CHAPTER 6

Discussion

The most common mechanism of resistance to β-lactam antibiotic among Enterobacteriaceae, predominantly *E. coli* and *K. pneumonia* is production of β-lactamases (Van Hoek *et al*., 2011). The resistance to third-generation cephalosporins and monobactams cause by two types of β-lactamase including ESBL and AmpC β-lactamase. These enzymes can degrade a broad range of β-lactam antibiotics. The plasmids containing genes-encoding ESBLs frequently also carry genes encoding resistance to other antimicrobial agents. Therefore, the choice of treatment option is limited (Paterson, 2000). Patients infected with ESBL-producing strain have to take more antibiotics and longer stay in hospital than those who are not infected with this strain. Currently, the spread of ESBL-producing *E. coli* was increased when compared with previous report in 1994 and 1996 (9.6%) (Lulitanond and Kaewkes, 1999) and this stain showed higher resistance to ceftazidime than cefotaxime. The *bla*$_{\text{SHV-12}}$ gene was the most prevalent genes in this strain while *bla*$_{\text{CTX-M}}$ was undetected (Chanawong *et al*., 2001). The *bla*$_{\text{CTX-M}}$ gene was first reported in Thailand during 1998 and 1999. The prevalence of *bla*$_{\text{CTX-M}}$ gene in Enterobacteriaceae was 52% (CTX-M-9 only) and increased to 65% in 2003 which comprising CTX-M-15 (44%), CTX-M-14 (11%) and CTX-M-9 (10%) (Chanawong, *et al*., 2007). In present study, the results showed that a highly incident of *bla*$_{\text{CTX-M}}$ genes (80.7%), which was in accordance with the previous study (Tribuddharat *et al*., 2007; Kiratisin *et al*., 2008; Maina *et al*., 2012). The major group of *bla*$_{\text{CTX-M}}$ genes was *bla*$_{\text{CTX-M-1subgroup}}$ including *bla*$_{\text{CTX-M-15}}$ 43 isolates (23.6%) and *bla*$_{\text{CTX-M-55}}$ 47 isolates (25.8%). CTX-M-15 and CTX-M-55 were first found in Thailand in 2007 (Chanawong *et al*, 2007; Kiratisin *et al*., 2007). In 2008, CTX-M-15 and CTX-M-55 were reported in 37 % and 17.4%, respectively (Kiratisin *et al*., 2008). These finding suggested that CTX-M-55 was increasingly detected. The results showed that the most gene combination was *bla*$_{\text{TEM}}$ and *bla*$_{\text{CTX-M-1subgroup}}$. These

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results support the hypothesis that $bla_{\text{CTX-M-15}}$ gene often coexists with $bla_{\text{TEM-1}}$ gene on the same plasmid (Carattoli, 2009). Moreover, the results of this study were found $bla_{\text{CTX-M-55}}$ gene coexists with bla_{TEM} gene in the same isolates.

The acquired resistant genes and impropriated use of third-generation cephalosporins has led to the antibiotic stress to select resistant strains and finally resulted to cause of resistant problem. Therefore, the association of ESBL genotypes with cephalosporin resistance may be advantageous in the selection of appropriate antimicrobial agents. The presence of the ESBL genes generally was correlated with various degrees of resistance to cefotaxime and ceftazidime, the genotypic information could be used to predict the susceptibility pattern to a particular antibiotic. The results demonstrated that the presence of $bla_{\text{CTX-M}}$ ($bla_{\text{CTX-M-1 subgroup}}$ and $bla_{\text{CTX-M-9 subgroup}}$) significantly correlated with cefotaxime-resistant strain. Especially, $bla_{\text{CTX-M-1 subgroup}}$ correlated with the high resistance to this drug. Corresponding, the study of Tribuddharat and colleagues has shown that ESBL-producing *K. pneumonia* from 30 patients hospitalized at Siriraj Hospital, Thailand. The presence $bla_{\text{CTX-M}}$ gene was correlated with resistance to cefotaxime and ceftriaxone (Tribuddharat *et al.*, 2007). The presence of bla_{SHV} and $bla_{\text{CTX-M-1subgroup}}$ were correlated with the resistance to ceftazidime, whereas previous study reported that the $bla_{\text{CTX-M}}$ gene did not correlated with the resistance to this drug (Tribuddharat *et al.*, 2007). The resistance to ceftazidime did not significantly associate with the presence of $bla_{\text{CTX-M-9subgroup}}$. In addition, the most of CTX-M-1 subgroup-producing isolates exhibited a higher level of resistance to ceftazidime than what appeared for the CTX-M-9 subgroup. Eleven of CTX-M-9 subgroup-producing isolates (84.6%) (without other ESBL type) were susceptible and intermediate to ceftazidime. In the previous study, ceftazidime can be used in the treatment of CTX-M ESBL-producing *E. coli* (CTX-M-3, CTX-M-14, or CTX-M-27) which cause of bacteremia due to urinary tract infection and biliary tract infection when the MIC of ceftazidime were ≤8 μg/ml (Bin *et al.*, 2006). Whereas, in some $bla_{\text{CTX-M-9 subgroup}}$ –carrying isolates were non-susceptible to ceftazidime. There was specific point mutation in the active site in these ESBL. They could confer an extraordinary substrate preference, for example the P167S mutation at the omega loop of CTX-M-19 or L169Q mutation in CTX-M-93 that differs from CTX-M-27 in single point mutation. These enzyme increased activity against ceftazidime more than other variants of CTX-M

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ESBL (Poirel *et al*., 2001; Kimura *et al*., 2004; Djamdjian *et al*., 2011). Accordingly, the appropriated use of antibiotic against ESBL-producing strain should be considered the susceptibility pattern and background of ESBL types. However, the presence of bla_{TEM} genes did not significantly associated with the resistance to cefotaxime and ceftazidime. The result was consistent with the previous studies (Tribuddharat *et al*., 2007; Maina *et al*., 2012). However, the result from this study cannot be concluded that the correlation between bla_{TEM} and antimicrobial susceptibility. To identify the ESBL type of bla_{TEM} gene, the DNA sequencing is required.

In recent years, the prevalence of plasmid-mediated AmpC β-lactamase-producing strains which resist to third-generation cephalosporins has increased among Enterobacteriaceae in many countries (Jacoby, 2009) The coproducing ESBL together with AmpC β-lactamase have been reported in Far East (Song *et al*., 2006; Yan *et al*., 2004). They demonstrate positive result by screening CLSI criteria for ESBL, while their commonly give a negative confirmatory test for ESBL production. Because AmpC β-lactamases were against to β-lactamase inhibitors so false-negative results were showed when using combined disk diffusion or double disc diffusion test. Therefore, genotypic method was accurate and useful for β-lactamase detection. AmpC β-lactamase detection is not routinely carried out in many microbiology units of service laboratories. A recently, disk diffusion test based on comparison of the inhibition zone diameters around a cefoxitin disk and a cefoxitin-cloxacillin disk were used to detect AmpC β-lactamase. (Peter-Getzlaff *et al*., 2011). The sensitivity and specificity of the disk diffusion test was 95% for the detection of AmpC β-lactamase in 127 strains of *E. coli*, *Klebsiella* spp., and *Proteus* spp. (Tan *et al*., 2009). Diagnostic laboratories should be concerned in different AmpC β-lactamase mediated resistance from other β-lactamase resistance mechanisms and need to use combination of phenotypic and molecular characterization methods. The multiplex PCR technique described in this study will be an important tool for the detection of AmpC β-lactamase and ESBL genes in Gram-negative bacteria. Thus, this finding provided the detection methods for AmpC enzymes in those isolates, which are already designated to be ESBL positive. The coexistence of different classes of β-lactamases in a single bacterial isolate shows a challenge both in diagnosis and therapy.

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The prevalence of plasmid AmpC β-lactamase is a few information in Thailand and not known in Chiang Mai. The first report of *ampC* genes were *bla*$_{CMY-2}$ (46 isolates), *bla*$_{CMY-8b}$ (4 isolates) and *bla*$_{DHA-1}$ (2 isolates) collected from patients who infected with *E. coli* and *K. pneumonia* in Srinagarind Hospital (Singtohin *et al*., 2010). In the present study, *ampC* genes was detected in 37% of cefotxitin resistance isolates, which all isolates carried a *bla*$_{CMY-2}$ gene (10 isolates) and this gene was higher found than in previous studies. The *ampC* genes were detected in 12.5 % of cefotxitin resistance isolates (Manoharan *et al*., 2012). In the study, some of cefoxitin resistant isolates were not positive for AmpC production by the disc synergy test or Multiplex PCR, Two reasons could explain this observation. First, these isolates exhibit cefoxitin-resistant due to other mechanisms and laboratory was not detected. Second, the recommendation of inhibition zone (< 18 mm) was inappropriate because it provided false positive results. Two isolates show that positive by double disc synergy test were not detectable *bla*$_{CMY}$ and *bla*$_{DHA}$, it might have other *bla* genes. In contrast, one isolate was negative by double disc synergy test but *bla*$_{CMY}$ gene was found. This result might be low level expression of this gene or technical error of phenotypic test.

AmpC β-lactamases also had differential activity on substrates. *E. coli* with ACC-1 could be resistant to ceftazidime but not to cefotaxime or cefotetan. DHA-2 may showed intermediate resistance to cefoxitin but they were susceptible to cefotaxime or ceftazidime. While isolates of CMY-2 could be resistant to cefotaxime, ceftazidime and cefoxitin but they were susceptible to meropenem and imipenem (Philippon *et al*., 2002). In our study, the results could not be discerned, further analysis of isolates including cephamycin hydrolysis assay is necessary to verify these effects. However, the results showed that the presences of *bla*$_{CMY-2}$ and *bla*$_{CMY-2}$ + *bla*$_{TEM}$ were usually found higher resistance to ceftazidime than cefotaxime.

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