Letters to the Editor
Are SHV β-Lactamases Universal in Klebsiella pneumoniae?

The status of SHV-1 or related β-lactamases as “typical” of Klebsiella pneumoniae prompted debate at the 39th International Conference of Antimicrobial Agents and Chemotherapy. To test whether SHV-type enzymes are ubiquitous in this species, as previously asserted by one of us, (2) we screened 20 isolates identified as K. pneumoniae with API 20E tests (Bio-Merieux, Lyons, France). The isolates were obtained in 1997–1998 from intensive care units across Europe (1) and were chosen as broadly susceptible to β-lactam antibiotics including aztreonam (MIC ≤ 0.25 μg/ml), ceftazidime (MIC ≤ 0.25 μg/ml), ceftriaxone (MIC ≤ 0.12 μg/ml), piperacillin (MIC ≤ 8 μg/ml), piperacillin plus tazobactam, 4 μg/ml (MIC ≤ 4 μg/ml), cefotaxime (MIC ≤ 8 μg/ml), cefotaxime (MIC ≤ 8 μg/ml), and meropenem (MIC ≤ 0.12 μg/ml). The MIC ratio of ceftazidime to ceftazidime plus clavulanic acid equalled unity, except for one isolate where it was 2, indicating the absence of extended-spectrum β-lactamases. The isolates were from 16 hospitals in eight European countries.

Screening for blaSHV genes was by PCR, initially using primers 5'-TCA GCG AAA AAC ACC TTG-3' and 5'-TCC CGC AGA TAA ATC ACC A-3' (5) to amplify a 475-bp fragment. These primers correspond to positions 509 to 526 and 962 to 980, respectively, in the blaSHV-1 sequence (GenBank accession number AF124984). Amplification of a DNA fragment of the expected size was achieved with 18 of the 20 isolates. The two isolates that failed to give amplification products were reidentified with biochemical tests (4). One was deduced to be K. oxytoca on the basis of pectate degradation, utilization of m-hydroxybenzoate, and the ability to grow at 10°C but was indole negative and so was not recognized as K. oxytoca by the API 20E system. The second isolate was confirmed as K. pneumoniae. This isolate was retested for blaSHV-related DNA using primers 5'-ATCGGTATAATTCGCTGGT-3' and 5'-GT TAGCGCTGCACTGCTCG-3', corresponding to positions 199 to 210 and 1041 to 1060, respectively, of blaSHV-1. These primers amplified an 865-bp fragment, corresponding to the entire coding region of blaSHV-1.

Phenotypic expression of blaSHV was examined by isoelectric focusing, performed as described by Livermore and Williams (3). β-Lactamases that cofocused with SHV-1 enzyme (pl 7.6) were detected in 16 of the 19 confirmed K. pneumoniae strains. No β-lactamase band was detected in extracts of the three remaining isolates, indicating that expression was absent or below the detection limit of the nitrocefin overlay. These three organisms gave inhibition zones 3 to 7 mm larger to discs containing amoxicillin plus clavulanate at 20 + 10 μg than to those containing ampicillin at 25 μg, suggesting that some slight β-lactamase activity was present despite the electrofocusing result. Surprisingly, and despite its lack of piperacillin resistance, one SHV-1-positive isolate also had a β-lactamase that cofocused with TEM-1 enzyme.

We conclude that blaSHV-related genes were present in all 19 confirmed K. pneumoniae isolates and were expressed in at least 16 of these strains, which were from diverse European sources and were selected as highly susceptible to β-lactams. These data support the view that SHV-related enzymes approach ubiquity in K. pneumoniae, at least in Europe.

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REFERENCES

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