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A 7-year surveillance for ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* at a university hospital in Taiwan: the increase of CTX-M-15 in the ICU

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SUMMARY

To monitor the changing trend of extended-spectrum β-lactamase (ESBL)-producing bacteria, a 7-year continuous study was launched in 2001 at the largest tertiary hospital in Taiwan. A significant increase over the study period was evident for ESBL-producing isolates of *Escherichia coli* (4·8–10·0%) and *Klebsiella pneumoniae* (15·0–23·4%). Molecular investigation conducted in three separate periods revealed the prevalent ESBL types and their genetic relatedness. CTX-M-producing isolates (73·8%) were more prevalent than SHV-type ESBLs (37·0%), the most frequent being CTX-M-14 (34·3%), CTX-M-3 (25·9%), and SHV-12 (25·7%). However, a marked increase of CTX-M-15-producing isolates from 2·1% in 2002 to 29·6% in 2007 was also noted. The increase of ESBL-producing isolates in both species may be mainly due to the horizontal transmission of resistance plasmids, while clonal expansion of some epidemic strains further added to the dispersion of ESBL-producing *K. pneumoniae*.

Key words: CTX-M-15, ESBL, ICUs.

INTRODUCTION

Extended-spectrum cephalosporins, such as ceftriaxone, cefotaxime, and ceftazidime, were approved for human therapy in the early to mid-1980s and are commonly used to treat infections caused by enterobacteria. Shortly after their release, clinical isolates of enterobacteria appeared that were able to produce extended-spectrum β-lactamases (ESBLs) [1–3]. These enzymes hydrolysed β-lactam antibiotics, thereby mediating resistance to these agents [4]. Today, infections by ESBL-producing enterobacteria are a serious clinical problem in Taiwan as they are often related to outbreaks in intensive care units (ICUs). Previous reports from Taiwan and other countries have indicated that the most predominant ESBL-producing species are *Klebsiella pneumoniae* and *Escherichia coli* [5–7] with the prevalence rate of these strains being relatively higher in Taiwan, ranging from 8·5% to 29·8% in *K. pneumoniae* and from 1·5% to 16·7% in *E. coli*, respectively [7].

At Chang Gung Memorial Hospital (CGMH), we have screened isolates for ESBL production according to the guidelines described by the Clinical and Laboratory Standards Institute (CLSI) since September 2000. Data from our previous report indicated that in the first year there was a relatively high prevalence of ESBL-producing enterobacteria (*E. coli*,

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4·6%; *K. pneumoniae*, 15·6%) [6]. We have continued to monitor trends of these bacteria up to 2007. Studies of the genetic relatedness of ESBL genes in ICU isolates were also undertaken in three separate time periods.

METHODS

Setting and laboratory-based surveillance

CGMH is a university-affiliated, tertiary hospital with 4000 beds located in northern Taiwan. There are 26 ICUs providing critical care to patients; the other 73 general wards are included in the inpatient department. A case of nosocomial infection was defined according to the updated criteria of the Centers for Disease Control and Prevention, USA [8].

Bacterial isolates

In total, 84458 *E. coli* and 31633 *K. pneumoniae* non-duplicate clinical isolates were identified by standard methods and their susceptibilities to the following antimicrobial agents were determined by a disk diffusion method according to CLSI guidelines [9]: amikacin, ampicillin, aztreonam, cefazolin, ceftazidime, ceftriaxone, cefuroxime, ciprofloxacin, gentamicin, imipenem, piperacillin, and trimethoprim/sulphamethoxazole. Duplicate isolates from the same body site of a patient within 30 days were excluded from statistical analysis. ESBL production was confirmed by a disk diffusion method using cefotaxime (30 μg) and ceftazidime (30 μg) in combination with 10 μg clavulanate as recommended [9].

A total of 568 non-duplicate ESBL-producing isolates of *E. coli* ($n=273$) and *K. pneumoniae* ($n=295$) were collected from ICU patients over three separate periods (I: August 2002–December 2003; II: January–June 2005; III: October–December 2007) and subjected to molecular investigation.

Molecular typing

Isolates were typed by infrequent-restriction-site PCR as previously described [10] and banding patterns were compared visually. Isolates showing >4-band difference in profile were categorized as different genotypes; those with identical band patterns or that differed by $\leqslant 3$ bands were considered indistinguishable or closely related, respectively, and designated as the same genotype.

Multiplex PCR and SHV melting curve mutation detection (MCMD) system

The multiplex PCR and SHV MCMD system was used to determine the ESBL genotypes of isolates [11]. Briefly, the multiplex PCR identified bla_{SHV}, $bla_{\mathrm{CTX\text{-}M\text{-}3}}$-like, and $bla_{\mathrm{CTX\text{-}M\text{-}14}}$-like genes and a modified SHV MCMD method was used to distinguish six prevalent bla_{SHV} genes ($bla_{\mathrm{SHV\text{-}1}}$, $bla_{\mathrm{SHV\text{-}2}}$, $bla_{\mathrm{SHV\text{-}2a}}$, $bla_{\mathrm{SHV\text{-}5}}$, $bla_{\mathrm{SHV\text{-}11}}$, $bla_{\mathrm{SHV\text{-}12}}$) in Taiwan. An 819-bp product of the bla_{SHV} was amplified over 50 cycles and two hybridization probes were used concomitantly in a single reaction tube in a LightCycler. Fluorescence signals were plotted automatically in real time *vs.* temperature (T) to produce melting curves for mutations at position 35 (F3 *vs.* T) and positions 238 and 240 (F2 *vs.* T) of the SHV gene. Melting curves were then converted into melting peaks by plotting the negative derivative of fluorescence *vs.* T ($-d\mathrm{F2}/dT$ *vs.* T and $-d\mathrm{F3}/dT$ *vs.* T). Isolates positive for CTX-M genes were subjected to further PCR and DNA sequencing analysis to identify the specific CTX-M types.

PCR amplification and DNA sequencing

During period I, six pairs of previously described primers were used to detect bla_{TEM} [12], bla_{SHV} [13], and four major groups of $bla_{\mathrm{CTX\text{-}M}}$ genes, including $bla_{\mathrm{CTX\text{-}M\text{-}2}}$-like, $bla_{\mathrm{CTX\text{-}M\text{-}3}}$-like, $bla_{\mathrm{CTX\text{-}M\text{-}8}}$-like, and $bla_{\mathrm{CTX\text{-}M\text{-}14}}$-like [11, 14, 15] in a collection of 149 *E. coli* and 177 *K. pneumoniae* isolates. As no other ESBLs except SHV, CTX-M-3, and CTX-M-14 types were identified during this period, the multiplex PCR and SHV MCMD system described above was adopted in the following two periods to determine the major ESBL types followed by direct PCR and sequencing of the identified SHV and CTX-M genes using three pairs of previously described primers [11, 13, 14]. All PCR products were purified using Microcon® PCR centrifugal filter devices (Millipore, USA) and sequences determined using an ABI 3100 Avant Genetic Analyzer (PerkinElmer, Applied Biosystems, USA). Homologous sequences in the GenBank database were searched for using the Basic Local Alignment Search Tool (BLAST) (http://www.ncbi.nlm.nih.gov/BLAST/).

Statistical analysis

The χ^2 test and Student's t test were used to determine the significance of differences. A difference was

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considered statistically significant with a P value $<0{\cdot}05$.

RESULTS

Prevalence of ESBL-producing isolates

A total of 7007 *E. coli* and 5729 *K. pneumoniae* isolates were identified as ESBL producers. The majority of isolates were from urine (56·3%; *E. coli*, 64·2%; *K. pneumoniae*, 46·7%), followed by the respiratory tract (16·8%; *E. coli*, 10·7%; *K. pneumoniae*, 24·2%) and sterile sites (13·4%; *E. coli*, 13·0%; *K. pneumoniae*, 13·8%). A significant increase was found in the proportions of urinary ESBL-producing *E. coli* isolates (from 59·5% in 2001 to 66·3% in 2007, $P<0{\cdot}01$), and of these isolates, a significant increase was evident for patients from the outpatient department (OPD) (from 22·1% in 2001 to 31·6% in 2007, $P<0{\cdot}005$). A similar situation was observed in the proportion of respiratory *K. pneumoniae* isolates (from 17·0% in 2001 to 26·2% in 2007, $P<0{\cdot}00005$); however, the increase was only found in respiratory isolates from patients in the IPD (from 32·0% in 2001 to 51·1% in 2007, $P<0{\cdot}001$).

Figure 1*a* shows that there was a significant increase in the number of ESBL-producing isolates of both species over the study period ($P<0{\cdot}0001$). The prevalence rate also increased significantly from 4·8% in 2001 to 10·0% in 2007 (average 8·3%) in *E. coli* and from 15·0% to 23·4% (average 18·1%) in *K. pneumoniae*; these rates were both highest in isolates from ICUs (reaching 24·3% in 2006 for *E. coli* and 36·9% in 2007 for *K. pneumoniae*), and lowest in those from OPD where there was a slight increase for *E. coli* and no change for *K. pneumoniae*.

Analysis of the distribution of ESBL-producing isolates in different ICUs (Fig. 1*b*) showed that the most significant increase ($P\ll0{\cdot}0001$) both annual numbers and prevalence rates occurred in medical ICUs (MICUs), with a peak prevalence rate of 35·9% (in 2006) for *E. coli* and 42·7% (in 2007) for *K. pneumoniae* isolates. The second largest group was from surgical ICUs (SICUs) although the increase in the prevalence in *E. coli* was more marked than in *K. pneumoniae*. There was a fall in the prevalence of ESBL-producing isolates in paediatric patients during 2003 and 2005 for *K. pneumoniae*, although that of *E. coli* remained constant at a low level during the period.

The infection density rates per 1000 patient-days for ESBL-producing isolates were much higher in MICUs in particular, than those in the IPD (Fig. 1*c*) but were relatively similar between SICUs and paediatric ICUs (PICUs). No difference in the trend of infection density between the two bacteria was evident, except that a sharp decrease occurred in *E. coli* in 2007, compared to the continuous increase in *K. pneumoniae*. The proportion of ESBL-producing nosocomial isolates was relatively constant in *E. coli* [IPD, 18·9% (range 16·5–23·5%); ICUs, 26·0% (range 23·8–26·6%); MICUs, 28·0%; SICUs, 23·8%; PICUs, 19·6%)], and for *K. pneumoniae*, the proportion in IPDs reduced significantly from 22·4% during 2001–2004 to 17·0% during 2005–2007 ($P<0{\cdot}0005$), but remained constant in ICUs [25·2% (range 22·8–29·0%); MICUs, 26·9%; SICUs, 21·5%; PICUs, 22·6%].

ESBL types

Sixteen ESBL types (9 SHV and 7 CTX-M) were identified (Table 1). CTX-M-14 was the most prevalent followed by CTX-M-3 and SHV-12. CTX-M-14 was also the major ESBL type in *E. coli* isolates, whereas SHV-12 was more prevalent in *K. pneumoniae*. Some rare or novel ESBL types were identified, but *E. coli* was more likely to be associated with CTX-M-type enzymes (CTX-M-13, CTX-M-24, CTX-M-27), while *K. pneumoniae* was characterized by SHV-2, SHV-26, SHV-27, SHV-28, SHV-31, and SHV-61. Significant differences were found in the proportion of various ESBL types in the PICUs with CTX-M-3-producing isolates (*E. coli*, $P<0{\cdot}05$; *K. pneumoniae*, $P<0{\cdot}0001$) being the most prevalent but fewer in CTX-M-14- (*E. coli*, $P<0{\cdot}000005$; *K. pneumoniae*, $P<0{\cdot}0001$) and SHV-12-producing isolates (*K. pneumoniae*, $P<0{\cdot}005$) (Table 1).

There was no significant difference in the changing trends of ESBL types in different ICUs over the three periods. The overall trends of the major types (Fig. 2) showed that for *E. coli*, the proportion of CTX-M producers increased gradually while SHV producers declined (Fig. 2*a*). Although CTX-M-14 was the most frequent type overall in *E. coli* isolates, the proportion of CTX-M-3 producers was very similar to that of CTX-M-14 producers during the first study period (April 2002–December 2003). Subsequently, the proportion of CTX-M-3-producing isolates reduced sharply and was accompanied by a sudden increase of CTX-M-15-producing isolates (Fig. 2*a*). Replacement of bacterial populations from predominant CTX-M-3 producers to CTX-M-14 producers in the

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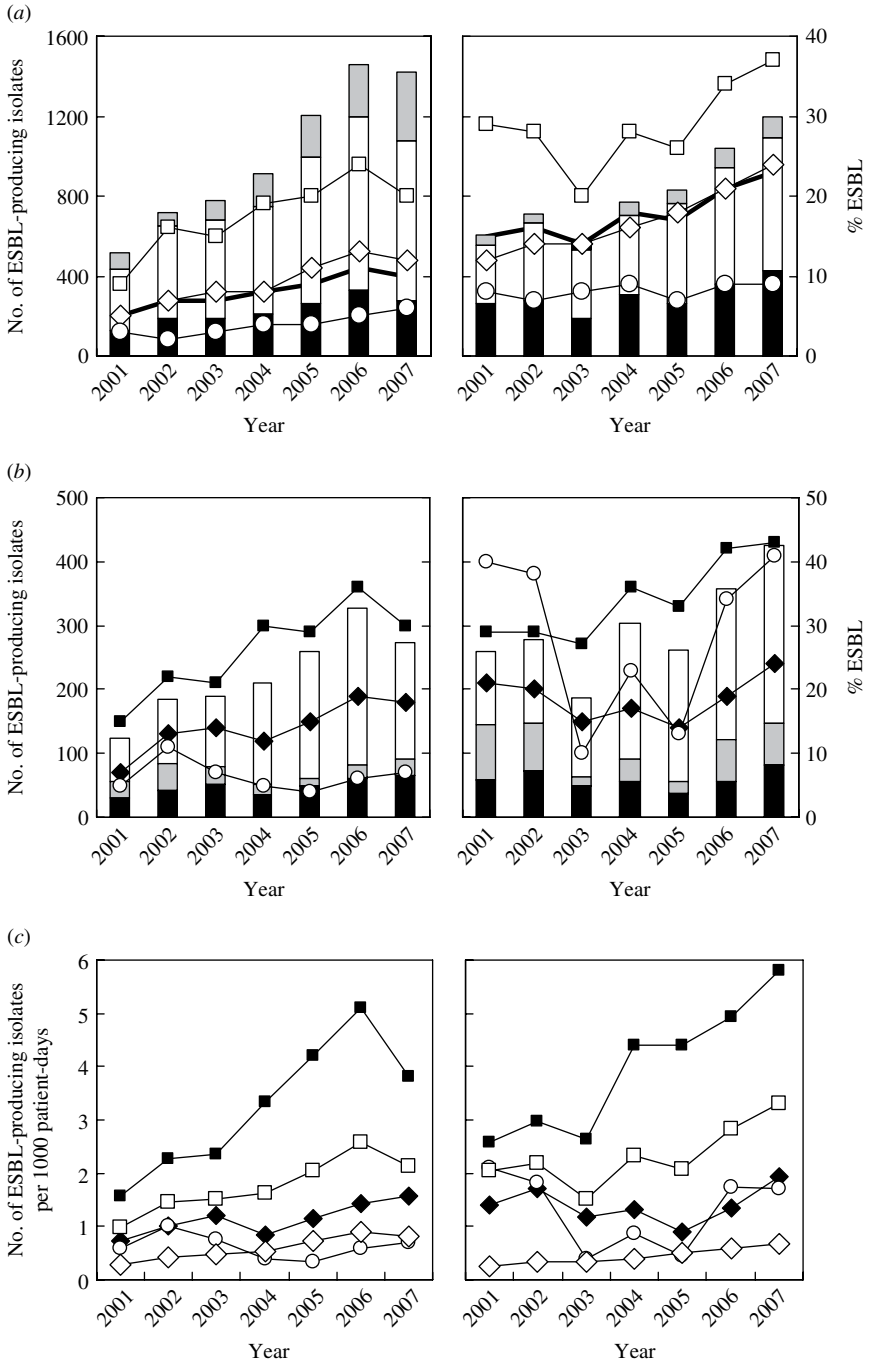

Fig. 1. Secular trends for the annual number (histograms) and prevalence/infection density (lines) of extended-spectrum β-lactamase (ESBL)-producing *E. coli* (left panels) and *K. pneumoniae* (right panels) isolated between 2001 and 2007 at Chang Gung Memorial Hospital. Panel (*a*) demonstrates the variation observed in isolates derived from intensive care units (ICUs) (–□– and black histograms), inpatient department (–◇– and white histograms), and outpatient department (–○– and grey histograms). Panel (*b*) demonstrates the variation observed in isolates derived from medical ICUs (–■– and white histograms), surgical ICUs (–◆– and black histograms), and paediatric ICUs (–○– and grey histograms). Panel (*c*) demonstrates the infection density per 1000 patient-days in isolates derived from inpatient department (–◇–) and ICUs (–□–), including medical ICUs (–■–), surgical ICUs (–◆–), and paediatric ICUs (–○–).

second and third study periods as well as the reverse trends of CTX-M-3- and CTX-M-15-producing isolates was also found in *K. pneumoniae* (Fig. 2*b*). The reduction of CTX-M-3-producing *E. coli* isolates was most significant in PICUs and SICUs where none of such isolates were recovered during the second and

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Table 1. *Distribution of various extended-spectrum β-lactamase* (*ESBL*)*-producing isolates of* E. coli *and* K. pneumoniae *in intensive care units* (*ICUs*)

		ESBL types, no. (%) of isolates																					
		SHV										CTX-M										SHV/CTX-M*		
All ICUs	No. tested	2	2a	5	12		26	27	28	31	61	3		9	13	14		15		24	27	S12/M3	S12/M14	Others
	568	7	9	43	146	(25·7)	1	1	1	1	1	147	(25·9)	12	3	195	(34·3)	55	(9·7)	6	1	15	18	28
E. coli																								
Medical ICUs	163	0	0	6	31	(19·0)	0	0	0	0	0	38	(23·3)	6	2	77	(47·2)	18	(11·0)	2	1	2	7	8
Paediatric ICUs	48	0	0	1	11	(22·9)	0	0	0	0	0	19	(39·6)	1	1	12	(25·0)	5	(10·4)	0	0	2	0	0
Surgical ICUs	62	0	1	2	12	(19·4)	0	0	0	0	0	13	(21·0)	4	0	24	(38·7)	8	(12·9)	1	0	1	2	1
Total	273	0	1	9	54	(19·8)	0	0	0	0	0	70	(25·6)	11	3	113	(41·4)	31	(11·3)	3	1	5	9	9
K. pneumoniae																								
Medical ICUs	187	4	3	20	66	(35·3)	1	1	0	0	1	37	(19·8)	1	0	58	(31·0)	18	(9·6)	3	0	6	8	12
Paediatric ICUs	43	2	1	9	5	(11·6)	0	0	0	0	0	22	(51·2)	0	0	1		6	(14·0)	0	0	1	0	2
Surgical ICUs	65	1	4	5	21	(32·3)	0	0	1	1	0	18	(27·7)	0	0	23	(35·4)	0		0	0	3	1	5
Total	295	7	8	34	92	(31·2)	1	1	1	1	1	77	(26·1)	1	0	82	(27·8)	24	(8·1)	3	0	10	9	19

S, SHV ESBLs; M, CTX-M ESBLs.

* The numbers of isolates producing dual ESBLs in the area are indicated. Other sporadic combinations are S2/M3, S2/M14, S2a/M3, S2a/M14, S2a/M15, S5/M3, S5/M14, S12/M13, S12/M15, S26/M14, S27/M14, S28/M3, S31/M14, S61/M15, M3/M14, and M14/M15.

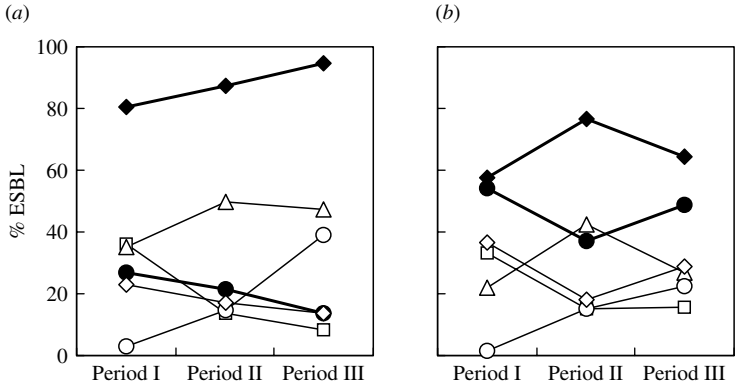

Fig. 2. Prevalence of various extended-spectrum β-lactamases (ESBLs) (CTX-M, –◆–; CTX-M-3, –□–; CTX-M-14, –△–; CTX-M-15, –○–; SHV, –●–; SHV-12, –◇–) in clinical isolates of *E. coli* (*a*) and *K. pneumoniae* (*b*) obtained in period I (August 2002–December 2003), period II (January 2005–June 2005), and period III (October 2007–December 2007).

third study periods. Compared to the other ESBL populations, the proportion of respiratory isolates was higher (32·7%; *E. coli*, 35·7%; *K. pneumoniae*, 29·6%), and lower or similar for urinary CTX-M-15-producing isolates (47·3%; *E. coli*, 46·4%; *K. pneumoniae*, 48·1%). Co-production of two different ESBLs by a single isolate was not unusual (*E. coli*, 8·4%; *K. pneumoniae*, 12·9%). Other than five isolates that produced two CTX-M-type enzymes, the others all produced various combinations of SHV/CTX-M ESBLs, with SHV-12/CTX-M-14 and SHV-12/CTX-M-3 being the most frequent combinations (Table 1).

Genotypes

Both species were highly diverse with 178 and 125 genotypes being defined for ESBL-producing isolates of *E. coli* and *K. pneumoniae*, respectively (Table 2). Multiple isolates were found only in genotypes 1–36 in *E. coli* and in genotypes 1–29 in *K. pneumoniae*. In *E. coli*, the largest cluster, genotype 1, was also the most predominant in MICU isolates, but it was sporadic in other ICUs. Similarly, isolates of other prevalent genotypes (2, 3, 4) were distributed in various ICUs, but genotype 5 was restricted to PICU isolates. Less genotype diversity was evident in *K. pneumoniae* isolates since >50% of the total fell into only ten genotypes. Of these, genotype 1 was the largest cluster and was also the most prevalent in all three ICUs. By contrast, the majority of genotype 2 isolates, the second largest group, were found in MICUs. For both species, isolates belonging to genotypes 1 and 2 could be found throughout the study periods.

Correlation between ESBL types and genotypes

Genotypes 1 and 2 isolates of *E. coli* were significantly associated with the production of CTX-M-14 in MICU isolates, as 18/21 isolates were recovered from MICUs over the three study periods ($P<0{\cdot}0005$). However, other significant correlations were revealed between SHV-5-producing isolates and genotype 1 ($P<0{\cdot}001$), and between CTX-M-3-producing isolates and genotype 5 ($P<0{\cdot}001$) (Table 2). The five genotype 5/CTX-M-3-producing isolates were all recovered from PICUs within 3 months during the first study period, while the other five genotype 1/SHV-5-producing isolates were all recovered from MICUs during the first ($n=2$) and second ($n=3$) study periods.

Significant correlations of genotypes and ESBLs were also found between isolates of *K. pneumoniae*: CTX-M-15 and genotype 2 ($P<0{\cdot}000001$), and SHV-12 and genotype 3 ($P<0{\cdot}0001$) (Table 2). The 12 genotype 3/SHV-12-producing isolates were all recovered during the first study period from MICUs ($n=6$) and SICUs ($n=6$) whereas the 11 genotype 2/CTX-M-15 producers were isolated from patients in MICUs during the second ($n=5$) and third ($n=6$) study periods. There were some differences between the distribution of isolates producing different ESBLs in the ICUs: CTX-M-14-producing isolates were found only in MICUs and SICUs throughout the study periods ($P<0{\cdot}001$), 14/15 CTX-M-3-producing isolates were recovered in the first study period and seven of them were from PICUs, and likewise five of the six CTX-M-15-producing isolates were found in PICUs during the second ($n=4$) and third ($n=1$) study periods ($P<0{\cdot}005$). Furthermore, five genotype

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Table 2. *Distribution of various genotypes in the extended-spectrum β-lactamase (ESBL)-producing isolates of* E. coli *and* K. pneumoniae *in different intensive care units (ICUs)*

Genotype	No. (%) tested		Medical ICUs		Paediatric ICUs		Surgical ICUs		ESBL types, no. (%) of isolates									
									SHV-5		SHV-12		CTX-M-3		CTX-M-14		CTX-M-15		Others
E. coli																			
1	26	(9·5)	20	(12·3)	4		2		5	(55·6)	8	(14·8)	2		13	(11·5)	0		0
2	11	(4·0)	8		2		1		0		5	(9·3)	1		8	(7·1)	1		0
3	8		6		0		2		0		4	(7·4)	3		3		0		0
4	5		2		0		3		0		2		1		2		0		0
5	5		0		5	(10·4)	0		0		0		5	(7·2)	0		0		0
6–178	218		127		37		54		4	(44·4)	35	(64·8)	58	(82·9)	87	(77·0)	30	(96·8)	19
All	273		163		48		62		9		54		70		113		31		19
K. pneumoniae																			
1	69	(23·4)	37	(19·8)	14	(32·6)	18	(27·7)	2		22	(23·9)	15	(19·5)	28	(34·1)	6	(25·0)	4
2	27	(9·2)	26	(13·9)	0		1		3		8	(8·7)	1		10	(12·2)	11	(45·8)	0
3	15	(5·1)	8		0		7		2		12	(13·0)	1		1		0		0
4	10		7		0		3		2		5	(5·4)	2		1		1		0
5	10		4		5		1		2		3		6	(7·8)	0		0		1
6–125	164		105		24		35		23	(67·6)	42	(45·7)	52	(67·5)	42	(51·2)	6	(25·0)	19
All	295		187		43		65		34		92		77		82		24		24

2 isolates recovered in MICUs within 7 months during the first study period were found to produce SHV-12 and CTX-M-14 concomitantly.

DISCUSSION

The data reported herein are the most up-to-date as well as the largest series of ESBL prevalence studies in Taiwan [2, 6, 7, 16]. In contrast to our earlier findings from this hospital [6], we have demonstrated a significant and sustained increase and substantial variations in ESBL production in *E. coli* and *K. pneumoniae* isolates. A report from eight countries in the Asia-Pacific region revealed that, during 1998–2002, the prevalence rates of ESBL-producing isolates varied from 0·5% to 24·5% (overall 5·9%) in *E. coli* and 3·7% to 35·6% (overall 17·3%) in *K. pneumoniae* [2]. Another report from the region focusing on intra-abdominal infections indicated that the prevalence of ESBL-producing isolates in *E. coli* and *Klebsiella* spp. was 19·6% and 22·9%, respectively, in 2004 [17]. They also showed that, although only 1 year apart, the ESBL rates appeared to increase slightly in 2004 [18]. Another report from Korea further demonstrated ESBL production in 10·2% of *E. coli* and 22·4% of *K. pneumoniae* isolates in 2005 [19]. Considered together these data indicate a generally growing trend of ESBL-producing population in the Asia/Pacific region. However, due to the difference in the participating countries or institutions in different studies, direct comparison of rates may not be feasible. In contrast, through the current longitudinal study, we have been able to demonstrate that the prevalence rates of ESBL producers in Taiwan were relatively similar to those of the neighbouring countries reported previously during the corresponding years, and that the prevalence of the ESBL producers indeed has continuously grown in the past few years.

A recent report from ICUs in Taiwan indicated that in 2005, the prevalence of ESBL production in *E. coli* and *K. pneumoniae* was 14% and 26%, respectively [16]. Our findings for ICUs during the same period showed a slightly higher rate in *E. coli* but similar in *K. pneumoniae*. As a result, the longitudinal surveillance reported here suggests that such a significant increase in the ESBL-producing bacteria may be not merely a local observation but may also be reflective of the Asia-Pacific region. Furthermore, the current study also clearly shows that the significant increase of ESBL-producing isolates was more likely observed in the hospital (ICUs and IPD) than in the community (OPD). It is known that the heavy use of antibiotics in hospitals may more easily induce the occurrence of resistant bacteria [20]. Hence, more prudent use of antimicrobial agents may be required to curtail the continuous increase of ESBL-producing populations.

Some ESBL types, such as TEM-, VEB-, and CTX-M-types other than CTX-M-3-like and CTX-M-14-like ESBLs, were only identified with low prevalence in Asian countries, including Taiwan [21]. We noted a similar situation in the first sampling period of this study, and as a consequence these rare ESBLs were not further investigated in the following two periods. In contrast, previous studies indicated that in Taiwan, the most prevalent ESBL types were SHV-5, SHV-12, CTX-M-3, and CTX-M-14 [7]. Compared to our earlier study [6], there has been a marked expansion (from 5 to 16 types) in the number of ESBL types circulating in the country. A significant and continuous increase in the proportion of CTX-M-producing isolates was clearly shown in *E. coli* from 69·2% previously [6] to 94·4% in the third period of this study; the figures for *K. pneumoniae* fluctuated between 58% and 77% in both studies [6]. On the other hand, a significant decline of SHV-producing isolates was also clear in *E. coli* from 35·9% previously [6] to 13·9% in our study, and for *K. pneumoniae* the proportion decreased from 60·8% previously [6] to between 37% and 54% in our study. Our results are consistent with those reported from European countries where the predominant ESBL types have changed dramatically from SHV and TEM types in the 1990s to CTX-M types at present [22]. Only in North America do SHV and TEM ESBLs still predominate [23].

A recent study from Thailand reported the almost universal prevalence (>99%) of $bla_{\text{CTX-M}}$ in ESBL-producing *E. coli* and *K. pneumoniae* [24]. Dissemination of the $bla_{\text{CTX-M}}$ genes was postulated to be probably due to the potential mobilization ability of some insertion sequence (IS) elements, especially IS*Ecp1*, which was present in the upstream region of $bla_{\text{CTX-M}}$ in most isolates [24]. Moreover, the 3′-end of the IS*Ecp1* insertion sequences was suggested to probably provide intact -35 and -10 promoter regions for the expression of the CTX-M enzymes [24–26]. The insertion of IS*Ecp1* upstream of the $bla_{\text{CTX-M}}$ genes has become a common phenomenon and has been reported frequently in many countries or institutions [27–30], including our hospital [31].

Investigation of recent isolates has revealed the widespread existence of IS*Ecp1* in the CTX-M-producing strains (L.-H. Su, unpublished data). Accordingly, before any strategies are implemented to control the increase, the occurrence in the near future of the high prevalence or endemicity of such resistant populations as observed in Thailand may be expected in Taiwan.

The continuous increase of CTX-M ESBLs was mostly found in the increase of isolates that produced CTX-M-14 between the first two periods of the current study. However, during the same period, a dramatic reverse change was also evident between the proportions of isolates that produced CTX-M-3 (decreasing) and CTX-M-15 (increasing). Although isolates that produced CTX-M-3 and CTX-M-14 both reduced in the third period, the increase of CTX-M-15-producing isolates continued and contributed to the overall increase in the proportion of CTX-M-producing isolates. This finding is in accord with reports from many European countries [22, 32] as well as from North America [33, 34] and Australia [30] where a rapid spread of CTX-M-15-producing isolates has been observed. In India, where CTX-M-15 was first reported in 2001 [25], the enzyme has now become the most predominant ESBL [35].

CTX-M-15-producing isolates in Taiwan were first reported in 2004 [36]. The gene encoding region of CTX-M-15 is only a single nucleotide substitution difference from CTX-M-3, leading to the higher ceftazidime-hydrolysing activity [25]. This modified activity appears to have a great impact on the superior prevalence of CTX-M-15 over CTX-M-3 [36]. However, the mechanisms responsible for its significant increase in our study appear to be different between the two bacterial species. Previous reports from Canada [37] and UK [26] indicated that some epidemic CTX-M-15-producing strains were responsible for the wide dissemination of such resistance in *E. coli*. This contrasts with our finding of diverse genotypes in CTX-M-15-producing *E. coli* isolates, suggesting that the prevalence of such resistant isolates may be due to the dissemination of resistance plasmids or other mobile elements carrying the $bla_{\text{CTX-M-15}}$ gene. Nevertheless, it is only recently that a pandemic urovirulent *E. coli* clone O25-ST131, as determined by serotyping and multilocus sequence typing, has been detected in many European countries as well as Canada, India, Japan, Korea, and Lebanon [32, 38–40]. Whether or not our CTX-M-15-producing *E. coli* isolates also belong to, or will be influenced by, this pandemic clone warrants a further investigation. On the other hand, over 70% of the CTX-M-15-producing *K. pneumoniae* isolates here were found in isolates of genotype groups 1 and 2, indicating that other than transmission of $bla_{\text{CTX-M-15}}$-containing plasmids, clonal spread may also play an important role in the further expansion of the $bla_{\text{CTX-M-15}}$ gene in *K. pneumoniae*. There was no apparent difference in the infection types associated with the two genotypes compared to other CTX-M-15-producing isolates.

Three rare ESBL types, SHV-31, SHV-61, and CTX-M-27, found here have not been reported previously in Taiwan. SHV-31 differs from SHV-1 by two amino-acid substitutions and has only been reported in an isolate of *K. pneumoniae* in The Netherlands [41]. SHV-61 was also only found in Portugal before this survey (M. M. M. Canica and N. R. Mendonca, GenBank AJ866284, http://www.lahey.org). On the other hand, CTX-M-27 is very similar (one amino- acid difference) to the prevalent CTX-M-14, and although it was not found previously in Taiwan, recent reports have indicated its widespread occurrence in many countries, including Thailand [24], Spain [42], China [43], Canada [37], and Egypt [44]. Whether or not isolates that produced this ESBL will propagate to be as prevalent as those observed nowadays in CTX-M-15 producers merits careful monitoring.

In conclusion, this large-scale, longitudinal surveillance for ESBL-producing isolates provides an excellent model to track changes of prevalence rates, ESBL types and epidemic strains over a 7-year period. The frequent use of β-lactam antibiotics may promote the growth in the prevalence of ESBL-producing isolates as well as the variation of ESBL types either from mutations of existent ESBL genes or transmissions of resistance genes or plasmids from other bacteria. The presence of some prevalent strains in both species also suggests that cross-transmission of these resistant pathogens may have occurred in patients. Therefore to effectively control the continuous increase of these resistant bacteria, not only should antibiotic prescribing be more strictly supervised, but also the compliance of infection control measures needs to be reinforced. The burst of CTX-M-15-producing isolates should be more cautiously monitored in Taiwan for its possible widespread dissemination as has already been observed in other countries.

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DECLARATION OF INTEREST

None.

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