Frequency of serovars and antimicrobial resistance in *Shigella* spp. from Brazil

Gisele Peirano+, Flávia dos Santos Souza, Dalia dos Prazeres Rodrigues, *Shigella* Study Group

Laboratório de Referência Nacional de Cólera e outras Enteroinfecções Bacterianas, Instituto Oswaldo Cruz-Fiocruz, Av. Brasil 4365, 21045-900 Rio de Janeiro, RJ, Brasil

A total of 296 Shigella *spp. were received from State Public Health Laboratories, during the period from 1999 to 2004, by National Reference Laboratory for Cholera and Enteric Diseases (NRLCED) - IOC/Fiocruz, Rio de Janeiro, Brazil. The frequency of* Shigella *spp. was:* S. flexneri *(52.7%),* S. sonnei *(44.2%),* S. boydii *(2.3%), and* S. dysenteriae *(0.6%). The most frequent* S. flexneri *serovars were 2a and 1b. The highest incidence rates of* Shigella *isolation were observed in the Southeast (39%) and Northeast (34%) regions and the lowest rate in the South (3%) of Brazil. Strains were further analyzed for antimicrobial susceptibility by disk diffusion method as part of a surveillance program on antimicrobial resistance. The highest rates of antimicrobial resistance were to trimethoprim-sulfamethozaxole (90%), tetracycline (88%), ampicillin (56%), and chloramphenicol (35%). The patterns of antimicrobial resistance among* Shigella *isolates pose a major difficulty in the determination of an appropriate drug for shigellosis treatment. Continuous monitoring of antimicrobial susceptibilities of* Shigella *spp. through a surveillance system is thus essential for effective therapy and control measures against shigellosis.*

Key words: *Shigella* - serovars - antimicrobial resistance surveillance - Brazil

Shigellosis is endemic throughout the world and it is among the most common causes of bacterial diarrhoeal diseases. It is responsible for approximately 165 million cases annually, of which 163 million are in developing countries and 1.5 million in industrialized ones. It is estimated that 1.1 million people die annually from *Shigella* infection and nearly 580,000 cases of shigellosis are reported among travelers from industrialized countries. The frequency of *S. flexneri* , *S. sonnei, S. boydii*, and *S. dysenteriae* were 60, 15, 6%, and 6% (30% of *S. dysenteriae* cases were type 1) in developing countries; and 16, 77, 2, and 1% in developed ones, respectively. In developing countries, the predominant serotype of *S. flexneri* is 2a, followed by 1b, 3a, 4a, and 6 (Kotloff et al. 1999).

Although epidemic Shiga dysentery is the most serious manifestation of *Shigella* infection in developing countries, the majority of *Shigella* infections are due to endemic shigellosis. *S. flexneri* is the endemic species and is responsible for approximately 10% of all diarrhoeal episodes among children younger than five years. *S. dysenteriae* type 1 causes epidemic and endemic disease, whereas, in developed countries, *S. sonnei* is predominantly involved in common source sporadic outbreaks. *S. boydii*, was first detected in India and up to now has been uncommonly found, excepting in the Indian subcontinent (Niyogi 2005).

Except for *S. sonnei*, each species contains multiple serotypes based on the structure of the O antigen, and, at least 49 serotypes of *Shigella* have been recognized, representing subtypes from three of the four groups; of which 15 belong to *S. flexneri* (Simmons & Romanowska 1987, Bopp et al. 2003).

Besides the self-limiting duration of disease, effective antimicrobial therapy reduces dysentery duration and severity and can also prevent potentially lethal complications. Concomitantly, the excretion of the pathogen in stools is shortened significantly, reducing spread of the infection (Bhattacharya & Sur 2003). However, *Shigella* spp. can easily become resistant to antibiotics (WHO 2001).

Indiscriminate use of drugs and horizontal gene transfer has led to *Shigella* species becoming resistant to commonly used antibiotics. Resistance patterns are influenced by geographic location, year that isolates were obtained, classes of antimicrobial agents, and pressure exerted by antimicrobial use. It was noticed that, over the past decades, *Shigella* strains have progressively become resistant to most of the widely use antimicrobials, such as ampicillin, chloramphenicol, tetracycline, and trimethoprim-sulfamethoxazole (Lima et al. 1995, Ashkenazi et al. 2003). The antimicrobials that remain effective are ciprofloxacin and other fluoroquinolones, ceftriaxone, and azithromycin (Anonymous 2004). There are also quite striking geographic differences in the corresponding resistance rates. The antimicrobial resistance pattern differs

Shigella Study Group: Aldo Proeitti Junior, Public Health Laboratory-PHL/AP, Cecília Camilo, PHL/RR, Lécia Maria SS Cosme PHL/MA, Christine K Fontana PHL/PR, Dauri S Rodrigues PHL/SC, Iracema S Miralles PHL/CE, Valdelúcia O Cavalcante PHL/PE, Maria da Penha AH Souza PHL/ES, Hilda Maria R Huf PHL/BA, Marizoneide Gomes PHL/DF, Marinalva Coelho, Children Reference Hospital-PE, Maria Cristina Lourenço, IPEC-Fiocruz.

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+Corresponding author: peirano@ioc.fiocruz.br

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between places. This may be due to the occurrence and spread of antimicrobial-resistant clones.

Surveillance programs on antimicrobial resistance not only identify pathogenic bacterial species, by reporting data like serotyping, microorganisms incidence rates, and susceptibility to the antimicrobial agents currently used for treatment, but also contribute to monitoring the intervention strategies used to control their spread (WHO 2001).

Since in Brazil shigellosis is not considered a compulsory notifiable disease, it has been difficult to estimate its incidence rate and respective importance on disseminating antimicrobial resistance.

This study was conducted to evaluate the frequency of species, serovars, and antimicrobial resistance profiles of *Shigella* spp. received from 1999 to 2004, by Brazilian *Shigella* Surveillance Program on Antimicrobial Resistance conducted by National Reference Laboratory for Cholera and Enteric Diseases, Fiocruz, Ministry of Health, Brazil.

MATERIALS AND METHODS

Bacterial isolates - This study included 296 Shigella isolates selected from NRLCED Strain Collection. Such strains were isolated from human fecal samples, from both hospitals and outbreaks, and sent by State Public Health Laboratories to NRLCED for further identification and serotyping.

Selective and differential media were used for isolating *Shigella* (xylose-lysine-desoxycholate – XLD Agar, Oxoid, UK). Isolates identification was confirmed by standard biochemical laboratory methods (Bopp et al. 2003). *Shigella* antisera was raised in NRLCED, Fiocruz, and serological identification was performed by slide agglutination with polyvalent somatic (O) antigen grouping sera followed by testing with monovalent antisera for specific serovar.

Strains were also submitted to antimicrobial susceptibility testing as part of a surveillance program on antimicrobial resistance and stored in NRLCED Strain Collection. All isolates were stored on phosphorous nutrient agar glass tubes and kept at room temperature (20-30ºC).

Antimicrobial susceptibility testing - Antimicrobial susceptibility testing was performed in 278 *Shigella* strains by standard disk diffusion method, following NCCLS guideline. Tests were performed on Mueller Hinton agar plates, using antimicrobial disks (Oxoid): ampicillin (AMP 10 µg), amikacin (AMK 30 µg), cefoxitin (FOX 30 µg), ceftriaxone (CRO 30 µg), cephalothin (CEF 30 µg), chloramphenicol (CHL 30 µmg), ciprofloxacin (CIP 5 µg), gentamicin (GEN 10 µg), imipenem (IPM 10 µg), nalidixic acid (NAL 30 µg), nitrofurantoin (NIT 300 µg), tetracycline (TET 30 µg), and trimethoprim-sulfamethoxazole (SXT 1,25/23,75 µg). Standard control strains of *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27852), *Enterococcus faecalis* (ATCC 29212), and *Staphylococcus aureus* (ATCC 29213) were used for monitoring the accuracy and precision of the disk diffusion test. The interpretation of zone diameters inhibition was that recommended by NCCLS (2003).

RESULTS

The serogroups prevalence of the 296 *Shigella* isolates was as follows: *S. flexneri*, *n* = 156 (52.7%), *S. sonnei*, *n* = 131 (44.2%), *S. boydii*, *n* = 7 (2.3%), and *S. dysenteriae*, *n* = 2 (0.6%). Table I shows the distribution of *Shigella* strains according to the year and to the Brazilian region from which they were isolated. It was observed the highest incidence rates of isolation in the Southeast (39%) and Northeast (34%) regions, and the lowest rate in the South (3%) region. It was additionally found that the number of *S. flexneri* was higher than that of *S. sonnei* isolates (65 and 33, respectively) in the Northeast; and that in the Southeast it was the opposite (74 *S. sonnei* and 42 *S. flexneri*).

The most frequent serotypes identified for *S. flexneri* were 2a and 1b. Both *S. boydii* and *S. dysenteriae* showed a very low number of isolates. Table II shows the distribution of *Shigella* species and serovars.

Overall, the highest rates of antimicrobial resistance among *Shigella* isolates were to trimethoprim-sulfamethozaxole (90%), tetracycline (88%), ampicillin (56%), and chloramphenicol (35%). Additionally twelve isolates and four isolates showed intermediate resistance to nalidixic acid and to ciprofloxacin, respectively. None of the isolates was resistant to imipenem (Table III).

S. flexneri and *S. sonnei* shared a similar antimicrobial resistance profile for most of the antibiotics. Among the 278 *Shigella* isolates tested, 97.8% were resistant to both one or more antimicrobial agents, and 91% to either two or more antimicrobial agents. Antimicrobial resistance profiles to TET and SXT resistance occurred in 30% of isolates and to AMP, CHL, TET, and SXT in 22% of isolates (Table IV).

DISCUSSION

Shigellosis is becoming an increasingly significant public health problem due to development of multiple antimicrobial resistance, frequently resulting in treatment failure, leading in turn to health complications and deaths (Sur et al. 2004).

In the Brazilian *Shigella* Surveillance Program, *S. flexneri* was the predominant isolated *Shigella* serogroup (≈ 53%) during the studied period, followed by *S. sonnei* (≈ 44%). This is in contrast with studies from other places in Brazil, which detected *S. sonnei* as the most frequent serogroups: Ribeirão Preto-SP (Medeiros et al. 2001), and Salvador-BA (Diniz-Santos et al. 2005). However, our finding is consistent with other reports from Northeastern Brazil (Lima et al. 1995), Porto Alegre-RS (Santos et al. 1997), and São José do Rio Preto-SP (Almeida et al. 1998), and those from other Latin America developing countries such as Argentina (Merino et al. 2004), Peru (Jones et al. 2004), and Chile (Fulla et al. 2005).

We also noticed some differences in the *Shigella* geographical isolation incidence among Brazilian regions. *S. flexneri* was observed to have higher isolation incidence in the Northeast, and *S. sonnei* were more frequently isolated in the Southeast. These two Brazilian regions are social and economically quite different, the Southeast being more likely to have developed cities. This may be

TABLE I

Regional distribution of *Shigella* species in Brazil (1999-2004)

	Year	*S. flexneri*	*S. sonnei*	*S. boydii*	*S. dysenteriae*
Southeast	1999	2	7	1	
(*n* = 118)	2000	4	6		
	2001	18	32		1
	2002	4	17		
	2003	4	9		
	2004	10	3		
Northeast	1999	8	11	1	
(*n* = 101)	2000	8			
	2001	9	5		
	2002	20	7	2	
	2003	1	1		
	2004	19	9		
North	1999	6	1	2	
(*n* = 38)	2000	6	2		
	2001	9			
	2002	6			
	2003	2	4		
	2004				
Middle-West	1999	5	3		1
(*n* = 30)	2000	3		1	
	2001				
	2002	8	2		
	2003	4	3		
	2004				
South	1999				
(*n* = 9)	2000				
	2001				
	2002				
	2003		8		
	2004		1		
Total		156	131	7	2

TABLE II

Distribution of *Shigella* spp. serotypes occurring in Brazil

Shigella spp.	Total
S. dysenteriae	
serotype 2	2
S. flexneri	
serotype 1a	2
serotype 1b	29
serotype 2a	79
serotype 2b	10
serotype 3a	3
serotype 4a	2
serotype 4c	7
serotype 6	1
Not determined	23
S. boydii	
serotype 4	7
S. sonnei	
serotype I	131

TABLE III

Antimicrobial resistance of *Shigella* spp. isolates

Antimicrobials	% of resistance (*n* resistant/*n* tested)
AMP	56 (155/278)
AMK	2 (5/278)
FOX	1 (2/278)
CRO	1 (2/278)
CEF	18 (55/278)
CHL	35 (97/278)
CIP	2 (4[a]/278)
GEN	5 (13/278)
IPM	0 (0/278)
NAL	5 (12[a]/278)
NIT	1 (1/278)
TET	88 (244/278)
SXT	90 (250/278)

AMP: ampicillin; AMK: amikacin; FOX: cefoxitin; CRO: ceftriaxone; CEF: cephalothin; CHL: chloramphenicol; GEN: gentamicin; NIT: nitrofurantoin; TET: tetracycline; SXT: trimethoprim/sulfamethoxazole; *a*: intermediate resistance; *n*: number of strains

TABLE IV
Antimicrobial resistance profiles of *Shigella* in Brazil (1999-2004)

Antimicrobial resistance profile	1999	2000	2001	2002	2003	2004
AMP			1		5	
GEN				1		
SXT	1		1		3	
TET	1		4	1	1	
AMP, CHL		1				
AMP, SXT	7		2		1	1
AMP, TET	2				1	
CEF, GEN				1		
CEF, TET				1		
CRO, SXT		1				
TET, SXT	23	11	24	7	9	9
AMP, CEF, SXT					2	
AMP, CHL, SXT	3	1	1			
AMP, CHL, TET	2	1	1	1		2
AMP, TET, SXT	2	2	7	2	3	3
AMK, TET, SXT					1	
CEF, TET, GEN				1		
CEF, TET, SXT			1	5	1	
AMP, AMK, CEF, SXT						1
AMP, CHL, TET, SXT	5	10	15	10	3	19
AMP, CEF, TET, SXT			2	15		
CEF, GEN, TET, SXT				5		
CEF, CHL, TET, SXT						1
CEF, NIT, TET, SXT				1		
FOX, GEN, TET, SXT					1	
AMP, CEF, CHL, TET, SXT				12		2
AMP, CHL, GEN, TET, SXT			1			
AMP, AMK, CHL, TET, SXT						2
CEF, CHL, GEN, TET, SXT				1		
AMP, CEF, CHL, FOX, TET, SXT					1	
AMP, AMK, CHL, GEN, TET, SXT					1	
AMP, AMK, CEF, CRO, TET, SXT						1
AMP, AMK, CEF, CHL, GEN, TET, SXT			1			

AMP: ampicillin; AMK: amikacin; FOX: cefoxitin; CRO: ceftriaxone; CEF: cephalothin; CHL: chloramphenicol; GEN: gentamicin; NIT: nitrofurantoin; TET: tetracycline; SXT: trimethoprim/sulfamethoxazole.

due to the modern lifestyle of developed cities, where the main ways of contracting shigellosis are by eating and/or drinking fecal contaminated food/water, and by person-to-person contact. The pathogens can be also transferred by flies, fingers, feces, food, and fomites. High-risk groups include children in day-care centers, homosexual men, people in custodial institutions, migrant workers, and travelers.

Our data showed very low occurrence of *S. boydii* and *S. dysenteriae*. Infections by *S. dysenteriae* usually occur in less developed countries, often reaching epidemic levels, with periodic outbreaks (Niyogi 2005).

Serotypes 2a and 1b were the most frequent serotypes of *S. flexneri* in this study, accounting for 59 and 21%, respectively, of the *S. flexneri* serotyped isolates. These are the most common serotypes occurring in developing countries (Kotloff et al. 1999, Souza 2001).

Appropriate antibiotic treatment of shigellosis depends on identifying *Shigella* resistance patterns that are circulating locally. These imply in local surveillance of antimicrobial resistance and its implication in empirical therapy (Ashkenazi 2004).

According to analyzed isolates, *S. flexneri* antimicrobial resistance patterns were mainly detected to ampicillin, chloramphenicol, tetracycline, and trimethoprim-sulfamethoxazole. *S. sonnei* presented a similar resistance profile, except for chlorampenicol, but with increasing resistance to cephalotin. Overall, it could be observed that such main resistance antimicrobial pattern (ampicillin, chloramphenicol, tetracycline, and trimethoprim-sulfamethoxazole) continues to be prevalent among *Shigella* isolates from Brazil (Lima et al. 1995, Rodrigues 2000). Also, in a recent study (Diniz-Santos et al. 2005) *Shigella* species presented a very high resistance rate to trimethoprim-sulfamethoxazole (90.1%), ampicillin (22%) alone or in combination with sulbactam, and also to piperacillin.

Antimicrobial agents as effective options for shigellosis treatment are becoming limited due to globally emerging drug resistance. Multiple resistant strains have oc-

curred in Europe (Maraki et al. 1998), Africa (Egah et al. 2003), Asia (Lee et al. 2001), and South America (Fulla et al. 2005). In the United States, the most common resistance rates among 620 *Shigella* isolates (86% of which were *S. sonnei*) were to ampicillin (77%), streptomycin (54%), trimethoprim-sulfamethoxazole (37%), sulfamethoxazole (32%), and tetracycline (31%) (CDC 2004). A similar resistance pattern was reported from England and Wales (Cheasty et al. 1998). Data from developing countries, such as Chile, indicate that most *Shigella* spp. are resistant to ampicillin (82%), cotrimoxazole (65%), tetracycline (53%), and chloramphenicol (49%) (Fulla et al. 2005). Reports from Bangladesh, where shigellosis is highly endemic, show a similar resistance pattern (Sur et al. 2003) compared to the ones in Latin America. Outbreaks caused by multiresistant *S. dysenteriae* type 1, including strains resistant to nalidixic acid, has also been reported. (Sarkar et al. 2003).

Thus, neither ampicillin, chloramphenicol, tetracyclines nor trimethoprim-sulfamethoxazole should be considered appropriate empiric therapy for shigellosis any longer. Nowadays, recommended therapy for people infected with *Shigella* includes fluoroquinolones (Bhattacharya & Sur 2003, Khan et al. 2004, Anonymous 2004), azithromycin (Basualdo & Ardo 2003), and third-generation cephalosporins (Niyogi et al. 2001).

However, it has been observed that *S. sonnei* have shown readily acquisition of resistance to ampicillin and cephalosporins through conjugative resistance-plasmids carrying resistance cassettes to beta-lactamases (Radice et al. 2001) and, in both *S. sonnei* and *S. flexneri*, chromosomal mutations that confer quinolones resistance (Jeong et al. 2003).

Continuous monitoring of antimicrobial susceptibilities of *Shigella* spp. through a surveillance system is thus essential for effective therapy and control measures against shigellosis. It is also of concern the use of nalidixic acid as a first step screening test to detect mutations causing fluoroquinolone resistance.

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