REVIEW

Defining an extended-spectrum β-lactamase

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ABSTRACT

The term 'extended-spectrum β-lactamase' (ESBL), initially 'extended-broad-spectrum β-lactamase', was first coined for derivatives of TEM and SHV enzymes able to hydrolyse oxyimino-cephalosporins. These all belonged to β-lactamase functional group 2be. Subsequently, the term has been stretched to include: (i) enzymes with spectra similar to those of TEM and SHV mutants but derived from other sources, e.g., the CTX-M and VEB types; (ii) TEM and SHV mutants with borderline ESBL activity, e.g., TEM-12; and (iii) various β-lactamases conferring wider resistance than their parent types but not meeting the definition for group 2be, e.g., OXA derivatives and mutant AmpC types with increased activity against cefepime. It seems best—and pragmatic—that the term 'ESBL' retains its broad modern usage, but that should always be accompanied by mention of the enzyme's family as, e.g., in 'TEM ESBL' or 'OXA ESBL', not as a sole moniker.

Keywords β-Lactamases, classification, CTX-M, ESBL, extended-spectrum β-lactamase, review, SHV, TEM

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THE BEGINNINGS OF A TERM

The development of broad-spectrum penicillins in the 1960s was swiftly followed by the emergence of resistance and by recognition that most of this resistance was due to β-lactamases. Multiple β-lactamase types were recognised and a classification was developed by Richmond and Sykes [1], based on hydrolytic profiles, susceptibility to inhibition by *p*-chloromercuri-benzoate and cloxacillin, and whether the enzymes were plasmid-mediated or chromosomal. Hydrolytic profile was based on relative activity against cephaloridine and benzylpenicillin and was defined as 'cephalosporinase', 'penicillinase', or 'broad spectrum', when both reference substrates were hydrolysed at similar rates. Among the broad-spectrum enzymes, placed in group III, were the TEM-1, TEM-2 and SHV-1 types, which rapidly became the major sources of acquired resistance to the broad-spectrum penicillins [2]. TEM-1 had already spread to 30–50% of *Escherichia coli* isolates and was well-established in other Enterobacteriaceae by the early 1970s; by the mid-1970s, it had spread also to *Haemophilus influenzae* and *Neisseria gonorrhoeae*. TEM-2 and SHV-1 enzymes remained ten-fold less prevalent than TEM-1 but also became widely scattered, generally scoring as the second and third most prevalent β-lactamases in surveys undertaken in the 1970s and early 1980s [3]. The origins of the TEM types remain uncertain, but SHV-1 was later realised to be derived from the chromosomal β-lactamase of *Klebsiella pneumoniae* [4].

The spread of plasmid-mediated TEM and SHV enzymes provided a major impetus for the development of 'β-lactamase-stable β-lactams' from the mid-1970s onwards. These included oxyimino-cephalosporins (Fig. 1), cephamycins, temocillin, aztreonam and carbapenems. For reasons of convenience, spectrum, cost and safety, the oxyimino-cephalosporins—principally cefuroxime, cefotaxime, ceftriaxone, ceftazidime and cefepime—became the most-used of these analogues, and are now standard therapies for pneumonias, intra-abdominal infections, and urinary infection in many hospitals worldwide. Ceftriaxone was the first injectible antibiotic to have achieved a turnover of $1 billion per annum, equating to about 10% of the global market.

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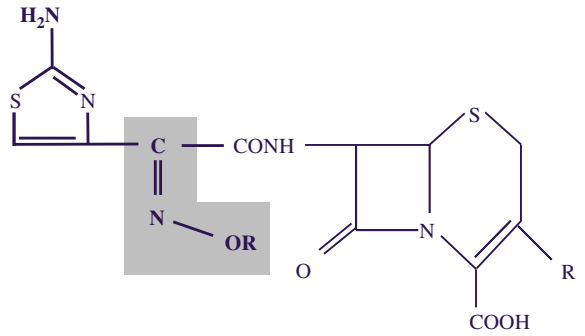

Fig. 1. Structure of an oxyimino-aminothiazolyl cephalosporin. The C=N–OR group, shaded, is held rigid and shields the β-lactam ring from attack by classic β-lactamases, but not by extended-spectrum β-lactamases. Cefuroxime, cefotaxime, ceftriaxone, ceftazidime, cefepime and cefpirome are all designed on this scaffold.

Needless to say, this heavy usage has exerted great selection pressure for resistance, which first became a concern in *Enterobacter*, *Citrobacter* and *Serratia* spp., where it is mostly contingent on mutational depression of chromosomal AmpC β-lactamases [5,6]. In 1982, however, Kliebe *et al.* [7] described a *K. pneumoniae* strain from Germany with SHV-2 β-lactamase, a mutant of SHV-1 capable of hydrolysing oxyimino-cephalosporins. Perhaps surprisingly, there were no more such mutants recorded for 5 years until Sirot *et al.* [8] reported an ongoing problem with multiple enterobacterial species in Clermont-Ferrand (France) producing a potent cephalosporinase, which they named CTX-1. Subsequent sequencing showed that CTX-1 was a three-amino-acid mutant of TEM-1 and it was renamed TEM-3 [9]. By this time, others, mostly in France, were describing series of different cefotaximases (CTX) and ceftazidimases (CAZ). These, too, largely proved to be TEM and SHV mutants and, once sequenced, were re-numbered within these families. Now, 20 years on, the catalogues of TEM and SHV variants have grown to 150 and over 88, respectively (see http://www.lahey.org/studies for an up-to-date listing), most of them with increased activity against oxyimino-cephalosporins.

The term 'extended-broad-spectrum β-lactamases' was applied to the oxyimino-cephalosporin-hydrolysing TEM and SHV enzymes in 1987 [10], based on the fact that they had 'extended broad-spectrum' activity as compared with the 'broad-spectrum' classic TEM and SHV enzymes in Richmond and Sykes' group III. Soon, however, the word 'broad' had been lost from the moniker and, by 1989, 'extended-spectrum β-lactamases' (ESBLs) was used in a seminal review [11] and ICAAC abstracts. Karen Bush, revising the β-lactamase classification of Richmond and Sykes in 1989, put ESBLs into her class 2be (initially 2b', renamed 2be in 1995 [12]), as broad-spectrum clavulanate-inhibited β-lactamases, able to hydrolyse oxyimino-cephalosporin at rates of at least 10% that for benzylpenicillin [13]. Class 2be also accommodated the chromosomal K1 (KOXY) β-lactamase of *Klebsiella oxytoca*, whereas further β-lactamases, able to hydrolyse oxyimino-cephalosporins and inhibited by clavulanate, were placed in group 2e on the basis of being substantially more active against cephaloridine than benzylpenicillin [14].

BLURRING THE EARLY DEFINITIONS

If ESBLs had remained exclusively clavulanate-inhibited TEM and SHV mutants with substitutive activity against oxyimino-cephalosporins, their definition would remain simple. Unfortunately, they have not.

First, other enzymes with similar hydrolytic profiles and inhibitor susceptibility to the TEM and SHV mutants, but with dissimilar evolutionary histories, have been recognised, notably the CTX-M, PER and VE13 groups. Second, some TEM mutants have increased cephalosporinase activity, as compared with TEM-1, and a greater ability to confer resistance, but fail to meet the criterion of hydrolysing any individual oxyimino-cephalosporin at 10% or more of the rate for benzylpenicillin. Third, mutation can extend the hydrolytic spectrum and/or the capacity to cause resistance among class C and D β-lactamases, which are inherently resistant to clavulanate and therefore cannot belong to functional group 2be. Fourth, some enzymes, particularly in the GES family, meet the criteria for inclusion in class 2be but, in addition, slowly hydrolyse carbapenems.

Depending on the author, some or all of these groups are included as ESBLs. Should they be? Underlying this confusion is the issue of whether 'ESBL' still serves as a descriptor of hydrolytic activity, as in the original 'extended-broad-spectrum,' or whether the term has evolved to become phylogenetic—indicating that an enzyme has an extended spectrum as compared with classic members of its family.

CLASS A ENZYMES SIMILAR TO TEM AND SHV ESBLS, BUT UNRELATED TO THEM

Several groups of acquired β-lactamases have hydrolytic profiles similar to those of the TEM and SHV mutants but have quite different evolutionary histories, although they also belong to molecular class A. Major examples include: (i) the CTX-M family, rapidly spreading worldwide [15]; (ii) the PER family, important in *Pseudomonas*, *Acinetobacter* and *Salmonella* in Turkey [16,17] and in *Salmonella* in Argentina [18]; (iii) the VEB family, which is most prevalent in the Far East [19] but which has also been seen in Western Europe, most notably in an *Acinetobacter baumannii* clone that spread widely in north-eastern France in 2003–2004 [20,21]; and (iv) various rare types, such as GES and TLE.

These are all acquired, generally plasmid-mediated, enzymes, inhibited by clavulanate and able to hydrolyse one or more oxyimino-cephalosporin(s) at 10% or more of the rate for benzylpenicillin, thus meeting the key criteria for inclusion in functional group 2be. Despite not having parents with narrower spectra, they should surely be included as ESBLs, as supported both by this author and by a show of hands at the conference.

What is more arguable is whether one should also include, as ESBLs, chromosomal types meeting the inhibition and hydrolytic criteria for group 2be. Enzymes that might be recruited to the class on this basis include the *K. oxytoca* K1/KOXY β-lactamase, which is placed in functional group 2be, and the *Proteus vulgaris*, *Citrobacter diversus* and *Stenotrophomonas maltophilia* L-2 chromosomal enzymes, placed in group 2e [14]. Perhaps most obviously, since the CTX-M family are genetic escapes from *Kluyvera* spp., [15] should not the corresponding chromosomal types in *Kluyvera* be counted as ESBLs?

My preference would be to exclude chromosomal types and to reserve the term 'ESBL' for *acquired* types, atypical of their host species, whether encoded by plasmids or chromosomal inserts. But this is an issue of preference, and many who attended the conference took the opposite view also accepting the term ESBL for enzymes that were inherent and chromosomal in a species.

TEM AND SHV MUTANTS WITH BORDERLINE ESBL ACTIVITY

Most TEM and SHV ESBLs have V_{max} rates for oxyimino-cephalosporins as high as those for benzylpenicillin or cephaloridine, whereas TEM-1 has 10 000-fold lower rates (Table 1). However, a few TEM mutants, e.g., TEM-12, have only slightly increased activity against the cephalosporins, with V_{max} rates much lower than 10% of that for benzylpenicillin [22]. They therefore fail to meet the criteria for inclusion in class 2be but nevertheless, are more active against the oxyimino-cephalosporins than TEM-1 itself, and confer low-level resistance to several cephalosporin analogues. Moreover, TEM-12 is the intermediate between TEM-1 and TEM-10, with only one of the two amino-acid substitutions present in TEM-10 which is a clear ESBL (Table 1) [13,22].

My preference, based on increased activity and the ability to confer resistance, is to include TEM-12 and variants like it as ESBLs. This was supported by a show of hands at the conference. Any other solution demands drawing a border at some arbitrary level of increased activity, such as the 10% figure used to define group 2be, and this would cause further anomalies. For example, an

Table 1. Hydrolytic profiles, sequences and resistance conferred for selected β-lactamases belonging to the TEM family

	Amino-acid at position					Relative V_{max}			MICs (mg/L) for *Escherichia coli* K-12 transconjugants		
	39	104	164	238	240	Benzylpenicillin	Cefotaxime	Ceftazidime	Ampicillin	Cefotaxime	Ceftazidime
None									2	0.06	0.06–0.12
TEM-1	Gln	Glu	Arg	Glu	Glu	100	0.01	<0.01	>128	0.06–0.12	0.12–0.25
TEM-3	**Lys**	**Lys**	Arg	**Ser**	Glu	100	170	8.3	>128	64–256	32–128
TEM-10	Gln	Glu	**Ser**	Glu	**Lys**	100	2.1	90	>128	0.25–4	32–256
TEM-12	Gln	Glu	**Ser**	Glu	Glu	100	0.75	0.10	>128	0.12	4–8

Data are from [13,22], and also http://www.lahey.org/studies
Bold font indicates changed amino acids

enzyme hydrolysing ceftazidime at 11% of the rate for benzylpenicillin, but hydrolysing cefotaxime at 1%, would count as an ESBL, whereas one hydrolysing both cephalosporins at 9% of the rate for penicillin would fail to count.

EXTENDED-SPECTRUM MUTANTS OUTSIDE MOLECULAR CLASS A

All the β-lactamase variants so far discussed belong to molecular class A and, to begin with, it was thought that ESBL-type activity could evolve only in this family. During the early 1990s, however, Danel and others worked extensively on class D β-lactamases, noting again that point mutation could extend the ability to confer resistance. Thus, OXA-15 is a mutant of OXA-2 [23], while OXA-11, -14, -16 and -17 are variants of OXA-10 (PSE-2) [24–27]. In each case, the mutants confer wider resistance to oxyimino-cephalosporins than their parent types (Table 2). These early OXA ESBLs were found in *Pseudomonas aeruginosa* from Turkey, although further examples, more recently, have been found in *P. aeruginosa* from France [28,29].

Although strains and transconjugants with OXA variants have increased resistance to one or more oxyimino-cephalosporins (Table 2), the extracted enzymes show little convincing increase in cephalosporinase activity, except perhaps that OXA-11 has a raised k_{cat}/K_m for ceftazidime [27]. Moreover, and like OXA enzymes in general, they are poorly inhibited by clavulanate. At one level, these considerations argue against counting the OXA mutants as ESBLs; nevertheless, and as with the borderline TEM types, their extended ability to confer resistance argues for their inclusion, and this view was supported by the majority of the conference audience.

The lack of a clear relationship between the ability to confer resistance and the kinetics of the extracted enzymes probably reflects the notorious difficulty of measuring the kinetics of OXA enzymes in general. Both OXA-2 and -10 and their variants exist as dimers at high concentration, as in the bacterial periplasm, but tend to dissociate into less active monomers when diluted into the assay mixture [30]. The result, in standard assays, is a hydrolysis 'rate' that declines more rapidly than can be explained by substrate depletion, and which may have little relevance to whatever cephalosporin inactivation

Table 2. The nature of 'extended-spectrum β-lactamase' activity in the OXA-10 (PSE-2) family

	Amino-acid at position				Relative V_{max}				MICs (mg/L) for transconjugants ± enzyme		
	73	**124**	**143**	**157**	**Penicillin G**	**Carbenicillin**	**Cefotaxime**	**Ceftazidime**	**Carbenicillin**	**Ceftazidime**	**Cefotaxime**
None[a]									32[a]	1[a]	16[a]
OXA-10	Asn	Ala	Asn	Gly	100	35	6.8	<0.1	1024[a]	2[a]	16[a]
OXA-11	Asn	Ala	**Ser**	**Asp**	100	3.8	1	0.6	512[a]	512[a]	32[a]
OXA-14	Asn	Ala	Asn	**Asp**	100	–	<1	<1[c]	512[a]	512[a]	32[a]
OXA-16	Asn	**Thr**	Asn	**Asp**	100	35	12	<1	128[a]	128[a]	32[a]
None									2[b]	0.25[b]	0.016[b]
OXA-17	**Ser**	Ala	Asn	Gly	100	40	440	<1	64[b]	0.25[b]	16[b]

[a]MICs for *Pseudomonas aeruginosa* PU21 recipient and its transconjugants.
[b]MICs for *Escherichia coli* JM109 recipient and its transformant.
[c]But high V_{max}/K_m.
Data are from [24–27]; Bold font indicates amino acids changed compared with OXA-10

rate the dimeric enzyme achieves in the periplasm.

Class C (AmpC) enzymes further complicate the definition of ESBLs. Typical AmpC enzymes, which belong to functional group 1, as clavulanate-resistant cephalosporinases, confer resistance to most oxyimino-cephalosporins if hyperproduced as a result of mutational derepression in, e.g., *Enterobacter* spp. [5,31], or if they become plasmid-encoded and constitutive [32,33]. This ability reflects high affinity (1ow K_m values) for oxyimino-cephalosporins, which confers catalytic efficiency despite low k_{cat} values [34,35].

By convention, AmpC enzymes are counted separately from ESBLs, but a taxonomic problem arises with AmpC mutants that have increased activity against cefepime and cefpirome, fourth-generation cephalosporins that have acceptable, if incomplete, stability to classic AmpC types. Such mutants have been seen among clinical *Serratia* and *Enterobacter* isolates [36,37], and have been obtained in laboratory selection experiments [38,39]; their increased activity may reflect greater affinity (lowered K_m) or an increased k_{cat} (Table 3). So far, such mutants have arisen from inherent chromosomal AmpC types, but they could equally evolve from the plasmidic AmpC types that are increasingly circulating in *Klebsiella* spp. and *E. coli*. Should such mutants be included as ESBLs, on the basis of having an extended spectrum as compared with their parent AmpC types? I would say 'yes', although a show of hands at the conference indicated that this was the minority view.

CARBAPENEM-HYDROLYSING β-LACTAMASES

β-Lactamases able to confer resistance to carbapenems are now recognised in classes A (e.g., IMI, KPC, NMC and SME), B (e.g., VIM, IMP, GIM, and SPM) and D (e.g., OXA-23-like, OXA-24-like, OXA-49, OXA-51-like and OXA-58) [40,41]. Most workers, myself included, count these separately from ESBLs, even though (i) most can also hydrolyse oxyimino-cephalosporins, and (ii) some of the class A types (which fall into Bush's group 2f) are inhibited, albeit rather weakly, by clavulanate [42]. The pragmatic reasons for separating carbapenemases from ESBLs are surely: (i) that carbapenemase activity is not a feature of ESBLs in general; and (ii) that, since carbapenems are often the last-resort β-lactams, any enzyme that confers resistance to them must be seen as a further threat, over and above that associated with an ESBL.

A problem does, however, arise with the GES family, where substitution of Gly170 with asparagine (GES-2) or serine (GES-3 and 4) confers the ability to cause low-level imipenem resistance, particularly in an impermeable host such as *P. aeruginosa* [43,44]. For GES-2, the increased activity reflects a reduced K_m and, hence, greater affinity, not a raised k_{cat} (Table 4) [44]. Such enzymes clearly meet the criterion of having an extended spectrum as compared with their parent type; moreover, they conform with the definitions for functional group 2be, as clavulanate-inhibited broad-spectrum enzymes that are able to hydrolyse

Table 3. 'Extended-spectrum' activity in chromosomal AmpC enzymes from *Serratia marcescens*, increasing the ability to confer cefepime resistance

	Kinetics of parent enzyme		**Kinetics of mutant enzyme, with four-amino-acid deletion in H-10 helix**		**MICs for *Escherichia coli* recipient and transconjugants**		
	k_{cat}[a] (/s)	**K_m (μM)**	**k_{cat} (/s)**	**K_m (μM)**	**R**	**Transconjugant with control enzyme**	**Transconjugant with mutant enzyme**
Benzylpenicillin	35	9	50	10	–	–	–
Cefotaxime	6	7	5	2	<0.06	8	16
Ceftazidime	5	>1000	270	20	<0.06	16	512
Cefepime	80	>1000	50	6	<0.06	2	512

Data are from [36].
[a]k_{cat} is a more fundamental measure of V_{max}, cited in other tables, being the maximum number of substrate molecules that one enzyme molecule can transform per unit time.

Table 4. Extending the spectrum of GES enzymes to include carbapenems

	Kinetics of GES-1		Kinetics of GES-2		MICs for *Escherichia coli* DH10B recipient and transconjugants		
	k_{cat} (/s)	K_m (μM)	k_{cat} (/s)	K_m (μM)	R	Transconjugant with GES-1	Transconjugant with GES-2
Benzylpenicillin	2.8	40	0.4	4	–	–	–
Amoxycillin	13	200	0.7	25.8	4	>512	>512
Cefotaxime	68	4600	2.5	890	0.06	4	1
Ceftazidime	380	2000	Not measurable	>1000	0.5	128	8
Cefepime	2.8	1800	1.1	1900	0.03	0.25	0.12
Imipenem	0.003	45	0.004	0.45	0.06	0.06	0.25

Data are from [44].

oxyimino-cephalosporins at 10% or more of the rate for benzylpenicillin … but they also stray into the realm of carbapenemases!

TOWARDS A PRAGMATIC DEFINITION

The term 'ESBL' was coined when fewer β-lactamases were known, and served well for the TEM and SHV mutants, both: (i) because they had high k_{cat} rates for oxyimino-cephalosporins; and (ii) because they had an extended spectrum as compared with their parent types. As the catalogue of β-lactamases has grown, enzymes have been called ESBLs on the basis of meeting *either* one of these criteria, or owing to the ability to confer resistance rather than to cause rapid hydrolysis. Several enzymes now included as ESBLs—notably the OXA variants—lack obvious cephalosporinase activity in standard assays, despite an ability to confer resistance, and are resistant to clavulanate.

If it is accepted that ESBLs are now a broad church and should remain so, then a definition that would still serve would be 'any β-lactamase,' generally acquired rather than inherent to a species, that is either able to confer resistance to oxyimino-cephalosporins (but not carbapenems), or that has an increased ability to do so, as compared with classic members of its genetic family. To be meaningful, such a definition demands that the ESBL class is also specified, as with 'TEM ESBL', 'OXA ESBL' or 'CTX-M ESBL', or 'extended-spectrum AmpC'. This would resolve most confusion, although the carbapenem-hydrolysing GES types still present a problem.

The alternative would be to restrict the term 'ESBL' to those enzymes meeting the criteria for functional group 2be, meaning CTX-M, VEB, SHV, most TEM types and a few obscure types. This, however, would exclude the 'borderline' TEM ESBLs and would demand the coining of a new term for the OXA ESBLs and those AmpC mutants with increased activity against cefepime. This surely would be a less satisfactory solution and—inappropriately—would exclude many of the enzymes included by presenters at this meeting.

Finally, it should be said that the great majority of the ESBLs encountered clinically belong to the TEM, SHV and CTX-M families. These are what are routinely sought in clinical laboratory ESBL tests, and it is their spread—and particularly that of the CTX-M types—which is causing public health concern. The OXA, GES and AmpC types that so complicate the definition remain extremely rare.

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