New β-Lactamases in Gram-Negative Bacteria: Diversity and Impact on the Selection of Antimicrobial Therapy

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Of the 340 discrete β-lactamases that have been identified, the most important groups of enzymes that are continuing to proliferate include the plasmid-encoded cephalosporinases, the metallo–β-lactamases, and the extended-spectrum β-lactamases. Resistance to specific β-lactam–containing antimicrobial agents frequently can be traced to a single β-lactamase, but this task is becoming more difficult for the clinical microbiology laboratory. Other factors, such as multiple β-lactamase production, transferable multidrug-resistance genes, alterations in outer-membrane porins, and possible antibiotic efflux, all may contribute to a resistance phenotype. Appreciation of these factors may help the physician make a more informed decision when choosing therapy to try to avoid selection of even more pathogenic strains.

In gram-negative pathogens, β-lactamase production remains the most important contributing factor to β-lactam resistance [1]. Penicillins, cephalosporins, monobactams, and carbapenems can all be hydrolyzed by multiple members of the β-lactamase family of enzymes, which results in microbiologically ineffective compounds [2]. Although numerous new β-lactams have been developed during the past 40 years in attempts to circumvent the activity of β-lactamases, it appears that the prime result has been the selection of more diverse and potentially more deleterious β-lactamases. When faced with this mélange of enzymes, the practicing physician may ask whether it is necessary to discriminate among specific enzymes in individual isolates or whether it is more important to look for trends in susceptibility profiles that may alert a clinical microbiology laboratory to a potential epidemiological problem in a specific hospital. These are not easy questions. However, knowledge about the types of enzymes that may be present can serve to guide the infectious disease physician toward choosing appropriate therapy without the need for extensive secondary testing.

At the latest count, at least 340 β-lactamases, originating from clinical isolates, have been described with unique amino acid sequences or differentiated phenotypic behavior (table 1). Many of these enzymes belong to closely related families with similar functions. Because of the relative ease of obtaining genetic sequence data, distinct enzymes are now readily distinguished on the basis of molecular structure, in contrast to early research, in which enzymes were sorted primarily on the basis of biochemical (functional) characteristics. As a result, the number of distinct TEM-related β-lactamases is approaching the century mark, and almost 30 SHV-derived enzymes have been described (http://www.lahey.org/studies/webt.htm). However, the clinical impact of β-lactamases is related to a combination of factors that rely more heavily on functional rather than structural characteristics, including specificity of hydrolysis and level of expression of enzymatic activity, together with the presence of additional resistance factors in the producing organism.

Although amino acid sequence data have provided an attractive means for differentiating β-lactamases, on the clinical level this approach suffers from the fact that we still cannot use molecular biology to predict enzyme function or the subsequent bacterial-susceptibility profile. When a clinician needs to decide how to treat a bacterial infection, the amino acid sequence of a β-lactamase is less important than knowing whether the producing organism is resistant or susceptible to this agent. However, in terms of epidemiology or long-term effects of antibiotic use, identification of specific β-lactamases can play an important role. Today, it is recommended that we determine the microbiological profile of the producing organism, the biochemical properties of a purified enzyme, and the
Table 1. Functional and molecular characteristics of the major groups of β-lactamases.

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<tbody>
<tr>
<td>1</td>
<td>C</td>
<td>Often chromosomal enzymes in gram-negative bacteria but may be plasmid-encoded. Confer resistance to all classes of β-lactams, except carbapenems (unless combined with porin changes). Not inhibited by clavulanic acid.</td>
<td>32</td>
<td>51</td>
<td></td>
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<tr>
<td>2</td>
<td>A, D</td>
<td>Most enzymes responsive to inhibition by clavulanic acid (unless otherwise noted).</td>
<td>136</td>
<td>256</td>
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<tr>
<td>2a</td>
<td>A</td>
<td>Staphylococcal and enterococcal penicillinases included. Confer high resistance to penicillins.</td>
<td>20</td>
<td>23</td>
<td></td>
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<tr>
<td>2b</td>
<td>A</td>
<td>Broad-spectrum β-lactamases, including TEM-1 and SHV-1, primarily from gram-negative bacteria.</td>
<td>16</td>
<td>16</td>
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<tr>
<td>2be</td>
<td>A</td>
<td>Extended-spectrum β-lactamases conferring resistance to oximino-cephalosporins and monobactams.</td>
<td>36</td>
<td>119</td>
<td></td>
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<tr>
<td>2br</td>
<td>A</td>
<td>Inhibitor-resistant TEM (IRT) β-lactamases; one inhibitor-resistant SHV-derived enzyme.</td>
<td>9</td>
<td>24</td>
<td></td>
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<tr>
<td>2c</td>
<td>A</td>
<td>Carbenicillin-hydrolyzing enzymes.</td>
<td>15</td>
<td>19</td>
<td></td>
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<tr>
<td>2d</td>
<td>D</td>
<td>Cloxacillin-(oxacillin)–hydrolyzing enzymes; modestly inhibited by clavulanic acid.</td>
<td>18</td>
<td>31</td>
<td></td>
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<tr>
<td>2e</td>
<td>A</td>
<td>Cephalosporinases inhibited by clavulanic acid.</td>
<td>19</td>
<td>20</td>
<td></td>
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<tr>
<td>2f</td>
<td>A</td>
<td>Carbapenem-hydrolyzing enzymes with active site serine, inhibited by clavulanic acid.</td>
<td>3</td>
<td>4</td>
<td></td>
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<tr>
<td>3</td>
<td>3a, 3b, 3c</td>
<td>Metallo-β-lactamases conferring resistance to carbapenems and all β-lactam classes except monobactams. Not inhibited by clavulanic acid.</td>
<td>13</td>
<td>24</td>
<td></td>
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<tr>
<td>4</td>
<td>?b</td>
<td>Miscellaneous unsequenced enzymes that do not fit into other groups.</td>
<td>7</td>
<td>9</td>
<td></td>
</tr>
</tbody>
</table>

NOTE. Table is adapted from [3, 4].

a Identified from clinical isolates.
b Unknown.

Group 1 cephalosporinases were known originally as the chromosomal enzymes in Pseudomonas aeruginosa or the Enterobacteriaceae that could be induced by β-lactams such as cefoxitin or ampicillin [7]. With the introduction of cephalosporins, such as cefotaxime and ceftazidime, selection of strains with derepressed (hyperproduced) group 1 β-lactamases became more common [8]. Hyperproduction of these enzymes is often associated with the loss of a porin in the outer membrane of the bacteria [9], leading in some cases to high-level resistance—not only to all cephalosporins, penicillins, and monobactams, but also to the carbapenems [10, 11].

Genes encoding group 1 enzymes are now appearing on high-copy-number plasmids and are being transmitted promiscuously among the Enterobacteriaceae, resulting in organisms such as Escherichia coli and Klebsiella pneumoniae with high levels of group 1 cephalosporinases. Some of the plasmid-encoded enzymes in this class include MIR-1 [12], ACT-1 [11], and the FOX series of enzymes [13], now numbered through FOX-5 [14]. Production of these enzymes has become especially problematic, because these plasmid-containing, cephalosporinase-

**a** Identified from clinical isolates.
**b** Unknown.
producing organisms frequently do not contain an endogenous group 1 β-lactamase but, instead, produce another set of β-lactamases with a substrate profile that complements that of the group 1 cephalosporinase. Group 3 metallo-β-lactamases pose a perplexing problem. These enzymes are capable of hydrolyzing β-lactams from all chemical classes except the monobactams, and they are not inhibited by the β-lactamase inhibitors, such as clavulanic acid or tazobactam. The IMP-1 metalloenzyme was initially confined to Japanese isolates and was thought to be a major threat when it appeared on a transposable element in 1991 [15]. Its dissemination throughout Japanese hospitals among a variety of Enterobacteriaceae and P. aeruginosa isolates alerted the infectious disease community to the possibility that this class of enzyme might spread rapidly to confer resistance worldwide to most antimicrobial agents that contain β-lactams [16].

However, no major Japanese epidemic has yet been traced to the production of a metallo-β-lactamase; all the IMP-1-producing organisms appeared to be nonclonal strains from scattered hospitals in varied areas of Japan. This situation is currently changing, since closely related enzymes such as IMP-2 [17], VIM-1 [18], and VIM-2 [19] have recently been identified in multiple European isolates from Italy and France, indicating that the problem is becoming global. As in Japan, however, no major epidemic of infection has been reported that has been related to these enzymes.

Although most metallo-β-lactamases can hydrolyze a broad range of β-lactam–containing agents, they may operate with low hydrolytic rates for key selected drugs in their spectrum [20]. This is emphasized by the fact that virtually every metallo-β-lactamase appears in an organism that produces at least 1 other unrelated β-lactamase from a different functional group [4]. For example, several strains of Aeromonas species produce a chromosomal group 1 cephalosporinase and a group 2 penicillinase, together with a narrow-spectrum group 3 carbapenem-hydrolyzing metallo-β-lactamase [4]. As a result, the producing organism exhibits resistance to cephalosporins, penicillins, and carbapenems, as well as to the β-lactamase inhibitor combinations, thereby making it more difficult for the clinical microbiology laboratory to identify the exact cause of cephalosporin or carbapenem resistance without conducting elaborate secondary tests.

ESBLs, currently the functional group with the greatest number of enzymes, are most often variants of the broad-spectrum TEM and SHV β-lactamases that can hydrolyze most penicillins, as well as cephalosporins, including the aminothiazoloxime–containing β-lactams such as cefotaxime, ceftazidime, aztreonam, and cefepime (to a lesser extent), thereby conferring resistance to these agents [3, 21]. They represent a very large and challenging group of enzymes that is being monitored closely by clinical microbiology laboratories [22]. When these β-lactamases were first recognized in the late 1980s, several hospitals realized that there was a serious resistance problem only after the number of cases had escalated to the point at which relatively large outbreaks of infection were reported [23, 24]. As a consequence, clinical microbiology laboratories began to look for definitive testing methods to identify the occurrence of ESBL-producing organisms. The assumption was made that restriction of the affected cephalosporin would arrest any further transfer of resistance among organisms with plasmid-encoded ESBLs.

Contributions of the enzymatic properties of the ESBLs to observed microbiological profiles of the producing organisms need to be considered. Most ESBLs have a specific set of penicillins and cephalosporins or monobactams that they can hydrolyze, and not all ESBLs hydrolyze the same cephalosporins equally well. Only those substrates that are hydrolyzed at measurable rates pose microbiological challenges. For example, ESBLs that are common in the United States, such as TEM-10 and TEM-26, have very high hydrolytic rates for ceftazidime and aztreonam and modest rates for cefotaxime, whereas the growing family of clavulanate-responsive CTX-M β-lactamases can hydrolyze cefotaxime at least 150 times more efficiently than can ceftazidime [25, 26]. Therefore, production of a CTX-M-type ESBL in organisms that have MICs of ceftazidime of 0.5–2 μg/mL (compared with MICs of cefotaxime of 8–256 μg/mL [27]) may cause a physician to consider the use of ceftazidime for treatment of an infection caused by a CTX-M-producing pathogen. However, no clinical data are available to support this.

Many ESBLs with low rates of cephalosporin hydrolysis, such as TEM-12 and SHV-2, represent first-step mutants that need only a second point mutation before graduating to become more efficient ESBLs. These enzymes may occur in organisms that would be expected to respond to third-generation cephalosporins in the absence of ESBL production. With increased exposure to the selective pressure of these agents, a strain with one additional mutation may then be selected, resulting in higher MICs of cephalosporins. This has been demonstrated with the identification of TEM-12, a low-level ceftazidime-hydrolyzing ESBL, in the same organism as the more catalytically efficient TEM-10 ESBL, in a hospital with an outbreak of infection with TEM-10–producing bacteria [28].

Identification of ESBLs based on phenotypic responses has been the topic of much discussion. A number of screening tests have been proposed, but the tests approved by the National Committee for Clinical Laboratory Standards (NCCLS) appear to be the most widely accepted procedures [22]. In these tests, an MIC ≥2 μg/mL noted for aztreonam or for the oximinocephalosporins (third-generation cephalosporins) cepodoxime, ceftazidime, cefotaxime, or ceftriaxone would trigger a phenotypic confirmatory test for ESBLs, in which a reduction of at least 8-fold in the MICs of ceftazidime or cefotaxime in
K6 (ATCC 700603) was shown to produce the production as many as 5 distinct enzymes in the same organism [11, 28]. Clinical isolates that cause of the increased penetrability of cefotaxime or increased the organism was more resistant to ceftazidime, possibly be- cause of 2 outer-membrane porins [31]. Although the isolated en-

Putative ESBL identification or nonidentification may lead to other erroneous phenotypic conclusions because of confounding factors, such as the production of multiple β-lacta-

Putative ESBL identification or nonidentification may lead to other erroneous phenotypic conclusions because of confounding factors, such as the production of multiple β-lactamases in one organism. Gram-negative bacteria have been iso-

developed together with a weak clavulanate-sensitive, cefotaxime-

Increased porin production coupled with overproduction of β-lactamase can result in another set of resistance characteristics. The recently described ESBL quality control strain K. pneumoniae K6 (ATCC 700603) was shown to produce the novel ESBL SHV-18 in an organism that also exhibited the loss of 2 outer-membrane porins [31]. Although the isolated en-

What does the clinical microbiology laboratory need to be aware of in its quest for reliable information concerning β-lactamase production? Does it really make a difference whether an isolate of K. pneumoniae produces an ESBL, a plasmid-encoded group 1 cephalosporinase, or a metallo-β-lactamase if the organism is resistant to an extended-spectrum cephalosporin? For the routine clinical laboratory, the answer is probably not. It may be more important to know whether the enzyme is plasmid-encoded or chromosomal, as transfer of plasmid-mediated resist-

This criterion was based partly on the fact that one can observe strong inoculum effects due to ESBL hydrolysis of cephalosporin substrates, so that even low MICs of cephalo-

In the near future, we may expect gene probes and PCR techniques to become routinely available in clinical micro-

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However, if a K. pneumoniae isolate is resistant to ceftazidime with an MIC of 128 μg/mL, it is probably not necessary to know whether the responsible β-lactamase is the ESBL TEM-26, the IMP-1 metalloenzyme, or the group 1 MIR-1. With regard to those isolates against which the MICs of cephalo-

This combination of enzyme level and internal drug concentration has also been implicated in resistance profiles of organisms that produce group 1 cephalosporinases [33].

In the ideal world we would be able to predict the direction of evolution expected from β-lactamase–producing organisms, because it is clear that new β-lactamases will continue to prolif-

The presence of clavulanic acid would be sought. (Note that comparable disk tests have also been approved.) Confirmation of ESBL production would result in a laboratory report of “resistant” for all penicillins, cephalosporins, and aztreonam.

In the ideal world we would be able to predict the direction of evolution expected from β-lactamase–producing organisms, because it is clear that new β-lactamases will continue to proliferate as long as there is selective pressure from β-lactam–containing agents. It is our responsibility to learn how we can respond to keep them from becoming much more diverse.
References


