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Surveillance of Antibiotic Susceptibility Patterns among *Shigella sonnei* Strains Isolated in Belgium during the 18-Year Period 1990 to 2007▽

M. Vrints,[1] E. Mairiaux,[2] E. Van Meervenne,[1] J.-M. Collard,[1,3] and S. Bertrand[1]*

Section of Bacteriology[1] and Bureau of Quality Assurance,[2] Scientific Institute of Public Health, Brussels, Belgium, and CERMES, Centre de Recherche Médicale et Sanitaire, Réseau International des Instituts Pasteur, B.P. 10887 Niamey, Niger[3]

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This study was conducted to determine the frequency and pattern of antimicrobial susceptibility of *Shigella sonnei*, the predominant species causing shigellosis in Belgium. Between 1990 and 2007, a total of 7,307 strains, mainly (98.2%) isolated from stools, were diagnosed by peripheral laboratories before being confirmed as *Shigella* strains by serotyping by the National Reference Center of *Salmonella* and *Shigella*. A significant increase in resistances to tetracycline, streptomycin, trimethoprim, sulfonamides, and cotrimoxazole (i.e., trimethoprim in combination with sulfonamides) was observed during this period. Since 1998, resistance to nalidixic acid also increased to reach a peak (12.8%) of resistant isolates in 2004. Concomitantly, multidrug resistance (MDR) in this species emerged in 2007, with 82% of total isolates being MDR. However, during this 18-year period, all isolates remained fully susceptible to ciprofloxacin and gentamicin. The work includes the molecular characterization of mechanisms of resistance to ampicillin, tetracycline, chloramphenicol, and cotrimoxazole and class 1 and class 2 integrons. *S. sonnei* acquired antimicrobial resistance to traditional antibiotics (ampicillin and tetracycline) by horizontal gene transfer, while the genetic stability of transposons was responsible for a high (89%) proportion of resistance to a commonly prescribed antibiotic (cotrimoxazole). Therefore, cotrimoxazole should no longer be considered appropriate as empirical therapy for treatment of shigellosis in Belgium when antibiotics are indicated. Rates of resistance to nalidixic acid should also be attentively monitored to detect any shift in fluoroquinolone resistance, because it represents the first line among antibiotics used in the treatment of shigellosis.

Shigella species are important pathogens that are responsible for 5 to 10% of diarrheal diseases and dysentery occurring all over the world (2). Human infections usually occur by food-borne, waterborne, or human-to-human transmission. Worldwide, the incidence of shigellosis is highest among children 1 to 4 years old (13).

Shigellosis produces inflammatory reactions and ulceration on the intestinal epithelium followed by bloody diarrhea and mucus in the stool. Infection may lead to dehydration and sometimes death, particularly in immunocompromised individuals.

Despite the disease being self-limiting, antibiotic treatment is recommended because it reduces the duration of illness and the transmission rate of the disease by shortening the period of excretion of the pathogen (4). Until 2007, fluoroquinolones, β-lactams, and a combination of trimethoprim (Tmp) and sulfamethoxazole (Sul) (cotrimoxazole [Sxt]) represented the drugs of choice to treat shigellosis (19). However, the therapy employing these drugs is becoming compromised by the emergence of strains resistant to these commonly used antibiotics.

The rapid emergence of multidrug-resistant (MDR) strains is largely due to their ability to acquire and disseminate exogenous genes associated with mobile genetic elements such as plasmids, transposons, integrons, and genomic islands (18). Based on the characteristics of their integrase genes, four classes of integrons (classes 1, 2, 3, and 4) have been identified to date (23). Class 1 and 2 integrons have been frequently reported in studies of *Shigella* spp. (7, 9).

The increasing incidence of MDR microorganisms has led to tremendous interest in the genetics and mechanisms of resistance evolved by bacteria to counteract the effects of antimicrobial agents.

Since 1990, the National Reference Centre for *Salmonella* and *Shigella* (NRCSS) has conducted a continuous surveillance program for monitoring the antimicrobial resistance of *Shigella* infections in Belgium. With approximately 400 cases of *Shigella* infections annually reported, shigellosis is the third leading bacterial gastrointestinal disease in Belgium. The majority (68%) of these infections are caused by the species *Shigella sonnei*. The current report presents the first analysis published since 1981 (6) of the evolution of the antimicrobial resistance of clinical *S. sonnei* strains isolated in Belgium. In the present study, the strains were isolated over an 18-year period (between 1990 and 2007). The work includes the determination of antimicrobial resistance frequencies for 13 antibiotics, the patterns of antimicrobial resistances, and molecular characterization of mechanisms of resistance to ampicillin (Amp), tetracycline (Tet), chloramphenicol (Chl), and Sxt and to class 1 and class 2 integrons.

MATERIALS AND METHODS

Bacterial isolates and serotyping. In Belgium, *Shigella* strains isolated from human patients by peripheral clinical laboratories (approximately 400 strains annually) are transferred on a voluntary basis to the NRCSS for serotyping. From 1 January 1990 to 31 December 2007, 7,307 *Shigella* strains were collected

* Corresponding author. Mailing address: National Shigella Reference Centre, Bacteriology Division, Scientific Institute of Public Health, J. Wytsman Street, 14-16, B-1050 Brussels, Belgium. Phone: 32 2 642 5081. Fax: 32 2 642 52 40. E-mail: sbertrand@iph.fgov.be.

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TABLE 1. PCR primers for detection of different antibiotic resistance genes

Primer	Nucleotide sequence (5′ to 3′)[a]	PCR product size (bp)	$MgCl_2$ concn (mM)	Annealing temp (°C)	GenBank accession no. or reference
Int 1F Int 1R	F: CCTCCCGCACGATGATC R: TCCACGCATCGTCAGGC	280	2	55	22
Int 2F Int 2R	F: TTATTGCTGGGATTAGGC R: ACGGCTACCCTCTGTTATC	233	3	50	22
5′-CS 3′-CS	F: GGCATCCAAGCAGCAAGC R: AAGCAGACTTGACCTGAT	Variable	3	55	U12338
Hep 74 Hep 51	F: CGGGATCCCGGACGGCATGCACGATTTGTA R: GATGCCATCGCAAGTACGAG	Variable	3	55	23
$Bla_{TEM}F$ $Bla_{TEM}R$	F: GTTGGGTGCACGAGTGGGTTACATC R: AAGGGCCGAGCGCAGAAGTGGTC	559	2	52	SFU48775
$Bla_{OXA}F$ $Bla_{OXA}R$	F: ACTGTCGCATCTCCATTATTTGA R: ACTGCATTTTTCTTGGCTTTTAT	713	3	52	AJ238349
CatF CatR	F: AACGACCCTGCCCTGAACCG R: TTGCGCCGAATAAATACCTG	1,003	3	58	U81140
Sul 1F Sul 1R	F: CGGCGTGGGCTACCTGAACG R: GCCGATCGCGTGAAGTTCCG	433	3	69	AF071413
Sul 2F Sul 2R	F: GCGCTCAAGGCAGATGGCATT R: GCGTTTGATACCGGCACCCGT	293	3	69	M36657
Tet A F Tet A R	F: GGCCTCAATTTCCTGACG R: AAGCAGGATGTAGCCTGTGC	372	3	59	X0006
Tet B F Tet B R	F: GAGACGCAATCGAATTCGG R: TTTAGTGGCTATTCTTCCTGCC	228	3	61	J01830
Tet C2 F Tet C2 R	F: TGCTCAACGGCCTCAACC R: AGCAAGACGTAGCCCAGCG	379	3	63	J01749
Tet C3 F Tet C3 R	F: TCCTTGCATGCACCATTCC R: AACCCGTTCCATGTGCTCG	635	3	63	J01749
Tet D F Tet D R	F: GGATATCTCACCGCATCTGC R: CATCCATCCGGAAGTGATAGC	436	3	61	X65876
DhfrIa F Dhfr Ia R	F: GGAGTGCCAAAGGTGAACAGC R: GAGGCGAAGTCTTGGGTAAAAAC	367	3	50	X00926

[a] F, forward; R, reverse.

and serotyped by slide agglutination with commercial antisera from the Denka Seiken Co. (Derbyshire, United Kingdom).

Antimicrobial susceptibility testing of *S. sonnei* isolates. From 1990 to 2007, the antibiotic susceptibility of *S. sonnei* strains isolated from humans was tested using a representative sampling of isolates (4,951 randomly selected isolates were tested, i.e., almost 90% of the isolates collected during the period 1990 to 1998 and 30% of those collected during 1999 to 2007).

The susceptibility of 13 antibiotics was determined by the disc diffusion (Kirby-Bauer) method following the recommendations of the Clinical and Laboratory Standards Institute (CLSI; formerly NCCLS) (12). The antibiotics tested were Amp, amoxicillin plus clavulanic acid (Amx), cefotaxime (Ctx), chloramphenicol (Chl), Tet, nalidixic acid (Nal), ciprofloxacin (Cip), streptomycin (Str), kanamycin (Kan), gentamicin (Gen), Sul, Tmp, and Sxt (tested during the period of 2000 to 2005) (Bio-Rad disks, Nazareth, Belgium). Interpretation of inhibition zones was performed according to the CLSI criteria, and quality control was performed using the *Escherichia coli* ATCC 25922 reference strain.

Molecular analysis of antibiotic-resistant determinants and integrons. Total DNA was extracted using a Qiagen DNA mini kit following the manufacturer's recommendations. Antibiotic resistance determinants and integrons were detected by PCR using the specific primers listed in Table 1. The PCR contained a 1.5 mM concentration of each primer (Table 1), 1× PCR buffer II (Applied Biosystems, Lennik, Belgium), different concentrations of $MgCl_2$ as indicated in Table 1, each of the four deoxynucleoside triphosphates at a concentration of 200 mM, and 1 unit of FastStart DNA polymerase (Roche, Vilvoorde, Belgium). Amplifications were performed with a final volume of 50 μl on an iCycler (Bio-Rad) using the following temperature programs: initial denaturation at 94°C for 4 min, 30 cycles of 94°C for 1 min, annealing for 30 s at different temperatures as indicated in Table 1, and extension for 1 min at 72°C, and a final extension step at 72°C for 10 min. PCR products (10 μl) were separated by electrophoresis using a 1% agarose gel and visualized under UV light after staining in a 1 μg ml^{-1} ethidium bromide solution.

The amplification of the gene cassettes from integrons 1 and 2 was performed with the primers 5′-CS and 3′-CS and the primers hep74 and hep51 (Table 1) in order to amplify the region between the two conserved segments of these integrons. Purified PCR fragments were sequenced using a Ceq 8000 DNA sequencer (Beckman Coulter, High Wycombe, United Kingdom) and a GenomeLab Dye Terminator cycle sequencing Quick Start kit. The BLASTN program of NCBI (http://www.ncbi.nlm.nih.gov) was used in order to identify the genes present in the gene cassette.

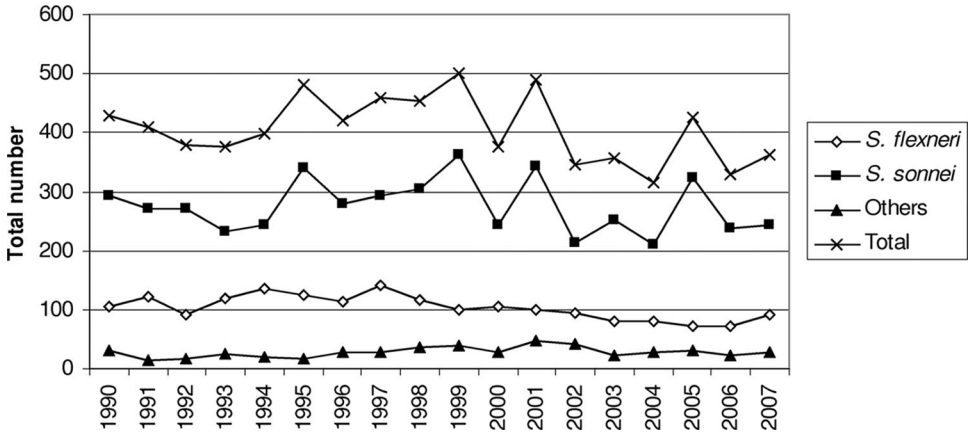

FIG. 1. Prevalence of *S. sonnei* and *S. flexneri* in Belgium from 1990 to 2007.

PFGE analysis. *S. sonnei* strains were analyzed by pulsed-field gel electrophoresis (PFGE) according to the PulseNet method (www.cdc.gov/pulsenet) and digested with the restriction endonuclease XbaI (New England Biolabs, Leusden, Netherlands). *Salmonella enterica* serovar Braenderup H9812 was used as a size marker. Fingerprinting II Informatix software (Bio-Rad) was used to compare the PFGE profiles. The generated bands were analyzed by using the Dice coefficient and the unweighted-pair group method using average linkages, with a tolerance of 1%.

Statistical analysis. Statistical analyses were conducted to evaluate the significance of the increasing antibiotic resistances. By using Statgraphics Plus version 5.1 statistical software (Statpoint Inc., Herndon, VA), a linear regression of the data was obtained and a *P* value was determined. *P* values < 0.05 were considered statistically significant.

RESULTS

Prevalence of serotypes. During the period 1990 to 2007, of the 7,307 strains of *Shigella* spp. isolated, 4,951 were serotyped as *S. sonnei* (67.8%), 1,856 as *S. flexneri* (25.4%), 244 as *S. boydii* (3.3%), and 163 as *S. dysenteriae* (2.2%) (Fig. 1). A total of 72 isolates were nonagglutinable (1%) but were biochemically confirmed as *Shigella* spp.

Over the 18-year period, the total number of isolated *S. sonnei* strains ranged from 209 to 362 a year, while the number of cases of *S. flexneri* infection diagnosed per year decreased slowly from 140 in 1997 to 90 in 2007 ($P < 0.01$). The number of *S. boydii* and *S. dysenteriae* strains isolated was always lower than 23 for each strain per year.

In approximately 13% of the *S. sonnei* cases, a clear link between a diagnosis of shigellosis and a trip abroad was established. In Belgium, the highest incidence per year of shigellosis (13.4 cases per 100,000 inhabitants) is detected among children 1 to 4 years old.

Antimicrobial resistance of *S. sonnei* isolates. Among the 4,951 *S. sonnei* strains isolated during the period of 1990 to 2007, 3,186 (64%) were randomly selected for antimicrobial susceptibility testing (Table 2). These *S. sonnei* isolates were resistant to Str (77.2%), Sul (71.6%), Tmp (75.2%), Sxt (85.9%; tested only from 2000 to 2007), and Tet (59.3%).

While in 1990 the rates of resistance to Str, Sul, and Tmp were 74.4%, 70.8%, and 61.1%, respectively, these reached respective levels of 90.4%, 79.8%, and 90% in 2007 ($P < 0.01$). The resistance to Sxt increased from 72.2% (2000) to 89.0% (2007), with a peak of 97.2% in 2005 ($P < 0.05$). On the other hand, the resistance to Tet fluctuated between 31.7% and 74.0% from 1990 to 2002, remaining stable until 2006 (76%) and finally reaching a value of 83.6% in 2007.

TABLE 2. Antimicrobial resistance of *S. sonnei* strains

Antimicrobial agent	% *S. sonnei* antimicrobial resistance in yr:																		Avg % antimicrobial resistance of *S. sonnei*
	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	
Amp	19.1	18.8	27.8	28.4	13.4	29.2	20.3	17.3	17.5	11	31.8	19.7	10	9.9	21.8	28	11.3	9.6	19.2
Amx	—[a]	—	—	—	—	—	—	0.4	1	5	0	0.8	0	0	1.3	0.9	1.3	1.4	0.7
Ctx	—	—	—	—	—	—	—	0	0	0	1.5	0	0	0	0	0	0	1.4	0.2
Tet	58.3	44.5	40.6	31.7	56	55	57.6	56.5	55.6	74	37.9	52	60	76.5	76.9	76.6	73.8	83.6	59.3
Chl	4.5	9.6	1.9	3.8	3.4	12.3	2.6	3.5	1.7	0	1.5	4.7	2	2.5	0	5.6	1.3	5.5	3.7
Cip	—	—	—	—	—	—	—	0	0	0	0	0	0	0	0	0	0	0	0
Nal	0.3	0	0	2.4	0	0.3	0	0.7	2	5	6.1	3.1	6	8.6	12.8	6.5	6.3	8.2	3.8
Str	74.7	63.6	65	53.4	72.4	67	73.8	75.3	75.1	86	72.7	81.1	76	88.9	96.2	92.5	85	90.4	77.2
Sxt	—	—	—	—	—	—	—	—	—	—	74.2	77.2	76	88.9	92.3	97.2	92.5	89	85.9
Sul	70.8	53.3	62.8	50	69.8	65	64.9	64	67	80	71.2	74	70	84	91	93.5	78.8	79.5	71.6
Tmp	61.1	47.4	62	49.5	61.6	63.5	71.2	72.4	72.1	88	74.2	81.9	78	91.4	96.2	98.1	93.8	90.4	75.2
Gen	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0
Kan	1.7	1.1	1.9	0	2.2	0.3	0.4	—	—	—	0	3.1	2	0	1.3	0.9	1.3	0	0.9

[a] —, not done.

TABLE 3. Antibiotic resistance profiles of *S. sonnei* strains

Antibiotic resistance profile	% *S. sonnei* antimicrobial resistance in yr:																		Avg % antimicrobial resistance of *S. sonnei*
	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	
Tet Str Sul Tmp (Sxt)	38.2	28.7	22.6	21.2	44.8	30.8	40.6	40.3	43.1	54	21.9	34	46	61.7	55.1	57.9	61.3	67.1	42.7
Amp Str Sul Tmp (Sxt)	0	2.3	10.9	8.2	3.4	8.2	3	4.2	6.4	1	17.2	6	2	0	12.8	12.2	1.3	0	5.5
Amp Tet Str Sul Tmp (Sxt)	9.4	3.8	11.3	3.4	2.6	7.9	11.8	5.3	5.4	2	7.8	9	6	3.7	7.7	5.6	3.8	2.7	6.1
Tet Nal Str Sul Tmp (Sxt)	0	0	0	0	0	0	0	0	1.3	4	4.7	2	6	8.6	11.5	4.7	6.3	5.5	3
MDR	55.9	46.4	48.5	37.5	58.2	59.7	59	58.3	60.6	69	58.1	57.7	62	76.6	88.5	89.6	76.3	82	63.6

From 9.9% to 31.8% of the *S. sonnei* isolates collected during this 18-year period also showed resistance to Amp. The surveillance of Ctx susceptibility in Belgium started in 1997. Over the period 1997 to 2007, only 1 (0.07%) of the 1,342 *S. sonnei* isolates tested was Ctx resistant. The β-lactam susceptibility phenotype was determined for that isolate. The *Shigella* strain represented by that isolate was highly resistant to Amp (MICs, ≥256 μg/ml), Ctx (MICs, ≥256 μg/ml), ceftazidime (MICs, ≥256 μg/ml), and, to a lesser extent, aztreonam (MICs, 32 μg/ml). The strain also remained susceptible to imipenem (MICs, 0.5 μg/ml).

Since 1998, a significant ($P < 0.01$) augmentation of the resistance to Nal was also noted, with values increasing from ≤2.5% in the 1990s to 12.8% in 2004 and falling back to ≤8.2% in 2005 to 2007. All these strains exhibited reduced susceptibility to Cip (MICs between 0.25 and 0.5 μg/ml).

From 1996 to 2007, the Chl resistance fluctuated between 0.0% and 5.5%. Before 1996, higher rates of resistance (12.3% in 1995 and 9.6% in 1991) were noted. Resistance to Kan was very seldom detected. All the isolates remained fully susceptible to Gen over the 18 years.

MDR profiles of *S. sonnei* isolates. MDR (i.e., resistance to at least four antibiotics) was common among *S. sonnei* strains, with an average of 63.5% between 1990 and 2007. The MDR frequency increased from 55.9% (1990) to 80.2% (2007) and reached a peak in 2004 and 2005 of 89% ($P < 0.01$). Dominant MDR profiles observed in *S. sonnei* were Tet Str Sul Tmp (Sxt) resistance (42.7%), Amp Tet Str Sul Tmp (Sxt) resistance (6.1%), Amp Str Sul Tmp (Sxt) resistance (5.5%), and Tet Nal Str Sul Tmp (Sxt) resistance (3.0%) (Table 3). All four of these MDR profiles were responsible together for 89.6% of the MDRs in *S. sonnei* over the period 1990 to 2007. The proportion of these four MDR profiles, already present in 1990 (47.6%), rose until 2007 (75.3%), with a peak in 2004 of 87.2% ($P < 0.01$).

Molecular analysis of the antibiotic-resistant determinants and integrons. A selection of 26 *S. sonnei* strains displaying different MDR profiles (1 strain with Str Tmp Sul Sxt resistance, 3 with Amp Str Tmp Sul Sxt resistance, 10 with Tet Str Tmp Sul Sxt resistance, 6 with Amp Tet Str Tmp Sul Sxt resistance, and 6 with Tet Nal Str Tmp Sul Sxt resistance) was made for the molecular characterization of antibiotic resistance determinants and integrons among members of the *S. sonnei* population (Table 4).

By using PCR and sequencing, the order and content of the antibiotic resistance gene cassettes inserted between the conserved regions of class 1 and class 2 integrons were determined.

In all strains, excepting one showing an Amp Tet Str Tmp Sul Sxt MDR profile, PCR fragments of approximately 1,500 bp ($n = 8$) and 2,000 bp ($n = 17$) were identified by the use of the class 2 integron primers. Sequencing of the 2,000-bp fragment revealed a class 2 integron of 2,224 bp containing three conserved resistance cassettes, *dfrA1*, *sat1*, and *aadA1* (100% identity with the class 2 integron [GenBank accession no. EU339237]), conferring resistance to Tmp, to streptothricin, and to Str and spectinomycin, respectively. All the strains containing a class 2 integron also harbored a *sul2* gene; among these, only two strains contained a *sul1* gene (Table 4).

Additionally, the nature of Amp resistance was examined by PCR testing and sequencing using universal primers for different β-lactamase-encoding genes. PCR screenings showed that all strains were negative for SHV and CTX-M β-lactamases

TABLE 4. Numbers of antibiotic resistance genes found in *S. sonnei* strains with different MDR profiles

MDR profile	No. of antibiotic resistance genes in *S. sonnei* strains with indicated MDR profile								
	Total	Integron		Sul		Tmp (*dfrA1*)	β-Lactamase (bla_{TEM-1})	Tet	
		Class 1	Class 2 (no. of bp)	*sul1*	*sul2*			*tetA*	*tetB*
Str Sul Tmp Sxt	1	0	1 (2,000)	0	1	1	—[a]	—	—
Tet Str Sul Tmp Sxt	10	0	7 (2,000), 3 (1500)	0	10	10	—	10	0
Amp Str Sul Tmp Sxt	3	0	3 (2,000)	0	3	3	3	—	—
Amp Tet Str Sul Tmp Sxt	6	0	6 (2,000)	2	6	5	6	0	6
Tet Nal Str Sul Tmp Sxt	6	0	1 (2,000), 5 (1,500)	0	6	6	—	6	0

[a] —, not present.

FIG. 2. Dendrogram generated by BioNumerics, showing the results of cluster analysis on the basis of PFGE fingerprinting. Similarity analysis was performed using the Dice coefficient, and clustering was by the unweighted-pair group method using average linkages. The different *Shigella* (Shig.) PFGE profiles and corresponding numbers of isolates are indicated.

but positive for TEM-type β-lactamase. Sequencing results using primers amplifying the whole TEM gene showed 100% identity with the $bla_{\text{TEM-1}}$ gene (GenBank accession no. EF125012). Two different *tet* genes were detected by PCR. Strains displaying the MDR profiles Tet Str Tmp Sul Sxt and Tet Nal Str Tmp Sul Sxt harbored the *tet*(A) gene, whereas the six strains with the Amp Tet Str Tmp Sul Sxt MDR profile harbored the *tet*(B) gene.

PFGE patterns. The 26 *S. sonnei* isolates characterized for their resistance genes were also subjected to a PFGE analysis. Seven distinct profiles were observed among the 26 isolates tested. The six isolates with the Tet Nal Str Tmp Sul Sxt MDR profiles and the three isolates with the Amp Tmp Str Sul Sxt MDR profiles displayed the same restriction fragment patterns (Xb-Shig 1 and Xb-Shig 2, respectively), confirming the relatedness of these resistant isolates (Fig. 2). For the other MDR profiles, no link between the PFGE profile and the antibiotic profile was able to be established. The six strains presenting the Amp Tet Str Tmp Sul Sxt MDR profile showed four different PFGE patterns (Xb-Shig 3, Xb-Shig 4, Xb-Shig 6, and Xb-Shig 7) (data not shown).

DISCUSSION

Four species of the genus *Shigella*, *S. dysenteriae*, *S. flexneri*, *S. boydii*, and *S. sonnei*, cause a wide spectrum of illnesses, ranging from watery diarrhea to fulminant dysentery. The low level required for an infectious inoculum (as few as 10 organisms) renders *Shigella* species highly contagious. The frequency of occurrence of *Shigella* spp. differs by country and in different populations within a country (10, 11). In Belgium, *S. sonnei* has always represented the predominant species, with a mean prevalence of 67.8%, followed by *Shigella flexneri* (25.4%) and *Shigella boydii* (3.5%), with *Shigella dysenteriae* (2.4%) being the least common.

Antimicrobial therapy for shigellosis reduces the duration and severity of the disease and can also prevent potentially lethal complications. However, over the past few decades *Shigella* spp. have become resistant to most of the widely used antimicrobials (14). This rise in bacterial resistance is probably partly due to the fact that, prior to 2000, no new classes of antibiotics had been discovered since 1960 and that only modifications of existing antibiotics have been launched since then (3). In Belgium, the NRCSS also recorded an increase of antibiotic resistances in *S. sonnei* strains during their surveillance program in the years 1990 to 2007. In particular, the resistance to Str and to Tmp and Sxt (Tmp plus Sul) substantially increased during this period to reach values for resistant isolates of 80% and 90%, respectively. The high prevalence of resistance to Sxt could be explained by the longtime use of this antibiotic to treat shigellosis, thereby ensuring selection pressure and maintenance of this resistance. Indeed, in the 1970s, the use of Sxt in the treatment of shigellosis was highly recommended due to the capacity of this antibiotic to be absorbed from the gastrointestinal tract. It was also proved that the use of this antibiotic significantly reduced the duration of the illness. In 2007, the Sanford guide to antimicrobial therapy (Belgium/Luxembourg version) still recommended the use of Sxt in cases of treatment failure with fluoroquinolones (19). Our study has proved that this antimicrobial is no longer appropriate for the treatment of shigellosis in Belgium. The same conclusion had already been reached a few years ago in the United States (21).

Together with the increase in Sxt resistance, the frequency of MDR isolates increased from approximately 50% in 1990 to 82% in 2007. This increase was clearly associated with the emergence of the Tet Str Sul Tmp Sxt MDR profile (47.2%) and, to a lesser extent, with that of the Amp Str Sul Tmp Sxt, Amp Tet Str Sul Tmp Sxt, and Tet Nal Str Sul Tmp Sxt MDR profiles. Interestingly, all these MDR profiles harbored resistance to Str and Sxt, regardless of whether this resistance was found in combination with Amp, Tet, and Nal resistance. Our data are in agreement with those of other studies showing the same trends of increases in incidence caused by similar MDR profiles (2, 17, 22). This increasing incidence of MDR has led to tremendous interest in the genetics and mechanisms of resistance evolved by bacteria to counteract the effects of antimicrobial agents. In gram-negative clinical isolates, capture and spread of antibiotic resistance determinants by integrons underlies the rapid evolution of MDR phenotypes (18). It is well known that in *S. sonnei*, class 2 integrons are most frequently detected (2, 7, 17). They are associated with transposon Tn*7* and are known to carry a classic gene cassette with three resistance genes, namely, dihydrofolate reductase (*dfrA1*), streptothricin acetyltransferase (*sat1*), and aminoglycoside adenyltransferase (*aadA1*), conferring resis-

tance to Tmp, to streptothricin, and to Str and spectinomycin, respectively (8). The promiscuous nature of Tn*7* is thought to have contributed to the rapid dissemination of Tmp resistance and spectinomycin-Str resistance determinants. The ability of Tn*7* to use both site-specific and nonspecific modes of transposition could explain why it has become so widespread and persistent in bacterial populations, as has been observed in studies of *S. sonnei* (17). In Belgium, the presence of class 2 integrons in almost all of the *S. sonnei* isolates tested could also explain the important increases in both Sxt resistance and Str resistance.

The genes responsible for Amp and Tet resistances in *S. sonnei* isolated in Belgium were shown to be $bla_{\text{TEM-1}}$ and *tet(*A) or *tet(*B), respectively. These genes are very common in different and distant countries (17, 20). In Korea, the TEM-type β-lactamase has been detected in a conjugally transferable R-plasmid, while the Tet resistance was located on a nontransferable plasmid which is genetically stable in *S. sonnei* (20).

In 2007, the Sanford guide to antimicrobial therapy (Belgium-Luxembourg version) recommended the use of fluoroquinolones or norfloxacin to treat shigellosis. It must be noted that, since 1998, the NRCSS has detected an increase in the resistance to Nal and that no full resistance against Cip (MICs between 0.25 and 0.5 μg/ml) has been detected so far in *S. sonnei* isolates collected in Belgium. In members of the *Enterobacteriaceae*, resistance to quinolones is generally acquired in a two-step mutation process. A first mutation in the quinolone resistance-determining region of the *gyrA* gene mediates full resistance to narrow-spectrum quinolones such as Nal and decreased susceptibility to fluoroquinolones. This is the reason that resistance to Nal is indicative of low-level resistance to Cip. A second mutation in one of the *gyrA*, *parC*, or *gyrB* genes mediates full resistance to fluoroquinolones (5). Another important mechanism accounting for decreased susceptibility to fluoroquinolones is the presence of an active efflux pump due to overproduction of the AcrAB proteins. This efflux pump has not yet been reported to be present in *Shigella* spp. thus far. The first *Shigella* strain with Nal resistance was reported in 1988 in India. Due to the extensively use of fluoroquinolones for the treatment of shigellosis, strains with full resistance to fluoroquinolones were consecutively detected in India, Nepal, and Bangladesh, beginning in the year 2002 (15, 16). More recently, a Japanese study published in 2006 mentioned the isolation of two Cip-resistant *S. sonnei* strains; however, these strains were not characterized at the molecular level (2).

At the present time, Cip is still one of the most potent antimicrobials available for medical treatment of invasive gastrointestinal infections in adults (7). Even a decrease in susceptibility to fluoroquinolones has become a major problem in medical practice. Aarestrup et al. (1) reported several treatment failures in patients infected by *Salmonella* isolates due to a decreased susceptibility to fluoroquinolones. Therefore, in Belgium, emergence of Nal resistances and possible decreased susceptibility to fluoroquinolones are attentively followed.

This report represents the first presentation of the results of long-term surveillance concerning antimicrobial resistances in clinical *S. sonnei* strains conducted in Belgium since 1978 (6). The emergence of MDR isolates strengthens the need for a continuous surveillance system. The follow-up of the evolutionary changes in antimicrobial resistance has also proven to be essential for the determination of an appropriate antimicrobial treatment for shigellosis.

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