CHAPTER II

LITERATURE REVIEWS

1. Extended Spectrum-Beta-lactamase producing *E.coli.*

Emergence of resistance to beta-lactam antibiotics began even before the first beta-lactam, penicillin, was developed. The most frequent and most efficient mechanism of resistance to beta-lactams is the production of beta-lactamase enzymes. (Sykes and Matthew, 1993; Sanders and Sanders, 1992). Beta-lactamases catalyze the hydrolysis of the bata-lactam ring, splitting the amide bond (Figure 2-1). As a result, the antibiotics can no longer inhibit bacterial cell wall synthesis.

Figure 2-1 Hydrolysis of the beta-lactam ring by beta-lactamases

Many genera of gram negative bacteria posses a naturally occurring, chromosomally-mediated beta-lactamase, which are inducible and are not inhibited by clavulanic acid. *E.coli* and *Shigella spp*, produce a small amout of chromosomal-medited AmpC beta-lactamase and are susceptible to ampicilin and other beta-lactam agents. Plasmid-mediated beta-lactamase, which are inhibited by clavulanic acid, can be transferred between various species of *Enterobacteriaceae.* The first plasmid-mediated beta-lactamase in gram-negative, TEM-1, was described in the early 1980s (Datta and Kontomichalou, 1965). The TEM-1 enzyme was originally found in a single strain of *E.coli* isolated from a blood culture from a patient named Temoniera in Greece, hence the designation TEM (Mederiros *et al.*, 1984). Being plasmid and transposon mediated has facilitated the spread of TEM-1 to other species of bacteria. Within a few years after its first isolation, the TEM-1 beta-lactamase spread worldwide and is now found in many different species of members of the family Enterobacteriaceae,

Pseudomonas aeruginosa, Haemophilus influenzae, and Neisseria gonorrhoeae. Another common plasmid-mediated beta-lactamase found in *Klebsiella pneumoniae* and *E.coli* is SHV-1 (for sulfhydryl variable). The SHV-1 beta-lactamase is chromosomally encoded in the majority of isolates of *K.pneumoniae* but is usually plasmid mediated in *E.coli.* Members of the family Enterobactericeae producing TEM-1 or SHV-1 beta-lactamase result in the resistance to ampicillin, ticarcillin, first-generation cephalosporins and piperacillin.

Over the last 20 years, many new beta-lactam antibiotics have been developed that were specificially designed to be resistant to the hydrolytic action of beta-lactamases. One of these new classes was the oxyimino-cephalosporins, which become widely used for the treatment of serious infections due to gram-negative bacteria in the 1980s.

Not surprisingly, resistance to these expanded-spectrum beta-lactam antibiotics due to beta-lactamases emerged quickly. The first of these enzymes capable of hydrolyzing the newer beta-lactams, SHV-2, was found in a single strain of *Klebsiella ozaenae* isolated in Germany (Kliebe *et al.*, 1985). Because of their increased spectrum of activity, especially against the oxyimino-cephalosporins, these enzymes were called extended-spectrum betalactamases (ESBLs). Today, over 150 different ESBLs have been described. Most ESBLs are derivatives of TEM or SHV enzyme and are most often found in *E.coli* and *K.pneumoniae*; however, they have also been found in *Proteus spp.*, *Providercia spp.*, and other genera of Enterobacteriaceae. These enzymes are plasmid mediated enzyme capable of hydrolyzing penicillins, oxymino-cephalosporin (e.g. cefuroxime, cefotaxime, ceftazidime and ceftriaxine) and monobactams (e.g. azetreonam), and thus are inhibited by beta-lactamase inhibitors such as clavulanic acid.

Plasmids are responsible for ESBL production, tend to be large (80 Kb or more in size), which can transfer the resistance to several agents, an important limitation in the design of treatment alternatives (Jacoby and Medeiros, 1991). The most frequent coresistances found in ESBL producing organisms are aminoglycosides, fluoroquinolones, tetracyclines, chloramphenicol and sulfamethoxazole-trimethoprim (Nathisuwan et al, 2001).

2. The prevalence of ESBL-producing *E.coli*

ESBL-producing strains have emerged among the Enterobacteriaceae, prominently in *Escherichia coli* and *Klebsiella pneumoniae*. They were first isolated in Germany in 1983 (Knothe et al., 1983), and the rapid dissemination has been responsible for numerous outbreaks of infections throughout the world, causing a major problem in many clinical setting especially in a tertiary-care medical center (Winokur et al., 2001). Although ESBL-producing *E.coli* resulting in beta-lactam antimicrobial resistance are commonly detected in nosocomial infection, recent study has reported the emergence of community-acquired infections caused by ESBL-producing *E.coli* in Canada, France, Israel, Spain, Italy, and United Kingdom (Rodriguez-Bano et al., 2004; Woodford et al., 2004; Church, 2005).

The prevalence of ESBL-producing Enterobacteriaceae especially *K. pneumoniae* and *E. coli*, among clinical isolates varies according to the type of hospital and country. For example, the prevalence of ESBL-producing *K.pneumoniae* was found to be highest from Latin America (45.4%), the Western Pacific region (24.6%), and Europe (22.6%),and lowest from the United States (7.6%), and Canada (5.9%) (Winokur et al., 2001). The prevalence of ESBL-producing *E.coli* was 8.5 %, 7.9 %, 5.3 %, 3.3 %, and 4.2 % in isolates from Latin America, the Western Pacific region and Europe, resprctively, while the lowest prevalence from the United States, and Canada, respectively.(Winokur et al., 2001).

In France, as many as 40% of *K.pneumoniae* isolates were found to be ceftazidime resistant (Branger et al., 1998). In Japan, the percentage of beta-lactam resistance due to ESBL production in *E. coli* and *K. pneumoniae* remains very low. In recent survey among the 196 institutions across the country, <0.1% of *E. coli* and 0.3% of *K. pneumoniae* strains possessed an ESBL (Yagi et al., 2000). Elsewhere in Asia, the percentage of ESBL production in *E. coli* and *K. pneumoniae* varies, from 4.8% in Korea (Pai et al., 1999) to 8.5% in Taiwan (Yan et al., 2000) and up to 12% in Hong Kong (Ho et al., 2000).

In Thailand, the data from National Antimicrobial Resistance Surveillance Thailand (NARST) showed the prevalence of ESBL-producing *K.pneumoniae* and *E.coli* from 2002 to 2003 were 33% and 15 %, respectively (Dejsirilert et al., 2004). An

increase in the prevalence of ampicillin- resistant *E.coli* has been observed from 1998-2006 as shown in Table 2-1 (http://narst.dmsc.moph.go.th/).

Table 2-1 Antibiogram Year 1998-2006 from 32 Hospitals in Thailand (http://narst.dmsc.moph.go.th/)

Year	Percentage of susceptible *Escherichia coli*														
	AMP			AMC			CAZ			NOR			SXT		
	Pus	Urine	Blood	Pus	Urine	Blood	Pus	Urine	Blood	Pus	Urine	Blood	Pus	Urine	Blood
1998	23.88	19.62	28.24	71.71	65.80	75.47	93.87	94.77	95.91	74.79	62.73	91.21	36.46	26.82	85.23
1999	25.73	18.46	24.79	68.19	64.01	74.86	93.35	94.35	97.41	72.97	64.76	78.75	37.23	27.22	33.71
2000	23.53	18.62	25.76	68.52	64.11	75.22	89.58	87.14	91.48	65.80	58.37	81.08	37.45	31.95	38.90
2001	25.39	19.36	26.51	72.83	68.04	81.09	91.09	89.23	94.40	71.22	58.69	78.43	38.01	30.46	38.13
2002	23.53	18.62	25.76	68.52	64.11	75.22	89.58	87.14	91.48	65.80	58.37	81.08	37.45	31.95	38.90
2003	25.11	17.74	25.88	66.85	60.82	72.53	86.80	83.97	89.92	60.35	54.95	72.02	39.73	34.30	49.54
2004	22.06	16.77	22.30	65.36	63.55	74.61	81.71	84.13	90.51	64.32	56.15	73.31	41.17	35.40	39.76
2005	17.84	15.88	23.26	60.27	62.34	73.85	78.83	82.46	88.96	70.54	53.99	78.45	37.26	34,23	41.58
2006	19.80	16.92	23.19	66.02	65.17	74.90	83.36	83.54	93.19	71.79	56.50	79.31	45.48	35.47	45.49

AMP=ampicillin; AMC=amoxicillin/clavulanic acid; CAZ=ceftazidime; SXT=co_trimoxazole, NOR = norfloxacin

The data from Songklanagarind Hospital showed that 32 % and 19 % ESBL-producing *K.pneumoniae* and *E.coli* were isolated in 2002 (Ingviya et al., 2003) and the susceptibility patterns of ESBL-producing *E.coli* isolated from patients with community acquired urinary tract infection (UTI) were detected in 6 out of 107 (6%) urine isolates; all of which were resistant to ampicillin, cefazolin, and cefuroxime. Of these 6 isolates, 67%, 50%, and 50 % were resistant to gentamicin, cefotaxime, and norfloxacin, respectively (Tunyapanit and Pruekprasert, 2006). A total of 15,530 bacterial isolates from 6,192 hospitalized patients in Chonburi Hospital, which were collected from January to December,2005, 1,589 isolates (10.23%) were *E.coli*, and 1,055 isolates (6.79%) were *K.pneumoniae*. All *E.coli*, and *K.pneumoniae* isolates were tested for the ESBL production and the antimicrobial susceptibility. 38.20 % (607 of 1,589) of *E.coli* and 50.90 % (537 of 1,055) of *K.pneumoniae* produced ESBLs. The prevalence of ESBL-producing bacteria in Trang Hospital, was determined during 2004. The majority of ESBL-producing *E.coli* isolates were recovered from urine (44%) and sputum (20.4%),

while the majority of ESBL-producing *K.pneumoniae* were isolated from sputum (53.3%) and urine (20.6%) (Kwanhian et al., 2005).

Serious infections from ESBL-producing pathogen are usually hospital-acquired. Therapy for infections caused by these pathogens is usually difficult , since they are not only also resistant to penicillins, cephalosporins, and the monobactam aztreonam but also to the other classes of antimicrobials (Schwaber et al., 2005). Therefore, the detection of ESBL-producing bacteria in clinical microbiology laboratory could provide useful information for both clinical treatment and epidemiologic control of the pathogen

3. Antimicrobial Therapy for ESBL – producing *E.coli*

Despite the increased recognition of ESBL-producing organisms as an important resistance threat, there have been no randomized controlled clinical trials on therapy of such infections. The therapeutic choice in infections caused by such strains remain extremely limited due to broad cross resistance (e.g. aminoglycosides, co-trimoxazole or fluoroquinolones) and due to the broad spectrum of the beta-lactamases produced by these organisms (Paterson et.al., 2000; Essack, 2000). At present, carbapenems (e.g. imipenem, meropenem) are regarded as the drug of choice against ESBL-producing organisms since they are uniformly active *in vitro* and *in vivo* against these strains (Bell et al., 2002; Jone et al., 1998). The details of these antimicrobials are as followed:

3.1 Beta-Lactam Antibiotics

Beta-lactam antibiotics, which are named for the beta-lactam ring in their chemical structure (Mandell and Perti, 1996), include the penicillins, cephalosporins, monobactam and carbapenemscompounds. These agents are active against many gram-positive, gram-negative and anaerobic organisms.

Mechanism of action and resistance

The cell walls of bacteria are essential for their normal growth and development. Peptidoglycan is a heteropolymeric component of the cell wall that provides rigid mechanical stability by virtue of its highly cross-linked lactticework. Gram-positive bacterial cell walls contain peptidoglycan and teichoic or teichuronic acid,

and the bacterium may or may not be surrounded by a protein or polysaccharide envelope. Gram-negative bacterial cell walls contain peptidoglycan, lipopolysaccharide, lipoprotein, phospholipid, and protein (Figure 2-2). The critical attack site of anti-cell-wall agents is the peptidoglycan layer. This layer is essential for the survival of bacteria in hypotonic environments; loss or damage of this layer destroys the rigidity of the bacterial cell wall, resulting in death.

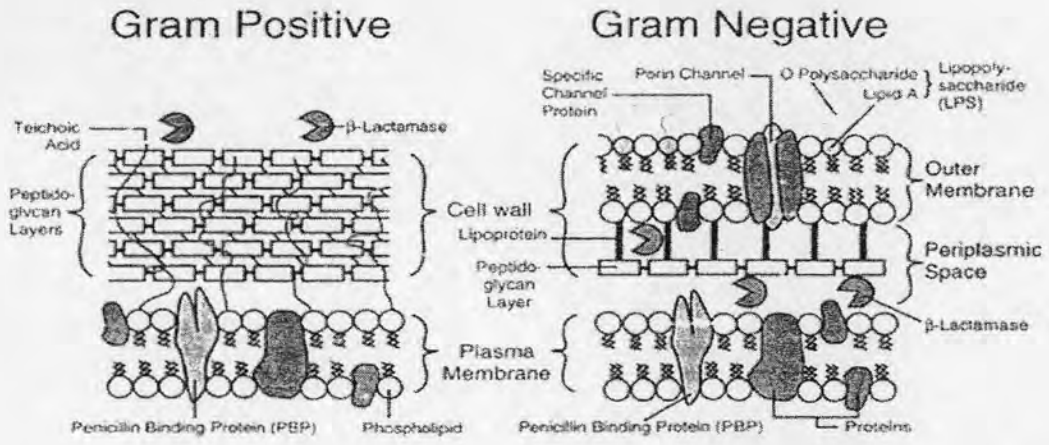


Figure 2-2 Outer wall of Gram-positive and Gram-negative species and dealof porin channels of Gram-negative bacteria. (Modified from Tortora et al., 1989)

Peptidoglycan synthesis that is inhibited by the beta-lactam antibiotics. The transpeptidase probably is acylated by penicillin; that is, penicilloyl enzyme apparently is formed, with cleavage of the –CO-N- bond of beta-lactam ring. Related targets for the actions of beta-lactam antibiotics; these are collectively termed penicillin-binding proteins (PBP; Spratt, 1980; Ghnysen, 1991). All bacteria have several such entities; for example, *S. aureus* has 4 PBPs, while *E.coli* has at least seven. The PBPs vary in their affinities for different beta-lactam antibiotics. The PBPs, to which a particular beta-lactam antibiotic binds, affects the morphologic response of the bacterium to the agent. For example, some antibiotics bind to a penicillin-binding protein that is involved in forming the septum between dividing cells; as a result, the bacteria continue to grow into long filaments, which eventually die. Binding to another penicillin-binding protein results in rapid lysis of a bacterium because the wall bulges and the bacterium bursts.

Although most or all bacteria contains PBPs, beta-lactam antibiotics cannot kill or even inhibit all bacteria, and various mechanism of bacterial resistance to these agents are operative. The microorganism may be intrinsically resistant because of structural differences in the PBPs that are the targets of these drugs. In gram negative bacteria beta-lactamases are found in relatively small amounts, but are located in periplasmic space between the inner and outer cell membranes (Figure 2-2). Since the enzyme of cell wall synthesis are on the outer surface of the inner membrane, these Beta-lactamases are strategically located for maximal protection of the microbe. Beta-lactamases of gram-negative bacteria are encoded either in chromosomes or in plasmids, and they maybe constitutive or inducible. The plasmids can be transferred between bacteria by conjugation. These enzymes may hydrolyze penicillins, cephalosporins, or both (Davies, 1994). Other instances of bacterial resistance to the beta-lactam antibiotics are caused by the inability of the agent to penetrate to its site of action or by energy-dependent efflux systems for pumping the antibiotic out of the bacteria (Jacoby, 1994; Nikaido, 1998) [Figure 2-3].

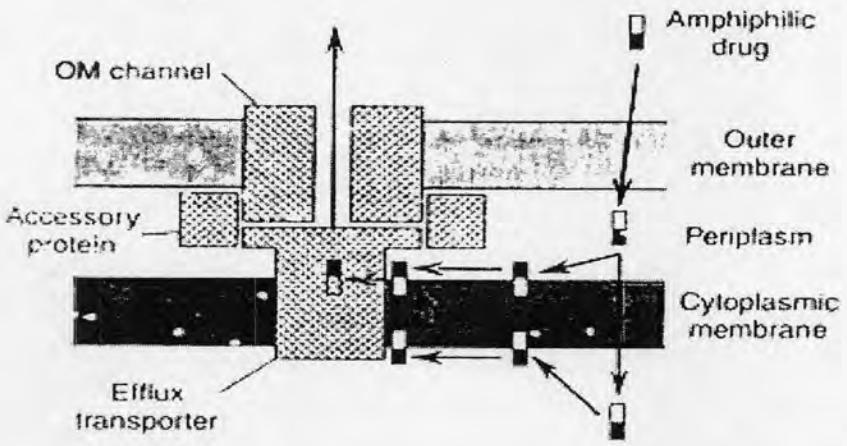


Figure 2-3 Antibiotic efflux pumps of gram-negative bacteria. (Nikaido,1998)

3.1.1 Ampicillin and amoxicillin have a wider spectrum of antibacterial activity than penicillin G in that they have greater activity against gram negative organism. They are all destroyed by beta-lactamase and are somewhat less potent than penicillin G against many gram positive cocci (Figure 2-4). however, ampicillin is readily cleaved by beta-lactamase and is useless in the treatment of

infections caused by *Staphylococcus aureus* or other organisms producing this enzyme. Plasmids conferring ampicillin resistance have appeared in *Salmonella typhi, Haemophilus influenzae*, and *N. gonorrhoeae*. Increasing resistance has appeared in strains of *E. coli, Streptococcus pneumoniae, Neisseria gonorrhea*, and nontyphoidal *Salmonella*

Broader-spectrum penicillins, such as ampicillin and amoxicillin, and the most of the cephalosporins diffuse through the pores in the *E.coli* outer membrane significantly more rapidly than can penicillin G. The number and size of pores in the outer membrane are variable among different gram-bacteria. Active efflux pumps serve as another mechanism of resistance, removing the antibiotic from its site of action before it can act (Nikaido, 1998). This is an important mechanism of beta-lactam resistance in *P. aeruginosa*, *E.coli*, and *N. gonorrhoeae*.

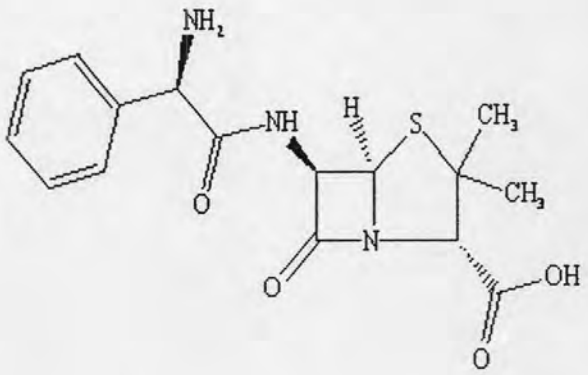


Figure 2-4 Structure of Ampicillin

Beta -lactamase inhibitors such as clavulanate and sulbactam are used to extend the spectrum of penicillins agents against beta-lactamase-producing organisms. These drugs essentially inactivates the enzyme by binding ti its active site, making these enzymes capable of beta-lactam ring cleavage (Figure 2-4c). In clinical situations in which there is increased development of beta-lactamase- producing organisms, amoxicillin-clavulanate may be the first choice for the treatment of otitis media, sinusitis, bronchitis, urinary tract infections and skin and soft tissue infections.

Because of its anaerobic coverage, amoxicillin-clavulanate is an excellent drug for treating infections caused by human and animal bites.

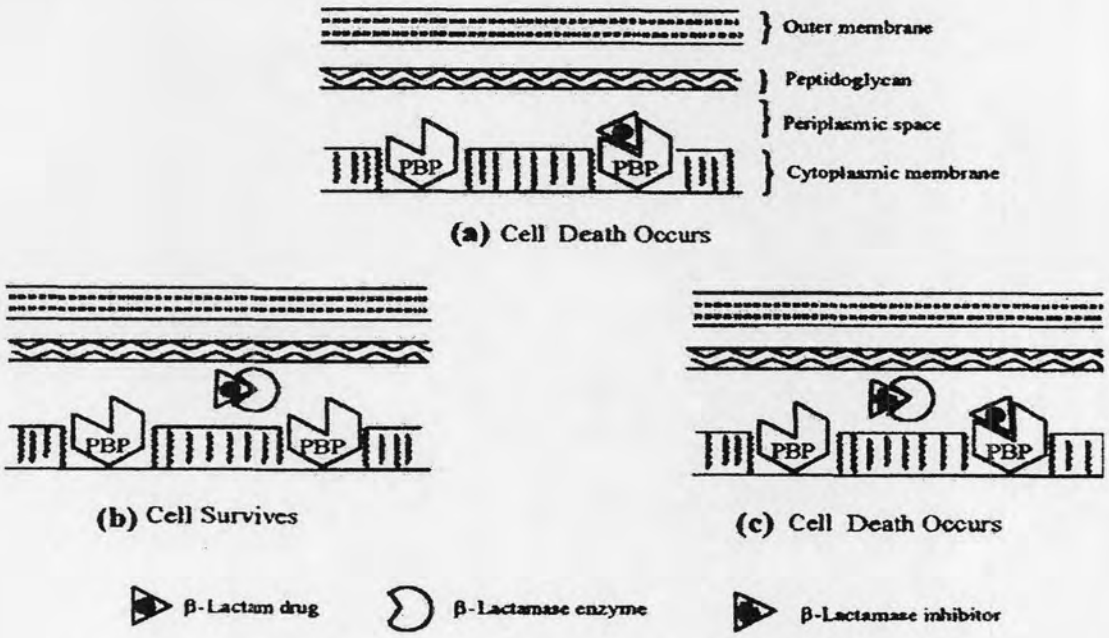


Figure 2- 5 Role of beta-lactam inhibitor (Sandanayaka and Prashad, 2002)

3.1.2 Carbapenames have the broadest antimicrobial spectrum of any antibiotic, e.g. imipenem and meropenem. Imipenem is derived from a compound produced by *Streptomyces cattleya*. Imipenem was effective against most clinically important gram-positive and gram-negative bacteria, including anaerobes. The activity of imipem is excellent in the Enterobacteriaceae, including microorganisms that are cephalosporin-resistant by virtue of expression of chromosomal or plasmid extended-spectrum beta-lactamase. Most strains of *Pseudomonas* and *Acinetobacter* are inhibited. The structure formula of imipenem is as follows (Figure 2-6):

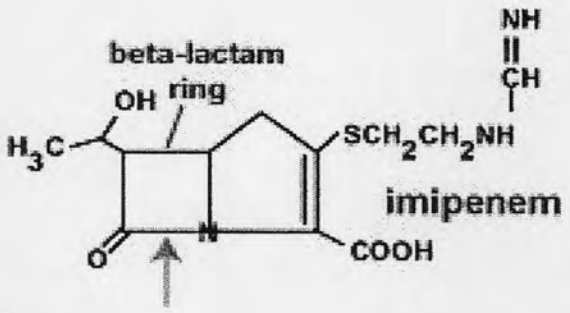


Figure 2-6 Structure of Imipenem

Resistance to imipenem can be caused by metallo-beta-lactamases, which these enzymes are capable of hydrolyzing beta-lactams from all chemical classes except monobactams, and they are not inhibited by the beta-lactamase inhibitors, such as clavulanic acid or tazobactam (Eliopoulos, 2001).

3.2 Fluoroquinolones

The fluoroquinolones have been useful clinically in a variety of infections, including urinary tract, genital, prostatic, GI, respiratory tract, soft tissue, and bone infections. The fluoroquinolones are broad-spectrum antimicrobials. Most enteric gram-negative bacilli, including *E. coli, Proteus, Klebsiella*, and *Enterobacter*, are highly susceptible. Norfloxacin is a fluorinated quinolone carboxylic acid derivative (Figure 2-7). Being one of the newer quinolone compounds, norfloxacin's antibacterial spectrum is very similar to that of enoxacin. It is active against most gram-negative bacteria which are resistant to nalidixic acid (Ito et al., 1980).

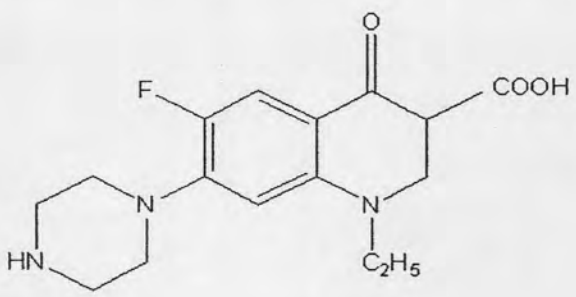


Figure 2-7 Structure of Norfloxacin

Mechanism of action

The quinolone antibiotics target bacterial DNA gyrase and topoisomerase IV (Drlica and Zhao, 1997). Many gram-negative bacteria (such as *E.coli*). DNA gyrase is the primary quinolone target (Hooper, 2000a; Alovero et al., 2000). The intracellular target for norfloxacin is the A subunit of DNA gyrase, and for bactericidal activity competent RNA and protein synthesis must be present (Crumplin et al., 1984; Benbrook and Miller, 1986). The greater susceptibility of bacteria to newer quinolones, like norfloxacin and ciprofloxacin, compared to nalidixic acid, may be related to their better penetration of the bacterial outer membrane (Hirai et al., 1986). Norfloxacin are potent bactericidal against *E.coli* and various species of *Salmonella*, *Shigella*, *Enterobacter*, *Campylobacter*, and *Neisseria* (Eliopoulos and Eliopoulos, 1993). Norfloxacin has been used mainly to treat urinary tract infections. It has a high activity against virtually all bacterial pathogens isolated from infected urine (Greenwood et al., 1984).

Resistance to quinolones can be caused by mutations in DNA gyrase subunits A or B, reduced outer membrane permeability in gram-negative cells, or to active efflux transporters found in many bacteria. The highest level of resistance to the newer fluoroquinolones is most frequently associated with chromosomal mutations, causing amino acid substitutions in a highly conserved region in the A subunit of DNA gyrase. Multiple-mechanisms of resistance can occur in a single isolate of bacteria, leading to a higher level of resistance to many fluoroquinolones.

3.3 Combination therapy

Antibiotics are frequently used in combination for treat a life-threatening infection, prevent emergence of bacterial resistance, treat mixed infections of aerobic and anaerobic bacteria, enhance antibacterial activity (synergy) and use lower doses of a toxic drug. Combined treatment is reasonable when the precise agents of a serious infection are unknown. Use of two or more drugs to prevent the emergence of resistance is effective for therapy of some infections.

Infection with ESBL-producing bacteria have demonstrated that beta-lactam/beta-lactamase inhibitor combinations may be effective but treatment with these drugs was clearly inferior to treatment with imipenem or a piperacillin/tazobactam-plus-

aminoglycoside combination which were the most effective regimens (Karadenizli et al., 2001; Thauvin-Eliopoulos et al., 1997; Rice et al., 1994; Mentec et al., 1992).

The data from the SENTRY Antimicrobial Surveillance Program Among ESBL-producing *E.coli* infections and ESBL-producing *K.pneumoniae* infections cases from January 2001 to December 2002 reported that carbapenem using as monotherapy (imipenem or meropenem) represented the antimicrobial class most often selected for treatment (32.6%). when carbapenem was combined combination (with either fluoroquinolones or aminoglycosides), the additive effect were observed in 237 cases (13.3%) (Bhavnani et al., 2006), while the use of cephalosporin monotherapy or was combined (with either fluoroquinolones or aminoglycosides), the additive effect were observed in 237 cases (8.1% and 8.9%, respectively). Therefore, investigations of combination therapy have become increasingly important as the prevalence of multidrug-resistant pathogens in patients continues rise.

3.4. Medicinal plant.

Nowadays multiple drug resistance has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious disease (Davies, 1994; Service, 1995). Given the alarming incidence of antibiotic resistance in bacteria of medical importance(Monroe and Polk, 2000), there is a constant need for new and effective therapeutic agents (Bhavnani, 2000). In Thailand, folkloric remedy is important not only in the up-country areas where modern medicine is almost unobtainable but also in big cities where people still prefer to see herbalists rather than physicians for their ailments, such as fever, intestinal disorder, stress and weakness (Craig, 1999).

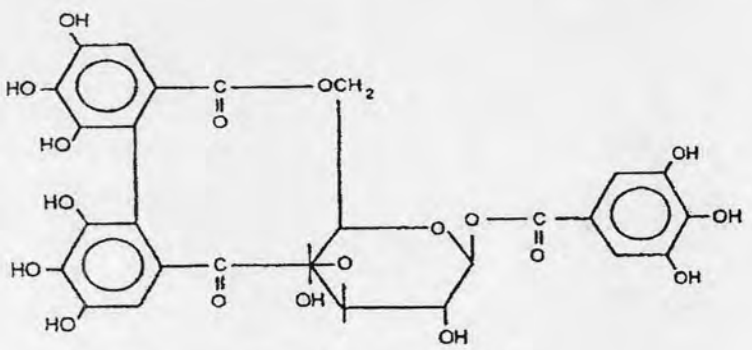
Plants have long provided mankind with herbal remedies for many infectious disease even today, they continue to play a major role in primary health care as therapeutic remedies in developing countries (Sokmen et al., 1999) .

Many medicinal plants produce a variety of compounds of known therapeutic properties. Substances that can either inhibit the growth of pathogens or kill them and have little or no toxicity to host cells are considered good candidates for developing new antimicrobial drugs. In recent years, antimicrobial properties of medical

properties of medicinal plants have been increasingly in different parts of the world. (Cowan, 1999).

Members of the Combretaceae are used for many medical purposes by traditional healers. This includes treating abdominal disorder, abdominal pain , backache, bilharziasis, coughs and chest, colds, conjunctivitis, diarrhea, hookworm, infertility in women, leprosy, pneumonia, scorpion bite , snake bite, swelling caused by mumps, syphilis, toothache, gastric ulcer, venereal diseases, heart diseases, cleanse the urinary system, dysentery, gallstones, sore throats, nosebleeds and general weakness (Hutching et al., 1996; Van et al., 1997).

Terminalia citrina ROXB. (Combretaceae), called "Sa-mo-de-ngu", has been used in traditional medicine in Thailand to treat diarrhea and skin infections. Five known tannins were isolated from the methanol extract of the fruits and identified as corilagin, punicalagin, 1,3,6-tri-O-galloyl-beta-D-glucopyranose, chebulagic acid and 1,2,3,4,6-penta-O-galloy-beta-D-glucopyranose. All compounds exhibited antibacterial activity against *Stapphylococcus aureus* (MIC values of 128-1024 μg/ml). Only corilagin (Figure 2-9) was active against *Esherichia coli* and *Klebsiella pneumoniae* (MIC values of 1024 μg/ml for both bacteria). Whereas both corilagin and punicalagin were active against *Pseudomonas aeruginosa* (MIC values of 1024 μg/ml). Except for 1,3,6-tri-O-galloyl-beta-D-glucopyranose, all compounds exhibited antifungal activity against *Candida albicans* (MIC values of 512-1024 μg/ml) (Burapadaja and Bunchoo, 1995).



ELLAGITANNIN (CORILAGIN)

Figure 2-9 Structure of Corilagin

Several investigations into the antimicrobial activity of members of the Combretaceae have been undertaken in recent years. Although the antibacterial properties of various *Terminalia* species (Silva et al., 1996; Eloff,1999; Fyhrquist et al., 2002) have been investigated in depth, this is not the case regarding their antifungal properties. Antifungal activity of *Terminalia* extracts was demonstrated, but no quantitative data was provided (Bhatt and Saxena, 1979; Baba-Moussa et al., 1998).

Various plant phenolics, including flavonoids and tannins, have been shown to have antibacterial effects (Mitscher et al., 1980; Kolodziej et al., 1999). This species is found distributing in Central: Bangkok (cultivated); South-Eastern: Trat (Koh Chang); South-Western: Ratchaburi, Prachuap khiri Khan; Peninsular: Chumphon, Nakorn Sri Thammarat, Trang. It has also found in India, Burma, Indo – China, Malay Peninsula, Philippines and New Guinea. The flowering buds flish in June – July. The fruits mature in September – November. The characteristic features are described as follow:- Large Tree, 20-30 m high, 150-210 cm girth, usually with small buttresses; bark smooth, grayish – brown with slightly shallow patches; young branches and young shoots rufous pubescent and lenticellate. Leaves: coriaceous, oblong-elliptic to elliptic, 3 – 14 by 2-6 cm, glabrous; apex shortly acuminate, base rounded or broadly cuneate; nerves 9 – 12 pairs, slightly raised with reticulate venation beneath. Petiole: 1.0 – 2.5 cm usually glabrous, with a pair of dotted glands near leaf-base. Inflorescence: axillary or terminal panicles, 3-6 cm, rachis rufous pubescent; flowers subtended by linear caducuous bracts, 2-4 mm. Calyx: outside glabrous. inside villous, 1-2 mm long by 2-4 mm in diameter, calyx – segments with acute apex. Stamens: 2-3 mm. Ovary: glabrous or slightly adpressed pubescent only at the base, 1-2 mm long; style 3-4; disc lobed, densely hairy. Fruit: drupe, ellipsoid rarely subglobose, 2-3 by 0.8 – 2 cm, glabrous usually slightly 5-angulate when fresh and prominently 5-angular when dry. Seed: rough, 0.6 – 1.7 cm., ellipsoid with 5-angulate ridges (Weerachai Nanakorn, 1985). [Fig 2-10]

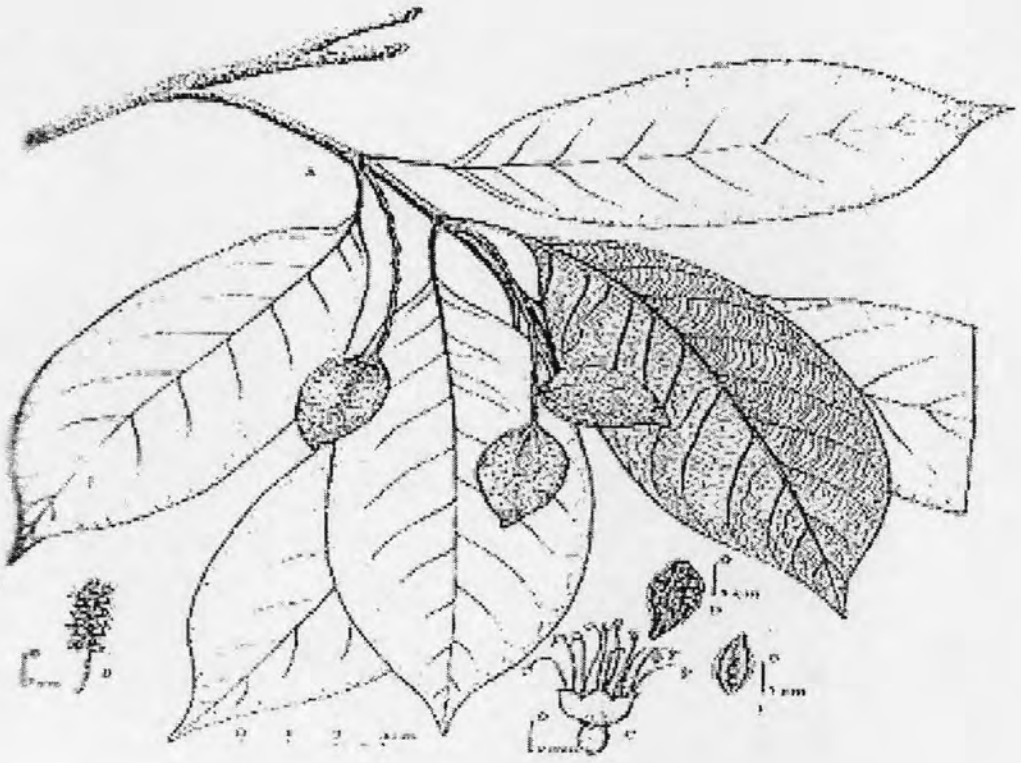


Figure 2-10 *Terminalia citrina* ROXB.

A. fruiting branches B. inflorescence C. flower

D. dry fruit E. seed

In individual part of this plant is used as the ethnomedicine in many countries. In Indonesia (Java), a decoction of fruit and "adaspoelasari" is taken as treatment for abdominal illness. In Philippines, use as astringent; a decoction is used in treating thrush and obstinate diarrhea (Perry and Metzger, 1980). The bark is endowed with both diuretic and cardiotonic properties (Kirtikar, Busu and An, 1935; CSIR, 1976).

The Thai traditional recipes enumerate the properties as:- The ripe fruits use to cure the complication caused by abnormal menstruation. Herbal medicine as an alternative for health care, the screening of medicinal plants for bioactive compounds is important (Wagnor et al., 1989).

Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious disease from medicinal plants (Clark, 1996; Cordell, 2000). Because of side effects and the resistance that pathogenic microorganisms build against antibiotics, much recent attention has been paid to the extracts and biologically active compounds isolated from plant species used in herbal medicine. Medicinal plants may offer a new source of antimicrobial agents for use (Essawi and Srour, 2000).