คุณลักษณะของยีนดื้อยากลุ่ม Quinolones ที่อยู่บนพลาสมิดในเชื้อกลุ่ม Enterobacteriaceae ที่แยกได้จากผู้ป่วยในประเทศไทย

นางสาวปัทมาพร เมาลีทอง

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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CHARACTERIZATION OF PLASMID-MEDIATED QUINOLONE RESISTANCE GENES IN ENTEROBACTERIACEAE CLINICAL ISOLATES IN THAILAND

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ปัทมาพร เมาลีทอง : คุณลักษณะของยีนดื้อยากลุ่ม Quinolones ที่อยู่บนพลาสมิดในเชื้อกลุ่ม Enterobacteriaceae ที่แยกได้จากผู้ป่วยในประเทศไทย. (CHARACTERIZATION OF PLASMID-MEDIATED QUINOLONE RESISTANCE GENES IN ENTEROBACTERIACEAE CLINICAL ISOLATES IN THAILAND) อ. ที่ปรึกษาวิทยานิพนธ์หลัก : อาจารย์ ดร . ธนิษฐา ฉัตรสุวรรณ , 184 หน้า.

การดื้อยากลุ่ม Quinolones มีกลไกหลัก คือ การเกิดการกลายพันธุ์ในบริเวณ Quinolone Resistance - Determining Region (QRDR) ของยีนที่อยู่บนโครโมโซมซึ่งสร้างเอนไซม์ DNA gyrase และ Topoisomerase IV นอกจากนี้ยังมี กลไกการดื้อยากลุ่ม Quinolones ที่ผ่านทางพลาสมิด (Plasmid-mediated quinolone resistance; PMQR) ได้แก่ การมียีน *qnr*, *aac(6')-Ib-cr* และ *qepA* วัตถุประสงค์ของการศึกษาครั้งนี้เพื่อศึกษาคุณลักษณะและความชุกของยีนดื้อยากลุ่ม quinolones ที่อยู่บนพลาสมิด ในเชื้อกลุ่ม Enterobacteriaceae จำนวน 671 สายพันธุ์ ที่แยกได้ผู้ป่วยในระหว่างปี พ .ศ. 2552 ถึง พ.ศ. 2554 พบว่าอัตราการดื้อยา nalidixic, norfloxacin, ciprofloxacin, cefoxitin, ceftazidime, cefotaxime และ ceftriaxone คือ 47.10%, 36.22%, 37.71%, 19.68%, 46.20%, 35.77% และ 44.11% ตามลำดับ จากการศึกษาพบว่าความชุกของยีน PMRQ เป็น 20.57% (138 สายพันธุ์) โดยพบยีน *qnr*, *aac(6')-Ib-cr* และ *qepA* คิดเป็น 12.82%, 7.30% และ 0.45% ตามลำดับ ในเชื้อกลุ่ม Enterobacteriaceae พบยีน *qnr*S พบมากที่สุด รองลงมาคือ *qnrA* (2.38%) และ *qnrD* (2.38%) ตามลำดับและไม่พบยีน *qnrB* และ *qnrC* เชื้อที่มียีน PMQR ทั้งหมด 138 สายพันธุ์พบ 83 สายพันธุ์ (60.14%)สร้างเอนไซม์ ESBLs, 5 สายพันธุ์ (3.62%) สร้างเอนไซม์ AmpC และ 9 สายพันธุ์ที่สร้าง ทั้ง ESBLs และ AmpC โดยพบยีน bla_{CTX-M} ได้มากที่สุด (31.16%, 43/138) เชื้อที่มียีน *aac(6')-Ib-cr* พบร่วมกับ ESBL คิดเป็น 87.76% (43/49) เชื้อที่มียีน *qnr* พบร่วมกับ ESBL คิดเป็น 43.02% (37/86) และเชื้อที่มียีน *qepA* ทั้งหมด 3 สายพันธุ์พบร่มกับยีน bla_{CTX-M} และ/หรือ bla_{TEM} เชื้อที่มียีนดื้อมียีน 23 สายพันธุ์ ถูกเลือกมาทำการตรวจหาการกลายพันธุ์ที่เป้าหมายของยากลุ่ม quinolones พบว่า เชื้อที่ดื้อต่อยา ciprofloxacin ในระดับสูงทั้ง 3 สายพันธุ์ พบการเปลี่ยนแปลงของกรดอะมิโน ใน GyrA ที่ตำแหน่ง S83L และ D87N และ ParC ที่ตำแหน่ง S80I ในขณะที่ไม่พบการกลายพันธุ์ในเชื้อ 20 สายพันธุ์ที่ให้ผล reduced susceptibility และ intermediate resistant ต่อยา ciprofloxacin โดยเชื้อที่ดื้อต่อยา ciprofloxacin ทั้ง 3 สายพันธุ์มียีน *qepA* และพบการทำงานของ efflux pump ยีน *qnrA, qnrS,* bla_{CIT}, bla_{CTX-M}, bla_{TEM} และ bla_{VEB} สามารถถูกถ่ายทอดให้กับ *E. coli* UB1637Az^{R} โดยวิธี conjugation โดยยีน *qnr*, ESBLและ *ampC* สามารถส่งผ่านร่วมกันได้ ในการศึกษานี้พบอัตราการดื้อยาสูงในกลุ่ม quinolones และ extended spectrum-cephalosporins ในเชื้อกลุ่ม Enterobacteriaceae โดยพบยีนดื้อยากลุ่ม quinolones ผ่านทางพลาสมิดเป็นชนิด *qnr* มากที่สุด ซึ่งพบว่าสามารถ ถ่ายทอดร่วมกับยีน ESBLและ *ampC* ได้ การศึกษานี้พบยีน *qepA* และ *qnrD* เป็นครั้งแรกในเชื้อกลุ่ม Enterobacteriaceae ที่แยกได้จากประเทศไทย

สาขาวิชา จุลชีววิทยาทางการแพทย์ ลายมือชื่อนิสิต .....

ปีการศึกษา 2554 ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก….....

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PATTAMAPORN MAOLEETHONG : CHARACTERIZATION OF PLASMID-MEDIATED QUINOLONE RESISTANCE GENES IN ENTEROBACTERIACEAE CLINICAL ISOLATES IN THAILAND. ADVISOR : TANITTHA CHATSUWAN, Ph.D., 184 pp.

Quinolone resistance is mainly caused by chromosomal mutations in the quinolone resistance-determining region (QRDR) of DNA gyrase and topoisomerase IV. Recently, plasmid-mediated quinolone resistance (PMQR) including target protection mechanism encoded by *qnr*, efflux pump encoded by *qepA* and fluoroquinolone-modifying enzyme encoded by *aac(6')-Ib-cr* has been described. This study characterized genes encoding PMQR and investigated the prevalence of quinolone and extended-spectrum cephalosporin resistance in Enterobactericeae isolates. A total of 671 Enterobacteriaceae isolated from patients during 2009-2011 were included in this study. The resistance rates to nalidixic, norfloxacin, ciprofloxacin, cefoxitin, ceftazidime, cefotaxime and ceftriaxone were 47.10%, 36.22%, 37.71%, 19.68%, 46.20%, 35.77% and 44.11%, respectively. The PMQR genes were detected in 20.57% (138 isolates).Of the 671 Enterobacteriaceae isolates, 86(12.82%), 49 (7.30%) and 3 (0.45%) carried *qnr, aac(6')-Ib-cr* and *qepA*, respectively. The *qnrS* gene (8.05%) was the most common *qnr* found in Enterobacteriaceae isolates followed by *qnrA* (2.38%) and *qnrD* (2.38%) genes, respectively. The *qnrB* and *qnrC* genes were not detected in any isolates. Of the138 PMQR-positive isolates, 60.14% (83/138) were ESBL producers, 3.62% (5/138) were AmpC producers and 6.52% (9/138) were both ESBL and AmpC producers. The *bla* $_{CTX-M}$ gene was the most common ESBL determinant (31.16%, 43/138). The *aac(6')-Ib-cr*-carrying isolates were commonly associated with ESBL genes (87.76%, 43/49) and *qnr* gene were found to be co-harboured with ESBL genes in 43.02% (37/86). All 3 *qepA*-positive isolates harboured ESBL genes (bla_{CTX-M} and/or *bla* $_{TEM}$). Mutations in quinolone target were investigated in 23 PMQR representative isolates. Three isolates which were highly resistant to ciprofloxacin (MICs of >256 µg/ml) had amino acid substitutions at S83L, D87N in GyrA and S80I in ParC, whereas no mutation was found in 20 isolates with reduced susceptibility and intermediate resistance to ciprofloxacin. All 3 ciprofloxacin-resistant isolates harbored the *qepA* gene and had efflux pump activity. The *qnrA, qnrS*, bla_{CIT}, bla_{CTX-M}, bla_{TEM} and bla_{VEB} genes were successfully transferred to *E. coli* UB1637Az^{R} by conjugation method. The *qnr*, ESBL and *ampC* genes were found to be co-transferred. Our study showed high rates of quinolone and extended spectrum-cephalosporin resistance in Enterobacteriaceae. The *qnr* genes were the most prevalent PMQR determinant and were able to be transferred with ESBL and *ampC* genes. This is the first report of the presence of *qepA* and *qnrD* genes in Enterobacteriaceae isolated from Thailand.

Field of Study : Medical Microbiology ..... Student's Signature .....

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LIST OF ABBREVIATIONS

Ala (A)	alanine
Asn (N)	asparagine
Asp (D)	aspartic acid
Arg (R)	arginine
bp	base pair
CLSI	Clinical and Laboratory Standards Institute
ºC	degree Celsius
dATP	deoxyadenosine 5'-triphosphate
dCTP	deoxycytidine 5'-triphosphate
dGTP	deoxyguanosine 5'-triphosphate
dTTP	deoxytymidine 5'-triphosphate
dNTPs	deoxynucleotide-tri-phosphate
DDW	double distilled water
DNA	deoxynucleic acid
DW	distilled water
EDTA	ethylenediamine tetraacetic acid
et al.	et alii
g	gram
Gly (G)	glycine
Glu (E)	glutamic acid
Gln (Q)	glutamine
HCl	hydrochloric acid
HPLC	high performance liquid chromatography
hr	hour
Lys (K)	lysine
Leu (L)	leucine
M	molar

mg	milligram
$MgCl_2$	magnesium chloride
MIC	minimum inhibitory concentration
min	minute (s)
ml	milliliter
mM	millimolar
mmol	millimole
NaCl	sodium chloride
Na_2HPO_4	sodium phosphate dibasic, anhydrous
NaOH	sodium hydroxide
Phe (F)	phenylalanine
PCR	polymerase chain reaction
pmol	picomol
Pro (P)	proline
sec	second
Ser (S)	serine
TAE	tris-acetate-EDTA
Thr (T)	threonine
Tris	Tris-(hydroxymethyl)-aminoethane
Trp (W)	tryptophan
Tyr (Y)	tyrosine
U	unit
µg	microgram
µl	microliter
µM	micromolar
UV	ultraviolet
V	volt

CHAPTER I

INTRODUCTION

Family Enterobacteriaceae includes many pathogens, such as *Escherichia coli*, *Klebsiella* species, *Enterobacter* species, *Salmonella* species and *Proteus* species. Most common infections include pneumonia, bacteremia and urinary tract infection. Quinolones are widely used for treatment of infections causes by Enterobacteriaceae [1]. They act by inhibiting the actions of drug targets, DNA gyrase and topoisomerase IV which affect DNA replication, transcription and recombination, leading to bacterial cell death[2]. The main mechanism of quinolone resistance is mutations in DNA gyrase (encoded by *gyrA* and *gyrB*) and topoisomerase IV (encoded by *parC* and *parE*) which cause high level of drug resistance.

Plasmid-mediated quinolone resistance (PMQR) has been discovered and has potential for horizontal transfer.. The first PMQR mechanism involves pentapeptide repeat proteins, encoded by *qnr* genes which confer low level fluoroquinolone resistance by binding to DNA gyrase and topoisomerase IV and protecting them from quinolones[3, 4]. In 1998, the first PMQR gene, named *qnrA*, was isolated from a clinical isolate of *Klebsiella pneumoniae* in USA[5]. In 2003, Martinez *et.al*.[6] found that *qnrA* introduced into fluoroquinolone-susceptible strains by conjugation caused an increase in the MIC of fluoroquinolones. In addition, the co-existance of *qnr* gene and other mechanism of quinolone resistance, including mutation in quinolone target, efflux pump activity or loss of outer membrane involved in high level of quinolone resistance [6]. Currently, *qnr* genes comprise five families, *qnrA*, *qnrB*, *qnrS*, *qnrC* and *qnrD* [7-10]. The *qnr* genes were found in member of Enterobacteriaceae, including *E. coli* [11-14], *Klebsiella* spp. [13-15], *Salmonella* spp. [14, 16, 17], *Enterobacter* spp. [13, 18, 19], *Proteus* spp.[12, 20], *Citrobacter* spp. [19, 21, 22], *M. morganii* [23] and *S. marcescens*[1] and were reported from many countries such as UK [11], USA [24], Hong Kong [16], French[1], Denmark, Thailand [17], Spain [15], China[10, 20], Korea[14, 25], Philippine [13], Singapore[26], Taiwan [18], Japan [27] and Vietnam

[28].The *bla* $_{VEB-1}$ and *qnr*A1 were found to be located in the same plasmid which molecular sizes ranged 130 to 200 kb in *E. cloacae* isolates [29] and *qnrB* and *bla* $_{DHA-1}$ were also present in the same plasmid which molecular size of 70 kb [30]. The association of *qnr* with ESBL and AmpC ß-lactamase genes, which were also plasmid-mediated antibiotic resistance genes, may facilitate the spread of these antibiotic resistance genes.

The second PMQR mechanism is the production of a fluoroquinolone-modifying enzyme, encoded by *aac(6')-Ib-cr*, the variant of *aac(6')-Ib* (aminoglycoside acetyltransferase gene) [31]. The prevalence of *aac(6')-Ib-cr* was 5% in Enterobacteriaceae isolates in Korea between 1998 and 2006 [32], 14% in the United States in 2006 [33] and 18.3% in China in 2008 [34]. Most Enterobacteriaceae isolates were *E. coli*, *Klebsiella* spp. and *Enterobacter* spp..During 2007-2009, the study from China showed that prevalence of *aac(6')-Ib-cr* was 37.1% in *S. enterica* serotype Typhimurium [35].

The last mechanism is efflux pump encoded by *qepA*, discovered in 2007. QepA protein, belonging to major facilitator superfamily (MFS), reduces susceptibility to hydrophilic fluoroquinolones (norfloxacin and ciprofloxacin)[36]. The study from Korea between 1998 and 2006 found that the prevalence of *qepA* was 0.2% in *E. coli* [32]. The study from China in 2008 showed that the prevalence of *qepA* was 4.8% in *E. coli* [37]. In 2009, *qepA* were found in 5.6% in ESBL-producing *E. coli* isolates from the United States [38]. Recently, the variant of *qepA* gene, *qepA2* was discovered in France , which has two amino acid differences from QepA [39].

PMQR determinants confer low level quinolone resistance and facilitates the selection of high level resistance during quinolone therapy. PMQR was high prevalent in members of Enterobacteriaceae. The majority of reported strains were isolates of *K. pneumoniae*, *E. coli*, *Salmonella* spp., *Enterobacter* spp., *Citrobacter freudii* and

Proteus spp.. PMQR has been increasingly reported in Europe, America and Asia [40, 41]. However, there is few data on the prevalence of PMQR in Thailand. Therefore, this study aimed to investigate the prevalence of plasmid-mediated quinolone resistance in Enterobacteriaceae clinical isolates and characterize the plasmid-mediated quinolone resistance genes.

CHAPTER II

OBJECTIVES

1. To investigate prevalence of plasmid-mediated quinolone resistance in Enterobacteriaceae clinical isolates in Thailand
2. To investigate the relationship between types of plasmid-mediated quinolone resistance gene and quinolone resistance level

CHAPTER III

LITERATURE REVIEW

1. BACTERIOLOGY

Enterobacteriaceae are Gram-negative, rod-shaped, non-spore-forming and facultative anaerobic bacteria. Enterobacteriaceae are large qroup of bacterial pathogens. The medically important members of Enterobacteriaceae include *Escherichia coli, Klebsiella* spp., *Enterobacter* spp., *Salmonella* spp., *Shigella* spp., *Citrobacter* spp. *Morganella* spp., *Proteus* spp., *Providencia* spp., *Serratia* spp. and *Yersinia* spp.. (Table 1). The virulence of Enterobacteriaceae is the ability to colonize, adhere, produce toxins and invade tissue. Some species also contain plasmids that are able to mediate resistance to antimicrobial agents.

Enterobacteriaceae are important nosocomial and community-acquied pathogens responsible for various in fections including urinary tract infections, pneumonia, bacteriamia, diarrhea, respiratory tract infections and wound infections. The habitat of *E. coli* is in normal bowel flora of humans and other animal. *E. coli* is an important pathogen in human and animal, which causes urinary tract infection, bacteremia and nosocomial infection. Many strains of *Klebsiella* species were found in gastrointestinal tract of humans and animals. *Klebsiella* species has produced polysaccharide capsule which protect the antimicrobial absorption and enhanced the virulence. *Klebsiella* species causes pneumonia , infection of respiratory tract, urinary tract, blood and normally sterile sites. The habitat of *Salmonella typhi* and *Salmonella paratyphi* only found in humans but not part of normal bowel flora, while other *Salmonella* species found disseminated in nature and various animals. *Salmonella* spp. are significant infections in humans and animals cause gastroenteritis, diarrhea, bacteremia and enteric fever. The habitat of *Shigella* spp. found in humans at times of infection but not part of normal bowel flora. *Shigella* spp. is an important pathogen

cause dysentery and diarrhea. The habitat of other species in Enterobacteriaceae, including *Enterobacter* species, Serratia species, Proteus species, *Morganella* species, Providencia species, and *Citrobacter* spp. found normal florain of gastrointestinal in human causes nosocomial infection of several type of body systems including respriratory tract, urinary tract, blood.

Table 1. Bacterial species and the infection in Enterobacteriaceae

Bacterial species	Diseases
Escherichia coli	Urinary tract infection, bacteremia, traveler's diarrhea
Shigella	Dysentery, diarrhea,
Salmonella	Gastroenteritis, diarrhea, bacteremia and enteric fever
Citrobacter	Opportunistic infection, wound and urinary infection
Klebsiella	Pneumonia, septicemia respiratory tract infection, urinary tract infection,
Enterobacter	Opportunistic infection, wound infection, bacteremia, Urinary tract infection
Serratia	Opportunistic infection, wound infection, Urinary tract infection
Proteus	Wound infection, bacteremia, Urinary tract infection
Morganella	Opportunistic infection
Providencia	Opportunistic infection, wound infection, bacteremia

2. QUINOLONES AND FLUOROQUINOLONES

In 1962 , nalidixic was discovered and was the first clinical useful quinolone. It was used for treatment of urinary tract infection caused by Gram-negative bacteria. This finding leads to the development of quinolones with broader clinical use. The addition of a fluorine atom at position 6 to the quinolone structure created fluoroquinolones. The addition of groups at N1, C-5, C-7 to the basic moleculer enhanced antibacterial activity (Figure 1.) [42]. The advantages of these quinolone including, broad spectrum activity of antibiotic, good tissue distribution, and reduced side effect [43].

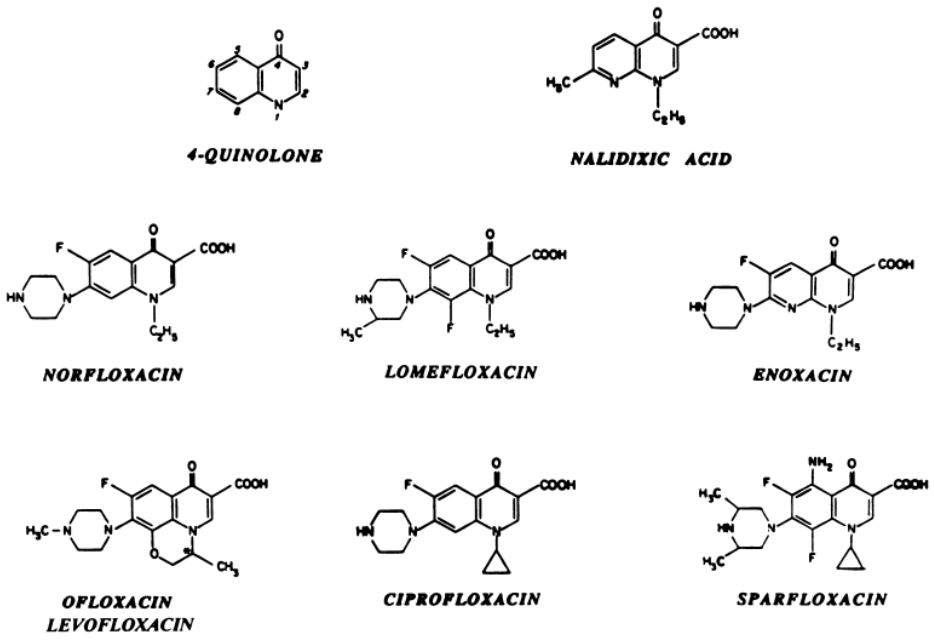

Figure 1. The structures of quinolone and fluoroquinolones for clinical use[42]

Quinolone Generations

The quinolones can be divided to four generations based on their chemical structures and activity against of bacterial species(Table 2) [44, 45].

First generation

First generation quinolones include nalidixic acid, cinoxacin and oxolinic acid. They were used to treate aerobic Gram-negative bacteria, including Enterobacteriaceae but no activity against Gram-positive bacteria and anaerobic bacteria.

Second generation

The addition of fluorine at the 6 position results in the development to fluoroquinolones. The second-generation quinolones include norfloxacin, ciprofloxacin, ofloxacin, lomefloxacin and pefloxacin. They were used to treated both aerobic Gram-positive and Gram-negative bacteria, but no activity against anaerobic bacteria.

Third generation

The third generation has been developed the activity against gram-positive bacteria. This generation comprises sparfloxacin, levofloxacin, grepafloxacin, gatifloxacin and moxifloxacin. They have activity against aerobic Gram-negative and Gram-positive bacteria including pneumococci.

Fourth generation

This generation is composed of clinafloxacin and trovafloxacin. They are more potent against anaerobes and have improved activity against pneumococci.

Table 2. Classification of quinolone antimicrobials

Generation	Quinolones
First generation	nalidixic acid, cinoxacin, oxolinic acid
Second generation	norfloxacin, ciprofloxacin, ofloxacin, lomefloxacin, pefloxacin
Third generation	sparfloxacin, levofloxacin, grepafloxacin, gatifloxacin, moxifloxacin
Fourth generation	clinafloxacin, trovafloxacin

3. MOLECULAR MECHANISMS OF ACTION OF QUINOLONES

The targets of quinolones are DNA gyrase and topoisomerase IV. DNA gyrase consists of two A subunits and two B subunits which were encoded by *gyrA* and *gyrB* genes, respectively. DNA gyrase is an enzyme that causes the negative supercoiling of DNA. Topoisomerase IV has two A subunits and two B subunits which were encoded by *parC* and *parE* genes,respectively. Topoisomerase IV acts by catenation and separation during DNA replication. Both enzymes are essential for bacterial DNA replication, transcription and recombination. Therefore, inhibition of these enzymes cause bacterial cell death.

Quinolones inhibit these enzymes by binding to gyrase/topoisomerase IV–DNA complex. The formation of quinolone-gyrase/topoisomerase IV-DNA complex prevents relegation and inhibits DNA synthesis leading to bacterial cell death [2, 46].

4. MECHANISMS OF FLUOROQUINOLONE RESISTANCE

4.1 Chromosomal-mediated Quinolone Resistance Mechanisms

4.1.1 Target Alterations

The major cause of quinolones and fluoroquinolones resistance in Gran-negative bacteria is alterations in DNA gyrase. The important region of target mutations is the quinolone-resistance determining region (QRDR) which the position of DNA-binding to the enzyme. In *E.coli*, QRDR of GyrA ranged from amino acid positions 67 to 106 [47]. Mutations at positions 83 and 87 in GyrA are predominantly for quinolones resistance in gram-negative bacteria. The amino acid substitutions included Ser 83->Phe, Tyr or Ala and Asp 87->Gly or Asn, Tyr or Ala [47-51]. ParC was the secondary target for quinolones in Gram-negative bacteria. The mutations in ParC at position 80 and 84, are frequently found in Gram-negative bacteria. The amino acid substitutions included Ser 80 -> Ile or Arg and Glu84->Lys, Val or Gly [47-49, 52, 53]. The alterations in GyrB were

found at position Asp 426->Asn and Lys 447->Glu in *E. coli* [54],while the mutation in ParE showed mutation at position Leu 445->His in *E. coli* [51].

The alterations in GyrA were reported more often than the mutations in GyrB, ParC and ParE . The single mutation in QRDR of GyrA commonly resulted in high level resistance to nalidixic acid, but fluoroquinolones resistance was required two or more mutations [2, 49, 51, 55-57]. The majority of amino acid substitutions of quinolone resistance in Enterobacteriaceae have been described for GyrA and ParC is shown in Table 3.

4.1.2 Decreased uptake

Decreased quinolone uptake was associated with increase the impermeability of antibacterial agents or overexpression of efflux pumps. Quinolones are able to through go porins and phospholipid bilayer. Thus, the change of the composition of porins and/orin the lipopolysaccharides may alter susceptibility profiles[58]. Efflux pump system was classified based on the amino acid into five groups including, the ATP binding cassette (ABC) family, the resistance nodulation cell division (RND) family, the major facilitator (MF)superfamily, the small multidrug resistance (SMR) protein family and the multidrug and toxic compound extrusion (MATE) family [59-61]. The multi-drug efflux system contribute to multi-drug resistance, including fluoroquinolones, ß-lactam, aminoglycosides, macrolides, tetracyclines, chiramphenical [62]. In *E. coli*, MarRAB or SoxRS were reported to be important to control the levels of expression of OmpF [63]. In *E. coli*, outer membrane comprised three main porins, including OmpA, OmpC and OmpF. The study of Aoyama [64] were found that the absence of OmpF showed decreased susceptibility to norfloxacin.

Table 3. Mutations in the GyrA and ParC associated with quinolone resistance [51, 57]

Species	GyrA			ParC		
	position	Wild	Mutation	position	Wild	Mutation
E. coli	67	Ala	Ser	78	Gly	Asp
	81	Gly	Cys, Asp	80	Ser	Leu, Ile, Arg
	82	Asp	Gly	84	Glu	Lys, Gly, Val
	83	Ser	Leu, Try, Ala			
	84	Ala	Pro			
	87	Asp	Asn, Val, Gly, Tyr, His			
	106	Gln	His, Arg			
Salmonella spp.	67	Ala	Pro			
	83	Ser	Phe, Tyr, Ala			
	87	Asp	Asn, Tyr, Gly			
	119	Ala	Val, Glu			
K. pneumoniae	83	Ser	Phe, Tyr	80	Ser	Ile, Arg
	87	Asp	Gly, Asn, Ala	84	Glu	Gly, Lys
K. oxytoca	83	Thr	Ile			
C. freundii	83	Thr	Ile	80	Ser	Ile
	87	Asp	Tyr, Val, Gly	84	Glu	Lys
E. cloacae	83	Ser	Phe, Tyr, Thr,Ile			
	87	Asp	Asn			
E. aerogenes	83	Thr	Ile			
S. marcessens	81	Gly	Cys			
	83	Ser	Arg, Ile			
	87	Asp	Tyr, Asn			
Shigella spp.	83	Ser	Leu			

4.2 Plasmid-mediated Quinolone Resistance (PMQR)

4.2.1 Qnr (Quinolone Resistance Protein)

Five Qnr determinants have been discovered including, QnrA, QnrB, QnrS, QnrC and QnrD. In 1998, the first PMQR gene named *qnrA* was isolated from a clinical isolate of *Klebsiella pneumoniae* in USA[5]. In 2003, Martinez *et.al.*[6] found that *qnrA* introduced into fluoroquinolone-susceptible strains by conjugation caused an increase in the MIC of fluoroquinolones. After that, in 2005, *qnrS* genes was discovered from a clinical isolate of *Shigella flexneri* 2b, which was an outbreak strain form food poisoning in Japan [8]. In 2006, *qnrB* was identified from clinical isolate of *K. pneumoniae* which produced CTX-M-15 ß-lactamase in South India[7]. In 2008, QnrC was first discoverd in clinical isolates of *Proteus miralbilis* which caused urinary tract infection in China [9].In 2009, qnrD genes was discovered from a clinical isolate of *Salmonella* enteric in China[10].

QnrB, QnrS, QnrC and QnrD share only about 43%, 44%, 64% and 48% amino acid identity with QnrA, respectively [65]. However, these Five determinants belong to the pentapeptide repeat family which composed 5-amino-acid tandem repeat with a semiconservative motif [Ser, Thr, Ala, or Val] [Asp or Asn] [Leu orPhe] [Ser, Thr, or Arg] [Gly]. Qnr protects the DNA gyrase and topoisomerase IV from the action of quinolones. The three-dimensional structure of Qnr and its charge distribution are closely to DNA. As a result, Qnr is inhibit DNA gyrase by competing with DNA for binding.[66, 67].

New variants of *qnr* genes have been increasingly reported worldwide. Qnr was found composed of QnrA1-QnrA6[68], QnrB1-QnrB39[69],and QnrS1-QnrS6 [70], while QnrC and QnrD [71] have been recently discovered and have only on type. The *qnr* genes were also found in chromosome of some gram positive or gram negative bacteria. They were termed as *qnr* from organism by creating the name of specific organisms such as from *Enterococcus faecalis, Vibrio parahaemolyticus* and *Vibrio cholerae* was called Efs*qnr* [72], Vp*qnr* [73]and Vc*qnr* [74], respectively.

4.2.1.1 Qnr and Enterobacteriaceae

There were many reports of *qnr* gene in Enterobacteriaceae. A study in 1994 from Alabama was the first identified *qnrA*1-positive strain in *K. pneumonia* [75]. After that there were many reports of qnr in members of Enterobacteriaceae worldwide. In China, *qnrA* were detected in 8% of ciprofloxacin-resistant *E. coli* isolates in 2000-2001 [76]. In German, *qnrA* were detected in 4% of integron-carrying Enterobacteriaceae isolates including *Enterobacter* spp. and *C. freundii* [77]. During 2005-2006, the new variants of *qnr* genes, *qnrS* and *qnrB*, were reported. The *qnrS* gene was discovered in *S. flexneri* 2b from Japan [8] and *qnrB* gene was discovered from *K. pneumoniae* in South India [7]. In 2006, of 335 non-typhi *Salmonella* isolated from USA, 8 (2.3%) and 2 (0.6%) isolates carried *qnrB* and *qnrS*, respectively [78]. In France, of 185 nalidixic acid resistant Enterobacteriaceae, *qnrA* was found in 0.5% in *K. pneumoniae* [1]. The study from Vietnam found *qnrS* in *Enterobacter* spp.(0.5%) [79]. In China, qnrA gene were detected in 1.3% of Gram-negative bacteria which were resistant or intermediate resistant to ciprofloxacin. These isolates were including *E. coli*, *E. cloacae* and *Citrobacter* spp.[80]. The study from Hong Kong was first detected *qnrA* gene in *Salmonella enterica* [16] In Algeria, *qnr* genes were detected in 3.5% of *E. cloacae* isolates which comprise *qnrS and qnrB* [81]. The study from UK, 32% of ciprofloxacin-resistant Enterobacteriaceae isolates found *qnrA* gene. *E. coli* was the common bacteria, followed by *C. freundii*, *K. pneumoniae* and *E. cloacae* [82]. The study by Cavaco *et al*., all 23 isolates of *S. enterica* serotype Corvallis with reduced susceptibility to ciprofloxacin from Denmark and Thailand were detected *qnrS* [17]. In USA, of *Salmonella* spp. with reduced susceptibility to ciprofloxacin isolates, 82.2% carried *qnrS* and 4.4% carried *qnrB* [83]. Thirty nine isolates of *Salmonella* spp. confer low-level ciprofloxacin resistance isolated from Netherlands which showed 87% carried *qnr* genes,including *qnrS1* (91.2%) and *qnrB* (8.8%) [84]. The study from Korea which showed *qnrB* gene (36.7%) was more common in 368 isolates of *Enterobacteriaceae* which resistant to nalidixic acid. Of *qnrB* positive isolates were found in *C. freundii*, *K. pneumonia, E. cloacae* and *E. coli* isolates, respectively [14]. The study from Spain ,

showed that low prevalence of *qnr* (0.3%) in ESBL-producing Enterobacteriaceae isolates[15].The prevalence of *qnr* in ESBL-producing *Enterobacteriaceae* isolated from Hungary in 3% for *qnrA*, 0.8% for *qnrB*, 0.4% for *qnrS*, respectively, *K. pneumoniae* was the most common bacteria, followed by *E. coli* and *C. freundii* [85]. While, the study from Philippines found *qnrB* in 8 (2.67%) isolates of ESBL-producing Enterobacteriaceae [13]. Recently, there has been reported the new variants of *qnr* gene were *qnrC* and *qnrD*. The *qnrC* has been first discovered in 2009 from clinical isolates of *P. miralbilis* [9], while *qnrD* genes was first detected in *Salmonella* enteric isolated from China in the same year [10]. The *qnrD* was recently reported in one *E. coli* [12] and twelve *P. mirabilis* [20] isolated from China. The study from Italy found *qnrD* gene in *P. mirabilis* and *M. morganii* isolates[23]. The *qnrC* gene was not found in ESBL-producing Enterobacteriaceae isolates from Philippines [13]. Similarly, *qnrC* was not detected in isolates from Japan [86], Israel [87]and Korea [32] .

There were many studies of genetic environment of *qnrA.* The plasmids carrying qnrA frequently carried antibiotic resistance genes and these plasmids was transferable. The study by Nordmann *et. al.* [88, 89], Wang *et. al.* [90] and Lascols *et. al.* [91] found that *qnrA* gene on plasmids pHSH1, pHSH2 and pQR1 from *E. coli* isolates and plasmid pHe96 from *K. pneumoniae* isolates were often embedded in complex *sul1*-type integrons. The *qnrA* gene were between duplicated 3`-conserved segments (3`-CS), containing *qacE*Δ*1* and *sul1* genes. The *orf513* genes, part of a IS*CR1,* were upstream of *qnrA* genes. The upstream of *orf513* may have different of gene cassettes. The Orf513 protein may act as a recombinase for mobilization of downstream-located antibiotic resistance genes (Figure 2).

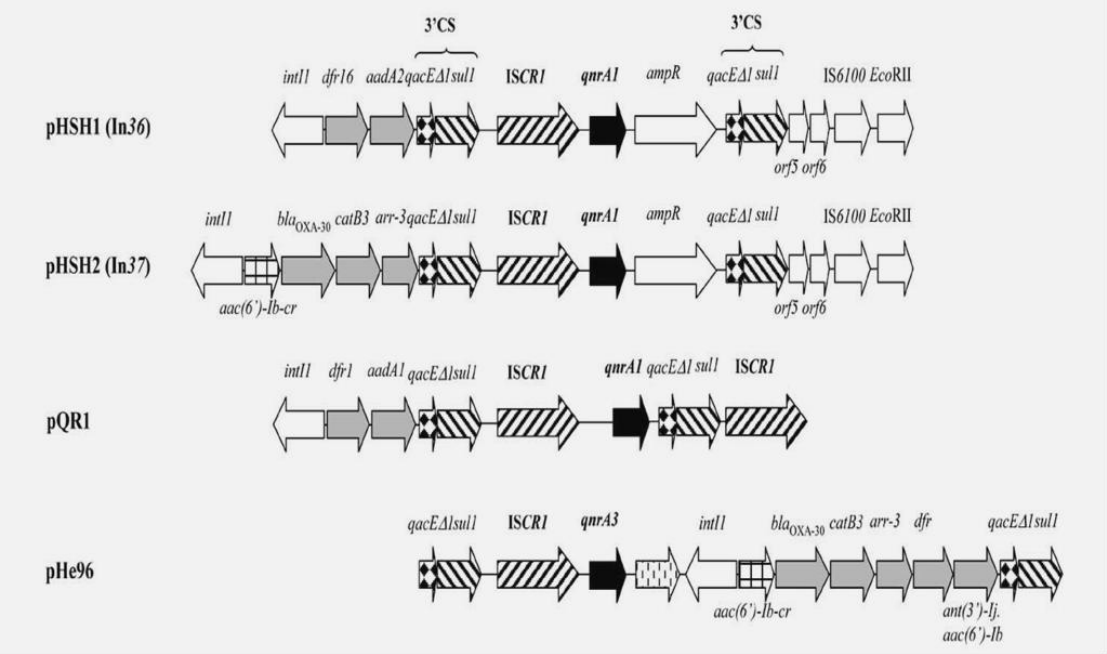

Figure 2. Genetic environment of *qnrA*

There were many studies of genetic environment of *qnrB* gene. The study by Jacoby *et.al.* [7] found that *qnrB1*gene was located in pMG298 and was associated with the *orf1005* gene encoding a putative transposase. Garnier *et.al.* [92] were reported that *qnrB2* was located in a complex *sul1*-type integron of *Salmonella* plasmid from Senegal. The *qnrB2* gene was between duplicated IS*CR1* element. Garnier *et.al.* [93] showed that the *qnrB4* gene on plasmid pRBDHA from clinical isolate of *K. pnemoniae* was located in a *sul1*-type integron with *orf1*, *bla*DHA-1*, ampR*, *psp* operon, and partial *qacEΔ1* upstream and IS*CR1* element*, qacEΔ1, sul1, bla*OXA-31 and aac(6')-Ib-cr genes downstream the *qnrB4* gene. The study by Quiroga *et al*. [94], showed that *qnrB10* had duplicated 3`-conserved segments (3`-CS) and *aac*(*6*')*-Ib-cr*, was downstream IS*CR1* (*orf513*).The *qnrB19* gene was associated with the IS*Ecp1* element [95]. However, thes gene was located in an opposite orientation (Figure 3).

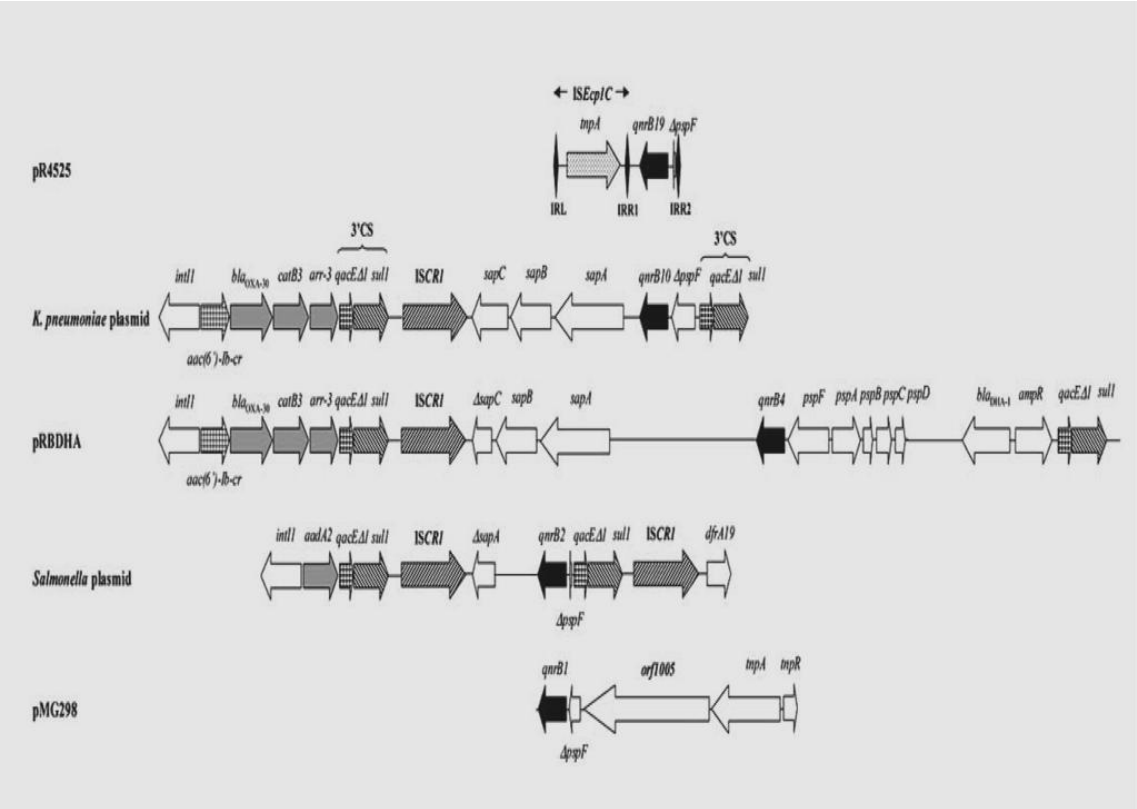

Figure 3. Genetic environment of *qnrB*

The genetic environment of *qnrS* genes were reported in many studies. The study by Hata *et al.* [8] showed that *qnrS1* was found upstream of Tn*3*-like transposon, containing bla_{TEM-1} in pAH0376 plasmid from *S. Flexneri* 2b isolate from Japan. *S. enterica* serovar Infantis isolated from Germany, *qnrS1* gene was found to locate in pINF5 plasmid upstream of Tn*3*-like transposon structures containing bla_{TEM-1} [96]. The In pS3-1 and pS5-1 plasmids from *E. cloacae* isolates from France had *qnrS1* located upstream of partial Tn*3*-like transposon and downstream the insertion sequence IS*Ecl*2 [97, 98]. The *qnrS2* gene in pGNB2 [99] and pMG308 [100] had no transposase gene (Figure 4).

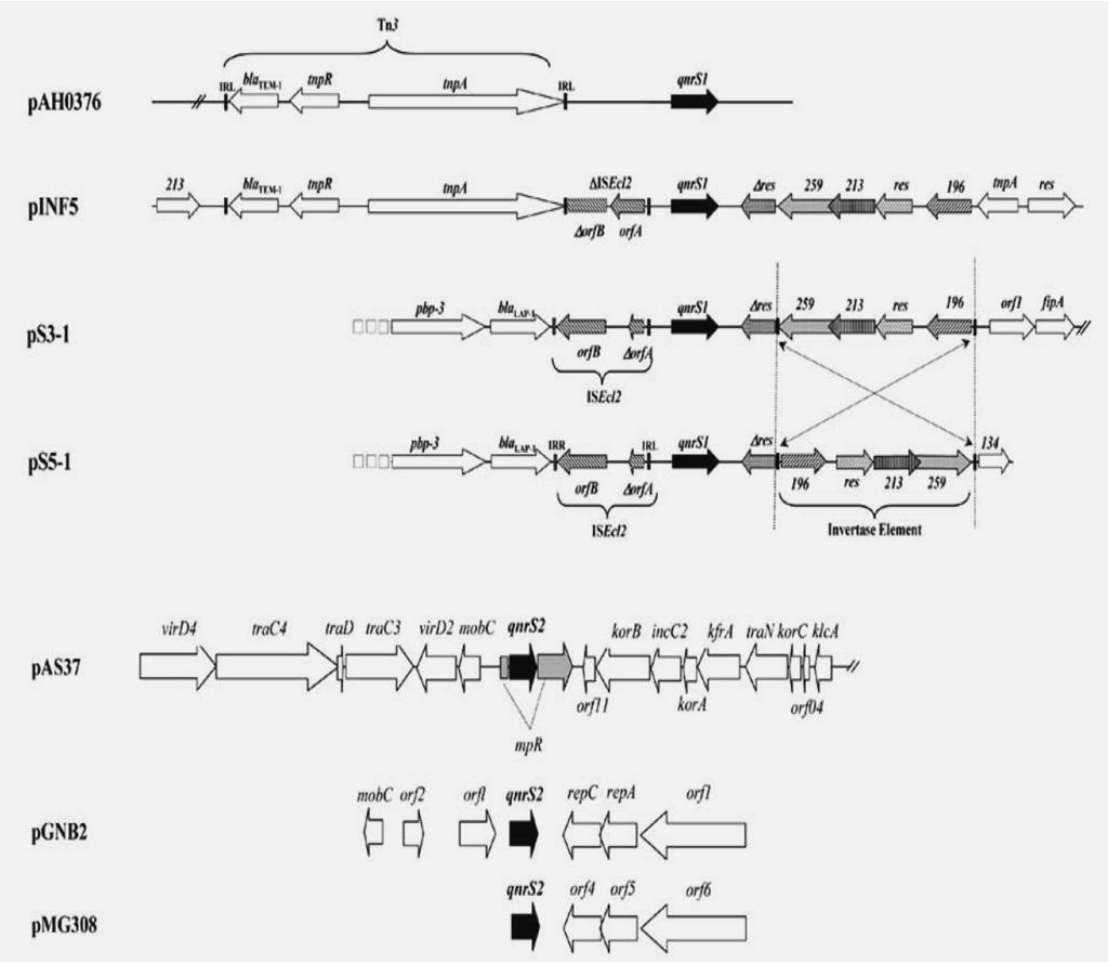

Figure 4. Genetic environment of *qnrS1* and *qnrS2*

The study of genetic environment in *qnrC* gene by Wang *et. al.* [9], showed that *qnrC* gene were located in HindIII fragment of plasmid pHS10 containing integrase upstream and amidase downstream (Figure 5). The genetic surrounding of *qnrD* was recently reported in small plasmid about 4.3 kb in *Salmonella enterica* isolates from China [10].

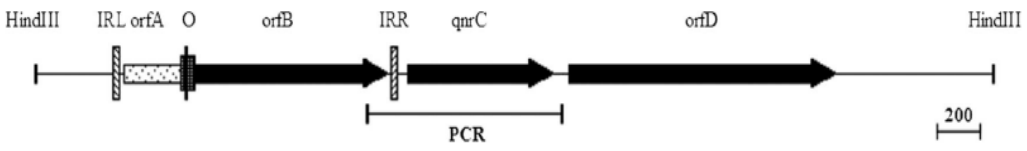

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Figure 5. Genetic environment of *qnrC*

Resistance levels mediated by Qnr determinants

The *qnrA* gene, introduced to *E. coli* J53, caused increased MICs of nalidixic acid and fluoroquinolone from 4 µg/ml to 32 µg/ml and from 0.008 µg/ml to 0.25µg/ml, respectively [101]. Cloning of *qnrA*, *qnrB*, and *qnrS* to *E. coli DH10B* showed the increase in quinolone resistance[102].

The co-existance of *qnr* and other mechanism of quinolone resistance including, mutation in quinolone target enzymes, efflux pump activity or loss of outer membrane was reported to be associated with high level of quinolone resistance [6].

The *qnr* genes were found to be linked to other resistance genes such as *aac(6')-Ib-cr, aadA1, aadA2, dfrA1, dfrA12, cmlA1* and *catB2* [103, 104]. There were reports of the correlation of *qnrA* genes with the genes encoding for ESBL and AmpC type ß-lactamase such as CTX-M-1[15], CTX-M-9[105], CTX-M-14[16], CTX-M-15 [106], SHV-5[107],SHV-7[105], SHV-92[15] , FOX-5[5, 105, 108] and VEB-1[109-111] . The *qnrB* genes were found to be located on the same plasmid with genes encoding for CTX-M-15[112], SHV-12[113], SHV-30 [19], KPC-2, KPC-3, IMP-4 and DHA-1 [111, 114]. The co-location with the genes encoding for CTX-M group [81, 115-117], TEM-1[8] and SHV-12[111] was also reported.

4.2.2 Plasmid-Mediated Quinolone Modifying Enzyme: *aac(6')-Ib-cr* genes

The *aac(6')-Ib-cr* has been discovered in *E.coli* J53 containing *qnrA1* genes from China. *E. coli* J53*,* wild type strains have MIC of ciprofloxacin at 0.008 µg/ml. Most of *E.coli* J53 containing *qnrA1* have MIC of ciprofloxacin at 0.25 µg/ml, while one isolates of *E.coli* J53 containing *qnrA1* showed MIC of ciprofloxacin at 1 µg/ml. They found that, this high-level of ciprofloxacin resistance was not caused by expression of *qnrA*. After that, they found the new variant of *aac(6')-Ib* genes was identified by transposon insertion analysis . Fluoroquinolone-modifying enzyme is encoded by *aac(6')-Ib-cr*, the variant of *aac(6')-Ib* (aminoglycoside acetyltransferase gene which confers resistance to aminoglycosides). Two mutations (Trp120Arg and Asp179Tyr) in *aac(6')-Ib*, enable the acetylation of the piperazinyl substituent of ciprofloxacin and norfloxacin, leading to the reduction of their activites (Figure 6) [31].

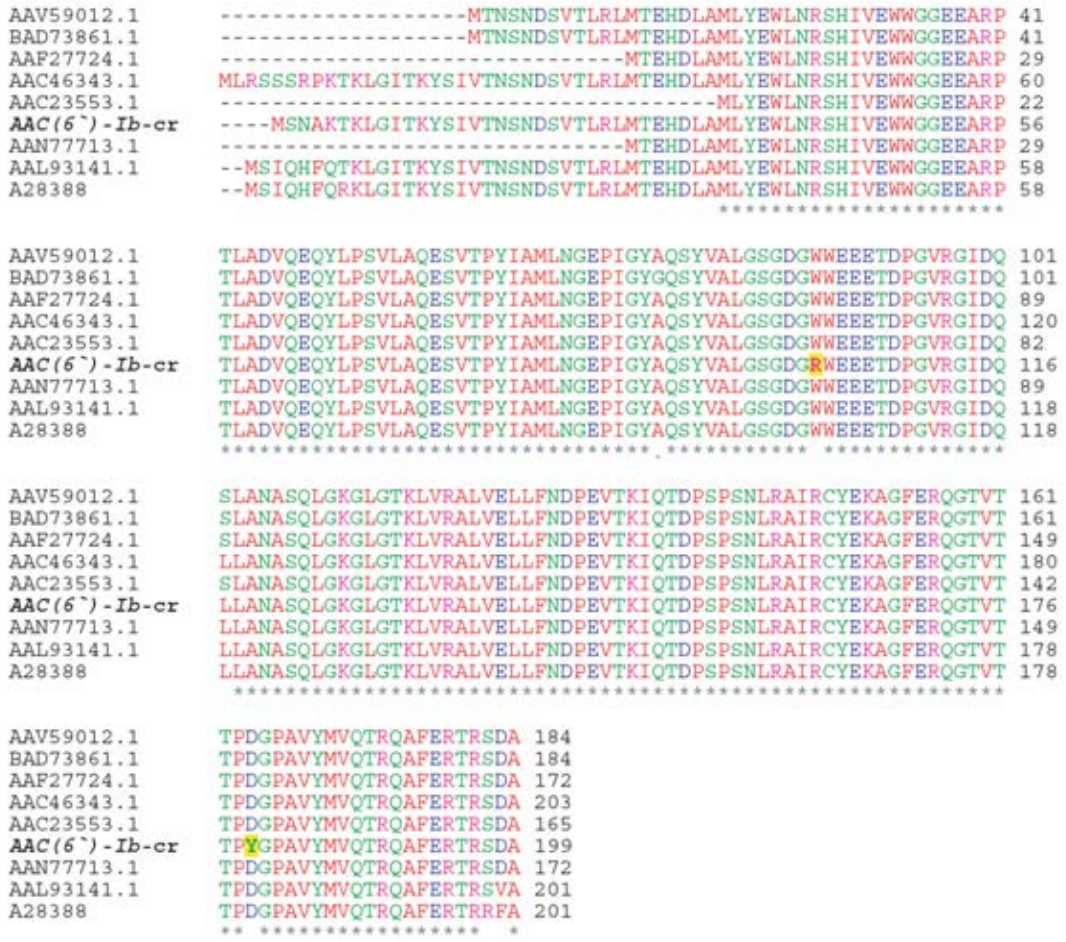

Figure 6. The Alignment of *aac(6')-Ib-cr* and eight variants of *aac(6')-Ib*.

The prevalence of *aac(6`)-Ib-cr* are most commonly found in clinical isolates of Gram-negative bacteria[88]. In the United States, in 2006, isolates, *aac(6')-Ib-cr* detected in 44 *Enterobacteriaceae* isolates(14%). It was common in *Klebsiella* spp., followed by *E. coli* and *Enterobacter* spp., respectively [118]. In Italy, *aac(6')-Ib-cr* was detected in 8% of VIM-1producing Enterobacteriaceae isolates [119].In China, *aac(6')-Ib-cr* was found in 9.9% of ESBL-producing *E. coli* and *K. pneumoniae* [117]. In Canada, *aac(6')-Ib-cr* was found in 10.8% of ciprofloxacin-resistant Enterobacteriaceae isolates. It was common in *E. coli*, followed by *Klebsiella* spp. and *P. mirabilis* [120]. In Slovenia, *aac(6')-Ib-cr* was detected in 34% of ESBL-producing *K.pneumoniae* [121]. The *aac(6')-Ib-cr* gene (37.1%) was commonly found in found *S. enterica* serotype

Typhimurium from China [35]. In Uruguay, *aac(6')-Ib-cr* were detected in 3% of ciprofloxacin-resistant Enterobacteriaceae isolates. All these isolates were *E.coli* [122]. In Korea, in 2009, *aac(6')-Ib-cr* was detected in 19 (3.4%) Enterobacteriaceae isolates. It was common in *Enterobacter* spp., *E. coli* and *K*. pneumoniae isolates, respectively [123]. In Turkey, *aac(6')-Ib-cr* was found in 45.9% of *E. coli* isolate [124].In Israel, *acc(6')-Ib-cr* was detected in 13% of KPC-producing *K. pneumoniae*[87]. It was found that eight isolates (4.7%) of AmpC-producing *E. cloacae* isolated from Korea had *aac(6')-Ib-cr* [19]. In Mexico, of 226 ESBL-producing Enterobacteriasece isolates, 49.5% had *aac(6')-Ib-cr* w [125]. The *aac(6')-Ib-cr* were associated with other resistance genes such PMQR and beta-lactamases genes, including *qnrB* [94, 120], *qnrS* [120], *qepA*, CTX-M group [87, 117, 120, 126], SHV-12 [33, 117, 126, 127], OXA-1 [123], TEM-1 [117], DHA-1and KPC-2 [87].

4.2.3 Plasmid-Mediated Fluoroquinolone Efflux Pumps; QepA

The first fluoroquinolone efflux pump, *qepA* gene, was identified from an *E. coli* isolate that caused urinary tract infection in Japan [36]. This strain showed resistance to antimicrobial agents including fluoroquinolones, aminoglycosides and extended-spectrum ß-lactams. The *qepA* gene located in conjugative plasmid pHPA, was introduced to *E. coliJ53*, leading to increased susceptibility to norfloxacin and ciprofloxacin. The QepA protein is composed of 511 amino acids , belonging to major facilitator superfamily (MFS) of 14-transmembrane segment efflux pump (Figure 7).The amino acid of QepA showed similarity to membrane transporters of gram-positive bacteria in order *Actinomycetales*. The QepA was disrupted by proton pump inhibitor, carbonyl cyanide m-chlorophenylhydrazone (CCCP). This indicated that QepA depended on the proton motive force which generated energy to dislodge of antimicrobial agents. QepA showed affects hydrophilic fluoroquinolones such as norfloxacin and ciprofloxacin[128].

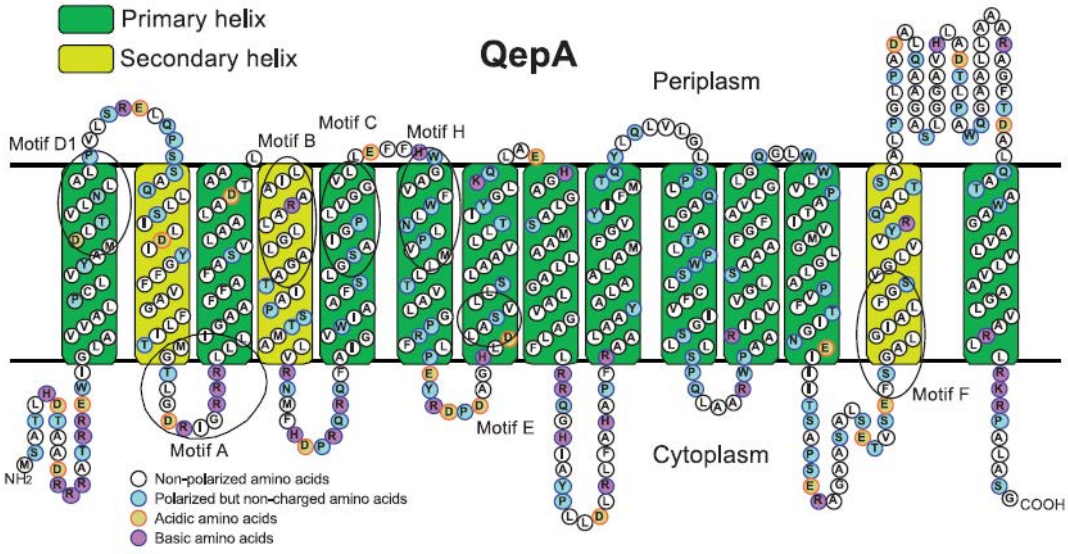

Figure 7. The 14-transmembrane structure of QepA

The genetic environment of *qepA* gene, studied by Yamane *et. al*., [36] showed that plasmid carrying *qepA* also harbored $bla_{CTX-M-12}$, *mphA* and *rmtB* genes. Cattoir *et. al.* [39] found the *qepA* gene was flanked by two copies of IS26 with *tnpA* and also contained *tnpR*, *blaTEM-1* and *rmtB* gene (Figure 8). The variant of *qepA* genes, discovered in France, had two amino acid changes at position 99 from Ala to Gly and position 134 from Val to Ile, but still confered the same phenotype as *qepA1*. The variant was named *qepA2* [39].

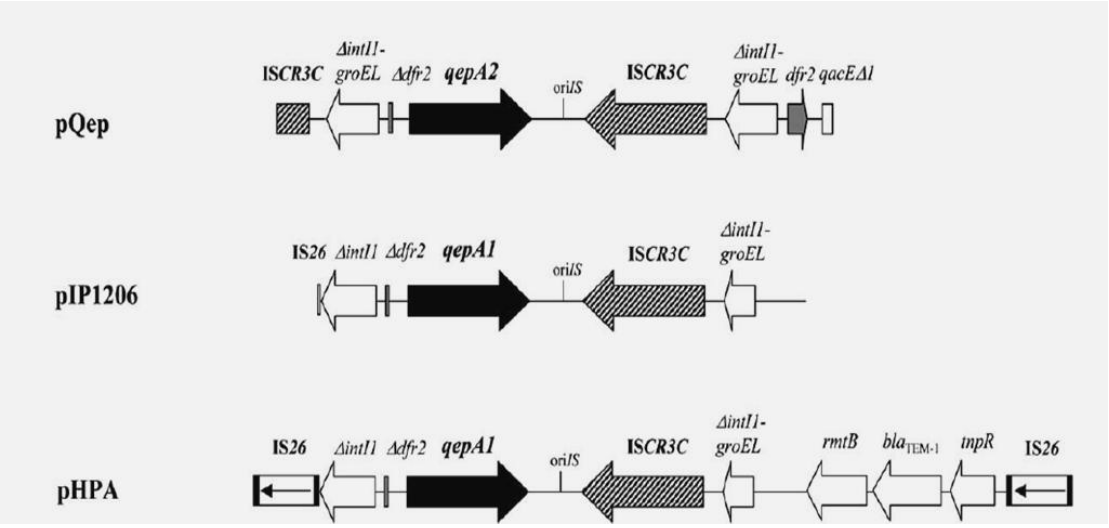

Figure 8. Genetic environment of *qepA*

The first report of *qepA* gene from Japan showed that 2 of 751 *E. coli* isolates (0.3%) carried *qepA*. Both isolates carried *rmtB* gene but negative for *qnr* gene[36]. PMQR was found in 58.3% for *qepA*, 2.1% for *qnrB,* 18.8% for *qnrS* and 12.5 % for *aac(6')-Ib-cr* in RmtB-producing *E. coli*. This results showed close relationship between *qepA* and *rmtB* genes[129]. Another study in Korea between1998 and 2006, showed that the prevalence of *qepA* was found 0.2% in *E. coli* [32]. The *qepA* gene was detectd 4.8% of *E. coli* isolates from china in 2008 [37]. In 2009, *qepA* were found in 5.6% in ESBL-producing *E. coli* isolates from the United States [38]. A study from Korea in 2011 in *qepA* was found in 0.6% of *Klebsiella* spp. and *E. coli* isolates[130].

5. EXTENDED-SPECTRUM ß-LACTAMASES (ESBLS)

Many species of gram-negative bacteria can express chromosome-mediated ß-lactamases, which are a major resistance mechanism to ß-lactam antibiotics[131]. TEM-1 are first plasmid-mediated ß-lactamases was described in the 1960s that found in *E. coli*. Isolated from Greece. After TEM-1 was discovered, It has spreaded in many species of Enterobacteriaceae worldwide[131]. TEM-type ESBLs are most often found in Enterobacteriaceae *such as E. coli, K. pneumonia, M. morganii, P. mirabilis* and *Salmonella* spp.[132, 133]. Another plasmid-mediated ß-lactamase, SHV-1 was derived from *Klebsiella* spp., It is commonly found in *K. pneumoniae* chromosome. Later, this gene was incorporated into a plasmid spreaded to other bacterial species. SHV-1 had activity similar to TEM-1. TEM-1 is able to hydrolyze penicillins and first-generation cephalosporins . SHV-1 confers resistance to penicillins and narrow-spectrum cephalosporin, but it had better activity against ampicillin In 1985, Kliebe *et al*.[134] found SHV-2 in *K. ozaenae* isolated from German which able to hydrolyze extended-spectrum cephalosporins. The majority of SHV-type ESBLs are found in *K. pneumoniae* and also found in *Citrobacter* spp. *and E. coli* [135].These enzymes called extended-spectrum ß-lactamase (ESBLs). The ESBLs have increaeingly and found in many species of Enterobacteriaceae[134]. ESBLs are molecular class A or D ß-lactamases, which are able to hydrolyze oxyimino, have serine for active-site, and are inhibited by ß-lactamase inhibitors such as clavulanic acid [131, 136]. In 1995, classification scheme based on four groups (1–4) and subgroups (a–f) by Bush *et al*.[137] (Table 4). The CTX-M (CTX-M-1) was found in a clinical isolate of *E. coli* from Germany in 1989. CTX-M enzume are able to hydrolyze cefotaxime and has become widespread in recent years. T e CTX-M-type enzymes are classified into five groups, containing CTX-M-1, CTX-M-2, CTX-M-9, CTXM-8, and CTX-M-25 group, based on the similarity of amino acid sequences[138]. The majority of CTX-M-type ESBLs are found in *K. pneumonia, E. coli, Salmonella* spp. and other species of Enterobacteriaceae [135].

Table 4. Functional grouping and molecular characteristics of major groups of-ß-lactamases [136, 137, 139]

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Functional group	Major subgroup	Molecular class	Attributes of ß-lactamases in functional group
1		C	Often found in chromosome may be found on plasmid, confer resistance to all classes of ß-lactams, except carbapenems, not inhibited by clavulanic acid
2		A, D	Most enzymes inhibited by clavulanic acid
	2a	A	Confer penicillin resistance
	2b	A	Broad-spectrum ß-lactamases, contain TEM-1 TEM-2 and SHV-1
	2be	A	Extended-spectrum ß-lactamases, confer oxymino-cephalosporins and monobactams resistance
	2br	A	Hydrolyze of penicillins and early cephalosporin, not well inhibited by clavulanic acid, contain IRTs; TEM-30, TEM-76,SHV-10 and SHV-26
	2c	A	Hydrolyze of carbenicillin, inhibited by clavulanic acid
	2d	D	hydrolyze of cloxacillin or oxacillin, not aways inhibited by clavulanic acid
	2e	A	hydrolyze of cephalosporin, inhibited by clavulanic acid.
	2f	A	Hydrolyze of Carbapenem with serine active site, inhibited by clavulanic acid.
3	3a, 3b,3c	B	metallo-ß-lactamases, confer carbapenem and all ß-lactam classes resistance except monobactams, not inhibited by clavulanic acid
4			Miscellaneous enzymes that do not fit into other groups

OXA-type enzymes confer resistance to penicillins cloxacillin and oxacillin, and weakly inhibited by clavulanic acid. The OXA are mostly found in *P. aeruginosa*, while most ESBLs are frequenty detected in member of Enterobacteriaceae such as *E. coli, K. pneumoniae*, *Salmonella* spp., etc. Other group of ESBLs are (VEB-1, PER and GES). The major of ESBLs are molecular class A ß-lactamases such as TEM, SHV and CTX-M-type,while OXA-type are molecular class D ß-lactamases.

There were many reports of ESBLs in Enterobacteriaceae. In 2002, 6.3% of Enterobacteriaceae isolated from Italy were ESBL-producers. *K. pneumoniae* was the most common, bacteria followed by *P. mirabilis*, *E. coli* and *E. aerogenes,* respectively. These isolates showed different genotypes including TEM, CTX, SHV and both TEM and SHV[140]. In 2003, the prevalence of ESBLs in *E. coli*, *K. pneumoniae*, *E. cloacae*, and *C. freundii* in China was 11.4%, 39.5%, 6% and 8%, respectively . The $bla_{CTX-M-3}$, bla_{TEM-1}, bla_{SHV-1}, and bla_{SHV-43} were detected in these isolates [141]. A study from spain in 2005, showed that 1.7% of Enterobacteriaceae isolates, including *E. coli, K. pneumoniae* and *C. freundii* were ESBL producers. Of these ESBL-producing isolates, bla_{CTX-M} was the most common ESBL, followed by bla_{SHV}, bla_{TEM} and bla_{PER-1} respectively [142]. The study from Portugal in 2007, showed that 39% of enterobacteriaceae isolates,including *K. pneumoniae E. coli* and *E. aerogenes* and *P. mirabilis* were ESBL-producing isolates, TEM was the most common ESBL, followed by SHV (30%) CTX-M (22%) and GES (2%) [143]. A study from Switzerland in 2010, showed that 5.8% Of Enterobacteriaceae isolates were ESBL producers., Of these ESBL-producing isolates $bla_{CTX-M-15}$ was the most common ESBL, followed by $bla_{CTX-M-1}$, $bla_{CTX-M-14}$, $bla_{CTX-M-2}$ and bla_{SHV-12}[144]. A study from Thailand in 2011, showed that 18.7% of Enterobacteriaceae isolates, including *C. freundii, E. aerogenes, E. cloacae, P. mirabilis , P. vulgaris, P. stuarti*., *Salmonella* spp. and S. marcescens, were ESBL-producers. The bla_{CTX-M} and bla_{TEM} were common ESBLgenes in these isolates [145].

6. PLASMID-MEDIATED AMPC ß-LACTAMASES

Many species of bacteria such as *Acinetobacter* spp., *Aeromonas* spp., *C. freundii*, *Enterobacter* spp., *E. coli, M. morganii , P. rettgeri*, *P. stuartii*, *P. aeruginosa S. marcescens*, etc. had AmpC ß-lactamases in their chromosomes [146]. In 1989, plasmid-encoded *ampC* genes were found in *K. pneumoniae* isolated from Korea, able to transfer plasmid pMVP-1 to *E. coli* which confered resistance to penicillins, cefoxitin, cefotetan, oxyimino-cephalosporins and monobactams. This enzyme named CMY-1, showed 82% similarity to chromosomal AmpC of *Aeromonas hydrophila* [147]. AmpC ß-lactamases are a second most common ß-lactamases. These enzymes can hydrolyze oximinocephalosporins (cefotaxime, ceftazidime and cefpodoxime), cephamycins (cefoxitin, cefotetan) and monobactams (aztreonam). They are not inhibited by ß-lactamases inhibitors such as clavulanic acid, sulbactam and tazobactam [146]. The plasmid-mediated AmpC ß-lactamases were reported in many species of bacterial including *K. pneumoniae*, *K. oxytoca, Salmonella* spp., *E. coli* and *P. mirabilis* which showed low level of chromosomal AmpC expression [146, 148].

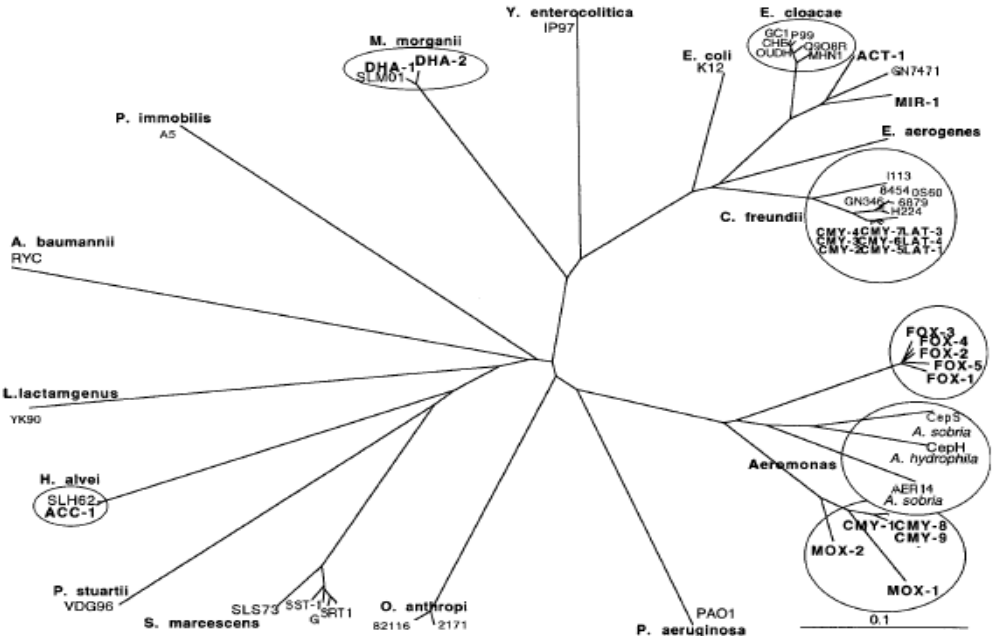

Figure 9. Dendrogram for chromosomal and plasmid-mediated AmpC ß-lactamases[146].

The plasmid-mediated AmpC ß-lactamases are closely related to chromosomal-AmpC ß-lactamases (Figure 9). These enzymes are classified into six families, base on amino acid sequences including MOX, CIT, DHA, ACC, EBC and FOX [146].The MOX-type ß-lactamases included CMY-1, CMY-8 to CMY-11, CMY-19,MOX-1 to MOX-8 [146, 148, 149]. The CIT-type ß-lactamases, included LAT-1, CMY-2 to CMY-7, CMY-12 to CMY-18, and CMY-20 to CMY-52. The DHA-type ß-lactamases included DHA-1 and DHA-2 [150]. The ACC-type ß-lactamases included ACC-1 to ACC-4[151]. The EBC-type ß-lactamases included ACT-1 to ACT-8 and MIR-1 to MIR-5. The FOX-type ß-lactamases included FOX-1 to FOX-7 [148].

The plasmid-mediated AmpC ß-lactamases were reported in member of Enterobacteriaceae. In 2007, of 3217 Enterobacteriaceae isolated from Switzerland, 17(0.5%) had plasmid-mediated AmpC ß-lactamases including CMY-2, DHA-1and CMY-31. The isolates were *E. coli, K. pneumoniae* and *P. mirabilis* [152]. In 2011, of 2129 Enterobacteriaceae isolated from Switzeland, 24(1.1%) had plasmid-mediated AmpC ß-lactamases including CIT(91.7%) and DHA(8.3%)[153]. In Algeria, 2.18% of Enterobacteriaceae isolates had plasmid-mediated AmpC ß-lactamases, including CMY-2(72.7%) and DHA-1(27.3%).These isolates were *E. coli, P. Stuartii, K. pneumoniae, S.marcescens, P.mirabilis and E. cloacae* [154].In Thailand, of 140 ESBL gene-carrying isolates in 2011, 96 (68.6%) carried plasmid-mediated AmpC ß-lactamases including EBC (75%), CIT (21.9%), ACC (13.5%), FOX (9.4%), DHA (5.2%) and MOX (1%). These isolates included *C. freundii, E. aerogenes, E. cloacae , P. vulgaris* and *Salmonella* spp.[145].

CHAPTER IV

MATERIALS AND METHODS

Methodology Scheme

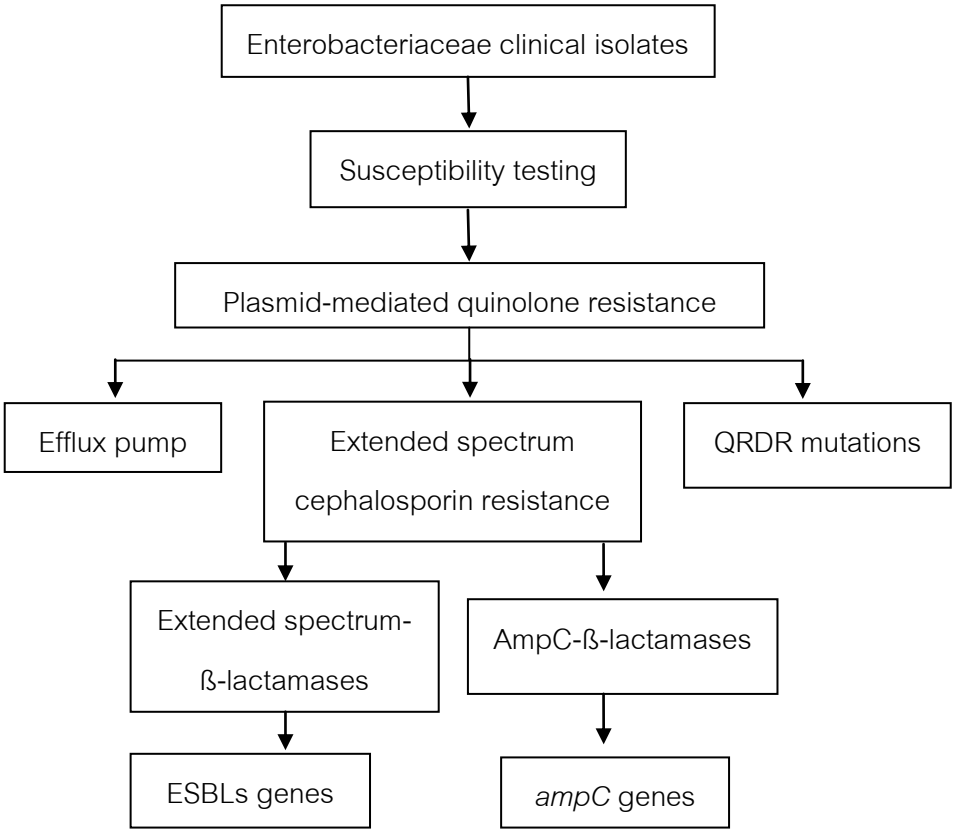

Figure 10. Methodology Scheme

PART I : BACTERIAL STRAINS

1. **Enterobacteriaceae isolates**

   A total of 674 non-replicated clinical isolates of Enterobacteriaceae collected between May 2009 and September 2011 from King Chulalongkorn Memorial Hospital, Bangkok, Thailand were included in this study.

2. **Quality control strains for MIC determination**

   Escherichia coli ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212 were used as control strains for MIC determination.

3. **Culture preservation**

   All Enterobacteriaceae isolates were cultured on trypticase soy agar (Oxiod, UK) at 37ºC for 18-24 hours. The fresh culture were collected in trypticase soy broth with 20% glycerol and stored at -80 ºC.

PART II : ANTIMICROBIAL SUSCEPTIBILITY TESTING

All Enterobacteriaceae isolates were determined for minimal inhibitory concentrations (MICs) of nalidixic acid, norfloxacin, ciprofloxacin, cefoxitin, ceftazidime, cefotaxime and ceftriaxone by agar dilution method and interpreted according to clinical and laboratory standard institute (CLSI 2011).

MICs were determined on Mueller-Hinton agar (BBL, Becton Dickinson and Company, Coskeysville, MD). Inoculum was prepared from a pure overnight culture in tryptic soy broth (BBL, Becton Dickinson and Company, Coskeysville, MD) and the turbidity was adjusted to a 0.5 McFarland standard in 0.85% NaCl. Then, the suspension was diluted 10-fold and the 1 ml of inoculum suspension was transferred to the multi-point inoculator wells which deliver 1-2 µl of inoculum suspension to agar dilution plates. The final inoculum was approximately 10^4 CFU/spot. The suspension was inoculated on Mueller-Hinton agar plates with two-fold dilution of antimicrobial agent at concentrations of 0.015625 to 256 µg/ml. The preparation for concentration of antimicrobial agents are shown in Table5. Plates were incubated at 35-37ºC for 18-24 hours. The MIC is the lowest concentration of antimicrobial agents that inhibit the growth of a microorganism (Figure 11).The MIC breakpoint criteria used in this study recommended by clinical and laboratory standard institute (CLSI) are shown in the Table 6. Accepted MIC determination for control strains are shown in the Table 7.

Table 5. Scheme for preparing dilutions of antimicrobial agents to be use in agar dilution susceptibility tests

	Antimicrobial solution					
Step	Conc. (µg/ml)	source	Volume (ml)	Diluent (ml)	Intermediate concentration (µg/ml)	Final conc. At 1:10 Dilution in agar (µg/ml)
	5120	stock	-	-	5120	512
1	5120	Stock	1.05	1.05	2560	256
2	5120	Stock	0.6	1.8	1280	128
3	5120	stock	0.6	4.2	640	64
4	640	Step 3	1.05	1.05	320	32
5	640	Step 3	0.6	1.8	160	16
6	640	Step 3	0.6	4.2	80	8
7	80	Step 6	1.05	1.05	40	4
8	80	Step 6	0.6	1.8	20	2
9	80	Step 6	0.6	4.2	10	1
10	10	Step 9	1.05	1.05	5	0.5
11	10	Step 9	0.6	1.8	2.5	0.25
12	10	Step 9	0.6	4.2	1.25	0.125
13	1.25	Step 12	1.05	1.05	0.625	0.0625
14	1.25	Step 12	0.6	1.8	0.3125	0.03125
15	1.25	Step 12	0.6	4.2	0.15625	0.015625

Note: This table is modified from Ericsson HM. Sherris JC. Antibiotic sensitivity testing. Report of an international collaborative study. (Acta Pathol Microbiol Scand.1971; 217 (suppl B): 1-98).

A B

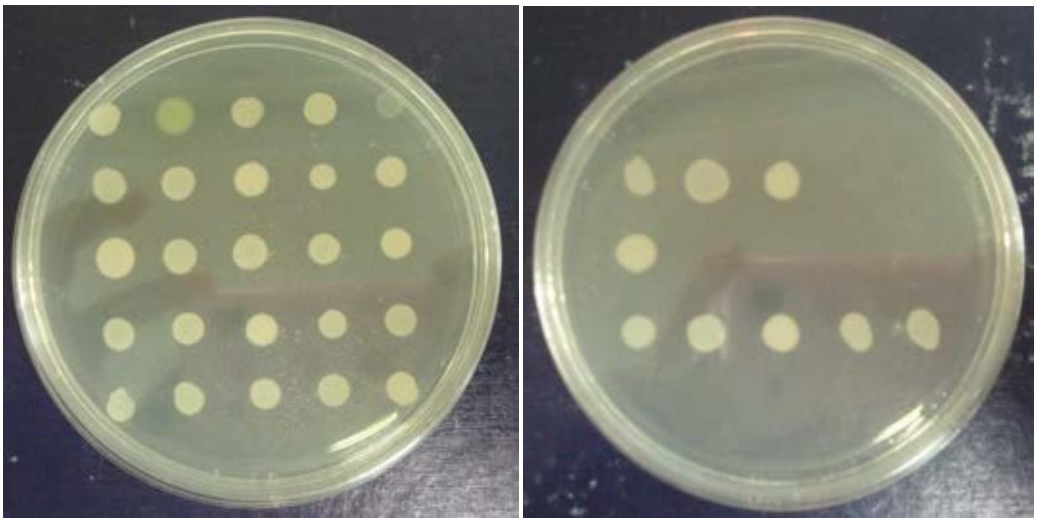

Figure 11. The inoculum plate by agar dilution method: A, control plate (no antibiotic); B, MIC plate with ciprofloxacin

Table 6. MIC interpretive standards for Enterobacteriaceae isolates

Antimicrobial agents	MIC interpretive(μg/ml)		
	Susceptible	Intermediate	Resistant
Nalidixic acid	≤ 16	-	≥ 32
Norfloxacin	≤ 4	8	≥16
Ciprofloxacin	≤ 1	2	≥ 4
Cefoxitin	≤ 8	16	≥ 32
Cefotaxime	≤ 1	2	≥ 4
Ceftazidime	≤ 4	8	≥ 16
Ceftriaxone	≤ 1	2	≥ 4

Table 7. Acceptable limits for quality control strains used to monitor accuracy of MICs

Antimicrobial agents	MIC (µg/ml)			
	Escherichia coli ATCC 25922	*Enterococcus faecalis* ATCC 29212	*Staphylococcus aureus* ATCC 29213	*Pseudomonas aeruginosa* ATCC 27853
Nalidixic acid	1-4	-	-	-
Norfloxacin	0.03 – 0.12	2 - 8	0.5 - 2	1 - 4
Ciprofloxacin	0.04 - 0.015	0.25 - 2	0.12 – 0.5	0.25 - 1
Cefoxitin	2 - 8	-	1 - 4	-
Ceftazidime	0.06 – 0.5	-	4 - 16	1 - 4
Cefotaxime	0.03 – 0.12	-	1 - 4	8 - 32
Ceftriaxone	0.03 – 0.12	-	1 - 8	8 - 64

PART III : SCREENING FOR PLASMID-MEDIATED QUINOLONE RESISTANCE GENES

A total of Enterobacteriaceae isolates were detected for PMQR genes by multiplex PCR.

1. DNA Extraction

Genomic DNA was prepared by boiling method. The 4-5 colonies of pure isolate in 200 µl of sterile distilled water were boiled for 10 min and centrifuged at 12,000 rpm for 5 min. The supernatant was used as the DNA template for PCR.

2. Primers

The presence of PMQR genes, including *qnrA, qnrB, qnrS, qnrC, qnrD, aac(6')-Ib* and *qepA* were screened by multiplex PCR using the primers as described in Table 8.

3. Amplification of *qnrA, qnrB, qnrS, qnrC* and *qnrD* genes by multiplex PCR

The primers specific for *qnrA, qnrB* and *qnrS* genes were based on those previously described by Kehrenberg *et al.* [96]. The primers specific for *qnrC* and *qnrD* were designed by this study. The PCR was performed in a final volume of 25 µl containing 1X buffer, 2 mM of $MgCl_2$, 0.2 mM of each deoxynucleotide triphosphates (dNTPs), 2 mM for qnrA-fw, qnrA-rv, qnrB-fw and qnrB-rv primers, 1 mM for qnrS-fw, qnrS-rv, qnrC-fw, qnrC-rv qnrD-fw and qnrD-rv primers, 0.1U of *Taq* polymerase (Fermentas, USA) and 2 µl of bacterial DNA template. The PCR conditions were 1 cycle of 94ºC for 3 min ; 30 cycles of 94ºC for 1 min, 56ºC for 1 min and 72ºC for 1 min ; and 1 cycle at 72ºC for 10 min.

Table 8 . Primers used for detection of PMQR genes

Gene	Primers	Sequence (5'-3')	Product size (bp)	Reference
qnrA	qnrA-fw	5'-TCAGCAAGAGGATTTCTCA-3'	627	[96]
	qnrA-rv	5'-GGCAGCACTATGACTCCCA-3'		
qnrB	qnrB-fw	5'-TCGGCTGTCAGTTCTATGATCG-3'	496	[96]
	qnrB-rv	5'-TCCATGAGCAACGATGCCT-3'		
qnrS	qnrS-fw	5'-TGATCTCACCTTCACCGCTTG-3'	566	[96]
	qnrS-rv	5'-GAATCAGTTCTTGCTGCCAGG-3'		
qnrC	qnrC-fw	5'-TTCCAAGGGGCAAACTGT-3'	277	This study
	qnrC-rv	5'-GCTCCCAAAAGTCATCAGAAA-3'		
qnrD	qnrD-fw	5'-TGTGATTTTTCAGGGGTTGA-3'	350	This study
	qnrD-rv	5'-GTGCCATTCCAGCGATTT-3'		
aac(6')-Ib	AAC-F	5'-GATCTCATATCGTCGAGTGGTGG-3'	435	[155]
	AAC-R	5'-GAACCATGTACACGGCTGGAC-3'		
qepA	Qep-F	5'-AACTGCTTGAGCCCGTAGAT-3'	198	[156]
	Qep-R	5'-CGT GTTGCTGGAGTTCTTCC-3'		

4. Amplification of *qepA* and *aac(6')-Ib* genes by multiplex PCR

The primers specific for *aac(6')-Ib* and *qepA* were those described by Kehrenberg *et. al* [155]. and Kang *et. al.* [156], respectively. The PCR was performed in a final volume of 25 µl, containing 1X buffer, 2 mM of $MgCl_2$, 0.2 mM of each deoxynucleotide triphosphates (dNTPs), 2 mM for AAC-F, AAC-R, Qep-F and Qep-R primers, 0.1U of *Taq* polymerase (Fermentas, USA) and 2 µl of bacterial DNA template. The PCR conditions were 1 cycle of 94ºC for 3 min ; 30 cycles of 94ºC for 1 min , 58ºC for 1 min , and 72ºC for 1 min ; and 1 cycle at 72ºC for 10 min.

5. Analysis of amplified DNA

The PCR products were analyzed on 1% agarose gel electrophoresis in 0.5X TBE buffer containing 0.5 µg/ml of ethidium bromide (Sigma, USA). PCR products were mixed with 6X of loading dye buffer. The electrophoresis was carried out at 100 volts for 50 minutes. The amplified products were visualized and photographed under UV light transilluminator. The PCR product sizes of *qnrA, qnrB, qnrS, qnrC, qnrD, aac(6')-Ib* and *qepA* were 627 bp, 496 bp, 566 bp, 277 bp, 350 bp, 435 bp and 198 bp, respectively.

6. Analysis of *aac(6')-Ib-cr*

The PCR products of *aac-6'-Ib* were were digested with BstCI (New England Biolabs) to identify *aac(6')-Ib-cr* [118]. The reaction mixture was performed in a final volume of 25 µl containing 1X buffer, 20 U of BstCI and 2 µl of PCR products. The mixture was incubated at 55ºC for 1 hr and analyzed on 1.5% agarose gel electrophoresis. PCR products encoding *aac(6')-Ib-cr* were not digested with enzyme which lacks the BtsCI restriction site. PCR products encoding *aac(6')-Ib* were digested by BstCI, producing two fragments of 254 and 181 bp.

PART IV : SCREENING FOR ESBL GENES

All PMQR positive isolates were detected for ESBL genes by multiplex PCR.

1. DNA Extraction

Genomic DNA was prepared by boiling method. The 4-5 colonies of pure isolate in 200 µl of sterile distilled water were boiled for 10 min and centrifuged at 12,000 rpm for 5 min. The supernatant was used as the DNA template for PCR

2. Primers

The presence of ESBL genes, including bla_{OXA}, bla_{TEM}, bla_{SHV}, bla_{CTX-M} and bla_{VEB} were screened by multiplex PCR using the primers as described in Table 9.

Table 9. Primers used for detection of ESBL genes

Specific for	Primers	Sequence (5'-3')	Product size (bp)	Reference
bla_{OXA}	OXA-F	5'-ATATCTCTAACTGTTGCATCTCC-3'	619	[157]
	OXA-R	5'-AAACCCTTCAAACCATCC-3'		
bla_{SHV}	SHV-F	5'-AGGATTGACTGCCTTTTTG-3'	392	[157]
	SHV-R	5'-ATTTGCTGATTTCGCTCG-3'		
bla_{TEM}	TEM-C	5'-ATCAGCAATAAACCAGC-3'	516	[158]
	TEM-H	5'-CCCCGAAGAACGTTTTC-3'		
bla_{CTX-M}	CTX-A	5'-CGCTTTGCGATGTGCAG-3'	550	[159]
	CTX-B	5'-ACCGCGATATCGTTGGT-3'		
bla_{VEB}	VEB-A	5'-CCTTTTGCCTAAAACGTGGA-3'	216	[160]
	VEB-B	5'-TGCATTTGTTCTTCGTTTGC-3'		

3. Amplification of *bla*$_{OXA}$, *bla*$_{SHV}$ and *bla*$_{TEM}$ by multiplex PCR

The primers specific for *bla*$_{OXA}$, *bla*$_{SHV}$ and *bla*$_{TEM}$ were based on those previously described by Colom *et al.* [157]and Mabilat *et al.* [158]. The PCR was performed in a final volume of 25 µl, containing 1X buffer, 2 mM of $MgCl_2$, 0.2 mM of each deoxynucleotide triphosphates (dNTPs), 0.06 mM of OXA-F and OXA-R primers, 0.04 mM of TEM-C and TEM-H primers, and 0.08 mM of SHV-F and SHV-R primers and 0.5 U *Taq* polymerase (Fermentas, USA), and 3 µL of DNA template. The PCR conditions were 1 cycle of 94ºC for 5 min ; 30 cycles of 94ºC for 30 sec , 54ºC for 30 sec , and 72ºC for 1 min ; and 1 cycle at 72ºC for 10 min.

4. Amplification of *bla*$_{CTX-M}$ and *bla*$_{VEB}$ by multiplex PCR

The primers specific for *bla*$_{CTX-M}$ and *bla*$_{VEB}$ were based on those previously described by Bonnet *et al.* [159] and Udomsantisuk *et al.* [160]. The PCR was performed in a final volume of 25 µl, containing 1X buffer, 2 mM of $MgCl_2$, 0.2 mM of each deoxynucleotide triphosphates (dNTPs), 0.1 mM of CTX-A and CTX-B primers, 0.05 mM of VEB-A and VEB-B primers and 0.5 U *Taq* polymerase (Fermentas, USA) and 3 µL of DNA template. The PCR conditions were 1 cycle of 94ºC for 5 min ; 30 cycles of 94ºC for 30 sec , 54ºC for 30 sec , and 72ºC for 1 min ; and 1 cycle at 72ºC for 10 min.

5. Analysis of amplified DNA

The PCR products were analyzed on 1% agarose gel electrophoresis in 0.5X TBE buffer containing 0.5 µg/ml of ethidium bromide (Sigma, USA). PCR products were mixed with 6X of loading dye buffer. The electrophoresis was carried out at 100 volts for 50 minutes. The amplified products were visualized and photographed under UV light transilluminator. The PCR product sizes of *bla*$_{OXA}$, *bla*$_{SHV}$, *bla*$_{TEM}$, *bla*$_{CTX-M}$ and *bla*$_{VEB}$ were 619 bp, 392 bp, 516 bp, 550 bp and 216 bp, respectively.

PART V : SCREENING FOR AMPC GENES

All PMQR positive isolates were detected for plasmid-mediated *amp*C genes by multiplex PCR.

1. DNA Extraction

Genomic DNA was prepared by boiling method. The 4-5 colonies of pure isolate in 200 µl of sterile distilled water were boiled for 10 min and centrifuged at 12,000 rpm for 5 min. The supernatant was used as the DNA template for PCR

2. Primers

The presence of plasmid-mediated *amp*C genes, including bla_{MOX}, bla_{CIT}, bla_{DHA}, bla_{EBC}, bla_{ACC} and bla_{FOX} were screened by multiplex PCR using the primers as described in Table 10.

3. Amplification of plasmid *amp*C genes by multiplex PCR

The primers specific for bla_{MOX}, bla_{CIT}, bla_{DHA}, bla_{EBC}, bla_{ACC} and bla_{FOX} genes were based on those previously described by Perez *et al*..[161] The PCR was performed in a final volume of 25 µl, containing 1X buffer, 2 mM of $MgCl_2$, 0.2 mM of each deoxynucleotide triphosphates (dNTPs), 0.8 mM for MOXMF, MOXMR, FOXMF and FOXMR primers, 0.6 mM for DHAMF and DHAMR primers, 0.5 mM for ACCMF and ACCMR primers, 0.4 mM for CITMF and CITMR primers, 0.3 mM for EBCMF and EBCMR primers, 1.25 U *Taq* polymerase (Fermentas, USA) and 2 µL of DNA template. The PCR conditions were 1 cycleof 94ºC for 3 min ; 25 cycles of 94ºC for 30 sec , 62ºC for 30 sec , and 72ºC for 1 min ; and 1 cycle at 72ºC for 7 min.

4. Analysis of amplified DNA

The PCR products were analyzed on 1.5% agarose gel electrophoresis in 0.5X TBE buffer containing 0.5 µg/ml of ethidium bromide (Sigma, USA). PCR products were mixed with 6X of loading dye buffer. The electrophoresis was carried out at 100 volts for 50 minutes. The amplified products were visualized and photographed under UV light transilluminator. The PCR product sizes of bla_{MOX}, bla_{CIT}, bla_{DHA}, bla_{EBC}, bla_{ACC} and bla_{FOX} were 520 bp, 462 bp, 405 bp, 302 bp, 346 bp, and 190 bp, respectively.

Table 10. Primers used for detection of plasmid *amp*C genes

Specific for	Primers	Sequence (5'-3')	Product size (bp)	Reference
MOX	MOXMF	5'-GCTGCTCAAGGAGCACAGGAT-3'	520	[161]
	MOXMR	5'-CACATTGACATAGGTGTGGTGC-3'		
CIT	CITMF	5'-TGGCCAGAACTGACAGGCAAA-3'	462	[161]
	CITMR	5'-TTTCTCCTGAACGTGGCTGGC-3'		
DHA	DHAMF	5'-AACTTTCACAGGTGTGCTGGGT-3'	405	[161]
	DHAMR	5'-CCGTACGCATACTGGCTTTGC-3'		
ACC	ACCMF	5'-AACAGCCTCAGCAGCCGGTTA-3'	346	[161]
	ACCMR	5'-TTCGCCGCAATCATCCCTAGC-3'		
EBC	EBCMF	5'-TCGGTAAAGCCGATGTTGCGG-3'	302	[161]
	EBCMR	5'-CTTCCACTGCGGCTGCCAGTT-3'		
FOX	FOXMF	5'-AACATGGGGTATCAGGGAGATG-3'	190	[161]
	FOXMR	5'-CAAAGCGCGTAACCGGATTGG-3'		

PART VI : DETECTION OF ESBLS AND AMPC ß-LACTAMASES

1. Detection of ESBL phenotype by combination disk test

All PMQR-positive isolates were investigated for ESBL phenotype, using the combination disk test. Adjusted bacterial suspension to 0.5 McFarland in 0.85% NaCl was inoculated on Mueller-Hinton agar plate by using a sterile swab.The disks containing 30 µg ceftazidime, 30 µg ceftazidime with 10 µg clavulanic acid and 30 µg cefotaxime 30 µg cefotaxime with 10 µg clavulanic acid (BBL, Becton Dickinson and Company, Coskeysville, MD) were placed on the same inoculated plate. Inhibition zones were measured after incubation at 35-37 ºC for 18-24 hours. Isolates that demonstrated the inhibition zone around the combination disk at least 5 mm larger than that of the cephalosporin alone were considered to have a positive for ESBL phenotype (Figure 12).

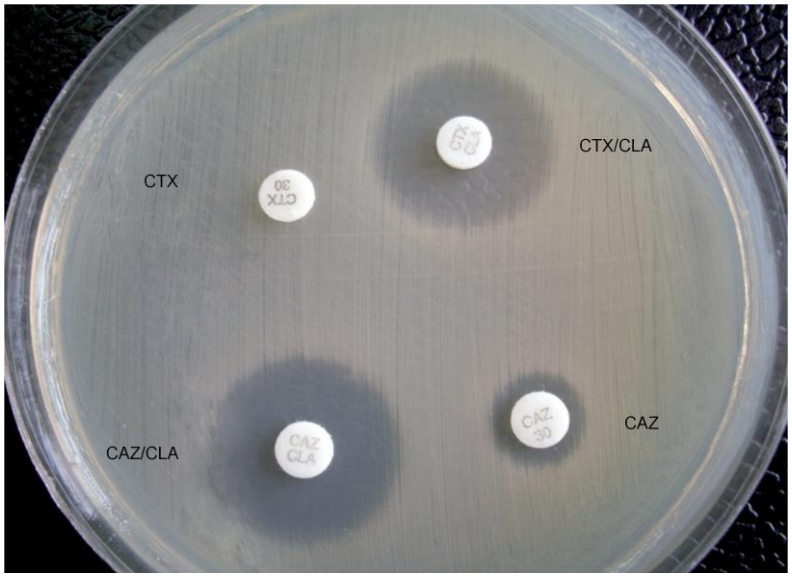

Figure 12. The combination disk test with clavulanic acid: cefotaxime (CTX), cefotaxime/ clavulanic acid (CTX/CLA), ceftazidime (CAZ),ceftazidime/clavulanic acid (CAZ/CLA)

2. Detection of AmpC ß-lactamase activity by modified Hodge test with cefoxitin

All PMQR-positive isolates were investigated for AmpC ß-lactamase activity by modified Hodge test. Suspension of cefoxitin-susceptible *E. coli* ATCC 25922, which was adjusted to 0.5 McFarland in 0.85% NaCl was spreaded on Mueller-Hinton agar plate. A 30 µg cefoxitin disk (BBL, Becton Dickinson and Company, Coskeysville, MD) was placed at the center of the plate. Two to three colonies of the overnight-cultured tested strains on tryptic soy agar were streaked outwards from the disk. The Mueller-Hinton agar plate was incubated at 37ºC for 18-24 hours. After 18 hours of incubation, the decreased radius of the inhibition zone along the growth of tested strain was considered a positive for modified Hodge test .(Figure 13)

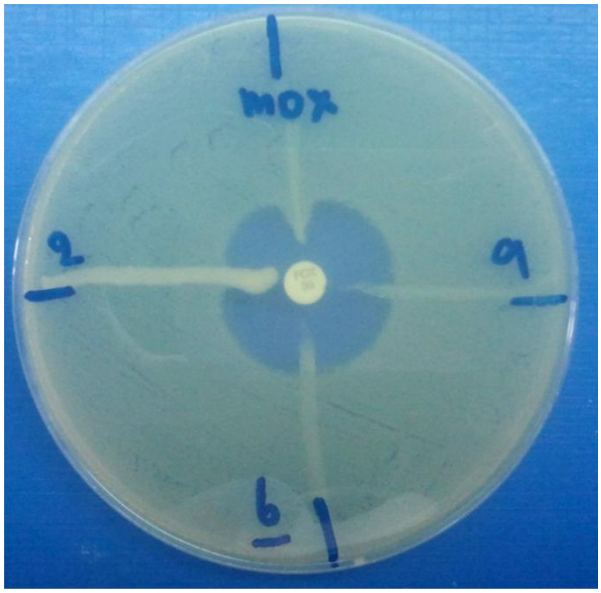

Figure 13 . Detection of AmpC ß-lactamases phenotype by modified Hodge test with 30 µg cefoxitin disk.

PART VII : DETECTION OF MUTAION IN QUINOLONE-RESISTANT DETERMINING REGION (QRDR) OF *GYRA* AND *PARC*

PMQR-positive isolates with lciprofloxacin resistance and reduced susceptibility to ciprofloxacin were investigated for the presence of *gyrA* and *parC* genes mutations.

1. DNA Extraction

Genomic DNA was prepared by boiling method. The 4-5 colonies of pure isolate in 200 µl of sterile distilled water were boiled for 10 min and centrifuged at 12,000 rpm for 5 min. The supernatant was used as the DNA template for PCR

2. Primers

The primers for the detection of *gyrA* and *parC* genes mutations were described in Table 11.

Table 11. Primers used for detection of *gyrA* and *parC* genes

Specific for	Primers	Sequence (5'-3')	Product size (bp)	Reference
gyrA	gyrA-F	5'-CGACCTTGCGAGAGAAAT-3'	626	[162]
	gyrA-R	5'-GTTCCATCAGCCCTTCAA-3'		
parC	parC-F	5'-AAGAAATCCGCCCGTACCGT-3'	150	[163]
	parC-R	5'-CGGTGCCCCCAGTTCCCCT-3'		

3. Amplification of *gyrA* and *parC* by PCR

The primers specific for *gyrA* and *parC* were based on those previously described by Weigel *et al.* [162] and Lascols *et al.* [163]. The PCR was performed in a final volume of 25 µl containing 1X buffer, 2 mM of $MgCl_2$, 0.2 mM of each deoxynucleotide triphosphates (dNTPs), 2 mM for forward and reverse primers and 0.5 U *Taq* polymerase (Fermentas, USA) and 3 µL of DNA template. The PCR conditions were 1 cycle of 94ºC for 5 min ; 30 cycles of 94ºC for 1 min , 52ºC for 1 min , and 72ºC for 1 min ; and 1 cycle at 72ºC for 10 min.

4. Analysis of amplified DNA

The PCR products were analyzed on 1% agarose gel electrophoresis in 0.5X TBE buffer containing 0.5 µg/ml of ethidium bromide (Sigma, USA). PCR products were mixed with 6X of loading dye buffer. The electrophoresis was carried out at 100 volts for 50 minutes. The amplified products were visualized and photographed under UV light transilluminator. The PCR product sizes of *gyrA* and *parC* were 626 bp and 150 bp, respectively.

5. **Purification of PCR Products**

The PCR products of *gyrA* and *parC* genes were purified by QIAquick PCR purification kit as described by the manufacturers (QIAGEN , Max-Volmer-StraBe4 , Hilden, Germany) Five volume of Buffer PBI were added into the 1 volume PCR products and mixed by vortexing. After that, the suspensions were placed into the 2 ml QIAquick column and centrifuged 13,000 rpm for 1 min. DNA was absorbed to the siliga-membrane in the presence of high salt while contaminants pass through the column. The filtrate was removed from the tube and 750 ml of PE buffer were added into the QIAquick column and centrifuged for 1 min. Flow-through was discarded and the QIAquick column was placed back in the same tube. The QIAquick columns were centrifuged for 1 min and placed the QIAquick column in a clean 1.5 ml microcentrifuge tube. The pure DNA was eluted with 30 µl of EB buffer (Elution buffer, 10mM Tris-Cl buffer, pH 8.5).The purified PCR products were stored at -20ºC.

6. **Preparation of sequencing reaction**

Automated sequencing was done at the Macrogen Inc. (Seoul, Korea). Sequencing was done by the chain termination method. Sequencing was conducted under BigDyeTM terminator cycling conditions. The reacted products were purified by ethanol precipitation and running using automatic sequencer, Applied Biosystems DNA sequencer model 3730xl (Rochester NY, USA)

7. **Sequence analysis**

The nucleotide and protein sequences were analyzed with the free software available over the internet at the national center for biotechnology information (http://www.ncbi.nlm.nih.gov/BLAST) and ExPASy (www.expasy.org/). Multiple sequence alignment of sequences was analyzed by Multilin (http://bioinfo.-genopoletoulouse.prd.fr/multalin/multalin.html).

PART VIII : DETECTION OF EFFLUX PUMP PHENOTYPE

Enterobacteriaceae isolates which carried *qepA*, a plasmid-mediated fluoroquinolone efflux pumps were detected for inhibitory effects of carbonyl cyanide *m*-chlorophenyl-hydrazone (CCCP), the efflux pump inhibitor. Susceptibility testing was carried out using agar dilution method. MIC of norfloxacin changes were observed in either the presence or the absence of CCCP (Sigma, USA) at a concentrations of 75 µM. Before test, all isolates were culture in presence and absence of 0.25 µg/ml of norfloxacin. The positive for efflux pump phenotype was detected by the decrease of norfloxacin MIC decreased at least 4-fold on the presenced of cccp compared with the MIC s in absence of CCCP.

PART IX : PLASMID ANALYSIS

Transfer of quinolone and/or cephalosporin resistance by conjugation method

Transconjugation experiments were performed by filter mating method to examine the ability to horizontally transfer quinolone resistance conferred by PMQR genes. Sodium azide-resistant *E. coli* (UB1637Az^{R}) was used as the recipient strain and MacConkey agar containing 150 µg/ml sodium azide, 3 µg/ml nalidixic acid and 2 µg/ml cefotaxime were used for selection of transconjugants.

The 4-5 colonies from donor and recipient were inoculated in 5 ml of TSB and incubated for 4 hr, at 37°C with shaking until OD 600 was 0.3 - 0.5. The donor and recipient was mixed in ratio 1 : 9 and collected on 0.45 µM sterilized filters. The filter was place on and incubated on Mueller-Hinton agar and incubated at 37°C. After 4-6 hr, the filters was suspended with 2 ml of TSB and spreaded on MacConkey agar containing 150 µg/ml sodium azide, 3 µg/ml nalidixic acid and 2 µg/ml cefotaxime for selection of transconjugants . Plates were incubated for 24-48 hr, at 37 °C. Single colony of transconjugants were picked and subculture on in plate were used for selection of transconjugants. After that, DNA of transconjugants was prepared by boiling method. Detection of PMQR genes in transconjugants was carried out by multiplex PCR. Susceptibility test on PMQR positive transconjugants was performed using agar dilution method.

CHAPTER V

RESULTS

PART I : BACTERIAL STRAINS

A total of 671 isolates of Enterobacteriaceae included 280 isolates of *E. coli* (41.73%), 147 isolates of *Klebsiella* spp.(21.91%), 94 isolates of *Salmonella* spp. (14.0%), 62 isolates of *Proteus* spp. (9.24%), 42 isolates of *Enterobacter* spp. (6.26%), 24 isolates of *Morganella morganii* (3.57%), 13 isolates of *Citrobacter* spp. (1.94%), 4 isolates of *Providencia rettgeri* (0.60%) and 5 isolates of *Serratia marcescens* (0.75%). All isolates collected between May 2009 and September 2011 from King Chulalongkorn Memorial Hospital. Each isolates were non-duplicate. One hundred and twenty (17.88%) isolates were from sterile sites including 85 (12.67%) from blood, 14 (2.09%) from body fluid, 12 (1.79%) from bile and 9 (1.34%) from tissue. Five hundred and fifty-one (82.12%) isolates from non-sterile sites included 314 (46.80%) from urine, 86 (12.82%) from stool, 67 (9.98%) from pus, 64 (9.54%) from sputum and 20 (2.98%) from other specimens, including tracheal secretion, rectal and cervical swab, respectively. A majority of Enterobacteriaceae isolates in this study were obtained from urine samples which were 46.80% of all isolates. Table 12 shows type of clinical specimen of Enterobacteriaceae isolates.

Table 12. Types of clinical specimen of 671 Enterobacteriaceae isolates

Source(n=671)	Specimen types	No. of isolates	%
Sterile sites (n=120)	Blood	85	12.67
	Body fluid	14	2.09
	Bile	12	1.79
	Tissue	9	1.34
Non- sterile sites(n=551)	Urine	314	46.80
	Stool	86	12.82
	Pus	67	9.98
	Sputum	64	9.54
	Other	20	2.98

PART II : ANTIMICROBIAL SUSCEPTIBILITY TESTING

The susceptibility of Enterobacteriaceae isolates to nalidixic, norfloxacin, ciprofloxacin, cefoxitin, ceftazidime, cefotaxime and ceftriaxone was determined by agar dilution method. The minimal inhibitory concentration (MIC) is the lowest concentration of antimicrobial agent able to inhibit the growth of an organism. The MIC_{50} and MIC_{90} are the lowest concentration of antimicrobial agents able to inhibit 50% and 90% of isolates tested, respectively. MIC breakpoints for resistance to nalidixic acid, norfloxacin and ciprofloxacin were $\geq$ 32, $\geq$ 16 and $\geq$ 4 µg/ml, respectively. MIC breakpoints for intermediate resistance to norfloxacin and ciprofloxacin were 8 and 2 µg/ml, respectively. MIC for ciprofloxacin between 0.125 and 1 µg/ml were classified as reduced susceptibility to ciprofloxacin. MIC breakpoints for resistance to ceftriaxone and cefotaxime were $\geq$ 4 µg/ml, while those of cefoxitin and ceftazidime were $\geq$32 and $\geq$ 16 µg/ml, respectively. MIC breakpoints for intermediate resistance to ceftriaxone and cefotaxime were 2 µg/ml, while those of cefoxitin and ceftazidime were 16 and 8 µg/ml, respectively. The results of susceptibility testing against Enterobacteriaceae isolates are summarized in Table 14.

Prevalence of nalidixic acid resistance was 47.10% (316/671). The results showed that 52.90% (355/671) of isolates were susceptible. The nalidixic acid MICs ranged from 0.125 to >256 µg/ml. MIC_{50} and MIC_{90} were 16 µg/ml and >256 µg/ml, respectively. Distribution of nalidixic acid MICs is shown in Figure 14. It was demonstrated that MICs of nalidixic acid-susceptible isolates ranged from 0.125 to 16 µg/ml.The MICs of nalidixic acid-susceptible isolates were peak at 4 µg/ml. Most nalidixic acid-resistant isolates had nalidixic acid MIC of >256 µg/ml (77.53%, 245/316).

Prevalence of norfloxacin resistance was 36.22% (243/671). The results showed that 61.10% (410/671) and 2.68% (18/671) of isolates were susceptible and intermediate resistant, respectively. The norfloxacin MICs ranged from 0.015 to >256 µg/ml. MIC_{50} and MIC_{90} were 2 µg/ml and >256 µg/ml, respectively. Distribution of norfloxacin MICs is shown in Figure 15. It was demonstrated that most norfloxacin-susceptible isolates had

norfloxacin MIC peak at 0.125 µg/ml. Most norfloxacin -resistant isolates had norfloxacin MIC of ≥256 µg/ml (65.84%, 160/243).

Prevalence of ciprofloxacin resistance was 37.71% (253/671). The results showed that 59.46% (399/671) and 2.83% (19/671) of isolates were susceptible and intermediate resistant, respectively. The results showed that 21.16% (142/671) of isolates had ciprofloxacin-reduced susceptibility. The ciprofloxacin MICs ranged from 0.015 to >256 µg/ml. MIC_{50} and MIC_{90} were 0.5 µg/ml and 128 µg/ml, respectively. Distribution of ciprofloxacin MICs is shown in Figure 16. It was demonstrated that most ciprofloxacin-susceptible isolates had ciprofloxacin MIC of 0.015 to 0.06 µg/ml (64.41%, 257/399). The majority of ciprofloxacin-resistant isolates had ciprofloxacin MIC of 32 to > 256 µg/ml (73.12%, 185/253).

Prevalence of cefoxitin resistance was 19.68% (132/671). The results showed that 62.44% (419/671) and 17.88% (120/671) of isolates were susceptible and intermediate resistant, respectively. The cefoxitin MICs ranged from 0.06 to >256 µg/ml. MIC_{50} and MIC_{90} were 8 µg/ml and 64 µg/ml, respectively. Distribution of cefoxitin MICs is shown in Figure 17. Normal distribution curve of cefoxitin MICs with peak at 8 µg/ml was demonstrated.

Prevalence of cefotaxime resistance was 46.20% (310/671). The results showed that 52.76% (354/671) and 1.04% (7/671) of isolates were susceptible and intermediate resistant, respectively. The cefotaxime MICs ranged from 0.015 to >256 µg/ml. MIC_{50} and MIC_{90} were 0.5 µg/ml and 256 µg/ml, respectively. Distribution of cefotaxime MICs is shown in Figure 18. It was demonstrated that most cefotaxime-susceptible isolates had cefotaxime MIC of 0.125 to 0.25 µg/ml (58.19%, 206/354). Most of cefotaxime-resistant isolates had cefotaxime MIC of 32 to >256 µg/ml (75.48%, 234/310).

Prevalence of ceftazidime resistance was 35.77% (240/671). The results showed that 61.10% (410/671) and 3.13% (21/671) of isolates were susceptible and intermediate resistant, respectively. The ceftazidime MICs ranged from 0.015 to >256 µg/ml. MIC_{50} and MIC_{90} were 1 µg/ml and 128 µg/ml, respectively. Distribution of ceftazidime MICs is shown in Figure 19. It was demonstrated that ceftazidime-susceptible isolates had the ceftazidime MIC peak at 0.5 µg/ml. Most ceftazidime-resistant isolates had ceftazidime MIC of 32 to >256 µg/ml (82.50%, 198/240).

Prevalence of ceftriaxone resistance was 44.11% (296/671). The results showed that 54.40% (365/671) and 1.49% (10/671) of isolates were susceptible and intermediate resistant, respectively. The ceftriaxone MICs ranged from 0.015 to >256 µg/ml. MIC_{50} and MIC_{90} were 0.5 µg/ml and 256 µg/ml, respectively. Distribution of ceftriaxone MICs is shown in Figure 20. It was demonstrated that ceftriaxone-susceptible isolates had ceftriaxone MIC peak at 0.125 µg/ml. Most of ceftriaxone-resistant isolates had ceftriaxone MIC of 32 to >256 µg/ml (77.36%, 229/296).

Table 13. The susceptibility of nalidixic acid, norfloxacin, ciprofloxacin, cefoxitin, ceftazidime, cefotaxime and ceftriaxone against 671 Enterobacteriaceae isolates

Antimicrobial agents	MIC (µg/ml)			%Susceptibility	%Intermediate	% Resistance	% Reduced susceptibility
	Range	MIC_{50}	MIC_{90}				
Nalidixic acid	0.125 - > 256	16	> 256	52.90	-	47.10	-
Norfloxacin	0.015 - > 256	2	> 256	61.10	2.68	36.22	-
Ciprofloxacin	0.015 - > 256	0.5	128	59.46	2.83	37.71	21.16
Cefoxitin	0.06 - > 256	8	64	62.44	17.88	19.68	-
Cefotaxime	0.015 - > 256	0.5	256	52.76	1.04	46.20	-
Ceftazidime	0.015 - > 256	1	128	61.10	3.13	35.77	-
Ceftriaxone	0.015 - ≥ 256	0.5	256	54.40	1.49	44.11	-

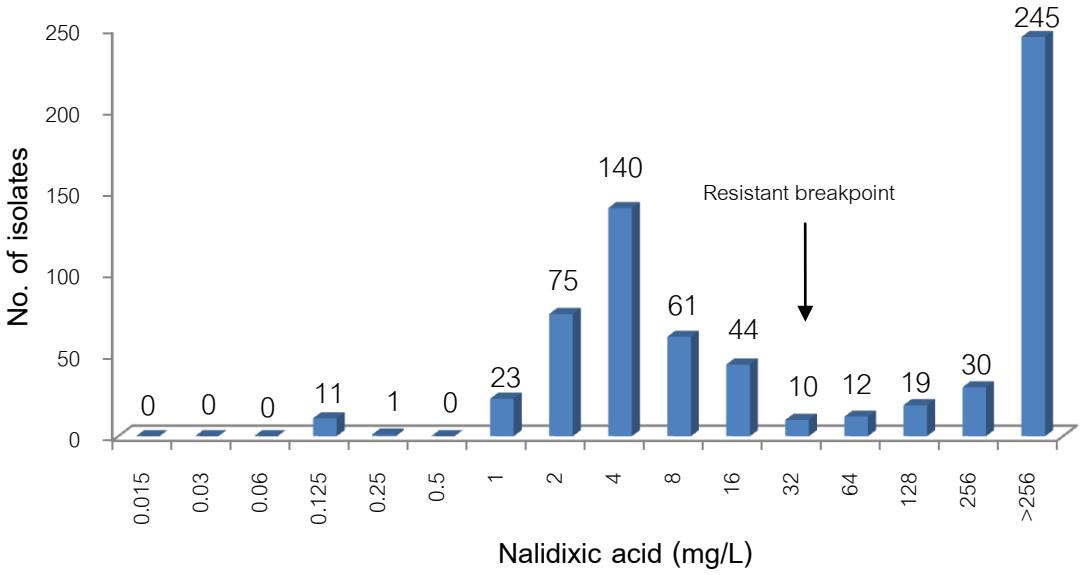

Figure 14. Distribution of nalidixic acid MICs among Enterobacteriaceae isolates

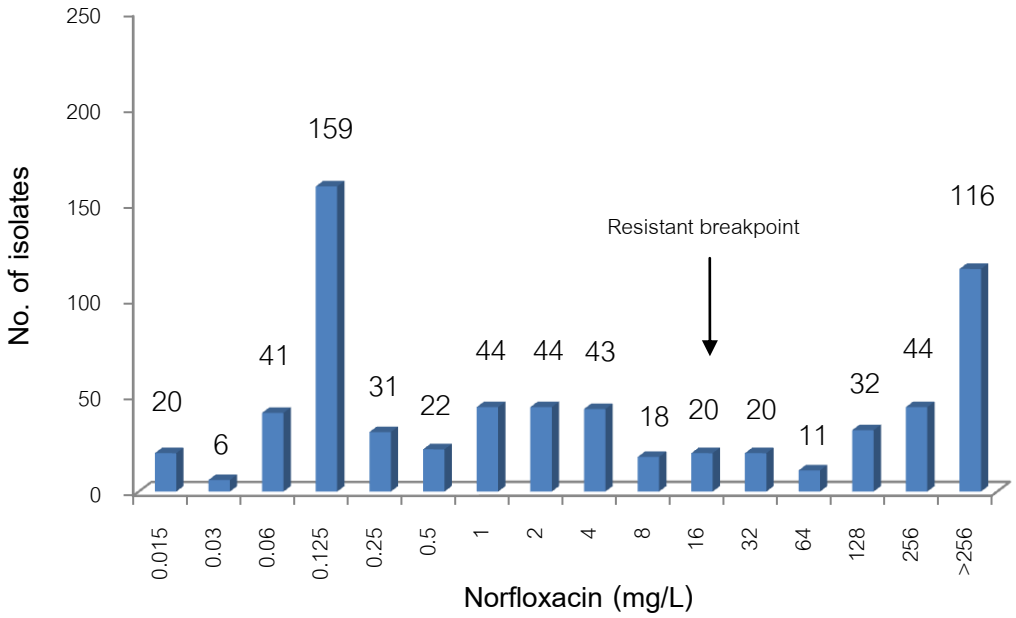

Figure 15 . Distribution of norfloxacin MICs among Enterobacteriaceae isolates

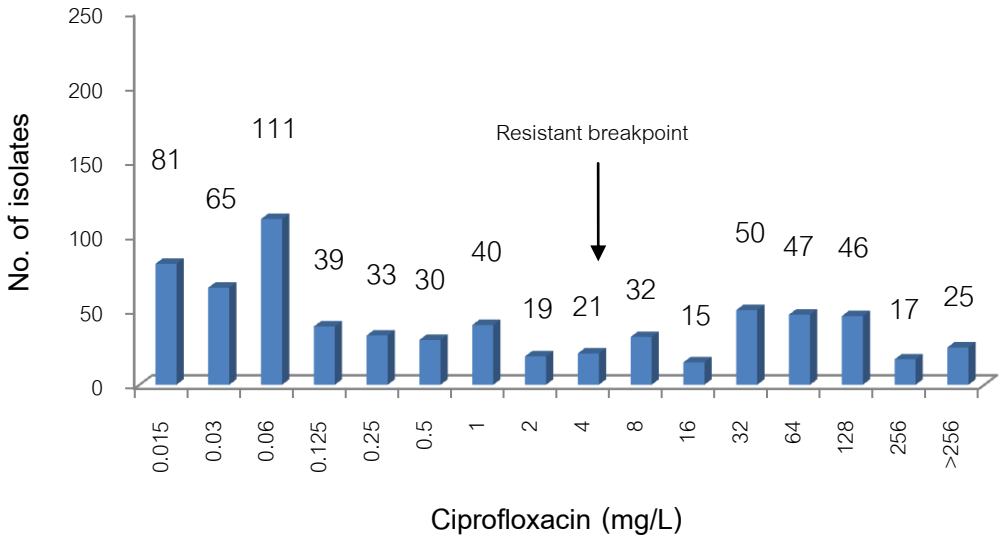

Figure 16 . Distribution of ciprofloxacin MICs among Enterobacteriaceae isolates

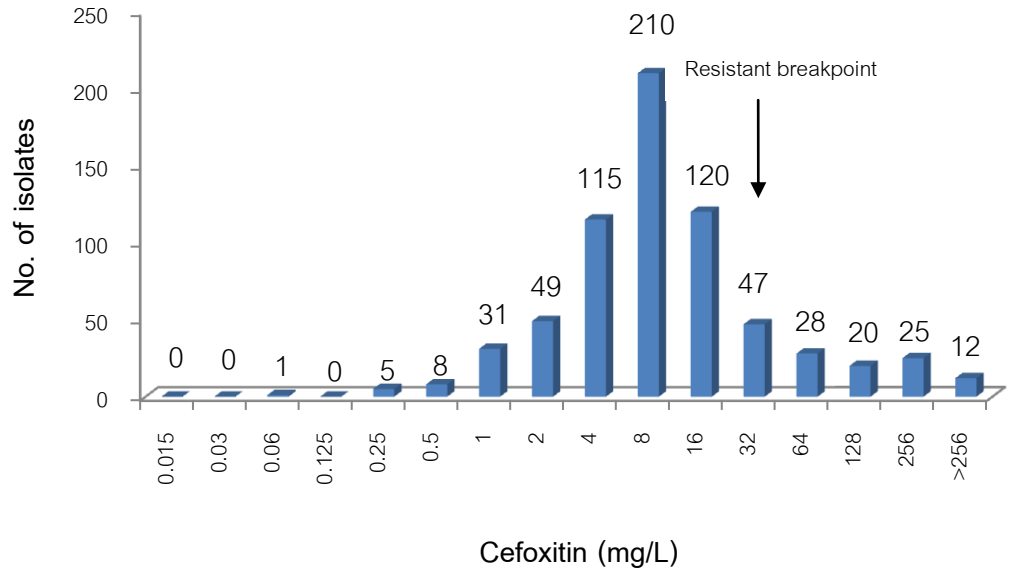

Figure 17 . Distribution of cefoxitin MICs among Enterobacteriaceae isolates

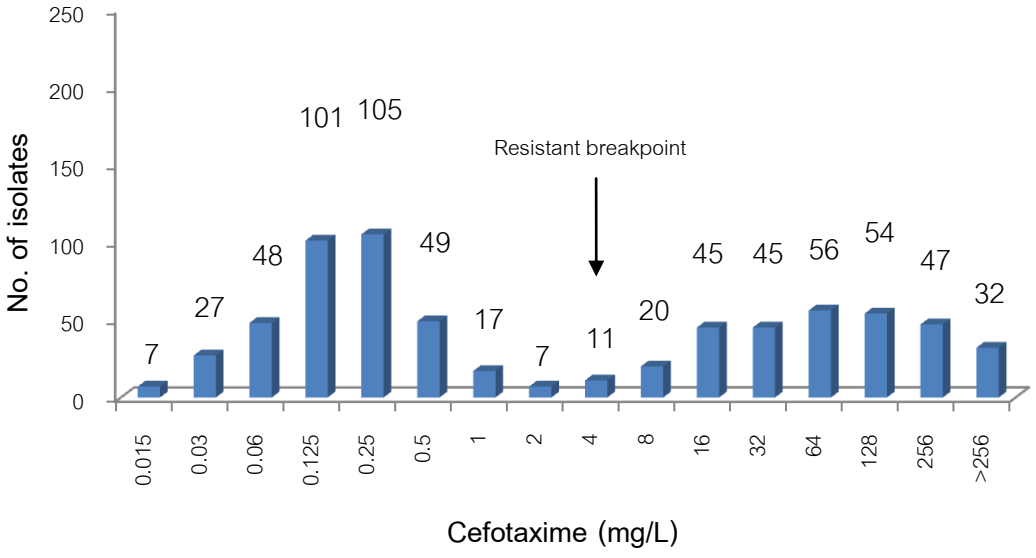

Figure 18. Distribution of cefotaxime MICs among Enterobacteriaceae isolates

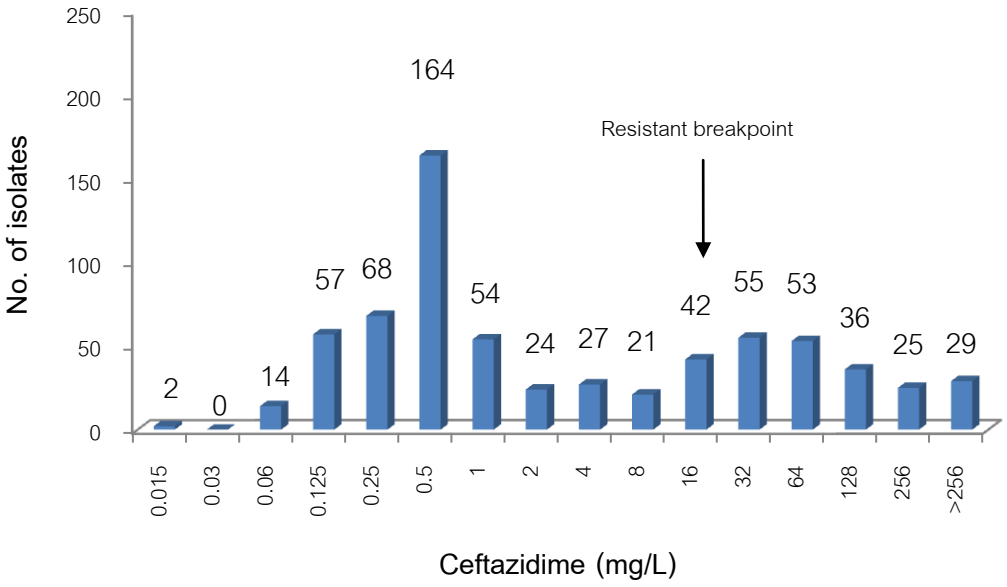

Figure 19 . Distribution of ceftazidime MICs among Enterobacteriaceae isolates

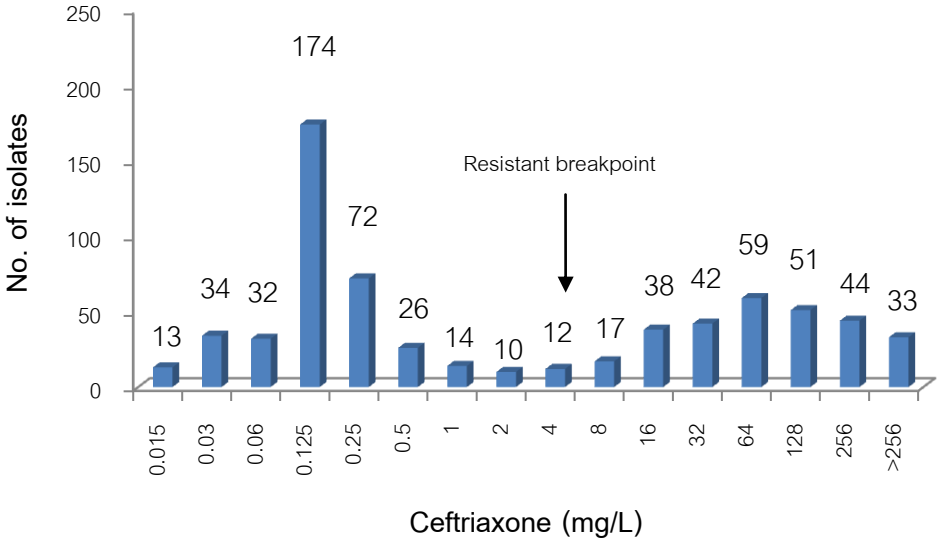

Figure 20. Distribution of ceftriaxone MICs among Enterobacteriaceae isolates

Table 14. The antibiotic susceptibility of 671 Enterobacteriaceae isolates from sterile and non-sterile sites

Type of specimen	No. isolates (%)	nalidixic acid(µg/ml)				norfloxacin(µg/ml)				ciprofloxacin(µg/ml)				
		MIC_{50}	MIC_{90}	range	%R	MIC_{50}	MIC_{90}	range	%R	MIC_{50}	MIC_{90}	range	%reduce	%R
Sterile sites(total = 120)		8	>256	0.125->256	40	1	>256	0.03->256	27.50	0.125	128	0.015->256	22.5	28.33
Blood	85(70.83)	8	>256	0.125->256	41.18	1	>256	0.03->256	29.41	0.125	128	0.015->256	20	29.41
Body fluid	14(11.67)	8	>256	2->256	28.57	2	16	0.06->256	14.29	1	8	0.015-64	50	21.43
Bile	12(10)	4	>256	2->256	41.67	0.125	256	0.06->256	33.33	0.06	128	0.03-256	8.33	33.33
Tissue	9(7.50)	4	>256	2->256	44.44	0.5	>256	0.06->256	22.22	0.06	>256	0.015-.256	22.22	22.22
Non-sterile sites(total = 551)		16	>256	0.125->256	48.64	2	>256	0.015->256	38.11	0.5	128	0.015->256	20.87	39.75
Urine	314(56.99)	>256	>256	0.125->256	61.47	32	>256	0.015->256	53.37	8	128	0.015->256	14.33	56.37
Stool	86(15.61)	4	256	1->256	26.74	0.125	4	0.015-4	0	0.06	1	0.015-2	34.88	0
Pus	67(12.16)	8	>256	0.125->256	37.31	2	>256	0.015->256	31.34	0.25	128	0.015->256	20.9	29.85
Sputum	64(11.62)	8	>256	1->256	26.56	1	128	0.015->256	21.88	0.125	32	0.015-256	28.13	26.56
Others	20(3.63)	8	>256	1->256	50	2	>256	0.015->256	30	0.125	128	0.015-128	40	25

Table 14. The antibiotic susceptibility of 671 Enterobacteriaceae isolates from sterile and non-sterile sites (cont.)

Type of specimen	No. isolates (%)	cefoxitin(µg/ml)				cefotaxime(µg/ml)				ceftazidime(µg/ml)				ceftriaxone(µg/ml)			
		MIC_{50}	MIC_{90}	range	%R	MIC_{50}	MIC_{90}	range	%R	MIC_{50}	MIC_{90}	range	%R	MIC_{50}	MIC_{90}	range	%R
Sterile sites(total = 120)		8	64	1->256	23.33	0.5	128	0.03->256	44.20	1	128	0.06->256	30.83	0.5	128	0.015->256	41.67
Blood	85(70.83)	8	64	1->256	22.35	0.5	128	0.03->256	42.35	1	64	0.125->256	29.40	0.25	128	0.03->256	41.18
Body fluid	14(11.67)	8	32	2-128	21.43	16	128	0.06-128	64.29	1	128	0.125-256	42.86	2	128	0.03-256	50
Bile	12(10)	8	256	4-256	25	0.25	256	0.125->256	41.67	0.5	128	0.125->256	33.33	1	256	0.125->256	41.67
Tissue	9(7.50)	16	128	4-128	33.33	0.25	64	0.03-64	33.33	0.5	256	0.06-256	22.22	0.125	64	0.015-64	33.33
Non-sterile sites(total = 551)		8	64	0.06->256	18.87	1	256	0.015->256	46.60	1	128	0.015->256	36.84	0.5	256	0.015->256	44.65
Urine	314(56.99)	8	64	0.5->256	21.34	8	256	0.015->256	53.20	4	256	0.015->256	41.72	4	256	0.015->256	51.91
Stool	86(15.61)	2	16	0.25->256	6.68	0.125	16	0.015->256	18.60	0.5	65	0.06->256	15.10	0.125	64	0.015->256	15.10
Pus	67(12.16)	8	128	0.5-256	25.37	8	256	0.03->256	52.24	2	128	0.06->256	43.28	2	256	0.015->256	47.80
Sputum	64(11.62)	8	64	0.06->256	18.75	0.5	128	0.015->256	48.44	1	128	0.015->256	37.50	0.5	256	0.015->256	48.40
Others	20(3.63)	8	16	2-256	10	0.5	64	0.03->256	40	1	64	0.125-256	30	0.25	64	0.03->256	35

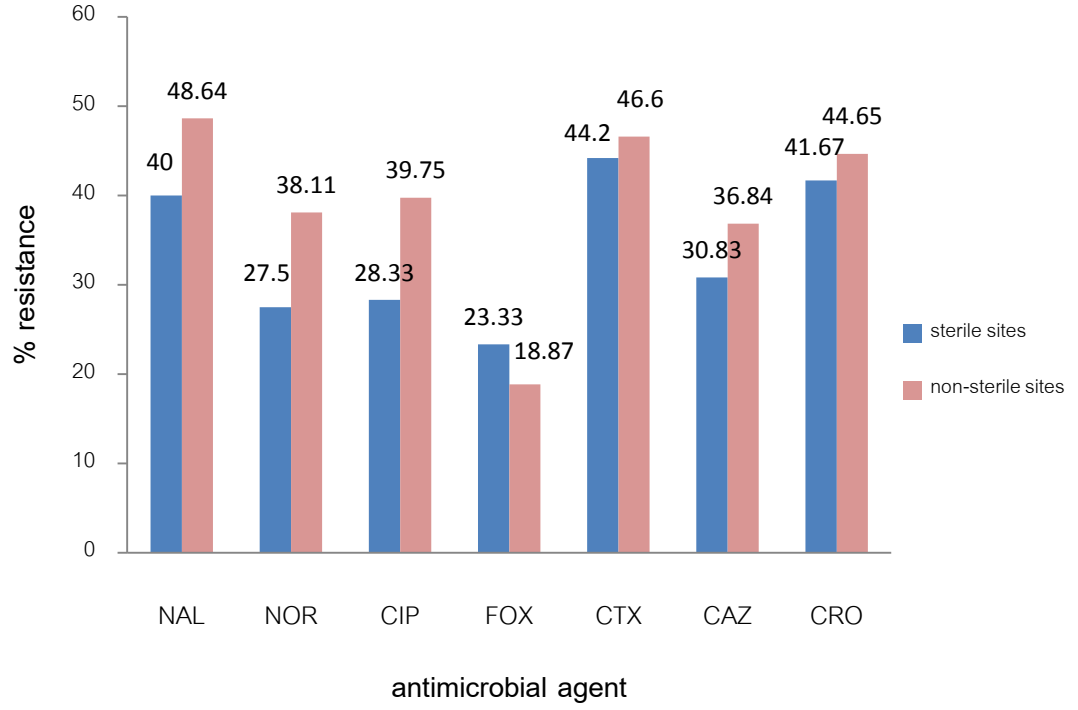

Figure 21. Comparion of antimicrobial resistance between isolates from sterile sites and non-sterile sites. NAL, nalidixic acid; NOR, norfloxacin; CIP, ciprofloxacin; FOX,cefoxitin; CTX, cefotaxime; CAZ, ceftazidime; CRO, ceftriaxone

The comparison between types of specimen and antibiotic susceptibility and resistance rates of isolates from sterile and non-sterile sites is shown in Table 14 and Figure 21. The results showed higher resistance rates to quinolones and third-generation cephalosporins, including nalidixic acid (48.64%), norfloxacin (38.11%), ciprofloxacin (39.75%), cefotaxime (46.6%), ceftazidime (36.84%) and ceftriaxone (44.65%) in isolates from non-sterile sites than those of sterile sites, whereas cefoxitin resistance was higher in isolates from sterile sites than non-sterile sites (Figure 21). There was no significant defference in antimicrobial resistance rates between isolates from sterile sites and non-sterile sites by using Chi-square test. Among isolates from sterile sites, body fluid isolates showed higher resistance rates to third-generation cephalosporins, including cefotaxime (64.29%), ceftazidime (42.86%) and ceftriaxone (50%) than isolates from other sterile sites, while bile isolates had higher rates of quinolone resistance including nalidixic acid (41.67%), norfloxacin (33.33%) and

ciprofloxacin (33.33%) than others. However, there was a small number of isolates from body fluid (n=14) and bile (n=12) included in the study. Blood isolates demonstrated high resistance to cefotaxime (42.35%), ceftriaxone (41.18%), ceftazidime (29.4%), nalidixic acid (41.18%), norfloxacin (29.41%) and ciprofloxacin (29.41%). Among non-sterile site sites, urine isolates demonstrated higher resistance rates to both quinolones and third-generation cephalosporins, including nalidixic acid (61.47%), norfloxacin (53.37%), ciprofloxacin (56.37%), cefotaxime (53.20%) and ceftriaxone (51.91%) than isolates from other non-sterile sites, whereas isolates from pus had higher resistance rates to cefoxitin (25.37%) and ceftazidime (43.28%) than others. The stool isolates showed the lowest resistance rates to fluoroquinolones (0%) and cephalosporins, including cefoxitin (6.68%), cefotaxime (18.60%), ceftazidime (15.10%) and ceftriaxone (15.10%). All these isolates were susceptible to ciprofloxacin but showed high rate of reduced susceptibility to ciprofloxacin (34.88%).

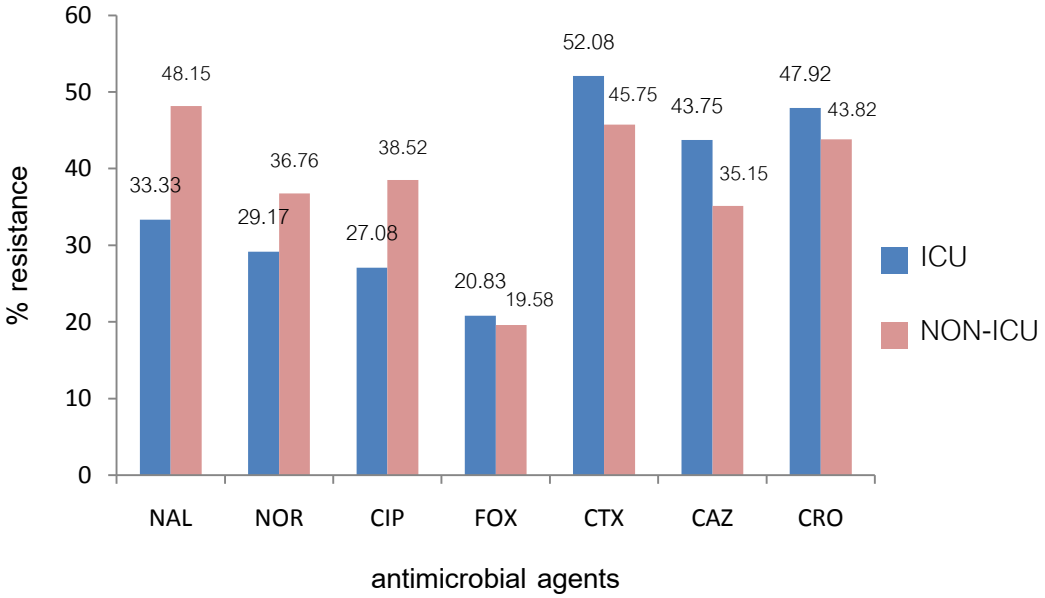

Figure 22. Comparison of antimicrobial resistance rates between isolates from ICUs and non-ICUs. NAL, nalidixic acid; NOR, norfloxacin; CIP, ciprofloxacin; FOX, cefoxitin; CTX, cefotaxime; CAZ, ceftazidime; CRO, ceftriaxone

Table 15. The antibiotic susceptibility of 671 Enterobacteriaceae isolates from ICUs and non-ICUs.

Ward	No. isolates (%)	% Resistance						
		NAL	NOR	CIP	FOX	CTX	CAZ	CRO
ICU(total=48)		33.33	29.17	27.08	20.83	52.08	43.75	47.92
Sterile sites	4(8.33)	0	0	0	50	50	50	50
Non- sterile sites	44(91.67)	36.36	31.82	29.55	18.18	52.27	43.18	47.73
non-ICU(total=623)		48.15	36.76	38.52	19.58	45.75	35.15	43.82
Sterile sites	116(18.62)	41.38	28.45	29.31	22.41	43.97	30.17	41.38
Non- sterile sites	507(81.38)	49.70	38.66	40.63	18.93	46.15	36.29	44.38

NAL, nalidixic acid; NOR, norfloxacin; CIP, ciprofloxacin; FOX, cefoxitin; CTX, cefotaxime; CAZ, ceftazidime; CRO, ceftriaxone

The comparison of quinolone and cephalosporin resistance rates between ICU and non-ICU isolates is shown in Table 15 and Figure 22. The isolates from non-ICUs showed higher rates of resistance to quinolones, including nalidixic acid (48.15%), norfloxacin (36.76%) and ciprofloxacin (38.52%) than those of ICU isolates. The isolates from ICUs showed higher rates of resistance to third-generation cephalosporins, including cefotaxime (52.08%), ceftazidime (43.75%) and ceftriaxone (47.92%) than those of non-ICU isolates. The cefoxitin resistance rate was similar among isolates from ICUs (20.83%) and non-ICUs (19.58%). There was no significant defference in antimicrobial resistance rates between isolates from ICUs and non-ICUs by using Chi-square test.

The non-sterile site isolates from ICU patients showed higher rates of resistance to cefotaxime (52.27%) and quinolones, including nalidixic acid (36.36%), norfloxacin (31.82%) and ciprofloxacin (29.55%) than those of isolates from sterile sites (0%). Ceftazidime and ceftriaxone resistance was more prevalent in sterile sites isolates than

non-sterile site isolates from ICU patients. Higher resistance rate of cefoxitin was found in isolates from sterile sites (50%) than that of isolates from non-sterile sites (18.18%).

The non-sterile site isolates from non-ICU patients had higher rates of resistance to quinolones and third-generation cephalosporins, including nalidixic acid (49.7%), norfloxacin (38.66%), ciprofloxacin (40.63%), cefotaxime(46.15%), ceftazidime(36.29%) and ceftriaxone (44.38%) than those of isolates from sterile sites. The sterile sites isolates showed higher resistance rate to cefoxitin (22.41%) than that of isolates from non-sterile sites(18.93%) in non-ICU patients.

PART III : SCREENING FOR PLASMID-MEDIATED QUINOLONE RESISTANCE GENES

A total of 671 isolates of Enterobacteriaceae were screened for *qnr* genes including *qnrA, qnrB, qnrS, qnrC, qnrD, aac(6')-Ib* and *qepA* by PCR. The PCR product sizes of *qnrA, qnrB, qnrS, qnrC, qnrD, aac(6')-Ib* and *qepA* were 627 bp, 496 bp, 566 bp, 277 bp, 350 bp, 435 bp and 198 bp, respectively. *aac(6')-Ib-cr* was identified by digestion of *aac(6')-Ib* PCR product with BtsCI. The PMQR genes were detected in 138 (20.57%) of Enterobacteriaceae isolates. Of the 671 Enterobacteriaceae isolates, 86(12.82%), 3 (0.45%) and 49 (7.30%) carried *qnr, qepA* and *aac(6')-Ib-cr*, respectively. All isolates had a single PMQR determinant. The prevalence of *qnrA, qnrS*, *qnrD*, *aac(6')-Ib-cr* and *qepA* were 2.38%, 8.05%, 2.38%, 7.30% and 0.45%, respectively. The *qnrB,* and *qnrC* genes were not detected in any isolates. The *qnrA* gene was commonly found in *Klebsiella* spp., followed by *E. coli*, *Enterobacter* spp., *Salmonella* spp. and *Citrobacter* spp.. The *qnrS* gene was the most prevalent PMQR determinant in our Enterobacteriaceae isolates and frequently identified in *Salmonella* spp., followed by *Klebsiella* spp., *E. coli*, *Enterobacter* spp. and *Citrobacter* spp.. The *qnrD* gene was commonly found in *Proteus* spp., followed by *Klebsiella* spp., *Enterobacter* spp. and *M. morganii.* The *aac(6')-Ib-cr* gene frequently identified in *E. coli*, followed by *Klebsiella* spp. and *Enterobacter* spp. All *qepA*-carrying isolates were *E. coli.* The prevalence of PMQR genes in 671 Enterobacteriaceae isolates are summarized in Table16.

Of the 280 *E. coli* isolates, *qnr* was found in 5.36%, including 1.79% for *qnrA* and 3.57% for *qnrS*, while 1.07% and 12.50% carried *qepA* and *aac(6')-Ib-cr*, respectively. The *aac(6')-Ib-cr* gene was the most common PMQR gene in *E. coli* isolates. Of the 147 *Klebsiella* spp. isolates, *qnr* was found in 17.69%, containing 4.76% for *qnrA*, 11.56% for *qnrS* and 1.36% for *qnrD*. The *aac(6')-Ib-cr* gene was found in 7.48%. The *qnrS* gene was the most prevalent PMQR determinant in *Klebsiella* spp. isolates. Of the 42 *Enterobacter* spp. isolates, *qnr* was detected in 23.81%, including 4.76% for *qnrA*, 16.67% for *qnrS* and 2.38% for *qnrD*, while 7.14% harbored *aac(6')-Ib-cr*. The *qnrS* gene was the most common PMQR determinant in *Enterobacter* spp.

isolates. Of the 94 *Salmonella* spp. isolates, *qnr* was found in 20.21%, containing 1.06% for *qnrA* and 19.15% for *qnrS*. The *qepA* and *aac(6')-Ib-cr* genes were not detected in any isolates. The *qnrS* gene was the most common PMQR genes in *Salmonella* spp. isolates. Of the 13 *Citrobacter* spp. isolates, *qnr* was detected in 23.08%, including 7.69% for *qnrA* and 15.38% for *qnrS*. The *qepA and aac(6')-Ib-cr* genes were not detected in any isolates. *Proteus* spp. and *M. morganii* carried only one PMQR gene which was *qnrD*. The prevalence of *qnrD* was 19.35% in *Proteus* spp. and 4.17% in *M. morganii*. PMQR genes were not detected in any isolates of *P. rettgeri* and *S. marcescens*.

Table 16. The prevalence of PMQR genes in 671 Enterobacteriaceae isolates

Bacterial species (n=671)	No. of isolates (%)							
	qnr						*qepA*	*aac(6')-Ib-cr*
	total	*qnrA*	*qnrB*	*qnrS*	*qnrC*	*qnrD*		
E. coli (n=280)	15 (5.36)	5 (1.79)	0	10 (3.57)	0	0	3 (1.07)	35 (12.50)
Klebsiella spp. (n=147)	26 (17.69)	7 (4.76)	0	17 (11.56)	0	2 (1.36)	0	11 (7.48)
Enterobacter spp. (n=42)	10 (23.81)	2 (4.76)	0	7 (16.67)	0	1 (2.38)	0	3 (7.14)
Salmonella spp. (n=94)	19 (20.21)	1 (1.06)	0	18 (19.15)	0	0	0	0
Proteus spp. (n=62)	12 (19.35)	0	0	0	0	12 (19.35)	0	0
M. morganii (n=24)	1 (4.17)	0	0	0	0	1 (4.17)	0	0
Citrobacter spp. (n=13)	3 (23.08)	1 (7.69)	0	2 (15.38)	0	0	0	0
P. rettgeri (n=4)	0	0	0	0	0	0	0	0
S. marcescens (n=5)	0	0	0	0	0	0	0	0
total (n=671)	86 (12.82)	16 (2.38)	0	54 (8.05)	0	16 (2.38)	3 (0.45)	49 (7.30)

Of the 138 PMQR-positive isolates, 54 (39.13%), 49 (35.51%), 16 (11.59%), 16 (11.59%) and 3 (2.17%) carried *qnrS, aac(6')-Ib-cr*, *qnrA*, *qnrD* and *qepA*, respectively (Table 17). The most prevalent PMQR gene was *qnrS*. The *qnrS* gene was the most common PMQR determinant in *Salmonella* spp. (94.74%), followed by *Citrobacter* spp. (66.67%), *Enterobacter* spp. (53.85%), *Klebsiella* spp. (45.95%) and *E. coli* (18.87%). The *qnrD* gene was most commonly detected in *Proteus* spp. (100%) and *M. morganii* (100%). The *aac(6')-Ib-cr* gene was the most prevalent PMQR determinant in *E. coli* isolates (66.04%).

Table 17. The prevalence of PMQR genes in 138 PMQR-positive isolates

Bacterial species	No. isolates (%)	*qnr*			*aac(6')-Ib-cr* (%)	*qepA*(%)
		qnrA (%)	*qnrS* (%)	*qnrD* (%)		
E. coli	53/138(38.41)	5 (9.43)	10 (18.87)	0	35 (66.04)	3 (5.66)
Klebsiella spp.	37/138 (26.81)	7(18.92)	17 (45.95)	2/37 (5.41)	11 (29.73)	0
Salmonella spp.	19/138 (13.77)	1 (5.26)	18 (94.74)	0	0	0
Proteus spp.	11/138 (7.97)	0	0	11/11 (100)	0	0
Enterobacter spp.	13/138 (9.42)	2 (15.38)	7 (53.85)	1/13 (7.69)	3(23.08)	0
M. morganii	2/138 (1.45)	0	0	2/2 (100)	0	0
Citrobacter spp.	3/138 (2.17)	1 (33.33)	2(66.67)	0	0	0
total (n=138)		16 (11.59)	54 (39.13)	16(11.59)	49 (35.51)	3 (2.17)

The susceptibility to nalidixic acid, norfloxacin and ciprofloxacin against PMQR-positive isolates is shown in Table 18. Of the 138 PMQR-positive isolates, the prevalence of nalidixic acid, norfloxacin and ciprofloxacin resistance in *qnrA*-positive isolates was 56.25% (9/16), 25% (4/16) and 31.25% (5/16), respectively. Reduced susceptibility to ciprofloxacin was found in 56.25% (9/16). Resistance to nalidixic acid, norfloxacin and ciprofloxacin in 54 *qnrS*-positive isolates was 42.59% (23/54), 22.22% (12/54) and

22.22% (12/54), respectively. The prevalence of reduced susceptibility to ciprofloxacin was 70.37% (38/54).

Of the 16 *qnrD*-positive isolates, the prevalence of nalidixic acid, norfloxacin and ciprofloxacin resistance were 75% (12/16), 68.75% (11/16) and 81.25% (13/16), respectively. Reduced susceptibility to ciprofloxacin was detected in 18.75% (3/16). All *qepA*-positive isolates were resistant to nalidixic acid, norfloxacin and ciprofloxacin. The prevalence of nalidixic acid, norfloxacin and ciprofloxacin resistance in isolates-carrying *aac(6')-Ib-cr* gene was 85.72% (42/49), 87.76% (43/49) and 89.80% (44/49), respectively. The prevalence of reduced susceptibility to ciprofloxacin was 6.12% (3/49).

Table 18. The susceptibility to nalidixic acid, norfloxacin and ciprofloxacin against 138 PMQR-positive isolates

Type of PMQR genes	Antimicrobial agents	No. of isolates (%)			
		Susceptible	Intermediate	Resistant	Reduced susceptible
qnrA(n=16)	nalidixic acid	7(43.75)	-	9 (56.25)	-
	norfloxacin	11 (68.75)	1 (6.25)	4 (25)	-
	ciprofloxacin	9 (56.25)	2 (12.5)	5 (31.25)	9 (56.25)
qnrS(n=54)	nalidixic acid	31 (57.41)	-	23 (42.59)	-
	norfloxacin	42 (77.78)	0	12 (22.22)	-
	ciprofloxacin	39 (77.22)	3 (5.56)	12 (22.22)	38 (70.37)
qnrD(n=16)	nalidixic acid	4 (25)	-	12 (75)	-
	norfloxacin	4 (25)	1 (6.25)	11 (68.75)	-
	ciprofloxacin	3 (18.75)	0	13 (81.25)	3 (18.75)
qepA(n=3)	nalidixic acid	0	-	3 (100)	-
	norfloxacin	0	0	3 (100)	-
	ciprofloxacin	0	0	3 (100)	0
aac(6')-Ib-cr	nalidixic acid	7 (14.29)	-	42 (85.72)	-
(n=49)	norfloxacin	4 (8.16)	2 (4.08)	43 (87.76)	-
	ciprofloxacin	3 (6.12)	2 (4.08)	44(89.80)	3(6.12)
Total PMQR	nalidixic acid	49 (35.50)	-	89 (64.49)	-
(n=138)	norfloxacin	61 (44.20)	4 (2.90)	73 (52.90)	-
	ciprofloxacin	54 (39.13)	7 (5.07)	77 (55.80)	53 (38.41)

Table 19. The antibiotic susceptibility among 671 Enterobacteriaceae isolates in PMQR-positive and PMQR-negative isolates

PMQR Type	No. isolates (%)	nalidixic acid(µg/ml)				norfloxacin(µg/ml)				ciprofloxacin(µg/ml)				
		MIC_{50}	MIC_{90}	range	%R	MIC_{50}	MIC_{90}	range	%R	MIC_{50}	MIC_{90}	range	%reduce	%R
Total (n=671)		16	>256	0.125->256	47.10	2	>256	0.015->256	36.22	0.5	128	0.015->256	21.16	37.71
PMQR-positive	138/671 (20.57)	256	>256	4->256	64.49	16	>256	0.25->256	52.90	8	>256	0.06->256	38.41	55.80
total qnr	86/671 (12.82)	32	>256	4->256	51.16	4	>256	0.25->256	31.40	1	>257	0.06->256	58.14	34.88
qnrA	16/671 (2.38)	32	>256	4->256	56.25	2	>256	0.25->256	25	1	128	0.125->256	56.25	31.25
qnrS	54/671 (8.05)	16	>256	4->256	42.60	2	256	0.25->256	22.22	1	256	0.06->256	70.37	22.22
qnrD	16/671 (2.38)	>256	>256	8->256	75	32	>256	0.25->256	68.75	8	>256	0.25->256	18.75	81.25
qepA	3/671 (0.45)	>256	>256	>256	100	>256	>256	>256	100	>256	>256	>256	0	100
aac(6')-Ib-cr	49/671 (7.3)	>256	>256	4->256	85.71	>256	>256	0.5->256	87.76	128	>256	0.125->256	6.12	89.80
PMQR-negative	533/671(79.43)	8	>256	0.125->256	42.59	0.5	>256	0.015->256	31.89	0.125	64	0.015->256	16.70	33.02

Table 19. The antibiotic susceptibility among 671 Enterobacteriaceae isolates in PMQR-positive and PMQR-negative isolates (cont.)

PMQR Type	No. isolates (%)	cefoxitin(µg/ml)				cefotaxime(µg/ml)				ceftazidime(µg/ml)				ceftriaxone(µg/ml)			
		MIC_{50}	MIC_{90}	range	%R	MIC_{50}	MIC_{90}	range	%R	MIC_{50}	MIC_{90}	range	%R	MIC_{50}	MIC_{90}	range	%R
Total(n=671)		8	64	0.06->256	19.68	8	64	0.015->256	46.21	1	128	0.015->256	35.77	0.5	256	0.015->256	44.11
PMQR-positive	138/671(20.57)	16	128	0.5->256	36.96	64	256	0.03->256	72.46	32	>256	0.06->256	62.32	64	>256	0.03->256	70.29
total qnr	86/671 (12.82)	16	256	0.5->256	45.35	16	128	0.03->256	56.98	4	256	0.06->256	44.19	8	256	0.03->256	53.49
qnrA	16/671 (2.38)	16	256	2->256	50	32	256	0.25-256	68.75	16	>256	0.5->256	56.25	32	256	0.125->256	68.75
qnrS	54/671 (8.05)	16	256	1->256	50	8	128	0.03->256	53.70	2	128	0.125->256	38.89	16	256	0.03->256	53.70
qnrD	16/671 (2.38)	4	64	0.5-64	25	16	64	0.06-128	56.25	4	256	0.06->256	50	0.25	128	0.03-128	37.50
qepA	3/671 (0.45)	32	32	4-32	66.67	128	256	128-256	100	64	64	32-64	100	128	256	128-256	100
aac(6')-Ib-cr	49/671 (7.3)	8	128	4->256	20.41	128	>256	0.25->256	97.96	64	>256	0.5->256	91.84	128	>256	0.125->256	97.96
PMQR-negative	533/671(79.43)	8	64	0.06->256	15.20	0.25	128	0.015->256	39.40	0.5	64	0.015->256	28.89	0.25	128	0.015->256	37.34

Table 20. The susceptibility test of quinolone in PMQR-positive and PMQR-negative isolates in 671 Enterobacteriaceae isolates

Bacterial species	No. isolates (%)	nalidixic acid(µg/ml)				norfloxacin(µg/ml)				ciprofloxacin(µg/ml)				
		MIC_{50}	MIC_{90}	range	%R	MIC_{50}	MIC_{90}	range	%R	MIC_{50}	MIC_{90}	range	%reduce	%R
PMQR positive (n=138)		256	>256	4->256	64.49	16	>256	0.25->256	52.9	8	>256	0.06->256	38.41	55.8
E. coli (n=53)	53/138(38.41)	>256	>256	4->256	68.79	>256	>256	1->256	83.02	128	>256	0.5->256	13.21	83.02
Klebsiella spp. (n=37)	37/138 (26.81)	16	>256	4->256	43.24	8	>256	0.25-.256	40.54	16	256	2->256	48.65	45.95
Salmonella spp. (n=19)	19/138 (13.77)	32	256	4->256	57.90	4	4	0.25-4	0	1	2	0.06-2	84.21	0
Proteus spp. (n=11)	11/138 (7.97)	>256	>256	8->256	81.82	32	>256	0.25->256	72.73	8	>256	0.25->256	9.09	90.91
Enterobacter spp. (n=13)	13/138 (9.42)	16	>256	4->256	38.46	2	256	1->256	30.77	1	64	0.25->256	61.54	30.77
M. morganii (n=2)	2/138 (1.45)	>256	>256	>256	100	>256	>256	>256	100	>256	>256	>256	0	100
Citrobacter spp. (n=3)	3/138 (2.17)	16	16	4-16	0	1	1	0.5-1	0	0.25	0.5	0.125-0.5	100	0
PMQR negative(n=533)		8	>256	0.125->256	42.59	0.5	>256	0.015->256	31.89	0.125	64	0.015->256	16.7	33.02
E. coli (n=227)	227/533(42.59)	>256	>256	0.125->256	64.76	64	>256	0.06->256	57.71	16	128	0.015->256	9.69	58.59
Klebsiella spp. (n=110)	110/533 (20.64)	4	>256	0.125->256	19.09	0.125	32	0.06->256	12.73	0.06	4	0.015->256	16.36	13.64
Salmonella spp. (n=75)	75/533 (14.07)	4	256	1->256	22.67	0.125	4	0.015-128	1.33	0.03	0.5	0.015-8	26.67	1.33
Proteus spp. (n=51)	51/533 (9.57)	8	>256	0.125->256	43.14	0.5	128	0.015->256	29.41	0.125	8	0.015->256	19.61	31.37
Enterobacter spp. (n=29)	29/533 (5.44)	4	>256	1->256	27.59	0.125	>256	0.015->256	13.79	0.06	32	0.015-256	24.14	13.79
M. morganii (n=22)	22/533 (4.13)	8	>256	1->256	50	2	>256	0.015->256	22.73	0.125	256	0.015->256	22.73	31.82
Citrobacter spp. (n=10)	10/533 (1.88)	4	8	1->256	10	0.125	0.5	0.015-2	0	0.015	0.125	0.015-0.5	30	0
P. rettgeri (n=4)	4/533 (0.75)	2	8	2-8	0	0.03	0.5	0.015-0.5	0	0.015	0.125	0.015-0.125	25	0
S. marcescens (n=5)	5/533 (0.94)	4	16	2-16	0	0.25	8	0.125-8	0	0.125	0.5	0.03-0.5	60	0

Table 21. The antibiotic susceptibility among 671 Enterobacteriaceae isolates from sterile/ non-sterile sites and ICUs/ non-ICUs.

Isolates	No. isolates (%)	% Resistance						
		NAL	NOR	CIP	FOX	CTX	CAZ	CRO
Sterile sites(total = 120)		40	27.50	28.33	23.33	44.20	30.83	41.67
PMQR-positive	27/120(22.50)	48.15	48.15	48.15	51.85	85.19	85.19	85.19
PMQR-negative	93/120(77.50)	37.63	21.51	22.58	15.05	32.26	18.28	29.03
Non-sterile sites(total = 551)		48.64	38.11	39.75	18.87	46.6	36.84	44.65
PMQR-positive	111/551(20.15)	68.47	54.05	57.66	33.33	69.37	59.46	66.67
PMQR-negative	440/551(79.85)	43.64	34.09	35.23	15.23	40.91	31.14	39.09
ICU(total=48)		33.33	29.17	27.08	20.83	52.08	43.75	47.92
PMQR-positive	11/48(22.92)	54.55	54.55	54.55	36.36	90.91	90.91	90.91
PMQR-negative	37/48(77.08)	27.03	21.62	18.92	16.22	40.54	29.73	35.14
non-ICU(total=623)		48.15	36.76	38.52	19.58	45.75	35.15	43.82
PMQR-positive	127/623(20.39)	65.35	52.76	55.91	37.01	70.87	59.84	68.50
PMQR-negative	496/623(15.41)	43.75	32.66	34.07	15.12	39.31	28.83	37.50

NAL, nalidixic acid; NOR, norfloxacin; CIP, ciprofloxacin; FOX, cefoxitin; CTX, cefotaxime; CAZ, ceftazidime; CRO, ceftriaxone

The comparison between quinolone and cephalosporin resistance rates in isolates with and without PMQR genes is shown in Table 19. The isolates carrying PMQR genes showed higher rates of resistance to quinolones and cephalosporins, including nalidixic acid (64.49%), norfloxacin (52.90%), ciprofloxacin (55.80%), cefoxitin (36.96%), cefotaxime (72.46%), ceftazidime (62.32%) and ceftriaxone (70.29%) than those of isolates without PMQR genes. $MICs_{90}$ of PMQR-positive isolates for quinolones and cephalosporins were similar/ or 2- to 4-fold higher than those of PMQR-negative isolates. However, $MICs_{50}$ for third-generation cephalosporins and quinolones of PMQR-positive isolates were 32- to 256-fold higher than those of PMQR-negative isolates. MIC_{50} for cefoxitin of PMQR-positive isolates was only 2-fold higher than that of PMQR-negative isolates. The *qnr*-carrying isolates showed resistance rates of nalidixic acid (51.16%),

norfloxacin (31.40%) and ciprofloxacin (34.88%) less than those of *qepA*-carrying isolates (100% for all quinolones) and *aac(6')-Ib-cr*-carrying isolates (85.71% for nalidixic acid, 87.76% for norfloxacin and 89.80% for ciprofloxacin). PMQR-carrying isolates either from sterile/non-sterile sites or from ICUs/non-ICUs showed higher rates of resistance to quinolones and cephalosporins than those of isolates without PMQR genes (Table. 21).

The comparison of quinolone resistance rates and the presence or absence of PMQR genes among bacterial species is shown in Table 20. The isolates of *E. coli*, *Klebsiella* spp., *Proteus* spp. and *Enterobacter* spp. habouring PMQR genes showed higher rates of resistance to quinolones, including nalidixic acid, norfloxacin and ciprofloxacin than those of isolates without PMQR genes. *Salmonella* isolates carrying PMQR genes showed high resistance rate to nalidixic acid (57.9%)but were susceptible to norfloxacin and ciprofloxacin. However, high rate of reduced susceptibility to ciprofloxacin was found in 84.21%. All PMQR-positive *M. morganii* isolates (n=2) were resistant to all quinolones. High rates of resistance to quinolones were found in PMQR-positive isolates of *E. coli* and *Proteus* spp..

PART IV : DETECTION OF ESBLS AND AMPC-ß-LACTAMASES

A total of 138 Enterobacteriaceae isolates carrying plasmid-mediated quinolone resistance were screened for ESBL phenotype by combination disc. Ninety-two isolates showed ESBL phenotype (66.67%). All ESBL-positive isolates had ceftazidime, cefotaxime and ceftriaxone MICs ranged from 2 to >256, 1 to >256 and 2 to >256 µg/ml, respectively. MIC_{50} of these isolates for ceftazidime, cefotaxime and ceftriaxone were 128, 64 and 128 µg/ml, respectively. MIC_{90} for ceftazidime, cefotaxime and ceftriaxone were >256 µg/ml. Of the 138 PMQR-positive, ESBL genes detected by multiplex PCR. ESBL genes, including, bla_{OXA}, bla_{TEM}, bla_{SHV}, bla_{CTX-M}, and bla_{VEB} were found in 108 isolates (78.26%). Sixteen of these isolates carried only bla_{TEM} and had no ESBL activity. The bla_{TEM} was found in these isolates may encode TEM-1 that does not exhibit the ESBL phenotype and were susceptible to ceftazidime, cefotaxime and ceftriaxone. Therefore, a total of 92 ESBL-producing isolates were further investigated for the co-existance of ESBL and PMQR determinants.

All 138 Enterobacteriaceae isolates carrying PMQR genes were screened for AmpC phenotype by modified Hodge test and were detected for plasmid-mediated *amp*C genes by multiplex PCR. Fourteen isolates (10.14%) carried plasmid-mediated *amp*C genes, including bla_{CIT}, bla_{DHA} and bla_{EBC}. The bla_{MOX}, bla_{ACC}, and bla_{FOX} genes were not detected. All 14 isolates also showed AmpC phenotype and had cefoxitin MIC ranging from 64 to >256 µg/ml.

The distribution of ESBL and *amp-C* genes in 138 PMQR-positive isolates is shown in table 22. The bla_{CTX-M} gene was the most common ESBL gene (71/130, 54.62%), followed by bla_{TEM} (35/130, 26.92%), bla_{SHV} (12/130,9.23%), bla_{VEB} (11/130, 8.46%) and bla_{OXA}(1/130,0.77%). The bla_{CIT} gene was the most common plasmid-mediated *amp*C gene (7/14, 50%), followed by bla_{DHA} (5/14, 35.71%) and bla_{EBC} (2/14,14.28%). The isolates harbouring only bla_{CTX-M} (43/138, 31.16%,) was most commonly found in PMQR-positive isolates, followed by the isolates carrying $bla_{CTX-M + TEM}$ (14/138,10.14%).

There were 70.29% (97/138) of PMQR-positive isolates carring ESBLand/or *ampC* genes. The resistance gene patterns of 138 PMQR-positive isolates with ESBL and/or *ampC* genes is showed in Table 23 and 24. Of the138 isolates, 60.14% (83/138) had ESBL gene, 3.62% (5/138) had *ampC* gene and 6.52% (9/138) had both ESBL and *ampC* genes. The *bla* $_{CTX-M}$ gene was the most common ESBL determinant (31.16%) in our PMQR-positive isolates. Fourty-one (29.71%) isolates carried only PMQR gene.

The distribution of PMQR, ESBL and *ampC* genes among 138 PMQR-positive isolates is showed in Table 22. The isolates harbouring only *qnrA* (5/16, 31.25%) were most common among *qnrA*-positive isolates, followed by the isolates carrying *bla*$_{CTX-M}$ (3/16, 18.75%) and *bla*$_{TEM}$ (2/16, 12.50%). One *qnrA*-positive isolates carried both ESBL and *ampC* genes, including *bla*$_{TEM + VEB +DHA}$. The isolates harbouring only *qnr*S (25/54, 46.30%) were most commonly found in *qnrS*-positive isolates, followed by the isolates carrying *bla*$_{TEM + CTX-M}$ (5/54, 9.30%), *bla*$_{TEM}$ (4/54, 7.41%), and *bla* $_{CTX-M}$(4/54, 7.41%). Four *qnr*S-positive isolates harbourd both ESBL and *ampC* genes. The resistance gene patterns were *bla*$_{DHA + TEM + CTX}$ (2/54, 3.70%), *bla*$_{CIT + TEM + CTX}$ (1/54, 1.85%) and *bla* $_{CIT + TEM + VEB}$ (1/54, 1.85%). Four *qnr*S-positive isolates carried *ampC* genes, including *bla*$_{CIT}$ (3/54, 5.56%) and *bla*$_{DHA}$(1/54, 1.85%). The isolates harbouring only *qnrD* (8/16, 50%) were most common among *qnrD*-positive isolates, followed by the isolates carrying *bla*$_{VEB}$(4/16, 25%). A *qnrD*-positive isolates had both ESBL and *ampC* genes which were *bla*$_{EBC + SHV}$. The *ampC* gene, *bla*$_{EBC}$, was found in one *qnrD*-positive isolates. All *qepA*-carrying isolates harboured ESBL genes (3/3, 100%). One qepA-positive isolate carried *bla*$_{CTX-M}$. Two *qnrA*-positive isolates harboured *bla*$_{CTX-M +TEM}$. The plasmid-mediated *amp*C genes were not found in any *qepA*-positive isolates. The isolates harbouring only *bla*$_{CTX-M}$ (35/49, 71.42%) were most commonly found in *aac(6')-Ib-cr* -positive isolates, followed by the isolates carrying *bla*$_{TEM + CTX-M}$ (6/49, 12.24%). Three *aac(6')-Ib-cr* -positive isolates carried both ESBL and *ampC* genes including *bla* $_{CIT+ CTX-M}$, *bla*$_{CIT +TEM + CTX-M}$ and *bla*$_{CIT +TEM+ CTX-M}$.

The *aac(6')-Ib-cr*-carrying isolates were commonly associated with ESBL genes (87.76%, 43/49). All 3 *qepA*-positive isolates harboured ESBL genes (bla_{CTX-M} and/or bla_{TEM}). The combination of PMQR, ESBL and *ampC* genes was found in 9 isolates (6.52%).The CTX-M + TEM +DHA pattern was found in three of nine isolates (33.33%).

Table 22 . The distribution of ESBL and *ampC* genes in 138 PMQR-positive isolates

ESBL and *ampC* genes	No. of isolates (%)	ESBL and *ampC* genes	No. of isolates (%)
CTX-M alone	43 (31.16%)	CTX-M + TEM + DHA	3 (2.17%)
TEM alone	8 (5.80%)	CTX-M + SHV	2 (1.45%)
SHV alone	2 (1.45%)	CTX-M + SHV + EBC	1 (0.72%)
VEB alone	4 (2.90%)	CTX-M + SHV + TEM	3 (2.17%)
DHA alone	1 (0.72%)	CTX-M + VEB	2 (1.45%)
CIT alone	3 (2.17%)	TEM + SHV	2 (1.45%)
EBC alone	1 (0.72%)	TEM + VEB + OXA	1 (0.72%)
CTX-M + CIT	1 (0.72%)	TEM + VEB + DHA	1 (0.72%)
CTX-M + TEM	14 (10.14%)	TEM + VEB + CIT	1 (0.72%)
CTX-M + TEM + CIT	2 (1.45%)	SHV + VEB	2 (1.45%)

Table 23. The resistance gene patterns of 138 PMQR-positive isolates with ESBL and/or *ampC* genes

PMQR type	Resistance determinants	No. of isolates (%)
Total *qnr* (n=86)	*qnr*S alone	38 (44.12%)
	*qnr*S + ESBL	37 (43.02%)
	*qnr*S + *ampC*	5 (5.81%)
	*qnr*S + ESBL + *ampC*	6 (6.98%)
qnrA (n=16)	*qnrA* alone	5 (31.25%)
	*qnr*A + ESBL	10 (62.50%)
	qnrA + ESBL + *ampC*	1 (6.25%)
qnrS (n=54)	*qnr*S alone	25 (46.30%)
	*qnr*S + ESBL	21 (38.89%)
	*qnr*S + *ampC*	4 (7.41%)
	qnrS + ESBL + *ampC*	4 (7.41%)
qnrD (n=16)	*qnrD* alone	8 (50%)
	qnrD + ESBL	6 (37.50%)
	qnrD + *ampC*	1 (6.25%)
	qnrD + ESBL + *ampC*	1 (6.25%)
q*epA* (n=3)	*qepA* + ESBL	3(100%)
aac(6')-Ib-cr (n=49)	*aac(6')-Ib-cr* alone	3 (6.12%)
	aac(6')-Ib-cr + ESBL	43 (87.76%)
	aac(6')-Ib-cr + ESBL + *ampC*	3 (6.12%)
Total (n=138)	PMQR alone	41 (29.71%)
	PMQR + ESBL	83 (60.14%)
	PMQR + ampC	5 (3.62%)
	PMQR + ESBL + *ampC*	9 (6.52%)

Table 24. Distribution of PMQR, ESBL and *ampC* genes among 138 PMQR-positive isolates

PMQR type	ESBL and AmpC pattern	N	PMQR type	ESBL and AmpC pattern	N
qnrS	-	25	*qnrA*	-	5
qnrS	CTX alone	4	*qnrA*	CTX alone	3
qnrS	CTX + VEB	1	*qnrA*	TEM alone	2
qnrS	SHV alone	1	*qnrA*	SHV alone	1
qnrS	SHV + CTX	1	*qnrA*	CTX + TEM	1
qnrS	SHV + TEM	2	*qnrA*	CTX + VEB	1
qnrS	SHV + TEM +CTX	2	*qnrA*	SHV +CTX	1
qnrS	SHV + VEB	1	*qnrA*	SHV+ VEB	1
qnrS	TEM alone	4	*qnrA*	DHA + VEB +TEM	1
qnrS	TEM + CTX	5	*aac(6')-Ib-cr*	-	3
qnrS	CIT alone	3	*aac(6')-Ib-cr*	CTX alone	35
qnrS	DHA alone	1	*aac(6')-Ib-cr*	TEM alone	2
qnrS	DHA + TEM + CTX	2	*aac(6')-Ib-cr*	TEM + CTX	6
qnrS	CIT + TEM + CTX	1	*aac(6')-Ib-cr*	CIT + CTX	1
qnrS	CIT + TEM + VEB	1	*aac(6')-Ib-cr*	CIT + TEM + CTX	1
qnrD	-	8	*aac(6')-Ib-cr*	DHA +TEM +CTX	1
qnrD	VEB alone	4	*qepA*	CTX alone	1
qnrD	SHV + TEM	1	*qepA*	CTX + TEM	2
qnrD	OXA + TEM +VEB	1			
qnrD	EBC alone	1			
qnrD	EBC +SHV	1			

PART V : DETECTION OF MUTATIONS IN QUINOLONE-RESISTANT DETERMINING REGION (QRDR) OF *GYRA* AND *PARC*

PMQR-positive isolates with ciprofloxacin resistance and reduced susceptibility to ciprofloxacin were investigated for the presence of *gyrA* and *parC* gene mutations. Twenty-three isolates were selected from Enterobacteriaceae carrying PMQR genes, including *qnrA* (5 isolates), *qnrS* (11 isolates), *qnrD* (1 isolates), *aac(6')-Ib-cr* (3 isolates) and *qepA* (3isolates). These isolates were classified into three groups, including reduced susceptibility, intermediate and resistance to ciprofloxacin. Twenty isolates had no amino acid substitutions in GyrA and ParC. These isolates had reduced susceptibility and intermediate resistance to ciprofloxacin. MICs ranges of nalidixic acid, norfloxacin and ciprofloxacin were 4 to 16 µg/ml, 0.5 to 8 µg/ml and 0.125 to 2 µg/ml, respectively (Table 25). Three isolates which were highly resistant to nalidixic acid, norfloxacin and ciprofloxacin (MICs of >256 µg/ml) had amino acid substitutions at S83L, D87N in GyrA and S80I in ParC . All these isolates harbored the *qepA* genes.

Table 25. Amino acid substitutions in QRDRs of GyrA and ParC and in 24 PMQR-positive isolates

Isolate No.	PMQR genes	amino acid substitutions			MIC(μg/ml)		
		GyrA		ParC	NAL	NOR	CIP
		S83	D87	S80			
L2EN-158	*qnrA*	-	-	-	8	1	0.125
L2EN-55	*qnrA*	-	-	-	16	1	0.5
L2EN-96	*qnrA*	-	-	-	8	2	1
L2EN-111	*qnrA*	-	-	-	16	2	1
L2EN-95	*qnrA*	-	-	-	16	2	1
L2EN-311	*qnrS*	-	-	-	8	1	0.25
L2EN-6	*qnrS*	-	-	-	4	1	0.5
L2EN-501	*qnrS*	-	-	-	8	2	0.5
L2EN-21	*qnrS*	-	-	-	16	1	0.5
L2EN-36	*qnrS*	-	-	-	16	1	0.5
L2EN-73	*qnrS*	-	-	-	16	2	0.5
L2EN-505	*qnrS*	-	-	-	16	2	0.5
L2EN-312	*qnrS*	-	-	-	8	4	0.5
L2EN-299	*qnrS*	-	-	-	8	1	1
L2EN-58	*qnrS*	-	-	-	8	2	1
L2EN-464	*qnrS*	-	-	-	32	4	2
L2EN-209	*qnrD*	-	-	-	8	2	0.25
L2EN-234	*aac(6')-Ib-cr*	-	-	-	4	0.5	0.125
L2EN-206	*aac(6')-Ib-cr*	-	-	-	16	4	0.5
L2EN-287	*aac(6')-Ib-cr*	-	-	-	16	8	2
L2EN-271	*qepA*	L	N	I	>256	>256	>256
L2EN-382	*qepA*	L	N	I	>256	>256	>256
L2EN-425	*qepA*	L	N	I	>256	>256	>256

PART VI : DETECTION OF EFFLUX PUMP PHENOTYPE

The activity of efflux pump was investigated by agar dilution with or without the proton pump inhibitor, carbonyl cyanide *m*-chlorophenylhydrazone (CCCP). The efflux pump activity was detected by the decrease of the MIC of norfloxacin at least 4-fold in the presence of CCCP compared with in the absence of CCCP. All 3 *qepA*-carrying isolates and four *qepA*-negative isolates with high level of norfloxacin resistance were tested for efflux pump activity. All isolates were cultured in presence and absence of 0.25 µg/ml of norfloxacin before detection of efflux pump phenotype. *Acinetobacter baumannii* Aci65 was used as control strains for efflux pump activity.

In the presence of CCCP, norfloxacin MICs were decreased for 8 to 32 fold in all *qepA*-carrying isolates. Four *qepA*-negative isolates had no change in norfloxacin MICs. Induction by 0.25 µg/ml norfloxacin before testing for efflux pump activity gave similar results. The results are summarized in Table 26. The efflux pump phenotype was found in all *qepA*-carrying isolates.

Table 26. The activity of efflux pump of *qepA*-positive and *qepA*-negative isolates.

Strains (No. of isolates)	Induction with norfloxacin	No. of fold decrease	MIC range (µg/ml)	
			Without CCCP	With CCCP
qepA-positive (n=3)	No	8-32	8,192-16,384	512-1,024
	Yes	8-16	8,192-16,384	1,024
qepA-negative (n=4)	No	-	256	256
	Yes	-	256	256
Control(Aci65)	No	8	512	64
	Yes	8	512	64

PART VII : PLASMID ANALYSIS

The horizontal transfer of quinolone resistance gene was examined by filter mating method. Sodium azide-resistant *E. coli* (UB1637Az^{R}) was used as the recipient strain and MacConkey agar containing 150 µg/ml sodium azide, 3 µg/ml nalidixic acid and/or 2 µg/ml cefotaxime were used for selection of transconjugants. PMQR-positive isolates were used as donors in conjugation experiment. *E. coli* (UB1637Az^{R}) demonstrated MIC of nalidixic acid, norfloxacin, ciprofloxacin, cefoxitin, ceftazidime, cefotaxime and ceftriaxone to be 2, 0.03, 0.015, 4, 0.25, 0.25 and 0.25 µg/ml, respectively.

Eighty-eight isolates were selected from Enterobacteriaceae isolates carrying PMQR genes, including *qnrA* (16 isolates), *qnrS* (30 isolates), *qnrD* (16 isolates), *aac(6')-Ib-cr* (23 isolates) and *qepA* (3 isolates). These isolates were investigated for the transfer of PMQR, ESBL and *ampC* genes to *E. coli* (UB1637Az^{R}) by conjugation method. Quinolone resistance and/or cephalosporin resistance were successfully transfered from 22 donor isolates (25%). The *qnr* genes were successfully transferred from 14 (63.64%) of the 22 donor isolates . The ESBL genes were successfully transferred from 20 of 22 donor isolates (90.91%) .The co-transfer of *qnr* and ESBL genes were successfully in 11 out of 22 donor isolates (50%). The co-transfer of *qnr*, ESBL and *ampC* genes were successfully in one donor isolate (4.55%). The *qnrA, qnrS*, bla_{CIT} and ESBL genes, including bla_{CTX-M}, bla_{TEM} and bla_{VEB} were successfully transferred by conjugation to *E. coli* (UB1637Az^{R}). The *qepA*, *aac(6')-Ib-cr* and *qnrD* were not successful in conjugation experiment.

The transconjugants carrying *qnr* had 4-fold increases in nalidixic acid MICs compared with recipient strain. These transconjugants showed reduced susceptibility to nalidixic, norfloxacin and ciprofloxacin. The transconjugants harbouring ESBL genes had 16- to 1024-fold increases in MICs of extended-spectrum cephalosporins compared with recipient strain. All transconjugants were resistant to cefotaxime, ceftazidime and ceftriaxone. The transconjugants carried both PMQR and ESBL genes had 4- to 1024-fold increases in extended-spectrum cephalosporin MICs and 2- to 32-fold increases in

nalidixic acid MICs compared with recipient strain. These transconjugants showed reduced susceptibility to nalidixic, norfloxacin and ciprofloxacin, and resistance to cefotaxime (90.91%), ceftazidime (72.73%) and ceftriaxone (90.91%). The transconjugants carried *qnr*, ESBL and *ampC* genes had 256- to 1024-fold increases in extended-spectrum cephalosporin MICs, 4-fold increases in nalidixic acid MICs and 64-fold increases in cefoxitin compared with recipient strain. The transconjugants showed reduced susceptibility to nalidixic acid, norfloxacin and ciprofloxacin, and resistance to extended-spectrum cephalosporins and cefoxitin (Table 27).

Table 27. Resistance gene and and MICs of quinolones and cephalosporin in 22 transconjugants (TC)

Resistance genes	No. of isolates (%)	No. of fold increases						
		NAL	NOR	CIP	FOX	CTX	CAZ	CRO
qnr alone	2 (9.09%)	4	8-16.67	2	-	-	-	-
ESBL alone	8 (36.36%)	-	-	-	-	128 - 1024	16-512	16-512
qnr + ESBL	11(50%)	2-32	4-66.67	2 - 8	-	8 – 512	32-1024	4- 1024
qnr + ESBL+*ampC*	1 (4.55%)	4	4	2	64	256	1024	256

NAL, nalidixic acid; NOR, norfloxacin; CIP, ciprofloxacin; FOX, cefoxitin; CAZ, ceftazidime; CTX, cefotaxime; CRO, ceftriaxone

The 16 *qnrA*-carrying isolates were tested for conjugation experiment. Only 6 isolates (37.50%) transferred *qnrA* and/or ESBL gene to UB1637Az^{R}. The *qnr* genes were successfully transferred from 4 of 6 (66.67%) *qnrA*-positive donors to UB1637Az^{R}.The four transconjugants named TC-55, TC-184, TC-158 and TC-358. These transconjugants had 2- to 8-fold increases in nalidixic acid MICs, 4- to 8-fold increases in norfloxacin MICs and 2- to 6-fold increases in ciprofloxacin MICs. The ESBL genes were successfully transferred from all 6 (100%) *qnrA*-positive donors to UB1637Az^{R}. The six transconjugants named TC-111, TC-218, TC-55, TC-184, TC-158

and TC-358. These transconjugants had 8- to 256-fold increases in cefotaxime MICs, 16- to 1024-fold increases in ceftazidime MICs and 4- to 256-fold increases ceftriaxone MICs . Furthermore, the *ampC* genes were successfully transferred from one (16.67%) *qnrA*-positive donor (L2EN-358) to UB1637Az^{R}. The transconjugants named TC-358. The MIC of cefoxitin increased from 4 to >256 µg/ml (64 folds) in TC-358 (Table 28).

Table 28. Resistance genes and MICs of quinolones and cephalosporins among 6 *qnrA*-positive donors and transconjugants

Isolate No.	PMQR determinants	ESBL or *ampC* ß-lactamase genes	MIC (µg/ml)						
			NAL	NOR	CIP	FOX	CTX	CAZ	CRO
UB^{AZ}	-	-	2	0.03	0.015	4	0.25	0.25	0.25
L2EN-111	*qnrA*	SHV, CTX	128	32	8	4	64	4	128
TC-111	-	CTX	4	0.06	0.03	4	64	4	128
L2EN-218	*qnrA*	CTX	256	8	4	8	> 256	64	> 256
TC-218	-	CTX	4	0.06	0.03	8	256	64	> 256
L2EN-55	*qnrA*	TEM	64	2	1	32	16	128	16
TC-55	*qnrA*	TEM	8	0.125	0.06	4	8	64	8
L2EN-184	*qnrA*	CTX, VEB	128	32	8	4	64	128	256
TC-184	*qnrA*	VEB	16	0.25	0.06	4	2	32	1
L2EN-158	*qnrA*	SHV, VEB	16	1	0.5	4	32	> 256	32
TC-158	*qnrA*	VEB	4	0.125	0.03	4	8	> 256	16
L2EN-358	*qnrA*	CIT, TEM, VEB	32	1	0.5	>256	128	256	64
TC-358	*qnrA*	CIT, VEB	8	0.125	0.03	> 256	64	256	64

NAL, nalidixic acid; NOR, norfloxacin; CIP, ciprofloxacin; FOX, cefoxitin; CAZ, ceftazidime; CTX, cefotaxime; CRO, ceftriaxone

The 30 *qnrS*-carrying isolates were selected for conjugation experiment. Only 13 (43.33%) of the total donor isolates transferred *qnrA* and/or ESBL genes. The *qnrS*

genes were successfully transferred from 10 of 13 (76.92%) *qnrS*-positive donors to UB1637Az^{R}. The ten transconjugants named TC-186 ,TC-288, TC-2, TC-51, TC-311, TC-9, TC-187, TC385, TC-487 and TC-501. These transconjugants had 4- to 32-fold increases in nalidixic acid MICs, 4- to 66.67-fold increases in norfloxacin MICs and 2- to 12-fold increases in ciprofloxacin MICs. The ESBL genes were successfully transferred from 11 of 13 (84.62%) *qnrS*-positive donors to UB1637Az^{R}. The eleven transconjugants named TC-349, TC-363, TC-595, TC-2, TC-51, TC-311, TC-9, TC-187, TC385, TC-487 and TC-501. These transconjugants had 32- to 1024-fold increases in cefotaxime MICs, 2- to 1024-fold increases in ceftazidime MICs, 16- to 1024- fold increases ceftriaxone MICs (Table 29).

Table 29. Resistance genes and MICs of quinolones and cephalosporins among 13 *qnrS*-positive donors and transconjugants

Isolate No.	PMQR determinants	ESBL or *ampC* ß- lactamase genes	MIC(µg/ml)						
			NAL	NOR	CIP	FOX	CTX	CAZ	CRO
UBaz	-	-	2	0.03	0.015	4	0.25	0.25	0.25
L2EN-349	*qnrS*	SHV, CTX	16	2	0.5	2	128	32	128
TC-349	0	CTX	2	0.03	0.01	2	128	32	16
L2EN-363	*qnrS*	TEM, VEB	16	1	0.25	32	128	256	32
TC-363	0	VEB	8	0.06	0.01	4	128	256	32
L2EN-595	*qnrS*	TEM, CTX	16	2	1	1	128	64	128
TC-595	0	TEM, CTX	2	0.015	0.015	4	32	16	128
L2EN-186	*qnrS*	SHV	> 256	> 256	256	32	32	16	16
TC-186	*qnrS*	0	8	0.5	0.03	4	0.125	0.5	0.125
L2EN-288	*qnrS*	TEM, CTX	> 256	> 256	64	32	> 256	256	> 256
TC-288	*qnrS*	0	8	0.25	0.03	2	0.125	0.25	0.125

Table 29. Resistance genes and MICs of quinolones and cephalosporins among 13 *qnrS*-positive donors and transconjugants (cont.)

Isolate No.	PMQR determinants	ESBL or *ampC* ß- lactamase genes	MIC(µg/ml)						
			NAL	NOR	CIP	FOX	CTX	CAZ	CRO
UBaz	-	-	2	0.03	0.015	4	0.25	0.25	0.25
L2EN-9	*qnrS*	CTX	32	1	0.5	4	> 256	64	128
TC-9	*qnrS*	CTX	8	1	0.125	4	8	16	128
L2EN-2	*qnrS*	DHA,TEM, CTX	> 256	> 256	256	256	256	128	256
TC-2	*qnrS*	CTX	8	0.5	0.125	4	64	16	32
L2EN-51	*qnrS*	CIT, TEM, CTX	> 256	> 256	256	256	128	128	256
TC-51	*qnrS*	CTX	16	0.5	0.06	4	128	8	256
L2EN-385	*qnrS*	CTX	16	1	0.25	4	128	16	128
TC-385	*qnrS*	CTX	8	0.5	0.125	4	128	16	128
L2EN-487	*qnrS*	CTX, VEB	8	4	1	32	64	4	256
TC-487	*qnrS*	CTX	8	0.25	0.06	4	32	2	64
L2EN-187	*qnrS*	TEM,SHV	> 256	128	32	32	16	16	32
TC-187	*qnrS*	TEM	64	2	0.125	2	16	2	4
L2EN-311	*qnrS*	TEM, SHV, CTX	8	2	0.5	2	16	1	16
TC-311	*qnrS*	TEM	8	0.25	0.03	2	8	0.5	16
L2EN-501	*qnrS*	TEM, CTX	16	2	0.5	2	256	64	> 256
TC-501	*qnrS*	TEM, CTX	8	0.125	0.06	2	64	16	128

NAL, nalidixic acid; NOR, norfloxacin; CIP, ciprofloxacin; FOX, cefoxitin; CAZ, ceftazidime; CTX, cefotaxime; CRO, ceftriaxone

All 3 *qepA*-carrying isolates were tested for conjugation experiment. Only the bla_{CTX-M} genes were successfully transferred from *qepA*-positive donors to UB1637Az^{R}. The transconjugants named TC-271, TC-382 and TC-425. The MIC of cefotaxime increased from 0.25 to 128 µg/ml (512 folds) in TC-271 and TC-425, from 0.25 to 8 µg/ml (32 folds) in TC-382. The MIC of ceftazidime increased from 0.25 to 32 µg/ml (128 folds) in TC-271 and TC-425, from 0.25 to 32 µg/ml (128 folds) in TC-382. The MIC of ceftriaxone increased from 0.25 to 128 µg/ml (512 folds) in TC-271 and TC-425, from 0.25 to 4 µg/ml (16 folds) in TC-382 (Table 30).

Table 30. Resistance genes and MICs of quinolones and cephalosporins among 3 *qepA*-positive donors and transconjugants

Isolate No.	PMQR determinants	ESBL or *ampC* ß - lactamase genes	MIC(µg/ml)						
			NAL	NOR	CIP	FOX	CTX	CAZ	CRO
UB^{AZ}	-	-	2	0.03	0.015	4	0.25	0.25	0.25
L2EN-271	*qepA*	TEM, CTX	> 256	> 256	> 256	8	128	32	256
TC-271	0	CTX	1	0.03	0.015	4	128	32	128
L2EN-382	*qepA*	CTX	> 256	> 256	> 256	8	128	32	256
TC-382	0	CTX	2	0.125	0.015	4	8	32	4
L2EN-425	*qepA*	TEM, CTX	>256	> 256	> 256	64	> 256	64	> 256
TC-425	0	CTX	1	0.03	0.01	4	128	32	128

NAL, nalidixic acid; NOR, norfloxacin; CIP, ciprofloxacin; FOX, cefoxitin; CAZ, ceftazidime; CTX, cefotaxime; CRO, ceftriaxone

CHAPTER VI
DISCUSSION

Fluoroquinolones and third-generation cephalosporins are drugs of choice for treatment of Enterobacteriaceae infection [164]. Increasing bacterial resistance to these antimicrobial agents has been reported worldwide and has become a serious therapeutic problem. The study of Enterobacteriaceae isolated from 12 European countries, including Belgium, the Czech Republic, France, Germany, Hungary, Italy, Netherlands, Poland, Slovakia, Spain, Sweden and the UK during 2005-2007 showed that ceftazidime and cefotaxime resistance were 11.8% and 12.9%, respectively [165]. A study from the United States in 2008 demonstrated that ciprofloxacin, ceftazidime and cefotaxime resistance in Enterobacteriaceae isolates were 18.3%, 7.7% and 5.7%,respectively [166]. During 2010-2011, the resistance rates to norfloxacin, ciprofloxacin, cefoxitin and ceftriaxone were 15.6%, 16.1%, 3.2% and 9.5%, respectively in Enterobacteriaceae isolated from Switzerland [167].

In Thailand, the susceptibility rates were decreased in ciprofloxacin, cefoxitin, ceftazidime, cefotaxime and ceftriaxone from 74.4%, 83.7%, 67.4%, 76.7% and 74.4%, respectively in 2008 [168] to 63.2%, 76.3%, 47.4%, 54.0% and 50.0%, respectively in 2009 [169]. In this study, antimicrobial susceptibility of nalidixic, norfloxacin, ciprofloxacin, cefoxitin, ceftazidime, cefotaxime and ceftriaxone against Enterobacteiaceae isolated from King Chulalongkorn Memorial Hospital between 2009 to 2011 was investigated. The results showed high prevalence of fluoroquinolone and extended-spectrum cephalosporin resistance among Enterobacteriaceae isolates. The resistance rates to nalidixic, norfloxacin, ciprofloxacin, cefoxitin, ceftazidime, cefotaxime and ceftriaxone were 47.10%, 36.22%, 37.71%, 19.68%, 46.20%, 35.77% and 44.11%, respectively. Similar to our study, the study of Enterobacteriaceae isolated from 12 Asian pacific countries, including India, Taiwan, China, New Zealand, Malaysia, Australia, Singapore, Hong Kong, Philippines, South Korea, Thailand and Vietnam showed resistance rates to ciprofloxacin, cefoxitin, ceftazidime, cefotaxime and ceftriaxone were 35.6%, 25.8%, 32%, 26.8% and 32.3%, respectively in 2008 [168] and 39.7%,

27.3%, 41%, 34.2% and 40.9% , respectively in 2009 [169].The study from Vietnam demonstrated that the resistance rates to cefotaxime, ceftazidime and ceftriaxone was 30.0%, 26.6% and 31.5%, respectively in Enterobacteriaceae [170]. The study from China [171] and Algeria [154] were reported high cefoxitin resistance rate of 65.4% and 35.6% in Enterobacteriaceae isolates, respectively. High resistance rates of ciprofloxacin in Enterobacteriaceae isolates was 55% in China [171] and 69.2%, in Spain [15], respectively. Le *et al.* [28] reported high rate of nalidixic acid resistance in Enterobacteriaceae isolates from Vietnam (74 %). Whereas, the study from France [97] and Cameroon [172] were reported low resistance rate of nalidixic acid in Enterobacteriaceae isolates at 16.0% and 25.7%, respectively. The high resistance rates of quinolones and cephalosporins in our Enterobacteriaceae isolates indicate the high consumption of these antimicrobial agents for treatment. The inappropriate use of antimicrobial agents should be monitored.

In our study, stool isolates showed high rate of reduced susceptibility to ciprofloxacin (34.88%) but no fluoroquinolone resistance was observed. The stool isolates were frequently identified as *Salmonella* spp. in 96.51%, which showed high rate (36.14%) of reduced susceptibility to ciprofloxacin. Previous studies demonstrated that infections with *Salmonella* isolates with reduced susceptibility to ciprofloxacin were associated with ciprofloxacin treatment failure [173-176].

The study by Rhomberg *et al.* [177] showed that resistance rate of ciprofloxacin was similar in isolates from ICU (5.7%) and non-ICU (5.6%) patients. The study by Polwichai *et al.* [178] showed higher rate of ciprofloxacin resistance in isolates from ICU (47.91%) than that of isolates from non-ICU patients (43.49%). In contrast to our study, higher rate of ciprofloxacin resistance was found in non-ICU isolates (38.52%) than that of ICU isolates (27.08%). Although high rates of ciprofloxacin resistance was found in non-ICU isolates, there was no statistical significant difference between ICU and non-ICU isolates by using Chi-square test. Rhomberg *et al.* [177] showed that the ICU isolates showed higher rates of resistance ceftazidime (3.1%) and ceftriaxone (8.1%) than that of non-ICU isolates (2.2% in ceftazidime and 4.2% in ceftriaxone). The study by

Polwichai *et al*. [178] showed the the isolates from ICU showed higher rates of resistance to ceftazidime (20.52%) and ceftriaxone (24.51%) than that of isolates from non-ICU (10.36% in ceftazidime and 13.77% in ceftriaxone). Similar to our results, ICU isolates showed high rates of resistance to ceftazidime (43.75%) and ceftriaxone (47.92%) than those of non-ICU isolates (35.15% for ceftazidime and 43.82% for ceftriaxone). Although high rates of ceftazidime and ceftriaxone resistance was found in ICU isolates, there was no statistical significant difference between ICU and non-ICU isolates by using Chi-square test.

Our results showed higher resistance rates to quinolones and third-generation cephalosporins, including nalidixic acid (48.64%), norfloxacin (38.11%), ciprofloxacin (39.75%), cefotaxime (46.6%), ceftazidime (36.84%) and ceftriaxone (44.65%) in non-sterile site isolates than those of sterile site isolates. Although high rates of nalidixic acid norfloxacin, ciprofloxacin, cefotaxime, ceftazidime and ceftriaxone resistance was found in non-sterile site isolates, there was no statistical significant difference between sterile site and non-sterile site isolates by using Chi-square test. The most common isolates from sterile and non-sterile sites were *E. coli* and *Klebsiella* spp.. The study by Dornbusch *et al.* [179] showed higher resistance rates to quinolones and third-generation cephalosporins, including ciprofloxacin (17%), cefotaxime (6%)and ceftazidime (3%) in *E. coli* urine isolates than *E. coli* blood isolates in Southern and Northern Europe, whereas *E. coli* blood isolates (10%) had higher cefoxitin resistance rates than *E. coli* urine isolates (7%) in Central Europe.

The study from Germany in 2005 demonstrated that *qnrA*-carrying Enterobacteriaceae isolates (4.8%) were resistant to ciprofloxacin, cefotaxime and ceftazidime [77]. The study from Peru and Bolivia in 2009 demonstrated that the *qnr* gene was present in Enterobacteriaceae isolates (0.6%). All *qnr*-harboring isolates showed high rate of nalidixic acid resistance (77%) and reduced susceptibility to ciprofloxacin (69%). The *qnrB* gene (0.38%) was most common *qnr* gene, followed by *qnrS* (0.28%), while *qnrA*, *qnrC* and *qnrD* were not detected in any isolates [180]. The study from Spain in 2011 showed that *qnr* gene was present in Enterobacteriaceae

isolates (0.64%). The *qnrS* gene (0.49%) was the most common PMQR gene, followed by *qnrA*(0.14%) and *qnrB*(0.01%), while *qnrC*, *qnrD, aac(6')-Ib-cr* and *qepA* were not detected in any isolates [181]. Le *et al*. [28] was reported high prevalence of *qnr* genes (26.9%) in Enterobacteriaceae isolated from Vietnam in 2009. The *qnrS* gene (20.3%) was most common *qnr* gene, followed by *qnrB* (3.4%) and *qnrA* (3.2%). The *aac(6')-Ib-cr* and *qepA* were detected in 5.6% and 0.18%, respectively. The study from Korea in 2011 demonstrated that the *qnr* gene (16.6%) was the most common PMQR gene, followed by *aac(6')-Ib-cr* (15.6%) and *qepA* (0.6%) in Enterobacteriaceae isolates[130]. Previous studies showed the prevalence of PMQR varied from less than 1% to 30-40%[41]. In this study, the presence of seven PMQR genes, including *qnrA, qnrB, qnrS, qnrC, qnrD, aac(6')-Ib-cr* and *qepA* was investigated. There were 138 (20.57%) out of 671 isolates which carried PMQR genes. The *qnr* genes (12.82%) was the most common PMQR determinant in Enterobacteriaceae isolates, followed by *aac(6')-Ib-cr* (7.30%) and *qepA* (0.45%). In contrast to the previous studies from France [182] and China [33], *aac(6')-Ib-cr* was the most common PMQR gene in Enterobacteriaceae isolates. In this study, *qnrS* (8.05%) was the most common *qnr* genes found in Enterobacteriaceae isolates, followed by *qnrA* (2.38%) and *qnrD* (2.38%). Similar to our study, *qnrS* was commonly found in Enterobacteriaceae isolates [1, 28, 181]. In contrast to the study from Spain, the *qnrA* gene(4.6%) was most commonly found in Enterobacteriaceae isolates, followed by *qnrS* (0.6%)[15]. Corkill *et al*. [11], reported high prevalence of *qnrA* (32%) in Enterobacteriaceae isolated from UK . Richter *et al.* [183] reported high prevalence of *qnrB* (15.5%) in Enterobacteriaceae isolated from Italy. The *qnrD* gene was first detected in *Salmonella enterica* isolated from China [10]. It was recently reported in one *E. coli* isolate [12] and twelve *P. mirabilis* isolates[20] in China. Similar to this study, *qnrD* gene was commonly found in *Proteus* spp. (19.35%), followed by *M. morganii* (4.17%), *Enterobacter* spp. (2.38%) and *Klebsiella* spp. (1.36%). The *qnrB* and *qnrC* genes were not detected in any isolates from this study. This is in contrast to a study from Korea which showed that *qnrB* gene was more common (36.7%) in Enterobacteriaceae, including *C. freundii*, *K. pneumonia, E.*

cloacae and *E. coli* isolates [14]. Low prevalence of *qnrB* and *qnrC* was reported from Philippines where *qnrB* was present in 2.67% of ESBL-producing Enterobacteriaceae and *qnrC* was not found in any isolates[13].

In our study, the *aac(6')-Ib-cr* was frequently identified in *E. coli* (12.50%), followed by *Klebsiella* spp.(7.48%) and *Enterobacter* spp.(7.14%). The study by Yang *et al.* in 2008 showed that *aac(6')-Ib-cr* was detected in 36 isolates (18.3%) of Enterobacteriaceae isolated from China. The *aac(6')-Ib-cr* gene was detected in Isolates of *C. freundii, K. pneumoniae, E. coli* and *E. cloacae* [33]. In contrast of this study, *aac(6')-Ib-cr* was commonly found in *S. enterica* serotype Typhimurium (37.1%) isolated from China, whereas *qnr* and *qepA* genes were not detected in any isolates[35].

All *qepA* genes were found in only 3 isolates of *E. coli* in this study. This is similar to a study from Vietnam which showed that *qepA* was found only in *E. coli* (1.8%)isolates [184] . A study from Korea found *qepA* in 3 isolates of *Klebsiella* spp. (0.8%) and *E. coli* (0.7%)[130].

Our results showed that in *E. coli, aac(6')-Ib-cr* gene was the most common PMQR gene (12.50%), followed by *qnr* (5.36%) and *qepA* (1.07%). A study from Turkey showed high prevalence of *aac(6')-Ib-cr* in *E. coli* (45.9%), while *qepA and qnr genes* were only found in 5.7% and 3.2%, respectively [124]. The *qnrD* gene was recently reported in one of 172 *E. coli* isolates [12], while *qnrD* genes were not detected in our *E. coli* isolates.

Our study showed that *qnr* (17.69%) was more predominant than *aac(6')-Ib-cr* (7.48%) in *Klebsiella* spp. isolates. Similar to the study from Korea, *qnr* gene (14.4%) was more comon than *aac(6')-Ib-cr* (1.6%) in *K. pneumoniae* [185]. The *qnrB* gene (6%) was most commonly found in *K. pneumoniae* isolated from Korea[185]. This was in contrast to our study which showed that *qnrS* (4.9%) was the most prevalent *qnr* gene in *Klebsiella* isolates and *qnrB* was not detected in our isolates.

The *qnr* gene (23.81%) was the most common PMQR in our *Enterobacter* spp. isolates, followed by *aac(6')-Ib-cr* (7.14%). Similar to this study, *qnr* (17.2%) in *Enterobacter* spp. was common in Taiwan. However, *qnrB*(10%) was the most

prevalent *qnr* gene [18]. In contrast to our study, *qnrS* (16.7%) was the most prevalent *qnr* gene in *Enterobacter* spp. isolates.

In this study, *Salmonella* isolates carried *qnr* genes in 20.21%. The *qnrS* gene (19.15%) was the most common *qnr* found in *Salmonella* isolates. Similar to our study, high prevalence of *qnrS* was found in *S. enterica* (38.9%) in a study from Denmark [17]. In contrast, *aac(6')-Ib-cr* (37.1%) was the most prevalent PMQR in *S.* Typhimurium isolates from China, whereas, *qnr* and *qepA* genes were not detected in any isolates[35]. The *qnrD* gene was reported in *S enterica* isolated from China in 2009 [10]. This was in contrast to our study which the *aac(6')-Ib-cr* and *qnrD* genes were not detected in any isolates of *Salmonella* spp..

The *qnr* gene was detected in our *Citrobacter* isolates in 23.0.8%, including 7.69% for *qnrA* and 15.38% for *qnrS* .The results was different from a study from Japan which found that *Citrobacter* spp. isolates carried only *aac(6')-Ib-cr* (1.7%) [27]. In present study, *qnrD* was found in 19.35% in *Proteus* spp. and 4.17% in *M. morganii*. This was in agreement with the study by Mazzariol *et al.,* which showed that *qnrD* was present in *P. mirabilis* (5.7%) and *M. morganii* (4.1%) isolates[23]. PMQR genes were not detected in any isolates of *P. rettgeri* and *S. marcescens* in this study. This may be due to a small number of these isolates included in this study. The results was different from the study of France and Korea which found *qnrD* in *P. rettgeri* isolates and *qnrB* in *S. marcescens* isolates, respectively [25, 186].

Prevalence of ESBL-producing Enterobacteriaceae has been reported to vary from country to country. Ben-Ami *et al.*[187] reported that ESBLs-production was detected 13.7% of Enterobacteriaceae isolated from Israel in 2005. The study from Philippine during 2006-2008 showed that 18.7% of Enterobacteriaceae isolates were ESBL producers [188]. The study from Korea found ESBL producers in 14.1% [189]. During 2006 to 2009, the study from Mexico showed that 29.82% of Enterobacteriace isolates produced ESBLs [190]. Low prevalence of ESBL producers was reported in Sweden (2-10%), Canada (4.9%), New Zealand (4.2%), French(1.7%) and Netherland (5.2%) [191-195]. In Thailand, the study in Hat Yai Hospital in 2004 in

Enterobacteriaceae isolates showed that 15.79 % of the isolates had ESBL production [196]. The study in Siriraj Hospital during 2006-2007 in Enterobacteriaceae isolates showed that 18.7% of the isolates produced ESBLs [145]. Udomsantisuk *et al.* showed that ESBL producers were found in 17% of *E. coli* and 34.5% of *K. pneumoniae* isolated from King Chulalongkorn Memorial Hospital[160].

In this study, 138 PMQR-carrying Enterobacteriaceae isolates were investigated for the presence of ESBL genes and plasmid-mediated *ampC* genes . Eighty-three of these isolates carried ESBL genes (60.14%). Five of PMQR-positive isolates harboring *ampC* gene (3.62%). Nine of PMPQR-positive isolates had both ESBL and *ampC* genes (6.52%). Similar to previous studies, the study by Jeong *et al.* [197] showed that *qnr*-positive isolates showed high rates of ESBLs and/or AmpC ß-lactamase production (48.94%). Wu *et al.* [198] reported that *E. cloacae* isolates carrying *qnr* gene showed high prevalence of ESBL genes(68.89%). Wang *et al.* [199] reported that *E. coli* and *K. pneumoniae* isolates harboring *qnr* gene showed high rates of ESBLs ß-lactamase production (84.21%). Frasson *et al.* [200] reported that ESBL production was detected in 87.5% of *aac(6')-Ib-cr* –positive isolates. *bla* $_{CTX-M}$ has been reported to be the most common ESBL gene among Enterobacteriaceae isolates. In the present study, *bla* $_{CTX-M}$ was the most common ESBL determinant (31.16%) in PMQR-positive isolates. The study from China in 2008 showed that $bla_{CTX-M-14}$ and $bla_{CTX-M-3}$ were the most prevalent ESBL types among Enterobacteriaceae harboring *qnr* gene [33]. Previous studies demonstrated the association between PMQR genes and ESBL and *ampC* genes [41, 201]. The *qnr* genes have been found to be associated with different types of ESBLs and AmpC ß-lactamases, including $bla_{CTX-M-group}$ [81, 97, 115-117, 202], bla_{TEM-1} [81, 117, 202], bla_{VEB-1}[97, 115], bla_{SHV-12} [68, 116, 202, 203], bla_{CMY-2} and bla_{DHA}[204]. The *aac(6')-Ib-cr* gene was reported to be co-harboring with bla_{CTX-M} bla_{TEM}, bla_{OXA} and bla_{DHA} in Canada, Portugal , Lebanon and the United Kingdom [120, 205-207]. The *qepA* gene was reported to be co-harboring with bla_{CTX-M} and bla_{TEM} [208].The results showed that *aac(6')-Ib-cr*-carrying isolates were commonly associated with ESBL genes. Our results showed that all 3 *qepA*-positive isolates harboured ESBL genes (bla_{CTX-M} and/or *bla* $_{TEM}$).

The results was in accordance with to a study from Mexico which detected $bla_{CTX-M-15}$ and bla_{TEM-1} in *E. coli* isolates carrying *qepA* [208]. PMQR genes were often reported to be found with ESBL and/or *ampC* genes on the same plasmid. The bla_{VEB-1} and *qnr*A1 were found to be located on the same plasmid ranging in size from 130 to 200 kb in *E. cloacae* isolates [29]. The *qnrB* and bla_{DHA-1} were also present on the same plasmid with molecular size of 70 kb[30]. Ruiz *et al*. [209] reported that *qepA* / $bla_{CTX-M-15}$ and the *aac(6')-Ib-cr/* $bla_{CTX-M-15}$ in *E. coli* were present on the same plasmids which with 145 and 120 kb, respectively by Southern blot analysis. Several studies showed that *qnrS* was located on large plasmids, with sizes ranging from 10 to 350 kb [210-212]. The *qnrS* gene was found on the same plasmid with bla_{TEM-1}[212], bla_{LAP-1}[213], $bla_{CTX-M-15}$[123], bla_{OXA-1}[123], *aac(6')-Ib-cr* [123, 214] and *qepA* [213]. Garnier *et.al.* [92] reported that *qnrB2* was located in a complex *sul1*-type integron of *Salmonella* plasmid from Senegal. Lascols *et. al.* [91] found that plasmid pHe96 from *K. pneumoniae* isolates was often embedded in complex *sul1*-type integrons. The results suggest that the co-location of PMQR and ESBL genes on the same plasmid may facilitate the rapid spread of both antibiotic resistance genes by horizontal gene transfer.

To investigate the role of target alteration in quinolone resistance, 23 PMQR-positive isolates with reduced susceptibility, intermediate and resistance to ciprofloxacin were investigated for the presence of *gyrA* and *parC* gene mutations. There were three *qepA*-carrying isolates which were highly resistant to all quinolones tested (MICs of >256 µg/ml) and twenty isolates with intermediate and reduced ciprofloxacin resistance. Mutations in GyrA (S83L, D87N) and ParC (S80I) were found in these 3 *qepA*-carrying isolates. However, quinolone resistance was not the effect of the presence of *qepA* alone as these isolates had mutations in GyrA and ParC which were previously reported to confer high-level quinolone resistance. We also could not exclude other resistance mechanisms associated with quinolone resistance in these 3 isolates. Similarly, the study by Kim *et al.* [215] in 2009 demonstrated that *E. coli* isolates carrying *qepA* which were highly resistant to fluoroquinolones showed Ser83Leu, Asp87Asn mutations in GyrA and Ser80Ile mutation in ParC, while their transconjugants showed reduced

susceptibility to hydrophilic fluoroquinolone suggesting that the presence of *qepA* may confer reduced susceptibility to hydrophilic fluoroquinolone. The results was similar to other studies, which found that high level of quinolone resistance was due to mutations in GyrA and ParC [36, 128, 162, 216, 217].

Twenty isolates with intermediate and reduced ciprofloxacin resistance had no alteration in GyrA and ParC. The results demonstrated that PMQR genes were associated with intermediate and reduced susceptibility to ciprofloxacin but did not play an important role in high-level ciprofloxacin resistance. The MICs of nalidixic acid, norfloxacin and ciprofloxacin for transconjugants that carried *qnrS* alone were 3- to 16.7-fold higher than recipient, indicating that the *qnrS* genes contribute to reduced susceptibility to quinolones tested. Similar to previous studies, PMQR genes conferred reduced susceptibility to quinolones and fluoroquinolones [6, 15, 17, 87, 182, 218, 219]. The study by Martinez *et.al.* [6] found that *qnrA* introduced into fluoroquinolone-susceptible strains by conjugation caused an increase in fluoroquinolone MIC but not resistance to fluoroquinolones according to CLSI breakpoints. The presence of PMQR determinants may facilitate the emergence of high-level quinolones resistance.

PMQR-negative isolates were also found to reduce susceptibility (16.70%) and resistance to fluoroquinolone (33.02%) which may caused by alteration of drug targets including GyrA, GyrB, ParC and ParE, overexpression of efflux pump and loss of outer membrane porins. However, these resistance mechanisms were not investigated in this study.

The efflux pump phenotype was found in all *qepA*-carrying isolates. The MICs of norfloxacin were decreased for 8- to 32-folds in the presence of CCCP in all isolates. However, the transfer of *qepA* genes were not successful in conjugation experiment. The efflux pump activity was not mediated only the *qepA* gene. Other efflux pump such as AcrAB-TolC, the chromosome-dependent efflux systems in RND family [36, 220] may play a role in the efflux pump activity in these isolates.

Eighty-eight PMQR-positive isolates were investigated for the transfer of PMQR, ESBL and *ampC* gene to *E. coli* (UB1637Az^{R}) by conjugation. Quinolone resistance

and/or cephalosporin resistance was successful transfer from 22 donor isolates (25%). The *qnrA, qnrS*, *bla* $_{CIT}$ and ESBL genes, including *bla* $_{CTX-M}$, *bla*$_{TEM}$ and *bla* $_{VEB}$ were successfully transferred by conjugation to *E. coli* (UB1637Az^{R}). The *qepA, qnrD* and *aac(6')-Ib-cr* were not successful in conjugation experiment. The co-transfer of *qnr* and ESBL genes indicates that these genes may be located on same plasmid. PMQR genes which failed to be transferred by conjugation method may be located on non-transferable plasmid or they are located on a large plasmid which could not be transferred by this method [17, 221, 222]. The co-transfer of *qnr* and ESBL indicated the linkage between plasmid-mediated quinolone and cephalosporin resistane. The results suggest that the co-transmission contributes to the rapid spread of antimicrobial resistance genes.

CHAPTER VII

CONCLUSION

Fluoroquinolones are widely used for treatment of infections causes by Enterobacteriaceae. Most common infections include pneumonia, bacteremia and urinary tract infection. Fluoroquinolone resistance is mainly caused by chromosomal mutations in the quinolone resistance-determining region (QRDR) of DNA gyrase and topoisomerase IV. Recently, plasmid-mediated quinolone resistance (PMQR) including target protection mechanism encoded by *qnr*, efflux pump encoded by *qepA* and fluoroquinolone-modifying enzyme encoded by *aac(6')-Ib-cr* has been described. In this study, A total of 671 non-replicated clinical isolates of Enterobacteriaceae collected between May 2009 and September 2011 from King Chulalongkorn Memorial Hospital, Bangkok, Thailand were included in this study. The results showed that the resistance rates to nalidixic, norfloxacin, ciprofloxacin, cefoxitin, ceftazidime, cefotaxime and ceftriaxone were 47.10%, 36.22%, 37.71%, 19.68%, 46.20%, 35.77% and 44.11%, respectively.

The results showed higher resistance rates to quinolones and third-generation cephalosporins in isolates from non-sterile sites than those of sterile sites. The isolates from non-ICUs showed higher rates of resistance to quinolones than those of ICU isolates, while the isolates from ICUs showed higher rates of resistance to third-generation cephalosporins than those of non-ICU isolates.

All 671 isolates were screened for seven PMQR genes, including *qnrA, qnrB, qnrS, qnrC, qnrD, aac(6')-Ib-cr* and *qepA* by PCR. The prevalence of *qnrA, qnrS*, *qnrD*, *aac(6')-Ib-cr* and *qepA* were 2.38%, 8.05%, 2.38%, 7.30% and 0.45%, respectively. All isolates had a single PMQR determinant. The *qnrB,* and *qnrC* genes were not detected in any isolates. The *qnrS* gene was the most prevalent PMQR determinant in our Enterobacteriaceae isolates. The *aac(6')-Ib-cr* gene was the most common PMQR determinants in *E. coli* isolates. The *qnrS* gene was frequently identified in *Salmonella* spp., followed by *Klebsiella* spp., *E. coli*, *Enterobacter* spp. and

Citrobacter spp.. *Proteus* spp. and *M. morganii* carried only one PMQR gene which was *qnrD*. The prevalence of *qnrD* was 19.35% in *Proteus* spp. and 4.17% in *M. morganii*. All 3 *qepA*-carrying isolates were *E. coli*.

The isolates carrying PMQR genes showed higher rates of resistance to quinolones and cephalosporins, including nalidixic acid (64.49%), norfloxacin (52.90%), ciprofloxacin (55.80%), cefoxitin (36.96%), cefotaxime (72.46%), ceftazidime (62.32%) and ceftriaxone (70.29%) than those of isolates without PMQR genes.

Of 138 Enterobacteriaceae isolates carrying PMQR genes, 92 (66.67%) and 14 (10.14%) were ESBL-producing and AmpC-producing isolates, respectively. There were 70.29% (97/138) of PMQR-positive isolates carring ESBL and/or *ampC* genes. Of the138 isolates, 60.14% (83/138) had ESBL genes, 3.62% (5/138) had *ampC* genes and 6.52% (9/138) had both ESBL and *ampC* genes. The $bla_{\text{CTX-M}}$ gene was the most common ESBL determinant (31.16%) in PMQR-positive isolates. Fourty-one (29.71%) isolates carried only PMQR gene. The *aac(6')-Ib-cr*-carrying isolates were commonly associated with ESBL genes (87.76%, 43/49). All 3 *qepA*-positive isolates harboured ESBL genes ($bla_{\text{CTX-M}}$ and/or bla_{TEM}). The combination of PMQR, ESBL and *ampC* genes was found in 9 isolates (6.52%).The $bla_{\text{CTX-M + TEM +DHA}}$ pattern was found in three of nine isolates (33.33%).

Of the 23 PMQR-positive representative, 20 isolates carried PMQR genes with reduced susceptibility and intermediate resistance to ciprofloxacin had no amino acid substitutions in GyrA and ParC. Three isolates carried PMQR genes which were highly resistant to ciprofloxacin (MICs of >256 µg/ml) also had amino acid substitutions at S83L, D87N in GyrA and S80I in ParC. Therefore, High-level quinolones resistance was associated with GyrA and ParC mutations, whereas the PMQR genes were associated with intermediate and reduced susceptibility to ciprofloxacin. All these quinolone resistant isolates harbored the *qepA* genes. The efflux pump phenotype was found in all *qepA*-carrying isolates

Eighty-eight PMQR-positive isolates were investigated for the transfer of PMQR, ESBL and *ampC* genes to *E. coli* (UB1637Az^R) by conjugation. Quinolone and/or

cephalosporin resistance was successful transfer from 22 donor isolates (25%). The *qnrA, qnrS*, *bla* $_{CIT}$ and ESBL genes, including *bla* $_{CTX-M}$, *bla*$_{TEM}$ and *bla* $_{VEB}$ were successfully transferred by conjugation to *E. coli* (UB1637Az^{R}). The transfer of *qepA*, *aac(6')-Ib-cr* and *qnrD* gene were fail conjugation experiment.

Our study showed high rates of quinolone and extended spectrum-cephalosporin resistance in Enterobacteriaceae. The *qnr* genes were the most prevalent PMQR determinant and were able to be transferred with ESBL and *ampC* genes. This is the first report of the presence of *qepA* and *qnrD* genes in Enterobacteriaceae isolated from Thailand.

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APPENDICES

APPENDIX A

REAGENTS AND INSTRUMENTS

Reagents

Absolute ethanol	(Merck, Germany)
Agarose	(Biorad, USA)
Boric acid	(Sigma, USA)
dNTPs	(Promega, USA)
EDTA	(Amresco, USA)
Ethidium bromide	(Amresco, USA)
NaCl	(Merck, Germany)
Taq DNA Polymerase	(Fermentas, USA)
Tris	(Amresco, USA)
100 bp DNA ladder	(Fermentas, USA)
100 bp plus DNA ladder	(Fermentas, USA)
Trytic soy agar	(BBL, USA)
Trytic soy broth	(BBL, USA)
Muller-Hinton II agar	(BBL, USA)
MacConkey agar	(Oxoid, England)
LB broth	(Pronadisa, Spain)
NaOH	(Sigma, USA)

Instruments

Automatic pipette	(Gilson, Lyon, France)
Camera Gel DocTM MZL	(BIO-RAD, USA)
Incubator	(Forma Scientific, USA)
Perkin Elmer GeneAmpPCR system 9600	(Perkin Elmer, USA)
Microcentrifuge	(Eppendorf, USA)
Spectrophotometer	(BIO-RAD, USA)
Water bath	(Memmert, USA)

APPENDIX B
MEDIA AND ANTIBIOTIC SOLUTION PREPARATION

1. Muller-Hinton II agar (BBL, USA)

Suspend 38 grams of the dehydrated medium in 1,000 ml of distilled water. Dissolve by heating until complete dissolution. Sterilize by autoclaving at 121ºC for 15 minutes. Once the medium is prepared, store at 4ºC.

2. Tryptic soy broth (BBL, USA)

Suspend 30 grams of the dehydrated medium in 1,000 ml of distilled water. Dissolve by heating until complete dissolution. Sterilize by autoclaving at 121°C for 15 minutes. Once the medium is prepared, store at 4ºC.

3. LB broth (Pronadisa, Spain)

Suspend 20 grams of the dehydrated medium in 1,000 ml of distilled water. Dissolve by heating until complete dissolution. Sterilize by autoclaving at 121ºC for 15 minutes. Once the medium is prepared, store at 4ºC.

4. Trytic soy agar (BBL, USA)

Suspend 40 grams in of the dehydrated medium in 1,000 ml of distilled water. Dissolve by heating until complete dissolution. Sterilize by autoclaving at 121ºC for 15 minutes. Once the medium is prepared, store at 4ºC.

5. MacConkey agar (Oxoid, England)

Suspend 51.5 grams in of the dehydrated medium 1,000 ml of distilled water. Dissolve by heating until complete dissolution. Sterilize by autoclaving at 121ºC for 15 minutes. Once the medium is prepared, store at 4ºC.

6. Sterile 0.85% NaCl (Merck, Germany)

NaCl 8.5 grams in 1,000 ml of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 121ºC for 15 minutes. Once the medium is prepared, store at room temperature.

7. Antibiotic solution preparation

Nalidixic acid, stock concentration 5120 mg/l

- Prepare a stock solution ; dissolve 0.0256 g in 50 µl of 0.1 N NaOH and 4.95 ml sterile distilled water

Ciprofloxacin, stock concentration 5120 mg/l

- Prepare a stock solution ; dissolve 0.0256 g in 100 µl of 0.1 N HCl and 4.9 ml sterile distilled water

Norfloxacin, stock concentration 5120 mg/l

- Prepare a stock solution ; dissolve 0.0256 g in 100 µl of 0.1 N HCl and 4.9 ml sterile distilled water

Cefoxitin, stock concentration 5120 mg/L

- Prepare a stock solution; dissolve 0.0269 g in 5 ml sterile distilled water

Ceftazidime, stock concentration 5120 mg/L

- Prepare a stock solution; dissolve 0.0256 g in 50 µl of 0.1 N NaOH and 4.95 ml sterile distilled water

Cefotaxime, stock concentration 5120 mg/L

- Prepare a stock solution; dissolve 0.0256 g in 5 ml sterile distilled water

Ceftriaxone, stock concentration 5120 mg/L

- Prepare a stock solution; dissolve 0.0256 g in 5 ml sterile distilled water

APPENDIX C

REAGENTS PREPARATION

1. 10x Tris-Borate buffer (TBE)

Tris base	108	g
Boric acid	55	g
0.5 M EDTA (pH 8.0)	40	ml

Adjust volume to 1 liter with distilled water. The solution was mixed and sterilized by autoclaving at 121ºC for 15 min.

2. 0.5 M EDTA (pH 8.0)

Disodium ethylene diamine tetra-acelate $2H_2O$	186.1	g

Adjust pH to 8.0 and volume to 1 liter. Store at room temperature for no longer than 1 year.

3. 10x TE buffer

Tris	12.11	g
0.5 M EDTA	20	ml

Adjust to pH 8.0 by adding conc. HCl. Adjust volume to 1,000 ml and sterilized by autoclaving at 121ºC for 15 min.

4. 1.5 % Agarose gel

Agarose	0.6	g
1x TBE	40	ml

Dissolve by heating in microwave oven and occasional mix unit no granules of agarose are visible.

5. 6x Loading buffer 40 ml

Bromphenol Blue	0.1	g
Sucrose	40	g

Adjust volume to 40 ml with distilled water. Mix the solution, aliquot into 1.5 microtubes and store at 4°C.

6. 1M NaOH

NaOH	20	g

Adjust volume to 500 ml with distilled water and sterilized by autoclaving at 121ºC for 15 min

7. Tris-EDTA

Tris-base	1.58	g
Disodium ethylene diamine tetra-acelate $2H_2O$	0.38	g

Adjust to pH 8.0 by adding conc. HCl. Adjust volume to 1,000 ml with Double distilled water and sterilized by autoclaving at 121ºC for 15 min.

APPENDIX D

THE RESULTS OF ALL TEST IN THIS STUDY

Results of Susceptibility test and the presence of PMQR , ESBL and AmpC genes of 671 Enterobacteriaceae isolates

No	isolates	MIC(µg/ml)							The presence of PMQR genes							ESBLs		AmpC	
		NAL	NOR	CIP	FOX	CTX	CAZ	CRO	*qnrA*	*qnrB*	*qnrS*	*qnrC*	*qnrD*	*aac(6')-Ib-cr*	*qepA*	phenotype	Genotype	phenotype	genotype
1	L2EN-1	>256	256	64	8	256	64	256	0	0	0	0	0	1	0	+	CTX	-	0
2	L2EN-2	>256	256	>256	256	128	128	256	0	0	1	0	0	0	0	+	TEM, CTX	+	DHA
3	L2EN-3	>256	>256	64	8	256	64	128	0	0	0	0	0	0	0	ND	ND	ND	ND
4	L2EN-4	2	0.25	0.03	128	0.5	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
5	L2EN-5	>256	>256	128	8	>256	128	256	0	0	0	0	0	1	0	+	CTX	-	0
6	L2EN-6	4	1	0.5	2	0.25	0.25	0.12	0	0	1	0	0	0	0	-	0	-	0
7	L2EN-7	>256	>256	64	8	256	32	64	0	0	0	0	0	1	0	+	CTX	-	0
8	L2EN-8	4	0.25	0.03	16	256	0.5	64	0	0	0	0	0	0	0	ND	ND	ND	ND
9	L2EN-9	16	1	0.5	32	128	32	128	0	0	1	0	0	0	0	+	CTX	-	0
10	L2EN-10	4	0.25	0.03	16	2	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND

No	isolates	MIC(μg/ml)							The presence of PMQR genes							ESBLs		AmpC	
		NAL	NOR	CIP	FOX	CTX	CAZ	CRO	*qnrA*	*qnrB*	*qnrS*	*qnrC*	*qnrD*	*aac(6')-Ib-cr*	*qepA*	phenotype	Genotype	phenotype	genotype
11	L2EN-11	4	0.25	0.03	16	0.5	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
12	L2EN-12	4	0.25	0.03	32	0.5	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
13	L2EN-13	>256	128	32	8	0.5	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
14	L2EN-14	>256	>256	128	32	256	128	128	0	0	0	0	0	0	0	ND	ND	ND	ND
15	L2EN-15	2	0.25	0.03	16	0.5	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
16	L2EN-16	>256	>256	64	16	32	16	16	0	0	0	0	0	0	0	ND	ND	ND	ND
17	L2EN-17	>256	32	16	32	32	256	64	0	0	0	0	0	0	0	ND	ND	ND	ND
18	L2EN-18	>256	64	8	32	32	4	16	0	0	0	0	0	0	0	ND	ND	ND	ND
19	L2EN-19	>256	>256	128	128	>256	128	>256	0	0	0	0	0	1	0	+	CTX	-	0
20	L2EN-20	8	0.25	0.03	16	1	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
21	L2EN-21	16	1	0.5	32	16	1	16	0	0	1	0	0	0	0	+	TEM	-	0
22	L2EN-22	2	0.25	0.03	16	256	16	64	0	0	0	0	0	0	0	ND	ND	ND	ND
23	L2EN-23	>256	>256	64	16	>256	128	128	0	0	0	0	0	1	0	+	CTX	-	0
24	L2EN-24	128	1	0.25	128	256	32	16	0	0	0	0	0	0	0	ND	ND	ND	ND
25	L2EN-25	>256	128	32	8	256	32	256	0	0	0	0	0	1	0	+	CTX	-	0

No	isolates	MIC(μg/ml)							The presence of PMQR genes							ESBLs		AmpC	
		NAL	NOR	CIP	FOX	CTX	CAZ	CRO	*qnrA*	*qnrB*	*qnrS*	*qnrC*	*qnrD*	*aac(6')-Ib-cr*	*qepA*	phenotype	Genotype	phenotype	genotype
26	L2EN-26	4	0.12	0.03	8	128	0.12	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
27	L2EN-27	>256	>256	>256	8	128	16	64	0	0	0	0	0	1	0	+	CTX	-	0
28	L2EN-28	>256	>256	>256	32	64	32	64	0	0	0	0	0	0	0	ND	ND	ND	ND
29	L2EN-29	2	2	0.06	8	1	1	0.25	0	0	0	0	0	0	0	ND	ND	ND	ND
30	L2EN-30	4	4	0.12	8	0.5	1	0.25	0	0	0	0	0	0	0	ND	ND	ND	ND
31	L2EN-31	>256	>256	64	8	2	1	0.25	0	0	0	0	0	0	0	ND	ND	ND	ND
32	L2EN-32	>256	>256	>256	8	128	32	256	0	0	0	0	0	1	0	+	CTX	-	0
33	L2EN-33	>256	128	1	8	64	2	32	0	0	0	0	0	0	0	ND	ND	ND	ND
34	L2EN-34	>256	>256	>256	8	64	>256	8	0	0	0	0	0	0	0	ND	ND	ND	ND
35	L2EN-35	>256	256	64	8	0.5	0.5	0.25	0	0	0	0	0	0	0	ND	ND	ND	ND
36	L2EN-36	16	1	0.5	32	0.5	0.5	0.25	0	0	1	0	0	0	0	-	TEM	-	0
37	L2EN-37	256	2	0.5	128	16	32	16	0	0	0	0	0	0	0	ND	ND	ND	ND
38	L2EN-38	256	256	64	8	64	16	64	0	0	0	0	0	0	0	ND	ND	ND	ND
39	L2EN-39	2	0.5	0.12	8	16	0.12	0.25	0	0	0	0	0	0	0	ND	ND	ND	ND
40	L2EN-40	64	4	0.12	8	0.5	1	0.25	0	0	0	0	0	0	0	ND	ND	ND	ND

No	isolates	MIC(μg/ml)							The presence of PMQR genes							ESBLs		AmpC	
		NAL	NOR	CIP	FOX	CTX	CAZ	CRO	*qnrA*	*qnrB*	*qnrS*	*qnrC*	*qnrD*	*aac(6')-Ib-cr*	*qepA*	phenotype	Genotype	phenotype	genotype
41	L2EN-41	128	32	0.12	8	0.5	0.5	0.25	0	0	0	0	0	0	0	ND	ND	ND	ND
42	L2EN-42	256	2	1	256	1	64	1	0	0	0	0	0	0	0	ND	ND	ND	ND
43	L2EN-43	64	2	0.12	8	0.5	1	0.25	0	0	0	0	0	0	0	ND	ND	ND	ND
44	L2EN-45	2	2	0.25	8	0.5	1	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
45	L2EN-46	32	4	2	4	0.5	1	0.25	0	0	0	0	0	0	0	ND	ND	ND	ND
46	L2EN-47	16	0.5	0.12	4	0.5	0.5	0.25	0	0	0	0	0	0	0	ND	ND	ND	ND
47	L2EN-48	>256	>256	32	64	16	16	16	0	0	0	0	0	0	0	ND	ND	ND	ND
48	L2EN-49	>256	>256	>256	8	128	32	64	0	0	0	0	0	1	0	+	CTX	-	0
49	L2EN-50	2	1	0.12	8	0.5	0.12	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
50	L2EN-51	>256	>256	>256	256	128	128	256	0	0	1	0	0	0	0	+	TEM, CTX	+	CIT
51	L2EN-52	>256	>256	32	4	256	32	256	0	0	0	0	0	0	0	ND	ND	ND	ND
52	L2EN-53	128	8	1	8	0.5	1	0.25	0	0	0	0	0	0	0	ND	ND	ND	ND
53	L2EN-54	4	0.06	0.06	2	0.06	0.25	0.5	0	0	0	0	0	0	0	ND	ND	ND	ND
54	L2EN-55	16	1	0.5	32	16	128	16	1	0	0	0	0	0	0	+	TEM	-	0
55	L2EN-56	>256	>256	256	128	>256	>256	>256	0	0	0	0	0	0	0	ND	ND	ND	ND

No	isolates	MIC(μg/ml)							The presence of PMQR genes							ESBLs		AmpC	
		NAL	NOR	CIP	FOX	CTX	CAZ	CRO	*qnrA*	*qnrB*	*qnrS*	*qnrC*	*qnrD*	*aac(6')-Ib-cr*	*qepA*	phenotype	Genotype	phenotype	genotype
56	L2EN-57	>256	256	64	16	128	64	128	0	0	0	0	0	0	0	ND	ND	ND	ND
57	L2EN-58	8	2	1	32	0.5	0.5	0.25	0	0	1	0	0	0	0	-	0	-	0
58	L2EN-59	>256	16	8	16	32	4	64	0	0	0	0	0	0	0	ND	ND	ND	ND
59	L2EN-60	4	0.06	0.06	2	2	8	1	0	0	0	0	0	0	0	ND	ND	ND	ND
60	L2EN-61	64	0.5	0.12	32	4	16	8	0	0	0	0	0	0	0	ND	ND	ND	ND
61	L2EN-62	>256	64	16	1	0.5	0.12	0.06	0	0	0	0	0	0	0	ND	ND	ND	ND
62	L2EN-63	>256	>256	64	16	32	16	32	0	0	0	0	0	0	0	ND	ND	ND	ND
63	L2EN-65	16	4	2	4	128	128	128	0	0	0	0	0	0	0	ND	ND	ND	ND
64	L2EN-66	8	0.25	0.06	4	0.01	0.12	0.01	0	0	0	0	0	0	0	ND	ND	ND	ND
65	L2EN-67	16	0.25	0.12	32	0.25	0.5	0.25	0	0	0	0	0	0	0	ND	ND	ND	ND
66	L2EN-68	>256	>256	>256	32	256	>256	256	0	0	1	0	0	0	0	+	SHV, TEM	-	0
67	L2EN-69	32	8	4	4	128	16	64	0	0	0	0	0	0	0	ND	ND	ND	ND
68	L2EN-70	>256	>256	>256	32	>256	>256	>256	0	0	0	0	0	0	0	ND	ND	ND	ND
69	L2EN-71	>256	2	0.5	4	0.06	0.12	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
70	L2EN-72	4	0.06	0.03	4	64	4	32	0	0	0	0	0	0	0	ND	ND	ND	ND

No	isolates	MIC(μg/ml)							The presence of PMQR genes							ESBLs		AmpC	
		NAL	NOR	CIP	FOX	CTX	CAZ	CRO	*qnrA*	*qnrB*	*qnrS*	*qnrC*	*qnrD*	*aac(6')-Ib-cr*	*qepA*	phenotype	Genotype	phenotype	genotype
71	L2EN-73	16	2	0.5	32	32	32	32	0	0	1	0	0	0	0	+	TEM	-	0
72	L2EN-74	4	0.25	0.06	4	0.25	0.25	0.25	0	0	0	0	0	0	0	ND	ND	ND	ND
73	L2EN-75	4	0.25	0.06	4	0.25	0.25	0.25	0	0	0	0	0	0	0	ND	ND	ND	ND
74	L2EN-76	>256	>256	64	128	32	64	32	0	0	0	0	0	0	0	ND	ND	ND	ND
75	L2EN-77	4	0.25	0.03	4	0.01	0.25	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
76	L2EN-78	16	2	0.5	4	64	16	128	0	0	0	0	0	0	0	ND	ND	ND	ND
77	L2EN-79	>256	>256	128	8	64	8	32	0	0	0	0	0	0	0	ND	ND	ND	ND
78	L2EN-80	>256	>256	64	64	8	16	8	0	0	0	0	0	0	0	ND	ND	ND	ND
79	L2EN-81	64	1	0.25	64	8	32	8	0	0	0	0	0	0	0	ND	ND	ND	ND
80	L2EN-82	8	1	0.12	8	128	64	256	0	0	0	0	0	0	0	ND	ND	ND	ND
81	L2EN-83	16	16	4	>256	>256	128	>256	0	0	0	0	0	0	0	ND	ND	ND	ND
82	L2EN-84	8	1	0.06	16	0.12	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
83	L2EN-85	2	0.12	0.03	8	0.12	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
84	L2EN-86	>256	32	8	8	32	4	32	0	0	0	0	0	0	0	ND	ND	ND	ND
85	L2EN-87	>256	32	8	16	0.12	0.5	0.25	0	0	0	0	0	0	0	ND	ND	ND	ND

No	isolates	MIC(μg/ml)							The presence of PMQR genes							ESBLs		AmpC	
		NAL	NOR	CIP	FOX	CTX	CAZ	CRO	*qnrA*	*qnrB*	*qnrS*	*qnrC*	*qnrD*	*aac(6')-Ib-cr*	*qepA*	phenotype	Genotype	phenotype	genotype
86	L2EN-88	4	1	0.06	16	0.12	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
87	L2EN-89	>256	>256	128	16	128	16	128	0	0	0	0	0	0	0	ND	ND	ND	ND
88	L2EN-90	>256	256	32	16	32	8	32	0	0	0	0	0	0	0	ND	ND	ND	ND
89	L2EN-91	4	0.12	0.06	16	0.12	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
90	L2EN-92	>256	>256	256	32	>256	>256	>256	0	0	0	0	0	1	0	+	CTX	-	0
91	L2EN-93	>256	256	64	8	8	0.5	8	0	0	0	0	0	0	0	ND	ND	ND	ND
92	L2EN-94	8	1	0.03	8	0.12	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
93	L2EN-95	16	2	1	32	0.5	0.5	0.25	1	0	0	0	0	0	0	-	0	-	0
94	L2EN-96	8	2	1	32	128	32	128	1	0	0	0	0	0	0	+	CTX	-	0
95	L2EN-97	256	4	1	16	128	32	256	0	0	0	0	0	0	0	ND	ND	ND	ND
96	L2EN-98	>256	256	32	16	16	4	16	0	0	0	0	0	0	0	ND	ND	ND	ND
97	L2EN-99	4	0.12	0.03	16	16	4	16	0	0	0	0	0	0	0	ND	ND	ND	ND
98	L2EN-100	>256	128	32	8	16	1	16	0	0	0	0	0	0	0	ND	ND	ND	ND
99	L2EN-101	>256	>256	128	8	64	32	64	0	0	0	0	0	1	0	+	CTX	-	0
100	L2EN-102	>256	>256	128	8	64	16	128	0	0	0	0	0	1	0	+	CTX	-	0

No	isolates	MIC(μg/ml)							The presence of PMQR genes							ESBLs		AmpC	
		NAL	NOR	CIP	FOX	CTX	CAZ	CRO	*qnrA*	*qnrB*	*qnrS*	*qnrC*	*qnrD*	*aac(6')-Ib-cr*	*qepA*	phenotype	Genotype	phenotype	genotype
101	L2EN-103	16	8	0.25	8	8	64	8	0	0	0	0	0	0	0	ND	ND	ND	ND
102	L2EN-104	128	32	4	8	128	64	256	0	0	0	0	0	1	0	+	CTX	-	0
103	L2EN-105	>256	>256	64	8	32	4	16	0	0	0	0	0	0	0	ND	ND	ND	ND
104	L2EN-106	4	0.25	0.06	8	0.12	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
105	L2EN-107	>256	128	32	16	8	0.5	8	0	0	0	0	0	0	0	ND	ND	ND	ND
106	L2EN-108	>256	256	64	16	>256	256	>256	0	0	0	0	0	0	0	ND	ND	ND	ND
107	L2EN-109	>256	128	32	8	64	16	64	0	0	0	0	0	0	0	ND	ND	ND	ND
108	L2EN-110	16	2	1	32	0.5	0.5	0.25	1	0	0	0	0	0	0	-	0	-	0
109	L2EN-111	64	16	4	32	32	4	128	1	0	0	0	0	0	0	+	SHV, CTX	-	0
110	L2EN-112	4	1	0.06	8	0.12	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
111	L2EN-113	8	0.12	0.01	8	0.12	0.5	0.25	0	0	0	0	0	0	0	ND	ND	ND	ND
112	L2EN-114	1	0.25	0.06	4	0.12	0.25	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
113	L2EN-115	>256	>256	128	32	>256	128	>256	0	0	0	0	0	1	0	+	CTX	-	0
114	L2EN-116	4	0.12	0.06	8	0.12	0.25	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
115	L2EN-117	>256	>256	128	16	0.25	2	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND

No	isolates	MIC(μg/ml)							The presence of PMQR genes							ESBLs		AmpC	
		NAL	NOR	CIP	FOX	CTX	CAZ	CRO	*qnrA*	*qnrB*	*qnrS*	*qnrC*	*qnrD*	*aac(6')-Ib-cr*	*qepA*	phenotype	Genotype	phenotype	genotype
116	L2EN-118	>256	256	64	4	64	32	64	0	0	0	0	0	1	0	+	CTX	-	0
117	L2EN-119	128	256	64	8	32	32	32	0	0	0	0	0	0	0	ND	ND	ND	ND
118	L2EN-120	>256	2	1	16	0.12	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
119	L2EN-121	4	0.12	0.06	16	0.12	4	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
120	L2EN-122	>256	128	64	32	32	>256	64	0	0	0	0	0	0	0	ND	ND	ND	ND
121	L2EN-123	>256	>256	128	128	>256	>256	>256	0	0	0	0	0	0	0	ND	ND	ND	ND
122	L2EN-124	>256	256	32	16	>256	256	>256	0	0	0	0	0	0	0	ND	ND	ND	ND
123	L2EN-125	8	0.5	0.06	16	0.12	2	0.25	0	0	0	0	0	0	0	ND	ND	ND	ND
124	L2EN-126	>256	>256	128	128	>256	>256	>256	0	0	0	0	0	0	0	ND	ND	ND	ND
125	L2EN-127	>256	>256	256	>256	>256	>256	>256	0	0	0	0	0	0	0	ND	ND	ND	ND
126	L2EN-128	4	0.12	0.06	16	0.12	2	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
127	L2EN-129	2	0.12	0.06	16	0.12	2	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
128	L2EN-130	8	0.5	0.06	16	0.12	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
129	L2EN-131	>256	4	2	16	0.12	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
130	L2EN-132	>256	128	64	16	32	64	32	0	0	0	0	0	0	0	ND	ND	ND	ND

No	isolates	MIC(μg/ml)							The presence of PMQR genes							ESBLs		AmpC	
		NAL	NOR	CIP	FOX	CTX	CAZ	CRO	*qnrA*	*qnrB*	*qnrS*	*qnrC*	*qnrD*	*aac(6')-Ib-cr*	*qepA*	phenotype	Genotype	phenotype	genotype
131	L2EN-133	2	0.25	0.06	16	0.25	2	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
132	L2EN-134	>256	>256	128	16	0.25	2	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
133	L2EN-135	2	0.12	0.06	8	0.12	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
134	L2EN-136	8	0.5	0.06	4	0.03	0.25	0.03	0	0	0	0	0	0	0	ND	ND	ND	ND
135	L2EN-137	4	0.12	0.06	16	0.12	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
136	L2EN-138	>256	>256	256	32	256	128	256	0	0	0	0	0	1	0	+	CTX	-	0
137	L2EN-139	4	0.12	0.06	16	0.12	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
138	L2EN-140	4	0.12	0.06	16	0.12	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
139	L2EN-141	2	0.12	0.06	4	64	32	64	0	0	0	0	0	0	0	ND	ND	ND	ND
140	L2EN-142	>256	>256	128	4	64	64	>256	0	0	0	0	0	1	0	+	CTX	-	0
141	L2EN-143	>256	128	32	8	256	64	256	0	0	0	0	0	0	0	ND	ND	ND	ND
142	L2EN-144	1	0.12	0.06	8	0.12	0.5	0.25	0	0	0	0	0	0	0	ND	ND	ND	ND
143	L2EN-145	>256	>256	128	16	>256	>256	256	0	0	0	0	0	0	0	ND	ND	ND	ND
144	L2EN-146	>256	128	16	8	32	64	16	0	0	0	0	0	0	0	ND	ND	ND	ND
145	L2EN-147	4	0.12	0.06	8	0.12	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND

No	isolates	MIC(μg/ml)							The presence of PMQR genes							ESBLs		AmpC	
		NAL	NOR	CIP	FOX	CTX	CAZ	CRO	*qnrA*	*qnrB*	*qnrS*	*qnrC*	*qnrD*	*aac(6')-Ib-cr*	*qepA*	phenotype	Genotype	phenotype	genotype
146	L2EN-148	2	0.12	0.01	16	0.12	0.5	0.5	0	0	0	0	0	0	0	ND	ND	ND	ND
147	L2EN-149	16	4	1	16	64	64	32	0	0	0	0	0	0	0	ND	ND	ND	ND
148	L2EN-150	2	0.12	0.03	128	0.5	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
149	L2EN-151	>256	128	32	128	8	64	16	0	0	0	0	0	0	0	ND	ND	ND	ND
150	L2EN-152	2	0.12	0.01	16	1	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
151	L2EN-153	>256	256	>256	32	0.5	2	0.25	0	0	1	0	0	0	0	-	TEM	-	0
152	L2EN-154	>256	>256	64	16	32	8	32	0	0	0	0	0	0	0	ND	ND	ND	ND
153	L2EN-155	16	4	1	>256	128	64	128	0	0	0	0	0	0	0	ND	ND	ND	ND
154	L2EN-156	2	0.12	0.06	32	0.12	4	16	0	0	0	0	0	0	0	ND	ND	ND	ND
155	L2EN-157	>256	>256	256	32	128	32	64	0	0	0	0	0	0	0	ND	ND	ND	ND
156	L2EN-158	8	1	0.12	16	32	>256	16	1	0	0	0	0	0	0	+	SHV, VEB	-	0
157	L2EN-159	>256	128	32	16	64	32	64	0	0	0	0	0	0	0	ND	ND	ND	ND
158	L2EN-160	>256	16	8	16	64	16	64	0	0	0	0	0	0	0	ND	ND	ND	ND
159	L2EN-161	>256	256	128	64	8	16	8	0	0	0	0	0	0	0	ND	ND	ND	ND
160	L2EN-162	2	0.25	0.01	8	0.12	1	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND

No	isolates	MIC(μg/ml)							The presence of PMQR genes							ESBLs		AmpC	
		NAL	NOR	CIP	FOX	CTX	CAZ	CRO	*qnrA*	*qnrB*	*qnrS*	*qnrC*	*qnrD*	*aac(6')-Ib-cr*	*qepA*	phenotype	Genotype	phenotype	genotype
161	L2EN-163	4	0.12	0.01	8	0.12	1	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
162	L2EN-164	>256	>256	128	16	64	16	64	0	0	0	0	0	0	0	ND	ND	ND	ND
163	L2EN-165	0.12	0.12	0.03	8	0.25	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
164	L2EN-166	256	>256	256	4	0.25	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
165	L2EN-167	256	>256	32	8	0.25	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
166	L2EN-168	0.12	0.12	0.03	8	32	16	32	0	0	0	0	0	0	0	ND	ND	ND	ND
167	L2EN-169	256	>256	64	8	0.25	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
168	L2EN-170	128	256	32	64	16	128	32	0	0	0	0	0	0	0	ND	ND	ND	ND
169	L2EN-171	2	2	0.25	8	0.5	2	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
170	L2EN-172	128	256	32	64	8	64	16	0	0	0	0	0	0	0	ND	ND	ND	ND
171	L2EN-173	0.12	0.12	0.03	8	0.25	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
172	L2EN-174	0.12	0.12	0.03	8	0.25	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
173	L2EN-175	64	128	16	8	64	4	64	0	0	0	0	0	0	0	ND	ND	ND	ND
174	L2EN-176	>256	>256	128	8	0.25	0.5	0.12	0	0	0	0	0	1	0	-	0	-	0
175	L2EN-177	256	8	8	16	256	64	256	1	0	0	0	0	0	0	+	CTX	-	0

No	isolates	MIC(μg/ml)							The presence of PMQR genes							ESBLs		AmpC	
		NAL	NOR	CIP	FOX	CTX	CAZ	CRO	*qnrA*	*qnrB*	*qnrS*	*qnrC*	*qnrD*	*aac(6')-Ib-cr*	*qepA*	phenotype	Genotype	phenotype	genotype
176	L2EN-178	0.25	0.25	0.03	8	0.25	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
177	L2EN-179	0.12	0.12	0.03	8	256	256	256	0	0	0	0	0	0	0	ND	ND	ND	ND
178	L2EN-180	0.12	0.12	0.03	8	0.25	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
179	L2EN-181	>256	>256	>256	32	0.5	1	0.25	1	0	0	0	0	0	0	-	0	-	0
180	L2EN-182	0.12	0.12	0.03	8	0.25	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
181	L2EN-183	256	>256	32	8	0.25	0.5	0.25	0	0	0	0	0	0	0	ND	ND	ND	ND
182	L2EN-184	64	32	8	16	128	64	256	1	0	0	0	0	0	0	+	CTX, VEB	-	0
183	L2EN-185	8	1	0.25	16	0.25	0.5	0.25	0	0	1	0	0	0	0	-	0	-	0
184	L2EN-186	>256	256	256	32	1	1	4	0	0	1	0	0	0	0	-	TEM	-	0
185	L2EN-187	>256	128	64	64	32	32	64	0	0	1	0	0	0	0	+	SHV, TEM	-	0
186	L2EN-188	>256	>256	64	8	64	16	64	0	0	0	0	0	0	0	ND	ND	ND	ND
187	L2EN-189	0.12	0.12	0.03	8	0.25	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
188	L2EN-190	>256	>256	256	4	256	256	256	0	0	0	0	0	1	0	+	TEM, CTX	-	0
189	L2EN-191	128	>256	16	8	32	8	32	0	0	0	0	0	0	0	ND	ND	ND	ND
190	L2EN-192	0.12	0.12	0.03	8	0.25	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND

No	isolates	MIC(μg/ml)							The presence of PMQR genes							ESBLs		AmpC	
		NAL	NOR	CIP	FOX	CTX	CAZ	CRO	*qnrA*	*qnrB*	*qnrS*	*qnrC*	*qnrD*	*aac(6')-Ib-cr*	*qepA*	phenotype	Genotype	phenotype	genotype
191	L2EN-193	8	1	0.25	64	128	64	128	0	0	0	0	1	0	0	+	SHV	+	EBC
192	L2EN-194	256	256	32	256	64	32	64	0	0	0	0	0	0	0	ND	ND	ND	ND
193	L2EN-195	256	>256	32	8	256	64	128	0	0	0	0	0	0	0	ND	ND	ND	ND
194	L2EN-196	0.12	0.12	0.03	8	0.25	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
195	L2EN-197	8	8	16	64	256	>256	256	0	0	0	0	0	1	0	+	TEM, CTX	-	0
196	L2EN-198	0.12	0.12	0.03	8	0.25	0.5	0.25	0	0	0	0	0	0	0	ND	ND	ND	ND
197	L2EN-199	>256	>256	64	8	256	64	128	0	0	0	0	0	1	0	+	CTX	-	0
198	L2EN-200	>256	128	16	4	16	16	8	0	0	0	0	0	0	0	ND	ND	ND	ND
199	L2EN-201	16	4	2	4	128	128	64	0	0	0	0	0	1	0	+	TEM, CTX	-	0
200	L2EN-202	>256	>256	128	16	64	64	16	0	0	0	0	0	0	0	ND	ND	ND	ND
201	L2EN-203	4	0.12	0.03	4	0.25	0.25	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
202	L2EN-204	>256	>256	256	16	256	256	256	0	0	0	0	0	0	0	ND	ND	ND	ND
203	L2EN-205	8	0.12	0.03	4	0.12	0.25	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
204	L2EN-206	16	4	0.5	8	256	>256	>256	0	0	0	0	0	1	0	+	TEM, CTX	-	0
205	L2EN-207	32	2	0.5	8	64	64	32	0	0	0	0	0	0	0	ND	ND	ND	ND

No	isolates	MIC(μg/ml)							The presence of PMQR genes							ESBLs		AmpC	
		NAL	NOR	CIP	FOX	CTX	CAZ	CRO	*qnrA*	*qnrB*	*qnrS*	*qnrC*	*qnrD*	*aac(6')-Ib-cr*	*qepA*	phenotype	Genotype	phenotype	genotype
206	L2EN-208	4	0.12	0.03	8	0.12	0.25	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
207	L2EN-209	8	2	0.25	64	64	64	128	0	0	0	0	1	0	0	-	TEM	+	EBC
208	L2EN-210	>256	>256	32	4	0.12	0.25	0.25	0	0	0	0	0	0	0	ND	ND	ND	ND
209	L2EN-211	>256	>256	128	16	0.25	0.25	0.5	0	0	0	0	0	0	0	ND	ND	ND	ND
210	L2EN-212	2	0.12	0.03	16	0.12	0.25	0.25	0	0	0	0	0	0	0	ND	ND	ND	ND
211	L2EN-213	>256	256	64	16	256	256	128	0	0	0	0	0	1	0	+	CTX	-	0
212	L2EN-214	>256	256	64	8	64	64	32	0	0	0	0	0	1	0	+	CTX	-	0
213	L2EN-215	4	0.12	0.03	8	0.12	0.25	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
214	L2EN-216	>256	16	4	64	8	16	64	0	0	0	0	0	0	0	ND	ND	ND	ND
215	L2EN-217	2	0.12	0.03	16	0.12	0.25	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
216	L2EN-218	256	4	2	16	256	64	>256	1	0	0	0	0	0	0	+	CTX	-	0
217	L2EN-219	4	0.12	0.03	16	0.12	0.25	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
218	L2EN-220	>256	256	32	4	256	256	64	0	0	0	0	0	0	0	ND	ND	ND	ND
219	L2EN-221	64	32	8	8	256	256	>256	0	0	0	0	0	1	0	+	CTX	-	0
220	L2EN-222	32	2	0.5	4	64	64	64	0	0	0	0	0	0	0	ND	ND	ND	ND

No	isolates	MIC(μg/ml)							The presence of PMQR genes							ESBLs		AmpC	
		NAL	NOR	CIP	FOX	CTX	CAZ	CRO	*qnrA*	*qnrB*	*qnrS*	*qnrC*	*qnrD*	*aac(6')-Ib-cr*	*qepA*	phenotype	Genotype	phenotype	genotype
221	L2EN-223	2	0.12	0.06	8	0.12	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
222	L2EN-224	>256	>256	256	8	128	64	128	0	0	0	0	0	1	0	+	CTX	-	0
223	L2EN-225	2	0.12	0.06	8	0.5	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
224	L2EN-226	2	0.12	0.06	8	0.5	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
225	L2EN-227	>256	>256	128	8	128	32	128	0	0	0	0	0	1	0	+	CTX	-	0
226	L2EN-228	>256	>256	128	8	>256	128	>256	0	0	0	0	0	1	0	+	CTX	-	0
227	L2EN-229	4	0.12	0.06	128	0.5	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
228	L2EN-230	2	0.12	0.06	8	0.25	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
229	L2EN-231	8	0.12	0.06	8	0.12	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
230	L2EN-232	256	4	4	8	256	64	256	0	0	0	0	0	0	0	ND	ND	ND	ND
231	L2EN-233	>256	>256	128	8	16	8	8	0	0	0	0	0	1	0	-	0	-	0
232	L2EN-234	4	0.5	0.12	8	128	32	128	0	0	0	0	0	1	0	+	CTX	-	0
233	L2EN-235	>256	256	32	8	0.5	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
234	L2EN-236	4	0.12	0.06	8	0.5	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
235	L2EN-237	>256	32	8	8	32	256	64	0	0	0	0	0	0	0	ND	ND	ND	ND

No	isolates	MIC(μg/ml)							The presence of PMQR genes							ESBLs		AmpC	
		NAL	NOR	CIP	FOX	CTX	CAZ	CRO	*qnrA*	*qnrB*	*qnrS*	*qnrC*	*qnrD*	*aac(6')-Ib-cr*	*qepA*	phenotype	Genotype	phenotype	genotype
236	L2EN-238	>256	>256	64	8	256	8	1	0	0	0	0	0	0	0	ND	ND	ND	ND
237	L2EN-239	4	0.12	0.06	4	0.12	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
238	L2EN-240	2	0.12	0.06	8	0.12	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
239	L2EN-241	2	0.12	0.06	8	0.12	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
240	L2EN-242	>256	256	32	8	32	2	64	0	0	0	0	0	0	0	ND	ND	ND	ND
241	L2EN-243	4	0.12	0.06	4	0.12	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
242	L2EN-244	256	4	4	64	256	64	256	0	0	0	0	0	0	0	ND	ND	ND	ND
243	L2EN-245	2	0.12	0.06	256	16	64	16	0	0	0	0	0	0	0	ND	ND	ND	ND
244	L2EN-246	4	0.12	0.06	4	0.12	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
245	L2EN-247	2	0.12	0.06	8	128	32	64	0	0	0	0	0	0	0	ND	ND	ND	ND
246	L2EN-248	8	1	0.25	8	8	2	16	0	0	0	0	0	0	0	ND	ND	ND	ND
247	L2EN-249	2	0.12	0.06	4	0.12	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
248	L2EN-250	2	0.12	0.06	4	0.12	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
249	L2EN-251	>256	>256	128	256	0.25	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
250	L2EN-252	4	0.12	0.06	4	0.25	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND

No	isolates	MIC(μg/ml)							The presence of PMQR genes							ESBLs		AmpC	
		NAL	NOR	CIP	FOX	CTX	CAZ	CRO	*qnrA*	*qnrB*	*qnrS*	*qnrC*	*qnrD*	*aac(6')-Ib-cr*	*qepA*	phenotype	Genotype	phenotype	genotype
251	L2EN-253	2	1	0.12	4	64	32	64	0	0	0	0	0	0	0	ND	ND	ND	ND
252	L2EN-254	1	0.12	0.06	4	0.25	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
253	L2EN-255	1	0.12	0.06	4	0.25	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
254	L2EN-256	>256	256	32	4	0.25	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
255	L2EN-257	>256	256	32	8	128	128	256	0	0	0	0	0	0	0	ND	ND	ND	ND
256	L2EN-258	2	0.12	0.06	8	0.5	0.5	0.25	0	0	0	0	0	0	0	ND	ND	ND	ND
257	L2EN-259	>256	128	64	8	32	2	64	0	0	0	0	0	0	0	ND	ND	ND	ND
258	L2EN-260	1	0.12	0.06	4	0.25	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
259	L2EN-261	>256	4	4	8	8	0.5	8	0	0	0	0	0	0	0	ND	ND	ND	ND
260	L2EN-262	4	0.12	0.06	4	0.25	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
261	L2EN-263	>256	>256	128	32	64	16	64	0	0	0	0	0	0	0	ND	ND	ND	ND
262	L2EN-264	>256	256	32	8	0.25	0.5	0.25	0	0	0	0	0	0	0	ND	ND	ND	ND
263	L2EN-265	256	16	4	8	8	0.5	8	0	0	0	0	0	0	0	ND	ND	ND	ND
264	L2EN-266	16	4	0.5	8	256	256	>256	0	0	0	0	0	0	0	ND	ND	ND	ND
265	L2EN-267	>256	>256	32	8	0.25	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND

No	isolates	MIC(μg/ml)							The presence of PMQR genes							ESBLs		AmpC	
		NAL	NOR	CIP	FOX	CTX	CAZ	CRO	*qnrA*	*qnrB*	*qnrS*	*qnrC*	*qnrD*	*aac(6')-Ib-cr*	*qepA*	phenotype	Genotype	phenotype	genotype
266	L2EN-268	>256	32	8	64	8	32	16	0	0	0	0	0	0	0	ND	ND	ND	ND
267	L2EN-269	2	0.12	0.06	4	0.25	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
268	L2EN-270	8	2	1	32	0.25	0.25	1	0	0	1	0	0	0	0	-	TEM	-	0
269	L2EN-271	>256	>256	>256	4	128	64	128	0	0	0	0	0	0	1	+	CTX, TEM	-	0
270	L2EN-272	2	0.25	0.12	4	0.25	0.5	0.25	0	0	0	0	0	0	0	ND	ND	ND	ND
271	L2EN-273	4	0.12	0.06	4	0.25	0.5	0.25	0	0	0	0	0	0	0	ND	ND	ND	ND
272	L2EN-274	>256	128	32	4	16	4	16	0	0	0	0	0	0	0	ND	ND	ND	ND
273	L2EN-275	32	4	1	16	16	4	32	1	0	0	0	0	0	0	+	TEM	-	0
274	L2EN-276	>256	>256	64	8	256	128	128	0	0	0	0	0	0	0	ND	ND	ND	ND
275	L2EN-277	>256	>256	128	16	128	16	>256	0	0	0	0	0	0	0	ND	ND	ND	ND
276	L2EN-278	4	0.12	0.06	8	0.25	1	0.25	0	0	0	0	0	0	0	ND	ND	ND	ND
277	L2EN-279	>256	>256	256	16	64	4	64	0	0	0	0	0	0	0	ND	ND	ND	ND
278	L2EN-280	128	4	2	16	0.5	1	0.25	0	0	0	0	0	0	0	ND	ND	ND	ND
279	L2EN-281	>256	16	8	8	0.25	1	0.25	0	0	0	0	0	0	0	ND	ND	ND	ND
280	L2EN-282	>256	16	8	8	0.25	1	0.25	0	0	0	0	0	0	0	ND	ND	ND	ND

No	isolates	MIC(μg/ml)							The presence of PMQR genes							ESBLs		AmpC	
		NAL	NOR	CIP	FOX	CTX	CAZ	CRO	*qnrA*	*qnrB*	*qnrS*	*qnrC*	*qnrD*	*aac(6')-Ib-cr*	*qepA*	phenotype	Genotype	phenotype	genotype
281	L2EN-283	4	0.12	0.06	16	0.12	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
282	L2EN-284	>256	256	128	64	1	2	1	0	0	0	0	1	0	0	-	TEM	-	0
283	L2EN-285	>256	256	32	8	0.12	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
284	L2EN-286	>256	32	8	8	>256	>256	>256	0	0	0	0	0	0	0	ND	ND	ND	ND
285	L2EN-287	16	8	2	16	64	32	128	0	0	0	0	0	1	0	+	TEM, CTX	-	0
286	L2EN-288	>256	256	64	>256	>256	>256	>256	0	0	1	0	0	0	0	+	TEM, CTX	-	0
287	L2EN-289	>256	64	16	8	16	4	16	0	0	0	0	0	0	0	ND	ND	ND	ND
288	L2EN-290	16	1	0.25	256	64	64	128	0	0	0	0	0	0	0	ND	ND	ND	ND
289	L2EN-291	2	0.12	0.06	8	0.12	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
290	L2EN-292	4	0.12	0.06	16	0.12	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
291	L2EN-293	2	0.12	0.06	4	0.25	0.5	0.5	0	0	0	0	0	0	0	ND	ND	ND	ND
292	L2EN-294	8	0.12	0.06	64	8	32	16	0	0	0	0	0	0	0	ND	ND	ND	ND
293	L2EN-295	>256	128	64	8	8	128	16	0	0	1	0	0	0	0	+	TEM	-	0
294	L2EN-296	4	0.12	0.06	4	0.12	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
295	L2EN-297	2	0.12	0.06	4	0.12	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND

No	isolates	MIC(μg/ml)							The presence of PMQR genes							ESBLs		AmpC	
		NAL	NOR	CIP	FOX	CTX	CAZ	CRO	*qnrA*	*qnrB*	*qnrS*	*qnrC*	*qnrD*	*aac(6')-Ib-cr*	*qepA*	phenotype	Genotype	phenotype	genotype
296	L2EN-298	4	0.12	0.06	8	0.12	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
297	L2EN-299	8	1	1	32	0.25	1	1	0	0	1	0	0	0	0	-	0	-	0
298	L2EN-300	>256	128	32	8	16	16	32	0	0	0	0	0	0	0	ND	ND	ND	ND
299	L2EN-301	2	0.12	0.06	8	0.12	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
300	L2EN-302	2	0.12	0.06	8	0.12	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
301	L2EN-303	>256	>256	128	16	64	16	64	0	0	0	0	0	0	0	ND	ND	ND	ND
302	L2EN-304	>256	32	8	8	64	16	64	0	0	0	0	0	0	0	ND	ND	ND	ND
303	L2EN-305	>256	>256	64	64	8	64	16	0	0	0	0	0	0	0	ND	ND	ND	ND
304	L2EN-306	>256	128	32	8	16	8	32	0	0	0	0	0	0	0	ND	ND	ND	ND
305	L2EN-307	>256	128	16	4	16	4	16	0	0	0	0	0	0	0	ND	ND	ND	ND
306	L2EN-308	4	0.12	0.03	8	16	2	32	0	0	0	0	0	0	0	ND	ND	ND	ND
307	L2EN-309	16	4	1	32	32	16	32	0	0	0	0	0	0	0	ND	ND	ND	ND
308	L2EN-310	4	0.25	0.12	16	0.25	1	0.25	1	0	0	0	0	0	0	-	0	-	0
309	L2EN-311	8	1	0.25	16	16	1	16	0	0	1	0	0	0	0	+	SHV, TEM, CTX	-	0
310	L2EN-312	8	4	0.5	16	16	2	32	0	0	1	0	0	0	0	+	SHV, TEM, CTX	-	0

No	isolates	MIC(μg/ml)							The presence of PMQR genes							ESBLs		AmpC	
		NAL	NOR	CIP	FOX	CTX	CAZ	CRO	*qnrA*	*qnrB*	*qnrS*	*qnrC*	*qnrD*	*aac(6')-Ib-cr*	*qepA*	phenotype	Genotype	phenotype	genotype
311	L2EN-313	8	0.25	0.25	8	0.5	0.25	0.12	0	0	0	0	1	0	0	-	0	-	0
312	L2EN-314	>256	>256	32	8	256	128	256	0	0	0	0	0	0	0	ND	ND	ND	ND
313	L2EN-315	2	0.12	0.03	4	0.06	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
314	L2EN-316	>256	>256	128	16	256	64	256	0	0	0	0	0	0	0	ND	ND	ND	ND
315	L2EN-317	>256	256	64	8	64	16	64	0	0	0	0	0	0	0	ND	ND	ND	ND
316	L2EN-318	>256	>256	128	32	>256	256	>256	0	0	0	0	0	0	0	ND	ND	ND	ND
317	L2EN-319	>256	256	64	8	64	16	64	0	0	0	0	0	0	0	ND	ND	ND	ND
318	L2EN-320	>256	64	16	16	128	32	128	0	0	0	0	0	0	0	ND	ND	ND	ND
319	L2EN-321	2	0.12	0.06	8	128	32	256	0	0	0	0	0	0	0	ND	ND	ND	ND
320	L2EN-322	2	0.12	0.06	4	0.06	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
321	L2EN-323	>256	256	64	4	64	16	64	0	0	0	0	0	0	0	ND	ND	ND	ND
322	L2EN-324	2	0.12	0.03	8	128	32	64	0	0	0	0	0	0	0	ND	ND	ND	ND
323	L2EN-325	2	0.12	0.03	8	0.06	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
324	L2EN-326	2	0.12	0.06	4	0.12	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
325	L2EN-327	2	0.12	0.06	4	0.12	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND

No	isolates	MIC(μg/ml)							The presence of PMQR genes							ESBLs		AmpC	
		NAL	NOR	CIP	FOX	CTX	CAZ	CRO	*qnrA*	*qnrB*	*qnrS*	*qnrC*	*qnrD*	*aac(6')-Ib-cr*	*qepA*	phenotype	Genotype	phenotype	genotype
326	L2EN-328	>256	32	8	8	128	64	128	0	0	0	0	0	0	0	ND	ND	ND	ND
327	L2EN-329	16	16	4	>256	>256	128	>256	0	0	0	0	0	1	0	+	TEM, CTX	+	DHA
328	L2EN-330	>256	>256	256	16	0.25	2	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
329	L2EN-331	2	0.12	0.06	4	0.12	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
330	L2EN-332	4	0.12	0.06	4	0.12	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
331	L2EN-333	>256	32	8	16	128	32	128	0	0	0	0	0	0	0	ND	ND	ND	ND
332	L2EN-334	>256	>256	128	8	256	32	128	0	0	0	0	0	1	0	+	CTX	-	0
333	L2EN-335	>256	>256	64	8	0.12	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
334	L2EN-336	>256	2	1	8	0.12	0.25	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
335	L2EN-337	8	1	0.25	4	0.25	0.25	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
336	L2EN-338	>256	8	8	16	32	2	16	0	0	0	0	0	0	0	ND	ND	ND	ND
337	L2EN-339	8	0.12	0.03	16	>256	32	128	0	0	0	0	0	0	0	ND	ND	ND	ND
338	L2EN-340	4	0.12	0.03	16	0.25	0.25	0.25	0	0	0	0	0	0	0	ND	ND	ND	ND
339	L2EN-341	>256	>256	128	8	256	128	256	0	0	0	0	0	0	0	ND	ND	ND	ND
340	L2EN-342	4	0.12	0.03	8	64	32	128	0	0	0	0	0	0	0	ND	ND	ND	ND

No	isolates	MIC(μg/ml)							The presence of PMQR genes							ESBLs		AmpC	
		NAL	NOR	CIP	FOX	CTX	CAZ	CRO	*qnrA*	*qnrB*	*qnrS*	*qnrC*	*qnrD*	*aac(6')-Ib-cr*	*qepA*	phenotype	Genotype	phenotype	genotype
341	L2EN-343	>256	4	1	16	0.25	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
342	L2EN-344	16	4	2	8	32	8	32	0	0	0	0	0	0	0	ND	ND	ND	ND
343	L2EN-345	4	0.12	0.03	8	0.25	0.25	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
344	L2EN-346	>256	>256	128	32	64	4	64	0	0	0	0	0	0	0	ND	ND	ND	ND
345	L2EN-347	>256	128	32	16	64	4	64	0	0	0	0	0	0	0	ND	ND	ND	ND
346	L2EN-348	8	2	0.5	2	0.5	1	0.12	0	0	1	0	0	0	0	-	TEM	-	0
347	L2EN-349	4	2	1	2	64	32	128	0	0	1	0	0	0	0	+	TEM	-	0
348	L2EN-350	4	0.12	0.06	8	0.25	0.25	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
349	L2EN-351	>256	>256	128	8	64	32	128	0	0	0	0	0	1	0	+	CTX	-	0
350	L2EN-352	>256	>256	64	16	128	64	256	0	0	0	0	0	1	0	+	CTX	-	0
351	L2EN-353	4	0.12	0.03	16	0.12	0.25	256	0	0	0	0	0	0	0	ND	ND	ND	ND
352	L2EN-354	4	0.12	0.03	64	0.12	0.25	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
353	L2EN-355	4	0.12	0.03	64	0.12	0.25	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
354	L2EN-356	16	4	2	16	>256	64	256	0	0	0	0	0	0	0	ND	ND	ND	ND
355	L2EN-357	4	0.12	0.03	16	0.25	0.25	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND

No	isolates	MIC(μg/ml)							The presence of PMQR genes							ESBLs		AmpC	
		NAL	NOR	CIP	FOX	CTX	CAZ	CRO	*qnrA*	*qnrB*	*qnrS*	*qnrC*	*qnrD*	*aac(6')-Ib-cr*	*qepA*	phenotype	Genotype	phenotype	genotype
356	L2EN-358	16	1	0.25	>256	128	>256	64	1	0	0	0	0	0	0	+	TEM, VEB	+	DHA
357	L2EN-359	>256	8	4	8	0.12	0.25	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
358	L2EN-360	4	0.12	0.03	8	0.12	0.25	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
359	L2EN-361	2	0.12	0.06	8	0.25	0.25	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
360	L2EN-362	8	0.12	0.06	16	0.5	1	0.25	0	0	0	0	0	0	0	ND	ND	ND	ND
361	L2EN-363	16	1	0.5	>256	128	>256	128	0	0	1	0	0	0	0	+	TEM, VEB	+	CIT
362	L2EN-364	>256	64	32	16	32	128	32	0	0	0	0	0	0	0	ND	ND	ND	ND
363	L2EN-365	8	1	0.5	32	0.25	2	0.25	0	0	0	0	0	0	0	ND	ND	ND	ND
364	L2EN-366	4	0.12	0.06	8	0.12	0.25	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
365	L2EN-367	>256	>256	128	8	>256	256	>256	0	0	0	0	0	1	0	+	CTX	-	0
366	L2EN-368	>256	>256	64	16	256	64	256	0	0	0	0	0	0	0	ND	ND	ND	ND
367	L2EN-369	>256	>256	256	16	64	32	64	0	0	0	0	0	0	0	ND	ND	ND	ND
368	L2EN-370	>256	16	8	2	32	4	32	0	0	0	0	1	0	0	+	SHV, TEM	-	0
369	L2EN-371	4	0.12	0.06	8	128	32	128	0	0	0	0	0	0	0	ND	ND	ND	ND
370	L2EN-372	2	0.12	0.06	8	0.25	0.25	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND

No	isolates	MIC(μg/ml)							The presence of PMQR genes							ESBLs		AmpC	
		NAL	NOR	CIP	FOX	CTX	CAZ	CRO	*qnrA*	*qnrB*	*qnrS*	*qnrC*	*qnrD*	*aac(6')-Ib-cr*	*qepA*	phenotype	Genotype	phenotype	genotype
371	L2EN-373	16	0.25	0.06	16	0.25	0.25	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
372	L2EN-374	>256	>256	128	16	128	16	128	0	0	0	0	0	1	0	+	CTX	-	0
373	L2EN-375	>256	256	32	16	128	32	128	0	0	0	0	0	0	0	ND	ND	ND	ND
374	L2EN-376	2	0.12	0.06	16	0.25	0.25	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
375	L2EN-377	>256	16	8	16	16	1	32	0	0	0	0	0	0	0	ND	ND	ND	ND
376	L2EN-378	4	0.12	0.06	8	0.25	0.25	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
377	L2EN-379	4	0.12	0.06	8	128	16	256	0	0	0	0	0	0	0	ND	ND	ND	ND
378	L2EN-380	2	0.25	0.06	2	0.25	1	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
379	L2EN-381	2	0.12	0.06	32	0.5	8	0.5	0	0	0	0	0	0	0	ND	ND	ND	ND
380	L2EN-382	>256	>256	>256	32	128	32	128	0	0	0	0	0	0	1	+	CTX	-	0
381	L2EN-383	>256	>256	256	256	128	128	128	0	0	1	0	0	0	0	+	TEM, CTX	+	DHA
382	L2EN-384	>256	>256	128	16	256	128	>256	0	0	0	0	0	0	0	ND	ND	ND	ND
383	L2EN-385	4	2	1	8	128	32	128	0	0	1	0	0	0	0	+	CTX	-	0
384	L2EN-386	>256	>256	128	8	128	32	128	0	0	0	0	0	0	0	ND	ND	ND	ND
385	L2EN-387	>256	256	32	8	64	8	64	0	0	0	0	0	0	0	ND	ND	ND	ND

No	isolates	MIC(μg/ml)							The presence of PMQR genes							ESBLs		AmpC	
		NAL	NOR	CIP	FOX	CTX	CAZ	CRO	*qnrA*	*qnrB*	*qnrS*	*qnrC*	*qnrD*	*aac(6')-Ib-cr*	*qepA*	phenotype	Genotype	phenotype	genotype
386	L2EN-388	>256	>256	128	8	64	32	64	0	0	0	0	0	0	0	ND	ND	ND	ND
387	L2EN-389	>256	>256	256	32	0.25	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
388	L2EN-390	>256	>256	128	32	256	32	128	0	0	0	0	0	0	0	ND	ND	ND	ND
389	L2EN-391	16	0.5	0.12	8	0.25	1	0.25	0	0	0	0	0	0	0	ND	ND	ND	ND
390	L2EN-392	>256	32	16	8	64	4	32	0	0	0	0	0	0	0	ND	ND	ND	ND
391	L2EN-393	>256	128	32	8	32	4	16	0	0	0	0	0	0	0	ND	ND	ND	ND
392	L2EN-394	8	2	1	8	128	32	128	0	0	0	0	0	0	0	ND	ND	ND	ND
393	L2EN-395	4	0.12	0.06	8	0.25	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
394	L2EN-396	8	8	2	8	>256	128	>256	0	0	0	0	0	0	0	ND	ND	ND	ND
395	L2EN-397	>256	>256	128	16	128	16	64	1	0	0	0	0	0	0	+	TEM, CTX	-	0
396	L2EN-398	64	2	2	2	0.5	0.5	0.12	1	0	0	0	0	0	0	-	TEM	-	0
397	L2EN-399	>256	128	32	8	128	64	256	0	0	0	0	0	1	0	+	CTX	-	0
398	L2EN-400	>256	64	16	8	0.25	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
399	L2EN-401	>256	>256	64	256	256	128	128	0	0	0	0	0	0	0	ND	ND	ND	ND
400	L2EN-402	>256	256	32	8	32	8	32	0	0	0	0	0	0	0	ND	ND	ND	ND

No	isolates	MIC(μg/ml)							The presence of PMQR genes							ESBLs		AmpC	
		NAL	NOR	CIP	FOX	CTX	CAZ	CRO	*qnrA*	*qnrB*	*qnrS*	*qnrC*	*qnrD*	*aac(6')-Ib-cr*	*qepA*	phenotype	Genotype	phenotype	genotype
401	L2EN-403	4	0.12	0.06	8	0.12	0.12	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
402	L2EN-404	4	0.12	0.06	8	0.25	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
403	L2EN-405	>256	>256	256	8	128	32	128	0	0	0	0	0	1	0	+	CTX	-	0
404	L2EN-406	8	2	0.25	16	32	16	32	0	0	1	0	0	0	0	+	TEM, CTX	-	0
405	L2EN-407	>256	>256	64	2	0.5	0.5	0.12	0	0	1	0	0	0	0	-	0	-	0
406	L2EN-408	>256	64	16	8	256	64	256	0	0	0	0	0	0	0	ND	ND	ND	ND
407	L2EN-409	>256	256	64	8	0.25	1	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
408	L2EN-410	8	4	2	4	32	16	32	0	0	0	0	0	0	0	ND	ND	ND	ND
409	L2EN-411	>256	>256	128	4	32	4	32	0	0	0	0	0	0	0	ND	ND	ND	ND
410	L2EN-412	>256	256	64	4	64	8	64	0	0	0	0	0	1	0	+	CTX	-	0
411	L2EN-413	>256	256	32	8	0.12	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
412	L2EN-414	4	0.12	0.03	4	0.12	0.25	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
413	L2EN-415	256	0.5	0.12	4	0.25	0.5	0.5	0	0	0	0	0	0	0	ND	ND	ND	ND
414	L2EN-416	>256	64	8	4	16	8	32	0	0	0	0	0	0	0	ND	ND	ND	ND
415	L2EN-417	>256	>256	64	4	0.25	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND

No	isolates	MIC(μg/ml)							The presence of PMQR genes							ESBLs		AmpC	
		NAL	NOR	CIP	FOX	CTX	CAZ	CRO	*qnrA*	*qnrB*	*qnrS*	*qnrC*	*qnrD*	*aac(6')-Ib-cr*	*qepA*	phenotype	Genotype	phenotype	genotype
416	L2EN-418	>256	256	64	4	64	16	64	0	0	0	0	0	0	0	ND	ND	ND	ND
417	L2EN-419	16	0.5	0.06	4	0.5	16	0.5	0	0	0	0	0	0	0	ND	ND	ND	ND
418	L2EN-420	>256	>256	64	16	4	8	4	0	0	0	0	0	1	0	-	TEM	-	0
419	L2EN-421	>256	>256	128	16	16	2	32	0	0	0	0	0	0	0	ND	ND	ND	ND
420	L2EN-422	>256	256	64	8	0.25	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
421	L2EN-423	>256	256	32	8	0.25	0.5	0.25	0	0	0	0	0	0	0	ND	ND	ND	ND
422	L2EN-424	>256	8	8	8	1	1	0.25	0	0	0	0	1	0	0	-	0	-	0
423	L2EN-425	>256	>256	>256	32	256	64	256	0	0	0	0	0	0	1	+	CTX, TEM	-	0
424	L2EN-426	4	0.12	0.06	8	0.25	0.5	0.25	0	0	0	0	0	0	0	ND	ND	ND	ND
425	L2EN-427	4	0.12	0.06	8	0.25	1	0.25	0	0	0	0	0	0	0	ND	ND	ND	ND
426	L2EN-428	>256	128	32	4	16	4	16	0	0	0	0	0	0	0	ND	ND	ND	ND
427	L2EN-429	2	0.12	0.03	4	0.25	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
428	L2EN-430	>256	128	32	4	128	32	128	0	0	0	0	0	0	0	ND	ND	ND	ND
429	L2EN-431	2	0.12	0.06	4	0.25	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
430	L2EN-432	16	2	1	4	256	256	256	0	0	0	0	0	0	0	ND	ND	ND	ND

No	isolates	MIC(μg/ml)							The presence of PMQR genes							ESBLs		AmpC	
		NAL	NOR	CIP	FOX	CTX	CAZ	CRO	*qnrA*	*qnrB*	*qnrS*	*qnrC*	*qnrD*	*aac(6')-Ib-cr*	*qepA*	phenotype	Genotype	phenotype	genotype
431	L2EN-433	>256	128	32	4	16	4	16	0	0	0	0	0	0	0	ND	ND	ND	ND
432	L2EN-434	4	0.12	0.06	8	32	2	32	0	0	0	0	0	0	0	ND	ND	ND	ND
433	L2EN-435	>256	>256	128	8	64	16	64	0	0	0	0	0	1	0	+	CTX	+	CIT
434	L2EN-436	4	0.12	0.06	8	0.25	1	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
435	L2EN-437	>256	>256	64	16	>256	32	>256	0	0	0	0	0	0	0	ND	ND	ND	ND
436	L2EN-438	128	1	0.25	8	0.25	0.5	0.25	0	0	0	0	0	0	0	ND	ND	ND	ND
437	L2EN-439	>256	128	32	128	16	32	32	0	0	0	0	0	0	0	ND	ND	ND	ND
438	L2EN-440	4	0.12	0.01	8	0.5	0.12	0.5	0	0	0	0	0	0	0	ND	ND	ND	ND
439	L2EN-441	4	0.12	0.01	8	256	256	128	0	0	0	0	0	0	0	ND	ND	ND	ND
440	L2EN-442	4	0.12	0.01	8	0.25	0.25	0.25	0	0	0	0	0	0	0	ND	ND	ND	ND
441	L2EN-443	4	0.12	0.01	8	0.25	0.12	0.25	0	0	0	0	0	0	0	ND	ND	ND	ND
442	L2EN-444	4	0.12	0.01	8	2	0.12	1	0	0	0	0	0	0	0	ND	ND	ND	ND
443	L2EN-445	>256	256	128	128	128	128	64	0	0	0	0	0	0	0	ND	ND	ND	ND
444	L2EN-446	>256	>256	128	32	128	128	64	0	0	0	0	0	1	0	+	CTX	-	0
445	L2EN-447	4	0.12	0.01	8	0.25	0.12	2	0	0	0	0	0	0	0	ND	ND	ND	ND

No	isolates	MIC(μg/ml)							The presence of PMQR genes							ESBLs		AmpC	
		NAL	NOR	CIP	FOX	CTX	CAZ	CRO	*qnrA*	*qnrB*	*qnrS*	*qnrC*	*qnrD*	*aac(6')-Ib-cr*	*qepA*	phenotype	Genotype	phenotype	genotype
446	L2EN-448	>256	128	32	4	16	16	4	0	0	0	0	0	0	0	ND	ND	ND	ND
447	L2EN-449	32	1	0.25	32	1	0.5	1	0	0	0	0	0	0	0	ND	ND	ND	ND
448	L2EN-450	8	1	0.12	4	16	32	2	0	0	0	0	0	0	0	ND	ND	ND	ND
449	L2EN-451	8	0.12	0.01	4	32	0.12	2	0	0	0	0	0	0	0	ND	ND	ND	ND
450	L2EN-452	4	0.12	0.01	32	0.5	0.12	0.5	0	0	0	0	0	0	0	ND	ND	ND	ND
451	L2EN-453	16	16	4	8	64	64	32	0	0	0	0	0	0	0	ND	ND	ND	ND
452	L2EN-454	8	1	0.12	8	32	32	4	0	0	0	0	0	0	0	ND	ND	ND	ND
453	L2EN-455	4	0.12	0.01	4	0.5	0.25	0.5	0	0	0	0	0	0	0	ND	ND	ND	ND
454	L2EN-456	4	0.25	0.03	8	0.5	0.5	1	0	0	0	0	0	0	0	ND	ND	ND	ND
455	L2EN-457	128	1	0.12	8	0.5	0.12	0.5	0	0	0	0	0	0	0	ND	ND	ND	ND
456	L2EN-458	8	2	1	2	32	2	32	0	0	1	0	0	0	0	+	SHV, CTX	-	0
457	L2EN-459	4	0.12	0.01	8	0.5	0.12	1	0	0	0	0	0	0	0	ND	ND	ND	ND
458	L2EN-460	4	0.12	0.01	4	0.5	0.12	0.5	0	0	0	0	0	0	0	ND	ND	ND	ND
459	L2EN-461	8	2	1	8	0.25	1	0.12	0	0	1	0	0	0	0	-	0	-	0
460	L2EN-462	4	0.12	0.01	4	0.5	0.12	0.5	0	0	0	0	0	0	0	ND	ND	ND	ND

No	isolates	MIC(μg/ml)							The presence of PMQR genes							ESBLs		AmpC	
		NAL	NOR	CIP	FOX	CTX	CAZ	CRO	*qnrA*	*qnrB*	*qnrS*	*qnrC*	*qnrD*	*aac(6')-Ib-cr*	*qepA*	phenotype	Genotype	phenotype	genotype
461	L2EN-463	256	128	0.12	4	0.25	0.12	0.5	0	0	0	0	0	0	0	ND	ND	ND	ND
462	L2EN-464	256	4	2	2	0.25	0.5	0.12	0	0	1	0	0	0	0	-	TEM	-	0
463	L2EN-465	4	0.12	0.03	8	0.25	0.12	0.5	0	0	0	0	0	0	0	ND	ND	ND	ND
464	L2EN-466	8	0.25	0.03	8	0.25	0.12	1	0	0	0	0	0	0	0	ND	ND	ND	ND
465	L2EN-467	4	0.12	0.03	8	256	256	128	0	0	0	0	0	0	0	ND	ND	ND	ND
466	L2EN-468	256	4	1	64	16	32	64	0	0	0	0	0	0	0	ND	ND	ND	ND
467	L2EN-469	256	1	0.12	8	0.25	0.12	0.5	0	0	0	0	0	0	0	ND	ND	ND	ND
468	L2EN-470	4	0.12	0.06	16	0.12	0.12	0.25	0	0	0	0	0	0	0	ND	ND	ND	ND
469	L2EN-471	256	4	1	64	16	64	16	0	0	1	0	0	0	0	-	TEM	+	CIT
470	L2EN-472	32	4	2	8	16	>256	256	0	0	0	0	0	0	0	ND	ND	ND	ND
471	L2EN-473	>256	0.5	0.12	8	0.12	0.12	0.25	0	0	0	0	0	0	0	ND	ND	ND	ND
472	L2EN-474	4	0.12	0.06	8	0.12	0.12	0.25	0	0	0	0	0	0	0	ND	ND	ND	ND
473	L2EN-475	4	0.12	0.06	8	0.25	0.12	0.25	0	0	0	0	0	0	0	ND	ND	ND	ND
474	L2EN-476	4	0.12	0.06	16	0.25	0.12	0.5	0	0	0	0	0	0	0	ND	ND	ND	ND
475	L2EN-477	4	0.12	0.06	16	0.25	0.12	0.5	0	0	0	0	0	0	0	ND	ND	ND	ND

No	isolates	MIC(μg/ml)							The presence of PMQR genes							ESBLs		AmpC	
		NAL	NOR	CIP	FOX	CTX	CAZ	CRO	*qnrA*	*qnrB*	*qnrS*	*qnrC*	*qnrD*	*aac(6')-Ib-cr*	*qepA*	phenotype	Genotype	phenotype	genotype
476	L2EN-478	4	0.12	0.06	16	0.25	0.12	0.5	0	0	0	0	0	0	0	ND	ND	ND	ND
477	L2EN-479	4	0.12	0.06	8	0.12	0.12	0.5	0	0	0	0	0	0	0	ND	ND	ND	ND
478	L2EN-480	16	0.5	0.5	8	128	64	64	0	0	0	0	0	0	0	ND	ND	ND	ND
479	L2EN-481	>256	1	0.12	16	0.25	0.12	0.5	0	0	0	0	0	0	0	ND	ND	ND	ND
480	L2EN-482	4	0.12	0.06	256	0.25	0.12	2	0	0	0	0	0	0	0	ND	ND	ND	ND
481	L2EN-483	64	0.12	0.12	16	8	32	4	0	0	0	0	0	0	0	ND	ND	ND	ND
482	L2EN-485	4	0.5	0.12	16	0.25	0.25	2	0	0	0	0	0	0	0	ND	ND	ND	ND
483	L2EN-486	>256	>256	>256	16	>256	>256	256	0	0	0	0	0	1	0	+	CTX	-	0
484	L2EN-487	8	4	2	>256	128	8	128	0	0	1	0	0	0	0	+	CTX, VEB	-	0
485	L2EN-488	>256	32	8	16	0.25	0.12	0.25	0	0	0	0	0	0	0	ND	ND	ND	ND
486	L2EN-489	4	0.12	0.06	16	0.25	0.12	0.25	0	0	0	0	0	0	0	ND	ND	ND	ND
487	L2EN-490	4	1	0.25	2	32	8	32	0	0	1	0	0	0	0	+	CTX	-	0
488	L2EN-491	16	4	2	8	32	64	8	0	0	0	0	0	0	0	ND	ND	ND	ND
489	L2EN-492	4	0.12	0.06	16	0.25	0.12	0.5	0	0	0	0	0	0	0	ND	ND	ND	ND
490	L2EN-493	2	0.06	0.01	1	0.03	0.25	0.03	0	0	0	0	0	0	0	ND	ND	ND	ND

No	isolates	MIC(μg/ml)							The presence of PMQR genes							ESBLs		AmpC	
		NAL	NOR	CIP	FOX	CTX	CAZ	CRO	*qnrA*	*qnrB*	*qnrS*	*qnrC*	*qnrD*	*aac(6')-Ib-cr*	*qepA*	phenotype	Genotype	phenotype	genotype
491	L2EN-494	4	0.06	0.03	2	0.03	0.12	0.01	0	0	0	0	0	0	0	ND	ND	ND	ND
492	L2EN-495	>256	64	16	4	0.06	0.5	0.06	0	0	0	0	0	0	0	ND	ND	ND	ND
493	L2EN-496	4	1	0.5	64	16	8	16	0	0	1	0	0	0	0	-	0	+	CIT
494	L2EN-497	4	0.12	0.03	2	0.03	0.25	0.03	0	0	0	0	0	0	0	ND	ND	ND	ND
495	L2EN-498	4	0.06	0.03	1	0.03	2	0.06	0	0	0	0	0	0	0	ND	ND	ND	ND
496	L2EN-499	4	1	0.12	256	64	128	128	0	0	0	0	0	0	0	ND	ND	ND	ND
497	L2EN-500	>256	>256	256	16	>256	>256	>256	0	0	0	0	0	1	0	+	TEM, CTX	+	CIT
498	L2EN-501	8	2	0.5	2	32	128	128	0	0	1	0	0	0	0	+	TEM, CTX	-	0
499	L2EN-502	>256	1	0.12	1	0.12	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
500	L2EN-503	4	0.06	0.01	4	0.12	1	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
501	L2EN-504	256	4	1	64	8	64	16	0	0	1	0	0	0	0	-	0	+	CIT
502	L2EN-505	16	2	0.5	64	8	64	16	0	0	1	0	0	0	0	-	0	+	DHA
503	L2EN-506	4	0.06	0.01	2	0.12	0.5	0.06	0	0	0	0	0	0	0	ND	ND	ND	ND
504	L2EN-507	8	1	0.25	4	0.25	0.5	0.12	0	0	1	0	0	0	0	-	TEM	-	0
505	L2EN-508	4	0.06	0.01	1	0.03	0.5	0.03	0	0	0	0	0	0	0	ND	ND	ND	ND

No	isolates	MIC(μg/ml)							The presence of PMQR genes							ESBLs		AmpC	
		NAL	NOR	CIP	FOX	CTX	CAZ	CRO	*qnrA*	*qnrB*	*qnrS*	*qnrC*	*qnrD*	*aac(6')-Ib-cr*	*qepA*	phenotype	Genotype	phenotype	genotype
506	L2EN-509	4	0.06	0.01	1	0.03	0.25	0.03	0	0	0	0	0	0	0	ND	ND	ND	ND
507	L2EN-510	4	0.06	0.01	1	0.03	0.5	0.03	0	0	0	0	0	0	0	ND	ND	ND	ND
508	L2EN-511	16	2	0.5	4	>256	256	>256	0	0	0	0	0	0	0	ND	ND	ND	ND
509	L2EN-512	4	0.06	0.01	4	0.12	1	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
510	L2EN-513	4	0.06	0.01	1	0.12	1	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
511	L2EN-514	4	0.12	0.01	8	0.12	0.5	0.06	0	0	0	0	0	0	0	ND	ND	ND	ND
512	L2EN-515	4	0.12	0.01	1	0.06	0.5	0.06	0	0	0	0	0	0	0	ND	ND	ND	ND
513	L2EN-516	>256	1	0.12	2	32	2	32	0	0	0	0	0	0	0	ND	ND	ND	ND
514	L2EN-517	8	0.5	0.12	64	0.25	1	0.25	0	0	0	0	0	0	0	ND	ND	ND	ND
515	L2EN-518	4	0.06	0.01	4	0.06	0.12	0.03	0	0	0	0	0	0	0	ND	ND	ND	ND
516	L2EN-519	2	0.12	0.12	16	4	1	2	0	0	0	0	0	0	0	ND	ND	ND	ND
517	L2EN-520	2	0.03	0.01	0.06	0.03	0.01	0.01	0	0	0	0	0	0	0	ND	ND	ND	ND
518	L2EN-521	1	0.25	0.12	16	0.25	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
519	L2EN-522	4	0.06	0.01	128	0.12	0.25	0.01	0	0	0	0	0	0	0	ND	ND	ND	ND
520	L2EN-523	4	0.12	0.01	4	0.25	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND

No	isolates	MIC(μg/ml)							The presence of PMQR genes							ESBLs		AmpC	
		NAL	NOR	CIP	FOX	CTX	CAZ	CRO	*qnrA*	*qnrB*	*qnrS*	*qnrC*	*qnrD*	*aac(6')-Ib-cr*	*qepA*	phenotype	Genotype	phenotype	genotype
521	L2EN-524	4	0.06	0.01	64	0.03	0.12	0.01	0	0	0	0	0	0	0	ND	ND	ND	ND
522	L2EN-525	8	2	1	128	128	256	128	0	0	0	0	0	1	0	+	TEM, CTX	-	0
523	L2EN-526	4	0.06	0.03	2	0.06	0.12	0.03	0	0	0	0	0	0	0	ND	ND	ND	ND
524	L2EN-527	2	0.06	0.01	256	0.06	0.25	0.06	0	0	0	0	0	0	0	ND	ND	ND	ND
525	L2EN-528	8	0.12	0.06	4	0.06	0.12	0.03	0	0	0	0	0	0	0	ND	ND	ND	ND
526	L2EN-529	8	0.06	0.03	2	0.06	0.12	0.03	0	0	0	0	0	0	0	ND	ND	ND	ND
527	L2EN-530	256	>256	128	4	0.25	1	0.12	0	0	0	0	1	0	0	-	TEM	-	0
528	L2EN-531	>256	2	0.5	8	>256	>256	>256	0	0	0	0	0	0	0	ND	ND	ND	ND
529	L2EN-532	2	0.06	0.01	8	0.06	0.12	0.03	0	0	0	0	0	0	0	ND	ND	ND	ND
530	L2EN-533	2	0.06	0.01	2	0.06	0.25	0.03	0	0	0	0	0	0	0	ND	ND	ND	ND
531	L2EN-534	>256	>256	>256	4	64	>256	8	0	0	0	0	1	0	0	+	OXA, TEM, VEB	-	0
532	L2EN-535	8	0.06	0.01	4	0.06	0.12	0.03	0	0	0	0	0	0	0	ND	ND	ND	ND
533	L2EN-536	2	0.06	0.01	1	0.06	0.12	0.03	0	0	0	0	0	0	0	ND	ND	ND	ND
534	L2EN-537	2	0.01	0.01	2	0.06	0.25	0.03	0	0	0	0	0	0	0	ND	ND	ND	ND
535	L2EN-538	2	0.12	0.12	16	0.25	0.5	0.25	0	0	0	0	0	0	0	ND	ND	ND	ND

No	isolates	MIC(μg/ml)							The presence of PMQR genes							ESBLs		AmpC	
		NAL	NOR	CIP	FOX	CTX	CAZ	CRO	*qnrA*	*qnrB*	*qnrS*	*qnrC*	*qnrD*	*aac(6')-Ib-cr*	*qepA*	phenotype	Genotype	phenotype	genotype
536	L2EN-539	4	0.06	0.01	4	0.06	0.12	0.03	0	0	0	0	0	0	0	ND	ND	ND	ND
537	L2EN-540	4	0.12	0.06	8	0.06	0.25	0.03	0	0	0	0	0	0	0	ND	ND	ND	ND
538	L2EN-541	4	0.06	0.01	2	0.12	1	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
539	L2EN-542	16	1	0.25	2	256	128	256	0	0	0	0	0	0	0	ND	ND	ND	ND
540	L2EN-543	128	0.5	0.12	2	0.12	1	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
541	L2EN-544	4	0.12	0.01	2	0.06	0.5	0.06	0	0	0	0	0	0	0	ND	ND	ND	ND
542	L2EN-545	4	0.06	0.01	1	0.06	0.5	0.06	0	0	0	0	0	0	0	ND	ND	ND	ND
543	L2EN-546	4	0.06	0.01	4	0.12	1	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
544	L2EN-547	>256	0.5	0.25	1	0.06	0.5	0.06	0	0	0	0	0	0	0	ND	ND	ND	ND
545	L2EN-548	4	0.06	0.01	4	0.12	1	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
546	L2EN-549	4	0.06	0.01	1	0.06	0.25	0.03	0	0	0	0	0	0	0	ND	ND	ND	ND
547	L2EN-550	8	1	0.25	4	16	64	16	0	0	0	0	0	0	0	ND	ND	ND	ND
548	L2EN-551	4	0.12	0.01	4	0.12	1	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
549	L2EN-552	4	0.06	0.01	2	0.06	0.5	0.03	0	0	0	0	0	0	0	ND	ND	ND	ND
550	L2EN-553	4	0.06	0.01	1	0.06	0.5	0.06	0	0	0	0	0	0	0	ND	ND	ND	ND

No	isolates	MIC(μg/ml)							The presence of PMQR genes							ESBLs		AmpC	
		NAL	NOR	CIP	FOX	CTX	CAZ	CRO	*qnrA*	*qnrB*	*qnrS*	*qnrC*	*qnrD*	*aac(6')-Ib-cr*	*qepA*	phenotype	Genotype	phenotype	genotype
551	L2EN-554	4	0.12	0.01	4	0.12	1	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
552	L2EN-555	4	0.12	0.01	2	0.12	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
553	L2EN-556	1	0.01	0.01	0.5	0.06	0.25	0.06	0	0	0	0	0	0	0	ND	ND	ND	ND
554	L2EN-557	1	0.01	0.01	1	0.12	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
555	L2EN-558	8	1	0.5	2	0.25	2	0.12	0	0	1	0	0	0	0	-	0	-	0
556	L2EN-559	1	0.01	0.01	0.25	0.12	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
557	L2EN-560	1	0.01	0.01	0.25	0.06	0.5	0.06	0	0	0	0	0	0	0	ND	ND	ND	ND
558	L2EN-561	2	0.5	0.12	1	0.06	0.5	0.06	0	0	0	0	0	0	0	ND	ND	ND	ND
559	L2EN-562	2	0.5	0.12	1	0.06	0.5	0.06	0	0	0	0	0	0	0	ND	ND	ND	ND
560	L2EN-563	8	1	0.5	4	0.25	0.5	0.12	0	0	1	0	0	0	0	-	0	-	0
561	L2EN-564	64	4	0.25	2	0.12	0.5	0.12	0	0	1	0	0	0	0	-	0	-	0
562	L2EN-565	32	4	1	4	1	1	0.25	0	0	1	0	0	0	0	-	0	-	0
563	L2EN-566	256	4	0.03	4	0.12	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
564	L2EN-567	128	4	1	4	1	1	0.25	0	0	0	0	0	0	0	ND	ND	ND	ND
565	L2EN-568	1	0.01	0.01	1	0.06	0.5	0.06	0	0	0	0	0	0	0	ND	ND	ND	ND

No	isolates	MIC(μg/ml)							The presence of PMQR genes							ESBLs		AmpC	
		NAL	NOR	CIP	FOX	CTX	CAZ	CRO	*qnrA*	*qnrB*	*qnrS*	*qnrC*	*qnrD*	*aac(6')-Ib-cr*	*qepA*	phenotype	Genotype	phenotype	genotype
566	L2EN-569	1	0.01	0.01	1	0.06	0.5	0.06	0	0	0	0	0	0	0	ND	ND	ND	ND
567	L2EN-570	32	4	1	8	1	1	0.25	0	0	1	0	0	0	0	-	0	-	0
568	L2EN-571	16	4	0.5	32	1	8	0.5	0	0	1	0	0	0	0	-	TEM	-	0
569	L2EN-572	>256	8	4	2	0.06	0.5	0.06	0	0	0	0	0	0	0	ND	ND	ND	ND
570	L2EN-573	>256	8	4	1	0.06	0.06	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
571	L2EN-574	>256	16	8	8	32	4	8	0	0	0	0	0	0	0	ND	ND	ND	ND
572	L2EN-575	16	0.12	0.06	1	2	0.06	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
573	L2EN-576	1	0.06	0.01	2	0.06	0.5	0.06	0	0	0	0	0	0	0	ND	ND	ND	ND
574	L2EN-577	128	0.06	0.03	2	0.12	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
575	L2EN-578	128	4	1	1	256	128	256	0	0	1	0	0	0	0	+	CTX	-	0
576	L2EN-579	1	0.01	0.01	1	0.06	0.5	0.06	0	0	0	0	0	0	0	ND	ND	ND	ND
577	L2EN-580	16	4	1	1	0.06	0.25	0.06	0	0	0	0	0	0	0	ND	ND	ND	ND
578	L2EN-581	>256	4	1	2	0.12	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
579	L2EN-582	2	0.06	0.06	2	0.06	0.5	0.06	0	0	0	0	0	0	0	ND	ND	ND	ND
580	L2EN-583	2	0.06	0.06	1	0.12	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND

No	isolates	MIC(μg/ml)							The presence of PMQR genes							ESBLs		AmpC	
		NAL	NOR	CIP	FOX	CTX	CAZ	CRO	*qnrA*	*qnrB*	*qnrS*	*qnrC*	*qnrD*	*aac(6')-Ib-cr*	*qepA*	phenotype	Genotype	phenotype	genotype
581	L2EN-584	256	4	2	2	4	1	0.25	0	0	1	0	0	0	0	-	TEM	-	0
582	L2EN-585	4	0.25	0.06	64	0.25	1	0.25	0	0	1	0	0	0	0	-	0	-	0
583	L2EN-586	128	4	1	1	2	1	0.25	0	0	1	0	0	0	0	-	0	-	0
584	L2EN-587	1	0.01	0.01	1	0.06	0.25	0.06	0	0	0	0	0	0	0	ND	ND	ND	ND
585	L2EN-588	16	0.5	0.25	2	0.12	0.5	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
586	L2EN-589	1	0.06	0.06	0.25	0.06	0.25	0.06	0	0	0	0	0	0	0	ND	ND	ND	ND
587	L2EN-590	64	0.06	0.01	1	0.06	0.25	0.06	0	0	0	0	0	0	0	ND	ND	ND	ND
588	L2EN-591	128	2	1	32	0.5	1	0.25	0	0	1	0	0	0	0	-	0	-	0
589	L2EN-592	1	0.01	0.01	2	0.12	0.5	0.5	0	0	0	0	0	0	0	ND	ND	ND	ND
590	L2EN-593	1	0.01	0.01	8	4	1	4	0	0	0	0	0	0	0	ND	ND	ND	ND
591	L2EN-594	1	0.01	0.01	0.25	4	1	0.5	0	0	0	0	0	0	0	ND	ND	ND	ND
592	L2EN-595	128	4	1	1	>256	128	256	0	0	1	0	0	0	0	+	TEM, CTX	-	0
593	L2EN-596	4	0.01	0.01	8	0.12	0.25	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
594	L2EN-597	8	0.01	0.01	0.5	0.01	0.06	0.01	0	0	0	0	0	0	0	ND	ND	ND	ND
595	L2EN-598	8	0.01	0.01	0.5	0.01	0.06	0.01	0	0	0	0	0	0	0	ND	ND	ND	ND

No	isolates	MIC(μg/ml)							The presence of PMQR genes							ESBLs		AmpC	
		NAL	NOR	CIP	FOX	CTX	CAZ	CRO	*qnrA*	*qnrB*	*qnrS*	*qnrC*	*qnrD*	*aac(6')-Ib-cr*	*qepA*	phenotype	Genotype	phenotype	genotype
596	L2EN-599	8	0.01	0.01	2	0.01	0.25	0.01	0	0	0	0	0	0	0	ND	ND	ND	ND
597	L2EN-600	4	0.01	0.01	0.25	0.01	0.25	0.01	0	0	0	0	0	0	0	ND	ND	ND	ND
598	L2EN-601	4	0.01	0.01	256	0.12	0.25	0.25	0	0	0	0	0	0	0	ND	ND	ND	ND
599	L2EN-602	>256	2	1	8	0.03	0.06	0.01	0	0	0	0	0	0	0	ND	ND	ND	ND
600	L2EN-603	8	0.06	0.01	128	0.25	0.5	0.25	0	0	0	0	0	0	0	ND	ND	ND	ND
601	L2EN-604	8	0.06	0.01	16	0.06	0.06	0.01	0	0	0	0	0	0	0	ND	ND	ND	ND
602	L2EN-605	8	0.06	0.01	0.5	0.03	0.06	0.01	0	0	0	0	0	0	0	ND	ND	ND	ND
603	L2EN-606	>256	0.25	0.06	128	256	64	256	0	0	0	0	0	0	0	ND	ND	ND	ND
604	L2EN-607	>256	8	8	0.5	4	32	4	0	0	0	0	0	0	0	ND	ND	ND	ND
605	L2EN-608	>256	2	1	0.5	0.06	0.12	0.06	0	0	0	0	0	0	0	ND	ND	ND	ND
606	L2EN-609	>256	16	4	0.5	0.06	0.06	0.06	0	0	0	0	1	0	0	-	0	-	0
607	L2EN-610	>256	2	1	16	16	16	4	0	0	0	0	0	0	0	ND	ND	ND	ND
608	L2EN-611	256	0.25	0.01	1	0.06	0.12	0.06	0	0	0	0	0	0	0	ND	ND	ND	ND
609	L2EN-612	>256	16	8	4	0.06	0.12	0.06	0	0	0	0	0	0	0	ND	ND	ND	ND
610	L2EN-613	>256	2	1	0.5	0.01	0.01	0.01	0	0	0	0	0	0	0	ND	ND	ND	ND

No	isolates	MIC(μg/ml)							The presence of PMQR genes							ESBLs		AmpC	
		NAL	NOR	CIP	FOX	CTX	CAZ	CRO	*qnrA*	*qnrB*	*qnrS*	*qnrC*	*qnrD*	*aac(6')-Ib-cr*	*qepA*	phenotype	Genotype	phenotype	genotype
611	L2EN-614	>256	2	0.12	4	0.03	0.12	0.06	0	0	0	0	0	0	0	ND	ND	ND	ND
612	L2EN-615	>256	2	0.25	256	64	256	32	1	0	0	0	0	0	0	+	CTX, VEB	-	0
613	L2EN-616	>256	0.03	0.03	1	0.12	0.5	0.06	0	0	0	0	0	0	0	ND	ND	ND	ND
614	L2EN-617	2	0.03	0.03	256	16	64	8	0	0	0	0	0	0	0	ND	ND	ND	ND
615	L2EN-618	2	0.03	0.03	256	16	128	16	0	0	0	0	0	0	0	ND	ND	ND	ND
616	L2EN-619	2	0.25	0.12	64	0.03	0.25	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
617	L2EN-620	4	0.5	0.12	2	0.03	0.12	0.03	0	0	1	0	0	0	0	-	0	-	0
618	L2EN-621	4	0.12	0.01	16	0.03	0.25	0.03	0	0	0	0	0	0	0	ND	ND	ND	ND
619	L2EN-622	4	0.12	0.03	64	16	0.25	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
620	L2EN-623	4	0.12	0.03	16	16	0.25	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
621	L2EN-624	1	0.03	0.01	16	0.03	0.25	0.03	0	0	0	0	0	0	0	ND	ND	ND	ND
622	L2EN-625	>256	32	4	32	16	128	8	0	0	0	0	0	0	0	ND	ND	ND	ND
623	L2EN-626	256	64	8	8	2	256	2	0	0	1	0	0	0	0	+	SHV	-	0
624	L2EN-627	256	32	4	256	16	>256	16	0	0	0	0	0	0	0	ND	ND	ND	ND
625	L2EN-628	>256	32	4	4	0.12	0.5	0.25	0	0	0	0	1	0	0	-	0	-	0

No	isolates	MIC(μg/ml)							The presence of PMQR genes							ESBLs		AmpC	
		NAL	NOR	CIP	FOX	CTX	CAZ	CRO	*qnrA*	*qnrB*	*qnrS*	*qnrC*	*qnrD*	*aac(6')-Ib-cr*	*qepA*	phenotype	Genotype	phenotype	genotype
626	L2EN-629	>256	128	8	256	64	>256	64	0	0	1	0	0	0	0	+	SHV, VEB	-	0
627	L2EN-630	256	32	2	>256	128	>256	256	0	0	0	0	0	0	0	ND	ND	ND	ND
628	L2EN-631	8	4	4	4	0.5	0.12	0.03	0	0	0	0	1	0	0	-	0	-	0
629	L2EN-632	>256	>256	>256	2	0.03	0.12	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
630	L2EN-633	>256	>256	>256	256	>256	>256	>256	0	0	0	0	0	1	0	+	TEM	-	0
631	L2EN-634	128	64	32	>256	256	>256	>256	0	0	0	0	0	1	0	+	TEM	-	0
632	L2EN-635	2	0.03	0.01	2	0.03	0.5	0.03	0	0	0	0	0	0	0	ND	ND	ND	ND
633	L2EN-636	32	0.12	0.01	>256	32	128	2	0	0	0	0	0	0	0	ND	ND	ND	ND
634	L2EN-637	1	0.01	0.01	2	0.03	0.06	0.03	0	0	0	0	0	0	0	ND	ND	ND	ND
635	L2EN-638	4	0.12	0.01	4	0.03	0.06	0.03	0	0	0	0	0	0	0	ND	ND	ND	ND
636	L2EN-639	2	0.12	0.01	128	16	64	4	0	0	0	0	0	0	0	ND	ND	ND	ND
637	L2EN-640	1	0.06	0.06	8	0.12	0.25	0.25	0	0	0	0	0	0	0	ND	ND	ND	ND
638	L2EN-641	>256	256	4	256	>256	>256	>256	0	0	0	0	0	0	0	ND	ND	ND	ND
639	L2EN-642	16	8	0.25	16	0.06	1	0.25	0	0	0	0	0	0	0	ND	ND	ND	ND
640	L2EN-643	16	8	0.25	256	1	1	1	0	0	0	0	0	0	0	ND	ND	ND	ND

No	isolates	MIC(μg/ml)							The presence of PMQR genes							ESBLs		AmpC	
		NAL	NOR	CIP	FOX	CTX	CAZ	CRO	*qnrA*	*qnrB*	*qnrS*	*qnrC*	*qnrD*	*aac(6')-Ib-cr*	*qepA*	phenotype	Genotype	phenotype	genotype
641	L2EN-644	>256	>256	32	256	>256	>256	>256	0	0	0	0	0	0	0	ND	ND	ND	ND
642	L2EN-645	>256	256	32	16	0.25	0.12	0.03	0	0	0	0	0	0	0	ND	ND	ND	ND
643	L2EN-646	4	0.5	0.25	8	1	0.12	0.5	0	0	0	0	0	0	0	ND	ND	ND	ND
644	L2EN-647	>256	16	8	2	4	8	4	0	0	0	0	0	0	0	ND	ND	ND	ND
645	L2EN-648	>256	>256	>256	4	64	256	0.12	0	0	0	0	1	0	0	+	VEB	-	0
646	L2EN-649	2	0.25	0.25	4	0.03	0.06	0.03	0	0	0	0	0	0	0	ND	ND	ND	ND
647	L2EN-650	>256	16	8	2	0.03	0.06	0.03	0	0	0	0	0	0	0	ND	ND	ND	ND
648	L2EN-651	16	16	8	2	4	16	2	0	0	0	0	0	0	0	ND	ND	ND	ND
649	L2EN-652	16	8	8	4	256	128	256	0	0	0	0	0	0	0	ND	ND	ND	ND
650	L2EN-653	>256	>256	>256	4	64	256	4	0	0	0	0	1	0	0	+	VEB	-	0
651	L2EN-654	>256	32	16	2	4	0.06	1	0	0	0	0	0	0	0	ND	ND	ND	ND
652	L2EN-655	2	0.12	0.06	128	0.03	0.25	0.12	0	0	0	0	0	0	0	ND	ND	ND	ND
653	L2EN-656	>256	>256	>256	4	16	256	0.12	0	0	0	0	1	0	0	+	VEB	-	0
654	L2EN-657	>256	2	0.5	4	8	16	4	0	0	0	0	0	0	0	ND	ND	ND	ND
655	L2EN-658	2	0.12	0.06	4	0.03	0.06	0.03	0	0	0	0	0	0	0	ND	ND	ND	ND

No	isolates	MIC(μg/ml)							The presence of PMQR genes							ESBLs		AmpC	
		NAL	NOR	CIP	FOX	CTX	CAZ	CRO	*qnrA*	*qnrB*	*qnrS*	*qnrC*	*qnrD*	*aac(6')-Ib-cr*	*qepA*	phenotype	Genotype	phenotype	genotype
656	L2EN-659	1	0.01	0.01	16	0.03	0.25	0.03	0	0	0	0	0	0	0	ND	ND	ND	ND
657	L2EN-660	>256	2	0.25	256	32	4	64	0	0	0	0	0	0	0	ND	ND	ND	ND
658	L2EN-661	4	1	0.25	128	16	8	64	0	0	1	0	0	0	0	+	TEM, CTX	-	0
659	L2EN-662	8	0.5	0.25	4	0.06	0.12	0.03	0	0	0	0	0	0	0	ND	ND	ND	ND
660	L2EN-663	>256	8	4	>256	1	4	2	0	0	0	0	0	0	0	ND	ND	ND	ND
661	L2EN-664	>256	16	8	4	0.03	0.12	0.03	0	0	0	0	0	0	0	ND	ND	ND	ND
662	L2EN-665	256	128	32	4	1	0.12	0.25	0	0	0	0	0	0	0	ND	ND	ND	ND
663	L2EN-666	4	1	0.25	256	0.5	0.5	0.25	0	0	1	0	0	0	0	-	TEM	-	0
664	L2EN-667	>256	>256	>256	2	4	0.25	1	0	0	0	0	0	0	0	ND	ND	ND	ND
665	L2EN-668	8	2	0.5	2	1	0.12	0.25	0	0	0	0	0	0	0	ND	ND	ND	ND
666	L2EN-669	8	2	0.5	4	0.06	0.5	0.06	0	0	0	0	0	0	0	ND	ND	ND	ND
667	L2EN-670	>256	>256	>256	4	32	256	0.12	0	0	0	0	1	0	0	+	VEB	-	0
668	L2EN-671	256	8	0.5	256	16	64	32	0	0	0	0	0	0	0	ND	ND	ND	ND
669	L2EN-672	>256	>256	>256	32	64	256	64	0	0	0	0	1	0	0	-	0	-	0
670	L2EN-673	>256	>256	>256	16	64	16	64	0	0	0	0	0	0	0	ND	ND	ND	ND

No	isolates	MIC(μg/ml)							The presence of PMQR genes							ESBLs		AmpC	
		NAL	NOR	CIP	FOX	CTX	CAZ	CRO	*qnrA*	*qnrB*	*qnrS*	*qnrC*	*qnrD*	*aac(6')-Ib-cr*	*qepA*	phenotype	Genotype	phenotype	genotype
671	L2EN-674	>256	16	2	2	0.12	0.25	0.03	0	0	0	0	0	0	0	ND	ND	ND	ND

NAL, nalidixic acid; NOR, norfloxacin; CIP, ciprofloxacin; FOX,cefoxitin; CTX, cefotaxime; CAZ, ceftrazidime; CRO, ceftriaxone; 1, positive; 0, negative; ND, not determined; +,positive; -, negative

Results of the antibiotic susceptibility of 671 Enterobacteriaceae isolates from ICUs and non-ICUs

ward	No. isolates (%)	nalidixic acid(μg/ml)				norfloxacin(μg/ml)				ciprofloxacin(μg/ml)				
		MIC_{50}	MIC_{90}	range	%R	MIC_{50}	MIC_{90}	range	%R	MIC_{50}	MIC_{90}	range	%reduce	%R
ICU(total=48)		8	>256	1->256	33.33	1	>256	0.015->256	29.17	0.25	128	0.015-256	27.08	27.08
Sterile sites	4(8.33)	4	16	4-16	0	1	4	0.125-4	0	0.5	1	0.03-1	50	0
Non- sterile sites	44(91.67)	8	>256	1->256	36.36	1	>256	0.015->256	31.82	0.25	64	0.015-256	22.73	29.55
non-ICU(total=623)		16	>256	0.125->256	48.15	2	>256	0.015->256	36.76	0.5	128	0.015->256	20.71	38.52
Sterile sites	116(18.62)	8	>256	0.125->256	41.38	1	>256	0.03->256	28.45	0.125	128	0.015->256	24.69	29.31
Non- sterile sites	507(81.38)	16	>256	0.125->256	49.7	2	>256	0.015->256	38.66	0.5	128	0.015->256	20.71	40.63

Results of the antibiotic susceptibility of 671 Enterobacteriaceae isolates from ICUs and non-ICUs (cont.)

ward	No. isolates (%)	cefoxitin(µg/ml)				cefotaxime(µg/ml)				ceftazidime(µg/ml)				ceftriaxone(µg/ml)			
		MIC_{50}	MIC_{90}	range	%R	MIC_{50}	MIC_{90}	range	%R	MIC_{50}	MIC_{90}	range	%R	MIC_{50}	MIC_{90}	range	%R
ICU(total=48)		8	256	0.06->256	20.83	8	256	0.03->256	52.08	2	128	0.015->256	43.75	2	128	0.015->256	47.92
Sterile sites	4(8.33)	4	>256	2->256	50	0.25	128	0.25-256	50	2	32	0.25-64	50	0.25	128	0.125-128	50
Non- sterile sites	44(91.67)	8	64	0.06->256	18.18	8	256	0.03->256	52.27	1	128	0.015->256	43.18	2	128	0.015->256	47.73
non-ICU(total=623)		8	64	0.25->256	19.58	0.5	256	0.015->256	45.75	1	128	0.015->256	35.15	0.5	256	0.015->256	43.82
Sterile sites	116(18.62)	8	64	1->256	22.41	0.5	128	0.03->256	43.97	1	128	0.06->256	30.17	0.5	128	0.015->256	41.38
Non- sterile sites	507(81.38)	8	64	0.25->256	18.93	1	256	0.015->256	46.15	1	128	0.015->256	36.29	0.5	256	0.015->256	44.38

Results of antibiotic susceptibility among 671 Enterobacteriaceae isolates from sterile/ non-sterile sites and ICUs/ non-ICUs

Type of specimen	No. isolates (%)	nalidixic acid(µg/ml)				norfloxacin(µg/ml)				ciprofloxacin(µg/ml)				
		MIC_{50}	MIC_{90}	range	%R	MIC_{50}	MIC_{90}	range	%R	MIC_{50}	MIC_{90}	range	%reduce	%R
sterile sites(total = 120)		8	>256	0.125->256	40	1	>256	0.03->256	27.50	0.125	128	0.015->256	22.50	28.33
PMQR-pos	27/120(22.50)	16	>256	4->256	48.15	8	>256	1->256	48.15	2	>256	0.125->256	40.74	48.15
PMQR-neg	93/120(77.50)	4	>256	0.125->256	37.63	0.25	128	0.03->256	21.51	0.06	32	0.015->256	17.20	22.58
Non-sterile sites(total = 551)		16	>256	0.125->256	48.64	2	>256	0.015->256	38.11	0.5	128	0.015->256	20.87	39.75
PMQR-pos	111/551(20.15)	256	>256	4->256	68.47	32	>256	0.25->256	54.05	8	>256	0.06->256	37.84	57.66
PMQR-neg	440/551(79.85)	8	>256	0.125->256	43.64	1	>256	0.015->256	34.09	0.125	64	0.015->256	16.59	35.23
ICU(total=48)		8	>256	1->256	33.33	1	>256	0.015->256	29.17	0.25	128	0.015-256	27.08	27.08
PMQR-pos	11/48(22.92)	64	>256	4->256	54.55	32	>256	0.5->256	54.55	8	128	0.125-256	45.45	54.55
PMQR-neg	37/48(77.08)	8	>256	1->256	27.03	1	256	0.015->256	21.62	0.125	32	0.015-128	21.62	18.92
non-ICU(total=623)		16	>256	0.125->256	48.15	2	>256	0.015->256	36.76	0.5	128	0.015->256	20.71	38.52
PMQR-pos	127/623(20.39)	256	>256	4->256	65.35	16	>256	0.25->256	52.76	8	>256	0.06->256	37.80	55.91
PMQR-neg	496/623(15.41)	8	>256	0.125->256	43.75	0.5	>256	0.015->256	32.66	8	64	0.015->256	16.33	34.07

Results of antibiotic susceptibility among 671 Enterobacteriaceae isolates from sterile/ non-sterile sites and ICUs/ non-ICUs (cont.)

Type of specimen	No. isolates (%)	cefoxitin(µg/ml)				cefotaxime(µg/ml)				ceftazidime(µg/ml)				ceftriaxone(µg/ml)			
		MIC_{50}	MIC_{90}	range	%R	MIC_{50}	MIC_{90}	range	%R	MIC_{50}	MIC_{90}	range	%R	MIC_{50}	MIC_{90}	range	%R
sterile sites(total = 120)		8	64	1->256	23.33	0.5	128	0.03->256	44.20	1	128	0.06->256	30.83	0.5	128	0.015->256	41.67
PMQR-pos	27/120(22.50)	32	>256	2->256	51.85	128	>256	0.25->256	85.19	32	>256	0.5->256	85.19	128	>256	0.25->256	85.19
PMQR-neg	93/120(77.50)	8	64	1->256	15.05	0.25	64	0.03->256	32.26	0.5	32	0.06->256	18.28	0.25	64	0.015->256	29.03
Non-sterile sites(total = 551)		8	64	0.06->256	18.87	1	256	0.015->256	46.6	1	128	0.015->256	36.84	0.5	256	0.015->256	44.65
PMQR-pos	111/551(20.15)	8	64	0.5->256	33.33	32	256	0.03->256	69.37	32	256	0.06->256	59.46	32	256	0.03->256	66.67
PMQR-neg	440/551(79.85)	8	64	0.06->256	15.23	0.25	128	0.015->256	40.91	0.5	64	0.015->256	31.14	0.25	128	0.015->256	39.09
ICU(total=48)		8	256	0.06->256	20.83	8	256	0.03->256	52.08	2	128	0.015->256	43.75	2	128	0.015->256	47.92
PMQR-pos	11/48(22.92)	16	256	2->256	36.36	128	>256	0.5->256	90.91	64	>256	0.5->256	90.91	128	>256	0.25->256	90.91
PMQR-neg	37/48(77.08)	8	256	0.06->256	16.22	0.25	64	0.03->256	40.54	0.5	64	0.015-128	29.73	0.25	64	0.015->256	35.14
non-ICU(total=623)		8	64	0.25->256	19.58	0.5	256	0.015->256	45.75	1	128	0.015->256	35.15	0.5	256	0.015->256	43.82
PMQR-pos	127/623(20.39)	16	128	0.5->256	37.01	64	256	0.03->256	70.87	32	256	0.06->256	59.84	64	256	0.03->256	68.50
PMQR-neg	496/623(15.41)	8	64	0.25->256	15.12	0.25	128	0.015->256	39.31	0.5	64	0.015->256	28.83	0.25	128	0.015->256	37.50

BIOGRAPHY

Miss Pattamaporn Maoleethonh was born on September 30, 1986 in Ayutthaya, Thailand. She graduated with a Bachelor degree of Science (Microbiology) from the Faculty of Sciences, KhonKaen University in 2008. She has studied in the Master degree in the Inter-Department of Medical Microbiology, Faculty of Graduate School, Chulalongkorn University since 2009.