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Identification of a Novel SHV-β-Lactamase Variant (SHV-144) in a Malaysian Multidrug-Resistant Klebsiella pneumoniae Isolate

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The emergence and spread of extended-spectrum β-lactamase (ESBL)-producing Klebsiella pneumoniae pose a serious problem in hospital settings worldwide (1). SHV is a chromosomally encoded or plasmid-borne β-lactamase that is most commonly found in K. pneumoniae. Point mutations in the SHV gene are frequently reported, and some are associated with alterations in the amino acid sequence, resulting in new β-lactamase variants (2).

Here, we report the identification of a novel SHV β-lactamase variant expressed by a multidrug-resistant K. pneumoniae isolate (designated as strain K24) recovered from the sputum of a 41-year-old breast cancer patient who was admitted to the oncology unit of the University of Malaya Medical Centre (UMMC), Kuala Lumpur, Malaysia in November 2010. The patient was being treated for post-chemotherapy neutropenic sepsis when she acquired a respiratory tract infection on the fourth day of hospitalization. A sputum culture revealed the growth of a Gram-negative rod, which was later confirmed as K. pneumoniae by standard biochemical methods and polymerase chain reaction (PCR) targeting the 16S–23S rRNA internal transcribed spacer unit (3).

The strain K24 was confirmed to produce ESBL using the E-test (cefepime/cefepime clavulanic acid; bioMérieux, Marcy l’Etoile, France) and a cefpodoxime combination disk kit (Oxoid, Hampshire, UK). Resistance to various antimicrobial agents was investigated using the disk diffusion and E-test methods in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines (4). Minimal inhibitory concentration (MIC) values are shown in Table 1. Plasmid-mediated CTX-M-15, TEM-1, qnrB6, aac(6’)-Ib-cr, and sul1, were also detected in the bacterial strain.

To test the transmissibility of the SHV-144 gene, a conjugation experiment was conducted by broth mating of strain K24 with Escherichia coli strain J53 AzR (9). Plasmid DNA was extracted from strain K24 and the E. coli transconjugant strain (strain K24-T) using a plasmid midi kit (Qiagen, Hilden, Germany). Three plasmids were identified from strain K24, but only one plasmid with an estimated size of approximately 100 kb was successfully transferred to the transconjugant strain. The SHV gene was not amplified, cloned into the pGEM-T Easy Vector (Promega, Madison, Wis., USA), and sequenced (8). The nucleotide and deduced protein sequences were analyzed using the BLAST search engine and online ESBL genotyping tool (http://sunlight.informatik.uni-kiel.de:8180/amino/) and compared with other SHV sequences available from the Lahey website (http://www.lahey.org/studies/webt.htm). Compared with the SHV-1 sequence (GenBank accession no. AF148850), the novel SHV sequence contained seven nucleotide substitutions, resulting in changes in two non-synonymous amino acids, Leu35Gln and Ala146Val. The amino acid changes have been reported either alone or in combination with other substitution(s) in several SHV types. The SHV gene demonstrated the highest sequence similarity with SHV-11 (GenBank accession no. X98101), SHV-38 (GenBank accession no. AY079099), and SHV-80 (GenBank accession no. AM176555), except for one amino acid difference. The novel SHV type was named SHV-144 (http://www.lahey.org/studies/webt.htm), and the nucleotide sequence was deposited in the GenBank database under the accession no. JQ926986. Besides SHV-144, antibiotic resistance genes, including CTX-M-15, TEM-1, qnrB6, aac(6’)-Ib-cr, and sul1, were also detected in the bacterial strain.

Sequence analysis of the partial fragment of the amplified SHV gene (713 bp) demonstrated several nucleotide modifications. Hence, the entire SHV gene was amplified, cloned into the pGEM-T Easy Vector (Promega, Madison, Wis., USA), and sequenced (8). The nucleotide and deduced protein sequences were analyzed using the BLAST search engine and online ESBL genotyping tool (http://sunlight.informatik.uni-kiel.de:8180/amino/) and compared with other SHV sequences available from the Lahey website (http://www.lahey.org/studies/webt.htm). Compared with the SHV-1 sequence (GenBank accession no. AF148850), the novel SHV sequence contained seven nucleotide substitutions, resulting in changes in two non-synonymous amino acids, Leu35Gln and Ala146Val. The amino acid changes have been reported either alone or in combination with other substitution(s) in several SHV types. The SHV gene demonstrated the highest sequence similarity with SHV-11 (GenBank accession no. X98101), SHV-38 (GenBank accession no. AY079099), and SHV-80 (GenBank accession no. AM176555), except for one amino acid difference. The novel SHV type was named SHV-144 (http://www.lahey.org/studies/webt.htm), and the nucleotide sequence was deposited in the GenBank database under the accession no. JQ926986. Besides SHV-144, antibiotic resistance genes, including CTX-M-15, TEM-1, qnrB6, aac(6’)-Ib-cr, and sul1, were also detected in the bacterial strain.

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Table 1. Antimicrobial minimum inhibitory concentration values (µg/ml) for *K. pneumoniae* strain K24, *E. coli* (strain J53 AzR) and K24-transconjugant strain

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th><em>K. pneumoniae</em> strain K24 (Donor)</th>
<th><em>E. coli</em> strain J53 AzR (Recipient)</th>
<th>K24-T (Transconjugant)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ceftazidime</td>
<td>16 (R)</td>
<td>0.5 (S)</td>
<td>8 (I)</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>&gt;256 (R)</td>
<td>0.125 (S)</td>
<td>&gt;256 (R)</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>16 (R)</td>
<td>0.094 (S)</td>
<td>16 (R)</td>
</tr>
<tr>
<td>Pipracillin/tazobactam</td>
<td>4 (S)</td>
<td>2 (S)</td>
<td>4 (S)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>1.5 (I)</td>
<td>0.023 (S)</td>
<td>0.5 (S)</td>
</tr>
<tr>
<td>Trimethoprim/sulfamethoxazole</td>
<td>&gt;4 (R)</td>
<td>0.125 (S)</td>
<td>&gt;4 (R)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>0.5 (S)</td>
<td>0.25 (S)</td>
<td>0.38 (S)</td>
</tr>
<tr>
<td>Amikacin</td>
<td>2 (S)</td>
<td>1 (S)</td>
<td>2 (S)</td>
</tr>
<tr>
<td>Meropenem</td>
<td>0.38 (S)</td>
<td>0.25 (S)</td>
<td>0.38 (S)</td>
</tr>
<tr>
<td>Cefoxitin, ertapenem*</td>
<td>Sensitive</td>
<td>Sensitive</td>
<td>Sensitive</td>
</tr>
</tbody>
</table>

*as indicated by disk diffusion method.

R, resistant; S, sensitive; I, intermediate susceptibility.

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Fig. 1. Pulsed-field gel electrophoresis (PFGE) profiles of *Xba*I-digested genomic DNA from *K. pneumoniae* isolates. KB, kilobase pair; λ, lambda PFGE ladder marker (New England BioLabs, Beverly, Mass., USA); S, *Salmonella enterica* serotype Braenderup (strain H9812) molecular size standard. Lanes 1–14, *Xba*I-digested genomic DNA from *K. pneumoniae* isolates. The isolate carrying SHV-144 is located in lane 10.

The transconjugant strain compared with those for the recipient strain. Although there were no apparent significant differences between the MICs of antimicrobials obtained for the donor and transconjugant strains, lower MICs of ceftazidime and ciprofloxacin were observed for the transconjugant strain, which could be due to other factors (such as drug permeability, efflux pumps, and chromosomal gene mutations) known to affect susceptibility to these antibiotics (10).

To investigate the genetic relationship between strain K24 and other multidrug-resistant *K. pneumoniae* clinical isolates, pulsed-field gel electrophoresis (PFGE) was performed for 92 multidrug-resistant *K. pneumoniae* isolates collected from 2010 to 2011 in our hospital (11). PFGE results indicated that strain K24 was genetically unrelated to any other isolates investigated during the study period (Fig. 1).

To date, there is little information regarding the epidemiology of nosocomial infections caused by *K. pneumoniae* in our hospital. An outbreak caused by SHV-5 ESBL-producing *K. pneumoniae* was reported in the pediatric oncology unit of the UMMC from December 1997 to January 1998 (12). In addition, sporadic cases of SHV-12 ESBL-producing *K. pneumoniae* have been reported, suggesting that the SHV-12 gene may have evolved from the SHV-5 gene by acquisition of an additional mutation (Leu35Gln) (13). Therefore, we propose that strain K24 is a sporadic isolate, considering that it originated from a patient with underlying diseases. Hereafter, expression, purification, and kinetics should be studied for further characterization of the SHV-144 enzyme. Next generation sequencing may
prove useful to provide further information regarding the genomic content of the isolate.

Antimicrobial selection pressure triggers the evolution of resistance in bacterial pathogens, which frequently results in the emergence of highly resistant strains. Furthermore, co-expression of multiple resistance genes allows bacteria to sustain antimicrobial selection pressure; hence, clonal expansion of multidrug-resistant strains is possible under favorable conditions (14). Combating multidrug-resistant bacteria remains a tremendous challenge; therefore, periodic surveillance of emerging antimicrobial resistance and effective infection control measures are important to prevent further spread of drug-resistant organisms in hospital settings.

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Conflict of interest None to declare.

REFERENCES