Tropical Biomedicine 35(2): 300–307 (2018)

Carbapenem-Resistant Enterobactericeae: Clinico-Epidemiological Perspective

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Received 26 September 2017; received in revised form 9 February 2018; accepted 10 February 2018

Abstract. Since its first discovery in 1996, Carbapenem-resistant Enterobactericeae (CRE) has been increasingly reported as a cause of infections particularly in immuno-compromised patients. With limited treatment options, these multidrug-resistant organisms are associated with high mortality rates and are now recognized as an important cause of health-care associated infections. This study aimed to determine the prevalence of CRE at a 500-bedded tertiary hospital in Selangor, Malaysia. This study identified and analyzed CRE culture results from January 2015 to December 2016. The isolates were identified by conventional and Vitek 2® methods. Susceptibility tests were done by disk diffusion technique and confirmed by E-test. Polymerase chain reaction was performed to identify NDM-1, KPC, OXA-48, VIM and IMP genes. Demographic data and clinical characteristics were collected from the Hospital Information System. The prevalence of CRE in 2015 and 2016 was 0.3% (5/1590) and 1.2% (17/1402) respectively. 65% of the patients had underlying haematological disorders. Majority (81.8%) of the isolates were *Klebsiella pneumoniae*, followed by *Serratia marcescens*, *Escherichia coli*, and *Citrobacter koseri*. *Klebsiella pneumoniae* that co-produced NDM-1 and OXA48 genes were the most common encounter (41%), followed by OXA-48 (35%), NDM-1 (12%) and KPC (6%). All isolates were resistant to all generations of cephalosporin and carbapenem. The rate of resistance to tigecycline, polymyxin B and colistin were quite high; 46% (5 from 12 isolates), 17% (2/12) and 17% (3/17) respectively. The prevalence of CRE in this institution was relatively low. However, there is a high prevalence of OXA-48 and NDM co-producer amongst CRE isolates. Physicians should have high index of CRE suspicion in hematological patients.

INTRODUCTION

Carbapenem, a family of beta lactam antibiotics has a broad-spectrum activity against gram-negative bacteria, particularly Enterobactericeae. It has been used as the agent of last resort in treating infections caused by resistant bacteria such as ESBL (extended spectrum beta lactamase) producing Enterobactericeae since 1980s (Moellering *et al.*, 1989).

Resistance to carbapenem developed not long after its introduction. The first reported case of *Klebsiella pneumoniae* CRE was detected in in 1993 (Naas & Nordmann, 1994). The rapid emergence of carbapenem-resistant Enterobactericeae (CRE) since the past decade has complicated the management of gram-negative infections. With limited treatment options, these multidrug-resistant organisms are associated with high mortality rate and are now recognized as an important cause of health-care associated infections (Falagas *et al.*, 2014).

Information on prevalence of CRE and antimicrobial susceptibility pattern are important to aid clinician in their patients' management. However, the data have not been well described in Malaysia. Therefore, we performed this study to determine the prevalence of CRE and to observe the

clinical and laboratory characteristics of CRE isolated from clinical samples.

MATERIALS & METHODS

This study was conducted in a tertiary government hospital with hematology specialty. It is a 500-bedded hospital with a total of 12 general wards, and 4 hematology wards. Laboratory culture results with Carbapenem-resistant Enterobactericeae (CRE) isolates from January 2015 until December 2016 were identified retrospectively.

The specimens were obtained from in-patients, during their acute clinical presentation. Identification of CRE was performed using standard methods in the microbiology laboratory within the hospital. Bacterial colonies grown on MacConkey agar were identified by its morphology, oxidase and basic biochemical tests. Genus identification was performed using Vitek 2® system (bioMerieux, Hazelwood, MO, USA). The initial antibiotic susceptibility test was done by Kirby Bauer disc diffusion technique. Minimum inhibitory concentration (MIC) to carbapenem, polymyxin B, polymyxin E and tigecycline was determined by E-test®. All susceptibility tests were done according to the Clinical Laboratory Standards Institute guidelines and breakpoints (CLSI, 2015 & CLSI, 2016). Isolates were then sent to the Institute of Medical Research (IMR), Kuala Lumpur for molecular study.

All CRE isolates were then subjected to PCR for the detection of blaKPC, blaIMP, blaNDM, blaVIM and blaOXA-48 β-lactamase genes. Genomic DNA was isolated from a single colony of each resistant isolate via boiling method. Five pairs of primers were designed to amplify the interest fragment with size ranging from 206 bp to 798 bp (Table 1). PCR reaction was performed in a DNA thermal cycler (Biometra, Germany) using the kit mytaqHS PCR Master Mix (2x) according to the manufacturer's recommendations. It is a ready-to-use mix containing mytaqHS hot start Taq DNA polymerase, optimized hot start PCR buffer, MgCl2 and dNTPS. The mix for detection was performed in 25 ul volume containing 12.5 ul mytaqHS Mix, the primer (20 uM each) and 1 ul of extracted DNA. Sterile nuclease free water was use to complete 25 ul volumes. Amplification was carried out with the following thermal cycling conditions: an initial denaturation at 95°C (1 min) and followed by 35 cycles of amplification consisting of denaturation at 95°C (15 sec), annealing at 60°C (15 sec), and extension at 72°C (15 sec). Positive and negative controls were amplified with each run. The presence of the correct amplicon size were separated and verified by electrophoresis in a 2% agarose gel at 100V for 100 minutes in 0.5X Tris Borate-EDTA buffer containing RedSafe staining for visualization under a gel documentation system (BIO-RAD). The DNA fragments size was compared to the molecular weight marker, Gene Ruler 1Kb plus ladder Cat.

Table 1. Oligonucleotides used in this study

Primer	Primer Sequence (5'-3')	Fragment size (bp)
NDM_F	ATT GGC ATA AGT CGC AAT CC	206
NDM_R	CTT CCA ACG GTT TGA TCG TC	
KPC_F	CGT CTA GTT CTG CTG TCT TG	798
KPC_R	CTT GTC ATC CTT GTT AGG CG	
OXA48_F	GCG TGG TTA AGG ATG AAC AC	438
OXA48_R	CAT CAA GTT CAA CCC AAC CG	
VIM3_F	GAT GGT GTT TGG TCG CAT A	390
VIM3_R	CGA ATG CGC AGC ACC AG	
IMP_F2	GGA ATA GAG TGG CTT AAT TCT C	287
IMP_R2	GCG GAC TTT GGC CAA GCT TCT A	

No. DM001-R500 (GeneDirex) (Poirela *et al.*, 2011).

Patients' clinical data including age, gender, co-morbidities and microbiologic investigation results were obtained from the 'Hospital Information System'. The data was analysed by using IBM Statistical Program for Social Sciences (SPSS) application version 22.0.

RESULTS

In 2015, 1590 Enterobactericeae was isolated from clinical samples, of which 5 (0.3%) were recorded as CRE. While 1.2% (17) from 1402 Enterobactericeae isolates in 2016 were CRE. In total, 22 non-duplicate CRE isolates were identified, 74% (n=17) were from 2016 and 26% (n=5) were from 2015. Most patients (68.2%) suffered haematological disorders that include multiple myeloma (n=3), acute myeloid leukemia (n=5) and lymphoma (n=4). Apart from haematological disorders, more than half (59.0%) of patients also suffered from at least one of chronic diseases such as diabetes mellitus (50%) and hypertension (31.8%). Table 2 shows demographic characteristic of all patients.

Most CRE were isolated from blood samples (73%), followed by tracheal aspirate (13.6), urine (9.1%) and pus aspirate (4.5%). Majority (81.8%) of the isolates were *Klebsiella pneumoniae*, followed by *Serratia marcescens* (9.1%), while the rate of *Escherichia coli*, and *Citrobacter koseri* were similar (4.5%). Table 3 shows the distribution of type of samples and organism isolated.

All isolates were resistant to ampicillin, amoxycillin-clavulonate, ceftazidime, cefepime, cefotaxime, ertapenem, imipenem, meropenem, cefoperazone and ampicillin-sulbactam. Approximately, half of isolates were resistant to gentamicin.

Table 2. Demographic characteristics and clinical data of the patients

Variable (n=22)	Frequency (n)	Percentage (%)
Gender		
Male	13	59.0
Female	9	41.0
Age		
20–40	7	31.8
41–60	8	36.3
61–88	7	31.8
Hematological disorders		
Yes	15	68.2
No	7	31.8
Co-morbid diseases		
1 disease	9	41.0
≥ 2 diseases	13	59.0
Reason for admission		
Chemotherapy	3	13.6
Peripheral blood stem cell transplant	5	22.7
Infection	4	18.2
Others	10	45.4
Antibiotic prior to CRE isolation		
Carbapenem	12	54.5
Other antibiotics	6	27.2
No antibiotic	4	18.2
Outcome		
Discharge well	8	36.3
Died	14	63.6

Table 3. Type of samples vs organisms for the 22 CRE cases

Type of specimen	Organisms	Frequency (Percentage)
Blood	*Klebsiella pneumoniae*	12 (54.5)
	Serratia marcescens	1 (4.5)
Blood (central line)	*Escherichia coli*	1 (4.5)
	Klebsiella pneumoniae	2 (9.1)
Tracheal aspirate	*Klebsiella pneumoniae*	2 (9.1)
	Serratia marcescens	1 (4.5)
Urine	*Klebsiella pneumoniae*	2 (9.1)
Pus	*Citrobacter koseri*	1 (4.5)

Antibiotic resistance pattern (disc diffusion technique) is shown in Figure 1.

Among major antibiotics of last resorts, resistance against polymyxin B, polymyxin E and tigecycline antibiotics were present but most were either sensitive or intermediate when tested using minimum inhibition concentration method, as shown in Figure 2. Only 18 isolates were subjected to molecular study for detection of NDM, OXA-48, KPC, VIM and IMP genes. Figure 3 shows the distribution on carbapenamase gene.

DISCUSSIONS

The isolation of Carbapenem-resistant Enterobactericeae (CRE) from clinical samples is on the rise. Data from the National Antibiotic Resistance Surveillance Report, Malaysia (2016) showed that the rate of *Klebsiella pneumoniae* resistance to imipenem and meropenem has increased from 3.2% and 3.7% respectively, in 2015 to 4% and 4.4% respectively, in 2016. This study showed that the prevalence of CRE in a tertiary hospital

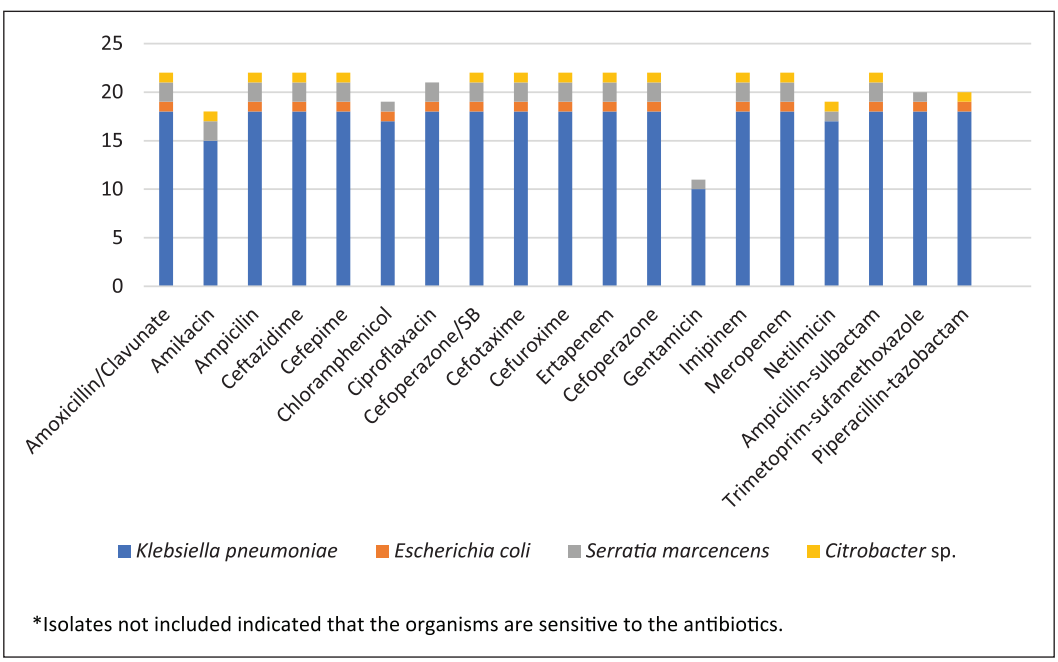

Figure 1. Antibiotic Resistance of the CRE Isolated From Patients Tested Using Disc Diffusion Method.

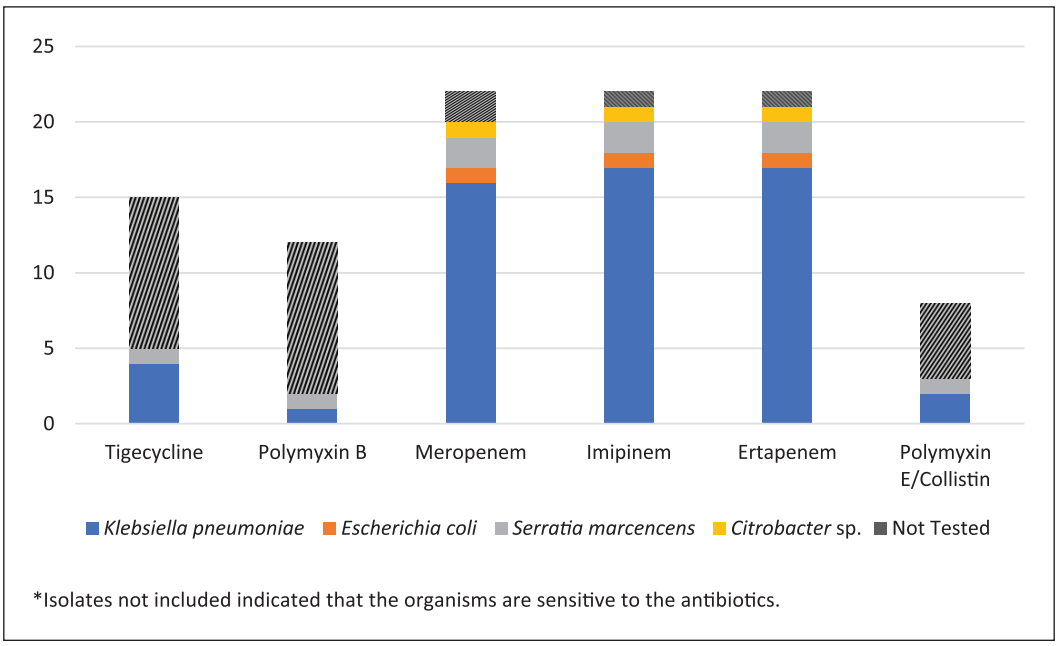

Figure 2. Antibiotic Resistance of the CRE Isolated From Patients Tested Using Minimum Inhibition Concentration (MIC) Method.

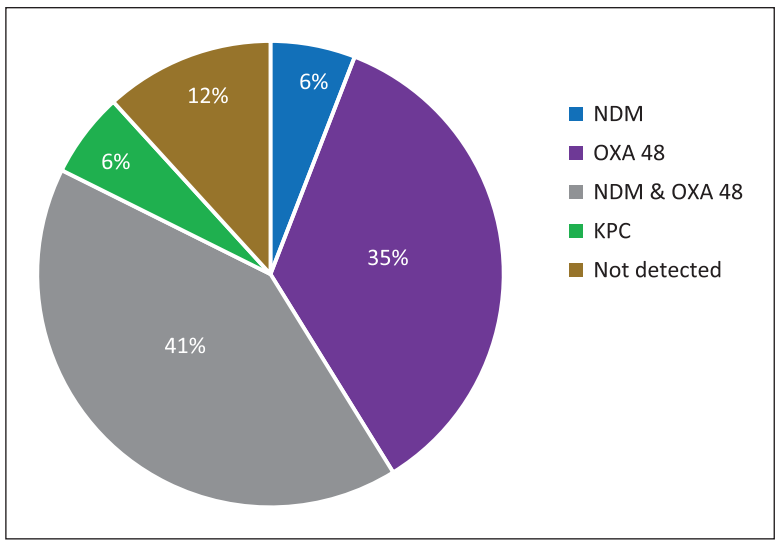

Figure 3. Genes for Carbapenemase Activity among CRE isolates.

showed increasing trend, from 0.3% in 2015 to 1.2% in 2016. This figure is relatively low compared to a similar study done in a university hospital in the East Coast of Peninsular Malaysia with prevalence of 5.74% (Zaidah *et al.*, 2017). The prevalence of CRE varies in different centers and countries, ranging from 0.04% in Spain (Miró *et al.*, 2013) to 22.4% in Uganda (Okoche *et al.*, 2015).

A cohort study in Mexico indicated that, the incidence of CRE fecal carriage was associated with transplant recipients, hematological malignancy, history of multidrug-resistant infection and transfer from other institution (Torres-Gonzalez

et al., 2015). In this study, more than one third of patients were suffering from hematological disorders, predominantly acute myeloid leukemia, multiple myeloma and lymphoma. Clinical data also revealed that about one third of them were admitted for either peripheral blood stem cell transplant (PBSCT) or chemotherapy, condition that might contribute to CRE infections due to the fact that the patients were immune-compromised.

The use of wide spectrum antibiotic such as carbapenem results in selection pressure and overgrowth of resistant organisms. Previous studies showed that carbapenem use was associated with CRE (Righi *et al*., 2017). More than half of our patients had prior carbapenem use for treatment of their underlying diseases. Nevertheless, the use of other classes of antibiotic namely third generation cephalosporin and fluroquinolones were also reported to be associated with CRE infections. This is agreeable with findings as shown by Patel *et al*. (2008) that carbapenem resistance is not attributed only to previous exposure to carbapenem but also exposure to other antibiotics.

In this study, *Klebsiella pneumoniae* accounted for majority of isolates, followed by *Serratia marcescens*, *Escherichia coli* and *Citrobacter koseri*. A review article found that *Klebsiella* spp. and *E. coli* accounted for the largest proportion of CRE, 39.3% and 22.0%, and then followed by *Serratia* spp. (19.8%), *Enterobacter* spp. (13.0%), *Proteus* spp. (4.0%), and *Citrobacter* spp. (2.0%) (Xu *et al.*, 2015).

Carbapenem resistance among Enterobacteriaceae is due to either a carbapenem-hydrolyzing enzyme (carbapenemase), or changes in outer membrane porins combined with overproduction of AmpC β-lactamases or ESBLs (extended-spectrum β-lactamase) (Nordmann, *et al*., 2009). Carbapenamase enzymes include NDM-1 (New Delhi metallo-β-lactamase-1), KPC (*Klebsiella pneumoniae* carbapenamase), OXA-48, VIM (Verona integron-encoded metallo-β-lactamase) and IMP (active on imipenem). From 18 isolates that were subjected to gene study, 16 (89%) were found to produce carbapenamase. *Klebsiella pneumoniae* that co-produced NDM-1 and OXA48 genes were the most common encounter (41%), followed by OXA-48 (35%), NDM-1 (12%) and KPC (6%). This result was almost similar to two studies done in the countries of the Gulf Cooperation Council (GCC) and Pakistan where OXA-48 predominated, followed by NDM gene. Both studies also reported presence of NDM-1 and OXA48 co-producer (Cizmeci *et al.*, 2017). In contrast, another two studies done in Malaysia revealed that NDM-1 gene outnumbered OXA-48 gene (Zaidah *et al.*, 2017). Other genes were found more commonly in other parts of the world; KPC (USA, Latin America and Europe), VIM (Greece) and IMP (Taiwan and Japan) (Satlin *et al.*, 2014).

All isolates from this study were resistant to all generations of cephalosporin and carbapenem. Aminoglycosides particularly gentamicin showed the least resistance pattern among other antibiotic classes. Despite the increasing burden of CRE infections, the most optimal treatment for CRE infections is largely unknown. A previous study concluded that treatment with single *in vitro* active agent resulted in mortality rates that are not significantly different from those treated with no active therapy, whereas combination therapy with two or more active agents was superior to monotherapy (Tzouvelekis *et al.*, 2014). Polymyxin B, colistin, tigecycline, fosfomycin and rifampin are among those suggested for treatment of CRE infections (Perez & Van Duin 2013). Unfortunately, resistances to these antibiotics have been reported from all over the world (Livermore *et al.*, 2011) (Capone *et al*., 2013). We also found that the rate of resistance to tigecycline, polymyxin B and colistin were quite high; 46% (5 from 12 isolates), 17% (2/12) and 17% (3/17) respectively. Management of infections due to resistant organisms not only relies on antibiotic treatment, it also involves infection control measures such as patient's isolation and contact precaution. Good infection control measures play paramount effect in interrupting the chain of transmission.

In conclusion, the prevalence of CRE in this institution was relatively low. However, there is a high prevalence of OXA-48 and NDM-1 co-producer amongst CRE isolates. Physicians should have high index of CRE suspicion in hematological patients.

Acknowledgement. The authors would like to thank the Director General of Health Malaysia for permission to publish this paper.

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