is affordable because its performance depends only on a meropenem disc and water. Interpretation of the result is also easy in the original protocol, i.e. lack of any zone around the disc is expressed as a positive result. Apart from our study, only one research on Enterobacteriaceae isolates has been carried out by this method.[6] In both studies, only one isolate was false negative. Positive predictive value and specificity of CIM are higher than those of MCNP.

CONCLUSION:-
In conclusion, we found the mCIM is a simple, inexpensive, accurate, and reproducible method for the identification of carbapenemase production among Enterobacteriaceae where automated systems are not available

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