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Original Research Article

COMPARISON OF VARIOUS PHENOTYPIC METHODS OF CARBAPENEMASE DETECTION AMONG KLEBSIELLA ISOLATED FROM CLINICAL SPECIMENS

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ABSTRACT

Background: Carbapenems are frequently used as a last resort antibiotic in the treatment of infections due to multidrug-resistant Enterobacteriaceae. Their alarming increase worldwide is worrisome and is left with very restricted therapeutic alternatives.

Objectives: This study compares the various phenotypic methods for detection of carbapenemase producers in Klebsiella species isolated from different clinical samples.

Methods: All specimens received for culture and sensitivity were processed as per standard guidelines. Identification of Klebsiella spp. was done using biochemical tests as per standard guidelines. Carbapenemase production was detected phenotypic screening methods as per CLSI guidelines. Confirmation done by using Modified Hodge Test, Modified carbapenem inactivation method (mCIM) and EDTA-modified carbapenem inactivation method (eCIM)

Results: Out of total 160 Klebsiella isolates test for carbapenemase production 101 (63.1%) was positive. MHT was positive in 76.2% isolates, whereas MCIM and ECIM were positive in 91.1% isolates. The sensitivity, specificity and PPV of MHT with MCIM were 82.6%, 88.9%, and 98.7% respectively, whereas sensitivity, specificity and PPV of ECIM with MCIM were 100%, 100%, and 100% respectively.

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Conclusion: MCIM in conjunction with ECIM test could be considered as more reliable phenotypic diagnostic methods for carbapenemase detection than the MHT in conjuction with the MCIM.

Keywords: MHT, MCIM, ECIM, Carbapenemase Producing Organisms

1. INTRODUCTION

Carbapenems are frequently used in the treatment of infections due to multidrug resistant Enterobacteriace. The emergence and spread of carbapenem-resistant gram-negative bacteria is a worldwide public health threat [1]. The mechanisms underlying carbapenem resistance in Enterobacteriace are complex and include both the production of carbapenem-hydrolyzing -lactamases (carbapenemase-producing CRE) and resistance due to the presence of a combination of other factors, such as hyperproduction of AmpC-lactamases or extended-spectrum -lactamases (ESBLs) combined with altered membrane permeability [2-3]. The major mechanism of beta‑ lactamase production is excessive use of beta‑ lactams in treating infections caused by the Enterobacteriace in recent years, [4]. Among the carbapenemases, Klebsiella pneumoniae carbapenemase (KPC, Class A), imipenemase (IMP, Class B), Verona integrin-encoded MBL (VIM, Class B), New Delhi metallo beta-lactamase (NDM, Class B), and oxacillinases (OXA, Class D) are the major types. These enzymes confer carbapenem resistance through hydrolysis [5-6]. MBL and KPC Carbapenemases are mostly encoded by mobile transposon and/or integron determinants resulting in faster dissemination to the other members of Enterobacteriaceae with limited therapeutic choices [7]. Bacterial isolates, which are capable of producing carbapenemase enzymes, have the ability to inactivate a wide range of β-lactams, including penicillins, cephalosporins, carbapenems, and monobactams [8]. Both phenotypic and molecular-based assays are available for the detection of carbapenemase producers from cultured isolates. Phenotypic assays currently used in clinical practice consist of the following: (i) modified Hodge test [MHT]), modified carbapenem inactivation method [mCIM]); Carba NP, matrix-assisted laser desorption–ionization time of light mass spectrometry [MALDI-TOF MS] methods); and lateral flow immunoassays [9-10]. The modified Carbapenem Inactivation Method (mCIM) is a simple phenotypic test that detects carbapenemases-producing gram- negative bacteria that have only been evaluated for use on bacterial colonies [11]. The Clinical and Laboratory Standards Institute (CLSI) lowered the carbapenem breakpoints for the Enterobacteriaceae in 2010 [12]. The revised breakpoints recommend meropenem or imipenem susceptibility with MICs of $\leq$ 1µg/ml and ertapenem susceptibility with MICs of $\leq$ 0.5 µg /ml.

Aims & objectives: this study evaluates the various phenotypic methods of the carbapenemase producers detection in Klebsiella isolated from clinical specimens in our tertiary care center.

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2. MATERIALS AND METHODS

This was a cross-sectional observational study carried out in the Department of Microbiology, MGM Medical College and associated M Y Hospital, Indore (M.P). Study was undertaken from June 2021 to June 2022 (01 years) after the approval from the ethics committee. A total of 160 Klebsiella species isolated from various clinical samples were enrolled in this study.

Inclusion Criteria:

- All Klebsiella spp. isolated from different specimen received for culture and sensitivity in microbiology department
- Patients who provide written informed consent for the study

Exclusion Criteria:

- Repeat isolation of Klebsiella spp. from same patient
- Patients who not provide consent for the study

All specimens received for culture and sensitivity were processed as per standard guidelines.

Isolation and identification of Klebsiella spp. was done using biochemical tests as per standard guidelines.

Antimicrobial sensitivity testing of isolated Klebsiella spp. for Imipenem and meropenem was done using Kirby Bauer disc diffusion and for Colistin using broth dilution method as per CLSI M100 2022 guidelines.

Screening criteria for carbapenemase production: Organism which were found resistant to imipenem and/or Meropenem were considered as probable Carbapenemase producers and considered as Screen positive.

Confirmation of carbapenemase production among screen positive Klebsiella was done using

- **Modified Hodge Test**
- **Modified carbapenem inactivation method (mCIM)**
- **EDTA-modified carbapenem inactivation method (eCIM)**

Carbapenemase positive: Zone diameter of 6–15 mm or presence of pinpoint colonies within a 16–18 mm zone

Carbapenemase negative: Zone diameter of ≥ 19 mm (clear zone)

Carbapenemase indeterminate: Zone diameter of 16–18 mm

Statistical analysis: SPSS 20 was incorporated in the analysis of the collected data (SPSS inc). The categorical or dichotomous variables were expressed as absolute values and percentages, and were compared with Pearson test. The continuous variables with a normal distribution were described as the mean (+/-SD). A P value less than .05 was considered Statistically Significant.

3. RESULTS

A total of 160 Klebsiella species isolated from various clinical specimens, out of that the higher proportion 81.9% was Klebsiella pneumoniae, 14.4% Klebsiella oxytoca and least 3.8% was Klebsiella Ozaenae. The higher proportion (38.1%) was urine followed by pus (33.2%) and the lower proportion (3.1%) was CSF respectively

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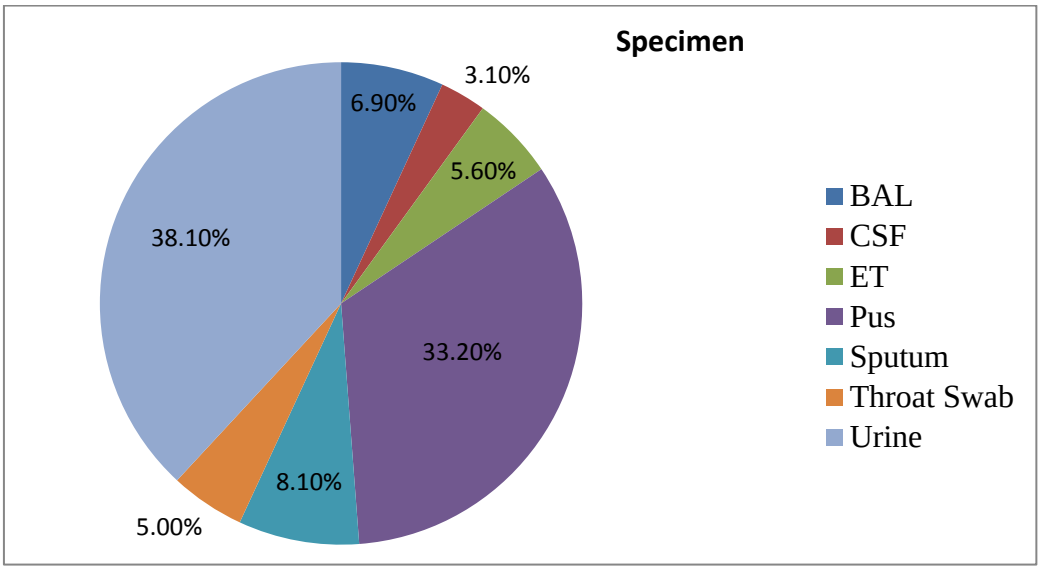
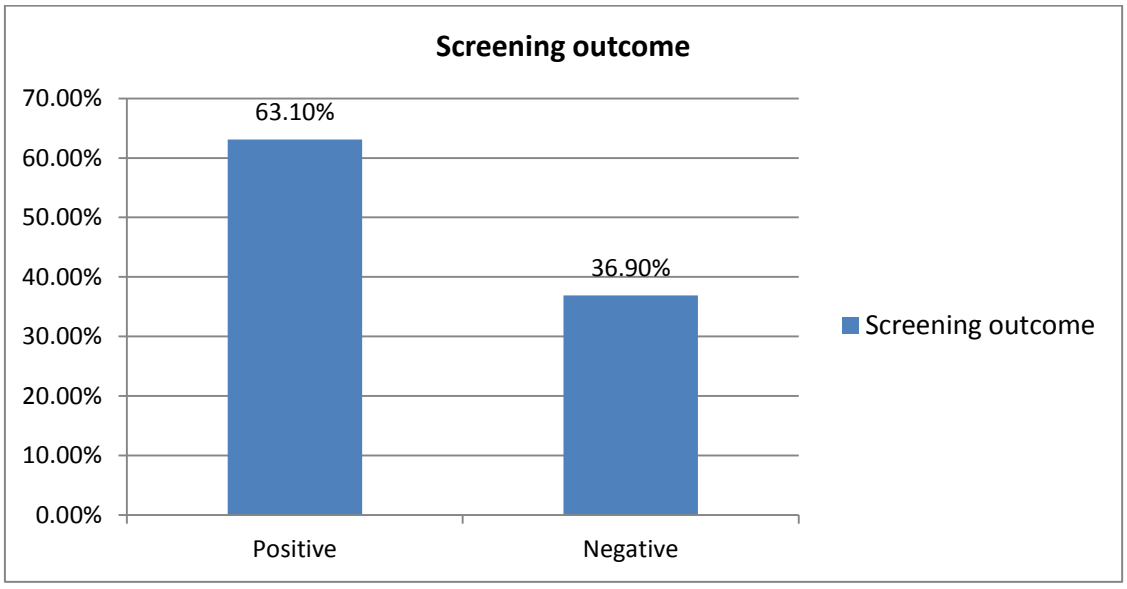
Figure 1: Distribution of subjects on the basis of specimens



All the Klebsiella isolated was screened for carbapenem resistance by production of carbapenemase. The KPC was used to confirm carbapenemase production among the carbapenem‑ resistant Klebsiella isolates. Among the screening test 101 (63.1%) isolates was carbapenemase production test positive [figure: 2].

Figure 2 Distribution on Basis of Screening for carbapenamase production



The carbapenemase screening test positive isolated was confirmed by Modified Hodge test (MHT), Modified Carbapenem inactivation method (MCIM) and EDTA Carbapenem inactivation method (ECIM). All these confirmatory methods were compared.

MHT was positive in 76.2% isolates, whereas MCIM and ECIM were positive in 91.1% isolates. Details of comparison between them was shown in table: 1

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Table 1 Comparisons between various methods of carbapenamase detection

Methods		**Frequency (n=101)**	**Percent**
Modified Hodge test (MHT)	Positive	77	76.2%
	Negative	24	23.8%
Modified Carbapenem inactivation method (MCIM)	Positive	92	91.1%
	Negative	9	8.9%
EDTA Carbapenem inactivation method (ECIM)	Positive	92	91.1%
	Negative	9	8.9%

The higher proportion 100% was for Positive outcome and the lower proportion 0.0% was for Negative outcome respectively. There was significant association between ECIM and MCIM outcomes (P<0.05).

Table 2: Association between ECIM and MCIM

ECIM	**MCIM**		**Total**
	Positive	**Negative**	
Positive	92 (100%)	0 (0.0%)	92 (91.1%)
Negative	0 (0.0%)	9 (100%)	9 (8.9%)
Total	92 (100%)	9 (100%)	101 (100%)
Chi-Square Value	**df**	**P Value**	**Result**
101.000	1	0.00	Significant

The higher proportion 98.7% was for Positive outcome and the lower proportion 1.3% was for Negative outcome respectively, association between MHT and MCIM outcome was significant (P<0.05).

Table 3: Distribution of MCIM Outcome for MHT Positive Outcome

MHT	**MCIM**		**Total**
	Positive	**Negative**	
Positive	76 (82.6%)	1 (11.1%)	77 (76.2%)
Negative	16 (17.4%)	8 (88.9%)	24 (23.8%)
Total	92 (100%)	9 (100%)	101 (100%)
Chi-Square Value	**df**	**P Value**	**Result**
23.133	1	0.00	Significant

4. DISCUSSION

Increased antibiotic resistance to carbapenems among Klebsiella isolates has become a major public health problem. The accurate and rapid detection of carbapenemase producing Klebsiella isolates is necessary for appropriate treatment, prevention of spreading, and control of infections. In the last decade, phenotypic methods were extensively used in clinical

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laboratories for a first-line detection of the isolates producing carbapenemases [13].
In our study majority of Klebsiella species were isolated from urine cultures followed by pus culture, similar observation reported by Parveen RM et al [14] and S K Pawar et al [15], discordance to our study, Valarmathi et al [16] observed maximum number of Klebsiella were isolated from pus, whereas Manikandan et al [17] showed that most of the Klebsiella species were isolated from sputum sample.
The organism found resistant to imipenem and/or Meropenem were considered as probable Carbapenemase producers and considered as Screen positive.
Current study found 63.1% of Klebsiella isolates were found to be Carbapenemase producers by screen test, in agreement with S Pandurangan, et al [18] and Wattal C, et al [19] reported Carbapenemase producers by screen test were 52% and 51% respectively. The major differences were likely due to geographic region, the testing method used, and the organism.
For confirmation of carbapenemase production among screen positive, Modified Hodge test, MCIM and ECIM was done.
Modified Hodge test (MHT) showed 76.2% positivity among the screening positive isolates in the present study, consistence finding observed by M Beig, et al [20] and C.G.Carvalhaes, et al [21].
Our study found Modified Hodge test was found to be 100% sensitive and specific for detection of carbapenem resistance in K. pneumoniae. Bashir H et al [22], reported modified Hodge test as a reliable and sensitive laboratory method for detection of carbapenem.
Current study reported MHT and MCIM are equally sensitive and effective methods for the detection of carbapenemase producer in Klebsiella species, our results comparable with the Tamma PD, et al [23].
ECIM and MCIM test showed similar sensitivity with (91.1%) positives and (8.9%) negatives, accordance to the Khare et al [24] and Bartolini A, et al [25].
Among various phenotypic methods for detection of carbapenem resistance, modified Hodge test (the Clover leaf test) is relatively simple and easy. It can be easily incorporated as a routine technique in laboratories with heavy workload. Since this test is recommended by CLSI, it is extensively used as a phenotypic method for detection of carbapenem resistance.

5. CONCLUSION

We have concluded that MCIM and ECIM test showed similar sensitivity, However, Modified Hodge test (MHT) showed lower sensitivity for detection of carbapenemase producers. As MCIM with ECIM can differentiate between both, it could be utilized as simple, reliable, cost-effective phenotypic method for carbapenemase detection which will contribute in the formulation of better treatment plan to curtail therapeutic failure.

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