

Original Research Article

Epidemiology of carbapenem-resistant Enterobacteriaceae colonization in ICU: a pilot study from a tertiary care hospital in Western Rajasthan, India

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ABSTRACT

Background: Carbapenem-resistant *Enterobacteriaceae* (CRE) is a serious global public health threat. Antibiotic overuse, improper sanitation and unhygienic practices lead to large scale carriage and rapid spread.

Methods: This is a prospective hospital based study planned for a period of 3 months including all patients admitted to 14 bedded Trauma ICU of a tertiary care hospital in Rajasthan. Rectal swabs were collected from admitted patients and carriage of carbapenem resistant *Enterobacteriaceae* looked for as per CDC guidelines. Screening of the *Enterobacteriaceae* colonies for carbapenemase production was done by Modified Hodge test. Carbapenem-resistant isolates were also tested for Metallo beta lactamase production by phenotypic disc confirmatory test.

Results: A total of 73 patients were screened and 27 CRE isolates were obtained, carriage rate being 37%. A high level of resistance was seen to aminoglycosides, fluoroquinolones and cephalosporins. 100% sensitivity was however seen to Colistin, Tigecycline and Fosfomycin. 5 out of 27 strains showed a positive MHT test. Metallo beta lactamase (MBL) production was seen in 21/25 strains as tested by meropenem and Meropenem-EDTA discs.

Conclusions: The current pilot study finds out the prevalence of CRE carriage among critically ill patients and stresses upon strong need for stringent infection control measures.

Keywords: Carbapenem resistant *Enterobacteriaceae*, ICU, Rectal swab, Surveillance

INTRODUCTION

Enterobacteriaceae constitute a large family of gram-negative rods, many of which are normal inhabitants of the human intestinal tract.¹ This occurrence of microorganisms as a part of normal flora in the human body is by and large a necessity for good health and well-being. However, if the carriage shifts in favour of multidrug resistant pathogens, it can lead to increased morbidity and mortality.^{1,2} They can cause invasive infections particularly in those with healthcare exposure and who are debilitated due to serious illness, old age,

invasive procedures, or indwelling devices.² This is especially true for the patients being admitted to intensive care units. With an increase in the number of people being exposed to antibiotics, intestinal microflora faces constant selection pressure. This has resulted in the emergence and spread of multidrug-resistant organisms including Carbapenem-resistant strains.^{1,2}

Carbapenems are the last resort of drugs for the treatment of multi-drug resistant organisms. Unfortunately, over the past decade resistance to this vital class of drugs is emerging and spreading fast. Carbapenem-resistant

Enterobacteriaceae (CRE) is a recognized serious global public health threat.³ They are extremely drug-resistant pathogens and are present in the community as well as in hospitals. Infections caused by them are associated with significant morbidity and mortality. Antibiotic overuse, improper sanitation and unhygienic practices lead to large scale carriage of these multidrug resistant organisms and their rapid spread.^{3,4}

The published literature from India reports the prevalence of Carbapenem resistant Gram negative bacilli among clinical samples to be varying from 5.3% to 59%.⁵ Clinical cultures done routinely in the laboratory detect only a small percentage of patients with CRE. Various studies have proven that carriers are more likely to spread the MDR organisms than clinical cases and that asymptomatic carriers are at a higher risk of developing an invasive infection. They can also act as a source of cross infection amongst other patients. Screening thus becomes an essential tool to identify this pool of unrecognized CRE colonization.⁶ There is a large scale consensus on conducting surveillance cultures in high risk areas, especially the intensive care units.⁵⁻⁸

There is a paucity of data on the prevalence of such organisms in our intensive care setup. Therefore, this prospective hospital based pilot study was planned for three months to evaluate the faecal carriage of CRE *Enterobacteriaceae* in patients admitted in the ICU of trauma centre of a tertiary care hospital in Jodhpur, Western India.

METHODS

This was a prospective hospital based study planned for a period of 3 months, from January to March 2018 and included all the patients admitted to 14 bedded Trauma ICU of Mathura Das Mathur Hospital (MDMH), Jodhpur. Jodhpur is the second largest city in the Indian state of Rajasthan and officially the second metropolitan city of the state. MDMH is a tertiary level care hospital and caters to a large population from in and around Jodhpur. For conducting the CRE surveillance, rectal swab was collected from each patient on day of their admission. A sterile swab was introduced approximately 1 inch into anal canal, rotated slowly for 10 seconds and put immediately in 5ml of Trypticase soy broth to which a 10µg of meropenem disc had already been added. This was incubated overnight at 37°C. The following day, incubated broth culture was vortexed and 100µl of the broth was subculture on MacConkey agar plate and streaked for isolation. Growth of lactose fermenting colonies (LF) if any was looked for identification and susceptibility testing performed as per the standard guidelines.⁹ Screening of the *Enterobacteriaceae* colonies for carbapenemase production was done by Modified Hodge test as per the CLSI guidelines.¹⁰ Carbapenem-resistant isolates were also tested for Metallobetactamase production by phenotypic disc confirmatory test (PDCT).²

Risk factors of patients with and without CRE colonization were also analysed. Authors have used t test to calculate the significance of proportion and Odds ratio to judge the strength of association of risk factors.

Ethical clearance for the study was obtained by Institutional Ethical committee (No. F1/ Acad/ MC/ JU/ 18/ 5152).

All the samples were tested under Mukhyamantri Nishulk Jaanch Yojana (MNJY) which stipulates that all road traffic accident cases are investigated and treated free of cost.

RESULTS

A total of 73 patients admitted in Trauma ICU were screened over a period of 3 months. The most common cause of admission was road traffic accident with resultant head injury. A total of 27 Carbapenem resistant *Enterobacteriaceae* isolates were obtained, carriage rate being 37%. The average age was 41 years and 30 years among colonized and non-colonized patients respectively. The patients were mostly males (Table 1).

Table 1: Demographic details of the patients.

	Pt colonized with CRE (n = 27)	Pt not colonized with CRE (n = 46)
Age (mean)	41.52	30.61
Male	25	40
Female	2	6
Total	27	46

All the patients needed urinary catheterization. About 95% of the patients also needed ventilator support and haemodynamic monitoring through a central line.

Table 2: Distribution of patients with and without CRE colonization as per certain associates.

Associates of CRE colonization	Patients Colonized With CRE	Patients not Colonized With CRE	OR	p-value
Older Age (40+)	14 (51.85%)	10 (21.74%)	3.88	0.009
Ventilator	25 (92.59%)	43 (93.47%)	0.87	0.885
Tracheostomy	15 (55.55%)	26 (56.52%)	0.96	0.936
Previous antibiotic use	14 (51.85%)	30 (65.21%)	0.57	0.262
Mortality	11 (40.74%)	15 (32.61%)	1.25	0.478

History of antibiotic usage was elicited from 14 (52%) of the CRE carriers and 30 (65%) non colonized patients.

History of previous hospitalization was elicited from 13 (48%) and 27 (59%) of colonized and non-colonized patients respectively. All the patients, except one were initially healthy to begin with and had no history of any co morbidity. No significant correlation was found with presence of co morbidity, history of previous hospitalization or antibiotic usage (Table 2).

Table 3: Break up of CRE strains.

Organisms isolated	N (%) (Total no of CRE isolated =27)
<i>Klebsiella spp.</i>	15 (56%)
<i>E. coli</i>	12 (44%)

There were Twenty seven CRE isolates included 15 *Klebsiella pneumoniae* and 12 *E. coli* strains. Table 3 Almost all the carriers had a single organism except one who showed the growth of an *E. coli* and *Klebsiella* both. Sensitivity testing of these 27 carbapenem resistant strains was done by Vitek. A high level of resistance was seen to aminoglycosides, fluoroquinolones and cephalosporins. 100% sensitivity was however seen to Colistin, Tigecycline and Fosfomycin (Table 4).

Five out of 27 strains showed a positive MHT test. Metallo beta lactamase (MBL) production was seen in 21/25 strains as tested by meropenem and Meropenem-EDTA discs.

Table 4: Antimicrobial sensitivity pattern of CRE strains.

Antibiotic sensitivity pattern	No. of resistant isolates (total CRE =27)	Percentage %
Amikacin (AK)	20	74.07
Meropenem (MRP)	27	100
Colistin (CL)	0	0
Cotrimoxazole (COT)	25	92.59
Ceftazidime (CAZ)	27	100
Tigecycline (TG)	0	0
Ceftriaxone (CTR)	27	100
Ciprofloxacin (CIP)	27	100
Ampi sulbactam (A/S)	26	96.29
Cefoxitin (CX)	27	100
Cefepime (CPM)	17	88.88
Norfloxacin (NX)	27	100
Fosfomycin (FOSFO)	0	0

DISCUSSION

The carbapenem group of antibiotics are considered last resort antibiotics. They offer broad spectrum antibiotic cover, enabling safe and effective treatment for severe infections. The mechanism of resistance to this class of drug can be varied. It may occur due to the acquisition of

carbapenemase enzymes (known as CPE carbapenemase-producing *Enterobacteriaceae*) or less commonly via other mechanisms (e.g. porin loss leading to decreased permeability of bacterial cell wall).^{2,3} Carbapenemase-producing *Enterobacteriaceae* are a particular infection prevention and control risk. Firstly, the genes encoding carbapenemase production are found on mobile genetic elements together with genes that code for resistance to other classes of antibiotics, such as fluoroquinolones and aminoglycosides. This makes CPE difficult to treat. Secondly, these are efficiently be transmitted between patients within a healthcare facility and are notorious to cause outbreaks.²⁻⁴

There is a lot of data available on increasing prevalence of CRE among clinical isolates but their carriage, especially asymptomatic, still needs to be explored.⁶ There are not many studies from India where work on surveillance cultures has been done. The colonization with these multi drug resistant organisms is associated with an increased incidence of clinical infections in the host and cross infections amongst other patients.^{5,6,8} Once acquired these pathogens continue to inhabit the ecosystem of hospitals for an indefinite period and are very difficult to eradicate.¹¹ Thus early identification and isolation of carriers are key components of an effective infection control strategy.¹² Keeping these points in mind, current study was designed as a pilot to find out the faecal carriage of CRE isolates among patients admitted in the ICU of trauma centre of a tertiary care hospital in Jodhpur.

A total of 27 CRE isolates were obtained. Modified Hodge test was positive in 5 strains signifying carbapenemase production. Since MHT is known to be highly sensitive for detection of KPC production, it is safe to presume that some other mechanisms were also responsible for resultant carbapenem resistance.⁹ 21 strains showed ≥ 7 mm increase in the zone of inhibition when tested by meropenem and meropenem-EDTA disc combination, thus signifying Metallo beta lactamase production. More conclusive data could have been obtained by molecular studies which could not be done because of lack of infrastructure.

Out of 73 cases admitted in Trauma ICU, 27(37%) CRE isolates were obtained, i.e. about $\frac{1}{3}$ of the patients have been detected with colonization of CRE. This percentage of CRE colonization was highly significant ($t=6.72$, $p<0.01$) as well. The mean age of patients found colonized with CRE was 41.52 while the mean age of patients not colonized with CRE was 30.61. The difference in age of patients (pts.) colonized with CRE and not colonized with CRE was also significant ($t=3.008$, $p<0.01$). Table 4 presents the analysis of some associates of CRE colonization. Data in row 1 of this table yields significant OR=3.88, $p=.009$ with respect to risk of colonization of CRE in older patients (Age 40+).

The risk of mortality among pts with CRE colonization is found a bit higher (OR=1.25, p=0.478) as read from data row 5 of this Table. The percentage of mortality among pts with CRE is also found higher than the percentage of mortality among pts without CRE colonization. This then suggests for better care for CRE colonized patients, in particular in the age group 40+. The other associates, namely; patients on ventilator, pts with tracheostomy or pts with previous history of antibiotic use did not show OR>1 as read from data rows 2, 3 and 4 of this Table. Also, the percentages of patients with colonization and without colonization are found well comparable.

Various studies have found that being on ventilator support is a significant risk factor for CRE colonization.^{2-4,12-14} However, in present study this is not the case. This may be due to the lesser number of study participants or the duration of study. However, even this small study makes it clear that in ICUs, the percentage of pts with CRE colonization is high, particularly in older age group patients and mortality is also more in patients with CRE colonization.

MDMH being a tertiary level care hospital caters to a large catchment area in and around Jodhpur. Almost all the patients with prior hospitalization or antibiotic usage included those that had received initial treatment after road traffic accident in one of the peripheral hospitals. They got transferred to MDMH only after no clinical improvement was seen. This may be one of the reasons why a higher percentage of CRE carriage is seen in present study as compared to other contemporary Indian studies. CRE carriage however did not affect the average stay in ICU which was 12 days in both the groups. The mortality rate was however, significantly higher in colonized as compared to non-colonized patients (OR=1.25).

In a study conducted in a tertiary care teaching hospital in Delhi, out of the 242 isolates tested from patients attending the OPD, 24 (9.9%) demonstrated carbapenemase activity.¹⁵ Lower rates found in this study may be explained by the fact that it was done on outpatients. The duration of hospital stays and antibiotic use has been shown to increase the risk of faecal carriage of CRE in many studies.^{12,16-19} Twelve (29%) *E. coli* and 8 (38%) *Klebsiella* spp isolated from rectal swabs were found to be positive for MBL production in a study by Aggarwal et al, Rohtak.²⁰ Mittal G et al, have reported faecal carriage of CPE in 11% and 22% in ICU patients on day 1 and day 4 respectively.²¹ In an active surveillance study conducted in 2011 in Chandigarh, no CRE isolate was reported in the rectal swab cultures in ICU patients.²²

However the carriage rate increased to 12% in similar settings in 2015.²³ These findings clearly suggest that the epidemiology of CRE is fast evolving and surveillance cultures become very important in today's scenario.

A higher faecal carriage rate of CRE has been reported from a study done in a tertiary care hospital in Mumbai, India. Twenty eight (52%) CRE isolates were obtained from 54 stool samples and *Klebsiella pneumonia* was more common followed by *E. coli*, like in present study. This study also showed a high level of sensitivity of CRE isolates to colistin and tigecycline.²⁴

On a global level, gastrointestinal carriage rate of CRE has been shown to be highly variable. Prevalence of CPE carriers admitted to ICUs in 11 acute care Spanish hospitals was 1.6% to 2.4%.²⁵ In a Korean study, faecal carriage of CRE was found in 0.3% patients upon admission. None of the CRE isolates was a carbapenemase producer.^{16,26} During a prospective observational study conducted on all patients admitted in an ICU in a hospital in Greece, 12.8% patients were found to be colonized. The associated risk factors were noted as previous ICU stay, chronic obstructive pulmonary disease, and previous use of carbapenems etc.²⁷ In a study conducted in a long term care facility in New York, CRE carriage was noticed in 18.9% patients.²⁸ Percentages of patients colonized upon ICU admission has been reported to be around 5 to 7% in Israel and 11% in Mexico.^{14,29,30} Almost negligible rates have been found from Germany and Algeria.^{31,32}

In a recent study conducted in Turkey, out of a total of 32 cases' clinical samples processed, 25 (78%) were found to have concomitant rectal CRE carriage.³³ Once found to be colonized, the median time to culture negativity was found to be 387 days on repeat culture.⁴ Reports also that a significant proportion of CRE carriers remain colonized for up to a year. During a national outbreak of carbapenem resistant *Klebsiella pneumonia* in Israel, out of 298 patients examined, 16 (5.4%) were found to be carriers.³⁰ During our study period, clinical samples were also collected from patients suspected to have a nosocomial infection. Interestingly, one CRE carrier developed Central line associated bloodstream infection (CLABSI) and the central line tip culture grew a *Klebsiella* strain which had a similar antibiotic sensitivity profile as that of *Klebsiella* isolated from the rectal swab. Likewise, another patient developed Catheter associated urinary tract infection (CAUTI) and the urine culture grew *Klebsiella* which again had an AST pattern similar to that isolated from rectal swab. It is a known fact that carriage of MDR organisms can lead to infection in the same as well as nearby patients by cross infection. It is a matter of speculation whether these were the same carriage strains that caused the nosocomial infections. Sequencing of both the strains could solve this dilemma.

Surveillance of multi drug resistant (MDR) organisms in a health care setting is necessary to have optimum treatment outcomes and less treatment failures.⁵⁻⁷ Once any health care setting gets colonized with these organisms, it is very difficult to decontaminate the environment. Hence, in addition to routine practices, additional precautions are needed including providing

single room accommodation. In a resource limited setting as ours where this isn't possible, number of visitors in the room should be minimized and patients may be advised to remain in the room except for emergency procedures. Transfer between facilities should only be done if medically necessary. Staff cohorting, strict adherence to hand hygiene, stringent use of personal protective equipment, rational use of antibiotics and minimizing device use are other effective measures to implement.

Present research is limited by a short time period and small sample size. Further detailed studies in this area are needed to generate more meaningful data and to understand the epidemiology of CRE carriage better. Also due to infrastructure limitations, molecular analysis of CRE strains could not be done. The current pilot study however, manages to find the prevalence of CRE carriage among critically ill patients. It also helps to sensitize the health care community about the existence of the problem and the strong need for stringent infection control measures.

CONCLUSION

This pilot study will guide clinicians and clinical microbiologists about colonization of such multidrug resistant strains in the human gastrointestinal tract. Early detection of asymptomatic rectal carriers of CRE is important for infection control purposes. Appropriate infection control interventions are required to prevent transmission of CRE not only within and among healthcare facilities but also between HCF and community. This study also emphasizes the need for formulation and absolute compliance to Antibiotic stewardship program in hospitals to control the emergence and spread of CRE.

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