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Original Research Article

Phenotypic Methods Used for Detection of Carbapenemase Producing *Klebsiella* Species and *Escherichia coli* in a Teaching Hospital in the Eastern Part of Bihar, India

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Abstract

Introduction: Clinical significance of carbapenemases in routine culture is underestimated, since most of the laboratories do not even perform the routine phenotypic tests for detection of carbapenemases. The present study had focused on isolates of carbapenemase producing *Escherichia coli* and *Klebsiella* species.

Material & Methods: The study was conducted in the Department of Microbiology, Katihar Medical College and Hospital, Bihar, India. 209 consecutive *E. coli* and *Klebsiella* species were selected for the study. Of these 55 strains were screening test positive, which were taken up for further testing for detection of carbapenemase production by various phenotypic test methods.

Result: *Klebsiella pneumoniae* carbapenemase **(**KPC) -producing *E. coli* was 3.4% (7/209) and Metallo-β-lactamase (MBL) producing *E. coli* was 9.6% (20/209) and KPC-producing *Klebsiella* species were 0.48% (1/209) and MBL -producing *Klebsiella* species were 7.2% (15/209). Some *E. coli* 2.4% (5/209) was found to produce both types of carbapenemases KPC and MBL.

Conclusion: This observation appears to be a warning sign against carbapenemase producing pathogenic profile of most commonly found Enterobacteriaceae *E. coli* and *Klebsiella* species and their constantly changing resistance pattern.

Keywords: Carbapenemase, *Escherichia coli*, *Klebsiella* species, KPC, MBL.

Introduction

One of the most observed resistance mechanism among Gram-negative pathogens are production of carbapenem inactivating enzymes, called carbapenemases which hydrolyse β-lactam antibiotics.

Emergence of carbapenemases in Enterobacteriaceae and non-fermentative Gram-negative bacteria poses a serious therapeutic problem in hospitals because carbapenems are often antibiotics of last resort for treatment of serious infections caused by multidrug-resistant Gram-negative bacteria. These bacteria have the potential to spread rapidly within the hospital environment and across countries and continents. [1]

Clinical significance of carbapenemases *viz*. KPC and MBL in routine culture is underestimated, since most of the laboratories do not even perform the routine phenotypic tests for detection of carbapenemases. The present study is focused on isolates of carbapenemase producing *E. coli* and *Klebsiella* species from indoor and outdoor patient departments in our hospital and evaluation of different phenotypic methods *viz.* Modified Hodge Test (MHT), Zone enhancement with EDTA-impregnated imipenem and ceftazidime discs, 2-MPA discs test and Boronic acid test for its detection. The present study was intended to generate knowledge on the occurrence of carbapenemase-producing strains in and around Katihar and of the possible threat to human health due to carbapenem resistance development. [2, 3]

Antibiotic-resistant strains can lead to serious problems in treatment of infection. Carbapenem antibiotics are the final treatment option for infections caused by serious and life-threatening multidrug-resistant Gram-negative bacteria. Therefore, an understanding of carbapenem resistance in this region was important for both microbiologists and clinicians to prevent, control and treat these infections in patients and also be helpful in smooth functioning of antimicrobial stewardship program.

Material and Methods

Data collection and processing:

Patient data including age, gender, date of admission, departments, discharge dates and clinical history was collected. After taking informed written consent, a proforma was filled up for each patient from whom the study strains were isolated. An ethical committee clearance was obtained from the institution. An online based calculator was used for statistical data calculation. [4]

Isolation and Identification: (Fig. 1)

209 consecutive samples of *Escherichia coli* and *Klebsiella* species which were isolated and identified in the department from January 2021 to June 2022, were selected for the study.

Standard culture techniques were used for isolation and routine biochemical media as well as VITEK®2 Compact (bioMérieux) was used for identification of these isolates. BacT/ALERT 3D system (bioMérieux) was used for blood cultures. [5, 6]

Isolates which could not be identified by standard biochemical tests were excluded from the study.

Antimicrobial susceptibility was done by modified Kirby-Bauer disc diffusion method using ampicillin (10µg), gentamicin(10µg), co-trimoxazole (25µg), levofloxacin (5µg), amikacin (30µg), cefuroxime (30µg), cefotaxime (30µg), cefoperazone/sulbactam (75/10µg), imipenem (10µg), cefepime (30µg) and piperacillin/tazobactam (100/10µg) was performed and interpretation of the result was done as sensitive, intermediately sensitive or resistant as per Clinical

Laboratory Standard Institute (CLSI) guidelines. **[7]**

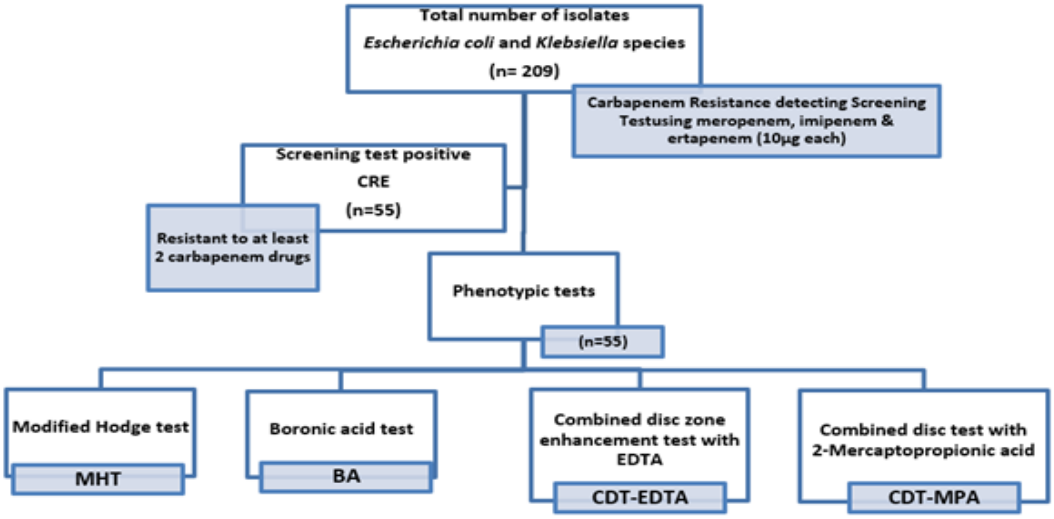

Figure 1. Showing Tests Performed in *Klebsiella* species and *Escherichia coli* Isolates

Carbapenemase Detection Screening Tests: [3]

Screening test was done by Kirby-Bauer disc diffusion method on Mueller-Hinton agar (MHA) media with three discs of carbapenem antibiotics containing imipenem (10µg), ertapenem (10µg) and meropenem (10µg) using *E. coli* ATCC 25922 as control strain. [3]

Carbapenemase Detection Phenotypic Confirmatory Tests: [3] (Fig. 2)

Phenotypic tests were performed with those isolates that showed resistance to at least 2 carbapenem discs in the screening test. 4 phenotypic tests were performed *viz.*

1. Modified Hodge test (MHT)
2. Zone enhancement with EDTA impregnated imipenem and ceftazidime discs (CDT-EDTA)
3. 2-Mercaptopropionic acid inhibition test (CDT-MPA)
4. Phenylboronic acid test (BA test)

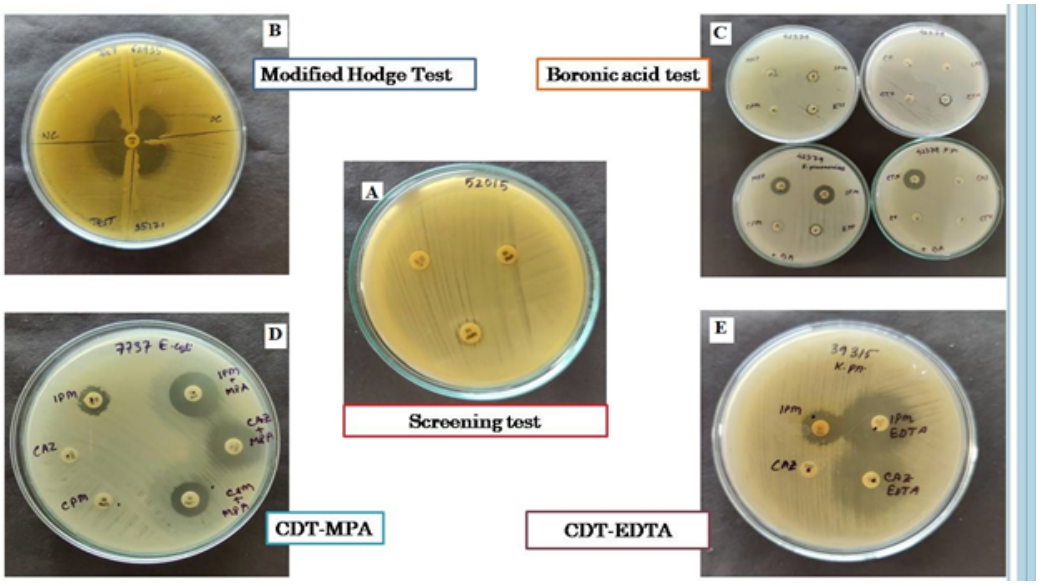

Figure 2. Carbapenemase Detection Tests *viz.* A. Screening test, B, C, D and E Phenotypic Detection Tests.

Results

The screening test was performed using 3 carbapenem discs - imipenem, meropenem and ertapenem discs of 10µg each. Out of the 209 strains selected for the study, 55 isolates of *E. coli* and *Klebsiella* were positive in the screening test. (Fig. 1).

E. coli 71.2% was the predominant species, followed by *Klebsiella* species 28.7%.

Majority of *Klebsiella* species were identified from Pediatrics 26.7% and Medicine department 25.0% and *E. coli* were identified from Medicine 34.9% and Surgery department 19.5%.

Maximum number of organisms were isolated from urine samples, 76.9% of *E. coli* and 23.1% of *Klebsiella* species were from urine samples followed by in pus samples from which 68.9% were *E. coli* and 31.1% were *Klebsiella* species.

Phenotypic test results:

Modified Hodge test was positive in 34.5% (19/55) whereas only 14.5% (8/55) were positive by the BA test for detection of KPC phenotypes. The MHT was found to be more sensitive than the BA test and this finding was found to be statistically significant (p = 0.01). (Table 1)

For MBL detection, 81.8% (45/55) of isolates were positive by CDT-EDTA test and 63.6% (35/55) isolates were positive by CDT-MPA test, this finding was also found to be statistically significant (p= 0.001). (Table 1)

Table 1. Evaluation of Various Methods for Detection of KPC and MBL Phenotypes: (n=55)

Tests	Positive test result (%)	Negative test result (%)	p value
MHT	19 (34.5)	36 (65.4)	0.01
BA Test	8 (14.5)	47 (85.5)	
CDT-EDTA	45 (81.8)	10 (18.2)	0.001
CDT-MPA	35 (63.6)	25 (45.5)	

In total, out of 55 screening test positive isolates, pure MBL-phenotype was found in 52.7% isolates followed by KPC-MBL co-producers which was seen in 10.9% and pure KPC phenotype was seen in only 3.6% isolates.

A total of 22 out of 36 screen test positive isolates of *E. coli* were carbapenemase producers (61.1%) out of which 41.7% (15/36) were MBL phenotype, 13.9% (5/36) were KPC-MBL co-producers and 5.6% (2/36) were KPC phenotype. (Table 2 and 3)

Amongst the *Klebsiella spp.* 78.9% (15/19) were carbapenemase producers out of which 73.7% (14/19) were MBL phenotype and 5.3% (1/19) were KPC-MBL-coproducers. (Table 2 and 3).

Table 2. Phenotypic Tests Positive for Carbapenemase Detection

Organisms	MHT (%)	BA (%)	CDT-EDTA (%)	CDT-MPA (%)	Total No of Carbapenemase Producers (%)
E. coli n=36	13 (63.1)	7 (19.4)	28 (77.8)	20 (55.6)	22 (61.1)
Klebsiella species n=19	6 (31.6)	1 (5.3)	17 (89.5)	15 (78.9)	15 (78.9)
Total n=55	19 (34.5)	8 (14.5)	45 (81.8)	35 (63.6)	37 (67.3)

Table 3. Organism-wise Distribution of KPC phenotypes and MBL phenotypes

Organisms	MHT and BA Positive (KPC) No. (%)	CDT-EDTA and CDT-MPA Positive (MBL) No. (%)	KPC-MBL co-producers No. (%)	Negative (KPC/MBL non-producers) No. (%)
Escherichia coli n=36	2 (5.6)	15 (41.7)	5 (13.9)	14 (38.9)
Klebsiella species n=19	0	14 (73.7)	1 (5.3)	4 (21.1)
Total n=55	2 (3.6)	29 (52.7)	6 (10.9)	18 (32.7)

Both the isolates with KPC phenotypes were isolated from urine samples. Majority of MBL phenotypes were also from urine samples 34.5% and blood and pus samples showed 31.0% each. Both KPC and MBL phenotypes were isolated from pus samples 50.0% and urine samples 33.3%. **(Fig 3).**

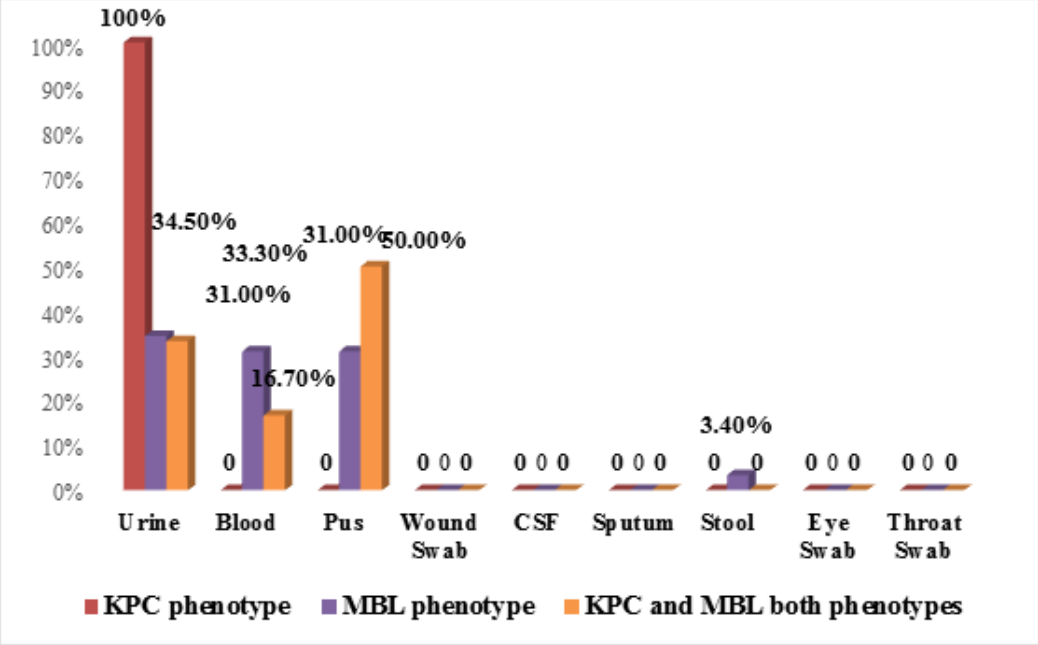

Figure 3. Clinical Sample-wise Distribution of KPC and MBL Phenotypes (n=55)

Discussion

Carbapenem resistance towards meropenem was 5.3% (11/209), imipenem 13.9% (29/209) and ertapenem 11.5% (24/209), among the 209 isolated *E. coli* and *Klebsiella* species strains. Reports from southern part of India found 58.4% (52/89) of *Klebsiella pneumoniae* strains were carbapenem resistant and maximum resistance was towards ertapenem was 51.7% (46/89) followed by imipenem 38.2% (34/89) and meropenem 31.5% (28/89). [8]

Only 34.5% (19/55) of 55 screen test positive isolates were Modified Hodge test positive. A South Indian study found that 84.3% (43/51) *E. coli* and *Klebsiella pneumoniae* isolates were Modified Hodge test positive, which differs from our study. [9]

The boronic acid test was less sensitive for KPC detection with only 14.5% being positive, while Doi et al (2008) found that

43.5 % of their strains were boronic acid positive. [10] While 81.8% of strains were positive for combined disc zone enhancement test with EDTA and ceftazidime test, a study in Nepal found this figure to be only 53.0%. [11]

Only 63.6% of isolates were positive for combined disc test with 2-Mercapto propionic acid test in the present study. Kim et al (2007) reported 40.0% positivity with the same test. [12]

Results of the present study revealed that 61.1% (22/36) *E. coli* were carbapenemase producers among which 41.7% (15/36) were MBL phenotype, 13.9% (5/36) were KPC-MBL co-producers and 5.6% (2/36) were KPC phenotype and 78.9% (15/19) of *Klebsiella spp.* were carbapenemase producers among which 73.7% (14/19) were MBL phenotype and 5.3% (1/19) were KPC-MBL-coproducers. In contrast, a study from Bangalore reported that out of 430 isolates, 13.9% (60/430) were carbapenem resistant *E. coli* and *Klebsiella* species, among them KPC producing isolates were 5.0% (3/60) and MBL producing isolates were 8.3% (5/60). [13,14]

Conclusion

Prevalence of MBL producing strains (53%) which is quite high in this region of Bihar is a threat for the clinicians. Broad-spectrum antibiotics like carbapenems need to be reserved for treating life threatening infections in critically-ill patients only as this antibiotic can escape certain β-lactamase resistance mechanism to which other β-lactam drugs are susceptible**.** The fact that carbapenem resistance was detected in the two most commonly isolated bacteria *i.e. E. coli* and *Klebsiella* clearly shows the need to review present antibiotic policies in order to rationalize the use of antibiotics and prevent their misuse.

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