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Detection of carbapenemase production in gram negative bacilli in medical and surgical intensive care unit patients in a tertiary care hospital

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Abstract

Background: Carbapenem-resistant Enterobacterales (CRE) are an urgent global public health problem. CRE infections are associated with high mortality and have limited available effective treatment. Carbapenemase enzymes are encoded by genes on mobile genetic elements, such as plasmids, which are highly transmissible between organisms and increase the potential spread of resistance. The study aim was to detect carbapenemase production in Gram negative bacilli by phenotypic (CarbaNP and mCIM only or in conjunction with eCIM) and genotypic (Xpert Carba-R) methods.

Material and methods: The various clinical samples were processed as per standard recommended procedures. Identification and antibiotic susceptibility test were done by using GN cards and AST N280 & AST N281 cards of Vitek 2 Compact (bioMérieux) respectively. Phenotypic (CarbaNP and mCIM only or in conjunction with eCIM) and genotypic (Xpert Carba-R) methods were used for detection of carbapenemases.

Result: 144 carbapenem-resistant Gram-negative bacilli isolated from various ICUs includes *Klebsiella pneumoniae* 52.7% (76/144) followed by *Escherichia coli* 18.05% (26/144) and 15.9% (23/144) *Acinetobacter baumannii.* Phenotypic test (CarbaNP and mCIM and or in conjunction with eCIM) showed sensitivity of 90%, 100% and 93.75% respectively. Genotypic test of 40 isolates showed predominant expression of NDM in 82.5% (33/40) isolates followed by OXA-48 in 40% (16/40).

Conclusion: The study showed mCIM as the most useful diagnostic test with less economic burden to the patients. There is an urgent need for more sensitive, rapid, highly precise and accurate genotypic test which is less expensive and less labor-intensive.

Keywords: carbapenem resistant; Enterobacterales; mCIM; eCIM; Xpert Carba-R

Introduction

Carbapenem-resistant Enterobacterales (CRE) are an urgent global public health problem. CRE infections are associated with high mortality and have limited available effective treatment [1-3]. Carbapenem are a group of β-lactam antimicrobial agents with an exceptionally broad spectrum of activity. Resistance to carbapenems can be brought about by various mechanisms. Enterobacterales (ertapenem, meropenem or imipenem), *Pseudomonas aeruginosa* (meropenem or imipenem) and *Acinetobacter baumannii* (meropenem or imipenem) that shows resistance to at least one of the carbapenems are called carbapenem-resistant Enterobacterales (CRE), carbapenem resistant *P. aeruginosa* (CRPA) and carbapenem resistant *A. bauamannii* (CRAB) respectively [4].

According to the report of ICMR AMR surveillance network, resistance to imipenem was found in 28% of *E. coli*, 55% of *K. pneumoniae*, and 80% of *A. baumannii* isolates. Hypervirulent carbapenem-resistant *K.*

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pneumoniae strains present an additional threat in Indian hospitals with a potential for global dissemination [5].

Production of carbapenemases, a class of enzymes capable of hydrolyzing carbapenems and other β-lactams is the most common mechanism. Other mechanisms include poor binding of carbapenems to penicillin-binding proteins present in the bacteria, over-expression of multidrug efflux pumps or lack of porins present in cell membrane. A combination of resistance mechanisms results in emergence of significant resistance [6]. Carbapenemase enzymes are encoded by genes on mobile genetic elements, such as plasmids, which are highly transmissible between organisms and increase the potential spread of resistance [7].

The present study is undertaken in a tertiary health care organization with medical, surgical and transplant cases in various ICUs. The magnitude of prevalence of multi drug resistant infection is relatively high as compared to primary and secondary health care setup. Hence the present study aims to detect carbapenemase production in Gram negative bacilli by phenotypic and genotypic methods. This will support clinician and institute to lay pavement path towards establishment of empirical treatment strategies, antibiotic stewardship program and further strengthening of infection prevention and control strategies.

Material and methods

This prospective observational study was conducted in the Department of Microbiology, Department of Laboratory Sciences, Krishna Institute of Medical Science, Secunderabad, from April 2021 to March 2022 on a sample size of 144 isolates. Approval for the study was obtained from Institutional Ethics Committee and Scientific Review/ Research Committee vide KIMS/IEC/2020/09-10 & KIMS/SRC/2020/01-13 respectively.

Inclusion criteria: Samples sent to microbiology lab for culture and sensitivity from patients admitted in intensive care units. Carbapenem resistant Gram-negative bacilli as identified by Vitek® 2 Compact System (Imipenem or meropenem MIC ≥ 4µg/mL or ertapenem MIC ≥2µg/mL for Enterobacterales, Imipenem or meropenem MIC ≥8µg/mL for *Pseudomonas aeruginosa* and *Acinetobacter baumannii*) [8]. Patients in the age group of ≥ 18 to ≤ 80 years were considered.

Exclusion criteria: Carbapenem sensitive or intermediate Gram-negative bacilli as identified by Vitek® 2 Compact System (Imipenem or meropenem MIC ≤2µg/mL or ertapenem MIC ≤1 µg/mL for Enterobacterales, imipenem or meropenem MIC ≤4µg/mL for *Pseudomonas aeruginosa* and *Acinetobacter baumannii*) [8]. Samples that grew isolates other than Enterobacterales, *Pseudomonas aeruginosa* and *Acinetobacter baumannii.* patients of age less than 18 years or more than 80 years, patients admitted in wards and out patients. Same patients growing same isolates from same samples i.e., duplicates are excluded.

Methods

The various clinical samples (blood, endotracheal secretion, pus, urine clean and catheter catch, bronchial wash and sputum) were processed as per standard recommended procedures. The sample processing was done in Biosafety Cabinet Class 2 (BSL-2) cabinet.

A routine Gram stain was done to note the presence of pus cells and microorganisms. The plates were incubated at 37°C for 36-48 hours. Following incubation, growths obtained on culture plates were examined for colony morphology and preliminary identification tests were done i.e., Gram stain, motility, catalase test & oxidase test. Based on the results of preliminary tests, further processing for identification and antimicrobial susceptibility testing was done using the respective VITEK cards.

Identification and antibiotic susceptibility were done by using GN card and AST N280 and AST N281 card of Vitex 2 Compact (bioMérieux) respectively. The quality control strains *Enterobacter hormaechei* ATCC 700323 and *Stenotrophomonas maltophilia* ATCC 17666 were included for GN card; *E. coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 35218 were included for AST N280 and AST N281 cards. Isolates with Imipenem or meropenem MIC ≤2µg/mL or ertapenem MIC ≤1 µg/mL for Enterobacterales, Imipenem or meropenem MIC ≤4µg/mL for *Pseudomonas aeruginosa* and *Acinetobacter baumannii* (according to CLSI M100 31st Edition, 2021), were subjected to phenotypic and genotypic tests.

Note: Samples that grew isolates other than Enterobacterales, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* were excluded from the study since test methods to detect carbapenemases in such organisms have not been validated by CLSI.

The phenotypic test done were as follows: (1) CarbaNP test [8], (2) Modified carbapenem inactivation method (mCIM) without or in conjunction with EDTA carbapenem inactivation method (eCIM) [8]. The genotypic test was done by Xpert Carba-R assay that detects *bla*NDM, *bla* KPC, *bla*VIM, *bla*OXA-48 & *bla*IMP [9].

Statistical analysis

Antibiotic susceptibility results from Vitek 2 compact were exported from Vitek to Microsoft excel. Data was analyzed using Microsoft-excel. The results were presented in term of frequency counts with percentages for categorical variables. The quantitative variable like age of patients was categorized using 10-year categories and expressed using percentage. The categorical variables such as gender, presence of carbapenamase enzyme, comorbidity was expressed as percentage. The statistical test of hypothesis was not applied to the present study. The clinical characteristics of patients included in this study by certain established risk factors (e.g., comorbidity, ICU admission, etc.) were identified from similar published studies.

Results

Carbapenem resistant Gram-negative bacilli as identified by Vitek® 2 Compact system (Imipenem or meropenem MIC ≥ 4µg/mL or ertapenem MIC ≥2µg/mL for Enterobacterales, imipenem or meropenem MIC ≥8µg/mL for *P. aeruginosa* and *A. baumannii*) were included in the study.

144 patient samples were included in the study. Out of 144 samples, 78 were of males (54%) while 66 were of females (46%) as shown in figure 1. In the study group, out of 144 patients, most number (n=33) of patients were of age group 40-49 years. Age distribution is given in figure 2.

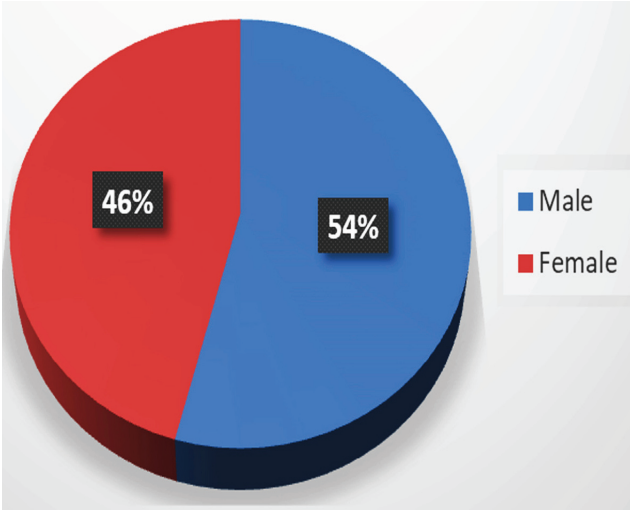

Figure 1: Gender distribution.

Of 144 isolates, 46 (31.94 %) have been recovered from medical ICU followed by 31 (21.25%) from heart lung transplant ICU, 30 (20.83 %) from surgical ICU, 14 (9.72 %) from transplant ICU, 13 (9.02%) from cardiac ICU and 10 (6.94%) from gastroenterology ICU as shown in figure 3.

As shown in the table 1, *Klebsiella pneumoniae* was the most commonly isolated organism (52.77%) followed by *Escherichia coli* (18.05%). Of the various samples processed, carbapenem resistant Gram-negative bacilli was most commonly isolated from blood (20.13%) and ET secretions (20.13%).

Table 1: Various clinical samples and organisms isolated.

Organism	*Blood*	*ET secretion*	*Pus*	*Urine clean catch*	*Urine catheter catch*	*BAL*	*Sputum*	*Total (%)*
K. pneumoniae	14	12	15	9	10	9	7	76 (52.77)
E. coli	3	3	3	11	4	1	1	26 (18.05)
A.baumannii	8	8	1	-	-	3	3	23 (15.9)
P. aeruginosa	2	3	2	-	1	-	2	10 (6.94)
P. mirabilis	-	-	-	2	1	-	-	3 (2.08)
S. marcescens	2	1	-	-	-	-	-	3 (2.08)
K. oxytoca	-	1	1	-	-	-	-	2 (1.38)
K. aerogenes	-	1	-	-	-	-	-	1 (0.69)
Total (%)	29 (20.13)	29 (20.13)	22 (15.2)	22 (15.2)	16 (11.11)	13 (9.02)	13 (9.02)	144 (100)

Phenotypic test results

Test results of CarbaNP, mCIM and eCIM were analyzed and tabulated in table 2 below. Out of 144 carbapenem resistant Gram-negative bacilli, 104 (72.22 %) were CarbaNP positive. *Klebsiella pneumoniae* accounted for the highest number of isolates (89 %) positive for CarbaNP.

Of 144 isolates, 113 (78.47 %) isolates tested positive for mCIM test, out of which, 98 were Enterobacterales. The majority of the isolates positive for mCIM were *Klebsiella pneumoniae* (93.42 %) followed by *E. coli* (88.46 %).

As mentioned in CLSI M100, 31st edition (8), 2021, Introduction to Tables 3B and 3C (page 119),

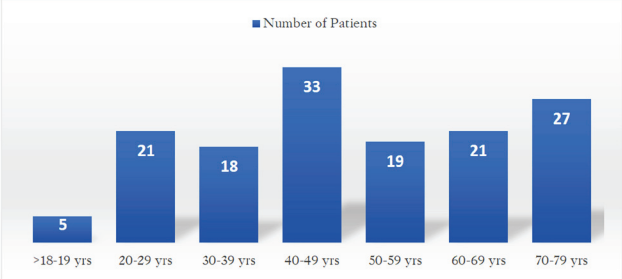

Figure 2: Age distribution of the patients.

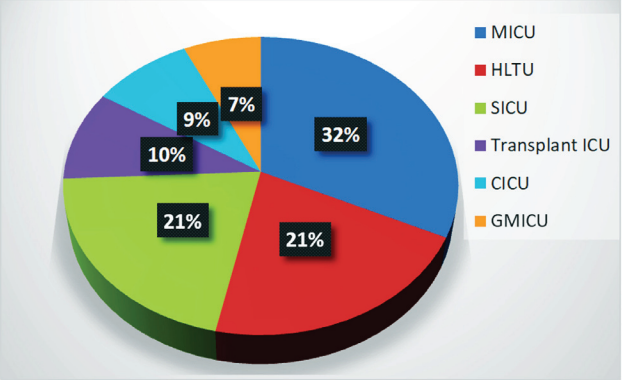

Figure 3: Distribution of patients from various ICUs.

Enterobacterales that were positive by mCIM were tested for eCIM. In this study, of 98 mCIM positive Enterobacterales, 86 (87.75 %) isolates were positive for eCIM test. The majority of the eCIM positive isolates were *Klebsiella pneumoniae* (66.32%) followed by *E. coli* (19.38%).

Table 2: Phenotypic test results of isolates.

Organism	*Number of isolates*	*CarbaNP*	*mCIM*	*eCIM*
Klebsiella pneumoniae	76	68	71	65
Escherichia coli	26	22	23	19
Proteus mirabilis	3	1	1	0
Serratia marcescens	3	1	2	1
Klebsiella oxytoca	2	1	1	1
Klebsiella aerogenes	1	0	0	0
Acinetobacter baumannii	23	5	8	-
Pseudomonas aeruginosa	10	6	7	-
Total	144 (100%)	104 (72.22%)	113 (78.47%)	86

As a pilot study, to establish a baseline genotypic profile for the institute and due to financial limitations, 40 carbapenem resistant isolates were tested by Xpert Carba-R method. In this study, carbapenemases were detected in all the 40 isolates (100%) tested.

Among the 40 isolates tested by Xpert Carba-R, 37 isolates were Enterobacterales (28 isolates were *Klebsiella pneumoniae*, 9 were *Escherichia coli)*, 2 were *Acinetobacter baumannii* and 1 was *Pseudomonas aeruginosa*. The most common genes identified were *bla*NDM (82.5%) followed by *bla*OXA-48 (40.0%). *Klebsiella pneumoniae* was the most common among the *bla*NDM gene positive organisms as shown in table 3.

Table 3: Organism wise distribution of genes (N= 40).

Gene	*K. pneumoniae (n=28)*	*E.coli (n=9)*	*A.baumannii (n=2)*	*P.aeruginosa (n=1)*	*Total*	*Percentage*
blaNDM	23	7	2	1	33	82.5
blaOXA-48	11	3	1	1	16	40
blaVIM	2	-	-	-	2	5
blaKPC	1	-	-	-	1	2.5
blaIMP	-	-	-	-	-	-

Of the 40 isolates tested by Xpert Carba-R, single gene detection was observed in 28 isolates (70.0%). Co-existence of carbapenemase genes were detected in 12 (30.0%) isolates, out of which *bla*NDM and *bla*OXA-48 were predominant (n=11) followed by *bla*VIM and *bla*OXA-48 (n=1) as shown in table 4.

Table 4: Co-existence of carbapenemase genes observed in the study.

Single gene		*>1 Gene*	
Gene	*Number*	*Co-existence*	*Number*
blaNDM	22	blaNDM + blaOXA-48	11
blaOXA-48	4	blaVIM + blaOXA-48	1
blaVIM	1		
blaKPC	1		
Total	28 (70%)	Total	12 (30 %)

Out of the 40 isolates tested by Xpert Carba-R, 36 (90%) were positive by CarbaNP test, as shown in table 5 below.

Table 5: Comparison of CarbaNP with Xpert Carba-R (n=40).

	CarbaNP test	*Xpert Carba R*
Number of isolates tested positive	36 (90%)	40 (100%)

Out of 40 isolates tested by Xpert Carba-R, 100% of the isolates (n=40) were positive for mCIM test. Out of 37

isolates of Enterobacterales tested by Xpert Carba- R, 32 were metallo-beta-lactamases (MBL) expressing Enterobacterales. Out of these, 30 were mCIM in conjunction with eCIM positive, as shown in table 6.

Table 6: Comparison of mCIM and eCIM test results with genotypically positive isolates (n=40).

	Xpert Carba R	*mCIM test (n=40)*	*mCIM in conjunction with eCIM test (n= 32)**
Number of isolates tested positive	40 (100%)	40 (100%)	30 (93.75%)

Note: *Out of 40 isolates tested by Xpert Carba R, results of eCIM test were compared with the 32 isolates of Enterobacterales which expressed carbapenemase genes belonging to Ambler molecular classification group B (metallo-beta-lactamases).

The statistical test of hypothesis was not applied to the present study. The clinical characteristics of patients included in this study by certain established risk factors (e.g., comorbidity, ICU admission, etc.) were identified from similar published studies.

The clinical characteristics of patients included in the study were divided into two sections as comorbidities and others. The comorbidity observed were variable therefore for better insight into the comorbidities, all conditions were categorized into 5 groups namely, Group 1 (metabolic disorder), Group 2 (pulmonary diseases), Group 3 (hematological malignancies), Group 4 (solid organ tumors) and Group 5 (autoimmune diseases) as shown in table 7.

Table 7: Different co-morbidities observed amongst patients.

Group 1: metabolic disorders	*Group 2: pulmonary diseases*	*Group 3: hematological malignancy*	*Group 4: solid organ tumors*	*Group 5: autoimmune diseases*
Hypertension n = 26 (18.0%)	Interstitial lung disease n= 9 (6.25%)	Hematolymphoid Neoplasm n= 9 (6.25%)	Carcinoma stomach n= 2 (1.38%)	Systemic lupus erythematous n= 5 (3.47%)
Diabetes mellitus n= 21 (14.5%)	Chronic obstructive disease n= 4 (2.7%)		Carcinoma prostate n= 2 (1.38%)	Celiac disease n= 1 (0.69%)
Chronic kidney disease n= 9 (6.25%)	Post covid fibrosis n= 4 (2.7%)		Carcinoma breast n= 1 (0.69%)	Rheumatoid arthritis n= 1 (0.69%)
Cerebrovascular accidents n= 3 (2.08%)			Carcinoma cervix n= 1 (0.69%)	
Coronary artery disease n= 1 (0.69%)			Carcinoma colon n= 1 (0.69%)	
Peripheral vascular disease n= 1 (0.69%)				
Total: 61 (42.36%)	Total: 17 (11.80%)	Total: 9 (6.25%)	Total: 7 (4.86%)	Total: 7 (4.86%)

Other clinical characteristics included in the study were presence of indwelling catheters, immune status of patients, history of surgery, history of ICU admission with or without mechanical ventilation and history of solid organ or hematological transplants are explained in Table 8.

Table 8: Various other clinical characteristics among patients.

Characteristics included under 'Other' group	*Number of patients*	*Percentage*
Indwelling catheters	144	100
Immunocompromised patient	46	31.94
H/O surgery	33	22.91
H/O previous ICU admission	51	35.41
Transplantation	45	31.25

Discussion

The emergence of carbapenem-resistant *Enterobacterales* (CRE), is a rapidly evolving global public health dilemma and calls for urgent action within the international scientific community [10, 11]. Over the last two decades, use of carbapenems have increased many folds and the same is reflected in imipenem susceptibility of *E. coli* dropping steadily from 86% in 2016 to 72% in 2020 and that of *Klebsiella pneumoniae* dropping steadily from 65% in 2016 to 45% in 2020 [5].

Methods for detecting carbapenem resistance in Gram negative bacilli vary from conventional disc diffusion test, determination of minimum inhibitory concentration to advanced methods like rapid card tests and genotypic tests. Advanced methods are not usually

used in routine laboratories, especially due to limitation of resources. This leads to missed or delayed detection of carbapenem resistance, causing therapeutic failure and spread of carbapenem resistant Gram-negative bacilli in hospitals. This study evaluated the phenotypic methods to detect carbapenem resistance.

In this study, the predominant gender was males (54.16 %). Similarly, in a study by Alebel et al. the predominant gender of the study group was males (36.7%) [12]. In the present study maximum isolates were recovered from patients admitted in Medical ICU (31.94%). Similar results were observed in a study by Yoo et al. where majority of the isolates were recovered from medical ICU (61.8%) [13].

In the present study, 20.13% of isolates were recovered from blood and ET secretions each, 15.2% from pus and urine clean catch each, 11.11% from urine catheter catch and 9.02% from BAL and sputum samples each. In a study by Govindaswamy et al. bacterial isolates were predominantly recovered from pus (37.8%), followed by urine (26.2%), endotracheal aspirate (26.2%), blood (6.7%) and sterile body fluids (2.9%) [14]. These findings do not correlate with the present study as Govindaswamy et al. studied beta-lactamase producers in only isolates of *Escherichia coli*. Pawar et al. conducted a study on detection of CRE in clinical specimens coming from both ICUs and wards, where urine (n=27) was commonest sample followed by pus (n=15) [15]. These findings are not comparable to the present study as the study group was only of ICU patients, who would be mostly in sepsis or on mechanical ventilation.

In the present study, of the 144 carbapenem resistant Gram-negative bacilli isolates *Klebsiella pneumoniae* was the predominant organism (52.77%). This is comparable to studies conducted by Pawar et al. and Han et al, where *Klebsiella pneumoniae* was the predominant organism accounting for 53%, 75.8% respectively [15, 16].

In this study, 72.22 % of the isolates were carbaNP test positive. This correlates with the findings of a study by Sinha et al. in which CarbaNP test was positive in 71% of their isolates [17]. Also, in a study by Datta et al. 86.4% of the isolates were CarbaNP test positive [18].

The performances of phenotypic tests were determined by comparing their result with performance of Xpert Carba-R. The sensitivity of CarbaNP was 90%. Table 9 shows the sensitivity of CarbaNP test in various studies.

Table 9: Sensitivity of CarbaNP test in various studies.

Reference	*Sensitivity %*	*Concordance or Discordance*
Zhou et al. [19]	99.6	Concordance
Shaikh et al. [20]	84	Concordance
Kumudunie et al. [21]	75.9	Discordance
Nordmann et al. [22]	100	Concordance
Present study	90	-

In this study, it was observed that CarbaNP test showed poor sensitivity (21.37%) in strains of *Acinetobacter baumannii*. This finding is in agreement with the CLSI M100, 2022 (21.3%) [23]. This is possibly due to weak carbapenemase activity compared to other enzymes.

It was also observed in this study that CarbaNP was negative for 4 isolates (*1 K. pneumoniae*, 1 *P. aeruginosa,* 1 *E. coli* and 1 *Acinetobacter baumanii*) for which OXA-48 was detected in Xpert Carba-R.

The observed results are in agreement with the study of Osterblad et al. where CarbaNP test gave a weak reaction in detection of OXA-48 (4/19) [24]. Also, in a multicentric study of 7 participating sites by Cunningham et al. sixteen carbapenemase-positive (OXA-48) isolates gave false-negative results by carbaNP test [25]. These results could be possibly due to incomplete lysis of bacterial isolate to extract enough active enzymes, less number of isolates, poor hydrolyzing capacity of OXA-48 or enzyme activity could be inhibited by lysis buffer.

This study concludes that though CarbaNP test is a rapid diagnostic test with a shorter turnaround time, the disadvantages like requirement for dedicated reagents (with associated costs and training needs), short shelf life of reagents, subjective result interpretation (based on color change), and certain carbapenemase types (e.g., OXA-type, chromosomally encoded) not being consistently detected must be considered before implementing in routine laboratories.

In the present study, 78.47 % of the isolates were mCIM positive. This coincides with the findings of Sinha et al. who found 80% of their isolates tested positive by mCIM method [17]. In a study by Pawar et al. 98.48% of the isolates were mCIM test positive [15]. In a study by Li et al. 97.5% of the isolates were mCIM positive [26].

In this study, out of the 40 isolates tested for Xpert Carba-R, all the 40 (100%) were mCIM positive. This shows that there was a high agreement between mCIM and Xpert Carba-R in the detection of carbapenemase enzyme. This finding is correlating with the results of previous studies by Patel et al. and Shaik et al. who

have reported nearly 100% sensitivity and specificity of mCIM for detecting carbapenemase types such as KPC, NDM, VIM, IMP, and OXA-48-like [20, 27]. These findings conclude that though mCIM test has a longer turnaround time due to overnight incubation, mCIM is suitable for a resource-poor microbiology laboratory as it is inexpensive, easy to perform, less subjective with a more reliable sensitivity compared to CarbaNP test.

In this study, of the mCIM positive isolates of Enterobacterales, 87.75% were eCIM positive. This is in concordance with Sinha et al. who also reported 87% of the mCIM positive Enterobacterales to be eCIM positive [17]. In an article by Li et al. 100% of the Enterobacterales they studied were eCIM positive [27].

Out of 40 isolates tested by Xpert Carba-R, 37 were Enterobacterales. Out of these, 32 were MBL (Ambler molecular classification group B; e.g., NDM, VIM) expressors. Considering CLSI M100 31st edition, table 3C, page 128, MBL expressing Enterobacterales were interpreted for assessing the performance of eCIM test. The sensitivity and specificity of eCIM in the present study is 93.75% and 100% respectively. Similar to this finding is the sensitivity (89.3%) and specificity (98.7%) of the eCIM test in a study by Tsai et al. [28]. The findings of this study also correlate with a study by Gill et al. in which eCIM diagnostic performance was evaluated in concordance to their genotypic profiles. All the VIM, NDM and KPC positive isolates evaluated had 100% sensitivity and specificity [29].

In this study, eCIM test was negative for one isolate which co-harbored VIM and OXA-48. As mentioned in CLSI M100 31st edition (table 3C, page 130-131), co-production of both serine carbapenemase and metallo-beta-lactamase will not be differentiated phenotypically, hence giving false negative eCIM result [8].

In the present study, the coincidence rate of mCIM in conjunction with eCIM was 87.75%. This correlates with a study by Gill et al. who had reported a coincidence rate of 89.3% [29]. A higher coincidence rate (97.5%) was reported in a study by Li et al. [26]. The variability in the co-incidence rates may be explained by variation in the genotypic profile of carbapenemase genes in different geographic distributions.

As a pilot study, due to financial limitations, 40 isolates were tested by Xpert Carba-R method. In this study, carbapenemase genes were detected in all the carbapenem resistant Gram-negative isolates (100%) tested. Likewise, 100% of the isolates were positive for carbapenemase genes in a study by Pawar et al [15]. Xpert Carba-R detected one or more genes of carbapenemases in 80.7% of the isolates in a study by Sheth et al [30]. Comparably, in a study by Vamsi et al. out of 207 carbapenemase producers, carbapenemase genes were detected in 92.7% of the isolates by genotypic method (real time PCR) [31].

In the present study genotypic test (Xpert carba-R) of 40 isolates showed predominant expression of NDM in 82.5% of the test isolates, followed by OXA-48 in 40 % of the test isolates. Comparably, in a study by Mohanty et al. NDM was the predominant gene detected (65.6%) and the second most common gene was OXA-48 (24.7%) [32]. These findings are comparable to the genotypic positive findings of Vamsi et al. where NDM was the predominant gene detected (47.3%) [31]. Similarly, in the study of Paudel et al [33], out of 45 carbapenemase-producing isolates, 24.4% and 15.5% were found to be positive for NDM and OXA-48 genes, respectively.

In this study, NDM and OXA-48 genes were detected predominantly in *Klebsiella pneumoniae* (69.6% and 68.7% respectively). This correlates with Mohanty et al. who found NDM (63.9%) expression followed by OXA-48 expression (24.7%) predominantly in *Klebsiella pneumoniae* [32]. Coinciding with these findings is that of Vamsi et al. who found that *Klebsiella pneumoniae* was the most common organism among the NDM gene positive organisms [31].

In the present study, 30 % of the genotypic positive organisms co-harbored more than 1 gene. NDM+OXA 48 co-existence accounted for 27.5% while 2.5% accounted for VIM+OXA-48. The presence of multiple genes has also been analyzed in various studies. This is similar to a study by Garg et al. NDM and OXA-48 were co-harbored in 21.1% of the isolates [34]. In the study by Mohanty et al. 5 out of 71 organisms co-harbored NDM and OXA-48 while 2 organisms co-harbored VIM and OXA-48 [32].

In the present study, 2 isolates of *K. pneumoniae* (1 VIM and 1 VIM+OXA-48) were false negative by eCIM method. In a study of Li et al. 2 KPC+NDM producing isolates of *K. oxytoca* were eCIM test negative [26]. In a study of Tsai et al. a total of 3 isolates were eCIM negative which were 2 *E. coli* isolates harboring both NDM and OXA-48, and 1 *K. pneumoniae* harboring IMP [28]. The false negative result of eCIM could be due to weak chelation of EDTA to inhibit metallo-β-lactamases.

As this study is a descriptive cross-sectional study, statistical test of hypothesis was not applied. The clinical characteristics of patients included in this study by certain established risk factors (e.g., comorbidity, ICU admission, etc.) were identified from similar published studies.

Comorbidities in the study were analyzed and compared to other research publications. In the present study, maximum comorbidity was contributed by metabolic disorders (42.36 %) with hypertension (18 %) being the most common condition among this group. Indwelling catheters were present in 100 % patients. In the study of Mariappan et al. male gender (p = 0.050), stay in ICU (p = 0.021), mechanical ventilation (p = 0.013), presence of multiple indwelling device (p = 0.011) including drains and central lines, presence of diabetes mellitus (p = 0.036), presence of focal infection or sepsis (p = 0.013), surgical interventions (p = 0.016), and usage of multiple antimicrobial agents (p = 0.007) were significant risk factors influencing the acquisition of CPE [35].

In the study of Patel et al. carbapenem-resistant *K. pneumoniae* infection was independently associated with recent organ or stem-cell transplantation (p = 0.008), receipt of mechanical ventilation (p = 0.04), longer length of stay before infection (p = 0.01) and exposure to cephalosporins (p = 0.02) [27].

The widespread distribution of carbapenem resistant Gram-negative bacilli is mainly attributable to their production of carbapenemases and the plasmid-mediated horizontal transmission of the encoding genes. The probable reasons for varied distribution of genes could be due to indiscriminate antibiotic prescription for self-limiting and non-bacterial infections with easy over the counter access to antibiotics with poor sales regulations. Inadequate infection control measures in healthcare facilities once carbapenem resistance has emerged and the use of non-therapeutic purpose of antibiotics for the promotion of animal growth in the animal husbandry is aggravating.

Conclusion

The study showed mCIM as the most useful phenotypic test with less economic burden to the patients. Genotypic test enables rapid detection and differentiation of the *bla*KPC, *bla*NDM, *bla*VIM, *bla*OXA-48, and *bla*IMP gene sequences which helps in optimization of patient management and therapeutic strategy. Active surveillance for carbapenem resistant Gram-negative bacilli using genotypic method provides an extra edge in rapid implementation of infection control practices so that their spread can be minimized in a health care setting.

Conflicts of interest

Authors declare no conflicts of interest.

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