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Phenotypic detection of Carbapenem Resistant Enterobacterales in tertiary care hospital.

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Abstract

Background: Antimicrobial resistance is being expressed in almost all most commonly isolated pathogens such as Enterobacteriales and Non fermenters. Antimicrobial-resistant infections are more difficult to treat, leading to treatment failure and complications, on top of huge financial costs to individuals. So we have undertaken this study by high lightening a few widely available and less expensive diagnostic techniques to detect carbapenem resistance. The study aims to detect and characterize Carbapenemase enzyme production among Enterobacterales using phenotyopic method. ***MATERIALS AND METHODS***: A total of 1164 patients attended various clinical departments with clinical manifestations of infection were included in this prospective observational study. All clinical specimens were processed for culture and sensitivity testing as per the standard guidelines. Enterobacterales isolates obtained from these samples were initially screened for carbapenem resistance using the Meropenem (10g) disc, further tested with Modified Carbapenem Inactivation Method (mCIM) and the EDTA –Modified Carbapenem Inactivation Method (eCIM). ***RESULTS***: 564 (48.4%) Enterobacteriaceae pathogens were isolated from 1164 clinical specimens. 293 carbapenem resistant isolates out of 564 Enterobacteriaceae isolates i.e., 51.9%. K.pneumoniae was the predominant isolate (47.09%) from various clinical specimens followed by Escherichia coli (31.7%), Klebsiella oxytoca (7.8%), Citrobacter (5.4%), Enterobacter (4.07%), and Proteus (3.7%). Out of 244 Carbapenem resistant isolates which are positive for either of tested methods, 240 (98.3%) were detected as MBL Carbapenemases and 1.63% were detected as Serine Carbapenemases. ***CONCLUSION***: Phenotypic characterization by mCIM and eCIM methods was performed and noted the type of carbapenemase production which will help in accurate management of infection and avoid failure of treatment due to antimicrobial resistance.

Keywords: Carbapenemases, Enterobacteriaceae, Antimicrobial resistance.

INTRODUCTION

Antimicrobial infections are becoming challenging aspect globally, with a great difficulty to treat at hospitals and have contributed to higher mortality. Antimicrobial resistance is being expressed in almost all most commonly isolated pathogens such as Enterobacteriales and Non fermenters. Antimicrobial-resistant infections are more difficult to treat, leading to treatment failure and complications, on top of huge financial costs to individuals. Escherichia coli, Klebsiella and Pseudomonas are most frequently isolated pathogens in ICU's raising concern because of marked resistance to all essential antibiotics such as 'Watch' antibiotic in AWaRe as per WHO – like carbapenems and fluoroquinolones which are most commonly used for severely infected critical patients [1].

Carbapenem resistant pathogens are associated with severe clinical outcomes and limited therapeutic options. Because of the increasing carbapenem resistance in bacteria, the empirical therapeutic choice for intensivists or clinicians is decreasing. Clinicians only hope to treat such extreme drug resistance pathogens is become a "last resort" antibiotics choice like colistin, polymyxin and amikacin [2].

Carbapenems are a class of broad-spectrum, "last resort" β lactam antibiotics. Their mode of action is inhibition of cell wall synthesis. Common carbapenems in clinical use are meropenem, imipenem, ertapenem ad doripenem [3].
Carbapenem resistance primarily occurs by three mechanisms by producing carbapenemases, by reducing outer membrane permeability and by efflux pumps which push antibiotic out of the bacterial cell. In addition, the resistance genes on mobile genetic elements transferred to other gram negative bacteria and share their resistance traits to other bacteria.

Carbapenem resistance mechanism detection does at laboratories to know how bacterial evade carbapenem antibiotics. Diagnostic techniques ranges from conventional methods like phenotypic detection of carbapenemases by Carba NP test, modified Carbapenem Inactivation method (mCIM), EDTA - modified Carbapenem Inactivation method (eCIM), to molecular assays like PCR, microassays, until advanced characterization such as WGS, MALDI-TOF MS. For fast detection of different types of Carbapenemases, Lateral flow Immunoassays are available. So we have undertaken this study by high lightening a few widely available and less expensive diagnostic techniques.

AIM:
The study aims to detect and characterize Carbapenemase enzyme production among Enterobacterales by phenotyopic methods.

OBJECTIVES:
1.To determine the prevalence of Carbapenemase-producing Enterobacterales (CPE) in clinical isolates.
2.To differentiate between serine carbapenemases and metallo β lactamases by phenotypic characterization.

MATERIALS AND METHODS

Study Settings & Design:
A Prospective observational study was conducted on clinical specimens received at the Department of Microbiology of Government General Hospital/Medical College, Anantapuram, Andhra Pradesh. A total of 1164 patients attended various clinical departments with clinical manifestations of infection were included in this study during the period of three months (February 2026 to April 2026). Ethical committee approved this study (IEC No. 15-11/2-25). Informed consent was taken from all the patients included in this study.

Inclusion criteria:
Clinical specimens collected from various body sites were received in the laboratory and processed using standard protocols.
Isolates showing reduced susceptibility to carbapenems.

Exclusion criteria:
Repetitive samples from same patients.

Sample collection and transportation:
A total number of 1164 samples were collected from patients presenting with clinical manifestations of infection. These specimens were collected as per the standard protocols either in a wide mouth, screw capped, leak proof container or sterile swabs or in BacTalert bottles. Specimens were advised to collect under aseptic precautions to avoid cross contamination. If there is any delay in transportation of samples, those were stored in refrigerator up to 24 hours.

Processing of samples:
Specimens were processed as per the standard guidelines [5]. All the specimens were streaked on appropriate culture plates and incubated at 370C. After 24 hours of incubation of streaked culture media if there is no growth then those plates were further incubated for another 24 hours. The colony characteristics observations, its gram stain and biochemical reactions were performed as per standard precautions [6].

Antimicrobial Susceptibility Testing:
Antimicrobial susceptibility of isolates was determined using the Modified Kirby-Bauer disk diffusion method. The results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [7].

Enterobacterales isolates obtained from these samples were initially screened for carbapenem resistance using the Meropenem (10g) disc [8]. Isolates exhibiting reduced susceptibility to meropenem were further tested for carbapenemase enzyme production using phenotypic methods, specifically the Modified Carbapenem Inactivation Method (mCIM) and the EDTA –Modified Carbapenem Inactivation Method (eCIM). eCIM results to be interpreted only if mCIM is positive (Table 1).

Statistical Analysis:
The data was collected from laboratory culture registers and the results were obtained in the Microsoft Excel and statistically analyzed by calculating the numbers and percentages of all descriptive variables.

RESULTS

Table 1. Interpretation of Carbapenemases

Test		Result	Interpretation
mCIM	Positive	Zone of inhibition 6-15 mm or colonies within 16-18 mm	Carbapenemase producer
	Negative	Zone of inhibition ≥19 mm	Non- Carbapenemase producer
eCIM		Increased zone compared to mCIM (≥5 mm difference)	Metallo β–lactamase (MBL) producer
		No significant change in zone	Serine Carbapenemase producer

During the study observation period prospectively we received 1164 various clinical specimens from patient's suspicious of infectious diseases. 564 (48.4%) Enterobacteriaceae pathogens were isolated from 1164 clinical specimens. A total of 293 Carbapenem Resistant isolates which were screened by meropenem disc of 10µg, it was 293 out of 564 Enterobacteriaceae isolates i.e., 51.9% (Fig 1).

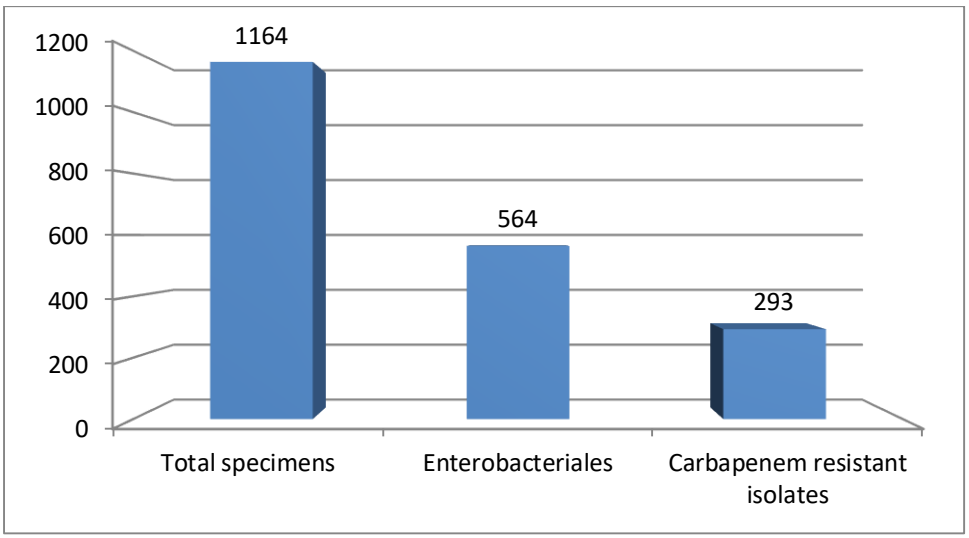

Fig 1. Distribution of specimens and isolates

Predominant clinical specimen processed for carbapenem resistant was urine (61.4%) followed by sputum (17.7%) and pus (7.8%). We also isolated carbapenem resistant pathogens from other specimens such as wound swabs, HVS, ET secretions and NG aspirate (Table 2).

Table 2. Percentage of various specimens processed

Specimen	No. of isolates	Percentage
Urine	180	61.4%
Sputum	52	17.7%
HVS	15	5.1%
Wound swab	11	3.75%
Pus aspirate	23	7.8%
ET tip	8	2.7%
NG aspirate	4	1.36%

K.pneumoniae was the predominant isolate (47.09%) from various clinical specimens followed by Escherichia coli (31.7%), *Klebsiella oxytoca* (7.8%), *Citrobacter* (5.4%), *Enterobacter* (4.07%), and *Proteus* (3.7%) (Fig 2).

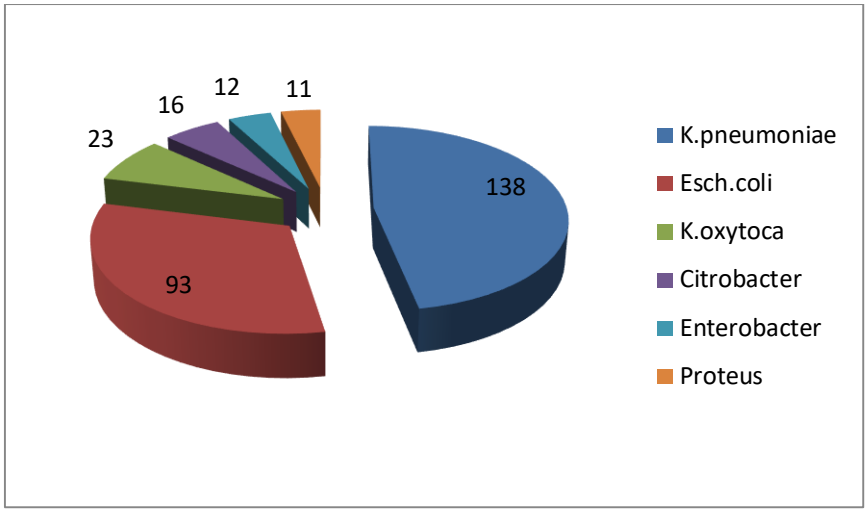

Fig 2. Distribution of various pathogens in study population

Out of 293 Enterobacteriaceae isolates, 244 (83.27%) were either mCIM or eCIM positive. 240 (81.9%) were positive by mCIM method and eCIM method , and 4 (1.36%) were positive by eCIM method and remaining 49 (16.7%) were undetected through either by mCIM and by eCIM methods (Fig 3 & Fig 4).

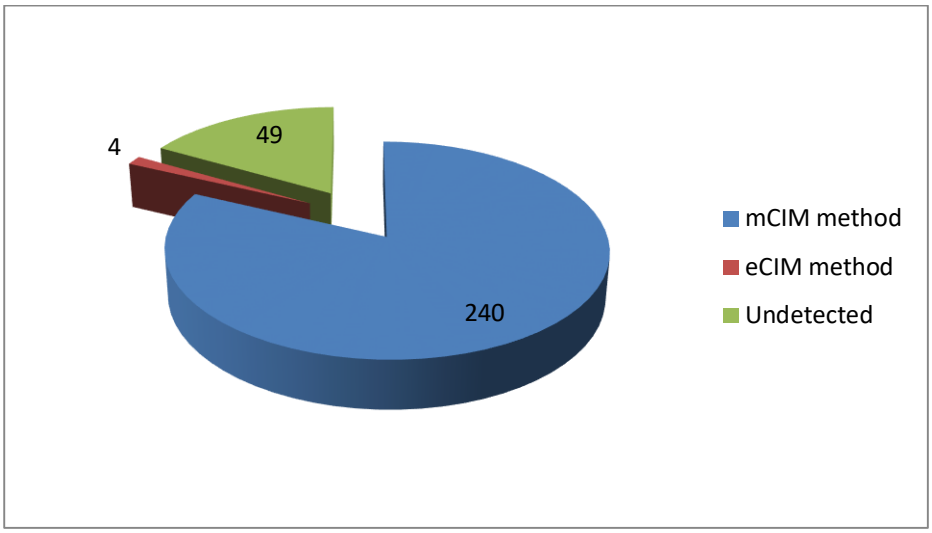

Fig 3. Isolates tested by mCIM and eCIM methods

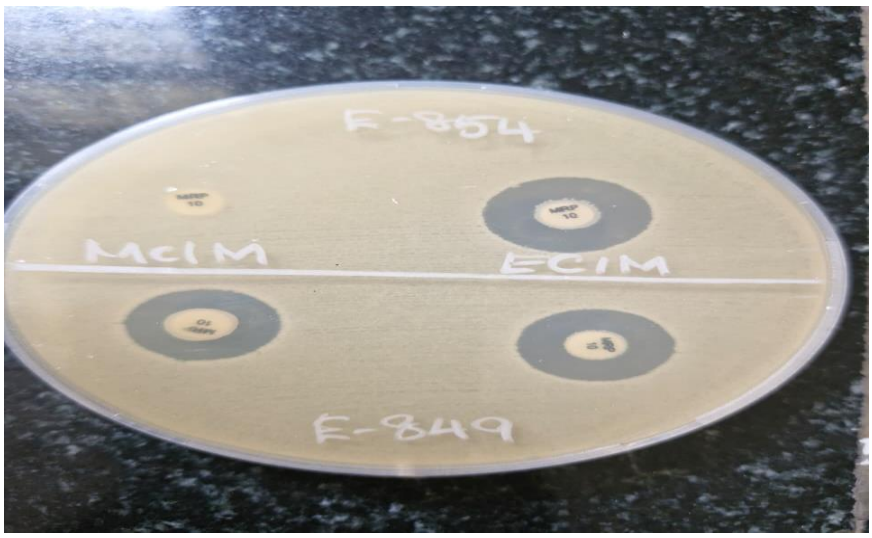

Fig 4. MCIM and eCIM method tested on a pathogen

Out of 244 Carbapenem resistant isolates which are positive for either of tested methods, 240 (98.3%) were detected as MBL Carbapenemases and 1.63% were detected as Serine Carbapenemases. Among 240 MBL positive isolates of Enterobacteriaceae 146 were isolated form urine predominantly. Out of 4 serine carbapenemase isolates 3 were from urine and one from sputum (Table 3).

Table 3. MBL and Serine Carbapenemases isolates

	Carbapenem resistant isolates tested positive for either mCIM or eCIM	MBL Carbapenemases - mCIM & eCIM positive	Serine Carbapenemases - mCIM positive & eCIM No significant change
Urine	149	146	3
Sputum	33	32	1
HVS	24	24	0
Wound swab	16	16	0
Pus aspirate	13	13	0
ET tip	7	7	0
NG aspirate	2	2	0
Total	244	240 (98.3%)	4 (1.63%)

Among 240MBL positive pathogens, majority were *Klebsiella pneumoniae* (52.08%), followed by *Escherichia coli* (33.3%), *Klebsiella oxytoca* (6.66%), *Citrobacter* (3.33%), *Enterobacter* (2.5%), and *Proteus* (2.08%). Out of 4 Serine Carbapenemases, 2 (50%) were Escherichia coli and 2 (50%) were *Klebsiella pneumoniae* (Table 4).

Table 4. MBL and Serine Carbapenemases detection in various pathogens

Organism	MBL Carbapenemase isolates	Percentage	Serine Carbapenemase isolates	Percentage
Escherichia coli	80	33.3%	2	50%
Enterobacter	6	2.5%	-	-
Citrobacter	8	3.33%	-	-
Klebsiella pneumoniae	125	52.08%	2	50%
Klebsiella oxytoca	16	6.66%	-	-
Proteus	5	2.08%	-	-
Total	240		4	

DISCUSSION

Carbapenemase Resistant Gram-Negative Bacilli (CR-GNB) expresses resistance against multiple β-lactam antibiotics including Carbapenems, they are typically reserved for severe, hospital-acquired or life-threatening infections when other treatments fail [9]. CR-GNB cause clinically significant resistance often, occurs by combination of mechanism of resistance. Among enzyme degradation Carbapenemases – common types include Klebsiella pneumoniae Carbapenemases (KPC), New Delhi Metallo β-lactamases (NDM), and OXA type enzymes.

564 (48.4%) Enterobacteriaceae pathogens were isolated from 1164 clinical specimens. A total of 293 Carbapenem Resistant isolates which were screened by meropenm disc of 10µg, it was 293 out of 1164 culture specimens i.e., 25.1%. Rajshekar et al [10] did a study on blood culture, noted 39.94% of multidrug resistant organisms and 32.11% isolates were Carbapenem resistant. In similar to the present study Bhatt et al [11] and Thomas Sarwat et al [12] noted the prevalence of CRE (Carbapenem resistant Enterobacterales) as 18.3% and 18.5% respectively.

K.pneumoniae was the predominant isolate (47.09%) from various clinical specimens followed by Escherichia coli (31.7%), Klebsiella oxytoca (7.8%), Citrobacter (5.4%), Enterobacter (4.07%), and Proteus (3.7%). In line with this study Rajshekar et al [10] observed out of 153 MDR GNB included, the majority was Klebsiella pneumoniae 88 (57.5%), followed by 32 (20.9%) E. coli, P. aeruginosa 29 (18.95%), and Serratia marcescens 4 (2.61%). Sinha Astha et al [13] observed 379 isolates of Enterobacterales, the maximum number of isolates belonged to Klebsiella pneumoniae at 44.85% (170/379), followed by Escherichia coli at 44.06% (167/379) and Klebsiella oxytoca at 11.08% (42/379). Kumari et al [14] also reported the highest carbapenem resistance in admitted patients in Klebsiella species. S RS et al [15] culture specimens yielded 211 isolates belonging to Enterobacteriaceae family, Klebsiella pneumoniae 66 (31.27%) was the predominant organism isolated followed by Klebsiella oxytoca 52 (24.64%), Escherichia coli 49 (23.22%), Citrobacter species 32(15.16%) and Enterobacter species 12(5.68%).

Out of 293 Enterobacteriaceae isolates, 244 (83.27%) were either mCIM or eCIM positive. 240 (81.9%) were positive by mCIM method, and 4 (1.36%) were positive by eCIM method and remaining 49 (16.7%) were undetected through either by mCIM and by eCIM methods. Rajshekar et al [10] reported that among 123 CRO's (Carbapenem Resistant Organisms) 67 (54%) isolates were Serine Carbapenemases which are mCIM positive and eCIM negative) and 1 isolate each of K. pneumoniae and P. aeruginosa showed indeterminate results. Of 123 CROs 54 (45.5%) were positive by both eCIM and mCIM and were determined as Metallo β-lactamases. Sinha Astha et al [13] documented out of total 70 CRE, 54.28% (38/70) were positive for carbapenemase production by either of the phenotypic methods. Isolates that were positive for carbapenemase production by both phenotypic methods were 34.29% (24/70). 18.57% (13/70) isolates were positive for Cabapenemase production by mCIM alone while 1.43% (1/70) isolates were Metallo-Beta-lactamase (MBL) positive by EDTA-DST alone. S RS et al [15] noted out of 211 isolates, 50 were CRE, where Escherichia coli (54%) was the predominant organism isolated followed by Klebsiella pneumoniae (20%).

Among 240MBL positive pathogens, majority were Klebsiella pneumoniae (52.08%), followed by Escherichia coli (33.3%), Klebsiella oxytoca (6.66%), Citrobacter (3.33%), Enterobacter (2.5%), and Proteus (2.08%). Out of 4 Serine Carbapenemases, 2 (50%) were Escherichia coli and 2 (50%) were Klebsiella pneumoniae. Rajshekar et al [10] documented that all serine carbapenemases are Klebsiella pneumoniae. Out of 54 metallo beta lactamases, 35 were Klebsiella pneumoniae, 16 were Escherichia coli, 2 Pseudomonas and 1 Serratia. A study from Jabalpu, India noted that the maximum number of CRE isolates were K. pneumoniae at 23.53% (40/170), followed by K. oxytoca at 16.67% (7/42) and E. coli at 13.77% (23/167) [13].

Serine (SBL's) and Metallo β-lactamases (MBL's) enzymes are responsible for antimicrobial resistance that destroy β-lactam antibiotics. SBL's can be effectively blocked by beta-lactamse inhibitors. MBLs are difficult to treat, as they are capable of hydrolyzing almost all β-lactam antibiotics. Resistance to carbapenems in K. pneumoniae (a very important pathogen causing bloodstream infection) and other Enterobacterales is ever-increasing. Identification of the carbapenemase (by molecular or immunochromatography tests) is of great importance.

CONCLUSION

From this study we conclude that the commonest specimens process to evaluate microbiotia are urine and sputum. Klebsiella species and Escherichia coli were predominantly isolated Enterobacteriaceae members. Carbapenem resistance percentage of clinical isolates varies from one region to another region which depends on antibiotic usage, susceptibility to infections and comorbdities, in this study the prevalence was around one-fifth of total specimens. Phenotypic characterization by mCIM and eCIM methods was performed and noted the type of carbapenemase production which will help in accurate management of infection and avoid failure of treatment due to antimicrobial resistance.

Phenotypic characterization can do it even in resource limited settings at a reasonable price which will guide clinicians to regulate the antibiotic usage. At present, the health care institutions should focus on implementation of hospital infection control and antimicrobial stewardship programme and the government authorities should monitor the mandatory implementation of this program. In future to address this antimicrobial resistance, the development of new antibiotics is urgently needed.

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