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The role of international travellers in the spread of CTX-M-15-producing *Shigella sonnei* in the Republic of Korea

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ABSTRACT

Background: Multidrug-resistant *Shigella* isolates have recently emerged as a serious public health threat worldwide. In particular, overseas travel is a risk factor for acquisition of antimicrobial-resistant *Shigella* strains. To explore the role of travel in the spread of cefotaxime-resistant *Shigella sonnei* in Korea, we screened 751 *Shigella* spp. isolates from 2007 to 2016 through the National Surveillance system, and 28 cephalosporin-resistant *S. sonnei* isolates were identified.

Methods: For cephalosporin-resistant *S. sonnei* isolates, epidemiological and molecular analyses (plasmid structure analysis, pulsed-field gel electrophoresis (PFGE) and high-quality single-nucleotide polymorphisms (hqSNPs) based on whole-genome sequencing (WGS)) were conducted to investigate the source of infection and transmission route.

Results: Among the 28 cefotaxime-resistant *S. sonnei* strains, 18 were isolated from travellers returning from Asia, including Vietnam ($n = 11$). Molecular analysis of 18 bla_{CTX-M}-type isolates revealed that 15 contain CTX-M-15; 50% of isolates from domestic patients contain CTX-M-14. Analysis of the genetic environments of the $bla_{CTX-M-14}$ and $bla_{CTX-M-15}$ genes revealed different genetic organization surrounding the bla_{CTX-M} genes. Additionally, PFGE and hqSNP results suggested a large phylogenetic distance between the *S. sonnei* isolates related to overseas travel and those acquired domestically in Korea.

Conclusion: Our study data demonstrates that two prevalent bla_{CTX-M} genes, $bla_{CTX-M-14}$ and $bla_{CTX-M-15}$, have been circulating in *S. sonnei* in Korea over the last 10 years. Recently, international travellers are at a high risk for acquisition of CTX-M-15-producing *S. sonnei* in Korea.

1. Introduction

Shigellosis is an acute diarrheal disease caused by *Shigella* bacteria of four species: *S. dysenteriae*, *S. flexneri*, *S. boydii*, and *S. sonnei* [1]. *Shigella* species are easily transmitted from person to person with a low infectious dose, as well as through contaminated food and recreational water [2]. Therefore, *Shigella* is the causative agent of one of the most communicable and severe forms of bacterial-induced diarrhoea [3]. Although antibiotic treatment is usually recommended to treat *Shigella*-associated diarrhoea [4], the emergence of multidrug resistant (MDR) strains over the last two decades has become a serious concern that can complicate the choice of therapeutic antimicrobial. Quinolones and cephalosporins are the drugs of choice [5]; however, resistance to fluoroquinolones and third- and fourth-generation cephalosporins has been reported among *Shigella* isolates acquired in southern and eastern Asia [6,7]. In addition, a recent increase in international travel and migration from high-risk areas to low-prevalence areas has accelerated the global spread of drug-resistant *Shigella* isolates to nonendemic regions [8].

In Korea, *S. flexneri* was the most common cause of shigellosis until the late 1980s, and *S. sonnei* has been the most prevalent agent since the 1990s [9]. Despite the marked decrease in the incidence of shigellosis in Korea due to consistent efforts to improve the national health care system and public sanitation, CTX-M-14-producing *Shigella* was first identified in 2001 [10], and extended spectrum β-lactamase (ESBL)-producing *Shigella* strains have been repeatedly reported [11–13]. It has been suggested that the continuous increase in ESBL-positive isolates is related to the spread of ESBLs throughout communities in Korea until the mid-2000s [14]. However, CTX-M-55-type ESBL *S. sonnei* was isolated

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from a traveller to China in 2013 [15], and the outbreaks of CTX-M-15-type ESBL *S. sonnei* that occurred in 2014 were associated with travellers returning from Vietnam [16]. These results suggest that the presence of *S. sonnei* with new types of CTX-M enzymes in Korea is due to international travel.

The lack of a population-based study limits estimation of the exact prevalence of ESBL-producing *Shigella* species in Korea. Here, we report the prevalence of ESBL-producing *S. sonnei* in Korea during 2007–2016 and characterize the phenotypic and genetic features of *S. sonnei* related to overseas travel and of that acquired domestically *via* analyses based on PFGE, pMLST, the genetic environment surrounding bla_{CTX-M} genes, and a whole-genome sequencing-based minimum spanning tree (MST).

2. Materials and methods

2.1. Bacterial isolates

From 2007 to 2016, *Shigella* strains were isolated from stool or rectal swab samples from diarrheal patients, which were collected from a national surveillance programme for acute diarrheal diseases as well as from outbreak cases. A single isolate per patient or outbreak case was included in this study. Isolates were serotyped (Denka-Seiken, Japan) and stocked according to serotype at −80 °C until use.

2.2. Antimicrobial susceptibility testing and beta-lactamase identification

Cefotaxime resistance was tested by the broth microdilution method using a customized Sensititre panel (KRCDC2F, TREK Diagnostics Technology, USA) and was confirmed using the ESB1F panel. The antimicrobial resistance of the isolates was interpreted according to CLSI guidelines [17].

2.3. Pulsed-field gel electrophoresis (PFGE)

PFGE was performed using a standardized protocol (www.pulsenetinternational.org). Plug-embedded genomic DNA from cefotaxime-resistant isolates was treated with *Xba*I nuclease and then processed using the PFGE Mapper II system (Bio-Rad, USA) with pulse times of 2.16 s and 54.17 s at 6 V/cm for 18 h. Genetic similarity among strains was assessed using DICE/UPGMA (optimization of 1.5% and tolerance of 1.5%) generated by BioNumerics software version 5.1 (Applied Math, USA).

2.4. Conjugation

Conjugation was carried out by solid mating using azide-resistant *Escherichia coli* J53 as a recipient strain. Transconjugants were selected by plating on MacConkey agar containing cefotaxime (1 μg/ml) and sodium azide (200 μg/ml) and then confirmed using the Analytical Profile Index (API) 20E system (bioMérieux, France) and PCR (negative for the *ipaH* gene but positive for beta-lactamase genes).

2.5. DNA extraction, library preparation, and whole-genome sequencing (WGS)

Genomic DNA was extracted using a DNeasy Blood & Tissue Kit (Qiagen, USA) according to the manufacturer's instructions. DNA concentrations were evaluated using a Qubit 3.0 Fluorometer (Invitrogen, USA) to determine DNA input. To assess the purity of DNA, the ratio of absorbance at 260 nm and 280 nm using a Nanodrop 2000 spectrophotometer (Thermo Fischer Scientific, USA) was employed, with values between 1.75 and 2.05. Index-tagged paired-end Illumina sequencing libraries were prepared using Nextera DNA CD Indexes (24 Indexes, 24 samples, Illumina, USA), and WGS was performed using the Illumina MiSeq platform with v2 reagent kits generating 2 × 250-bp paired-end reads (Illumina, USA). The resulting sequence reads were trimmed using Trim_galore v0.5.0 to remove adapters and remaining reads with lengths <50 bp. Ultimately, we obtained high-quality sequence reads with at least 100x average genome coverage.

2.6. In silico identification of antibiotic resistance genes and typing of plasmids

ResFinder [18] was employed for the *in silico* prediction of acquired antibiotic resistance genes in the genomes under study. Plasmid incompatibility groups and specific plasmid multi-locus sequence type (pMLST) were identified using PlasmidFinder and pMLST, respectively [19]. Insertion sequences were identified using ISFinder [20]. Additionally, using PCR, we double-checked the results of *in silico* analysis.

2.7. Evolutionary lineage analysis

For hqSNP analysis, high-quality Illumina reads were mapped to *S. sonnei* reference genome SS46 (GenBank accession: NC_007384.1) using gsMapper version 2.8. The BAM file was sorted using Samtools version 1.3.1, and a pileup file for each genome was produced. BCFtools version 1.3.1 was utilized to identify high-quality variants. The Python script was applied to parse the .vcf file and construct an initial hqSNP matrix. Based on the initial hqSNP matrix data, only hqSNPs satisfying conditions such as a nonrepeatable region with a read depth of 100 or more and nonsynonymous genetic variation were selected. In total, 166 SNPs were produced from 158 loci and converted into allelic profiles. MST for evolutionary lineage analysis was performed using PHYLOVIZ version 2.0.

3. Results

3.1. Bacterial isolates and antimicrobial susceptibility testing

A total of 751 *Shigella* spp. were isolated from diarrheal patients from 2007 to 2016. *S. sonnei* constituted the greatest number of isolates, 433 isolates were isolated during study period, and 272 were isolated from returning travellers. A total of 28 cefotaxime-resistant *S. sonnei* were isolated during the study period. Among the 28 cefotaxime-resistant *S. sonnei* strains, 18 were isolated from travellers returning from Asia, including Vietnam ($n = 11$), Thailand ($n = 2$), Laos ($n = 1$), India ($n = 1$), Cambodia ($n = 1$), China ($n = 1$) and an unknown country ($n = 1$) (Table 1, Fig. 1). Except for 2009, 2012 and 2015, years when there was no or just one case of cefotaxime-resistant *S. sonnei* from a returning traveller, the incidence rate of cefotaxime-resistant *S. sonnei* isolated from travellers was more than 50% for the entire period.

3.2. PFGE

All of the CTX-M-14-producing *S. sonnei* isolates showed the same PFGE pattern (SZNX01.008). Twelve (71%) of the 17 isolates of CTX-M-15-producing *S. sonnei* in the PFGE banding pattern were SZNX01.173 (Table 1, Fig. 2).

3.3. Identification of beta-lactamase genes

All cefotaxime-resistant isolates of *S. sonnei* were found to be positive for bla_{CTX-M}-type genes (Table 1). DNA and deduced amino acid sequence analyses revealed $bla_{CTX-M-15}$ to be the most

Table 1
Characteristics of CTX-M-producing *S. sonnei* isolates in Korea during 2007–2016.

Year of isolation	Strain ID	MIC (μg/ml) value of cefotaxime[a]	β-lactamae genes	Travel history	Plasmid replicon	Plasmid ST	*bla*CTX structure	PFGE type
2007	20077407	128	CTX-M-15	Vietnam	IncI1	ST16	IS*Ecp1*-$bla_{CTX\text{-}M\text{-}15}$-*orf477*	SZNX01.173
2008	20084469	64	CTX-M-15, TEM-1	Thailand	IncI1	ST16	IS*Ecp1*-$bla_{CTX\text{-}M\text{-}15}$-*orf477*	SZNX01.173
2009	20090829	64	CTX-M-27	India	IncI1	new	IS*Ecp1*-$bla_{CTX\text{-}M\text{-}27}$-IS*903D*	SZNX01.173
	2009N3	32	CTX-M-14	Domestic patient	IncF	-	IS*Ecp1*-$bla_{CTX\text{-}M\text{-}14}$-ΔIS*903D*	SZNX01.008
	2009N4	32	CTX-M-14	Domestic patient	IncF	-	IS*Ecp1*-$bla_{CTX\text{-}M\text{-}14}$-ΔIS*903D*	SZNX01.008
	2009N5	128	CTX-M-55, TEM-1	Domestic patient	IncI2	-	IS*Ecp1*-$bla_{CTX\text{-}M\text{-}55}$-*orf477*	SZNX01.239
2010	20100368	128	CTX-M-15, TEM-1	Vietnam	IncI1	ST16	IS*Ecp1*-$bla_{CTX\text{-}M\text{-}15}$-*orf477*	SZNX01.173
	20100786	128	CTX-M-15, TEM-1	Laos	IncI1	ST16	IS*Ecp1*-$bla_{CTX\text{-}M\text{-}15}$-*orf477*	SZNX01.173
	20100989	128	CTX-M-15, TEM-1	Vietnam	IncI1	ST16	IS*Ecp1*-$bla_{CTX\text{-}M\text{-}15}$-*orf477*	SZNX01.173
	20102064[b]	128	CTX-M-15, TEM-1	Vietnam	IncI1	ST16	IS*Ecp1*-$bla_{CTX\text{-}M\text{-}15}$-*orf477*	SZNX01.173
	20102065[b]	128	CTX-M-15, TEM-1	Vietnam	IncI1	ST16	IS*Ecp1*-$bla_{CTX\text{-}M\text{-}15}$-*orf477*	SZNX01.173
	20102070	128	CTX-M-15, TEM-1	Vietnam	IncI1	ST16	IS*Ecp1*-$bla_{CTX\text{-}M\text{-}15}$-*orf477*	SZNX01.173
	20102346	64	CTX-M-14	Domestic patient	Non-typeable	–	IS*Ecp1*-$bla_{CTX\text{-}M\text{-}14}$-ΔIS*903D*	SZNX01.008
	20102545	64	CTX-M-14	Domestic patient	Non-typeable	–	IS*Ecp1*-$bla_{CTX\text{-}M\text{-}14}$-ΔIS*903D*	SZNX01.008
	20102590	32	CTX-M-14	Domestic patient	Non-typeable	–	IS*Ecp1*-$bla_{CTX\text{-}M\text{-}14}$-ΔIS*903D*	SZNX01.008
2011	20110267	64	CTX-M-14	China	Non-typeable	–	IS*Ecp1*-$bla_{CTX\text{-}M\text{-}14}$-ΔIS*903D*	SZNX01.008
	20110278	128	CTX-M-15, TEM-1	Thailand	IncI1	ST31	IS*Ecp1*-$bla_{CTX\text{-}M\text{-}15}$-*orf477*	SZNX01.216
	20110771	128	CTX-M-15, TEM-1	Vietnam	IncI1	ST16	IS-$bla_{CTX\text{-}M\text{-}15}$-*orf477*	SZNX01.173
	20111513	128	CTX-M-15, TEM-1	Vietnam	IncI1	ST16	IS*Ecp1*-$bla_{CTX\text{-}M\text{-}15}$-*orf477*	SZNX01.173
	20111539	128	CTX-M-15, TEM-1	Vietnam	IncI1	ST16	IS*Ecp1*-$bla_{CTX\text{-}M\text{-}15}$-*orf477*	SZNX01.173
2013	20130673	>128	CTX-M-15, TEM-1	Vietnam	IncI1	ST16	IS*Ecp1*-$bla_{CTX\text{-}M\text{-}15}$-*orf477*	SZNX01.173
	20131929	128	CTX-M-15, TEM-1	Cambodia	IncI1	ST16	IS*Ecp1*-$bla_{CTX\text{-}M\text{-}15}$-*orf477*	SZNX01.176
	20132123	128	CTX-M-55	Domestic patient	IncI1	ST16	IS*Ecp1*-$bla_{CTX\text{-}M\text{-}15}$-*orf477*	SZNX01.236
2014	20143520	128	CTX-M-15, OXA-1	Vietnam	IncF	–	IS*26*-ΔIS*Ecp1*-$bla_{CTX\text{-}M\text{-}15}$-*orf477*	SZNX01.240
	20145220	128	CTX-M-15, TEM-1	Domestic patient	IncI1	ST16	IS*Ecp1*-$bla_{CTX\text{-}M\text{-}15}$-*orf477*	SZNX01.183
2015	20153135	128	CTX-M-55	domestic patient	IncZ	–	ΔIS*Ecp1*-IS*5*-$bla_{CTX\text{-}M\text{-}55}$-*orf477*	SZNX01.249
	20153330	128	CTX-M-15	domestic patient	IncK	–	Δbla_{TEM}-ΔIS*Ecp1*-$bla_{CTX\text{-}M\text{-}15}$-*orf477*	SZNX01.237
2016	20161384	128	CTX-M-55	Unknown country	IncI1	ST16	IS*Ecp1*-$bla_{CTX\text{-}M\text{-}55}$-*orf477*	SZNX01.290

[a] The EUCAST break point of cefotaxime (S <1 μg/ml and R >2 μg/ml).
[b] The consecutive number (20102064 and 20102065) indicated that they were isolated in one outbreak.

frequently detected gene ($n = 17$), followed by $bla_{CTX\text{-}M\text{-}14}$ ($n = 6$), $bla_{CTX\text{-}M\text{-}55}$ ($n = 4$) and $bla_{CTX\text{-}M\text{-}27}$ ($n = 1$). CTX-M-15 was prevalent among travellers (83%), whereas CTX-M-14 (50%) was a major type among domestic isolates. Three of four CTX-M-55-producing *S. sonnei* strains were isolated domestically, and a CTX-M-27-producing *S. sonnei* strain was isolated from travellers returning from India. In addition, bla_{TEM} and bla_{OXA} were found in 15 and 1 isolates, respectively.

3.4. Plasmid analysis

Conjugation experiments showed that cefotaxime resistance in both imported and domestically isolated *S. sonnei* was successfully transferred to *E. coli* J53. The most plasmids (89%) isolated from strains of travellers belong to the IncI1 plasmid incompatibility group. In contrast, 20% of the plasmids isolated from strains of domestic patients belong to the IncI1 or IncF incompatibility

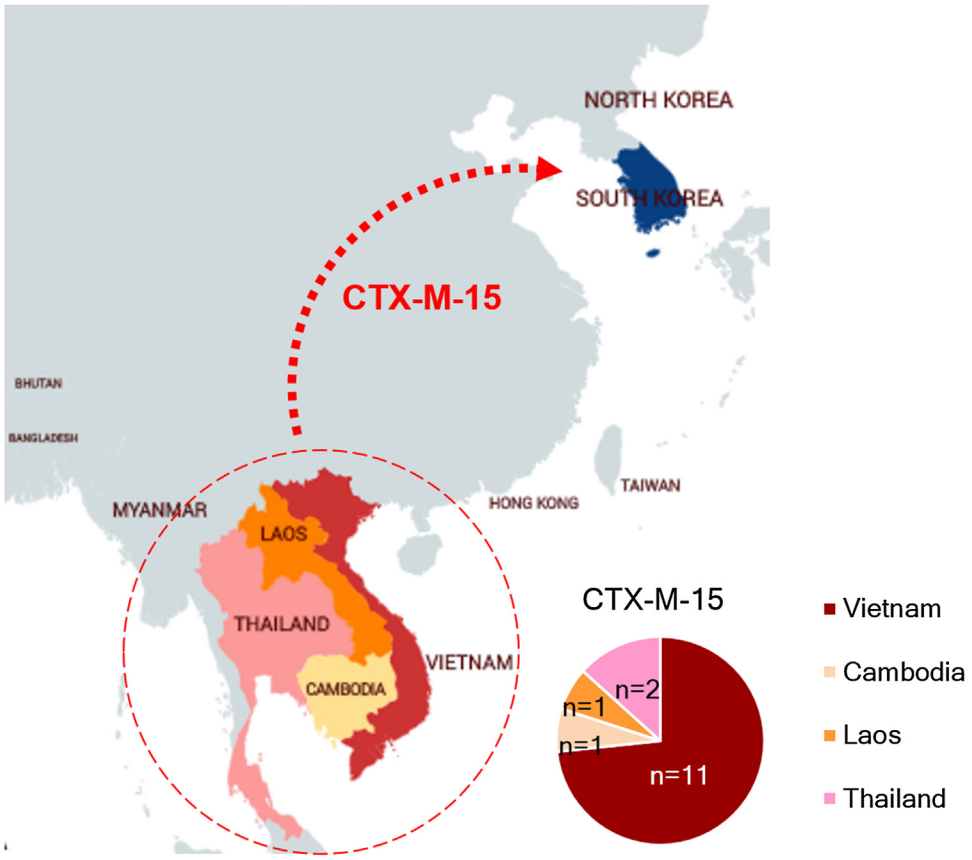
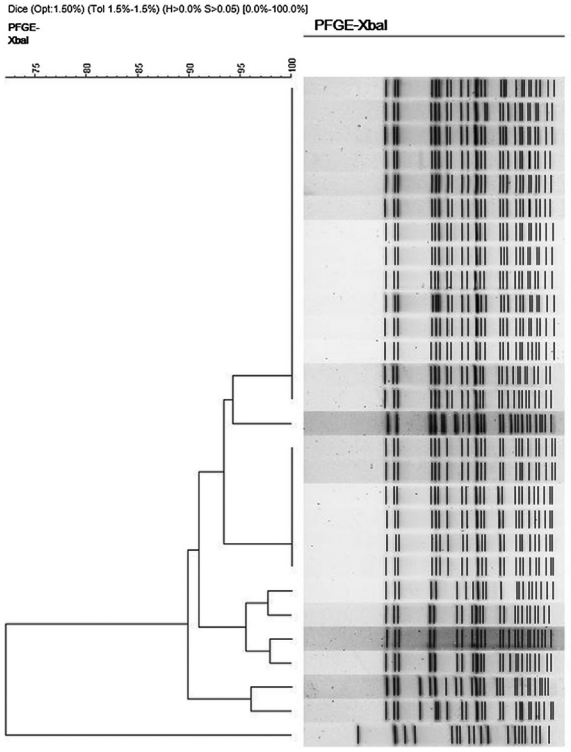


Fig. 1. Importation of CTX-M-15 producing *S. sonnei* by international travel, especially to Southeast Asia. Note that 88% of CTX-M-15 isolates were isolated from international travellers returning from Southeast Asia. For clarity, only Vietnam, Thailand, Laos, and Cambodia are highlighted in the world map. The number of CTX-M-15 isolates is displayed as a pie graph with the same colour scheme as the map.



Strain ID	Travel history	β-lactamase genes	Year	PFGE type
20077407	Vietnam	CTX-M-15	2007	SZNX01.173
20084469	Thailand	CTX-M-15	2008	SZNX01.173
20090829	India	CTX-M-27	2009	SZNX01.173
20100368	Vietnam	CTX-M-15	2010	SZNX01.173
20100786	Laos	CTX-M-15	2010	SZNX01.173
20100989	Vietnam	CTX-M-15	2010	SZNX01.173
20102064	Vietnam	CTX-M-15	2010	SZNX01.173
20102065	Vietnam	CTX-M-15	2010	SZNX01.173
20102070	Vietnam	CTX-M-15	2010	SZNX01.173
20110711	Vietnam	CTX-M-15	2011	SZNX01.173
20111513	Vietnam	CTX-M-15	2011	SZNX01.173
20111539	Vietnam	CTX-M-15	2011	SZNX01.173
20130673	Vietnam	CTX-M-15	2013	SZNX01.173
20131929	Cambodia	CTX-M-15	2013	SZNX01.176
20161384	Unknown country	CTX-M-55	2016	SZNX01.290
2009N3	domestic patient	CTX-M-14	2009	SZNX01.008
2009N4	domestic patient	CTX-M-14	2009	SZNX01.008
20102346	domestic patient	CTX-M-14	2010	SZNX01.008
20102545	domestic patient	CTX-M-14	2010	SZNX01.008
20102590	domestic patient	CTX-M-14	2010	SZNX01.008
20110267	China	CTX-M-14	2011	SZNX01.008
20110278	Thailand	CTX-M-15	2011	SZNX01.216
20153135	domestic patient	CTX-M-55	2015	SZNX01.249
20145220	domestic patient	CTX-M-15	2014	SZNX01.183
20132123	domestic patient	CTX-M-55	2013	SZNX01.236
20153330	domestic patient	CTX-M-15	2015	SZNX01.237
2009N5	domestic patient	CTX-M-55	2009	SZNX01.239
20143520	Vietnam	CTX-M-15	2014	SZNX01.240

Fig. 2. Representative PFGE patterns of CTX-resistant *S. sonnei*. The dendrogram (left) was obtained from cluster analysis based on *Xba*I macrorestriction patterns of CTX-resistant *S. sonnei* isolates using BioNumerics software.

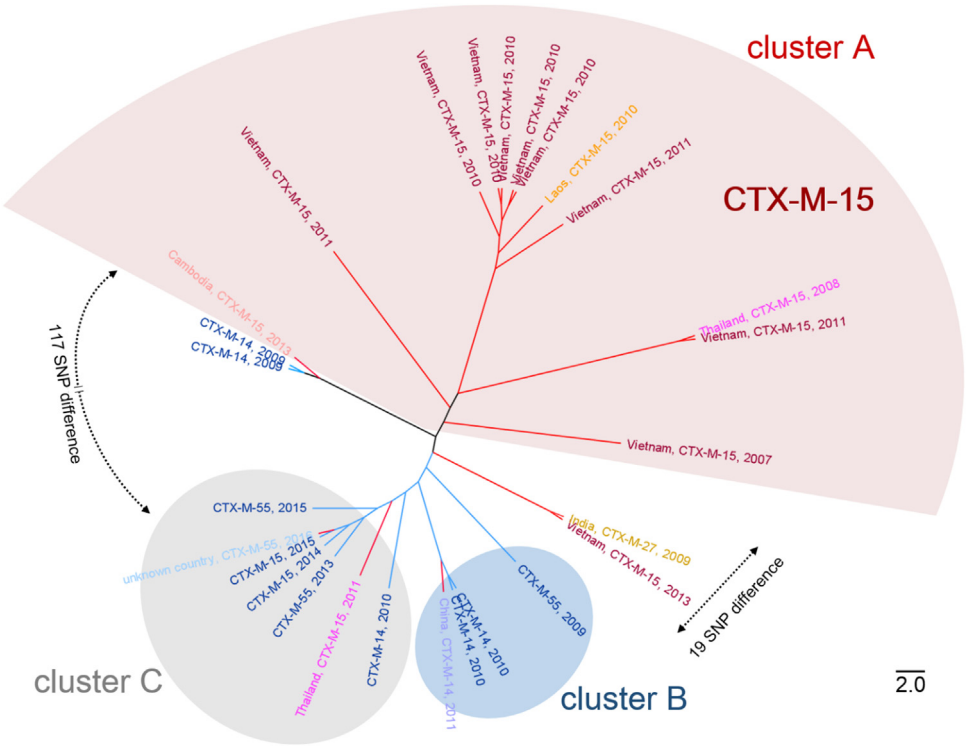


Fig. 3. Phylogenetic distribution of CTX-M-producing *S. sonnei* isolates in the Republic of Korea from 2007 to 2016. CTX-M-producing isolates from international travel and domestic patients are coloured red and blue, respectively. The same colour scheme used in Fig. 1 was applied to indicate the visited countries.

group. Moreover, analysis of the sequence type of the IncI1 incompatibility group by pMLST indicated that 88% of the cefotaxime-resistant *S. sonnei* strains isolated from travellers are sequence type 16 (Table 1).

In addition, analysis of the genetic environment of the $bla_{\text{CTX-M}}$ gene revealed different genetic organization surrounding $bla_{\text{CTX-M}}$ genes in the two groups (travellers and domestic patients). As shown in Fig. 3, 83% of the plasmids isolated from returning travellers harbour the insertion sequence IS*Ecp1* and a truncated *orf477*, which were detected in the downstream and upstream regions of $bla_{\text{CTX-M}}$ genes (Types C and D), respectively. However, in domestic patients, only 30% of plasmids had an identical structure with those of international travellers, and 50% of plasmids belong to Type G, which has an IS*Ecp1*-$bla_{\text{CTX-M}}$-IS*903D* structure (Table 1).

3.5. MST analysis of S. sonnei genomes

MST analysis of cefotaxime-resistant *S. sonnei* isolates was conducted to visualize their relatedness, whereby clustering based on the geographic origin of the strains was expected to be the main driver of phylogenetic segmentation. The major group included isolates from international travel (cluster A). The other two groups exhibit 19 (cluster B) and 117 (cluster C) SNP differences compared to cluster A isolates, respectively, and they were mostly isolated from domestic patients. Therefore, CTX-resistant *S. sonnei* appears to cluster according to the geographic origin of the strain (Fig. 3).

4. Discussion

Our study showed that, since 2012, CTX-M-14-producing *S. sonnei* was no longer being isolated in the Republic of Korea and that CTX-M-15 has been a prevalent CTX-M-type since then. This recent increase in the incidence of CTX-M-15-producing *S. sonnei* in Korea is associated with increasing international travel. CTX-M-15-producing *S. sonnei* was first isolated in 2008 from an outbreak in Gyeongbuk Province [21]. However, according to our data, CTX-M-15 was first isolated in 2007 from a traveller to Vietnam. From 2010 to 2015, there were 16 cases of CTX-M-15-producing *S. sonnei*, accounting for 89% of all CTX-M-15 cases over the course of ten years in Korea. Notably, 13 of the 16 cases were isolated from travellers returning from the Asian continent, 10 of whom visited Vietnam during this period.

Many studies regarding travellers from Europe, North America and Australia have shown that international travel is a risk factor for colonization with ESBL-producing Enterobacteriaceae [22–25]. In those studies, travel to the Indian subcontinent was associated with a high risk of infection. These findings agree with the results of our study that international travel is a major risk factor for acquisition of CTX-M-15-producing *S. sonnei*, though the highest risk factor for Korean travellers was Vietnam and not the Indian subcontinent.

A study by von Wintersdorff et al. [26] that investigated acquisition of antimicrobial resistance genes associated with travel to a specific region in the Netherlands showed that acquisition of the CTX-M-1 group increased dramatically after travel to the Indian subcontinent. Additionally, comparison of the prevalence CTX-M groups before and after travel clearly demonstrated that international travel contributed to the spread of a specific CTX-M group, the CTX-M-1 group, in the Netherlands. In Korea, CTX-M-15-producing *S. sonnei* isolated from travellers returning from Vietnam has increased explosively since 2010; in 2014, there was an outbreak of CTX-M-15-producing *S. sonnei* associated with travel to Vietnam [16]. Moreover, a sudden dominance of $bla_{\text{CTX-M-15}}$-harbouring plasmids in *Shigella* spp. circulating in Southern Vietnam was reported in 2010 [27]. These findings suggest that the increase in CTX-M-15-producing *S. sonnei* among travellers to Vietnam is associated with the dissemination of these strains in Vietnam in the same period. In addition, analysis of the PFGE pattern of CTX-M-14, which was isolated from domestic patients in our study, revealed a pattern identical to that of CTX-M-14 isolated from an outbreak in 2004 in Korea [12]. This indicates that *S.*

sonnei-harbouring plasmids that contain two different bla_{CTX-M} genes have been circulating in Korea; one is $bla_{CTX-M-14}$, which is responsible for predominantly community-wide infection, and the other is $bla_{CTX-M-15}$, which has recently increased due to the return of international travellers from Vietnam.

We investigated the phenotypic and genetic features of CTX-M-15-producing *S. sonnei* isolated mainly from overseas travellers and CTX-M-14-producing *S. sonnei*, which was prevalent in domestic patients. As the CTX-M type distinguishes international travellers from domestic patients, all of the phenotypic and genetic characteristics between *S. sonnei* related to overseas travel and those acquired domestically in Korea differed. Furthermore, pMLST revealed that most of the plasmids of cefotaxime-resistant *S. sonnei* isolated from international travel belong to the IncI1 incompatibility group, grouping as sequence type 16. Additionally, the genetic environment of $bla_{CTX-M-15}$ commonly consists of an intact IS*Ecp1* 48 bp upstream of the $bla_{CTX-M-15}$ open reading frame and *orf477* downstream. This organization is frequently observed in plasmids harbouring $bla_{CTX-M-15}$ worldwide [28–31]. In contrast, only 20% of the plasmids from cefotaxime-resistant *S. sonnei* isolated from domestic patients belong to the IncI1 incompatibility group, and the sequence type for most of them was unclassified. In general, the genetic environment of $bla_{CTX-M-14}$ consists of an intact IS*Ecp1* 42 bp upstream of the $bla_{CTX-M-14}$ open reading frame and *IS903D* downstream.

We also performed hqSNP analysis by WGS to analyze the evolutionary lineage of the isolates. MST analysis without the putative plasmid sequences indicated that the majority of isolates clustered in accordance with geographical origin. Most isolates associated with international travel clustered into one major group, suggesting that overseas travel likely resulted in the diffusion of CTX-M-15-producing *S. sonnei* in the Republic of Korea. The large phylogenetic distances between the *S. sonnei* isolates related to overseas travel and those acquired domestically in Korea support the idea that there is no link between these two groups, which is consistent with the epidemiological data.

Our study data demonstrate that two prevalent bla_{CTX-M} genes, $bla_{CTX-M-14}$ and $bla_{CTX-M-15}$, have been circulating in *S. sonnei* in Korea over the last 10 years. Recently, there has been an increase in the number of CTX-M-15-producing *S. sonnei* isolates, which is associated with international travel. These findings contribute to increasing evidence that travel represents a major risk factor for the spread of antimicrobial drug resistance. Thus, more comprehensive surveillance is required to prevent further spread of resistant clonal strains.

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Conflict of interests

There are no conflicts of interest for any of the authors.

Ethical approval

Not required.

References

[1] DuPont HL, Levine MM, Hornick RB, Formal SB. Inoculum size in shigellosis and implications for expected mode of transmission. J Infect Dis 1989;159:1126–8.
[2] Leggett HC, Cornwallis CK, West SA. Mechanisms of pathogenesis, infective dose and virulence in human parasites. PLoS Pathog 2012;8:e1002512.
[3] Krilov LR, Barone SR, Mandel FS, Cusack TM, Gaber DJ, Rubino JR. Impact of an infection control program in a specialized preschool. Am J Infect Control 1996;24:167–73.
[4] Mendez Arancibia E, Pitart C, Ruiz J, Marco F, Gascón J, Vila J. Evolution of antimicrobial resistance in enteroaggregative *Escherichia coli* and enterotoxigenic *Escherichia coli* causing traveller's diarrhoea. J Antimicrob Chemother 2009;64:343–7.
[5] Niyogi SK. Shigellosis. J Microbiol 2005;43:133–43.
[6] Taneja N. Changing epidemiology of shigellosis and emergence of ciprofloxacin-resistant shigellae in India. J Clin Microbiol 2007;45:678–9.
[7] Qu M, Zhang X, Liu G, Huang Y, Jia L, Liang W, et al. An eight-year study of Shigella species in Beijing, China: serodiversity, virulence genes, and antimicrobial resistance. J Infect Dev Ctries 2014;8:904–8.
[8] Pitout JD, Campbell L, Church DL, Gregson DB, Laupland KB. Molecular characteristics of travel-related extended-spectrum-β-lactamase-producing *Escherichia coli* isolates from the Calgary Health Region. Antimicrob Agents Chemother 2009;53:2539–43.
[9] Lee JC, Jeong YS, Oh JY, Kang HY, Kim KH, Kim J, et al. Epidemiology of shigellosis in Korea. J Bacteriol Virol 2006;36:41–9.
[10] Pai H, Choi EH, Lee HJ, Hong JY, Jacoby GA. Identification of CTX-M-14 extended-spectrum β-lactamase in clinical isolates of *Shigella sonnei*, *Escherichia coli*, and *Klebsiella pneumoniae* in Korea. J Clin Microbiol 2001;39:3747–9.
[11] Kim S, Kim J, Kang Y, Park Y, Lee B. Occurrence of extended-spectrum β-lactamases in members of the genus *Shigella* in the Republic of Korea. J Clin Microbiol 2004;42:5264–9.
[12] Hong SJ, Lee CH, Wang JH, Song W, Jung SH. Clinical characteristics of extended-spectrum β-lactamase producing *Shigella sonnei* infection outbreaked in Chungju Area. Korean J Lab Med 2006;26:168–73.
[13] Lim HT, Lee SH, Lee JH, Kim JE, Kim KS, Jeong EJ, et al. A domestic outbreak of bacterial dysentery caused by extended-spectrum β-lactamase (ESBL)-producing *Shigella sonnei*. Korean J Pediatr 2005;48:1107–15.
[14] 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.
[15] Lee W, Chung HS, Lee H, Yum JH, Yong D, Jeong SH, et al. CTX-M-55-type extended-spectrum β-lactamase-producing *Shigella sonnei* isolated from a Korean patient who had travelled to China. Ann Lab Med 2013;33:141–4.
[16] Kim JS, Kim JJ, Kim SJ, Jeon SE, Seo KY, Choi JK, et al. Outbreak of ciprofloxacin-resistant *Shigella sonnei* associated with travel to Vietnam, Republic of Korea. Emerg Infect Dis 2015;21:1247–50.
[17] CLSI. Performance standards for antimicrobial susceptibility testing. 24nd Informational Supplement. Clinical and Laboratory Standards (M100-S24), Wayne, P.A. CLSI Doc M100-S16CLSI. .
[18] Kim J, Jeon S, Rhie H, Lee B, Park M, Lee H, et al. Rapid detection of extended spectrum β-lactamase (ESBL) for Enterobacteriaceae by use of a multiplex PCR-based method. Infection and Chemotherapy 2009;41:181–4.
[19] Carattoli A, Bertini A, Villa L, Falbo V, Hopkins KL, Threlfall EJ. Identification of plasmids by PCR-based replicon typing. J Microbiol Methods 2005;63:219–28.
[20] Lv L, Partridge SR, He L, Zeng Z, He D, Ye J, et al. Genetic characterization of IncI2 plasmids carrying bla CTX-M-55 spreading in both pets and food animals in China. Antimicrob Agents Chemother 2013;57:2824–7.
[21] Moran RA, Anantham S, Pinyon JL, Hall RM. Plasmids in antibiotic susceptible and antibiotic resistant commensal *Escherichia coli* from healthy Australian adults. Plasmid 2015;80:24–31.
[22] Barton BM, Harding GP, Zuccarelli AJ. A general method for detecting and sizing large plasmids. Anal Biochem 1995;266:235–40.
[23] Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, et al. Identification of acquired antimicrobial resistance genes. J Antimicrob Chemother 2012;67:2640–4.
[24] Carattoli A, Zankari E, Garciá-Fernández A, Larsen MV, Lund O, Villa L, et al. In Silico detection and typing of plasmids using plasmidfinder and plasmid multilocus sequence typing. Antimicrob Agents Chemother 2014;58:3895–903.
[25] Siguier P. ISfinder: the reference centre for bacterial insertion sequences. Nucleic Acids Res 2006;34:D32–6.
[26] Kim JS, Kim J, Jeon S-E, Kim SJ, Kim N-O, Hong S, et al. Complete nucleotide sequence of the IncI1 plasmid pSH4469 encoding CTX-M-15 extended-spectrum beta-lactamase in a clinical isolate of *Shigella sonnei* from an outbreak in the Republic of Korea. Int J Antimicrob Agents 2014;44:533–7.
[27] Weisenberg SA, Mediavilla JR, Chen L, Alexander EL, Rhee KY, Kreiswirth BN, et al. Extended spectrum beta-lactamase-producing enterobacteriaceae in international travelers and non-travelers in New York City. PLoS One 2012;7:e45141.
[28] Lübbert C, Straube L, Stein C, Makarewicz O, Schubert S, Mössner J, et al. Colonization with extended-spectrum beta-lactamase-producing and carbapenemase-producing Enterobacteriaceae in international travelers returning to Germany. Int J Med Microbiol 2015;305:148–56.
[29] Kennedy K, Collignon P. Colonisation with *Escherichia coli* resistant to "critically important" antibiotics: a high risk for international travellers. Eur J Clin Microbiol Infect Dis 2010;29:1501–6.
[30] Tängdén T, Cars O, Melhus Å, Löwdin E. Foreign travel is a major risk factor for colonization with *Escherichia coli* producing CTX-M-type extended-spectrum β-lactamases: a prospective study with Swedish volunteers. Antimicrob Agents Chemother 2010;54:3564–8.
[31] von Wintersdorff CJ, Penders J, Stobberingh EE, Oude Lashof AML, Hoebe CJPA, Savelkoul PHM, et al. High rates of antimicrobial drug resistance gene acquisition after international travel, the Netherlands. Emerg Infect Dis 2014;20:649–57.