SHV-28, AN EXTENDED-SPECTRUM β-LACTAMASE PRODUCED BY A CLINICAL ISOLATE OF KLEBSIELLA PNEUMONIAE IN SOUTH INDIA

*SA Jemima, S Verghese

Abstract

SHV-28, an extended spectrum β-lactamase from a clinical isolate of Klebsiella pneumoniae, had an isoelectric point of 7.6 and a substrate profile showing preferential hydrolysis for cefotaxime over ceftazidime. It differed from SHV-1 by one amino acid substitution. The conserved S–T–F–K and K–T–G motifs were identified by SHV-28 protein sequencing.

Key words: Extended-spectrum β-lactamase, SHV-28, K. pneumoniae

Materials and Methods

Case history

The patient was a 65-year-old male patient who had undergone coronary artery bypass graft surgery. An isolate of K. pneumoniae was obtained from the urine sample of the patient. The organism was found to be highly resistant to penicillins, aminoglycosides, quinolones and cephalosporins, including third-generation cephalosporins such as CTX, CTZ, cefaperazone and ceftriaxone. The organism was susceptible to carbapenems, both imipenem and meropenem.

MIC testing and confirmation of ESBL activity

Organisms were tested by agar dilution method as described in the CLSI standard M100-S15. Escherichia coli ATCC 25922 and K. pneumoniae ATCC 700603 were used as quality control strains. The double-disk synergy test for the detection of ESBL activity was performed essentially as recommended by the CLSI M100-S15 by screening for synergism between clavulanate (represented by a disk of amoxicillin–clavulanic acid) and CTZ and CTX. The disks were placed at distance of 15–20mm (centre to centre). A potentiation of the inhibitory zones of any of the expanded spectrum β-lactams by clavulanate was considered suggestive of ESBL production.

β-lactamase assays

Bacteria exponentially growing at 37°C in Luria–Bertani medium were harvested and cell-free lysate was prepared by the lysozyme and EDTA treatment method. Analytical isoelectric focusing was performed using broad range soluble amphotelys (Biorad, Hercules, CA, USA) covering a pH range of 3.5–10. β-lactamases were visualized by overlaying gels with filter paper moistened with a 0.25mg/mL solution of nitrocefin (Oxoid, UK) in 0.1 M sodium phosphate buffer (pH 7.0) containing dimethylsulphoxide (1%v/v). The intact substrate molecule is yellow, but becomes pink when the
β-lactam bond is broken such that the focused bands with β-lactamase activity appear pink on a yellow background.

**Molecular analysis**

The presence of bla \textit{svr} resistance genes was detected by PCR. A 1052-bp PCR product that included the bla \textit{svr} structural gene was amplified from \textit{K. pneumoniae} with oligonucleotide primers using the template DNA.\[^{9}\] Cycling parameters for the amplification of the bla \textit{svr} gene included a 5-min denaturation at 96°C followed by 35 cycles of denaturation (96°C for 1 min), annealing (55°C for 1 min) and extension (72°C for 1 min) and ending with a final extension period of 72°C for 10 min.

**Direct sequencing of PCR products**

PCR products were used as templates for nucleotide sequence determination. A fragment of 1052 bp within bla \textit{svr} was amplified. The amplification products were purified with the Ultra Clean PCR purification kit (Mo bio Lab, CA, USA) and their nucleotide sequences were sequenced in an automated sequencing machine at the Medical Research Foundation, Tamil Nadu, India. Nucleotide sequence determinations were performed on both DNA strands and on two independently generated amplimers.

**Nucleotide sequence accession number**

The DNA sequence and deduced amino acid sequence of SHV-28 has been deposited in GenBank and assigned the accession number EU441172.

**Results**

**Antimicrobial susceptibility**

In the disc agar diffusion test, the isolate producing ESBL was resistant to all β-lactams tested, which included cefazolin, cefuroxime, CTX, CTZ, cefoparazone and ceftriaxone.

The isolate showed a very high MIC to CTZ of > 1024 μg/mL and the MIC to CTX was 1024 μg/mL. There was a strong synergistic effect observed when the combination drugs were used, with CTZ–CLA showing an MIC of 256 μg/mL and CTX–CLA showing an MIC of 128 μg/mL.

When the discs were placed 15–20 mm apart, a synergistic effect was observed due to resistance of the isolate to β-lactam antibiotics alone and in combination with β-lactam inhibitors like clavulanic acid.

**Isoelectric focusing**

The isolate producing an SHV-28 ESBL was analysed for its β-lactamase content. The pink band was produced at 7.6. No additional band of activity was detected with chromogenic cephalosporin as a substrate.

**Identification of the SHV-encoding gene by PCR and DNA sequencing**

By using SHV-specific primers, a DNA fragment of 1052 bp was amplified from the sample of the isolate using colony PCR. Sequence analysis revealed that the SHV gene showed a 100% similarity (Fig. 1) with SHV-28 (GenBank AF299299).

**Sequence analysis of the SHV-28 β-lactamase gene in \textit{K. pneumoniae}**

The SHV-28 gene comprises an initiation codon ATG (positions 57–59) and the stop codon TAA (positions 915–917), which translates into a protein of 286 amino acids (Fig. 1) Within this protein, two highly conserved motifs, serine–threonine–phenyalanine–lysine tetrad (S–T–F–K) at positions 66–69 and lysine–threonine–arginine (K–T–G) at positions 230–232 were found. This included the conserved serine and lysine residues characteristic of β-lactamases possessing a serine active site. The amino acid sequence of SHV-28 differed from the amino acid sequence of SHV-1 by one amino acid substitution: tyrosine to phenylalanine at position 3.

**Discussion**

SHV-28 was reported at the Southwest Hospital of the Third Military Medical College in China in 2002 (GenBank AF538324), and it was the first report of SHV-28. Kim et al. reported the presence of SHV-28 in two strains in Korea.\[^{10}\] SHV-28 was also demonstrated by Tofteland et al. in Norway in 2006.\[^{11}\] Ndugulile et al. reported the presence of SHV-28 in Africa for the first time in 2005.\[^{12}\]

In the present study, the patient had undergone a catheterization and had an urinary tract infection subsequently. The urine sample was collected on the 4th post-operative day during his stay in the intensive care unit. On Gram’s staining, the urine showed > 25 pus cells/low-power field, with numerous Gram-negative bacilli and moderate budding yeasts with pseudohyphae. It showed a significant growth of \textit{K. pneumoniae} and \textit{Candida} sps, both in excess of 1,00,000 colonies/mL. The \textit{K. pneumoniae} was identified as an ESBL producer and it was only susceptible to carbapenems. The patient was treated with Imipenem 0.5 g thrice a day for 5 days and fluconazole 200 mg once daily for 5 days. Many of these patients are particularly vulnerable to infection as they are on multiple invasive lines that prove to be an access to bacteria.

SHV-28 has not been previously reported from India. To the best of our knowledge, this is the first report of the detection of SHV-28 from India. The DNA sequence of the gene showed 100% homology to that of the gene detected from \textit{K. pneumoniae} that was isolated at China (GenBank AF299299). The gene, encoding β-lactamase SHV-28, detected in this study is a genotype that differs from...
SHV-1 only by a single amino acid substitution (tyrosine to phenylalanine) at position 3 of SHV-1. It is very important to prevent resistant bacteria by correctly identifying ESBL-producing *K. pneumoniae* and treating infected patients with appropriate antibiotics. The intensive use of penicillin-inhibitor combinations in the hospital settings and in the treatment of community-acquired infections may facilitate the sporadic appearance of enzymes like the one described here. Studies from other parts of the world reported that the SHV-5 gene was common in *K. pneumoniae* isolates.[13] Similar studies need to be performed in different parts of our country to know the genotypes of ESBL enzymes in a particular geographical area for epidemiological purpose.

### References


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