Frequency of High Antimicrobial Susceptibility Carbapenemase Producing Enterobacteriaceae in Clinical Isolates of a Set of Population

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Author’s Contribution
1,4 Conception of study
1 Experimentation/Study conduction
1,2,3 Analysis/Interpretation/Discussion
1,2,3 Manuscript Writing
3 Critical Review
1 Facilitation and Material analysis

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Abstract

Objective: The objective of the study was to determine the frequency and antimicrobial susceptibility pattern of Carbapenem-Resistant Enterobacteriaceae among clinical isolates in Karachi, Pakistan.

Materials and Methods: This observational study was conducted at Ziauddin University Hospital, Karachi, Pakistan, from January to December 2017. The consecutive clinical isolates of the Enterobacteriaceae were collected and analyzed, using established conventional microbiological techniques. Antimicrobial susceptibility testing and Carbapenem resistance were detected by the initial screen test using the CLSI method. Statistical analysis was performed by SPSS version 17.

Results: The total of 2100 clinical isolates of Enterobacteriaceae attained during this study time period, were predominantly from female patients 1300/2100 (61.9%), while male patients were 800/2100 (38.1%). The female-to-male ratio was 1.6:1. The overall frequency of CRE among isolates of Enterobacteriaceae was 136/2100 (6.5%). Within individual organism group Klebsiella species had highest (108/136 (79.41%)) frequency, followed by E.coli 22 (18.38%) and Enterobacter species 03 (2.21%). All CRE isolates were sensitive to Colistin and Fosfomycin. No resistance was observed against Tigecycline among Carbapenem-resistant isolates of E. coli and Enterobacter species.

Conclusion: Both Colistin and Fosfomycin antimicrobials can be considered empirical treatment options for CRE suspected infections. Better practices and changes in policy matters are required to improve the deteriorating situation of health care.

Keywords: Antimicrobial Susceptibility, Carbapenem-Resistant Enterobacteriaceae, Klebsiella. Escherichia coli.
Introduction

Enterobacteriaceae is a family of gram-negative organisms that cause a wide range of diseases in the community as well as in the hospital setting. A large share of clinical isolates from the blood, the peritoneal cavity, the respiratory tract, and the urinary tract are reported to have these organisms. Enterobacteriaceae have the predisposition to quickly propagate among humans by transferring through hands, infected food, and water, thereby acquiring genetic material by horizontal gene transfer, often through plasmids and transposons.2

Beta-lactams are extensively used to treat Enterobacteriaceae, especially extended-spectrum cephalosporins (Cefotaxime or Ceftriaxone) for life-intimidating infections such as bloodstream infections (BSI) as well as common infections like pneumonia and urinary tract infections (UTI). As a result of the overuse of Beta-lactams especially extended-spectrum cephalosporins, a worldwide increasing trend of extended-spectrum beta-lactamases (ESBLs), is observed.3 A study from Pakistan also reported the overall prevalence of 65.7% for ESBL producing Enterobacteriaceae among urinary pathogen isolates.4 Carbapenems are considered the most appropriate drug for serious infections due to ESBL-producing organisms like Escherichia coli (E. coli) and Klebsiella pneumoniae (K. pneumoniae). The mounting frequency of ESBL producers is sufficient to constrain a greater dependence on carbapenems. As a result, there is a selection pressure for the drug to prescribe in cases of carbapenems resistance Enterobacteriaceae, and this is a global public health concern because there are few antibiotics in backup against CRE.5

Over the past decade, the occurrence of Carbapenem-resistant Enterobacteriaceae (CRE) has become a challenging threat to public health. According to the National Nosocomial Infection Surveillance System/National Healthcare Safety Network, carbapenem resistance raised tenfold between 2001 and 2011, from 1.6% to 10.4% in Klebsiella isolates, and four times from 1.2% to 4.2% in Enterobacter isolates.6 The prevalence of CRE varied between 5.74 percent and 29.8 percent according to several Asian institutions. The frequency of CRE in Southeast Asia, on the other hand, is poorly documented and underreported.7

In Pakistan, data from hospitals show a steady increase in the rates of carbapenem-resistant E. coli, Enterobacter species, and Klebsiella pneumoniae from 5%, 7%, and 15%, respectively in 2013 to 6%, 23%, and 22% by 2015, respectively.8,11 There are considerable gaps in data and lacking of proper surveillance programs universally which has made it challenging to identify the origin, propagation, and level of the problem in depth.9 Likewise, owing to a lack of epidemiological local data within Pakistan, the precise frequency of CRE is not well known.10,11 A variety of factors such as unnecessary usage of antibiotics, inefficient and overburdened health services, and increased foreign travel (especially the increasing rise of medical tourism) have led to the increase and spread of these bacterial strains worldwide.12 CRE are resistant to all β-lactam medications, and also bear pathways that impart resistance to certain types of antimicrobials, thus restricting therapeutic choices.

The primary aim of this study is to evaluate the frequency of CRE in our tertiary care system and to determine the pattern of antimicrobial susceptibility to CRE. Precise detection of CRE in the clinical laboratory is an important first stage in prevention. It's also important to consider how common CRE is in our setup of medical care. Through this study, we can implement aggressive infection control measures, limit the non-judicious use of antimicrobials, practice antimicrobial stewardship, and increase laboratory capacities to control the spread of these difficult-to-treat pathogens. Applying appropriate infection control measures will help to control the mortality rates due to infections with these superbugs.

Materials and Methods

This observational study was conducted in the Department of Clinical Microbiology at Ziauddin University Hospital, Karachi. Two thousand one hundred consecutive Enterobacteriaceae clinical isolates were obtained by convenient sampling from various clinical samples. These 2100 isolates of Enterobacteriaceae of in-patients (hospitalized patients) were included in the study and tested for Carbapenem resistance. Isolates from out-patients (Out-patient department) were excluded from the study. Repeated and duplicate isolates were also excluded. Sources of isolates included samples from blood, urine, respiratory secretions, wounds swabs, tips of central venous pressure (CVP) lines, sterile body fluids, and pus, etc. Ethical approval of the study was obtained from the Ziauddin Ethics Review Committee. Informed consent was taken from either the patients or their close relatives.
**Identification of Enterobacteriaceae**

The samples were received by the Microbiology laboratory in sterile containers or in an Amies transport medium. In accordance with the standard microbiological techniques, these samples were processed and incubated at 37°C ± 2°C in ambient air for 24–28 hours for the growth of Enterobacteriaceae. The members of Enterobacteriaceae were recognized by using conventional techniques including colony morphology, gram staining, cytochrome oxidase test, differential growth on MacConkey’s agar medium, and routine biochemical tests with additional usage of API 20E (Procop et al., 2017).

Antimicrobial susceptibility testing was performed based on Clinical and Laboratory Standards Institute (CLSI) 2016 guidelines on Mueller Hinton agar (MHA) medium (Oxoid Ltd., England) using modified Kirby Bauer’s disk diffusion method (CLSI, 2016). A 0.5 McFarland equivalent suspension of the organism was prepared and inoculated onto MHA plates with subsequent application of antimicrobial discs. The plates were then incubated overnight at 37°C in an ambient air incubator.

Carbapenem resistance is detected by screening test using Meropenem (10 µg) disk. Isolates showing a Meropenem zone of inhibition of ≤ 19 mm were considered resistant, while a zone of inhibition of ≥ 23 mm was considered sensitive, shown in Figure 1. Antimicrobial susceptibility results were interpreted according to CLSI criteria. *E. coli* American Type Culture Collection (ATCC®) 25922, *E. coli* ATCC® 35218, and *Pseudomonas aeruginosa* ATCC® 27853 were used as control strains. The disk diffusion sensitivity criteria for Colistin against the isolates of CRE is taken as ≥ 11 mm (sensitive) as given for *Pseudomonas aeruginosa* in the CLSI 2016. Tigecycline disk diffusion sensitivity criteria for Enterobacteriaceae is taken as ≥ 19 mm (sensitive).

**Statistical analysis**

All the important data were documented using a study proforma. Analysis of data was performed using version 17 of the Statistical System for Social Sciences (SPSS). Frequencies and percentages for the representation of all categorical variables such as microorganisms, class, sensitivity, and resistance were measured. Mean and standard deviation was calculated for quantitative variables like the age of patients.

**Ethical Consideration**

All the samples were obtained via clinical practice procedures and submitted to the laboratory. Before analysis, the results were anonymized and no socio-demographic variables are analyzed.

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**Results**

A total of 2100 clinical isolates of Enterobacteriaceae were attained during the study period. Distributions of isolates of Enterobacteriaceae from different clinical samples are shown in Table 1.

**Frequency of Different Species of Enterobacteriaceae**

Among these 2100 isolates, the majority of isolates were identified as *E. coli* 1260/2100 (60%), followed by *Klebsiella species* 462/2100 (22%), *Enterobacter species* 210/2100 (10%), *Proteus species* 63/2100 (3%), *Morganella morgannii* 21/2100 (1%), and others 84/2100 (4%). Predominantly, the isolates were from female patients 1300/2100 (61.9%), while the isolates from male patients were 800/2100 (38.1%). Female to male ratio was 1.6:1. The overall frequency of CRE among isolates of Enterobacteriaceae is 136/2100 (6.5%).

**Frequency of CRE in various clinical samples**

Out of 136 CRE isolates, from blood were 57 (41.91%), urine 49 (36.03%), pus 10 (7.35%), and other clinical samples 20 (14.7%). The frequency of Carbapenemase positive in different clinical samples is shown in Table 1. Within individual organism group frequency of CRE positivity was highest amongst *Klebsiella species* 108/136 (79.41%), followed by *E. coli* 22 (18.38%) and *Enterobacter species* 03 (2.12%).

**Antimicrobial sensitivity testing**

All isolates of CRE were sensitive to Colistin and Fosfomycin (Table 2). Furthermore, no resistance was observed against Tigecycline among Carbapenem-resistant isolates of *E. coli* and *Enterobacter species*.

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**Table 1: Frequency of carbapenemase-positive isolates in different clinical samples**

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Clinical Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Urine</td>
</tr>
<tr>
<td>Carbapenemase positive</td>
<td>49</td>
</tr>
<tr>
<td><em>Klebsiella species</em></td>
<td>24</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>22</td>
</tr>
<tr>
<td><em>Enterobacter</em></td>
<td>3</td>
</tr>
</tbody>
</table>

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Reference:

- CLSI guidelines (2016) for antimicrobial susceptibility testing.
- Procop et al. (2017) for identification of Enterobacteriaceae.

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Table 2: Resistant pattern of CRE among clinical isolates of Enterobacteriaceae

<table>
<thead>
<tr>
<th>Antimicrobials</th>
<th>Klebsiella species (n=108)</th>
<th>Escherichia coli (n=25)</th>
<th>Enterobacter species (n=03)</th>
<th>Total (n=136)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. (Percent) Resistant to</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amikacin</td>
<td>97/108 (89.91%)</td>
<td>13/25 (52%)</td>
<td>03/03 (100%)</td>
<td>113/136 (83.08%)</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>Not Tested</td>
<td>25/25 (100%)</td>
<td>Not Tested</td>
<td>25/25 (100%)</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>108/108 (100%)</td>
<td>25/25 (100%)</td>
<td>03/03 (100%)</td>
<td>136/136 (100%)</td>
</tr>
<tr>
<td>Cefixime</td>
<td>108/108 (100%)</td>
<td>25/25 (100%)</td>
<td>03/03 (100%)</td>
<td>136/136 (100%)</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>108/108 (100%)</td>
<td>25/25 (100%)</td>
<td>03/03 (100%)</td>
<td>136/136 (100%)</td>
</tr>
<tr>
<td>Cefoperazone/Sulbactam</td>
<td>108/108 (100%)</td>
<td>25/25 (100%)</td>
<td>03/03 (100%)</td>
<td>136/136 (100%)</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>76/108 (70.37%)</td>
<td>06/25 (24%)</td>
<td>01/03 (33.33%)</td>
<td>83/136 (61.02%)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>99/108 (91.66%)</td>
<td>25/25 (100%)</td>
<td>03/03 (100%)</td>
<td>127/136 (93.38%)</td>
</tr>
<tr>
<td>Co-amoxiclav</td>
<td>108/108 (100%)</td>
<td>25/25 (100%)</td>
<td>03/03 (100%)</td>
<td>136/136 (100%)</td>
</tr>
<tr>
<td>Colistin</td>
<td>00/108 (00%)</td>
<td>00/25 (00%)</td>
<td>00/03 (00%)</td>
<td>00/136 (00%)</td>
</tr>
<tr>
<td>Co-trimoxazole</td>
<td>92/108 (85.18%)</td>
<td>20/25 (80%)</td>
<td>01/03 (33.33%)</td>
<td>113/136 (83.08%)</td>
</tr>
<tr>
<td>Fosfomycin</td>
<td>00/108 (00%)</td>
<td>00/25 (00%)</td>
<td>00/03 (00%)</td>
<td>00/136 (00%)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>98/108 (90.74%)</td>
<td>21/25 (84%)</td>
<td>03/03 (100%)</td>
<td>122/136 (89.70%)</td>
</tr>
<tr>
<td>Meropenem</td>
<td>108/108 (100%)</td>
<td>25/25 (100%)</td>
<td>03/03 (100%)</td>
<td>136/136 (100%)</td>
</tr>
<tr>
<td>Piperacillin/ tazobactam</td>
<td>108/108 (100%)</td>
<td>25/25 (100%)</td>
<td>03/03 (100%)</td>
<td>136/136 (100%)</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>59/108 (54.62%)</td>
<td>00/25 (00%)</td>
<td>00/03 (00%)</td>
<td>59/136 (43.38%)</td>
</tr>
</tbody>
</table>

Table 3: Klebsiella species resistance worldwide reported by various studies

<table>
<thead>
<tr>
<th>Authors (year)</th>
<th>Data collected</th>
<th>Country</th>
<th>Percentage increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present study</td>
<td>2017</td>
<td>Pakistan</td>
<td>5.14%</td>
</tr>
<tr>
<td>Hu et al. (2015)</td>
<td>2005-2014</td>
<td>China</td>
<td>2.9-9.4%</td>
</tr>
<tr>
<td>Jacob et al. (2013)</td>
<td>2001-2011</td>
<td>USA</td>
<td>1.6-10.4%</td>
</tr>
<tr>
<td>Sievert et al. (2013)</td>
<td>2007-2010</td>
<td>USA</td>
<td>3.1-4.1%</td>
</tr>
<tr>
<td>Durrani et al. (2014)</td>
<td>2011</td>
<td>Pakistan</td>
<td>9.5%</td>
</tr>
<tr>
<td>Qamar et al. (2017)</td>
<td>2013-2015</td>
<td>Pakistan</td>
<td>13-22%</td>
</tr>
<tr>
<td>Yannick et al (2018)</td>
<td>2012-2015</td>
<td>Cambodia</td>
<td>28%-35%</td>
</tr>
</tbody>
</table>

Discussion

The highest frequency of CRE positivity within the individual organism group was found amongst Klebsiella species (79.41%), followed by E. coli (18.38%) and Enterobacter species (2.21%) in this study. Related research performed in Cambodia observed E. coli (63.9%) as the most prevalent isolate relative to K. pneumoniae (19.8%), however, the majority of the study samples in their study were urine samples and the presence of E. coli in urine samples is well known and it is the more frequent source of mild urinary tract infection.14 Urine, in accordance with the above study, was also the most common sample in this study. An overall 6.5% frequency of CRE is found in our study samples. Although, antibiotic resistance is reported to be increasing every year among clinical isolates...
worldwide. However, this is much less compared to the 15.2% frequency of carbapenems resistant among Enterobacteriaceae reported by a Nigerian study. This higher resistance might be attributed to the small sample size compared to our study. In a study from a university hospital in Jakarta in 2011, Indonesia reported 27.6% of CRE among isolates. However, an Indian study reported a gradual increase in CRE from 0-8% from 2006 to 2009, whereas, a 5% prevalence was reported in Taiwan.

Earlier in 2011, a study conducted at Pakistani military hospitals laboratories showed CRE carriage rates of 18.5% (n=200) in stool samples, similarly, the carriage rate was found to be 8.6% and 18.3% in two laboratory-based studies among diarrhea patients. These figures signify that they are the most prevalent cause of both community and hospital-acquired infections. In this study, the Klebsiella species 108/136 (79.41%) was the most common among individual organism groups, followed by E.coli (18.38%) and Enterobacter species (2.21%). Studies worldwide report an overall increase in resistance of Klebsiella species from the year 2001 to 2017, year wise tabulation is presented below in Table 3.

During the period 2009 - 2011 (Table III) CRE of Klebsiella species in the US was high, compared to Asian studies where it was progressing as reported by Singapore Formal National Antimicrobial Resistance Surveillance Program. In CVP-associated bloodstream infections, CRE was 12.8% of Klebsiella species, whereas, for catheter-associated urinary tract infections the frequency was 12.5%, but, the overall hospital-admitted patients reported 33.3% of Klebsiella species infection. Centers for Disease Control and Prevention USA (2001-2011) reports the CRE increase from 1.2% to 4.2% with the incidence of Carbapenem-resistant Klebsiella species, from 1.6% to 10.4%, during the same time period (Centers for Disease and Prevention, 2013). While Hu et al. (2005-2014) report an increase in CRE Klebsiella strain from 2.9-9.4% in China. Similar emergence of K. pneumoniae with carbapenem resistance has been reported in Australia as well. However, in Pakistan, the resistance is reported from 9.5%-22% in comparison to the current study which observed 5.14% CRE strains of Klebsiella species. This shows that the resistance also increased in Pakistan from 2011 to 2017. The less frequency observed in our study might be due to increased awareness and practices of the constant maintenance of infection control and antimicrobial stewardship activity.

National Healthcare Safety Network reports the frequencies of Carbapenem-resistant E. coli as 1.9% for bloodstream infections, while 2.3% for catheter-associated urinary tract infections. With E. coli, carbapenem resistance was comparatively lesser 18.38% (25/136) in our samples, than a study from Nigeria reported resistant strains for E.coli at 30.8% (33/107). Comparing Enterobacter species, in our samples, the Carbapenem-resistant was 2.21% which differed from a study conducted in the US showing 4.6% prevalence. However, a Taiwanese retrospective study reported Enterobacter cloacae (25/37) as the major causative and resistant pathogen. Carbapenems are the main antimicrobial drug prescribed against Enterobacteriaceae (CRE) strains, but the upsurge of Carbapenem-hydrolyzing activity has led to an augmented occurrence of Carbapenem-resistant strains of Enterobacteriaceae (CRE). The frequency of CRE poses a significant threat since Carbapenems are the last line of defense against extended-spectrum β-lactamase-producing Enterobacteriaceae (ESBL-E), and Enterobacteriaceae. Enterobacteriaceae, being the cause of multiple types of infections, with increased CRE frequency is creating an alarming situation for a potential widespread transmission of Carbapenem resistance and the higher mortality associated with it. All isolates of CRE were observed sensitive to Colistin and Fosfomycin in this current study. CRE limits the treatment options to combinations of Polymyxin, Tigecycline, Aminoglycosides, or Carbapenem, in general. One of the European surveys reported 67 isolates in nine of the ten Hospital Laboratories, (61 K. pneumoniae and 6 E. coli) with a non-susceptibility rate for meropenem and imipenem of 64 and 59%, respectively, for K. pneumoniae, and of 100% for E. coli. CRE strains reported higher rates of non-susceptibility for all the antibiotic groups evaluated as compared to the control isolates.

Many approaches have been suggested to maximize the efficacy of such antibiotics, notably aerosolized antibiotics for colistin management and higher colistin and tigecycline maintenance doses. Such regimens also enhanced the beneficial results although there is sparse convincing evidence. Combination treatments do occur in this way. In this context, combination therapies for treating multidrug-resistant CRE infections have been recommended. Nonetheless, modern antibiotics against CRE have a stronger susceptibility and protection profile and are anticipated to become the medication of preference in the immediate future.
The control of the augmenting spread of highly resistant bacteria tends to be centered on a dual policy of the antibiotics prescription; one is to reduce the selection burden and the second is to deter carrier spread. In order to combat cross-transmission by CRE variants and resistance genes, interventions may incorporate hygiene steps in hospitals as well as in the community. The prescription of third-generation cephalosporins and fluoroquinolones should be strictly and continuously supervised. It is indeed worth making 'better usage' of antibiotics, devising the guidelines or protocols for the use of antimicrobials that exert the weakest selection pressure on these highly resistant organisms. Antibiotic consumption must be regulated strictly in hospitals and in the community, not only for humans but as well as for animal treatment.

### Conclusion

The highest frequency of CRE positivity within the individual organism group was found amongst Klebsiella species (79.41%), followed by E. coli (18.38%) and Enterobacter species (2.21%) in this study. Taken as a whole, very few hospitals in Pakistan have antibiotic resistance control units or monitoring programs. An important first step and the key to the clinical microbiology laboratory control of CRE infections is to identify the producers of Carbapenemase more efficiently. We found all CRE isolates sensitive to Colistin and Fosfomycin. Although treatment for CRE includes a combination of Aminoglycoside, Polymyxin, Tigecycline, or Carbapenem, but new better antibiotics with superior activity against CRE are required in the future which should have improved safety as well.

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