

## Extended Spectrum Beta- Lactamases: A Minireview of Clinical Relevant Groups

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### Abstract

Extended spectrum beta-lactamases are enzymes that hydrolyze the beta-lactam ring of Beta-lactam antibiotics rendering the organism resistant. ESBL prevalence is still increasing across the globe and have been implicated in hospital acquired infections and complicated urinary tract infections in Canada. TEM, SHV and CTX-M are becoming more common with CTX-M becoming more of importance as it is associated with complicated urinary tract infections. A variety of detection methods can be used including phenotypical methods, molecular methods and automated methods. Several automated instruments are commercially available to detect phenotypic resistance, recently the Clinical Laboratory Standards Institute has altered their breakpoints negating the necessity to perform confirmatory tests. Unfortunately not all of the commercial panels have the ability to detect lower breakpoints. Due to the importance of ESBL producing organisms in both hospital and community acquired infections, the associated increase in cost treating these infections and expanding spread across the globe, there is a need for further research into these enzymes.

**Keywords:** Extended spectrum beta-lactamases; CTX-M; TEM; SHV; CLSI Cephalosporin breakpoints; Automated detection instruments

### Introduction

ESBLs are enzymes that are most commonly found in *Escherichia coli* and *Klebsiella pneumoniae* and are becoming more predominant in *Enterobacteriaceae* [1]. These genes are carried on plasmids and include temoneira (TEM  $\beta$ -lactamase), sulfhydryl variable (SHV  $\beta$ -lactamase) and cefotaximase (CTX-M  $\beta$ -lactamase) [2]. Resistance genes to other antibiotic classes are often carried on these plasmids [3].

### Specific Gene Variations within ESBL

Within the ESBL family the most common genes are temoneira (*bla*TEM) and sulfhydryl variable (*bla*SHV) with increasing reports of cefotaximase (*bla*CTX-M) [4]. The *bla*SHV and the *bla*TEM subgroups arise from mutational changes due to point mutations in the genes resulting in subtypes. Examples include *bla*SHV which differs from *bla*SHV-1 due to a single mutation of an amino acid from glycine to serine at position 238 (G238S) [5] while the same mutation in the *bla*TEM gene is referred to as *bla*TEM-2 [6]. *Bla*CTX-M is becoming more prevalent, acquired on a plasmid from *Kluyvera* species, over 125 different subtypes of the *bla*CTX-M gene have been described with *bla*CTX-M-15 most predominant around the globe associated with clonal spread [7,8]. Other less familiar genes within the ESBLs include *bla*OXA, *bla*PER, *bla*VEB, *bla*CME, *bla*TLA, *bla*SFO and *bla*GES to name a few. *Bla*OXA differs from the *bla*OXA-10 by one amino acid mutation, either S73D or G157D. The *bla*PER enzyme is mainly found in Turkey and South America, the genes *bla*VEB, *bla*CME and *bla*TLA are closely related to the *bla*PER gene [5], *bla*SFO is closely related to class A beta-lactamases [5]. ESBLs can be categorized into four different classes: A, B, C and D. Class A denotes a serine enzyme beta-lactamase, class B refers to metallo-beta-lactamases, class C represents cephalosporinases and class D enzymes are oxacillin-hydrolyzing enzymes [2,9].

### Prevalence of ESBL

There have been a large number of outbreaks reported in the past decade in Africa, Asia, Europe, and North America [10,11], mostly

originating in hospitals or in nursing homes [10,11]. A prevalence study was done involving 11 hospitals across Canada from 2005 to 2009, the prevalence increased in Canada from 0.12 in 2005 to 0.47 per 1000 inpatient days in 2009 [10].

A study in Manitoba showed the increase of the prevalence of ESBL in *E. coli* and *K. pneumoniae* from 2007 to 2011. In 2007 the prevalence of ESBL in *E. coli* was 3.4%, *K. pneumoniae* 1.5% and *AmpC E. coli* 0.7% which increased in 2011 for *E. coli* to 7.1%, *K. pneumoniae* to 4.0% and *AmpC E. coli* to 2.9% [11]. The prevalence of ESBL producing organisms is likely to continue to increase, as these organisms survive on surfaces and benches for prolonged periods of time and has been linked with clonal spread across the globe [10,12].

### Detection Methods

The detection methods include phenotypical methods and molecular methods. Examples of the former include the double disc synergy test and antibiotic gradient strips, in principle synergistic activity between the clavulanate component and another beta-lactam antibiotic is considered to be positive for an ESBL. Synergy is indicated by the enlargement of the zone around the Kirby-Bauer discs or inhibition of growth also seen on the strips [13]. The E-tests and Liofilchem strips are similar, the minimum inhibitory concentration (MIC) are read in  $\mu$ g/ml and a positive result is defined as a two double dilution reduction between the clavulanate and non-clavulanate containing strips [12,14].

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Although they are similar Liofilchem strips are a high-quality paper product that is easier to read, the E-test are a plastic strip which is often associated with air bubbles being trapped under the strip [14].

Molecular methods include polymerase chain reactions (PCR) and gene sequencing. These methods offer different approaches to the detection of ESBLs. Limiting factors to the implementation of these methods in routine laboratory testing includes cost, technical expertise and regional genetic variations. As these methods are dependent on a specific target sequence and local prevalence of a specific gene it is often difficult to develop an all-encompassing molecular algorithm to detect these genes. Reporting the clinical significance remains problematic as the question arises if these genes are expressed as a clinical significant entity [15]. On the other hand ESBLs may not be detected due to primer-probe target mismatch.

More recent testing involve phenotypic tests like the NDP-ESBL test and the use of Matrix-Assisted Laser Desorption/Ionization (MALDI) Time-of-Flight instruments. Both these methods relies on the beta-lactam ring being hydrolyzed by the ESBL enzyme. With the NDP-ESBL the resulting acidity is detected by the addition of a pH indicator, phenol red, and inhibition through the addition of tazobactam [16]. This method is faster (less than 1 hour) than PCR testing due to the ability to test from isolated colonies or clinical samples and the test has 100% specificity [17,18]. By analyzing the peaks on the MALDI-TOF scatter plot, hydrolysis of the beta-lactam ring can be detected [14,19]. The beta-lactam antibiotic gives a unique peak which disappears if the bacteria produces an ESBL enzyme (Table 1).

## Automated Instruments Used for Detection

Automated instruments detect the growth in antibiotic reaction wells, comparing these to a databank of bacterial identities and corresponding susceptibility profiles. Software compares and analyzes the data to generate the final susceptibility results. Cefotaxime, ceftazidime and cefepime together with clavulanate are tested in most automated systems. The automated systems include VITEK 2 (BioMérieux, France), BD Phoenix (Becton Dickinson, United States) and MicroScan WalkAway (Siemens Healthcare Diagnostics, United States) the performance of these systems varies and differs depending on the species investigated with a much higher sensitivity (80-99%) than specificity (50-80%) [20]. These tests are time consuming and confirmatory tests are often required [21-24]. The MicroScan WalkAway is considered to give the most comparable results to micro broth dilutions [23,25].

## Cephalosporin Breakpoints

All of the detection methods Double Disc Synergised determines susceptibility or resistance to  $\beta$ -lactam antibiotics. The revised Clinical Laboratory Standards Institute (CLSI) breakpoints for cefuroxime, cefotaxime, ceftriaxone, ceftazidime, cefpodoxime and cefepime [20] allows for detection of cephalosporin resistance negating the need to perform confirmation tests for ESBL detection. Rational for lowering the breakpoints include a greater margin of safety and better correlation between clinical achievable serum level concentrations of the cephalosporin and the MIC of the isolate [20]. Unfortunately this will in all likelihood lead

Test	General Procedure	Advantages	Disadvantages
<b>Phenotypic methods</b>			
Double Disc Synergy test	Phenotypic method. Clavunulate is used to detect the presence of ESBL enzyme by having a synergistic effect.	Simple test to perform. Inexpensive compared to other methods.	Sensitivity depends on inoculum, quantity of enzyme produced. Synergy or inhibition of the enzyme can be overlooked by inexperienced technologists. Time consuming: needs at least 18 hours of incubation.
E-tests and Liofilchem gradient strips	Gradient antibiotic strip. Enhanced ratio of inhibition is determined by reading the MIC.	Phenotypic expression detected. Sensitivity is high if ESBLs is actively produced.	Does not detect the presence of genes. More expensive than other phenotypic methods. The E-test is plastic strips and tends to form air bubbles if not applied by experienced staff.
<b>Molecular methods</b>			
PCR and Genome Sequencing	Determine and amplify sequence of specific gene primer. Detection by Real-time melting curve analysis or agarose gel detection.	Sensitive method with high efficiency; small amounts of sample needed; amenable to high throughput. Less time consuming than phenotypic methods.	Specificity depends on the primer selected; this may result in unidentified phenotypically expressed ESBL. Expensive compared to phenotypic methods. Highly qualified technical staff needed to perform assays.
MALDI-ToF	Presence or absence of antibiotic is detected.	Modified phenotypic method. Cost effective if the initial equipment purchase is excluded.	Very accurate results in experienced hands. Methods are not yet accepted by accreditation bodies like EUCAST or CLSI. Variable results can be obtained.
<b>Automated instruments</b>			
Vitek Systems	Use identification and susceptibility cards. Wells in card is monitored for growth using light attenuation measurement.	Automated and can easily be integrated with modern laboratory workflow.	Some results may have to be verified with another test (ie. Indeterminate results); isolates cannot be older than 24 hours. The results are determined as projection of growth, this method can be less accurate than micro broth dilutions
BD Phoenix System	Use identification and susceptibility cards with redox indicator to determine growth in wells.	The instrument is automated and can be integrated with modern laboratory workflow. Results are more comparable to micro broth dilution methods.	The inoculation of the cards can be onerous. The addition of an Automated preparation station is required for easy workflow integration.
MicroScan WalkAway	Use photometric or fluorogenic reader to determine growth in wells.	Considered to be the closest instrument to provide micro broth dilution comparable results.	Longer incubation time compared to other automated systems. The inoculation of the wells are extremely time consuming and labour intensive.

**Table 1:** Summary Table of Detection Methods of ESBLs including major advantages and disadvantages.

Drug	MIC						Disk					
	New			Old			New			Old		
	S	I	R	S	I	R	S	I	R	S	I	R
Cefotaxime	≤ 1	2	≥ 4	≤ 8	16- 32	≥ 64	≥ 26	23- 25	≤ 22	≥ 23	15- 22	≤ 14
Ceftriaxone	≤ 1	2	≥ 4	≤ 8	16- 32	≥ 64	≥ 23	20- 22	≤ 19	≥ 21	14- 20	≤ 13
Ceftazidime	≤ 4	8	≥ 16	≤ 8	16	≥ 32	≥ 21	18- 20	≤ 16	≥ 18	15- 17	≤ 14
Ceftizoxime	≤ 1	2	≥ 4	≤ 8	16- 32	≥ 64	≥ 25	22- 24	≤ 21	≥ 21	14- 20	≤ 13

†S: Susceptible; I: Intermediate; R: Resistant

**Table 2:** Revised and new CLSI breakpoints

to an increased use of carbapenems, associated with increased cost and potential selection of resistance to carbapenems (Table 2).

## Conclusion

Although primarily in hospitals, community-acquired ESBLs are starting to become more frequent [1]. Many gene variations, and subtypes of genes, occur as a result of point mutations [26]. The *bla*TEM and the *bla*SHV subtypes are most common with the *bla*CTX-M becoming more frequent [1]. Automated instruments are frequently used in laboratories due to their limited labour requirement. Continued research is mandated as there is an ever expanding variation and spread of the ESBL genes.

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