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Review Article

Extended-spectrum beta-lactamases in Taiwan: epidemiology, detection, treatment and infection control

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Extended-spectrum beta-lactamases (ESBLs) efficiently hydrolyze extended-spectrum beta-lactams such as cefotaxime, ceftriaxone, ceftazidime, and aztreonam. ESBLs are most often plasmid-mediated. In Taiwan, the prevalence of ESBLs in bacteria has risen, ranging from 8.5 to 29.8% in *Klebsiella pneumoniae* and 1.5 to 16.7% in *Escherichia coli* isolates. The most prevalent types of ESBLs are SHV-5, SHV-12, CTX-M-3, and CTX-M-14 in isolates of *K. pneumoniae* and *E. coli*, with differences between institutions. SHV-12 and CTX-M-3 have been reported as the most common ESBLs in isolates of *Enterobacter cloacae* and *Serratia marcescens*, respectively. Molecular epidemiology studies suggest that the ESBL-encoding genes have been disseminated either by proliferation of epidemic strains or by transfer of plasmids carrying the resistance traits. The current ESBL screen guidelines of the Clinical and Laboratory Standards Institute (formerly National Committee for Clinical Laboratory Standards) are issued for *E. coli*, *Klebsiella* spp., and *Proteus mirabilis.* Owing to the lack of standard methods, it remains difficult to assure the presence of ESBL in an isolate co-harboring an AmpC beta-lactamase, particularly in cases where the latter is produced in larger amounts than the former. Empirical therapy with piperacillin-tazobactam to replace third-generation cephalosporins may help to reduce the occurrence of ESBLs in an institution with a high prevalence of ESBL producers. Carbapenems remain the drugs of choice for serious infections caused by ESBL-producing organisms. To retard the selection for carbapenem-resistant bacteria, 7-alpha-methoxy beta-lactams or fourth-generation cephalosporins can be therapeutic alternatives for mild-to-moderate infections provided that the pharmacokinetic and pharmacodynamic target can be easily achieved.

Key words: AmpC beta-lactamase, beta-lactam resistance, beta-lactamases, *Enterobacteriaceae* infections, prevalence, review

Introduction

According to their chemical structure, beta (β)-lactam antibiotics can be divided into different groups which include penicillins, cephalosporins, carbapenems, and monobactams. The antibiotics have the β-lactam ring in common, but apart from the monobactams, differences between the groups are due to the secondary ring structures fused to the β-lactam ring. β-Lactamases are bacterial enzymes that are encoded by chromosomal or by plasmid-borne genes, the enzymes protecting the microorganisms against the lethal effects of β-lactam antibiotics by hydrolyzing the β-lactam ring, thus rendering the drugs inert [1]. Bacterial enzymes are the most important resistance mechanism to β-lactam antibiotics [2]. Based on their amino acid sequences, β-lactamases can be classified into 4 different molecular groups, the Ambler classes A, B, C, and D [3]. Class A, C (AmpC), and D β-lactamases possess an active-site serine residue that is essential for the inactivation of β-lactam drugs, whereas class B β-lactamases are metallo-enzymes requiring zinc for their activity. Class A β-lactamases, which are predominantly represented

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by the plasmid-encoded TEM and SHV families, are the most common molecular group of β-lactamases produced by the *Enterobacteriaceae*, such as *Escherichia coli* and *Klebsiella pneumoniae*.

The chromosomally encoded AmpC β-lactamases are naturally occurring in many members of *Enterobacteriaceae*, such as *Enterobacter cloacae*, *Citrobacter freundii*, *Morganella morganii*, and *Serratia marcescens* [4]. An *ampC* gene is present in the genome of both *E. coli* and *Shigella* spp., but owing to the lack of the *ampR* regulator gene, the *ampC* gene is typically not transcribed. The genomes of *K. pneumoniae* and *Salmonella* spp. seem to be deficient of an *ampC* gene [4,5].

In the early to mid-1980s, extended-spectrum cephalosporins, such as cefotaxime, ceftriaxone, and ceftazidime, were approved for clinical use and shortly after their release, bacteria resistant to these cephalosporins began to appear. The first identified class A extended-spectrum β-lactamase (ESBL), SHV-2, conferred resistance to cefotaxime, and the enzyme was found in isolates of *K. pneumoniae*, *S. marcescens*, and *Klebsiella ozaenae* recovered in Germany [6,7]. The extended-spectrum cephalosporins are not hydrolyzed by the broad-spectrum β-lactamases TEM-1, TEM-2 and SHV-1, which are the progenitors of the TEM- and SHV-type ESBLs [3,4].

According to the Bush-Jacoby-Medeiros classification scheme, a class A ESBL is defined as a β-lactamase hydrolyzing cefotaxime, ceftazidime, or aztreonam at a rate >10% that for benzylpenicillin [3], and like many other class A β-lactamases, ESBLs are also inactivated by the β-lactamase inhibitor, clavulanate [3]. The class A ESBLs do not hydrolyze 7-alpha-methoxy β-lactams including cephamycins such as cefoxitin and cefotetan, and oxacephems such as moxalactam and flomoxef, to detectable extents. Carbapenems, such as imipenem, meropenem, and ertapenem are also stable to ESBLs. As AmpC β-lactamases also hydrolyze not only extended-spectrum β-lactams, but also cephamycins and oxacephems, the latter property may be used to distinguish phenotypically between class A ESBLs and AmpC β-lactamases [4]. Another difference between the class A ESBL and the AmpC enzyme is the inhibition of the former by clavulanate [3].

Most of the genes encoding class A ESBLs are plasmid-borne, which may facilitate the intra- or interspecies exchange of the resistance genes, resulting in acquisition of new resistance phenotypes in the recipients. Since the identification of the first class A ESBL, a number of new TEM and SHV ESBL variants have evolved [8], and entirely new families of class A ESBLs have emerged, such as the CTX-M-ases, the GES/IBC enzymes, the PER enzymes and the VEB enzymes [9-12].

Chromosomal *ampC* genes have recently been recruited by plasmids [13,14], and these plasmid-borne *ampC* genes predominantly have been transferred to bacterial species that naturally lack a chromosomal AmpC β-lactamase, such as *K. pneumoniae* and *Salmonella* spp., thus providing these species with new resistance traits. These plasmid-mediated AmpC β-lactamases are closely related to chromosomal AmpC β-lactamases of either *Enterobacter asburiae* (ACT-1 and MIR-1), *C. freundii* (CMY-2 and LAT-1), or *M. morganii* (DHA-1 and DHA-2).

The plasmid-borne class A ESBLs and AmpC β-lactamases have now spread to many important clinical pathogens, including *K. pneumoniae*, *E. coli*, *Salmonella* spp., *Proteus mirabilis*, and *Pseudomonas aeruginosa*, and isolates harboring these enzymes have been collected worldwide [8-15]. In Taiwan, the incidence of bacteria resistant to extended-spectrum β-lactam antibiotics has risen, a development which has been shown to be related to an increased usage of these β-lactam drugs [16]. Numerous reports of ESBL identification in Taiwan have been published. This review will deal briefly with the epidemiology and characterization of ESBLs produced by enterobacterial isolates collected in Taiwan, while devoting more emphasis to the detection methods of ESBL-producing isolates. Treatment and antibiotic strategies to prevent and control infections caused by ESBL-producing microorganisms are also discussed.

Epidemiology in Taiwan: Prevalence and Types of ESBLs Reported

Prevalence

The prevalence of ESBL producers among enterobacterial isolates differs between institutions and varies in geographic regions, as compiled in Table 1 [17-28]. Two large surveys from a medical center in southern Taiwan showed that 8.5% and 1.5%, respectively, of the collected *K. pneumoniae* [20] and *E. coli* [21] isolates produced an ESBL. The incidence of ESBLs in enterobacterial isolates collected from three medical centers in northern Taiwan was 13.5% in *K. pneumoniae* and 5.6% in *E. coli* isolates [25]. In a large medical center in north Taiwan, the occurrence of ESBLs in *K. pneumoniae* increased from 3.4% in 1993 to 10.3%

Table 1. Prevalence data for extended-spectrum beta-lactamases (ESBLs) producers among enterobacterial isolates in Taiwan

Reference	Isolate(s)	No.	Prevalence (%)	MIC method	Institution	Geography
Liu et al [17]	*Klebsiella pneumoniae*	104	29.8	Agar dilution + Etest ESBL screen	District hospital	Central
Jan et al [18]	*K. pneumoniae*	NA	3.4-10.3	None (disk diffusion)	Medical center	North
Biedenbach et al [19]	*Escherichia coli*	60	16.7	Etest	Six medical centers	North, central and south
	K. pneumoniae	60	21.7			
Yan et al [20]	*K. pneumoniae*	234	8.5	Etest	Medical center	South
Yan et al [21]	*E. coli*	1210	1.5	Agar dilution	Medical center	South
Hsueh et al [22]	*E. coli*	177	11.9	Agar dilution	Five medical centers	North and south
	K. pneumoniae	124	11.3			
Lin et al [23]	*K. pneumoniae*	422	14	None (disk diffusion)	District hospital	North
Su et al [24]	Non-typhoid *Salmonella*	3592	0.2	Broth microdilution	Medical center	North
Hirakata et al [25]	*E. coli*	319	5.6	Broth microdilution	Three medical centers	North
	K. pneumoniae	222	13.5			
	Klebsiella oxytoca	17	23.5			
	Citrobacter koseri	5	20			
	Salmonella spp.	104	0			
Wu et al [26]	*Proteus mirabilis*	111	30.6	Etest ESBL screen + Etest	3 district hospitals	Central
Yu et al [27]	*Enterobacter cloacae*	110	15	Agar dilution	District hospital	Central
Cheng et al [28]	*Serratia marcescens*	123	12	Agar dilution	Medical center	Central

Abbreviations: MIC = minimal inhibitory concentration; NA = not available

in 1997 [18]. Factors that potentially may affect the frequency of ESBLs include methods of detection for ESBL (Table 1) and the proportion of nosocomial strains or epidemic clones in the collected bacterial samples [29-32].

ESBL types

The most prevalent types of ESBLs are SHV-5, SHV-12, CTX-M-3, and CTX-M-14, all of which have been identified in isolates of *K. pneumoniae* and *E. coli*. The different types of ESBLs found in enteric bacteria collected in Taiwan are summarized in Table 2 [17, 20,21,24,26-28,33-48]. However, the predominant ESBL type varies from species to species and from institution to institution. In one hospital, the SHV-5 β-lactamase was reported as the most common ESBL in *K. pneumoniae* [17], while SHV-12 or CTX-M-3 were most common in *K. pneumoniae* isolates from other hospitals [20,37]. Other studies have shown that CTX-M-3 was the most common ESBL in *E. coli* [21,37, 40], *S. marcescens* [28,47], and non-typhoid *Salmonella* [24] recovered from other hospitals. CTX-M-14 was the predominant ESBL in *P. mirabilis* according to a recent study [26]. In *E. cloacae*, SHV-12 has consistently been reported as the most common ESBL [27,37,40,48]. The high occurrence of the broad-spectrum SHV-1 and SHV-11 in *K. pneumoniae* isolates that also harbour SHV-2, SHV-5 or SHV-12 suggests that the latter SHV ESBLs may have evolved from SHV-1 or SHV-11 through stepwise non-synonymous mutations [35]. TEM-type ESBLs have rarely been reported in isolates from Taiwan, and only TEM-10 has so far been identified [21]. Two *K. pneumoniae* isolates possessed TEM-1, SHV-1, SHV-5 and a new CTX-M-1 variant, the CTX-M-15 β-lactamase, and in a transconjugant, the latter enzyme conferred resistance to both cefotaxime and ceftazidime [38]. This resistance phenotype differs from that conferred by typical CTX-M-ases, which bring about resistance to cefotaxime and ceftriaxone, but not to ceftazidime [9,10]. A novel SHV-variant, SHV-57, identified in *E. coli* conferred a unique resistance profile to the isolate, which was resistant to ceftazidime, but susceptible to cefazolin [43]. Other rarely identified ESBLs, such as SHV-2 [17], CTX-M-9 [27], CTX-M-24 [39], and non-ESBL-β-lactamases such as SHV-25, SHV-26 [35], SHV-33 [40] and TEM-31 [37], may suggest that the β-lactamases in many members of *Enterobacteriaceae* have evolved to a number of different variants.

The co-existence of different ESBL variants of SHV with a CTX-M-type, AmpC (DHA or CMY) or metallo-β-lactamase (IMP) in a single strain has been described for *K. pneumoniae* [20,33,34,40,49-51] and *E. coli* [21]. The most frequent plasmid-mediated

Table 2. Types of extended-spectrum beta-lactamases (ESBLs) identified in Taiwanese *Enterobacteriaceae*

Isolates/beta-lactamases (no.)	Source(s)	Reference
Klebsiella pneumoniae		
SHV-5 (22), SHV-2 (2)	Sputum, urine, blood, pus	Liu et al [17]
SHV-12 (11), SHV-5 (4). Non-ESBL: CMY-8 (3)	Not available	Yan et al [20]
SHV-12 (1). Non-ESBL: TEM-1 (1), IMP-8 (1)	Catheter tip	Yan et al [33]
SHV-12 (36). Non-ESBL: IMP-8 (40), TEM-1 (40), SHV-11 (4)	Wound, sputum, blood, catheter tip, urine	Yan et al [34]
SHV-12 (31), SHV-5 (8), SHV-2 (2). Non-ESBL: SHV-1 (37), SHV-11 (33), SHV-25 (1), SHV-26 (1)	Blood	Chang et al [35]
CTX-M-3 (28), CTX-M-14 (22)	Not available	Yu et al [36]
CTX-M-3 (36), SHV-12 (6), SHV-2 (4), SHV-2a (3), SHV-5 (1), CTX-M-14 (1); Non-ESBL: TEM-31 (2), OXY-1 (1)	Not available	Wu et al [37]
CTX-M-15 (2)	Urine, blood	Yu et al [38]
CTX-M-24 (2)	Blood, stool	Lee et al [39]
SHV-12 (24), CTX-M-3 (19), SHV-5 (10), CTX-M-14 (1). Non-ESBL: TEM-1 (11), SHV-11 (5), SHV-1 (4), SHV-33 (2), LEN-1 (1), OXY-1a (1)	Blood, urine, sputum, others	Ma et al [40]
Escherichia coli		
SHV-5 (4). Non-ESBL: TEM-1 (4)	Blood	Siu et al [41]
CTX-M-3 (10), SHV-12 (2), SHV-5 (1), TEM-10 (1). Non-ESBL: TEM-1 (35), CMY-2 (5)	Blood, urine, sputum, others	Yan et al [21]
CTX-M-14 (4)	Not available	Ma et al [42]
CTX-M-3 (13), SHV-12 (3)	Not available	Wu et al [37]
CTX-M-3 (15), SHV-12 (7), CTX-M-14 (4). Non-ESBL: TEM-1 (26), SHV-1 (1)	Blood, urine, sputum, others	Ma et al [40]
SHV-57 (1)	Urine	Ma et al [43]
Non-typhoid *Salmonella*		
CTX-M-3 (1)	Necrotic tissue	Su et al [44]
CTX-M-3 (6), SHV-2a (1), SHV-12 (1). Non-ESBL: CMY-2 (14)	Blood, stool, wound	Su et al [24]
CTX-M-14 (1). Non-ESBL: CMY-2 (7)	Stool	Li et al [45]
Serratia marcescens		
CTX-M-3 (4). Non-ESBL: TEM-1 (4)	Blood	Yu et al [46]
CTX-M-3 (22), SHV-5 (1)	Pus, urine, blood, sputum, others	Wu et al [47]
CTX-M-3 (15)	Urine, blood, sputum, others	Cheng et al [28]
Enterobacter cloacae		
SHV-12 (26), CTX-M-3 (3)	Not available	Wu et al [37]
SHV-12 (25)	Blood	Liu et al [48]
SHV-12 (27), CTX-M-3 (3). Non-ESBL: TEM-1 (24)	Blood, urine, sputum, others	Ma et al [40]
SHV-12 (17), CTX-M-9 (2), CTX-M-3 (1)	Pus, sputum, blood, Catheter tip	Yu et al [27]
Proteus mirabilis		
CTX-M-14 (33), CTX-M-3 (6). Non-ESBL: TEM-1 (25), DHA-1 (1), CMY-2 (1)	Urine, sputum, pus	Wu et al [26]

AmpC β-lactamases in Taiwan are DHA-1 in *K. pneumoniae* [49,50] and CMY-2 in *E. coli* [21], *K. pneumoniae* [50], and non-typhoid *Salmonella* [24,45]. The novel DHA-3, a new variant of DHA-1, was identified in an SHV-5-producing *K. pneumoniae* collected in central Taiwan [51]. Co-existence of SHV-12 and IMP-8 in *K. pneumoniae* isolates has been found in a medical center in southern Taiwan [33,34]. In a recent study, only 2 isolates were found to harbour an additional plasmid-mediated DHA-1 or CMY-2 β-lactamase among 34 CTX-M-producing *P. mirabilis* isolates [26].

Molecular epidemiology

The increasing prevalence of ESBL producers among enterobacterial isolates may be caused by the dissemination of the resistance traits either by proliferation of epidemic strains or by transfer of the plasmids carrying the resistance genes. The first pilot study of the epidemiology of ESBL-producing *K. pneumoniae* isolates recovered from central Taiwan indicated that the plasmid-borne bla_{SHV-5} gene most likely has disseminated by transfer of the carrying plasmid rather than by spread of an epidemic clone [17]. Likewise, all CTX-M-3-producing *E. coli* isolates

collected from a medical center in southern Taiwan were found to be genetically unrelated [21]. Both pilot studies used pulsed-field gel electrophoresis (PFGE) to compare the genetic relationship between the isolates. A large investigation of *K. pneumoniae* isolates from ten hospitals in northern Taiwan could not conclusively document that a major ribotype of the SHV-producers had disseminated in any of the hospitals [35]. In a recent nationwide study of ESBL-producing *E. coli*, *K. pneumoniae* and *E. cloacae* from 22 hospitals, 28 and 37 different ribotype profiles were identified among 48 *K. pneumoniae* and 60 *E. coli* isolates, respectively [40]. Some of the studied strains shared the same ribotype, which may indicate that clonal spread had occurred among a limited number of the strains. However, it was the view of the authors that the number of similar strains was too small to decide whether any of the investigated strains was epidemic. These reports suggest that plasmids carrying the resistance determinants may have been transferred between these genetically unrelated strains and thus have been responsible for the spread of the resistance traits. The presence of a common highly transmissible plasmid was, however, not documented [17,21,35,40].

Other mobile elements such as integrons, transponsons or insertion sequences have been documented to be involved in the horizontal transmission of the ESBL-encoding genes [9,10]. In Taiwan, so far only a few preliminary studies have been conducted in this field [52]. In contrast, a large number of ESBL-producing *K. pneumoniae* isolates from 24 hospitals in another nationwide surveillance study using automated ribotyping followed by PFGE showed that several major clones were distributed between hospitals and inside hospitals [32]. During a 16-month period, two predominant genotypes of *E. cloacae* producing SHV-12 and one of *K. pneumoniae* producing CTX-M-3 predominately were prevalent within the pediatric intensive care units in a large medical center [37]. In addition, a predominant genotype of SHV-12-producing *E. cloacae* isolates collected from patients with bacteremia was reported from another medical center in northern Taiwan [48]. The genotypes of *E. cloacae* isolates from the two investigations [37,48] were not compared. Furthermore, clonal dissemination of *K. pneumoniae* and *P. mirabilis* strains producing CTX-M-3 and CTX-M-14 was reported [26]. Based on the above reports, some enterobacterial clones harboring SHV- or CTX-M-ESBLs seem to have the capabilities to spread wider than previously thought, which highlights the need to continue the nationwide surveillance for the spread of ESBL producers by use of molecular techniques.

Detection of ESBL Phenotype in Clinical Microbiology Laboratories: Standard Methods and Controversy

Standard methods

The disk diffusion methods for ESBL detection are used by clinical microbiology laboratories in Taiwan as recommended by the Clinical and Laboratory Standards Institute (CLSI), formerly the National Committee for Clinical Laboratory Standards. Currently, CLSI has recommended that more than one of the 5 indicators (cefpodoxime, ceftazidime, cefotaxime, aztreonam, and ceftriaxone) should be used to screen for expression of ESBL in *K. pneumoniae*, *Klebsiella oxytoca*, *E. coli*, and *P. mirabilis* [53]. If the zone diameter of any of the indicators meets the screening criteria (cefpodoxime, ≤17 mm; ceftazidime, ≤22 mm; cefotaxime, ≤27 mm; aztreonam, ≤27 mm; ceftriaxone, ≤25 mm), additional phenotypic tests are mandatory, in order to ascertain the production of an ESBL. The CLSI advocates for the use of cefotaxime or ceftazidime disks with and without clavulanate for phenotypic confirmation of the presence of ESBLs. An increase in zone diameter (≥5 mm) for either cefotaxime or ceftazidime in the presence of clavulanate compared to its zone without clavulanate is interpreted as positive for the presence of an ESBL. According to the CLSI comments for confirmed ESBL-producing strains, a positive test should be reported as resistant for all penicillins, cephalosporins, and aztreonam.

In addition to the disk diffusion method, investigators in research microbiology laboratories in Taiwan may also perform the minimal inhibitory concentration (MIC)-based method to verify an ESBL production (Table 1). The CLSI recommends that if the MIC of any of the indicators meets the screening criteria (i.e., MIC ≥2 μg/mL for cefotaxime, ceftazidime, aztreonam, or ceftriaxone; or MIC ≥8 μg/mL for cefpodoxime), the isolate should undergo a phenotypic corroboration for ESBL production. An 8-fold or larger reduction of the MIC determined by the standard broth dilution method for either cefotaxime or ceftazidime in the presence of clavulanate (4 μg/mL) is defined as a positive result for an ESBL-producing isolate [54].

In a large nationwide survey of 3211 enterobacterial isolates collected from 22 hospitals in Taiwan, 171 strains (*E. coli*, 60; *K. pneumoniae*, 48; and *E. cloacae*, 63) met the CLSI screening criteria (MIC ≥2 μg/mL for either cefotaxime or ceftazidime) for likely ESBL production. Among these 171 isolates, Ma et al found

that a total of 91 isolates (53.2%), including 26 *E. coli* (43.3%), 44 *K. pneumoniae* (91.7%), and 21 *E. cloacae* (33.3%) isolates were positive ESBL producers according to CLSI phenotypic confirmation test using the broth microdilution method [40].

The most common MIC methods used by investigators in Taiwan — the agar dilution method, the Etest and/or Etest ESBL screen (Table 1) — are not recommended by CLSI as methods suitable for verification of ESBL production. However, compared to the CLSI disk diffusion tests and a genotypic detection of ESBL encoding genes, these methods seem reliable in most series (Table 2). The Etest ESBL screen strips (AB Biodisk, Solna, Sweden) are useful for both screening and phenotypic confirmation of ESBL production. However, due to a relatively narrow MIC range (ceftazidime gradient, 0.5-32 μg/mL and cefotaxime gradient, 0.25-16 μg/mL), off-scale MICs may occur for some isolates, which could result in a non-determinable outcome of the test. In these cases, a separate conventional Etest strip containing broad MIC range for only ceftazidime (0.016-256 μg/mL) or cefotaxime (0.016-256 μg/mL) with or without clavulanate may be helpful [29].

False-positive test

Increased expression of the non-ESBL SHV-1 β-lactamase may occasionally result in elevated MICs to some of the extended-spectrum cephalosporins, and these MICs may be reduced by 8-fold or more in the presence of clavulanate. The apparent synergistic effect between these antibiotics and clavulanate may give false-positive confirmatory tests of an ESBL production [55]. Similarly, Wu and co-workers have reported from an outbreak of *K. pneumonaie* that co-existence of TEM-1 and SHV-1 as well as the reduced expression of an outer membrane protein in the same strain may lead to false-positive identification as an ESBL producer [56]. They found that a cefotaxime zone diameter of 26-27 mm increased to 32 mm for a cefotaxime/clavulanate disk, indicating a positive test for the presence of an ESBL. However, attempts to detect and identify an ESBL encoding gene failed.

False-negative test

The co-existence of ESBLs with plasmid-mediated AmpC β-lactamases (DHA or CMY) in strains of *K. pneumoniae* [20,49-51] or *E. coli* [21] has been reported in Taiwan. The AmpC enzymes which are not inhibited by clavulanate may potentially interfere with the inhibitory effect of clavulanate on ESBLs and thus contribute to maintaining elevated MICs to all groups of cephalosporins, provided that the AmpC enzymes are produced in larger amounts. This may result in a false-negative result for ESBL production. The *K. pneumoniae* isolate harboring both SHV-5 and DHA-3 showed MICs of 64/64 μg/mL for cefotaxime/cefotaxime plus clavulanate and 128/128 μg/mL for ceftazidime/ceftazidime plus clavulanate [51]. In a recent survey for ESBL-producing *P. mirabilis* [26], a strain harboring DHA-1 and CTX-M-3 showed positive phenotypic confirmation of ESBL production, whereas another strain carrying CMY-2 and CTX-M-14 gave negative results of ESBL expression. The reason for this discrepancy may be explained by the net effect of each enzyme in terms of elevating the MICs to all groups of cephalosporins. False-negative results are supposed to occur if the AmpC activity is larger than the ESBL activity. All plasmid-borne DHA-1 encoding genes seem to be associated with an adjacent and upstream located *ampR* gene, and the expression of the $bla_{\text{DHA-1}}$ gene is thus inducible [50]. Accordingly, the isolate producing both CTX-M-3 and the inducible DHA-1 should give a false-negative result for ESBL. Nevertheless, if CTX-M-3 is produced in amounts far exceeding those of DHA-1, this may result in a positive result for an ESBL (i.e., a net effect of significant reduction in the MICs of cefotaxime in the presence of clavulanate). A similar linkage to *ampR* has not been found for the $bla_{\text{CMY-2}}$ genes, and the expression of the gene is thus not inducible. However, a stable high-level production may be caused by a high copy number of the plasmid, several copies of the gene on the plasmid, or the presence of a more potent promoter. Therefore, the isolate producing CTX-M-14 and a high-level expression of CMY-2 may also have given a false-negative result for ESBL. In this case, a double-disk diffusion test using a cefepime disk separated by an amoxicillin-clavulanate disk may improve the detection of ESBL, since cefepime is only marginally affected by AmpC, and consequently ESBL production may be disclosed [57]. Although cefepime is not specified as an ESBL indicator in the CLSI recommendations, application of CLSI ESBL disk diffusion or broth dilution confirmatory tests using cefepime with and without clavulanate may be helpful to verify the presence of ESBLs [29].

Controversy

Although the current CLSI ESBL recommendations have only been issued for *E. coli*, *Klebsiella* spp., and

P. mirabilis [53,54], the occurrence of ESBLs in other enterobacteria from Taiwan, including *E. cloacae*, *S. marcescens*, *C. freundii*, and non-typhoid *Salmonella*, has also been reported (Table 2). Similar to the previously mentioned false-negative CLSI ESBL tests for co-produced ESBLs and plasmid-mediated AmpCs in *E. coli* or *K. pneumonaie*, such false-negative tests may also occur for other isolates, such as *E. cloacae* and *S. marcescens*, producing concomitantly both an ESBL and an intrinsically chromosomal AmpC β-lactamase. Due to the lack of standard methods, clinical microbiology laboratories do not routinely screen for ESBLs in genera of *Enterobacteriaceae* other than *E. coli*, *Klebsiella* spp. and *P. mirabilis*, such as *Serratia* and *Enterobacter*, albeit they may be potential carriers of ESBLs. The consequences of neglecting ESBLs in these organisms may leave patients and hospitals at risk for treatment failure and nosocomial outbreaks [37,48]. The fact that no reliable detection methods are available, together with the rare occurrence of ESBLs among the intrinsic AmpC producers in the USA, may explain why the CLSI does not find screening for ESBLs in AmpC-producing *Enterobacteriaceae* to be warranted [58]. In Taiwan, several research laboratories extended the CLSI ESBL standard methods and/or modified the tests [56] to detect ESBLs in *S. marcescens* [28,46,47] and *E. cloacae* [27,37,40]. Although the modified test methods are not totally reliable, because isolates that are false-negative or undeterminable for ESBL production would be overlooked, the occurrence of ESBL-producing *C. freundii* or *E. cloacae* or *S. marcescens* isolates is not unusual (Tables 1 and 2), which should deserve similar attention as *E. coli* or *K. pneumoniae*. These studies underline the need to establish practical guidelines for ESBL screening, confirmation and reporting for chromosomal AmpC producers in Taiwan.

Antimicrobial Susceptibility Testing for ESBL-producing Organisms

The 'susceptibility' in this section refers to the original phenotype before its revision according to the CLSI reporting rules for organisms expressing ESBLs [53, 54]. Based on the CLSI MIC susceptibility breakpoints, a large study of ESBL-producing *K. pneumoniae* showed that 4% of isolates were ceftriaxone-susceptible, 39% ceftazidime-susceptible, 53% cefepime-susceptible, 79% piperacillin-tazobactam-susceptible, and 100% carbapenem (imipenem and meropenem)-susceptible [29]. Seventy seven percent of the isolates were also susceptible to the fluoroquinolone ciprofloxacin. However, only treatment with a cephalosporin against an ESBL-producer with MIC ≤1 μg/mL for the cephalosporin has shown a lower failure rate (27%) than for that with an MIC of 2 to 8 μg/mL (75%) [59]. Among apparently effective cephalosporins (MIC ≤8 μg/mL), MICs were mostly in the range 2 to 8 μg/mL, including ceftriaxone (all of the 4% susceptible strains), ceftazidime (77% of susceptible strains) and cefepime (83% of susceptible strains). This indicates a likely failure of treatment if these drugs were the choice for treatment of serious infections caused by ESBL-producing organisms. Therefore, these organisms should be revised as resistant to the apparently effective cephalosporins according to the CLSI rules for reporting ESBLs. But there is a concern with reporting ESBL producers as resistant to cefepime at some clinical microbiology laboratories in Taiwan, if multidrug or pandrug-resistant *Acinetobacter baumannii* (PDRAB) has been prevalent in their hospitals. Routinely regarding cefepime as an ineffective drug will further reduce the therapeutic options, and thus may potentially promote indiscriminate usage of carbapenems against ESBL producers. Increased use of carbapenems and ciprofloxacin as well as clonal dissemination may have contributed to the wide spread of PDRAB in a medical center in northern Taiwan [60].

Another study of antimicrobial susceptibility testing for ESBL-producing *E. coli* and *K. pneumoniae* isolates showed similar results, as the susceptibility rate of isolates for ciprofloxacin was about 53 to 56%, ceftriaxone 10 to 17%, ceftazidime 56 to 57%, cefepime 60 to 61%, and imipenem 100% [25]. In a recent study of antimicrobial susceptibility testing for ESBL-producing *E. coli* and *K. pneumoniae*, about 30 to 37% of the isolates were susceptible to ciprofloxacin, 73 to 78% to cefepime, 99% to ertapenem, and 100% were susceptible to both imipenem and meropenem [61]. Isolates collected from different hospitals showed that the susceptibilities to several antimicrobials diverged [61]. In general, carbapenem-resistant ESBL-producing isolates remain extremely rare in Taiwan. A clonal outbreak of *K. pneumoniae* that harbored SHV-12 and the metallo-β-lactamase, IMP-8, circulated within a medical center in southern Taiwan [34]. It is noteworthy that 15 (88%) of the 17 non-repetitive bla_{IMP-8}-positive isolates appeared susceptible to imipenem (MICs <4 μg/mL) and meropenem (MICs <1 μg/mL), suggesting that current routine susceptibility tests may

not always reflect the presence of the metallo-β-lactamase in *K. pneumoniae* isolates provided that its quantities are relatively low.

Owing to different hydrolyzing abilities of ESBLs against various β-lactam antibiotics, certain cephalosporin-resistance profiles may be helpful as a guide to the type of ESBL present. CTX-M-3- or CTX-M-14-producing *K. pneumoniae* isolates generally have higher MICs for cefotaxime (or ceftriaxone), cefepime and aztreonam than for ceftazidime, whilst SHV-5- or SHV-12-producing *K. pneumoniae* generally have higher MICs for ceftazidime, cefotaxime (or ceftriaxone) and aztreonam than for cefepime [32,41,62]. At present, it is uncertain whether the above rules can be applied to CTX-M-ase-, SHV-5- or SHV-12-producing species other than *K. pneumoniae*. For the CTX-M-3- and -14-producing *E. coli* isolates reported in Taiwan, the MIC data for cefepime were lacking [21,37,40,42]. For the CTX-M-3- and CTX-M-14-producing *P. mirabilis* strains, MICs for cefepime are lower than those of the *K. pneumoniae* strains [26]. Co-existence of SHV- and CTX-M-type ESBLs or a unique CTX-M-15 alone may confer resistance to all tested cephalosporins, including cefotaxime (or ceftriaxone), ceftazidime, cefepime and aztreonam [32,38,62].

A nationwide molecular epidemiology study has demonstrated that isolates belonging to the same genomic clone (PFGE-based genotype) may exhibit different types of antibiograms due to the presence of different β-lactamases, as shown by diverse patterns of isoelectric focusing points [32]. This study suggests that it is unreliable to screen for the presence of outbreaks or dissemination of epidemic clones among collected isolates by the use of antibiogram-based methods, which have been used traditionally by hospital infection control personnel in Taiwan.

Clinical Impact and Therapeutic Strategy of Infections Caused by ESBL-producing Organisms

Clinical impact

Few studies in Taiwan have compared the differences in outcome between patients infected by ESBL-producing pathogens and those infected by non-ESBL producers. Liu et al [48] reported that the mortality of patients with *E. cloacae* bacteremia was associated with multiresistant isolates (mostly harboring SHV-12, TEM-1 and AmpC β-lactamases) and infections caused by multiple species. However, excluding the patients infected by a major clone, only "infections caused by multiple species" became a significant risk factor associated with mortality. This suggested that sporadic cases with ESBL-producing *E. cloacae* infection were not coupled with a higher mortality compared to the infections caused by non-ESBL producers. This result is consistent to several other studies in other countries in which no significant differences in terms of mortality between patients infected by organisms with and without ESBLs were found [12, 63]. In contrast, other studies have reported that the mortality rates of bacteremia due to ESBL-producing strains were significantly higher than for ESBL-negative strains [64,65]. Because some virulence factors may potentially confound the clinical outcome, there is a significant uncertainty in these types of outcome studies since the ESBL producers may not necessarily be more virulent than non-ESBL producers [66,67].

The second question is the clinical impact of appropriate antibiotic therapy on the infections caused by ESBL producers. The data on this issue are also sparse in Taiwan. Siu and co-workers [41] have described the clinical features of 13 pediatric patients with underlying malignancies (12 also with neutropenia). The children had 16 episodes of bacteremia caused by ESBL producers (4 *E. coli* and 12 *K. pneumoniae*), and 9 of the patients received an imipenem-containing regimen. Two of the patients died and both were infected by *K. pneumoniae*. Among the other 4 patients who received treatment with extended-spectrum β-lactams, only 1 patient died as a result of the *K. pneumoniae* infection. The 3 surviving patients all had urinary tract infections or catheter-related infections. The infected catheters were removed, and the antimicrobial regimens already contained additional effective amikacin. The case number is too small to achieve a statistical significance. However, provision of adequate antimicrobial agents and removal of the involved catheters may replace the use of carbapenems to treat patients with bacteremia due to urinary tract infections or catheter-related infections caused by ESBL producers, even in immunocompromised patients. By multivariate analysis, a prospective worldwide study found that the use of a carbapenem during the 5-day period after onset of bacteremia due to an ESBL-producer was independently associated with lower mortality [68]. In contrast, a clinical study of bacteremia caused by ESBL-producing *K. pneumoniae* in Korea reported no significant difference in mortality between patients who received appropriate empiric antibiotic therapy and those who did not [63].

Therapeutic strategy

Due to their stability against the hydrolytic effects of ESBLs, consistently low MICs for the majority of isolates, and broadly-based clinical evidence, carbapenems have been recommended as the drugs of choice for serious infections with ESBL-producing organisms [11,12,25,59,68-70]. The term 'serious infection' generally refers to bacteremia, hospital-acquired pneumonia, intra-abdominal infection or meningitis, excluding urinary tract infection [12]. However, without specified criteria or severity scores to define the 'serious infection', the above statement could easily promote to the use of carbapenems, a development that has been associated with the emergence of carbapenem-resistant bacterial species, particularly *A. baumannii* in Taiwan [16,60].

As mentioned above, ESBL producers should be reported as resistant to all third-generation cephalosporins, and consequently these drugs should not be used to treat serious infections caused by ESBL producers. Other β-lactam antibiotics (β-lactam/β-lactamase inhibitor combinations or 7-alpha-methoxy β-lactams such as cephamycins and oxacephems) and non-β-lactam drugs, such as quinolones, trimethoprim-sulfamethoxazole and aminoglycosides, may be useful options to treat mild-to-moderate infections, but are not adequate as first-line therapy for serious infections. The high rates of co-resistance to quinolones, trimethoprim-sulfamethoxazole and aminoglycosides among ESBL producers collected from Taiwan have greatly limited the therapeutic role of these classes of antibiotics against ESBL producers [25,29,61].

Yang et al reported of a patient receiving intermittent peritoneal dialysis who developed dialysis-related peritonitis caused by ESBL-producing *K. pneumoniae* [71]. The patient was successfully treated with a 3-week course of intraperitoneal flomoxef without subsequent relapse. Flomoxef may be regarded as a third-generation oxacephem, and possesses good in vitro activity against several ESBL producers [18,61]; however, resistance to this antibiotic can be enhanced either by the combined effects of an ESBL and reduced outer membrane permeability or by hyperproduction of AmpC β-lactamase in the isolates [72]. Because co-existence of ESBL and AmpC enzymes has been reported in Taiwan [21,49-51], this class of antibiotic should be used cautiously if indicated.

Although piperacillin-tazobactam and cefepime may have good in vitro activities against several ESBL producers collected from some institutions [29,61], an 8-fold or more MIC increase to these antibiotics may usually be obtained if the inoculum of ESBL-producing organisms rises concomitantly (inoculum effect) [73]. It was the opinion of the authors that high inocula occur in endocarditis, meningitis, septic arthritis, osteomyelitis, abscesses, and other deep-seated infections. However, it is difficult to discern whether a patient has a truly 'high inoculum' disease. Besides, compared with non-ESBL producers, recent animal model studies have demonstrated that ESBLs per se have no impact on the determination of the pharmacokinetic (PK) and pharmacodynamic (PD) target for therapy [74]. The PK/PD target is usually defined as the desired proportion (%) of the dosing interval (T) that the drug concentration remains above the MIC (designated as %T>MIC). Achievement of the PK/PD target can predict drug efficacy. For example, if the PK/PD target of cefepime for a non-ESBL producer (MIC 0.1 μg/mL) is 60%T>MIC, then the PK/PD target of cefepime for an ESBL producer (MIC 4 μg/mL) should remain 60%T>MIC. Thus, regardless of the inoculum effect of the ESBL producer, the cefepime level should remain above 4 μg/mL for over 60% of the dosing interval. Therefore, the inoculum effects of ESBL producers may be overcome by increasing drug exposure to raise the likelihood of achieving the PK/PD target with specific cephalosporin dosage regimens. A study in the USA reported that continuous infusion of 4 g cefepime could achieve the highest probability (77%) of PK/PD target attainment against ESBL producers (for cefepime, ≥60% T>MIC), followed by 1 g every 8 h (65%) and 2 g every 12 h(58%) [75]. The PK/PD profiles of cefepime were generally superior to those of piperacillin-tazobactam in terms of predicting clinical success against ESBL-producing *E. coli* and *K. pneumoniae* [75,76]. Based on PK/PD profiles demonstrated in another study in the USA, a regimen of 2 g every 8 h for cefepime should be preferred as the empirical treatment of Gram-negative pulmonary infections in the intensive care unit (ICU) [77].

Although this study focussed on ICU patients, it is presumed that patients in other settings would also benefit from this treatment. The combination of cefepime and sulbactam may be an option for the treatment of bacteremia caused by SHV-12-producing *E. cloacae* [48]. Use of third-generation cephalosporins such as ceftazidime or cefotaxime in combination with the β-lactamase inhibitor sulbactam could result in improved in vitro activity against ESBL-producing enterobacteria [78].

According to CLSI recommendations, ESBL producers should be reported as resistant to fourth-generation cephalosporins. These antibiotics may have the potential to treat mild-to-moderate infections caused by organisms with an SHV-related phenotype, whereas the drugs are not suitable for eradication of CTX-M-producers [62]. Meanwhile, considering the increasing threat of carbapenem-resistant *A. baumannii* in Taiwan and limited alternative choices of drug against ESBL producers, some clinical laboratories continue to report the susceptibility to cefepime of an ESBL-producing organism.

Nosocomial Infection Control: Risk Factor Analysis and Strategy for Prevention of Proliferation of ESBL-producing Organisms

As mentioned previously, several molecular epidemiology studies have demonstrated that some epidemic clones have disseminated or distributed regionally or nationwide in Taiwan [26,32,37,48]. Because a linkage of time and geography between these isolates with the same genotype cannot be established, these epidemic clones have been regarded as already 'endemic' after several generations of dissemination, but not as real 'epidemic' outbreaks with direct patient-to-patient transmission [32]. Infection control of endemic ESBL producers should include changes in the policy of antibiotic prescription and continuous infection control programs, such as surveillance, hand washing, and contact isolation procedures [12].

Risk factors

A case-control study was performed in a district hospital in Taiwan in order to identify the risk factors for acquiring ESBL-producing *K. pneumoniae* [23]. Univariate analysis showed that tracheostomy, insertion of a Foley catheter, endotracheal tube, nasogastric tube and central venous catheter use were risk factors, whereas only tracheostomy and prior use of ceftazidime remained as risk factors by multivariate analysis. Ceftazidime was the main prescribed third-generation cephalosporin in that hospital, whereas utilization of other third-generation cephalosporins was quite low. Indeed, a worldwide study has reported that previous use of oxyimino-β-lactam antibiotics (cefuroxime, cefotaxime, ceftriaxone, ceftazidime, or aztreonam) appeared to be a risk factor for nosocomial bacteremia caused by *K. pneumoniae* with production of an ESBL [31].

Infection control by intervention in antibiotic use

A prospective study was conducted in a medical ICU (MICU) of a regional hospital by the use of rectal swab surveillance in order to evaluate the efficacy of piperacillin-tazobactam in reducing acquisition rate for ESBL-producing *E. coli* or *K. pneumoniae* [79]. Ceftazidime, which was the most commonly used extended-spectrum antibiotic in the MICU in the period prior to the intervention, was replaced mainly by piperacillin-tazobactam. The results demonstrated that the shift from primarily ceftazidime use to primarily piperacillin-tazobactam use caused a significant decrease in the incidence of ESBL-producing isolates [79]. Other studies have substantiated these findings [80-82].

Nosocomial infections caused by *A. baumannii* also seem to pose another serious problem in Taiwan, with a general mortality of patients with bacteremia ranging from 22 to 45% [83,84], and 60% mortality of patients with the PDRAB bacteremia [85]. The antibiotic policy for routine use of carbapenems to treat 'serious infections' caused by ESBL-producing microorganisms would potentially trigger PDRAB problems in an institution with existing highly endemic ESBL producers. Therefore, it is necessary to define a good strategy to control and treat infections caused by ESBL-producing pathogens, and at the same time prevent an increasing occurrence of PDRAB infections in hospitals. ICUs or institutions with high prevalences of ESBL-producing microorganisms and/or multidrug-resistant *A. baumannii*, carbapenems should be the drugs of choice for combating severe infections judged by a cautiously defined severity scoring system, such as the presence of septic shock, acute respiratory distress syndrome or multiple sites of organ failure. Empirical therapy with a fourth-generation cephalosporin or piperacillin-tazobactam using a dosage regimen of high probability of PK/PD target attainment may be helpful for treatment of infections of mild-to-moderate severity [76]. The likelihood of ertapenem achieving the required PK/PD target attainment (>40%T>MIC) against ESBL producers was 78%, equivalent to cefepime [86]. If a culture turns out to be an ESBL producer, antibiotic replacement by a carbapenem (imipenem or meropenem) could be withheld if clinical improvement has already been documented, although clinical outcome data for cefepime or piperacillin-tazobactam have been limited. Switching to ertapenem or adding sulbactam to either piperacillin or a fourth-generation cephalosporin may be alternative choices. However, the long-term impact of the use of ertapenem on the emergence of PDRAB

needs to be evaluated further. These antibiotic usage policies might be included in strategies to restrict overuse of carbapenems to impede selection for carbapenem-resistant bacteria.

Conclusions

The types of ESBLs and their host species have increased in Taiwan hospitals. Detection of ESBL-producing microorganisms poses an ongoing difficulty for scientists in clinical microbiology laboratories. To treat infections caused by ESBL producers and to reduce their occurrence continue to represent great challenges to physicians and infection control personnel. In addition to clonal spread and plasmid dissemination, the environment of ESBL-encoding genes should be investigated further, in order to elucidate the potential modes of horizontal transmission. Carbapenems and other alternative drugs of therapeutic choice should be given according to the severity of infectious diseases defined by use of detailed scoring systems rather than by sites of infections. For example, bacteremia per se may not always refer to serious infection, provided that it is catheter-related and the involved catheter is rapidly removed. Based on local MIC data of ESBL producers, it is necessary to simulate the PK of Taiwanese population with specific drug dosage regimens to predict a high probability of achieving the PD target.

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