Molecular epidemiology of carbapenem resistant Enterobacteriaceae in Sri Lanka: First report of KPC-producing *Klebsiella pneumoniae*

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Research article

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Abstract

Background Extended spectrum β -lactamase producing Enterobacteriaceae (ESBL-PE) and carbapenemase producing Enterocacteriaceae (CPE) are widely disseminated globally creating a huge public health threat. Even though incidence of multidrug-resistant Enterobacteriaceae is rapidly growing, the epidemiological data regarding the occurrence of CRE in Sri Lanka is scarce. In this study, we determined the prevalence of ESBL-PE and CRE and the genetic determinants of CPE. **Methods** A total of 593 clinically significant Enterobacteriaceae was isolated from different clinical samples (urine, pus/wound, respiratory, blood, and other sterile specimens) at a tertiary care hospital in Sri Lanka from December 2017 – February 2018. Antimicrobial susceptibility and identification of ESBL-PE, CRE were done by disc diffusion method. CRE were identified to species level using a rapid identification kit and carbapenemase production was determined by modified carbapenem inactivation method. The presence of blaKPC, blaNDM, blaOXA-48-like genes were detected by PCR. **Results** The overall prevalence of ESBL-PE and CRE were found to be 26.0% and 9.6%, respectively. The rate of ESBL-PE in different sample types ranged from 18.2% to 30.8% with the highest prevalence among uropathogenic Enterobacteriaceae. The occurrence of CRE ranged from 6.7% to 20.8% with the highest prevalence among respiratory Enterobacteriaceae. CRE species identified were *K. pneumoniae* (80.7%), *E. coli* (5.3%), *C. freundii* (7.0%), *P. rettgeri* (3.5%), *E. cloacae* (1.7%), and *E. aerogenes* (1.7%). The carbapenemase production was detected in 94.7% of CRE isolates. The carbapenemases found were OXA-48-like (88.9%), NDM (14.8%), and KPC (3.7%). **Conclusions** The prevalence of CRE in Sri Lanka is alarming. Carbapenemase production was the major mechanism of carbapenem resistance and *K. pneumoniae* was the predominant CRE. Presence of KPC enzyme was detected in addition to the previously reported NDM and OXA-48-like carbapenemases in Sri Lanka. The rapid spread of CPE, necessitates the prompt implementation of preventive measures in Sri Lanka.

Background

Members of family Enterobacteriaceae, especially *Escherichia coli* and *Klebsiella pneumoniae* are amongst the most common causes of both community-acquired and healthcare associated infections (1). Many of these infections have been successfully treated with β -lactam antibiotics for years. Unfortunately the emergence of multidrug-resistant (MDR) bacteria, particularly extended spectrum β -lactamase producing Enterobacteriaceae (ESBL-PE) and carbapenem-resistant Enterobacteriaceae (CRE) have limited the therapeutic utility of β -lactams for the infections caused by these microorganisms (2).

ESBL-PE are resistant to all commonly prescribed β -lactam antibiotics (i.e. penicillins, cephalosporins and monobactam) except carbapenems, leaving carbapenems as the “last-resort” antibiotics against ESBL producers (3). These organisms often show resistant to the antibiotics in the other classes of antimicrobial agents as well. The evolution of carbapenem resistance has made nearly all available antibiotics ineffective against the infections caused by CRE (1, 2). Further, CRE has been ranked as an urgent threat for human health by the Centers for Disease Control and Prevention (CDC) (4) and the exponential increase of infections caused by CRE has resulted in high morbidity and mortality worldwide

(5, 6). Enterobacteria confer carbapenem resistance by several modes, but carbapenemase mediated mechanism is the most common. These carbapenemase producing Enterobacteriaceae (CPE) are more worrisome due to the existence of several powerful transmissible carbapenem inactivating enzymes (1 , 7).

ESBL-PE and CPE are widespread and are endemic in many countries including the countries of Indian subcontinent (8-10). Prevalence of MDR Enterobacteriaceae in the most of South Asian countries is variable, but high rates of ESBL-PE (> 50%) and CRE (> 10%) have been reported (9, 11-13). Though the occurrence of CRE has increased dramatically during the last decade in neighboring India (12), a little is known about the prevalence of CRE in Sri Lanka, which appears to be growing (14-16). Additionally, the CPE in Sri Lanka was first reported in 2013 and the existence of several different carbapenemase types has been revealed thereafter (17-20).

Sri Lanka has always been at a greater risk of being affected by ESBL-PE and CPE outbreaks due to the liberal antibiotic usage in the country and the increased travel from MDR Enterobacteriaceae endemic countries like India, China, Middle East, etc. (21-23). Hence, baseline epidemiological data of MDR Enterobacteriaceae is essential to streamline the national antibiotic policy and the infection control protocols. Present study sought to determine the prevalence of MDR Enterobacteriaceae with the emphasis on ESBL-PE and CRE at a tertiary healthcare facility in Sri Lanka and to explore the genetic determinants of carbapenem resistance.

Methods

Study design

A laboratory based, prospective cross-sectional study was conducted on clinically significant Enterobacteria isolated from patients attended at the Colombo North Teaching Hospital (CNTH), Sri Lanka over a ten week period from December 2017 to February 2018. CNTH is a major public tertiary care hospital with 1477 beds located in the suburbs of Colombo, the capital of Sri Lanka. Enterobacteriaceae were isolated from variety of clinical specimens (blood, urine, other sterile fluids, respiratory specimens, and wound/pus swabs) received consecutively to the microbiology laboratory at CNTH.

Bacterial isolates and antimicrobial susceptibility testing

In order to isolate Enterobacteria, clinical materials were cultured on blood agar. Each isolated organism was screened by standard microbiological procedures including Gram staining, oxidase test, lactose fermentation and other characteristics on Kligler Iron Agar. Antimicrobial susceptibility was performed by disc diffusion method on Muller-Hinton agar according to M100-S28 of Clinical and Laboratory Standards Institute (CLSI) guidelines. Antibiotic discs were from Oxoid, UK. Any isolate resistant to at least one antibiotic in 3 or more antimicrobial classes was considered as MDR (24). ESBL-PE and CRE were identified by disk diffusion method as per CLSI guidelines. Accordingly, Enterobacteria, resistant to

cefotaxime were further tested for ESBL production by double disc synergy test using cefotaxime (30 µg) and amoxicillin-clavulanic acid (20/10 µg) and by combined disc containing ceftazidime-clavulanic acid (30/10 µg). Organisms resistant to meropenem and / or imipenem were considered as CRE. ESBL-PE and CRE isolates were stored in peptone broth containing 20% glycerol at -80 °C until used. Before further experiments, the frozen bacterial samples were recovered on blood agar and were subsequently sub cultured on Muller-Hinton agar (Oxoid, UK).

Phenotypic and genotypic characterization

All CRE isolates were identified up to species level by Rap ID One system Enterobacteriaceae identification kit (Remel, Thermo scientific) according to manufacturer's instructions. Phenotypic carbapenemase production in CPE was determined by modified carbapenem inactivation method (mCIM) described by Pierce V.M. *et. al.*(25). Briefly, 1 µl loop full of overnight grown test organism was inoculated in 2 ml of tryptic soy broth and it was incubated with a 10 µg meropenem disc (Oxoid, UK) at 37 °C for 4 h. After incubation, meropenem disc was removed and placed on Muller-Hinton agar plate inoculated with *E. coli* (ATCC 25922) strain and was incubated at 37°C for 18 – 24 h. A zone diameter of < 15 mm was considered as positive for carbapenemase production. *K. pneumoniae* ATCC BAA-1705 and BAA-1706 strains were used as positive and negative controls, respectively.

Genotypic characterization was performed by the multiplex PCR assay for *bla*_{KPC}, *bla*_{NDM}, *bla*_{IMP}, *bla*_{VIM}, and *bla*_{OXA-48-like} genes using the oligonucleotide primers described by Poirel L. *et. al.* (26). Briefly, bacterial DNA was extracted by heat lysis method. PCR was carried out with 2 units of GoTaq Flexi DNA polymerase (Promega, USA), 3 mM of MgCl₂, 0.2 mM of each deoxy ribonucleotide triphosphate, and 0.2 µM of each primer (Integrated DNA technologies, USA). Five microliters of crude DNA extract was used in the 50 µl PCR reaction. The thermal cycling conditions were as follows: initial denaturation at 94 °C for 10 minutes, followed by 35 cycles of amplification with 95 °C for 30 seconds, 52 °C for 40 seconds, and 72 °C for 60 seconds, and a final extension at 72 °C for 5 minutes. The amplified gene products were visualized on a 2% agarose gel after staining with ethidium bromide. The amplicons in the multiplex PCR were further confirmed by simplex PCR.

Statistics

Data were stored in Microsoft Excel and analysis were conducted using SPSS version 22 (IBM corporation, USA). The categorical variables were compared using Chi-squared test. *p*-values < 0.05 were considered statistically significant.

Results

Epidemiology of multidrug-resistance Enterobacteriaceae

A total of 593 clinically significant Enterobacteriaceae was isolated from the consecutive patient samples received to the microbiology laboratory at a tertiary care hospital in Sri Lanka. Of them, 328 (55.3%) were resistant to cefotaxime and 154 (26.0%) were identified as ESBL-PE, whereas 57 (9.6%) were identified as CRE. Table 1 summarizes the epidemiological characteristics of the study sample. The study population consisted of nearly equal number of male and female patients with the median age of 56 years (range 4 days to 89 years). ESBL-PE were predominantly recovered from female patients (n = 87, 56.5%, p=0.063) and CRE were predominate among male patients (n = 36, 63.2%, p=0.046). Enterobacteriaceae were primarily found in urine samples (n = 311, 52.4%) and pus / wound swabs (n = 164, 27.7%). ESBL-PE were mainly recovered from urine samples (n = 96, 62.3%) and pus / wound swabs (n = 35, 22.7%), while CRE were mostly isolated from urine samples (n = 27, 47.4%), pus / wound swabs (n = 11, 19.3%), and respiratory specimens (n = 10, 17.5%). Further, 31 (5.2%) of the total Enterobacteriaceae were isolated from the clinical samples received from the intensive care units (ICUs). Among ICU isolates, 7 (22.6%) were ESBL-PE and 12 (38.7%) were CRE (p=0.017).

The sample-wise distribution of ESBL-PE and CRE is shown in Figure 1. Of 311 urinary Enterobacteriaceae isolates, 96 (30.8%) were ESBL-PE and 27 (8.7%) were CRE (p=0.000). Among 164 Enterobacteriaceae isolated from pus/wound swabs, 35 (21.3%) were ESBL-PE and 11 (6.7%) were CRE (p=0.073). Of 48 respiratory Enterobacteriaceae isolates, 9 (18.7%) were ESBL-PE and 10 (20.8%) were CRE (p=0.088). Of 37 blood-born Enterobacteriaceae, 8 (21.6%) were ESBL-PE and 5 (13.5%) were CRE (p=0.207). Among 33 Enterobacteriaceae isolated from sterile specimens, 6 (18.2%) were ESBL-PE and 4 (12.1%) were CRE (p=0.315). In addition, six out of 18 (33.3%) respiratory specimens and 5 of 6 (83.3%) blood samples received from ICUs were found to harbor CRE.

Figure 1: Distribution of ESBL-PE and CRE in each sample type

Phenotypic characterization of CRE

CRE isolates were identified up to species level using a commercial Enterobacteriaceae identification kit. Carbapenem resistance was most prevalent among *Klebsiella pneumoniae* (n = 46, 80.7%, p=0.000) (Figure 2A). The other identified CRE were; *Escherichia coli* (n = 3, 5.3%), *Citrobacter freundii* (n = 4, 7.0%), *Providencia rettgeri* (n = 2, 3.5%), *Enterobacter cloacae* (n = 1, 1.7%), and *Enterobacter aerogenes* (n = 1, 1.7%). Figure 2B shows the relative distribution of different CRE species in each sample type. All CRE isolated from respiratory, blood and other sterile specimens were of *K. pneumoniae*. In addition to *K. pneumoniae* (n = 7, 63.6%), *C. freundii* (n = 2, 18.2%), *E. coli* (n = 1, 9.1%), and *P. rettgeri* (n = 1, 9.1%) were also found among CRE isolated from pus / wound swabs. Uropathogenic CRE were more diverse and consisted of *K. pneumoniae* (n = 20, 74.1%), *C. freundii* (n = 2, 7.4%), *E. coli* (n = 2, 7.4%), *P. rettgeri* (n = 1, 3.7%), *E. cloacae* (n = 1, 3.7%), and *E. aerogenes* (n = 1, 3.7%). All CRE isolates from ICU patients were *K. pneumoniae*.

The modified carbapenem inactivation method (mCIM) was used to explore whether the carbapenem resistance is conferred by the carbapenem degrading enzymes. The carbapenemase production was

detected in 54 (94.7%) of 57 CRE isolates. Two *K. pneumoniae* and one *P. rettgeri* isolates were of non CPE. Moreover, Eleven out of 12 (91.7%) CRE isolated from ICU samples were carbapenamase producers. All the Enterobacteriaceae identified as CPE had a zone diameter of 6 mm for the meropenm disc incubated with the test organism in the mCIM study.

Figure 2: Species identification (A) and relative distribution of CRE species among different samples (B)

Molecular characterization of carbapenemases

Presence of five most common carbapenamase encoding genes (*bla*_{KPC}, *bla*_{NDM}, *bla*_{OXA-48-like}, *bla*_{IMP}, and *bla*_{VIM}) among CRE cohort was evaluated by PCR. The distribution of the identified carbapenamase genes among CPE is summarized in Table 2. A total of 58 carbapenamase encoding genes were detected in 54 CPE isolates. Forty eight (88.9%) CPE isolates [42 (95.45%) *K. pneumoniae*, 3 (100%) *E. coli*, 2 (50.0%) *C. freundii*, and 1 (100%) *E. aerogenes*] carried the *bla*_{OXA-48-like} gene, whereas 8 (14.8%) isolates [3 (6.8%) *K. pneumoniae*, 2 (50.0%) *C. freundii*, 1 (100%) *P. rettgeri*, 1 (100%) *E. cloacae*, and 1 (100%) *E. aerogenes*] harbored *bla*_{NDM} gene. Two *K. pneumoniae* (3.7%) were KPC type carbapenamase producers. Four (7.4%) CPE isolates [3 (6.8%) *K. pneumoniae* and 1 (100%) *E. aerogenes*] co-harbored both *bla*_{NDM} and *bla*_{OXA-48-like} genes. Interestingly, all three *bla*_{NDM} containing *K. pneumoniae* were found to carry *bla*_{OXA-48-like} gene. None of the CPE isolates were positive for IMP and VIM carbapenemases. All non CPE identified by mCIM test showed negative results for the tested carbapenemase genes.

Discussion

In this prospective study, we determined the prevalence of ESBL-PE and CRE in one of the major public tertiary care hospitals in Sri Lanka based on 593 clinically significant Enterobacteriaceae isolated during December 2017 – February 2018. The patients involved in the study group comprised of nearly equal number of males and females with the age ranged from 4 days to 89 years. Enterobacteria were recovered from a diverse spectrum of microbiological samples from patients (Table 1), the majority being from urine (52.4%) and pus/wound swabs (27.7%). Yet, this is in parallel with the types of infections caused by Enterobacteriaceae in clinical settings.

Probing into the antibiotic resistance data in the present study, the incidence of ESBL-PE was 26.0%. Although the ESBL-PE occurrence was highest (30.8%) among urinary coliforms (Figure 1), it is still lower than the uropathogenic ESBL-PE reported in Sothern Sri Lanka in 2013 as 40.2% (27). This disparity could be attributed to the demographic variations in the two regions of the country. Besides, alarmingly high rates of ESBL-PE (> 60 %) have been reported in the neighboring India and Pakistan (11, 12, 28, 29). Though the overall prevalence of ESBL-PE was low among our study isolates, cefotaxime resistance was found to be high (55.3%), highlighting the potential risk of transmission of antibiotic resistance to the susceptible isolates.

This study identified the overall prevalence of CRE as 9.6%, which was comparable to the CRE prevalence reported in many regions of South Asia (13). Further, sample-wise analysis noted that the occurrence of CRE was highest among respiratory specimens (20.8%) and that was lowest among the Enterobacteriaceae isolated from pus/wound swabs (6.7%) (Figure 1). The prevalence of CRE among the urinary Enterobacteriaceae isolates in our study was found to be 8.7%, which is in agreement with the rate of meropenem resistance (9.0%) reported in the coliforms isolated from urine cultures in a multi-center study conducted in the Western Province of Sri Lanka (14). Additionally, Fernando *et. al.* has documented that the prevalence of meropenem resistance among ESBL producing uropathogenic Enterobacteriaceae as 4.9% (16). However, the carbapenem resistance was not detected within the ESBL-PE cohort of the present study.

Intensive care units (ICUs) are known reservoirs for multi-drug resistant bacteria in healthcare settings due to the presence of high antibiotic pressure and higher propensity for cross-contamination (30, 31). Considering the antibiotic resistance in the cohort of Enterobacteriaceae isolated from the clinical samples of the ICUs, we found that high overall prevalence of CRE (38.7%; 12/31), which was significantly higher than the prevalence of ESBL-PE (22.6%; 7/31) detected in the same group. However, this difference needs to be further explored with the antibiotic usage pattern of the relevant ICU settings since frequent use of third-generation cephalosporins drives the emergence of ESBL-PE whereas CRE selection is driven by the use of carbapenems. Further, six out of 10 (60.0%) of respiratory CRE were found to be of ICU origin and the prevalence of CRE among the ICU respiratory Enterobacteriaceae isolates was 33.3%. These observations may at least partly be associated with the frequent usage of carbapenems and ventilator equipment in ICU settings. Similarly, high rate of respiratory CRE (38.1%; 2/7 *E.coli* and 6/14 *K. pneumoniae*) has been reported in the ICUs of a tertiary care hospital in the Central Province of Sri Lanka (30). Additionally, in the present study, an alarmingly high prevalence of CRE (83.3%) was found among the blood culture Enterobacteriaceae isolates of ICU origin. However, these observations need to be confirmed by further studies with larger sample sizes.

In this study, we encountered surprising species diversity within CRE isolates. Enterobacteriaceae is a diverse family of Gram negative rods which includes diverse spectrum of human pathogens with *K. pneumoniae* being the most frequent CRE species found globally (8, 22). Earlier studies have reported the presence of carbapenem resistant *K. pneumoniae*, *E. coli*, and *E. cloacae* in Sri Lanka (18, 19, 30). In addition to the previously documented Enterobacteriaceae species in the country, carbapenem resistance was also recognized in *C. freundii*, *P. rettgeri*, and *E. aerogens* in our study (Figure 2). Further, *K. pneumoniae* was the predominant CRE detected and was isolated as the only CRE species from respiratory and blood samples. In contrast, *E. coli* has been reported as the predominant organism among urinary ESBL-PE in Sri Lanka (16, 27).

Investigation of the ability of CRE to inactivate meropenem by carbapenem inactivation method, we found that the majority of CRE were carbapenemase producers (94.7%). This is in agreement with the fact that carbapenemase production is the most common mechanism of carbapenem resistance in Enterobacteriaceae (32). Further, genomic identification of CRE by polymerase chain reactions found that

KPC, NDM, and OXA-48-like carbapenemases as the determinants of carbapenem resistance in our CPE cohort (Table 2). Of them, OXA-48-like was identified as the most common carbapenemase (88.9%). Previously, OXA-181 has been reported as the predominant carbapenemase type in Sri Lanka (19, 20). Since *bla*_{OXA-181} is a variant of *bla*_{OXA-48} gene, nucleotide sequence analysis is necessary to determine the exact isoform of OXA-48-like enzyme. Additionally, NDM carbapenemase was found only in 14.8% of CPE. In contrast, NDM-1 is the most prevalent carbapenemase in India and other South Asian countries (8, 33). Besides, previous studies have reported the presence of NDM-1 and NDM-4 in Sri Lanka (18, 19). In addition, 7.4% of CPE in our study were found to coproduce NDM and OXA-48-like enzymes. Similarly, *K. pneumoniae* carrying *bla*_{NDM-1} and *bla*_{OXA-181} genes have been previously documented in Sri Lanka by Hall *et al.* (19). Most interestingly, the existence of the *bla*_{KPC} gene was found in two CRE isolates as the first such finding in Sri Lanka. Of the two KPC harboring *K. pneumoniae*, one was recovered from a sputum sample of 71 year old female patient and the other was isolated from a urine sample of 55 year old male patient. KPC has been reported internationally and has been identified as the most common carbapenemase type in China (22). Yet, other two major carbapenemases; IMP and VIM have not been reported in Enterobacteriaceae in Sri Lanka to date and in the present study as well.

A number of limitations were recognized in our study. The initial screening of CRE isolates was done using only imipenem and/or meropenem depending on the availability of antibiotic discs, which might have left out some of the CRE according to the 2015 CDC CRE surveillance definition (i.e. any Enterobacteriaceae resistant to imipenem, meropenem, doripenem, or ertapenem are considered as CRE) (34). Additionally, the DNA sequence analysis was not performed to determine the exact genotype of identified carbapenemase genes. Prevalence of ESBL-PE and CRE were not compared in all sample types due to small sample number.

Conclusions

Our study emphasizes the burden of multidrug-resistant Enterobacteriaceae in a tertiary healthcare setting in the country by providing baseline data for overall and the sample-wise prevalence of the ESBL-PE and CRE. *K. pneumoniae* was identified as the predominant CPE organism. Carbapenemase production was found to be the main mechanism behind carbapenem resistance and *bla*_{KPC}, *bla*_{NDM}, and *bla*_{OXA-48-like} genes were identified as the molecular determinants. To our knowledge, this is the first document, which shows the occurrence of KPC type carbapenemase in Sri Lanka. Since carbapenemase encoding genes are transmissible and prevalent, implementation of preventive measures are urged to minimize the spread of CRE that could otherwise lead to future epidemics of difficult to treat infections in the country.

List Of Abbreviations

ATCC: the American Type Culture Collection

CDC: Centers for Disease Control and Prevention

CLSI: Clinical and Laboratory Standards Institute

CNTH: Colombo North Teaching Hospital

CPE: Carbapenemase producing Enterobacteriaceae

CRE: Carbapenem-resistant Enterobacteriaceae

ESBL-PE: Extended spectrum β -lactamase producing Enterobacteriaceae

ICU: Intensive care unit

IMP: Imipenemase

KPC: *Klebsiella pneumoniae* carbapenemase

mCIM: Modified carbapenem inactivation method

MDR: Multidrug-resistant

NDM: New Delhi metallo-beta-lactamase

OXA: Oxacillinase

PCR: Polymerase chain reaction

SPSS: Statistical package for the social sciences

VIM: Verona integron-encoded metallo- β -lactamase

Declarations

Ethics approval and consent to participate

The ethical approval for this study was granted (P/215/08/2017) by the ethics review committee, Faculty of Medicine, University of Kelaniya, Sri Lanka. During the initial screening, the organisms were collected from the microbiology laboratory at the CNTH. The data (age and gender) were obtained from the laboratory request form and no personal identifiers were collected. The demographic and clinical data were collected from patients, who were found to have infected with ESBL-PE and CRE after obtaining informed written consent. In case of children (<18 years) or critically ill patients, informed written consent was obtained from their parents/guardians.

Consent for publication

Not applicable.

Availability of data and materials

Data of the current study are available from the corresponding author upon a reasonable request.

Competing interest

The authors declare that they have no competing interest.

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Author's contribution

YSW, WRPLIW, KDN, NPS conceptualized and designed the study. WGMK, YSW, WRPLIW, KDN were involved in data collection and interpretation. WGMK, YSW, WRPLIW drafted the manuscript. All authors read and approved the final manuscript.

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Tables

Table 1: Epidemiological characteristics of the study sample

	Total population n = 593	ESBL-PE n = 154	CRE n = 57
Age, median in years (range)	56 (4 days – 89)	60 (10 months – 89)	54 (20 – 82)
Gender			
Male	300 (50.5%)	67 (43.5%)	36 (63.2%)
Female	293 (49.5%)	87 (56.5%)	21 (36.8%)
Sample type			
Urine	311 (52.4%)	96 (62.3%)	27 (47.4%)
Pus/wound swabs	164 (27.7%)	35 (22.7%)	11 (19.3%)
Respiratory specimens	48 (8.1%)	9 (5.8%)	10 (17.5%)
Blood	37 (6.2%)	8 (5.2%)	5 (8.8%)
Sterile specimens	33 (5.6%)	6 (3.9%)	4 (7.0%)

Table 2: Distribution of carbapenemase genes in CPE cohort

	<i>bla</i> KPC	<i>bla</i> NDM	<i>bla</i> OXA-48-like
<i>K. pneumoniae</i> , n= 44 (%)	2 (4.5%)	3 (6.8%)	42 (95.4%)
<i>E. coli</i> , n= 3 (%)	0	0	3 (100%)
<i>C. freundii</i> , n= 4 (%)	0	2 (50.0%)	2 (50.0%)
<i>P. rettgeri</i> , n= 1 (%)	0	1 (100%)	0
<i>E. cloacae</i> , n= 1 (%)	0	1 (100%)	0
<i>E. aerogenes</i> , n= 1 (%)	0	1 (100%)	1 (100%)

Figures

Note: Non RE are Enterobacteriaceae isolates that are not either ESBL-PE or CRE.

Figure 1

Distribution of ESBL-PE and CRE in each sample type

A **B** **Figure 2**

Species identification (A) and relative distribution of CRE species among different samples (B)