CHAPTER 1

Introduction

Escherichia coli (*E. coli*) is a Gram-negative bacterium in the family Enterobacteriaceae. *E. coli* are commonly found in the human intestinal tract but some pathotypes are competent of causing disease in healthy people. This bacterium causes the most common infections in the urinary tract. Some strains can cause intestinal disease such as diarrhea and extraintestinal diseases such as septicemia and neonatal meningitis. During the past 20 years, the antibiotic most widely used to treat *E. coli* infection are extended spectrum cephalosporins (third generation cephalosporins), such as cefotaxime, ceftazidime and ceftriaxone. The introduction of the extended spectrum cephalosporin into clinical practice in the early 1980s was regarded discovery in the fight against β-lactamase-mediated bacterial resistance to antibiotic. After clinical use of the extended spectrum cephalosporin, resistant isolates have emerged in many countries (Bradford, 2001). The tendency of resistance to extended spectrum cephalosporins in *E. coli* is increasing due to the major mechanisms of resistance are the β-lactamase enzymes produced by the organism, especially extended spectrum β-lactamase and AmpC β-lactamase. The enzymes are known to protect Gram-negative bacteria against the effect of β-lactams including penicillins, cephalosporins or monobactams. The increasing resistance to extended spectrum cephalosporin is due to acquiring the resistance genes together with selective pressure of the overuse and misuse of this group of beta-lactam.

Extended spectrum β-lactamase (ESBLs) are enzymes that cause resistance to a broad range of β-lactam antibiotic including penicillins, aztreonam, and cephalosporins except cephamycins. ESBL-type enzymes are developed from original TEM and SHV beta-lactamase enzymes, with substitution of one or more amino acid residue around the active site (Paterson *et al*., 2001). These changes alter the catalytic site allowing the hydrolysis of extended-spectrum cephalosporins. In early 1980s, TEM-ESBL and SHV-

ESBL were dominant among ESBL types. In the past decade, CTX-M- ESBL has emerged and become the most common ESBL worldwide (Caton and Coque, 2006). Insertion of IS*Ecp1* element frequently present in the upstream region of the $bla_{\text{CTX-M}}$ genes play an important role in mobilization and expression of these gene may explain the current spread of CTX-M-type enzymes worldwide (Poirel *et al*., 2003). ESBL are most commonly found in Enterobacteriaceae, especially infections strain of *E. coli* and *Klebsiella* sp. (Livermore, 1995). ESBL genes are usually encoded on plasmids, which can easily be transferred between isolates (Jacoby and Medeiros, 1991). In the last decade, AmpC β-lactamase has increased drastically among clinical isolates of *E. coli* and *K. pneumonia* (Jacoby, 2009). AmpC β-lactamases are enzymes that confer resistance to many β-lactam antibiotics. AmpC β-lactamase are the enzymes that hydrolyze third-generation cephalosprins, but unlike ESBL, they are also resistant to group of cephamycins, such as cefotetan, cefoxitin, or cefmetazole, and are active against inhibition by clavulanate or other β-lactamase inhibitor (Rupp and Fey, 2003; Jacoby and Munoz-Price, 2005). Moreover, in contrast to ESBL, AmpC β-lactamases are inhibited by boronic acid and cloxacillin (Beesley *et al*., 1983; Jacoby, 2009; Tan *et al*., 2009). These enzymes are typically encoded on the chromosomes of many gram negative bacteria including *E. coli*, *Citrobactor freundii* and *Enterobacter* spp. but can also been found on plasmid (Philippon *et al*., 2002). In *E. coli,* overproductions of their chromosomal AmpC β-lactamase cause of cefoxitin resistance more often than plasmid-mediated AmpC β-lactamases (Sasirekha and Shivakumar, 2012). Some ESBL-producing Enterobacteriaceae also express AmpC β-lactamases and clinical data show that the prognosis of infections with strain that produce ESBL and/or AmpC β-lactamase is worse that non-producing strain (Ramphal and Ambrose, 2006; Pai *et al*., 2004; Park *et al*., 2009; Sidjabat *et al*., 2009). Although Clinical and Laboratory Standards Institute (CLSI) has promulgation recommendation for screening and confirmation protocols of ESBL-producing organisms are usually reliable for identity ESBL, sometime false-negative results can occur when those strains carrying both ESBLs and AmpC β-lactamases because this enzyme can interfere ESBLs detection. Most clinical laboratories did not detect AmpC β-lactamases producer. While AmpC β-lactamases were positive in screening CLSI criteria for ESBLs but they commonly give a negative confirmatory test for ESBL production. Because this test is based on the

principle that ESBL are inhibited by Clavanulate. This proposes that subsequent test must design to insist and differentiate AmpC β-lactamases from ESBL. Pai and colleagues reported treatment failure and high mortality rate of patients with septicemia caused by plasmid-mediated AmpC β-lactamases producing *K. pneumonia* and receive third-generation cephalosporins (Pai *et al*., 2004). Therefore, it is of clinical importance to detect AmpC β-lactamases in *E. coli* and *K. pneumonia* which are frequently encountered as nosocomial pathogens. In Thailand, the study in Srinagarind Hospital, Khon Kaen, found that the prevalence of ESBL-producing Enterobacteriaceae was 26% among the clinical isolates collected and 35% of the isolates were resistant to ceftazidime with MIC ≥ 8μg/ml (Chanawong *et al*., 2007; Lulitanond and Kaewkes, 1999). Subsequently, beta-lactamase genes were detected in these clinical isolates of Enterobacteriaceae. Among 48 isolates collected between 1998 and 1999, there were bla_{SHV} (79%), $bla_{\text{CTX-M-9}}$ (52%), $bla_{\text{TEM-1}}$ (48%) and bla_{VEB} (33%) whereas isolates collected in 2003 were found to carry $bla_{\text{TEM-1}}$ (79%), $bla_{\text{CTX-M-15}}$ (44%), bla_{SHV} (36%), bla_{VEB} (36%), $bla_{\text{CTX -M-14}}$ (11%) and $bla_{\text{CTX-M-9}}$ (10%) (Chanawong *et al*., 2007). At Maharaj Nakorn Chiang Mai Hospital, the largest university hospital in northern Thailand, the number of patients who were infected with ESBL-producing *E. coli* has been increasing every year. The percentage of ESBL increased from 13% (398 isolates) in 2003 (Tharavichitkul *et al*., 2005) to 44.3% (2,166 isolates) in 2012 (Data from Microbiology Laboratory, Maharaj Nakorn Chiang Mai Hospital). However, AmpC β-lactamases producing strains are not detectable in routine susceptibility test and and there is no established guidelines from the CLSI are available for the detection of AmpC β-lactamases. In Chiang Mai, there are a few information or report on the prevalence of ESBL and AmpC- β lactamase genes. Therefore, The purposes of this study are i) to examine the incidence of ESBL and AmpC β-lactamases from clinical isolates, and ii) to investigate the correlation between the phenotype regarding minimal inhibitory concentration (MIC) and the ESBL genotypes of ESBL-producing isolates from patients in Maharaj Nakorn Chiang Mai Hospital between April to May, 2010. The association of ESBL genotypes with cephalosporin resistance may be useful in providing the guidelines for clinicians in the selection of appropriate antimicrobial agents for treatment of patients infected with ESBL producing *E. coli*.