**SHV-5 EXTENDED-SPECTRUM β-LACTAMASES IN CLINICAL ISOLATES OF ESCHERICHIA COLI IN MALAYSIA**

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**Abstract**

*Escherichia coli* isolates resistant to ceftazidime isolated in the University Malaya Medical Center (UMMC) Kuala Lumpur, Malaysia, between the years 1998 and 2000 were studied for extended-spectrum β-lactamase (ESBL) production. All strains were analysed phenotypically and genotypically and found to be ESBL-producing organisms harbouring SHV-5 β-lactamase. This was confirmed by PCR-SSCP and nucleotide sequencing of the blaSHV amplified gene. As there was no evidence of ESBL activity in *E. coli* prior to this, coupled with the fact that there was a predominance of SHV-5 β-lactamases in *Klebsiella pneumoniae* isolates in UMMC, we postulate that the *E. coli* obtained the SHV-5 β-lactamase genes by plasmid transfer from the ESBL-producing *K. pneumoniae*.

**Key words:** *E. coli*, extended-spectrum β-lactamases, SHV-5

β-lactams are widely used in the treatment of infections and this has resulted in considerable selection pressure for emergence of resistance to the β-lactams. Although several species of bacteria including *Escherichia coli* are naturally susceptible to extended-spectrum cephalosporins, these organisms acquire resistance to these antibiotics by several mechanisms that include the production of extended-spectrum β-lactamases (ESBLs) under the selection pressure of the use of expanded-spectrum cephalosporins in clinical practice. Sequence analysis of the β-lactamases has allowed them to be grouped into four classes, class A to D. Most ESBLs found in *E. coli* and *K. pneumoniae* belong to class A which include the TEM- and SHV-type of β-lactamases. These enzymes are generally located on large, transferable plasmids and the increasing incidence and spread of β-lactam resistance can be attributed to the dissemination of these plasmids. Among the SHV-type of β-lactamases, SHV-5 was found to be responsible for outbreaks of nosocomial infections in several countries.

In this study, we analyzed 11 isolates of ESBL-producing *E. coli* in order to characterize the β-lactamases produced and to determine if any transfer of genetic determinants had occurred between *K. pneumoniae* and *E. coli*.

**Materials and Methods**

A total of 11 sporadic isolates of ceftazidime-resistant *E. coli* from clinical samples (blood, pus and urine) were obtained from the University Malaya Medical Center (UMMC) over a 2-year period between 1998 to the year 2000 (Table).

Minimum inhibition concentration (MIC) for each isolate against a range of antibiotics was determined using the agar dilution technique as described in the National Committee for Clinical Laboratory Standards (NCCLS) guidelines (Table). Isoelectric focusing (IEF) assay was done to determine the type of β-lactamase produced. The presence of the enzymes were confirmed by polymerase chain reaction-single stranded conformational polymorphism (PCR-SSCP) analysis.

**Results**

All *E. coli* isolates were resistant to ceftazidime with 70% of the isolates displaying high level of resistance (64-128 µg/mL). There was a 8-fold reduction in the MIC values of ceftazidime when combined with clavulanic acid and this phenotypically confirmed the isolates as ESBL producers. All isolates were highly susceptible to the carbapenems, imipenem and meropenem. Analytical isoelectric focusing revealed the presence of more than one β-lactamase with pl values ranging from 5.1 to > 9.0 (Table). Ninety-one percent of these isolates produced a β-lactamase with a pl value of 8.2 which is similar to the pl value of the SHV-5 β-lactamase. Most of the isolates also produced β-lactamases with pl values of between 5.1 to 6.0 which are characteristic of TEM enzymes. Two of the isolates produced a β-lactamase with the pl value of > 9.0 corresponding to that of the AmpC type. The presence of the SHV-type, TEM-type or AmpC-type enzyme was further confirmed by PCR and the results correlated with the data obtained from the IEF assay. PCR-SSCP analysis of the SHV gene carried out on the 11 isolates which produced enzymes with pl values of 8.2 indicative of the SHV-5 type β-lactamase gene. The identity of this gene was confirmed by sequencing the entire coding region of the amplified *blaSHV* genes. Plasmid profiles of the 14 isolates harbouring the SHV-5 gene revealed the uniform presence of a large molecular plasmid of more than 147 kb in size. Almost 80% of the isolates contained multiple plasmids.
plasmids ranging from <5 kb to >147 kb in size. The plasmid profiles for each isolate were unique. PCR amplification of the SHV gene from the purified large plasmid of each isolate and subsequent sequence analysis confirmed that the SHV-5 gene in these ESBL-producing E. coli isolates was located on the plasmid.

**Discussion**

The escalating incidence of ESBL-producing organisms has been attributed to the increased use of expanded-spectrum cephalosporins in clinical practice. Plasmid-mediated resistance to the third generation cephalosporins and the ease by which resistant plasmids are able to transfer from one genus to another, further complicates the control of these resistant organisms and are the major cause of various outbreaks of nosocomial infections. Although several studies have addressed the issue of the emergence of SHV ESBL-producing strains of E. coli worldwide, no such published data is available in Malaysia.

The incidence of nosocomial infections and outbreaks seemed largely due to K. pneumoniae in many cases. In UMMC, a study was carried out between the years 1995 to 1996 whereby 28 isolates of ESBL-producing K. pneumoniae were obtained and characterised. A majority of these harbour a SHV-5 type β-lactamase gene and a large molecular weight plasmid of more than 100 kb. Until 1998, there were no reports of ESBL-producing E. coli in UMMC. The isolates analysed in this study were obtained from 1998 to the year 2000 and were shown to be ESBL-producing isolates. Thus, the isolation of ESBL-producing E. coli strains harbouring SHV-5 type β-lactamases two years after detection of the prevailing SHV-5 type β-lactamases in K. pneumoniae led us to postulate that the normally sensitive E. coli isolates acquired these SHV-5 genes from K. pneumoniae via transfer of plasmids. This postulation was also aided by the presence of large molecular weight plasmids harbouring the SHV-5 gene in both the E. coli and K. pneumoniae isolates tested. Furthermore, these findings were concurrent with reports from other countries on the spread of SHV-5 through the transfer of plasmids from K. pneumoniae to E. coli as well as other members of the family Enterobacteriaceae.

The ease by which these organisms transmit resistance plasmids to other Enterobacteriaceae that are normally sensitive to broad spectrum cephalosporins, poses a serious problem in the control of antibiotic resistance. Furthermore, the rapid accumulation of point mutations at the active site of these enzymes has given rise to an entire family of SHV-type β-lactamases. This poses an even more serious threat in that the bacteria harbouring these genes will always be able to survive the changing treatment regime simply by mutation of the β-lactamase genes.

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References


