

## Carbapenem –resistant *Enterobacteriaceae* : Prevalence and bacteriological profile in a tertiary teaching hospital from rural western India

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### Abstract

**Introduction:** In last few years, Gram-negative bacilli are isolated capable of producing various classes of carbapenemases with ability of hydrolyzing the  $\beta$ -lactam antimicrobial. Carbapenem Resistant *Enterobacteriaceae* (CRE) have been reported worldwide. There is a serious threat to public health due to the emergence and rapid spread of CRE.

**Aims:** To find prevalence and bacterial profile of CRE in clinical isolates in indoor patients from a rural tertiary care centre.

**Materials and Methods:** The study was conducted in a tertiary care teaching hospital in Western India, June 2016 to April 2018. The clinical specimens received in microbiology laboratory were processed by the standard method. Bacteria were identified by VITEK 2 compact (Biomerieux) automation system, and antimicrobial susceptibility testing was done with the same system to detect minimum inhibitory concentrations for carbapenem group of antimicrobials. CLSI 2016 guidelines were used to detect CRE.

**Results:** Total 535 *Enterobacteriaceae* clinical isolates were included in the study. Of these, 31.77% (n=170) were CRE. Specimens like urine, pus/ wound swab and endotracheal tube secretion were the major contributors for CRE isolates. 82% of CRE were *Klebsiella pneumoniae* (63%) and *E.coli* (19%).

**Conclusions:** A high prevalence of 31.77% carbapenem resistance was observed among *Enterobacteriaceae* isolates. Early detection, isolation and contact precaution of CRE patients will help to prevent rapid dissemination of CRE infection.

**Keywords:** Carbapenem Resistant *Enterobacteriaceae*, CRE, Carbapenemase, *Klebsiella pneumoniae*.

### Introduction

Antibiotic resistance has emerged as a major health-related issue in last few years. There have been growing epidemics of infections due to gram negative bacteria especially from *Enterobacteriaceae* family, which are resistant to many classes of antibiotics.<sup>1</sup>

$\beta$ -lactam antimicrobial agents are the major group of antimicrobials used for the treatment of gram-negative bacterial infections. Besides extended spectrum penicillins and cephalosporins, the carbapenems have become important options for medication especially in intensive care unit patients.<sup>2</sup> Carbapenems are agents with an exceptionally broad spectrum of activity.<sup>3</sup> Due to extended spectrum beta-lactamase (ESBL) and AmpC enzyme producing *Enterobacteriaceae*, carbapenems are used as a last resort against many multi drug resistant, gram – negative bacteria.<sup>4</sup>

But in last few years, gram negative bacilli are producing various classes of carbapenemases capable of hydrolyzing the  $\beta$ -lactam antimicrobial.<sup>5</sup> Besides *Pseudomonas* spp. and *Acinetobacter* spp., Carbapenem Resistant *Enterobacteriaceae* (CRE) have been reported worldwide.<sup>6</sup> Carbapenem Resistant *Enterobacteriaceae* can be defined as *Enterobacteriaceae* that are resistant to one or all of the following carbapenems: ertapenem, meropenem, imipenem or doripenem.<sup>7</sup> Besides production of carbapenemase enzyme the other mechanism of

resistance include over-expression of efflux pumps by the bacteria, lack of porins present in the bacterial cell membrane and poor binding of carbapenems to penicillin-binding proteins.<sup>4</sup> Carbapenemase encoding gene responsible for this resistance mechanism is spread rapidly within different gram negative bacterial species.<sup>8</sup> This inter and intra species spread occurs through horizontal plasmid mediated transmission which is common among CRE.<sup>8</sup>

There is a serious threat to public health due to the emergence and rapid spread of Carbapenem-resistant bacteria especially CRE. These CRE infections are associated with high mortality and rapid dissemination.<sup>8</sup> Because of rapid rise in the prevalence of CRE, it is essential for early detection. Also epidemiology and bacteriology of CRE will help any health care institute for rational therapy and isolation and contact precautions of such infectious patient preventing further spread. There have been studies on detection of carbapenemase producing bacteria, from many part of the country, but not from this region of western rural India prompting to undertake the study.

### Materials and Methods

The institutional ethical clearance was taken before the study project.

**Study Design:** Laboratory based prospective, observational study.

**Study Centre:** Department of Microbiology, KIMS and KH&MRC, Karad.

**Inclusion Criteria:** Non-repetitive *Enterobacteriaceae* strains which are Carbapenem-resistant isolated from clinical specimens.

**Exclusion Criteria:** *Enterobacteriaceae* strains which are Carbapenem sensitive.

**Methodology:** Total 2518 clinical specimens, received in the laboratory over a period of three years, for culture and sensitivity were included for study purpose. Specimens were pus, endotracheal secretions, sputum, urine, cerebrospinal fluid, blood, and body fluids like ascitic fluid, peritoneal fluid, pleural fluid and other specimens like catheter tips. Processing of the specimens was done as per standard methods on blood agar, Chocolate agar, and MacConkey's agar.<sup>9</sup> Bacterial colonies were identified by VITEK 2 compact (Biomérieux) automation system and antimicrobial susceptibility testing was done with the same system to detect MIC.<sup>10</sup> For this gram negative antimicrobial panel was used which included Imipenem, Meropenem and Ertapenem besides other antimicrobials.

Interpretation of test was done as per CLSI (2016) guidelines. MIC value of  $\geq 4$   $\mu\text{g/ml}$  for Imipenem, Meropenem and  $\geq 2$   $\mu\text{g/ml}$  for Ertapenem was

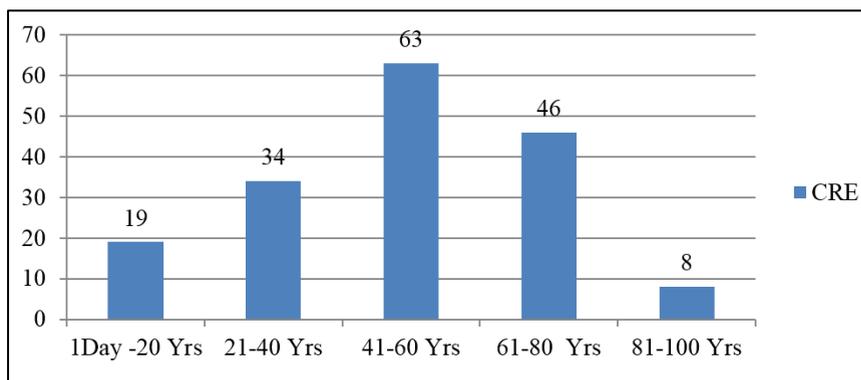
considered as resistant to carbapenem antimicrobials.<sup>7</sup> Quality control for bacterial identification was done by standard ATCC strain *Escherichia coli* 25922, *Pseudomonas aeruginosa* 27853, and *Staphylococcus aureus* 29213.<sup>7</sup> For CRE *Klebsiella pneumoniae* ATCC strain BAA -1705 & 1706 were used as positive and negative controls respectively.<sup>7</sup>

### Statistical Analysis

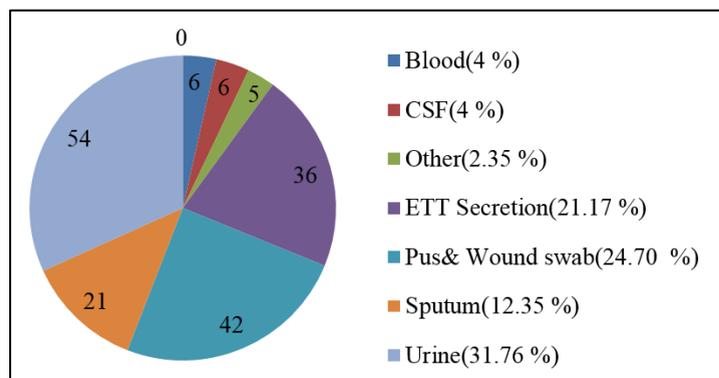
Data entry was done in Excel format and was analyzed using SPSS in the form of Tables, Figures and Charts.

### Results

Total 2518 specimens were processed over a period of three years. Of these clinical specimens total 1024 showed growth of either gram positive or gram negative organism. From these 535 *Enterobacteriaceae* clinical isolates were included for the study. According to CLSI guidelines, 170 (31.77%) of these isolates were CRE. In sex distribution male prevalence (n=111) outnumbered female prevalence (n=59). 41-60 age group showed maximum CRE prevalence. (Fig.1)



**Fig. 1: Age distribution of CRE isolates**



**Fig. 2: Distribution of CRE in clinical specimens**

**Other:** CVP tip, ICDT tip, Tracheostomy secretion, peritoneal fluid

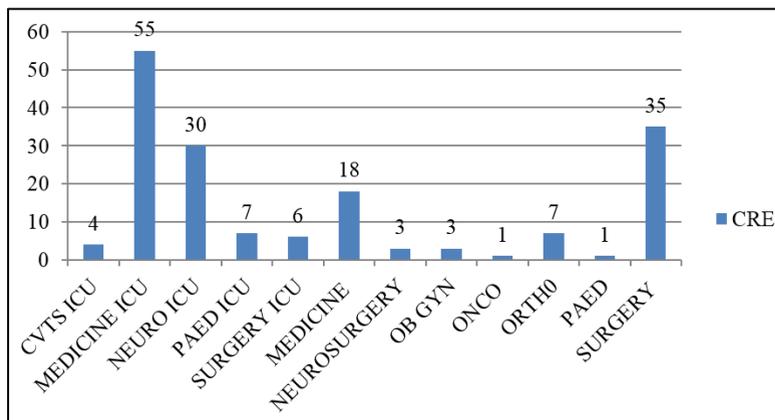
Maximum isolates were from urine (n=54), followed by pus and wound swab (n=42). ETT secretion was the third most number of isolates. (Fig. 2)

60% of CRE (n=102) were from ICU while 40% (n=68) of isolates were from wards (Table 1).

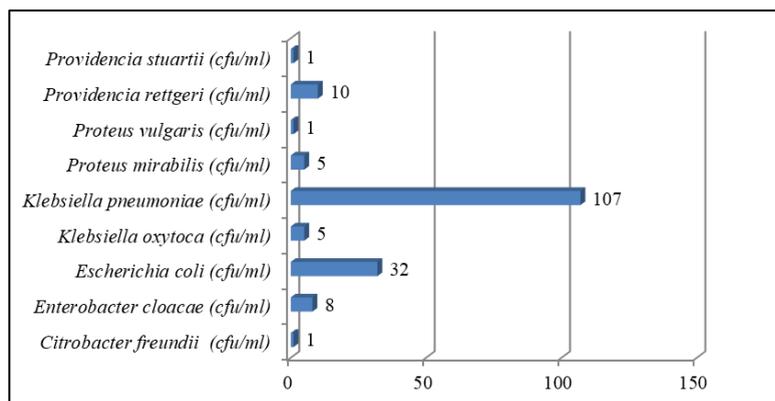
As shown in Fig. 3 Medicine ICU was among highest CRE contributor while among wards most CRE (n=35) were isolated from Surgery.

**Table 1 : Distribution of CRE in hospital**

	CRE (n)	Percentage (%)
ICU	102	60
Ward	68	40
<b>Total</b>	<b>170</b>	<b>100</b>



**Fig. 3: ICU and ward wise distribution of CRE isolates**



**Fig. 4 Distribution of bacterial species among CRE**

63% (n=107) of all the CRE isolates were *Klebsiella pneumoniae* while 19% (n=32) were *Escherichia coli*. (Fig. 4) The other isolates in decreasing order were *Providencia rettgeri*,

*Enterobacter cloacae*, *Klebsiella oxytoca*, *Proteus mirabilis*, *Proteus vulgaris*, *Citrobacter freundii*, *Providencia stuartii*.

**Table 2: Distribution of CRE isolates among different clinical specimens**

Bacterial species	Peritoneal fluid	CVP Tip	Sputum	Urine	Pus & Wound swab	Blood	ETT Secretion	Tracheostomy Secretion	CSF	ICDT Tip
<i>Citrobacter freundii</i> (cfu/ml)	0	0	0	0	1	0	0	0	0	0
<i>Enterobacter cloacae</i> (cfu/ml)	0	0	0	2	2	0	2	0	2	0
<i>Escherichia coli</i> (cfu/ml)	0	0	3	17	10	0	2	0	0	0
<i>Klebsiella oxytoca</i> (cfu/ml)	0	0	1	1	3	0	0	0	0	0
<i>Klebsiella pneumoniae</i> (cfu/ml)	1	1	17	23	22	6	30	2	4	1

<i>Proteus mirabilis</i> (cfu/ml)	0	0	0	1	3	0	1	0	0	0
<i>Proteus vulgaris</i>	0	0	0	0	1	0	0	0	0	0
<i>Providencia rettgeri</i> (cfu/ml)	0	0	0	10	0	0	0	0	0	0
<i>Providencia stuartii</i> (cfu/ml)	0	0	0	0	0	0	1	0	0	0
<b>Total (n=170)</b>	1	1	21	54	42	6	36	2	6	1

Most *Klebsiella pneumoniae* were isolated from ETT secretion (n=30) followed by urine (n=23) and pus and wound swab (n=22). (Table 2). Maximum *E.coli* (n=17) were isolated from urine. While all the CRE from blood were *Klebsiella pneumoniae*, all

*Providencia rettgeri* isolated were from urine specimens. (Table 2). These total 170 CRE were isolated over a period of three years. One of the important findings, was prevalence reduced from 37.20% in 2016 to 21.33% in 2018. (Table 3).

**Table 3: Year wise distribution of CRE**

Year	<i>Enterobacteriaceae</i> (n)	CRE(n)	CRE (%)
2016	129	48	37.20
2017	293	98	33.44
2018	113	24	21.23
<b>Total</b>	535	170	31.77

## Discussion

Prevalence of CRE in the present study was 31.77%. Kumarasami et al found 23.7% prevalence rate of CRE from isolates from Hariyana.<sup>11</sup> 13 to 51% prevalence rate was noted by Wattal et al in a tertiary care hospital from Delhi.<sup>12</sup> In a study in Mumbai, Nair et al found it around 12.26%.<sup>13</sup> A range of 17 to 22% was observed by Gupta et al in a study in northern India.<sup>14</sup> The present study was carried out in 1125 bedded multispecialty tertiary care hospital from rural western India. Most patients are referred after already receiving antimicrobials. Also 60% of the isolates in the study were from ICU where patients are likely to undergo invasive procedure.<sup>15</sup> These factors and longer duration of hospital stay might have contributed for the high prevalence of CRE in the study.<sup>15</sup>

In India like developed countries noncommunicable disease, accidents and injury are becoming the leading cause for hospitalisation.<sup>16</sup> This explains the reason for more number of patients having age more than 40 years in the study. Also, they are likely to get intervention as a part of indoor patient management, contributing to CRE infection. These include healthcare and long term care exposure, invasive devices, drains and endotracheal intubation.<sup>17</sup> Urine (31.76%) was the leading specimen for CRE isolates. In the study of Nair et al<sup>13</sup> (42%) and Singh et al<sup>18</sup> (39.4%) again urine was leading specimen contributing carbapenemase producing isolates. Medicine ICU (n=55) while among wards surgery ward (n=35) showed maximum CRE isolates. In study by Nagaraj et al most carbapenemase producing isolates were from general surgery, general medicine, and ICU.<sup>19</sup>

The majority of organisms in human gut flora are from *Enterobacteriaceae* family. Further carbapenemase

producing *Enterobacteriaceae* are spread rapidly because of horizontal transmission of plasmid encoding genes responsible for carbapenemase production. This occurs mainly by faeco-oral route in community-acquired infections as well as in hospitalized patients.<sup>20</sup> Because of this close proximity urine specimens show more CRE prevalence. Also poor health care person and patients hand hygiene compliance may contribute for high prevalence in surgery patients.<sup>17,20</sup> Longer duration of stay and invasive procedure which is common in ICU patients, are major risk factors for CRE infections.<sup>21</sup>

In the present study 82% of CRE were *Klebsiella pneumoniae* (63%) and *E.coli* (19%). Similar findings were observed in northern Indian study by Chattergy et al where 66% of total CRE were *Klebsiella pneumoniae* and *E.coli*.<sup>22</sup> Lorenzoni et al found 95.7% of strains of *Klebsiella pneumoniae* responsible for carbapenemase production.<sup>23</sup> Major gene responsible for carbapenemase production in India associated in these organisms is *bla<sub>NDM1</sub>*. Universal presence of this gene in CRE has been proven in various Indian studies.<sup>11,22</sup> *bla<sub>NDM1</sub>* gene is present in many unrelated gram negative bacilli including *Enterobacteriaceae* family. Also more worrisome is population of more than 1.4 billion in Indian subcontinent is a major threat for rapid dissemination of CRE.<sup>20</sup>

All the blood CRE isolates in present study were *Klebsiella pneumoniae*. Sekhar et al has documented significant proportion of *Klebsiella pneumoniae* compared to *E.coli* in blood stream infections caused by CRE.<sup>24</sup> Problem with carbapenemase producing bacteria is delay in its confirmation in average laboratory set up. Though molecular methods are gold standard, has its own limitation in the form of time consumption and cost effectiveness. In a laboratory setup with automation

facility like ours, MIC detection will aid in early detection of CRE.<sup>13</sup> This will help in providing contact precautions for the patients. Infact, in the present study, it was found that prevalence of CRE reduced from 37.2% in 2016 to 21.33% in 2018. The hospital staff has undergone continued medical education on hand hygiene as part of training process of National Accreditation Board of Hospital and Health Care Providers. This has improved hand hygiene compliance of the health care persons.<sup>25</sup> This may be one of the factor responsible for decreasing CRE prevalence over period of three years although it requires to be confirmed by epidemiological study.

For prevention of CRE infection various control measures have been proposed.<sup>26</sup> These include early laboratory detection, infection control measures in the form of contact precaution, hand hygiene, isolation proper medical waste disposal, restricted use of invasive devices, epidemiological screening of rectal and perirectal swabs and antibiotic stewardship.<sup>26</sup>

### Conclusion

The emergence of CRE has become major public health issue. A high prevalence of 31.77% carbapenem resistance was observed among *Enterobacteriaceae* isolates. 82% of these CRE, were *Klebsiella pneumoniae* and *E.coli*. Improved compliance in hand hygiene technique may be one of the findings, responsible for reduced prevalence of the CRE over a period of three year in the study. This further emphasizes role of early detection, isolation and contact precaution of CRE patients to prevent rapid dissemination of carbapenemase encoded genes present on the plasmids. There should be restricted use of Carbapenem antimicrobials to prevent further escalation of carbapenem-resistance, which underlines the importance of strict implementation of Antimicrobial Stewardship Programme.

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