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Epidemiology of multidrug-resistant *Enterobacteriaceae* in Sri Lanka: First evidence of bla_{KPC} harboring *Klebsiella pneumoniae*

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ABSTRACT

Background: Extended-spectrum β-lactamase producing *Enterobacteriaceae* (ESBL-PE) and carbapenem-resistant *Enterobacteriaceae* (CRE) are disseminated worldwide posing a serious public health concern. Although, the presence of ESBL-PE and CRE in Sri Lanka has been reported, the prevalence is unknown. This study aimed to provide up-to-date epidemiological data on multidrug-resistant *Enterobacteriaceae* and to characterize the molecular determinants of carbapenemase-producing *Enterobacteriaceae* (CPE) in Sri Lanka.

Methods: A prospective cross-sectional study was conducted at a tertiary care hospital in Sri Lanka between December 2017 and February 2018. ESBL-PE and CRE were identified by disc diffusion method. Carbapenemase production was determined by carbapenem inactivation method and the presence of selected carbapenemase genes were detected by PCR.

Results: Five hundred and ninety three *Enterobacteriaceae* were isolated from variety of clinical samples. Overall prevalence of ESBL-PE and CRE were 26.0% (n = 154) and 9.6% (n = 57), respectively. The highest rate of ESBL-PE (30.8%) was found in urine samples, while the highest occurrence of CRE (20.8%) was seen in respiratory specimens. The most common CRE species identified was *K. pneumoniae* (n = 46, 80.7%), followed by *C. freundii* (n = 4, 7.0%), *E. coli* (n = 3, 5.3%), *P. rettgeri* (n = 2, 3.5%), *E. cloacae* (n = 1, 1.7%), and *K. aerogenes* (n = 1, 1.7%). Carbapenemase production was observed in 54 (94.7%) of CRE isolates. Fifty eight carbapenemase encoding genes were identified in 54 CPE. The most prevalent carbapenemase gene was $bla_{\text{OXA-48-like}}$ (n = 48, 88.9%), followed by bla_{NDM} (n = 8, 14.8%), and bla_{KPC} (n = 2, 3.7%).

Conclusion: This study reports an alarming rate of CRE and the emergence of bla_{KPC} harboring *K. pneumoniae* in Sri Lanka. The need for preventive measures is highlighted to limit the spread of these difficult-to-treat bacteria in the country.

Introduction

Members of family *Enterobacteriaceae* that includes *Klebsiella pneumoniae* and *Escherichia coli* are the most common causes of both community-acquired and healthcare associated infections globally [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 almost all frequently 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 resistance to the antibiotics in the other classes of antimicrobial agents as well. The evolution of carbapenem resistance has made

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nearly all available antibiotics ineffective against the infections caused by CRE [1,2]. Further, CRE has been continuously ranked as an urgent treat 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 acquire carbapenem resistance by several ways, but the production of carbapenemase is the most common resistant mechanism. The emergence of carbapenemase-producing *Enterobacteriaceae* (CPE) is a serious concern due to the existence of several powerful and transmissible carbapenem inactivating enzymes [1,7].

ESBL-PE and CPE are widespread and endemic in many countries including the countries of Indian subcontinent [8–10]. Moreover, high prevalence of ESBL-PE (>50%) and CRE (>10%) have been reported in most regions in South Asia [9,11–13]. Though the occurrence of CRE has increased dramatically during the last decade in neighboring India [12], not much is known about the prevalence of CRE in Sri Lanka, which appears to be growing [14–16]. Incidence of clinical carbapenemase-producing *Enterobacteriaceae* in Sri Lanka was first documented in 2013 [17,18]. Thereafter, the occurrence of metallo-β-lactamase NDM-1 and oxacillinase OXA-181 carbapenemase types have been reported in a couple of hospitals in the county [19,20]. Yet, no other major carbapenemase types, such as KPC, IMP, and VIM have been identified in Sri Lanka.

Sri Lanka has always been at a greater risk of being affected by ESBL-PE and CPE outbreaks due to the liberal usage of antibiotics [21,22] and the increased travel from MDR *Enterobacteriaceae* endemic countries like India, China, Middle East, etc. [23–25]. Hence, baseline epidemiological data of MDR *Enterobacteriaceae* is essential to streamline the national antibiotic policy and the infection control protocols. Present study sought to provide updated prevalence of MDR *Enterobacteriaceae* with the emphasis on ESBL-PE and CRE at a public tertiary healthcare facility in Sri Lanka and to explore the genetic determinants of carbapenemases.

Materials and methods

Study design

A laboratory based, prospective cross-sectional study was conducted on clinically significant Enterobacteria (i.e. *Enterobacteriaceae* recovered from respiratory specimens are from the patients with respiratory tract infection) 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 suburb 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. The repeated sample/s from the same patient and the samples with contaminations were excluded.

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. The data (age and gender) were obtained from the laboratory request form. The demographic and clinical data were collected from patients, who were found to have infected with ESBL-PE or 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.

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 using ampicillin, amoxicillin/clavulanate, ceftriaxone, cefotaxime, cefuroxime, ciprofloxacin, amikacin, gentamicin, nitrofurantoin, imipenem, meropenem, doripenem, and ertapenem discs 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 three or more antimicrobial classes was considered as MDR [26]. ESBL-PE and CRE were identified by disk diffusion method as per CLSI guidelines. Accordingly, Enterobacteria, resistant to cefotaxime or ceftriaxone 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. *K. pneumoniae* ATCC 700603 and *E.coli* ATCC 25922 were used as positive and negative controls for the identification of ESBL-PE respectively, while *K. pneumoniae* ATCC BAA-1705 and ATCC BAA-1706 were used as positive and negative controls for the identification of CRE, respectively. 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 subcultured 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 et al. [27]. 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 $bla_{OXA\text{-}48\text{-}like}$ genes using the oligonucleotide primers described by Poirel et al. [28]. 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 $MgCl_2$, 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 min, followed by 35 cycles of amplification with 95 °C for 30 s, 52 °C for 40 s, and 72 °C for 60 s, and a final extension at 72 °C for 5 min. 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 amplification. *K. pneumoniae* ATCC BAA-1705, ATCC BAA-2146, and ATCC BAA-2524 were used as positive controls for the detection of bla_{KPC}, bla_{NDM}, $bla_{OXA\text{-}48\text{-}like}$ genes, respectively. *K. pneumoniae* ATCC BAA-1706 was used as the negative control.

Statistics

Data were stored in Microsoft® Excel and analysis was conducted using SPSS version 22 (IBM® corporation, USA). The

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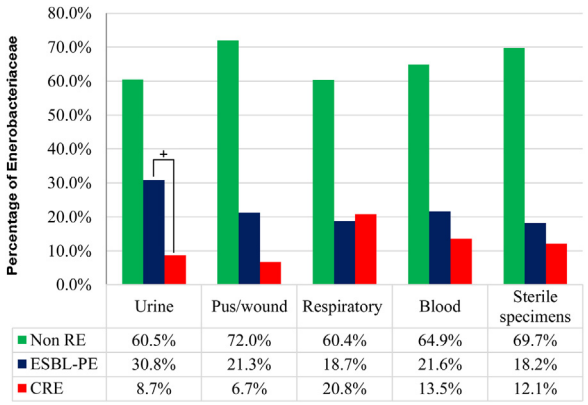


	Urine	Pus/wound	Respiratory	Blood	Sterile specimens
Non RE	60.5%	72.0%	60.4%	64.9%	69.7%
ESBL-PE	30.8%	21.3%	18.7%	21.6%	18.2%
CRE	8.7%	6.7%	20.8%	13.5%	12.1%

Fig. 1. Distribution of ESBL-PE and CRE in each sample type.
Note: Non RE are *Enterobacteriaceae* isolates that are neither ESBL-PE or CRE.
+ Statistically significant ($p < 0.05$).

categorical variables were compared using Chi-squared test. *P*-values < 0.05 were considered statistically significant.

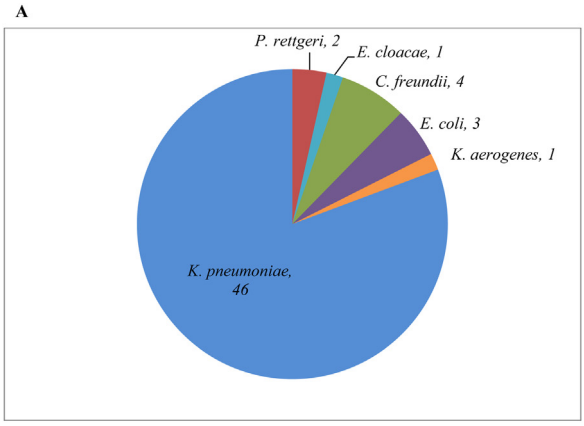
Results

Epidemiology of multidrug-resistant 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, 499 (84.1%) were from inpatients and 94 (15.9%) were from outpatients. Among total (i.e. inpatient and outpatient) *Enterobacteriaceae* isolates, 328 (55.3%) were resistant to cefotaxime/ceftriaxone and 154 (26.0%) were identified as ESBL-PE, whereas 57 (9.6%) were identified as carbapenem-resistant *Enterobacteriaceae*. Among the *Enterobacteriaceae* isolated from inpatients, 120 (24.0%) and 56 (11.2%) were ESBL-PE and CRE, respectively. Of outpatient *Enterobacteriaceae* isolates, 34 (36.2%) were ESBL-PE and one (1.1%) isolate was found to be CRE.

Table 1 summarizes the epidemiological characteristics of overall 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 mostly recovered from female patients (n = 87, 56.5%, $p = 0.063$). This may be partly due to a high proportion of ESBL-PE (96/154) were isolated from urine samples and a large number of ESBL-PE positive urine samples (n = 59; 61.5%) were received from female patients. However, CRE were predominate among male patients (n = 36, 63.2%, $p = 0.046$). ESBL-PE were predominantly recovered from urine samples (n = 96, 62.3%; inpatients, n = 64) and pus/wound swabs (n = 35, 22.7%; inpatients, n = 33), 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%). The single outpatient carbapenem-resistant *Enterobacteriaceae* was isolated from a urine sample. Further, 31 (6.2%) of inpatient *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 Fig. 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 form 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, 6 out of 18 (33.3%) respiratory specimens and 5 of 6 (83.3%) blood samples received from ICUs were found to harbor CRE.



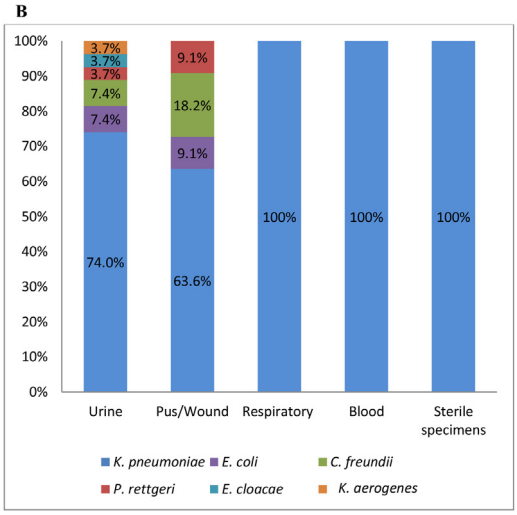


Fig. 2. Species identification (A) and relative distribution of CRE species among different samples (B).

Phenotypic characterization of CRE

Carbapenem-resistant *Enterobacteriaceae* 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%, $p = 0.000$) (Fig. 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 *Klebsiella aerogenes* (n = 1, 1.7%). Fig. 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%), *K. cloacae* (n = 1, 3.7%), and *K. aerogenes* (n = 1, 3.7%). All CRE isolates from ICU patients and the single outpatient CRE isolate were of *K. pneumoniae*.

The modified carbapenem inactivation method was used to explore whether the carbapenem resistance is conferred by

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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, n (%)	300 (50.5%)	67 (43.5%)	36 (63.2%)*
Female, n (%)	293 (49.5%)	87 (56.5%)	21 (36.8%)*
Sample type			
Urine, n (%)	311 (52.4%)	96 (62.3%)	27 (47.4%)
Pus/wound swabs, n (%)	164 (27.7%)	35 (22.7%)	11 (19.3%)
Respiratory specimens, n (%)	48 (8.1%)	9 (5.8%)	10 (17.5%)
Blood, n (%)	37 (6.2%)	8 (5.2%)	5 (8.8%)
Sterile specimens, n (%)	33 (5.6%)	6 (3.9%)	4 (7.0%)

* Statistically significant ($p < 0.05$).

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, 11 out of 12 (91.7%) CRE isolated from ICU samples were carbapenemase producers. All the *Enterobacteriaceae* identified as CPE had a zone diameter of 6 mm for the meropenem disc incubated with the test organism in the mCIM study.

Molecular characterization of carbapenemases

Presence of five most common carbapenemase encoding genes (bla_{KPC}, bla_{NDM}, $bla_{OXA\text{-}48\text{-}like}$, bla_{IMP}, and bla_{VIM}) among CRE cohort was evaluated by PCR. All three non-CPE identified by mCIM test showed negative results for the tested carbapenemase genes in PCR amplification. A total of 58 carbapenemase encoding genes were detected in 54 carbapenemase-producing *Enterobacteriaceae* isolates, which included co-occurrence of two carbapenemase genes (bla_{NDM} and $bla_{OXA\text{-}48\text{-}like}$) in four isolates. The distribution of the identified carbapenemase genes among CPE is summarized in Table 2. The most common carbapenemase gene identified was $bla_{OXA\text{-}48\text{-}like}$ (n = 48, 88.9%), followed by bla_{NDM} (n = 8, 14.8%), and bla_{KPC} (n = 2, 3.7%). Of 44 *K. pneumoniae* isolates, 2 (4.5%) harbored bla_{KPC} and 39 (88.6%) harbored $bla_{OXA\text{-}48\text{-}like}$ genes. None of the *K. pneumoniae* found to carry bla_{NDM} as the only carbapenmase gene. However, 3 (6.8%) *K. pneumoniae* isolates co-harbored both bla_{NDM} and $bla_{OXA\text{-}48\text{-}like}$ genes. All carbapenemase-producing *E. coli* (n = 3) were OXA-48-like carbapenemase producers. Out of four *C. freundii*, 2 isolates (50.0%) harbored bla_{NDM} and the other 2 isolates (50.0%) harbored $bla_{OXA\text{-}48\text{-}like}$ genes. Interestingly, both *P. rettgeri* isolate and *E. cloacae* isolate were NDM carbapenemase producers. The one and only *K. aerogenes* isolate co-produced NDM and OXA-48-like carbapenemases. However, none of the CPE isolates carried bla_{IMP} or bla_{VIM} carbapenemase genes.

Discussion

In this prospective epidemiological 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 clinical samples from patients, 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 prevalence of ESBL-PE was 26.0%. Although the ESBL-PE occurrence was highest (30.8%) among urinary coliforms (Fig. 1), it is still lower than the uropathogenic ESBL-PE reported in Sothern Sri Lanka in 2013 as 40.2% [29]. 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 [11,12,30,31]. Though the overall prevalence of ESBL-PE was low among our study isolates, cefotaxime/ceftriaxone resistance was found to be high (55.3%), highlighting the potential risk of transmission of antibiotic resistance to the susceptible isolates.

We have also determined that the overall prevalence of CRE as 9.6% and the rate of CRE among inpatients as 11.2%. These numbers were comparable to the prevalence of CRE reported in many regions of South Asia [13]. However, the observed high prevalence of CRE in this study imposes a serious public health concern in the country. Even though, similar number of *Enterobacteriaceae* were isolated form male and female patients, it was not clear why CRE was significantly high among males. Further, sample-wise analysis noted that the occurrence of CRE was highest among respiratory specimens (20.8%). 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]. Further, carbapenem resistance was not detected within the ESBL-PE cohort of the present study. In contrast, Fernando et al. has documented that the prevalence of meropenem resistance among ESBL producing uropathogenic *Enterobacteriaceae* as 4.9% [16].

Intensive care units 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 [32,33]. 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 need 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, 6 out of 10 (60.0%) 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 [32]. 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 using a large sample together with appropriate adjustment for confounding factors.

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Table 2
Distribution of carbapenemase genes in CPE cohort.

	bla_{KPC}	bla_{NDM}	$bla_{OXA-48-like}$	bla_{NDM} + $bla_{OXA-48-like}$
K. pneumoniae, n = 44 (%)	2 (4.5%)	0	39 (88.6%)	3 (6.8%)
E. coli, n = 3 (%)	0	0	3 (100%)	0
C. freundii, n = 4 (%)	0	2 (50.0%)	2 (50.0%)	0
P. rettgeri, n = 1 (%)	0	1 (100%)	0	0
E. cloacae, n = 1 (%)	0	1 (100%)	0	0
K. aerogenes, n = 1 (%)	0	0	0	1 (100%)

In this study, we encountered a 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 [8,24]. Earlier studies have reported the presence of carbapenem resistant *K. pneumoniae*, *E. coli*, and *E. cloacae* in Sri Lanka [18,19,32]. In addition to the previously documented *Enterobacteriaceae* species in the country, carbapenem resistance was also recognized in *C. freundii*, *P. rettgeri*, and *K. aerogens* for the first time in our study. 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 [16,29].

Investigating 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* [34]. Further, genomic identification of CPE by polymerase chain reactions found that bla_{KPC}, bla_{NDM}, and $bla_{OXA-48-like}$ carbapenemase genes as the determinants of carbapenem resistance in our CPE cohort. Of them, OXA-48-like was identified as the most common carbapenemase type (88.9%). Previously, $bla_{OXA-181}$ has been reported as the predominant carbapenemase gene present 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 detected in the present study. 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 [8,35]. 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 bla_{KPC} harboring *K. pneumonia,* 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 [24]. 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. This study included the *Enterobacteriaceae* isolated in a single hospital. However, this hospital is one of the largest public tertiary care hospitals in Sri Lanka and it receives patients form many areas of the country and from several public and private hospitals. In addition, the initial screening of CRE isolates was performed 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) [36]. Further, presence of inducible AmpC producing *Enterobacteriaceae* in the study cohort was not investigated and the exact genotype and sequence type of CPE isolates were not determined due to lack of resources.

Conclusions

Our study established the previously unknown prevalence of CRE as 9.6%, which emphasizes the burden of difficult to treat *Enterobacteriaceae* in Sri Lanka. Carbapenemase production was found to be the main mechanism behind carbapenem resistance. Our data indicated the emergence of bla_{KPC} harboring *K. pneumoniae* in Sri Lanka. In addition, the occurrence of carbapenemase genes (bla_{NDM}, and $bla_{OXA-48-like}$) in a number of clinically important *Enterobacteriaceae* was found and *K. pneumoniae* was identified as the predominant CPE organism. Since carbapenemase encoding genes are transmissible and prevalent, implementation of preventive measures are urged to minimize the spread of carbapenemase-producing *Enterobacteriaceae* that could otherwise lead to future epidemics of difficult to treat infections in the country.

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Conflict of interest

The authors declare that there are no conflicts of interest.

Ethical approval

This study was approved by the ethics review committee of the Faculty of Medicine, University of Kelaniya, Sri Lanka.

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