ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, June 2002, p. 2056–2057 Vol. 46, No. 6
0066-4804/02/$04.00+0 DOI: 10.1128/AAC.46.6.2056–2057.2002

The Extended-Spectrum K1 β-Lactamase from *Klebsiella oxytoca* SC 10,436 Is a Member of the bla_{OXY-2} Family of Chromosomal *Klebsiella* Enzymes

Bacteria in the genus *Klebsiella* have had a curious history of multiple names, together with multiple names for the chromosomal β-lactamases they produce. These enzymes have played a major role in the development of new classes of β-lactams but are less widely recognized than some of the more prominent TEM or SHV plasmid-encoded β-lactamases.

Within this family of enzymes is the K1 β-lactamase, first identified from a sick child infected with *Klebsiella aerogenes* (*Klebsiella pneumoniae*) 1082E in Glasgow, Scotland (8, 9). This enzyme is also known in the literature as the chromosomal enzyme from *K. aerogenes* NCTC 418 that Beecham scientists used to characterize the β-lactamase inhibitor clavulanic acid from *Streptomyces clavuligerus* fermentation broths (3). The K1 β-lactamase from *K. pneumoniae* SC 10,436, a strain documented as originating from *K. aerogenes* 1082E, became a pivotal enzyme in the Squibb monobactam program, based on the observation that it hydrolyzed many monobactams more rapidly than cephalosporins or penicillins; aztreonam was selected for clinical development because it was the most β-lactamase-stable monobactam in the Squibb library in 1980 (11). At that time, the K1 β-lactamase was the only bacterial enzyme that hydrolyzed aztreonam at a relative rate that was at least 10% greater than that for cephaloridine, hence its designation as the first extended-spectrum β-lactamase in the functional β-lactamase classification by Bush in 1989 (4). In 1986, this strain was renamed *Klebsiella oxytoca* SC 10,436 based on changes in *Klebsiella* nomenclature (2).

During the 1990s, systematic analyses of *K. oxytoca* clinical isolates have revealed two distinct groups of K1 β-lactamase genes, bla_{OXY-1} and bla_{OXY-2}. These groups have approximately 87% sequence identity (7). However, when this analysis was completed, no historical K1-producing strain was included. In this report, we describe the identification of the K1 *bla* gene from *K. oxytoca* SC 10,436.

The coding and promoter regions were amplified using the primers OXY-A (5′-TCGGTAACTGTGACGGGA-3′) and OXY-B (5′-CCGAATTTCGGGAAGCCA-3′) and then sequenced with an automated cycle-sequencing system.

The sequence analysis (GenBank accession no. AY055205) showed that the gene encoding K1 from strain SC 10,436 is very similar to the bla_{OXY-2} group. However, it differs from the reference $bla_{OXY-2-1}$ gene (GenBank accession no. Z49084) by five mutations. Two of these mutations are silent (A603G and A855G) while three of the mutations (G94A, G622A, and G790A) are responsible for the following amino acid substitutions: Gly20Ser located in the sequence signal and Asp197Asn and Asp255Asn located in the mature protein. As indicated in Table 1, these three substitutions have been previously published but not in the same association as found in K1 from strain SC 10,436 (6, 10).

In addition to the mutations in the coding gene, two others were observed in the promoter region: GATAGT → GATAAT (−10 region) and TTGTCA → TTGACA (−35 region). The association of these two mutations has been previously shown as creating the strongest promoter among those tested after having been cloned upstream of the *cat* gene (5). The presence of such a promoter explains the increased hydrolytic activity against monobactams that is observed in the K1-producing strain SC 10,436.

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TABLE 1. Amino acid substitutions in K1 protein of *Klebsiella oxytoca* strain SC 10,436 compared with previously published K1 OXY-2

Strain	β-Lactamase	pI	Amino acid at codon:						Reference(s) (1)
			20	35	130	197	223	255	
SL911	OXY-2-1	5.2	Gly	Asp	Ser	Asp	Ala	Asp	1, 6
K1794	OXY-2-2	5.7				Asn			6
D488	OXY-2-3	6.4				Asn	Val		6
11V	OXY-2-4	6.8		Ala		Asn		Asn	6
KER		6.3	Ser		Gly	Asn		Asn	10
SC 10,436		6.5	Ser			Asn		Asn	4; this study

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Sophie A. Granier
Véronique Leflon-Guibout
Marie-Hélène Nicolas-Chanoine*
Service de Microbiologie-Hygiène
Microbiology Department
Hôpital Ambroise Paré AP-HP
9 avenue Charles de Gaulle
Université Versailles-Saint-Quentin-en-Yvelines-UFR
Médicale Paris-Ile-de-France-Ouest
Boulogne-Billancourt, France

Karen Bush
Johnson & Johnson Pharmaceutical Research & Development
Raritan, New Jersey

Fred W. Goldstein
Laboratoire de Microbiologie Médicale
Fondation Hôpital Saint-Joseph
Paris, France

*Phone: 33-1-49 09 55 40
Fax: 33-1-49 09 59 21
E-mail: marie-helene.nicolas-chanoine@apr.ap-hop-paris.fr