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Original Research Article

Detection of carbapenem resistant enterobacteriaceae from various clinical samples: A record based study in a tertiary care hospital in Mandya

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ABSTRACT

Introduction: Carbapenem Resistant Enterobacteriaceae (CRE) has gradually evolved as one of the serious global health concern due to its high mortality and limited treatment options. Overuse of the antibiotic and improper sanitation has led to its rapid spread.

Aim: To determine the proportion of Carbapenem resistant Enterobacteriaceae from various clinical samples received in the Department of Microbiology, MIMS Mandya for Culture and sensitivity by using Meropenem and Imipenem disk.

Materials and Methods: This is a retrospective study conducted over a period of 6 months from March 2021 to august 2021. The samples tested were all the clinical specimens like sputum, pus, urine, body fluids coming to our Microbiology laboratory. The specimens were processed by the standard laboratory methods. Bacteria was isolated and identified by standard biochemical reactions and antimicrobial susceptibility testing was done on Mueller Hinton Agar by Kirby Bauer disk diffusion method and interpreted according to CLSI guidelines.

Results: A total of 1624 samples were included in the study, among which 211 isolates were identified as members of Enterobacteriaceae family. 50 out of 211 isolates were confirmed as Carbapenem resistant giving a prevalence rate of 23.69%. Urine (42%) was the major contributor of CRE, followed by pus (34%). Among CRE, *Escherichia coli* (54%) was the major organism isolated followed by *Klebsiella pneumoniae* (20%).

Conclusion: Our study showed high CRE prevalence rate of 23.69%, indicating the rapid emergence of CRE. Hence, a strict adherence to antibiotic policy and basic infection control measures to be applied in view of reducing the spread of CRE in the community.

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1. Introduction

Members of Enterobacteriaceae are a group of non-sporing, non-acid fast, Gram-negative bacilli that are found in gut of humans and animals. They belong to a complex family that exhibit general morphological and biochemical similarities. They are the common pathogens encountered in the community and health care associated infections. In case of severe infections with Enterobacteriaceae,

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Carbapenems, were the main stay of treatment.³ Due to their unique structure and wide spectrum of activity, they were suggested as the final choice of drug for treating ESBLs and AmpC producers.⁴ Unfortunately, in the past few years, Carbapenem resistance among Enterobacteriaceae is one of the foremost challenges that the medical world is facing.⁵ Centre for disease control and prevention (CDC) classifies Carbapenem resistant Enterobacteriaceae (CRE) as an urgent threat to public health.⁶ CDC also defines it as any member of the family Enterobacteriaceae resistant to carbapenems

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like meropenem, imipenem, ertapenem or doripenem.⁷ Carbapenemase producing organisms are also resistant to other beta lactam antibiotics thereby leaving a very limited treatment option like tigecycline and polymyxins.⁸

Klebsiella pneumoniae Carbapenemases (KPC) was first identified in the United States of America in the year 2000. The presence of New Delhi metallo beta lactamases was demonstrated in United Kingdom from the clinical isolate of *E coli* and *Klebsiella pneumoniae* in a Swedish patient who had travelled India. 9

Carbapenem resistance in bacteria is brought about by mechanisms like changes in outer membrane proteins over expression of efflux pumps and by carbapenem hydrolyzing enzymes. ¹⁰

The mobile genetic elements carry the drug resistant genes and hence they can easily transmit from person to person via the healthcare personnel hands or through contaminated medical equipments. High level of resistance to Carbapenem and many other antimicrobial agents (fluoroquinolones and aminoglycosides) is caused by these genes. ¹¹

Early detection can prevent the spread of Carbapenemases. Hence, this study was conducted to detect the Carbapenem Resistant Enterobacteriaceae in our hospital and to evaluate a cost-effective method for carbapenem production detection.

2. Aim

The aim of the present research is to determine the proportion of Carbapenem resistant Enterobacteriaceae from various clinical samples received in the Department of Microbiology, MIMS Mandya for Culture and sensitivity by using Meropenem and Imipenem disk.

3. Materials and Methods

3.1. Study design

Record based.

3.2. Study period

Months from March 2021 to August 2021.

3.3. Inclusion criteria

All Enterobacteriaceae isolates obtained from clinical samples received for culture and sensitivity in the Department of Microbiology

Data regarding demography, culture findings and antibiotic susceptibility pattern will be collected from the laboratory record maintained in the department of Microbiology.

Data will be entered in excel sheet and analyzed for descriptive statistics like percentage.

3.4. Methodology

A total of 1624 clinical specimens received at the laboratory over a period of 6 months were included for study purposes. Clinical specimens were sputum, pus, urine, cerebrospinal fluid, body fluids like ascitic fluid, pleural fluid and others.

Processing of the specimens was done on MacConkey agar, Blood agar as per standard methods and incubated overnight at 37°C. Isolated colonies were identified by using standard laboratory methods. 12 Antimicrobial susceptibility testing was performed on Muller Hinton agar by Kirby Bauer disc diffusion method as recommended by the Clinical Laboratory Standards Institute (CLSI) guidelines. 13 Organisms showing resistance to any one of the Carbapenem drugs including Meropenem($10\mu g$) and Imipenem($10\mu g$) with the susceptibility zones of </=23mm were identified as carbapenem resistant.



Fig. 1: Screening Test of CRE: Isolate showing zone of inhibition around Meropenem disk

4. Results

Among 1624 clinical specimens 566 urine, 514 pus, 362 sputum, 182 body fluids were received, 211 isolates were identified as members of Enterobacteriaceae family. 50 out of 211 isolates were confirmed as Carbapenem resistant giving a prevalence rate of 23.69%. Male predominance (58%) was seen. Among 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%). Among these 211 isolates, 50 were CRE, where *Escherichia coli* (54%) was the predominant

organism isolated followed by *Klebsiella pneumoniae* (20%).

Table 1: Distribution of CRE in various clinical samples

Sample	Number(n=50)	Percentage	
Urine	21	42%	
Pus	17	34%	
Sputum	12	24%	
Body Fluid	0	0	

From various samples tested CRE was predominant in urine 21(42%) followed by pus sample 17(34%), sputum 12(24%).

Table 2: Gender wise distribution of CRE

Species	Number (n=50)	Percentage
Male	29	58
Female	21	42

The prevalence of CRE was more in males (58%) compared to females (42%).

Table 3: Distribution of CRE among different species

Species	Number of Isolates (n=50)	Percentage (%)
Escherichia coli	27	54
Klebsiella pneumoniae	10	20
Klebsiella oxytoca	9	18
Citrobacter species	3	6
Enterobacter species	1	2

Among CRE, Escherichia coli (27) was the predominant organism isolated.

Among 50 CRE, all isolates were resistant towards Meropenem whereas 47 (94%) isolates were resistant towards Imipenem.

CRE strains showed high level resistance towards Fluoroquinolones, Aminoglycosides and Cephalosporins. 100% sensitivity was shown towards Colistin and Tigecycline.

5. Discussion

The prevalence of CRE in our study is 23.69% (50/211). Pawar SK et al ¹⁰ found a rate of 31.77% in Western hospital during the year 2016-2018. While a study conducted by Srivastava P et al ¹⁴ found CRE prevalence rate of 29.35% from a study conducted in Uttar Pradesh.

In our study, male (58%) predominance was observed. Similar male predominance was seen in other studies, where Thomas N et al⁵ showed the prevalence of 53.75% in males. Pawar SK et al ¹⁰ showed 65.3% prevalence in males.

In the present study CRE isolates were predominantly obtained from urine (42%), followed by Pus (34%), sputum

Table 4: Antimicrobial susceptibility pattern of CRE strains(n=50)

Antibiotics	Sensitive (%)	Resistant (%)
Gentamycin	10(20%)	40(80%)
Amikacin	11(22%)	39(78%)
Ceftriaxone	16(32%)	34(68%)
Cefotaxime	12(24%)	38(76%)
Ceftazidime	13(26%)	37(74%)
Cefepime	11(22%)	39 (78%)
Ciprofloxacin	7(14%)	43(86%)
Ampicillin	9(18%)	41(82%)
Amoxicillin -Clavulinic	12(24%)	38(76%)
acid		
Piperacillin-	10(20%)	40(80%)
Tazobactam		
Ceftazidime-Clavulinic	11(22%)	39(78%)
acid		
Cefaperazone-	10(20%)	40(80%)
Sulbactam		
Imipenem	3(6%)	47(94%)
Meropenem	0	50(100%)
Cotrimoxazole	8(16%)	42(84%)
Colistin	50(100%)	0
Tigecycline	50(100%)	0

Table 5: Prevalence of CRE in various studies

Author	Percentage (%)
Pawar SK et al ¹⁰	31.77
Srivastava P et al ¹⁴	29.69
Present study	23.69

(24%). Similar findings were obtained from Nair et al, ¹⁵ where 46% of the isolates were isolated from urine samples. Srivastava P et al ¹⁴ also observed that maximum number of isolates were obtained from urine samples (58.86%).

In our study *Escherichia coli* (54%) was the predominant organism, followed by *Klebsiella pneumoniae* (20%). Similar findings were observed in a study conducted by Parimala et al, ¹⁶ where *Escherichia coli* (63.04%) was the predominant organism isolated. Srivastava P et al ¹⁴ also observed that *Escherichia coli* was the predominant organism isolated 68.13%. The predominance of *Escherichia coli* could be due to the increased urine samples and *Escherichia coli* being a major pathogen in the urinary tract infection. ¹⁷

Major part of the gut flora is contributed by Enterobacteriaceae. They also serve as reservoirs for spreading infections or contaminating the environment and fomites, especially in healthcare settings. Disinfection measures need to be followed to control the spread. Appropriate use of carbapenems will also prevent selecting resistant bacteria in a geographical area. ⁷

For treating invasive and life-threatening conditions, carbapenems are preferred, due to their wide spectrum of activity and concentration independent killing of bacteria.

Currently Carbapenem Resistant Enterobacteriaceae infections are one of the major challenges the health care setting is facing due to its limited treatment options. Hence this study was conducted to assess the prevalence rate of CRE in our hospital.

6. Conclusion

The high CRE prevalence rate of 23.69% suggests a major public health issue. This emphasizes the need for control of CRE spread in the community. Early identification and isolation of CRE patients with infection control practices and a strict implementation of antimicrobial stewardship programme with restricted use of carbapenems are of paramount importance in view of prevention of further increase in carbapenem resistance.

7. Limitation

The limitation of our study was the lack of confirmatory test for the CRE.

8. Source of Funding

None.

9. Conflict of Interest

None.

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