

Identification of *Shigella sonnei* Biotype *g* Isolates Carrying Class 2 Integrons in Italy (2001 to 2003)

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Phenotyping and genotyping have been carried out on 64 epidemic and sporadic isolates of *Shigella sonnei* identified in Italy in the years 2001 to 2003. Class 2 integron carriage has been also investigated. Isolates from four of the five outbreaks and four of six sporadic cases were biotype *g*, pulsed-field gel electrophoresis type B, and class 2 integron positive, suggesting emergence and spread of an epidemic clone in Italy.

Bacillary dysentery caused by *Shigella* species is an important cause of acute diarrheal disease in both developing and industrialized countries (5, 9, 12).

Several phenotypic and molecular techniques have been used to type *Shigella sonnei* (6). Pulsed-field gel electrophoresis (PFGE), a broadly applicable typing method with a high degree of intra- and interlaboratory reproducibility, has been previously applied in studies of this organism in several countries and allowed the identification of epidemiologically relevant strains (2, 3, 11). Mobile genetic elements, including plasmids, transposons, and gene cassettes in integrons, are frequently reported in *Shigella* isolates (11). Moreover, recent studies suggest that class 2 integrons predominate in *S. sonnei* (4). Class 2 integrons seem to be specifically associated with an epidemic strain of *S. sonnei* biotype *g* that has been recently described in Australia, western Ireland, and southwestern Korea (2, 4, 8). More recently, a prolonged multistate increase in shigellosis occurring in the years 2001 to 2003 in the South and mid-Atlantic areas of the United States has been attributed to rhamnose-negative strains of *S. sonnei*; lack of rhamnose fermentation is the distinctive biochemical characteristic of biotype *g* (1).

In the years 1991 to 2000, *S. sonnei* has been very infrequently isolated in Italy from immigrants or from travelers from endemic areas (Enter-Net Italia annual surveillance reports). Moreover, data from the Centre of Enteric Pathogens of Southern Italy illustrate that biotype *g* had been quite infrequent (2

out of 779 isolates in the decade 1971 to 1980) among strains of *S. sonnei* identified in this geographic area (6).

In the years 2001 to 2003, community outbreaks in Lombardy (northern Italy) and in Sicily (southern Italy) prompted us to investigate the apparent reemergence of *S. sonnei*. We have examined 64 isolates identified in this period by analyzing their biotype, PFGE profile, drug susceptibility pattern, and carriage of class 2 integrons.

The outbreak isolates were from five different events (Table 1). (i) Eleven isolates were from an outbreak that occurred in August and September 2001 in a Kosovo refugee camp in the province of Bergamo (Lombardy). (ii) Six isolates, from a cluster of 28 culture-confirmed cases (25 children and three adults), were from an outbreak that occurred in a child care center in Montello, a small town in the province of Bergamo (Lombardy), in September 2001. (iii) Eight isolates were from an outbreak involving three families living in a small suburban community in the province of Bergamo, in September 2001. (iv) Fourteen isolates, 12 from children and 2 from adults, were from a further outbreak that occurred in October 2001 in a child care center in Melegnano, a small town in the province of Milan (Lombardy). The possible inclusion of the three events in a larger outbreak was hypothesized, but epidemiological investigation could not detect a common source for the above events. (v) Twenty-nine isolates were from hospitalized patients identified in Palermo, Sicily, in May to December 2003. Shigellosis in Palermo sickened mostly children and young adults (median age, 13 years; range, 4 to 32 years). Epidemiological investigation was unable to identify a common source or vehicle of infection.

Furthermore, six sporadic isolates were identified in the years 2001 to 2003, respectively, from one refugee child from Kosovo resident in Lombardy who was frequenting a child care center; an epidemiological relationship with the refugee camp outbreak was not apparent; three travelers, one returning from a visit to Egypt, one to Kenya, and one to Morocco, respectively; one Tunisian child hospitalized in Palermo and one apparently sporadic case identified in Brindisi, Apulia (Table 1).

Biotyping was performed by the method of Nastasi et al. (6). Antimicrobial susceptibility testing was performed by the disk

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TABLE 1. Characteristics of isolates included in this study

Region, date of isolation	Source	Antimicrobial resistance phenotype ^a						No. of isolates	Bio-type	PFGE		Class 2 integron
		Ap ^r	Cm ^r	Sm ^r	Su ^r	Tp ^r	Tc ^r			Type	Subtype	
Outbreak isolates												
Lombardy, August–September 2001	Kosovo refugee camp	R	S	S	R	R	S	10	<i>a</i>	A		–
		S	S	R	R	R	S	1	<i>a</i>	A		–
Lombardy, September 2001	Child care center	S	S	R	R	R	S	5	<i>g</i>	B	1	+
		S	R	R	R	R	S	1	<i>g</i>	B	1	+
Lombardy, September 2001	Cluster of three families	S	S	R	R	R	S	7	<i>g</i>	B	1	+
		S	S	R	S	R	S	1	<i>g</i>	B	1	+
		S	S	R	S	R	S	7	<i>g</i>	B	1	+
Lombardy, October 2001	Child care center	R	S	R	R	R	S	4	<i>g</i>	B	1	+
		S	S	R	R	R	S	3	<i>g</i>	B	1	+
Sicily, August–December 2003	Metropolitan area of Palermo	S	S	R	R	R	R	29	<i>g</i>	B	2	+
Sporadic isolates												
Melegnano (province of Milan), Lombardy, October 2001	Kosovo refugee child	S	S	S	R	R	S	1	<i>a</i>	A		–
Palermo, Sicily, June 2002	Hospitalized Tunisian child	S	S	R	R	R	R	1	<i>a</i>	A		–
Bergamo, Lombardy, October 2002	Adult returning from visit to Egypt	S	S	R	S	R	S	1	<i>g</i>	B	2	+
Brindisi, Apulia, 2003	Adult	S	S	R	R	R	R	1	<i>g</i>	B	3	+
Palermo, Sicily, August 2003	Adult returning from visit to Kenya	S	S	R	R	R	R	1	<i>g</i>	B	2	+
Palermo, Sicily, August 2003	Adult returning from visit to Morocco	S	S	R	S	R	S	1	<i>g</i>	B	2	+

^a Susceptibilities were determined by testing the following antimicrobial agents (the disk content is in parentheses): ampicillin, Ap (10 µg); chloramphenicol, Cm (30 µg); streptomycin, Sm (10 µg); sulfonamides, Su (300 µg); tetracycline, Tc (30 µg); trimethoprim, Tp (5 µg); ciprofloxacin (5 µg); kanamycin (30 µg); and nalidixic acid (30 µg). R, resistant; S, susceptible.

diffusion method as recommended by the National Committee for Clinical Laboratory Standards (7). Genomic DNA was digested with XbaI and separated in a 1% agarose gel with a contour-clamped homogeneous electric field apparatus (CHEF-DRIII; Bio-Rad Laboratories). The conditions for electrophoresis were 6 V/cm for 21 h with the pulse time increasing from 5 to 40 s (8). PFGE patterns were initially visually assessed and interpreted by using the criteria established by Tenover et al. (10). Computer-assisted analysis of PFGE banding patterns was performed with the Diversity Database software (Bio-Rad Laboratories). Similarity between isolates was calculated using the Dice coefficient after clustering was performed by the unweighted-pair group method using average linkages.

To detect the class 2 integrons, PCR was performed with primers hep74 (5'-CGGGATCCCGGAGGCATGCACGATT TGTA-3') and hep51 (5'-ATGCCATCGCAAGTACGAG-3') (2, 13). Tn7-containing strain *Escherichia coli* K-12 J62 (ColE1::Tn7) was used as the positive control strain. The similarity among the 2.2-kb amplicons of the *E. coli* reference strain and *S. sonnei* isolates was investigated by restriction analysis with the endonucleases AvaI, HincII, and HinfI.

Two biotypes were identified among the strains under investigation. Biotype *a* included the 11 isolates from the outbreak in the Kosovo refugee camp in 2001 and two apparently sporadic isolates identified in 2001 and 2002, respectively, from a child living in a refugee community from Kosovo and from a pediatric patient of Tunisian origin. Biotype *g* included all the remaining isolates.

The antimicrobial resistance patterns are summarized in Table 1. PFGE assigned the *S. sonnei* strains under study into two clusters: PFGE type A (*n* = 13) and PFGE type B (*n* = 51). PFGE type A patterns were indistinguishable; PFGE type B isolates were divided into three variants named B1, B2, and B3, differing from each other by fewer than three bands (Fig. 1).

Isolates from southern Italy appeared closely related to those from the imported cases from African countries (Fig. 1).

Class 2 integrons of 2.2 kbp were identified in all isolates of *S. sonnei* biotype *g* and PFGE type B (Fig. 2a). The sequence similarity among the 2.2-kb amplicons was confirmed by restriction analysis with the restriction endonucleases AvaI, HincII, and HinfI. All amplicons gave identical restriction patterns, and the sum of the sizes of the digestion fragments was consistent with the size of the undigested amplicons (Fig. 2b). All isolates positive for class 2 integrons were also phenotypically resistant to trimethoprim and streptomycin, which is consistent with the resistance genes contained within the gene cassettes.

Our study supports the existence within the isolates under study of two well-defined patterns. The PFGE type A, biotype *a* pattern was confined, with the exception of one isolate from a Tunisian child, to the epidemiological context of the Kosovo refugees. The PFGE type B, biotype *g*, class 2 integron-positive pattern contained isolates from four epidemic clusters occurring in Italy in the years 2001 and 2003 and from five apparently sporadic cases, including three cases acquired in different African countries in the same period.

These data suggest the attribution of *S. sonnei* isolates circulating in recent years in different regions of Italy and, presumably, in some Africa countries to a clonal spread of a well-defined strain.

Furthermore, visual comparison between PFGE type pattern B, obtained with XbaI from our isolates, and the PFGE pattern from Korean isolates that had been obtained under the same electrophoretic conditions, suggests a close similarity (8). XbaI pattern B from western Ireland isolates and XbaI pattern A from Japanese human and oyster isolates appear also very similar, but with the cautiousness imposed by a visual assessment (2, 11).

Recently, some papers have reported that *S. sonnei* strains of

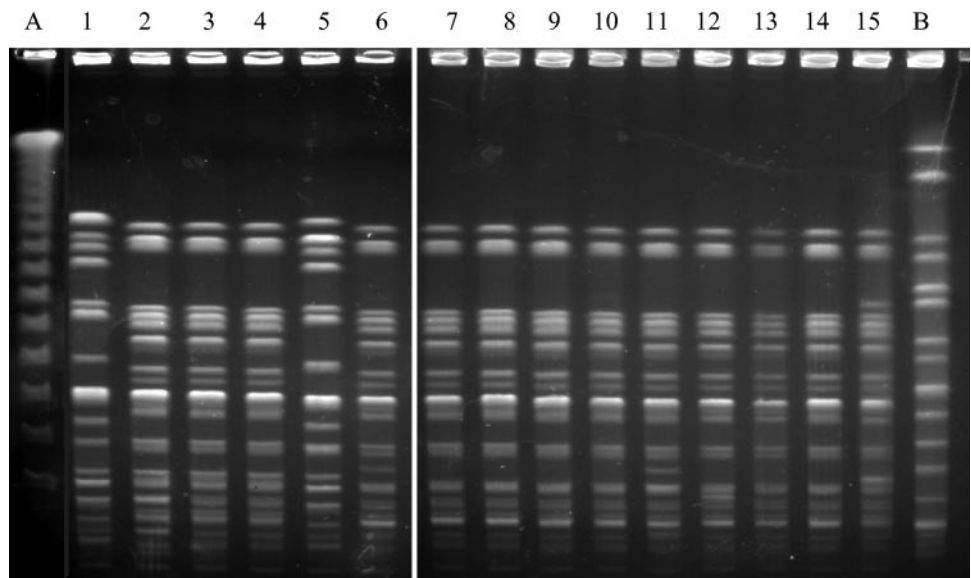


FIG. 1. PFGE patterns of *S. sonnei* strains in Italy from 2001 to 2003. Lanes: A, supercoiled ladder DNA; 1, PFGE type A outbreak strain from Kosovo refugee camp; 2 to 4, PFGE type B1 strains from three outbreaks that occurred in Lombardy, northern Italy, in 2001; 5, PFGE type A sporadic strain from a Tunisian child isolated in Palermo, southern Italy, in 2001; 6 to 11, PFGE type B2 strains from a prolonged community outbreak that occurred in Palermo, southern Italy, in 2003; 12 to 14, PFGE type B2 strains from imported cases of shigellosis in travelers returning from visits to Egypt, Kenya, and Morocco, respectively, in 2003; 15, PFGE type B3 sporadic strain isolated in Brindisi, southern Italy, in 2003; B, *Salmonella* Braenderup PFGE global standard. For more details about *S. sonnei* strains, see Table 1.

biotype *g* carrying class 2 integrons are prevalent since 1998 in Australia and southwestern Korea (4, 8). Moreover, emergence of phage type 6 in England and Wales, where it accounts in recent years for 80% of *S. sonnei* isolates, suggests an additional involvement of these countries in the spreading phase of this clone because of the closest correlation among phage type 6, biotype *g*, and PFGE type B among *S. sonnei* isolates identified in western Ireland (2). Finally, the recent report of

rihamnose-negative *S. sonnei* responsible for multistate outbreaks in 2001 to 2003 in the United States could probably be interpreted as further evidence of the circulation of this clone on a worldwide scale (1).

Although the above findings are suggestive of the spread of a pandemic clone of *S. sonnei*, further work, including a phenotypic and genotypic direct comparison of the strains isolated in the different geographic areas, is needed to assess the true extent

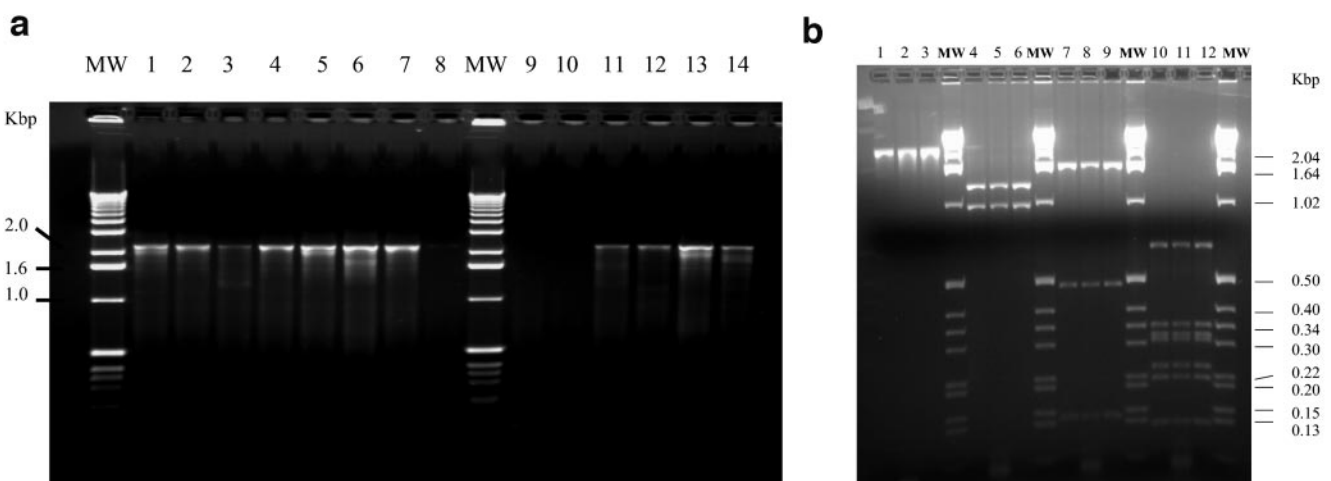


FIG. 2. (a) Class 2 integrons in *S. sonnei* strains in Italy in 2001 to 2003. Lanes: MW, 1-kbp DNA ladder; 1 to 8, strains from southern Italy; 1 to 4, outbreak strains isolated in Palermo, Sicily, in 2003; 5, sporadic strain from Brindisi, Apulia, in 2003; 6 to 7, strains imported from Kenya and Morocco in 2003; 8, sporadic strain from a Tunisian child in 2001. 9 to 14, strains from northern Italy; 9, strain from a Kosovo refugee camp outbreak in 2001; 10, sporadic strain from a Kosovo child in 2001; 11 to 13, strains from three different outbreaks in Lombardy in 2001; 14, strain imported from Egypt in 2003. For more details about *S. sonnei* strains, see Table 1. (b) Results of restriction analysis of the 2.2-kb amplicons of ColE1::Tn7, RP4-2, and two representative class 2 integron-positive *S. sonnei* strains with *Ava*I (lanes 4, 5, and 6), *Hinc*II (lanes 7, 8, and 9), and *Hinf*I (lanes 10, 11, and 12). Lanes 1, 2, and 3 contain the undigested amplicons. Lane MW, 1-kbp DNA ladder.

of the problem within the wider context of the genetic diversity of *S. sonnei*.

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