Epidemic Strains of *Shigella sonnei* Biotype g Carrying Integrons

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Class 2 integrons (Tn7) were found in all randomly selected epidemic (*n* = 27) and preepidemic (*n* = 13) strains of multiresistant *Shigella sonnei* biotype g. A class 1 integron was also found in two epidemic strains. Gene cassettes within these integrons account for resistance to commonly used therapeutic agents.

Shigella species are a common cause of severe gastroenteritis characterized by excretion of stools containing white cells and blood. Infection may lead to dehydration and sometimes death, particularly in immunocompromised individuals. *Shigella* is a highly infectious agent, and antibiotic treatment may be necessary to manage infection and reduce fecal excretion of the bacterium to prevent further transmission. Treatment of shigellosis has been confounded by widespread resistance to commonly used antibiotics such as ampicillin, trimethoprim-sulfamethoxazole (cotrimoxazole), nalidixic acid, and tetracycline (5, 10, 12, 13, 17, 20). Resistance to these antibiotics may be plasmid, transposon, and/or chromosome mediated (5, 18). Mobile genetic elements (integrons) are found on these genetic entities and are responsible for the horizontal transfer of antibiotic resistance among gram-negative bacilli (11, 26). Integrons containing the resistance genes *dfrA1* and *oxa-1* have been shown to confer *Shigella sonnei* resistant to trimethoprim (*dfrA1*) and ampicillin (*oxa-1*), respectively (17). In this study, we focused on multiresistant strains of *S. sonnei* biotype g isolated in Sydney, Australia. Isolates collected during an epidemic of shigellosis among homosexual men were compared with strains from the previous 3 years for resistance phenotypes and genotypes. The aim of this study was to determine whether integrons are present in a random selection of strains representative of this epidemic and the antecedent 3-year period and whether these mobile genetic elements contribute to the antibiotic susceptibility pattern detected.

In Sydney, between January and July 2000, 148 cases of gastrointestinal infections caused by *S. sonnei* biotype g were reported to the South Eastern Sydney Public Health Unit (1). In the previous 10 years there had been an average of 95 cases of shigellosis in this state, approximately 50% of which were attributable to *S. sonnei* biotype g. Over 90% of cases during the epidemic occurred in homosexual men aged 20 to 40 years and were strongly linked with having attended sex-on-premises venues (B. O'Sullivan, V. Delpech, G. Pontivivo, and J. McAnulty, Abstr. Communicable Diseases Control Conf., Canberra,

abstr. 31, 2001). A number of these patients were HIV-positive and required hospitalization (1). Antibiotic susceptibility surveillance since 1997 by a laboratory within the eastern Sydney area (St Vincent's Hospital, Darlinghurst) detected a change in susceptibility pattern to commonly used therapeutic agents. Of 126 strains tested, those isolated during 2000 (epidemic strains) were more likely to be resistant to ampicillin than strains isolated during the previous 3 years (86 versus 16%) (Table 1). In these strains, there was a smaller, nonsignificant increase in the proportion of strains resistant to cotrimoxazole during the epidemic, from 84 to 94%. All strains were sensitive to ciprofloxacin. Furthermore, these same strains have been shown to be clonal by molecular fingerprinting (14) by PCR with enterobacterial repetitive intergenic consensus primers (T. Karagiannis, D. Marriott, P. Kearney, M. Poynten, V. Delpech, G. Pontivivo, M. Ferson, and J. Harkness, Abstr. 11th Annu. Conference, ASHM, abstr. P52, 1999; T. Karagiannis, personal communication).

In this study, a total of 60 strains (from the above collection) were randomly selected from the epidemic (*n* = 27) and the 3-year antecedent (*n* = 33) periods. The identifications of all strains were confirmed by biochemical methods (8), and strains were typed by a reference laboratory. Susceptibility to an extended range of antibiotics was undertaken by using the calibrated dichotomous sensitivity method (4). The aim of this experiment was to assess phenotypic diversity with regard to a range of pharmacologically different antibiotics rather than to explore therapeutic options. Here, five patterns of resistance were found for sensitivities to the aminoglycosides (gentamicin, kanamycin, streptomycin, and tobramycin), antifolates (sulfafurazole and trimethoprim), cephalosporins (cefotaxime and cephalexin), quinolones (nalidixic acid and norfloxacin), chloramphenicol, imipenem, nitrofurantoin, and tetracycline (Table 2). Preepidemic strains were predominantly resistant to streptomycin, sulfafurazole, trimethoprim, and tetracycline (97.0%). Strains isolated during the epidemic period were predominantly resistant to streptomycin, sulfafurazole, trimethoprim, tetracycline, and ampicillin (96.3%). The emergence of resistance to ampicillin, which is commonly used for *Shigella* infection, had significant therapeutic implications.

Forty of the above strains, including all 27 epidemic and 13 randomly selected preepidemic strains, were examined for in-

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TABLE 1. Comparison of the number of *S. sonnei* biotype g strains resistant to cotrimoxazole and ampicillin isolated during 2000 and 1997–1999

Antibiotic	No. (%) of resistant strains[a]		P[b]	Relative risk	95% confidence interval
	2000 ($n = 88$)	1997–1999 ($n = 38$)			
Cotrimoxazole	83 (94)	32 (84)	0.08	1.12	0.967–1.297
Ampicillin	76 (86)	6 (16)	<0.0001	5.47	2.612–11.454

[a] Antibiotic susceptibilities were determined by the NCCLS method (16).
[b] Probability was calculated by using Fisher's exact test.

tegrons to determine their contribution to the observed resistance patterns. The methods used to detect and characterize integrons were the same as those used in previous studies to demonstrate a correlation between integrons and antibiotic resistance in urinary isolates of the *Enterobacteriaceae* (25, 26). Briefly, a PCR with degenerate primers was used to amplify conserved regions of integron integrase genes *intI1*, *intI2*, and *intI3*. The PCR products were subjected to restriction fragment length polymorphism analysis to discern class 1, 2, and 3 integrons. Class 1 and 2 cassette regions were amplified and characterized by PCR sequencing (26).

All 40 strains harbored a class 2 integron with a gene cassette array analogous to that found in Tn7, namely, *dfrA1*, *sat1*, and *aadA1*, conferring resistance to trimethoprim, streptothricin, and streptomycin/spectinomycin, respectively. Tn7 is a transposon and class 2 integron containing a defective integrase gene that is unable to alter this array of gene cassettes (19). The promiscuous nature of Tn7 is thought to have contributed to the rapid dissemination of trimethoprim- and streptomycin/spectinomycin-resistant bacteria (13, 22). Although *dfrA1* has been reported to be prevalent in *S. sonnei* (6, 13) and is found in Tn7, the exact role of this transposon in conferring antibiotic resistance in shigellae remains undefined.

Two of the 27 epidemic isolates also contained a class 1 integron harboring *dfrA12* and *aadA2*, which code for trimethoprim and streptomycin/spectinomycin resistance, respectively. The class 1 integrons differ from class 2 in that they are able to integrate and excise gene cassettes, and also contain a sulfonamide resistance gene (*sul1*) in the 3′ conserved segment (11). Analysis of the cassette regions of both integron classes did not identify gene cassettes associated with ampicillin resistance.

In recent studies, more clinical isolates of gram-negative enterobacteria contained class 1 integrons than class 2 integrons (9, 15, 26). The presence of class 2 integrons in all *Shigella* isolates in this study contradicts this trend but explains

the evolution of streptomycin- and trimethoprim-resistant phenotypes in these strains during the past 3 decades. These two antibiotics were introduced in the 1950s as alternative treatments for sulfonamide-resistant *Shigella* spp. and were used extensively in the Australian population (24). Streptomycin resistance is strongly associated with integrons because of the high prevalence of *aadA* cassettes within class 1 and 2 integrons (26). Streptomycin has long been excluded for treatment of shigellosis, and the same fate is envisaged for cotrimoxazole because of the rapid dissemination of dihydrofolate reductases, encoded by *dfrA1* cassettes in integrons.

Resistance to the fluoroquinolones ciprofloxacin and norfloxacin was not detected among the strains studied here, which is different from the trend seen in some developing countries (21). Only one of the 60 strains tested was resistant to nalidixic acid, reflecting the limited use to date of this antibiotic for the treatment of shigellosis in Australia. Nalidixic acid is not a recommended therapeutic agent for shigellosis in this country (2) and was recently withdrawn from distribution (3). Three of the 60 strains were also resistant to cephalexin, and none were resistant to gentamicin, tobramycin, kanamycin, cefotaxime, chloramphenicol, imipenem, or nitrofurantoin. Gentamicin has been recommended only for the treatment of severe shigellosis (23). However, clinical isolates of enterobacteria containing class 1 integrons from the same geographic area contain aminoglycoside-inactivating gene cassettes, as reported previously (26). This may act as a reservoir of resistance genes that can be transferred to other species within and outside the family *Enterobacteriaceae* (27). Gentamicin resistance could rapidly be established in strains containing integrons with aminoglycoside resistance genes if gentamicin was used more extensively, in the same way that integrons in shigellae containing *dfrA1* and *aadA1* have been selected during periods of high usage of trimethoprim and streptomycin. The treatment of shigellosis is often empirical due to the delay in obtaining an antibiotic susceptibility profile of the isolate. Thus, the inappropriate use of ampicillin during this epidemic based on previous favorable experience enhanced the selection and dissemination of strains resistant to this antibiotic.

The resistance of shigellae to the recommended therapeutic agents cotrimoxazole and ampicillin restricts current treatment options to the quinolones (Table 1). These antibiotics are more expensive and are contraindicated for treating infections in children, pregnant women, and breastfeeding women. The endemicity of a sexually transmitted disease in a defined epidemiologic setting has been described to be a function of transmissibility, the rate of interaction between infected individuals, and the duration of infectivity of the organism (7). Use of

TABLE 2. Resistance patterns of preepidemic (1997–1999) and epidemic (2000) isolates of *S. sonnei* biotype g

No. of isolates		Result for antimicrobial agent[a]						
1997–1999 ($n = 33$)	2000 ($n = 27$)	Streptomycin	Sulfafurazole	Trimethoprim	Ampicillin	Tetracycline	Nalidixic acid	Cephalexin
3	0	R	R	R	R	R	S	R
2	26	R	R	R	R	R	S	S
0	1	R	R	R	S	R	R	S
27	0	R	R	R	S	R	S	S
1	0	S	R	S	S	S	S	S

[a] Susceptibilities were determined using calibrated dichotomous sensitivity criteria (4). R, resistant; S, sensitive.

recommended antibiotics (ampicillin or cotrimoxazole) to treat infections caused by the shigellae studied here will contribute to endemicity, as inappropriate treatments extend infectivity. Thus, the presence of integrons in shigella strains, with the potential to acquire antibiotic resistance genes and the ability to spread in epidemics, is a strong imperative for intervention at a public health level. Controlling the level of infection within a high-risk group such as promiscuous homosexual men could limit the spread of infection. Within such a defined group, treatment with the appropriate antibiotic with short and effective doses limits the environmental pressure for selection and dissemination of resistance genes. Such targeted interventions could also delay the spread of integron-mediated resistance between *Shigella* strains.

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