Extended-Spectrum β-Lactamase Genes of Klebsiella pneumoniae Strains in Taiwan: Recharacterization of shv-27, shv-41, and tem-116

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ABSTRACT

Klebsiella pneumoniae causing primary liver abscess (PLA) is emerging. This study identified the β-lactamases genes of K. pneumoniae isolates in Taiwan. The susceptibilities of β-lactam antibiotics of 30 K. pneumoniae strains associated with primary liver abscess and 30 noninvasive strains were analyzed. The β-lactamase genes of randomly selected 24 strains from community-acquired infection and 7 extended-spectrum β-lactamases (ESBL) strains were identified by PCR and DNA sequencing. Protein expression and the ESBL phenotype of β-lactamase were determined. All 60 strains were ampicillin resistant and cefotaxime susceptible, whereas no strain was ESBL producing. In the 24 selected strains, shv-1a was found in 14, shv-1 in 7; shv-26, shv-27, and shv-41 were detected in one. However, all of these 24 strains had the tem-116 gene. In 7 ESBL-producing K. pneumoniae strains, shv-5a was found in 5, whereas shv-5 and ctx-m-9 group were detected in 1 strain. Two previously reported ESBL genes, shv-27 and tem-116, as well as a suspected ESBL gene, shv-41, were found in non-ESBL-producing strains. Transformation of these genes conferred ampicillin resistance but not the ESBL-producing phenotype in Escherichia coli. β-Lactamase protein expression of these strains was further confirmed by western blotting. In conclusion, ESBL is rare in community-acquired K. pneumoniae infection and is not associated with PLA in Taiwan. The shv-5a, shv-5, and ctx-m-9 groups are present in ESBL-producing strains in Taiwan, but shv-27, shv-41, and tem-116 are not ESBL genes.

INTRODUCTION

Klebsiella pneumoniae commonly causes hospital-acquired infections and is also an important pathogen in community-acquired infections such as community-acquired pneumonia.1,10,19 K. pneumoniae-caused primary liver abscess (PLA) is an important emerging infection in Taiwan.7,12,14,21 The invasive K. pneumoniae infections caused a community-acquired PLA with sepsis and bacteremia and sometimes complicated with metastatic meningitis or endophthalmitis. This disease is also a global concern, as attested by reports from North America, Europe, and Asia.2,5,20

Resistance to β-lactam antibiotics of many Gram-negative bacteria was as a result of beta-lactamases. The first plasmid-mediated β-lactamase in Gram-negative bacteria, tem-1, was described in the early 1960s. Another common plasmid-mediated β-lactamase found in K. pneumoniae and E. coli is shv-1. Because these β-lactamases were developing, many new β-lactam antibiotics have been designed to be resistant to hydrolytic action. Resistance to these new β-lactam antibiotics due to extended-spectrum β-lactamases (ESBLs) also have emerged subsequently. ESBLs were commonly derived from tem-1 and shv-1 β-lactamases by mutations to alter the hydrolytic abilities and spectrums.4 Over 100 tem and shv types of β-lactamases have been characterized (http://www.lahey.org/studies/web/asp).

In this study, we analyzed the susceptibilities to β-lactam antibiotics of community-acquired K. pneumoniae PLA and noninvasive strains. The β-lactamase genes of these strains in Taiwan were also identified.

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MATERIALS AND METHODS

Bacterial strains

From 1997 to 2001, a total of 60 K. pneumoniae clinical isolates from blood culture (30 strains from patients with PLA and 30 from patients with community-acquired sepsis without any tissue-invasive disease) were randomly selected from the National Taiwan University Hospital. K. pneumoniae MGH78578 was obtained from the American Type Culture Collection (ATCC) as a control that was selected by the Washington University for full genome sequencing (http://genome.wustl.edu/projects/bacterial/kpneumoniae/).

Susceptibility tests

MICs were determined by the broth and agar dilution methods of the National Committee for Clinical Laboratory Standards (NCCLS). Both ampicillin and cefotaxime were tested. ESBL production was defined phenotypically by disk diffusion as a ≥5-mm increase in a zone diameter for either cefotaxime (30 µg) or ceftazidime (30 µg) tested in combination with clavulanic acid (10 µg) compared to the zone when tested alone without clavulanic acid.17

PCR and sequencing

The β-lactamase genes of these strains were identified. shv-1a (shv-1, shv-5) and shv-5a were differentiated by their locations in the genome. According to the finished K. pneumoniae MGH78578 full genome sequence, the location of shv-1a was B_KPN.Contig3591, nucleotides 1,104,698–1,105,558, and shv-5a was located in the B_KPN.Contig3511, nucleotides 1,229–2,089. shv-1a (shv-1, shv-5) was amplified with 408t7aa (5′-CTGAATCTATTGCGTCCGG-3′) and 402t3aa (5′-CACCACCATCATTACCGAC-3′). shv-5a was amplified with 5a-contig-f (5′-CCGACTATTGGAACAGGTC-3′) and m01t7aa (5′-GTTGCTACTTATCGTGATGGCC-3′). Tem-1 (5′-CGCTCATGAGACATACACC-3′) and tem-829r (5′-CAGTGAGGACCTATCCT-3′) were used to amplify tem. These PCR products were sequenced directly by an automatic sequencer (Applied Biosystems, Weiterstadt, Germany).

Cloning of the β-lactamase genes

shv-27, shv-41, and tem-116 were amplified and cloned into a TA vector, pGEM-T easy (Promega, Madison, WI). These β-lactamase genes were subcloned into pBK-CMV (Stratagene, La Jolla, CA) with EcoRI digestion and then transformed into an E. coli DH10B strain. The susceptibilities to β-lactam antibiotics of E. coli DH10B transformants were analyzed. ESBL production was also identified as previously described.

Western detection of expressed β-lactamases

The expression of β-lactamase was detected sequentially with a commercial anti-β-lactamase antibody (Chemicon, Temecula, CA) and goat horseradish peroxidase–conjugated anti-rabbit immunoglobulin G (IgG) antibodies before development with an enhanced chemiluminescence system.

RESULTS

Susceptibilities of PLA and noninvasive K. pneumoniae strains

All of these 60 K. pneumoniae strains (30 PLA strains and 30 noninvasive strains) were ampicillin resistant (≥512 mg/L) and cefotaxime susceptible (≤2 mg/L). None of these isolates was ESBL-producing.

The β-lactamase genes of PLA and noninvasive strains

PCR and sequencing results of shv and tem genes of 12 randomly selected strains causing PLA and 12 noninvasive strains were listed in Table 1. shv-1a was detected in 14 strains, shv-1 was found in 7 strains, while shv-26, shv-27, and shv-41 were found in one strain, respectively.6,9,16 Interestingly, the tem-116 gene, which was not reported in Taiwan before, was found in all of these 24 isolates.13

Table 1. shv and tem Genes of 12 PLA and 12 Noninvasive K. pneumoniae Strains

<table>
<thead>
<tr>
<th>PLA strains</th>
<th>shv</th>
<th>tem</th>
<th>Noninvasive strains</th>
<th>shv</th>
<th>tem</th>
</tr>
</thead>
<tbody>
<tr>
<td>A01</td>
<td>shv-1</td>
<td>tem-116</td>
<td>N01</td>
<td>shv-1a</td>
<td>tem-116</td>
</tr>
<tr>
<td>A02</td>
<td>shv-1a</td>
<td>tem-116</td>
<td>N02</td>
<td>shv-1</td>
<td>tem-116</td>
</tr>
<tr>
<td>A03</td>
<td>shv-1</td>
<td>tem-116</td>
<td>N03</td>
<td>shv-1</td>
<td>tem-116</td>
</tr>
<tr>
<td>A04</td>
<td>shv-1</td>
<td>tem-116</td>
<td>N04</td>
<td>shv-27</td>
<td>tem-116</td>
</tr>
<tr>
<td>A05</td>
<td>shv-1a</td>
<td>tem-116</td>
<td>N05</td>
<td>shv-1a</td>
<td>tem-116</td>
</tr>
<tr>
<td>A06</td>
<td>shv-1</td>
<td>tem-116</td>
<td>N06</td>
<td>shv-1a</td>
<td>tem-116</td>
</tr>
<tr>
<td>A07</td>
<td>shv-1a</td>
<td>tem-116</td>
<td>N07</td>
<td>shv-41</td>
<td>tem-116</td>
</tr>
<tr>
<td>A08</td>
<td>shv-1a</td>
<td>tem-116</td>
<td>N08</td>
<td>shv-1a</td>
<td>tem-116</td>
</tr>
<tr>
<td>A09</td>
<td>shv-26</td>
<td>tem-116</td>
<td>N09</td>
<td>shv-1a</td>
<td>tem-116</td>
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<tr>
<td>A10</td>
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<td>N10</td>
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<td>tem-116</td>
<td>N11</td>
<td>shv-1a</td>
<td>tem-116</td>
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<tr>
<td>A12</td>
<td>shv-1a</td>
<td>tem-116</td>
<td>N12</td>
<td>shv-1a</td>
<td>tem-116</td>
</tr>
</tbody>
</table>

PLA strains were strains isolated from patients with primary liver abscesses.
Noninvasive strains were strains isolated from patients without PLA, endophthalmitis, and meningitis.

aAll 24 strains are non-ESBL-producing strains.

Cloning of β-lactamase genes

shv-27 and tem-116 were both previously reported as ESBL, however these two genes were detected in the non-ESBL-pro-
dicing strains in our study. Especially, tem-116 was found in all of 24 non-ESBL-producing strains. Therefore, we cloned these two genes into a pBK-CMV plasmid and then transformed into an E. coli DH10B strain. The E. coli DH10B strain was converted into an ampicillin-resistant strain by transformation of the shv-27- and tem-116-containing plasmid, but did not produce an ESBL. E. coli DH10B transformed with cloned shv-5 produced an ESBL, whereas cloned tem-1 produced ampicillin resistance but not an ESBL-producing phenotype. shv-41 was also found in the ESBL-producing strain previously but not yet proven as ESBL. However, shv-41 was detected in our non-ESBL-producing isolate, and transformation of a shv-41-containing plasmid also converted E. coli DH10B strain into an ampicillin-resistant strain but did not produce ESBL. We conclude that shv-27, shv-41, and tem-116 are not ESBLs. Furthermore, the expression of β-lactamase in these E. coli DH10B strains was confirmed by western blotting (Fig. 1).

![Protein expression of β-lactamases of K. pneumoniae strains](image)

**FIG. 1.** Protein expression of β-lactamases of _K. pneumoniae_ strains. Protein expression of β-lactamases was detected by western blotting using anti-β-lactamase antibody (Chemicon). Lane 1, _E. coli_ DH10B strain carrying pBK-CMV vector without insert; lane 2, pBK-CMV vector carrying tem-116 of _K. pneumoniae_ A01 strain in _E. coli_ DH10B strain; lane 3, pBK-CMV vector carrying tem-116 of _K. pneumoniae_ NTUH-K2044 strain in _E. coli_ DH10B strain; lane 4, pBK-CMV vector carrying tem-1 of _K. pneumoniae_ MGH78578 strain in _E. coli_ DH10B strain; lane 5, pBK-CMV vector carrying shv-27 of _K. pneumoniae_ N04 strain in _E. coli_ DH10B strain; lane 6, pBK-CMV vector carrying shv-41 of _K. pneumoniae_ N07 strain in _E. coli_ DH10B strain; lane 7, pBK-CMV vector carrying shv-5 of _K. pneumoniae_ C18 strain in _E. coli_ DH10B strain. The arrow indicates 31 kD (expected molecular weight of β-lactamase).

**TABLE 2. β-Lactamase Genes of 7 Nosocomial _K. pneumoniae_ Strains**

<table>
<thead>
<tr>
<th>Strains</th>
<th>shva</th>
<th>shvb</th>
<th>tem</th>
<th>ctx-m</th>
<th>per</th>
<th>Source or reference</th>
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<tr>
<td>MGH78578</td>
<td>shv-1a</td>
<td>shv-5a&lt;sup&gt;c&lt;/sup&gt;</td>
<td>tem-1</td>
<td>—</td>
<td>—</td>
<td>ATCC</td>
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<tr>
<td>NTUH2044</td>
<td>shv-1a</td>
<td>—</td>
<td>tem-116</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>C4</td>
<td>—</td>
<td>shv-5a&lt;sup&gt;c&lt;/sup&gt;</td>
<td>tem-1</td>
<td>—</td>
<td>—</td>
<td>This study</td>
</tr>
<tr>
<td>C6</td>
<td>shv-1</td>
<td>shv-5a&lt;sup&gt;c&lt;/sup&gt;</td>
<td>tem-1</td>
<td>—</td>
<td>—</td>
<td>This study</td>
</tr>
<tr>
<td>C9</td>
<td>shv-1a</td>
<td>—</td>
<td>tem-1</td>
<td>—</td>
<td>—</td>
<td>This study</td>
</tr>
<tr>
<td>C10</td>
<td>shv-1a</td>
<td>shv-5a&lt;sup&gt;c&lt;/sup&gt;</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>This study</td>
</tr>
<tr>
<td>C19</td>
<td>shv-1a</td>
<td>—</td>
<td>tem-31</td>
<td>ctx-m-9 group&lt;sup&gt;c&lt;/sup&gt;</td>
<td>—</td>
<td>This study</td>
</tr>
<tr>
<td>C20</td>
<td>shv-1a</td>
<td>shv-5a&lt;sup&gt;c&lt;/sup&gt;</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>This study</td>
</tr>
</tbody>
</table>

According to the finished _K. pneumoniae_ MGH78578 full genome sequence, shv-1a (shv-1, shv-5) and shv-5a were differentiated by the location in the genome.

<sup>a</sup>The location of shv-1a was B_KPN.Contig3591, nucleotides 1,104,698–1,105,558.

<sup>b</sup>The location of shv-5a was B_KPN.Contig3511, nucleotides 1,229–2,089.

<sup>c</sup>ESBL genes.

**DISCUSSION**

We have identified three specific genome regions in PLA strains, therefore, the genomic heterogeneity might also associated with antibiotic resistance pattern. However, all PLA strains and noninvasive strains were ampicillin resistant and ceftaxime susceptible, and none was ESBL-producing. Therefore, ESBL is not associated with PLA.

As shown by previous studies, shv-1 and shv-1a were detected in most non-ESBL-producing _K. pneumoniae_ strains and shv-5a was detected in most ESBL-producing isolates. The shv-5a, shv-5, and ctx-m-9 groups were detected in ESBL-producing strains in Taiwan. Interestingly, tem-116 was found in all of the community-acquired _K. pneumoniae_ strains but in none of the 7 nosocomial ESBL isolates. The tem-116 gene that has been identified in Korea recently was first reported in _K. pneumoniae_ strains of Taiwan.

In our study, shv-27 and tem-116 were detected in non-ESBL-producing isolates; especially, tem-116 was found in 24 community-acquired non-ESBL-producing strains. These two β-lactamases were all identified as ESBLs previously because they were found in ESBL-producing isolates. shv-41 was found in ESBL isolates before; however, its role regarding ESBL was not defined. By transformation of these 3 β-lactamase genes into the non-ESBL-producing _E. coli_ DH10B strain, they did not produce the ESBL phenotype. However, they conferred ampicillin resistance, and protein expression was further confirmed. Therefore, they are not real ESBL genes. Be-
cause no knock-out/complementation or transformation studies were done to confirm the ESBL gene function in the previous reports, there may be other genes responsible for ESBL production in their strains.

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REFERENCES


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