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Characterization of Trimethoprim-Sulfamethoxazole Resistance Genes and Their Relatedness to Class 1 Integron and Insertion Sequence Common Region in Gram-Negative Bacilli

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Trimethoprim-sulfamethoxazole (TMP-SMX) has been used for the treatment of urinary tract infections, but increasing resistance to TMP-SMX has been reported. In this study, we analyzed TMP-SMX resistance genes and their relatedness with integrons and insertion sequence common regions (ISCRs) in uropathogenic gram-negative bacilli. Consecutive non-duplicate TMP-SMX nonsusceptible clinical isolates of *E. coli*, *K. pneumoniae*, *Acinetobacter* spp., and *P. aeruginosa* were collected from urine. The minimal inhibitory concentration was determined by Etest. TMP-SMX resistance genes (*sul* and *dfr*), integrons, and ISCRs were analyzed by PCR and sequencing. A total of 45 *E. coli* (37.8%), 15 *K. pneumoniae* (18.5%), 12 *Acinetobacter* spp. (70.6%), and 9 *Pseudomonas aeruginosa* (30.0%) isolates were found to be resistant to TMP-SMX. Their MICs were all over 640. In *E. coli* and *K. pneumoniae*, *sul*1 and *dfr* genes were highly prevalent in relation with integron1. The *sul*3 gene was detected in *E. coli*. However, in *P. aeruginosa* and *Acinetobacter* spp., only *sul*1 was prevalent in relation with class 1 integron; however, *dfr* was not detected and *sul*2 was less prevalent than in *Enterobacteriaceae*. ISCR1 and/or ISCR2 were detected in *E. coli*, *K. pneumoniae*, and *Acinetobacter* spp. but the relatedness with TMP-SMX resistance genes was not prominent. ISCR14 was detected in six isolates of *E. coli*. In conclusion, resistance mechanisms for TMP-SMX were different between *Enterobacteriaceae* and glucose non-fermenting gram-negative bacilli. Class 1 integron was widely disseminated in uropathogenic gram-negative baciili, so the adoption of prudent use of antimicrobial agents and the establishment of a surveillance system are needed.

Keywords: Gram negative bacteria, insertion sequence common regions, integrons, trimethoprim-sulfamethoxazole

Introduction

Urinary tract infections (UTIs) have been reported to be the most common hospital acquired infection, which are associated with significant morbidity and mortality [9, 10]. The predominant causative pathogen of UTIs is *Escherichia coli*; however, there have been increasing reports of other *Enterobacteriaceae* such as *Klebsiella pneumoniae* and gram-negative non-fermenters such as *Acinetobacter* spp., and *Pseudomonas aeruginosa* as causes of UTIs [5, 7, 15]. Trimethoprim-sulfamethoxazole (TMP-SMX) has been used for several decades as efficient antibiotics for the treatment of UTIs [11]. In many countries, however, the presence of resistance to TMP-SMX can lead to treatment failure in cases of UTIs [9]. Sulfonamide resistance in gram-negative bacilli generally arises from the acquisition of dihydropteroate synthase (DHPS) genes in integrons that are not inhibited by the drug [11]. Currently, three different types of DHPS genes (*sul*1, *sul*2, and *sul*3) are known [9]. The *sul*1 gene is found linked to other resistance genes in class 1 integrons and on large conjugative plasmids [25], while *sul*2 is usually located on small nonconjugative plasmids [21],

large transmissible multiresistance plasmids [9], or through insertion element common region (ISCR2) element [24]. Although rare, *sul*3, a plasmid-borne sulfonamide resistance gene, is also present [25].

TMP affects bacterial folic acid synthesis by the inhibition of dihydrofolate reductase (DHFR), which catalyzes the reduction of dihydrofolate to tetrahydrofolate [11]. There are several mechanisms of TMP resistance, such as development of permeability barriers, efflux pumps, existence of naturally insensitive target DHFR enzymes, mutational and regulation changes in target enzymes, and the acquirement of drug-resistant target enzymes [11]. Among them, the acquirement of DHFR variants encoded by *dfr* genes is the most common mechanism for TMP resistance, which results in high-level resistance in various bacteria [20]. To date, more than 30 different *dfr* genes are known, which are usually found in gene cassettes within integrons [3, 20] and are also associated with ISCR1 [12].

Although several literatures studied *sul* and/or *dfr* genes in relation to class 1 integron in *E. coli* [9, 13, 19, 20], there are limited reports investigating the prevalence of TMP-SMX resistance genes in relation to integrons and ISCRs in other *Enterobacteriaceae* such as *Klebsiella pneumoniae* and gram-negative glucose non-fermenters such as *Acinetobacter* spp., and *P. aeruginosa* in Korea. Therefore, in this present study, we investigated the prevalence of TMP-SMX resistance and *sul* genes and *dfr* genes in various uropathogenic gram-negative bacilli and their association with class 1 integrons and ISCRs.

Materials and Methods

Bacterial Isolates

Consecutive non-duplicate TMP-SMX nonsusceptible clinical isolates of *E. coli*, *K. pneumoniae*, *Acinetobacter* spp., and *P. aeruginosa* were obtained from urine specimens collected at Chungnam National University Hospital from September 2011 to September 2013. All isolates were identified using the Vitek 2 automated ID system (bioMèrieux Vitek Inc., Hazelwood, MO, USA).

Antimicrobial Susceptibility Testing

The MIC for trimethoprim-sulfamethoxazole was determined by the Etest, conducted in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines [16]. *E. coli* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853 were used as control strains.

PCR Amplification and Sequencing for *sul* Genes and ISCRs

Bacterial genomic DNA was obtained from each target strain by using a genomic DNA extraction kit (Bioneer, Daejeon, Korea) according to the manufacturer's instructions.

Bacterial genomic DNA was amplified by PCR. The PCR was performed using 50 ng of bacterial whole DNA, 2.5 µl of 10× Taq buffer, 0.5 µl of 10 mM dNTP mix, 20 pmol of each primer, and 0.7 U of Taq DNA polymerase (SolGent, Daejeon, Korea), in a total volume of 25 µl. Each target site was amplified in a SEEAMP (Seegene, Seoul, Korea). The amplified products were separated by electrophoresis on 1.5% (w/v) agarose gels containing ethidium bromide, and visualized using a BioDoc-14 imaging system (UVP, Cambridge, UK). The amplicons were purified with a PCR purification kit (SolGent), and sequenced using a BigDye Terminator Cycle Sequencing kit (PE Applied Biosystems, Foster City, CA, USA) and an ABI PRISM 3730XL DNA analyzer (PE Applied Biosystems).

The following primers were used for the PCR and sequencing of *sul* genes: sul1F (5′-CTTCGATGAGAGCCGGCGGC-3′) and sul1R (5′-GCAAGGCGGAAACCCGCGCC-3′) [1], sul2F (5′-GCGCTCAAGGCAGATGGCATT-3′) and sul2R (5′-GCGTTTGATACCGGCACCCGT-3′) [1], and sul3F (5′-GAGCAAGATTTTTGGAATCG-3′) and sul3R (5′-CTAACCTAGGGCTTTGGATAT-3′) [18]. For detection of all ISCRs, the following primers were used: CRF (5′-CACTWCCACATGCTGTKKC-3′) and CRFF-r (5′-CGCTTGAGSCGTTGCRYCC-3′) [24].

Identification of Integrons and *dfr* Genes

Class 1 integrons were amplified using the primers hep58 (5′-TCATGGCTTGTTATGACTGT-3′) and hep59 (5′-GTAGGGCTTATTATGCACGC-3′). Class 2 integrons were amplified using the primers hep51 (5′-GATGCCATCGCAAGTACGAG-3′) and hep74 (5′-CGGGATCCCGGACGGCATGCACGATTTGTA-3′) [26]. Class 3 integrons were amplified using the primers Int3F (5′-GCCTCCGGCAGCGACTTTCAG-3′) and Int3R (5′-ACGGATCTGCCAAACCTGACT-3′) [22].

Only trimethoprim resistance genes, *dfr*, located inside class 1 integrons were characterized. Sequencing of purified class 1 integron amplicons were performed using the forward primer hep58. When sequencing with the forward primer revealed no *dfr* alleles, samples were resubmitted with the reverse primer to provide a complete sequence. Class 1 integron amplicons sequence were compared with published *dfr* allele sequences from GenBank.

Results

Bacterial Isolates and Antimicrobial Susceptibility Testing

During the study period, 119 *E. coli*, 81 *K. pneumoniae*, 17 *Acinetobacter* spp. isolates, and 29 *P. aeruginosa* isolates were collected from urine specimens. Among these isolates, 45 *E. coli* (37.8%), 15 *K. pneumoniae* (18.5%), 12 *Acinetobacter* spp. (70.6%), and 9 *Pseudomonas aeruginosa* (30.0%) isolates were found to be resistant to TMP-SMX. MIC90 values were >640 in all of the isolates.

Table 1. Prevalence of *sul* and *dfr* genes among trimethoprim-sulfamethoxazole resistant isolates from urine specimens.

Isolates	*sul*						*dfr*						
	sul	*sul*1 only	*sul*2 only	*sul*1 + *sul*2	*sul*3 only	None	*dfr*	*dfr*A17	*dfr*A12	*dfr*A1	*dfr*A27	*dfr*A5	None
E. coli (45)	45	10 (22%)	10 (22%)	24 (53.3%)	1	0	34	27 (60%)	3	2	1	1	11 (24.4%)
K. pneumoniae (15)	15	2 (18.8%)	5 (31.2%)	8 (50.0%)	0	0	7	0	0	7 (43.8%)	0	0	7 (43.8%)
Acinetobacter spp. (13)	13	9 (69.2%)	3 (23.1%)	1 (7.7%)	0	0	0	0	0	0	0	0	12 (100%)
P. aeruginosa (9)	9	9 (100.0%)	0	0	0	0	0	0	0	0	0	0	28 (100%)

Characterization of TMP-SMX Resistance Genes and Their Relatedness to Class 1 Integrons and ISCRs

In 45 *E. coli* isolates, 10 isolates contained only the *sul*1 gene and 10 other isolates contained only the *sul*2 gene. Twenty-four isolates carried both the *sul*1 and *sul*2 genes (Table 1). Class 1 gene cassettes were detected in 34 (75.6%) of 45 isolates. Various gene cassette arrays of *dfr*A17–*aad*A5 (27 isolates), *dfr*A12-*aad*A2 (3 isolates), *dfr*A1-*aad*A1 (2 isolates), *aac*A4-*arr*3-*dfr*A27 (1 isolate), *aad*A2 (1 isolate) were found and are shown in Table 2. Among 34 isolates with the *sul*1 gene, 33 isolates (97.1%) were found to carry class 1 integrons. Thirty-four isolates (75.6%) carried *dfr* genes, most of which were found within gene cassette arrays of class 1 integron. An ISCR was detected in 4 isolates with *sul*1, 1 isolate with *sul*2, and 5 isolates with *sul*1 and *sul*2. Among 10 isolates with an ISCR, one isolate with ISCR1 had class 1 integron, and 2 isolates with ISCR2 had class 1 integron and *sul*1 and/or *sul*2. One isolate had ISCR3, and 6 isolates contained ISCR14 (Table 3).

In 15 *K. pneumoniae* isolates, *sul*1 and *sul*2 were detected in 2 and in 5 isolates, respectively, and 8 isolates contained both *sul*1 and *sul*2 (Table 1). All isolates with *sul*1 had class 1 integron with various gene cassette arrays of *dfr*A1-*ofr*C (6), *aad*A2 (2), *aac*(6')-Ib-*oxa*-1-*aad*A2 (1), and *dfr*A1-*aad*A1 (1) (Table 2). Eight isolates contained *dfr* genes, all of which were within the gene cassette array of class 1 integron. ISCR2 was detected in one isolate with the *sul*2 gene.

In 13 *Acinetobacter* spp., 9 and 3 isolates had *sul*1 and *sul*2, respectively, and one isolate had both *sul*1 and *sul*2 (Table 1). All 9 isolates with *sul*1 carried class 1 integron with gene cassette arrays with *aac*A4-*cat*B8-*aad*A1 (6) and *aac*A44-IMP1-*oxa*-2 (3) (Table 2). Three and 2 isolates had ISCR1 and ISCR2, respectively, and 2 isolates with ISCR1 contained the class 1 integon and *sul*1 gene, and 1 isolate with ISCR2 contained the *sul*2 gene.

In 9 *P. aeruginosa* isolates, 9 isolates contained *sul*1 (Table 1). Among those isolates, 8 carried the class 1 integron with cassette arrays of *aad*B–*cml*A-*oxa*-10-*aad*A1

Table 2. Gene cassette arrays in class 1 integrons from trimethoprim-sulfamethoxazole resistant isolates from urine specimens.

Isolates	Gene cassette arrays (No. of isolates)	*sul*1 only	*sul*2 only	*sul*1 + *sul*2	*sul*3
E. coli (34)	*dfr*A17-*aad*A5 (27)	5		22	
	*dfr*A12-*aad*A2 (3)	3			
	*dfr*A1-*aad*A1 (2)	2			
	*aad*A2 (1)				1
	*aac*A4-*arr*3-*dfr*A27 (1)	1			
K. pneumoniae (10)	*dfr*A1-*ofr*C (6)	1		5	
	*aad*A2 (2)		1	1	
	aac(6′)-Ib-*oxa*-1-*aad*A2 (1)			1	
	*dfr*A1-*aad*A1 (1)	1			
Acinetobacter spp. (10)	*aac*A4-*cat*B8-*aad*A1 (7)	6		1	
	*aac*A44-IMP1-*oxa*-2 (3)	3			
P. aeruginosa (8)	*aad*B-*cml*A-*oxa*-10-*aad*A1 (7)	7			
	*aad*A2 (1)	1			

Table 3. Distribution of ISCR, integrons, and *sul* genes among trimethoprim-sulfamethoxazole resistant isolates from urine specimens.

Isolates	Class I integron	ISCR	*sul*1	*sul*2	*sul*1 + *sul*2	*sul*3
E. coli	*dfr*A17-*aad*A5	ISCR3			1	
		ISCR14			2	
		ISCR2			1	
		None	4	1	18	
	*dfr*A12-*aad*A2	ISCR14	2			
		ISCR2	1			
	*aac*A4-*arr*3-*dfr*A27	ISCR1	1			
	*aad*A2	None				1
	*dfr*A1-*aad*A1	None	2			
	None	ISCR14			1	
		ISCR14		1		
	None	None		7	1	

Isolates	Class I integron	ISCR	*sul*1	*sul*2	*sul*1 + *sul*2	*sul*3
K. pneumoniae	*dfr*A1-*ofr*C	ISCR2	1			
		None			5	
	*aad*A2	None			2	
	*dfr*A1-*aad*A1	None	1			
	aac(6')-Ib-*oxa*-1-*aad*A2	None			1	
	None	ISCR2		1		
		None		4		

Isolates	Class I integron	ISCR	*sul*1	*sul*2	*sul*1 + *sul*2	*sul*3
Acinetobacter spp.	*aac*A4-*cat*B8-*aad*A1	ISCR1	1			
		ISCR2	1			
		None	6		1	
	*aac*A44-IMP1-*oxa*-2	ISCR1	1			
		None	2			
	None	ISCR1		1		
		ISCR2		1		
		None		1		

(7) and *aad*A2 (1) (Table 2). No isolates were found to carry the *sul*2 gene and ISCR.

The *dfr* gene was not found in the any *Acinetobacter* spp. and *P. aeruginosa* isolates.

Discussion

Recent rise in TMP-SMX resistance is thought to be due to horizontal gene transfer and clonal expansion [2]. In this study, we evaluated genes related to TMP-SMX resistance and their relatedness with the class 1 integron and ISCR in uropathogenic gram-negative bacilli.

According to our study, the prevalence of the class 1 integron in TMP-SMX-resistant *E. coli* isolates was 73.3% (34 out of 45 isolates), which was increased in comparison with previous reports in Korea [13, 28]. The most prevalent class 1 gene cassette array was *drf*A17-*aad*A5 (79.4%), which was found to be the most prevalent one in Korea [13, 28] and also in China [23], but different from the gene cassette array found in other parts of the world [2]. Class 1 integrons carrying a single gene cassette were prevalent in clinical *E. coli* isolates from the 1980s, whereas class 1 integrons carrying multigene cassettes were prevalent in clinical isolates from the 1990s and 2000s in Korea [13, 28],

indicating that class 1 integrons in *E. coli* facilitated acquisition of resistance to a broad spectrum of antibiotic agents [23]. Therefore, it was found that the class 1 integron with multiple resistance genes was more widely distributed in Korea, which directly contributed to the resistance not only to trimethoprim and sulfamethoxazole, but also to other antibiotics. The TMP-SMX resistance genes *sul*1, *sul*2, and *dfr* were found to be widely disseminated in *E. coli* (Table 1). Isolates with *sul*1 and *dfr* genes showed significant relatedness with class 1 integron in accordance with other studies [2, 8, 13, 20, 23], whereas *sul*2 did not show any relationship with class 1 integron, suggesting other transfer mechanisms. Interestingly, in most of the *E. coli* isolates in our study, the allele frequencies of the *sul*1 and *sul*2 genes were exactly the same. This finding was somewhat different from the previous literature in which the gene frequency distribution was reported as *sul*2 > *sul*1 > *sul*3 [2]. However, in recent study, the pattern of increased *sul*1 frequency over *sul*2 was observed [9]. This might be caused by wide dissemination of the class 1 integron, which is in close relationship with the *sul*1 gene. The *sul*3 gene was also detected in association with class 1 integron (*aad*A2), which was the first report in South Korea.

With *K. pneumoniae*, the prevalence of *sul*1 and *sul*2 genes was similar to that in *E. coli*. The class 1 integron was also highly prevalent in *K. pneumoniae* (73.3%) and was strongly associated with the *sul*1 and *dfr* genes as in *E. coli*, while different gene cassette arrays were shown. The dominant gene cassette array was *dfr*A1-*ofr*C, which was similar in other literature [14]. Thus, class 1 integrons with various gene cassette arrays in association with *sul*1 and *dfr* genes were highly prevalent in *Enterobacteriaceae*, and the variation of the gene cassettes in class 1 integrons may reflect the horizontal transfer of integrons among members of the *Enterobacteriacea*e family [14].

In contrast to *Enterobacteriacea*e, the prevalence of *sul*1 was much higher than that of *sul*2 in glucose non-fermenting gram-negative bacilli. Class 1 integron was also widely disseminated with different gene cassette arrays among different species as in *Enterobacteriacea*e, but *dfr* genes were not detected in any of the isolates. Thus, the mechanism of resistance to TMP-SMX in gram-negative non-fermenting bacilli was significantly different from *Enterobacteriacea*e.

The ISCR has been found in numerous gram-negative organisms and identified as being closely associated with the spread of many antibiotic resistance genes. ISCR elements in relation to TMP-SMX resistance genes are ISCR1 in relation to *dfr*A1, 12, and 17 gene cassettes in class 1 integrons, and ISCR2 in relation to the *sul2* gene [26]. In our study, the prevalence of ISCRs in *E. coli* was 22.2% (10 out of 45 isolates), 13.3% (2 out of 15) in *K. pneumoniae*, and 38.5% (5 out of 13) in *Acinetobacter* spp. Among 6 isolates with ISCR2, 3 isolates harbored *sul*2, but among 3 isolates with ISCR1, we could not find related gene cassettes of *dfr*A1, 12, and 17. Therefore, in terms of TMP-SMX resistance, ISCRs did not show a strong association than class 1 integron did in our study. Interestingly, ISCR14, known to be related to *rmt*D and *erm*(B), was detected in 6 *E. coli* isolates [17]. In *K. pneumoniae* and *P. aeruginosa*, *rmt*D, a 16S ribosomal RNA methyltransferase gene conferring high level of resistance to aminoglycosides, was found [4, 27]. The existence of ISCR14 raises the possibility of the presence of *rmt*D in *E. coli*. Consequently, further study is warranted to uncover the possibility of ISCRs for carrier of resistance genes, especially in *E. coli* and other gram-negative bacilli.

In conclusion, resistance to TMP-SMX in *Enterobacteriacea*e, *E. coli*, and *K. pneumoniae* was explained by the acquisition of *sul*1, *sul*2, and *dfr* genes. In most of the cases, class 1 integrons with various multigene cassette arrays in association with *sul*1 and *dfr* genes were widely disseminated in uropathogenic gram-negative bacilli. Dominant gene cassette arrays were different between *E. coli* and *K. pneumoniae*. On the other hand, resistance to TMP-SMX in glucose non-fermenting gram-negative bacilli, *P. aeruginosa* and *Acinetobacter* spp., was explained by either the *sul*1 gene in *P. aeruginosa* or the *sul*1 and *sul*2 genes in *Acinetobacter* spp. Class 1 integrons were also widely distributed among these isolates in relation to the *sul*1 gene. Dominant gene cassette arrays were also different among species. Interestingly, the *dfr* gene, which was observed in relation to the class 1 integron in *Enterobacteriaceae*, was not observed in any isolates of glucose non-fermenting gram-negative bacilli. Several ISCRs were observed in *Enterobacteriaceae* and glucose non-fermenting gram-negative bacilli, but we could not find strong relatedness between TMP-SMX resistance genes.

In summary, the class 1 integron carrying multigene was highly prevalent among gram-negative bacilli, but mechanisms of TMP-SMX resistance were different between *Enterobacteriaceae* and glucose non-fermenting gram-negative bacilli. The wide dissemination of integrons may be because of the horizontal transfer of antibiotic resistance gene cassettes, so the adoption of prudent use of antimicrobial agents and the establishment of a surveillance system are needed for further dissemination of the class 1 integron in gram-negative bacilli.

References

1. Aarestrup FM, Lertworapreecha M, Evans MC, Bangtrakulnonth A, Chalermchaikit T, Hendriksen RS, *et al*. 2003. Antimicrobial susceptibility and occurrence of resistance genes among *Salmonella enterica* serovar Weltevreden from different countries. *J. Antimicrob. Chemother.* **54:** 715-718.
2. Blahna MT, Zalewski CA, Reuer J, Kahlmeter G, Foxman B, Marrs CF. 2006. The role of horizontal gene transfer in the spread of trimethoprim-sulfamethoxazole resistance among uropathogenic *Escherichia coli* in Europe and Canada. *J. Antimicrob. Chemother.* **57:** 666-672.
3. Cambray G, Guerout AM, Mazel D. 2010. Integrons. *Annu. Rev. Genet*. **44:** 141-166.
4. Castanheira M, Fritsche TR, Sader HS, Jones RN. 2008. RmtD 16S RNA methylase in epidemiologically unrelated spm-1-producing *Pseudomonas aeruginosa* isolates from Brazil. *Antimicrob. Agents Chemother*. **52:** 1587-1588.
5. Djordjevic Z, Folic MM, Zivic Z, Markovic V, Jankovic SM. 2013. Nosocomial urinary tract infections caused by *Pseudomonas aeruginosa* and *Acinetobacter* species: sensitivity to antibiotics and risk factors. *Am. J. Infect. Control* **41:** 1182-1187.
6. Enne VI, Livermore DM, Stephens P, Hall LM. 2001. Persistence of sulphonamide resistance in *Escherichia coli* in the UK despite national prescribing restriction. *Lancet* **357:** 1325-1328.
7. Foxman B. 2010. The epidemiology of urinary tract infection. *Nat. Rev. Urol.* **7:** 653-660.
8. Frank T, Gautier V, Talarmin A, Bercion R, Arlet G. 2007. Characterization of sulphonamide resistance genes and class 1 integron gene cassettes in *Enterobacteriaceae*, Central African Republic (CAR). *J. Antimicrob. Chemother.* **59:** 742-745.
9. Gundogdu A, Long YB, Vollmerhausen TL, Katouli M. 2011. Antimicrobial resistance and distribution of *sul* genes and integron-associated *intI* genes among uropathogenic *Escherichia coli* in Queensland, Australia. *J. Med. Microbiol.* **60:** 1633-1642.
10. Hsueh PR, Hoban DJ, Carmeli Y, Chen SY, Desikan S, Alejandria M, *et al*. 2011. Consensus review of the epidemiology and appropriate antimicrobial therapy of complicated urinary tract infections in Asia-Pacific region. *J. Infect.* **63:** 114-123.
11. Huovinen P. 2001. Resistance to trimethoprim-sulfamethoxazole. *Clin. Infect. Dis*. **32:** 1608-1614.
12. Ilyina TS. 2012. Mobile ISCR elements: structure, functions, and role in emergence, increase, and spread of blocks of bacterial multiple antibiotic resistance genes. *Mol. Gen. Mikrobiol. Virusol*. **4:** 3-13.
13. Kang HY, Jeong YS, Oh JY, Tae SH, Choi CH, Moon DC, *et al*. 2005. Characterization of antimicrobial resistance and class 1 integrons found in *Escherichia coli* isolates from humans and animals in Korea. *J. Antimicrob. Chemother*. **55:** 639-644.
14. Li B, Hu Y, Wang Q, Yi Y, Woo PC, Jing H, *et al*. 2013. Structural diversity of class 1 integrons and their associated gene cassettes in *Klebsiella pneumoniae* isolates from a hospital in China. *PLoS One* **30:** e75805.
15. Mittal R, Aggarwal S, Sharma S, Chhibber S, Harjai K. 2009. Urinary tract infections caused by *Pseudomonas aeruginosa*: a minireview. *J. Infect. Public Health* **2:** 101-111.
16. National Committee for Clinical Laboratory Standards. 2012. *Performance Standards for Antimicrobial Disk Susceptibility Tests*; Approved Standard—Eleventh Edition. M02-A11. Wayne, PA.
17. Partridge SR. 2011. Analysis of antibiotic resistance regions in gram-negative bacteria. *FEMS Microbiol Rev*. **35:** 820-855.
18. Perreten V, Boerlin P. 2003. A new sulfonamide resistance gene (*sul3*) in *Escherichia coli* is widespread in the pig population of Switzerland. *Antimicrob. Agents Chemother*. **47:** 1169-1172.
19. Rao AN, Barlow M, Clark LA, Boring JR 3rd, Tenover FC, McGowan JE Jr. 2006. Class 1 integrons in resistant *Escherichia coli* and *Klebsiella* spp., US hospitals. *Emerg. Infect. Dis*. **12:** 1101-1104.
20. Seputiené V, Povilonis J, Ruzauskas M, Pavilonis A, Suziedéliené E. 2010. Prevalence of trimethoprim resistance genes in *Escherichia coli* isolates of human and animal origin in Lithuania. *J. Med. Microbiol.* **59:** 315-322.
21. Skold O. 2000. Sulfonamide resistance: mechanisms and trends. *Drug Resist.* **3:** 155-160.
22. Srinivasan VB, Rajamohan G, Pancholi P, Stevenson K, Tadesse D, Patchanee P, *et al*. 2009. Genetic relatedness and molecular characterization of multidrug resistant *Acinetobacter baumannii* isolated in central Ohio, USA. *Ann. Clin. Microbiol. Antimicrob.* **8:** 21.
23. Su J, Shi L, Yang L, Xiao Z, Li X, Yamasaki S. 2006. Analysis of integrons in clinical isolates of *Escherichia coli* in China during the last six years. *FEMS Microbiol. Lett.* **254:** 75-80.
24. Toleman MA, Bennett PM, Bennett DM, Jones RN, Walsh TR. 2007. Global emergence of trimethoprim/sulfamethoxazole resistance in *Stenotrophomonas maltophilia* mediated by acquisition of *sul* genes. *Emerg. Infect. Dis.* **13:** 559-565.
25. Trobos M, Christensen H, Sunde M, Nordentoft S, Agersø Y, Simonsen GS, *et al*. 2009. Characterization of sulphonamide-resistant *Escherichia coli* using comparison of *sul2* gene sequences and multilocus sequence typing. *Microbiology* **155:** 831-836.
26. White PA, McIver CJ, Rawlinson WD. 2001. Integrons and gene cassettes in the *Enterobacteriaceae*. *Antimicrob. Agents Chemother*. **45:** 2658-2661.
27. Yamane K, Rossi F, Barberino MGMA, Adams-Haduch JM, Doi Y, Paterson DL. 2008. 16S ribosomal RNA methylase RmtD produced by *Klebsiella pneumoniae* in Brazil. *J. Antimicrob. Chemother.* **61:** 746-747.
28. Yu HS, Lee JC, Kang HY, Jeong YS, Lee EY, Choi CH, *et al*. 2004. Prevalence of *dfr* genes associated with integrons and dissemination of *dfrA*17 among urinary isolates of *Escherichia coli* in Korea. *J. Antimicrob. Chemother.* **53:** 445-450.