

Review Article

Extended-Spectrum Beta-Lactamases (ESBLs): A Global Problem

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Kuwait Medical Journal 2006, 38 (3): 171-185

ABSTRACT

Extended-spectrum beta-lactamases (ESBLs) constitute a growing class of plasmid-mediated β -lactamases which confer resistance to broad spectrum beta-lactam antibiotics. They are commonly expressed by *Enterobacteriaceae* but the species of organisms producing these enzymes are increasing and this is a cause for great concern. The prevalence of ESBL - producing organisms is increasing worldwide and several outbreaks have been reported. Serious infections with these organisms are

associated with high mortality rates as therapeutic options are limited. The emergence of ESBLs creates a real challenge for both clinical microbiology laboratories and clinicians because of their dynamic evolution and epidemiology, wide substrate specificity with its therapeutic implications, their significant diagnostic challenges and their prevention and infection control issues. The aim of this review is to increase awareness about this serious antibiotic resistance threat.

KEY WORDS: broad spectrum beta-lactam antibiotics, *Enterobacteriaceae*, plasmidmediated β -lactamases

INTRODUCTION

The accelerated emergence of antibiotic resistance among the prevalent pathogens is the most serious threat to the management of infectious diseases. β -lactam antibiotics are the most common treatment for bacterial infections^[1]. Production of β -lactamases is the main mechanism of bacterial resistance to these classes of antibiotics^[2]. The first β -lactamase was identified in *Escherichia coli* prior to the release of penicillin for use in medical practice^[3]. Many Gram-negative bacteria possess naturally occurring, chromosomally mediated β -lactamases (e.g., AmpC cephalosporinases of *Enterobacteriaceae*). These enzymes may have some physiological role in peptidoglycan assembly or may arise to defend bacteria against β -lactams produced by environmental bacteria and fungi^[2]. The first plasmid-mediated β -lactamase in Gram - negatives, TEM-1, was reported in 1965 from an *Escherichia coli* isolate belonging to a patient in Athens, Greece, named Temoniera (hence the designation TEM)^[4]. The TEM-1 β -lactamase has spread worldwide and is now found in different species of members of *Enterobacteriaceae*, *Pseudomonas aeruginosa*, *Haemophilus influenzae* and *Neisseria gonorrhoeae*^[2]. Another common plasmid-mediated β -lactamase found in *Klebsiella pneumoniae* and *Escherichia coli* is SHV-1 (named after the sulfhydryl "variable" active site)^[2].

The extended-spectrum β -lactams (ESBLs) became widely used in the treatment of serious infections due to Gram-negative bacteria in the 1980's^[5]. Resistance to these newer β -lactams due to β -lactamases emerged quickly^[5,6]. The first report of plasmid-encoded β -lactamases capable of hydrolyzing the extended-spectrum cephalosporins was published in 1983^[7]. A *Klebsiella ozaenae* isolate from Germany possessed a β -lactamase, SHV-2, which efficiently hydrolyzed cefotaxime and to a lesser extent ceftazidime^[7]. Sequencing showed that this β -lactamase differed from the parent enzyme SHV-1, by replacement of glycine by serine at the 238th position. Later on, many plasmid-encoded extended-spectrum β -lactamases were recognized^[5,9]. They spread relatively quickly worldwide and became well entrenched in many hospitals^[5,8,9,10].

DEFINITION

ESBLs are known as extended-spectrum because they are able to hydrolyze a broader spectrum of β -lactam antibiotics than the simple parent β -lactamases from which they are derived. They are acquired plasmid-mediated β -lactamases. They have the ability to inactivate β -lactam antibiotics containing an oxyimino-group such as oxyimino-cephalosporins (e.g., ceftazidime, ceftriaxone, cefotaxime) as well as oxyimino-monobactam

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(aztreonam)^{15,91}. They are not active against cephamycins and carbapenems. Generally, they are inhibited by β -lactamase-inhibitors such as clavulanate and tazobactam.

FUNCTIONAL AND MOLECULAR GROUPING

In the β -lactamase functional classification scheme by Bush, Jacoby and Medeiros, ESBLs are located in two subgroups of group 2, namely subgroups 2be (extended-spectrum β -lactamases, Ambler's class A enzymes) and 2d (cloxacillin-hydrolyzing β -lactamases, Ambler's class D ESBLs)¹¹¹.

ESBL PRODUCING ORGANISMS

ESBLs have been found in a wide range of Gram-negative rods. However, the vast majority of strains expressing these enzymes belong to the family *Enterobacteriaceae*⁵¹. *Klebsiella pneumoniae* seems to remain the major ESBL producer. Another very important organism is *Escherichia coli*. It is important to note the growing incidence of ESBLs in *Salmonella spp*¹²¹. ESBLs have become more prevalent among species with inducible AmpC β -lactamases¹³¹.

Non-*Enterobacteriaceae* ESBL producers are relatively rare with *Pseudomonas aeruginosa* being the most important organism¹⁴¹. ESBL has also been reported in *Acinetobacter spp*, *Burkholderia cepacia* and *Alcaligenes fecalis*⁵¹.

THE ORIGIN AND GENETIC DETERMINANTS OF ESBLs

ESBL activity is demonstrated by enzymes with substantial diversity in terms of structure and evolutionary origin^{15,161}. The most prevalent ESBL types have evolved through point mutations of key amino acid substitutions in the parent TEM and SHV enzymes¹⁵¹. TEM-1 is the most commonly encountered: β -lactamase in Gram-negative bacteria. Upto 90% of ampicillin resistance in *E.coli* is due to production of TEM-1¹²¹. TEM-1 is able to hydrolyze penicillin and early cephalosporin. TEM-2, the first derivative of TEM-1, has a single amino acid substitution when compared to the original β -lactamase¹⁷¹. 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. The SHV-1 β -lactamase is most commonly found in *K.pneumoniae* and is responsible for up to 20% of the plasmid-mediated ampicillin resistance in this species¹⁸¹. The changes that have been observed to give rise to SHV variants occur in fewer positions within the

structural gene. Mutations in the OXAenzymes can also give the ESBL phenotype, which is the only ESBL belonging to class D. The OXA-type β -lactamases are so named because of their oxacillin-hydrolyzing abilities. These β -lactamases are characterized by hydrolysis rates for cloxacillin and oxacillin greater than 50% that for benzylpenicillin and the fact that they are poorly inhibited by clavulanic acid¹¹¹. They predominantly occur in *Pseudomonas aeruginosa*, but have been detected in many other Gram-negative bacteria¹⁴¹. The genealogy of other ESBL types is more mysterious, however, genetic studies revealed similarities between some of them and certain species specific β -lactamases belonging to Bush subgroup 2be and 2e^{19,201}.

The amino acid substitutions have been found to affect enzyme structures and activity in different ways^{15,151}. These substitutions that occur within TEM, SHV and OXA enzymes occur at a limited number of positions. The combination of these amino acid changes results in various subtle alterations in the ESBL phenotype such as the ability to hydrolyze specific oxyimino-cephalosporins or changes in their isoelectric points. The most important are the spectrum-extending mutations which result in the expansion of the active site that allows the increased activity against expanded spectrum cephalosporins and may result in the increased susceptibility to β -lactamase inhibitors^{15,16,211}.

The selection pressure that drives the emergence of ESBLs has usually been attributed to the intense use of oxyimino-beta lactams, mainly the third-generation cephalosporins^{15,22,231}. However, the constant or fluctuating pressure of various β -lactam antibiotics including diverse oxyimino-compounds as well as pencillins and early generation cephalosporins has recently been proposed to affect ESBL variation²⁴¹. ESBL is characterized by highly selective substrate preference. The selection of a particular enzyme variant in a given center has frequently been attributed to the specific profile of antibiotic use but such a correlation has not always been observed²⁵¹.

The strong selective pressure for the use of β -lactam drugs exerted on ESBL producer strains may lead to the selection of strains that hyperproduce ESBL, the emergence of strains expressing different types of ESBLs, the selection of complex mutant enzymes with inhibitor resistant phenotype or porin alteration which lead to the development of resistance to cephamycins and other antimicrobials^{10,26-281}. ESBL producing isolates are frequently resistant to other antimicrobials²⁶¹. The plasmids that harbor genes encoding ESBLs frequently contain other genes encoding mechanisms of resistance to aminoglycoside and cotrimoxazole²⁹¹. Quinolone resistance is frequently found in ESBL

producer strains although the mechanism of co-resistance is not clear^[30].

The total number of ESBLs that are characterized exceeds 200. These are detailed on the authoritative website on the nomenclature of ESBLs hosted by George Jacoby and Karen Bush. (<http://www.lahey.org/studies/webt.htm>).

ESBL TYPES

TEM - beta - lactamases :

The TEM-type ESBL are derivatives of TEM-1 and TEM-2. *Klebsiella pneumoniae* isolates detected in France as early as 1984 were found to harbor a novel plasmid-mediated β -lactamases originally named CTX-1 because of its enhanced activity against cefotaxime^[31]. This enzyme, now termed TEM-3, differs from TEM-2 by two amino acid substitutions^[32]. More than 100 TEM-type β -lactamases have been described, the majority of which are ESBLs. The amino acid changes in comparison with TEM-1 and TEM-2 are documented at <http://www.lahey.org/studies/temtable.htm>. Some mutants of TEM β -lactamases are being recovered. They maintain the ability to hydrolyze third-generation cephalosporins but also demonstrate an inhibitor resistance. These are referred to as complex mutants of TEM (CMT-1 to 4)^[33]. Although TEM-type ESBLs are most often found in *E.coli* and *K.pneumoniae*, they are also found in other species of Gram-negative bacteria with increasing frequency^[2]. TEM-type ESBLs have been reported in other genera of *Enterobacteriaceae* such as *Enterobacter aerogenes*, *Enterobacter cloacae*, *Morganella morganii*, *Proteus mirabilis* and *Salmonella* spp^[34-36]. Furthermore, TEM-type ESBL have been found in non-*Enterobacteriaceae* gram-negative bacteria, e.g., *Pseudomonas aeruginosa*^[14].

SHV - beta - lactamases:

The SHV-type of ESBL may be found in clinical isolates more frequently than any other type of ESBLs^[37]. Unlike the TEM-type β -lactamases, there are relatively few derivatives of SHV-1. The majority of SHV variants possessing an ESBL phenotype are characterized by the substitution of a serine for glycine at position 238. Also some have a substitution of lysine for glutamate at position 240. The serine residue at position 238 is critical for efficient hydrolysis of ceftazidime and lysine residue is critical for the efficient hydrolysis of cefotaxime^[38]. More than 50 SHV varieties are described worldwide. The majority possess the ESBL phenotype and inhibitor-resistant phenotype have been reported in few of the SHV enzymes^[39]. SHV-type of ESBLs has been detected in a wide range of *Enterobacteriaceae*^[37,39,40]. Outbreaks of SHV-

producing *Pseudomonas aeruginosa* and *Acinetobacter* spp. have been reported^[41,42].

CTX - M and Toho- beta - lactamases:

CTX-M is a recently described family of the extended-spectrum β -lactamases^[43]. The name CTX reflects the potent hydrolytic activity of these β -lactamases against cefotaxime^[44]. These enzymes hydrolyze cephalothin better than benzylpenicillin and they preferentially hydrolyze cefotaxime over ceftazidime. While ceftazidime MICs are usually in the apparently susceptible range, some of the CTX-M-type β -lactamases confer resistance to this drug^[45]. Aztreonam MICs have been found to be variable. CTX-M-type β -lactamases hydrolyze cefipime with high efficiency^[43]. They are inhibited better by the β -lactamase inhibitor tazobactam than by sulbactam and clavulanate^[46]. Rather than arising by mutation, they represent examples of plasmid acquisition of beta - lactamase genes that are normally found on the chromosome of *Kluyvera* species^[47]. CTX-M-ESBLs were predominantly found in three geographic areas: South America, the Far East and Eastern Europe^[43,48-50]. However, in recent years, CTX-M-type ESBLs have been reported in Western Europe, North America, China, Japan and India^[51-55]. CTX-M type β -lactamases may be the most frequent type of ESBLs worldwide. The number of CTX-M-type β -lactamases is rapidly expanding. More than 40 CTX-M variants are currently known^[43]. They have been found in different *Enterobacteriaceae* including *Salmonella* spp^[56]. Toho-1 and Toho-2 are β -lactamases that are structurally related to CTX-M-type β -lactamases and they have similar hydrolytic activity against cefotaxime (Toho refers to the Toho-University School of Medicine, Omari Hospital in Tokyo, where a child who was infected with Toho-1 β -lactamase-producing *Escherichia coli* was hospitalized)^[57,58].

OXA - beta - lactamases :

The OXA-type β -lactamases are another growing family of ESBLs. The OXA-type ESBLs were originally discovered in *Pseudomonas aeruginosa* isolates from a single hospital in Ankara, Turkey^[59]. Several of the OXA-type ESBLs have been derived from the original OXA-10 β -lactamase (e.g., OXA -11, 14, 6 and 17)^[59,60,61]. In contrast to the majority of OXA-type ESBLs, which confer resistance to ceftazidime, the OXA-17 β -lactamase confers resistance to cefotaxime and ceftriaxone but provides only marginal protection against ceftazidime^[62]. A novel ESBL (OXA-18) was reported to be inhibited by clavulanic acid^[63]. Many of the newer members of OXA-type of ESBLs have been found mainly in *Pseudomonas aeruginosa* isolates originating from Turkey and France^[59, 63].

Other ESBLs:

A variety of other β -lactamases which are plasmid-mediated or integron-associated class A enzymes have been recently discovered. They are not simple point mutations of any known β -lactamases. They are characterized by their geographic diversity.

PER:

The PER-type-ESBLs share only around 25 to 27% homology with the known TEM- and SHV-type ESBLs^[64]. PER-1 β -lactamase efficiently hydrolyzes penicillins and cephalosporins and is susceptible to clavulanic acid inhibition. The PER-1 β -lactamase was first detected in strains of *Pseudomonas aeruginosa* isolated from Turkey^[65]. Later, it was found among isolates of *Salmonella enterica* serovar Typhimurium and *Acinetobacter baumannii*, *Porteus mirabilis* and *Alcaligenes fecalis*^[9,66,67]. Although PER-1 enzyme has been predominantly found in Turkey, it was detected also in France, Italy, Belgium and Korea^[5,9,66]. PER-2, which shares 86% homology to PER-1, has been detected in *Salmonella enterica* serovar Typhimurium, *E.coli*, *K.pneumonia*, *Proteus mirabilis*, and *Vibrio cholera* O1, El Tor^[9,68,69]. However, PER-2 has only been found in South America^[68, 69].

VEB:

VEB-1 has greatest homology to PER-1 and PER-2 (38%). It confers high level of resistance to ceftazidime, cefotaxime and aztreonam, which is reversed by clavulanic acid. VEB-1 was first found in a single isolate of *E.coli* from Vietnam^[70]. An identical beta-lactamase has also been found in *K.pneumonia*, *Enterobacter cloaca* and *Pseudomonas aeruginosa* isolates in Thailand^[71]. Other VEB enzymes have also been detected in Kuwait and China^[72].

A few ESBLs have been reported but are uncommon and are found at a limited number of geographic sites^[5,9]. GES and IBC beta-lactamases are found mainly in *P.aeruginosa*^[73,74]. Other rare ESBLs found in *Enterobacteriaceae* are BES, SFO and TLA^[75,76,77].

EPIDEMIOLOGY

ESBL-producing organisms have been increasingly detected worldwide. Their prevalence varies from one country to another and from institution to institution. A survey on 81,213 bloodstream infection pathogens during 1997 - 2002 showed that the *Klebsiella* spp. with an ESBL phenotype was isolated at a rate of 42.7% in Latin America, 21.7% in Europe and 5.8% in North America^[78]. The Pan European Antimicrobial Resistance using Local Surveillance (PEARLS) study (2001 - 2002) showed that the percentages of ESBL production among *E.*

coli and *K. pneumoniae* and *Enterobacter* spp. were 5.4, 18.2 and 8.8% respectively for all the study sites. The overall ESBL production rate for the combined *Enterobacteriaceae* was 10.5%. The highest rates were encountered in Egypt (38.5%) and Greece (27.4%) and lowest in the Netherlands (2%) and Germany (2.6%)^[79]. In Japan, the percentage of ESBL production in *E.coli* and *K. pneumoniae* remains low^[80]. Elsewhere in Asia the percentage varies from 4.8% in Korea to 12% in Hong Kong^[81, 82]. Although the exact prevalence of ESBL in the Kingdom of Saudi Arabia (KSA) is currently unknown, the PEARLS study (2001 - 2002) showed that the overall ESBL production rate from *Enterobacteriaceae* was (18.6%) and other reports from KSA suggest that ESBL producers are common and began to disseminate between hospitals^[79,83-86]. In a tertiary care hospital in Riyadh, 48.4% of *K. pneumoniae* and 15.8% of *E.coli* blood culture isolates collected from January 2003 through December 2004 were ESBL producers^[86].

Infection and colonization with ESBL-producing organisms are usually hospital-acquired especially in intensive care units (ICUs)^[51,87]. Other hospital units that are at increased risk include surgical wards, pediatrics and neonatology, rehabilitation units and oncology wards^[88-90]. Community clinics and nursing homes have also been identified as a potential reservoir^[91]. Recent studies have demonstrated the danger of ESBL producers in livestock^[12]. Risk factors for infection or colonization with ESBL-producing organisms include: length of hospital or ICU stay, presence of vascular or urinary catheters, undergoing hemodialysis, emergency abdominal surgery, gut colonization, low birth weight, prior exposure to any antibiotic (e.g., quinolones, trimethoprim-sulfamethoxazole, aminoglycoside and metronidazole), prior ceftazidime or aztreonam administration and prior residence in a long term care facility^[5,8,9,23,24,87].

It is interesting that specific ESBLs appear to be unique to a certain country or region although recent reports suggest worldwide dissemination^[5,8]. An ESBL variant may appear in a Center due to *de novo* selection which may result in a novel type of enzyme or in one that have been previously identified in another institution (convergent evolution)^[92]. Once selected, the ESBL variant may spread in the Center by different means that include clonal dissemination of producer strain or horizontal transmission of the ESBL-gene carrying plasmid among non-related strains^[15,25,29].

Several outbreaks have been reported and they mostly occurred in tertiary hospitals where patients transfer rate is high^[8,25,27]. Transfer of a colonized ICU patient in the hospital has enormous opportunity for dissemination. These outbreaks

may be large, often start in ICUs and then they spread to other parts of the hospital^[93,94]. Very often the exact source of outbreak is never identified. However, the lower digestive tract of colonized patients has been recognized as the major source of ESBL-producing organisms and their cross-transmission among patients has been attributed to the hands of medical and nursing personnel^[93,94,95]. Environmental foci have also been reported but they are rare. They include: ultrasound gel, thermometers, blood pressure cuffs and contaminated bronchoscopes^[97-100]. Other studies demonstrated that cockroaches infesting a neonatal ICU in South Africa carried the same ESBL strain responsible for an outbreak^[101]. Many investigators are using molecular methods such as pulse field gel electrophoresis (PFGE) to examine the epidemiology of the outbreaks^[97,101-103].

SPECTRUM OF CLINICAL DISEASE

Initially ESBL-producing organisms were only seen to cause nosocomial infections^[103,104]. Later on they were shown to cause a long-term carriage in the community^[92,95]. Recently there have been several reports of true community-acquired infections (e.g., urinary tract infections) with ESBL-producing *E.coli*^[105]. It was found that diabetes mellitus, prior quinolone use, recurrent urinary tract infections, prior hospital admission and older age were independent risk factors^[105].

ESBLs-producing organisms cause a wide spectrum of clinical diseases ranging from colonization to serious infections^[15,103,104]. The common types of infections include urinary tract infections, peritonitis, cholangitis and intra-abdominal abscess. They are a common cause of nosocomial pneumonia and central venous line-related bacteremia^[89,101,103,104]. In hospitalized patients undergoing neurosurgical procedures, ESBL producers may also cause meningitis^[106].

LABORATORY DETECTION

The significance of ESBL detection:

The accurate detection of ESBL production in clinical isolates is crucial^[107,108]. The concern for this is two fold:

1. The therapeutic implications: Infections with ESBL producers have an important impact on clinical outcomes^[108,109]. They are associated with high rates of morbidity and mortality, a prolonged hospital stay and a higher cost^[109,111]. Generally patients infected with ESBL producer are at increased risk of treatment failure with extended spectrum - beta-lactams^[112]. The failure rate is high and may exceed 90% when cephalosporins were used for serious infections caused by ESBL-

producing organisms, particularly when the MICs for used cephalosporins are elevated (e.g., 4 or 8 µg/ml) but are still within susceptible range^[112-114]. The Clinical Laboratory Standard Institute (CLSI) indicates that cephalosporin susceptibility is indicated by MICs 8µg/ml^[115]. The reporting of cephalosporin resistance varies and depends on the national breakpoints^[9]. ESBL detection originated because some ESBL-producing organisms appeared susceptible to cephalosporins using conventional breakpoints^[116].

Many strains of the ESBL-producing organisms demonstrate an inoculum effect in that (MICs) of the extended-spectrum cephalosporins rises as the inoculum increases. Therefore, MIC determined with standard inoculum (10 CFU/ml)^[5] may remain below the standard breakpoints for resistance^[116,117]. The inoculum effect has been demonstrated in some animal models of endocarditis and intra-abdominal abscess^[118]. This effect may be clinically relevant in similar type of infections or in infections at sites in which drug penetration is poor (e.g., meningitis). The inoculum effect will increase MICs to levels unattainable even with aggressive dosing leading to treatment failure^[87,112]. The co-existence of resistance to other antimicrobial classes has led to the availability of few treatment options^[110]. Therefore, accurate *in vitro* detection of ESBL is essential to guide therapy selection^[119,120].

2. The epidemiological and infection control aspects: This is an important reason in favor of ongoing efforts aimed at ESBL detection. Although several reports show that there is an increasing prevalence of ESBLs worldwide, the extent of the problem is under-recognized due to unawareness and poor laboratory detection and reporting^[121]. Monitoring prevalence is important to define the magnitude of the problem and may help to implement appropriate infection control measures. These measures can control endemic situations as well as arrest outbreaks^[93,94].

Diagnostic problems of ESBL detection:

Detection of ESBL is not straight forward for many reasons:

1. There is no simple marker for its presence unlike methicillin-resistant *Staphylococcus aureus* (MRSA) or vancomycin resistant *Enterococcus* (VRE). Ceftazidime resistance is no longer a suitable marker for the presence of ESBLs since in many hospitals ceftazidime is being replaced by cefipime and therefore, no longer tested. Also CTX-M ESBLs are not in general ceftazidime hydrolysers.

2. Not all ESBL producers are universally resistant to any one of extended spectrum β-lactams. They vary in their substrate specificity and

may not phenotypically express resistance to its own substrate^[15].

3. An ESBL producer may harbor multiple ESBLs or other different enzymes which may alter the antibiotic resistance phenotype (e.g., AmpC β -lactamases, metallo- β -lactamases)^[13,26,28,33,104,116,122].

4. The importance of the inoculum effect on MICs determination^[117].

Many clinical microbiology laboratories make no effort to detect ESBL production by Gram-negative bacteria. There is an ongoing argument by many investigators that detection of ESBLs is too complex and costly since many diagnostic problems are encountered. They suggest that changes of cephalosporins breakpoints for *Enterobacteriaceae* is a more appropriate approach than expanding efforts to detect ESBLs and dispute that the inoculum effect is important^[123]. However, such an approach would require a substantial effort by antimicrobial susceptibility testing committees and the MICs alone may give erroneous information as a result of the inoculum effect.

It is still recommended by CLSI that clinical microbiology laboratories perform specialized tests for detection of ESBLs^[9,115]. Clinical laboratories which look for ESBLs vary in their success in identifying these enzymes since criteria for ESBL detection have changed over time and the need for improved detection is well recognized^[124,125]. It may be necessary to detect ESBL producers in all or specific clinical isolates especially those associated with serious infections without having to identify them specifically^[9]. There is no widely accepted screening test but an inexpensive and an easy to use screening test may be introduced into the routine susceptibility testing. Positive screening results must still be verified with a confirmatory test^[5,9,115]. A comprehensive study needs to be carried out initially comparing the abilities of all available tests, their merits and shortcomings and their suitability for a given laboratory before adopting any of them^[5,9,125].

DETECTION METHODS

The detection methods can be divided into:

- a) Phenotypic methods
- b) Molecular methods

a) Phenotypic methods:

They are based upon the resistance that ESBLs confer to oxyimino-beta-lactams (e.g. ceftriaxone, cefotaxime, ceftazidime and aztreonam) and the ability of a beta-lactamase inhibitor, usually clavulanate, to block this resistance. Several tests have been proposed.

Double disk diffusion test: The Jarlier double disk approximation or double disk synergy (DDS) was the first detection test described in 1980's^[126]. DDS is a disk diffusion test in which 30 μ g antibiotic disks of ceftazidime, ceftriaxone, cefotaxime and aztreonam are placed on the plate, 30 mm (center to center) from the amoxicillin/clavulanate (20 μ g/10 μ g) disk. A clear extension of the edge of the antibiotic's inhibition zone toward the disk containing clavulanate is interpreted as synergy, indicating the presence of an ESBL. The use of cefpodoxime as the expanded spectrum cephalosporin of choice has been suggested as evaluation of DDS has shown sensitivities and specificities ranging from 79% to 97% and 94% to 100% respectively^[127,128]. False-negative results have been observed with isolates harboring SHV-2, SHV-3 and TEM-12^[128-130]. In isolates which are suspicious for harboring ESBLs but are negative using the standard distance of 30 mm between disks, the test may be repeated with closer (e.g., 20 mm) or more distant (e.g., 40 mm) disks^[128,129]. The test remains a reliable, convenient and inexpensive method of screening for ESBLs. However, the interpretation of the test is quite subjective. Sensitivity may be reduced when ESBL activity is very low leading to wide inhibition zones around the cephalosporin and aztreonam^[130].

Cephalosporin/clavulanate combination: The British Society for Antimicrobial Chemotherapy has recommended the disk diffusion method for phenotypic confirmation of ESBL presence using ceftazidime/clavulanate and cefotaxime/clavulanate combination disks with semi-confluent growth on Iso-Sensitest agar^[131]. The zone diameter of each combination is compared with zone diameter of cephalosporin alone and a ratio of cephalosporin/clavulanate zone size to cephalosporin zone size is calculated. A ratio of 1.5 or greater indicates the presence of ESBL. Once the sensitivity of the test is increased to 93% for both antibiotics, it is considered that the test does not detect ESBL production by strains producing SHV-6.

Agar supplemented with clavulante: Antibiotic disks of ceftazidime (30 μ g), cefotaxime (30 μ g), ceftriaxone (30 μ g) and aztreonam (30 μ g) are placed on Mueller-Hinton agar supplemented with 4 μ g/ml clavulanate and on clavulanate free Mueller-Hinton agar plate. A difference in β -lactam zone width of 10 mm on the two media is considered positive for ESBL production^[130]. The sensitivity is 93-96% and specificity is 100% for the ceftazidime^[129]. A major disadvantage of test is the need to freshly prepare clavulanate containing media.

Disk replacement method: Three amoxicillin/clavulanate disks are applied to a Mueller-Hinton plate inoculated with the test organism. After one hour at room temperature, these antibiotic disks are removed and replaced on the same spot by disks containing ceftazidime, cefotaxime and aztreonam. Control disks of these three antibiotics are simultaneously placed at least 30 mm from these locations. A positive test is indicated by a zone increase of 5 mm for the disks which have replaced the amoxicillin/clavulanate disks compared to the control disks^[132].

Three dimensional test: The three dimensional test was described by Thomson and Sanders^[128]. It gives phenotypic evidence of ESBL-induced inactivation of extended-spectrum cephalosporins or aztreonam without relying on the demonstration of inactivation of the β -lactamases by a β -lactamase inhibitor^[128]. The test depends on the ability of a culture of the test organism to distort the zone of inhibition around an oxyimino-beta lactam disk. This test was determined to be sensitive but it is more technically challenging and labor intensive than other methods.

Etest for ESBL: The Etest ESBL strip is a two-sided strip in which clavulanate is added to one side of a dual oxyimino-beta lactam gradient looking for a reduction in the MIC of cephalosporins in the presence of clavulanate^[133]. The availability of cefotaxime as well as ceftazidime strips improves the ability to detect ESBL types which preferentially hydrolyze cefotaxime such as CTX-M-types enzymes. This method is useful for both screening and phenotypic confirmation of ESBL production. The reported sensitivity as a phenotypic confirmatory test for ESBL is 87 to 100% and specificity is 95 to 100%^[129,132,133]. The test is limited by its indeterminate results, difficulties in recognizing subtle zone deformities and cost.

ESBL detection methods by the automated antimicrobial susceptibility test systems: The automated antimicrobial susceptibility test systems (Vitek, MicroScan and BD phoenix) have also produced ESBL tests. The Vitek ESBL test utilizes cefotaxime and ceftazidime alone and in combination. A predetermined reduction in the growth of the cefotaxime or ceftazidime wells containing clavulanate, compared with the level of growth in the well with cephalosporin alone indicates a positive test^[135]. Sensitivity and specificity of the method exceed 90%^[135;136]. False-negative results have been observed in *Klebsiella pneumoniae* isolates producing both an ESBL and AmpC-type beta-

lactamase^[137]. *Klebsiella oxytoca* strains hyperproducing the K1 β -lactamase will usually be recorded as positive on the Vitek ESBL test^[135]. MicroScan panels which contain combinations of ceftazidime or cefotaxime plus β -lactamase inhibitors have appeared highly reliable^[138]. The Phoenix ESBL test uses growth response to cefpodoxime, ceftazidime, ceftriaxone and cefotaxime with or without clavulanate to detect the production of ESBLs. The results are usually available within six hours^[139]. The Phoenix ESBL method detects ESBL production in greater than 90% of strains genotypically confirmed to produce ESBL^[136,139]. The method correctly detects ESBL production by *Klebsiella*, *E.coli*, *Enterobacter*, *Proteus* and *Citrobacter spp*^[139].

In the laboratory of the Riyadh Armed Forces Hospital, which is a major tertiary care hospital in Riyadh, Saudi Arabia, ESBL detection in clinical isolates was initially done by the E-test method. Later on, MicroScan panels for ESBL detection were introduced.

CLSI recommended methods for ESBL detection: The CLSI has provided guidelines for ESBL detection for both disk diffusion and standard broth microdilution methods^[115].

Disk diffusion screening methods: CLSI proposed disk diffusion methods for screening for ESBL production by *E. coli*, *Klebsiella spp* and *Proteus mirabilis*. Ceftriaxone, cefotaxime, ceftazidime, cefpodoxime and aztreonam are used. If any of the zone diameters indicate suspicion for ESBL production, phenotypic confirmatory tests should be used to verify the diagnosis^[115]. The use of more than one antimicrobial agent for screening improves the sensitivity of detection.

Screening by dilution tests: CLSI has proposed dilution methods for screening for ESBL production by *E.coli* and *Klebsiella spp*^[115]. CLSI recommends the use of ESBL breakpoints for indicator drugs (ceftriaxone, cefotaxime, ceftazidime, cefpodoxime or aztreonam) to screen for ESBL. When the initial screen is positive, CLSI recommends a phenotypic confirmatory test.

Phenotypic confirmatory tests for ESBL production: Cephalosporin/clavulanate combination disks are used. The CLSI recommend use of cefotaxime (30 μ g) or ceftazidime (30 μ g) disks with or without clavulanate for phenotypic confirmation for the presence of ESBLs in *Klebsiella* and *E.coli*. The CLSI recommends that the disk test performed with confluent growth on Mueller-Hinton agar. A difference of 5 mm between the zone diameters of either of the cephalosporin disks and their

respective cephalosporin/clavulanate disk is considered to be phenotypic confirmation of ESBL production^[115]. The use of both antibiotic disks is advisable since the use of ceftazidime alone has resulted in the inability to detect CTX-M-producing organisms^[140].

Phenotypic confirmatory testing can also be performed by broth microdilution assays using ceftazidime (0.25 to 128 µg/ml), ceftazidime plus clavulanic acid (0.25/4, 128/4), cefotaxime (0.25 µg to 64 µg/ml) and cefotaxime plus clavulanic acid (0.25/4 to 64/4). The use of both antibiotics is recommended. The test is done using standard methods. Phenotypic confirmation is considered as 3-twofold-serial-dilution decrease in MIC of either cephalosporin in the presence of clavulanic acid to its MIC when used alone.

Quality control recommendations of CLSI should be followed in both screening and confirmatory tests^[115]. For all phenotypically confirmed ESBL producing strains according to CLSI criteria, the test should be reported as resistant for all penicillins, cephalosporins (except the cephamycins, cefoxitin and the cefotetan) and aztreonam regardless of the routine susceptibility test results. β-lactam / β-lactamase inhibitor combinations (for example: piperacillin/tazobactam and ticarcillin/clavulanate) are reported as susceptible, if MICs or zone diameters are within the appropriate range.

The phenotypic confirmatory tests are highly sensitive and specific compared to genotypic confirmatory tests. However, false positive confirmatory tests have been reported in *Klebsiella pneumoniae* or *E.coli* isolates which lack ESBLs but hyperproduce SHV-1^[141]. The coexistence of both ESBLs and plasmid-mediated AmpC-type β-lactamases in *Klebsiella pneumoniae* may result in false negative tests. AmpC-type β-lactamases resist inhibition by clavulanate and hence obscure the synergistic effect of clavulanate and cephalosporin against ESBL^[137]. There are a number of instances whereby the screening tests are positive but the confirmatory tests are negative or indeterminate^[140]. The use of cefipime alone and cefipime plus clavulanate or the utilization of CLSI ESBL disk diffusion confirmatory tests may sometimes determine whether clavulanate effect truly occurs in such cases^[140]. Also cefoxitin susceptibility may be used as a means of deducing mechanism of resistance. Cefoxitin resistant isolates may produce AmpC-type enzymes or possess porin changes, although it must be recognized that these can coexist with ESBL production^[140].

An evaluation of the use of CLSI methods for *Enterobacteriaceae* other than *E.coli* and *Klebsiella* spp. has shown that they might apply quite well to

Salmonella spp. but not to the other *Enterobacteriaceae* or non-fermentative bacteria such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii*^[142].

There have been numerous reports of both *Enterobacter* spp. harboring ESBLs in addition to chromosomal AmpC-type β-lactamases^[13,139,143]. The inhibitor-based ESBL detection methods are less reliable in detecting ESBL in *Enterobacter* spp. In organisms that produce both ESBL and AmpC, clavulanate may induce hyperproduction of the AmpC β-lactamase leading to hydrolysis of the third generation cephalosporin thus masking any synergy arising from inhibition of the ESBL. Modification of the conventional DDS in which a 30 µg cefipime (or cefpirome) disks are placed at distance of 30 or 20 mm (center to center) from a disk containing 20 µg amoxicillin plus 10 µg clavulanate has been used to detect ESBLs in *Enterobacter* spp.^[144]. Enhancement of the zone of inhibition in the area between amoxicillin/clavulanate disk and the cefipime may still be observed since cefipime is less subject to hydrolysis by AmpC β-lactamases than third generation cephalosporins. The sensitivity and specificity are higher with disks spacing 20 mm apart (90% and 97% respectively) rather than 30 mm (61% and 92%)^[144].

b) Molecular Methods:

There are a number of methods which can be used to characterize ESBLs. The most fundamental of these is iso-electric focusing as used by D'Agata *et al* which can give a presumptive identification since many of them possess identical isoelectric points^[145]. Early detection of β-lactamase genes was performed using DNA probes that were specific for TEM and SHV enzymes^[146]. The first ESBLs studied with probes belong to the TEM family^[146,147]. The using of DNA probes can sometimes be labor intensive.

PCR with oligonucleotide primers that are specific for a β-lactamase gene is the easiest and most common molecular methods used to detect the presence of a β-lactamase belonging to a family of enzymes^[5]. However, PCR will not discriminate among different variants of TEM or SHV^[147].

Several molecular methods that will aid in the detection and differentiation of ESBLs without sequencing have been suggested. The oligotyping method was used to discriminate between TEM-1 and TEM-2^[148]. This method used oligonucleotide probes that are designed to detect point mutations under stringent hybridization conditions. Several new TEM variants were identified using this method^[147]. These probes are less sensitive for the detection of mutations which are responsible for the extended substrate range^[148]. In some cases these

mutations lead to the appearance or disappearance of restriction sites. Amplification of the relevant part of the gene by PCR followed by restriction enzyme analysis can thus indicate the presence or absence of specific TEM or SHV derived ESBLs^[149].

PCR-single-strand conformation polymorphism (PCR-SSCP) has also been applied to the study of ESBLs with satisfactory results^[150]. This method has been used to detect a single base mutation at specific location within the beta-lactamase gene. The combination of PCR-SSCP with PCR-restriction fragment length polymorphism (PCR-RFLP) allows the identification of newer SHV variants. The ligase chain reaction (LCR) is used for the identification of SHV genes. LCR allows the discrimination of DNA sequences that differ by a single base pair^[151].

A novel sequence-specific peptide nucleic acid (PNA)-based multiplex PCR detection method provides an accurate means of identification of *bla* (GES-2) compared to the standard PCR and the gene sequencing techniques^[73].

In Turkey, the distribution of PER-1 ESBL was investigated by southern blot analysis with a PER-1 gene-specific probe^[67]. Nucleotide sequencing remains the standard for determination of the specific β -lactamase gene present in a strain^[5,147,152]. However, this too can give variable results depending on the method used.

TREATMENT OPTIONS

In the absence of data from randomized controlled trials to guide optimal therapy, the choice of treatment option is based on data from *in vitro* and observational studies^[110,120]. These studies suggest that carbapenems should be regarded as drugs of choice for serious life-threatening infections due to ESBL-producing organisms since they have been associated with the best outcome in terms of survival and bacteriologic clearance^[110,120]. The choice between imipenem and meropenem is difficult. Published experience is greatest with imipenem, but MICs are slightly lower for meropenem. In nosocomial meningitis, meropenem should be regarded as the drug of choice. Intrathecal polymyxin B should also be considered along with removal of neurosurgical hardware in cases of CSF shunt infections^[106]. There is no evidence that combination therapy with a carbapenem and antibiotics of other classes is superior to the use of carbapenem alone^[153]. Synergy has been exhibited in some but not in all studies. Unfortunately carbapenem resistance has been observed in organisms commonly harbouring ESBLs. Therefore, the appropriate use of these valuable agents should be strictly followed^[74,154]. For non-life threatening infections with ESBL-producers, therapy should be streamlined based on

the initial treatment response and the sensitivity results^[120].

The third generation cephalosporins should not be used to treat serious infections with ESBL-producing organisms because clinical outcome is poor even in the presence of apparent susceptibility^[112,120]. The clinical experience with use of cefipime and cephamycins is limited. Cefipime should not be used as the first line therapy against ESBL-producing organisms^[110,120,155]. If it is to be used (for example against organisms with cefipime MIC < 2 μ g/ml), it should be given in high dosage, at least 2 g twice a day^[9,120,123]. *In vitro* synergy may be achievable between cefipime and amikacin^[9,120,156]. Cefipime resistance may be more frequent in strains which produce the CTX-M-type ESBLs^[157].

Cephamycins are not recommended as first line therapy for ESBL-producing organisms despite their good *in vitro* activity^[9,120]. In one of the reports, selection of porin resistant mutants occurred during therapy, resulting in cefoxitin resistance and relapse of infection^[28]. In addition, combined cephamycins and carbapenem resistance in *Klebsiella pneumoniae* has been observed in the setting of widespread cephamycin use in response to an outbreak of infection with ESBL-producing organisms^[154].

However, β -lactam / β -lactamase inhibitors (for example piperacillin-tazobactam) are not regarded as suitable first line therapy for serious infections caused by ESBL producer^[9,120]. Data regarding their use in the treatment of serious infections is sparse and treatment failures have been reported^[120]. The activity of β -lactam / β -lactamase inhibitors is inoculum-dependent^[117]. Some animal studies have shown that β -lactam / β -lactamase inhibitor to be less active than carbapenem against ESBL-producing organisms^[158]. They are usually active against organisms producing a single ESBL. Their effectiveness may be reduced in organisms producing multiple ESBLs^[26]. The hyper-production of the parent enzymes (for example, TEM-1 or SHV-1) in ESBL-producing organisms or the combination of β -lactamase production and porin loss can also lead to a reduction in activity of β -lactamase inhibitor^[159]. *In vitro* resistance to β -lactam / β -lactamase inhibitors is increasing^[51]. Amoxicillin/clavulanate may be regarded as second line therapy for urinary tract infection^[9,120]. It is noteworthy that the use of beta-lactamase inhibitors has been shown to be a protective factor for infection or colonization with ESBL-producing *K. pneumoniae*^[160].

Treatment with non- β -lactam containing regimens was described in a small number of patients^[110,120]. Quinolones may be considered as therapy of choice for urinary tract infection and second line therapy for bacteremia, hospital

acquired pneumonia and intra-abdominal infections caused by ESBL-producing organisms provided the organism is susceptible^[9,120]. The observed increase in quinolones resistance will limit their role in treatment options^[161]. In general, the newer quinolones are unlikely to provide great additional benefits over ciprofloxacin. An observational clinical study found that carbapenems were superior to quinolones, whereas another study found that they were equivalent in effectiveness^[162,163]. It is possible that suboptimal dosing of quinolones in the presence of strains with elevated quinolone MICs, but still in the susceptible range, may account for those differences^[9]. *In vitro* studies have suggested that synergy may occur when ciprofloxacin is added to β -lactam antibiotic against ESBL-producers (e.g., cefipime, cefotaxime or imipenem)^[164]. The use of aminoglycosides for serious infection should be probably limited to combination with β -lactam antibiotics^[9,120]. More outcome studies are still needed to optimize therapy selection.

PREVENTION AND CONTROL

Many of the reported outbreaks were managed using two types of interventions: implementation of infection control measures and restriction of use of oxyimino-cephalosporins^[93,94,164]. However, it has been reported that a long lasting outbreak was successfully controlled by isolation measures^[94]. This emphasize the importance of infection control measures and the necessity to ensure compliance with them^[94]. ESBL - producing organisms may be endemic in many hospitals and measures to control their spread should be considered^[9].

Laboratory detection and reporting: Clinical microbiology laboratory plays a vital role in the control of ESBL-producing organisms. The implementation of appropriate ESBL detection methods is recommended by CLSI and several other studies^[107,115]. It is recommended by some investigators that laboratories should also report the presence of ESBL to the infection control practitioners and some suggest to the clinicians also^[124,125]. This approach is implemented in our institution in an attempt to increase awareness, guide therapy and to institute appropriate infection control precautions.

Surveillance systems: The surveillance systems help to establish a baseline prevalence data and monitor changing of rates. These data will be used to identify selective pressures and determinants which are crucial for follow up in the intervention programs^[121].

Admission screening policies: These policies will help to identify colonized or infected patients with ESBL producing organisms especially those admitted to ICUs or other high risk areas^[94,125].

Antibiotic policies: The use of third generation cephalosporins especially at widespread empiric level should be restricted either by formal restriction of availability or by education and increased availability of alternatives^[125,164]. The judicious use of other antimicrobials is essential for the control of ESBL-producing organisms.

Infection control measures: Implementation of appropriate infection control measures and monitoring the adherence to them are crucial to control spread of antibiotic resistant organisms. The importance of hand hygiene should be reinforced and the recommended isolation precautions for patients colonized or infected with ESBL producer should be followed^[93,94,125].

Education programs: Continuous education programs are necessary to address the problem of ESBLs and their control measures.

Research projects: Research studies which include different aspects of ESBLs will help in their treatment and control.

CONCLUSION

ESBLs have become a widespread serious problem and several aspects of them are worrying. These enzymes are becoming increasingly expressed by many strains of pathogenic bacteria with a potential for dissemination. They compromise the activity of wide-spectrum antibiotics creating major therapeutic difficulties with a significant impact on the outcome of patients. The continued emergence of ESBLs presents diagnostic challenges to the clinical microbiology laboratories, who need to be more aware of the need for their detection. ESBLs occurrence and spread need to be controlled. Appropriate antimicrobial selection, surveillance systems and effective infection control procedures are the key partners in their control.

REFERENCES

1. Kotra LP, Samama J, Mobashery S. Beta-lactamases and resistance to beta-lactam antibiotics. In: Lewis K, Slayters AA, Taber HW, Wax RG, editors. Bacterial resistance to antimicrobials. New York: Marcel Decker; 2002:123-160.
2. Livermore DM. Beta-lactamases in laboratory and clinical resistance. Clin Microbiol Rev 1995; 8:557-584.
3. Abraham EP, Chain E. An enzyme from bacteria able to destroy penicillin. Nature 1940; 146:837.
4. Datta N, Kontomichalou P. Penicillinase synthesis controlled by infectious R factors in *Enterobacteriaceae*. Nature 1965; 208:239-241.

5. Bradford PA. Extended spectrum beta-lactamases in the 21st century: characterization, epidemiology and the detection of this important resistance threat. *Clin Microbiol Rev* 2001; 14:933-951.
6. Samaha-kfoury JN, Araj GF. Recent developments in β -lactamases and extended spectrum β -lactamases. *Br Med J* 2003; 327:1209-1213.
7. Knothe H, Shah P, Krcmery V, Antal M, Mitsuhashi S. Transferable resistance to cefotaxime, cefoxitin, cefamandole and cefuroxime in clinical isolates of *Klebsiella pneumoniae* and *Serratia marcescens*. *Infection* 1983; 11:315-317.
8. Patterson JE. Extended spectrum beta - lactamases: the European experience. *Curr Opin Infect Dis* 2001; 14:697-701.
9. Paterson DL, Bonomo RA. Extended-spectrum β -lactamases: a clinical update. *Clin Microbiol Rev* 2005; 18:657-686.
10. Sirot D. Extended-spectrum plasmid mediated β -lactamases. *J Antimicrob Chemother* 1995; 36:19-34.
11. Bush K, Jacoby GA, Medeiros AA. A functional classification scheme for β -lactamases and its correlation with molecular structure. *Antimicrob Agents Chemother* 1995; 39:1211-1233.
12. Winokur PL, Brueggemann, DeSalvo DL, et al. Animal and human multidrug - resistant, cephalosporin-resistant *Salmonella* isolates expressing a plasmid-mediated CMY-2 AmpC β -lactamase. *Antimicrob Agents Chemother* 2000; 44: 2777-2783.
13. Levison ME. Plasmid-mediated extended-spectrum beta-lactamases in organisms other than *Klebsiella pneumoniae* and *Escherichia coli*: a hidden reservoir of transferable resistance genes. *Curr Infect Dis Rep* 2002; 4:181-183.
14. Nordmann P, Guibert M. Extended spectrum β -lactamases in *Pseudomonas aeruginosa*. *J Antimicrob Chemother* 1998; 42:128-131.
15. Gniadkowski M. Evolution and epidemiology of extended-spectrum β -lactamases (ESBLs) and ESBL-producing microorganisms. *Clin Microbiol Infect* 2001; 7:597-608.
16. Bush K. New β -lactamases in gram - negative bacteria: diversity and impact on the selection of antimicrobial therapy. *Clin Infect Dis* 2001; 32:1085 -1089.
17. DuBois SK, Marriot MS, Amyes SG. TEM-SHV-derived extended-spectrum β -lactamases:relationship between selection ,structure and function. *J Antimicrob Chemother* 1995; 35:7-22.
18. Tzouveleki LS,Bonomo RA. SHV-type β -lactamases. *Curr Pharm Des* 1999; 5:847-864
19. Jacoby GA. Genetic of extended - spectrum Beta lactamases. *Eur J Clin Microbiol Infect Dis* 1994; 13:2-11.
20. Philippon A, Arlet G, Lagrange PH. Origin and impact of plasmid - mediated extended spectrum beta - lactamases. *Eur J Clin Microbiol Infect Dis* 1994; 13:17-29.
21. Jacoby GA, Medeiros AA. More extended-spectrum β -lactamases. *Antimicrob Agents Chemother* 1991; 35:1697-1704.
22. Rasheed JK, Jay C, Metchock B, et al. Evolution of extended spectrum β -lactam resistance (SHV-8) in a strain of *Escherichia coli* during multiple episodes of bacteremia. *Antimicrob Agents Chemother* 1997; 41:647-653.
23. Medeiros AA. Evolution and dissemination of beta-lactamases accelerated by generations of beta-lactam antibiotics. *Clin Infect Dis* 1997; 24:19-45.
24. Blazquez J, Morosini M-I, Negri M-C, Baquero F. Selection of naturally occurring extended-spectrum TEM β -lactamase variants by fluctuating β -lactam pressure. *Antimicrob Agents Chemother* 2000; 44:2182-2184.
25. Palucha A, Mikiewicz B, Hryniewicz W, Gniadkowski M. Concurrent outbreaks of extended-spectrum β -lactamases producing organisms of the family *Enterobacteriaceae* in a Warsaw hospital. *J Antimicrob Chemother* 1999; 44: 489-499.
26. Bradford PA, Cherubin CE, Idemyor V, Rasmussen BA, Bush K. Multiply resistant *Klebsiella pneumoniae* from two Chicago hospitals : identification of the extended-spectrum TEM-12 and TEM-10 ceftazidime-hydrolyzing β -lactamases in a single isolates. *Antimicrob Agents Chemother* 1994; 38:761-766.
27. French GL, Shannon KP, Simmons N. Hospital outbreak of *Klebsiella pneumoniae* resistant to broad-spectrum cephalosporins and beta-lactam-beta-lactamase inhibitor combinations by hyperproduction of SHV-5 β -lactamase. *J Clin Microbiol* 1996; 34:358-363.
28. Pangon B, Bizet C, Bure A, et al. In vivo selection of a cephamycin resistant, porin deficient mutant of *klebsiella pneumoniae* producing a TEM-3-beta- lactamase. *J Infect Dis* 1989; 159:1005-1006.
29. Villa L, Pezzella C, Tosini F, Visca P, Petrucca A, Carattoli A. Multiple-antibiotic resistance mediated by structurally related IncL/M plasmids carrying an extended spectrum β -lactamase gene and class 1 integron. *Antimicrob Agents Chemother* 2000; 44:2911-2914.
30. Paterson D, Mulazimoglu L, Casellas J M, et al. Epidemiology of ciprofloxacin resistance and its relationship to extended-spectrum beta-lactamase production in *Klebsiella pneumoniae* isolates causing bacteremia. *Clin Infect Dis* 2000; 30: 473-478.
31. Burn-Buisson C, Legrand P, Philippon A, Montravers F, Asquer M, Duval J. Transferable enzymatic resistance to third generation cephalosporins during nosocomial outbreak of multiresistant *Klebsiella pneumoniae*. *Lancet* 1987; 11:302-306.
32. Sougakoff W, Goussard S, Gerbaud G, Courvalin P. Plasmid-mediated resistance to third-generation cephalosporins caused by point mutations in TEM-type penicillinase genes. *Rev Infect Dis* 1988; 10:879-884.
33. Poir L,Mammeri H,Nordmann P. TEM-121, a novel complex mutant of TEM-type beta-lactamases from *Enterobacter aerogenes*. *Antimicrob Agents Chemother* 2004; 48:4528-4531.
34. Marchandin H, Carriere C, Sirot D, Jean-Pierre H, Darbas H. TEM-24 produced by four different species of *Enterobacteriaceae*, including *Providencia rettgeri*, in a single patient. *Antimicrob Agents Chemother* 1999; 43:2069-2073.
35. Perilli M,Segatore B,Massis MRD, et al. TEM-72,a new extended-spectrum β -lactamase detected in *Proteus mirabilis* and *Morganella morganii* in Italy. *Antimicrob Agents Chemother* 2000; 44:2537-2539.
36. Morosini MI, Canton R, Martinez-Beltran J, Negri MC, Perez-Diaz JC, Baquero F, Blazquez J. New extended-spectrum TEM-type β -lactamase from *Salmonella enterica* subsp. *enterica* isolated in a nosocomial outbreak. *Antimicrob Agents Chemother* 1995; 39:458-461.
37. Jacoby GA. Extended-spectrum beta-lactamases and other enzymes providing resistance to oxymino-beta-lactams. *Infect Dis Clin N Am* 1997; 11:875-887.
38. Huletsky A, Knox JR, Levesque RC. Role of ser-238 and Lys-240 in the hydrolysis of 3rd generation cephalosporins by SHV-type beta-lactamases probed by site-directed mutagenesis and 3-dimensional modeling. *J Biol Chem* 1993; 268:3690-3697.
39. Prinarakis EE, Miriagou V, Tzelepi E, Gazouli M, Tzouveleki LS. Emergence of an inhibitor-resistant β -lactamase(SHV-10) derived from an SHV-5 variant. *Antimicrob Agents Chemother* 1997; 41:838-840.
40. El Harrif-Heraud Z, Arpin C, Benliman S,Quentin C. Molecular epidemiology of a nosocomial outbreak due to SHV-4 producing strain of *Citrobacter diversus*. *J Clin Microbiol* 1997; 35:2561-2567.
41. Huang ZM, Mao PH,Chen Y, Wu L,Wu J. Study on

- molecular epidemiology of SHV type beta-lactamase-encoding genes of multiple-drug-resistant *Acinetobacter baumannii*. *Zhonghua Liu Xing Bing Xue Za Zhi* 2004; 25:425-427.
42. Poirel L, Lebessi E, Castro M, Fevre C, Foustokou M, Nordmann P. Nosocomial outbreak of extended-spectrum beta-lactamase SHV-5-producing isolates of *Pseudomonas aeruginosa* in Athens, Greece. *Antimicrob Agents Chemother* 2004; 48:2277-2279.
 43. Tzouveleakis LS, Tzelepi E, Tassios PT, Legakis NJ. CTX - M - type - beta-lactamases: an emerging group of extended spectrum enzymes. *Int J Antimicrob Agents* 2000; 14: 137-142.
 44. Bonnet R. Growing group of extended-spectrum beta-lactamases: the CTX-M enzymes. *Antimicrob Agents Chemother* 2004; 48:1-14.
 45. Poirel L, Gniadkowski M, Nordmann P. Biochemical analysis of the ceftazidime-hydrolysing extended-spectrum beta-lactamase CTX-M-15 and of its structurally related beta-lactamase CTX-M-3. *J Antimicrob Chemother* 2002; 50:1031-1034.
 46. Bush K, Macalintal C, Rasmussen BA, Lee VJ, Yang Y: Kinetic interactions of tazobactam with beta-lactamases from all major structural classes. *Antimicrob Agents Chemother*.1993; 37:851-858.
 47. Humeniuk C, Arlet G, Gautier V, Grimont R, Labia R, Philippon A. Beta-lactamases of *Kluyvera ascorbata*; probable progenitors of some plasmid-encode CTX-M types. *Antimicrob Agents Chemother* 2002; 42:1084-1094.
 48. Radice M, Power P, DiConza J, Gutkind G. Early dissemination of CTX-M-derived enzymes in South America. *Antimicrob Agents Chemother* 2002; 46:602-604.
 49. Baraniak A, Fielt J, Hryniewicz W, Nordmann P, Gniadkowski M. Ceftazidime- hydrolyzing CTX-M-15 extended-spectrum beta-lactamase (ESBL) in Poland. *J Antimicrob Chemother* 2002; 50:393-396.
 50. Cao V, Lambert T, Nhu DQ, *et al.* Distribution of extended-spectrum beta-lactamases in clinical isolates of *Enterobacteriaceae* in Vietnam. *Antimicrob Agents Chemother* 2002; 46:3739-3743.
 51. Babini GS, Livermore DM. Antimicrobial resistance amongst *Klebsiella* spp.collected from intensive care units in Southern and Western Europe in 1997-1998. *J Antimicrob chemother* 2000; 45:183-189.
 52. Moland ES, Black JA, Hossain A, *et al.* Discovery of CTX-M-like extended-spectrum-beta-lactamases in *Escherichia coli* isolates from five U.S.states. *Antimicrob Agents Chemother* 2003; 47:2382-3383.
 53. Wang H, Kelkar S, Wu W, Chen M, Quinn JI. Clinical isolates of *Enterobacteriaceae* producing extended-spectrum beta-lactamases: prevalence of CTX-M-3 at a Hospital in china. *Antimicrob Agents Chemother* 2003; 47:790-793.
 54. Yamasaki Y, Komatsu M, Yamashita T, *et al.* Production of CTX-M-3 extended-spectrum beta-lactamases and IMP-1 metallo-beta-lactamase by five Gram-negative bacilli:survey of clinical isolates from seven laboratories collected in 1998 and 2000 in the Kinki region of Japan. *J Antimicrob Chemother* 2003; 51:631-638.
 55. Karim A, Poirel L, Nagarajan S, Nordmann P. Plasmid - mediated extended-spectrum beta-lactamase (CTX-M-3 like) from India and gene association with insertion sequence ISEcp1. *FEMS Microbiol Lett* 2001; 201:237-241.
 56. Bradford PA, Yang Y, Sahn D, Grope I, Gardovska D, Storch G. CTX-M-5, a novel cefotaxime-hydrolyzing beta-lactamase from an outbreak of *Salmonella typhimurium* in Latvia. *Antimicrob Agents Chemother* 1998; 42:1980-1984.
 57. Ishii Y, Ohno A, Taguchi H, Imajo S, Ishiguro M, Matsuzawa H. Cloning and sequence of the gene encoding cefotaxime-hydrolyzing class A β -lactamase isolated from *Escherichia coli*. *Antimicrob Agents Chemother* 1995; 39:2269-2275.
 58. Ma L, Ishii Y, Ishiguro M, Matsuzawa H, Yamaguchi K. Cloning and sequencing of the gene encoding Toho-2, a class A β -lactamase preferentially inhibited by tazobactam. *Antimicrob Agents Chemother* 1998; 42:1181-1186.
 59. Hall LM, Livermore DM, Gur D, Akova M, Akalin HE. OXA-11, an extended-spectrum variant of OXA-10 (PSE-2) beta-lactamase from *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 1993; 37:1637-1644.
 60. Danel F, Hall LMC, Gur D, Livermore DM. OXA-16, a further extended-spectrum variant of OXA-10 β -lactamase, from two *Pseudomonas aeruginosa* isolates. *Antimicrob Agents Chemother* 1998; 42:3117-3122.
 61. Danel F, Hall LMC, Gur D, Livermore DM. OXA-14, another extended - spectrum variant of OXA-10(PSE-2) β -lactamase from *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 1995; 39:1881-1884.
 62. Danel F, Hall LMC, Duke B, Gur D, Livermore DM. OXA-17, a further extended-spectrum variant of OXA-10 β -lactamase, isolated from *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 1999; 43:1362-1366.
 63. Philippon LN, Nass T, Bouthor A-T, Barakett V, Nordmann P.OXA-18, a class D clavulanic acid-inhibited extended-spectrum β -lactamase from *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 1997; 41:2188-2195.
 64. Nordmann P, Nass T. Sequence analysis of PER-1 extended-spectrum beta -lactamase from *Pseudomonas aeruginosa* and comparison with class A beta-lactamases. *Antimicrob Agents Chemother* 1994; 38:104-114.
 65. Nordmann P, Ronco E, Nass T, Duport C, Michel-Briand Y, Labia R. Characterization of a novel extended-spectrum beta-lactamase from *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 1993; 37:962-969.
 66. Vahaboglu H, Ozturk R, Aygun G, *et al.* Widespread detection of PER-1-type extended-spectrum β -lactamases among nosocomial *Acinetobacter* and *Pseudomonas aeruginosa* isolates in Turkey: a nationwide multicentre study. *Antimicrob Agents Chemother* 1997; 41:2265-2269.
 67. Vahaboglu H, Hall LMC, Mulazimoglu L, Dodanlı S, Yildirim I, Livermore DM. Resistance to extended-spectrum cephalosporins, caused by PER-1 β -lactamase, in *Salmonella typhimurium* from Istanbul. Turkey *J Med Microbiol* 1995; 43:294-299.
 68. Bauernfeind A, Stemplinger I, Jungwirth R, *et al.* Characterization of beta - lactamase gene blaPER-2, which encodes an extended-spectrum class A β -lactamase. *Antimicrob Agents Chemother* 1996; 40:616-620.
 69. Petroni A, Corso A, Melano R *et al.* Plasmidic extended - spectrum beta-lactamase in *Vibrio cholera* O1 El Tor isolates in Argentina. *Antimicrob Agents Chemother* 2002; 46:1462-1468.
 70. Poir L, Nass T, Guibert M, Chaibi B, Labia R, Nordmann P. Molecular and biochemical characterization of VEB-1, a novel class A extended-spectrum beta-lactamase encoded by an *Escherichia coli* integron gene. *Antimicrob Agents Chemother* 1999; 43:573-581.
 71. Girlich D, Poirel L, Leelaporn A, Karim A, Tribuddharat C, Fennewald M, Nordmann P. Molecular epidemiology of the integron-located VEB-1 extended - spectrum beta-lactamase in nosocomial enterobacterial isolates in Bangkok, Thailand. *J Clin Microbiol* 2001; 39:175-182.
 72. Poirel L, Rotimi VO, Mokaddas EM, Karim A, Nordmann P. VEB-1 like extended - spectrum beta-lactamases in *Pseudomonas aeruginosa*, Kuwait. *Emerg Infect Dis* 2001; 7:468-470.
 73. Weldhagen GF. Sequence selective recognition of extended-spectrum beta-lactamase GES-2 by a competitive peptide

- nucleic acid based multiplex PCR. *Antimicrob Agents Chemother* 2004; 48:3402-3406.
74. Galani I, Souli M, Chryssouli Z, Katsala D, Giamarellou H. First identification of an *Escherichia coli* clinical isolate producing both metallo-beta-lactamase VIM-2 and extended-spectrum beta-lactamase IBC-1. *Clinical Microbiol Infect* 2004; 10:757-760.
 75. Bonnet R, Sampaio JL, Chanal C, et al. A novel class A extended-spectrum beta-lactamase (BES-1) in *Serratia marcescens* isolated in Brazil. *Antimicrob Agents Chemother* 2000; 44:3061-3068.
 76. Matsumoto Y, Inoue M. Characterization of SFO-1, a plasmid-mediated inducible class A beta-lactamase from *Enterobacter cloaca*. *Antimicrob Agents Chemother* 1999; 43:307-313.
 77. Silva J, Aguiler C, Ayla G, et al. TLA-1: a new plasmid-mediated extended-spectrum beta-lactamase from *Escherichia coli*. *Antimicrob Agents Chemother* 2000; 44:997-1003.
 78. Beidenbach DJ, Moet G J, Jones RN. Occurrence and antimicrobial resistance pattern comparisons among bloodstream infection isolates from the SENTRY Antimicrobial surveillance program (1997 - 2002). *Diagn Microb Infect Dis* 2004; 50:59-69.
 79. Bouchillon SK, Johnson BM, Hoban DJ, et al. Determining incidence of extended - spectrum beta-lactamase producing *Enterobacteriaceae*, vancomycin-resistant *Enterococcus faecium* and Methicillin-resistant *Staphylococcus aureus* in 38 Centres from 17 countries: the PEARLS study 2001 - 2002. *Int J Antimicrob Agents* 2004; 24:119-124.
 80. Yagi T, Kruokawa H, Shibata N, Shibayama K, Arakawa Y. A preliminary survey of extended-spectrum beta-lactamases (ESBLs) in clinical isolates of *Klebsiella pneumoniae* and *Escherichia coli* in Japan. *FEMS Microbiol Lett* 2000, 184:53-56.
 81. Pai H, Lyu S, Lee JH, et al. Survey of extended -spectrum beta-lactamases in clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae* : prevalence of TEM-52 in Korea. *J Clin Microbiol* 1999; 37:1758-1763.
 82. Ho PL, Tsang DNC, Que TL, Ho M, Yuen KY. Comparison of screening methods for detection of extended-spectrum beta-lactamases and their prevalence among *Escherichia coli* and *Klebsiella* species in Hong Kong. *APMIS* 2000; 108:237-240.
 83. Karsh T, Taufik A, AL shammery F, Al Soleh S, Kambal A, Shibl A. Antimicrobial resistance and prevalence of extended spectrum beta-lactamase among clinical isolates of gram negative bacteria in Riyadh. *J Chemother* 1995; 7: 509-514.
 84. Baby M. Detection of extended spectrum beta-lactamases in members of the family *Enterobacteriaceae* at a teaching hospital, Riyadh, Kingdom of Saudi Arabia. *Saudi Med J* 2002; 23:186-190.
 85. Kader A, Kumar A. Prevalence of extended spectrum beta-lactamase among multidrug resistant gram negative isolates from a general hospital in Saudi Arabia. *Saudi Med J* 2004, 25:570 - 574.
 86. El-Khizzi NA, Bakheshwain SM. Prevalence of extended-spectrum beta-lactamases among *Enterobacteriaceae* isolated from blood culture in a tertiary care hospital. *Saudi Med J* 2006; 27:37-40.
 87. Lautenbach E, Patel JB, Bilker WB, Edelstein PH, Fishman NO. Extended-spectrum beta-lactamases producing *Escherichia coli* and *Klebsiella pneumoniae*: risk factors for infection and impact of resistance on outcomes. *Clin Infect Dis* 2001; 32:1162-1171.
 88. Bermudes H, Arpin C, Jude F, El-Harrif Z, Bebear C, Quentin C. Molecular epidemiology of an outbreak due to extended-spectrum beta-lactamase-producing enterobacteria in a French hospital. *Eur J Clin Microbiol Infect Dis* 1997; 16:533-529.
 89. Martinez-Aguilar G, Alpuche-Aranda CM, Anaya C, et al. Outbreak of nosocomial sepsis and pneumonia in a newborn intensive care unit by multiresistant extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* :high impact on mortality. *Infect Control Hosp Epidemiol* 2001; 22:725-728.
 90. Hibbert-Rogers LC, Heritage J, Gascoyne-Binzi DM, et al. Molecular epidemiology of ceftazidime resistant *Enterobacteriaceae* from patients on a paediatric oncology ward. *J Antimicrob Chemother* 1995; 36:65-68.
 91. Wiener J, Quinn JP, Bradford PA, et al. Multiple antibiotic resistant *Klebsiella* and *Escherichia coli* in nursing homes. *JAMA*1999, 281: 517- 523.
 92. Hibbert -Rogers LC, Heritage J, Todd N, Hawkey PM. Convergent evolution of TEM-26, a beta-lactamase with extended-spectrum activity. *J Antimicrob Chemother* 1994; 33:707-720
 93. Pena C, Pujol M, Ardanuy C, et al. Epidemiology and successful control of a large outbreak due to *Klebsiella pneumoniae* producing extended-spectrum beta lactamases. *Antimicrob Agents chemother* 1998; 42: 53-58.
 94. Lucet JC, Decré D, Fichelle A, et al. Control of a prolonged outbreak of extended spectrum beta-lactamase producing *Enterobacteriaceae* in a university hospital. *Clin Infect Dis* 1999; 20:1411-1418.
 95. Pena C, Pujol M, Ricart AJ, et al. Risk factors for faecal carriage of *Klebsiella pneumoniae* producing extended-spectrum beta-lactamase(ESBL-KP) in the intensive care unit. *J Hosp Infect* 1997; 35:9-16.
 96. Lucet JC, Chevret S, Decre D, et al. Outbreak of multiply resistant enterobacteriaceae in intensive care unit: epidemiology and risk factors for acquisition. *Clin Infect Dis* 1996; 22:430-436.
 97. Gaillot O, Maruejols C, Abachin E, et al. Nosocomial outbreak of *Klebsiella pneumoniae* producing SHV-5 extended-spectrum beta-lactamase, originating from a contaminated ultrasonography coupling gel. *J Clin Microbiol* 1998; 36:1357-1360.
 98. Rougues AM, Boulard G, Allery A, et al. Thermometers as a vehicle for transmission of extended-spectrum producing beta-lactamase-producing *Klebsiella pneumoniae*. *J Hosp Infect* 2000; 45:76-77.
 99. Bureau-Chalot F, Drieux L, Pierrat-Solans C, Forte D, deChamp C, Bajolet O. Blood pressure cuffs as a potential reservoir of extended-spectrum beta-lactamase VEB-1-producing isolates of *Acinetobacter baumannii*. *J Hosp Infect* 2004; 58:91-92.
 100. Branger C, Bruneau B, Lesimple AL, et al. Epidemiological typing of extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* isolates responsible for five outbreaks in a university hospital. *J Hosp Infect* 1997; 36:23-36.
 101. Cotton MF, Wasserman E, Pieper CH, et al. Invasive disease due to extended-spectrum beta-lactamase producing *Klebsiella pneumoniae* in a neonatal unit: the possible role of cockroaches. *J Hosp Infect* 2000; 44:13-17.
 102. Eisen DE, Russell EG, Tymms M, Roper EJ, Grayson ML, Turnidge J. Random amplified polymorphic DNA and plasmid analyses used in investigation of an outbreak of multiresistant *Klebsiella pneumoniae*. *J Clin Microbiol* 1995; 33:713-717.
 103. Schiappa DM, Hayden MK, Matushek MG, et al. Ceftazidime-resistant *Klebsiella pneumoniae* and *Escherichia coli* bloodstream infection: case-control and molecular epidemiologic investigation . *J Infect Dis* 1996; 174:529-536.
 104. Alcantar-Curiel D, Tinoco JC, Gayosso C, et al. Nosocomial bacteremia and urinary tract infections caused by extended-spectrum-beta-lactamase-producing *Klebsiella pneumoniae* with plasmids carrying both SHV-5 and TLA-1, genes. *Clin Infect Dis* 2004; 38:1067-1074.

105. Rodriguez-Bano, Navarro JMD, Romero L, et al. Epidemiology and clinical features of infections caused by extended-spectrum beta-lactamase-producing *Escherichia coli* in nonhospitalized patients. *J Clin Microbiol* 2004; 42:1089-1094.
106. Segal-Maurer S, Mariano N, Qavi A, Urban C, Rahal jr JJ. Successful treatment of ceftazidime-resistant *Klebsiella pneumoniae* ventiliculitis with intravenous meropenem and intraventricular polymyxin B: case report and review. *Clin Infect Dis* 1999; 28:1134-1138.
107. Bush K. Is it important to identify extended - spectrum beta lactamase producing isolates? *Eur J Clin Microbiol Infect Dis* 1996; 15: 361-364.
108. Quinn JP. Clinical significance of extended-spectrum beta-lactamase. *Eur J Clin Microbiol Infect Dis* 1994; 13:39-42.
109. Mackenzie FM, Gould IM. Extended spectrum β -lactamases. *J Infect* 1998; 36: 255-258.
110. Paterson DL, Ko WC, VonGottberg A, et al. Antibiotic therapy for *Klebsiella pneumoniae* bacteremia: implications of production of extended-spectrum beta-lactamases. *Clin Infect Dis* 2004; 39:31-37.
111. Anonymous. The cost of antibiotic resistance: effect of resistance among *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa* on length of hospital stay. *Infect Control Hosp Epidemiol* 2002; 23:106-108.
112. Paterson DL, Ko WC, Von Gottberg A, et al. Outcome of Cephalosporin treatment for serious infections due to apparently susceptible organisms producing extended-spectrum beta-lactamases: implications for the clinical microbiology laboratory. *J Clin Microbiol* 2001; 39: 2206-2212.
113. Ho PL, Chan WM, Tsang KW, Wang SS, Young K. Bacteremia caused by *Escherichia coli* producing extended-spectrum β -lactamase: a case-control study of risk factors and outcomes. *Scand J Infect Dis* 2002; 34:567-563.
114. Kim YK, Pai H, Lee HJ, et al. Bloodstream infections by extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in children: epidemiology and clinical outcome. *Antimicrob Agents Chemother* 2002; 46:1481-1491.
115. National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial susceptibility testing; 15th informational supplement (M100-S15), 2005 National Committee for Clinical Laboratory Standards, Wayne, PA.
116. Philippon A, Labia R, Jacoby G. Extended-spectrum beta-lactamases. *Antimicrob Agents Chemother* 1989; 33:1131-1136.
117. Thomson ks, Moland ES. Cefipime, piperacillin-tazobactam and the inoculum effect in tests with extended spectrum beta-lactamase producing *Enterobacteriaceae*. *Antimicrob Agents Chemother* 2001; 45:3548-3554.
118. Rice LB, Yao JD, Klmm K, Eliopoulos GM, Moellering Jr RC. Efficacy of different beta-lactams against an extended-spectrum beta-lactamase producing *Klebsiella pneumoniae* strain in the rat intra-abdominal abscess model. *Antimicrob Agents Chemother* 1991; 35:1243-1244.
119. Bush K. New β -lactamases in gram-negative bacteria: diversity and impact on the selection of antimicrobial therapy. *Clin Infect Dis* 2001; 32:1085-1089.
120. Patterson DL. Recommendations for treatment of severe infections caused by *Enterobacteriaceae* producing extended-spectrum beta-lactamases (ESBL). *Clin Microbiol Infect* 2000; 6:460-463.
121. Rahal JJ. Extended spectrum β -lactamases: how big is the problem? *Clin Microbiol Infect* 2000; 6:2-6.
122. Docquier JD, Luzzaro F, Amicosante G, Toniolo A, Rossolini GM. Multidrug-resistant *Pseudomonas aeruginosa* producing PER-1 extended-spectrum serine-beta-lactamase and VIM-2 metallo-beta-lactamase. *Emerg Infect Dis* 2001; 7:910-911.
123. Maglio D, Ong C, Banevicius MA, Geng Q, Nightingale CH, Nicolau DP. Determination of the in vivo pharmacodynamic profile of cefepime against extended-spectrum-beta-lactamase-producing *Escherichia coli* at various inocula. *Antimicrob Agents Chemother* 2004; 48:1941-1947.
124. Tenover FC, Mohammed MJ, Gorton TS, Dembek ZF. Detection and reporting of organisms producing extended spectrum beta-lactamases: survey for laboratories in Connecticut. *J Clin Microbiol* 1999; 37:4065-4070.
125. Patterson JE, Yu VL. Extended spectrum β -lactamases; a call for improved detection and control. *Clin infect Dis* 1999; 29:1419 -1422.
126. Jarlier V, Nicolas MH, Fournier G, Philippon A. Extended-spectrum beta-lactamases conferring transferable resistance to newer beta-lactam agents in *Enterobacteriaceae*: hospital prevalence and susceptibility patterns. *Rev Infect Dis* 1988; 10:867-878.
127. Thomson KS, Sanders CC. A simple and reliable method to screen isolates of *Escherichia coli* and *Klebsiella pneumoniae* for the production of TEM- and SHV- derived extended-spectrum beta-lactamases. *Clinical Microbiol Infect* 1997; 3:549-554.
128. Thomson KS, Sanders CC. Detection of extended -spectrum beta-lactamases in members of the family *Enterobacteriaceae*: comparison of the double-disk and three-dimensional tests. *Antimicrob Agents Chemother* 1992; 36:1877-1882.
129. Ho PL, Chow KH, KW, Yuen KY, Ng WS, Chau PY. Comparison of a novel, inhibited-potentiated disc-diffusion test with other methods for the detection of extended-spectrum beta-lactamases in *Escherichia coli* and *Klebsiella pneumoniae*. *J Antimicrob Chemother* 1998; 42:49-54.
130. Vercauteren E, Descheemaeker P, Ieven M, Sanders CC, Goossens H. Comparison of screening methods for detection of extended-spectrum beta-lactamases and their prevalence among blood isolates of *Escherichia coli* and *Klebsiella* spp. in a Belgian teaching hospital. *J Clin Microbiol* 1997; 35:2191-2197.
131. M'Zali FH, Chanawong A, Kerr KG, Birkenhead D, Hawkey PM. Detection of extended-spectrum beta-lactamases in members of the family *enterobacteriaceae*: comparison of the MAST DD test, the double disc and the Etest ESBL. *Antimicrob Agents Chemother* 2000; 45:881-885.
132. Schooneveldt JM, Nimmo GR, Giffard P. Detection and characterisation of extended- spectrum beta-lactamases in *Klebsiella pneumoniae* causing nosocomial infection. *Pathology* 1998; 39:164-168.
133. Cormican MG, Marshall SA, Jones RN. Detection of extended-spectrum beta-lactamase (ESBL)-producing strains by the Etest ESBL screen. *J Clin Microbiol* 1996; 34:1880-1889.
134. Florjin A, Nijssen S, Schmitz FJ, Verhoef J. Comparison of E test and double disc diffusion test for detection of extended spectrum beta lactamases. *Eur J Clin Microbiol Infect Dis* 2002; 21:241-243.
135. Sanders CC, Barry AL, Washington JA, et al. Detection of extended-spectrum -beta-lactamase-producing members of the family *Enterobacteriaceae* with Vitek ESBL test. *J Clin Microbiol* 1996; 34:2997-3001.
136. Liverstein - van Hall MA, Fluit AC, Paauw A, Box AT, Brisse S, Verhoef J. Evaluation of the Etest ESBL and the BD phoenix, VITEK 1 and VITEK 2 automated instruments for detection of extended-spectrum beta-lactamases in multiresistant *Escherichia coli* and *Klebsiella* spp. *J Clin microbiol* 2002; 40:3703 -3711.
137. Tzouveleakis LS, Vatopoulos AC, Katsanis G, Tzelepi E. Rare case of failure by an automated system to detect extended-

- spectrum beta-lactamases in a cephalosporin-resistant *Klebsiella pneumoniae* isolate. *J Clin Microbiol* 1999; 37:2388.
138. Sturenberg E, Lang M, Horstkotte MA, Laufs R, Mack D. Evaluation of the MicroScan ESBLplus confirmation panel for detection of extended-spectrum beta-lactamases in clinical isolates of oxyimino-cephalosporin-resistant Gram-negative bacteria. *J Antimicrob Chemother* 2004; 54:870-875.
 139. Sanguinetti M, Posteraro B, Spanu T, et al. Characterization of clinical isolates of *Enterobacteriaceae* from Italy by the BD Phoenix extended-spectrum beta-lactamase detection method. *J Clin Microbiol* 2003; 41:1463-1468.
 140. Steward CD, Rasheed JK, Hubert SK, et al. Characterization of clinical isolates of *Klebsiella pneumoniae* from 19 laboratories using the National Committee for Clinical Laboratory Standards extended-spectrum beta-lactamase detection methods. *J Clin Microbiol* 2001; 39:2864-2872.
 141. Rice LB, Carias LL, Hujer AM, et al. High-level expression of chromosomally encoded SHV-1 beta-lactamase and an outer membrane protein change confer resistance to ceftazidime and piperacillin-tazobactam in a clinical isolate of *Klebsiella pneumoniae*. *Antimicrob Agents Chemother* 2000; 44:362-367.
 142. Schwaber MJ, Raney PM, Rasheed JK, et al. Utility of National Committee for Clinical Laboratory Standards guidelines for identifying extended-spectrum beta-lactamases in non-*Escherichia coli* and non-*Klebsiella* spp of *Enterobacteriaceae*. *J Clin Microbiol* 2004; 42:294-298.
 143. Pitout JD, Thomson KS, Hanson ND, Ehrhardt AF, Coudron P, Sanders CC. Plasmid-mediated resistance to expanded-spectrum cephalosporins among *Enterobacter aerogenes* strains. *Antimicrob Agents Chemother* 1998; 42:596-600.
 144. Tzelepi E, Giakkoupi P, Sofianou D, Loukova V, Kemeroglou A, Tsakris A. Detection of extended-spectrum beta-lactamases in clinical isolates *Enterobacter cloacae* and *Enterobacter aerogenes*. *J Clin Microbiol* 2000; 38:542-546.
 145. D'Agata E, Venkataraman L, DeGirolami P, Weigel L, Samore M, Tenover F. The molecular and clinical epidemiology of enterobacteriaceae-producing extended-spectrum beta-lactamases in a tertiary care hospital. *J Infect* 1998; 36: 279-285.
 146. Huovinen S, Huovinen P, Jacoby GA. Detection of plasmid-mediated beta-lactamases with DNA probes. *Antimicrob Agents Chemother* 1988; 32:175-179.
 147. Fluit AC, Visser MR, Schmitz F-J. Molecular detection of antimicrobial resistance. *Clin Microbiol Rev* 2001; 14:836-871.
 148. Tham TN, Mabilat C, Courvalin P, Guesdon JL. Biotinylated oligonucleotide probes for the detection and the characterization of TEM-type extended broad spectrum beta-lactamases in *Enterobacteriaceae*. *FEMS Microbiol Lett* 1990; 69:109-116.
 149. Arlet G, Brami J, Dècère D, Flippo A, Galtotol O, Lagrange PH, Philippon A. Molecular characterization by PCR-restriction fragment length polymorphism of TEM beta-lactamases. *FEMS Microbiol Lett* 1995; 134:1498-1500.
 150. M'Zali FH, Gascoyne-Binzi DM, Heritage J, Hawakey M. Detection of mutations conferring extended-spectrum activity on SHV beta-lactamases using polymerase chain reaction single strand conformational polymorphism (PCR-SSCP). *J Antimicrob Chemother* 1996; 37:797-802.
 151. Kim J, Lee HJ. Rapid discriminatory detection of genes coding for SHV-beta-lactamases by ligase chain reaction. *Antimicrob Agents Chemother* 2000; 44:1860-1864.
 152. Bradford PA. Automated thermal cycling is superior to traditional methods for nucleotide sequencing of *bla*SHV genes. *Antimicrob Agents Chemother* 1999; 43:2960-2963.
 153. Pattharachayakul S, Neuhauser MM, Quinn JP, Pendland SL. Extended-spectrum beta-lactamase (ESBL)-producing *Klebsiella pneumoniae*: activity of single versus combination agents. *J Antimicrob Chemother* 2003; 51:737-739.
 154. Bradford PA, Urban C, Mariano N, Projan SJ, Rahal JJ, Buch K. Imipenem resistance in *Klebsiella pneumoniae* is associated with the combination of ACT-1, a plasmid-mediated AmpC beta-lactamase and the loss of outer membrane protein. *Antimicrob Agents Chemother* 1997; 41:563-569.
 155. Wong-Beringer A. Therapeutic challenges associated with extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae*. *Pharmacotherapy* 2001; 21:583-592.
 156. Elkhail H, Kamili N, Linger L, et al. In vitro time-kill curves of cefepime and ceftazidime combined with amikacin, gentamicin or ciprofloxacin against *Klebsiella pneumoniae* producing extended-spectrum beta-lactamase. *Chemotherapy* 1997; 43:245-253.
 157. Yu WL, Pfaller MA, Winokur PL, Jones RN. Cefepime MIC as a predictor of the extended-spectrum beta-lactamase type in *Klebsiella pneumoniae*, Taiwan. *Emerg Infect Dis* 2002; 8:522-524.
 158. Karadenzili A, Mutlu B, Okay E, Kolayi F, Vahaboglu H. Piperacillin with and without tazobactam against extended-spectrum beta-lactamase-producing *Pseudomonas aeruginosa* in a rat thigh abscess model. *Chemotherapy* 2001; 47:292-296.
 159. Akhan S, Coskuncan F, Tansel O, Vahaboglu H. Conjugative resistance to tazobactam plus piperacillin among extended-spectrum beta-lactamase-producing nosocomial *Klebsiella pneumoniae*. *Scand J Infect Dis* 2001; 33:512-515.
 160. Piroth L, Aube H, Doise JM, Vincent - Martin M. Spread of extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae*: are beta-lactamase inhibitors of therapeutic value? *Clin Infect Dis* 1998; 27:76.
 161. Martinez-Martines L, Pascual A, Coejo Mdel C, et al. Energy-dependent accumulation of norfloxacin and porin expression in clinical isolates of *Klebsiella pneumoniae* and relationship to extended-spectrum beta-lactamase-production. *Antimicrob Agents Chemother* 2002; 46:3926-3932.
 162. Endimiani A, Luzzaro F, Perilli M, et al. Bacteremia due to *Klebsiella pneumoniae* isolates producing the TEM-52 extended-spectrum beta-lactamase: treatment outcome of patients receiving imipenem or ciprofloxacin. *Clin Infect Dis* 2004; 38:243-251.
 163. Kang CI, Kim SH, Park WB, et al. Bloodstream infections due extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae*: risk factors for mortality and treatment outcome, with special emphasis on antimicrobial therapy. *Antimicrob Agents Chemother* 48:4574-4581.
 164. Archambaud M, Labau E, Clave D, Suc C. Bactericidal effect of cefotaxime-sulbactam and imipenem combined with gentamicin and/or ciprofloxacin against CTX-1 producing *Klebsiella pneumoniae*. *Pathol Biol (Paris)* 1989; 37:534-539.
 165. Rahal JJ, Urban C, Horn D. Class restriction of cephalosporin use to control total cephalosporin resistance in nosocomial *klebsiellae*. *JAMA* 1998; 280:1233-1237.