



ELSEVIER

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.e-jmii.com



ORIGINAL ARTICLE

Epidemiology and risk factors of community-onset urinary tract infection caused by extended-spectrum β -lactamase-producing Enterobacteriaceae in a medical center in Taiwan: A prospective cohort study



Che-Hsuan Kung^{a,b}, Wen-Wei Ku^a, Chi-Hung Lee^{a,c},
Chang-Phone Fung^{a,d}, Shu-Chen Kuo^{a,d,e}, Te-Li Chen^d,
Yi-Tzu Lee^{a,d,f,*}

^a Division of Infectious Diseases, Taipei Veterans General Hospital, Taipei, Taiwan

^b Department of Internal Medicine, Taipei City Hospital, Zhongxing Branch, Taipei, Taiwan

^c Department of Medicine, National Yang-Ming University Hospital, Yilan, Taiwan

^d Institute of Clinical Medicine, School of Medicine, National Yang-Ming University, Taipei, Taiwan

^e National Institute of Infectious Diseases and Vaccinology, National Health Research Institutes, Miaoli County, Taiwan

^f Department of Emergency Medicine, Taipei Veterans General Hospital, Taipei, Taiwan

Received 25 April 2013; received in revised form 21 July 2013; accepted 13 August 2013

Available online 21 September 2013

KEYWORDS

Community-onset;
Extended-spectrum
 β -lactamase;
Urinary tract
infection

Background: Extended-spectrum β -lactamase (ESBL)-producing pathogens have been increasingly identified in community-onset urinary tract infection (UTI). This study was conducted to determine the epidemiology and risk factors of community-onset UTI caused by ESBL-producing pathogens, and to determine the correlation of antimicrobial resistance with ESBL detected by phenotypic and genotypic methods.

Methods: The study was conducted from December 2010 to January 2012. Patients with community-onset UTI caused by Enterobacteriaceae were enrolled from the emergency department. The production of ESBL was determined by the phenotypic method (using the combined disk test) or by the genotypic method (using polymerase chain reaction detection). The patients' medical records were reviewed and risk factors were analyzed by multivariate analysis.

* Corresponding author. Division of Infectious Diseases, Department of Medicine, Taipei Veterans General Hospital, No. 201, Section 2, Shih-Pai Road, Taipei 112, Taiwan.

E-mail address: s851009@yahoo.com.tw (Y.-T. Lee).

Results: A total of 376 patients were enrolled and 393 isolates from urine culture were analyzed. *Escherichia coli* was the most commonly isolated species (259/393 isolates; 65.9%), followed by *Klebsiella pneumoniae* (42/393 isolates; 10.7%). Fifty-three (13.5%) isolates were phenotypically positive for ESBL production. Nine (2.3%) isolates were phenotypically positive for both ESBL and AmpC β -lactamase (AmpC) production. Nasogastric tube placement [odds ratio (OR) 2.230; 95% confidence interval (CI) 1.244–3.997; $p = 0.007$] and hospitalization within the previous 3 months (OR 2.567, 95% CI 1.448–4.551, $p = 0.001$) were independently associated with the acquisition of ESBL-producing pathogens in community-onset UTI. The ESBL phenotype had a better correlation with resistance to third-generation cephalosporins, compared to the ESBL-positive genotype.

Conclusion: In our study, nasogastric tube placement and hospitalization within the previous 3 months were significantly associated with the acquisition of ESBL-producing pathogens in community-onset UTI.

Copyright © 2013, Taiwan Society of Microbiology. Published by Elsevier Taiwan LLC. All rights reserved.

Introduction

Multidrug-resistant bacteria are an emerging challenge for clinicians with respect to antimicrobial treatment and infection control in hospitals. Extended-spectrum β -lactamase (ESBL)-producing Gram-negative bacteria are one of the most annoying groups of pathogens that have become a major problem in hospitalized patients since the early 1990s.¹ However, these pathogens have further spread to nursing homes and the community in recent years.^{2–4}

Since 1998, community-onset urinary tract infection (UTI) caused by ESBL-producing Enterobacteriaceae has been increasingly reported.^{3,4} Patients with community-acquired UTI caused by ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* had longer hospital stays and higher costs for antimicrobial use.⁵ However, the case numbers used in previous studies were rather small.^{5–8} Accurately identifying ESBLs by using conventional phenotypic methods is sometimes difficult in practice. Identification is also made more difficult because ESBL-producing Enterobacteriaceae increasingly overproduce AmpC β -lactamases (AmpC) concomitantly.^{9–12} All equivocations indicate a need for further investigation. The aim of this large-scale prospective cohort study is to determine the epidemiology and risk factors of ESBL-producing Enterobacteriaceae, which were tested by phenotypic methods and by genotypic methods in community-onset UTI in a medical center in northern Taiwan.

Methods

Hospital setting and patients

The Taipei Veterans General Hospital is a 2900-bed tertiary care teaching hospital in northern Taiwan. Adult patients who were admitted to the Taipei Veterans General Hospital with a diagnosis of community-onset UTI caused by Enterobacteriaceae were eligible for inclusion in this study if they required initial parental antimicrobial therapy. Patients were initially enrolled in the study, based on a positive urinalysis result at the Emergency Department. They were then evaluated only if they met the criteria for a

positive urine culture [i.e., at least 1×10^5 colony-forming units per milliliter (CFU/mL) of Enterobacteriaceae]. A single isolate was included from a patient, unless the culture grew more than one species. The exclusion criteria were pregnancy or lactation in women, complete obstruction of the urinary tract, and perinephric or intrarenal abscess. Male patients with a history or physical findings suggestive of acute or chronic prostatitis were also excluded. This study was approved by the Institutional Review Board of the Taipei Veterans General Hospital (VGHIRB; No. 201003025IC).

Definitions

Community-onset infection was defined as an infection diagnosed within 48 hours after admission. Community onset infections included infections that were classified as community-acquired or as healthcare-associated, if either of the following criteria were present¹³: (1) the patient received intravenous therapy, wound care, or specialized nursing care at home or in an outpatient clinic, or received renal dialysis in the previous 30 days; and (2) the patient had more than 48 hours of hospital admission or had resided in a nursing home or long-term care facility within the previous 90 days. The criteria for UTIs were clinical signs and/or symptoms of UTIs (e.g., fever $> 38^\circ\text{C}$, urgency, frequency, dysuria, or suprapubic tenderness) with no other recognized cause; pyuria (i.e., urine specimen with ≥ 10 white blood cells/mm³) and a positive urine culture ($\geq 1 \times 10^5$ CFU/mL of a uropathogen).

Bacteriology and antimicrobial susceptibility testing

Enterobacteriaceae were identified by using the VITEK 2 system (bioMérieux, Marcy l'Etoile, France). *In vitro* susceptibility testing to commonly used antibiotics was performed by using agar dilution methods. The results were interpreted in accordance with the Clinical Laboratory Standards Institute (CLSI).⁹ We used CLSI ESBL confirmatory test with disks containing cefotaxime (CTX; 30 μg) and ceftazidime (CAZ; 30 μg) with and without clavulanic acid (CLA; 10 μg) on Mueller–Hinton agar.⁹ An increase in the

Table 1 ESBL phenotypes of Enterobacteriaceae causing community-onset urinary tract infections

Bacteria species	Phenotype		
	N	ESBL only, N (%)	ESBL + AmpC, N (%)
<i>Citrobacter amalonaticus</i>	1	0	0
<i>Citrobacter diversus</i>	6	0	0
<i>Citrobacter freundii</i>	4	0	0
<i>Enterobacter aerogenes</i>	3	0	1 (33.3)
<i>Enterobacter cloacae</i>	6	0	0
<i>Enterobacter gergoviae</i>	1	0	0
<i>Escherichia coli</i>	259	36 (13.9)	2 (0.8)
<i>Klebsiella oxytoca</i>	1	0	0
<i>Klebsiella pneumoniae</i>	42	11 (26.2)	6 (14.3)
<i>Morganella morganii</i>	9	1 (11.1)	0
<i>Proteus mirabilis</i>	36	3 (8.3)	0
<i>Proteus penneri</i>	1	0	0
<i>Proteus vulgaris</i>	1	0	0
<i>Providencia alcalifaciens</i>	1	0	0
<i>Providencia rettgeri</i>	5	2 (40.0)	0
<i>Providencia stuartii</i>	3	0	0
<i>Serratia marcescens</i>	13	0	0
<i>Serratia species</i>	1	0	0
Total	393	53 (13.5)	9 (2.3)

AmpC = AmpC β -lactamase; ESBL = extended-spectrum β -lactamase.

zone diameter of ≥ 5 mm in the presence of CLA was considered phenotypic confirmation of ESBL production. *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603 were used as the negative and positive controls, respectively, and were the routine quality control. Because ESBL can be masked by the expression of AmpC, a modified disk potentiation test using 3-aminophenylboronic acid (APB; 400 μ g) was conducted to enhance the detection of ESBLs in isolates that may simultaneously harbor plasmid-borne AmpCs or chromosomal AmpC gene.^{14–16} A positive result for ESBLs was a ≥ 5 mm increase in the zone diameter of CTX/CLA and/or CAZ/CLA disks that were tested in combination with APB (i.e., CTX/CLA/APB and/or CAZ/CLA/

APB) versus CTX and/or CAZ disks containing APB (i.e., CTX/APB and/or CAZ/APB). Polymerase chain reaction (PCR) testing of ESBL-positive isolates for known ESBL genes was performed by using specific primers for *bla*_{TEM}, *bla*_{SHV}, *bla*_{CTX-M}, *bla*_{Toho}, *bla*_{VEB}, and *bla*_{PER}.^{17,18}

Statistical analysis

Descriptive statistics were calculated for baseline demographic and clinical variables and the prevalence of ESBL (including those with and without AmpC) among Enterobacteriaceae that cause community-onset UTIs. Univariate analysis was performed by using two-sided Student *t* tests, Chi square tests, and Fisher's exact tests, as appropriate. Univariate analysis, followed by multivariable logistic regression analysis, was performed to identify the risk factors of community-onset UTIs caused by ESBL-positive Enterobacteriaceae. We also used univariate analysis, followed by multivariable logistic regression analysis, to identify the genes that were relevant to the ESBL-positive phenotype. The variables included in multivariate analysis were identified in the univariate analysis ($p < 0.10$) and were implicated in previous studies or were biologically plausible. Prior to analysis, the variables were assessed for collinearity. All analyses were performed with the SPSS version 19.0 software (SPSS, Chicago, IL, USA). All statistical tests were two-tailed. A p value < 0.05 was considered statistically significant.

Results

A total of 376 patients with community-onset UTI caused by Enterobacteriaceae were enrolled and 393 isolates from the patients' urine cultures were collected and analyzed. *E. coli* was the most commonly isolated species (259/393 isolates; 65.9%), followed by *K. pneumoniae* (42/393 isolates; 10.7%), *Proteus mirabilis* (35/393 isolates; 8.9%), and *Serratia marcescens* (13/393 isolates; 3.3%). Fifty three (13.5%) isolates were phenotypically positive for ESBL production. *E. coli* (36 isolates, 67.9%) was the most common pathogen and *K. pneumoniae* (11 isolates; 20.8%) was the second-most common pathogen (Table 1). Nine (2.3%) isolates—six *K. pneumoniae*, two *E. coli*, and one *Enterobacter aerogenes*—were phenotypically positive for ESBL

Table 2 Genotype analysis of phenotypical ESBL-positive Enterobacteriaceae causing community-onset urinary tract infections

Bacterial species	No. of isolates carrying						
	Total no. of isolates	PER	SHV	TEM	VEB	CTX-M/Toho	No gene detected
<i>Enterobacter aerogenes</i>	1	0	0	0	0	0	1
<i>Escherichia coli</i>	38	0	6	8	2	31	4
<i>Klebsiella pneumoniae</i>	17	0	16	3	0	12	1
<i>Morganella morganii</i>	1	0	0	1	1	1	0
<i>Proteus mirabilis</i>	3	0	0	0	0	3	0
<i>Providencia rettgeri</i>	2	0	0	0	0	1	1
Total	62	0	22	13	3	48	6

ESBL = extended-spectrum β -lactamase.

Table 3 Risk factors for the acquisition of phenotypically ESBL-positive Enterobacteriaceae

Characteristic	Univariate analysis		Multivariate analysis	
	OR (95% CI)	<i>p</i>	OR (95% CI)	<i>p</i>
Nasogastric tube use	2.763 (1.575–4.847)	<0.001	2.230 (1.244–3.997)	0.007
Chronic kidney disease	2.053 (1.104–3.816)	0.023		
Cerebral vascular accident	1.809 (1.031–3.175)	0.039		
Bedridden	1.848 (1.057–3.231)	0.031		
Foley catheter	2.075 (1.198–3.596)	0.009		
Diagnosis of UTI within 1 year	2.205 (1.265–3.844)	0.005		
Antibiotics exposure within 3 months ^a				
Penicillins with/without a β-lactamase inhibitor	2.412 (1.287–4.519)	0.006		
Cephalosporins	2.158 (1.230–3.787)	0.007		
Fluoroquinolones	3.038 (1.554–5.940)	0.001		
Hospitalization within the previous 3 months	3.034 (1.743–5.284)	<0.001	2.567 (1.448–4.551)	0.001

^a Aminoglycosides, carbapenems, tetracyclines (including tigecycline), trimethoprim/sulfamethoxazole, colimycin, sulbactam, monobactam are excluded because of the small case number.

CI = confidence interval; ESBL = extended-spectrum β-lactamase; OR = odds ratio; UTI = urinary tract infection.

production and AmpC β-lactamase production. There were 138 ESBL genes detected in 62 phenotypical ESBL-positive isolates. Of the 62 phenotypical ESBL-positive isolates, six isolates did not have the ESBL gene. The genes *bla*_{CTX-M} plus *bla*_{Toho} were the most commonly detected genes (100/138 isolates; 72.5%), followed by *bla*_{SHV} (22/138 genes; 15.9%; Table 2).

Table 3 shows the risk factors for phenotypically ESBL-positive Enterobacteriaceae in community onset UTI. Multivariate analysis showed that nasogastric tube placement [odds ratio (OR) 2.230; 95% confidence interval (CI)

1.244–3.997; *p* = 0.007] and hospitalization within the previous 3 months (OR 2.567; 95% CI 1.448–4.551; *p* = 0.001) were independently associated with ESBL-producing pathogens in community-onset UTI. The ESBL-positive phenotype showed a better correlation with resistance to third-generation cephalosporins, compared to bacteria with the ESBL-positive genotype (Table 4). In addition, phenotypically positive ESBL Enterobacteriaceae exhibited resistance not only to cephalosporins but to noncephalosporin antibiotics such as the fluoroquinolones and trimethoprim/sulfamethoxazole.

Table 4 Correlation of ESBL phenotype and genotype with antimicrobial susceptibilities->

	Phenotype		<i>p</i>	Genotype		<i>p</i>
	ESBL(-)	ESBL(+)		ESBL(-)	ESBL(+)	
Cefazolin	99/330 (30.0)	57/62 (91.9)	<0.001	77/202 (38.1)	79/191 (41.4)	0.580
Cefuroxime	60/315 (19.0)	57/61 (93.4)	<0.001	50/192 (26.0)	67/184 (36.4)	0.039
Cefmetazole	46/316 (14.6)	17/61 (27.9)	0.018	34/193 (17.6)	29/184 (15.8)	0.730
Cefoxitin	0/14	0/1		0/9	0/7	
Flomoxef	30/316 (9.5)	13/61 (21.3)	0.015	22/193 (11.4)	21/184 (11.4)	> 0.99
Cefotaxime/ceftriaxone	39/330 (11.8)	52/62 (83.9)	<0.001	31/202 (15.3)	60/191 (31.4)	<0.001
Ceftazidime	31/329 (9.4)	48/62 (77.4)	<0.001	23/202 (11.4)	56/190 (29.5)	<0.001
Cefepime/cefpirome	6/329 (1.8)	46/61 (75.4)	<0.001	7/202 (3.5)	45/189 (23.8)	<0.001
Ampicillin	252/330 (76.4)	60/62 (96.8)	<0.001	145/202 (71.8)	167/191 (87.4)	<0.001
Ampicillin/sulbactam	89/329 (27.1)	32/61 (52.5)	<0.001	63/202 (31.2)	58/189 (30.7)	> 0.99
Piperacillin/tazobactam	5/315 (1.6)	10/60 (16.7)	<0.001	2/193 (1.0)	13/183 (7.1)	0.006
Gentamicin	60/330 (18.2)	24/62 (38.7)	0.001	38/202 (18.8)	46/191 (24.1)	0.250
Amikacin	0/14	0/1		0/9	0/7	
Ciprofloxacin	62/327 (19.0)	42/61 (68.9)	<0.001	42/201 (20.9)	62/188 (33.0)	0.010
Levofloxacin	60/329 (18.2)	41/61 (67.2)	<0.001	40/202 (19.8)	61/189 (32.3)	0.007
Ertapenem	1/330 (0.3)	0/62 (0.0)	> 0.99	1/202 (0.5)	0/191 (0.0)	> 0.99
Imipenem	1/328 (0.3)	1/60 (1.7)	0.286	1/200 (0.5)	1/188 (0.5)	> 0.99
Tigecycline	0/13	0/1		0/9	0/6	
Trimethoprim/sulfamethoxazole	133/316 (42.1)	42/60 (70.0)	<0.001	76/193 (39.4)	99/183 (54.1)	0.006

ESBL = extended-spectrum β-lactamase.

Table 5 Univariate and multivariate analyses of gene type correlation with positive phenotype in the genotypical positive isolates

ESBL gene	Univariate analysis		Multivariate analysis	
	OR (95% CI)	<i>p</i>	OR (95% CI)	<i>p</i>
<i>bla</i> _{SHV}	0.785 (0.416–1.480)	0.454		
<i>bla</i> _{TEM}	0.316 (0.156–0.640)	0.001		
<i>bla</i> _{VEB}	7.585 (0.772–74.557)	0.082	16.476 (1.194–227.294)	0.036
<i>bla</i> _{CTX-M/Toho}	13.286 (5.779–30.543)	<0.001	14.699 (6.143–35.172)	<0.001

CI = confidence interval; ESBL = extended-spectrum β -lactamase; OR = odds ratio.

There were 56 phenotypical positive isolates in 191 genotypical positive isolates. In the 191 genotypically ESBL-positive isolates, the presence of *bla*_{VEB} (OR 16.476; *p* = 0.036) or *bla*_{CTX-M/Toho} (OR 14.699; *p* < 0.001) were independent risk factors for the ESBL-positive phenotype (Table 5).

Discussion

This prospective study, which was conducted in a tertiary medical center, found that approximately 15% of *E. coli* and 40% of *K. pneumoniae* produced ESBL β -lactamases. This indicates that ESBL-producing Enterobacteriaceae are no longer a nosocomial problem, but are a growing comprehensive issue in community-onset UTIs. Among the ESBL-producing isolates, pathogens carrying the *bla*_{CTX-M} genes were the most prevalent in the community. The ESBL phenotype, rather than the ESBL genotype, had a better predictive value for *in vitro* susceptibility to the third-generation cephalosporins. The use of nasogastric tubes and hospitalization within the previous 3 months were the most important risk factors for the acquisition of ESBL-positive pathogens in people with community-onset UTIs.

Our study showed a higher prevalence of ESBL-positive *E. coli* and *K. pneumoniae*, compared to a previous study conducted in 2010 at another medical center in northern Taiwan.⁵ One Korean study showed an even higher prevalence rate in ESBL-positive *E. coli*.¹⁹ In this study, the ESBL-positive rate of Enterobacteriaceae causing community-onset UTIs was higher than the ESBL-positive rate of pathogens causing nosocomial infections in Spain (12% in 2003), Italy (7.4% in 2006), and Denmark (2.8% in 2012).^{20–22} The results indicated that ESBL-producing Enterobacteriaceae have emerged as important pathogens in community settings in Taiwan and in other countries.

In our study, CTX-M was the most popular β -lactamase carried by the Enterobacteriaceae that cause community-onset UTIs; this was consistent with the results of past studies conducted in four continents.^{23–32}

The gene *bla*_{Toho} was considered with *bla*_{CTX-M} because of their similar structure.¹ In a previous review in Taiwan, *bla*_{SHV} and *bla*_{CTX-M} were the most common genes identified from Enterobacteriaceae isolated from various clinical samples of nosocomial and community-acquired infections.³³ Our study was confined to community-onset urinary tract infections, which provides a valuable molecular epidemiological data of emerging ESBL-producing Enterobacteriaceae in northern Taiwan hospitals. Isolates

carrying *bla*_{VEB} or *bla*_{CTX-M/Toho} had a higher frequency of the ESBL-positive phenotype. Further investigation may be needed to determine its mechanism and clinical significance.

Phenotypical ESBL-positive bacteria exhibited resistance not only to cephalosporins but also to noncephalosporins such as the fluoroquinolones and trimethoprim/sulfamethoxazole because ESBL encoding plasmids may carry genes that encode resistance to other classes of antibiotics such as the fluoroquinolones and sulfonamides.^{34,35} Thus, limited antibiotic choices are available for treating infections caused by these multidrug-resistant strains. In this circumstance, the antibiotics piperacillin/tazobactam, ertapenem, or imipenem are indicated for treatment.^{7,36}

Nasogastric tube usage and hospitalization within the previous 3 months were independent risk factors for ESBL-producing isolates in our study. One study from southern Taiwan showed hospitalization within the previous 6 months and antibiotic use within the previous 60 days are risk factors for ESBL-producing Enterobacteriaceae causing community-onset UTIs.³⁷ Nasogastric tube usage and hospitalization in the previous 3 months are two of many risk factors that have been mentioned previously.¹ Previous studies found that nasogastric tube usage was a risk factor for infections caused by multidrug-resistant microbes such as ESBL pathogens and methicillin-resistant *Staphylococcus aureus*.^{1,38–40} Patients with nasogastric tubes are prone to have more manipulations (e.g., regular replacement of a new tube) performed by nursing home or emergency department staff. These manipulations and frequent hospital visits may provide ESBL pathogens an opportunity for further colonization. Further studies are needed to establish possible mechanisms of ESBL pathogen colonization via indwelling catheters such as a nasogastric tube.

This prospective study provided the risk factors for community-onset urinary tract infection caused by extended-spectrum β -lactamase-producing Enterobacteriaceae, but it still had some limitations.

First, the clinical samples were screened by a double-disk synergy test and then confirmed by PCR assay. However, no known ESBL genes were detected in six of the 62 phenotypical ESBL-positive isolates. A more specific method such as an isoelectric focusing (IEF) study may be needed to confirm the presence of any β -lactamase. We will consider this in subsequent studies. Second, patients using oral antibiotics for community-onset UTI were not included in this study. Third, we excluded special groups such as children, pregnant or lactating women, people with complete obstruction of the urinary tract, and patients

with a perinephric or intrarenal abscess, or male patients with prostatitis. Therefore, our findings could only apply to the specific group of adults with a diagnosis of urinary tract infection caused by ESBL-producing Enterobacteriaceae under parental antibiotic use.

Choosing empirical antibiotics appropriately can be challenging in a busy, high-volume emergency department of a tertiary hospital. Our study may help clinicians in such a setting to identify patients with urinary tract infection that is likely caused by ESBL-producing pathogens.

In conclusion, in the community, ESBL pathogens have become a serious issue. Clinicians should be aware of the emerging problem of antibiotic resistance. Further studies on the epidemiological trend of ESBL pathogens in community-acquired UTI are crucial.

Conflicts of interest

Dr. Chang-Phone Fung received a research grant from the Investigator-initiated Studies Program of Merck & Co., Inc. This does not alter the authors' adherence to all of the policies of the *Journal of Microbiology, Immunology and Infection* concerning sharing data and materials.

Acknowledgments

This study was supported by a research grant from the Merck Investigator-Initiated Studies Program of Merck & Co., Inc. (grant no. MISP#37925). The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

References

- Paterson DL, Bonomo RA. Extended-spectrum β -lactamases: a clinical update. *Clin Microbiol Rev* 2005;18:657–86.
- Wiener J, Quinn JP, Bradford PA, Goering RV, Nathan C, Bush K, et al. Multiple antibiotic-resistant *Klebsiella* and *Escherichia coli* in nursing homes. *JAMA* 1999;281:517–23.
- Pitout JD, Nordmann P, Laupland KB, Poirel L. Emergence of Enterobacteriaceae producing extended-spectrum β -lactamases (ESBLs) in the community. *J Antimicrob Chemother* 2005;56:52–9.
- Rodríguez-Baño J, Navarro MD. Extended-spectrum β -lactamases in ambulatory care: a clinical perspective. *Clin Microbiol Infect* 2008;14(Suppl. 1):104–10.
- Yang YS, Ku CH, Lin JC, Shang ST, Chiu CH, Yeh KM, et al. Impact of extended-spectrum β -lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* on the outcome of community-onset bacteremic urinary tract infections. *J Microbiol Immunol Infect* 2010;43:194–9.
- Calbo E, Romani V, Xercavins M, Gomez L, Vidal CG, Quintana S, et al. Risk factors for community-onset urinary tract infections due to *Escherichia coli* harbouring extended-spectrum beta-lactamases. *J Antimicrob Chemother* 2006;57:780–3.
- Rodríguez-Baño J, Alcalá JC, Cisneros JM, Grill F, Oliver A, Horcajada JP, et al. Community infections caused by extended-spectrum β -lactamase-producing *Escherichia coli*. *Arch Intern Med* 2008;168:1897–902.
- Lee DS, Lee CB, Lee SJ. Prevalence and risk factors for extended spectrum beta-lactamase-producing uropathogens in patients with urinary tract infection. *Korean J Urol* 2010;51:492–7.
- Clinical and Laboratory Standards Institute. *Performance standards for antimicrobial susceptibility testing*. Supplement M100–S19. Wayne, PA: Clinical and Laboratory Standard Institute; 2009.
- Munier GK, Johnson CL, Snyder JW, Moland ES, Hanson ND, Thomson KS. Positive extended-spectrum-beta-lactamase (ESBL) screening results may be due to AmpC beta-lactamases more often than to ESBLs. *J Clin Microbiol* 2010;48:673–4.
- Bell JM, Chitsaz M, Turnidge JD, Barton M, Walters LJ, Jones RN. Prevalence and significance of a negative extended-spectrum beta-lactamase (ESBL) confirmation test result after a positive ESBL screening test result for isolates of *Escherichia coli* and *Klebsiella pneumoniae*: results from the SENTRY Asia-Pacific Surveillance Program. *J Clin Microbiol* 2007;45:1478–82.
- Steward CD, Rasheed JK, Hubert SK, Biddle JW, Raney PM, Anderson GJ, et al. Characterization of clinical isolates of *Klebsiella pneumoniae* from 19 laboratories using the National Committee for Clinical Laboratory Standards extended-spectrum beta-lactamase detection methods. *J Clin Microbiol* 2001;39:2864–72.
- Kjerulf A, Hansen DS, Sandvang D, Hansen F, Frimodt-Møller N. The prevalence of ESBL-producing *E. coli* and *Klebsiella* strains in the Copenhagen area of Denmark. *APMIS* 2008;116:118–24.
- Jeong SH, Song W, Park MJ, Kim JS, Kim HS, Bae IK, et al. Boronic acid disk tests for identification of extended-spectrum beta-lactamase production in clinical isolates of Enterobacteriaceae producing chromosomal AmpC beta-lactamases. *Int J Antimicrob Agents* 2008;31:467–71.
- Song W, Jeong SH, Kim JS, Kim HS, Shin DH, Roh KH, et al. Use of boronic acid disk methods to detect the combined expression of plasmid-mediated AmpC beta-lactamases and extended-spectrum beta-lactamases in clinical isolates of *Klebsiella* spp., *Salmonella* spp., and *Proteus mirabilis*. *Diagn Microbiol Infect Dis* 2007;57:315–8.
- Song W, Bae IK, Lee YN, Lee CH, Lee SH, Jeong SH. Detection of extended-spectrum beta-lactamases by using boronic acid as an AmpC beta-lactamase inhibitor in clinical isolates of *Klebsiella* spp. and *Escherichia coli*. *J Clin Microbiol* 2007;45:1180–4.
- Poirel L, Le Thomas I, Naas T, Karim A, Nordmann P. Biochemical sequence analyses of GES-1, a novel class A extended-spectrum beta-lactamase, and the class 1 integron In52 from *Klebsiella pneumoniae*. *Antimicrob Agents Chemother* 2000;44:622–32.
- Brasme L, Nordmann P, Fidel F, Lartigue MF, Bajolet O, Poirel L, et al. Incidence of class A extended-spectrum beta-lactamases in Champagne-Ardenne (France): a 1 year prospective study. *J Antimicrob Chemother* 2007;60:956–64.
- Kang CI, Wi YM, Lee MY, Ko KS, Chung DR, Peck KR, et al. Epidemiology and risk factors of community onset infections caused by extended-spectrum β -lactamase-producing *Escherichia coli* strains. *J Clin Microbiol* 2012;50:312–7.
- Luzzaro F, Mezzatesta M, Mugnaioli C, Perilli M, Stefani S, Amicosante G, et al. Trends in production of extended-spectrum β -lactamases among Enterobacteria of medical interest: report of the second Italian Nationwide Survey. *J Clin Microbiol* 2006;44:1659–64.
- Coque TM, Baquero F, Canton R. Increasing prevalence of ESBL-producing Enterobacteriaceae in Europe. *Euro Surveill* 2008;13 pii=19044.
- Hansen DS, Schumacher H, Hansen F, Stegger M, Hertz FB, Schonning K, et al. Extended-spectrum beta-lactamase (ESBL) in Danish clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae*: prevalence, beta-lactamase distribution,

- phylogroups, and co-resistance. *Scand J Infect Dis* 2012;**44**:174–81.
23. Yamasaki K, Komatsu M, Yamashita T, Shimakawa K, Ura T, Nishio H, et al. Production of CTX-M-3 extended-spectrum β -lactamase and IMP-1 metallo β -lactamase by five Gram-negative bacilli: survey of clinical isolates from seven laboratories collected in 1998 and 2000, in the Kinki region of Japan. *J Antimicrob Chemother* 2003;**51**:631–8.
 24. Bonnet R. Growing group of extended-spectrum β -lactamases: the CTX-M enzymes. *Antimicrob Agents Chemother* 2004;**48**:1–14.
 25. Wang H, Kelkar S, Wu W, Chen M, Quinn JP. Clinical isolates of Enterobacteriaceae producing extended-spectrum β -lactamases: prevalence of CTX-M-3 at a hospital in China. *Antimicrob Agents Chemother* 2003;**47**:790–3.
 26. Radice M, Power P, Di Conza J, Gutkind G. Early dissemination of CTX-M-derived enzymes in South America. *Antimicrob Agents Chemother* 2002;**46**:602–4.
 27. Palucha A, Mikiewicz B, Hryniewicz W, Gniadkowski M. Concurrent outbreaks of extended-spectrum β -lactamase-producing organisms of the family Enterobacteriaceae in a Warsaw hospital. *J Antimicrob Chemother* 1999;**44**:489–99.
 28. Cantón R, Coque TM. The CTX-M β -lactamase pandemic. *Curr Opin Microbiol* 2006;**9**:466–75.
 29. Chanawong A, M'Zali FH, Heritage J, Xiong J-H, Hawkey PM. Three Cefotaximases, CTX-M-9, CTX-M-13, and CTX-M-14, among Enterobacteriaceae in the People's Republic of China. *Antimicrob Agents Chemother* 2002;**46**:630–7.
 30. Yan JJ, Ko WC, Tsai SH, Wu HM, Jin YT, Wu JJ. Dissemination of CTX-M-3 and CMY-2 beta-lactamases among clinical isolates of *Escherichia coli* in southern Taiwan. *J Clin Microbiol* 2000;**38**:4320–5.
 31. Tofteland S, Haldorsen B, Dahl KH, Simonsen GS, Steinbakk M, Walsh TR, et al. Effects of phenotype and genotype on methods for detection of extended-spectrum β -lactamase-producing clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae* in Norway. *J Clin Microbiol* 2007;**45**:199–205.
 32. Pitout JD, Laupland KB. Extended-spectrum beta-lactamase-producing Enterobacteriaceae: an emerging public-health concern. *Lancet Infect Dis* 2008;**8**:159–66.
 33. Yu WL, Chuang YC, Walther-Rasmussen J. Extended-spectrum beta-lactamases in Taiwan: epidemiology, detection, treatment and infection control. *J Microbiol Immunol Infect* 2006;**39**:264–77.
 34. Rupp ME, Fey PD. Extended spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae: considerations for diagnosis, prevention and drug treatment. *Drugs* 2003;**63**:353–65.
 35. Medeiros AA. Evolution and dissemination of beta-lactamases accelerated by generations of beta-lactam antibiotics. *Clin Infect Dis* 1997;**24**(Suppl. 1):S19–45.
 36. Ramphal R, Ambrose PG. Extended-spectrum β -lactamases and clinical outcomes: current data. *Clin Infect Dis* 2006;**42**:S164–72.
 37. Wu YH, Chen PL, Hung YP, Ko WC. Risk factors and clinical impact of levofloxacin or cefazolin nonsusceptibility or ESBL production among uropathogens in adults with community-onset urinary tract infections. *J Microbiol Immunol Infect* 2014;**47**:197–203.
 38. Asensio A, Oliver A, Gonzalez-Diego P, Baquero F, Pérez-Díaz JC, Ros P, et al. Outbreak of a multiresistant *Klebsiella pneumoniae* strain in an intensive care unit: antibiotic use as risk factor for colonization and infection. *Clin Infect Dis* 2000;**30**:55–60.
 39. Dziekan G, Hahn A, Thüne K, Schwarzer G, Schäfer K, Daschner FD, et al. Methicillin-resistant *Staphylococcus aureus* in a teaching hospital: investigation of nosocomial transmission using a matched case-control study. *J Hosp Infect* 2000;**46**:263–70.
 40. Graffunder EM, Venezia RA. Risk factors associated with nosocomial methicillin-resistant *Staphylococcus aureus* (MRSA) infection including previous use of antimicrobials. *J Antimicrob Chemother* 2002;**49**:999–1005.