One New LEN Enzyme and Two New OKP Enzymes in *Klebsiella pneumoniae* Clinical Isolates and Proposed Nomenclature for Chromosomal β-Lactamases of This Species

Three families of chromosomally encoded beta-lactamases have been identified so far in *Klebsiella pneumoniae* clinical isolates: SHV (7), LEN (1), and very recently, OKP (5). These enzymes are closely related to OHIO-1 (10). We determined the sequences of the *bla* genes of nine isolates of *K. pneumoniae* producing a penicillinase with an isoelectric point different from 7.6, the pI of the most commonly encountered SHV-1 (6). The strains were susceptible to cephalosporins (except for one isolate that also produced CTX-M-3). The *bla* gene was amplified with primers Pro3-F (9) and Gra2-R (8).

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**FIG. 1.** Alignment of the amino acid sequences of OKP-1, OKP-2, OKP-3, OKP-4 (truncated sequences) (5), OKP-5, and OKP-6 (this study). The amino acid position is shown above the sequence. The consensus sequence (C) is shown below the other sequences. In the consensus sequence, asterisks indicate positions of amino acid substitutions. Gaps introduced to maximize alignment are indicated by dashes.
Sequencing of the entire bla gene was performed with the use of additional primers: P1-F (2), P1-Rev (CAAGGTTTTT CGCTGA), and P3-F (CCACTACCCGGCCACCATG) (this study). Primers Pro3-F, Gra2-R, P1-F, P1-Rev, and P3-F were located at positions 132 to 151, 1060 to 1041, 509 to 526, 526 to 509, and 725 to 744 of blaSHV-1 (GenBank accession number AF124984), respectively. A new blaLEN gene and two new blaOKP genes were located at positions 132 to 151, 1060 to 1041, 509 to 526, 526 to 509, and 725 to 744 of blaOKP-5 (GenBank accession number AY512506 for blaOKP-5 and AY850171 for blaOKP-6). The alignment of the amino acid sequences of the OKP enzymes. Like the other members of the family, OKP-5 and OKP-6 differed from SHV-1 and LEN-1 by 27 to 33 amino acids. It is noteworthy that three substitutions occurred within the first 16 amino acids. Unfortunately, this region was not sequenced in the case of the four OKP variants described so far, because the primers used by the authors do not encompass the beginning of the sequence of the bla gene (5).

Our results confirm the diversity of class A chromosomal beta-lactamase genes among K. pneumoniae isolates. Haeggman et al. noticed in their study that the plS of the OKP beta-lactamases (7.8, 8.1, 7.0, and 6.5) are different from the plS of the chromosomal SHV (7.6) and LEN (7.1) enzymes and therefore proposed using the pl as the first tool to identify the beta-lactamase family of K. pneumoniae (5). The new LEN-10 enzyme had a pl somewhat different from 7.1, indicating the possibility of variation in the plS among the members of this family. Moreover, OKP-5 and OKP-6 had a pl of 7.1, identical to that of multiple LEN variants. These two observations undermine the proposal of Haeggman et al. We propose grouping the LEN and the OKP enzymes in a single new family “K. pneumoniae-specific chromosomal beta-lactamases,” excluding SHV on the basis of the following major observations. (i) LEN and OKP enzymes have been characterized only in K. pneumoniae isolates, unlike SHV-1. (ii) The blaLEN and blaOKP genes are chromosomally located, whereas blaSHV-1 is sometimes carried by a plasmid (3, 4). (iii) LEN and OKP have been unable to evolve the ability to hydrolyze extended-spectrum cephalosporins.

In conclusion, this work pointed out the necessity of obtaining the complete sequence of the bla gene with the appropriate primers to identify the phylogenic group of a K. pneumoniae chromosomal beta-lactamase.

REFERENCES

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