

Beta-Lactamases and ESBL

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Abstract

Microbial resistance through extended-spectrum β -lactamase (ESBL) was first reported in the early 1980s in Europe and subsequently in the United States soon after the introduction of third-generation cephalosporins in clinical practice.

β -Lactamases are enzymes that inactivate β -lactam antibiotics by opening the β -lactam ring of penicillins and cephalosporin and abolish their antimicrobial activity. Beta-lactamases have been described for many species of gram-positive and gram-negative bacteria. Some β -lactamases are plasmid mediated while others are chromosomally mediated.

Keywords: Esbl-B -Lactamases-Tem; Shv; Ctx-M; Toho; Oxa; Per; Veb-1; Bes-1 Beta Lactamases

Introduction

Bacterial resistance to antibacterial agents is a condition in which there is no or decreased susceptibility to antibacterial agents that ordinarily cause inhibition of bacterial cell growth or death. Bacterial resistance to antibacterial agents is a quantitative measurement of the efficiency (concentration expressed in micrograms per millilitre or as inhibition zones in millimetres for the diffusion techniques) of an antibacterial agent against a specific bacterium. *In vitro* measurements of antibacterial activity are based on testing increasing concentrations of an antibacterial agent against a bacterial isolate to find out at which concentration the growth of the bacterium is inhibited. This is known as the minimum inhibitory concentration (MIC) of the drug [1].

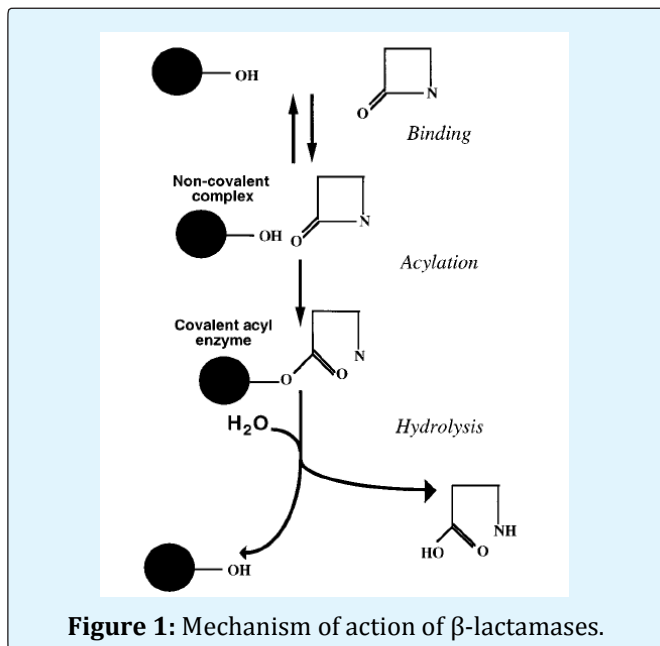
The resistance mechanism may be expressed continuously whether an inciting challenge is present or not. This state is referred to as constitutive expression. In contrast, some genes must be "induced" by exposure to the challenge substance before they produce the induced gene product [2].

Some enzymes are secreted actively into the extracellular environment where they can exert their antibacterial action, such as the β -lactamases of staphylococci. In contrast, most of the enzymes of Gram-negative bacteria are cell-bound so that they exert their effects only if the antimicrobial agent enters the bacterial cell wall [3].

Beta-Lactamases

Definition: β -lactamases are bacterial enzymes that are encoded by chromosomal or by plasmid-borne genes, they protect the microorganisms against lethal effects of β -lactam antibiotics by hydrolyzing the β -lactam ring, thus rendering the drugs inert. Based on their amino acid sequences, β -lactamases can be classified into 4 different molecular groups, the Ambler classes A, B, C, and D [4].

Mechanism of Action of B-Lactamases: The action of serine β -lactamases depends on the association of the enzyme noncovalently with the antibiotic to yield the noncovalent Michaelis complex (Figure 1). The β -lactam ring is then attacked by the free hydroxyl on the side chain of a serine residue at the active site of the enzyme, yielding a covalent acyl ester. Hydrolysis of the ester finally liberates active enzyme and the hydrolyzed inactive drug. This mechanism is followed by β -lactamases of molecular classes A, C, and D but class B enzymes (Metallo β -lactamases) utilize a zinc ion to attack the β -lactam ring [5].



Nomenclature: B-Lactamase nomenclature has not followed rational rules. Some enzymes have three or four-letter abbreviations. Some were named for properties related to some of the substrates they hydrolyze, such as

CARB (for activity against carbenicillin) and OXA and IMP (for activity against oxacillins and imipenem respectively), and some were named for their biochemical properties, such as SHV (for “sulphydryl variable”). NMC-A stands for “nonmetallo carbapenemase of class A.” The name CTX is an abbreviation of cefotaximase and refers to the potent hydrolytic activity of these enzymes against cefotaxime. The AmpC β -lactamases were named for the genes that encode them and hence for ampicillin hydrolysis. Other enzymes were named for a particular bacterium; for example, PSE refers to *Pseudomonas*-specific enzymes and Sme refers to the *Serratia marcescens* enzyme [6].

TEM was named after the patient from whom it was isolated in 1963 in Athens, Greece, a young female called Temoniera. The names of some other enzymes refer to a hospital, for example, MIR (for “Miriam Hospital”). The names of other β -lactamases refers to the pharmaceutical company and bacteria with which they are associated, such as FPM (for “Fujisawa’s *Proteus mirabilis*”), and others refer to the researchers who discovered them, such as HMS (for “Harris, Matthew, Sykes”). Thus the nomenclature does not always refer to the properties of these particular enzymes [7].

Classification: β -lactamases are most commonly classified according to two general schemes: the Ambler molecular classification scheme and the Bush-Jacoby-Mederos functional classification system.

The Ambler scheme divides β -lactamases into four major classes (A to D). The basis of this classification scheme rests upon protein homology (amino acid similarity), and not phenotypic characteristics. In the Ambler classification scheme, β -lactamases of classes A, C and D are active-site serine β -lactamases. But class B or metallo β -lactamases need zinc to attack the β -lactam ring [8].

The Bush-Jacoby-Medeiros classification scheme (Table 1) groups β -lactamases according to functional similarities (substrate and inhibitor profile). There are four main groups and multiple subgroups in this system. This classification scheme is of much more immediate relevance to the physician or microbiologist in a diagnostic laboratory because it considers β -lactamase inhibitors and β -lactam substrates that are clinically relevant [9].

Group	Enzyme type	Inhibition by clavulanate	Molecular class (Ambler)	No. of enzymes (Minimum)	Predominant location of enzyme	Predominant expression	Examples
1	Cephalosporinase	No	C	53	Chromosomal or plasmid	Chromosomal enzymes usually inducible; plasmid enzymes usually constitutive	Resistance to all β -lactams except carbapenems (unless combined with porin changes)
2a	Penicillinase	Yes	A	23	Plasmid or transposon	Inducible in Staph.aureus	Staph. aureus; S.epidermidis; Enterococcus spp.; predominantly penicillins
2b	Broad-spectrum	Yes	A	16	Chromosomal or plasmid	Inducible or constitutive	TEM-1, SHV-1; penicillins and cephalosporins
2be	Extended-spectrum	Yes	A	119	Plasmid	Constitutive	TEM-3; SHV-2; Klebsiella oxytoca K-1; penicillins and cephalosporins
2br	Inhibitor-resistant	Diminished	A	24			Primarily derived from TEM type
2c	Carbenicillinase	Yes	A	19			PSE-1; CARB-3; BRO-1; Carbenicillin
2d	Cloxacillinase	Yes	D or A	31			OXA-1; PSE-2; Streptomyces cacaioi; cloxacillin/oxacillin
2e	Cephalosporinase	Yes		20			Proteus vulgaris Bacteriodes fragilis; Cep A; Cephalosporins
2f	Carbapenemase	Yes	A	4			Enterobacter cloacae IMI-1; NMC-A; carbapenems
3	Metalloenzyme	No	B	24			Stenotrophomonas maltophilia L1; all β -lactam classes except monobactams
4	Penicillinase	No		9			Burkholderia cepacia; miscellaneous unsequenced enzymes that do not fit into other groups

Table 1: Bush-Jacoby-Medeiros Functional Classification Scheme for β -lactamases (With Correlation to the Ambler Molecular Classification Scheme) [10].

Extended Spectrum Beta-Lactamases

Definition: In Gram-negative pathogens, β -lactamases production remains the most important contributing factor to β -lactam resistance. β -lactamases are bacterial enzymes that inactivate β -lactam antibiotics by hydrolysis, which results in ineffective compounds. One group of β -lactamases, extended-spectrum β lactamases (ESBLs), have the ability to hydrolyse and cause resistance to various types of the newer β -lactam antibiotics, including the expanded-spectrum (or third-generation) cephalosporins (eg, cefotaxime, ceftriaxone, ceftazidime) and monobactams (eg, aztreonam), but not the cephamycins (eg, cefoxitin and cefotetan) and carbapenems (eg, imipenem, meropenem and

ertapenem). ESBLs are also inhibited by the β -lactamase inhibitors (clavulanate, tazobactam, sulbactam) Organisms that produce ESBLs remain an important reason for therapy failure with cephalosporins and have serious consequences for infection control. Therefore it is important that clinical microbiology laboratory detects and reports ESBLs [11].

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 [12]. 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 (25.8%) and Greece (27.4%) and lowest in the Netherlands (2%) and Germany (2.6%) [13,14]. In Japan, the percentage of ESBL production in *E.coli* and *K. pneumoniae* is low (5%) [15]. Elsewhere in Asia the percentage varies from 4.8% in Korea to 12% in Hong Kong [16]. In the Kingdom of Saudi Arabia 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 [17]. ESBLs exist in every region of the world and in most genera of enterobacteria [18].

In Cairo, studied the presence of β -lactamases with an extended spectrum of activity in clinical *Escherichia coli* isolates [19]. Forty-six *E.coli* isolates were collected from patients with urinary tract infections at Al-Azhar University Hospital 2001. Phenotypic characterisation identified a very high ESBLs rate of 60.9%. Pulsed-field gel electrophoresis and plasmid profiles revealed eight different clonal groups. All ESBL producers were polymerase chain reaction-positive for *bla*TEM and *bla*CTX-M genes. Within the CTX-M family, three different enzymes, CTX-M-14, CTX-M-15 and CTX-M-27, were found. The ESBL producers carried multiple plasmids and further plasmid-encoded resistances. In several strains, genes for up to six aminoglycoside-modifying enzymes were detected. A linkage to fluoroquinolone resistance was not observed. This study confirms the high rate of ESBLs in Egypt and further demonstrates the worldwide spread of genes coding for CTX-M enzymes in clinical isolates.

ESBL Types

TEM Beta-Lactamases (class A): The TEM-type ESBL are derivatives of TEM-1 and TEM-2. TEM-1, TEM-2 and TEM-13 are not ESBLs. TEM-1 was first reported in 1965 from an *E.coli* isolate from a patient in Athens, Greece, named Temoneira (hence the designation TEM). More than 100 TEM-type β -lactamases have been described, the majority of which are ESBLs. 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. TEM-type ESBLs have been reported in other genera of *Enterobacteriaceae* such as *Enterobacter aerogenes*, *Enterobacter cloacae*,

Morganella morganii, *Proteus mirabilis* and *Salmonella* spp. Furthermore, TEM-type ESBL have been found in non-*Enterobacteriaceae* Gram - negative bacteria, e.g., *Pseudomonas aeruginosa* [20].

SHV Beta-Lactamases (Class A): The SHV-type of ESBL may be found in clinical isolates more frequently than any other type of ESBLs. 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. More than 50 SHV varieties are described worldwide. SHV-type of ESBLs has been detected in a wide range of *Enterobacteriaceae*. Outbreaks of SHV-producing *Pseudomonas aeruginosa* and *Acinetobacter* spp. have been reported [21].

CTX-M Beta-Lactamases (Class A) & Toho Beta-Lactamases: These enzymes were named for their greater activity against cefotaxime than other oxyimino-beta-lactam substrates (eg, ceftazidime, ceftriaxone, or cefepime). Rather than arising by mutation, they represent examples of plasmid acquisition of β -lactamase genes normally found on the chromosome of *Kluyvera* species, a group of rarely pathogenic commensal organisms. These enzymes are not very closely related to TEM or SHV β -lactamases in that they show only approximately 40% identity with these two commonly isolated β -lactamases. More than 40 CTX-M enzymes are currently known. Despite their name, few are more active on ceftazidime than cefotaxime. They have mainly been found in strains of *Salmonella enterica* serovar Typhimurium and *E. coli*, but also have been described in other species of *Enterobacteriaceae* and are the predominant ESBL type in parts of South America [22].

Toho-1 and Toho-2 are β -lactamases related structurally to CTX-M-type β -lactamases. Toho refers to the Toho University School of Medicine Omori Hospital in Tokyo, where a child who was infected with Toho-1 β -lactamase-producing *Escherichia coli* was hospitalized. Like most CTX-M-type β -lactamases, the hydrolytic activity of the Toho-1 and Toho-2 enzymes is more potent against cefotaxime than ceftazidime [23].

OXA Beta-Lactamases (Class D): 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. Several of the OXA-type ESBLs have been derived from the original OXA-10 β -lactamase (e.g., OXA -11, 14, 6 and 17). 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. A novel ESBL (OXA-18) was reported to be inhibited by clavulanic acid. Many of the newer members of OXA-type of ESBLs have been found mainly in *Pseudomonas aeruginosa* isolates originating from Turkey and France [24].

PER-Type Beta-Lactamases: The PER-type ESBLs share only around 25 to 27% homology with known TEM- and SHV-type ESBLs. PER-1 β -lactamase efficiently hydrolyses penicillins and cephalosporins and is susceptible to clavulanic acid inhibition. PER-1 was first detected in *Pseudomonas aeruginosa*, and later in *Salmonella enteric* serovar Typhimurium and *Acinetobacter* isolates as well. In Turkey, as many as 46% of nosocomial isolates of *Acinetobacter* spp. and 11% of *Pseudomonas aeruginosa* were found to produce PER-1. Isolates of *Pseudomonas aeruginosa* producing PER-1 have been detected in France, Italy, and Belgium. Additionally, a high prevalence of PER-1 in *Acinetobacter* spp. from Korea has been noted. PER-2, which shares 86% homology to PER-1, has been detected in *S. enterica* serovar Typhimurium, *Escherichia coli*, *Klebsiella pneumonia*, *Proteus mirabilis*, and *Vibrio cholera* O1 El Tor. PER-2 has only been found in South America [18].

VEB-1, BES-1 and Other ESBLs: A variety of other β -lactamases which are plasmid-mediated or integron-associated class A enzymes have been discovered. They are not simple point mutant derivatives of any known β -lactamases. They are remarkable for their geographic diversity. They include VEB-1 which has greatest homology with PER-1 and PER-2 (38%). It confers high-level resistance to ceftazidime, cefotaxime, and aztreonam, which is reversed by clavulanic acid. The gene encoding VEB-1 was found to be plasmid mediated; such plasmids also confer resistance to non- β -lactam antibiotics. The patient from whom the β -lactamase was originally described was a Vietnamese infant hospitalized in France. An identical β -lactamase has also been found in *Escherichia coli*, *Klebsiella pneumonia*, *Enterobacter cloacae*, *Enterobacter sakazakii*, and *Pseudomonas aeruginosa* isolates in Thailand. Other VEB enzymes have also been detected in Kuwait and China [25].

GES, BES, TLA, SFO, and IBC are other examples of non-TEM, non-SHV ESBLs and have been found in a wide range of geographic locations [26].

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