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Extended Spectrum Beta Lactamases- Important Resistance Threat

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ABSTRACT

β-Lactamases continue to be the leading cause of resistance to β-lactam antibiotics among gram-negative bacteria. In recent years there has been an increased incidence and prevalence of extended-spectrum β-lactamases (ESBLs), enzymes that hydrolyze and cause resistance to oxyimino-cephalosporins and aztreonam. The majority of ESBLs are derived from the widespread broad-spectrum β-lactamases TEM-1 and SHV-1. There are also new families of ESBLs, including the CTX-M and OXA-type enzymes as well as novel, unrelated β-lactamases. Several different methods for the detection of ESBLs in clinical isolates have been suggested. While each of the tests has merit, none of the tests is able to detect all of the ESBLs encountered. ESBLs have become widespread throughout the world and are now found in a significant percentage of Escherichia coli and Klebsiella pneumoniae strains in certain countries. They have also been found in other Enterobacteriaceae strains and Pseudomonas aeruginosa. Strains expressing these β-lactamases will present a host of therapeutic challenges as we head into the 21st century. This review will focus on the characterization of ESBLs, the importance of detection of these enzymes, and their epidemiology.

Introduction and history

Fifty years ago the antibiotic era began with the discovery of penicillin. Within a few years of introduction of penicillin into clinical use, penicillinase producing Staphylococcus aureus started to proliferate in hospitals. To overcome this problem, penicillinase resistant penicillins came into picture. Shortly afterward, the broad spectrum penicillins and first generation cephalosporins were introduced. They remained a first line of defense against microbes for over 20 years, before resistance due to b-lactamases produced by gram negative bacilli became a serious problem.**(1)** To counter this threat, the pharmaceutical industry marketed six novel classes of b lactam antibiotics (cephamycins, oxyimino cephalosporins, carbapenems, monobactams and clavam and penicillianic acid sulfone inhibitors) within a relatively short span of 7-8 years. Although, novel b-lactamases had emerged gradually after the introduction of new b lactam agents, their number and variety accelerated at an alarming rate **(1,2).** More than 170 b-lactamases have been recognised**(1).**

The first plasmid-mediated β-lactamase in gram-negatives, TEM-1, was described in the early 1960s **(3).** The TEM-1 enzyme was originally found in a single strain of *E. coli* isolated from a blood culture from a patient named Temoniera in Greece, hence the designation TEM**(4).** Being plasmid and transposon mediated has facilitated the spread of TEM-1 to other species of bacteria. Within a few years after its first isolation, the TEM-1 β-lactamase spread worldwide and is now found in many different species of members of the family *Enterobacteriaceae, Pseudomonas aeruginosa, Haemophilus influenzae*, and *Neisseria gonorrhoeae.* Another common plasmid-mediated β-lactamase found in *Klebsiella pneumoniae* and *E. coli* is SHV-1 (for sulphydryl variable). The SHV-1 β-lactamase is chromosomally encoded in the majority of isolates of *K. pneumoniae* but is usually plasmid mediated in *E. coli.*

The first of these enzymes capable of hydrolyzing the newer β-lactams, SHV-2, was found in a single strain of *Klebsiella ozaenae* isolated in Germany **(5).** Because of their increased spectrum of activity, especially against the oxyimino-cephalosporins, these enzymes were called extended-spectrum β-lactamases (ESBLs)... They are a worrying global public health issue as infections caused by such enzyme producing organisms are associated with a higher morbidity and mortality and greater fiscal burden. ESBLs are primarily produced by the Enterobacteriaceae family of Gram-negative organisms, in particular *Klebsiella pneumonia* and *Escherichia coli* **(6,7).** They are also produced by nonfermentative Gram-negative organisms, such as *Acinetobacter baumannii* and *Pseudomonas aeruginosa* **(8).**

Functional and Molecular Grouping

The original method of *β*-lactamase categorisation is the Ambler classification **(9)** which orders the enzymes into 4 classes (A, B, C, and D) based on molecular structure**(9).** Class A enzymes are characterized by an active-site serine, a molecular mass of approximately 29,000 Da, and the preferential hydrolysis of penicillins **(10).** Class A β-lactamases include such enzymes as TEM-1, SHV-1, and the penicillinase found in *S. aureus*. ESBLs are Class A *β*-lactamases and may be defined as plasmid-mediated enzymes that hydrolyse oxyimino-cephalosporins, and monobactams but not cephamycins or carbapenems **(11).** They are inhibited in vitro by clavulanate **(12).** There are various genotypes of ESBLs. Of these, the most common are the SHV, TEM, and CTX-M types **(13).** Other clinically important types include VEB, PER, BEL-1, BES-1, SFO-1, TLA, and IBC **(14).** In 1995, Bush et al. devised a classification of *β*-lactamases based upon their functional characteristics and substrate profile, a classification which is widely used **(15).** The enzymes are divided into three major groups: group1 cephalosporinases which are not inhibited by clavulanic acid, the larger group 2 broad spectrum enzymes which are generally inhibited by clavulanic acid (except for the 2d and 2f groups) and the group 3 metallo-*β*-lactamases. Most ESBLs are assigned to group 2be, that is, hydrolyse penicillins, cephalosproins, and monobactams, and inhibited by clavulanic acid (as per the Ambler classification).

TABLE-1 (BUSH JACOBY CLASSIFICATION) (15)

Bush Jacoby Medeiros Group	Molecular class (Ambler)	*β*-lactamase inhibitors	Representative enzymes	Resistance or susceptibility
1	C	Cephalosporins	Amp-C	Resistant
2b	A	Penicillins, Cephalosporins	TEM, SHV	Susceptible
2be	A	Penicillins, Extended spectrum Cephalosporins, Monobactams	TEM, SHV	Susceptible
2d	D	Penicillins, Cloxacillin	OXA	Resistant
2e	A	Cephalosporins	Inducible cephalosporinases from Proteus vulgaris	Susceptible
2f	A	Penicillins, Cephalosporins, Carbapenems	NMC-A from Enterobacter clocae	Resistant
3	B	Most β-lactams including Carbapenems	L1 from Stenotrophomonas maltophila	Resistant

Multidrug-resistance profiles in ESBL-producing isolates

ESBL producers are commonly resistant to different antibiotic families besides beta-lactams –fluoroquinolones, aminoglycosides and trimethoprim-sulfamethoxazole, which contribute to the selection and persistence of multidrug-resistant ESBL strains and plasmids in both clinical and community settings

(16, 17). The proportion of ESBL-producing isolates resistant to fluoroquinolones has increased over time, initially in *K. pneumoniae* and later also in *E. coli* **(16,18,19).** This increase has apparently occurred in parallel to the increase in plasmid-mediated resistance mechanisms.

A high level of fluoroquinolone resistance is often due to additional loss of outer membrane proteins or efflux pump overexpression in clones that already contain *gyrA* and *parC* chromosomal mutations and plasmid-mediated mechanisms **(21).** Genes that encode resistance to aminoglycosides (different modifying enzymes and ArmA methylase), trimethoprim or sulfonamides and are located on a wide range of genetic elements such as class 1, 2 and 3 integrons or transposable elements have been associated with different multidrug-resistant ESBL plasmids from human and animal origin **(20-33).**

Laboratory Detection of ESBL
The methods for detection of ESBLs can be broadly divided into two groups: phenotypic methods that use non-molecular techniques, which detect the ability of the ESBL enzymes to hydrolyse different cephalosporins; and genotypic methods, which use molecular techniques to detect the gene responsible for the production of the ESBL. Clinical diagnostic laboratories use mostly phenotypic methods because these tests are easy to do, are cost effective, and have been incorporated in most automated susceptibility systems, making them widely accessible **(24).** However, phenotypic methods are not able to distinguish between the specific enzymes responsible for ESBL production (SHV, TEM, and CTX-M types). Several research or reference laboratories use genotypic methods for the identification of the specific gene responsible for the production of the ESBL, which have the additional ability to detect low-level resistance (ie, can be missed by phenotypic methods) **(25).**Furthermore, molecular assays also have the potential to be done directly on clinical specimens without culturing the bacteria, with subsequent reduction of detection time **(26).** The detection of ESBL-producing bacteria in laboratories is a crucial step for appropriate management of patients, but genotypic identification of these enzymes provides essential information for infection prevention and control efforts, as well as the tracking of these organisms in surveillance systems.

Double Disk Approximation Test
In this test, first described by Jarlier et al. in 1988, the organism to be tested is spread onto a Mueller-Hinton Agar plate**(27).** Next, two antimicrobial disks are placed 30mm apart (centre to centre). One of the disks contains amoxicillin/ clavulanic acid and the other contains an expanded- spectrum cephalosporin (for example, ceftriaxone, cefotaxime or ceftazidime). The test is positive if, after 24-hour incubation, the zone of inhibition in between the disks is enhanced. The enhancement is due to the inhibition of the ESBL by clavulanic acid (provided by the amoxicillin/ clavulanic acid disk) and the subsequent action of the extended-spectrum cephalosporin**(28).**

Inhibitor potentiated disc diffusion test
Cephalosporin disc is placed on clavulanate containing and without clavulanate containing MHA plates. More than10 mm increase in the zone of inhibition on the clavulanate containing MHA plate indicates ESBL production **(49).**

Three-Dimensional Test
In this test, the organism to be tested is spread onto a Mueller-Hinton agar plate and a slit is cut into the agar the length of the plate **(28).** The test organism is then inoculated into the slit and an expanded- spectrum cephalosporin is placed 3mm from the slit. A distorted zone on the side of the slit is considered a positive test. The test was shown to be as sensitive at detecting ESBLs as the double disk approximation test but is more technically challenging **(28,29).**

E-Test
AB Biodisk (Solna, Sweden) has introduced a two-sided ESBL E-test strip that contains either a combination of ceftazidime and ceftazidime/clavulanic acid or cefotaxime and cefotaxime/clavulanic acid. Both strips have a decreasing gradient of ceftazidime or cefotaxime alone on one end and a decreasing gradient of ceftazidime or cefotaxime plus a fixed gradient of clavulanic acid on the other end. A >3log reduction in the MIC of cefotaxime or ceftazidime in the presence of clavulanic acid is considered a positive test.

Vitek
The Vitek automated susceptibility system (Biomerieux, Hazlewood, Missouri, USA) has introduced an ESBL test on their system whereby ceftazidime and cefotaxime are tested alone and in combination with clavulanic acid. Logarithmic reduction in growth within the well containing clavulanic acid compared to the well not containing clavulanic acid indicates expression of an ESBL. **(30-32).**

Molecular Typing
A variety of molecular methods have been used to study the epidemiology of ESBL-producing bacteria. These methods include plasmid profiles, pulsed-field gel electrophoresis (PFGE), ribotyping, arbitrarily primed polymerase chain reaction (PCR) and random amplified polymorphic DNA (RAPD)**(33).**

Epidemiology
Large number of outbreaks of infection due to ESBL-producing organisms have been described on every continent of the globe except Antarctica**(33).**

Outbreak populations(33)
Adults
Intensive care units
Solid organ transplantation
Bone marrow transplantation
Long-term care units
Paediatrics
Neonatal intensive care units
Paediatric intensive care units
Solid organ transplantation

Risk factors for acquisition of ESBL-producing Enterobacteriaceae(33)
Severity of illness
Length of hospital stay
Length of intensive care unit stay
Invasive procedures
Intravascular devices
Arterial catheters
Central venous catheters
Administration of total parenteral nutrition
Mechanical ventilatory assistance
Urinary catheters
Gastrostomy, jejunostomy or nasogastric tubes
Age
Haemodialysis
Decubitus ulcers
Poor nutritional status
Low birth weight
Antibacterialadministration

Reservoirs/vectors (33)
Healthcare worker hand colonisation
Contaminated ultrasonography gel
Thermometers
Cockroaches

Management
With regards to the antimicrobial treatment of the septic patient, there is a lack of options against ESBL-producing organisms. As well as hydrolysing the β-lactam ring found in penicillin, cephalosporins (except cephamycins), and aztreonam, ESBL producers often have other mechanisms that confer resistance to other classes of antimicrobials, as described earlier. *Carbapenems.* Carbapenems are regarded as the antibiotic of choice and mainstay against severe infections caused by ESBLs **(34,35).** They also have the added benefit of being effective against other classes of β-lactamases such as the Amp C class.

*Fluoroquinolones.*A good clinical outcome can be achieved using quinolones **(36).** In UTIs caused by susceptible ESBLs, quinolones may be regarded as an excellent treatment option **(37).** However, the empirical use of fluoroquinolones to treat these infections is generally not recommended, due to the concern of resistance, the rates of which are increasing worldwide **(36).**

Aminoglycosides. As per the quinolones, if an organism is susceptible on antibiotic testing to aminoglycosides, they are effective. Aminoglycosides can be a useful adjunct due their rapidly bactericidal activity; however, their use as monotherapy should be avoided where possible particularly in serious infection **(38).**

Fosfomycin. Fosfomycin has excellent in vitro activity against ESBL-producing Enterobacteriaceae **(39).** It is one of the few antibiotics active against ESBLs that can be administered orally. It has been proven effective against susceptible ESBL producing isolates causing cystitis **(40).**

Tigecycline. Tigecycline is a derivative of minocycline with a broad spectrum of activity. It has excellent in vitro activity against ESBL producers, especially *E. coli* isolates**(41).**

β-lactamase Inhibitor Combinations. These agents may be active against organisms possessing a single ESBL **(37).** Whilst it should never be used for serious infections, amoxicillin/clavulanate may be effective in community acquired UTIs caused by susceptible ESBLs **(40, 42).** Tazobactam has been shown to be more effective against CTX-M ESBLs compared with clavulanate whilst both appear superior to sulbactam against SHV and TEM types**(43,44).**

Polymixins (Colisitin and Polymixin B). Colistin is often used to combat multidrug-resistant organisms, in particular *Acinetobacter baumannii* and *Pseudomonas aeruginosa* **(45).** It has excellent efficacy against ESBL producers **(46)** and with the emergence of carbapenem resistant organisms (e.g., KPCs), it has been used (albeit rarely) to treat such infections and curb outbreaks **(47).**

Nitrofurantoin. Nitrofurantoin can be effective in uncomplicated UTIs caused by ESBL producers **(48)**.

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