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Incidence of Extended-Spectrum β-Lactamases and Characterization of Integrons in Extended-Spectrum β-Lactamase-producing *Klebsiella pneumoniae* Isolated in Shantou, China

Fen YAO[1], Yuanshu QIAN[1]*, Shuzhen CHEN[2], Peifen WANG[2], and Yuanchun HUANG[2]

[1] *Department of Pharmacology, Shantou University Medical College, Shantou 515041, China;*
[2] *Department of Clinical Laboratory, First Affiliated Hospital, Shantou University Medical College, Shantou 515041, China*

Abstract This study is concerned with the level of antibiotic resistance of extended-spectrum β-lactamase (ESBL)-producing *Klebsiella pneumoniae*, isolated in Shantou, China, and its mechanism. Seventy-four non-repetitive clinical isolates of *K. pneumoniae* producing ESBLs were isolated over a period of 2 years. Antibiotic susceptibility, carried out by Epsilometer test, showed that most of the isolates were multiresistant. Polymerase chain reaction showed that, among the several types of β-lactamases, SHV was the most prevalent, TEM was the second most prevalent, and CTX-M was the least prevalent. Sixty-nine isolates were positive for integrase gene *IntI1*, but no *IntI2* or *IntI3* genes were found. The variable region of class 1 integrons were amplified and further identified by sequencing. Thirteen different gene cassettes and 11 different cassette combinations were detected. *Dfr* and *aadA* cassettes were predominant and cassette combinations *dfrA12*, *orfF* and *aadA2* were most frequently found. No gene cassettes encoding ESBLs were found. Integrons were prevalent and played an important role in multidrug resistance in ESBL-producing *K. pneumoniae*.

Key words *Klebsiella pneumoniae*; extended-spectrum β-lactamase (ESBL); integron; gene cassette

Klebsiella pneumoniae is an important hospital or community-acquired pathogen that is naturally susceptible to extended-spectrum cephalosporins (ESCs). However, strains resistant to these antibiotics mediated by extended-spectrum β-lactamases (ESBLs) have now spread worldwide. ESBLs contain several types of β-lactamases, including SHV, TEM, CTX-M and OXA [1]. Dissemination of antibiotic resistance genes by horizontal transfer has led to the rapid emergence of antibiotic resistance among clinical isolates. In the 1980s, genetic elements termed integrons were identified [2]. To date, at least eight classes of integrons, with different *Int* genes, have been described [3]. Among the different integron families, class 1 integrons are found to be most prevalent in drug-resistant bacteria [4]. Class 1 integrons are mobile DNA elements with a specific structure consisting of two conserved segments flanking a central region containing "cassettes" that usually code for resistance to specific antimicrobials [5]. The 5′-conserved segment contains the integrase gene (*IntI1*), a promoter region, and the *IntI1*-specific integration site *attI1*. The 3′-conserved segment usually contains a combination of the three genes *qacEΔ1* (antiseptic resistance), *sulI* (resistance to sulfonamides), and an open reading frame (*orf5*) of unknown function [6]. Between the two conserved segments, the central variable region can contain from zero to multiple cassettes [7]. The acquisition of resistance genes in bacteria is often facilitated by integrons. The presence of integrons among clinical *K. pneumoniae* isolates might account for multiple-antibiotic resistance.

In this study, we determined the incidence of ESBL-coding genes and characterized the different variable regions of the class 1 integrons in order to identify the

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*Corresponding author: Tel, 86-754-8900432; Fax, 86-754-855-7562; E-mail, g_fyao2000@yahoo.com.cn

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mechanism of resistance in clinical *K. pneumoniae* isolates.

Materials and Methods

Clinical isolates

From February 2001 to June 2003, 74 non-repetitive (one per patient) clinical isolates of *K. pneumoniae* producing ESBLs were isolated from hospitalized patients in the First Affiliated Hospital, Shantou University Medical College (Shantou, China). Twenty-three strains were isolated from the Department of Neurosurgery, 14 from the Neonatology Center, 11 from the Surgery Intensive Care Ward, 7 from the Department of Pediatrics, 5 from the Department of Neurology and 14 from other wards. Sputum was the most frequent type of sample (68 strains), followed by exudates (three strains), blood (one strain), urine (one strain), and stool (one strain). Production of ESBLs was determined by an agar dilution method and the double-disk synergy test by ceftazidime/cefotaxime with and without clavulanate on Mueller-Hinton agar. The results were interpreted according to Clinical and Laboratory Standards (CLSI) antimicrobial susceptibility testing standards (2006) [8].

Antimicrobial susceptibility determination

Minimal inhibitory concentrations to antimicrobial agents including cefotaxime, ceftazidime, ceftriaxone, cefepime, imipenem, gentamicin, amikacin, ciprofloxacin and tetracycline were determined. Epsilometer test (E-test) was carried out according to the manufacturer's recommendations with E-test strips (AB BIODISK, Solna, Sweden). *Escherichia coli* ATCC 35218 was used as the quality control strain.

Polymerase chain reaction (PCR), cloning, sequencing and protein analysis

Template DNA was prepared as follows: a cell pellet from 1.5 ml of overnight culture was resuspended in 500 μl of TE (10 mM Tris, 1 mM EDTA, pH 8.0) after centrifugation and boiling for 10 min. After centrifugation, the supernatant was used for PCR. The primers and conditions for PCR are listed in **Table 1** [9−15]. Strains containing the *IntI1* gene were subsequently subjected to PCR for amplification of the class 1 integron gene cassettes

Table 1 Primers and conditions of polymerase chain reaction used in this study

Primer	PCR primers (5′→3′)	Expected size (bp)	PCR conditions	PCR product	Ref.
SHV-F	GGGTTATTCTTATTTGTCGC	928	94 ºC, 5 min; 35 cycles of 94 ºC,	SHV-1, -2, -5, -7, -11,	9
SHV-R	TTAGCGTTGCCAGTGCTC		1 min, 58 ºC, 1 min, 72 ºC, 1 min	-12, -18, -26, -32, -33, -38, -44, -46, -49	
TEM-F	ATAAAATTCTTGAAGACGAAA	1080	94 ºC, 5 min; 35 cycles of 94 ºC,	TEM-1, -52, -71, -104,	10
TEM-R	GACAGTTACCAATGCTTAATCA		1 min, 58 ºC, 1 min, 72 ºC, 1 min	-105, -138, -151, -152	
CTX-M-F	ACGCTGTTGTTAGGAAGTG	759	94 ºC, 5 min; 35 cycles of 94 ºC,	CTX-M-1, -3, -12, -15,	11
CTX-M-R	TTGAGGCTGGGTGAAGT		45 s, 58 ºC, 45 s, 72 ºC, 1 min	-22, -30, -32, -33, -38, -52, -57, -58, -60, -61	
OXA-1-F	ACACAATACATATCAACTTCGC	813	94 ºC, 5 min; 35 cycles of 94 ºC,	OXA-1, -4, -30, -31, -47	12
OXA-1-R	AGTGTGTTTAGAATGGTGATC		1 min, 58 ºC, 1 min, 72 ºC, 1 min		
OXA-2-F	TTCAAGCCAAAGGCACGATAG	814	94 ºC, 5 min; 35 cycles of 94 ºC,	OXA-2, -3, -15, -21, -32	12
OXA-2-R	TCCGAGTTGACTGCCGGGTTG		45 s, 61 ºC, 45 s, 72 ºC, 1 min		
IntI1-F	CCTCCCGCACGATGA	281	94 ºC, 5 min; 35 cycles of 94 ºC,	*IntI1*	13
IntI1-R	TCCACGCATCGTCAG		45 s, 64 ºC, 45 s, 72 ºC, 1 min		
RB201	GCAAACGCAAGCATTCATTA	393	94 ºC, 5 min; 35 cycles of 94 ºC,	*IntI2*	14
RB202	ACGGATATGCGACAAAAAGG		1 min, 40 ºC, 1 min, 72 ºC, 1 min		
RB317	AACCTTGACCGAACGCAG	Uncertain	35 cycles of 94 ºC, 45 s, 59 ºC,	Variable region of	14
RB320	AGCTTAGTAAAGCCCTCGCTAG		45 s, 72 ºC, 3 min	class 1 integron	
IntI3-F	GCAGGGTGTGGACGAATACG	760	94 ºC, 5 min; 35 cycles of 94 ºC,	*IntI3*	15
IntI3-R	ACAGACCGAGAAGGCTTATG		1 min, 40 ºC, 1 min, 72 ºC, 1 min		

with primers RB317 and RB320 as described [13]. Amplicons of the same size obtained with primers RB317 and RB320 were digested with *Eco*RI, *Hin*dIII and *Bsp*I. PCR product with different restriction profiles was purified with a UNIQ-10 column PCR product purification kit (Sangon, Shanghai, China) and cloned into pUCm-T vector by T_4 ligase (Sangon). After incubation at 16 ºC for 1 h, ligation mixtures were used to transform into *E. coli* JM109. Transformants containing inserts were screened by blue/white colony on a Mueller-Hinton agar plate containing ampicillin (100 μg/ml), IPTG plus X-gal, then identified by PCR analysis. Recombinant plasmid DNA extracted from transformants was sequenced by Invitrogen (Shanghai, China). DNA sequences were translated into protein sequences using Web-based analysis tools (http://www.expasy.ch/tools/dna.html) then compared with the protein sequence of the GenBank database using the BLAST network service (http://www.ncbi.nlm.nih.gov/blast).

Results

Antimicrobial susceptibility determination

Most of the isolates were highly resistant (minimal inhibitory concentration>128 μg/ml) to gentamicin and amikacin. More than half of the isolates showed resistance or decreased susceptibility (intermediate resistance) to ESCs except cefepime. Although most of the isolates were multi-resistant (resistant to more than two classes of antibiotics), they all remained susceptible to imipenem (**Table 2**).

Table 2 Antibiotic susceptibility of extended-spectrum β-lactamase-producing *Klebsiella pneumoniae*

Antibiotic	S (%)	I (%)	R (%)
Cefotaxime	33 (44.6)	30 (40.6)	11 (14.9)
Ceftazidime	13 (17.6)	32 (43.2)	29 (39.2)
Ceftriaxone	32 (43.2)	3 (4.1)	39 (52.7)
Cefepime	52 (70.3)	10 (13.5)	12 (16.2)
Imipenem	74 (100.0)	0 (0.0)	0 (0.0)
Gentamicin	12 (16.2)	0 (0.0)	62 (83.8)
Amikacin	19 (25.7)	0 (0.0)	55 (74.3)
Ciprofloxacin	37 (50.0)	10 (13.5)	27 (36.5)
Tetracycline	13 (17.6)	0 (0.0)	61 (82.4)

I, intermediate resistant; R, resistant; S, susceptible.

Prevalence of ESBL-coding *IntI1*, *IntI2* and *IntI3* genes

Most of the isolates contained either *bla*SHV, *bla*TEM, or both. The *bla*SHV was amplified from 63 isolates, *bla*TEM was amplified from 39 isolates, *bla*CTX-M was amplified from 21 isolates, *bla*OXA-1 was amplified from six isolates, and *bla*OXA-2 was amplified from only one isolate. The combinations of genotypes of ESBLs are listed in **Table 3**. The *IntI1* gene was detected in 69 of the 74 isolates included in this study. *IntI2* and *IntI3* genes were not detected.

Characterization of cassette arrays

Twelve isolates containing the *IntI1* gene failed to produce an amplicon by RB317 and RB320. Thirteen different gene cassettes and 11 groups of variable segment were detected within the integrons (**Fig. 3**).

Table 4 showed an overview of the ESBLs and various cassettes arrays detected in isolates of different resistance phenotypes.

Discussion

The introduction of ESCs has facilitated effective treatment of severe infections caused by gram-negative bacteria. However, resistance to these agents increased in recent years and this correlated with the increasing use of ESCs [16]. According to the susceptibility test, imipenem and the fourth-generation cephalosporin, cefepime, showed better *in vitro* activity than third-generation cephalosporin, such as cefotaxime, ceftazidime and ceftriaxone to ESBL-producing *K. pneumoniae*.

Table 3 Genotypes of extended-spectrum β-lactamases (ESBLs) in ESBL-producing *Klebsiella pneumoniae*

Genotype	No. of strains (%)	Genotype	No. of strains (%)
*bla*SHV+*bla*TEM+*bla*CTX-M	8 (10.81)	*bla*TEM+*bla*CTX-M	1 (1.35)
*bla*SHV+*bla*TEM+*bla*OXA	4 (5.41)	*bla*SHV	12 (16.22)
*bla*SHV+*bla*TEM	25 (33.78)	*bla*TEM	1 (1.35)
*bla*SHV+*bla*CTX-M	11 (14.86)	*bla*CTX-M	1 (1.35)
*bla*SHV+*bla*OXA	3 (4.05)	Others	8 (10.81)

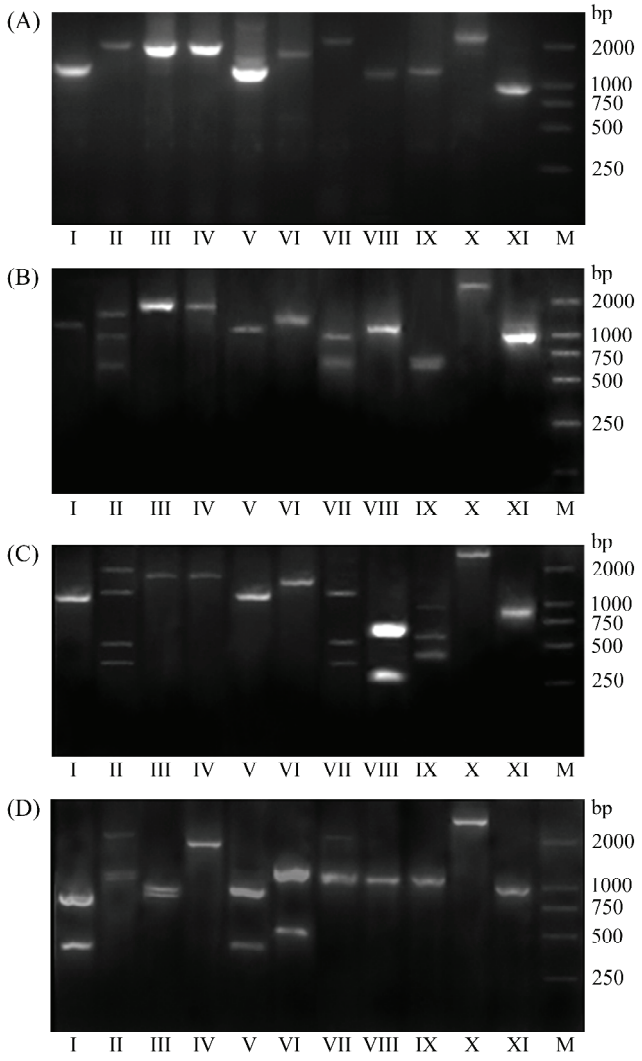

Fig. 1 Polymerase chain reaction (PCR) and restriction profiles of variable region of class 1 integron

PCR results (A) and *Eco*RI (B), *Hin*dIII (C) and *Bsp*I (D) restriction profiles of 11 groups of the variable segment of class 1 integron. M, marker.

Resistance to ESCs is primarily mediated by β-lactamases especially ESBLs and AmpC β-lactamases. To date, although a variety of ESBLs have been described, SHV, TEM and CTX-M enzymes are the three main types of EBSLs among members of the family *Enterobacteriaceae* [17]. In our study, SHV β-lactamase was most prevalent, TEM β-lactamase was the second most prevalent, and CTX-M β-lactamase was less than both. This prevalence of ESBLs appeared to be different from those seen in other areas of China [18,19]. In fact, ESBL-encoding genes in our study were not sequenced. Because primers for SHV and TEM β-lactamases can amplify non-ESBLs SHV-1 and TEM-1 β-lactamases, respectively, some SHV-positive and TEM-positive isolates might produce SHV-1 and TEM-1 β-lactamases [9,10].

The dissemination of antibiotic resistance genes among bacterial strains is an increasing problem in bacterial infections. Integron had become an important horizontal gene transfer system of resistance genes in clinical isolates. Incidence of class 1 integron was high in ESBL-producing *K. pneumoniae*. Twelve isolates containing the *IntI1* gene failed to produce an amplicon using primers RB317 and RB320. This was probably due to the lack of a 3′ conserved segment or the variable region was too long to be amplified in these isolates. This phenomenon had been reported previously [14].

Integron-positive isolates were more likely to be multi-resistant than integron-negative isolates [20]. Multiresistant integrons are considered to be important contributors to the development of antibiotic resistance among Gram-negative bacteria [21,22]. In our study, high prevalence of class 1 integron contributed to the multiresistance in most isolates. PCR sequencing analysis of the cassette arrays revealed a predominance of *dfr* and *aadA* cassettes that confer resistance to trimethoprim and aminoglycosides. The high incidence of *aadA* and *aacA* gene cassettes, confering resistance to aminoglycosides, was an important reason for the high prevalence of resistance to gentamicin and amikacin. The cassette combinations *dfrA*12, *orfF* and *aadA*2 were most frequently found in this study and also very prevalent in other areas. The reason for the wide distribution of some integrons with a specific cassette combination is so far unknown [23,24].

To date, genes resistant to nearly every major class of antibiotics including ESBL-coding genes such as *blaCTX-M*, *blaGES*, *blaOXA* and *blaVEB* integrated into integron had been reported, but *blaSHV* and *blaTEM* had not been found within integron [25–29]. In our study, although all the isolates exhibited ESBLs activity, no cassette encoding ESBLs was found, indicating that ESBL genes were not spread by integron. In our previous study, 37 isolates in this study had been typed by pulsed-field gel electrophoresis. Data showed that most of the isolates belong to a different genotype. Isolates in the same pulsed-field gel electrophoresis type had different resistance profiles, and most of them contained different types of ESBL-coding genes and different gene cassettes [30]. It seemed that clonal spread was not important for the dissemination of ESBLs and integron. As many ESBLs and integrons are on conjugative plasmids, horizontal spread by conjugation might be a major mechanism for their dissemination.

These data indicated that integrons were very prevalent

Table 4 Extended-spectrum β-lactamases (ESBLs) and various cassette arrays in isolates of different resistance phenotypes

Resistance phenotypes	Resistance profile	No. of isolates	PCR for ESBLs	Gene cassettes
I	CTX/CAZ/CRO, CFP,	2	SHV, TEM, CTX-M	*dfrA12-orfF-aadA2*
	GEN/AMK, CIP, TTC	1	SHV, TEM	*dfrA12-orfF-aadA2*
		1	SHV, CTX-M	*dfrA12-orfF-aadA2*
		1	CTX-M	*dfrA12-orfF-aadA2*
		1	SHV, TEM, OXA	*orfD-aacA4*
		1	SHV, TEM	*orfD-aacA4*
		1	SHV, OXA	*dfr17- aadA5*
		2	SHV, TEM, OXA	*dfr17- aadA4/aadA5*
		1	SHV, TEM	*drfA25*
		1	SHV, CTX-M	*dfrA12-aadA2*
		1	None	None
II	CTX/CAZ/CRO,	5	SHV, TEM	*dfrA12-orfF-aadA2*
	GEN/AMK, TTC	1	SHV, TEM	*drfA25*
		1	SHV, OXA	*aadA1*
		1	SHV, TEM	*orfD-aacA4*
		1	SHV	None
		1	SHV, TEM, OXA	*dfr17- aadA4/aadA5*
		2	SHV, TEM	None
		1	SHV, TEM, CTX-M	None
		1	SHV, CTX-M	*dfr17- aadA4/aadA5*
		1	SHV, TEM, CTX-M	*aadA2*
III	CTX/CAZ/CRO, CFP, TTC	1	TEM, CTX-M	None
IV	CTX/CAZ/CRO, CIP	1	SHV	None
V	CTX/CAZ/CRO	1	None	*arr3-aacA4*
VI	CTX/CAZ/CRO, GEN/AMK	2	SHV, CTX-M	*dfrA12-orfF-aadA2*
		1	SHV	None
		1	SHV	*drfA25*
VII	CTX/CAZ/CRO, CIP, TTC	1	None	*arr3-aacA4*
		1	None	None
VIII	CTX/CAZ/CRO, CFP	1	SHV, TEM	*dfrA12-orfF-aadA2*
IX	CTX/CAZ/CRO,CEP,	1	SHV, TEM, CTX-M	*dfrA12-orfF-aadA2*
	GEN/AMK	1	SHV, CTX-M	*dfrA12-orfF-aadA2*
X	CTX/CAZ/CRO, TTC	2	SHV	None
		1	SHV, TEM, CTX-M	None
		1	SHV	*aadA1*
		1	SHV	*aacA4-cmlA* variant
XI	CIP	1	SHV, TEM	*dfr17- aadA4/aadA5*
XII	GEN/AMK, TTC	1	None	*dfrA12-orfF-aadA2*
XIII	CTX/CAZ/CRO, CFP,	1	SHV, CTX-M	*dfrA12-orfF-aadA2*
	GEN/AMK, CIP			
XIV	TTC	1	None	*dfrA12-orfF-aadA2*
XV	CTX/CAZ/CRO,	3	SHV, TEM	*dfr17- aadA4/aadA5*
	GEN/AMK, CIP, TTC	3	SHV, CTX-M	*dfrA12-orfF-aadA2*
		1	SHV, TEM, CTX-M	*dfrA12-orfF-aadA2*
		2	SHV, TEM	*dfrA12-orfF-aadA2*
		1	SHV, TEM	*aadA2*
		1	None	*orfD-aacA4*
		1	SHV, OXA	*dfrA12-orfF-aadA2*
		1	SHV, TEM	*dhfrV*
		2	SHV	None
		1	SHV, TEM	None
		1	None	*dfrA12-orfF-aadA2*
		1	SHV, TEM	*dfrA12-aadA2*
XVI	GEN/AMK, CIP, TTC	1	TEM	*dfrA12-orfF-aadA2*
		1	SHV	*dfrA12-orfF-aadA2*
XVII	GEN/AMK	1	SHV	*dfrA12-orfF-aadA2*
XVIII	CTX/CAZ/CRO, CFP,	1	SHV, TEM	*dfrA12-aadA2*
	GEN/AMK, TTC	1	SHV, TEM, CTX-M	None
		1	SHV	*aadA1*
		1	SHV, CTX-M	None

AMK, amikacin; CAZ, ceftazidime; CFP, cefepime; CIP, ciprofloxacin; CRO, ceftriaxone; CTX, cefotaxime; GEN, gentamicin; PCR, polymerase chain reaction; TTC, tetracycline.

and played an important role in multidrug resistance in ESBL-producing *K. pneumoniae*. The production of ESBLs and integrons will continue to threaten the usefulness of antibiotics as therapeutic agents.

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References

1 Paterson DL, Bonomo RA. Extended-spectrum β-lactamases: A clinical update. Clin Microbiol Rev 2005, 18: 657–686
2 Stokes HW, Hall RM. A novel family of potentially mobile DNA elements encoding site-specific gene-integration functions: Integrons. Mol Microbiol 1989, 3: 1669–1683
3 Nield BS, Holmes AJ, Gillings MR, Recchia GD, Mabbutt BC, Nevalainen KM, Stokes HW. Recovery of new integron classes from environmental DNA. FEMS Microbiol 2001, 195: 59–65
4 Jones ME, Peters E, Weersink AM, Fluit A, Verhoef J. Widespread occurrence of integrons causing multiple antibiotic resistance in bacteria. Lancet 1997, 349: 1742–1743
5 Hall RM, Stokes HW. Integrons: Novel DNA elements which capture genes by site-specific recombination. Genetica 1993, 90: 115–132
6 Paulsen IT, Littlejohn TG, Radstrom P, Sundstrom L, Skold O, Swedberg G, Skurray RA. The 3′ conserved segment of integrons contains a gene associated with multidrug resistance to antiseptics and disinfectants. Antimicrob Agents Chemother 1993, 37: 761–768
7 Recchia GD, Hall RM. Gene cassettes: A new class of mobile element. Microbiology 1995, 141: 3015–3027
8 Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing, 16th informational supplement. CLSI/NCCLS M100-S16. Clinical and Laboratory Standards Institute, Wayne, Pennsylvania, USA. 2006
9 Rasheed JK, Jay C, Metchock B. Evolution of extended-spectrum beta-lactamase resistance (SHV-8) in a strain of *Escherichia coli* during multiple episodes of bacteremia. Antimicrob Agents Chemother 1997, 41: 647–653
10 Chang FY, Siu LK, Fung CP, Huang MH, Ho M. Diversity of SHV and TEM β-lactamases in *Klebsiella pneumoniae*: Gene evolution in Northern Taiwan and novel β-lactamases, SHV-5 and SHV-6. Antimicrob Agents Chemother 2001, 45: 2407–2413
11 Lu J, Tang YC, Wu BQ, Zhang KX, Zhang TT, Bi XG, Zhu JX *et al*. Genotype characterization of plasmid mediated extended-spectrum β-lactamases in Southern China. Chin J Microbiol Immunol 2002, 22: 638–643
12 Steward CD, Rasheed JK, Hubert SK, Biddle JW, Raney PM, Anderson GJ, Williams PP *et al*. Characterization of clinical isolates of *Klebsiella pneumoniae* from 19 laboratories using the National Committee for Clinical Laboratory Standards extended-spectrum β-lactamase detection methods. J Microbiol 2001, 39: 2864–2872
13 Zhao S, White DG, Ge B, Ayers S, Friedman S, English L, Wagner D *et al*. Identification and characterization of integron-mediated antibiotic resistance among Shiga toxin-producing *Escherichia coli* isolates. Appl Environ Microbiol 2001, 67: 1558–1564
14 Barlow RS, Pemberton JM, Desmarchelier PM, Gobius KS. Isolation and characterization of integron-containing bacteria without antibiotic selection. Antimicrob Agents Chemother 2004, 48: 838–842
15 Senda K, Arakawa Y, Ichiyama S, Nakashima K, Ito H, Ohsuka S, Shimokata K *et al*. PCR detection of metallo-β-lactamase gene (*bla*IMP) in gram-negative rods resistant to broad-spectrum β-lactams. J Clin Microbiol 1996, 34: 2909–2913
16 Muller A, Lopez-Lozano JM, Bertrand X, Talon D. Relationship between ceftriaxone use and resistance to third-generation cephalosporins among clinical strains of *Enterobacter cloacae*. J Antimicrob Chemother 2004, 54: 173–177
17 Bradford PA. Extended-spectrum β-lactamases in the 21st century: Characterization, epidemiology, and detection of this important resistance threat. Clin Microbiol Rev 2001, 14: 933–951
18 Xiong Z, Zhu D, Wang F, Zhang Y, Okamoto R, Inoue M. Investigation of extended-spectrum β-lactamase in *Klebsiella pneumoniae* and *Escherichia coli* from China. Diagn Microbiol Infect Dis 2002, 44: 195–200
19 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: 790–793
20 Martinez-Freijo P, Fluit AC, Schmitz FJ, Grek VS, Verhoef J. Class I integrons in Gram-negative isolates from different European hospitals and association with decreased susceptibility to multiple antibiotic compounds. J Antimicrob Chemother 1998, 42: 689–696
21 Carattoli A. Importance of integrons in the diffusion of resistance. Vet Res 2001, 32: 243–259
22 Fluit AC, Schmitz FJ. Class 1 integrons, gene cassettes, mobility, and epidemiology. Eur J Clin Microbiol Infect Dis 1999, 18: 761–770
23 Lee JC, Oh JY, Cho JW, Park JC, Kim JM, Seol SY, Cho DT. The prevalence of trimethoprim-resistance-conferring dihydrofolate reductase genes in urinary isolates of *Escherichia coli* in Korea. J Antimicrob Chemother 2001, 47: 599–604
24 Sunde M. Prevalence and characterization of class 1 and class 2 integrons in *Escherichia coli* isolated from meat and meat products of Norwegian origin. J Antimicrob Chemother 2005, 56: 1019–1024
25 Bonnet R. Growing group of extended-spectrum β-lactamases: The CTX-M enzymes. Antimicrob Agents Chemother 2004, 48: 1–4
26 Giuliani F, Docquier JD, Riccio ML, Pagani L, Rossolini GM. OXA-46, a new class D β-lactamase of narrow substrate specificity encoded by a bla_{VIM-1}-containing integron from a *Pseudomonas aeruginosa* clinical isolate. Antimicrob Agents Chemother 2005, 49: 1973–1980
27 Poirel L, Brinas L, Fortineau N, Nordmann P. Integron-encoded GES-type extended-spectrum β-lactamase with increased activity toward aztreonam in *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 2005, 49: 3593–3597
28 Naas T, Aubert D, Lambert T, Nordmann P. Complex genetic structures with repeated elements, a *sul*-type class 1 integron, and the bla_{VEB} extended-spectrum β-lactamase gene. Antimicrob Agents Chemother 2006, 50: 1745–1752
29 Machado E, Canton R, Baquero F, Galan JC, Rollan A, Peixe L, Coque TM. Integron content of extended-spectrum-β-lactamase-producing *Escherichia coli* strains over 12 years in a single hospital in Madrid, Spain. Antimicrob Agents Chemother 2005, 49: 1823–1829
30 Yao F, Chen SZ, Cai YM, Qian YS. Antimicrobial resistance of extended-spectrum β-lactamases-producing *Klebsiella pneumoniae* and their genotyping by pulsed-field gel electrophoresis. Chin J Antibiotics 2004, 29: 290–292

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Jan WALTHER-RASMUSSEN