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SURVEILLANCE FOR ANTIMICROBIAL RESISTANCE PROFILES AMONG *SHIGELLA* SPECIES ISOLATED FROM A SEMIRURAL COMMUNITY IN THE NORTHERN ADMINISTRATIVE AREA OF SANTIAGO, CHILE

NOELIA FULLÁ, VALERIA PRADO, CLAUDIA DURÁN, ROSANNA LAGOS, AND MYRON M. LEVINE

Programa de Microbiología, Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile, Santiago, Chile; Centro para Vacunas en Desarrollo, Santiago, Chile; Center for Vaccine Development, University of Maryland, School of Medicine, Baltimore, Maryland

Abstract. Variations in antibiotic resistance patterns were studied among 178 *Shigella* strains isolated from 1997 to 2001 in children less than five years of age with acute diarrhea from Colina, a semi-rural community in Santiago, Chile. The minimal inhibitory concentration of several commonly used antibiotics was determined by the agar dilution method. *Shigella* strains showed high rates of resistance to ampicillin (82%), cotrimoxazole (65%), tetracycline (53%), and chloramphenicol (49%). Furthermore, 51% of the strains showed resistance patterns to multiple antibiotics. Only 9% of the strains were resistant to amoxicillin-clavulanic acid and no resistance was observed to ciprofloxacin, nalidixic acid, or cefotaxime. Continuous monitoring of resistance patterns in *Shigella* is essential for establishing and updating guidelines for antibiotic treatment in shigellosis.

INTRODUCTION

Shigella is recognized by the World Health Organization as a major global public health problem. It is one of the principal causes of diarrhea in pediatric patients in developing countries, but remains as an occasional cause of diarrhea among children in industrialized countries, particularly in settings such as day care. As with most enteric infections, the risk of contracting shigellosis is associated with deficiencies in environmental sanitation and personal hygiene.[1–4] In Chile, *Shigella* is responsible for 4–12% of the cases of acute diarrhea and 22–30% of the cases of bloody diarrhea in children less than five years of age.[1,3]

Shigellosis is one of the few enteric infections for which antimicrobials are prescribed, with both clinical and epidemiologic benefits, but increasing resistance has been observed.[3–6]

Since 1995, we have maintained epidemiologic and bacteriologic surveillance for *Shigella* infections in Colina, a location in the outskirts of the Northern Administrative Area of the Metropolitan Region of Santiago, Chile. This location has a population of 77,647 inhabitants, but socioeconomic indicators and sanitary conditions are lower than the national mean. Previously, Prado and others examined *Shigella* strains isolated from Colina during the period 1995–1997 and reported high and increasing levels of resistance, as well as patterns of multi-resistance to antibiotics commonly used for treating shigellosis in Colina.[3]

In this study, we extended our previous surveillance studies by examining antibiotic resistance patterns among *Shigella* strains isolated from 1997 to 2001 from children less than five years of age with acute diarrhea.

MATERIALS AND METHODS

Surveillance study period. Shigellosis is highly seasonal in Santiago, predominating during the warm months. Thus, the surveillance study for each year was initiated on November 1 and terminated on April 30 to coincide with this period. During the four successive surveillance seasons, a stool specimen from each diarrheal consultation at the two outpatient clinics in Colina was collected with a cotton swab, transported to the laboratory in buffer glycerol phosphate medium, and tested using standard procedures for the isolation of *Shigella*. The same procedures were used through all four study periods. Strains were stored in trypticase soy broth with 15% glycerol at –80°C. The four surveillance periods used in this study were period I: November 1997 to May 1998; period II: November 1998 to May 1999; period III: November 1999 to May 2000; and period IV: November 2000 to May 2001.

The surveillance study protocol was included within a more comprehensive protocol that was reviewed and approved by the Institutional Review Board of the University of Maryland and the Ethics Committee of the Servicio de Salud Metropolitano Norte (SSMN) of the metropolitan region of Santiago. Stool samples were obtained from patients as a routine procedure of standard care in the Consultorio Colina. Bacterial strains were archived and tested in an anonymous manner, and authorization for susceptibility testing was obtained from the Ethics Committee of the SSMN.

***In vitro* susceptibility to antibiotics.** The minimal inhibitory concentrations of antimicrobials were determined by the agar dilution method according to the 2003 recommendations of the National Committee for Clinical Laboratory Standards.[7] The antibiotics used included ampicillin, amoxicillin-clavulanic acid, cotrimoxazole, chloramphenicol, tetracycline, ciprofloxacin, nalidixic acid, and cefotaxime. Each antimicrobial was serially diluted and tested at the following ranges of concentrations: 1.0–64 μg/mL for ampicillin, amoxicillin-clavulanic acid, chloramphenicol and nalidixic acid; 0.25–4.75 to 16–304 μg/mL for cotrimoxazole; 0.03–64 μg/mL for cefotaxime; 0.5–32 μg/mL for tetracycline; and 0.004–8.0 μg/mL for ciprofloxacin. *Escherichia coli* strain ATCC 25922 (American Type Culture Collection, Manassas, VA) was used as a control.

RESULTS

In the four surveillance seasons from 1997 to 2001, 4,080 episodes of acute diarrhea among children less than five years of age were observed at the health centers in Colina. *Shigella* was isolated from 178 of these diarrhea episodes and the distribution by species is shown in Table 1.

TABLE 1
Isolation of *Shigella flexneri* and *S. sonnei* in the community of Colina between 1997 and 2001

Period of study*	No. of episodes	Frequency of isolation			
		S. flexneri	*S. sonnei*	Total	
		No. of strains	No. of strains	No.	%
I	916	12	34	46	5.0
II	629	10	2	12	1.9
III	697	18	21	39	5.6
IV	1,838	37	44	81	4.4
Total	4,080	77	101	178	4.4

* Period I = November 1997 to May 1998; Period II = November 1998 to May 1999; Period III = November 1999 to May 2000; Period IV = November 2000 to May 2001.

Susceptibility patterns of *S. flexneri* strains. The 77 strains of *S. flexneri* isolated in the four surveillance periods showed a high proportion of resistant strains to tetracycline (78%), ampicillin (74%), cotrimoxazole (71%), and chloramphenicol (69%), and a low proportion of resistant strains to amoxicillin-clavulanic acid (12%). All *S. flexneri* strains were susceptible to ciprofloxacin, nalidixic acid, and cefotaxime. Over the study period, *S. flexneri* exhibited some variations in the resistance patterns. A significant decrease in resistance to ampicillin and tetracycline ($P < 0.05$) in period III with reemergence of high levels of resistance in period IV, and an increase in resistance to cotrimoxazole in period IV with respect to previous *Shigella* seasons were observed (Figure 1).

Susceptibility patterns of *S. sonnei* strains. Of the 101 strains of *S. sonnei* isolated during the study period, 88% were resistant to ampicillin, 60% to cotrimoxazole, 36% to tetracycline, 34% to chloramphenicol, and 7% to amoxicillin-clavulanic acid. All *S. sonnei* strains were susceptible to ciprofloxacin, nalidixic acid, and cefotaxime. There were annual variations in antimicrobial resistance of *S. sonnei*, showing significant decreases in resistance to ampicillin, chloramphenicol, tetracycline, and amoxicillin-clavulanic acid, and a significant increase in resistance to cotrimoxazole in periods III and IV ($P < 0.05$) (Figure 2).

Overall, we observed that 155 (87%) strains were resistant to one or more antibiotic distributed into 13 distinct patterns (Table 2). Of these, 91 strains (51%) exhibited resistance to three or more antibiotics (multi-resistance). The most frequent multi-resistance pattern observed among *S. flexneri* strains was the combined resistance to ampicillin, cotrimoxazole, chloramphenicol, and tetracycline, which was seen in 35 (53.8%) strains, followed by resistance to ampicillin, chloramphenicol and tetracycline, which was observed in 9 strains (13.8%). The most frequent multi-resistance pattern among *S. sonnei* strains was to ampicillin, chloramphenicol, and tetracycline (23.3%).

A comparison of resistance levels observed in this study with those previously reported for the 1995–1997 period showed a significant increase in resistance to ampicillin and cotrimoxazole and a decrease in resistance to amoxicillin-clavulanic acid ($P < 0.05$).

DISCUSSION

The emergence and dissemination of antimicrobial resistance among *Shigella* strains is an increasing global health problem that is complicating the therapeutic management of severe shigellosis cases. Studies from many regions of Chile have already reported an increase in isolation of *Shigella* that are resistant or exhibiting multi-resistance to clinically important and commonly used antibiotics.[8–10] Our findings from the semirural study area of Colina, are consistent with those reports in that we also observed an alarmingly high prevalence of *Shigella* that are resistant to antibiotics frequently used for treating shigellosis by clinicians in Santiago.

The changes in the resistance profiles observed among *S. sonnei* strains from Colina cannot be explained, to our knowledge, by changes in practice of antibiotic usage. A plausible explanation may be that there are selective pressures that are causing the disappearance and reemergence of specific clones, as shown by the virtual absence of *S. sonnei* during period II of the study, followed by resurgence during periods III and IV (Table 1). Molecular typing would help clarify this issue.

Consistent with other studies, our results confirmed the high prevalence of resistance to tetracycline and ampicillin in *S. flexneri*, despite the fact that ampicillin has not been used during the past 18 years in Chile for treatment of suspected

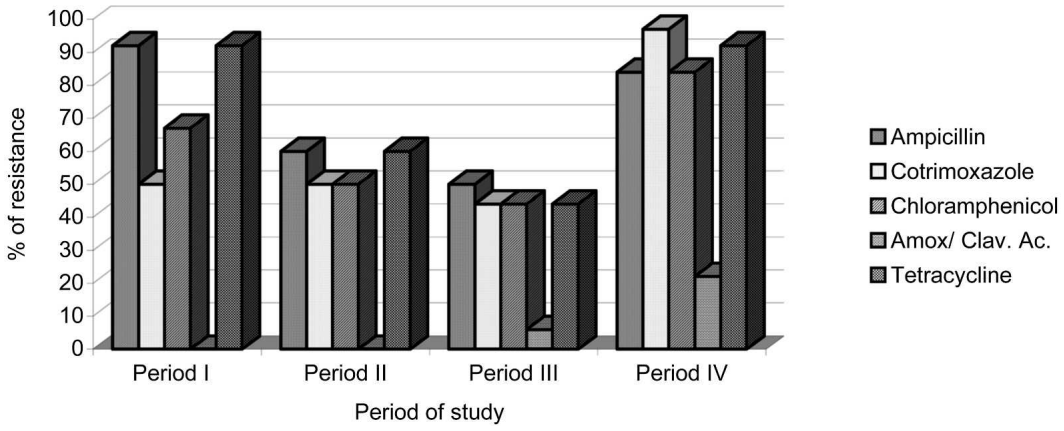

FIGURE 1. Variations in the frequency of resistance to five antimicrobials among strains of *Shigella flexneri* isolated in Colina, Santiago, Chile between 1997 and 2001. $P = 0.0007$ for cotrimoxazole period I versus period IV; the global P value was not significant. Amox/Clav. Ac. = amoxicillin/clavulanic acid.

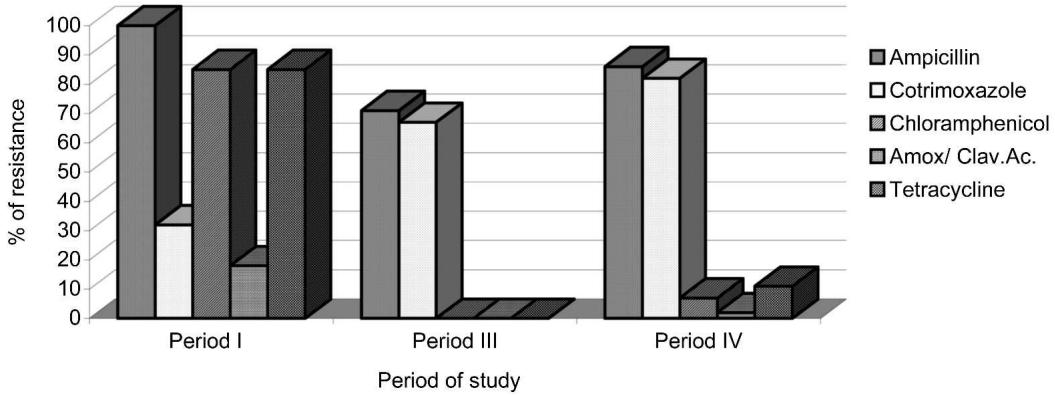

FIGURE 2. Variations in the frequency of resistance to five antimicrobials among strains of *Shigella sonnei* isolated in Colina, Santiago, Chile between 1997 and 2001. In period II, only two *S. sonnei* strains were isolated. $P < 0.05$ for period I versus period IV percentage of resistance for all antimicrobials. Amox/Clav. Ac. = amoxicillin/clavulanic acid.

cases of shigellosis and that tetracycline is not used in children.[11,12] The persistence of ampicillin resistance may be explained by the wide spread use of β-lactams for diverse infections in Chilean children, causing selection of resistant strains and propagation of β-lactamase-resistant genes in the normal intestinal flora. It has been reported that antimicrobial resistance genes can be readily transmitted between commensal Enterobacteriaceae and enteropathogens *in vivo* and *in vitro*.[13]

It is also noteworthy that none of the *Shigella* strains were resistant to nalidixic acid and ciprofloxacin. Several studies show that ciprofloxacin offers advantages in the treatment of shigellosis, reaching high concentrations in serum and feces. Short courses of ciprofloxacin therapy in pediatric patients with specific enteric infections is becoming a common practice among pediatricians worldwide.[14,15] Other antibiotics that remain highly active against *Shigella* are the third-generation cephalosporins. However, these are generally reserved for severe systemic infections such as bacterial meningitis or for clinically severe shigellosis caused by multiresistant strains. We discourage widespread use of cephalosporins for treating all *Shigella* infections to avoid selection of resistant strains.

In conclusion, antimicrobial resistance patterns of *Shigella* observed in this study confirm reports from other countries, showing widespread resistance of *Shigella* to multiple, clinically relevant antimicrobials. For areas where shigellosis is endemic, recommendations on antibiotic selection must be periodically updated depending on surveillance of antimicrobial resistance.

TABLE 2
Distribution of resistance patterns among 65 *Shigella flexneri* and *90 S. sonnei* isolated in Colina between 1997 and 2001

Resistance patterns*	No. of strains		
	S. flexneri	*S. sonnei*	*Shigella* spp.
AM	0	4	4
STX	3	1	4
AM, AMC	0	1	1
AM, STX	2	48	50
AM, TET	1	0	1
STX, TET	4	0	4
AM, STX, TET	1	2	3
AM, CL, TET	9	21	30
AM, AMC, STX	1	0	1
STX, CL, TET	1	0	1
AM, AMC, CL, TET	0	4	4
AM, STX, CL, TET	35	6	41
AM, STX, AMC, TET, CL	8	3	11

* AM = ampicillin; STX = cotrimoxazole; AMC = amoxicillin/clavulanic acid; TET = tetracycline; CL = chloramphenicol.

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Authors' addresses: Noelia Fullá, Valeria Prado, and Claudia Durán, Programa de Microbiología, Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile, Correo 7, Clasificador 7, Santiago, Chile, Telephone: 56-2-678 6641, Fax: 56-2-735 5855, E-mails: promicro@med.uchile.cl and vprado@med.uchile.cl. Rosanna Lagos, Centro para Vacunas en Desarrollo, Santiago, Chile, Telephone: 56-2-737 5022, Fax: 56-2-777 5766, E-mail: rlagos@adsl.tie.cl. Myron M. Levine, Center for Vaccine Development, University of Maryland, School of Medicine, Baltimore, MD 21201, Telephone: 410-706-7588, Fax: 410-706-6205, E-mail: Mlevine@medicine.umaryland.edu.

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