Evolution of extended-spectrum β-lactamases by mutation

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ABSTRACT

Antimicrobial resistance genes in pathogenic bacteria belong to the most rapidly evolving DNA sequences, which results in an enormous structural diversity of resistance effectors. Structural modifications of resistance genes by mutation and recombination, together with a multitude of events that stimulate their mobility and expression, allow microorganisms to survive in environments saturated with antimicrobial agents of various types and generations. Genes coding for β-lactamases in Gram-negative bacteria are a fascinating example of this multifocal and multidirectional evolution, with the extended-spectrum β-lactamases (ESBLs) being one of the most spectacular 'achievements'. Some of the ESBLs known today are 'ready-to-use' enzymes in their natural producers but these are often of low pathogenic potential, or none at all. The problem appears upon mobilisation of a gene encoding such an ESBL, and its acquisition and sufficient expression by a more virulent organism. Many ESBLs are generated by mutations in genes coding for broad-spectrum enzymes, which have been mobile since at least the 1960s and which have disseminated very widely in populations of pathogenic bacteria. Strong selection pressure exerted by antimicrobial use, especially with newer-generation β-lactam antibiotics, efficiently promotes these two modes of ESBL emergence and subsequent spread. It also stimulates further evolution of ESBLs by accumulation of other mutations with an astonishing variety of effects on β-lactamase structure and activity. Remarkably, more than 300 natural ESBL variants have been identified since the mid-1980s but in-vitro studies suggest that ESBL evolution has certainly not come to an end; they may also help in predicting future developments. The aim of this review is to briefly overview the role of various mutations in ESBL evolution.

Keywords β-lactamase, ESBL, evolution, mutation, review

Clin Microbiol Infect 2008; **14** (Suppl. 1): 11–32

INTRODUCTION

Evolution at the molecular level involves the gradual accumulation of mutations (and other changes) in DNA sequences under the selective constraints of the environment [1]. Once a mutation affects the structure of a gene product, or gene expression, it may cause loss of function, cause gain of function, or be neutral. The spectrum of possible mutational effects may be wide, and a loss-of-function mutation may lead to the full or partial reduction of a gene product activity, whereas a gain-of-function mutation may confer either a new activity or enhance an existing one. Moreover, a single mutation may cause both a loss and gain of function, due to a structure-based 'trade-off' between the two functions, or between stability and activity of the gene product [2].

The rate of evolution of DNA sequences is highly variable [1]. Among the DNA sequences that evolve most rapidly are the bacterial genes responsible for antimicrobial resistance. Resistance often evolves rapidly after a new antibiotic enters into clinical practice, with this rapidity reflecting several characteristics of microorganisms, such as their large population sizes, short generation times, and the expansion of resistance by transmission of mobile genes. Other key factors include the strong selective pressure of antimicrobial use in humans, animals, and agriculture [3], the presence of mutational hot-spots

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in resistance genes, and the high structural flexibility of many resistance effectors [4].

β-Lactamases, especially those of Gram-negative bacteria, provide an excellent example of the evolution of resistance mechanisms. Their history has been extensively analysed on various time scales. Long-term analyses, based on protein sequences, have resulted in views of genealogy of the fundamental lineages of β-lactamase classes A, B, C and D [5] and their evolutionary relationship to penicillin-binding proteins [6], or of β-lactamase families [7]. Shorter-term studies aim to reveal the evolution of variants, often via single sequence changes (microevolution) [8], sometimes in specific geographical regions [9,10]. These analyses are carried out on naturally occurring β-lactamase variants by protein or DNA sequence comparisons. In the former case, the studies reveal only the evolutionary trends within β-lactamase families; in the latter case, the studies precisely reconstruct the history of particular enzyme lineages, considering also silent mutations and genetic context. Studies on laboratory mutants, obtained by in-vitro mutagenesis or via directed evolution, contribute to our understanding of the evolutionary past, and allow future predictions [11].

The largest structural/evolutionary group of β-lactamases is Ambler class A [12], which includes the vast majority of the Bush, Jacoby and Medeiros group 2 with 'penicillinases, cephalosporinases and broad-spectrum β-lactamases that are generally inhibited by active site-directed β-lactamase inhibitors' [5]. Intensive evolution of class A β-lactamases has resulted in great structural diversity, with numerous families and subfamilies, as well as recent modifications distinguishing multiple variants. Minor structural diversity can confer major differences in biochemical properties, affecting substrate spectra, inhibition profiles and, consequently, the phenotypes of resistant pathogens. Class A includes species-specific and/or acquired β-lactamases that are expressed constitutively or inducibly [13].

TEM AND SHV β-LACTAMASES: CONVERTING A BROAD-SPECTRUM ENZYME INTO AN EXTENDED-SPECTRUM β-LACTAMASE (ESBL)

TEM and SHV β-lactamases are the most studied class A enzymes. Both families have had a relatively long period of evolution in the 'clinical' era, resulting in the observation of high numbers of structural modifications, many of them affecting biochemical properties [14,15]. Moreover, owing to the very high prevalence of TEM and SHV β-lactamases in Gram-negative bacteria (mostly the Enterobacteriaceae), the enzymes have had a strong impact on the clinical context and the epidemiology of bacterial infections all over the world. The TEM and SHV families, as we now observe them, may be traced back to a few parental enzymes, namely TEM-1, TEM-2, SHV-1 and SHV-11. The deeper origins of TEM β-lactamases remain unrevealed, with all TEM variants being identified as acquired enzymes, encoded by mobile genes [13,15]. On the other hand, the parental SHV β-lactamases are specific for *Klebsiella pneumoniae*, and the mobile bla_{SHV} genes are the descendants of several escapes from the chromosome of this species [16]. The microevolution of TEM and SHV β-lactamases has been extremely intensive in recent years, as demonstrated by the almost exponential growth in numbers of variants identified since the mid-1980s. The number of recognised polymorphic sites in amino-acid sequences has rapidly increased as well, especially in the case of SHV enzymes, with *c.* 160 TEM and 100 SHV amino-acid sequences submitted to the http://www.lahey.org/studies/webt.asp website. These differ from each other in *c.* 50 positions. This number seems likely to increase, since, in a saturation mutagenesis study, 220 out of 263 amino-acid positions of the mature TEM-1 protein tolerate mutations, with the enzyme retaining good hydrolytic activity against ampicillin [17]. Among both the TEM and SHV families, three major types of activity have been identified: those of the broad-spectrum β-lactamases, the ESBLs, and the inhibitor-resistant β-lactamases.

ESBLs are the most 'spectacular' result of this microevolution [5,13,15,18–21]. These enzymes have an expanded substrate spectrum towards oxyimino-β-lactams (oxyiminocephalosporins and aztreonam). The responsible mutations, therefore, result in gain of function, but because the parent broad-spectrum enzymes still have trace activity against oxyimino-compounds, the ESBL activity should be seen as an improved activity rather than as something entirely new. This gain is almost always accompanied by partial loss of activity against penicillins [22].