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RESEARCH ARTICLE

PREVALENCE OF CARBAPENEM RESISTANT ENTEROBACTERIACEAE AT A TERTIARY CARE INSTITUTE: NEED FOR POLICY

***Sarita Yadav**

Associate Professor, BPS GMC (W) Govt. Medical College

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ABSTRACT

Introduction: Resistance to broad-spectrum antimicrobials is a well recognized problem among Enterobacteriaceae. Carbapenems have served as an important antimicrobial class for the treatment of these organisms. The emergence and dissem¬ination of carbapenem resistant enterobacteriaceae (CRE) in recent times represents a serious threat to public health.

Aim: The study was conducted to detect the prevalence of carbapenem resistance in members of Enterobacteriaceae and to detect the enzymes *Klebsiella pneumoniae* carbapenemase (KPC) and Metallo-Beta lactamases (MBL) in resistant isolates

Material and methods: The study was carried out in the Department of Microbiology, at Bhagat Phool Singh Govt. Medical College for Women, Haryana. The bacterial isolates, obtained from various clinical samples, were identified according to standard microbiological procedures. All isolates belonging to Enterobacteriaceae family like Escherichia coli, *Klebsiella* species and *Citrobacter* species were included in the study. All these isolates were screened for carbapenem resistance by disc diffusion method according to CLSI.

Results: A total of 54 (13.77%) isolates of CRE or¬ganisms were obtained among 392 Enterobacteriaceae isolates. The carbapenem resistance rate was highest among ward patients. Maximum CRE was cultured from urine samples (50.0%), followed by pus samples (24.0%). The most predominant species among CRE isolates was Escherichia coli in the present study.

Conclusion: Over the past decade, the emergence of carbapenem resistant Enterobacteriaceae has become a formidable threat to public health. As a first step towards control, the capacity for resistance detection and surveillance in low-resource countries needs to be improved in order to collect more reliable data on the worldwide distribution of CRE.

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INTRODUCTION

Carbapenems are a group of β-lactam antimicrobial agents with an exceptionally broad spectrum of activity. Resistance to broad-spectrum antimicrobials, such as the extended-spectrum cephalosporins, is a well recognised problem among Enterobacteriaceae (Jacoby, 2005). Carbapenems have served as an important antimicrobial class for the treatment of these organisms. The emergence and dissemination of carbapenem resistant bacteria in recent times represents a serious threat to public health (Bansal, 2013). Carbapenem Resistant Enterobacteriaceae (CRE) can be defined as Enterobacteriaceae that are resistant to one or all of the following carbapenems: ertapenem, meropenem, imipenem or doripenem; and resistant to all of the following third-generation cephalosporins: ceftriaxone, cefotaxime, and ceftazidime (Nair, 2013). Resistance to carbapenems can be brought about by various mechanisms, the most common being the production of carbapenemases, a class of enzymes capable of hydrolyzing carbapenems and other β-lactams. Carbapenemase enzymes fall into Ambler classification- A, B and D. Class A (serine carbapenemase) enzymes include enzymes such as *Klebsiella pneumoniae* carbapenemase (KPC), IMI, SME etc. Class B enzymes are Metallo-Beta lactamases (MBL) including VIM, IMP and SPM (Bush, 1995). Molecular techniques can easily differentiate between these classes of carbapenemases but need of the hour is a rapid, practical, phenotypic method which can detect these enzymes easily. CRE are particularly problematic given the frequency with which Enterobacteriaceae cause infections, the high mortality associated with infections, and the potential for widespread transmission of carbapenem resistance via mobile genetic elements (Nair, 2013; Tsakris, 2010 and Patel, 2008). With the increasing incidence of CRE in hospitals, a rapid and accurate routine protocol for CRE screening and detection is

Corresponding author: **Sarita Yadav,**
Associate Professor, BPS GMC (W) Govt. Medical College

required. Appropriate detection of CRE is vital in patient care and infection control in order to institute correct, targeted treatment and to reduce the escalation of resistance. So,the present study was designed to assess the prevalence of carbapenem resistance in members of Enterobacteriaceae and to detect the enzymes KPC and MBL in carbapenem resistant isolates.

MATERIAL AND METHODS

The study was carried out in the Department of Microbiology, at Bhagat Phool Singh Govt. Medical College for Women, Haryana. A total of 392 bacterial isolates obtained from various clinical samples like pus, wound swabs, body fluids, sputum, throat swab, endotracheal secretions etc. were included in the study. The bacterial isolates were identified according to standard microbiological procedures (Collee, 1996). All isolates belonging to Enterobacteriaceae family like *Escherichia coli*, *Klebsiella* species and *Citrobacter* species were included in the study. All these isolates were subjected to initial screening for carbapenem resistance ertapenem (10µg)/ meropenem (10 µg), ceftriaxone (30 µg), cefotaxime (30 µg) and ceftazidime (30 µg) (HiMedia lab.) by disc diffusion method according to CLSI (Clinical and Laboratory Standards Institute, 2012). Those strains showing reduced susceptibility were further tested for the presence of KPC and MBL enzymes.

Detection of enzymes (Datta, 2012)

Use of inhibitor phenylboronic acid (PBA), EDTA or both along with meropenem disc was used for detection of KPC and MBL, respectively. The stock solution of PBA in the concentration of 20 mg/ml was prepared by dissolving PBA (Sigma-Aldrich, Germany) in DMSO. Twenty microliters (400 µg of PBA) from this solution was dispensed onto meropenem discs. The stock solution of EDTA was prepared by dissolving anhydrous EDTA (Sigma-Aldrich) in distilled water at a concentration of 0.1 M. Ten microliters (292 µg of EDTA) from this solution was dispensed onto meropenem discs. The meropenem discs with inhibitor added was dried and used within 60 min. On Mueller Hinton agar plate inoculated with test strain, four discs of meropenem were used. One disc of meropenem was without any inhibitor, one disc had PBA (400 µg) only, one disc had EDTA (292 µg) only and fourth disc of meropenem had both PBA plus EDTA. The agar plates were incubated at 37° C overnight and the diameter of the growth inhibitory zone around these meropenem discs with inhibitor added was compared with that around the plain meropenem disc.

The isolate was considered to be KPC producer when the growth inhibitory zone diameter around the meropenem disc with PBA and meropenem disc with both PBA and EDTA was increased >5mm compared with growth-inhibitory zone diameter around the disc containing meropenem alone. The isolate was considered MBL producing when the growth-inhibitory zone diameter around the meropenem disc with EDTA and the meropenem disc with both PBA and EDTA was increased >5mm compared with the growth-inhibitory zone diameter around the disc containing meropenem alone. The isolate was considered negative for MBL and KPC production, when none of the three combined-disc tests was positive. PBA and EDTA disc alone and *Klebsiella pneumoniae* ATCC BAA-1705 were used as negative control and positive control respectively.

RESULTS

A total of 54 (13.77%) isolates of CRE organisms were obtained among 392 Enterobacteriaceae isolates (Table 1). The carbapenem resistance rate was highest among ward patients (53.70%), followed by ICU patients (25.92%) and OPD patients (20.37%) (Figure 1).

Table 1. Distribution of bacterial isolates

Bacteria	Number	Percentage of CRE
Escherichia coli	263	9.88%
Klebsiella species	62	29.03%
Citrobacter species	35	22.85%
Enterobacter species	13	7.69%
Proteus species	19	5.26%
Total	392	13.77%

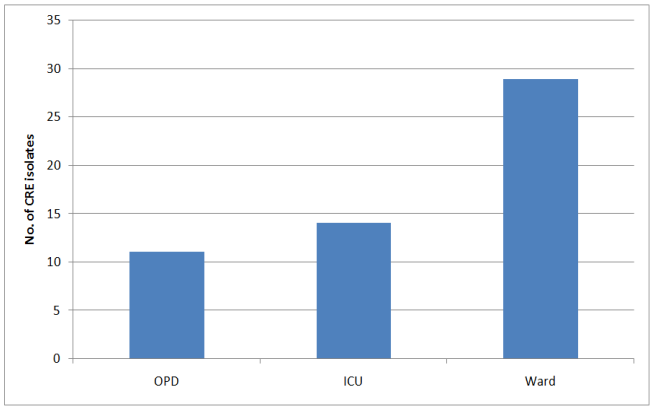

Figure 1. Proportion of Carbapenem Resistant Enterobacteriaceae (CRE) isolates across different units of hospital

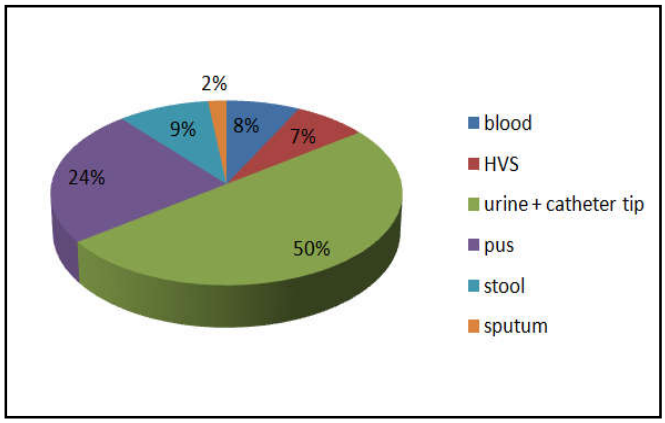

Figure 2. Sample wise distribution of Carbapenem Resistant Isolates

Maximum CRE was cultured from urine samples (51.0%), followed by pus samples (24.0%), stool samples (9.0%), blood (7.0%), high vaginal swabs (7.0%) and sputum (2.0%). (Figure 2). The most predominant species among CRE isolates was *Escherichia coli* in the present study (Figure 3). The distribution of CRE species is shown in Figure 4. Twenty six isolates were MBL producers (Table 2). None of the isolates were KPC producers.

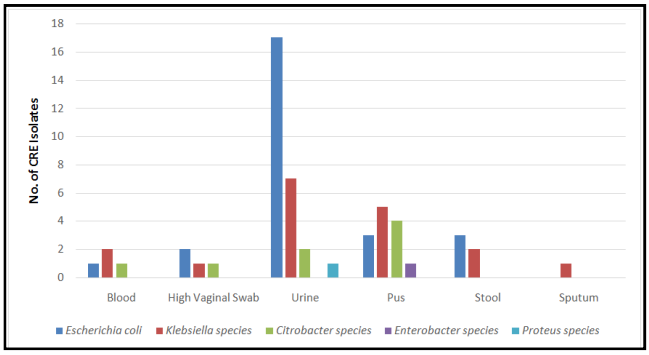

Figure 3. Distribution of CRE species in clinical samples

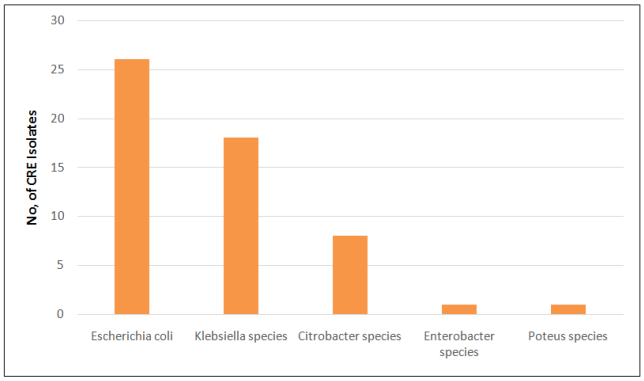

Figure 4. Species-wise distribution of Carbapenem Resistant Enterobacteriaceae

Table 2. Distribution of bacterial isolates producing MBL

Bacterial species	No. of strains resistant to carbapenem	No. of strains showing inhibition by EDTA
Escherichia coli	26	14
Klebsiella spp.	18	8
Citrobacter spp.	8	2

DISCUSSION

Over the past decade, the emergence of carbapenem resistant Enterobacteriaceae has become a formidable threat to public health. The prevalence of CRE in the present study was found to be 13.37%. This is similar to the rates obtained by Nair *et al* at a tertiary care hospital in Mumbai (Nair, 2013). Gupta *et al* reported carbapenem resistance varying from 17-22% among Enterobacteriaceae strains (Gupta, 2006). However, 44.33% CRE isolates were detected by Parimala in their study from South India (Parimala, 2017). Similarly, Sachin and colleague found 57 out of 80 isolates (71.25%) to be carbapenem resistant (Kumar, 2014). This current and increasing spread is an important cause of concern since these carbapenemases are, in most cases, combined with non-beta-lactam resistance mechanisms, therefore, leading to multidrug resistant isolates.
Carbapenem resistance among the isolates is highest in wards followed by ICUs in our study which is comparable with the study of Nair and co-authors where a higher number of carbapenemase producers were from the wards (42%) as compared to ICUs (26%) (Nair, 2013). This information can be used to identify areas that may require increased infection prevention and control efforts to prevent further dissemination of these resistant pathogens. We observed that the carbapenem resistant organisms were isolated mainly from urine samples (51.0%) followed by pus samples (24.0%). It has been shown previously that majority of the CRE isolates were obtained from urine samples (Nair, 2013; Gupta, 2006; Parimala, 2017 and Kumar, 2014). Among the CRE, *E.coli* accounted for the largest proportion of the isolates. Similar trends has also been observed by other workers (Nair, 2013 and Parimala, 2017). However, few studies have also demonstrated *Klebsiella* spp.as the leading cause of carbapenem resistance and a notorious collector of multidrug resistance plasmids (Datta, 2012; Kumar, 2014; Nagaraj, 2012 and Xu, 2015). The prevalence rates varies by the geographic regions.

The regional differences observed in various studies support the understanding that infection control of resistant pathogens needs to be based on local epidemiology. Tsakris *et al* in his study showed that use of PBA and EDTA for phenotypic detection of carbapenemase to be a very sensitive method.[5] MBL production was demonstrated in 24 out of the 54 CRE isolates. No strain of the CRE isolates showed production of KPC in the present study. Similar findings have been reported by Datta *et al*. (Datta, 2006). While carbapenem-resistant *K. pneumoniae* are currently more frequent and more likely to cause healthcare-associated outbreaks, carbapenem-resistant *E. coli* pose a greater risk for spread in the community (Thaden, 2014). We believe tertiary care hospitals have much more work to do to prepare for and respond to CRE. Specifically, policies must be developed for improved laboratory detection, prevent CRE transmission and infection control.

The CDC has outlined basic strategies to decrease transmission, including hand hygiene, contact precautions, healthcare personnel education, limitation of medical device use, patient and staff cohorting, laboratory notification strategies, antimicrobial stewardship, and CRE active screening (Thaden, 2014). Of note, tertiary care centres face significant difficulty implementing these recommendations because of resource limitations. National guidelines, national surveillance systems, national reference laboratories, mandatory reporting of CRE and national campaigns to promote infection control and prudent antimicrobial use are the cornerstones for effective CRE control. Analysis of routinely collected local microbiology data provides an opportunity to improve our knowledge and understanding of antimicrobial resistance. Regular analysis of antimicrobial resistance data is crucial to ensure that policy, infection control measures and surveillance strategies are effective in addressing the increasing threat of carbapenem resistance in Gram-negative bacteria.

Conclusion

The rapidly increasing prevalence of Enterobacteriaceae harbouring carbapenemases is alarming. Molecular techniques are the gold standard method for the detection of carbapenemase production in Enterobacteriaceae but it is not suitable for daily testing in resource limited clinical laboratories due to the cost and inconvenience. The need of the hour is simple, rapid and cost effective tests which will be able to identify drug resistant pathogens at a tertiary care institute. These centres, caters most of the rural population that accounts for 65-70% of total burden. CRE patients serve as reservoirs for community acquired infection. CRE can become an issue not only in individual institutions but also across an entire community, thus highlighting a role for public health in CRE-prevention efforts. So, the present study was designed to know the prevalence of CRE at root level, in order to initiate antimicrobial stewardship at the peripheral centres, which will further prevent dissemination of the resistance.

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