CHAPTER II

LITERATURE REVIEWS

1. Characteristics of *Klebsiella* species (spp.)

Klebsiella named for Edwin Klebs (1834 - 1913), an early German bacteriologist (12) The genus *Klebsiella* is straight rod, 0.3 - 1.0 μm. in diameter and 0.6 - 6.0 μm in length, arranged singly, in pairs, or short chain. *Klebsiella* species are non motile, they are facultative anaerobe. It belongs to a group of bacteria classified in the family *Enterobacteriaceae.* (1) The outer most layer of *Klebsiella* spp. consists of a large polysaccharide capsule and hence produce large mucoid lactose fermenting colonies, the character which distinguishes member of this genus from most other bacteria in the family *Enterobacteriaceae.* The colony morphology of *Klebsiella pneumoniae* is large, smooth, elevated, mucoid colonies on blood agar and MacConkey agar. Most strains can use citrate and glucose as a sole carbon source. Glucose is fermented with the production of acid and gas. Most strians produce 2,3 - butanediol as a major end product of glucose fermentation and the Voges - Proskauer test is usually positive, lactic, acetic and formic acids are formed in smaller amounts and ethanol in larger amounts than in a mixed acid fermentation. Fermentation of inositol, hydrolysis of urea, and lack of production of ornithine decarboxylase are further distinctive character. Almost all strains can growth at 10°C. The genus *Klebsiella*; *K. pneumoniae*, *K. ozaenae,* and *K. rhinoscleromatis* belong to the same DNA relatedness group. Thus, *K. ozaenae,* and *K. rhinoscleromatis* are considered as

subspecies of *K pneumoniae*. Both subspecies (subsp.) may be considered as metabolically inactive biogroups of *K. pneumoniae*. *K. rhinoscleromatis* is the most metabolically inactive, while the metabolic activity in *K. ozaenae* strains is variable. However, There are 3 subspecies of *K. pneumoniae*; compose of *K. pneumoniae* subsp. *pneumoniae*, *K. pneumoniae* subsp. *ozaenae*, and *K. pneumoniae* subsp. *rhinoscleromatis*. Other species were also recognized; *K. oxytoca*, *K. terrigena* and *K. planticola*. *K. pneumoniae* and *K. oxytoca* is normally found in the intestinal tract and nasopharynx in man and animals. *K. terrigena* are derived mainly form aquatic and soil environments. It can growth at 10°C, fermented of melizitose and can not produce gas production from lactose when incubated 44.5°C that distinguishes *K. terrigena* form *K. pneumoniae* and *K. oxytoca*. *K. planticola* is isolated primarily from botanical and soil environments. It can growth 10°C and by inability to produce gas from lactose at 44.5°C that distinguishes from *K. pneumoniae*. Its inability to ferment melizitose distinguishes *K. planticola* from *K. terrigena*.

2. Pathogenesis and Epidemiology of *K. pneumoniae*

Almost all (95 percent) clinical isolates are *K. pneumoniae*, which is present in the intestinal and respiratory tracts of about 10 percent of healthy persons. (2) *K. pneumoniae* are associated with upper respiratory disease, urinary tract infection and other septic complications of the patients admitted in hospitals (abscesses, meningitis, wound infections). *K. ozaenae* caused ozaena and *K. rhinoscleromatis*

causes rhinoscleroma, both are destructive disease of the nose. *K. pneumoniae* is well known to most clinicians as a cause of the community acquired bacterial pneumonia, occurring particularly in chronic alcoholics. It is associated with hospitalization as opportunistic pathogen primarily attack immunocompromised individuals who are hospitalized and suffer from severe underlying diseases such as diabetes mellitus or chronic pulmonary obstruction. The carrier rates change drastically in the hospital environment, where colonization rates increase in direct proportion to the length of stay. Even hospital personnel have elevated rates of *Klebsiella* carriage. (13) Reported carrier rates in hospitalized patients are 77 % in stool, 19 % in the pharynx and 42 % on the hand of patients. (8,9) The high rate of nosocomial *Klebsiella* colonization appears to be associated with the use of antibiotics rather than with factors connected with delivery of care in the hospital. (9) The nosocomial *Klebsiella* colonization occurred primarily in patients receiving antibiotics, especially in persons receiving broad - spectrum or multiple antibiotics. Furthermore, widespread use of antimicrobial therapy has often been held responsible for occurrence of the multiple resistant *Klebsiella* strains in many hospitals. (10,14) Apart from medical equipment (contaminated due to faulty hygienic procedure) (15), the principal reservoirs for transmission of *Klebsiella* in the hospital setting are the gastrointestinal tract of patients and the hands of hospital personnel. (16) The ability of this organism to spread rapidly often leads to nosocomial outbreaks, especially in neonatal units. (17)

3. Antimicrobial drug resistance with focus on β - lactam resistance

β - lactam antibiotics are the most frequently prescribed antibiotic worldwide. Therefore, the resistance to these agents has become a major problem for physicians. β - lactam antibiotics exert their antimicrobial effect by interfering with cell wall synthesis. This is accomplished by the drugs attaching covalently to their targets, the penicillin - binding proteins (PBPs). The PBPs are diverse enzymes involved cell wall synthesis, and are anchored in the cytoplasmic membrane of the bacterium. The site at which β - lactam drugs bind to PBPs is located on the portion of the PBP that extends into the periplasmic space of gram - negative bacteria. Covalent binding to PBPs interferes with synthesis of cell wall and ultimately leads to cell death. Resistance to β - lactam antibiotics arises through one or more of the following mechanisms:

A) PBP Modifications

Resistance to β - lactam antibiotics due to PBP modification, occur either through mutations in the chromosomal genes encoding the PBPs or through the acquisition of supplementary foreign genes encoding new PBPs. (18) This mechanism of resistance is important in gram - positive cocci such as *Staphylococcus aureus* and *Streptococcus pneumoniae*, but it occurs in much less frequently in gram - negative bacteria. Among fastidious gram - negative bacteria, resistance arising through altered PBPs is seen among species of *Neisseria* and rarely with *Haemophilus*. Among nonfastidious gram - negative bacteria, resistance via altered PBPs is

exceeding rare although resistance to imipenem among species *Proteus* due to alteration of PBPs which has been reported recently.

B) Drug impermeability

Bacteria are unicellular organism and their cytoplasm is separated from the external environment by the cytoplasmic membrane. Gram - negative bacteria surrounded themselves with an additional membrane, the outer membrane, that functions as an effective barrier for chemotherapeutic agents and shields the PBPs from the external environment. Nutrients and antibiotics must cross this membrane to reach the inside of the bacterium. This happens passively by diffusion through protein channels called porins. (19) Alterations in these porins diminish the amount of β - lactam antibiotics that can enter the cell. This form of resistance usually leads to multiple antibiotics resistance that may cross drug class lines as many different drugs often share the same porin. Alternatively, it can lead to specific resistance when only a single drug uses a specific porin. Impermeability can also contribute to imipenem resistance in *Enterobacter* spp. and *Serratia* spp., where both changes in cell permeability for imipenem and the enhanced production of chromosomal beta-lactamase combine to cause resistance to this drug.

C) β - lactamase production

The most prevalent mechanism of resistance to β - lactam among clinical isolates of gram - negative bacteria is the production of β - lactamase. (20) These enzymes inactivate β - lactam antibiotics by hydrolyzing the β - lactam ring of the

drug. In gram - negative organisms, β - lactamase are located in the periplasmic space between the outer and cytoplasmic membrane. The β - lactamase of gram - negative bacteria are diverse and numerous. Some are characteristically encoded by chromosomal genes, whereas others are characteristically encoded by plasmid genes.

A number of schemes have been proposed for the classification of β - lactamase. The most recent and complete scheme developed by Bush et al. (38) attempted to combine elements of all previous schemes and correlate this with molecular structure of the enzyme was shown as followed:

Group 1 β - lactamase including the AmpC enzymes that are instrinsically resistance to β - lactamase inhibitors and are found in a variety of gram - negative bacteria such as *Enterobacter* spp., *Citrobacter freundii*, *Serratia marcescens*, *P. aeruginosa, E. coli* and *K. pneumoniae.* This group of enzymes are resist to β - lactamase inhibitor including when it is combined with penicillins, cephamycins, first, second, and third generation cephalosporins and monobactams is seen in these strains. However, they do not show their activities against cefepime and imipenem.

Group 2 β - lactamase including a variety of enzymes all of which are instrinsically susceptible to the β - lactamase inhibitors. These enzymes is the plasmid mediated β - lactamase that found in *E. coli* and *K. pneumoniae*, responsible for resistance to ampicillin and first generation cephalosporins in these species. Mutant forms of these enzymes are now appearing isolates of *E. coli* and *K. pneumoniae,* which are responsible for expanded spectrum of resistance to cephalosporins and

aztreonam called extended spectrum β - lactamases, ESBLs and also to β - lactamase inhibitor / β - lactam drug combinations.

Group 3 β - lactamase including the metallo - β - lactamase capable of hydrolyzing the carbapenems. Such enzymes are found in *Stenotrophomonas maltophilia,* species of *Aeromonas*, some strains of *Bacteriodes* and *P. aeruginosa*

Group 4 β - lactamases are not commonly encountered.

4. Types of β - lactamase

4. 1) TEM - type β - lactamase

The first plasmid mediated β - lactamase in gram - negative bacteria, TEM - 1, was described in the early 1960s. The TEM - 1 enzyme was originally found in a single strain of *E. coli* isolated from blood culture from a patient named Temoniera in Greece, hence the designation was TEM. TEM - 1 is the most commonly encountered β - lactamase in gram - negative bacteria. Up to 90 % ampicillin reisitance in *E. coli* is due to the production of TEM - 1. (24) This enzymes is also responsible for the ampicilin and penicillin resistance that is seen in *H. influenzae* and *N. gonorrhoeae* is increasing numbers. TEM - 1 is able to hydrolyze penicillin and early cephalosporins such as cephalothin and cephalodine. TEM - 2, the first derivative of TEM - 1, had a single amino substitution from the original β - lactamase. TEM - 3, originally reported in 1989, was the first TEM - type β - lactamase that displayed the ESBL phenotype. As shown in the Fig 2.1, the amino acid substitutions that occur within the TEM enzyme occur at a limited number of

positions. The combinations of these amino acid changes result in various subtle 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 can range from pI of 5.2 to 6.5 as shown in the Table 2.1. A number of amino acid residues are especially important for producing the ESBL phenotype when substitutions occur at that position. They include glutamate to lysine at position 104, arginine to either serine or histidine at position 164, glycine to serine at position 238, and glutamate to lysine at position 240 (Fig 2.1). In addition to β - lactamase TEM - 1 through TEM - 92 shown in the Fig. 2.1 and the Table 2.1, there has been a report of a naturally occuring TEM - like enzyme, TEM - AQ, that contained a number of amino acid substitutions and one amino acid deletion that have not been noted in other TEM - enzymes. Although TEM - type β - lactamase are often found in *E. coli* and *K. pneumoniae*, they are also found in other species of gram - negative bacteria with increasing frequency. TEM - type ESBLs have been reported in genera of *Enterobacteriaceae* such as *Enterobacter aerogenes*, *Moganella morganii*, *Proteus milabilis*, *Proteus rettgeri*, and *Salmonella* spp..(25-27) Furthermore, TEM - type ESBLs have been found in non - *Enterobacteriaceae* gram - negative bacteria. The TEM - 42 β - lactamase was found in a strain of *P. aeruginosa* (28). Additionally, a recent report found the TEM -17 β - lactamase being expressed from a plasmid in a blood culture isolate of *Capnocytophaga orchracea* .(29)

The majority of ESBLs are derived through single amino acid substitution in three non - ESBL parental enzymes, TEM - 1, TEM - 2 and SHV - 1. (22) These enzymes capable of hydrolyzing the β - lactam ring of penicillin, cephalosporin and monobactam such as aztreonam rending them inactive but have no detectable activity against cephmycin and carbapenem. Since TEM - and SHV - had been uniformly susceptible to β - lactamase inhibitor such as clavulanic acid, sulbactam and tazobactam, inhibitor β - lactam drug combinations were advocaated as potential theraputic alternatives. Beside mainly found in *E. coli* and *K. pneumoniae*. ESBLs have also been recovered from other species of *Enterobacteriaceae* such as *Serratia marcesens*, *Enterobacter* spp., *Salmonella* spp., *P. aeruginosa*, *K. oxytoca*, *P. mirabilis* and *Citrobacter* spp.. Gene encoding of these extended spectrum β - lactamase were typically carried on self - transferable plasmid that often carried other determinants of antibitoic resistance. (23) The nosocomial outbreaks of organisms producing ESBLs were often occurred in the ICUs, oncology, burn, and neonatal ward. (6) Patients at risk of acquiring an infection with an organism producing an ESBL include those prolong stays, surgery, previous antibiotic exposure, instrumentation, admission to an intensive care unit or oncology unit and admission to a nursing home. (21) Extended spectrum β - lactamase producing organisms associated with high morbidity and mortality.

Most ESBLs are the derivatives of TEM or SHV enzymes.(6,23) There are now more than 90 TEM - type - β - lactamase and more than 25 SHV - type enzymes.

With both of these groups of enzymes, a few point mutations at selected loci within the gene give rise to extended - spectrum phenotype.

4.2) Inhibitor - resistant β - lactamase

Although the inhibitor - resistant β - lactamase are not ESBLs, they are often discussed with ESBLs because they are also derivatives of classical TEM - or SHV - type enzymes. In the early 1990s, β - lactamases that were resistant to the inhibition by clavulanic acid were discovered. Nucleotide sequencing revealed that these enzymes were variants of the TEM - 1 or TEM - 2 β - lactamase. These enzymes were at first given the designation IRT for inhibitor - resistant TEM β - lactamase; however, all have subsequently been renamed with numerical TEM designations. There are at least 19 distinct inhibitor resistant TEM β - lactamase which have been found mainly in clinical isolates of *E. coli*, but also some strains of *K. pneumoniae*, *K. oxytoca*, *P. mirabilis*, and *Citrobacter freundii*. (30,31) Although the inhibitor - resistant TEM variants are resistant to inhibition by clavulanic acid and sulbactam, thereby showing clinical resistance to the β - lactam - β - lactamase inhibitor combinations of amoxicillin - clavulanate, ticarcillin - clavulanate, and ampicillin - sulbactam, they remain susceptible to inhibition by tazobactam and subsequently the combination of piperacillin and tazobactam. (32) As shown in the Fig 2.2, point mutations that lead to the inhibitor - resistant phenotype occur at a few specific amino acid residues within the structural gene for the TEM enzyme, Met - 69, Arg - 244, Arg - 275, and Asn - 276 (33). The sites of these amino acid substitutions are distinct from those that lead to the ESBL phenotype.

4. 3) SHV - type β - lactamase

The SHV - 1 (for sulphydryl variable) is most commonly found in *K. pneumoniae* and is responsible for up to 20 % of the plasmid mediated ampicillin resistance in this species. Unlike the TEM - type β - lactamases, there are relatively few derivatives of SHV - 1 as shown in the Table 2.2. The majority of SHV variants possessing an ESBL phenotype are characterized by the substitution of a serine for glycine at position 238. A number of variants related to SHV - 5 also have a substitution of lysine for glutamate at position 240. The serine residue at position 238 is critical for the efficient hydrolysis of ceftazidime, and the lysine residue is critical for the hydrolysis of cefotaxime. The majority of SHV - type ESBLs are found in strains of *K. pneumoniae*. However, these enzymes have also been found in *Citrobacter diversus*, *E. coli*, and *P. aeruginosa* (34-36).

4. 4) CTX - M

In recent years a new family of plasmid - mediated ESBLs, called CTX - M, that preferentially hydrolyze cefotaxime has arisen. They have mainly found in strains of *Salmonella enterica* serova Typhimurium and *E. coli*, but have also been described in other species of *Enterobacteriaceae* (Table 2.3). They include the CTX - M type enzymes CTX - M - 1, CTX - M - 2 through CTX - M - 10 as well as Toho enzymes 1 and 2. (37) These enzymes are not very closely related to TEM or SHV β - lactamases in that they show only approximately 40 % identity with these two commonly isolated β - lactamases.(37) A phylogenetic study of the

CTX - M family of β - lactamases showned four major types: the CTX - M - 1 type including CTX - M - 1, and CTX - M - 3 ; the CTX - M - 2 type including CTX - M - 2, CTX - M - 4, CTX - M - 5,CTX - M - 6, CTX - M - 7, and Toho - 1; Toho - 2; and CTX - M - 8, the latter two groups containing only one member to date.(38) The CTX - M type β - lactamases hydrolyze cephalothin or cephalodine better than benzylpenicillin and they preferentially hydrolyze cefotaxime over ceftazidime. (37) It has been suggested that the serine residue at position 237, which is present in all of the CTX - M enzymes, play an impotant role in the extended - spectrum activity of the CTX - M - type β - lactamase. (37) Strains expressing CTX - M - type β - lactamases have been isolated from many parts of the world, but have most often been associated with focal outbreaks in eastern Europe, South America , and Japan. (39)

4.5) OXA - type β - lactamase

The OXA - type enzymes are another growing family of ESBLs. The OXA - type β - lactamases confer resistance to ampicillin and cephalothin and are characterized by their hydrolytic activity against oxacillin and cloxacillin and the fact that they are poorly inhibited by clavulanic acid. (23) The OXA - type ESBLs have been found mainly in *P. aeruginosa* (Table 2.4) Several of the OXA - type ESBLs have been derived from OXA - 10 (OXA - 11, OXA - 14, OXA - 16, and OXA - 17). Among the enzymes related to OXA - 10, the ESBL variants have one of two amino acid substitutions; an asparagine for serine at position 73, or an aspartate for glycine at

position 157. In particular, the Gly157 Asp substitution may be necessary for high - level resistance to ceftazidime. (40)

4.6) Other β - lactamase

While the majority of ESBLs are derived from TEM or SHV β - lactamases and others can be catagorized with one of the newer families of ESBLs, a few ESBLs have been reported that are not closely related to any of the established families of β - lactamase (Table 2.5). The PER - 1 β - lactamase was first discovered in strain of *P. aeruginosa* isolated from patients in Turkey. (41) Later, it was also found among isolates of *S. enterica* serova Typhimurium and *A. baumanii*. (42) A related enzyme, PER - 2, was found among *S. enterica* serova Typhimurium strains in Argentina. (43) Another enzyme that is somewhat related to PER - 1 is the VEB - 1 β - lactamase . VEB - 1 was first found in a single isolate of *E. coli* in a patient from Vietnam, but was subsequently also found in a *P. aeruginosa* isolate from a patient from Thailand. A third related enzyme is CME - 1, which was isolated from *Chryseobacterium meningosepticum* . A fourth enzyme in this group is TLA - 1, which was identified in an *E. coli* isolate from a patient in Mexico. (44) The PER - 1, PER - 2, VEB - 1, CME - 1, and TLA - 1 β - lactamases are related but show only 40 to 50 % homology. These enzymes all confer resistance to oxyimino - cephalosporins, especially ceftazidime, and aztreonam.

An unusual feature of SFO - 1 isolated from *Serratia fonticola*. It is a transferable β - lactamase that can be induced to high - level production of β - lactamase by imipenem .(45) GES - 1 is another uncommon ESBL enzyme that is not closely related to any other plasmid - mediated β - lactamase. This enzyme isolated from *Proteus milabilis*.

5. The prevalence and epidemiology of extended spectrum β - lactamase producing organisms

Several ESBLs derived from TEM or SHV appear to be widely disseminated and have been reported in many countries. Most of the clinical isolates that produce ESBLs come from hospitalized patients and have frequently caused nosocomial outbreak

In France, the reported outbreaks involving ESBL producing strains were due to *K. pneumonaie* strains which is most caused produced TEM - 3 or SHV - 4. (46) Recently, outbreak due to *Enterobacter aerogines* producing TEM - 24 β - lactamase have been observed in France. In Tunisia, a nosocomial outbreak of acute gastroenteritis was caused by *Salmonella wein* producing SHV - 2 β - lactamase. SHV - 2 have been reported in Germany, Argentina, Chile, China, Greece, and France. (47) In Germany, spread of *K. pneumoniae* strain producing SHV - 5 enzyme was reported in patients hospitalized in different wards of a single hospital. (48) In the USA, the enzyme which occur commonly in outbreak caused by

ceftazidime resistance *K. pneumoniae* were TEM -10, TEM - 12, and TEM - 26. (49-50)

Sporadic nosocomial outbreak due to strains producing an ESBL have led to an endemic problem in some hospitals resulting in a concurrent dissemination of genes, plasmid and strains.(21) Risk factor for the acquisition of ESBL are length of stay in ICUs, invasive procedures and selective pressure from the wide spread use of broad spectrum cephalosporins which enhances colonization of the digestive or respiratory tract.

Sader HS, et al. studied the intra and interlaboratory dissemination of ESBL producing *Enterobacteriaceae*. The incidence of extended - spectrum β - lactamases (ESBL) producing *K. pneumoniae* and *E. coli* has been increasing rapidly, and they are probably even more prevalent than is currently recognized because of difficulties in their detection by the clinical microbiology laboratory. In addition, several outbreaks associated with these multiresistant strains have been reported. They evaluated 30 clinical isolates (27 *K. pneumoniae* from 11 hospitals and 3 *E. coli* from three hospitals) that were resistant or intermediately susceptible ceftazidime and / or cefuroxime by testing for the susceptibility to ceftazidime, cefuroxime, gentamicin, and ofloxacin. The production of ESBL was assessed by the disk approximately synergy test and the typing was performed by pulsed - field gel electrophoresis (PFGE) of chromosomal DNA. ESBL production was demonstrated in 15 *K pneumoniae* (from 7 hospitals) and in one *E. coli* strain. Most ESBL producing

isolates were cross resistant with gentamicin and ofloxacin. Chromosomal DNA analysis by PFGE exhibited a great genomic variability among ESBL - producing isolates. This study confirmed the extensive strain diversity among ESBL producing *K. pneumoniae* and the discovery of isolates with similar PFGE patterns in two different hospital as well as indicating the outbreak of interhospital transmission of ESBL producing strain in one geographic area. (51)

Royle J, et al. studied the outbreak of extended spectrum β - lactamase producing *K. pneumoniae* in a neonatal unit. An outbreak of extended spectrum β - lactamase producing *K. pneumoniae* in a neonatal unit was controlled using simple measures. Normally, the control of such infections can be time consuming and expensive. Seven cases of septicemia resulted in two deaths. Extended spectrum β - lactamase producing *K. pneumoniae* isolates were subtyped by PFGE , and four isolates typed were identical. Control of the outbreak was achieved by altered empiric antibiotic treatment for late onset sepsis and the prevention of cross infection by strict attention to hand washing. Widespread colonization of babies in the unit was presumed, so initial surveillance cultures were not performed. No further episodes of sepsis occurred. (52)

Yan JJ, et al. studied the prevalence of SHV - 12 among clinical isolates of extended spectrum β – lactamase producing *K. pneumoniae*. Twenty of 234 nonrepetitive clinical isolates of *K. pneumoniae* from Southern Taiwan were found to produce extended spectrum β - lactamases by double disk synergy test and

phenotypic confirmatory test recommended by NCCLS. Ten strains produced SHV - 12, four produced SHV - 5, two produced a non - TEM and non - SHV ESBL with pI of 8.3, three produced a novel Amp C β - lactamase designated CMY - 8 with pI of 8.25, and one produced SHV - 12 and unidentified AmpC enzyme with pI of 8.2 by IEF analysis and gene sequencing. Plasmid and PFGE analyses revealed that all isolates harboring an SHV - derived ESBL were genetically unrelated, indicating that dissemination of resistance plasmids is responsible for spread of SHV - ESBL among *K. pneumoniae* in this area. All three isolates carrying CMY- 8 had identical genotypic patterns, suggesting the presence of an epidemic strains. (53)

6. The detection of Extended - spectrum β – lactamase producing strains

Katsanis GP, et al. detected *K. pneumoniae* and *E. coli* strains producing extended spectrum β - lactamases by 5 different test systems included I) Agar dilution, II) E - test strips, III) disk diffusion test, IV) The microscan, V) The Vitek rapid automated. Although MICs, as determined by agar dilution or E - test strips, were increased and disk diffusion zone diameters were diminished, breakpoints for resistance to oxyimino - β - lactams were not reached, and neither approach was sensitive in detecting resistance to oxyimino - beta - lactam antibiotics. The microscan 18 h microdilution or vitek rapid automated procedured were similarly insensitive. Ceftazidime was the best single test antibiotic for detecting extended spectrum β - lactamase production. (54)

Cormican MG, et al. detected extended spectrum β - lactamase producing strains by the E - test ESBL screen. The E - test ESBL screen uses stable gradient technology to evaluate the MIC of ceftazidime alone compared with the MIC of ceftazidime with clavulanic acid (2 μg /ml) to facilitate the recognition strains expressing inhibitable enzyme. ESBL - producing strains (17 *E. coli* transconjugants) were studied to define " sensitive " interpretive criteria for the E - test ESBL screen. These criteria (reduction of ceftazidime MIC by > 2 $\log_2$ dilution steps in the presence of clavulanic acid) defined a group of 92 probable ESBL - positive organisms among the 225 tested strains of *Klebsiella* spp. and *E. coli* having suspicious antibiogram phenotype. A subset (82 organisms) of the 225 strains was tested by the disk approximation method and with the E - test ESBL screen method. The result of disk approximation method compared with the result of E - test ESBL screen. The E - test ESBL screen was more sensitive (100%) than the disk approximation test (87%) and was more convenient. The E - test ESBL screen test with the ceftazidime substrate appears to be a useful method for detecting or validating the presence of enteric bacilli potentially producing extended - spectrum β - lactamase. (55)

Vercauteren E, et al. compared the screening methods for detection of extended spectrum β - lactamases of *E . coli* and *Klebsiella* spp. isolated from blood culture in a Belgian teaching hospital. Extended spectrum β - lactamase producing

strains of *E. coli* and *K. penumoniae* 33 isolates compared three screening methods for ESBL detection: (I) a double disk synergy test, (II) a three dimensional test, Both the double disk synergy test and the three dimensional test were performed with ceftriaxone, ceftazidime, aztreonam and cefepime, and (III) the E - test ESBL screen, based on the recognition of a reduction in the ceftazidime MIC in the presence of clavulanic acid. In double disk test, all four indicator antibiotics scored equally and 31 of 33 isolates were recognized. In three dimensional test, ceftriaxone was the only satisfactory indicator and 30 ESBL positive strains were detected by this antibiotics. The E - test ESBL screen detected 26 of 33 ESBL - positive strains. The researchers proposed the use of the combination of the double disk and the three dimensional test to detected ESBL producing strains because used it was easier for the investigator to interpret test results. (56)

Jan IS, et al. investigated the usefulness of three screening methods; the E - test ESBL screen, the double disk synergy, and the ceftazidime disk test for identifying ESBL producing *Klebsiella pneumoniae* strains. The agar dilution method was used as the standard method. A total of 93 isolates of *K. pneumoniae* were found to be resistance to at least one of the third - generation cephalosporins (cefotaxime and ceftazidime) or aztreonam using the routine disk diffusion method. Among these isolates, 35 were classified as having an ESBL phenotype using agar dilution method. Thirty - two (91 %) of the 35 isolates of *K. pneumoniae* with the ESBL related resistance phenotype were detected by the E - test ESBL screen , while the ceftazidime disk screen test detected 77 % of these isolates, and the double disk

synergy test detected 74 %. Therefore, the E - test ESBL screen is suitable method to detect ESBL producing organism because it has been shown to be more sensitive than the double disk synergy test and the ceftazidime disk screen test. (57)

Derek FJ, et al. detected extended - spectrum β - lactamases produicng organism by E - test and double - disk potentiation methods. These tests are based on antagonism by clavulanic acid of the action ESBLs on newer cephalosporins, particularly ceftazidime and/or aztreonam. Eighteen ESBL - producing strains tested were *E. coli*, *Klebsiella* spp., and *Citrobacter freundii*. Strains were tested by the E - test method with ceftazidime ESBL strips and the double disk potentiation method with ceftazidime 30μg and co - amoxiclav 30μg disks. Fifteen of the 18 ESBL producing strain were correctly reported with both methods. This study showed that the reproducibility of both the E - test and double disk potentiation tests was generally good, therefore, both methods more reliable tests appropriate for use in routine laboratories. (58)

Hadziyannis E, et al. studied the screening and confirmatory testing for extended spectrum β - lactamases (ESBL) in *E. coli*, *K. pneumoniae*, and *K. oxytoca* clinical isolates. *E. coli* and *Klebsiella* spp. were screened for ESBL based on routine susceptibility testing results. Isolates with intermediate or resistant susceptibilities for extended spectrum cephalosporins or aztreonam were reported as probable ESBL producers by using the NCCLS proposed ESBL confirmatory method. They tested 61 screen - positive isolates from 42 patients, 30 randomly selected susceptible

isolates and 12 isolates with previously characterized β - lactamases. Ceftazidime contributed to 97 % of screen - positive isolates, whereas aztreonam added a single patient isolate. An ESBL was confirmed in 86 % of *K. pneumoniae*, 100% of *K. oxytoca,* and 20% of *E. coli* screen positive single patient isolates. None of the susceptible isolates were shown to produce ESBL Based on these findings a comment regarding the presence of ESBL seems sufficient for *Klebsiella* spp., but confirmatory testing is indicated for *E. coli*. There was 85% agreement between the type of β - lactamase and the result of the ESBL confirmatory test. When a cefotaxime MIC > 0.25 μg/ml was used indicate the presence of ESBL, the specificity of these assay increased to 100% The researchers showed that NCCLS ESBL phenotypic confirmatory method was reproducible and accurate enough to be used in the clinical laboratory . (59)

Carter MW, et al. detected extended β - lactamases in *Klebsiella* with the oxoid combination disk method. One hundred and eighty isolates of *Klebsiella pneumoniae* and *Klebsiella oxytoca* collected from intensive care unit patients were deteced ESBL producing by the oxoid combination disk test of the NCCLS method and the British disk method. The oxoid combination disk method for detecting extended spectrum β - lactamases depends on comparing the inhibition zones of cefpodoxime (10μg) and cefpodoxime plus clavulanic acid (10 - plus 1 μg)disks. The presence of clavulante enlarged the zone for all of 180 ESBL - producing *Klebsiella*

by ≥ 5 mm. Good discrimination was achieved with either the NCCLS or the British disk method. (60)

Zali FH, et al. studied the detection of extended spectrum β - lactamases in members of the family *Enterobacteriaceae* by the three method I) the MAST double disk test, II) the double disk, and III) The E - test ESBL screen. A total 100 - epidemiologiclally distinct strains including ESBL and non - ESBL producing strains were included in the study. The MAST double disk test, which uses disks containing ceftazidime and a complementary disk containing ceftazidime and clavulanic acid and a second pair containing cefotaxime and cefotaxime and clavulanic acid was compared with the double disk diffusion test and the E - test ESBL screen. Both the E - test ESBL screen and the MAST double disk method correctly identified 93 % of ESBL producers. The investigators concluded that the MAST double disk was an inexpensive alternative to current methods for the detection of ESBL production. (61)

7. The molecular epidemiology of extended spectrum β - lactamase producing organisms

Gouby A, et al. studied the epidemiological by pulsed - field gel electrophoresis of an outbreak of extended spectrum β - lactamase producing *Klebsiella pneumoniae* in the Geriatric hospital. They included 12 cases of infection caused by extended spectrum β - lactamase producing *Klebsiella pneumoniae* at the same ward between August 1991 and March 1993 and strains isolated in other hospital

in Nimes University hospital at the same time . Restriction profiles a total genomic DNAs cleaved by *Xba* I and *Spe* I were compared by pulsed - field gel electrophoresis. The restriction profiles of the 12 isolates and those recovered from asymptomatic patients in the same ward were very similar. Over a period of more than 1 year, extended spectrum β - lactamase producing were not detect in *K. pneumoniae* isolates with restriction pattern different from that of the epidemic strain. It seemed, therefore, that there was no transfer of a plasmid or a gene coding for ESBL to strains of *K. pneumoniae* that were different from the epidemic strain. At the same time, ESBL producing *K. pneumoniae* isolates exhibiting restriction endonuclease profile which were very different from that of the epidemic strain were isolated from other hospitals in Nimes. None of these strains cause an outbreak. The researchers concluded that pulsed - field gel electrophoresis was a useful tool for studying the ESBL producing *K. pneumoniae* strains involved in nosocomial outbreaks. (62)

Arlet G, et al. studied the epidemiology of *K. pneumoniae* produce SHV - 4 β – lactamase in 14 French hospitals during 1987-1989 based on various phenotypic and genotypic markers to compare *K. pneumoniae* strains producing this enzyme isolated in 14 French hospital . Twelve of 14 isolates were the same biotype (weak urease activity and no sucrose fermentation). Among the six plasmid profiles observed, one accounted for eight strains. Large plasmids of 170 kb encoding SHV - 4 β - lactamase were present in all strains of *K pneumoniae*. The result was confirmed by PFGE and ribotyping which showed concordant result. The conclusion of the study indicated that the dissemination in France of the SHV - 4 extended spectrum β -

lactamase was thus essentially due to the diffusion of a single *K. pneumoniae* clone. (63)

Gori A, et al. studied extended spectrum β - lactamase producing *K. pneumoniae* by PFGE compared with randomly amplified DNA polymorphism (RAPD). The incidence and transmission patterns of ESBL producing *K. pneumoniae* isolates from patients who were admitted to the intensive care unit were investigated for over a 3 - year period. *K. pneumoniae* were tested by the antibiotype, capsular serotyping, plasmid profiles, and PFGE. with *Xba* I, and the results were compared with those obtained by typing with the randomly amplified polymorphic DNA (RAPD) pattern. Isolates from ICU patients were subdivided into six capsular serotypes and into four clonal groups based on antibiotypes, plasmid content, and both PFGE and RAPD patterns. Two clones were associated with the clusters of cross infection, involving 5 and 12 patients, respectively. PFGE and RAPD analysis showed concordant results and comparable discrimination for differentiation between groups of epidemiologically related strain of ESBL produicng *K. pneumoniae*. More subclonal variants were determined among epidemic clones by PFGE analysis than by RAPD analysis. PFGE has been shown to be more discriminatory than other genotypic techniques for typing *Klebsiella* species and other bacteria. (64)

Branger C, et al. studied the epidemiological typing of extended spectrum β - lactamase producing *K. pneumoniae* isolates responsible for five outbreaks in the university hospital. Thirty - seven isolates of extended spectrum β - lactamase

producing *K. pneumoniae* implicated in five nosocomial outbreaks on three distinct wards of the hospital were compared using capsular typing, biotyping, antibiotyping, enzyme electrophoresis typing and DNA macrorestriction analysis with *Xba*I resolved by PFGE. The isolates from each outbreak had common phenotypic and genotypic characteristics indicating that they were related epidemiolically. Isolates from outbreak I (4 patients) and V (13 patients) produced the same ESBL (SHV- 4). The isolates of outbreak II (7 patients), III (4 patients), and IV (7 patients), which occurred in a single surgical intensive care unit, produced an ESBL (TEM - 3). Isolates from outbreaks III and IV had similar *Xba* I patterns suggesting that the two outbreaks were due to a single strain which persisted endemically in the ward. PFGE analysis was a useful complement to phenotypic method for identifying *K. pneumoniae* strains responsible for outbreaks harboring a common ESBL. (65)

Gazouli M, et al. studied an outbreak of cefoxitin resistant *K. pneumoniae* in the general hospital. Six *K. pneumoniae* isolates which were resistant to cefoxitin and penicillin - inhibitor combinations were derived from patients in the intensive care unit of a hospital in Athens, Greece during a 3 - month period. The study an outbreak by PCR and PFGE was performed. Both methods provided evidence of the clonal origin of the isolates. Conventional techniques and ribotyping were inadequate in proving that the isolates were related. Resistance was due to a plasmid class C β - lactamase. These investigators indicated that molecular techniques based on inherently stable bacterial characteristics provide more detailed and accurate typing result. (66)

Nuesch MT, et al. surveyed the molecular genetics of SHV - β - lactamases in *Enterobacteriaceae* isolated in Switzerland. Sixty isolates of *Enterobacteriaceae* resisted to β - lactam antibiotics were collected over a period of 2 years and were screened by the hybridization technique for the carriage of SHV - genes. Thirty - four positive strains were found, and their SHV - genes were amplified and sequenced. SHV extended spectrum β - lactamases were found thirteen strain contained SHV - 2a, 12 strains harbored SHV - 2, and SHV - 5 was found twice. Four strains were shown to contain SHV - 1. In addition, they reported two new SHV variants, termed SHV - 11 and SHV -12. (67)

Pena C, et al. studied the epidemiology of the large outbreak due to ESBL producing *K. pneumoniae* by PFGE. An outbreak due to ESBL producing *K. pneumoniae* was detected from May 1993 to June 1995. A total 145 patients, particularly 107 patients in ICU, were colonized or infected. Infection developed in 92 (63%) patients and primary bacteremia caused by ESBL producing *K. pneumoniae* was the most frequent infection. A single clone of ESBL producing *K. pneumoniae* was identified by PFGE analysis throughout the whole period, and no molecular epidemiological relationship could be found between the epidemic strain and non ESBL producing *K. pneumoniae* isolates. A major PFGE pattern was found in as analysis of the 50 ESBL producing *K. pneumoniae* isolates with the same anibiotypes, although slight differences in the restriction patterns of some of them were found, they were considered subtypes of the epidemic clone. The PFGE patterns of the isolates from clinical sample and feces from the same patient were identical. (68)

Decre D, et al. studied the epidemiology of extended spectrum β - lactamase producing *K. pneumoniae* in the Medical intensive care unit. They conducted an epidemiologic study of strain ESBL producing *K. pneumoniae* that were isolated in one ICU during a - 16 month period. The ESBL producing *K. pneumoniae* were typed according to the biotypes, antimicrobial susceptibility patterns, isoelectric point, plasmid profile, and PFGE. In addition, they compared phenotypic method with genotypic method and compared PFGE with PCR - mediated fingerprint with primers based on repetitive extragenic palmdromic (REP - PCR). In this study were consistent with ICU acquired origin of most cases and showed that epidemic clone and its subclonal variants producing SHV - 4 β - lactamase was responsible for 85% of ICU acquired cases. Other sporadic cases were related to strains producing various ESBL types such as SHV - 5, SHV - 2, SHV - 3, and TEM - 3. PFGE was used for epidemiological study and was reported to be superior over other genotypic methods for typing various bacterial genera including *Klebsiella*. REP - PCR fingerprint was shown to be less accurate for differentiating strains belonging to the same subclonal population than PFGE. The investigators concluded that genotypic method are accurate for analyzing dissemination of epidemic strain over extended period. (69)

Gaillot O, et al. studied the nosocomial outbreak of *K. pneumoniae* producing SHV - 5 extended spectrum β - lactamase *K. pneumoniae* resistant to ceftazidime was isolated from six adult women and two neonates hospitalized between July and November 1993 in the department of Obstetrics and Gynecology of

Baucicaut Hospital. by phenotypic (biotyping, antibiotyping) and genotypic (plasmid profile and pulsed - field gel electrophoresis). The epidemiological investigation revealed a notably short delay between admission and contamination of the six adults and peripartum transmission to the neonates. The only environmental source of ceftazidime resistance *K. pneumoniae* was the ultrasonography coupling gel used in the emergency room. Phenotypic and genotypic analysis of all the clinical isolates indicated the spread of a single strain which produced SHV - 5 and TEM - 1 β - lactamases, as demonstrated by isoelectric focusing and gene sequencing. This was the first time an epidemic of SHV - 5 ESBL producing *K. pneumoniae* has been reported from French hospital. PFGE has been showed to be an excellent tool for typing ESBL producing *K pneumoniae* strain and enabled the identification of the source of contamination (70)

Ahmad M, et al. studied epidemiology associated with imipenem resistant *K. pneumoniae*. Eight patients were infected or colonized with imipenem resistant *K. pneumoniae* from December 1994 to November 1995. Initial *K. pneumoniae* were susceptible to imipenem but resistant to all cephalosporins, aminoglycosides, and β - lactam inhibitor combinations. All patients had been in the surgical intensive care unit and had undergone abdominal surgery or tracheostomy during hospitalization. The average age of the patients was 71 years (range 41-81 years). All patients were tested with imipenem for 5 to 36 days and imipenem resistant *K. pneumoniae* was recovered from each during or after therapy. PFGE of the imipenem resistant *K. pneumoniae* isolates revealed three distinct three clonal patterns. Paired sequential isolates of

imipenem susceptible *K. pneumoniae* and imipenem - resistant *K. pneumoniae* from the two patients had identical PFGE patterns. The imipenem resistance in *K. pneumoniae* may occur when this agent is used for treatment of infection due to ceftazidime and aminoglycoside resistant strains. (71)

Sui LK, et al. studied the bacteremia due to extended spectrum β – lactamase producing *E. coli* and *K. pneumoniae* in a pedriatic oncology ward. Thirteen patients who had 16 episodes of bacteremia were observed between 1993 and 1997 Four blood isolates were *E. coli* and 12 were *K. pneumoniae*, and these isolates harbored extended spectrum β - lactamase (ESBLs). All isolates were screened for ESBL producing strains using the double disk synergy test and the E - test ESBL strip and determined the antimicrobial susceptibility patterns and PFGE. The result showed fourty - nine (56.3 %) of 87 isolates of *K. pneumoniae* and 21 (18.6 %) of 113 *E. coli* isolates obtained from all unselected clinical specimens were resistant or intermediately susceptible to aztreonam or broad spectrum cephalosporins. Of the blood isolates 12 (46.2 %) of 26 isolates of *K. pneumoniae* and 6 (23.1 %) of 26 isolates of *E. coli* were not susceptible to the extended spectrum β - lactams. PFGE revealed that four SHV - 2 β - lactamase producing *K. pneumoniae* isolates from 1994 were of the same clone. Other ESBL producer, including six that carried both TEM - 1 and SHV - 5, five that carried SHV - 5, and one that carried SHV - 2 alone, were unrelated. The researchers concluded that SHV - 5 was present in 11 of the 16 isolates and coexisted with TEM - 1 in 6 isolates.(72)

Mongeney N, et al. studied the epidemiology of extended spectrum β - lactamase producing *K. pneumoniae* isolates neurologuical unit. Thirty - eight different strains of extended spectrum β - lactamase producing *K. pneumoniae* isolated from urine and pus sample of 38 patients hospitalized in a medium and long stay neurology department between 1 January 1992 and 31 December 1996, were analyzed by antibiotic resistance phenotyping, isoelectric focusing β - lactamase, and PFGE. The 38 isolates were distributed into 13 antibiotypes. The PFGE pattern identified 15 genotypes. A combination of the two typing method revealed several epidemic clones that emerged consecutively. Two main types of ESBL (SHV - 2 and CTX - 1) were identified by isoelectric focusing. In this study showed that the length of hospital stay, degree of malnutrition and dependency, and urinary sphincter status were the main factors significantly associated with ESBL producing *K. pneumoniae* isolation.(73)

Girlich D, et al. studied molecular epidemiolgy of an outbreak due to extended spectrum β - lactamase producing *K. pneumoniae* in geriatric department. In February 1998, 195 patients in the geriatric department of French hospital were screened for the presence of co - amoxiclav - resistance *K. pneumoniae* using the antimicrobial susceptibilty test, isoelectric focusing, PCR, PFGE, and hybridization. All of the eleven co - amoxiclav resistance isolates obtained produced an identical IRT - 2 β - lactamase. These *K. pneumoniae* isolates were also clonally related. This

study underlines that geriatric department may be a reservoir for the antibiotic resistant strains and that IRT β - lactamase producing strains might be nosocomial pathogens. (74)

Baraniak A, et al. studied the epidemiology of CTX - M - 3 extended spectrum β - lactamase producing microorganisms of the family *Enterobacteriaceae* in Poland. Eighty - four clinical isolates of the family *Enterobacteriaceae*, recovered from 1998 to 2000 in 15 hospitals in 10 Polish cities were analyzed. All the isolates produced β -lactamases with pIs of 8.4 and 5.4, and the pI 8.4 enzymes were demonstrated to hydrolyzed cefotaxime but not ceftazidime in the *in vitro* bioassy. PCR analysis and DNA sequencing have revealed that in all cases the pI 8.4 β - lactamase was probably the CTX - M - 3 extended - spectrum β - lactamase (ESBL) variant. The dissemination of plasmid was probably preceded by center to center transmission of several strains, as indicated by the identification by PFGE of closely related or possibly related *K. pneumoniae*, *E. coli,* and *Citrobacter freundii* isolates in five different hospitals. CTX - M - 3 producing organisms revealed a very high degree of diversity in β - lactam resistance levels and patterns.(75)

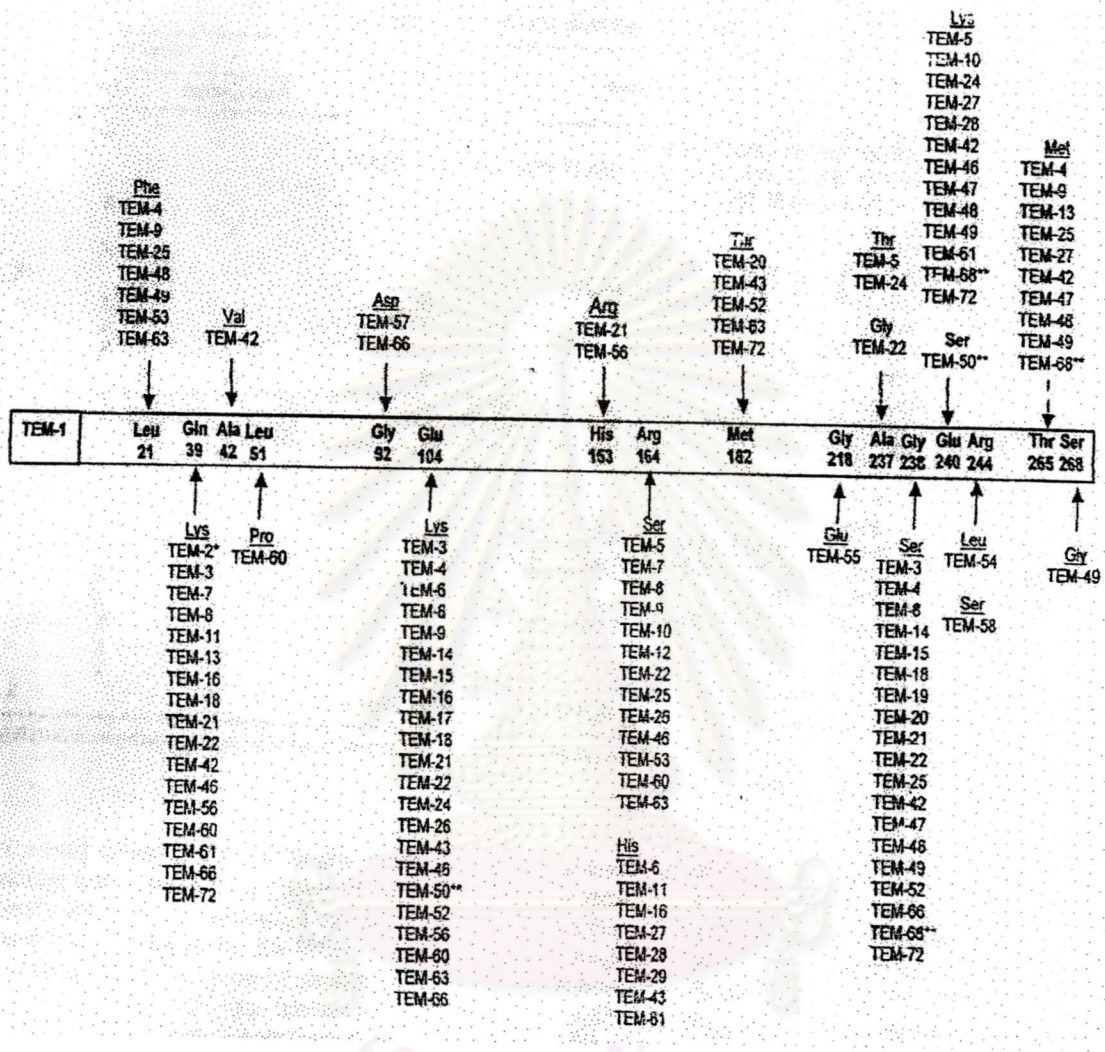

Figure 2.1 Amino acid substitutions in TEM ESBL derivatives. The amino acids listed within the bar are those found in the structural gene of TEM -1 β - lactamase. Substitutions found in TEM - types ESBL derivatives are shown under the amino acids of TEM - 1. TEM types variants may contains more than one amino acid substitution. Only the amino acid substitutions that are common to TEM - type ESBLs are shown in this figure. (21)

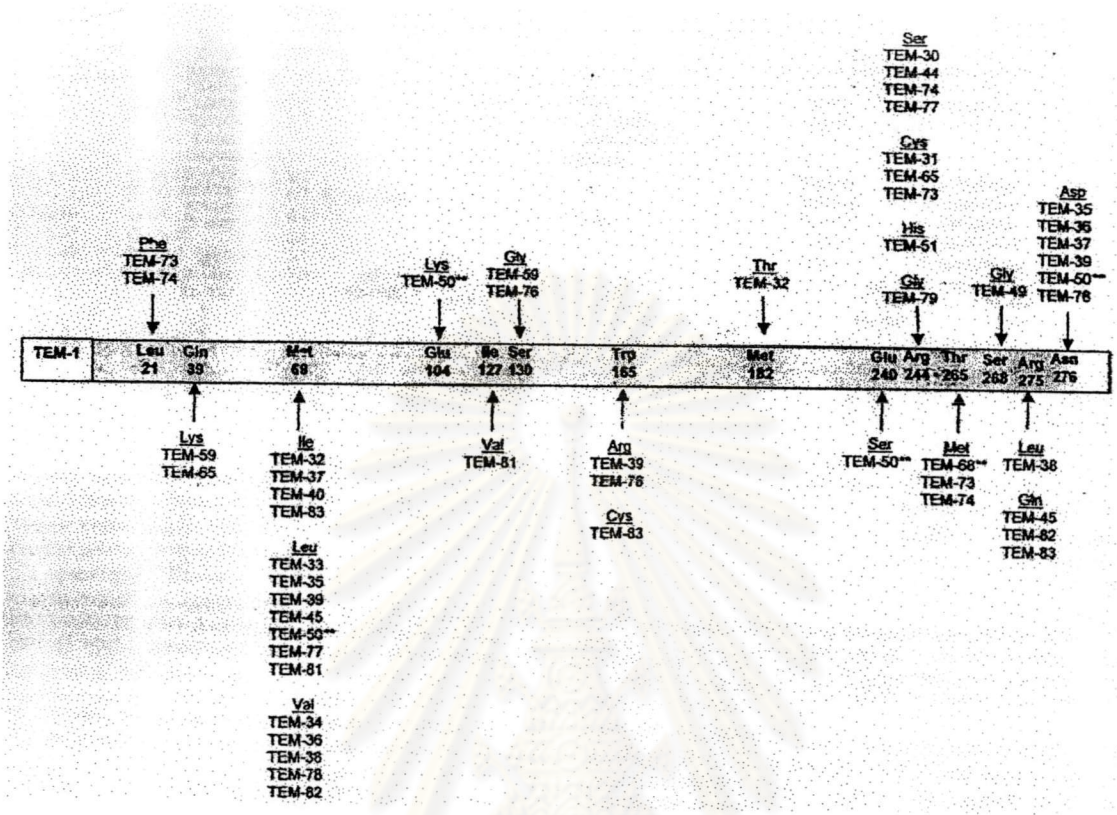

Figure 2.2 Amino acid substitutions in TEM IRT derivatives. The amino acids listed within the bar are those found in the structural gene of TEM -1 β - lactamase. Substitutions found in TEM - types IRT derivatives are shown under the amino acids of TEM - 1. TEM types variants may contains more than one amino acid substitution. Only the amino acid substitutions that are common to TEM - type IRTs are shown in this figure. (21)

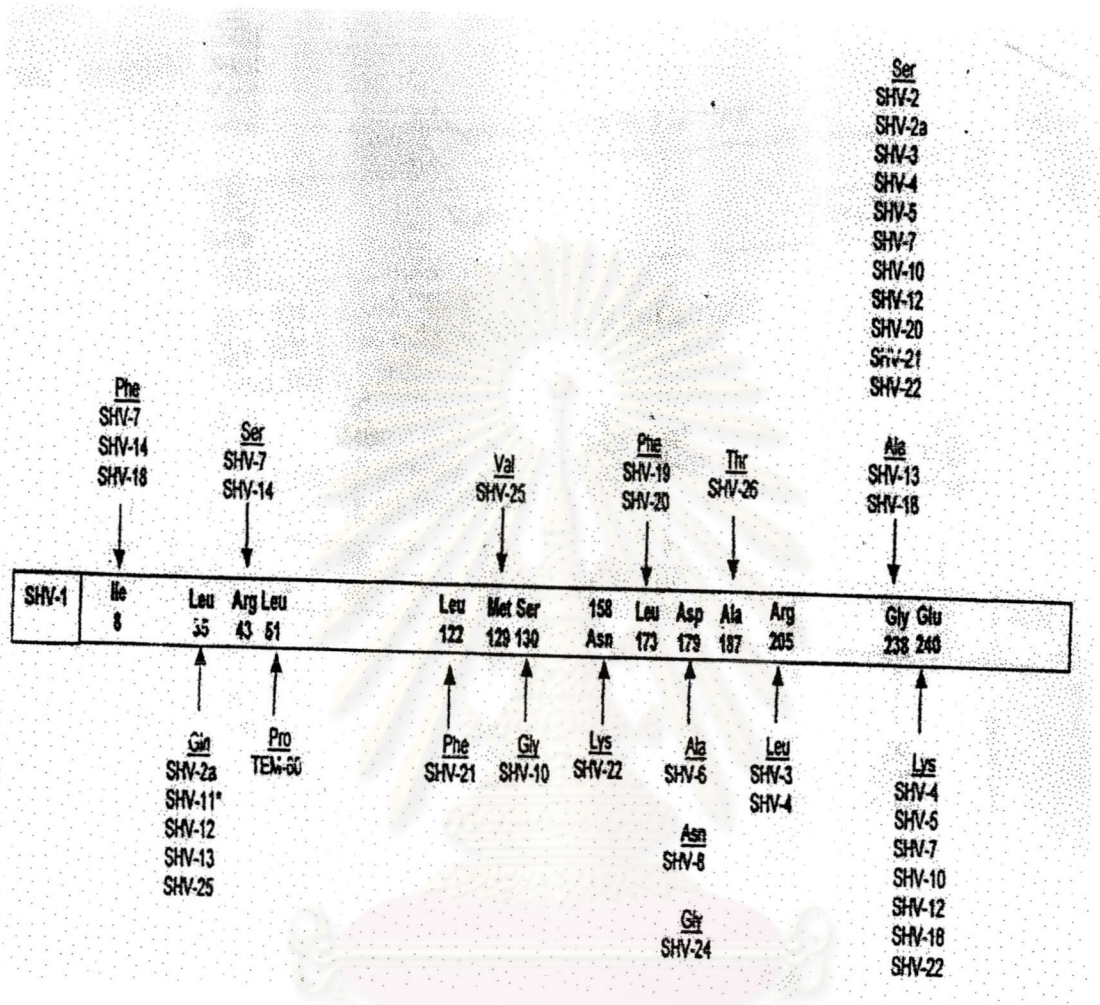

Figure 2.3 Amino acid substitutions in SHV - ESBL derivatives. The amino acids listed within the bar are those found in the structural gene of SHV - 1 β - lactamase. Substitutions found in SHV - types ESBL derivatives are shown under the amino acids of SHV -1. SHV - types variants may contains more than one amino acid substitution. SHV - 11 is not an ESBL but is in included in the figure as a derivatives of SHV - 1 (21)

Table 2.1 Characteristics of TEM - type β - lactamase

pI	Enzymes	Enzyme type		
		Broad spectrum	ESBL	IRT
5.2	TEM-12, TEM-55, TEM-57, TEM-58		X	
	TEM-30, TEM-31, TEM-35, TEM-36, TEM-37, TEM-38, TEM-41, TEM-45, TEM-51, TEM-73, TEM-74			X
5.3	TEM-25		X	
5.4	TEM-1	X		
	TEM-7, TEM-19, TEM-20, TEM-65		X	
	TEM-32, TEM-33, TEM-34, TEM-39, TEM-40, TEM-44			X
5.42	TEM-29		X	
5.55	TEM-5, TEM-17		X	
5.59	TEM-9		X	
5.6	TEM-2	X		
	TEM-10, TEM-11, TEM-13, TEM-26, TEM-63		X	
	TEM-50		X	X
	TEM-59			X
5.7	TEM-68		X	X
5.8	TEM-42		X	
5.9	TEM-4, TEM-6, TEM-8, TEM-27, TEM-72		X	
6.0	TEM-15, TEM-47, TEM-48, TEM-49, TEM-52, TEM-66, TEM-92		X	
6.1	TEM-28, TEM-43		X	
6.3	TEM-3, TEM-16, TEM-21, TEM-22		X	
6.4	TEM-56, TEM-60		X	
6.5	TEM-24, TEM-46, TEM-61		X	
Not determined	TEM-14, TEM-53, TEM-54		X	
	TEM-76, TEM-77, TEM-78, TEM-79, TEM-81, TEM-82, TEM-83, TEM-84			X

Table 2.2 Characteristics of SHV - type β - lactamase

pI	Enzymes	Enzyme type		
		Broad spectrum	ESBL	Inhibitor resistant
7.0	OHIO-1, LEN-1	X		
	SHV-3, SHV-14		X	
7.5	SHV-24		X	
7.6	SHV-1, SHV-11	X		
	SHV-2, SHV-2a, SHV 6, SHV-8, SHV-13, SHV-19, SHV-20, SHV-21, SHV-22		X	
7.8	SHV-4, SHV-7[b], SHV-18		X	
8.2	SHV-5, SHV-9, SHV-12		X	
	SHV-10			X

Table 2.3 Characteristics of CTX - M type ESBLs

β-Lactamase	Alternative name	pI	Country of origin	Bacterial species	Reference(s)
CTX-M-1	MEN-1	8.9	Germany, Italy	*E. coli*	12, 13
CTX-M-2		7.9	Argentina	*S. enterica*[a]	11, 13
CTX-M-3		8.4	Poland	*C. freundii, E. coli*	64
CTX-M-4		8.4	Russia	*S. enterica*	57, 59
CTX-M-5	CTX-M-3	8.8	Latvia	*S. enterica*	29
CTX-M-6		8.4	Greece	*S. enterica*	58, 173
CTX-M-7	CTX-M-5	8.4	Greece	*S. enterica*	58, 173
CTX-M-8		7.6	Brazil	*P. mirabilis, E. cloacae, E. aerogenes, C. amalonaticus*	21
CTX-M-9		8.0	Spain	*E. coli*	148
CTX-M-10		8.1	Spain	*E. coli*	Oliver et al.[b]
Toho-1		7.8	Japan	*E. coli*	72
Toho-2		7.7	Japan	*E. coli*	88

Table 2.4 Characteristics of OXA type ESBLs

β-Lactamase	Derivation	pI	Amino acid substitutions vs. OXA-10	Country of origin	Bacterial species	Reference
OXA-11	OXA-10	6.4	Asn143Ser, Gly157Asp	Turkey	*P. aeruginosa*	65
OXA-13	OXA-10	8.0	Ile10Thr, Gly20Ser, Asp55N, Asn73Ser, Thr107Ser, Tyr174Phe, Glu229Gly, Ser245Asn, Glu259Ala	France	*P. aeruginosa*	104
OXA-14	OXA-10	6.2	Gly157Asp	Turkey	*P. aeruginosa*	45
OXA-15	OXA-2	8.7, 8.9 doublet	NA[a]	Turkey	*P. aeruginosa*	46
OXA-16	OXA-10	6.2	Ala124Thr, Gly157Asp	Turkey	*P. aeruginosa*	47
OXA-17	OXA-10	6.1	Asn73Ser	Turkey	*P. aeruginosa*	44
OXA-18	OXA-9, OXA-12	5.5	NA	France	*P. aeruginosa*	131
OXA-19	OXA-10	7.6	Ile10Thr, Gly20Ser, Asp55Asn, Thr107Ser, Gly157Asp, Tyr174Phe, Glu229Gly, Ser245Asn, Glu259Ala	France	*P. aeruginosa*	102
OXA-28	OXA-10	7.6	Ile10Thr, Gly20Ser, Thr107Ser, Trp154Gly, Gly157Asp, Tyr174Phe, Glu229Gly, Ser245Asn, Glu259Ala	France	*P. aeruginosa*	134

Table 2.5 Characteristics of novel, unrelated ESBLs

β-Lactamase	Closest relative	pI	Preferred substrate[a]	Country of origin	Bacterial species	Reference
BES-1	Penicillinase from *Yersinia enterocolitica*	7.5	CTX, CAZ, ATM	Brazil	*S. marcescens*	20
FEC-1		8.2	CTX	Japan	*E. coli*	93
GES-1	Penicillinase from *P. mirabilis*	5.8	CAZ	French Guiana	*K. pneumoniae*	136
CME-1	VEB-1	>9.0	CAZ	Isolated from reference strain	*Chryseobacterium meningosepticum*	147
PER-1	PER-2	5.4	CAZ	France	*P. aeruginosa*	113
PER-2	PER-1	5.4	CAZ	Argentina	*S. enterica* serovar Typhimurium	14
SFO-1	AmpA from *S. fonticola*	7.3	CTX	Japan	*E. cloacae*	94
TLA-1	CME-1	9.0	CAZ, CTX, ATM	Mexico	*E. coli*	153
VEB-1	PER-1, PER-2	5.35	CAZ, ATM	Vietnam/Thailand	*E. coli*	135