Carbapenem-Resistant *Enterobacteriaceae* at Rajavithi Hospital: Results of a Microbiology Laboratory Program (2009-2015)

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Background: *Enterobacteriaceae are the most commonly-found pathogens in humans, and they are an important cause of many infections. In the past few decades, extended-spectrum beta-lactamases (ESBL-) producing Enterobacteriaceae have spread worldwide. Carbapenem is the drug of choice for ESBL organism treatment, but carbapenem-resistant Enterobacteriaceae (CRE) have increasingly been reported worldwide. Unfortunately, treatment of CRE organisms has been limited by the emergence of multidrug resistance, and this has led to increasing mortality rates.*

Objective: *To investigate the occurrence of CRE isolated from patients in Rajavithi Hospital between 2009 and 2015.*

Material and Method: *CRE strains isolated in Rajavithi Hospital between 2009 and 2015 were studied. All specimens were screened for CRE isolates using inhibition of carbapenem disks.*

Results: *In 2015, the prevalence of CRE at Rajavithi Hospital was 5.8% (411/7,039). Of 411 CRE isolates, Klebsiella pneumoniae was the species with the highest number of isolates (n = 290); however, Providencia rettgeri had the highest proportion of CRE isolates per species at 34.4%. Between 2009 and 2015, the percentage of CRE isolates of each species rose dramatically especially in K. pneumoniae, which increased from 1.3% in 2009 to 1.6% in 2010, 3.8% in 2011, 5.7% in 2012, 11.1% in 2013, 11.8% in 2014 and 16.4% in 2015. Higher numbers of CRE isolates were found in IPD (88.5%) than in OPD (11.5%), and they were most commonly identified in the medical wards (55.6%). The specimens with the greatest numbers of CRE isolates were urine at 48.3%, followed by pus, sputum, blood and genital tract, at 23.3%, 15.3%, 11.5% and 1.6% respectively. Most CRE strains were resistant to all antimicrobial agents; however, tigecycline (90%S) was active against CRE in E. coli strains and colistin (75%S) was effective against CRE in K. pneumoniae.*

Conclusion: *The occurrence of CRE organisms in Rajavithi Hospital increased at a worrying rate, and early CRE reporting is an important step in halting its further proliferation. Prevention of both CRE transmission and CRE infections has become an important infectious control (IC) objective.*

Keywords: *Enterobacteriaceae, Carbapenem, Carbapenem-Resistant Enterobacteriaceae, CRE, Antimicrobial susceptibility test, AST*

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Enterobacteriaceae are a family of gram-negative bacilli that include many genera, but common organisms are the *Klebsiella* species (spp.), *Escherichia* spp., *Enterobacter* spp., *Providencia* spp., *Citrobacter* spp., *Morganella* spp., *Salmonella* spp., *Serratia* spp., *Proteus* spp., and *Shigella* spp.. *Enterobacteriaceae* are the most common human pathogens which cause many diseases including urinary tract infections, skin and soft tissue infections, bloodstream infections and pneumonia, and they are also the source of nosocomial and community-acquired infections[1,2]. The past several decades have seen the spread of extended-spectrum beta-lactamases (ESBL-) producing *Enterobacteriaceae* which are resistant to most beta-lactam antibiotics (penicillins: ampicillin, cephalosporins: cephalothin, ceftriaxone, ceftazidime, ceftriaxone, monobactams: aztreonam) apart from carbapenem (imipenem, meropenem, ertapenem and doripenem). Carbapenem has been used to treat ESBL organism infections[3], but now carbapenem-resistant

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Enterobacteriaceae (CRE) isolates have been reported worldwide[2].

CRE has at least two mechanisms: 1) reduced outer membrane permeability by porin loss in combination with the production of ESBL or of Amp C-type beta-lactamases, and 2) production of carbapenamases (carbapenemases-producing *Enterobacteriaceae*, CPE) which is mediated by several enzymes. The carbapenamases can be divided into 3 Ambler categories: class A types KPC (*Klebsiella pneumoniae* carbapenamase); class B (metallo-beta-lactamases) types IMP, VIM and NDM (New Delhi metallo-beta-lactamase); and class D, type OXA-23, -48 and -58. However, KPC, NDM and OXA-48 are the most common types of CRE found[1]. Most carbapenamases are plasmid-encoded; thus, they can be transferred from isolate to isolate and between bacterial species, causing nosocomial infections and triggering outbreaks of CRE in many hospitals worldwide[4-6]. Since first being identified at the beginning of 1996[7], KPC-type carbapenemase CRE organisms, especially *K. pneumoniae*, have spread worldwide and have proliferated dramatically in recent years[8]. A recent report described the emergence of CRE types in different geographical regions[2], and KPC producers have been reported in the USA, Europe, South America, China[2] and Canada[9], while NDM carbapenemases have been described in India[8], Pakistan[8], England[10], the United States[11] and Canada[12], and OXA-48 has been reported in Europe[13] and the Middle East[14].

These resistant organisms are clinically important because inappropriate antibiotic treatment can lead to increasing treatment failure, the spread of the organisms, and rising morbidity and mortality rates[15,16].

In Thailand, there have been a few reports of CRE, the first of which was described in Maharat Nakhon Ratchasima Hospital in 2014[17], followed by one in Siriraj Hospital, Bangkok in 2015[18].

At Rajavithi Hospital, the prevalence of ESBL-producing organisms was high due to the frequent use of carbapenem. The more carbapenem is used for ESBL treatment, the greater the chance of carbapenem resistance in *Enterobacteriaceae*, and the prevalence of CRE isolates rose dramatically in 2014. The aim of this study was to evaluate the proportion of CRE per species isolated at Rajavithi Hospital between 2009 and 2015, to determine the distribution of these organisms in outpatient and inpatient departments, to identify the sources of infection, and to establish the patterns of susceptibility of these isolates to different antimicrobial agents. These data are very important, and can be used for empirical therapeutics and infectious control for CRE infections.

Material and Method

The protocol of this research was reviewed and approved by the ethics committee of Rajavithi Hospital (010/2015).

Bacterial isolates

Between January 2009 and December 2015, all *Enterobacteriaceae* isolates (including *Klebsiella* spp., *Escherichia coli*, *Enterobacter* spp., *Providencia* spp., *Citrobacter* spp., *Morganella morgannii*, *Serratia* spp. and *Proteus* spp.) were described. All CRE isolates of *Enterobacteriaceae* which were intermediate or resistant to carbapenem discs (imipenem, meropenem, ertapenem or doripenem) were collected from various clinical specimens (with the exception of stools) of patients in the outpatient and inpatient departments of Rajavithi Hospital, a 1,200-bed general hospital in Bangkok, Thailand.

Before 2014, all *Enterobacteriaceae* had their genus and species identified by conventional biochemical tests. More recently, the matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS), MALDI Biotyper (Bruker, Germany) has been used.

All *Enterobacteriaceae* and CRE isolates were defined as being from different patients or from different susceptibility patterns in the same patient. An isolate was defined as being from the same patient, from the same species, with the same susceptibility patterns, and from the same site in each year. Outpatient cases were defined as patients with an infection identified while in the emergency room or observation wards, and cases admitted to the hospital were classified as inpatients.

Antimicrobial agents

The antimicrobial agents were as follows: beta-lactam disks consisting of penicillin; ampicillin (AM) (10 microgram (mcg)), beta-lactam-beta-lactamase inhibitor combinations (BLBI); amoxicillin-clavulanic acid (AMC) (20/10 mcg), ampicillin-sulbactam (SAM) (10/10 mcg), piperacillin-tazobactam (TZP) (100/10 mcg) and cefoperazone-sulbactam (SCF) (75/30 mcg); cephalosporins; cephalothin (CF) (30 mcg), cefotaxime (CTX) (30 mcg), ceftriaxone (CRO) (30 mcg) and ceftazidime (CAZ) (30 mcg), carbapenem; imipenem

(IPM) (10 mcg), meropenem (MEM) (10 mcg), ertapenem (ETP) (10 mcg) and doripenem (DOR) (10 mcg), aminoglycosides; gentamicin (GM) (10 mcg), amikacin (AN) (30 mcg) and netilmicin (NET) (30 mcg), quinolones; ciprofloxacin (CIP) (5 mcg), levofloxacin (LEV) (5 mcg) and satifloxacin (STFX) (10 mcg), trimethoprim-sulfamethoxazole (SXT) (1.25/23.75 mcg), tetracycline (TE) (30 mcg), chloramphenicol (C) (30 mcg), and glycylcyclines; tigecycline (TGC) (50 mcg); and the gradient diffusion MIC (minimum inhibitory concentration) method of polymixins; colistin (CL) (0.016-256 mcg/mL), MIC of CIP and LEV (0.002-32 mcg/mL) (Liofilchem srl., Italy).

Quality control isolates, including *Escherichia coli* American Type Culture Collection (ATCC) 25922 and *Pseudomonas aeruginosa* ATCC 27853 isolates were used in this study.

Susceptibility tests

Antimicrobial susceptibility testing of all antibiotics was performed by the Kirby-Bauer disk diffusion method and the results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) recommendations. Briefly, the organism was suspended in sterile normal saline (0.5 McFarland turbidity), after which inoculum was swabbed onto a Mueller-Hinton agar plate (Oxoid, Milan, Italy).

The antibiotic susceptibility patterns were determined by the disk diffusion method in a Mueller-Hinton agar plate. The agar plate was then incubated at 35°C for 24 hour. Inhibition zones for disks and MIC of colistin were compared and interpreted according to CLSI guidelines[19]. For tigecycline, interpretation was performed according to the European Committee for Antimicrobial Susceptibility Testing (EUCAST) breakpoints[20]. For cefoperazone-sulbactam and satifloxacin were followed by the manufactory's.

For this study, percentage of resistance, including both intermediate and resistant categories, was used in calculations.

Detection of phenotypic CRE isolates

All *Enterobacteriaceae* isolates were tested for phenotypic CRE. If they were found to be resistant or intermediate to one or more carbapenem discs (imipenem (IPM), or meropenem (MEM), or ertapenem (ETP) or doripenem (DOR)), they were considered to be CRE[19].

Statistical analysis

The Chi-square (χ^2) was used for statistical analysis, and a *p*-value of <0.05 was considered significant.

Results

Prevalence and proportion of CRE isolates at Rajavithi Hospital in 2015

In 2015, all of the 7,039 *Enterobacteriaceae* isolates were studied (data not shown) of which 411 were found to be resistant to meropenem. The prevalence of CRE in our hospital was therefore 5.8% (411/7,039). Of the 411 CRE isolates, the most common CRE species was *Klebsiella pneumoniae* (290), followed by 47 *Escherichia coli*, 31 *Enterobacter cloacae* complex, 21 *Providencia rettgeri*, 13 *Citrobacter freundii*, two *Enterobacter aerogenes*, *Serratia marcescens*, and *Salmonella* serotype Cholerasuis, and one each of *Providencia stuartii*, *Proteus mirabilis* and *Klebsiella oxytoca*. However, the proportion per species of CRE varied by organisms, with *P. rettgeri* having the highest frequency of CRE at 34.4% (21/61), followed by *C. freundii* at 20.0% (13/65), *K. pneumoniae* at 16.0% (290/1,814), *E. cloacae* complex at 7.5% (31/411), *S.* Choleraesuis at 6.9% (2/29), *K. oxytoca* at 3.0% (1/33), *E. aerogenes* at 2.7% (2/74), *E. coli* at 1.4% (47/3,366), *P. stuartii* at 0.9% (1/115), and *P. mirabilis* at 0.2% (1/488) (Table 1).

CRE isolates between 2009 and 2015

During a 7-year period (2009-2015), all 1,358 CRE isolates were collected from the 13 bacterial species of family *Enterobacteriaceae* including 819 (60.3%) *K. pneumoniae* (CRKP), 198 (14.5%) *E. coli* (CREC), 253 (18.6%) *E. cloacae* complex (CRECC), 33 (2.4%) *P. rettgeri* (CRPR), 32 (2.4%) *C. freundii* (CRCF), and 5 (0.4%) *Morganella morgannii* (CRMM), 4 (0.3%) *E. aerogenes* (CREA), 3 (0.2%) *P. stuartii* (CRPS), 3 (0.2%) *P. mirabilis* (CRPM), 3 (0.2%) *K. oxytoca* (CRKO), 2 (0.15%) *S. marcescens* (CRSM), 2 (0.15%) *S. cholerasuis* (CRSC), and 1 (0.1%) *Citrobacter koseri* (CRCK) (Table 1 and 3).

Between 2009 and 2015, the percentage of CRE isolates identified per year rose 9.5-fold from 3.2% (43/1,358) in 2009 to 3.7% (50/1,358) in 2010, 9.9% (135/1,358) in 2011, 12.5% (170/1,358) in 2012, 19.0% (258/1,358) in 2013, 21.4% (291/1,358) in 2014 and 30.3% (411/1,358) in 2015 (Table 1 and 2).

The percentage of CRE isolates of each species per year from 2009 through 2015 rose dramatically, especially in CRKP, CREC, CRECC, CRPR and CRCF isolates as follows: in a total of 819 CRKP isolates, 1.3% (n = 15) in 2009, 1.6% (n = 21) in 2010,

Table 1. Numbers of CRE isolates isolated at Rajavithi Hospital, 2009-2015

Year	*Klebsiella pneumoniae*		*Escherichia coli*		*Enterobacter cloacae*		*Providencia rettgeri*		*Citrobacter freundii*		*Morganella morgannii*	
	Total No. of isolates	No. (%) of CRE isolates (CRKP)	Total No. of isolates	No. (%) of CRE isolates (CREC)	Total No. of isolates	No. (%) of CRE isolates (CRECC)	Total No. of isolates	No. (%) of CRE isolates (CRPR)	Total No. of isolates	No. (%) of CRE isolates (CRCF)	Total No. of isolates	No. (%) of CRE isolates (CRMM)
2009	1,130	15 (1.3)	2,660	8 (0.3)	312	19 (6.1)	14	0 (0)	31	1 (3.2)	106	0 (0)
2010	1,309	21 (1.6)	2,879	13 (0.5)	396	15 (3.8)	5	0 (0)	42	0 (0)	131	0 (0)
2011	1,418	54 (3.8)	2,882	19 (0.7)	419	59 (14.1)	13	1 (7.7)	54	2 (3.7)	135	0 (0)
2012	1,566	89 (5.7)	2,893	35 (1.2)	405	39 (9.6)	15	3 (20.0)	57	2 (3.5)	131	0 (0)
2013	1,488	165 (11.1)	2,808	31 (1.1)	267	49 (18.4)	25	4 (16.0)	28	6 (21.4)	133	1 (0.8)
2014	1,562	185 (11.8)	3,154	45 (1.4)	366	41 (11.2)	20	4 (20.0)	60	8 (13.3)	154	4 (2.6)
2015	1,814	290 (16.0)	3,366	47 (1.4)	411	31 (7.5)	61	21 (34.4)	65	13 (20.0)	219	0 (0)
Total		819		198		253		33		32		5

Table 1. Numbers of CRE isolates isolated at Rajavithi Hospital, 2009-2015 (Cont.)

Year	*Enterobacter aerogenes*		*Providencia stuartii*		*Proteus mirabilis*		*Klebsiella oxytoca*		*Serratia marcescens*		*Salmonella* Choleraesuis		*Citrobacter koseri*	
	Total No. of isolates	No. (%) of CRE isolates (CREA)	Total No. of isolates	No. (%) of CRE isolates (CRPS)	Total No. of isolates	No. (%) of CRE isolates (CRPM)	Total No. of isolates	No. (%) of CRE isolates (CRKO)	Total No. of isolates	No. (%) of CRE isolates (CRSM)	Total No. of isolates	No. (%) of CRE isolates (CRSC)	Total No. of isolates	No. (%) of CRE isolates (CRCK)
2009	45	0 (0)	53	0 (0)	292	0 (0)	20	0 (0)	67	0 (0)	30	0 (0)	61	0 (0)
2010	64	0 (0)	89	0 (0)	345	1 (0.3)	25	0 (0)	55	0 (0)	18	0 (0)	60	0 (0)
2011	56	0 (0)	118	0 (0)	325	0 (0)	32	0 (0)	78	0 (0)	12	0 (0)	62	0 (0)
2012	47	1 (2.1)	163	1 (0.6)	339	0 (0)	32	0 (0)	94	0 (0)	22	0 (0)	52	0 (0)
2013	56	0 (0)	95	1 (1.1)	310	1 (0.3)	30	0 (0)	99	0 (0)	17	0 (0)	52	0 (0)
2014	42	1 (2.4)	82	0 (0)	366	0 (0)	44	2 (4.5)	67	0 (0)	26	0 (0)	86	1 (1.2)
2015	74	2 (2.7)	115	1 (0.9)	488	1 (0.2)	33	1 (3.0)	131	2 (1.5)	29	2 (6.9)	53	0 (0)
Total		4		3		3		3		2		2		1

Table 2. Numbers (%) of CRE isolates identified in outpatient and inpatient departments and different wards of inpatient department at Rajavithi hospital, 2009-2015

Year	Total No. of CRE isolates	OPD	IPD	IPD wards			
				Medical	Surgical	ICU	Others
2009	43 (3.2)	7 (16.3)	36 (83.7)	16 (44.4)	10 (27.8)	5 (13.9)	5 (13.9)
2010	50 (3.7)	4 (8.0)	46 (92.0)	18 (39.2)	14 (30.4)	0 (0)	14 (30.4)
2011	135 (10.0)	14 (10.4)	121 (89.6)	52 (43.0)	40 (33.1)	9 (7.4)	20 (16.5)
2012	170 (12.5)	14 (8.2)	156 (91.8)	94 (60.3)	31 (19.9)	22 (14.1)	9 (5.7)
2013	258 (19.0)	16 (6.2)	242 (93.8)	146 (60.2)	65 (26.9)	14 (5.9)	17 (7.0)
2014	291 (21.4)	27 (9.3)	264 (90.7)	154 (58.3)	52 (19.7)	12 (4.6)	46 (17.4)
2015	411 (30.3)	74 (18.0)	337 (82.0)	188 (55.8)	54 (16.0)	54 (16.0)	41 (12.2)
Total	1,358	156 (11.5)	1,202 (88.5)	668 (55.6)	266 (22.1)	116 (9.7)	152 (12.6)

Table 3. Species and specimen distribution of 1,358 CRE isolates at Rajavithi Hospital, 2009-2015

Organisms	Specimen type (n (%)					
	Urine	Pus	Sputum	Blood	Genital Tract	Total
Klebsiella pneumoniae (CRKP)	299 (36.5)	211 (25.7)	184 (22.5)	113 (13.8)	12 (1.5)	819 (60.3)
Escherichia coli (CREC)	85 (42.9)	69 (34.9)	9 (4.6)	28 (14.1)	7 (3.5)	198 (14.6)
Enterobacter cloacae complex (CRECC)	208 (82.2)	21 (8.3)	10 (4.0)	11 (4.3)	3 (1.2)	253 (18.6)
Providencia rettgeri (CRPR)	28 (84.9)	4 (12.1)	0	1 (3.0)	0	33 (2.4)
Citrobacter freundii (CRCF)	28 (87.5)	4 (12.5)	0	0	0	32 (2.4)
Morganella morgannii (CRMM)	3	1	0	1	0	5 (0.4)
Enterobacter aerogenes (CREA)	2	2	0	0	0	4 (0.3)
Providencia stuartii (CRPS)	1	1	1	0	0	3 (0.2)
Proteusmirabilis (CRPM)	2	1	0	0	0	3 (0.2)
Klebsiella oxytoca (CRKO)	0	1	2	0	0	3 (0.2)
Serratia marcescens (CRSM)	0	0	2	0	0	2 (0.15)
Salmonella Choleraesuis (CRSC)	0	0	0	2	0	2 (0.15)
Citrobacter koseri (CRCK)	0	1	0	0	0	1 (0.1)
Total	656 (48.3)	316 (23.3)	208 (15.3)	156 (11.5)	22 (1.6)	1,358

3.8% (n = 54) in 2011, 5.7% (n = 89) in 2012, 11.1% (n = 165) in 2013, 11.8% (n = 185) in 2014 and 16.0% (n = 290) in 2015; and in 198 CREC isolates, 0.3% (n = 8) in 2009, 0.5% (n = 13) in 2010, 0.7% (n = 19) in 2011, 1.2% (n = 35) in 2012, 1.1% (n = 31) in 2013, 1.4% (n = 45) in 2014 and 1.4% (n = 47) in 2015. There was a significant yearly increase in the proportion of CRE isolates found during the 7 years studied (2009-2015), and worryingly, the rate of CRE isolates rose significantly from 14.7% (twelve-fold from 1.3% in 2009 to 16.0% in 2015) among CRKP; by 1.1% (five-fold, from 0.3% to 1.4%) among CREC; by 1.4% (from 6.1% to 7.5%) among CRECC; by 34.4% (from 0% to 34.4%) among CRPR; and by 16.8% (six-fold, from 3.2% to 20.0%) among CRCF (Table 1).

Distribution of CRE isolates

Table 2 shows the distribution rates of CRE isolates obtained from the specimens collected from outpatients and inpatients at Rajavithi Hospital between 2009 and 2015. The total of 1,358 isolates consisted of 156 (11.5%) from outpatient (OPD) and 1,202 (88.5%) from inpatient departments (IPD). The CRE rate from IPD was significantly higher than that of OPD; however, the number of CRE isolates in OPD rose by 11.8% from 6.2% in 2013 to 18.0% in 2015.

IPD CRE isolates were most commonly found in the medical wards at 55.6% (668/1,202), followed by surgical, intensive care units (ICUs) and other wards at 22.1% (266/1,202), 9.7% (116/1,202) and 12.6% (152/

Table 4. Percentage of antimicrobial susceptibilities of CRE isolates identified at Rajavithi Hospital, 2009-2015

OrganismsAM	AMC	SAM	TZP	CF	CTX	CRO	CAZ	SCP	IPM	MEM	ETP	DOR	GM	AN	NET	CIP	LEV	STFX	SXT	TE	C	TGC	CL*	
Klebsiella	0	0	0	4	0	0	0	0	1	40	5	0	3	44	61	62	3	7	12	6	22	12	-	75
pneumoniae	(810)	(811)	(811)	(731)	(811)	(811)	(811)	(810)	(699)	(329)	(788)	(298)	(148)	(808)	(807)	(207)	(299)	(696)	(59)	(811)	(808)	(516)		(28)
(CRKP)																								
Escherichia	0	2	0	19	0	0	0	0	7	44	22	0	0	46	94	86	3	7	40	27	7	48	90	
coli (CREC)	(191)	(191)	(66)	(156)	(191)	(191)	(191)	(190)	(177)	(94)	(184)	(109)	(21)	(190)	(190)	(66)	(87)	(171)	(5)	(191)	(191)	(111)	(133)	-
Enterobacter	0	0	0	6	0	0	0	0	3	80	60	0	7	22	50	19	2	7	29	15	21	53		
cloacae complex	(252)	(252)	(141)	(180)	(252)	(252)	(252)	(252)	(229)	(179)	(237)	(194)	(15)	(251)	(249)	(105)	(172)	(209)	(17)	(251)	(252)	(49)	-	-
(CRECC)																								
Providencia	0	0	0	30	0	0	0	0	0	0	0	0	0	0	38	14	0	0	0	3	0	0	-	-
rettgeri(CRPR)	(34)	(34)	(10)	(33)	(34)	(34)	(34)	(34)	(29)	(7)	(34)	(9)	(8)	(34)	(34)	(7)	(22)	(28)	(3)	(34)	(34)	(5)		
Citrobacter	0	0	0	0	0	0	0	0	0	20	3	0	0	59	88	100	5	4	0	3	28	20	-	-
freundii(CRCF)	(31)	(32)	(10)	(29)	(32)	(32)	(32)	(32)	(30)	(10)	(32)	(11)	(7)	(32)	(32)	(3)	(22)	(28)	(4)	(32)	(32)	(5)		
Morganella	0	0	0	0	0	0	0	0	0	0	0	-	-	50	100	-	-	0	-	0	0	50	-	-
morgannii	(4)	(4)	(1)	(4)	(4)	(4)	(4)	(4)	(3)	(1)	(4)			(4)	(4)			(4)		(4)	(4)	(2)		
(CRMM)																								
Enterobacter aero	0	0	0	0	0	0	0	25	0	100	25	0	-	25	25	0	0	67	-	50	25	50	-	-
genes(CREA)	(4)	(4)	(1)	(3)	(4)	(4)	(4)	(4)	(3)	(1)	(4)	(1)		(4)	(4)	(1)	(2)	(3)		(4)	(4)	(2)		
Providencia	0	0	0	33	0	0	0	33	33	0	33	-	-	33	100	100	0	0	-	33	0	0	-	-
stuarttii(CRPS)	(3)	(3)	(1)	(3)	(3)	(3)	(3)	(3)	(3)	(2)	(3)			(3)	(3)	(2)	(1)	(3)		(3)	(3)	(2)	-	
Proteus	0	0	0	33	25	25	50	50	100	50	33	0	0	75	100	0	0	67	-	75	0	0	-	-
mirabilis(CRPM)	(4)	(3)	(1)	(3)	(4)	(4)	(4)	(4)	(3)	(2)	(3)	(2)	(1)	(4)	(3)	(1)	(1)	(3)	-	(4)	(4)	(2)	-	-
Klebsiella	0	0	-	0	0	0	0	0	0	0	0	0	-	33	67	-	-	50	-	67	67	67	-	-
oxytoca(CRKO)	(3)	(3)		(3)	(3)	(3)	(3)	(3)	(2)	(1)	(3)	(1)		(3)	(3)			(2)		(3)	(3)	(3)		
Serratia	0	0	-	0	0	0	0	0	50	-	100	-	0	100	50	-	-	50	-	100	0	50	-	-
marcescens(CRSM)	(2)	(2)		(2)	(2)	(2)	(2)	(2)	(2)		(2)		(2)	(2)	(2)			(2)		(2)	(2)	(2)		
Salmonella	0	100	-	100	-	0	0	0	0	-	100	-	0	-	-	-	0	0	-	0	0	0	-	-
Choleraesuis	(2)	(2)		(2)		(2)	(2)	(2)	(2)		(2)		(2)				(2)*	(2)*		(2)	(2)	(2)		
(CRSC)																								
Citrobacter	0	0	-	0	0	0	0	100	100	-	0	0	-	0	100	100	-	0	-	0	0	0	-	-
koseri (CRCK)	(1)	(1)		(1)	(1)	(1)	(1)	(1)	(1)		(1)	(1)		(1)	(1)	(1)		(1)		(1)	(1)	(1)		

AM-ampicillin, AMC-amoxiciilin-clavulanic acid, SAM-ampicillin-sulbactam, TZP- piperacillin-tazobactam, CF- cephalothin, CTX-cefotaxime, CRO-ceftriaxone, CAZ-ceftazidime, SCP- cefoperazone-sulbactam, IPM- imipenem, MEM- meropenem, ETP-ertapenem, DOR-doripenem, GM-gentamicin, AN-amikacin, NET-netilmicin, CIP-ciprofloxacin, LEV-levofloxacin, STFX-satifloxacin, SXT- trimethoprim-sulfamethoxazole, TE-tetracycline, C-chloramphenicol, TGC-tigecycline, CL-colistin.
( ): Number of tests., *MIC, minimum inhibitory concentration

1,202) respectively.

Although the numbers of CRE isolates found in the medical wards were high (39.2 to 60.3%), their frequency rates for the 7-year period remained relatively constant.

Sources of CRE isolates

CRE isolates were most often found in urine (48.3%, 656/1,358), followed by pus (23.3%, 316/1,358), sputum (15.3%, 208/1,358), blood (11.5%, 156/1,358), and genital tract (1.6%, 22/1,358).

CRKP isolates were most commonly found in urine (36.5%, 299/819), pus (25.7%, 211/819), sputum (22.5%, 184/819), blood (13.8%, 113/819) and genital tract (1.5%, 12/819), while CREC isolates were mostly found in urine 42.9%, followed by pus, blood, sputum and genital tract at 34.9%, 14.1%, 4.6% and 3.5% respectively. CRECC, CRPR and CRCF were mostly found in urine specimens, at 82.2% (208/253), 84.9% (28/33) and 87.5% (28/32) respectively (Table 3).

Susceptibility patterns

The action of antibiotics against CRE isolates are summarized in Table 4 which shows similar resistance patterns in all CRE isolates, which were resistant to almost all antibiotics including AM, AMC, SAM, TZP, CR, CTX, CRO, CAZ and SCP (except for AMC in CRSC isolates, 100% susceptible (%S; n = 2)), TZP in CREC, CRPR, CRPS, CRPM and CRSC isolates, 19%, 30%, 33%, 33% and 100%, respectively, and SCP in CRPS, CRSM and CRPM 33%, 50% and 100% respectively, With regard to carbapenem, almost all CRE isolates were resistant (100%R) to IPM, MEM, ETP and DOR with the exception of CRKP, CREC and CRECC. The resistance rates (%R) of IPM, MEM, ETP and DOR among CRE isolates were variable, 68%, 97%, 100% and 97% respectively for CRKP, 73%, 91%, 100% and 100% respectively for CREC, and 20%, 44%, 100% and 100% respectively for CRECC.

While these antimicrobial agents were ineffective against CRE isolates, aminoglycosides, GM, AN, and NET were highly active against CRE isolates at 44%, 61% and 62% respectively for CRKP and 46%, 94% and 86% respectively for CREC.

All CRE isolates showed a high rate of resistance to quinolones CIP, LEV and STFX. High resistance to SXT and TE was common in CRKP (94% and 78% respectively), CREC (73% and 93%R respectively) and CRECC (85% and 79%R, respectively) isolates. Although CRKP had a high resistance (88%) to chloramphenicol (C), CREEC remained susceptible (53%).

Tigecycline (TGC) was highly effective (at 90%) against CREC.

Two CRE of *Salmonella* serotype Cholerasuis isolates were susceptible to meropenem but resistant to doripenem.

Table 5 shows the MIC level of colistin in CRKP isolates, demonstrating that the percentage of susceptibility of CRKP isolates to colistin was very high (MIC≤2 mcg/mL, 75%, 21/28) however; some CRKP strains were found that were resistant to colistin (MICs3-32 mcg/mL).

Discussion

Our data revealed that the prevalence of CRE in Rajavithi Hospital was 5.8%. This report showed that the percentage of CRE isolates identified was higher than in previous reports in Thailand by Singpoltan N (3.8%)[16] and Netikul T (1.4%)[17]; however, it was lower than in those conducted by Datta P et al[20] and Gupta E et al[21]. Regrettably, this study found CRE isolates in many (13) pathogen species of *Enterobacteriaceae*, especially in *K. pneumoniae*, *E. coli*, *E. cloacae* complex, *P. rettgeri* and *C. freundii*, with the exception of only two common pathogen species, *Proteus vulgaris* and *Edwardsiella tarda*, and this is in line with the reports of Singpoltan N[16], and Baran I[22].

The prevalence of CRE organisms at Rajavithi Hospital was significantly higher in inpatients than in outpatients, but rates of CRE isolates identified in outpatients have risen dramatically. The data from outpatients in this study reflected no truly community-acquired infections, as some isolates were from patients staying in nursing homes and other healthcare institutes. As in other reports, the majority of infections caused by CRE isolates were described as nosocomially acquired[23], but some recent data suggest that

Table 5. MIC of colistin to CRE *K. pneumoniae* isolates

	MIC Level of Colistin															
mcg/mL	0.047	0.094	0.125	0.19	0.25	0.38	0.50	0.75	1.0	1.5	2	3	16	24	32	Total
N	1	1	5	1	2	3	1	1	1	3	2	3	2	1	1	28

infections due to CRE organisms, especially *K. pneumoniae* isolates, might be an emergent problem in community-acquired infection in different countries[30]. A large number of CRE organisms have been found in medical wards.

The highest occurrence of CRE isolates was in urine specimens, in keeping with the findings of the reports of Bratu S[16]and Marchaim D[23].

As in the research by Hussein K[24], most CRE isolates showed resistance to many antimicrobial agents, including beta-lactams and quinolones. Fortunately, carbapenem-resistant *E. coli* isolates remain susceptible to aminoglycosides, gentamicin, amikacin and netilmicin, as found in the reports by Livermore DM and Castanheira M[25,26]. In contrast to the findings of Livermore DM[27], tigecycline was ineffective against most CRE isolates, with the exception of carbapenem-resistant *E.coli*, which remain susceptible at 90%. Gales AC[28], reported that colistin remains the most active in vitro against CRE isolates; however, our hospital was tested for carbapenem-resistant *K. pneumoniae* only (75%).

Finally, infections caused by carbapenem-producing organisms have limited treatment options and have been associated with high mortality rates. Early detection by the microbiology laboratory and infection control team will help in eliminating and controlling the spread of CRE organisms.

Conclusion

The findings of our study indicate that the incidence of CRE in Rajavithi Hospital, Bangkok, Thailand is rising dramatically throughout many *Enterobacteriaceae* species, especially in *K. pneumoniae* and *E. coli*. CRE organisms were found in both OPD and IPD, and were most commonly discovered in urine specimens. Most CRE are resistant to the majority of antibiotic agents, and therefore CRE present treatment challenges, and it is essential to take infection control precautions to control their proliferation.

What is already known on this topic?

CRE represent an emerging problem at Rajavithi Hospital.

CRE are significantly more commonly found in inpatients than in outpatients.

A large number of CRE have been identified in medical wards.

Most CRE are found in urine.

Early and accurate detection by the microbiology laboratory and infection control team will help in eliminating and controlling the spread of CRE organisms.

What this study adds?

This study used only carbapenem disks for CRE phenotypic identification.

Future studies are required using other genotypic techniques (such as PCR techniques) for classification of CRE into different types such as KPC-, NDM- or OXA-48 types.

Acknowledgements

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Potential conflict of interest

None.

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เชื้อ Enterobacteriaceae ดื้อต่อยา Carbapenem ในโรงพยาบาลราชวิถี: ผลของโปรแกรม ห้องปฏิบัติการ (พ.ศ. 2552 ถึง 2558)

ประกายทิพย์ ทองคุ้ม, สุวัฒนา กาญจนหฤทัย, สุริวรรณ จันทรคุปตังกูร, สมศักดิ์ ราหุล, มาริน่า ปูปาน, พิชญา ตันตระกูล, นิตยา มะสันต์, เลิศทิพย์ เทียมมงคลรัตน์

ภูมิหลัง: *เชื้อ Enterobacteriaceae เป็นเชื้อส่วนใหญ่ที่ก่อโรคในมนุษย์และเป็นเชื้อสำคัญในการก่อโรคติดเชื้อต่าง ๆ เมื่อหลายสิบปีที่ผ่านมา ทั่วโลกพบเชื้อ Enterobacteriaceae ผลิตเอนไซม์ extended-spectrum beta-lactamases (ESBL) ยากลุ่ม Carbapenem จึงเป็นยาที่ใช้สำหรับการรักษาการติดเชื้อ ESBL จนปัจจุบันพบเชื้อ Enterobacteriaceae ดื้อต่อยากลุ่ม Carbapenem (CRE) พบรายงานเชื้อนี้เพิ่มขึ้นและกระจายไปทั่วโลก สิ่งที่น่าวิตกกังวลคือการรักษาโรคติดเชื้อดื้อยา CRE นี้ถูกจำกัดเนื่องจากเชื้อนี้ดื้อต่อยาจำนวนมากหลายกลุ่มยาและการติดเชื้อนี้ ยังเป็นสาเหตุการเสียชีวิตที่เพิ่มขึ้น*

วัตถุประสงค์: *เพื่อประเมินสถานการณ์ของเชื้อ CRE ที่แยกได้จากผู้ป่วยในโรงพยาบาลราชวิถีระหว่าง ปี พ.ศ. 2552 ถึง 2558*

วัสดุและวิธีการ: *เชื้อสายพันธุ์ CRE ที่แยกได้ในโรงพยาบาลราชวิถีระหว่างปี พ.ศ. 2552 ถึง 2558 ถูกนำมาศึกษา วิธีการตรวจหาเชื้อสายพันธุ์ CRE เบื้องต้นด้วยการดูการยับยั้งการดื้อยาของเชื้อต่อแผ่นยากลุ่ม carbapenem*

ผลการศึกษา: *ในปี พ.ศ. 2558 ความชุกของเชื้อ CRE ในโรงพยาบาลราชวิถี พบร้อยละ 5.8 (411/7,039) ในเชื้อ CRE จำนวน 411 สายพันธุ์ เชื้อ Klebsiella pneumoniae พบว่าเป็นเชื้อสปีชีส์ที่พบ CRE มากที่สุด (จำนวน 290 สายพันธุ์) แต่อย่างไรก็ตาม เชื้อ Providencia rettgeri กลับเป็นเชื้อที่เป็น CRE ต่อสปีชีส์มากที่สุด พบร้อยละ 34.4 ในระหว่างปี พ.ศ. 2552-2558 เปอร์เซ็นต์การพบเชื้อ CRE ที่พบต่อปีนั้นเพิ่มขึ้นเรื่อย ๆ อย่างน่ากังวล โดยเฉพาะอย่างยิ่ง เชื้อ K. pneumoniae โดยพบร้อยละ 1.3 ใน พ.ศ. 2552, ร้อยละ 1.6 ใน พ.ศ. 2553, ร้อยละ 3.8 ใน พ.ศ. 2554, ร้อยละ 5.7 ใน พ.ศ. 2555, ร้อยละ 11.1 ใน พ.ศ. 2556, ร้อยละ 11.8 ใน พ.ศ. 2557 และร้อยละ 16.4 ใน พ.ศ. 2558 เชื้อ CRE พบแยกได้ในผู้ป่วยใน ร้อยละ 88.5 มากกว่า ผู้ป่วยนอก ซึ่งพบร้อยละ 11.5 และพบเชื้อนี้ในหอผู้ป่วย อายุรกรรมมากที่สุด ร้อยละ 55.6 เชื้อ CRE พบมากที่สุดในสิ่งส่งตรวจประเภทปัสสาวะถึงร้อยละ 48.3 รองลงมาพบใน หนอง เสมหะ เลือด และสิ่งส่งตรวจจากอวัยวะสืบพันธุ์ ร้อยละ 23.3, 15.3, 11.5 และ 1.6 ตามลำดับ ส่วนใหญ่ของเชื้อ CRE ดื้อต่อยาปฏิชีวนะทุกกลุ่มยา อย่างไรก็ตาม เชื้อ E. coli ที่สร้าง CRE ยังคงไวต่อยา tigecycline ถึงร้อยละ 90 และ เชื้อ K. pneumoniae ที่สร้าง CRE ยังคงไวต่อยา colistin ร้อยละ 75*

สรุป: *สถานการณ์ของเชื้อ CRE ในโรงพยาบาลราชวิถีเพิ่มขึ้นอย่างวิกฤติ การรายงาน CRE อย่างเร่งด่วนเป็นขั้นตอน ช่วยป้องกันการป้องกันการแพร่กระจายของเชื้อและการติดเชื้อนี้จึงเป็นน่าจะเป็นเข็มมุ่งของคณะกรรมการควบคุมโรคติดเชื้อ*
