MICROBIAL DRUG RESISTANCE
Volume 12, Number 1, 2006

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/webt.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).[17] 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.Cotig3511, nucleotides 1,229–2,089. *shv-1a* (*shv-1*, *shv-5*) was amplified with 408t7aa (5′-CTGAATCATTATGCGTCCGG-3′) and 402t3aa (5′-CACCACCATCATTACCGAC-3′). *shv-5a* was amplified with 5a-contig-f (5′-CCGACTATTTGCAACAGTGC-3′) and m01t7a (5′-GTTGCATCTATCTGGATGCC-3′). Tem-f (5′-CGCTCATGAGACAATAACCC-3′) and tem-829r (5′-CAGTGAGGCACCTATCTC-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 *Eco*RI 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]

The β-lactamase genes of nosocomial strains

Because none of the 60 community-acquired *K. pneumoniae* strains was an ESBL-producing strain, 7 ESBL-producing nonblood nosocomial *K. pneumoniae* strains in 1997–2001 were selected for ESBL gene identification. Four ESBL genes in *K. pneumoniae*, *shv*, *tem*, *ctx*, and *per*, were tested by PCR and DNA sequencing.[18] Primers used to detect *ctx* and *per* were designed as previously described.[18] The genes responsible for ESBL phenotype were identified (Table 2). The ESBL, *shv-5a*, was detected in 5 of the 7 strains. The remaining 2 strains contained *shv-5* and *ctx-m-9* group, respectively.[3]

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-

TABLE 1. *shv* AND *tem* GENES OF 12 PLA AND 12 NONINVASIVE *K. pneumoniae* STRAINS[a]

PLA strains	shv	tem	*Noninvasive strains*	shv	tem
A01	*shv-1*	*tem-116*	N01	*shv-1a*	*tem-116*
A02	*shv-1a*	*tem-116*	N02	*shv-1*	*tem-116*
A03	*shv-1*	*tem-116*	N03	*shv-1*	*tem-116*
A04	*shv-1*	*tem-116*	N04	*shv-27*	*tem-116*
A05	*shv-1a*	*tem-116*	N05	*shv-1a*	*tem-116*
A06	*shv-1*	*tem-116*	N06	*shv-1a*	*tem-116*
A07	*shv-1a*	*tem-116*	N07	*shv-41*	*tem-116*
A08	*shv-1a*	*tem-116*	N08	*shv-1a*	*tem-116*
A09	*shv-26*	*tem-116*	N09	*shv-1a*	*tem-116*
A10	*shv-1*	*tem-116*	N10	*shv-1a*	*tem-116*
A11	*shv-1a*	*tem-116*	N11	*shv-1a*	*tem-116*
A12	*shv-1a*	*tem-116*	N12	*shv-1a*	*tem-116*

PLA strains were strains isolated from patients with primary liver abscess.

Noninvasive strains were strains isolated from patients without PLA, endophthalmitis, and meningitis.

[a]All 24 strains are non-ESBL-producing strains.

TABLE 2. β-LACTAMASE GENES OF 7 NOSOCOMIAL *K. pneumoniae* STRAINS

strains	shv[a]	shv[b]	tem	ctx-m	per	*Source or reference*
MGH78578	*shv-1a*	*shv-5a*[c]	*tem-1*	—	—	ATCC
NTUH2044	*shv-1a*	—	*tem-116*	—	—	20
C4	—	*shv-5a*[c]	*tem-1*	—	—	This study
C6	*shv-1*	*shv-5a*[c]	*tem-1*	—	—	This study
C9	*shv-1a*	*shv-5a*[c]	*tem-1*	—	—	This study
C10	*shv-1a*	*shv-5a*[c]	—	—	—	This study
C18	*shv-5*[c]	—	*tem-1*	—	—	This study
C19	*shv-1a*	—	*tem-31*	*ctx-m-9 group*[c]	—	This study
C20	*shv-1a*	*shv-5a*[c]	—	—	—	This study

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.

[a]The location of *shv-1a* was B_KPN.Contig3591, nucleotides 1,104,698–1,105,558.

[b]The location of *shv-5a* was B_KPN.Contig3511, nucleotides 1,229–2,089.

[c]ESBL genes.

ducing strains in our study.[9,13] 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* conferred 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.[16] 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).

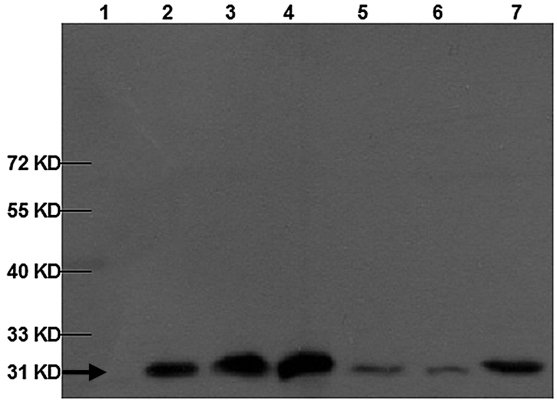

FIG. 1. Protein expression of β-lactamases of *K. pneumoniae* strains. Protein expression of β-latamases was detected by western blotting using anti-β-lactamase antibody (Chemicon). Lane 1, *E. coli* DH10B strain carring pBK-CMV vector without insert; lane 2, pBK-CMV vector carring *tem-116* of *K. pneumoniae* A01 strain in *E. coli* DH10B strain; lane 3, pBK-CMV vector carring *tem-116* of *K. pneumoniae* NTUH-K2044 strain in *E. coli* DH10B strain; lane 4, pBK-CMV vector carring *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 carring *shv-41* of *K. pneumoniae* N07 strain in *E. coli* DH10B strain; lane 7, pBK-CMV vector carring *shv-5* of *K. pneumoniae* C18 strain in *E. coli* DH10B strain. The arrow indicates 31 kD (expected molecular weight of β-lactamase).

DISCUSSION

We have identified three specific genome regions in PLA strains, therefore, the genomic heterogeneity might also associated with antibiotic resistance pattern.[8,11,15] However, all PLA strains and noninvasive strains were ampicillin resistant and cefotaxime 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.[9,13] *shv-41* was found in ESBL isolates before; however, its role regarding ESBL was not defined.[16] 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.

ACKNOWLEDGMENTS

This work was supported by the grants from the National Science Council in Taiwan and the National Taiwan University Hospital (NTUH-98-S-002).

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