



Occurrence of Extended-Spectrum Beta-Lactamases in *Shigella sonnei* and *S. flexneri* isolated after 2007 in the Korea

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ABSTRACT

Objectives; A nationwide survey was carried out in the Republic of Korea to know the prevalent type of extended-spectrum beta-lactamases (ESBLs) those produced by *S. sonnei* and *S. flexneri*.

Methods; From 2007 to 2010, 507 clinical strains were isolated and screened for resistance to extended-spectrum cephalosporins based on the synergistic effect between clavulanate and selected beta-lactams (ceftazidime and cefotaxime) by the Clinical and Laboratory Standards Institute (CLSI) criteria. Classification of beta-lactamases was performed using multiplex PCR. Sequence analysis of the beta-lactamases including ESBL encoding genes was carried out.

Results; Twenty six *S. sonnei* and 2 *S. flexneri* isolates were found to be ESBL positive by their phenotype. 24 strains including 2 *S. flexneri* isolates, found to possess CTX-M ESBLs. Among these, 12 *S. sonnei* strains also contained TEM beta-lactamases. All 26 isolates found to possess CMY beta-lactamases by multiplex PCR. Nucleotide sequence analysis revealed that they harbored *bla*_{CTX-M-15} (13 *S. sonnei* and 2 *S. flexneri* strains), *bla*_{CTX-M-14} (10 *S. sonnei* strains), *bla*_{CTX-M-27} (1 *S. sonnei* strain), and *bla*_{TEM-1} (12 *S. sonnei* strains). In one strain, SS084469, we found two new TEM beta-lactamases, did not reported so far. One contained a substitution (Phe55Leu), and the other had two substitutions (Ser253Pro and His255Arg) in the *bla*_{TEM}.

Conclusion; From these results, We found that, after 2007, the common type of ESBL in *S. sonnei* in Korea, changed from TEM type to CTX-M type. The predominance of CTX-M types in *S. sonnei* appears to be similar to that seen in other Enterobacteriaceae. This is the first report of the finding of two new TEM type beta-lactamases

INTRODUCTION

The dominant pathogenic *Shigella* species found in Korea are *S. sonnei* and *S. flexneri*. They were pandemic from the end of 1990s to 2000s, but not after 2005. The most commonly used antibiotics for the treatment of shigellosis in Korea are ceftriaxone, trimethoprim-sulfamethoxazole and ciprofloxacin. We had reported the isolation of *S. sonnei* strains producing extended spectrum beta-lactamases (ESBL). Recently, it seems that cephalosporin-resistant *Shigella* spp. produce ESBLs. That resistance to cephalosporins started from 0.95% in 2007, 4.7% in 2008, 4.8% in 2009 to 10% in 2010. In this study, a nationwide survey was carried out in the Republic of Korea to know the prevalent type of extended-spectrum beta-lactamases (ESBLs) those produced by *S. sonnei* and *S. flexneri*.

MATERIAL AND METHOD

From 2007 to 2010, 507 clinical strains were isolated and screened for resistance to extended-spectrum cephalosporins based on the synergistic effect between clavulanate and selected beta-lactams (ceftazidime and cefotaxime) by the Clinical and Laboratory Standards Institute (CLSI) criteria

- The MICs for ESBLs producing strains and their transconjugants were determined by the Sensititer.
- Plasmid transfer of cefotaxime resistance markers was performed by a broth culture conjugation method. *E. coli* J53 Azi^r was used as a recipient.
- Classification of beta-lactamases was performed using multiplex PCR.
- Sequence analysis of the beta-lactamases including ESBL encoding genes was carried out
- PFGE was conducted by PulseNet standardized protocol and computer-assisted analysis of the PFGE banding patterns was performed with BioNumerics software.

RESULT

- The 507 clinical strains for this study were selected on the basis of their resistance to cephalosporins and all the strains were screened by CLSI ESBL phenotypic confirmatory tests. 26 (5.1%) *S. sonnei* and 2 (0.3%) *S. flexneri* strains were found to be ESBL positive by their phenotypes.

Table 1. MICs for antimicrobial agents of tested strains and their transconjugants.

strains	MIC (μg/ml) of antimicrobial agent									
	CIP	IMI	CEP	TET	AMP	AXO	STR	AMI	NAL	SXT
SS077407	<0.12	<2	>64	64	>64	>32	>128	<4	<2	>16/304
C*-SS077407	<0.12	<2	>=64	2	>=64	>=32	4	<4	4	<1/19
SF080279	16	<2	>64	64	>64	8	>128	8	>128	>16/304
SF082227	4	<2	16	128	>64	<1	64	<4	>128	<1/19
SS4469	<0.12	<2	>64	<2	>64	>32	128	<4	64	4/76
C*-SS084469	<0.12	<2	>=64	<2	>=64	>=32	8	<4	4	<1/19
SS4470	<0.12	<2	>64	<2	>64	>32	64	<4	32	4/76
C*-SS084470	<0.12	<2	>=64	<2	>=64	>=32	4	<4	4	<1/19
SS4471	<0.12	<2	>64	128	>64	>32	64	<4	32	4/76
C*-SS084471	<0.12	<2	>=64	<2	>=64	>=32	4	<4	4	<1/19
SS4472	<0.12	<2	>64	<2	>64	>32	64	<4	32	2/38
C*-SS084472	<0.12	<2	>=64	<2	>=64	>=32	4	<4	4	<1/19
SS4473	<0.12	<2	>64	<2	>64	>32	64	<4	32	2/38
C*-SS084473	<0.12	<2	>=64	<2	>=64	>=32	4	<4	4	<1/19
SS829	0.25	<2	>64	128	>64	>32	>128	<4	128	>16/304
C*-SS090829	<0.12	<2	>=64	<2	>=64	>=32	4	<4	4	<1/19
SSN26	<0.12	<2	>64	64	>64	>32	>128	<4	<2	>16/304
C*-SS09N26	<0.12	<2	>=64	<2	>=64	>=32	4	<4	4	<1/19
SSN27	<0.12	<2	>64	16	>64	>32	>128	<4	64	>16/304
C*-SS09N27	<0.12	<2	>=64	<2	>=64	>=32	4	<4	4	<1/19
SS09N31	<0.12	<2	8	64	<2	<1	>128	<4	<2	>16/304
SS10N28	<0.12	<2	4	128	4	<1	>128	<4	<2	>16/304
SS368	<0.12	<2	>64	<2	>64	>32	64	<4	64	8/152
C*-SS100368	<0.12	<2	>=64	<2	>=64	>=32	4	<4	4	<1/19
SS100786	<0.12	<2	>64	>128	>64	>32	>128	<4	64	16/304
SS100989	<0.12	<2	>64	>128	>64	>32	>128	<4	64	>16/304
SS102064	<0.12	<2	>64	>128	>64	>32	>128	<4	32	>16/304
SS102065	<0.12	<2	>64	>128	>64	>32	>128	<4	32	>16/304
SS102070	<0.12	<2	>64	64	>64	>32	>128	<4	32	>16/304
SS102346	0.25	<2	>64	128	>64	32	>128	<4	64	>16/304
SS102545	0.25	<2	>64	128	>64	32	>128	<4	64	>16/304
SS102546	0.25	<2	>64	128	>64	32	>128	<4	64	>16/304
SS102590	0.25	<2	>64	128	>64	32	>128	<4	64	>16/304
SS102591	0.25	<2	>64	128	>64	>32	>128	<4	64	>16/304
SS102592	<0.12	<2	>64	64	>64	32	>128	<4	64	>16/304
SS102593	0.25	<2	>64	64	>64	32	>128	<4	64	>16/304
SS102594	<0.12	<2	>64	64	>64	32	>128	<4	64	>16/304
SS102595	0.25	<2	>64	64	>64	32	>128	<4	64	>16/304

C* ; Transconjugant

- Among these, 12 *S. sonnei* strains also contained TEM beta-lactamases. All 26 isolates found to possess CMY beta-lactamases by multiplex PCR. Nucleotide sequence analysis revealed that they harbored *bla*_{CTX-M-15} (13 *S. sonnei* and 2 *S. flexneri* strains), *bla*_{CTX-M-14} (10 *S. sonnei* strains), *bla*_{CTX-M-27} (1 *S. sonnei* strain), and *bla*_{TEM-1} (12 *S. sonnei* strains).
- In the *S. sonnei* isolate, SS084469, we found two new TEM beta-lactamases, did not reported so far. One contained a substitution (Phe55Leu), and the other had two substitutions (Ser253Pro and His255Arg) in the *bla*_{TEM}. They are named as below.
 - bla*_{TEM-196} (GenBank: JQ34306)
 - bla*_{TEM-197} (GenBank: JQ34307)

Table 2. Representative ESBLs type of *S. sonnei* and *S. flexneri* strains from 2007 to 2010

Strains	ESBL type	TEM sequencing	CTX sequencing
SS077407	CTX MI, CMY II		CTX M 15
SF080279	TEM, CTX MI	TEM1	CTX M 15
SF082227	TEM, CTX MI	TEM1	CTX M 15
SS4469	TEM, CTX MI, CMYII	TEM196, TEM197	CTX M 15
SS4470	TEM, CTX MI, CMYII	TEM1	CTX M 15
SS4471	TEM, CTX MI, CMYII	TEM1	CTX M 15
SS4472	TEM, CTX MI, CMYII	TEM1	CTX M 15
SS4473	TEM, CTX MI, CMYII	TEM1	CTX M 15
SS829	CTX M IV, CMY II		CTX M 14
SSN26	CTX M IV, CMY II		CTX M 27
SSN27	TEM, CTX MI, CMYII	TEM1	CTX M 15
SS09N31	CMYII		
SS10N28	CMYII		
SS368	TEM, CTX MI, CMYII	TEM1	CTX M 15
SS100786	TEM, CTX MI, CMYII	TEM1	CTX M 15
SS100989	TEM, CTX MI, CMYII	TEM1	CTX M 15
SS102064	TEM, CTX MI, CMYII	TEM1	CTX M 15
SS102065	TEM, CTX MI, CMYII	TEM1	CTX M 15
SS102070	TEM, CTX MI, CMYII	TEM1	CTX M 15
SS102346	CTX M IV, CMY II		CTX M 14
SS102545	CTX M IV, CMY II		CTX M 14
SS102546	CTX M IV, CMY II		CTX M 14
SS102590	CTX M IV, CMY II		CTX M 14
SS102591	CTX M IV, CMY II		CTX M 14
SS102592	CTX M IV, CMY II		CTX M 14
SS102593	CTX M IV, CMY II		CTX M 14
SS102594	CTX M IV, CMY II		CTX M 14
SS102595	CTX M IV, CMY II		CTX M 14

- Twenty one *S. sonnei* and 2 *S. flexneri* strains were examined by PFGE using *Xba*I and *Not*I. This study observed 4 different *Xba*I and 2 different *Not*I patterns, their were assumed that these PFGE patterns were genetically related to each other.

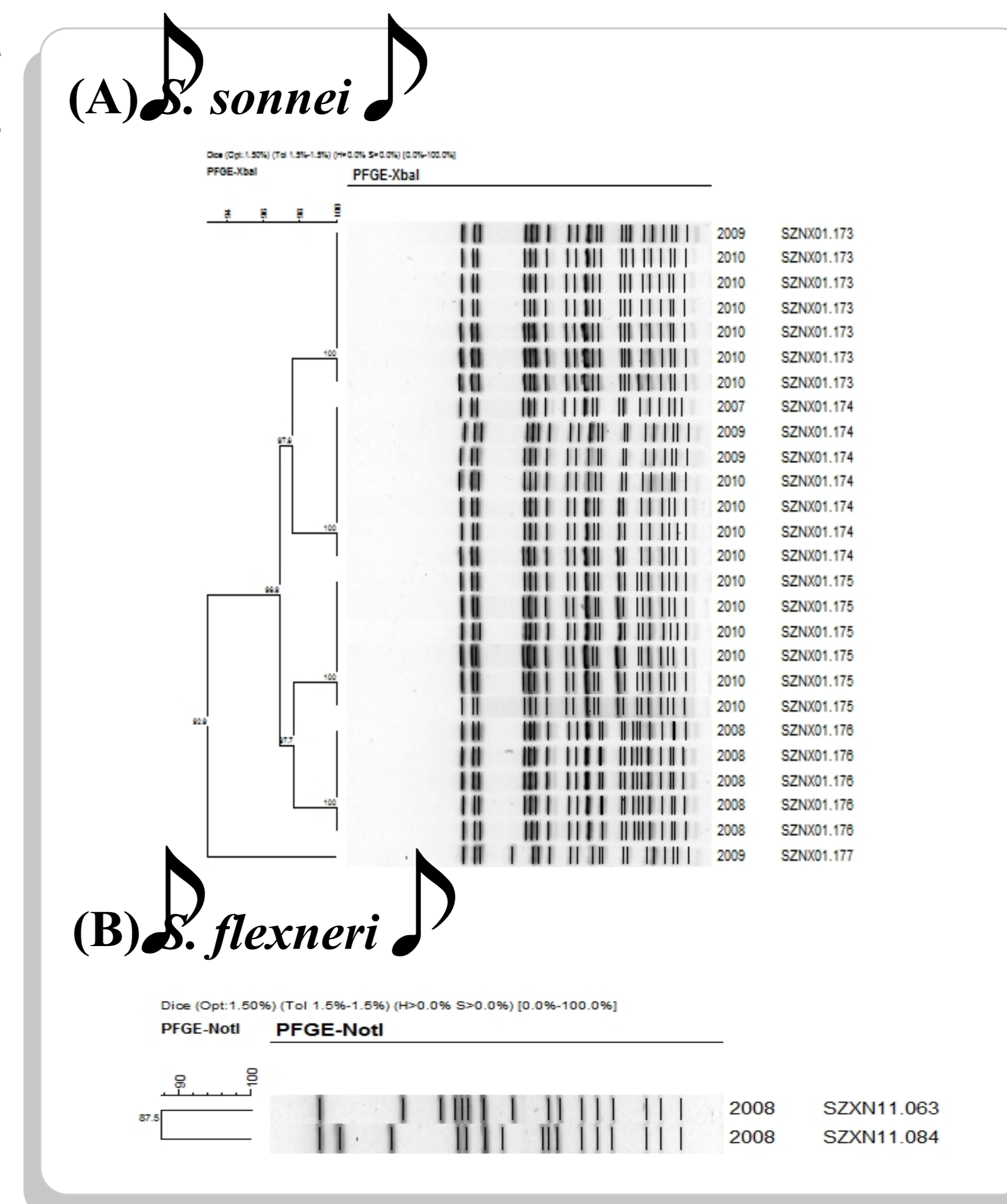


Fig. 1. Clustering of PFGE patterns for ESBL producing *S. sonnei* and *S. flexneri*

CONCLUSION

- Twenty six *S. sonnei* and 2 *S. flexneri* strains were found to be ESBL positive by their phenotype.
- We found that, after 2007, the common type of ESBL producing *S. sonnei* in Korea, changed from TEM type to CTX-M type.
- The comparison with PFGE patterns of ESBL producing *S. sonnei* and *S. flexneri* between 2007-2010, *S. sonnei* strains were showed above 92% of relatedness.
- These results suggest the limit of the commonly used antibiotics for the shigellosis.