คุณลักษณะของยีนที่สร้างเอ็นไซม์ Extended-spectrum β-lactamases และ AmpC β-lactamases ในเชื้อ Nontyphoidal *Salmonella* ที่แยกได้จากผู้ป่วยในประเทศไทย

นางสาว สิริรัตน์ ลูกอินทร์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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CHARACTERIZATION OF EXTENDED-SPECTRUM β-LACTAMASE AND AMPC β-LACTAMASE GENES IN NONTYPHOIDAL *SALMONELLA* CLINICAL ISOLATES IN THAILAND

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สิริรัตน์ ลูกอินทร์ : คุณลักษณะของยีนที่สร้างเอ็นไซม์ Extended-spectrum β-lactamases และ AmpC β-lactamases ในเชื้อ Nontyphoidal *Salmonella* ที่แยกได้จากผู้ป่วยในประเทศไทย. (Characterization of Extended-spectrum β-lactamase and AmpC β-lactamase Genes in Nontyphoidal *Salmonella* Clinical Isolates in Thailand) อ. ที่ปรึกษาวิทยานิพนธ์หลัก : อาจารย์ ดร. ธนิษฐา ฉัตรสุวรรณ, อ. ที่ปรึกษาวิทยานิพนธ์ร่วม รองศาสตราจารย์ นายแพทย์ วันล่า กุลวิชิต 192 หน้า.

ปัจจุบันมีรายงานการสร้าง extended-spectrum β-lactamases (ESBLs) และ plasmid-mediated AmpC β-lactamases ในเชื้อ nontyphoidal *Salmonella* เพิ่มสูงขึ้น ในหลายประเทศทั่วโลก ซึ่งนำไปสู่ปัญหาการรักษาโรคติดเชื้อ *Salmonella* อย่างไรก็ตามยังไม่มีข้อมูลการสร้างเอ็นไซม์เหล่านี้ ในเชื้อ nontyphoidal *Salmonella* ที่แยกได้จากผู้ป่วยในประเทศไทย การศึกษานี้มีวัตถุประสงค์เพื่อตรวจหาคุณลักษณะของยีนที่สร้างเอ็นไซม์ ESBLs และ AmpC β-lactamases และตรวจหาความชุกของการดื้อยากลุ่ม Extended-spectrum cephalosporin (ESC) ในเชื้อ nontyphoidal *Salmonella* โดยทำการศึกษาในเชื้อ nontyphoidal *Salmonella* จำนวน 560 สายพันธุ์ซึ่งแยกได้จากผู้ป่วยระหว่าง ปี พ.ศ. 2548 ถึง ปี พ.ศ. 2550 พบว่า อัตราการดื้อยา cefoxitin, ceftazidime, cefotaxime, และ ceftriaxone คือ 11.79%, 14.64%, 9.46%, และ 12.50% ตามลำดับ เชื้อดื้อยากลุ่ม ESC ทั้งหมด 119 สายพันธุ์ ถูกนำมาตรวจหาลักษณะทาง phenotype ของ ESBL และ AmpC พบว่า เชื้อสร้าง ESBLs 52 สายพันธุ์ (43.70%), สร้าง non-inducible AmpC 66 สายพันธุ์ (55.46%), และ สร้างเอ็นไซม์ ESBL และ AmpC ร่วมกัน 1 สายพันธุ์ (0.84%) การตรวจคัดกรองยีน *bla* พบ $bla_{CIT\text{-}like}$ 66 สายพันธุ์ (55.46%), $bla_{CTX\text{-}M\text{-}9}$ group 31 สายพันธุ์ (26.05%), $bla_{CTX\text{-}M\text{-}1}$ group ร่วมกับ $bla_{TEM\text{-}like}$ 14 สายพันธุ์ (11.76%), $bla_{CTX\text{-}M\text{-}9}$ group ร่วมกับ $bla_{TEM\text{-}like}$ 7 สายพันธุ์ (5.88%), และ พบ $bla_{CTX\text{-}M\text{-}9}$ group $bla_{TEM\text{-}like}$ และ $bla_{CIT\text{-}like}$ ร่วมกัน 1 สายพันธุ์ (0.84%) การวิเคราะห์ลำดับนิวคลีโอไทด์ของยีน *bla* จากตัวแทนเชื้อ พบว่าเชื้อที่มียีน $bla_{CTX\text{-}M\text{-}1}$ group ทั้งหมด 14 สายพันธุ์ พบ เป็น $bla_{CTX\text{-}M\text{-}55}$ 13 สายพันธุ์ และ $bla_{CTX\text{-}M\text{-}15}$ 1 สายพันธุ์ ในขณะที่ตัวแทนของเชื้อที่มียีน $bla_{CTX\text{-}M\text{-}9}$ group 10 สายพันธุ์, $bla_{TEM\text{-}like}$ 22 สายพันธุ์, และ $bla_{CIT\text{-}like}$ 10 สายพันธุ์ พบเป็น $bla_{CTX\text{-}M\text{-}14}$, $bla_{TEM\text{-}1}$, และ $bla_{CMY\text{-}2}$ ทั้งหมด ตามลำดับ จากการศึกษาบริเวณ upstream ของยีน $bla_{CTX\text{-}M}$ และ bla_{CIT} พบ IS*Ecp1* element ในเชื้อทุกสายพันธุ์ และ พบ -35 และ -10 putative sequences อยู่บน IS*Ecp1* ในตัวแทนเชื้อทั้งหมด 34 สายพันธุ์ การศึกษานี้เป็นรายงานแรกของความชุกของยีนที่สร้าง ESBL และ AmpC ในเชื้อ nontyphoidal *Salmonella* ที่แยกได้จากผู้ป่วยในประเทศไทย โดยพบว่าอัตราการดื้อยากลุ่ม ESC ที่สูงในเชื้อ nontyphoidal *Salmonella* มีสาเหตุมาจากการสร้างเอ็นไซม์ ESBLs ชนิด CTX-M และ plasmid-mediated AmpC โดย ESBLs ชนิด CTX-M-9 group และ AmpC ชนิด CIT เป็นเอ็นไซม์ β-lactamases ที่พบมากที่สุด การศึกษาครั้งนี้เป็นรายงานแรกที่พบ CTX-M-14 ใน *S.* Choleraesuis และเป็นรายงานแรกในเชื้อ nontyphoidal *Salmonella* ที่พบการสร้างเอ็นไซม์ CMY-2, CTX-M-14, และ TEM-1 ร่วมกัน

สาขาวิชา จุลชีววิทยาทางการแพทย์ ลายมือชื่อนิสิต .....

ปีการศึกษา 2552 ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก.....

ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์ร่วม .....

5087209720 : MAJOR MEDICAL MICROBIOLOGY

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SIRIRAT LUK-IN : CHARACTERIZATION OF EXTENDED-SPECTRUM β-LACTAMASE AND AMPC β-LACTAMASE GENES IN NONTYPHOIDAL *SALMONELLA* CLINICAL ISOLATES IN THAILAND. ADVISOR : TANITTHA CHATSUWAN, Ph.D., CO-ADVISOR : ASSOCIATE PROFESSOR WANLA KULWICHIT, M.D., 192 pp.

The production of extended-spectrum β-lactamases (ESBLs) and plasmid-mediated AmpC β-lactamases in nontyphoidal *Salmonella* are increasingly reported worldwide and pose a serious threat for salmonellosis. Nevertheless, no prevalence data on ESBLs and AmpC β-lactamases in nontyphoidal *Salmonella* in Thailand have been reported. This study characterized genes encoding ESBLs and plasmid-mediated AmpC β-lactamases and investigated the prevalence of extended-spectrum cephalosporin (ESC) resistance in nontyphoidal *Salmonella* isolates. A total of 560 nontyphoidal *Salmonella* isolated from patients during 2005-2007 were included in this study. The resistance rates to cefoxitin, ceftazidime, cefotaxime, and ceftriaxone were 11.79%, 14.64%, 9.46%, and 12.50%, respectively. A total of 119 ESC-resistant isolates were detected for ESBL and AmpC phenotypes. The results showed that 52 isolates (43.70%), 66 isolates (55.46%), and 1 isolate (0.84%) were ESBL producers, AmpC producers, and ESBL and AmpC co-producer, respectively. Screening for the presence of *bla* genes revealed that 66 isolates (55.46%) carried $bla_{CIT\text{-}like}$ followed by 31 isolates (26.05%) with $bla_{CTX\text{-}M\text{-}9}$ group, 14 isolates (11.76%) with $bla_{CTX\text{-}M\text{-}1}$ group together with $bla_{TEM\text{-}like}$, 7 isolates (5.88%) with $bla_{CTX\text{-}M\text{-}9}$ group together with $bla_{TEM\text{-}like}$, and 1 isolate (0.84%) with $bla_{CTX\text{-}M\text{-}9}$ group $bla_{TEM\text{-}like}$ and $bla_{CIT\text{-}like}$. DNA sequencing analysis of the entire *bla* genes from representative isolates showed that of the 14 $bla_{CTX\text{-}M\text{-}1}$ group, 13 were $bla_{CTX\text{-}M\text{-}55}$ and one was $bla_{CTX\text{-}M\text{-}15}$ whereas all 10 $bla_{CTX\text{-}M\text{-}9}$ group, 22 $bla_{TEM\text{-}like}$, and 10 $bla_{CIT\text{-}like}$ were $bla_{CTX\text{-}M\text{-}14}$, $bla_{TEM\text{-}1}$, and $bla_{CMY\text{-}2}$, respectively. IS*Ecp1* was present in the upstream regions of $bla_{CTX\text{-}M}$ and bla_{CIT} in all isolates. IS*Ecp1*-mediated -35 and -10 promoter sequences were found in all 34 representative isolates. This is the first report of the prevalence of ESBLs and plasmid-mediated AmpC β-lactamases in nontyphoidal *Salmonella* isolates in Thailand. Our results showed that the high rate of ESC resistance was attributed to the production of CTX-M-type ESBL and plasmid-mediated AmpC which CTX-M-9 group ESBLs and CIT-type AmpC were the most frequent β-lactamases. This was the first report of CTX-M-14 in *S.* Choleraesuis and also the first report of co-carrying CMY-2, CTX-M-14, and TEM-1 in nontyphoidal *Salmonella*.

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

Academic Year : 2009 Advisor's Signature .....

Co-Advisor'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
^{o}C	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
pI	isoelectric point

CHAPTER I

INTRODUCTION

Nontyphoidal *Salmonella* is a common cause of human gastroenteritis that may be complicated by extraintestinal infections such as bacteremia, meningitis, and osteomyelitis (1). The conventional antimicrobial agents, such as ampicillin, chloramphenicol and sulfamethoxazole/trimethoprim, were the drugs of choice in the treatment of salmonellosis. Lately, nontyphoidal *Salmonella* with resistance to these drugs has been reported from many countries (2). Third-generation or extended-spectrum cephalosporins (ESCs) and fluoroquinolones are recommended as alternatives (3). However, nontyphoidal *Salmonella* isolates resistant to extended-spectrum cephalosporins and fluoroquinolones have been increasingly reported in worldwide (4-7).

Cephalosporins are members of β-lactam antibiotics which act by inhibiting bacterial cell wall synthesis by inactivating peptidoglycan transpeptidases known as penicillin-binding proteins which catalyze the cross-linking of the peptidoglycan polymers in the bacterial cell wall. Cephalosporins are classified to first to fourth generation based on their antibacterial activities.

Extended-spectrum cephalosporin resistance in nontyphoidal *Salmonella* has been reported to be increasing in worldwide. A study in nontyphoidal *Salmonella* isolated from 17 states of United States demonstrated that ceftriaxone-resistant nontyphoidal *Salmonella* was increasing from 0.1% in 1996 to 0.4% in 1997, and 0.5% in 1998 (4) and was increased to be 3.2% in 2000 (8). The study in nontyphoidal *Salmonella* isolated from 10 European countries, Austria, Denmark, England and Wales, Germany, Ireland, Italy, Luxembourg, the Natherlands, Scotland, and Spain showed that ceftriaxone resistance were 0.7% in 2000, 0.3% in 2001, 0.4% in 2002, 0.2% in 2003, and 0.2% in 2004 (9). In Taiwan, ceftriaxone resistance in nontyphoidal *Salmonella* isolated during 1999-2003 increased significantly from 0.8% in 1999 to 1.5% in 2003 (10). In 2004, the prevalence of resistance to ESC in *Salmonella* increased to be 3.3% and high rate of resistance was 17.8% in *S.* Choleraesuis in Taiwan (5). During 2003-

2005, a study of nontyphoidal *Salmonella* from seven Asian countries, including Philippines, Hong Kong, Singapore, Sri Lanka, Korea, Thailand, and Taiwan showed that 3.0% were ceftriaxone resistance (11). In addition, it was reported that 3% and 2.3% of nontyphoidal *Salmonella* isolated from China in 2006 and Singapore during 2003-2006, respectively were ceftriaxone resistant (12, 13).

The major cause of resistance to extended-spectrum cephalosporins in nontyphoidal *Salmonella* is production of β-lactamases which destroy β-lactam ring structure of drug, resulting in loss of antibiotic activity. The production of extended-spectrum β-lactamases (ESBLs) and plasmid-mediated AmpC β-lactamases are the important resistance mechanisms to extended-spectrum cephalosporins in nontyphoidal *Salmonella* (14, 15). Recently, the CTX-M-type β-lactamases are rapidly growing group of ESBLs and have been reported in several countries (15). The CTX-M β-lactamase family are sub-classified into five groups (16). Only three groups including CTX-M-1, CTX-M-2, and CTX-M-9 groups have been reported in nontyphoidal *Salmonella* (14). ESBLs which were reported in nontyphoidal *Salmonella* included TEM-3 (17), TEM-52 (18), SHV-2a (10), SHV-5 (19), SHV-12 (10), CTX-M-1 (6), CTX-M-2 (20), CTX-M-3 (10), CTX-M-5 (21), CTX-M-9 (22), CTX-M-14 (23), and CTX-M-15 (24) which were reported in many countries such as Morocco (17), Hungary (18), Taiwan (10), Romania (19), Argentina (20), France (22), China (12), Kuwait and the United Arab Emirates (24). The plasmid-mediated AmpC can be divided into six families based on nucleotide sequences including MOX, CIT, DHA, ACC, EBC, and FOX family (25). Most AmpC β-lactamases are derivatives of CIT-type β-lactamases, including LAT-1, CMY-2 to CMY-7, CMY-12 to CMY-18 and CMY-20 to CMY-50 (26, 27). Recently, CMY-2, CMY-4, CMY-7, ACC-1, and DHA-1 have been found in nontyphoidal *Salmonella* (28-32). CMY-2 was commonly identified worldwide such as England and Wales (28), France (6), United States (8), Taiwan (5, 23), South Korea (33), China (12), and Singapore (13).

The genetic element, IS*Ecp1* has been implicated in the mobilization of *ampC* genes. The IS*Ecp1*, a member of the IS1380 family was found to be associated with many CMY alleles including CMY-2 (34), CMY-4 (35), CMY-5 (36), CMY-7 (29), CMY-12 (37), CMY-14 (37), CMY-15 (37), CMY-16 (38), CMY-21 (39), CMY-31, and CMY-36 as well as ACC-1 (40) and ACC-4 (41). It was reported the putative promoter of IS*Ecp1*

played a role in the high-level expression of bla_{CMY-4} (35). IS*Ecp1* was found in the upstream regions of several CTX-M genes belonging to the CTX-M-1, CTXM-2, CTX-M-9, and CTX-M-25 groups (16). Poirel *et al.* described that IS*Ecp*1 element may involve in the mobilization and provided -35 and -10 promoter sequences, contributing to the high-level expression of the $bla_{CTX-M-19}$ gene (42, 43).

In Thailand, nontyphoidal *Salmonella* isolated from blood were examined in Siriraj Hospital during 2005 and *Salmonella* group C was found to be the most common serogroup (47%) (44). The results showed that 17.8%, 14.1%, 17.8% of isolates were resistant to ceftriaxone, ceftazidime, and cefotaxime, respectively. Likewise, high rate of ceftriaxone resistance was reported in *S.* Choleraesuis isolated from bacteremic patients at King Chulalongkorn Memorial Hospital and from the WHO National *Salmonella* and *Shigella* Center during 2003-2005 (45).

Emergence of extended-spectrum cephalosporin resistance in nontyphoidal *Salmonella* has become a global concern (46, 47). The production of ESBLs and plasmid-mediated AmpC β-lactamases in nontyphoidal *Salmonella* have been reported from many parts of the world. Nevertheless, no data on these β-lactamases in nontyphoidal *Salmonella* has been reported in Thailand. The purpose of this study is to investigate the prevalence of extended-spectrum cephalosporin resistance and characterize genes encoding ESBLs and plasmid-mediated AmpC β-lactamases in nontyphoidal *Salmonella* clinical isolates in Thailand during 2005 and 2007.

CHAPTER II

OBJECTIVES

1. To characterize ESBL and plasmid-mediated AmpC β-lactamase genes in nontyphoidal *Salmonella* from Thailand
2. To investigate prevalence of ESBL and plasmid-mediated AmpC β-lactamases among nontyphoidal *Salmonella* isolated from patients in Thailand
3. To study the effect of IS*Ecp*1 upstream of *bla* genes on cephalosporin resistance level
4. To investigate the prevalence of extended-spectrum cephalosporin resistance in nontyphoidal *Salmonella* isolated from patients in Thailand

CHAPTER III

LITERATURE REVIEW

PART I : MECHANISM OF EXTENDED-SPECTRUM CEPHALOSPORIN RESISTANCE

1. Extended-spectrum β-lactamases (ESBLs)

Many genera of gram-negative bacteria can express chromosome-mediated β-lactamases, which are a major resistance mechanism to β-lactam antibiotics. The first plasmid-mediated β-lactamase to be discovered, TEM-1, was described in 1965 by Datta and Kontomichalou (48). The subsequently discovered TEM-2 was derived from TEM-1 with a similar hydrolytic spectrum (49). The later, SHV-1 was discovered, is another common plasmid mediated β-lactamase. Its hydrolytic spectrum of activity was similar to that of TEM-1, but it had better activity against ampicillin (50). These early plasmid-mediated β-lactamases are broad-spectrum β-lactamases, which are capable of hydrolyzing both penicillins and narrow-spectrum cephalosporins (50). Over the last 20 years, many new β-lactams have been developed that were purposely designed to be resistant to the hydrolytic action of β-lactamases. One of these new classes was the oxyimino-cephalosporins (ceftazidime, cefotaxime, and ceftriaxone) in third-generation cephalosporins, which became extensively used for the treatment of serious infections caused by gram-negative bacteria in the 1980s. Not surprisingly, in 1985, Kliebe *et al.* described the first plasmid-mediated β-lactamase, termed SHV-2 in *Klebsiella ozaenae* isolated in Germany and it was able to hydrolyze the extended-spectrum cephalosporins. Thus, this enzyme was called extended-spectrum β-lactamase (51). Then, other extended-spectrum β-lactamases (ESBLs) have been discovered and increasingly described in several species of Enterobacteriaceae (15, 52).

By definition, ESBLs are molecular class A or D β-lactamases, which (i) are able to hydrolyze oxyimino, (ii) have an active-site serine, and (iii) are inhibited by β-lactamase inhibitors such as clavulanic acid (50, 53). ESBLs contain a number of

mutations that allow them to include its criteria. The major of ESBLs are molecular class A β-lactamases such as TEM, SHV, and CTX-M-type derivatives (15). Class A β-lactamases commonly found four motifs including Ser70-X-X-Lys73 (X is any amino acid), Ser130-X-Asn, Asp233-Lys234-Thr/Ser-Gly, and Ω-loop (Glu166-Pro-Glu-Leu-Asn) which Ser70 was the main catalytic residue in their active site (Ambler numbering (54)) (55, 56) (Figure 1.). The OXA–type β-lactamases are molecular class D β-lactamases and contain an active site serine. In the Bush functional classification scheme, ESBLs are placed in two subgroups of group 2 which are inhibited by clavulanate, including subgroups 2be for mainly TEM and SHV-derived ESBLs and 2d for OXA-derived ESBLs (50).

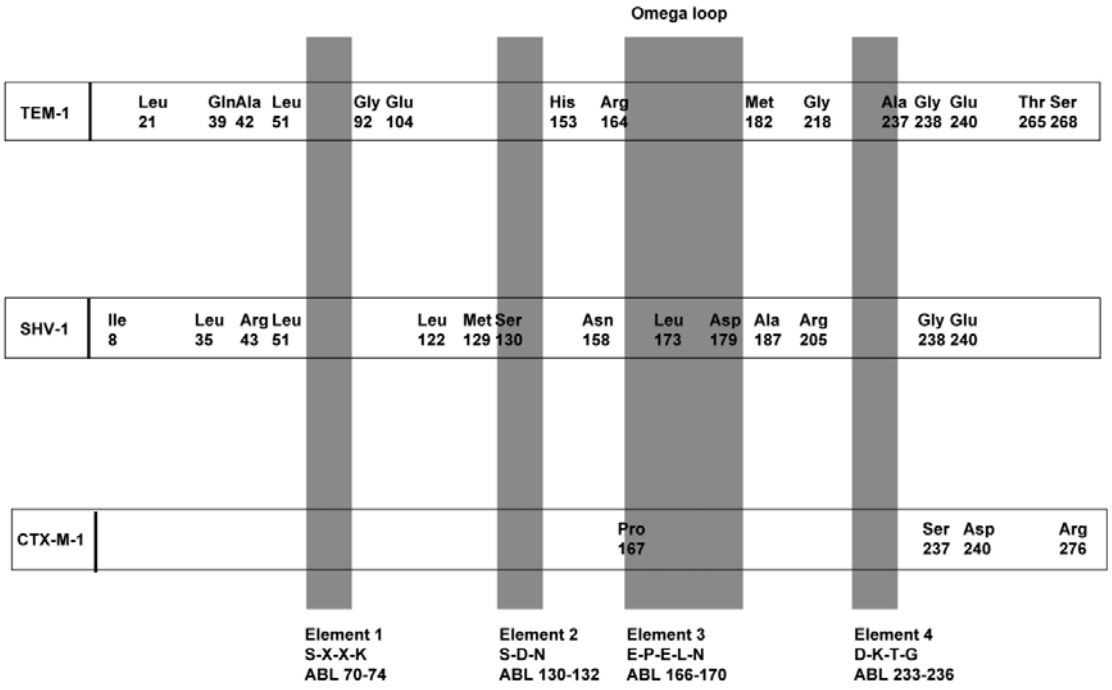

Figure 1. The key amino acid positions in molecular class A β-lactamases TEM, SHV, and CTX-M (52). The grey shaded areas represent the evolutionary conserved structural elements that limit the active site and the numbering is according to the scheme of Ambler *et al.* (57).

ESBLs differ from their parent TEM-1, TEM-2 and SHV-1 enzymes by 1 to 7 amino acid substitutions that alter the configuration and the properties of the active site (Figure 1.). The most important substitutions for extending spectrum activity are position

164 in TEM, 179 in SHV and 238 in both, which enlarge the active site to provide enough space for containing the oxyimino side-chains (52, 58). ESBLs have the ability to hydrolyze the oxyimino-cephalosporins at rates at least 10% of that observed for benzylpenicillin, in contrast to their parent, which poorly hydrolyze the extended-spectrum β-lactams (52). In addition, ESBL enzymes are consistently resistant to penicillins and are not active against cephamycins (cefoxitin and cefotetan) and carbapenems (imipenem, meropenem, and ertapenem) (52, 59). However, it has been reported that ESBL producing strains can become resistant to cephamycins or carbapenems due to the loss of outer membrane (60, 61).

ESBLs have been reported frequently in *Escherichia coli* and *Klebsiella* species (52). Furthermore, these enzymes have been found in other bacterial species including *Salmonella* spp., *Pseudomonas aeruginosa,* and *Proteus mirabilis* (15, 62). The early predominance of TEM- and SHV-type variants among ESBLs appears to reflect the widespread distribution of their plasmid-mediated ancestor enzymes (TEM-1 and SHV-1) in the 1970s. On the other hand, the CTX-M-type β-lactamases have increased dramatically in recent years and are the most rapidly growing group of ESBLs (15).

1.1 TEM

TEM-1 was discovered from *E. coli* isolate from a Greek patient named Temoniera, thus termed TEM (63). The TEM-1 with isoelectric point (pI) of 5.4 is able to hydrolyze penicillins and first-generation cephalosporins and spread worldwide. It is now the most common mechanism of β-lactam resistance in gram-negative bacilli. In *E. coli* up to 50–60% of ampicillin-resistant was due to the production of TEM-1 (53). TEM-2 with pI of 5.6, the first derivative of TEM-1, has a single amino acid substitution Gln39Lys from TEM-1 and has an almost identical hydrolytic profile with TEM-1 (59). The first TEM variant with increased activity against extended-spectrum cephalosporins was TEM-3 that defined as the ESBL phenotype (64). TEM-3 has two amino acid substitutions from TEM-2, Glu104Lys and Gly238Ser and shows pI of 6.3 (59). After that, a rapid increase of TEM variants and the number of derivatives have been described from TEM-1 reach to TEM-178 in recent years (26). Some of them are inhibitor-resistant enzymes, but the majority of the new derivatives are ESBLs. The broader spectrum of

resistance is related to the amino acid substitutions in certain areas which are adjacent to the four evolutionary conserved structural elements of the enzyme (55) (Figure 1.). The amino acid substitutions that occur within TEM variants occur at a limited number of positions (26, 59). The combinations of these amino acid changes result in various alterations in the ESBL phenotypes, such as the ability to hydrolyze specific oxyimino-cephalosporins such as ceftazidime and cefotaxime, or a change in their isoelectric points, which range from a pI of 5.2 to 6.5 (26, 59).

Inhibitor-resistant TEM β-lactamases (IRT) have TEM variants ESBL-type mutations which have been identified together with those that determine resistance to β-lactamase inhibitors. The key amino acid residues are positions 69, 130, 244, 275 and 276 (52). Most of the inhibitor-resistant TEM β-lactamases are inefficient at hydrolyzing oxyimino-cephalosporins. However, in rare cases variants (e.g. TEM-50 and TEM-68) it has been found to retain both activities at significant levels (65).

TEM-type ESBLs are most often found in *E. coli* and *K. pneumoniae* and have been reported in Enterobacteriaceae such as *Morganella morganii, Proteus mirabilis,* and *Salmonella* spp. (66, 67). Furthermore, TEM-type ESBLs have been found in non-Enterobacteriaceae gram-negative bacteria such as TEM-42 β-lactamase which was found in a strain of *P. aeruginosa* (103).

1.2 SHV

SHV β-lactamases appear to be derived from *Klebsiella* spp. The progenitor of SHV enzymes, SHV-1, is commonly found in *K. pneumoniae* within the chromosome, so it may be that the gene for SHV-1 β-lactamase was evolved as a chromosomal gene in *Klebsiella* and was later incorporated into a plasmid which has spread to other bacterial species (53). SHV-1 with pI of 7.6 confers resistance to penicillins and narrow-spectrum cephalosporins such as cephalothin and cephaloridine but not to the oxyimino-cephalosporins (53). Then, SHV-2 with the same pI, the first derivative of SHV-1 was demonstrated to be the first plasmid-mediated resistance mechanism for oxyimino-cephalosporins. Therefore, the first reported ESBL belonging to the SHV family, SHV-2, differs from SHV-1 by a single amino acid, Gly238Ser (51). The amino acid substitution resulted in an enhanced affinity for oxyimino-cephalosporins, with a

significant increase in the MIC to cefotaxime and a more limited increase in the MIC to ceftazidime (51). Consequently, a number of ESBL variants have been reported to contain additional amino acid substitutions which mostly occur in positions 179, 205, and 240 (52) (Figure 1.). Most of SHV-type ESBL enzymes have a Gly238Ser substitution in common. In addition, a number of variants related to SHV-5 also have a Glu240Lys substitution. Ser-238 is important to cefotaxime hydrolysis whereas additional Glu240Lys substitution increases the hydrolytic activity against ceftazidime (68). Recently, the number of SHV derivatives have been described from SHV-1 reach to SHV-131 which are over 40 variants of SHV-type ESBL enzymes (26).

Unlike the situation in the TEM-type enzymes, there has been only one report of a SHV variant that has an inhibitor-resistant phenotype. In SHV-10, position 130 serine was replaced by glycine. The enzyme partially retained its ability to hydrolyze penicillins, but its activity against cephalosporins was significantly reduced (69).

The majority of SHV-type ESBLs are found in *K. pneumoniae* (59). However, these enzymes have also been found in *Citrobacter diversus, E. coli*, and *P. aeruginosa* (70-72).

1.3 CTX-M

The new family of plasmid-mediated ESBLs, called CTX-M, that preferentially hydrolyzes cefotaxime, has become particularly widespread in recent years. The first CTX-M (CTX-M-1), with an pI of 8.9, was found in a clinical isolate of *E. coli* from Germany in 1989 (73, 74). CTX-M-2, with an pI of 7.9, was described from an isolate of multidrug-resistant *Salmonella enterica* serovar Typhimurium and had 84% amino acid identity to CTX-M-1 (74, 75). In recent years, the number of CTX-M derivatives have been described from CTX-M-1 to CTX-M-92 but the amino acid sequences of CTX-M-14 and CTX-M-18 of CTX-M-55 and CTX-M-57 are identical (26). CTX-M-type enzymes are divided into five groups, namely CTX-M-1, CTX-M-2, CTX-M-9, CTXM-8, and CTX-M-25, according to the similarity of their amino acid sequences (16, 26). CTX-M derivatives were: the CTX-M-1 group, including CTX-M-1, -3, -10, -11, -12, -15, -22, -23, -29, -30, -32, -33, -28, -36, and CTX-M-54; the CTX-M-2 group, including CTX-M-2, -4, -6, -7, -20, -31,and CTX-M-44; the CTX-M-9 group, including CTX-M-9, -13, -14, -16, -17, -19, -24,

-27, -45, -46, -47, -48, -49, and CTX-M-50; the CTX-M-8 group, including CTX-M-8 and CTX-M-40; the CTX-M-25 group, including CTX-M-25, -26, -39, and CTX-M-41 (16). The gene sequences encoding CTX-M enzymes show a high similarity to β-lactamases of *Kluyvera* species. Moreover, the gene sequences adjacent to the CTX-M genes of Enterobacteriaceae are also similar to those surrounding the β-lactamase genes on the chromosomes of *Kluyvera* species. Thus, it is considered that the CTX-M-1 and CTX-M-2 groups were originate from the chromosomal β-lactamase of *Kluyvera ascorbata*, as the CTX-M-8 and CTX-M-9 groups are derived from the chromosomal β-lactamase of *Kluyvera georgiana* (76-79). CTX-M-producing strains are resistant to cefotaxime, but they often appear to be susceptible to ceftazidime. The crystal structure of CTX-M enzymes has shown that the active sites of CTX-M enzymes are not large enough to recognize ceftazidime, which is larger than cefotaxime (80). Moreover, most of CTX-M enzymes hydrolyze cefepime effectively and MIC values of cefepime for bacteria producing CTX-M enzymes tend to be higher than those for bacteria producing other types of ESBLs (81). The capabilities of the CTX-M enzymes to hydrolyze extended-spectrum cephalosporins are 'intrinsic' and not the result of a few amino acid substitutions enabling an ancestral enzyme to expand its substrate spectrum towards oxyimino-cephalosporins (52). Nevertheless, a key role in extended-spectrum activity has been attributed to Ser-237, Asp-240, and Arg-276 (82-84) (Figure 1.). It has been suggested that the serine residue at position 237, which is present in all of the CTX-M enzymes, plays an important role in the extended-spectrum activity of the CTX-M-type β-lactamases (82). Furthermore, substitutions of Asp240 and Pro167 are known to lead to enhance hydrolysis to ceftazidime. Asp240Gly substitution appears to increase the flexibility of B3 β-strand, allowing an increase in the activity against ceftazidime (85). Mutation in the Ω-loop at position Pro167 modifies the interaction between β-lactams and the binding sites and enhances significantly ceftazidime hydrolytic activity (86, 87).

Inhibition by β-Lactamase inhibitors such as sulbactam, clavulanate, and tazobactam are commonly known as inactivators of class A ESBLs. Interestingly, CTX-M-14 is capable of hydrolyzing sulbactam, while clavulanate and tazobactam retain their ability to inactivate this enzyme (88). Toho-1 (CTX-M-44) also possesses a similar hydrolytic activity against sulbactam (80).

The major organisms producing TEM-type and SHV-type ESBLs are identified from hospitalized patients especially in strain of *Klebsiella* spp., however a growing number of infections caused by CTX-M producing organisms in the community setting have been reported typically in *E. coli* since the late 1990s (89). Moreover, these enzymes have commonly been found in strains of *Salmonella* spp. in recent years, and have also been described in other species of Enterobacteriaceae (59). Interestingly, many of new variant of CTX-M enzymes have been found among isolates of *S.* Typhimurium (90, 91). The outbreaks of CTX-M producing strains of *S.* Typhimurium have been reported in South America and Eastern Europe. It has also been found to express a variety of CTX-M type variants (20, 90, 92).

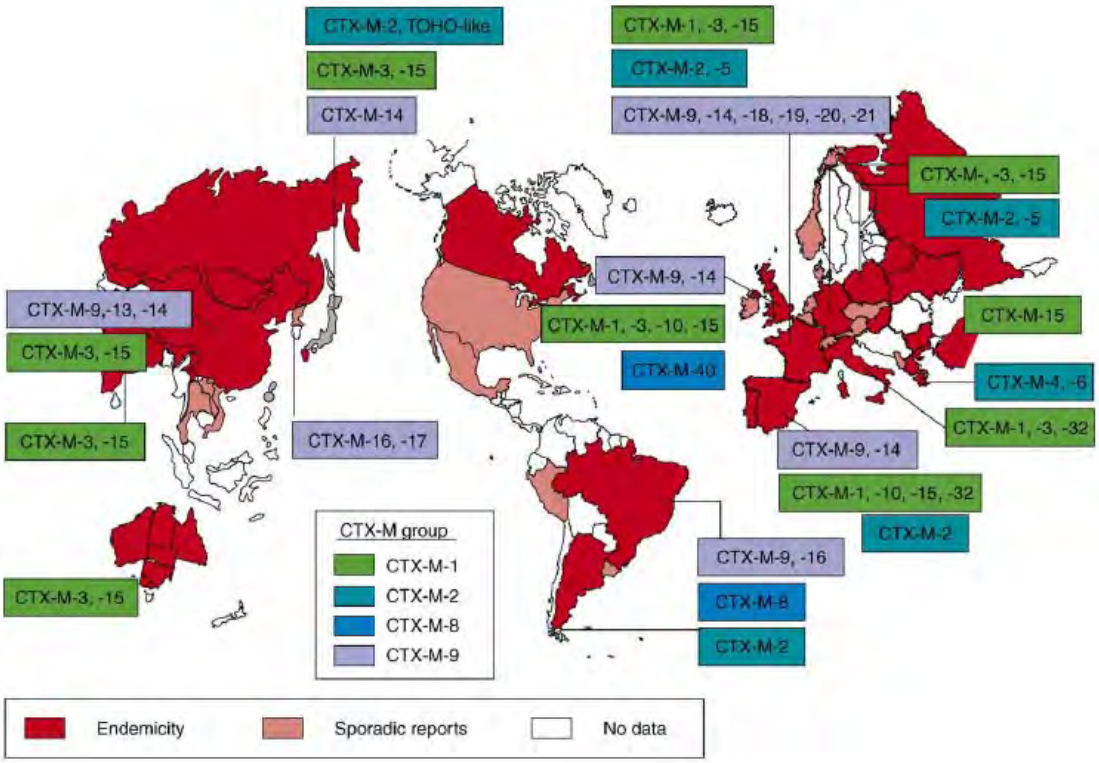

Figure 2. The current situation of CTX-M type ESBLs in different geographic areas (16).

Recently, it is interesting that an endemic situation is dominant in most countries in Europe, Asia and South America (16) (Figure 2.). In the USA, there are only sporadic reports have been published (93). Different enzymes are not similarly represented in all geographic areas (16) (Figure 2.). The enzymes from the CTX-M-9 group are well represented in the countries surrounding the Mediterranean Sea (94, 95). The CTX-M-2

has been mainly isolated in South America and Japan (96, 97) whiles CTX-M-15 is spread nearly worldwide (98, 99). CTX-M enzymes have been reported in many outbreaks worldwide, e.g. in China (CTX-M-3, CTX-M-9, CTXM-13, and CTX-M-14) (100, 101), Vietnam (CTX-M-14 and CTX-M-17) (102), Taiwan (CTX-M-3 and CTX-M-14) (103), Korea (CTX-M-14) (104), Poland (CTX-M-3 and CTX-M-15) (105, 106). This suggests that they are widely dispersed. There is a concern that CTX-M β-lactamases confer resistance to all cephalosporins, but are not detectable by detection tests which are based on using only ceftazidime.

The genetic elements have been demonstrated to be involved in the mobilization of $bla_{\text{CTX-M}}$ such as two insertion sequence IS*Ecp1* and the IS*CR1* element (formerly recognized as CR1 element or ORF513) (16). IS*Ecp1* has been found upstream of several CTX-M genes belonging to the CTX-M-1, CTXM-2, CTX-M-9, and CTX-M-25 groups (16). IS*Ecp1* belongs to the IS1380 family and is capable of mobilizing the neighboring genes by one-ended transposition mechanism (107). A *in vitro* study revealed that the mobilization of $bla_{\text{CTX-M-2}}$ from *K. ascorbata* to *E. coli* was achievable in the presence of IS*Ecp1* (108). A study by Poirel *et al.* demonstrated that IS*Ecp1* element may involve in the mobilization that generated a 5 bp duplication at the target site and provided -35 and -10 promoter sequences, contributing to the high-level expression of the $bla_{\text{CTX-M-19}}$ gene (42, 43). Genes encoding the CTX-M-2 and CTX-M-9 groups have also been observed within IS*CR1* associated with class 1 integron. It has been shown that both IS*Ecp1* and IS*CR1* provide promoter sequences for high-level expression of CTX-M enzymes (42, 109).

1.4 Other ESBLs

The OXA type (oxacillin-hydrolyzing) enzymes are another widespread family of β-lactamases. These enzymes are classified into class D in the Ambler scheme and were placed in group 2d in the Bush functional scheme (50, 57). Their preferred substrates are penicillins and cloxacillin, rather than oxyimino-cephalosporins and poorly inhibited by clavulanic acid (59). There have been only rare reports of ESBL-type members of the OXA family. They are most often found in *P. aeruginosa,* rather than in members of the Enterobacteriaceae. ESBL phenotypes have been observed mostly as a

consequence of point mutations of OXA-2 (OXA-15, and OXA-32) and of OXA-10 derivatives (OXA-11, OXA-13, OXA-14 to OXA-17, OXA-19, OXA-28, and OXA-35) (52). In addition, novel groups of ESBLs, unusual enzymes that also have extended-spectrum activity have been reported (e.g. BES-1, CME-1, VEB-1, PER, SFO-1). These novel enzymes are found infrequently (59).

2. Plasmid-mediated AmpC β-lactamases

AmpC β-lactamases, verified to be chromosomally mediated since 1981, have been described in many genera of bacteria such as *Acinetobacter* spp., *Aeromonas* spp., *Chromobacterium violaceum*, *C. freundii*, *Enterobacter* spp., *E. coli*, *Hafnia alvei*, *Lysobacter lactamgenus*, *Morganella morganii*, *Ochrobactrum anthropi*, *Proteus rettgeri*, *Providencia stuartii*, *P. aeruginosa*, *Psychrobacter immobilis*, *Rhodobacter sphaeroides*, *S. marcescens*, and *Yersinia enterocolitica* (25, 110).

Until 1989, Bauernfeind *et al.* demonstrated *K. pneumoniae* isolate from South Korea that could transfer resistance to penicillins, cefoxitin, cefotetan, oxyimino-cephalosporins, and monobactams to *E. coli*. The enzyme was identified as plasmid-mediated AmpC β-lactamase, termed CMY-1 which showed 82% identity to chromosomal AmpC from *Aeromonas hydrophila* (111, 112). This enzyme had cephamycinase activity, with an pI of 8.0 and was poorly inhibited by clavulanate or tazobactam (111). Lately, the plasmid-mediated AmpC β-lactamases have been reported in several bacterial species that lack chromosomal AmpC enzymes, such as *K. pneumoniae*, *K. oxytoca, Salmonella* spp., and *P. mirabilis* as well as *E. coli* , which has a low level expression of chromosomal AmpC enzymes (25, 27).

Plasmid-mediated AmpC β-lactamases have molecular masses of 38 to 42 kDa and isoelectric points between 6.4 to 9.4 (25). The three-dimensional structures of AmpC enzymes are shown in Figure 3.

A B

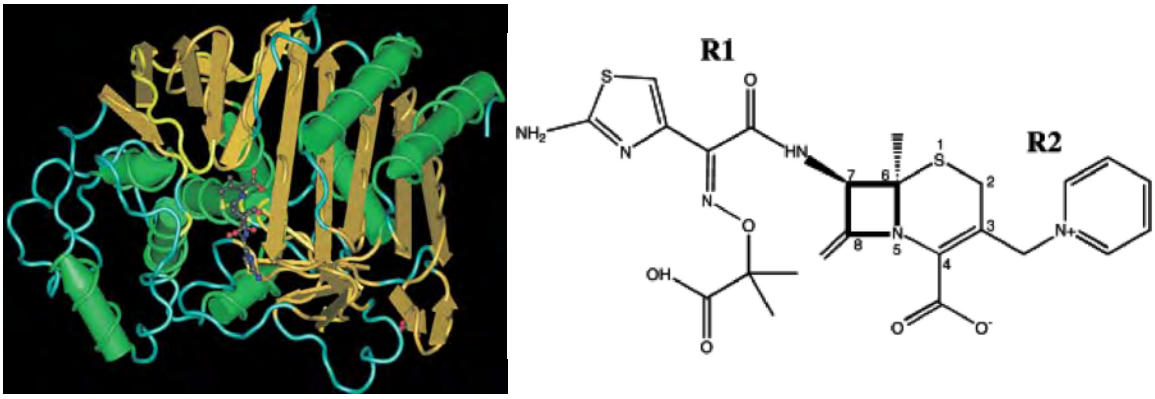

Figure 3. Diagram of AmpC from *E. coli* complexed with acylated ceftazidime (PDB accession number 1IEL): (A), The conserved residues S64, K67, Y150, N152, K315, and A318 are shown in yellow (27).; (B), Schematic representation of ceftazidime with the R1 side chain at C7 and the R2 side chain at C3 (27).

There is α-helical domain and α/β domain, which the active site lies in the center of two domains (27, 56). The key catalytic residues are found in active site pocket other than Ser64 for class C enzymes include Lys67, Tyr150, Asn152, Lys315, and Ala318, with substitutions at these sites lowering enzymatic activity significantly (27). The enzymes showed that the motif Ser-X-X-Lys (X is any amino acid) at residues 64 to 67 were a serine active site of the mature protein. A Lys-Ser/Thr-Gly motif has been found at residues 315 to 317 and plays an essential role in forming the tertiary structure of the active site. A tyrosine residue at position 150 forms motif Tyr-X-Asn and is also important for catalysis of β-lactam hydrolysis (113, 114). The active site is subdivided into an R1 site, accepting the R1 side chain at C7 of the β-lactam nucleus, and an R2 site for the R2 side chain at C3 (27) (Figure 3.). AmpC enzymes were classified to class C in the Ambler structural classification of β-lactamases (57) and were assigned to group 1 in the functional classification scheme of Bush *et al.* (50). AmpC enzymes were consistently resistant to penicillins and provided resistance to cephalosporins in the oxyimino-cephalosporins (ceftazidime, cefotaxime, ceftriaxone) and the cephamycins (cefoxitin, cefotetan) (25). MICs were usually higher for ceftazidime than for cefotaxime and for cefoxitin than for cefotetan (25). These enzymes were also resistant to the monobactam and aztreonam and were poorly inhibited by β-lactamase inhibitors such

as clavulanic acid, sulbactam, and tazobactam (25). However, the enzymes were susceptible to cefepime, cefpirome and carbapenems (imipenem, meropenem) (25).

It has been suggested that plasmid-mediated AmpC β-lactamases originate from the transfer of chromosomal genes onto plasmids (25, 27, 115). This transfer has resulted in plasmid-mediated AmpC enzymes in several members of the family Enterobacteriaceae (25). The plasmid-mediated AmpC β-lactamases are classified base on amino acid sequences into six families that are closely related to chromosomal-mediated AmpC β-lactamases as indicated in Figure 4. (25, 116).

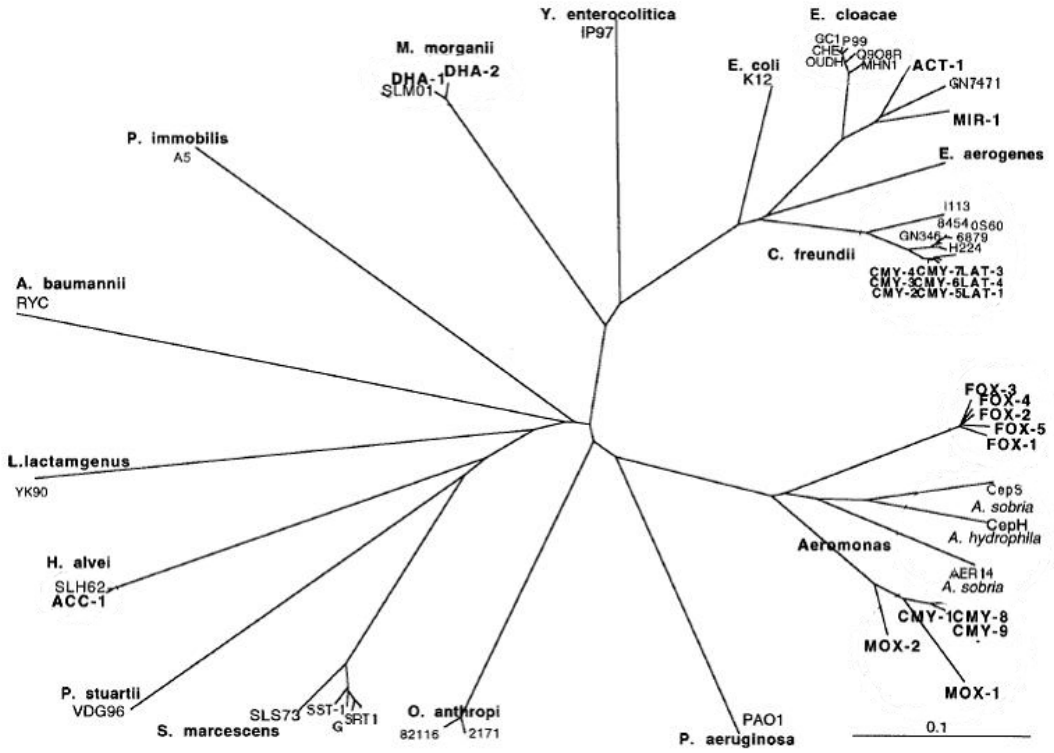

Figure 4. Dendrogram for chromosomal and plasmid-mediated AmpC β-lactamases: Plasmid AmpC are shown in boldfaces and branch lengths are proportional to the number of amino acid exchanges (25).

2.1 MOX

The MOX-type β-lactamases included CMY-1, CMY-8 to CMY-11, CMY-19, MOX-1 to MOX-8 (25, 26, 116). The percentage of amino acid identities among the family members within these family were more than 83.4% and were related to chromosomal-mediated AmpC enzymes of *Aeromonas* spp. (25, 27, 116, 117).

Horii *et al.* demonstrated MOX-1 with pI of 8.4 in *K. pneumoniae* isolate from Japan. This enzyme showed 80% identity to chromosomal AmpC from *A. hydrophila* (118).

2.2 CIT

Most of AmpC β-lactamases were derivatives of the CIT-type β-lactamases, including LAT-1, CMY-2 to CMY-7, CMY-12 to CMY-18, and CMY-20 to CMY-52 (25, 26, 116). The relationship among the family members within these group (LAT-1 and CMY-2 to CMY-7) were more than 96% identity with each other and were more than 94% identity with chromosomal-mediated AmpC enzymes of *C. freundii* (25). LAT-1 was described to be the first enzyme in this family with pI of 9.4 and showed 95% identiy to chromosomal AmpC from *C. freundii*. This enzyme was found in *K. pneumoniae* isolates from Greece in 1993 (119). In 1996, Bauernfeind *et al.* demonstrated CMY-2 with pI of 9.0 in *K. pneumoniae* isolate from Greece. It showed 96% identity to chromosomal AmpC from *C. freundii* (120). In addition, AmpC linked to *ampR* gene, termed CFE-1 was described in this family and was found in *E. coli* isolate from Japan by Nakano *et al.* showed 99% identity to chromosomal AmpC from *C. freundii* (121).

2.3 DHA

The DHA-type β-lactamases included DHA-1 (30, 122) and DHA-2 (123). The percentage of amino acid identity between DHA-1 and DHA-2 was 98%. These enzymes were related to chromosomal-mediated AmpC enzymes of *M. morganii* with 99% amino acid identity (25, 122, 123). Gaillot *et al.* demonstrated DHA-1 with pI of 7.8 in *S.* Enteritidis isolate from Saudi Arabia. DHA-1 showed 99% identity to chromosomal AmpC from *M. morganii* (122).

2.4 ACC

The ACC-type β-lactamases included ACC-1 to ACC-4 (26). Bauernfeind *et al.* first described ACC-1 in *K. pneumoniae* isolate from Germany and showed that *E. coli* transconjugants with ACC-1 were resistant to ceftazidime but not cefoxitin or cefotetan and had an pI of 7.7 (124). Girlich *et al.* demonstrated that ACC-1 showed 99% identity to chromosomal AmpC from *H. alvei* (125).

2.5 EBC

The EBC-type β-lactamases included ACT-1 to ACT-8 and MIR-1 to MIR-5 (26). ACT-1 and MIR-1 shared 91.4% amino acid identity with each other (25). Papanicolaou *et al.* and Jacoby *et al.* described MIR-1 with pI of 8.4 in *K. pneumoniae* isolate from United States. This enzyme showed 99% identity to chromosomal AmpC from *E. cloacae* (126, 127). Bradford *et al.* and Rottman *et al.* showed that ACT-1 from *K. pneumoniae* isolate from United States had pI of 9.0 and showed 98% identity to chromosomal AmpC from *Enterobacter asburiae* (128, 129).

2.6 FOX

The FOX-type β-lactamases included FOX-1 to FOX-7 (26). The percentage of amino acid identity among the family members within these family was more than 96% (117). Gonzalez Leiza *et al.* and Fosse *et al.* demonstrated that FOX-1 in *K. pneumoniae* isolate from Argentina showed 99% identity to chromosomal AmpC from *A. caviae* (130, 131).

Variations in β-lactam MICs resulted from substitution of amino acid in different type of plasmid-mediated AmpC β-lactamases and level of AmpC expression (27). Most *amp*C genes on plasmids were considered to be noninducible because of the constitutively high-level expression of AmpC enzymes (25). Thus, these enzymes were resistant to all cephalosporins except the fourth-generation cephalosporins. Most of chromosomal AmpC were inducible enzymes, which were not resistant to the third-generation cephalosporins unless the AmpC β-lactamase was induced to be a high-level expression (132). However, the inducible plasmid-mediated AmpC β-lactamases of *E. cloacae*, *M. morganii,* and *C. freundii* origins, including ACT-1, DHA-1, DHA-2, and CMY-13 have been described (30, 123, 133, 134). The genes for ACT-1, DHA-1, DHA-2, and CMY-13 are linked to *ampR* genes of their original organisms.

The induction of AmpC β-lactamase is controlled by the activity of three genes linked to the cell wall recycling pathway. The *ampR* encodes a transcriptional regulator of the LysR family, *ampD* encodes a cytoplasmic *N*-acetyl-muramyl-L-alanine amidase,

and *ampG* encodes cytoplasmic membrane-bound permease which allows entry of cell wall degradation products (27, 135). The *ampC* and *ampR* genes are species specific which are present only in members of the family Enterobacteriaceae with inducible β-lactamases. The *ampD* and *ampG* genes are very common and highly conserved in all members of the family Enterobacteriaceae because they encode enzymes involved in cell wall recycling pathway. In the cell wall recycling pathway, AmpG transports the cell wall degradation products, GlcNAc-anhMurNAc-tripeptide (GlcaMurTp) into the cytosol and the GlcNAc residue is removed by the cytosolic N-β-acetylglucosaminidase. The resulting anhMurNAc-tripeptide (aMurTp) is hydrolyzed by AmpD into 1,6-anhydromuramic acid and peptide (135). The peptide is processed into tripeptide, which is reused by the enzymes of the cell wall recycling pathway, finally resulting in the formation of the cell wall precursor, UDP-MurNAc-pentapeptide (135). The disruption of cell wall biosynthesis by a β-lactam agent leads to an intracellular accumulation of the AmpD substrate, anhMurNAc-tripeptide (aMurTp). These oligopeptides compete with UDP-MurNAc-pentapeptide for a binding site on AmpR, which binds to a 38-bp sequence within the intercistronic region between *ampR* and *ampC*. Displacement of the UDP-MurNAc-pentapeptide signals a conformational change in AmpR, which changes from repressor to activator for activation of the *ampC* transcription (27, 135) (Figure 5.).

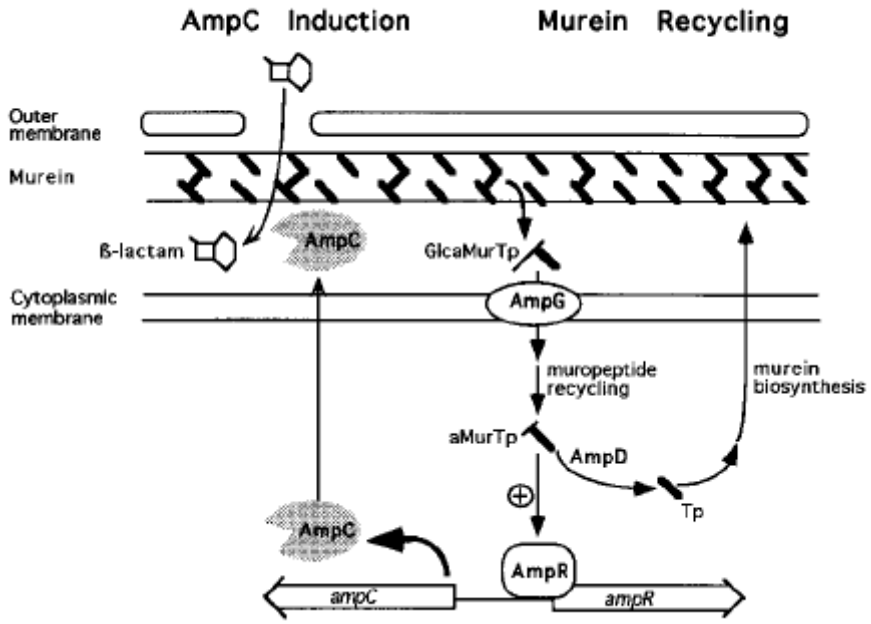

Figure 5. The pathway of Murein recycling (Cell wall recycling) and their role in AmpC β-lactamase induction (135)

β-Lactams are different in their inducing abilities (136-138). Benzylpenicillin, ampicillin, amoxicillin, and cephalosporins such as cefazolin and cephalothin are strong inducers and good substrates for AmpC β-lactamases. Cefoxitin and imipenem are also strong inducers but are much more stable for hydrolysis. However, cefoxitin are commonly used to detect inducible AmpC β-lactamase phenotype because a rapid bacteriocidal action of imipenem will kill the organism before induction (132, 139). Moreover, β-Lactamase inhibitors are also inducers, particularly clavulanate, which has little inhibitory effect on AmpC β-lactamase activity (140) but can paradoxically appear to increase AmpC mediated resistance in an inducible organism (141). The mutation in the genes for regulation of AmpC expression causes AmpC overexpression. The most common cause of AmpC overexpression is a mutation in *ampD*, leading to AmpC hyperinducibility or constitutive hyperproduction (142). AmpR mutations are less common but can also cause high-constitutive or hyperinducible phenotypes (143-145). Least common are mutations in AmpG, which cause constitutive low-level expression (146). Although the *ampC* encoded on plasmids lack *ampD* genes, the level of

expression can also be increased with the loss of chromosomal AmpD function (142, 147).

Nevertheless, the level of expression of both inducible and noninducible plasmid-mediated AmpC enzyme; is higher than the level of expression of the chromosomal-mediated AmpC. This is due to a higher gene copy number for the plasmid-encoded enzymes, greater promoter strength for the plasmid genes, and the absence of *ampR* (27). Reisbig *et al.* described that the expression of both inducible ACT-1 and noninducible MIR-1 plasmid-mediated *ampC* genes were 33- to 95-fold higher than the level of expression of the chromosomal-mediated *ampC* genes of *E. cloacae* because of a higher gene copy number for the plasmid-mediated enzymes (2 copies for bla_{ACT-1} and 12 copies for bla_{MIR-1}) and greater promoter strength for the plasmid genes (8-fold increased from the hybrid MIR-1 promoter and 17-fold increased because of a single base change relative to the wild type in the ACT-1 promoter) (147, 148).

Genes for the AmpC enzymes have been located on plasmids of sizes varying from 7 to 180 kb (25). Most of them are self-transmissible and the remaining are transferable by transformation (36, 127) or mobilization (119, 149). Plasmids encoding AmpC enzymes often carry multiple resistance, including resistance to aminoglycosides, chloramphenicol, sulfonamide, tetracycline, and trimethoprim (27). *K. pneumoniae* strains with loss of outer membrane porin channels and carrying plasmid-mediated AmpC were resistant to imipenem (128, 150). The plasmid *ampC* genes have been found to coexist with the *bla* gene encoded other β-lactamases such as TEM-1, PSE-1 (151), CTX-3 (152), SHV varieties (153), and VIM-1 (133). The *bla* genes may be on different plasmids, but often coexist on the same plasmid. In addition, the same *ampC* gene can be incorporated into different backbones on different plasmids (154).

A variety of genetic elements have been implicated in the mobilization of *ampC* genes onto plasmids. The insertion sequence IS*Ecp1* is associated with many CMY alleles including CMY-2 (34), CMY-4 (35), CMY-5 (36), CMY-7 (29), CMY-12 (37), CMY-14 (37), CMY-15 (37), CMY-16 (38), CMY-21 (39), CMY-31, and CMY-36 as well as ACC-1 (40) and ACC-4 (41). Other *ampC* genes including several CMY varieties (CMY-1, -8, -9, -10, -11, and -19), DHA-1, and MOX-1 are found adjacent to an insertion

sequence common region (IS*CR1*) involved in gene mobilization into complex class 1 integrons (155). On the other hand, the gene for CMY-13 with *ampR* gene are bounded by two directly repeated IS*26* elements (133).

Recently, nontyphoidal *Salmonella* isolates have been reported worldwide to have both inducible and noninducible plasmid-mediated AmpC β-lactamases including CMY-2, CMY-4, CMY-7, ACC-1, and DHA-1 (28-31). CIT derivatives especially CMY-2 has been commonly reported to be responsible for ceftriaxone resistance in nontyphoidal *Salmonella* (12, 23, 154). CMY producers were found in several serovars of *Salmonella enterica*, with *S.* Typhimurium and Newport being the most common (14, 62, 156).

PART II : EPIDEMIOLOGY OF EXTENDED-SPECTRUM CEPHALOSPORIN RESISTANCE IN NONTYPHOIDAL *SALMONELLA*

Extended-spectrum cephalosporins (ESCs), especially ceftriaxone, are commonly used to treat invasive infections and severe diarrhea caused by nontyphoidal *Salmonella*. However, ceftriaxone resistant nontyphoidal *Salmonella* in human has frequently been reported worldwide in recent years, including United States, Europe, South America, and Asia (6-8, 11). The prevalence of ESC resistance among nontyphoidal *Salmonella* in different geographic areas is shown in Table 1.

In three-year period study, 1996-1998, by the National Antimicrobial Resistance Monitoring System (NARMS) the prevalence of ceftriaxone resistance in 4,093 nontyphoidal *Salmonella* isolates from 17 states of the United States was increasing from 0.1% in 1996 to 0.4% in 1997, and 0.5% in 1998, respectively (4). 15 isolates, were ceftriaxone MIC of $\geq$ 16 mg/L and 12 (80%) of isolates were *S.* Typhimurium. Thirteen (86.7%) of isolates produced CMY-2 enzyme and 2 (13.3%) of isolates produced ESBL with pI of 5.4 and 7.6. Later, the 1,378 isolates of 2000 NARMS collection were examined and ceftriaxone resistance increased to be 3.2% (44/1,378). All isolates produced CMY-2 and 27 (61%) of 44 CMY-2-producing *Salmonella* were *S.* Newport (8). A study in Taiwan, the 3,592 nontyphoidal *Salmonella* isolated in 1999-2003 which were examined (10). The results showed that, 0.6% (22/3,592) of isolates were resistant to ceftriaxone and rate of resistance increased in several serogroups (0.8-2.1%; average, 1.5%). Moreover, the ceftriaxone resistance increased significantly from 0.8% in 1999 to 1.5% in 2003. All 22 ceftriaxone-resistant isolates were associated with CMY-2 in 14 (63.64%) isolates and with ESBLs in 8 (36.36%) isolates, including CTX-M-3 (6 isolates), SHV-2a (1 isolate), and SHV-12 (1 isolate). In 2004, the prevalence of resistance to ESC in nontyphoidal *Salmonella* in Taiwan were increased to be 3.3% (20/600) and 17.8% high rate resistance were detected in *S.* Choleraesuis. All ESC-resistant isolates produced CMY-2 (5). During 2003-2005, a study of nontyphoidal *Salmonella* isolates in seven Asian countries (Philippines, Hong Kong, Singapore, Sri Lanka, Korea, Thailand, and Taiwan) showed that 3.0% were resistant to ceftriaxone (11).

In Thailand, the study in Siriraj Hospital during 2005, 135 nontyphoidal *Salmonella* isolated from blood were examined (44). *Salmonella* serogroup C was most common serogroup (47%) followed by group D and group B and 37% of the patients were HIV seropositive. The results showed that 17.8%, 14.1%, and 17.8% of isolates were resistant to ceftriaxone, ceftazidime, and cefotaxime, respectively. Likewise, Kulwichit *et al.* reported high rate of ceftriaxone resistance in nontyphoidal *Salmonella* isolates, especially *S.* Choleraesuis which were isolated from bacteremic patients at King Chulalongkorn Memorial Hospital and from bacteremic patients in Thailand sent to the WHO National *Salmonella* and *Shigella* Center during 2003-2005 (45).

Table 1. The prevalence of ESC resistance among nontyphoidal *Salmonella* in different geographic areas

Country/region	Year (collected)	No. of test isolates	ESC resistance (%)	Serotypes	Mechanism of ESC resistance	Reference
America						
United States	1996	1,326	1 (0.1%)	*S.* Thompson	CMY-2	(4)
	1997	1,301	5 (0.4%)	*S.* Typhimurium		
	1998	1,466	7 (0.5%)	*S.* Cubana		
				S. Newport		
United States	2000	1,378	44 (3.2%)	*S.* Typhimurium	CMY-2	(8)
				S. Newport		
				S. Heidelberg		
United States	2003	1,864	1 (<0.1%), 105(5.6%)[b]	*S.* Typhimurium	CTX-M-5	(157)
North America	1997-2000	116	0 (0%)	*S.* Typhimurium	CMY-2	(7)
	2001-2003	192[a]	4 (2.1%)	*S.* Newport		
	2004	31	1 (3.2%)			
North America	2003	57[c]	0 (0%), 2(3.5%)[b]	*Salmonella* spp.		(21)
Latin America	1997-2000	161	0 (0%)	*S.* Typhimurium	CMY-2	(7)
	2001-2003	234[a]	1 (0.4%)	*S.* Newport		
	2004	47	0 (0%)			
Latin America	2003	170	1 (0.6%)	*Salmonella* spp.	CTX-M-2	(21)

Table 1. The prevalence of ESC resistance among nontyphoidal *Salmonella* in different geographic areas (cont.)

Country/region	Year (collected)	No. of test isolates	ESC resistance (%)	Serotypes	Mechanism of ESC resistance	Reference
Europe						
England and Wales	1992-2003	278,308	106 (<0.1%)	*S.* Senftenberg	CMY-2	(28)
				S. Typhimurium	DHA-1	
				S. Anatum	CMY-4	
				S. Worthington		
Europe	2003	664	15 (2.4%)	*S.* Typhimurium	CTX-M-5	(21)
Europe	2000	24,413	171(0.7%)	*S.* Typhimurium	ND	(9)
	2001	27,800	83 (0.3%)	*S.* Enteritidis		
	2002	26,734	107 (0.4%)	*S.* Virchow		
	2003	26,673	53 (0.2%)			
	2004	23,811	48 (0.2%)			
France	2000-2005	585[d]	46 (7.9%)	*S.* Newport	CMY-2	(6)
					CTX-M-1	

Table 1. The prevalence of ESC resistance among nontyphoidal *Salmonella* in different geographic areas (cont.)

Country/region	Year (collected)	No. of test isolates	ESC resistance (%)	Serotypes	Mechanism of ESC resistance	Reference
Asia						
Taiwan	1997-2000	384[a]	6 (1.6%)	*S.* Typhimurium	CMY-2	(158)
Northern Taiwan	1999	549	2 (0.36%)	*S.* Typhimurium	CMY-2	(23)
	2000	870	13 (1.49%)	*S.* Senftenberg	CTX-M-14	
	2001	893	7 (0.78%)	*S.* Panama		
	2002	715	9 (1.26%)	*S.* Derby		
Taiwan	1999-2003	3,592	22 (0.6%)	*S.* Typhimurium	CMY-2	(10)
				S. Enteritidis	CTX-M-3	
				S. Choleraesuis	SHV-2a	
				Others	SHV-12	
Taiwan	2004	600	20 (3.3%)	*S.* Choleraesuis	CMY-2	(5)
				S. Stanley		
				Others		

Table 1. The prevalence of ESC resistance among nontyphoidal *Salmonella* in different geographic areas (cont.)

Country/region	Year (collected)	No. of test isolates	ESC resistance (%)	Serotypes	Mechanism of ESC resistance	Reference
Korea	2002-2004	130	1 (0.8%)	*Salmonella* spp.	DHA-1	(32)
Asia	2003-2005	400	12 (3%)	*S.* Typhimurium	ND	(11)
				S. Panama		
Singapore	2003-2006	640	15 (2.3%)	*S.* Typhimurium	SHV-5	(13)
				S. Enteritidis	CMY-2 group	
				Others	CTX-M-9 group	
					CTX-M-1 group	
					DHA	
China	2006	221[a]	7 (3%)	*S.* Derby	CTX-M-14	(12)
				S. Agona	CTX-M-83	
				Others	CTX-M-84	
					CTX-M-84	
					CTX-M-86	

[a], The isolates were collected from stool specimens.; [b], The percentages were based on ESC MIC of $\geq$ 2mg/L

; [c], The isolates were collected from blood specimens.; [d], All isolates were *S.* Newport.; ND, Not done

CHAPTER IV

MATERIALS AND METHODS

Methodology Scheme

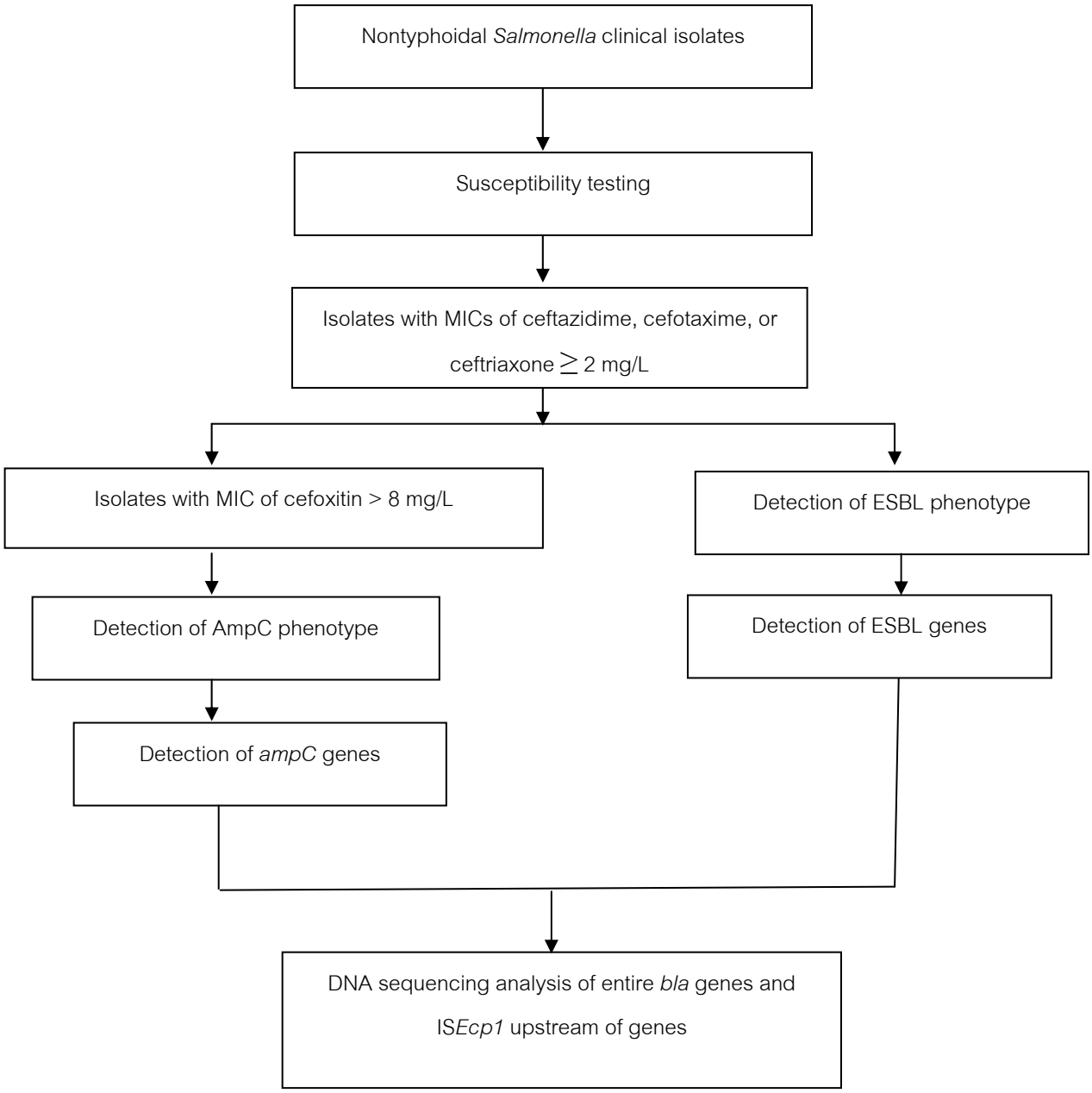

Figure 6. Methodology Scheme

PART I : CLINICAL ISOLATES

1. Bacterial strains

1.1 Nontyphoidal *Salmonella* isolates

Five hundred and sixty nontyphoidal *Salmonella* isolated from clinical specimens included 496 isolates from WHO National *Salmonella* and *Shigella* Center in 2005 and 2007 and 64 isolates from Department of Microbiology, King Chulalongkorn Memorial Hospital (Bangkok) between August 2005 and May 2006. The isolates were collected from blood, stool, rectal swab, urine, pus, tissue, CSF, and sputum.

1.2 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 quality control strains for MIC determination.

2. Culture preservation

All culture isolates were grown on tryptic soy agar (BBL, Becton Dickinson and Company, Cockeysville, MD) at 35-37^{o}C for 18-24 hours. The overnight cultures were transferred to cryogenic vials of 1 ml trypticase soy broth containing 10% glycerol and were kept at -70^{o}C.

PART II : ANTIMICROBIAL SUSCEPTIBILITY TEST

All 560 nontyphoidal *Salmonella* isolates were determined for minimal inhibitory concentrations (MICs) of cefoxitin, ceftazidime, cefotaxime, and ceftriaxone by agar-dilution technique and interpreted according to CLSI (Clinical and Laboratory Standards Institute, 2008) (159). *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213, and *Enterococcus faecalis* ATCC 29212 were used for quality control. The antimicrobial agents used in this study were cefoxitin, ceftazidime (Sigma Chemical Co., St. Louis, Mo, USA), cefotaxime (Merck & Co.,USA), and ceftriaxone (Roche, Switzerland)

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 (approximately $1.5x10^8$ CFU/mL) in 0.85% NaCl. After adjusting the turbidity of inoculum, the suspension was diluted 10-fold to yield the final inoculum suspension and the 1 mL of inoculum suspension was transferred to the multi-point inoculator wells. The suspension was inoculated on Mueller-Hinton agar plates with two-fold dilution of antimicrobial agent at concentrations of 0.015625 to 256 mg/L. The preparation for two-fold dilution of antimicrobial agent is shown in Table 2. (159). A multipoint inoculator was used to deliver 1-2 µl of inoculum suspension to the agar dilution plates. The final inoculum was approximately 10^4 CFU/spot (Figure 7.). The plates were incubated at 35-37 ºC for 18-24 hours. The suspension was inoculated on Mueller-Hinton agar plates without antibiotic for the growth control. The MIC is defined as the lowest concentration of antimicrobial agent at which there is no visible growth (Figure 7.).

MICs interpretation used breakpoint criteria recommended by CLSI (Clinical and Laboratory Standards Institute, 2008) (159) are shown in the Table 3. Ceftriaxone and cefotaxime MICs of $\geq$ 64 mg/L, cefoxitin and ceftazidime MICs of $\geq$ 32 mg/L were classified as resistant. Acceptable MIC limits for quality control reference strains are listed in Table 4. (159).

Table 2. Scheme for preparing dilutions of antimicrobial agents to used in agar dilution susceptibility tests

Step	Antimicrobial solution						
	Conc. (mg/L)	Source	Vol. (mL)	Diluent (mL)	Intermediate concentration(mg/L)	Final conc. at 1:10 dilution in agar (mg/L)	Log $_2$
	5,120 (mg/L)	Stock	-	-	5,120	512	9
1	5,120	Stock	2	2	2,560	256	8
2	5,120	Stock	1	3	1,280	128	7
3	5,120	Stock	1	7	640	64	6
4	640	Step 3	2	2	320	32	5
5	640	Step 3	1	3	160	16	4
6	640	Step 3	1	7	80	8	3
7	80	Step 6	2	2	40	4	2
8	80	Step 6	1	3	20	2	1
9	80	Step 6	1	7	10	1	0
10	10	Step 9	2	2	5	0.5	-1
11	10	Step 9	1	3	2.5	0.25	-2
12	10	Step 9	1	7	1.25	0.125	-3
13	1.25	Step 10	2	2	0.625	0.0625	-4
14	1.25	Step 10	1	3	0.3125	0.03125	-5
15	1.25	Step 10	1	7	0.15625	0.015625	-6

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

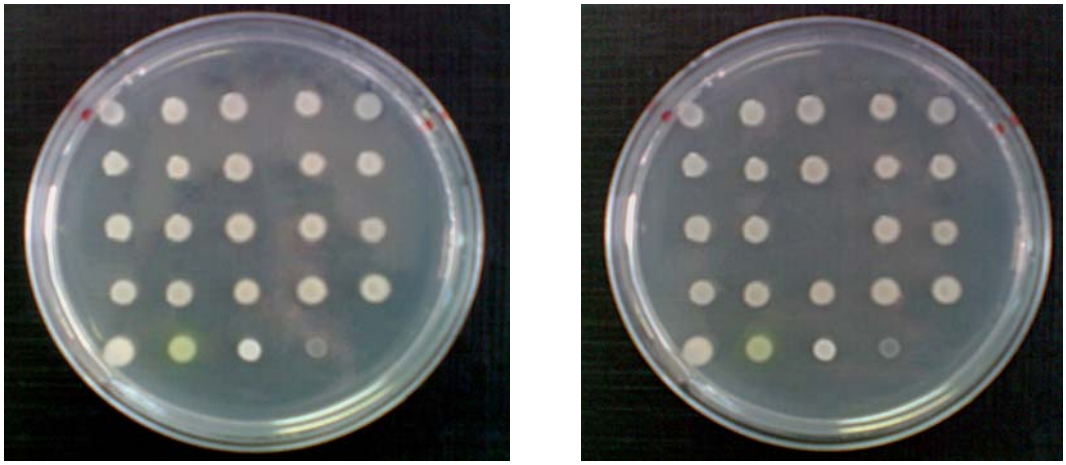

Figure 7. The inoculum plate of agar dilution method for nontyphoidal *Salmonella* isolates: A, control plate (no antibiotic); B, MIC plate with 0.06 mg/L of cefotaxime

Table 3. MIC interpretive standards (mg/L) for Enterobacteriaceae

Antimicrobial agents	MIC interpretive standard (mg/L)		
	Susceptible	Intermediate	Resistant
Cefoxitin	≤8	16	≥32
Ceftazidime	≤8	16	≥32
Cefotaxime	≤8	16-32	≥64
Ceftriaxone	≤8	16-32	≥64

Table 4. Acceptable limits for quality control strains used to monitor accuracy of MICs

Antimicrobial agents	*Escherichia coli* ATCC 25922	*Pseudomonas aeruginosa* ATCC 27853	*Staphylococcus aureus* ATCC 29213	*Enterococcus faecalis* ATCC 29212
Cefoxitin	2-8	-	1-4	-
Ceftazidime	0.06-0.5	1-4	4-16	-
Cefotaxime	0.03-0.12	8-32	1-4	-
Ceftriaxone	0.03-0.12	8-64	1-8	-

PART III : DETECTION OF ESBL PHENOTYPE

1. Detection of ESBL phenotype by combination disk test

All isolates for which the MICs of either ceftazidime, cefotaxime, or ceftriaxone $\geq$ 2 mg/L were considered to have a positive screening test for ESBL phenotype and subjected to clavulanate confirmatory testing using the combination disk test (159).

An overnight culture suspension of isolate, which was adjusted to 0.5 McFarland in 0.85% NaCl was inoculated on Mueller-Hinton agar plate by using a sterile swab. Pairs of disks containing 30 µg ceftazidime (BBL, Becton Dickinson and Company, Coskeysville, MD), 30 µg ceftazidime with 10 µg clavulanic acid (BBL, Becton Dickinson and Company, Coskeysville, MD) and 30 µg cefotaxime (BBL, Becton Dickinson and Company, Coskeysville, MD), 30 µg cefotaxime with 10 µg clavulanic acid (BBL, Becton Dickinson and Company, Coskeysville, MD) were placed on the opposite sides of the same inoculated plate. Inhibition zones were measured following 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 confirmatory test for ESBL phenotype (Figure 8.).

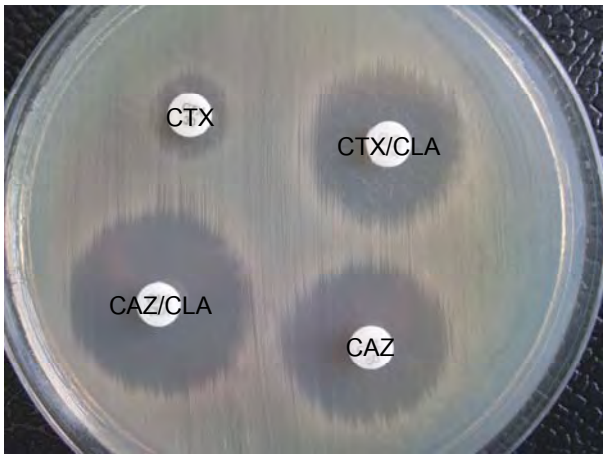

Figure 8. The combination disk test with clavulanate: cefotaxime (CTX), cefotaxime/clavulanic acid (CTX/CLA), ceftazidime (CAZ), ceftazidime/clavulanic acid (CAZ/CLA)

PART IV : DETECTION OF AMPC PHENOTYPE

1. Detection of AmpC β-lactamase activity by modified Hodge test with cefoxitin

All isolates, with the MICs of ≥ 2 mg/L for ceftazidime or cefotaxime or ceftriaxone and with cefoxitin MICs of > 8 mg/L were subjected to detect for AmpC β-lactamase activity by modified Hodge test with cefoxitin disk, previously described by Yong *et al.* (160). A Mueller-Hinton agar plate was inoculated with an overnight culture suspension of cefoxitin-susceptible *E. coli* ATCC 25922, which was adjusted to 0.5 McFarland in 0.85% NaCl. 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 picked and heavily streaked outwards from the disk. The Mueller-Hinton agar plate was incubated at 35-37°C 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 of modified Hodge test (Figure 9.). CMY-2-producing *E.coli* isolate and *E. coli* ATCC 25922 were used for positive and negative control strains, respectively.

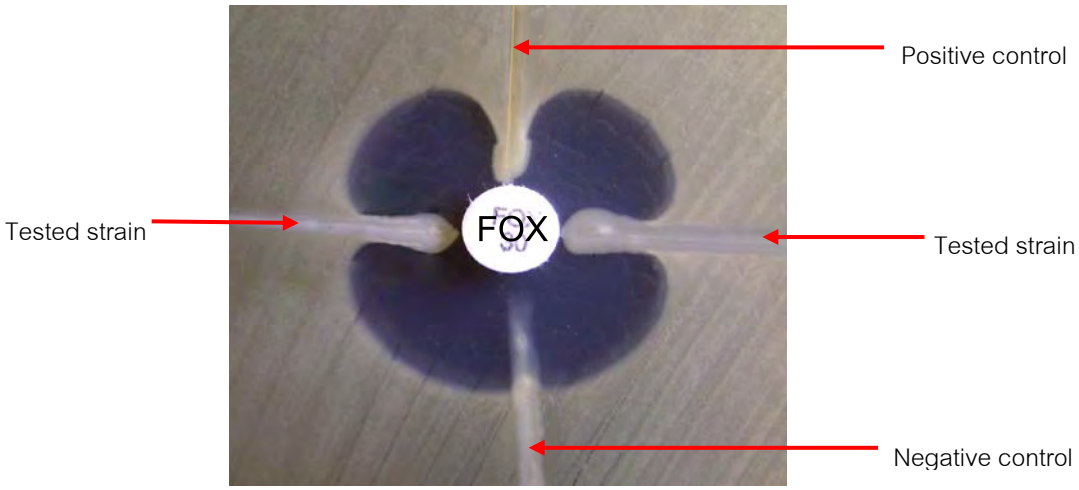

Figure 9. Detection of AmpC β-lactamase phenotype by modified Hodge test with 30 µg cefoxitin disk. The decreased radius of the inhibition zone along the growth of tested strain showed a positive of AmpC β-lactamase activity.

2. Detection of inducible AmpC phenotype by double-disk diffusion test with cefoxitin and ceftazidime

All isolates with AmpC β-lactamase activity were determined for inducible AmpC phenotype by double-disk diffusion test with cefoxitin and ceftazidime disks, which was based on those previously described by Livermore *et al.* (139).

The tested isolate grown overnight on tryptic soy agar was adjusted to 0.5 McFarland in 0.85% NaCl and inoculated on a Mueller-Hinton agar plate by using a sterile swab. After drying, a 30 µg cefoxitin disk (BBL, Becton Dickinson and Company, Coskeysville, MD) and 30 µg ceftazidime disk (BBL, Becton Dickinson and Company, Coskeysville, MD) were placed 15-20 mm apart on Muller-Hinton agar. The Mueller-Hinton agar plate was incubated at 35-37°C for 18-24 hours. After overnight incubation, the presence of a "D"-shaped zone around the ceftazidime disk defined as the inducible AmpC phenotype and resistance to two antibiotics disks defined as the constitutive AmpC phenotype (Figure 10.). DHA-1 producing *E.coli* isolate were used for positive control strain.

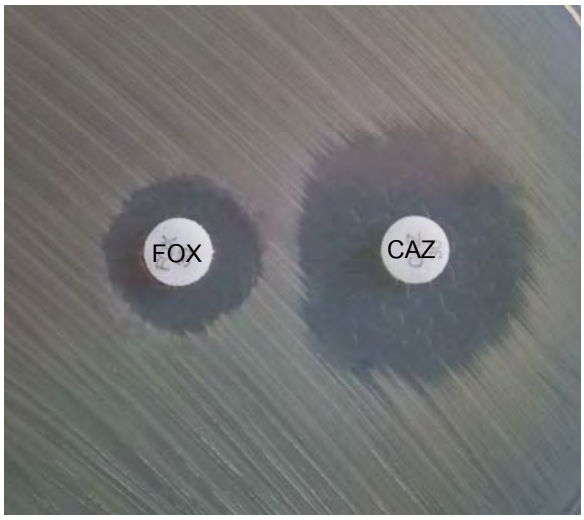

Figure 10. Double-disk diffusion test with cefoxitin (FOX) and ceftazidime (CAZ) for detection of inducible AmpC phenotype

PART V : SCREENING FOR THE PRESENCE OF ESBL GENES

The nontyphoidal *Salmonella* isolates resistant to extended-spectrum cephalosporins with ESBL phenotype were investigated for the presence of ESBL genes, including bla_{OXA}, bla_{TEM}, bla_{SHV}, bla_{CTX-M}, and bla_{VEB} by multiplex PCR.

1. DNA extraction

The overnight culture suspension, 4-5 colonies of pure culture nontyphoidal *Salmonella* isolate in 200 µl of steriled nuclease-free water was boiled for 10 min and centrifuged at 12,000 rpm at room temperature for 5 min. The supernatant was used as the DNA template in the PCR experiments and stored at -20^{o}C.

2. Primers

The presence of bla_{OXA}, bla_{TEM}, bla_{SHV}, bla_{CTX-M}, and bla_{VEB} was screened by multiplex PCR using OXA-F, OXA-R, TEM-C, TEM-H, SHV-F, SHV-R, CTX-A, CTX-B, VEB-1, and VEB-2 primers. The primers are described in Table 5. and are based on those previously described by Colom *et al.* (161), Mabilat *et al.* (162), Bonnet *et al.* (163), and Udomsantisuk *et al*. (unplublished data).

Table 5. Primers of the multiplex PCR used for amplification of *bla* genes encoded for ESBLs

Specific for	Primer	Primer sequence (5'- 3')	Product size (bp)	Reference
bla_{SHV}	SHV-F	AGGATTGACTGCCTTTTTG	392	(161)
	SHV-R	ATTTGCTGATTTCGCTCG		
bla_{TEM}	TEM-C	ATCAGCAATAAACCAGC	516	(162)
	TEM-H	CCCCGAAGAACGTTTTC		
bla_{VEB}	VEB-A	CCTTTTGCCTAAAACGTGGA	216	Udomsantisuk *et al.*
	VEB-B	TGCATTTGTTCTTCGTTTGC		
bla_{CTX-M}	CTXM-A	CGCTTTGCGATGTGCAG	550	(163)
	CTXM-B	ACCGCGATATCGTTGGT		
bla_{OXA}	OXA-F	ATATCTCTAACTGTTGCATCTCC	619	(161)
	OXA-R	AAACCCTTCAAACCATCC		

3. Amplification of bla_{OXA}, bla_{TEM}, and bla_{SHV} genes by multiplex PCR

The presence of bla_{OXA}, bla_{TEM}, and bla_{SHV} was screened using OXA-F, OXA-R, TEM-C, TEM-H, SHV-F, and SHV-R primers as described by Colom *et al.* (161) and Mabilat *et al.*(162). The PCR was performed in 25 µl PCR reaction mixture containing 1X *Taq* buffer, 2.0 mM $MgCl_2$, 0.2 mM dNTPs (Fermentas, USA), 0.06 µM of OXA-F and OXA-R primers, 0.04 µM of TEM-C and TEM-H primers, and 0.08 µM of SHV-F and SHV-R primers, and 0.5 U *Taq* polymerase (Fermentas, USA), and 3 µL of DNA template. The amplification conditions were, initial denaturation at 94ºC for 5 minutes, 30 cycles of 94ºC for 30 seconds, 54ºC for 30 seconds and 72ºC for 1 minute, and a final elongation at 72ºC for 10 minutes.

4. Amplification of bla_{CTX-M} and bla_{VEB} genes by multiplex PCR

The presence of bla_{CTX-M} and bla_{VEB} was screened using CTX-A, CTX-B, VEB-1, and VEB-2 primers described by Bonnet *et al*. (163) and Udomsantisuk *et al*.

(unplublished data). The PCR was performed in 25 µl PCR reaction mixture containing 1X *Taq* buffer, 2.0 mM $MgCl_2$, 0.2 mM dNTPs (Fermentas, USA), 0.1 µM of CTX-A and CTX-B primers, 0.05 µM of VEB-1 and VEB-2 primers, and 0.5 U *Taq* polymerase (Fermentas, USA), and 1 µL of DNA template. Multiplex PCR conditions were performed as described previously (161). The amplification conditions were, initial denaturation at 94ºC for 5 minutes, 30 cycles of 94ºC for 30 seconds, 54ºC for 30 seconds and 72ºC for 1 minute, and a final elongation at 72ºC for 10 minutes.

5. Analysis of amplified DNA

The PCR products were analyzed on 1.5% agarose gel electrophoresis (Pronalisa, Spain) 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 (20% ficoll, 0.05% bromphenol blue). The electrophoresis was carried out at 100 volts for 60 minutes. The amplified products were visualized and photographed under UV light transilluminator. The PCR product sizes of bla_{OXA}, bla_{TEM}, bla_{SHV}, bla_{CTX-M}, and bla_{VEB} were 619 bp, 516 bp, 392 bp, 550 bp, and 216 bp, respectively. A 100 bp DNA ladder (Fermentus, USA) was used as a DNA size marker.

6. Quality control

The clinical strains of *Klebsiella pneumoniae* harbouring bla_{OXA}, bla_{TEM}, bla_{SHV}, bla_{CTX-M}, and bla_{VEB} were used as positive control strains.

PART VI : SCREENING FOR GROUP OF *BLA*-CTX-M GENES

The nontyphoidal *Salmonella* isolates carrying bla_{CTX-M} were investigated for group of bla_{CTX-M} genes encoding CTX-M β-lactamase by multiplex PCR. The CTX-M are sub-classified into four groups, including CTX-M-1, CTX-M-2, CTX-M-9, and CTX-M-8/25 groups (164).

1. DNA extraction

The overnight culture suspension, 4-5 colonies of pure culture nontyphoidal *Salmonella* isolate in 200 µl of steriled nuclease-free water was boiled for 10 min and centrifuged at 12,000 rpm at room temperature for 5 min. The supernatant was used as the DNA template in the PCR experiments and stored at -20°C.

2. Primers

The multiplex PCR classified bla_{CTX-M} genes into four groups, including CTX-M-1, CTX-M-2, CTX-M-9, and CTX-M-8/25 groups using CTXM7, CTXM8, CTXM17, CTXM18, CTXM19, CTXM20, CTXM11, and CTXM12 primers. The primers are described in Table 6., as those previously described by Li Xu *et al*. (164).

Table 6. Primers of the multiplex PCR used for amplification of *bla*$_{CTX-M}$ groups

Specific for	Primer	Primer sequence (5'- 3')	Product size (bp)	Reference
bla$_{CTX-M-1}$ group	CTXM7	GCGTGATACCACTTCACCTC	260	(164)
	CTXM8	TGAAGTAAGTGACCAGAATC		
bla$_{CTX-M-2}$ group	CTXM17	TGATACCACCACGCCGCTC	341	
	CTXM18	TATTGCATCAGAAACCGTGGG		
bla$_{CTX-M-8/25}$ group	CTXM19	CAATCTGACGTTGGGCAATG	207	
	CTXM20	ATAACCGTCGGTGACAATT		
bla$_{CTX-M-9}$ group	CTXM11	ATCAAGCCTGCCGATCTGGTTA	293	
	CTXM12	GTAAGCTGACGCAACGTCTGC		

3. Amplification of *bla*$_{CTX-M}$ groups by multiplex PCR

The multiplex PCR classified *bla*$_{CTX-M}$ genes into four groups using CTXM7, CTXM8, CTXM17, CTXM18, CTXM19, CTXM20, CTXM11, and CTXM12 primers. The primers and conditions are based on those previously described by Li Xu *et al*. (164). The PCR was performed in 25 µl PCR reaction mixture containing 1X *Taq* buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs (Fermentas, USA), 0.4 µM of CTXM7, CTXM8, CTXM17, CTXM18, CTXM19, CTXM20, CTXM11, and CTXM12 primers, and 1.25 U *Taq* polymerase (Fermentas, USA), and 2 µL of DNA template. The amplification conditions were, initial denaturation at 95ºC for 2 minutes, 25 cycles of 95ºC for 1 minute, 55ºC for 1 minute and 72ºC for 1 minute, and a final elongation at 72ºC for 10 minutes.

4. Analysis of amplified DNA

The PCR products were analyzed on 2% agarose gel electrophoresis (Pronalisa, Spain) 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 (20% ficoll, 0.05% bromphenol blue). The electrophoresis was carried out at 100 volts for 60 minutes. The amplified products were visualized and photographed under UV light transilluminator. The PCR product sizes of $bla_{\text{CTX-M-1}}$ group, $bla_{\text{CTX-M-2}}$ group, $bla_{\text{CTX-M-8/25}}$ group, and $bla_{\text{CTX-M-9}}$ group were 260 bp, 341 bp, 207 bp, and 293 bp, respectively. A 100 bp DNA ladder (Fermentus, USA) was used as a DNA size marker.

5. Quality control

The clinical strains of *Klebsiella pneumoniae* harbouring $bla_{\text{CTX-M-1}}$ group and $bla_{\text{CTX-M-9}}$ group were used as positive control strains.

PART VII : SCREENING FOR THE PRESENCE OF *AMPC* GENES

The nontyphoidal *Salmonella* isolates with AmpC phenotype were investigated for the presence of plasmid-mediated *amp*C genes by using multiplex PCR. The primers and PCR conditions were modified from those previously described by Perez *et al*. (116).

1. DNA extraction

The 4-5 colonies of nontyphoidal *Salmonella* isolate were suspended in 200 µl of steriled nuclease-free water and boiled for 10 min and centrifuged at 12,000 rpm at room temperature for 5 min. The supernatant was used as the DNA template in the PCR experiments and stored at -20^{o}C.

2. Primers

The multiplex PCR used specific primers for plasmid *amp*C, encoding six groups of AmpC β-lactamases, including MOX, CIT, DHA, ACC, EBC, and FOX. The primers are described in Table 7. and are based on those previously described by Perez *et al.* (116).

Table 7. Primers of the multiplex PCR used for amplification of plasmid *amp*C genes

Specific for	Primer	Primer sequence (5'to 3')	Product size (bp)	Reference
MOX	MOXMF	GCTGCTCAAGGAGCACAGGAT	520	(116)
	MOXMR	CACATTGACATAGGTGTGGTGC		
CIT	CITMF	TGGCCAGAACTGACAGGCAAA	462	
	CITMR	TTTCTCCTGAACGTGGCTGGC		
DHA	DHAMF	AACTTTCACAGGTGTGCTGGGT	405	
	DHAMR	CCGTACGCATACTGGCTTTGC		
ACC	ACCMF	AACAGCCTCAGCAGCCGGTTA	346	
	ACCMR	TTCGCCGCAATCATCCCTAGC		
EBC	EBCMF	TCGGTAAAGCCGATGTTGCGG	302	
	EBCMR	CTTCCACTGCGGCTGCCAGTT		
FOX	FOXMF	AACATGGGGTATCAGGGAGATG	190	
	FOXMR	CAAAGCGCGTAACCGGATTGG		

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

The presence of plasmid *amp*C, including MOX, CIT, DHA, ACC, EBC, and FOX groups was screened using MOXMF, MOXMR, CITMF, CITMR, DHAMF, DHAMR, ACCMF, ACCMR, EBCMF, EBCMR, FOXMF, and FOXMR primers by multiplex PCR. The PCR was performed in 25 µl PCR reaction mixture containing 1X *Taq* buffer, 1.5 mM $MgCL_2$, 0.2 mM dNTPs (Fermentas, USA), 0.8 µM for MOXMF, MOXMR, FOXMF, and FOXMR primers, 0.6 µM for DHAMF and DHAMR primers, 0.5 µM for ACCMF and ACCMR primers, 0.4 µM for CITMF and CITMR primers, 0.3 µM for EBCMF and EBCMR primers, 1.25 U of *Taq* polymerase (Fermentas, USA) and 2 µl of bacterial

DNA template. The amplification conditions were, initial denaturation at 94ºC for 3 minutes, 25 cycles of 94ºC for 30 seconds, 62ºC for 30 seconds and 72ºC for 1 minute, and a final elongation at 72ºC for 7 minutes.

4. Analysis of amplified DNA

The PCR products were analyzed on 2% agarose gel electrophoresis (Pronalisa, Spain) 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 (20% ficoll, 0.05% bromphenol blue). The electrophoresis was carried out at 100 volts for 80 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. A 100 bp DNA ladder (Fermentus, USA) was used as a DNA size marker.

5. Quality control

The *E. coli* transconjugant strains carryring bla_{CMY-2}, bla_{DHA-1}, bla_{ACC-1}, bla_{MIR-1}, and bla_{FOX-4} were used as the positive control strains for CIT, DHA, ACC, EBC, and FOX family The *E. coli* transconjugant strains were positive control strains obtained from Associate Professor Dr. Aroonwadee Chanawong, Khon Kaen University, Thailand. *Aeromonas caviae* clinical isolated harbouring bla_{MOX-8} was used as the positive control strain for MOX family.

PART VIII : SCREENING FOR THE PRESENCE OF IS*ECP1* UPSTREAM OF *BLA* GENES BY POLYMERASE CHAIN REACTION (PCR)

The nontyphoidal *Salmonella* isolates carrying bla_{CTX-M} or bla_{CIT} were investigated for IS*Ecp1* in the upstream region of *bla* genes by PCR. The isolates were screened by PCR using the forward primer specific for IS*Ecp1* and the reverse primers specific for bla_{CTX-M} or bla_{CIT} genes.

1. DNA extraction

The overnight culture suspension, 4-5 colonies of pure culture nontyphoidal *Salmonella* isolate in 200 µl of steriled nuclease-free water was boiled for 10 min and centrifuged at 12,000 rpm at room temperature for 5 min. The supernatant was used as the DNA template in the PCR experiments and stored at -20^{o}C.

2. Primers

The forward primer specific for IS*Ecp1* was previously described by Eckert *et al*. (165). The reverse primers specific for bla_{CTX-M} or bla_{CIT} genes were designed by Primer 3 program (http://www.genome.wi.mit.edu/cgibin/primer/primer3_www.cgi) based on the sequence data in GenBank under accession no. AY458016, GQ385324, and FJ621588. The primers for screening of IS*Ecp1* upstream of of *bla* genes are shown in Table 8.

Table 8. Primers for amplification of IS*Ecp1* upstream of *bla* genes

Specific for	Primer	Primer sequence (5'- 3')	Product size (bp)	Reference
IS*Ecp1* and $bla_{CTX\text{-}M\text{-}1}$ group	IS*Ecp1*-CMY-F	AAAAATGATTGAAAGGTGGT	1030	(165)
	CTXM1-R	ACCGTYGGTGACGATTTT*		This study (AY458016)
IS*Ecp1* and $bla_{CTX\text{-}M\text{-}9}$ group	IS*Ecp1*-CMY-F	AAAAATGATTGAAAGGTGGT	1023	(165)
	CTXM9-R	CCTTCGGCGATGATTCTC		This study (GQ385324)
IS*Ecp1* and bla_{CIT}	IS*Ecp1*-CMY-F	AAAAATGATTGAAAGGTGGT	1338	(165)
	IS*Ecp1*-CMY-R	GACACGGACAGGGTTAGGAT		This study (FJ621588)

*, Y: C or T

3. Amplification of IS*Ecp1* upstream of *bla* genes by PCR

The presence of IS*Ecp*1 upstream of *bla* genes was screened using IS*Ecp1*-CMY-F forward-primer and reverse primers including CTXM1-R, CTXM9-R, and IS*Ecp1*-CMY-R primers. The PCR was performed in 50 µl PCR reaction mixture containing 1X *Taq* buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs (Fermentas, USA), 0.4 µM of IS*Ecp1*-CMY-F forward-primer and 0.4 µM of CTXM1-R or CTXM9-R or IS*Ecp1*-CMY-R reverse-primers, and 1.25 U *Taq* polymerase (Fermentas, USA), and 2 µL of DNA template. The amplification conditions were, initial denaturation at 95ºC for 2 minutes, 30 cycles of 95ºC for 1 minute, 55ºC for 1 minute and 72ºC for 1 minute, and a final elongation at 72ºC for 10 minutes.

4. Analysis of amplified DNA

The PCR products were analyzed on 1.0% agarose gel electrophoresis (Pronalisa, Spain) 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 (20% ficoll, 0.05% bromphenol blue). The electrophoresis was carried out at 100 volts for 60 minutes. The amplified products were visualized and photographed under UV light transilluminator. The PCR product sizes for presence of IS*Ecp1* upstream of $bla_{\text{CTX-M-1}}$ group, $bla_{\text{CTX-M-9}}$ group, and bla_{CIT} genes were 1030 bp, 1023 bp, and 1338 bp, respectively. A 100 bp plus DNA ladder (Fermentus, USA) was used as a DNA size marker.

PART IX : ANALYSIS OF ENTIRE *BLA* GENES AND IS*ECP1* UPSTREAM REGION BY PCR AND DNA SEQUENCING

The nontyphoidal *Salmonella* isolates carrying *bla* genes were characterized by PCR of entire *bla* genes and IS*Ecp1* upstream region and automated DNA sequencing.

1. Plasmid DNA extraction

The nontyphoidal *Salmonella* was extracted plasmid DNA for amplifying entire *bla* genes those performed by Plasmid Mini Kit (GmbH & Co. KG, Germany) according to the manufacturers. Nontyphoidal *Salmonella* isolate was cultured in Luria-Bertani broth (Pronadisa, Spain) and measured the density of bacterial cells up to 12 OD/mL (OD600). Bacterial cells were transferred to a microcentrifuge tube and centrifuged at 11,000 g for 30 seconds. The supernatant was removed. The 250 µL of resuspension solution were added and mixed by vortexing. The 250 µL of lysis solution were added to the sample and mixed by inverting 6-8 times. The 350 µL of neutralizing solution were added to the sample and mixed by inverting 6-8 times. After that, the sample was centrifuged for 5-10 min. The supernatant contained the plasmid DNA was transferred into a plasmid mini column and was centrifuged for 1 min. The filtrate was removed from the tube and was replaced into the same wash tube. The 750 µL of wash solution were added to the column and centrifuged for 1 min. The wash solution was discarded. The column was replaced into the same wash tube and centrifuged for 1 additional minute to remove residual wash solution. Finally, the plasmid mini column was transferred to a 1.5 mL microcentrifuge tube and the 50 µL of elution solution was added onto the base of the column and allowed for 1 min. After that, the column was centrifuged for 1 min. to elute the plasmid DNA. The eluted plasmid DNA samples were stored at -20^{o}C.

2. Primers for PCR and DNA sequencing

The primers for PCR and sequencing of IS*Ecp1* upstream of *bla* genes and entire *bla* genes were designed by Primer 3 program (http://www-genome.wi.mit.edu/cgibin/primer/primer3_www.cgi) based on the sequence data in GenBank under accession no. AY458016, GQ385324, and FJ621588. The IS*Ecp1*-CMY-F were specific for IS*Ecp*1 (165). The IS*Ecp1*-CMY-F was used for PCR and sequencing to determine nucleotide sequence of IS*Ecp1* upstream of *bla* genes and were also used for amplified entire bla_{CTX-M} and bla_{CIT} genes for DNA sequencing. The primers used for PCR and sequencing are shown in Table 9.

Table 9. Sequence of the oligonucleotides used as primers for PCR and DNA sequencing entire *bla* genes and IS*Ecp*1 upstream region

Specific for	Primer name	Primer sequence (5'- 3')	Product size (bp)	Reference
PCR primers				
Entire bla_{CTX-M} group 1	IS*Ecp1*-CMY-F	AAAAATGATTGAAAGGTGGT	1398	(165)
	ORF477-R	CCCTCACACCTTCGAGCTAC		This study (AY458016)
Entire bla_{CTX-M} group 9	IS*Ecp1*-CMY-F	AAAAATGATTGAAAGGTGGT	1281	(165)
	IS903-R	TCGTGATGGCAAGGTCAG		This study (GQ385324)
Entire bla_{CMY}	IS*Ecp1*-CMY-F	AAAAATGATTGAAAGGTGGT	1596	(165)
	CMY-R	CAGGTTCCCAGATAGCGTTT		This study (FJ621588)
Entire bla_{TEM}	TEM-F	CAGGAAGCAAAGCTGAAAGG	1349	This study (AY458016)
	TEM-R	CGCTCAGTGGAACGAAAACT		This study (AY458016)
Sequencing primers				
Entire bla_{CTX-M} group 1	IS*Ecp1*-CMY-F	AAAAATGATTGAAAGGTGGT		(165)
	ORF477-R	CCCTCACACCTTCGAGCTAC		This study (AY458016)
Entire bla_{CTX-M} group 9	IS*Ecp1*-CMY-F	AAAAATGATTGAAAGGTGGT		(165)
	IS903-R	TCGTGATGGCAAGGTCAG		This study (GQ385324)
Entire bla_{CMY}	IS*Ecp1*-CMY-F	AAAAATGATTGAAAGGTGGT		(165)
	CMY-R	CAGGTTCCCAGATAGCGTTT		This study (FJ621588)
	AmpC-1	ATGATGAAAAAATCGTTATGC		(166)
Entire bla_{TEM}	TEM-F	CAGGAAGCAAAGCTGAAAGG		This study (AY458016)
	TEM-R	CGCTCAGTGGAACGAAAACT		This study (AY458016)

3. Amplification of the entire *bla* genes and IS*Ecp1* upstream region by PCR

The entire $bla_{CTX\text{-}M\text{-}1}$ group, $bla_{CTX\text{-}M\text{-}9}$ group, bla_{CIT}, and bla_{TEM} genes were amplified by PCR. The PCR was performed in 50 µl PCR reaction mixture containing 1X *Taq* buffer, 2.0 mM $MgCl_2$, 0.2 mM dNTPs (Fermentas, USA), 0.4 µM of each forward and reverse primer, and 1.25 U *Taq* polymerase (Fermentas, USA), and 2 µL of DNA template. The amplification conditions were, initial denaturation at 95ºC for 2 minutes, 30 cycles of 95ºC for 1 minute, 55ºC for 1 minute and 72ºC for 1 minute, and a final elongation at 72ºC for 10 minutes.

4. Analysis of amplified DNA

The PCR products were analyzed on 1.0% agarose gel electrophoresis (Pronalisa, Spain) 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 (20% ficoll, 0.05% bromphenol blue). The electrophoresis was carried out at 100 volts for 60 minutes. The amplified products were visualized and photographed under UV light transilluminator. The PCR product sizes of entire $bla_{CTX\text{-}M\text{-}1}$ group, $bla_{CTX\text{-}M\text{-}9}$ group, bla_{CIT}, and bla_{TEM} genes were 1398 bp, 1281 bp, 1596 bp, and 1349 bp, respectively. A 100 bp plus DNA ladder (Fermentus, USA) was used as a DNA size marker.

5. Purification of PCR products

The PCR products of entire $bla_{CTX\text{-}M\text{-}1}$ group, $bla_{CTX\text{-}M\text{-}9}$ group, bla_{CIT}, and bla_{TEM} genes were purifired using 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 pulse-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 60 sec 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 concentration of DNA was measured by spectrophotometer (BIO RAD, Smart Spec tm 3000, U.S.A) and approximately adjusted to 50-100 ng/µl for preparation of sequencing reaction. The purified PCR products were stored at -20^{o}C.

6. Preparation of sequencing reaction

Automated sequencing was done at the Macrogen Inc. (Seoul, Korea). Sequencing was done by the chain termination method. DNA samples were sequenced using four primer sets, entire $bla_{CTX\text{-}M\text{-}1}$ group, $bla_{CTX\text{-}M\text{-}9}$ group, bla_{CIT}, and bla_{TEM} genes (Table 9.). Sequencing was conducted under BigDye^{TM} 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). The sequencing primers are shown in Table 9.

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.genopole-toulouse.prd.fr/multalin/multalin.html).

CHAPTER V

RESULTS

PART I: BACTERIAL STRAINS

A total of 560 nontyphoidal *Salmonella* isolated from clinical specimens were used in this study. This included 496 isolates from WHO National *Salmonella* and *Shigella* Center in 2005 and 2007 and 64 isolates from the Department of Microbiology, King Chulalongkorn Memorial Hospital between August 2005 and May 2006. Each isolate was from different patient. Of the 560 isolates, 289 (51.61%), 205 (36.61%), 64 (11.43%), and 2 isolates (0.36%) were *Salmonella* serogroup D, C, B, and E, respectively. Three hundred and seventy-five (66.96%) isolates were from sterile sites, including 374 isolates from blood and one isolate from CSF. One hundred and eighty-five (33.04%) isolates from non-sterile sites including stool, rectal swab, urine, pus, tissue, and sputum were 119, 36, 14, 12, 3, and 1 isolates, respectively. Thus, a majority of nontyphoidal *Salmonella* isolates in this study were recovered from blood specimens which were accounted for 66.79% of all isolates. The results are shown in Table 10.

Table 10. Types of clinical specimen of 560 nontyphoidal *Salmonella* isolates

Source	Specimen types	No. of isolates	Total (%)
Sterile sites	Blood	374	66.79
(n=375)	CSF	1	0.18
Non- sterile sites	Stool	119	21.25
(n=185)	Rectal swab	36	6.43
	Urine	14	2.50
	Pus	12	2.14
	Tissue	3	0.53
	Sputum	1	0.18
	Total	**560**	

PART II : ANTIMICROBIAL SUSCEPTIBILITY TEST

The susceptibility of nontyphoidal *Salmonella* to cefoxitin, ceftazidime, cefotaxime, and ceftriaxone was determined by agar dilution method. The MIC is the lowest concentration of antimicrobial agent required to inhibit the growth of a microorganism *in vitro*. The MIC_{50} and MIC_{90} are the lowest concentration of anitimicrobial agents required to inhibit 50% and 90% of isolates tested, respectively. Ceftriaxone and cefotaxime MICs of $\geq$ 64 mg/L, cefoxitin and ceftazidime MICs of $\geq$ 32 mg/L were classified as resistant. Ceftriaxone and cefotaxime MICs of 16 or 32 mg/L, cefoxitin and ceftazidime MICs of 16 mg/L were classified as intermediate resistant. The results of susceptibility testing and resistance rates of cefoxitin, ceftazidime, cefotaxime, and ceftriaxone against 560 nontyphoidal *Salmonella* isolates are summarized in Table 11.

Prevalence of ceftriaxone resistance was 12.50% (70/560). The results showed that 8.75% (49/560) of isolates were intermediate resistant. The ceftriaxone MIC ranged from 0.016 to >256 mg/L. MIC_{50} and MIC_{90} were 0.125 mg/L and 64 mg/L, respectively. Distribution of ceftriaxone MICs of 560 nontyphoidal *Salmonella* isolates is shown in Figure 11. It was demonstrated that two groups of distribution included 78.75% (441/560) for ceftriaxone-susceptible isolates with ceftriaxone MIC range of 0.016 to 1 mg/L and 21.25% (119/560) for ceftriaxone-intermediate-resistant isolates with ceftriaxone MIC range of 16 to >256 mg/L. Most of ceftriaxone-susceptible isolates (81.86%, 316/441) had ceftriaxone MICs of 0.06 and 0.125 mg/L. Of the 70 ceftriaxone-resistant isolates, 75.71% (53/70) had high-level resistance with ceftriaxone MIC range of 128 to >256 mg/L. The ceftriaxone-resistant isolates had ceftazidime MIC range of 2 to >256 mg/L. Of all 70 ceftriaxone-resistant isolates, 51.43% (36/70) were also resistant to ceftazidime, 7.14 % (5/70) were intermediate resistant to ceftazidime and 41.43% (29/70) were susceptible to ceftazidime. The ceftriaxone-resistant isolates had cefotaxime MIC range of 8 to >256 mg/L. Of all ceftriaxone-resistant isolates, 72.86% (51/70), 21.43% (15/70), and 5.71% (4/70) were resistant, intermediate resistant, and susceptible to cefotaxime, respectively.

The susceptibility to ceftazidime showed that 14.64% (82/560) of isolates were resistant and 1.25% (7/560) were intermediate resistant. The MIC of ceftazidime ranged from 0.125 to >256 mg/L. MIC_{50} and MIC_{90} were 0.5 mg/L and 64 mg/L, respectively. Distribution of ceftazidime MICs is shown in Figure 12. It was demonstrated that MICs of ceftazidime-susceptible nontyphoidal *Salmonella* isolates ranged from 0.125 to 8 mg/L. Most ceftazidime-susceptible isolates (59.87%, 282/471) had ceftazidime MIC of 0.5 mg/L. Of the 82 ceftazidime-resistant isolates, 54.88% (45/82) had high-level resistance with MIC range of 128 to >256 mg/L. Moreover, the ceftazidime-resistant isolates had ceftriaxone MIC range of 16 to >256 mg/L. Of all ceftazidime-resistant isolates, 43.90% (36/82) were also resistant to ceftriaxone and 56.10% (46/82) were intermediate resistant to ceftriaxone. In addition, the ceftazidime-resistant isolates had cefotaxime MIC range of 4 to >256 mg/L. Of all 82 ceftazidime-resistant isolates, 21.95% (18/82), 48.78% (40/82), and 29.27% (24/82) were resistant, intermediate resistant, and susceptible to cefotaxime, respectively.

Cefotaxime susceptibility showed that 9.46% (53/560) of isolates were resistant and 7.50% (42/560) were intermediate resistant. The MIC of cefotaxime ranged from 0.016 to >256 mg/L. MIC_{50} and MIC_{90} were 0.125 mg/L and 32 mg/L, respectively. Distribution of cefotaxime MICs is shown in Figure 13. It was shown that MICs of cefotaxime-susceptible nontyphoidal *Salmonella* isolates ranged from 0.016 to 8 mg/L. Most of cefotaxime-susceptible isolates (70.12%, 326/465) had cefotaxime MIC of 0.125 mg/L. Of the 53 cefotaxime-resistant isolates, 73.58% (39/53) had high-level resistance with MIC range of 128 to >256 mg/L. Most of cefotaxime-resistant isolates (96.23%, 51/53) were resistant to ceftriaxone with MIC range of 64 to >256 mg/L. Only two of cefotaxime-resistant isolates were intermediate resistant to ceftriaxone with the MIC of 32 mg/L. However, cefotaxime-resistant isolates had ceftazidime MIC range of 2 to >256 mg/L. Of all 53 cefotaxime-resistant isolates, 33.96% (18/53), 13.21% (7/53), and 52.83% (28/53) were resistant, intermediate resistant, and susceptible to ceftazidime, respectively.

Cefoxitin susceptibility showed that 11.79% (66/560) of isolates were resistant and 0.36% (2/560) were intermediate resistant. The MIC of ceftazidime ranged from 0.5 to 128 mg/L. MIC_{50} and MIC_{90} were 2 mg/L and 32 mg/L, respectively. Distribution

of cefoxitin MICs is shown in Figure 14. It was demonstrated that MICs of cefoxitin-susceptible nontyphoidal *Salmonella* isolates ranged from 0.5 to 8 mg/L. Most of cefoxitin-susceptible isolates (87.40%, 430/492) had cefoxitin MIC of 2 and 4 mg/L. Moreover, of the 66 cefoxitin-resistant isolates, all were resistant to ceftazidime with MIC range of 32 to >256 mg/L and 31.82% (21/66) and 4.46% (3/66) were resistant to ceftriaxone and cefotaxime, respectively.

The results showed that 31.71% (65/205), 32.68% (67/205), 18.05% (37/205), and 26.34% (54/205) of *Salmonella* serogroup C were resistant to cefoxitin, ceftazidime, cefotaxime, and ceftriaxone, respectively. *Salmonella* serogroup B showed that 1.56% (1/64), 23.44% (15/64), 25.0% (16/64), and 25.0% (16/64) were resistant to cefoxitin, ceftazidime, cefotaxime, and ceftriaxone, respectively. *Salmonella* serogroup D and E isolates were susceptible to all testing cephalosporins. The results showed that *Salmonella* serogroup C and serogroup B had a high rate of cephalosporin resistance.

Eighteen isolates (3.21%) were resistant to all third-generation cephalosporins. These isolates showed MIC ranges for ceftazidime, cefotaxime, and ceftriaxone were 32 to >256, 64 to >256, and 64 to >256 mg/L, respectively. Three isolates (0.54%) were resistant to all third generation cephalosporins and cefoxitin with MIC ranges for cefoxitin, ceftazidime, cefotaxime, and ceftriaxone of 32 to 128, 128 to 256, 64 to 128, and 64 to >256 mg/L, respectively.

Table 11. The susceptibility of cefoxitin, ceftazidime, cefotaxime, and ceftriaxone against 560 nontyphoidal *Salmonella* isolates

Antimicrobial agents	MICs (mg/L)			Susceptibility (%)		
	MIC_{50}	MIC_{90}	Range	R	I	S
Cefoxitin	2	32	0.5-128	66 (11.79)	2 (0.36)	492 (87.82)
Ceftazidime	0.5	64	0.125->256	82 (14.64)	7 (1.25)	471 (84.11)
Cefotaxime	0.125	32	0.016->256	53 (9.46)	42 (7.50)	465 (83.04)
Ceftriaxone	0.125	64	0.016->256	70 (12.50)	49 (8.75)	441 (78.75)

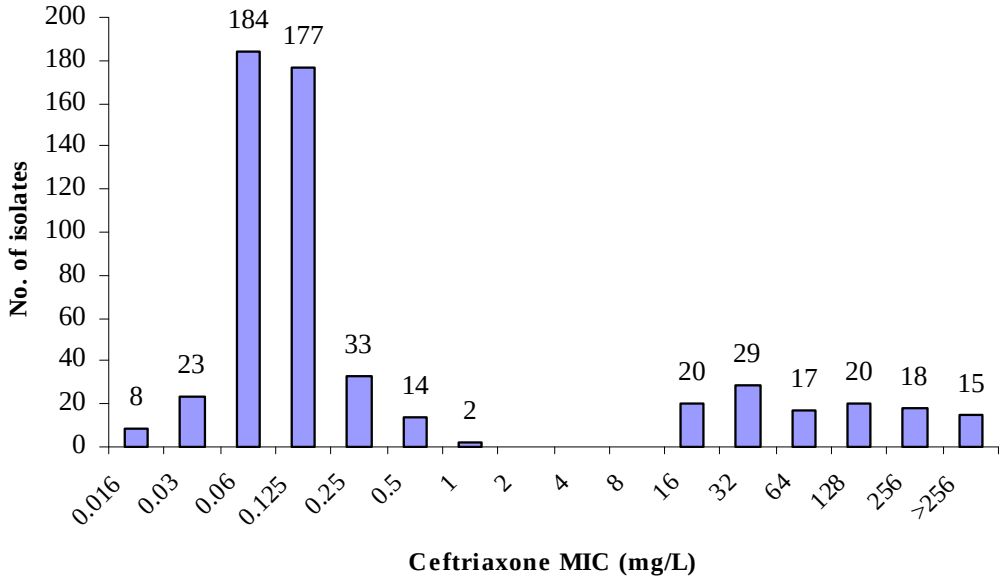

Figure 11. Distribution of ceftriaxone MICs among 560 nontyphoidal *Salmonella* isolates

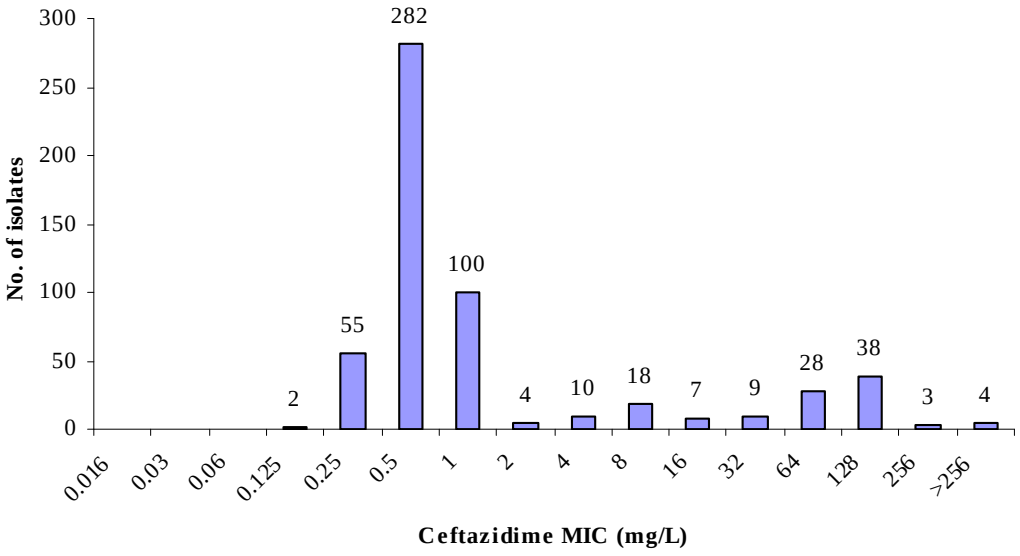

Figure 12. Distribution of ceftazidime MICs among 560 nontyphoidal *Salmonella* isolates

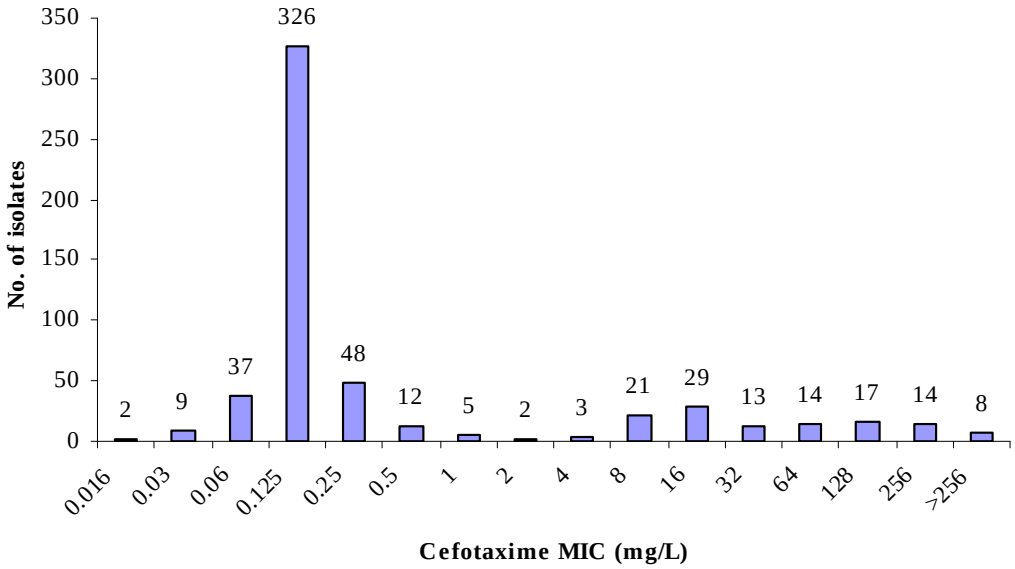

Figure 13. Distribution of cefotaxime MICs among 560 nontyphoidal *Salmonella* isolates

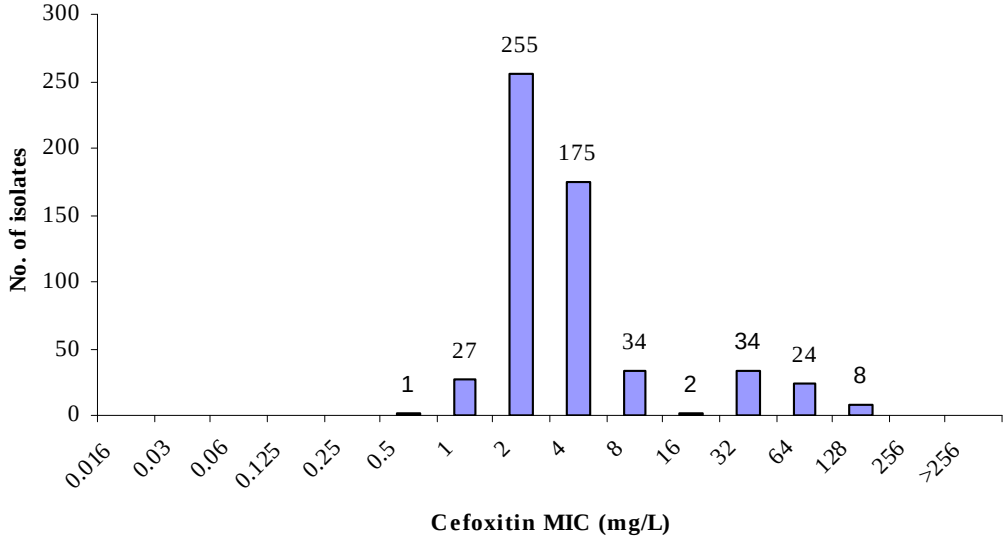

Figure 14. Distribution of cefoxitin MICs among 560 nontyphoidal *Salmonella* isolates

PART III : DETECTION OF ESBL PHENOTYPE

A total of 560 nontyphoidal *Salmonella* isolates were screened for ESBL phenotype by the MICs of third-generation cephalosporins. The isolates which the MICs of either ceftazidime, cefotaxime, or ceftriaxone $\geq$ 2 mg/L were considered to have a positive screening test for ESBL phenotype by CLSI and subjected to clavulanate confirmatory testing using the combination disk test. One hundred and twenty four of nontyphoidal *Salmonella* isolates, were positive for ESBL screening test. The 42.74% (53/124) of all isolates were positive for ESBL phenotype by combination disk test (Figure 15.). Of the 124 isolates, 42.74% (53/124) showed positive results with cefotaxime and cefotaxime/clavulanic acid disk and 38.71% (48/124) showed positive results with ceftazidime and ceftazidime/clavulanic acid disk. Five isolates showed negative results with ceftazidime and ceftazidime/clavulanic acid disk. All isolates with ESBL phenotype showed that inhibition zone of cefotaxime/clavulanic acid were $\geq$ 5 mm larger than that of cefotaxime disk alone and showed a defference of 6 to 27 mm zone size. Most isolates (98.11%) showed a defference of $\geq$ 14 mm zone size. Only one isolate showed a difference in zone diameter of 6 mm for cefotaxime and cefotaxime/clavulanic acid disk and had negative result with ceftazidime and ceftazidime/clavulanic acid disk (Figure 15.). A total of 48 isolates with positive results for ceftazidime and ceftazidime/clavulanic acid disk showed a defference of 5 to 13 mm zone size. Most isolates (62.50%) showed a defference of < 10 mm zone size. The 18 isolates (37.50%) showed a defference of 10 to 13 mm zone size.

The susceptibility of cefoxitin, ceftazidime, cefotaxime, and ceftriaxone against 53 nontyphoidal *Salmonella* isolates with ESBL phenotype are summarized in Table 12. All isolates with ESBL phenotype showed high rates of cefotaxime and ceftriaxone resistance of 96.23% (51/53) and 94.34% (50/53), respectively. The MIC of cefotaxime ranged from 16 to 256 mg/L. MIC_{50} and MIC_{90} were 128 mg/L and >256 mg/L, respectively. However, 30.9% (16/53) of isolates were resistant to ceftazidime. The MIC of ceftazidime ranged from 0.5 to >256 mg/L. MIC_{50} and MIC_{90} were 8 mg/L and 128 mg/L, respectively. Only one isolate was resistant to cefoxitin with the MIC of

64 mg/L and was also resistant to ceftazidime, cefotaxime, and ceftriaxone. Distribution of the MICs for cefoxitin, ceftazidime, cefotaxime, and ceftriaxone among 53 nontyphoidal *Salmonella* isolates with ESBL phenotype are shown in Figure 16.

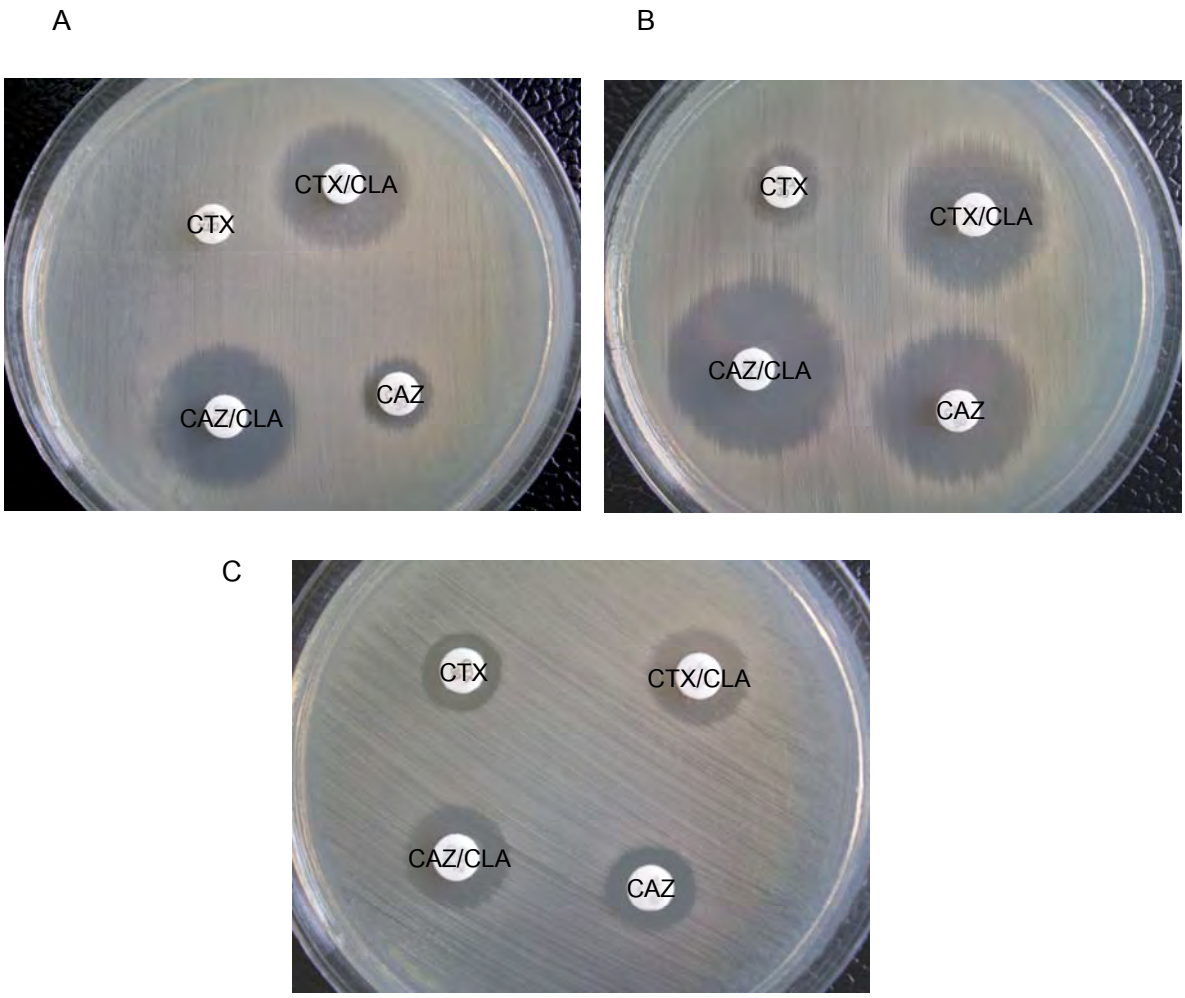

Figure 15. The combination disk test: cefotaxime (CTX), cefotaxime/clavulanic acid (CTX/CLA) and ceftazidime (CAZ), ceftazidime/clavulanic acid (CAZ/CLA): (A), The isolate was positive for both cefotaxime and ceftazidime combination disk test.; (B), The isolate was positive for cefotaxime combination disk test and was negative for ceftazidime combination disk test. (C) The isolate was weakly positive for cefotaxime combination disk test and was negative for ceftazidime combination disk test.

Table 12. The susceptibility of cefoxitin, ceftazidime, cefotaxime, and ceftriaxone against 53 nontyphoidal *Salmonella* isolates with ESBL phenotype

Antimicrobial agents	MIC (mg/L)			Suscetibility (%)		
	MIC_{50}	MIC_{90}	Range	R	I	S
Cefoxitin	2	4	1-64	1 (1.89)	0	52 (98.11)
Ceftazidime	8	128	0.5->256	16 (30.19)	7 (13.21)	30 (56.60)
Cefotaxime	128	>256	16->256	51 (96.23)	2 (3.77)	0
Ceftriaxone	256	>256	32->256	50 (94.34)	3 (5.66)	0

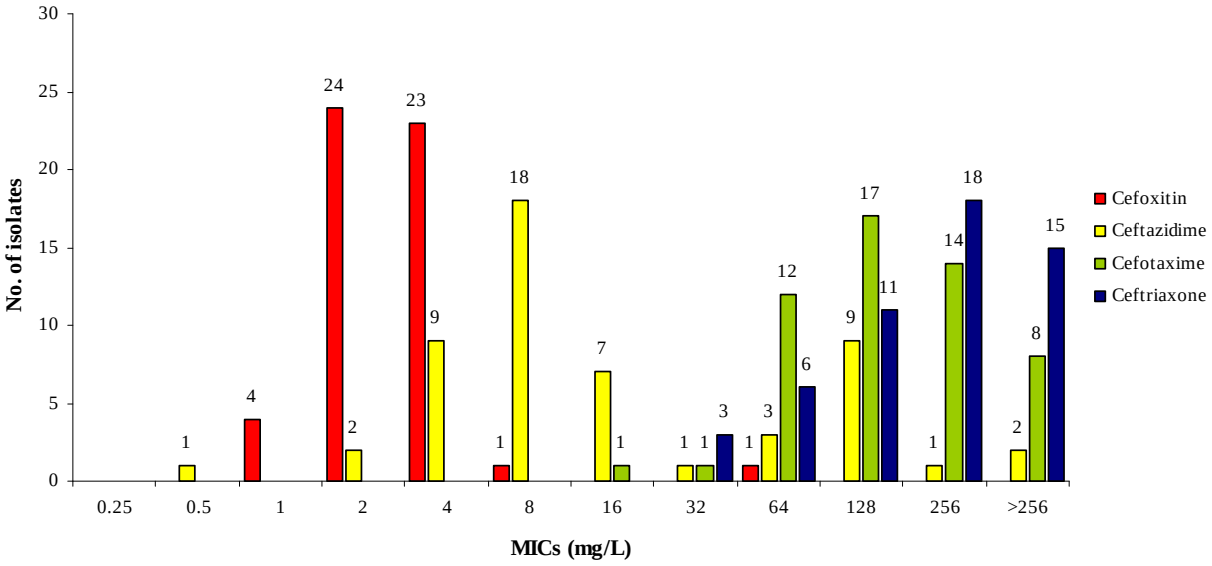

Figure 16. Distribution of MICs for cefoxitin, ceftazidime, cefotaxime, and ceftriaxone among 53 nontyphoidal *Salmonella* isolates with ESBL phenotype

PART IV : DETECTION OF AMPC PHENOTYPE

1. Detection of AmpC β-lactamase activity

A total of 560 nontyphoidal *Salmonella* isolates were screened for suspected AmpC phenotype isolates which had MICs of ≥2 mg/L for ceftazidime or cefotaxime or ceftriaxone and cefoxitin MIC of >8 mg/L. Sixty-seven of nontyphoidal *Salmonella* isolates had ceftazidime or cefotaxime or ceftriaxone MICs of ≥2 mg/L and cefoxitin MICs of >8 mg/L and were subjected to detect for AmpC β-lactamase activity by modified Hodge test with cefoxitin disk. All 67 tested isolates produced AmpC β-lactamases which inhibited cefoxitin activity and showed the decreased radius of the inhibition zone of cefoxitin-susceptible organism (*E. coli* ATCC 25922) along the growth of tested isolates. All isolates were positive for modified Hodge test and had AmpC β-lactamase activity (Figure 17.).

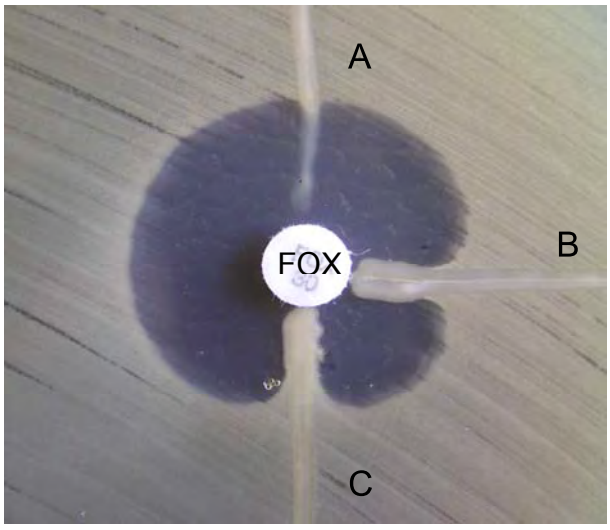

Figure 17. The modified Hodge test with cefoxitin (FOX) for detection of AmpC activity: A, negative control (*E. colli* ATCC 25922); B, positive control (CMY-2-producing *E.coli*); C, tested strain with AmpC enzyme activity

2. Detection of inducible AmpC phenotype

A total of 67 nontyphoidal *Salmonella* isolates with AmpC β-lactamase activity were subjected to detect for inducible AmpC phenotype by double-disk diffusion test with cefoxitin and ceftazidime disks. The presence of a "D"-shaped zone around the ceftazidime disk showed inducible activity (Figure 18.). All of 67 AmpC-producing isolates were negative for inducible AmpC phenotype.

A B

Figure 18. The double-disk diffusion test with cefoxitin (FOX) and ceftazidime (CAZ) for detection of inducible AmpC phenotype: (A), inducible phenotype (DHA-1-producing *E.coli*) and (B), non-inducible phenotype.

The susceptibility of cefoxitin, ceftazidime, cefotaxime, and ceftriaxone against 67 nontyphoidal *Salmonella* isolates with AmpC β-lactamase activity with non-inducible phenotype are summarized in Table 13. Sixty-six isolates (98.51%) were resistant to cefoxitin and only one isolate was intermediate resistant to cefoxitin. All isolates were resistant to ceftazidime with MIC range of 32 to >256 mg/L and 31.34% (21/67) and 4.48% (3/67) were resistant to ceftriaxone and cefotaxime, respectively. Distribution of the MICs for cefoxitin, ceftazidime, cefotaxime, and ceftriaxone among 67 nontyphoidal *Salmonella* isolates with non-inducible AmpC enzyme are shown in Figure 19. The isolates with non-inducible AmpC enzyme

showed high-level MICs to cefoxitin, ceftazidime and ceftriaxone but showed low-level MICs to cefotaxime.

Table 13. The susceptibility of cefoxitin, ceftazidime, cefotaxime, and ceftriaxone against 67 of non-inducible AmpC-producing nontyphoidal *Salmonella* isolates

Antimicrobial agents	MICs (mg/L)			Susceptibility (%)		
	MIC_{50}	MIC_{90}	Range	R	I	S
Cefoxitin	32	128	16-128	66 (98.51)	1 (1.49)	0
Ceftazidime	128	128	32->256	67 (100.00)	0	0
Cefotaxime	16	32	4-128	3 (4.48)	40 (59.70)	24 (35.82)
Ceftriaxone	32	128	16->256	21 (31.34)	46 (68.66)	0

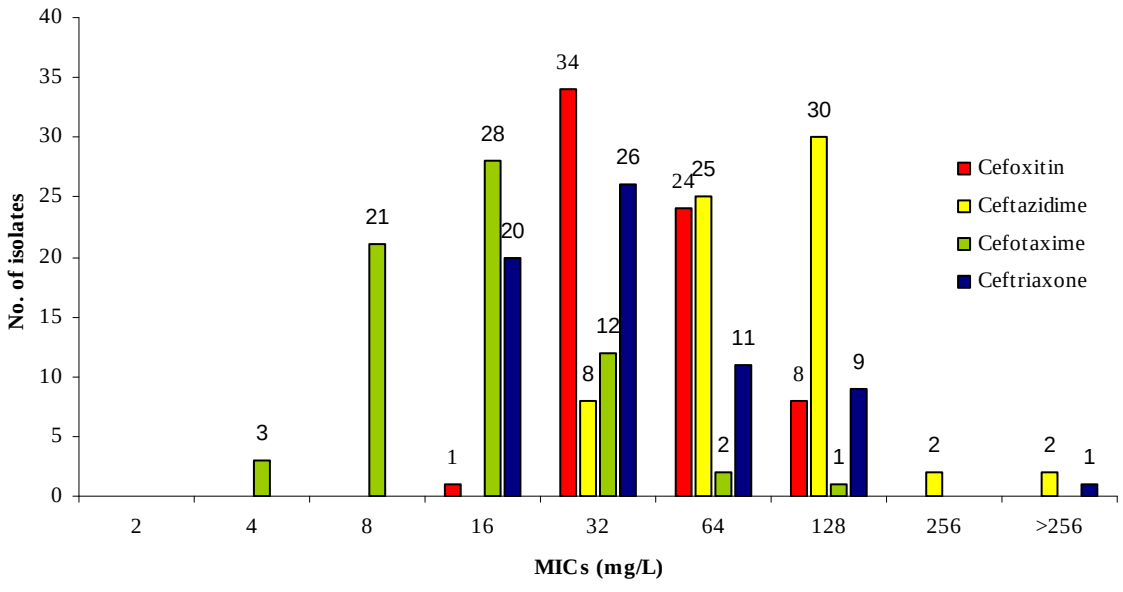

Figure 19. Distribution of MICs for cefoxitin, ceftazidime, cefotaxime, and ceftriaxone among 67 non-inducible AmpC-producing nontyphoidal *Salmonella* isolates

PART V : SCREENING FOR THE PRESENCE OF ESBL GENES

1. Screening for the presence of *bla*$_{OXA}$, *bla*$_{TEM}$, *bla*$_{SHV}$, *bla*$_{CTX-M}$, and *bla*$_{VEB}$

A total of 53 nontyphoidal *Salmonella* isolates with positive for ESBL phenotype were screened for the presence of ESBL genes including, *bla*$_{OXA}$, *bla*$_{TEM}$, *bla*$_{SHV}$, *bla*$_{CTX-M}$, and *bla*$_{VEB}$ by multiplex PCR. The expected PCR product sizes of *bla*$_{OXA}$, *bla*$_{TEM}$, *bla*$_{SHV}$, *bla*$_{CTX-M}$, and *bla*$_{VEB}$ were 619 bp, 516 bp, 392 bp, 550 bp, and 216 bp, respectively (Figure 20.). The *bla*$_{CTX-M-like}$ was detected in all 53 isolates. Of the 53 isolates, 31 (58.49%) had only *bla*$_{CTX-M-like}$ and 22 (41.51%) had both *bla*$_{CTX-M-like}$ and *bla*$_{TEM-like}$. The *bla*$_{OXA}$, *bla*$_{SHV}$, and *bla*$_{VEB}$ genes were not detected.

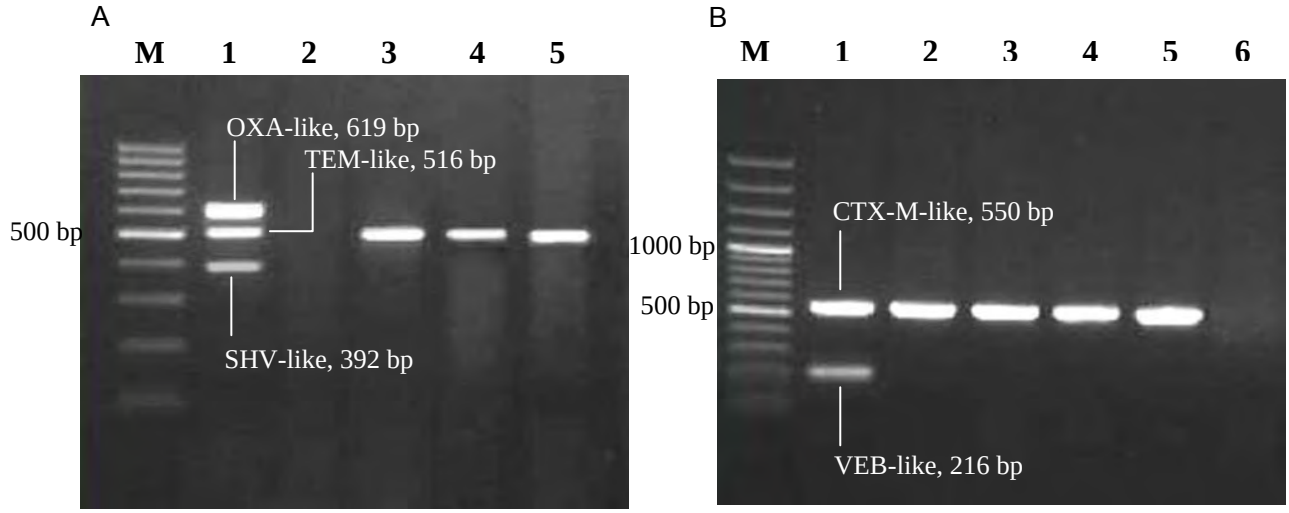

Figure 20. (A), The multiplex PCR analysis for *bla*$_{OXA}$, *bla*$_{TEM}$, and *bla*$_{SHV}$ genes: M, 100-bp DNA ladder; Lanes 1, 3 Templates, *bla*$_{OXA-like}$ (619 bp), *bla*$_{TEM-like}$ (516 bp), and *bla*$_{SHV-like}$ (392 bp); Lanes 2, negative control (sterile DDW); Lanes 3-5, Nontyphoidal *Salmonella* isolates harboring the *bla*$_{TEM-like}$ gene and (B), The multiplex PCR analysis of *bla*$_{CTX-M}$ and *bla*$_{VEB}$ genes: M, 100-bp plus DNA ladder; Lanes 1, 2 Templates, *bla*$_{CTX-M-like}$ (550 bp) and *bla*$_{VEB-like}$ (216 bp); Lanes 2-5, Nontyphoidal *Salmonella* isolates harboring the *bla*$_{CTX-M-like}$ gene; Lanes 6, negative control (sterile DDW).

2. Screening for group of bla_{CTX-M} genes

All 53 of nontyphoidal *Salmonella* isolates carrying $bla_{CTX-M-like}$ were investigated for the group of bla_{CTX-M} genes encoding CTX-M β-lactamase, including CTX-M-1, CTX-M-2, CTX-M-9, and CTX-M-8/25 groups The expected PCR product sizes of $bla_{CTX-M-1}$ group, $bla_{CTX-M-2}$ group, $bla_{CTX-M-8/25}$ group, and $bla_{CTX-M-9}$ group were 260 bp, 341 bp, 207 bp, and 293 bp, respectively (Figure 21.).

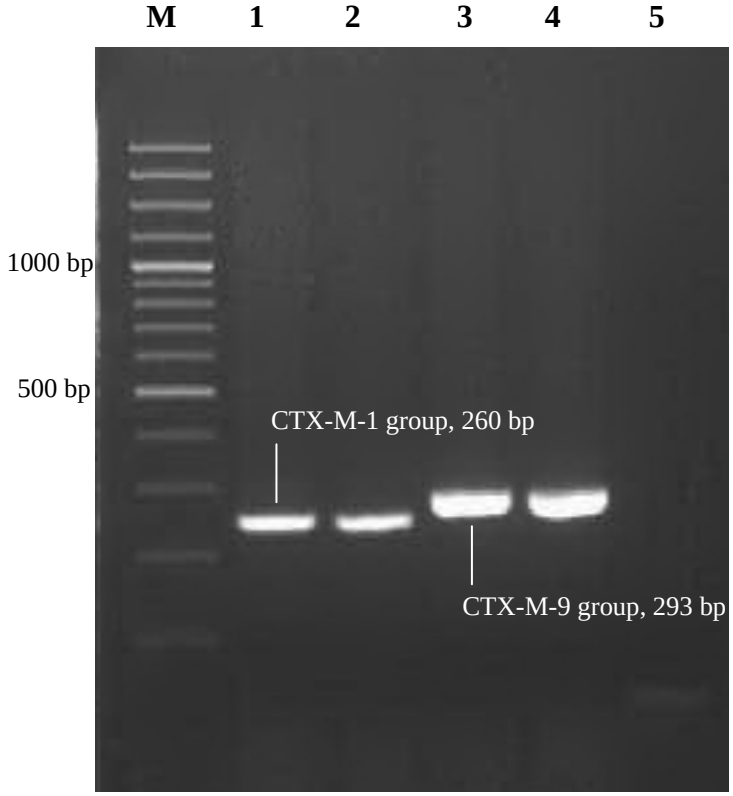

Figure 21. The multiplex PCR analysis of $bla_{CTX-M-1}$ group, $bla_{CTX-M-2}$ group, $bla_{CTX-M-8/25}$ group, and $bla_{CTX-M-9}$ group genes: M, 100-bp plus DNA ladder; Lanes 1, Template $bla_{CTX-M-1}$ group (260 bp); Lanes 2, Nontyphoidal *Salmonella* isolates harboring the $bla_{CTX-M-1}$ group gene; Lanes 3, Template $bla_{CTX-M-9}$ group (293 bp); Lanes 4, Nontyphoidal *Salmonella* isolates harboring the $bla_{CTX-M-9}$ group gene; Lanes 5, negative control (sterile DDW)

Of 53 isolates carrying *bla*$_{\text{CTX-M-like}}$, 73.58% (39/53) had *bla*$_{\text{CTX-M-9}}$ group and 26.42% (14/53) had *bla*$_{\text{CTX-M-1}}$ group. Thirty-one isolates (58.49%) carried only *bla*$_{\text{CTX-M-9}}$ group. Eight isolates (15.10%) carried both *bla*$_{\text{CTX-M-9}}$ group and *bla*$_{\text{TEM-like}}$. Fourteen isolates harbored both *bla*$_{\text{CTX-M-1}}$ group and *bla*$_{\text{TEM-like}}$. The *bla*$_{\text{CTX-M-2}}$ group and *bla*$_{\text{CTX-M-8/25}}$ group were not detected.

PART VI : SCREENING FOR THE PRESENCE OF PLASMID-MEDIATED *AMPC* GENES BY PCR

The nontyphoidal *Salmonella* isolates with AmpC phenotype were investigated for the presence of plasmid-mediated *ampC* genes, including bla_{MOX}, bla_{CIT}, bla_{DHA}, bla_{EBC}, bla_{ACC}, and bla_{FOX} by using multiplex PCR. The expected 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 (Figure 22.). The bla_{CIT} was detected in all 67 isolates. The bla_{MOX}, bla_{DHA}, bla_{EBC}, bla_{ACC}, and bla_{FOX} were not detected.

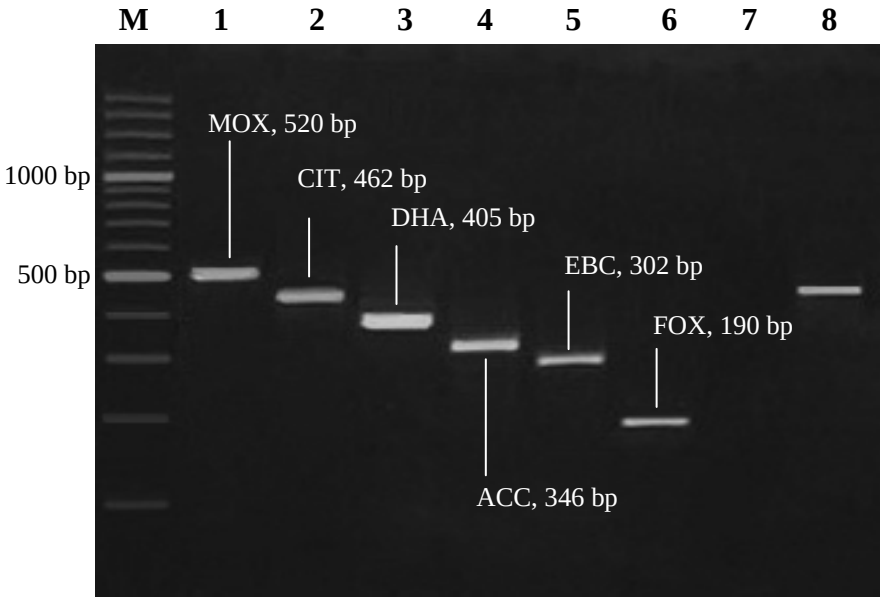

Figure 22. The multiplex PCR analysis of *ampC* genes: M, 100-bp plus DNA ladder; Lanes 1, Template bla_{MOX-8} (bla_{MOX}, 520 bp); Lanes 2, Template bla_{CMY-2} (bla_{CIT}, 462 bp); Lanes 3, Template bla_{DHA-1} (bla_{DHA}, 405 bp); Lanes 4, Template bla_{ACC-1} (bla_{ACC}, 346 bp); Lanes 5, Template bla_{MIR-1} (bla_{EBC}, 302 bp); Lanes 6, Template bla_{FOX-4} (bla_{FOX}, 190 bp); Lanes 7, negative control (sterile DDW); Lanes 8, Nontyphoidal *Salmonella* isolate harboring $bla_{CIT-like}$.

PART VII : CHARACTERIZATION OF ESBL AND AMPC ENZYMES

Of the 560 nontyphoidal *Salmonella* isolates, 119 (21.25%) were resistant to third-generation cephalosporins, including ceftriaxone, ceftazidime, and cefotaxime. Of the 119 third-generation cephalosporin-resistant isolates, 104 (87.39%) and 15 (12.61%) isolates were *S.* Cholerasuis (serogroup C) and *S.* Typhimurium (serogroup B), respectively. The results showed that *S.* Choleraesuis followed by *S.* Typhimurium had a higher frequency of ceftriaxone resistance than other serotypes. Of the 119 isolates, 43.70% (52/119) were ESBL producers, 55.46% (66/119) were AmpC producers, and 0.84% (1/119) was ESBL and AmpC co-producer. Sixty-six isolates (55.46%) carried $bla_{\text{CIT-like}}$ and all of these isolates were *S.* Cholerasuis. Fourteen isolates (11.76%) carried both $bla_{\text{CTX-M-1}}$ group and $bla_{\text{TEM-like}}$ and all of these isolates were *S.* Typhimurium. All 39 isolates carried $bla_{\text{CTX-M-9}}$ group. Thirty-one isolates (26.05%) carried $bla_{\text{CTX-M-9}}$ group and were *S.* Cholerasuis. Seven isolates (5.88%) carried both $bla_{\text{CTX-M-9}}$ group and $bla_{\text{TEM-like}}$. Of these isolates, 6 isolates were *S.* Cholerasuis and 1 isolate was *S.* Typhimurium. One isolate (0.84%) carried $bla_{\text{CTX-M-9}}$ group, $bla_{\text{TEM-like}}$ of *ESBL* genes and $bla_{\text{CIT-like}}$ of *ampC* gene. The isolate was *S.* Typhimurium. The results are summarized in Table 14.

Table 14. Type of *bla* genes in the 119 third-generation cephalosporin-resistant nontyphoidal *Salmonella* isolates

Type of *bla* genes	No. of isolates (%)	Serotype (No. of isolates)
ESBL producers (n=52)		
$bla_{\text{CTX-M-9}}$ group	31 (26.05)	*S.* Cholerasuis (31)
$bla_{\text{CTX-M-9}}$ group and $bla_{\text{TEM-like}}$	7 (5.88)	*S.* Cholerasuis (6)
		S. Typhimurium (1)
$bla_{\text{CTX-M-1}}$ group and $bla_{\text{TEM-like}}$	14 (11.76)	*S.* Typhimurium(14)
AmpC producers (n=66)		
$bla_{\text{CIT-like}}$	66 (55.46)	*S.* Cholerasuis (66)
ESBL and AmpC co-producer (n=1)		
$bla_{\text{CTX-M-9}}$ group, $bla_{\text{TEM-like}}$, and $bla_{\text{CIT-like}}$	1 (0.84)	*S.* Typhimurium (1)

The correlation between *bla* gene patterns and the susceptibility, MIC range, MIC_{50}, and MIC_{90} of cephalosporins are summarized in Table 15.

Thirty-one isolates carrying $bla_{CTX\text{-}M\text{-}9}$ group showed low-level MICs to ceftazidime with MIC range of 0.5 to 32 mg/L. All of these isolates were susceptible to cefoxitin and had low-level MICs to cefoxitin with MIC range of 1 to 8 mg/L. MIC_{50} of cefoxitin and ceftazidime were 2 and 8 mg/L, whereas MIC_{90} of cefoxitin and ceftazidime were 4 and 16 mg/L, respectively. However, these isolates showed high-level MICs to cefotaxime and ceftriaxone, ranging from 16 to 256 mg/L and 32 to >256 mg/L, respectively. MIC_{50} of these isolates for cefotaxime and ceftriaxone were 128 and 256 mg/L, respectively, whereas MIC_{90} for both cefotaxime and ceftriaxone were 256 mg/L. The isolates carrying $bla_{CTX\text{-}M\text{-}9}$ group showed high-level MICs to cefotaxime and ceftriaxone but showed low-level MICs to ceftazidime and cefoxitin.

Similar to the $bla_{CTX\text{-}M\text{-}9}$ group carrying isolates, seven isolates carrying both $bla_{CTX\text{-}M\text{-}9}$ group and $bla_{TEM\text{-}like}$ showed low-level MICs to cefoxitin and ceftazidime with MIC range of 2 to 4 mg/L and 4 to 16 mg/L, respectively. MIC_{50} of these isolates for cefoxitin and ceftazidime were 2 and 8 mg/L, respectively. MIC_{90} for cefoxitin and ceftazidime were 4 and 8/16 mg/L, respectively. These isolates showed high-level MICs to cefotaxime and ceftriaxone ranged from 64 to 256 mg/L and 32 to 256 mg/L, respectively. MIC_{50} for both of cefotaxime and ceftriaxone were 128 mg/L, whereas MIC_{90} of cefotaxime and ceftriaxone were 128/256 and 256 mg/L, respectively. Similar to the $bla_{CTX\text{-}M\text{-}9}$ group carrying isolates, the isolates carrying both $bla_{CTX\text{-}M\text{-}9}$ group and $bla_{TEM\text{-}like}$ showed high-level MICs to cefotaxime and ceftriaxone but showed low-level MICs to ceftazidime and cefoxitin.

All 14 isolates carrying both $bla_{CTX\text{-}M\text{-}1}$ group and $bla_{TEM\text{-}like}$ were resistant to cefotaxime, ceftriaxone, and ceftazidime but were susceptible to cefoxitin. These isolates showed high-level MICs to cefotaxime and ceftriaxone with MIC range of 256 to >256 mg/L. MIC_{50}/MIC_{90} of cefotaxime and ceftriaxone were >256/>256 mg/L. These isolates demonstrated high-level MICs to ceftazidime ranged from 64 to >256. MIC_{50} and MIC_{90} of ceftazidime were 128 mg/L and 256/>256 mg/L, respectively. The susceptibility demonstrated that 57.12% (8 isolates) of these isolates had ceftazidime MIC of 128 mg/L, 92.86% (13 isolates) showed high-level MIC of >256 mg/L for

cefotaxime and 57.12% (8 isolates) had high-level MIC of >256 mg/L for cefriaxone. However, cefoxitin MIC ranged from 2 to 4 mg/L and MIC_{50}/MIC_{90} of cefoxitin were 4/4 mg/L. Most of the isolates (85.71%, 12 isolates) had cefoxitin MIC of 2 mg/L. The isolates carrying both $bla_{CTX\text{-}M\text{-}1}$ group and $bla_{TEM\text{-}like}$ showed high-level MICs to all third-generation cephalosporins, including cefotaxime, ceftriaxone, and ceftazidime but showed low-level MICs to cefoxitin.

Of the 66 isolates carrying $bla_{CIT\text{-}like}$, 65 (98.48%) were resistant to cefoxitin and only one isolate was intermediate resistant to cefoxitin. The cefoxitin susceptibility showed that MIC ranged from 16 to 128 mg/L and MIC_{50}/MIC_{90} of cefoxitin were 32/128 mg/L. Most isolates (51.52%, 34 isolates) had cefoxitin MIC of 32 mg/L. In addition, all isolates were resistant to ceftazidime. Ceftazidime MIC ranged from 32 to >256 mg/L. MIC_{50} and MIC_{90} of ceftazidime were 64/128 mg/L and 128 mg/L, respectively. Most of the isolates (43.94%, 29 isolates) had high-level cefoxitin MIC of 128 mg/L. However, cefotaxime MICs of these isolates ranged from 4 to 64 mg/L. MIC_{50}/MIC_{90} of cefotaxime were 16/32 mg/L. Most isolates (60.61%, 40 isolates) were intermediate resistant to cefotaxime and had MIC of 16 to 32 mg/L. The ceftriaxone susceptibility showed that MIC ranged from 16 to 128 and MIC_{50}/MIC_{90} were 32/128 mg/L. Most isolates (69.70%, 46 isolates) were intermediate resistant to ceftriaxone and had MIC of 16 to 32 mg/L. The isolates carrying $bla_{CIT\text{-}like}$ showed high-level MICs to ceftazidime and cefoxitin but showed low-level MICs to cefotaxime and ceftriaxone.

One isolate was ESBL and AmpC co-producer carrying $bla_{CTX\text{-}M\text{-}9}$ group, $bla_{TEM\text{-}like}$, and $bla_{CIT\text{-}like}$. The susceptibility demonstrated that MICs of cefoxitin, ceftazidime, cefotaxime, and ceftriaxone were 64, 128, 128, and >256 mg/L, respectively. This isolate had high-level MICs to all third-generation cephalosporins and cefoxitin and showed weakly positive result for cefotaxime combination disk test with a difference in zone diameter of 6 mm for cefotaxime and cefotaxime/clavulanic acid disk and had negative result with ceftazidime and ceftazidime/clavulanic acid disk.

Eighteen isolates (3.21%) were resistant to all third-generation cephalosporins. The $bla_{CIT\text{-}like}$ was found in 2 isolates, the $bla_{CTX\text{-}M\text{-}1}$ group together with $bla_{TEM\text{-}like}$ were found in 14 isolates, the $bla_{CTX\text{-}M\text{-}9}$ group was found in 1 isolate, and the

bla$_{CTX-M-9}$ group *bla*$_{TEM-like}$ and *bla*$_{CIT-like}$ was found in 1 isolate. Three isolates (0.54%) were resistant to all third-generation cephalosporins and cefoxitin. Two of these isolates carried *bla*$_{CIT-like}$ and one carried *bla*$_{CTX-M-9}$ group, *bla*$_{TEM-like}$ and *bla*$_{CIT-like}$.

Table 15. The *bla* gene patterns and the MICs of cephalosporins in the 119 third-generation cephalosporin-resistant nontyphoidal *Salmonella* isolates

Pattern of *bla* genes	No. of isolates (%)	ESBL phenotype	AmpC phenotype	Cefoxitin MIC (mg/L)			Ceftazidime MIC (mg/L)			Cefotaxime MIC (mg/L)			Ceftriaxone MIC (mg/L)		
				MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range
$bla_{CTX-M-9}$ group	31 (26.05)	+	-	2	4	1-8	8	16	0.5-32	128	256	16-256	256	256	32->256
$bla_{CTX-M-9}$ group and $bla_{TEM-like}$	7 (5.88)	+	-	2	4	2-4	8	8/16	4-16	128	128/256	64-256	128	256	32-256
$bla_{CTX-M-1}$ group and $bla_{TEM-like}$	14 (11.76)	+	-	4	4	2-4	128	256/>256	64->256	>256	>256	256->256	>256	>256	256->256
$bla_{CIT-like}$	66 (55.46)	-	+	32	128	16-128	64/128	128	32->256	16	32	4-64	32	128	16-128
$bla_{CTX-M-9}$ group, $bla_{TEM-like,}$ and $bla_{CIT-like}$	1 (0.84)	+	+			64			128			128			>256

+, positive result; -, negative result

PART VIII : CORRELATION BETWEEN ESBL AND AMPC PRODUCERS AND TYPE OF CLINICAL SAMPLES

Comparison between type of clinical specimen, cephalosporin MIC and *bla* genes are shown in Table 16. Of the 560 nontyphoidal *Salmonella* isolates, 375 (66.94%) were isolated from sterile sites including 374 (99.73%) isolates from blood and one (0.27%) isolate from CSF. These isolates showed MICs of cefoxitin, ceftazidime, cefotaxime, and ceftriaxone, ranging from 0.5 to 128, 0.25 to >256, 0.016 to >256, and 0.016 to >256 mg/L, respectively. MIC_{50}/MIC_{90} of cefoxitin, ceftazidime, cefotaxime, and ceftriaxone were 2/32, 0.5/64, 0.125/32, and 0.125/64 mg/L, respectively. The isolates from sterile sites showed high-level MICs to cefoxitin, ceftriaxone, and ceftazidime but showed low-level MICs to cefotaxime. Of the 185 isolates from non-sterile sites included isolates from stool, rectal swab, urine, pus, tissue, and sputum. Most (64.32%, 119/185) isolates were recovered from stool specimen. The isolates showed MICs of cefoxitin, ceftazidime, cefotaxime, and ceftriaxone ranging from 1 to 64, 0.125 to >256, 0.06 to >256, and 0.03 to >256 mg/L, respectively. MIC_{50}/MIC_{90} of cefoxitin, ceftazidime, cefotaxime, and ceftriaxone were 4/4, 0.5/64, 0.125/64, and 0.125/128 mg/L, respectively. The isolates from non-sterile sites showed high-level MICs to cefotaxime, ceftriaxone, and ceftazidime but showed low-level MICs to cefoxitin.

The comparison between cephalosporin resistance rates of isolates from sterile site and non-sterile sites are shown in Figure 23. The isolates from sterile sites showed high rate of resistance to cephalosporins. The resistance rate of cefoxitin, ceftazidime, cefotaxime, and ceftriaxone were 15.47% (58/375), 16.0% (60/375), 8.53% (32/375), and 13.07% (49/375), respectively. The isolates from non-sterile sites showed resistance rate of cefoxitin, ceftazidime, cefotaxime, and ceftriaxone to be 4.32% (8/185), 11.89% (22/185), 11.35% (21/185), and 11.35% (21/185), respectively. The 24.27% (91/375) of isolates from sterile sites were resistant to third-generation cephalosporins whereas 15.14% (28/185) of non-sterile site isolates were resistant to these antimicrobial agents (Figure 23.). By statistical analysis using Chi-square test, there was significant difference in third-generation cephalosporin resistance rates

between isolates from sterile and non-sterile site ($P < 0.05$). There was a significant difference in cefoxitin resistance rates between isolates from sterile and non-sterile site ($P < 0.01$).

Of the 91 third-generation cephalosporin-resistant isolates from sterile sites, 59 (64.84%) were AmpC producers, and 39 (42.86%) were ESBL producers. Most (64.84%, 59/91) isolates carried $bla_{\text{CIT-like}}$ gene and 31.87% (29/91) isolates carried $bla_{\text{CTX-M-9}}$ group gene (Table 17.). Of the 28 third-generation cephalosporin-resistant isolates from non-sterile sites, 20 (71.43%) were ESBL producers, 7 (25%) were AmpC producers, and 1 (3.57%) was ESBL and AmpC co-producer, carring $bla_{\text{CTX-M-9}}$ group, $bla_{\text{TEM-like}}$, and $bla_{\text{CIT-like}}$. This isolate was recovered from stool specimen and had cefoxitin, ceftazidime, cefotaxime, and ceftriaxone MICs of 64, 128, 128, and >256 mg/L, respectively. Fifty percent of the isolates (14/28) carried $bla_{\text{CTX-M-1}}$ group together with $bla_{\text{TEM-like}}$ genes and 25% (7/28) carried $bla_{\text{CIT-like}}$ gene (Table 17.).

The results demonstrated that $bla_{\text{CIT-like}}$ was the most prevalent gene in isolates from sterile sites whereas both $bla_{\text{CTX-M-1}}$ group and $bla_{\text{TEM-like}}$ were the most prevalent gene in non-sterile site isolates.

Table16. The susceptibility and *bla* genes of 560 nontyphoidal *Salmonella* isolates from sterile and non-sterile sites

Type of specimen	No. of isolates (%)	Cefoxitin MIC (mg/L)			Ceftazidime MIC (mg/L)			Cefotaxime MIC (mg/L)			Ceftriaxone MIC (mg/L)			Presence of *bla* genes			
		MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	$bla_{CTX-M-1}$ group	$bla_{CTX-M-9}$ group	$bla_{TEM-like}$	$bla_{CIT-like}$
Sterile sites (Total=375)	**375**	**2**	**32**	**0.5-128**	**0.5**	**64**	**0.25->256**	**0.125**	**32**	**0.016->256**	**0.125**	**64**	**0.016->256**	**0**	**32**	**3**	**59**
Blood	374(99.73)	2	32	0.5-128	0.5	64	0.25->256	0.125	32	0.016->256	0.125	64	0.016->256		31	3	59
CSF	1 (0.27)			1			16			128			64		1		
Non-sterile sites (Total=185)	**185**	**4**	**4**	**1-64**	**0.5**	**64**	**0.125->256**	**0.125**	**64**	**0.06->256**	**0.125**	**128**	**0.03->256**	**14**	**7**	**19**	**8**
Stool	119(64.32)	4	8	1-64	0.5	32	0.125-256	0.125	32	0.06->256	0.125	32	0.03->256	7	5	12	7
Rectal swab	36 (19.46)	2	4	1-8	0.5	128	0.125->256	0.125	256	0.06->256	0.125	>256	0.06->256	6		6	
Urine	14 (7.57)	2	4	1-64	0.5	4/128	0.5-128	0.125	16/64	0.125->256	0.125	32/64	0.06->256	1	1	1	1
Pus	12 (6.49)	2	4	2-8	0.5	1	0.25-8	0.125	1	0.06-64	0.125	0.25	0.06-128		1		
Tissue	3 (1.62)	1	1/4	1-4	0.5	0.5/1	0.5-1	0.125	0.125	0.125	0.06	0.06	0.06				
Sputum	1 (0.54)			4			0.5			0.125			0.125				

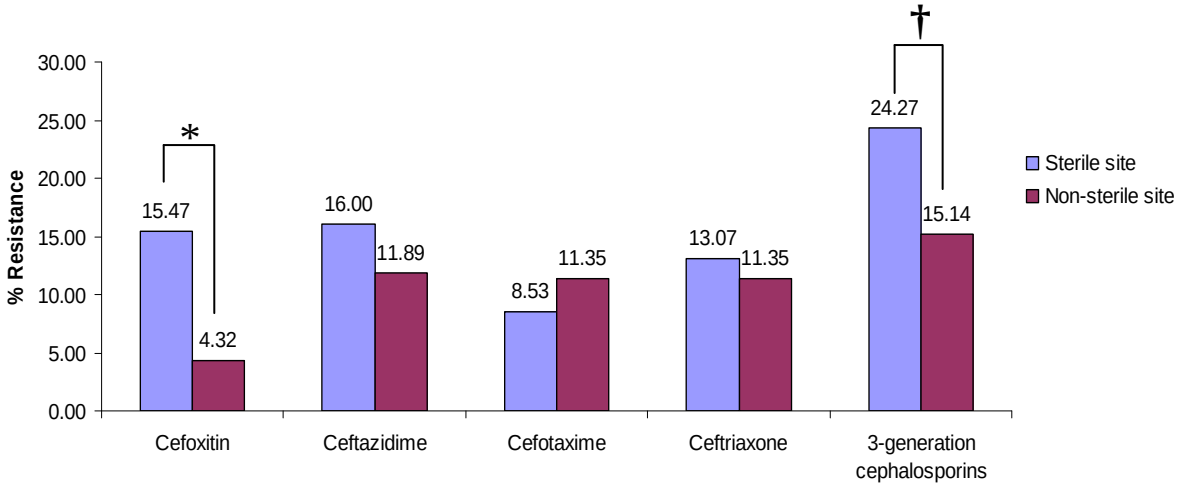

Figure 23. Distribution of resistance rates of cefoxitin, ceftazidime, cefotaxime, ceftriaxone, and either of third-generation cephalosporins compared with the sources of clinical samples: *, significant (P<0.01); †, significant (P<0.05)

Table 17. The *bla* genes of 119 third-generation cephalosporin-resistant isolates from sterile (n=91) and non-sterile sites (n=28)

Type of *bla* genes	Number of resistant isolates (%)	
	Sterile site (n=91)	Non-sterile site (n=28)
$bla_{CTX\text{-}M\text{-}9}$ group	29 (31.87)	2 (7.14)
$bla_{CTX\text{-}M\text{-}9}$ group, $bla_{TEM\text{-}like}$	3 (3.30)	4 (14.29)
$bla_{CTX\text{-}M\text{-}1}$ group, $bla_{TEM\text{-}like}$	0	14 (50.00)
$bla_{CIT\text{-}like}$	59 (64.84)	7 (25.00)
$bla_{CTX\text{-}M\text{-}9}$ group, $bla_{TEM\text{-}like}$, $bla_{CIT\text{-}like}$	0	1 (3.57)
Total	100%	100%

PART IX : DNA SEQUENCING ANALYSIS OF ENTIRE *BLA* GENES

The entire $bla_{CTX\text{-}M\text{-}1}$ group, $bla_{CTX\text{-}M\text{-}9}$ group, bla_{TEM}, and bla_{CIT} genes were amplified by PCR. DNA sequencing was performed in which 22, 14, 10, and 10 isolates with different MIC level were selected randomly from isolates carrying $bla_{TEM\text{-}like}$, $bla_{CTX\text{-}M\text{-}1}$ group, $bla_{CTX\text{-}M\text{-}9}$ group, and $bla_{CIT\text{-}like}$ genes, respectively. DNA sequences were analyzed by the software available over the internet at the National Center for Biotechnology Information (http://blast.ncbi.nlm.nih.gov /Blast.cgi), Multalin (http://multalin.toulouse.inra.fr/multalin/multalin.html), and ExPASy Proteomics Server (http://au.expasy.org/tools/dna.html). The results of DNA sequencing are summarized in Table 18.

All 22 isolates carrying $bla_{TEM\text{-}like}$ including S17-1, S19, S31, S32, S33, S39, S49, S53, S434, S437, S19-1, S29-1, S17, S20, S21, S22, S23, S38, S40, S45, S51, and S323 were performed DNA sequencing. These isolates carried various combinations of *bla* genes and showed MIC ranges of cefoxitin, ceftazidime, cefotaxime, and ceftriaxone to be 2 to 64, 4 to >256, 64 to >256, and 32 to >256 mg/L, respectively. DNA sequence analysis of the 1349 bp DNA fragments of $bla_{TEM\text{-}like}$ revealed an open reading frame of 861 bp, encoding 286 amino acids. All of these isolates showed 100% nucleotide and amino acid sequences identity to $bla_{TEM\text{-}1}$ and TEM-1 enzyme, respectively (GenBank accession no. FN568351 and NP608310, respectively). Alignments of amino acid sequences of TEM-1 (GenBank accession no. NP608310) compared with TEM-like from our isolates are shown in Appendix E.

All 14 isolates carrying $bla_{CTX\text{-}M\text{-}1}$ group including S17, S19, S20, S21, S22, S23, S31, S38, S39, S40, S45, S49, S51, and S53 were performed DNA sequencing. All isolates carried $bla_{TEM\text{-}1}$ genes (Table 18.). MIC ranges of cefoxitin, ceftazidime, cefotaxime, and ceftriaxone of these isolates were 2 to 4, 64 to >256, 256 to >256, and 256 to >256 mg/L, respectively (Table 18.). DNA sequence analysis of the 1398 bp DNA fragments of $bla_{CTX\text{-}M\text{-}1}$ group revealed an open reading frame of 876 bp, encoding 291 amino acids. Of the 14 isolates, 13 isolates carried $bla_{CTX\text{-}M\text{-}1}$ group which showed 100% nucleotide identity to $bla_{CTX\text{-}M\text{-}55}$ and $bla_{CTX\text{-}M\text{-}57}$ (GenBank accession no. DQ885477 and DQ810789, respectively) and 100% amino acid

sequences identity to CTX-M-55 and CTX-M-57 enzyme (GenBank accession no. ABI34705 and ABG80523, respectively). CTX-M-55 shared 100% amino acid identity with CTX-M-57 which shared 99% amino acid identity with CTX-M-15 and was different from CTX-M-15 by an Ala80Val substitution (Ala77Val, Ambler numbering). Alignments for amino acid sequences of CTX-M-55 (GenBank accession no. ABI34705), and CTX-M-57 (GenBank accession no. ABG80523) compared with CTX-M-1 group from our isolates are showed in Figure 24. Only one isolate (S53) carrying $bla_{\text{CTX-M-1}}$ group showed 100% nucleotide and amino acid sequences identity to $bla_{\text{CTX-M-15}}$ and CTX-M-15 enzyme, respectively (GenBank accession no. AY458016 and NP957562, respectively). The MICs of cefoxitin, ceftazidime, cefotaxime, and ceftriaxone of S53 were 4, 64, 256, and >256 mg/L, respectively. Alignments of amino acid sequences of CTX-M-15 (GenBank accession no. NP957562) compared with CTX-M-1 group from our isolate are shown in Figure 25.

The 10 representative isolates including S19-1, S33, S299, S307, S384, S400, S412, S429, S438, and S32 were selected for $bla_{\text{CTX-M-9}}$ group sequencing. These isolates carried various combinations of *bla* genes and showed cefoxitin, ceftazidime, cefotaxime, and ceftriaxone MICs of 1 to 4, 2 to 32, 16 to 256, and 64 to >256 mg/L, respectively (Table 18.). Two isolates (S19-1 and S33) carrying $bla_{\text{CTX-M-9}}$ group together with $bla_{\text{TEM-1}}$ had MIC ranges of cefoxitin, ceftazidime, cefotaxime, and ceftriaxone to be 4, 4 to 8, 128, and 128 to 256 mg/L, respectively. DNA sequence analysis of the 1281 bp DNA fragments of $bla_{\text{CTX-M-9}}$ group revealed an open reading frame of 876 bp, encoding 291 amino acids. All of these isolates carried $bla_{\text{CTX-M-9}}$ group which showed 100% nucleotide identity to $bla_{\text{CTX-M-14}}$ and $bla_{\text{CTX-M-18}}$ (GenBank accession no. AF252622 and AF325133, respectively) and 100% amino acid sequences identity to CTX-M-14 and CTX-M-18 enzyme (GenBank accession no. AAF72530 and AAK55533, respectively). CTX-M-14 shared 100% amino acid identity with CTX-M-18. Alignments of amino acid sequences of CTX-M-14 (GenBank accession no. AAF72530) and CTX-M-18 (GenBank accession no. AAK55533) compared with CTX-M-9 group from our isolates are showed in Appendix E.

The 10 representative isolates including S23-1, S278, S285, S306, S331, S339, S383, S394, S453, and S32 were selected for $bla_{\text{CIT-like}}$ sequencing. These isolates showed MIC ranges of cefoxitin, ceftazidime, cefotaxime, and ceftriaxone to be 16 to 128, 32 to >256, 8 to 64, and 16 to 128 mg/L, respectively (Table 18.). DNA sequence analysis of the 1596 bp DNA fragments of $bla_{\text{CIT-like}}$ revealed an open reading frame of 1146 bp, encoding 381 amino acids. All of these isolates carried $bla_{\text{CIT-like}}$ which showed 100% nucleotide and amino acid sequences identity to $bla_{\text{CMY-2}}$ and CMY-2 enzyme, respectively (GenBank accession no. AB525688 and YP001101962, respectively). Alignments for amino acid sequences of CMY-2 (GenBank accession no. YP001101962) compared with CIT-like from our isolates are shown in Appendix E.

One isolate (S32) carrying $bla_{\text{CTX-M-9}}$ group together with $bla_{\text{TEM-like}}$ and $bla_{\text{CIT-like}}$ had MICs of cefoxitin, ceftazidime, cefotaxime, and ceftriaxone to be 64, 128, 128, and >256 mg/L, respectively (Table 18.). DNA sequence analysis of the DNA fragments of $bla_{\text{CTX-M-9}}$ group, $bla_{\text{TEM-like}}$, and $bla_{\text{CIT-like}}$ revealed that this isolate had $bla_{\text{CTX-M-14}}$, $bla_{\text{TEM-1}}$, and $bla_{\text{CMY-2}}$ genes, respectively.

Table 18. Sequencing analysis of entire $bla_{CTX\text{-}M\text{-}1}$ group, $bla_{CTX\text{-}M\text{-}9}$ group, bla_{TEM}, and bla_{CIT} genes

Isolates	ESBL phenotype	AmpC phenotype	MICs (mg/L)				Presence of *bla* genes				Type of *bla* gene for DNA sequencing
			Cefoxitin	Ceftazidime	Cefotaxime	Ceftriaxone	$bla_{CTX\text{-}M\text{-}9}$ group	$bla_{CTX\text{-}M\text{-}1}$ group	bla_{TEM}	bla_{CIT}	
S17	+	-	4	64	256	>256	-	+	+	-	$bla_{CTX\text{-}M\text{-}55}$ $bla_{TEM\text{-}1}$
S19	+	-	4	>256	>256	>256	-	+	+	-	$bla_{CTX\text{-}M\text{-}55}$ $bla_{TEM\text{-}1}$
S20	+	-	4	128	256	>256	-	+	+	-	$bla_{CTX\text{-}M\text{-}55}$ $bla_{TEM\text{-}1}$
S21	+	-	4	128	256	>256	-	+	+	-	$bla_{CTX\text{-}M\text{-}55}$ $bla_{TEM\text{-}1}$
S22	+	-	4	>256	>256	>256	-	+	+	-	$bla_{CTX\text{-}M\text{-}55}$ $bla_{TEM\text{-}1}$
S23	+	-	2	128	>256	>256	-	+	+	-	$bla_{CTX\text{-}M\text{-}55}$ $bla_{TEM\text{-}1}$
S31	+	-	4	256	>256	>256	-	+	+	-	$bla_{CTX\text{-}M\text{-}55}$ $bla_{TEM\text{-}1}$
S38	+	-	4	128	>256	>256	-	+	+	-	$bla_{CTX\text{-}M\text{-}55}$ $bla_{TEM\text{-}1}$
S39	+	-	4	128	>256	>256	-	+	+	-	$bla_{CTX\text{-}M\text{-}55}$ $bla_{TEM\text{-}1}$
S40	+	-	4	64	>256	>256	-	+	+	-	$bla_{CTX\text{-}M\text{-}55}$ $bla_{TEM\text{-}1}$
S45	+	-	2	128	256	>256	-	+	+	-	$bla_{CTX\text{-}M\text{-}55}$ $bla_{TEM\text{-}1}$
S49	+	-	4	128	256	256	-	+	+	-	$bla_{CTX\text{-}M\text{-}55}$ $bla_{TEM\text{-}1}$
S51	+	-	4	64	256	>256	-	+	+	-	$bla_{CTX\text{-}M\text{-}55}$ $bla_{TEM\text{-}1}$
S53	+	-	4	128	>256	>256	-	+	+	-	$bla_{CTX\text{-}M\text{-}15}$ $bla_{TEM\text{-}1}$

Table 18. Sequencing analysis of entire *bla*$_{CTX-M-1}$ group, *bla*$_{CTX-M-9}$ group, *bla*$_{TEM}$, and *bla*$_{CIT}$ genes (cont.)

Isolates	ESBL phenotype	AmpC phenotype	MICs (mg/L)				Presence of *bla* genes				Type of *bla* gene for DNA sequencing
			Cefoxitin	Ceftazidime	Cefotaxime	Ceftriaxone	*bla*$_{CTX-M-9}$ group	*bla*$_{CTX-M-1}$ group	*bla*$_{TEM}$	*bla*$_{CIT}$	
S19-1	+	-	4	8	128	128	+	-	+	-	*bla*$_{CTX-M-14}$ *bla*$_{TEM-1}$
S33	+	-	4	4	128	256	+	-	+	-	*bla*$_{CTX-M-14}$ *bla*$_{TEM-1}$
S299	+	-	4	4	16	64	+	-	-	-	*bla*$_{CTX-M-14}$
S307	+	-	4	16	256	>256	+	-	-	-	*bla*$_{CTX-M-14}$
S384	+	-	1	8	256	256	+	-	-	-	*bla*$_{CTX-M-14}$
S400	+	-	1	4	64	64	+	-	-	-	*bla*$_{CTX-M-14}$
S412	+	-	2	2	64	256	+	-	-	-	*bla*$_{CTX-M-14}$
S429	+	-	2	32	128	128	+	-	-	-	*bla*$_{CTX-M-14}$
S438	+	-	1	16	128	64	+	-	-	-	*bla*$_{CTX-M-14}$
S23-1	-	+	64	32	8	16	-	-	-	+	*bla*$_{CMY-2}$
S278	-	+	128	128	16	128	-	-	-	+	*bla*$_{CMY-2}$
S285	-	+	128	256	64	128	-	-	-	+	*bla*$_{CMY-2}$
S306	-	+	32	64	32	32	-	-	-	+	*bla*$_{CMY-2}$
S331	-	+	32	128	32	64	-	-	-	+	*bla*$_{CMY-2}$
S339	-	+	128	>256	32	128	-	-	-	+	*bla*$_{CMY-2}$
S383	-	+	32	128	64	64	-	-	-	+	*bla*$_{CMY-2}$
S394	-	+	16	32	16	16	-	-	-	+	*bla*$_{CMY-2}$
S453	-	+	32	64	16	16	-	-	-	+	*bla*$_{CMY-2}$
S32	+	+	64	128	128	>256	+	-	+	+	*bla*$_{CMY-2}$ *bla*$_{CTX-M-14}$ *bla*$_{TEM-1}$

+, positive result; -, negative resul

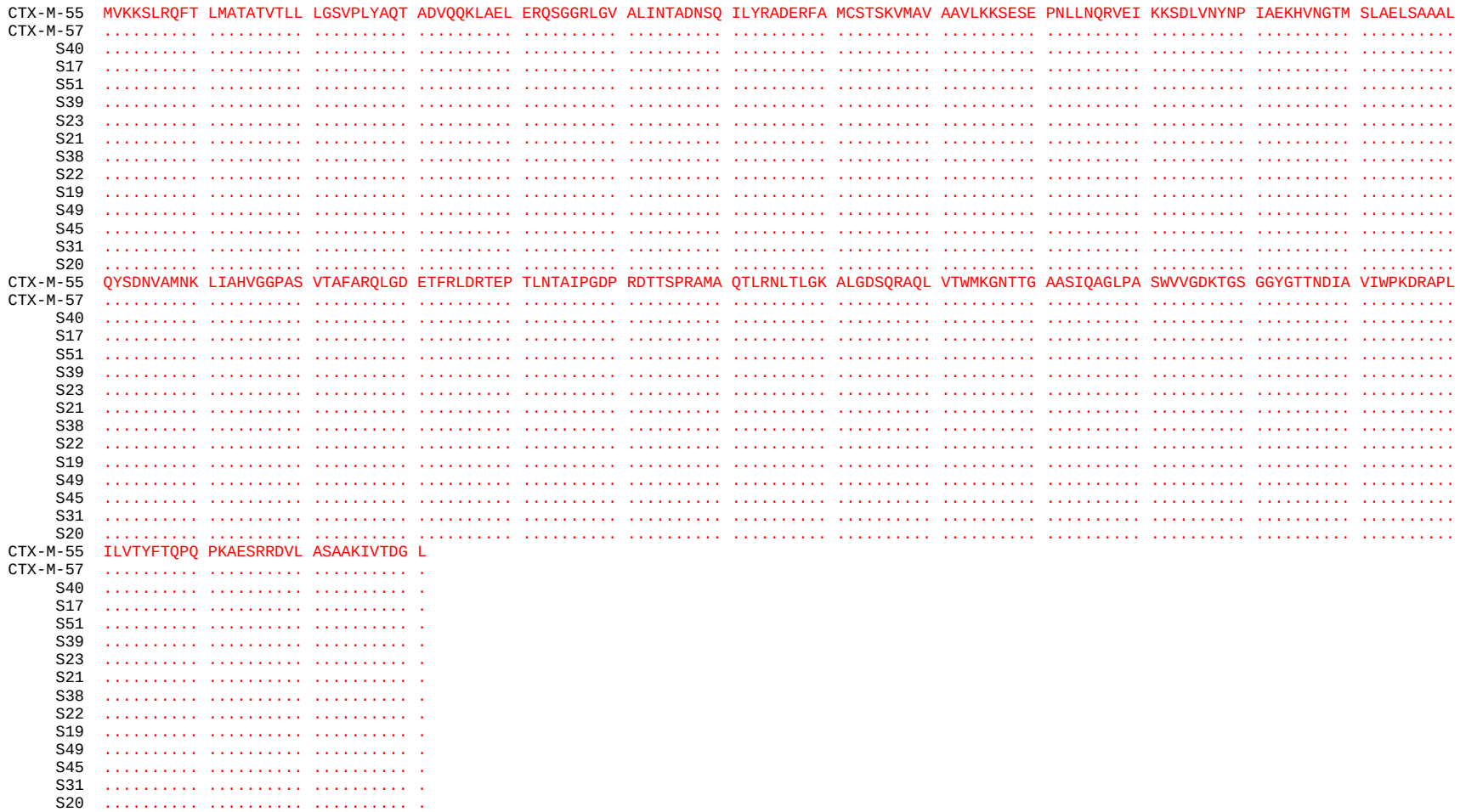

Figure 24. Alignments for amino acid sequences of CTX-M-55 (GenBank accession no. ABI34705), CTX-M-57 (GenBank accession no. ABG80523), and CTX-M-1 group from nontyphoidal *Salmonella* isolates

```
           1                                                                                                                                130
CTX-M-15   MVKKSLRQFT LMATATVTLL LGSVPLYAQT ADVQQKLAEL ERQSGGRLGV ALINTADNSQ ILYRADERFA MCSTSKVMAA AAVLKKSESE PNLLNQRVEI KKSDLVNYNP IAEKHVNGTM SLAELSAAAL
      S53  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Consensus  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

           131                                                                                                                              260
CTX-M-15   QYSDNVAMNK LIAHVGGPAS VTAFARQLGD ETFRLDRTEP TLNTAIPGDP RDTTSPRAMA QTLRNLTLGK ALGDSQRAQL VTWMKGNTTG AASIQAGLPA SWVVGDKTGS GGYGTTNDIA VIWPKDRAPL
      S53  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Consensus  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

           261                           291
CTX-M-15   ILVTYFTQPQ PKAESRRDVL ASAAKIVTDG L
      S53  .......... .......... .......... .
Consensus  .......... .......... .......... .
```

Figure 25. Alignments for amino acid sequences of CTX-M-15 (GenBank accession no. NP957562) and CTX-M-1 group from nontyphoidal *Salmonella* isolate (S53)

PART X : SCREENING AND DNA SEQUENCING OF IS*ECP1* UPSTREAM *BLA* GENES

A total of 119 isolates carrying bla_{CTX-M} and bla_{CIT} were screened for the presence of IS*Ecp1* in the upstream region of *bla* genes. IS*Ecp1* was identified in the upstream region of *bla* genes in all isolates. DNA sequences of IS*Ecp1* in the upstream region of bla_{CTX-M} and bla_{CIT} were analyzed for 34 representative isolates.

1. IS*Ecp1* upstream $bla_{CTX-M-15}$ and $bla_{CTX-M-55}$ genes

All 13 isolates carrying $bla_{CTX-M-55}$ including S17, S19, S20, S21, S22, S23, S31, S38, S39, S40, S45, S49, S51 and one isolate (S53) carrying $bla_{CTX-M-15}$ had IS*Ecp1* in the upstream region and revealed 1398 bp of PCR fragments. DNA sequences of all isolates showed that IS*Ecp1* was present upstream from $bla_{CTX-M-55}$ and $bla_{CTX-M-15}$ was 48 bp long and had IS*Ecp1*-mediated promoter sequence including -35 (TTGAAA) and -10 (TACAAT) regions (Figure 26-27.).

2. IS*Ecp1* upstream $bla_{CTX-M-14}$ gene

The 10 representative isolates carrying $bla_{CTX-M-14}$ including S19-1, S32, S33, S299, S307, S384, S400, S412, S429, and S438 had IS*Ecp1* in the upstream region and revealed 1281 bp of PCR fragments. DNA sequences of all isolates revealed that IS*Ecp1* was present upstream from $bla_{CTX-M-14}$ was 42 bp long and had IS*Ecp1*-mediated promoter sequence including -35 (TTGAAA) and -10 (TACAAT) regions (Figure 28.).

3. IS*Ecp1* upstream bla_{CMY-2} gene

The 10 representative isolates harbouring bla_{CMY-2} including S23-1, S32, S278, S285, S306, S331, S339, S383, S394, and S453 had IS*Ecp1* in the upstream region and revealed 1596 bp of PCR fragments. DNA sequences of all isolates showed that IS*Ecp1* was present upstream from bla_{CMY-2} was 116 bp long and had

IS*Ecp1*-mediated promoter sequence including -35 (TTGAAA) and -10 (TACAAT) regions (Figure 29.).

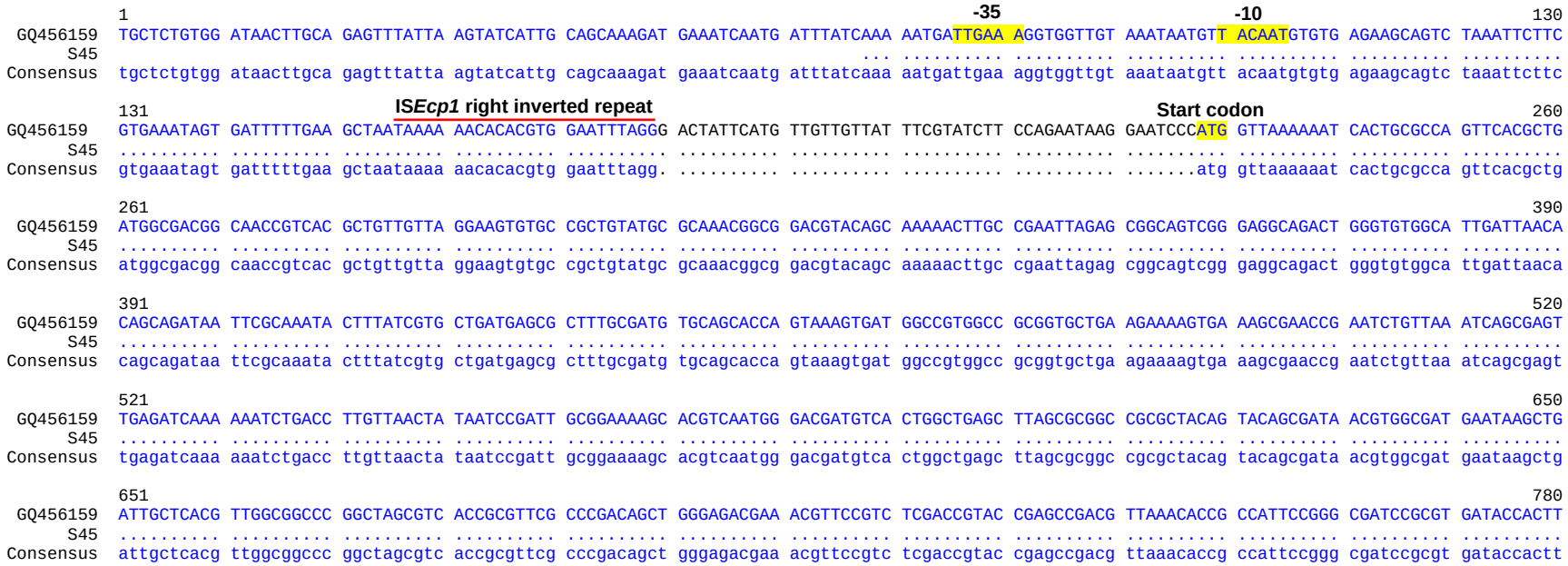

Figure 26. Comparison of upstream region of *bla*$_{CTX-M-55}$ gene of S45 and sequences in GenBank accession no. GQ456159

Figure 27. Comparison of upstream region of *bla*$_{CTX-M-15}$ gene of S53 and sequences in GenBank accession no. AY458016

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          1431                                                                                                                  1560
                                                                                                                              -35
GQ892052  TGAAAAATGT CTGGTATAAT AAGAATATCA TCAATAAAAT TGAGTGTTGC TCTGTGGATA ACTTGCAGAG TTTATTAAGT ATCATTGCAG CAAAGATGAA ATCAATGATT TATCAAAAAT GATTGAAAGG
    S299                                                                                                                   ...... ..........
Consensus .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ....aaaaat gattgaaagg

          1561                  -10                                                            ISEcp1 right inverted repeat           1690
GQ892052  TGGTTGTAAA TAATGTTACA ATGTGTGAGA AGCAGTCTAA ATTCTTCGTG AAATAGTGAT TTTTGAAGCT AATAAAAAAC ACACGTGGAA TTTAGGGAAT ACTGATGTAA CACGGATTGA CCGTATTGGG
    S299  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Consensus tggttgtaaa taatgttaca atgtgtgaga agcagtctaa attcttcgtg aaatagtgat ttttgaagct aataaaaaac acacgtggaa tttagg.... .......... .......... ..........

          1691 Start codon                                                                                                      1820
GQ892052  AGTTTGAGAT GGTGACAAAG AGAGTGCAAC GGATGATGTT CGCGGCGGCG GCGTGCATTC CGCTGCTGCT GGGCAGCGCG CCGCTTTATG CGCAGACGAG TGCGGTGCAG CAAAAGCTGG CGGCGCTGGA
    S299  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Consensus ........at ggtgacaaag agagtgcaac ggatgatgtt cgcggcggcg gcgtgcattc cgctgctgct gggcagcgcg ccgctttatg cgcagacgag tgcggtgcag caaaagctgg cggcgctgga

          1821                                                                                                                  1950
GQ892052  GAAAAGCAGC GGAGGGCGGC TGGGCGTCGC GCTCATCGAT ACCGCAGATA ATACGCAGGT GCTTTATCGC GGTGATGAAC GCTTTCCAAT GTGCAGTACC AGTAAAGTTA TGGCGGCCGC GGCGGTGCTT
    S299  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Consensus gaaaagcagc ggagggcggc tgggcgtcgc gctcatcgat accgcagata atacgcaggt gctttatcgc ggtgatgaac gctttccaat gtgcagtacc agtaaagtta tggcggccgc ggcggtgctt

          1951                                                                                                                  2080
GQ892052  AAGCAGAGTG AAACGCAAAA GCAGCTGCTT AATCAGCCTG TCGAGATCAA GCCTGCCGAT CTGGTTAACT ACAATCCGAT TGCCGAAAAA CACGTCAACG GCACAATGAC GCTGGCAGAA CTGAGCGCGG
    S299  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Consensus aagcagagtg aaacgcaaaa gcagctgctt aatcagcctg tcgagatcaa gcctgccgat ctggttaact acaatccgat tgccgaaaaa cacgtcaacg gcacaatgac gctggcagaa ctgagcgcgg

          2081                                                                                                                  2210
GQ892052  CCGCGTTGCA GTACAGCGAC AATACCGCCA TGAACAAATT GATTGCCCAG CTCGGTGGCC CGGGAGGCGT GACGGCTTTT GCCCGCGCGA TCGGCGATGA GACGTTTCGT CTGGATCGCA CTGAACCTAC
    S299  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Consensus ccgcgttgca gtacagcgac aataccgcca tgaacaaatt gattgcccag ctcggtggcc cgggaggcgt gacggctttt gcccgcgcga tcggcgatga gacgtttcgt ctggatcgca ctgaacctac
```

Figure 28. Comparison of upstream region of *bla*$_{CTX\text{-}M\text{-}14}$ gene of S299 and sequences in GenBank accession no. GQ892052

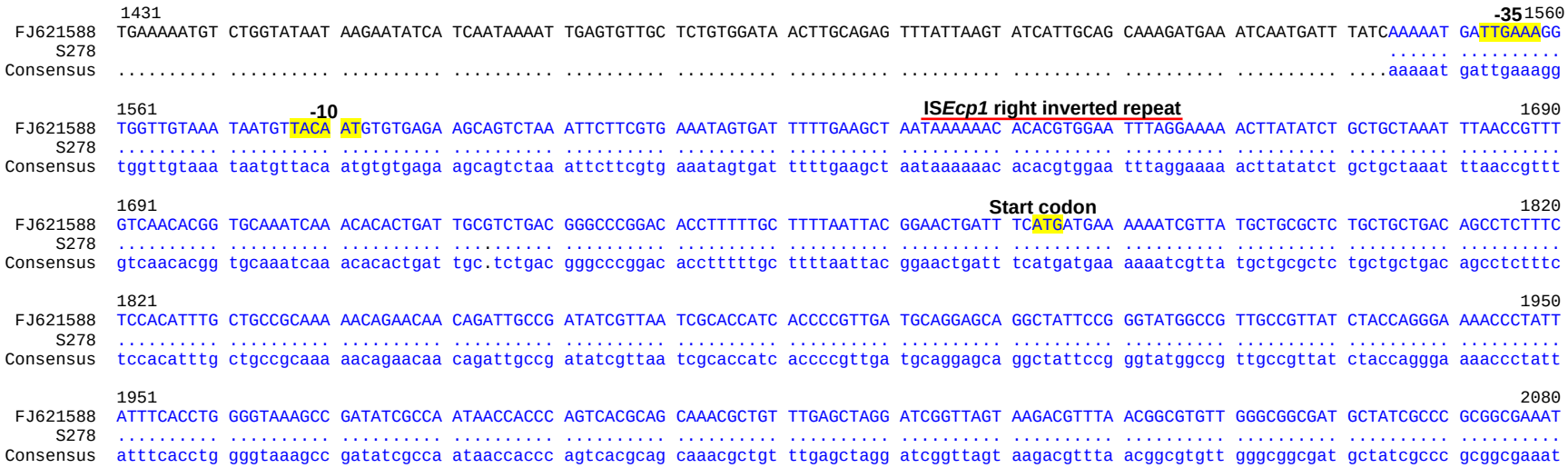

Figure 29. Comparison of upstream region of *bla*$_{CMY-2}$ gene of S278 and sequences in GenBank accession no. FJ621588

CHAPTER VI

DISCUSSION

Resistance to extended-spectrum cephalosporins in nontyphoidal *Salmonella* has become a serious therapeutic problem. A study from 17 states of the United States demonstrated that ceftriaxone resistance in nontyphoidal *Salmonella* was increasing from 0.1% in 1996 to 0.4% in 1997, 0.5% in 1998 (4) and 3.2% in 2000 (8). The study of nontyphoidal *Salmonella* isolated from 10 European countries, including Austria, Denmark, England and Wales, Germany, Ireland, Italy, Luxembourg, the Natherlands, Scotland, and Spain showed that ceftriaxone resistance were 0.7% in 2000, 0.3% in 2001, 0.4% in 2002, 0.2% in 2003, and 0.2% in 2004 (9). In Asian countries, ceftriaxone resistance in nontyphoidal *Salmonella* isolated during 1999-2003 increased from 0.8% in 1999 to 1.5% in 2003 in Taiwan (10) and resistance to ESC was increased to be 3.3% in 2004 (5). During 2003-2005, ceftriaxone resistance was reported to be 3.0% in nontyphoidal *Salmonella* collected from seven Asian countries, including Philippines, Hong Kong, Singapore, Sri Lanka, Korea, Thailand, and Taiwan (11). It has been reported that 3% and 2.3% of nontyphoidal *Salmonella* isolated from China in 2006 and from Singapore during 2003-2006 were resistant to ceftriaxone, respectively (12, 13). The prevalence of ESC resistance in nontyphoidal *Salmonella* in Asian countries, especially Taiwan has been reported to be higher than those in European and American countries.

In Thailand, the study in Siriraj Hospital during 2005 in nontyphoidal *Salmonella* isolated from blood showed that *Salmonella* group C was the most common serogroup (47%) and 37% of the patients were HIV seropositive. It was demonstrated that 17.8% of the isolates were resistant to ceftriaxone (44). Likewise, Kulwichit *et al.* reported high rate of ceftriaxone resistance in *S.* Choleraesuis isolates from bacteremic patients at King Chulalongkorn Memorial Hospital and from bacteremic patients in Thailand sent to the WHO National *Salmonella* and *Shigella* Center (45). In this study, we investigated 560 nontyphoidal *Salmonella* isolated from patients in King Chulalongkorn Memorial Hospital and from WHO National *Salmonella*

and *Shigella* Center which were collected from patients in Thailand during 2005-2007. Our results showed high prevalence of extended-spectrum cephalosporin resistance among nontyphoidal *Salmonella* isolates. The resistance rates to ceftazidime, cefotaxime and ceftriaxone were 14.64%, 9.46%, and 12.50%, respectively. In addition, the results showed high prevalence of cefoxitin resistance (11.79%). High rate of ceftriaxone resistance in *S.* Choleraesuis has been reported from Taiwan (5) and Thailand (45). In Taiwan, the prevalence of ESC resistance in *Salmonella* in 2004 were 3.3% and 17.8% of *S.* Choleraesuis isolates were resistant to ESCs (5). A study in Thailand reported that approximately 15% of *S.* Choleraesuis isolates were resistant to ceftriaxone (45). Similar to previous studies (44, 45), our results demonstrated a high rate of ceftriaxone resistance in nontyphoidal *Salmonella* and *S.* Choleraesuis was the major serotype (76.14%).

Recently, antimicrobial agents such as β-lactams have been licensed for use in the food animal industry. This provided opportunity for selection pressure in development of β-lactam resistance (167). In Thailand, ceftiofur, a third-generation cephalosporin is used extensively in swine farming for treatment and growth promotion (45, 167, 168). Moreover, *S.* Cholerasuis is relatively a host-specific in pig (169). Kulwichit *et al.* observed that *Salmonella* Choleraesuis isolates showed a higher frequency of resistance to ceftriaxone than non-*Salmonella* Choleraesuis isolates, suggesting that increasing ceftriaxone resistance in this serotype may be associated with inappropriate use of ceftiofur in swine farm (45).

Many studies demonstrated that *S.* Typhimurium and *S.* Newport were common serotypes for ceftriaxone resistance worldwide (4, 5, 7, 8, 11, 21). The National Antimicrobial Resistance Monitoring System (NARMS) in the United States demonstrated that 0.37% of nontyphoidal *Salmonella* isolates during 1996-1998 had ceftriaxone MIC of $\geq$ 16 mg/L and 80% of these isolates were *S.* Typhimurium (4). The rate of ceftriaxone resistance increased to be 3.2% in 2000 (8) which 61% and 25% of resistant isolates were *S.* Newport and *S.* Typhimurium, respectively. Yan *et al.* demonstrated that 1.6% of nontyphoidal *Salmonella* isolates from Taiwan during 1997-2000 were resistant to ceftriaxone and all resistant isolates were *S.* Typhimurium (21). The SENTRY Antimicrobial Surveillance Program showed that 2.4% of

nontyphoidal *Salmonella* isolates from European countries in 2003 were resistant to ceftriaxone and all of these isolates were *S.* Typhimurium (158). *S.* Typhimurium has a comparatively wide host range and has been reported to be the most common serotype (17%) of nonhuman isolates and second most common (12%) of human isolates from 49 countries in 6 regions including Africa, Asia, Latin America and the Caribbean, Europe, North America, and Oceania by the WHO Global *Salmonella*-Surveillance during 2000 to 2002 (170). In the United States, *S.* Typhimurium was the most common source of human salmonellosis and was in the top 5 most detected for each major food animal species including swine (36%), chicken (10.30%), and turkey (2%) (171). In Thailand, *S.* Typhimurium was in the top 10 most detected for human salmonellosis isolates during 1993-2002 (172). The NARMS in USA demonstrated that *S.* Typhimurium had high level of ceftiofur resistance (14.4%) from animal isolates during 1999-2003 (173). In addition, ESC resistance has been reported in *S.* Typhimurium isolates from animals and animal source foods in many countries (31, 174-176). In the United States, ceftiofur has been approved to use in food animals such as swine, turkey, and cattle (177). Paul *et al.* demonstrated the relationship between ceftriaxone-resistant *S.* Typhimurium isolate from human and cattles (178). This suggested that increasing ceftriaxone resistance in *S.* Typhimurium from human isolates may be associated with inappropriate use of ceftiofur in farm animals (178). Similar to previous studies, our study demonstrated a high rate of ceftriaxone resistance in *S.* Choleraesuis and *S.* Typhimurium isolates, suggesting that inappropriate use of antimicrobial agents in food animal should be concerned in Thailand to control the ceftriaxone resistance in *Salmonella* (45, 167, 173, 174, 178).

ESC resistance in nontyphoidal *Salmonella* is commonly due to the production of ESBLs and AmpC β-lactamases. In this study, we found that 119 (21.25%) isolates were resistant to ESCs (ceftazidime, or cefotaxime, or ceftriaxone). The results showed that 43.70%, 55.46%, and 0.84% were ESBL producers, non-inducible AmpC producers, and ESBL and AmpC co-producer, respectively. Most AmpC β-lactamases are derivatives of CIT-type β-lactamases, including LAT-1, CMY-2 to CMY-7, CMY-12 to CMY-18, and CMY-20 to CMY-50 (26, 27). This study revealed that the majority of ESC-resistant isolates (55.46%) carried $bla_{\text{CIT-like}}$, suggesting that the

spread of $bla_{CIT\text{-}like}$ played a major role in ESC resistance in nontyphoidal *Salmonella* in Thailand. The $bla_{CMY\text{-}2}$ was found in all 10 representative isolates by DNA sequencing of entire $bla_{CIT\text{-}like}$ gene. CMY-2 were most commonly found in nontyphoidal *Salmonella* which have been reported worldwide such as England and Wales (28), France (6), United States (8), Taiwan (5, 23), South Korea (33), China (12), and Singapore (13). This is the first report which demonstrated that CIT AmpC was the major cause of high rate ESC resistance in nontyphoidal *Salmonella* isolates in Thailand. All of our CMY-2-producing isolates were *S.* Choleraesuis. The results were in agreement with a study in Taiwan which reported that ceftriaxone resistance in *S.* Choleraesuis was due to the production of CMY-2 AmpC enzyme (5, 10). Recently, AmpC enzymes including CMY-2, CMY-4, CMY-7, ACC-1, and DHA-1 have been found in nontyphoidal *Salmonella* isolates (28-32).

Our results showed that all ESBL-producing isolates were found to produce the CTX-M type of ESBLs, including 73.58% for CTX-M-9 group and 26.42% for CTX-M-1 group. CTX-M-1 group included CTX-M-1, -3, -10, -11, -12, -15, -22, -23, -29, -30, -32, -33, -28, -36, -54, -55 and CTX-M-57 (16, 26, 179, 180). CTX-M-9 group included CTX-M-9, -13, -14, -16, -17, -18, -19, -24, -27, -45, -46, -47, -48, -49, and CTX-M-50 (16, 26, 87, 101). CTX-M-1 (6), CTX-M-3 (10), CTX-M-9 (22), CTX-M-14 (23), and CTX-M-15 (24) have been reported in nontyphoidal *Salmonella* in many countries such as Taiwan (10), France (22), China (12), Kuwait and the United Arab Emirates (24). CTX-M-2 group including CTX-M-2 (20), CTX-M-5 (21), and CTX-M-6 (91) have been reported in nontyphoidal *Salmonella*. Distribution of CTX-M in nontyphoidal *Salmonella* has been reported to be CTX-M-3, CTX-M-1, CTX-M-15, CTX-M-2, CTX-M-5, CTX-M-9, CTX-M-14 in non-Asian countries (6, 14, 21, 157) and CTX-M-3, CTX-M-15, CTX-M-14 in Asian countries (10, 14, 23, 181). In addition, CTX-M-15 and CTX-M-14 have been reported to be the major ESBLs responsible for extended-spectrum cephalosporin resistance in *Escherichia coli* and *Klebsiella pneumoniae* in Thailand (182, 183). Likewise, we found that most of ESBL producers were CTX-M-9 group β-lactamases, which all 10 representative isolates carrying $bla_{CTX\text{-}M\text{-}9}$ group isolates showed 100% identity to $bla_{CTX\text{-}M\text{-}14}$ and $bla_{CTX\text{-}M\text{-}18}$. CTX-M-14 (GenBank accession no. AAF72530) was found in the first time from *E. coli* isolates

from China (101). CTX-M-14 shared 99% amino acid identity with CTX-M-9 and differed from CTX-M-9 by only one amino acid change, at position 234 from Ala to Val (Ambler numbering: Ala231Val) (54). Furthermore, CTX-M-14 shared 100% amino acid identity with CTX-M-18 (GenBank accession no. AAK55533) which were found in *K. pneumoniae* isolate from France (87). These enzymes can hydrolyze ceftazidime poorly as most of CTX-M-type β-lactamases (59, 88, 101). Our results demonstrated that CTX-M-14 which had poorly hydrolytic activity to ceftazidime was commonly found in nontyphoidal *Salmonella* isolated from Thailand. CTX-M-14 confers resistance to all cephalosporins, but are not detectable by detection tests which are based on using only ceftazidime (101). Moreover, in the present study, most of CTX-M-14-producing isolates were *S.* Choleraesuis. However, It has been reported that CTX-M-3 was identified in ceftriaxone-resistant *S.* Choleraesuis isolates in previous study by Su *et al.* (10). CTX-M-14 has not yet been reported in *S.* Choleraesuis. Therefore, this study demonstrated for the fist time for the CTX-M-14 in *S.* Choleraesuis.

We found that CTX-M-55 was the dominant type of CTX-M-1 group and this was the first report of CTX-M-55 in nontyphoidal *Salmonella* isolates in Thailand. CTX-M-55 (GenBank accession no. ABI34705) which was reported as a novel ESBL in *E. coli* and *K. pneumoniae* clinical isolates from Thailand and shared 99% amino acid identity with CTX-M-15 and differentiated from CTX-M-15 by an Ala80Val substitution (Ambler numbering: Ala77Val) (179). Furthermore, CTX-M-55 shared 100% amino acid identity with CTX-M-57 (GenBank accession no. ABG80523) (26). CTX-M-57 was identified as a novel ESBL from *Salmonella* Typhimurium H06 058 0162 which was isolated from the faeces of a patient who had previously been admitted to hospital during a visit to Thailand in 2006 (180). This enzyme showed high level of resistance to cefotaxime, ceftriaxone and had good catalytic activity against ceftazidime similar to CTX-M-15. This suggested that this enzyme carried Asp242Gly substitution (Ambler numbering: Asp240Gly) from CTX-M-1 and CTX-M-3 (184). In this study, we found that all CTX-M-55-producing isolates were *S.* Typhimurium and were resistant to all ESCs and also to ceftazidime. A CTX-M-55 has been found in *K. pneumoniae* and *E. coli* isolates from Thailand and Korea (183, 185).

The results demonstrated that $bla_{CTX\text{-}M\text{-}1}$ group or $bla_{CTX\text{-}M\text{-}9}$ group genes were found together with $bla_{TEM\text{-}1}$. However, The $bla_{TEM\text{-}1}$ encoded TEM-1 enzymes that is able to hydrolyze penicillins and early cephalosporins such as cephalothin and cephaloridine (59, 63). TEM-1 is only broad-spectrum β-lactamase and it dose not exhibit the ESBL phenotype (59). Therefore, ESC resistance in our nontyphoidal *Salmonella* isolates was caused by the production of CTX-M enzymes. In this study, $bla_{CTX\text{-}M\text{-}55}$ and $bla_{CTX\text{-}M\text{-}15}$ were found to be co-carrying with $bla_{TEM\text{-}1}$ in all isolates. Boyd *et al.* demonstrated that both $bla_{CTX\text{-}M\text{-}15}$ and $bla_{TEM\text{-}1}$ genes were co-localized on the same plasmid (99). However, there has been not report about plasmid co-carrying $bla_{CTX\text{-}M\text{-}55}$ and $bla_{TEM\text{-}1}$.

Furthermore, we found one isolate which was ESBL and AmpC co-producer carrying $bla_{CTX\text{-}M\text{-}14}$, $bla_{TEM\text{-}1}$, and the $bla_{CMY\text{-}2}$ gene. This isolate was recovered from stool specimen. The isolate had high level of resistance to cefoxitin (MIC=64 mg/L) and ceftazidime (MIC=128 mg/L). CMY-2 has been commonly reported to be responsible for ceftriaxone resistance in nontyphoidal *Salmonella* (12, 23, 154). Moreover, this isolate showed weakly positive for ESBL confirmatory test by clavulanate, suggesting that clavulanate could not inhibit CMY-2 activity (27, 139). The combination of ESBL and AmpC enzymes has been described to be diagnostic problem for the ESBL confirmatory test (139). Yan *et al.* demonstrated that 47.4% of the isolates with co-existence of AmpC enzyme (DHA-1, CMY-2, or CMY-8) and one or two ESBLs (CTX-M and/or SHV-type ESBLs) in *K. pneumoniae* isolates could be classified as ESBL producers by the double disk synergy test (186). The ESBL and plasmid-AmpC co-producers have been reported to have high frequency in *E. coli* and *K. pneumoniae* isolates and were commonly found SHV-12 together with DHA-1(185, 187), and SHV-2a together with DHA-1 (187, 188). Others included CTX-M-15 together with DHA-1 or CMY-2 or CMY-10 and CTX-M-14 together with CMY-2 in *E. coli* and CTX-M-3 together with DHA-1 or CMY-2 or CMY-8 and CTX-M-14 together with DHA-1 in *K. pneumoniae* (185, 186). However, the co-producing ESBLs and plasmid-AmpC enzymes has been rarely reported in nontyphoidal *Salmonella*. Nancy *et al.* reported that *S.* Typhimurium isolate produced SHV-9, CMY-7, and OXA-30 (189). Shelley *et al.* described that *S.* Newport isolate produced CMY-2,

SHV-12, and TEM-1b (190). The combination of CTX-M-14 and CMY-2 was detected for the first time in nontyphoidal *Salmonella* isolates in the present study.

Nontyphoidal *Salmonella* isolates from sterile sites which most 99.73% of isolates were from blood showed that 24.27% were resistant to ESC which were significantly higher than that of 15.14% from non-sterile site isolates ($P<0.05$). The isolates from sterile sites showed high rate of resistance to ceftazidime (16.00%), ceftriaxone (13.07%), and cefoxitin (15.47%). All 91 ESC-resistant isolates from sterile sites were *S.* Choleraesuis and most 64.84% of these isolates carried $bla_{\text{CIT-like}}$ and 31.87% carried $bla_{\text{CTX-M-9}}$ group which all representative isolates showed DNA sequences to be identical with $bla_{\text{CMY-2}}$ and $bla_{\text{CTX-M-14}}$, respectively. A study in 2005 in Thailand showed that *Salmonella* serogroup C was a causative agent of nontyphoidal *Salmonella* bacteraemia in up to 58.1% of HIV infected patients that increased 6.4 fold from 1993 and these isolates showed high rate of ceftriaxone resistance (17.8%) (44). This is in agreement with a study during 2003-2005 in Thailand which demonstrated that *S.* Choleraesuis isolates from bacteremic patients showed high rate of ceftriaxone resistance (45). Jean *et al.* reported that the incidence of *S.* Choleraesuis bacteraemia was increasing from 1996 through 2004 in Taiwan (191). The ceftriaxone resistance in *S.* Choleraesuis has been reported to be high and attributed to the production of CMY-2, CTX-M-3, SHV-12, and SHV-2a (5, 10).

The isolates from non-sterile sites showed that high rate of ceftazidime and cefotaxime resistance of 11.89% and 11.35%, respectively. Of the 28 third-generation cephalosporin-resistant isolates from non-sterile sites, 71.43% were ESBL producers, 25% were AmpC producers, and 3.57% were ESBL and AmpC co-producer. Most isolates (46.43%) carried $bla_{\text{CTX-M-55}}$ together with $bla_{\text{TEM-1}}$ genes and were *S.* Typhimurium, suggesting that the production of ceftazidime-resistant CTX-M-55 ESBL was the dominant cause of ESC resistance in isolates from non-sterile sites. CTX-M-55 was first detected in *S.* Typhimurium isolates from feces but was termed CTX-M-57 (180). However, CTX-M-55 and CTX-M-57 were found to have 100% amino acid identity. Samilar to preveious studies, CTX-M-15, CTX-M-9, CTX-M-14, and CTX-M-5 have been reported in *S.* Typhimurium isolates from stool (24, 157, 192).

Our results showed that the IS*Ecp1* element was identified in the upstream regions of bla_{CTX-M} and bla_{CIT} in all isolates. Analysis of the DNA sequence upstream of $bla_{CTX-M-55}$, $bla_{CTX-M-15}$, $bla_{CTX-M-14}$, and bla_{CMY-2} gene showed IS*Ecp1*-mediated -35 and -10 promoter sequences, suggesting that IS*Ecp1*-mediated putative promoter for the expression of these *bla* genes. The IS*Ecp1*, a member of the IS1380 family was found to be related to three clusters of CTX-M including CTX-M-1, CTX-M-2, and CTX-M-9 (16, 42, 193, 194) and associated with many CMY alleles including CMY-2 (34), CMY-4 (35), CMY-5 (36), CMY-7 (29), CMY-12 (37), CMY-14 (37), CMY-15 (37), CMY-16 (38), CMY-21 (39), as well as ACC-1 (40) and ACC-4 (41). Poirel *et al.* demonstrated that IS*Ecp1* element may involve in the mobilization that generated a 5 bp duplication at the target site and provided -35 and -10 promoter sequences, contributing to the high-level expression of the $bla_{CTX-M-19}$ gene (42, 43). Nakano *et al.* described that there were 18.9-fold increased in bla_{CMY-4} expression by the putative promoter of IS*Ecp1* which was higher than that of *C. freundii*. This suggested that the putative promoter element of IS*Ecp1* played a role for the high-level expression of bla_{CMY-4} (35). It was demonstrated that IS*Ecp1*-incorporated promoter sequences contributed to the expression of these genes by providing its promoter, suggesting that it played an important role in the mobilization and expression of these genes (42, 195). However, we didn't find any isolates with no IS*Ecp1* element in the upstream region of *bla* genes. Therefor, the comparison of cephalosporin resistance level cound not be evaluate for the effect of the presence of IS*Ecp1* in this study.

The mechanism of ESC resistance are mainly due to ESBLs and plasmid-mediated AmpC-type β-lactamases (14, 62). However, a few reports have described the reduction in permeability or increased export of the drug as a resistance mechanism in *Salmonella* (196-198). Moreover, it has reported the combination AmpC-type β-lactamase productions and the loss of porin in outer membrane caused carbapenem resistance in *K. pneumoniae* isolate (199, 200).

Our results showed that most extended-spectrum cephalosporin resistant isolates (49.58%) were sterile site isolates carrying $bla_{CIT-like}$ genes. Moreover, the high rate of extended-spectrum cephalosporins resistance in nontyphoidal *Salmonella* in Thailand was attributed to the dissemination of CIT-type AmpC and CTX-M-type

β-lactamases. Thus, the CIT-type AmpC and CTX-M-type ESBLs should be taken seriously surveillance. However, other mechanisms including the loss of porin proteins and efflux pump may be involved in ESC resistance and should be further investigated.

CHAPTER VII

CONCLUSION

Third-generation or extended-spectrum cephalosporins (ESCs) are the drugs of choice in the treatment of salmonellosis in recent years. However, emergence of extended-spectrum cephalosporin resistance in nontyphoidal *Salmonella* has been reported worldwide. The production of extended-spectrum β-lactamases (ESBLs) and plasmid-mediated AmpC β-lactamases in nontyphoidal *Salmonella* are increasingly reported worldwide and pose a serious threat for Salmonellosis. A total of 560 nontyphoidal *Salmonella* isolates from King Chulalongkorn Memorial Hospital and WHO National *Salmonella* and *Shigella* Center during 2005-2007 were included in this study. These isolates included 51.61%, 36.61%, 11.43%, and 0.36% were *Salmonella* serogroup D, C, B, and E, respectively.

The results showed that the resistance rates to cefoxitin, ceftazidime, cefotaxime, and ceftriaxone were 11.79%, 14.64%, 9.46%, and 12.50%, respectively. The 21.25% of isolates were resistant to third-generation cephalosporins. The results showed that 31.71%, 32.68%, 18.05%, and 26.34% of *Salmonella* serogroup C were resistant to cefoxitin, ceftazidime, cefotaxime, and ceftriaxone, respectively. *Salmonella* serogroup B showed that 1.56%, 23.44%, 25.0%, and 25.0% were resistant to cefoxitin, ceftazidime, cefotaxime, and ceftriaxone, respectively. The results showed that *Salmonella* serogroup C and serogroup B had a high rate of cephalosporin resistance. The ESC resistance of isolates from sterile sites and from non-sterile sites were 24.27% and 15.14%, respectively. The resistance rate of nontyphoidal *Salmonella* isolates from sterile sites were significantly higher than that of from non-sterile site isolates ($P<0.05$). There was a significant difference in cefoxitin resistance rates between isolates from sterile and non-sterile site ($P < 0.01$).

Of the 119 ESC-resistant isolates, 43.70%, 55.46%, and 0.84% were ESBL producers, non-inducible AmpC producers, and ESBL and AmpC co-producer, respectively. Screening for *bla* genes by PCR revealed that $bla_{\text{CIT-like}}$ was found in 55.46%, $bla_{\text{CTX-M-9}}$ group was found in 26.05%, $bla_{\text{CTX-M-1}}$ group together with $bla_{\text{TEM-like}}$

was found in 11.76%, *bla*$_{CTX-M-9}$ group together with *bla*$_{TEM-like}$ was found in 5.88%, and *bla*$_{CTX-M-9}$ group *bla*$_{TEM-like}$ and *bla*$_{CIT-like}$ was found in 0.84%. This suggested that *bla*$_{CIT-like}$ AmpC gene played a major role in ESC resistance in nontyphoidal *Salmonella* in Thailand. DNA sequencing analysis of the entire *bla* genes from representative isolates showed that of the 14 *bla*$_{CTX-M-1}$ group, 13 were *bla*$_{CTX-M-55}$ and one was *bla*$_{CTX-M-15}$ whereas all 10 *bla*$_{CTX-M-9}$ group, 22 *bla*$_{TEM-like}$, 10 *bla*$_{CIT-like}$ were *bla*$_{CTX-M-14}$, *bla*$_{TEM-1}$, and *bla*$_{CMY-2}$, respectively. A combination of *bla*$_{CMY-2}$, *bla*$_{CTX-M-14}$, and *bla*$_{TEM-1}$ was detected in one isolate. This combinationtion was identified for the first time in nontyphoidal *Salmonella*.

The IS*Ecp1* element was identified in the upstream regions of *bla*$_{CTX-M}$ and *bla*$_{CIT}$ in all isolates. DNA sequences upstream from *bla*$_{CTX-M-55}$, *bla*$_{CTX-M-15}$, *bla*$_{CTX-M-14}$, and *bla*$_{CMY-2}$ gene showed IS*Ecp1*-mediated -35 and -10 promoter sequences in all representative isolates, suggesting that IS*Ecp1* played an important role in the mobilization and expression of these genes.

This is the first report of the prevalence of ESBLs and plasmid-mediated AmpC β-lactamases in nontyphoidal *Salmonella* isolated in Thailand. Our results showed that the high rate of extended-spectrum cephalosporin resistance in nontyphoidal *Salmonella* was attributed to the production CTX-M-type ESBL and plasmid-mediated AmpC. CTX-M-9 group was the most common ESBLs and CIT-type was the most frequent AmpC β-lactamases. This was the first report of CTX-M-14 in *S.* Choleraesuis and also the first report of co-carrying CMY-2, CTX-M-14, and TEM-1 in nontyphoidal *Salmonella* isolate.

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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)
LB broth	(Pronadisa, Spain)
NaOH	(Sigma, USA)

Instruments

Automatic pipette	(Gilson, Lyon, France)
Camera Gel Doc™ MZL	(BIO-RAD, USA)
Incubator	(Forma Scientific, USA)
Perkin Elmer GeneAmp PCR 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 with frequent agitation until complete dissolution. Adjust final volume to 1,000 ml. Sterilize at 121°C (15 lbs. sp) for 15 minutes. Once the medium is prepared, store at 4°C.

2. Tryptic soy broth (BBL, USA)

Suspend 30 grams in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 121°C for 15 minutes. Once the medium is prepared, store at 4°C.

3. LB broth (Pronadisa, Spain)

Suspend 20 grams of the dehydrated medium in 900 ml of distilled water. Dissolve by heating with frequent agitation until complete dissolution. Adjust final volume to 1,000 ml. Sterilize at 121°C (15 lbs. sp) for 15 minutes. Once the medium is prepared, store at 4°C.

4. Trytic soy agar (BBL, USA)

Suspend 40 grams in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 121°C for 15 minutes. Once the medium is prepared, store at 4°C.

5. Sterile 0.85% NaCl (Merck, Germany)

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

6. Antibiotic solution preparation

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/L
Boric acid	55 g/L
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 121°C for 15 min.

2. 0.5 M EDTA (pH 8.0)

Disodium ethylene diamine tetra-acelate $2H_2O$	186.1 g/L
Distilled water	1 L

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/L
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 121°C 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 100 ml

Tris HCl	0.6 g
EDTA	1.68 g
SDS	0.5 g
Bromphenol Blue	0.1 g
Sucrose	40 g

Adjust volume to 100 ml with distilled water. Mix the solution, aliquot into 1.5 microtubes and store at 4°C.

6. Reagent for DNA extraction

6.1 Protease K

Reconstituted of protease K (lyophilized) with 1.25 ml protease solvent, stored at -20°C

6.2 Buffer AL (Ready to used)

6.3 Buffer AW1

Buffer AW1 is supplied as a concentrate. Before using for the first time, add the 25 ml of ethanol (96-100%) to buffer AW1 concentrate as indicated on the bottle.

6.4 Buffer AW2

Buffer AW2 is supplied as a concentrate. Before using for the first time, add the 30 ml of ethanol (96-100%) to buffer AW2 concentrate as indicated on the bottle.

6.5 Buffer AE (Ready to used)

7. Reagent for PCR product purification

7.1 Buffer PB (Ready to used)

7.2 Buffer PE

Buffer PE is supplied as a concentrate. Before using for the first time, add the 55 ml of ethanol (96-100%) to buffer PE concentrate as indicated on the bottle.

APPENDIX D

THE RESULTS OF ALL TESTS IN THIS STUDY

Results of cephalosporin susceptibility, ESBL phenotype, AmpC phenotype, and the presence of *bla* genes of 560 nontyphoidal *Salmonella* isolates

No.	Isolate	MICs (mg/L)				ESBL phenotype	AmpC phenotype	The presence of *bla* genes			
		FOX	CAZ	CTX	CRO			$bla_{\text{CTX-M-1}}$ group	$bla_{\text{CTX-M-9}}$ group	$bla_{\text{TEM-like}}$	$bla_{\text{CIT-like}}$
1	S1-1	4	1	0.06	0.03	-	-	-	-	-	-
2	S2-1	2	0.5	0.06	0.06	-	-	-	-	-	-
3	S3-1	2	1	0.125	0.06	-	-	-	-	-	-
4	S4-1	2	0.5	0.125	0.06	-	-	-	-	-	-
5	S5-1	4	1	0.125	0.03	-	-	-	-	-	-
6	S6-1	2	1	0.25	0.06	-	-	-	-	-	-
7	S7-1	4	1	0.5	0.125	-	-	-	-	-	-
8	S8-1	4	1	0.125	0.06	-	-	-	-	-	-
9	S9-1	4	1	0.125	0.06	-	-	-	-	-	-
10	S10-1	4	0.5	0.125	0.06	-	-	-	-	-	-
11	S11-1	4	0.5	0.125	0.125	-	-	-	-	-	-
12	S12-1	8	0.5	0.5	0.25	-	-	-	-	-	-
13	S13-1	4	1	0.125	0.06	-	-	-	-	-	-
14	S14-1	4	1	0.125	0.125	-	-	-	-	-	-
15	S15-1	4	1	0.125	0.06	-	-	-	-	-	-
16	S16-1	4	0.5	0.125	0.06	-	-	-	-	-	-
17	S17-1	2	8	128	256	1	-	0	1	1	-
18	S18-1	4	1	0.125	0.06	-	-	-	-	-	-
19	S19-1	4	8	128	128	1	-	0	1	1	-
20	S20-1	4	1	0.125	0.125	-	-	-	-	-	-
21	S21-1	4	0.5	0.125	0.03	-	-	-	-	-	-
22	S22-1	4	1	0.125	0.06	-	-	-	-	-	-
23	S23-1	64	32	8	16	0	1	-	-	0	1
24	S24-1	4	1	0.125	0.125	-	-	-	-	-	-
25	S25-1	4	0.5	0.25	0.06	-	-	-	-	-	-
26	S26-1	2	1	0.125	0.06	-	-	-	-	-	-
27	S27-1	4	1	0.25	0.06	-	-	-	-	-	-
28	S28-1	2	0.5	0.125	0.06	-	-	-	-	-	-
29	S29-1	2	8	128	128	1	-	0	1	1	-
30	S30-1	2	0.5	0.125	0.125	-	-	-	-	-	-
31	S31-1	32	64	8	32	0	1	-	-	-	1

No.	Isolate	MICs (mg/L)				ESBL phenotype	AmpC phenotype	The presence of *bla* genes			
		FOX	CAZ	CTX	CRO			$bla_{CTX\text{-}M\text{-}1}$ group	$bla_{CTX\text{-}M\text{-}9}$ group	$bla_{TEM\text{-}like}$	$bla_{CIT\text{-}like}$
32	S32-1	4	1	0.25	0.06	-	-	-	-	-	-
33	S33-1	4	1	0.25	0.06	-	-	-	-	-	-
34	S34-1	4	1	0.125	0.06	-	-	-	-	-	-
35	S35-1	2	0.5	0.125	0.125	-	-	-	-	-	-
36	S36-1	1	0.5	0.06	0.125	-	-	-	-	-	-
37	S37-1	8	1	0.5	0.25	-	-	-	-	-	-
38	S38-1	8	1	0.5	0.25	-	-	-	-	-	-
39	S39-1	4	1	0.125	0.125	-	-	-	-	-	-
40	S40-1	2	1	0.125	0.125	-	-	-	-	-	-
41	S41-1	2	1	0.125	0.125	-	-	-	-	-	-
42	S42-1	2	1	0.125	0.125	-	-	-	-	-	-
43	S43-1	2	1	0.25	0.06	-	-	-	-	-	-
44	S44-1	2	0.5	0.125	0.06	-	-	-	-	-	-
45	S45-1	4	0.5	0.25	0.03	-	-	-	-	-	-
46	S46-1	2	0.5	0.25	0.06	-	-	-	-	-	-
47	S47-1	2	1	0.125	0.06	-	-	-	-	-	-
48	S48-1	2	0.5	0.125	0.06	-	-	-	-	-	-
49	S49-1	4	1	0.25	0.06	-	-	-	-	-	-
50	S50-1	2	0.5	0.06	0.03	-	-	-	-	-	-
51	S51-1	2	0.5	0.06	0.03	-	-	-	-	-	-
52	S52-1	2	0.5	0.03	0.015	-	-	-	-	-	-
53	S53-1	0.5	0.25	0.015	0.015	-	-	-	-	-	-
54	S54-1	2	0.25	0.125	0.06	-	-	-	-	-	-
55	S55-1	1	0.25	0.03	0.015	-	-	-	-	-	-
56	S56-1	2	1	0.125	0.06	-	-	-	-	-	-
57	S57-1	4	1	0.25	0.125	-	-	-	-	-	-
58	S58-1	1	0.25	0.03	0.015	-	-	-	-	-	-
59	S59-1	1	0.25	0.03	0.06	-	-	-	-	-	-
60	S60-1	2	0.25	0.125	0.03	-	-	-	-	-	-
61	S61-1	1	0.25	0.03	0.015	-	-	-	-	-	-
62	S62-1	1	0.25	0.06	0.03	-	-	-	-	-	-
63	S63-1	2	0.5	0.125	0.06	-	-	-	-	-	-
64	S64-1	2	0.5	0.06	0.03	-	-	-	-	-	-
65	S65-1	8	1	0.25	0.25	-	-	-	-	-	-
66	S66-1	4	0.5	0.25	0.06	-	-	-	-	-	-
67	S67-1	2	0.25	0.03	0.03	-	-	-	-	-	-
68	S68-1	2	0.5	0.125	0.125	-	-	-	-	-	-
69	S69-1	4	0.5	0.015	0.015	-	-	-	-	-	-

No.	Isolate	MICs (mg/L)				ESBL phenotype	AmpC phenotype	The presence of *bla* genes			
		FOX	CAZ	CTX	CRO			$bla_{CTX\text{-}M\text{-}1}$ group	$bla_{CTX\text{-}M\text{-}9}$ group	$bla_{TEM\text{-}like}$	$bla_{CIT\text{-}like}$
70	S70-1	4	0.5	0.125	0.03	-	-	-	-	-	-
71	S71-1	4	0.5	0.03	0.015	-	-	-	-	-	-
72	S72-1	2	0.5	0.125	0.06	-	-	-	-	-	-
73	S73-1	4	0.5	0.125	0.06	-	-	-	-	-	-
74	S74-1	4	1	0.125	0.25	-	-	-	-	-	-
75	S75-1	4	0.5	0.125	0.06	-	-	-	-	-	-
76	S76-1	4	1	0.06	0.06	-	-	-	-	-	-
77	S77-1	2	0.25	0.06	0.06	-	-	-	-	-	-
78	S78-1	4	1	1	0.25	-	-	-	-	-	-
79	S79-1	4	0.5	0.125	0.06	-	-	-	-	-	-
80	S80-1	8	1	0.125	0.25	-	-	-	-	-	-
81	S81-1	2	1	0.125	0.06	-	-	-	-	-	-
82	S82-1	2	0.5	0.06	0.03	-	-	-	-	-	-
83	S83-1	2	0.5	0.125	0.125	-	-	-	-	-	-
84	S84-1	2	1	0.125	0.06	-	-	-	-	-	-
85	S85-1	32	64	8	32	0	1	-	-	-	1
86	S86-1	4	0.5	0.06	0.06	-	-	-	-	-	-
87	S87-1	4	1	0.25	0.125	-	-	-	-	-	-
88	S88-1	8	1	0.25	0.5	-	-	-	-	-	-
89	S89-1	8	1	0.125	0.125	-	-	-	-	-	-
90	S90-1	2	0.5	0.06	0.06	-	-	-	-	-	-
91	S91-1	8	1	0.25	0.125	-	-	-	-	-	-
92	S92-1	2	0.5	0.125	0.06	-	-	-	-	-	-
93	S93-1	4	1	0.06	0.06	-	-	-	-	-	-
94	S94-1	4	1	0.125	0.06	-	-	-	-	-	-
95	S95-1	8	1	0.125	0.06	-	-	-	-	-	-
96	S96-1	8	1	0.125	0.06	-	-	-	-	-	-
97	S97-1	8	0.5	0.25	0.06	-	-	-	-	-	-
98	S98-1	4	1	0.25	0.25	-	-	-	-	-	-
99	S99-1	4	1	0.25	0.125	-	-	-	-	-	-
100	S100-1	8	1	0.25	0.125	-	-	-	-	-	-
101	S101-1	4	1	0.03	0.25	-	-	-	-	-	-
102	S102-1	8	1	0.06	0.03	-	-	-	-	-	-
103	S103-1	8	1	0.03	0.06	-	-	-	-	-	-
104	S104-1	8	1	0.06	0.125	-	-	-	-	-	-
105	S105-1	2	0.5	0.125	0.125	-	-	-	-	-	-
106	S106-1	8	1	0.125	0.06	-	-	-	-	-	-
107	S107-1	8	1	0.125	0.06	-	-	-	-	-	-

No.	Isolate	MICs (mg/L)				ESBL phenotype	AmpC phenotype	The presence of *bla* genes			
		FOX	CAZ	CTX	CRO			$bla_{CTX-M-1}$ group	$bla_{CTX-M-9}$ group	$bla_{TEM-like}$	$bla_{CIT-like}$
108	S108-1	8	0.5	0.25	0.06	-	-	-	-	-	-
109	S1	4	1	0.125	0.25	-	-	-	-	-	-
110	S2	2	0.25	0.125	0.06	-	-	-	-	-	-
111	S3	4	0.25	0.125	0.125	-	-	-	-	-	-
112	S4	4	0.5	0.125	0.125	-	-	-	-	-	-
113	S5	4	0.5	0.125	0.25	-	-	-	-	-	-
114	S6	4	0.5	0.125	0.125	-	-	-	-	-	-
115	S7	4	0.5	0.125	0.125	-	-	-	-	-	-
116	S8	4	0.5	0.125	0.125	-	-	-	-	-	-
117	S9	2	1	0.125	0.125	-	-	-	-	-	-
118	S10	4	0.5	0.125	0.25	-	-	-	-	-	-
119	S11	2	0.5	0.125	0.06	-	-	-	-	-	-
120	S12	4	0.5	0.125	0.125	-	-	-	-	-	-
121	S13	4	0.5	0.125	0.125	-	-	-	-	-	-
122	S14	2	0.25	0.125	0.5	-	-	-	-	-	-
123	S15	4	0.25	0.125	0.06	-	-	-	-	-	-
124	S16	2	0.25	0.125	0.06	-	-	-	-	-	-
125	S17	4	64	256	>256	1	-	1	0	1	-
126	S18	4	0.5	0.125	0.25	-	-	-	-	-	-
127	S19	4	>256	>256	>256	1	-	1	0	1	-
128	S20	4	128	256	>256	1	-	1	0	1	-
129	S21	4	128	256	>256	1	-	1	0	1	-
130	S22	4	>256	>256	>256	1	-	1	0	1	-
131	S23	2	128	>256	>256	1	-	1	0	1	-
132	S24	4	0.5	0.125	0.125	-	-	-	-	-	-
133	S25	2	0.5	0.125	0.125	-	-	-	-	-	-
134	S27	8	1	0.5	0.25	-	-	-	-	-	-
135	S28	4	0.5	0.125	0.125	-	-	-	-	-	-
136	S29	4	0.5	0.125	0.5	-	-	-	-	-	-
137	S30	4	0.5	0.125	0.06	-	-	-	-	-	-
138	S31	4	256	>256	>256	1	-	1	0	1	-
139	S32	64	128	128	>256	1	1	0	1	1	1
140	S33	4	4	128	256	1	-	0	1	1	-
141	S34	8	1	0.125	0.06	-	-	-	-	-	-
142	S35	4	0.5	0.125	0.06	-	-	-	-	-	-
143	S36	4	0.5	0.125	0.125	-	-	-	-	-	-
144	S37	4	0.5	0.125	0.5	-	-	-	-	-	-
145	S38	4	128	>256	>256	1	-	1	0	1	

No.	Isolate	MICs (mg/L)				ESBL phenotype	AmpC phenotype	The presence of *bla* genes			
		FOX	CAZ	CTX	CRO			$bla_{CTX\text{-}M\text{-}1}$ group	$bla_{CTX\text{-}M\text{-}9}$ group	$bla_{TEM\text{-}like}$	$bla_{CIT\text{-}like}$
146	S39	4	128	>256	>256	1	-	1	0	1	-
147	S40	4	64	>256	>256	1	-	1	0	1	-
148	S41	4	0.5	0.125	0.125	-	-	-	-	-	-
149	S42	4	0.5	0.125	0.125	-	-	-	-	1	-
150	S43	4	0.5	0.125	0.5	-	-	-	-	-	-
151	S44	4	0.5	0.125	0.125	-	-	-	-	-	-
152	S45	2	128	256	>256	1	-	1	0	1	-
153	S46	2	1	0.125	0.5	-	-	-	-	-	-
154	S47	8	0.5	0.125	0.125	-	-	-	-	-	-
155	S48	4	0.5	0.25	0.125	-	-	-	-	-	-
156	S49	4	128	256	256	1	-	1	0	1	-
157	S50	4	0.5	0.125	0.06	-	-	-	-	-	-
158	S51	4	64	256	>256	1	-	1	0	1	-
159	S53	4	128	>256	>256	1	-	1	0	1	-
160	S54	2	0.5	0.125	0.25	-	-	-	-	-	-
161	S55	4	0.5	0.125	0.25	-	-	-	-	-	-
162	S56	4	0.5	0.125	0.25	-	-	-	-	-	-
163	S57	1	0.5	0.06	0.06	-	-	-	-	-	-
164	S58	2	0.5	0.125	0.125	-	-	-	-	-	-
165	S59	2	0.5	0.125	0.125	-	-	-	-	-	-
166	S60	4	0.5	0.125	0.125	-	-	-	-	-	-
167	S61	8	1	0.5	0.25	-	-	-	-	-	-
168	S62	2	0.5	0.125	0.125	-	-	-	-	-	-
169	S63	4	0.5	0.125	0.125	-	-	-	-	-	-
170	S64	4	0.5	0.125	0.06	-	-	-	-	-	-
171	S65	2	0.25	0.125	0.06	-	-	-	-	-	-
172	S66	4	0.5	0.125	0.125	-	-	-	-	-	-
173	S67	2	0.5	0.125	0.125	-	-	-	-	-	-
174	S68	4	0.5	0.125	0.125	-	-	-	-	-	-
175	S69	4	0.5	0.125	0.125	-	-	-	-	-	-
176	S70	2	0.5	0.125	0.06	-	-	-	-	-	-
177	S71	2	0.5	0.125	0.125	-	-	-	-	-	-
178	S72	4	0.5	0.125	0.125	-	-	-	-	-	-
179	S73	2	0.5	0.125	0.125	-	-	-	-	-	-
180	S74	2	0.5	0.06	0.125	-	-	-	-	-	-
181	S75	4	0.5	0.125	0.125	-	-	-	-	-	-
182	S76	2	0.5	0.125	0.125	-	-	-	-	-	-
183	S77	2	0.5	0.125	0.125	-	-	-	-	-	-

No.	Isolate	MICs (mg/L)				ESBL phenotype	AmpC phenotype	The presence of *bla* genes			
		FOX	CAZ	CTX	CRO			$bla_{CTX\text{-}M\text{-}1}$ group	$bla_{CTX\text{-}M\text{-}9}$ group	$bla_{TEM\text{-}like}$	$bla_{CIT\text{-}like}$
184	S78	2	0.5	0.125	0.06	-	-	-	-	-	-
185	S79	2	0.5	0.06	0.125	-	-	-	-	-	-
186	S80	4	0.5	0.5	0.125	-	-	-	-	-	-
187	S81	2	0.5	0.06	0.125	-	-	-	-	-	-
188	S82	2	0.25	0.125	0.06	-	-	-	-	-	-
189	S83	4	0.5	0.125	0.125	-	-	-	-	-	-
190	S84	4	0.5	0.125	0.125	-	-	-	-	-	-
191	S85	4	0.5	0.25	0.125	-	-	-	-	-	-
192	S86	4	0.5	0.125	0.125	-	-	-	-	-	-
193	S87	2	0.25	0.125	0.125	-	-	-	-	-	-
194	S88	2	0.25	0.125	0.125	-	-	-	-	-	-
195	S89	4	0.5	0.125	0.125	-	-	-	-	-	-
196	S90	4	0.5	0.25	0.125	-	-	-	-	-	-
197	S91	4	0.5	0.125	0.125	-	-	-	-	-	-
198	S92	2	0.5	0.125	0.125	-	-	-	-	-	-
199	S93	2	0.25	0.125	0.06	-	-	-	-	-	-
200	S94	4	0.5	0.25	0.06	-	-	-	-	-	-
201	S95	2	0.5	0.125	0.06	-	-	-	-	-	-
202	S96	2	0.5	0.125	0.125	-	-	-	-	-	-
203	S97	2	0.25	0.125	0.06	-	-	-	-	-	-
204	S98	2	0.5	0.125	0.06	-	-	-	-	-	-
205	S99	4	0.5	0.125	0.06	-	-	-	-	-	-
206	S100	4	0.5	0.125	0.125	-	-	-	-	-	-
207	S101	4	0.5	0.25	0.125	-	-	-	-	-	-
208	S102	2	0.5	0.125	0.125	-	-	-	-	-	-
209	S103	4	0.5	0.25	0.125	-	-	-	-	-	-
210	S104	2	0.5	0.125	0.125	-	-	-	-	-	-
211	S105	2	0.5	0.25	0.125	-	-	-	-	-	-
212	S106	2	0.5	0.125	0.06	-	-	-	-	-	-
213	S107	2	0.5	0.125	0.06	-	-	-	-	-	-
214	S108	2	0.5	0.125	0.125	-	-	-	-	-	-
215	S109	4	0.5	0.125	0.125	-	-	-	-	-	-
216	S110	4	0.5	0.125	0.06	-	-	-	-	-	-
217	S111	2	0.5	0.25	0.125	-	-	-	-	-	-
218	S112	2	0.5	0.125	0.06	-	-	-	-	-	-
219	S113	2	0.5	0.125	0.06	-	-	-	-	-	-
220	S114	4	0.5	0.125	0.125	-	-	-	-	-	-
221	S115	4	0.5	0.125	0.06	-	-	-	-	-	-

No.	Isolate	MICs (mg/L)				ESBL phenotype	AmpC phenotype	The presence of *bla* genes			
		FOX	CAZ	CTX	CRO			$bla_{CTX\text{-}M\text{-}1}$ group	$bla_{CTX\text{-}M\text{-}9}$ group	$bla_{TEM\text{-}like}$	$bla_{CIT\text{-}like}$
222	S116	4	0.5	0.125	0.125	-	-	-	-	-	-
223	S117	2	0.5	0.125	0.06	-	-	-	-	-	-
224	S118	2	0.5	0.125	0.06	-	-	-	-	-	-
225	S119	2	0.5	0.125	0.125	-	-	-	-	-	-
226	S120	4	0.5	0.125	0.06	-	-	-	-	-	-
227	S121	2	0.25	0.125	0.03	-	-	-	-	-	-
228	S122	2	0.5	0.125	0.06	-	-	-	-	-	-
229	S123	2	0.5	0.125	0.125	-	-	-	-	-	-
230	S124	4	0.5	0.125	0.06	-	-	-	-	-	-
231	S125	2	0.5	0.125	0.06	-	-	-	-	-	-
232	S126	2	0.5	0.125	0.06	-	-	-	-	-	-
233	S127	4	0.5	0.125	0.06	-	-	-	-	-	-
234	S128	8	1	0.5	0.125	-	-	-	-	-	-
235	S129	2	0.5	0.125	0.125	-	-	-	-	-	-
236	S130	2	0.5	0.125	0.125	-	-	-	-	-	-
237	S131	2	0.25	0.125	0.06	-	-	-	-	-	-
238	S132	2	0.5	0.125	0.06	-	-	-	-	-	-
239	S133	2	0.5	0.125	0.06	-	-	-	-	-	-
240	S134	2	0.5	0.125	0.06	-	-	-	-	-	-
241	S135	4	0.5	0.125	0.06	-	-	-	-	-	-
242	S136	4	0.25	0.125	0.03	-	-	-	-	-	-
243	S137	2	0.5	0.125	0.06	-	-	-	-	-	-
244	S138	2	0.5	0.125	0.06	-	-	-	-	-	-
245	S139	2	0.5	0.125	0.06	-	-	-	-	-	-
246	S140	4	0.5	0.125	0.06	-	-	-	-	-	-
247	S141	4	0.5	0.125	0.06	-	-	-	-	-	-
248	S142	2	0.25	0.125	0.06	-	-	-	-	-	-
249	S143	2	0.25	0.06	0.06	-	-	-	-	-	-
250	S144	4	0.5	0.125	0.06	-	-	-	-	-	-
251	S145	4	0.5	0.125	0.06	-	-	-	-	-	-
252	S146	4	0.25	0.125	0.06	-	-	-	-	-	-
253	S147	2	0.5	0.06	0.06	-	-	-	-	-	-
254	S148	2	0.25	0.125	0.06	-	-	-	-	-	-
255	S149	4	0.5	0.125	0.06	-	-	-	-	-	-
256	S150	2	0.5	0.125	0.06	-	-	-	-	-	-
257	S151	4	0.5	0.125	0.06	-	-	-	-	-	-
258	S152	4	0.5	0.125	0.06	-	-	-	-	-	-
259	S153	2	0.25	0.06	0.06	-	-	-	-	-	-

No.	Isolate	MICs (mg/L)				ESBL phenotype	AmpC phenotype	The presence of *bla* genes			
		FOX	CAZ	CTX	CRO			$bla_{CTX\text{-}M\text{-}1}$ group	$bla_{CTX\text{-}M\text{-}9}$ group	$bla_{TEM\text{-}like}$	$bla_{CIT\text{-}like}$
260	S154	2	0.25	0.125	0.06	-	-	-	-	-	-
261	S155	2	0.5	0.125	0.06	-	-	-	-	-	-
262	S156	2	0.5	0.125	0.06	-	-	-	-	-	-
263	S157	2	0.5	0.125	0.06	-	-	-	-	-	-
264	S158	2	0.25	0.06	0.06	-	-	-	-	-	-
265	S159	2	0.25	0.125	0.06	-	-	-	-	-	-
266	S160	2	0.5	0.125	0.06	-	-	-	-	-	-
267	S161	2	0.125	0.125	0.06	-	-	-	-	-	-
268	S162	2	0.25	0.06	0.06	-	-	-	-	-	-
269	S163	2	0.25	0.06	0.125	-	-	-	-	-	-
270	S164	4	0.5	0.125	0.125	-	-	-	-	-	-
271	S165	2	0.5	0.125	0.125	-	-	-	-	-	-
272	S166	2	0.5	0.125	0.06	-	-	-	-	-	-
273	S167	2	0.5	0.125	0.06	-	-	-	-	-	-
274	S168	4	0.5	0.06	0.125	-	-	-	-	-	-
275	S169	4	0.25	0.125	0.125	-	-	-	-	-	-
276	S170	4	0.5	0.125	0.06	-	-	-	-	-	-
277	S171	4	0.5	0.125	0.125	-	-	-	-	-	-
278	S172	2	0.5	0.125	0.06	-	-	-	-	-	-
279	S173	2	0.25	0.125	0.06	-	-	-	-	-	-
280	S174	16	0.5	0.25	0.25	-	-	-	-	-	-
281	S175	2	0.25	0.125	0.125	-	-	-	-	-	-
282	S176	8	1	0.25	0.125	-	-	-	-	-	-
283	S177	4	0.5	0.125	0.125	-	-	-	-	-	-
284	S178	4	0.5	0.125	0.125	-	-	-	-	-	-
285	S179	4	0.5	0.125	0.125	-	-	-	-	-	-
286	S180	2	0.5	0.125	0.125	-	-	-	-	-	-
287	S181	2	0.5	0.125	0.125	-	-	-	-	-	-
288	S182	2	0.25	0.125	0.125	-	-	-	-	-	-
289	S183	4	0.5	0.125	0.125	-	-	-	-	-	-
290	S184	4	0.5	0.125	0.06	-	-	-	-	-	-
291	S185	4	0.5	0.125	0.06	-	-	-	-	-	-
292	S186	4	0.5	0.125	0.06	-	-	-	-	-	-
293	S187	4	0.25	0.125	0.125	-	-	-	-	-	-
294	S188	2	0.25	0.125	0.06	-	-	-	-	-	-
295	S189	4	0.5	0.125	0.06	-	-	-	-	-	-
296	S190	4	0.5	0.125	0.125	-	-	-	-	-	-
297	S191	4	0.125	0.125	0.06	-	-	-	-	-	-

No.	Isolate	MICs (mg/L)				ESBL phenotype	AmpC phenotype	The presence of *bla* genes			
		FOX	CAZ	CTX	CRO			$bla_{CTX\text{-}M\text{-}1}$ group	$bla_{CTX\text{-}M\text{-}9}$ group	$bla_{TEM\text{-}like}$	$bla_{CIT\text{-}like}$
298	S192	4	0.5	0.125	0.06	-	-	-	-	-	-
299	S193	4	0.25	0.125	0.125	-	-	-	-	-	-
300	S194	2	0.5	0.25	0.125	-	-	-	-	-	-
301	S195	2	0.5	0.125	0.06	-	-	-	-	-	-
302	S196	4	0.5	0.125	0.125	-	-	-	-	-	-
303	S197	4	0.5	0.125	0.06	-	-	-	-	-	-
304	S198	4	0.5	0.125	0.125	-	-	-	-	-	-
305	S199	4	0.25	0.125	0.125	-	-	-	-	-	-
306	S200	2	0.25	0.125	0.06	-	-	-	-	-	-
307	S201	4	0.5	0.125	0.06	-	-	-	-	-	-
308	S202	4	0.5	0.125	0.06	-	-	-	-	-	-
309	S203	2	0.5	0.125	0.06	-	-	-	-	-	-
310	S204	2	0.5	0.25	0.125	-	-	-	-	-	-
311	S205	2	0.5	0.125	0.06	-	-	-	-	-	-
312	S206	2	0.5	0.125	0.06	-	-	-	-	-	-
313	S207	2	0.5	0.25	0.125	-	-	-	-	-	-
314	S208	2	0.5	0.125	0.125	-	-	-	-	-	-
315	S209	2	0.5	0.125	0.125	-	-	-	-	-	-
316	S210	2	0.5	0.125	0.06	-	-	-	-	-	-
317	S211	2	0.5	0.125	0.125	-	-	-	-	-	-
318	S212	2	0.5	0.125	0.125	-	-	-	-	-	-
319	S213	2	0.5	0.125	0.125	-	-	-	-	-	-
320	S214	2	0.5	0.125	0.06	-	-	-	-	-	-
321	S215	4	1	0.125	0.25	-	-	-	-	-	-
322	S216	2	0.5	0.125	0.06	-	-	-	-	-	-
323	S217	2	0.5	0.125	0.125	-	-	-	-	-	-
324	S218	2	0.5	0.125	0.125	-	-	-	-	-	-
325	S219	2	0.5	0.125	0.06	-	-	-	-	-	-
326	S220	2	0.5	0.125	0.06	-	-	-	-	-	-
327	S221	2	0.5	0.25	0.125	-	-	-	-	-	-
328	S222	2	1	0.125	0.125	-	-	-	-	-	-
329	S223	2	1	0.5	0.5	-	-	-	-	-	-
330	S224	2	0.5	0.125	0.06	-	-	-	-	-	-
331	S225	2	0.5	0.125	0.125	-	-	-	-	-	-
332	S226	2	1	0.125	0.125	-	-	-	-	-	-
333	S227	2	0.5	0.125	0.06	-	-	-	-	-	-
334	S228	2	0.5	0.125	0.125	-	-	-	-	-	-
335	S229	2	0.5	1	0.5	-	-	-	-	-	-

No.	Isolate	MICs (mg/L)				ESBL phenotype	AmpC phenotype	The presence of *bla* genes			
		FOX	CAZ	CTX	CRO			$bla_{CTX\text{-}M\text{-}1}$ group	$bla_{CTX\text{-}M\text{-}9}$ group	$bla_{TEM\text{-}like}$	$bla_{CIT\text{-}like}$
336	S230	2	0.5	0.125	0.125	-	-	-	-	-	-
337	S231	2	0.5	0.25	0.5	-	-	-	-	-	-
338	S232	4	0.5	0.125	0.06	-	-	-	-	-	-
339	S233	2	0.5	0.125	0.06	-	-	-	-	-	-
340	S234	2	1	0.125	0.06	-	-	-	-	-	-
341	S235	2	1	0.25	1	-	-	-	-	-	-
342	S236	2	0.25	0.125	0.03	-	-	-	-	-	-
343	S237	4	0.5	0.125	0.06	-	-	-	-	-	-
344	S238	2	0.5	0.125	0.06	-	-	-	-	-	-
345	S239	2	0.5	0.125	0.125	-	-	-	-	-	-
346	S240	4	0.5	0.125	0.06	-	-	-	-	-	-
347	S241	1	0.5	0.125	0.125	-	-	-	-	-	-
348	S242	2	1	0.125	0.5	-	-	-	-	-	-
349	S243	2	1	0.125	0.06	-	-	-	-	-	-
350	S244	2	0.5	0.06	0.015	-	-	-	-	-	-
351	S245	2	1	0.125	0.125	-	-	-	-	-	-
352	S246	4	0.5	0.125	0.06	-	-	-	-	-	-
353	S247	2	1	0.125	0.125	-	-	-	-	-	-
354	S248	2	1	0.125	0.125	-	-	-	-	-	-
355	S249	2	0.5	0.125	0.06	-	-	-	-	-	-
356	S250	2	0.5	0.25	0.125	-	-	-	-	-	-
357	S251	2	0.5	0.125	0.125	-	-	-	-	-	-
358	S252	2	0.5	0.125	0.06	-	-	-	-	-	-
359	S253	2	0.5	0.125	0.125	-	-	-	-	-	-
360	S254	2	0.5	0.125	0.125	-	-	-	-	-	-
361	S255	2	1	0.125	0.03	-	-	-	-	-	-
362	S256	2	0.5	0.125	0.125	-	-	-	-	-	-
363	S257	2	0.5	0.125	0.06	-	-	-	-	-	-
364	S258	2	0.5	0.125	0.06	-	-	-	-	-	-
365	S259	2	0.5	0.125	0.125	-	-	-	-	-	-
366	S260	8	1	1	0.25	-	-	-	-	-	-
367	S261	8	1	0.125	0.125	-	-	-	-	-	-
368	S262	2	1	0.125	0.125	-	-	-	-	-	-
369	S263	2	0.5	0.125	0.125	-	-	-	-	-	-
370	S264	2	1	0.125	0.125	-	-	-	-	-	-
371	S265	64	128	16	128	0	1	-	-	-	1
372	S266	32	64	8	128	0	1	-	-	-	1
373	S267	32	64	16	128	0	1	-	-	-	1

No.	Isolate	MICs (mg/L)				ESBL phenotype	AmpC phenotype	The presence of *bla* genes			
		FOX	CAZ	CTX	CRO			$bla_{CTX\text{-}M\text{-}1}$ group	$bla_{CTX\text{-}M\text{-}9}$ group	$bla_{TEM\text{-}like}$	$bla_{CIT\text{-}like}$
374	S268	2	1	0.125	0.125	-	-	-	-	-	-
375	S269	2	0.25	0.125	0.125	-	-	-	-	-	-
376	S270	4	8	128	256	1	-	0	1	-	-
377	S271	32	64	16	128	0	1	-	-	-	1
378	S272	2	0.25	0.06	0.125	-	-	-	-	-	-
379	S273	2	1	0.125	0.125	-	-	-	-	-	-
380	S274	2	0.25	0.125	0.125	-	-	-	-	-	-
381	S275	64	64	8	64	0	1	-	-	-	1
382	S276	2	0.5	0.06	0.125	-	-	-	-	-	-
383	S277	2	1	0.06	0.125	-	-	-	-	-	-
384	S278	128	128	16	128	0	1	-	-	-	1
385	S279	2	1	0.125	0.125	-	-	-	-	-	-
386	S280	2	1	0.06	0.125	-	-	-	-	-	-
387	S281	2	8	64	256	1	-	0	1	0	-
388	S282	32	64	32	64	0	1	-	-	-	1
389	S283	8	1	0.125	0.125	-	-	-	-	-	-
390	S284	4	16	256	256	1	-	0	1	0	-
391	S285	128	256	64	128	0	1	-	-	-	1
392	S286	2	8	128	256	1	-	0	1	0	-
393	S287	2	1	0.125	0.125	-	-	-	-	-	-
394	S288	2	1	0.25	0.125	-	-	-	-	-	-
395	S289	8	0.5	0.25	0.125	-	-	-	-	-	-
396	S290	8	0.5	0.06	0.03	-	-	-	-	-	-
397	S291	128	64	16	32	0	1	-	-	-	1
398	S292	1	0.25	0.25	0.06	-	-	-	-	-	-
399	S293	4	4	64	128	1	-	0	1	0	-
400	S294	8	0.5	0.125	0.125	-	-	-	-	-	-
401	S295	64	128	8	16	0	1	-	-	-	1
402	S296	2	0.5	0.125	0.06	-	-	-	-	-	-
403	S297	2	8	256	256	1	-	0	1	0	-
404	S298	4	0.5	0.06	0.06	-	-	-	-	-	-
405	S299	4	4	16	64	1	-	0	1	0	-
406	S300	64	64	8	64	0	1	-	-	-	1
407	S301	2	8	64	256	1	-	0	1	0	-
408	S302	2	8	128	256	1	-	0	1	0	-
409	S303	2	0.5	0.125	0.06	-	-	-	-	-	-
410	S304	2	0.25	0.125	0.06	-	-	-	-	-	-
411	S305	128	64	16	32	0	1	-	-	-	1

No.	Isolate	MICs (mg/L)				ESBL phenotype	AmpC phenotype	The presence of *bla* genes			
		FOX	CAZ	CTX	CRO			$bla_{CTX-M-1}$ group	$bla_{CTX-M-9}$ group	$bla_{TEM-like}$	$bla_{CIT-like}$
412	S306	32	64	32	32	0	1	-	-	-	1
413	S307	4	16	256	>256	1	-	0	1	0	-
414	S308	32	32	4	16	0	1	-	-	-	1
415	S309	2	4	128	128	1	-	0	1	0	-
416	S310	2	4	64	128	1	-	0	1	0	-
417	S311	4	0.5	0.125	0.25	-	-	-	-	-	-
418	S312	32	64	8	32	0	1	-	-	-	1
419	S313	2	0.25	0.06	0.5	-	-	-	-	-	-
420	S314	32	128	32	64	0	1	-	-	-	1
421	S315	2	0.5	0.125	0.25	-	-	-	-	-	-
422	S316	2	1	0.125	0.06	-	-	-	-	-	-
423	S317	32	32	4	32	0	1	-	-	-	1
424	S318	2	0.5	0.125	0.06	-	-	-	-	-	-
425	S319	2	1	0.125	0.125	-	-	-	-	-	-
426	S320	4	2	0.125	0.125	0	-	-	-	1	-
427	S321	2	1	1	0.03	-	-	-	-	-	-
428	S322	32	32	8	32	0	1	-	-	-	1
429	S323	2	4	64	128	1	-	0	1	1	-
430	S324	2	4	0.125	0.06	0	-	-	-	-	-
431	S325	4	1	0.125	0.06	-	-	-	-	-	-
432	S326	4	1	0.125	0.06	-	-	-	-	-	-
433	S327	2	0.5	0.125	0.06	-	-	-	-	-	-
434	S328	64	128	8	64	0	1	-	-	-	1
435	S329	64	64	16	64	0	1	-	-	-	1
436	S330	2	8	128	256	1	-	0	1	0	-
437	S331.	32	128	32	64	0	1	-	-	-	1
438	S332	32	64	32	32	0	1	-	-	-	1
439	S333	2	0.5	0.125	0.5	-	-	-	-	-	-
440	S334	8	16	128	256	1	-	0	1	0	-
441	S335	2	8	64	256	1	-	0	1	0	-
442	S336	2	0.5	0.125	0.125	-	-	-	-	-	-
443	S337	128	256	32	128	0	1	-	-	-	1
444	S338	32	128	32	32	0	1	-	-	-	1
445	S339	128	>256	32	128	0	1	-	-	-	1
446	S340	32	64	32	32	0	1	-	-	-	1
447	S341	2	8	128	256	1	-	0	1	0	-
448	S342	32	64	16	32	0	1	-	-	-	1
449	S343	2	0.5	0.125	0.125	-	-	-	-	-	-

No.	Isolate	MICs (mg/L)				ESBL phenotype	AmpC phenotype	The presence of *bla* genes			
		FOX	CAZ	CTX	CRO			$bla_{CTX\text{-}M\text{-}1}$ group	$bla_{CTX\text{-}M\text{-}9}$ group	$bla_{TEM\text{-}like}$	$bla_{CIT\text{-}like}$
450	S345	2	0.5	0.125	0.125	-	-	-	-	-	-
451	S346	2	0.5	0.125	0.125	-	-	-	-	-	-
452	S347	4	0.5	0.125	0.125	-	-	-	-	-	-
453	S348	4	0.5	0.125	0.125	-	-	-	-	-	-
454	S349	4	0.5	0.125	0.125	-	-	-	-	-	-
455	S350	2	0.5	0.125	0.06	-	-	-	-	-	-
456	S351	8	1	0.5	0.25	-	-	-	-	-	-
457	S352	2	0.5	0.125	0.125	-	-	-	-	-	-
458	S353	2	0.5	0.125	0.125	-	-	-	-	-	-
459	S354	2	0.25	0.125	0.06	-	-	-	-	-	-
460	S355	2	0.5	0.125	0.125	-	-	-	-	-	-
461	S356	2	0.5	0.125	0.125	-	-	-	-	-	-
462	S357	2	0.5	0.125	0.125	-	-	-	-	-	-
463	S358	4	0.5	0.25	0.25	-	-	-	-	-	-
464	S359	4	0.5	0.5	0.125	-	-	-	-	-	-
465	S360	2	0.5	0.125	0.125	-	-	-	-	-	-
466	S361	2	0.5	0.25	0.125	-	-	-	-	-	-
467	S363	1	0.5	0.125	0.06	-	-	-	-	-	-
468	S364	1	0.5	0.125	0.06	-	-	-	-	-	-
469	S365	1	0.5	0.125	0.06	-	-	-	-	-	-
470	S367	4	1	0.125	0.06	-	-	-	-	-	-
471	S368	4	1	0.125	0.06	-	-	-	-	-	-
472	S369	1	0.5	0.125	0.06	-	-	-	-	-	-
473	S370	1	1	0.125	0.06	-	-	-	-	-	-
474	S371	1	0.5	0.125	0.06	-	-	-	-	-	-
475	S372	32	128	8	32	0	1	-	-	-	1
476	S373	64	>256	32	128	0	1	-	-	-	1
477	S374	32	64	16	16	0	1	-	-	-	1
478	S375	2	0.5	0.125	0.25	-	-	-	-	-	-
479	S376	32	32	16	32	0	1	-	-	-	1
480	S377	1	8	128	128	1	-	0	1	0	-
481	S378	1	0.5	0.125	0.06	-	-	-	-	-	-
482	S379	2	4	64	64	1	-	0	1	0	-
483	S380	1	0.5	0.25	0.06	-	-	-	-	-	-
484	S381	2	8	64	128	1	-	0	1	0	-
485	S382	64	128	16	64	0	1	-	-	-	1
486	S383	32	128	64	64	0	1	-	-	-	1
487	S384	1	8	256	256	1	-	0	1	0	-

No.	Isolate	MICs (mg/L)				ESBL phenotype	AmpC phenotype	The presence of *bla* genes			
		FOX	CAZ	CTX	CRO			$bla_{CTX\text{-}M\text{-}1}$ group	$bla_{CTX\text{-}M\text{-}9}$ group	$bla_{TEM\text{-}like}$	$bla_{CIT\text{-}like}$
488	S385	64	128	16	32	0	1	-	-	-	1
489	S386	1	0.5	0.25	0.125	-	-	-	-	-	-
490	S387	8	0.5	2	0.125	0	-	-	-	-	-
491	S388	1	0.5	0.25	0.125	-	-	-	-	-	-
492	S389	1	0.5	0.125	0.06	-	-	-	-	-	-
493	S390	1	0.5	0.125	0.06	-	-	-	-	-	-
494	S391	8	1	0.5	0.25	-	-	-	-	-	-
495	S392	1	1	0.25	0.125	-	-	-	-	-	-
496	S393	1	1	0.125	0.125	-	-	-	-	-	-
497	S394	16	32	16	16	0	1	-	-	-	1
498	S395	2	0.5	0.125	0.125	-	-	-	-	-	-
499	S396	4	1	0.125	0.25	-	-	-	-	-	-
500	S397	128	128	32	64	0	1	-	-	-	1
501	S398	64	128	16	32	0	1	-	-	-	1
502	S399	2	0.25	0.125	0.5	-	-	-	-	-	-
503	S400	1	4	64	64	1	-	0	1	0	-
504	S401	64	128	16	32	0	1	-	-	-	1
505	S402	2	0.5	0.25	0.125	-	-	-	-	-	-
506	S403	4	0.25	0.25	1	-	-	-	-	-	-
507	S404	2	0.5	0.125	0.25	-	-	-	-	-	-
508	S405	2	1	0.125	0.125	-	-	-	-	-	-
509	S406	64	32	8	16	0	1	-	-	-	1
510	S407	2	0.5	0.06	0.06	-	-	-	-	-	-
511	S408	2	0.5	0.125	0.03	-	-	-	-	-	-
512	S409	2	0.5	2	0.06	0	-	-	-	-	-
513	S410	4	0.5	32	32	1	-	0	1	0	-
514	S411	4	16	128	32	1	-	0	1	0	-
515	S412	2	2	64	256	1	-	0	1	0	-
516	S413	2	0.5	0.125	0.25	-	-	-	-	-	-
517	S414	2	0.5	0.125	0.06	-	-	-	-	-	-
518	S415	64	64	16	16	0	1	-	-	-	1
519	S416	64	64	16	32	0	1	-	-	-	1
520	S417	32	128	32	64	0	1	-	-	-	1
521	S418	2	2	64	64	1	-	0	1	0	-
522	S419	64	128	16	32	0	1	-	-	-	1
523	S420	2	2	0.125	0.25	0	-	-	-	-	-
524	S421	32	64	8	32	0	1	-	-	-	1
525	S422	32	64	16	32	0	1	-	-	-	1

No.	Isolate	MICs (mg/L)				ESBL phenotype	AmpC phenotype	The presence of *bla* genes			
		FOX	CAZ	CTX	CRO			$bla_{CTX-M-1}$ group	$bla_{CTX-M-9}$ group	$bla_{TEM-like}$	$bla_{CIT-like}$
526	S423	32	128	8	16	0	1	-	-	-	1
527	S424	128	64	8	16	0	1	-	-	-	1
528	S425	32	128	8	16	0	1	-	-	-	1
529	S426	2	0.25	0.06	0.5	-	-	-	-	-	-
530	S427	32	128	4	32	0	1	-	-	-	1
531	S428	2	8	256	128	1	-	0	1	0	-
532	S429	2	32	128	128	1	-	0	1	0	-
533	S430	32	64	16	32	0	1			0	1
534	S431	4	8	64	128	1	-	0	1	0	-
535	S432	2	0.5	0.125	0.125	-	-	-	-	-	-
536	S433	2	0.5	0.06	0.25	-	-	-	-	-	-
537	S434	4	16	256	32	1	-	0	1	1	-
538	S435	2	8	256	256	1	-	0	1	0	-
539	S436	2	16	256	256	1	-	0	1	0	-
540	S437	2	4	128	64	1	-	0	1	1	-
541	S438	1	16	128	64	1	-	0	1	0	-
542	S439	64	32	16	16	0	1	-	-	-	1
543	S440	2	0.25	0.125	0.06	-	-	-	-	-	-
544	S441	32	128	16	32	0	1	-	-	-	1
545	S442	4	0.5	1	0.25	-	-	-	-	-	-
546	S443	2	0.5	0.125	0.03	-	-	-	-	-	-
547	S444	2	0.5	0.125	0.03	-	-	-	-	-	-
548	S445	64	128	16	32	0	1	-	-	-	1
549	S447	64	128	16	16	0	1	-	-	-	1
550	S448	32	128	16	16	0	1	-	-	-	1
551	S449	32	128	16	16	0	1	-	-	-	1
552	S450	32	128	8	16	0	1	-	-	-	1
553	S451	64	128	8	16	0	1	-	-	-	1
554	S452	64	128	8	32	0	1	-	-	-	1
555	S453	32	64	16	16	0	1	-	-	-	1
556	S454	2	0.5	0.125	0.06	-	-	-	-	-	-
557	S455	2	0.5	0.125	0.03	-	-	-	-	-	-
558	S456	32	128	16	16	0	1	-	-	-	1
559	S457	64	128	8	16	0	1	-	-	-	1
560	S458	64	64	8	16	0	1	-	-	-	1

FOX, cefoxitin; CAZ, ceftazidime; CTX, cefotaxime; CRO, ceftriaxone

;1, positive; 0, negative; -, not done

APPENDIX E

NUCLEOTIDE SEQUENCES ALIGNMENT OF SELECTED ISOLATES

All 14 isolates with *bla*$_{\text{CTX-M-1}}$ group genes

13 isolates with *bla*$_{\text{CTX-M-55}}$ gene

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           1                                                                                                                                  130
CTX-M-55   ATGGTTAAAA AATCACTGCG CCAGTTCACG CTGATGGCGA CGGCAACCGT CACGCTGTTG TTAGGAAGTG TGCCGCTGTA TGCGCAAACG GCGGACGTAC AGCAAAAACT TGCCGAATTA GAGCGGCAGT
CTX-M-57   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S51        .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S49        .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S45        .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S40        .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S39        .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S38        .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S31        .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S23        .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S22        .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S21        .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S20        .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S19        .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S17        .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Consensus  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

           131                                                                                                                                260
CTX-M-55   CGGGAGGCAG ACTGGGTGTG GCATTGATTA ACACAGCAGA TAATTCGCAA ATACTTTATC GTGCTGATGA GCGCTTTGCG ATGTGCAGCA CCAGTAAAGT GATGGCCGTG GCCGCGGTGC TGAAGAAAAG
CTX-M-57   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S51        .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S49        .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S45        .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S40        .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S39        .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S38        .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S31        .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S23        .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S22        .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
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      S21 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S20 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S19 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S17 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Consensus .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

          261                                                                                                                                        390
 CTX-M-55 TGAAAGCGAA CCGAATCTGT TAAATCAGCG AGTTGAGATC AAAAAATCTG ACCTTGTTAA CTATAATCCG ATTGCGGAAA AGCACGTCAA TGGGACGATG TCACTGGCTG AGCTTAGCGC GGCCGCGCTA
 CTX-M-57 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S51 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S49 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S45 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S40 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S39 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S38 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S31 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S23 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S22 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S21 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S20 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S19 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S17 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Consensus .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

          391                                                                                                                                        520
 CTX-M-55 CAGTACAGCG ATAACGTGGC GATGAATAAG CTGATTGCTC ACGTTGGCGG CCCGGCTAGC GTCACCGCGT TCGCCCGACA GCTGGGAGAC GAAACGTTCC GTCTCGACCG TACCGAGCCG ACGTTAAACA
 CTX-M-57 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S51 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S49 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S45 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S40 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S39 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S38 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S31 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S23 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S22 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S21 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S20 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S19 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S17 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Consensus .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

          521                                                                                                                                        650
 CTX-M-55 CCGCCATTCC GGGCGATCCG CGTGATACCA CTTCACCTCG GGCAATGGCG CAAACTCTGC GGAATCTGAC GCTGGGTAAA GCATTGGGCG ACAGCCAACG GGCGCAGCTG GTGACATGGA TGAAAGGCAA
 CTX-M-57 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S51 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S49 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S45 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S40 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S39 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
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      S38  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S31  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S23  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S22  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S21  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S20  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S19  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S17  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Consensus  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

           651                                                                                                                                        780
 CTX-M-55  TACCACCGGT GCAGCGAGCA TTCAGGCTGG ACTGCCTGCT TCCTGGGTTG TGGGGGATAA AACCGGCAGC GGTGGCTATG GCACCACCAA CGATATCGCG GTGATCTGGC CAAAAGATCG TGCGCCGCTG
 CTX-M-57  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S51  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S49  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S45  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S40  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S39  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S38  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S31  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S23  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S22  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S21  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S20  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S19  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S17  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Consensus  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

           781                                                                                 876
 CTX-M-55  ATTCTGGTCA CTTACTTCAC CCAGCCTCAA CCTAAGGCAG AAAGCCGTCG CGATGTATTA GCGTCGGCGG CTAAAATCGT CACCGACGGT TTGTAA
 CTX-M-57  .......... .......... .......... .......... .......... .......... .......... .......... .......... ......
      S51  .......... .......... .......... .......... .......... .......... .......... .......... .......... ......
      S49  .......... .......... .......... .......... .......... .......... .......... .......... .......... ......
      S45  .......... .......... .......... .......... .......... .......... .......... .......... .......... ......
      S40  .......... .......... .......... .......... .......... .......... .......... .......... .......... ......
      S39  .......... .......... .......... .......... .......... .......... .......... .......... .......... ......
      S38  .......... .......... .......... .......... .......... .......... .......... .......... .......... ......
      S31  .......... .......... .......... .......... .......... .......... .......... .......... .......... ......
      S23  .......... .......... .......... .......... .......... .......... .......... .......... .......... ......
      S22  .......... .......... .......... .......... .......... .......... .......... .......... .......... ......
      S21  .......... .......... .......... .......... .......... .......... .......... .......... .......... ......
      S20  .......... .......... .......... .......... .......... .......... .......... .......... .......... ......
      S19  .......... .......... .......... .......... .......... .......... .......... .......... .......... ......
      S17  .......... .......... .......... .......... .......... .......... .......... .......... .......... ......
Consensus  .......... .......... .......... .......... .......... .......... .......... .......... .......... ......
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All 14 isolates with *bla*$_{\text{CTX-M-1}}$ group genes (cont.)

1 isolate (S53) with *bla*$_{\text{CTX-M-15}}$ gene

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           1                                                                                                                   130
CTX-M-15   ATGGTTAAAA AATCACTGCG CCAGTTCACG CTGATGGCGA CGGCAACCGT CACGCTGTTG TTAGGAAGTG TGCCGCTGTA TGCGCAAACG GCGGACGTAC AGCAAAAACT TGCCGAATTA GAGCGGCAGT
      S53  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Consensus  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

           131                                                                                                                 260
CTX-M-15   CGGGAGGCAG ACTGGGTGTG GCATTGATTA ACACAGCAGA TAATTCGCAA ATACTTTATC GTGCTGATGA GCGCTTTGCG ATGTGCAGCA CCAGTAAAGT GATGGCCGCG GCCGCGGTGC TGAAGAAAAG
      S53  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Consensus  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

           261                                                                                                                 390
CTX-M-15   TGAAAGCGAA CCGAATCTGT TAAATCAGCG AGTTGAGATC AAAAAATCTG ACCTTGTTAA CTATAATCCG ATTGCGGAAA AGCACGTCAA TGGGACGATG TCACTGGCTG AGCTTAGCGC GGCCGCGCTA
      S53  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Consensus  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

           391                                                                                                                 520
CTX-M-15   CAGTACAGCG ATAACGTGGC GATGAATAAG CTGATTGCTC ACGTTGGCGG CCCGGCTAGC GTCACCGCGT TCGCCCGACA GCTGGGAGAC GAAACGTTCC GTCTCGACCG TACCGAGCCG ACGTTAAACA
      S53  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Consensus  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

           521                                                                                                                 650
CTX-M-15   CCGCCATTCC GGGCGATCCG CGTGATACCA CTTCACCTCG GGCAATGGCG CAAACTCTGC GGAATCTGAC GCTGGGTAAA GCATTGGGCG ACAGCCAACG GGCGCAGCTG GTGACATGGA TGAAAGGCAA
      S53  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Consensus  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

           651                                                                                                                 780
CTX-M-15   TACCACCGGT GCAGCGAGCA TTCAGGCTGG ACTGCCTGCT TCCTGGGTTG TGGGGGATAA AACCGGCAGC GGTGGCTATG GCACCACCAA CGATATCGCG GTGATCTGGC CAAAAGATCG TGCGCCGCTG
      S53  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Consensus  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

           781                                                                                                  876
CTX-M-15   ATTCTGGTCA CTTACTTCAC CCAGCCTCAA CCTAAGGCAG AAAGCCGTCG CGATGTATTA GCGTCGGCGG CTAAAATCGT CACCGACGGT TTGTAA
      S53  .......... .......... .......... .......... .......... .......... .......... .......... .......... ......
Consensus  .......... .......... .......... .......... .......... .......... .......... .......... .......... ......
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10 representarive isolates with *bla*$_{CTX\text{-}M\text{-}9}$ group gene

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            1                                                                                                                                      130
 CTX-M-14   ATGGTGACAA AGAGAGTGCA ACGGATGATG TTCGCGGCGG CGGCGTGCAT TCCGCTGCTG CTGGGCAGCG CGCCGCTTTA TGCGCAGACG AGTGCGGTGC AGCAAAAGCT GGCGGCGCTG GAGAAAAGCA
 CTX-M-18   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     S438   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     S429   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     S412   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     S400   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     S384   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     S307   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     S299   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S33   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
    S19-1   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Consensus   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

            131                                                                                                                                    260
 CTX-M-14   GCGGAGGGCG GCTGGGCGTC GCGCTCATCG ATACCGCAGA TAATACGCAG GTGCTTTATC GCGGTGATGA ACGCTTTCCA ATGTGCAGTA CCAGTAAAGT TATGGCGGCC GCGGCGGTGC TTAAGCAGAG
 CTX-M-18   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     S438   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     S429   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     S412   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     S400   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     S384   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     S307   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     S299   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S33   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
    S19-1   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Consensus   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

            261                                                                                                                                    390
 CTX-M-14   TGAAACGCAA AAGCAGCTGC TTAATCAGCC TGTCGAGATC AAGCCTGCCG ATCTGGTTAA CTACAATCCG ATTGCCGAAA AACACGTCAA CGGCACAATG ACGCTGGCAG AACTGAGCGC GGCCGCGTTG
 CTX-M-18   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     S438   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     S429   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     S412   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     S400   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     S384   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     S307   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     S299   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S33   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
    S19-1   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Consensus   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

            391                                                                                                                                    520
 CTX-M-14   CAGTACAGCG ACAATACCGC CATGAACAAA TTGATTGCCC AGCTCGGTGG CCCGGGAGGC GTGACGGCTT TTGCCCGCGC GATCGGCGAT GAGACGTTTC GTCTGGATCG CACTGAACCT ACGCTGAATA
 CTX-M-18   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     S438   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     S429   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     S412   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
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     S400 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     S384 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     S307 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     S299 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S33 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
    S19-1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Consensus .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

          521                                                                                                                                                        650
 CTX-M-14 CCGCCATTCC CGGCGACCCG AGAGACACCA CCACGCCGCG GGCGATGGCG CAGACGTTGC GTCAGCTTAC GCTGGGTCAT GCGCTGGGCG AAACCCAGCG GGCGCAGTTG GTGACGTGGC TCAAAGGCAA
 CTX-M-18 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     S438 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     S429 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     S412 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     S400 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     S384 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     S307 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     S299 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S33 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
    S19-1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Consensus .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

          651                                                                                                                                                        780
 CTX-M-14 TACGACCGGC GCAGCCAGCA TTCGGGCCGG CTTACCGACG TCGTGGACTG TGGGTGATAA GACCGGCAGC GGCGACTACG GCACCACCAA TGATATTGCG GTGATCTGGC CGCAGGGTCG TGCGCCGCTG
 CTX-M-18 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     S438 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     S429 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     S412 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     S400 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     S384 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     S307 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     S299 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S33 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
    S19-1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Consensus .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

          781                                                                                                                                                  876
 CTX-M-14 GTTCTGGTGA CCTATTTTAC CCAGCCGCAA CAGAACGCAG AGAGCCGCCG CGATGTGCTG GCTTCAGCGG CGAGAATCAT CGCCGAAGGG CTGTAA
 CTX-M-18 .......... .......... .......... .......... .......... .......... .......... .......... .......... ......
     S438 .......... .......... .......... .......... .......... .......... .......... .......... .......... ......
     S429 .......... .......... .......... .......... .......... .......... .......... .......... .......... ......
     S412 .......... .......... .......... .......... .......... .......... .......... .......... .......... ......
     S400 .......... .......... .......... .......... .......... .......... .......... .......... .......... ......
     S384 .......... .......... .......... .......... .......... .......... .......... .......... .......... ......
     S307 .......... .......... .......... .......... .......... .......... .......... .......... .......... ......
     S299 .......... .......... .......... .......... .......... .......... .......... .......... .......... ......
      S33 .......... .......... .......... .......... .......... .......... .......... .......... .......... ......
    S19-1 .......... .......... .......... .......... .......... .......... .......... .......... .......... ......
Consensus .......... .......... .......... .......... .......... .......... .......... .......... .......... ......
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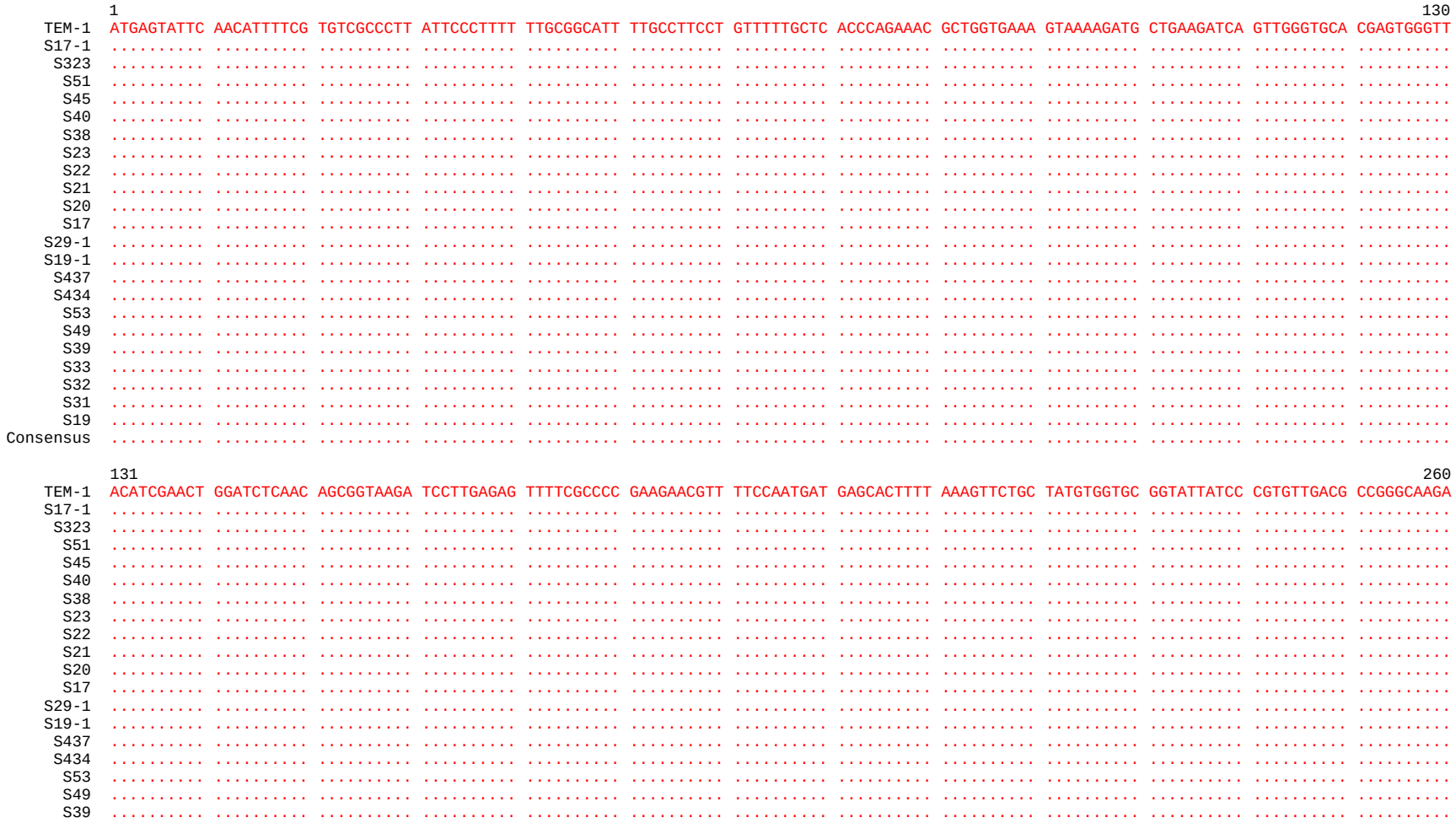
All 22 isolates with *bla*$_{TEM}$ gene



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      S33 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S32 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S31 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S19 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Consensus .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

          261                                                                                                                                         390
    TEM-1 GCAACTCGGT CGCCGCATAC ACTATTCTCA GAATGACTTG GTTGAGTACT CACCAGTCAC AGAAAAGCAT CTTACGGATG GCATGACAGT AAGAGAATTA TGCAGTGCTG CCATAACCAT GAGTGATAAC
    S17-1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     S323 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S51 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S45 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S40 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S38 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S23 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S22 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S21 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S20 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S17 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
    S29-1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
    S19-1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     S437 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     S434 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S53 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S49 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S39 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S33 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S32 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S31 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S19 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Consensus .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

          391                                                                                                                                         520
    TEM-1 ACTGCTGCCA ACTTACTTCT GACAACGATC GGAGGACCGA AGGAGCTAAC CGCTTTTTTG CACAACATGG GGGATCATGT AACTCGCCTT GATCGTTGGG AACCGGAGCT GAATGAAGCC ATACCAAACG
    S17-1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     S323 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S51 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S45 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S40 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S38 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S23 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S22 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S21 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S20 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S17 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
    S29-1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
    S19-1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     S437 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     S434 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S53 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
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      S49 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S39 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S33 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S32 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S31 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S19 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Consensus .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

          521                                                                                                                                                       650
   TEM-1  ACGAGCGTGA CACCACGATG CCTGCAGCAA TGGCAACAAC GTTGCGCAAA CTATTAACTG GCGAACTACT TACTCTAGCT TCCCGGCAAC AATTAATAGA CTGGATGGAG GCGGATAAAG TTGCAGGACC
   S17-1  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
    S323  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
    S51   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
    S45   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
    S40   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
    S38   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
    S23   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
    S22   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
    S21   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
    S20   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
    S17   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
   S29-1  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
   S19-1  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
    S437  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
    S434  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
    S53   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
    S49   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
    S39   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
    S33   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
    S32   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
    S31   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
    S19   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Consensus .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

          651                                                                                                                                                       780
   TEM-1  ACTTCTGCGC TCGGCCCTTC CGGCTGGCTG GTTTATTGCT GATAAATCTG GAGCCGGTGA GCGTGGGTCT CGCGGTATCA TTGCAGCACT GGGGCCAGAT GGTAAGCCCT CCCGTATCGT AGTTATCTAC
   S17-1  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
    S323  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
    S51   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
    S45   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
    S40   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
    S38   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
    S23   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
    S22   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
    S21   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
    S20   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
    S17   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
   S29-1  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
   S19-1  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
    S437  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
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      S434  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
       S53  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
       S49  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
       S39  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
       S33  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
       S32  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
       S31  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
       S19  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
 Consensus  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

            781                                                                                                                                 861
    TEM-1   ACGACGGGGA GTCAGGCAAC TATGGATGAA CGAAATAGAC AGATCGCTGA GATAGGTGCC TCACTGATTA AGCATTGGTA A
    S17-1   .......... .......... .......... .......... .......... .......... .......... .......... .
     S323   .......... .......... .......... .......... .......... .......... .......... .......... .
     S51    .......... .......... .......... .......... .......... .......... .......... .......... .
     S45    .......... .......... .......... .......... .......... .......... .......... .......... .
     S40    .......... .......... .......... .......... .......... .......... .......... .......... .
     S38    .......... .......... .......... .......... .......... .......... .......... .......... .
     S23    .......... .......... .......... .......... .......... .......... .......... .......... .
     S22    .......... .......... .......... .......... .......... .......... .......... .......... .
     S21    .......... .......... .......... .......... .......... .......... .......... .......... .
     S20    .......... .......... .......... .......... .......... .......... .......... .......... .
     S17    .......... .......... .......... .......... .......... .......... .......... .......... .
    S29-1   .......... .......... .......... .......... .......... .......... .......... .......... .
    S19-1   .......... .......... .......... .......... .......... .......... .......... .......... .
     S437   .......... .......... .......... .......... .......... .......... .......... .......... .
     S434   .......... .......... .......... .......... .......... .......... .......... .......... .
      S53   .......... .......... .......... .......... .......... .......... .......... .......... .
      S49   .......... .......... .......... .......... .......... .......... .......... .......... .
      S39   .......... .......... .......... .......... .......... .......... .......... .......... .
      S33   .......... .......... .......... .......... .......... .......... .......... .......... .
      S32   .......... .......... .......... .......... .......... .......... .......... .......... .
      S31   .......... .......... .......... .......... .......... .......... .......... .......... .
      S19   .......... .......... .......... .......... .......... .......... .......... .......... .
 Consensus  .......... .......... .......... .......... .......... .......... .......... .......... .
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10 representarive isolates with *bla*$_{\text{CIT-like}}$ gene

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          1                                                                                                                 130
CMY-2     ATGATGAAAA AATCGTTATG CTGCGCTCTG CTGCTGACAG CCTCTTTCTC CACATTTGCT GCCGCAAAAA CAGAACAACA GATTGCCGAT ATCGTTAATC GCACCATCAC CCCGTTGATG CAGGAGCAGG
S23-1      .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S32        .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S453       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S394       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S383       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S339       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S331       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S306       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S285       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S278       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Consensus  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

          131                                                                                                               260
CMY-2     CTATTCCGGG TATGGCCGTT GCCGTTATCT ACCAGGGAAA ACCCTATTAT TTCACCTGGG GTAAAGCCGA TATCGCCAAT AACCACCCAG TCACGCAGCA AACGCTGTTT GAGCTAGGAT CGGTTAGTAA
S23-1      .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S32        .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S453       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S394       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S383       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S339       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S331       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S306       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S285       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S278       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Consensus  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

          261                                                                                                               390
CMY-2     GACGTTTAAC GGCGTGTTGG GCGGCGATGC TATCGCCCGC GGCGAAATTA AGCTCAGCGA TCCGGTCACG AAATACTGGC CAGAACTGAC AGGCAAACAG TGGCAGGGTA TCCGCCTGCT GCACTTAGCC
S23-1      .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S32        .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S453       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S394       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S383       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S339       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S331       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S306       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S285       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S278       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Consensus  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

          391                                                                                                               520
CMY-2     ACCTATACGG CAGGCGGCCT ACCGCTGCAG ATCCCCGATG ACGTTAGGGA TAAAGCCGCA TTACTGCATT TTTATCAAAA CTGGCAGCCG CAATGGACTC CGGGCGCTAA GCGACTTTAC GCTAACTCCA
S23-1      .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S32        .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
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      S453 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S394 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S383 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S339 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S331 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S306 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S285 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S278 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Consensus .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

           521                                                                                                                                                  650
    CMY-2 GCATTGGTCT GTTTGGCGCG CTGGCGGTGA AACCCTCAGG AATGAGTTAC GAAGAGGCAA TGACCAGACG CGTCCTGCAA CCATTAAAAC TGGCGCATAC CTGGATTACG GTTCCGCAGA ACGAACAAAA
    S23-1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S32 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
    S453  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
    S394  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
    S383  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
    S339  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
    S331  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
    S306  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
    S285  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
    S278  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Consensus .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

           651                                                                                                                                                  780
    CMY-2 AGATTATGCC TGGGGCTATC GCGAAGGGAA GCCCGTACAC GTTTCTCCGG GACAACTTGA CGCCGAAGCC TATGGCGTGA AATCCAGCGT TATTGATATG GCCCGCTGGG TTCAGGCCAA CATGGATGCC
    S23-1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S32 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
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    S331  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
    S306  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
    S285  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
    S278  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Consensus .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

           781                                                                                                                                                  910
    CMY-2 AGCCACGTTC AGGAGAAAAC GCTCCAGCAG GGCATTGCGC TTGCGCAGTC TCGCTACTGG CGTATTGGCG ATATGTACCA GGGATTAGGC TGGGAGATGC TGAACTGGCC GCTGAAAGCT GATTCGATCA
    S23-1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S32 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
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    S394  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
    S383  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
    S339  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
    S331  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
    S306  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
    S285  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
    S278  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
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Consensus  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

           911                                                                                                                                  1040
    CMY-2  TCAACGGCAG CGACAGCAAA GTGGCATTGG CAGCGCTTCC CGCCGTTGAG GTAAACCCGC CCGCCCCCGC AGTGAAAGCC TCATGGGTGC ATAAAACGGG CTCCACTGGT GGATTTGGCA GCTACGTAGC
    S23-1  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S32  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     S453  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     S394  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     S383  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     S339  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     S331  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     S306  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     S285  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     S278  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Consensus  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

           1041                                                                                  1146
    CMY-2  CTTCGTTCCA GAAAAAAACC TTGGCATCGT GATGCTGGCA AACAAAAGCT ATCCTAACCC TGTCCGTGTC GAGGCGGCCT GGCGCATTCT TGAAAAGCTG CAATAA
    S23-1  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ......
      S32  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ......
     S453  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ......
     S394  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ......
     S383  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ......
     S339  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ......
     S331  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ......
     S306  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ......
     S285  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ......
     S278  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ......
Consensus  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ......
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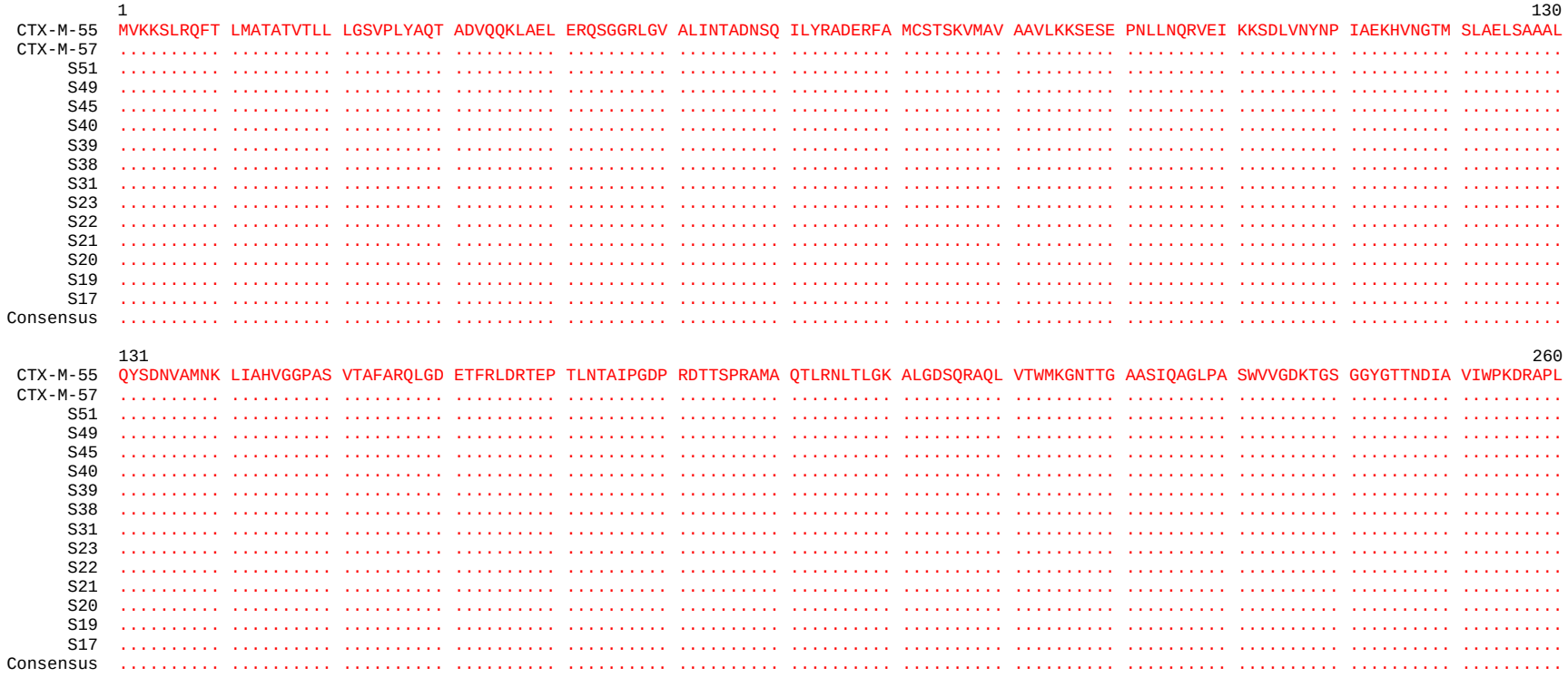
AMINO ACID SEQUENCES ALIGNMENT OF SELECTED ISOLATES

All 14 isolates with CTX-M-1 group

13 isolates with CTX-M-55



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           261                                291
CTX-M-55   ILVTYFTQPQ PKAESRRDVL ASAAKIVTDG L
CTX-M-57   .......... .......... .......... .
     S51   .......... .......... .......... .
     S49   .......... .......... .......... .
     S45   .......... .......... .......... .
     S40   .......... .......... .......... .
     S39   .......... .......... .......... .
     S38   .......... .......... .......... .
     S31   .......... .......... .......... .
     S23   .......... .......... .......... .
     S22   .......... .......... .......... .
     S21   .......... .......... .......... .
     S20   .......... .......... .......... .
     S19   .......... .......... .......... .
     S17   .......... .......... .......... .
Consensus  .......... .......... .......... .
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All 14 isolates with CTX-M-1 group (cont.)

1 isolate (S53) with CTX-M-15

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           1                                                                                                                                                 130
CTX-M-15   MVKKSLRQFT LMATATVTLL LGSVPLYAQT ADVQQKLAEL ERQSGGRLGV ALINTADNSQ ILYRADERFA MCSTSKVMAA AAVLKKSESE PNLLNQRVEI KKSDLVNYNP IAEKHVNGTM SLAELSAAAL
     S53   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Consensus  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

           131                                                                                                                                               260
CTX-M-15   QYSDNVAMNK LIAHVGGPAS VTAFARQLGD ETFRLDRTEP TLNTAIPGDP RDTTSPRAMA QTLRNLTLGK ALGDSQRAQL VTWMKGNTTG AASIQAGLPA SWVVGDKTGS GGYGTTNDIA VIWPKDRAPL
     S53   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Consensus  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

           261                                291
CTX-M-15   ILVTYFTQPQ PKAESRRDVL ASAAKIVTDG L
     S53   .......... .......... .......... .
Consensus  .......... .......... .......... .
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10 representarive isolates with CTX-M-9 group

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           1                                                                                                                                130
CTX-M-14   MVTKRVQRMM FAAAACIPLL LGSAPLYAQT SAVQQKLAAL EKSSGGRLGV ALIDTADNTQ VLYRGDERFP MCSTSKVMAA AAVLKQSETQ KQLLNQPVEI KPADLVNYNP IAEKHVNGTM TLAELSAAAL
CTX-M-18   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S438       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S429       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S412       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S400       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S384       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S307       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S299       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S33        .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S19-1      .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Consensus  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

           131                                                                                                                              260
CTX-M-14   QYSDNTAMNK LIAQLGGPGG VTAFARAIGD ETFRLDRTEP TLNTAIPGDP RDTTTPRAMA QTLRQLTLGH ALGETQRAQL VTWLKGNTTG AASIRAGLPT SWTVGDKTGS GDYGTTNDIA VIWPQGRAPL
CTX-M-18   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S438       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S429       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S412       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S400       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S384       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S307       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S299       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S33        .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S19-1      .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Consensus  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

           261                           291
CTX-M-14   VLVTYFTQPQ QNAESRRDVL ASAARIIAEG L
CTX-M-18   .......... .......... .......... .
S438       .......... .......... .......... .
S429       .......... .......... .......... .
S412       .......... .......... .......... .
S400       .......... .......... .......... .
S384       .......... .......... .......... .
S307       .......... .......... .......... .
S299       .......... .......... .......... .
S33        .......... .......... .......... .
S19-1      .......... .......... .......... .
Consensus  .......... .......... .......... .
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All 22 isolates with TEM-like

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          1                                                                                                                                130
TEM-1     MSIQHFRVAL IPFFAAFCLP VFAHPETLVK VKDAEDQLGA RVGYIELDLN SGKILESFRP EERFPMMSTF KVLLCGAVLS RVDAGQEQLG RRIHYSQNDL VEYSPVTEKH LTDGMTVREL CSAAITMSDN
S17-1     .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S323      .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S51       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S45       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S40       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S38       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S23       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S22       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S21       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S20       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S17       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S29-1     .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S19-1     .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S437      .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S434      .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S53       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S49       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S39       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S33       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S32       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S31       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S19       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Consensus .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

          131                                                                                                                              260
TEM-1     TAANLLLTTI GGPKELTAFL HNMGDHVTRL DRWEPELNEA IPNDERDTTM PAAMATTLRK LLTGELLTLA SRQQLIDWME ADKVAGPLLR SALPAGWFIA DKSGAGERGS RGIIAALGPD GKPSRIVVIY
S17-1     .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S323      .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S51       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S45       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S40       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S38       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S23       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S22       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S21       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S20       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S17       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S29-1     .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S19-1     .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S437      .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S434      .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S53       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S49       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S39       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
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      S33  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S32  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S31  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S19  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Consensus  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

           261                    286
    TEM-1  TTGSQATMDE RNRQIAEIGA SLIKHW
    S17-1  .......... .......... ......
     S323  .......... .......... ......
      S51  .......... .......... ......
      S45  .......... .......... ......
      S40  .......... .......... ......
      S38  .......... .......... ......
      S23  .......... .......... ......
      S22  .......... .......... ......
      S21  .......... .......... ......
      S20  .......... .......... ......
      S17  .......... .......... ......
    S29-1  .......... .......... ......
    S19-1  .......... .......... ......
     S437  .......... .......... ......
     S434  .......... .......... ......
      S53  .......... .......... ......
      S49  .......... .......... ......
      S39  .......... .......... ......
      S33  .......... .......... ......
      S32  .......... .......... ......
      S31  .......... .......... ......
      S19  .......... .......... ......
Consensus  .......... .......... ......
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10 representarive isolates with CIT-like

```
          1                                                                                                                                130
CMY-2     MMKKSLCCAL LLTASFSTFA AAKTEQQIAD IVNRTITPLM QEQAIPGMAV AVIYQGKPYY FTWGKADIAN NHPVTQQTLF ELGSVSKTFN GVLGGDAIAR GEIKLSDPVT KYWPELTGKQ WQGIRLLHLA
S23-1     .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S32       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S453      .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S394      .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S383      .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S339      .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S331      .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S306      .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S285      .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S278      .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Consensus .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

          131                                                                                                                              260
CMY-2     TYTAGGLPLQ IPDDVRDKAA LLHFYQNWQP QWTPGAKRLY ANSSIGLFGA LAVKPSGMSY EEAMTRRVLQ PLKLAHTWIT VPQNEQKDYA WGYREGKPVH VSPGQLDAEA YGVKSSVIDM ARWVQANMDA
S23-1     .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S32       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S453      .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S394      .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S383      .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S339      .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S331      .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S306      .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S285      .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S278      .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Consensus .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

          261                                                                                                                          381
CMY-2     SHVQEKTLQQ GIALAQSRYW RIGDMYQGLG WEMLNWPLKA DSIINGSDSK VALAALPAVE VNPPAPAVKA SWVHKTGSTG GFGSYVAFVP EKNLGIVMLA NKSYPNPVRV EAAWRILEKL Q
S23-1     .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .
S32       .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .
S453      .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .
S394      .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .
S383      .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .
S339      .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .
S331      .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .
S306      .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .
S285      .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .
S278      .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .
Consensus .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .
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NUCLEOTIDE SEQUENCES ALIGNMENT OF IS*ECP1* IN UPSTREAM REGION OF *BLA* GENES OF SELECTED ISOLATES

13 isolates with IS*Ecp1* in upstream region of *bla*$_{\text{CTX-M-55}}$ gene

```
           1                                                                                                                              130
 GQ456159  TGCTCTGTGG ATAACTTGCA GAGTTTATTA AGTATCATTG CAGCAAAGAT GAAATCAATG ATTTATCAAA AATGATTGAA AGGTGGTTGT AAATAATGTT ACAATGTGTG AGAAGCAGTC TAAATTCTTC
      S17                                                                         ... .......... .......... .......... .......... .......... ..........
      S45                                                                         ... .......... .......... .......... .......... .......... ..........
      S51                                                                         ... .......... .......... .......... .......... .......... ..........
      S49                                                                         ... .......... .......... .......... .......... .......... ..........
      S40                                                                         ... .......... .......... .......... .......... .......... ..........
      S39                                                                         ... .......... .......... .......... .......... .......... ..........
      S38                                                                         ... .......... .......... .......... .......... .......... ..........
      S31                                                                         ... .......... .......... .......... .......... .......... ..........
      S23                                                                         ... .......... .......... .......... .......... .......... ..........
      S22                                                                         ... .......... .......... .......... .......... .......... ..........
      S21                                                                         ... .......... .......... .......... .......... .......... ..........
      S20                                                                         ... .......... .......... .......... .......... .......... ..........
      S19                                                                         ... .......... .......... .......... .......... .......... ..........
Consensus                                                                         ... .......... .......... .......... .......... .......... ..........

           131                                                                                                                            260
 GQ456159  GTGAAATAGT GATTTTTGAA GCTAATAAAA AACACACGTG GAATTTAGGG ACTATTCATG TTGTTGTTAT TTCGTATCTT CCAGAATAAG GAATCCCATG GTTAAAAAAT CACTGCGCCA GTTCACGCTG
      S17  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S45  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S51  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S49  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S40  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S39  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S38  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S31  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S23  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S22  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S21  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S20  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S19  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Consensus  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
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1 isolate (S53) with IS*Ecp1* in upstream region of *bla*$_{\text{CTX-M-15}}$ gene

```
          1431                                                                                                                1560
AY458016  AAGGGAGTGT ATGAAAAATG TCTGGTATAA TAAGAATATC ATCAATAAAA TTGAGTGTTG CTCTGTGGAT AACTTGCAGA GTTTATTAAG TATCATTGCA GCAAAGATGA AATCAATGAT TTATCAAAAA
     S53  ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... .....
Consensus ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... .....

          1561                                                                                                                1690
AY458016  TGATTGAAAG GTGGTTGTAA ATAATGTTAC AATGTGTGAG AAGCAGTCTA AATTCTTCGT GAAATAGTGA TTTTTGAAGC TAATAAAAAA CACACGTGGA ATTTAGGGAC TATTCATGTT GTTGTTATTT
     S53  ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... .....
Consensus ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... .....

          1691                                                                                                                1820
AY458016  CGTATCTTCC AGAATAAGGA ATCCCATGGT TAAAAAATCA CTGCGCCAGT TCACGCTGAT GGCGACGGCA ACCGTCACGC TGTTGTTAGG AAGTGTGCCG CTGTATGCGC AAACGGCGGA CGTACAGCAA
     S53  ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... .....
Consensus ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... .....

          1821                                                                                                                1950
AY458016  AAACTTGCCG AATTAGAGCG GCAGTCGGGA GGCAGACTGG GTGTGGCATT GATTAACACA GCAGATAATT CGCAAATACT TTATCGTGCT GATGAGCGCT TTGCGATGTG CAGCACCAGT AAAGTGATGG
     S53  ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... .....
Consensus ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... .....

          1951                                                                                                                2080
AY458016  CCGCGGCCGC GGTGCTGAAG AAAAGTGAAA GCGAACCGAA TCTGTTAAAT CAGCGAGTTG AGATCAAAAA ATCTGACCTT GTTAACTATA ATCCGATTGC GGAAAAGCAC GTCAATGGGA CGATGTCACT
     S53  ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... .....
Consensus ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... .....

          2081                                                                                                                2210
AY458016  GGCTGAGCTT AGCGCGGCCG CGCTACAGTA CAGCGATAAC GTGGCGATGA ATAAGCTGAT TGCTCACGTT GGCGGCCCGG CTAGCGTCAC CGCGTTCGCC CGACAGCTGG GAGACGAAAC GTTCCGTCTC
     S53  ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... .....
Consensus ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... .....
```

10 representarive isolates with IS*Ecp1* in upstream region of *bla*$_{\text{CTX-M-14}}$ gene

```
          1431                                                                                                                1560
GQ892052  TGAAAAATGT CTGGTATAAT AAGAATATCA TCAATAAAAT TGAGTGTTGC TCTGTGGATA ACTTGCAGAG TTTATTAAGT ATCATTGCAG CAAAGATGAA ATCAATGATT TATCAAAAAT GATTGAAAGG
   S19-1                                                                                                                ...... ..........
     S299                                                                                                               ...... ..........
     S438                                                                                                               ...... ..........
     S429                                                                                                               ...... ..........
     S412                                                                                                               ...... ..........
     S400                                                                                                               ...... ..........
     S384                                                                                                               ...... ..........
     S307                                                                                                               ...... ..........
      S33                                                                                                               ...... ..........
      S32                                                                                                               ...... ..........
Consensus

          1561                                                                                                                1690
GQ892052  TGGTTGTAAA TAATGTTACA ATGTGTGAGA AGCAGTCTAA ATTCTTCGTG AAATAGTGAT TTTTGAAGCT AATAAAAAAC ACACGTGGAA TTTAGGGAAT ACTGATGTAA CACGGATTGA CCGTATTGGG
   S19-1  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     S299  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     S438  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     S429  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     S412  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     S400  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     S384  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     S307  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S33  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S32  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Consensus

          1691                                                                                                                1820
GQ892052  AGTTTGAGAT GGTGACAAAG AGAGTGCAAC GGATGATGTT CGCGGCGGCG GCGTGCATTC CGCTGCTGCT GGGCAGCGCG CCGCTTTATG CGCAGACGAG TGCGGTGCAG CAAAAGCTGG CGGCGCTGGA
   S19-1  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     S299  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     S438  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     S429  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     S412  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     S400  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     S384  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     S307  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S33  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
      S32  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Consensus
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10 representarive isolates with IS*Ecp1* in upstream region of *bla*$_{\text{CMY-2}}$ gene

```
          1431                                                                                                                1560
FJ621588  TGAAAAATGT CTGGTATAAT AAGAATATCA TCAATAAAAT TGAGTGTTGC TCTGTGGATA ACTTGCAGAG TTTATTAAGT ATCATTGCAG CAAAGATGAA ATCAATGATT TATCAAAAAT GATTGAAAGG
   S23-1                                                                                                                ...... ..........
     S32                                                                                                                ...... ..........
    S453                                                                                                                ...... ..........
    S394                                                                                                                ...... ..........
    S383                                                                                                                ...... ..........
    S339                                                                                                                ...... ..........
    S331                                                                                                                ...... ..........
    S306                                                                                                                ...... ..........
    S285                                                                                                                ...... ..........
    S278                                                                                                                ...... ..........
Consensus

          1561                                                                                                                1690
FJ621588  TGGTTGTAAA TAATGTTACA ATGTGTGAGA AGCAGTCTAA ATTCTTCGTG AAATAGTGAT TTTTGAAGCT AATAAAAAAC ACACGTGGAA TTTAGGAAAA ACTTATATCT GCTGCTAAAT TTAACCGTTT
   S23-1  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     S32  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
    S453  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
    S394  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
    S383  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
    S339  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
    S331  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
    S306  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
    S285  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
    S278  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Consensus

          1691                                                                                                                1820
FJ621588  GTCAACACGG TGCAAATCAA ACACACTGAT TGCGTCTGAC GGGCCCGGAC ACCTTTTTGC TTTTAATTAC GGAACTGATT TCATGATGAA AAAATCGTTA TGCTGCGCTC TGCTGCTGAC AGCCTCTTTC
   S23-1  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     S32  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
    S453  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
    S394  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
    S383  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
    S339  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
    S331  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
    S306  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
    S285  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
    S278  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Consensus
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APPENDIX F

DNA CODON

One- and Three-Letter symbols for the amino acids

A	Ala	Alanine
B	Asx	Asparagine or aspartic acid
C	Cys	Cysteine
D	Asp	Aspartic acid
E	Glu	Glutamic acid
F	Phe	Phenylalanine
G	Gly	Glycine
H	His	Histidine
I	Ile	Isoleucine
K	Lys	Lysine
L	Leu	Leucine
M	Met	Methionine
N	Asn	Asparagine
P	Pro	Proline
Q	Gln	Glutamine
R	Arg	Arginine
S	Ser	Serine
T	Thr	Threonine
V	Val	Valine
W	Trp	Trytophan
Y	Tyr	Tyrosine
Z	Glx	Gln or Glu

The standard genetic code

First position (5'end)	Second position				Third position (3' end)
	U	C	A	G	
U	UUU Phe	UCU Ser	UAU Tyr	UGU Cys	U
	UUU Phe	UCC Ser	UAC Tyr	UGC Cys	C
	UUA Leu	UCA Ser	UAA Stop	UGA Stop	A
	UUG Leu	UCG Ser	UAG Stop	UGG Trp	G
C	CUU Leu	CCU Pro	CAU His	CGU Arg	U
	CUC Leu	CCC Pro	CAC His	CGC Arg	C
	CUA Leu	CCA Pro	CAA Gln	CGA Arg	A
	CUG Leu	CCG Pro	CAG Gln	CGG Arg	G
A	AUU Ile	ACU Thr	AAU Asn	AGU Ser	U
	AUC Ile	ACC Thr	AAC Asn	AGC Ser	C
	AUA Ile	ACA Thr	AAA Lys	AGA Arg	A
	AUG Met[a]	ACG Thr	AAG Lys	AGG Arg	G
G	GUU Val	GCU Ala	GAU Asp	GGU Gly	U
	GUC Val	GCC Ala	GAC Asp	GGC Gly	C
	GUA Val	GCA Ala	GAA Glu	GGA Gly	A
	GUG Val	GCG Ala	GAG Glu	GGG Gly	G

[a]AUG forms part of the initiation signal as well as coding for internal Met residues.

APPENDIX G

SALMONELLA SEROTYPE

Serogroup	Serotype
B	Agona
	Brandenburg
	Derby
	Heidelberg
	I 4,[5],12:i:-
	Kiambu
	Paratyphi B
	Reading
	Saintpaul
	Sandiego
	Schwarzengrund
	Stanley
	Typhimurium
C1	Bareilly
	Braenderup
	Choleraesuis
	Hartford
	Infantis
	Mbandaka
	Montevideo
	Ohio
	Oranienburg
	Tennessee
	Thompson
	Virchow

Serogroup	Serotype
C2	Blockley
	Hadar
	I 8,20:-:z6
	Litchfield
	Manhattan
	Muenchen
	Newport
C3	Kentucky
D1	Berta
	Dublin
	Enteritidis
	Javiana
	Panama
	Typhi
E1	Anatum
	Meleagridis
	Muenster
	Uganda
E4	Senftenberg
F	Rubislaw
G1	Poona
	Worthington

Serogroup	Serotype
G2	Cubana
	Havana
	Mississippi
K	Cerro
	IIIa 18:z4,z32:-
L	Minnesota
O	Adelaide
R	Johannesburg

BIOGRAPHY

Miss Sirirat Luk-in was born on April 29, 1985 in Bangkok, Thailand. She graduated with the Bachelor degree of Science Science (Medical Technology) from the Faculty of Allied Health Sciences, Chulalongkorn University in 2006. She is currently a student in the Inter-Department of Medical Microbiology, Faculty of Graduate School, Chulalongkorn University since 2007.