

Dissemination of Extended-Spectrum β -Lactamase-Producing *Enterobacteriaceae* in Pediatric Intensive Care Units

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To study the growing trend of extended-spectrum β -lactamase (ESBL)-producing *Enterobacteriaceae* in pediatric intensive care units (PICUs), 88 nonrepetitive ESBL-producing isolates were prospectively collected and analyzed by molecular methods during a 16-month period. The emergence and dissemination of ESBL-producing *Enterobacteriaceae* in PICUs are the consequence of the clonal dissemination of a few epidemic strains along with the horizontal transmission of resistance gene-carrying plasmids among bacterial organisms.

Since the initial description of extended-spectrum β -lactamase (ESBL) production by *Klebsiella pneumoniae* and *Escherichia coli* in the 1980s (1, 10), strains of enterobacteria that are resistant to extended-spectrum cephalosporins are increasingly being recognized (3, 6). ESBL-producing enterobacteria have been frequently implicated in outbreaks in pediatric intensive care units (PICUs) and neonatal intensive care units (NICUs) (2, 9, 11). We started to screen for ESBL-producing enterobacteria by using the method recommended by the NCCLS since September 2000. In our hospital, we found a marked increase in the number of the ESBL-producing enterobacteria in the PICUs and NICUs. To further delineate the mode of dissemination of ESBLs, we collected clinical isolates of ESBL-positive members of the family *Enterobacteriaceae* from the ICUs during the period of September 2000 to December 2001. The relatedness of these isolates and the types of ESBLs was investigated.

In the Chang Gung Children's Hospital at Kweishan, there are one PICU and four NICUs. PICU 2L admitted all critical pediatric patients beyond the neonatal stage. NICUs 3L1, 3L2, 5L1, and 5L2 admitted only neonatal and premature patients. 3L1 and 3L2 were separate units located in the same floor, and so were 5L1 and 5L2. Pediatricians and nursing staff members could move freely among these ICUs, provided regulations of hospital infection control were obeyed. A geographically independent ward, Taipei PICU (TPICU), is located in the Taipei branch of Chang Gung Memorial Hospital, which is about 20 kilometers away from Chang Gung Children's Hospital at Kweishan.

A total of 88 nonrepetitive ESBL-producing isolates of *E. coli* ($n = 16$), *Enterobacter cloacae* ($n = 26$), and *K. pneumoniae* ($n = 46$) were collected from specimens from various sources during the study period. All isolates were screened for the presence of ESBLs by the disk diffusion method with cefotaxime (30 μ g) and ceftazidime (30 μ g), and the results were

confirmed by the phenotypic confirmatory test recommended by the NCCLS (14, 15).

In the present study, the genetic relatedness of the clinical isolates was investigated by the method of infrequent restriction site PCR (IRS-PCR) (19). Isolates with identical banding patterns or pattern difference of fewer than four bands were arbitrarily assigned to the same genotypes or subtypes of an existing genotype. Isolates with more than four band differences were considered different and assigned to different genotypes.

To determine the ESBL types, three primer sets previously described for the detection of the *bla*_{TEM}, *bla*_{SHV}, and *bla*_{CTX-M} genes were used in the amplification procedure (5, 13, 16). PCR products were purified with Microcon PCR centrifugal filter devices (Millipore) and sequenced by an ABI 377 automatic sequencer (Perkin-Elmer Applied Biosystems). A search for homologous sequences in the GenBank database was done with the FASTA software through the internet.

The presence of plasmids was checked by the modified alkaline lysis method described earlier (8). DNA-DNA hybridization was performed by the method of Southern (18), except that the probe for the target β -lactamase gene was prepared from a *bla*_{SHV-12} or *bla*_{CTX-M-3} PCR product and labeled with digoxigenin-11-dUTP (Roche).

As shown in Table 1, a total of 88 nonrepetitive ESBL-producing isolates were collected during the study period. CTX-M-3 (52 isolates) was the most prevalent type of ESBL identified, followed in the order of frequency by SHV-12 (35 isolates). SHV-12 was present in all isolates of *E. cloacae*, and CTX-M-3 was found coexisting with SHV-12 in three *E. cloacae* isolates. CTX-M-3 was also the most common type of ESBL produced by *E. coli* (81%) and *K. pneumoniae* (78%). These were the most common ESBLs found with the published TEM, SHV, and CTX-M sets of primers. Other ESBLs, including OXY-1, TEM-31, and SHV-2, -2a, -5, and -12, were found in sporadic isolates of *E. coli* and *K. pneumoniae* (Table 1). Eleven representative isolates of *E. coli*, 10 of *E. cloacae*, and 13 of *K. pneumoniae* were examined for their plasmid patterns. All of the isolates tested carried one or multiple large plasmids greater than 95 kb in size. DNA-DNA hybridization

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TABLE 1. Number and distribution of various ESBLs among strains of *E. cloacae*, *E. coli*, and *K. pneumoniae*

Bacteria	Total no.	No. of different ESBLs							
		SHV-12	SHV-2	SHV-2a	SHV-5	CTX-M-3	CTX-M-14	TEM-31	OXY-1
<i>E. cloacae</i>	26	26	0	0	0	3	0	0	0
<i>E. coli</i>	16	3	0	0	1	13	0	0	0
<i>K. pneumoniae</i>	46	6	4	3	1	36	1	2	1

with a *bla*_{SHV-12} or *bla*_{CTX-M-3} PCR product as the probe showed that these *bla* genes were located on the large plasmids in the majority of the representative isolates. Only two isolates of *E. coli*, two of *E. cloacae*, and one of *K. pneumoniae* did not carry plasmid-mediated *bla*.

Results of the genotyping and their relationship to the ICUs where the clinical isolates were collected are shown in Fig. 1. Molecular typing identified 17, 10, and 14 different genotypes among *K. pneumoniae*, *E. cloacae*, and *E. coli* isolates, respectively. Two predominant genotypes, types 1 and 8, were found in 18 (69%) of the 26 *E. cloacae* isolates. Isolates with genotype 8 were mainly derived from NICUs 3L1 and 3L2, while genotype 1 was from the TPICU and NICU 3L2. The most common genotype found in *K. pneumoniae* isolates (23 of 46, 50%) was genotype 2, most of which were collected from 3L2, 5L2, and the TPICU. Diverse genotypes were found among the 16 *E. coli* isolates.

Infection by ESBL-producing gram-negative bacteria has become a serious problem in Taiwan. According to a report from a medical center in northern Taiwan, the proportion of isolates of *K. pneumoniae* exhibiting ESBL phenotypes has increased progressively from 3.4% in 1993 to 10.3% in 1997 (7). From 1998 through 2000, several reports from different hospitals in Taiwan showed that ESBL production accounts for

8 to 30% of the total number of clinical isolates of *K. pneumoniae* (4, 7, 12, 17, 23, 25), and SHV-5 and SHV-12 were the most prevalent ESBLs. In addition, four novel β-lactamases (CMY-8, SHV-25, SHV-26, and IMP-8) were identified in 2000 in Taiwan (4, 12, 17, 23, 24). Among the ESBL-producing *E. coli* isolates, which accounted for 1.6 to 6.7% of the total number of *E. coli* isolates, strains expressing CTX-M-3 and CMY-2 were disseminated in Taiwan (25).

In the present study, the most common types of ESBL identified among clinical isolates were SHV-12 and CTX-M-3. SHV-12 was present in all isolates of *E. cloacae*, while CTX-M-3 was predominant in *E. coli* and *K. pneumoniae*. The ESBL genes were located on the large plasmids in most of the isolates. Previous studies have shown that bacteria could acquire resistance from other organisms through the exchange of genetic materials, such as plasmids, in vivo (19, 22). While clonal spread could not entirely explain the rapid and wide dissemination of ESBLs among different species of the family *Enterobacteriaceae*, our results provide evidence that the increase in ESBL-producing *Enterobacteriaceae* in hospitals is in part due to the widespread dissemination of resistance plasmids.

Clonal spread was detected by the molecular typing method in the present study. Two distinct strains of *E. cloacae* and one of *K. pneumoniae* were prevalent within the PICUs, as demonstrated by IRS-PCR. Genotyping is very important when epidemic strains are resistant to many antimicrobial agents. Pulsed-field gel electrophoresis is considered the most reliable molecular typing method. IRS-PCR, the method used in this study, is simpler and less time-consuming. It has been applied in the investigation of outbreaks caused by many different bacterial organisms, including *K. pneumoniae*, *Serratia marcescens*, and *Salmonella enteritidis* (20, 21, 26). By use of this method, we were able to identify major genotypes of *K. pneumoniae* and *E. cloacae* circulating in the hospital wards studied.

In conclusion, the increase in the number of ESBL-producing isolates of *Enterobacteriaceae* in PICUs is due to the occurrence of multiple consecutive outbreaks of infections associated with ESBL-producing bacteria. The dissemination of ESBL-producing *Enterobacteriaceae* is a consequence of the clonal expansion of a few epidemic strains and the spread of resistance plasmids among bacterial organisms. Since the resistance displayed by bacteria reflects the environment in which the organism thrives, immediate action, including reinforcement of infection control measures, should be taken to prevent further spread of the resistant bacteria.

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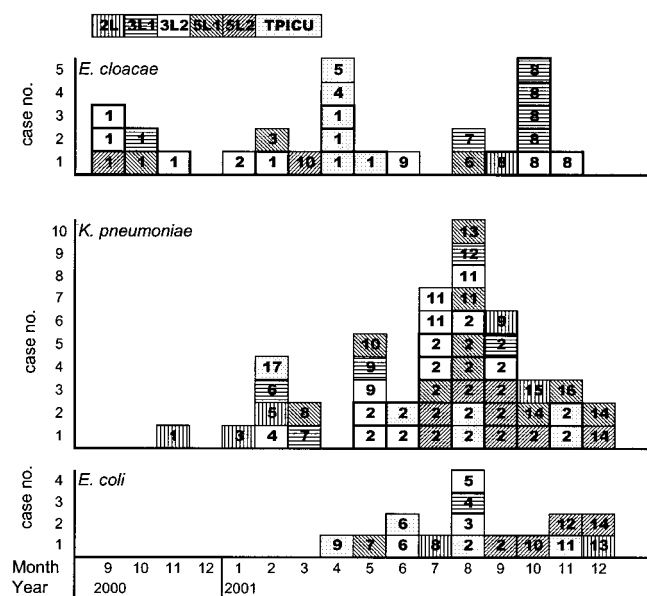


FIG. 1. Incidence of ESBL-producing clinical isolates of *E. cloacae*, *E. coli*, and *K. pneumoniae* in the six ICUs studied between September 2000 and December 2001. Every square represents one isolate, and genotypes are shown as arabic numbers. Different ICUs are differentiated by different patterns.

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