Article/Artigo

Antimicrobial Resistance of *Shigella* spp. isolated in the State of *Pará*, Brazil

Resistência Antimicrobiana de *Shigella* spp. isoladas no Estado do Pará

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ABSTRACT

Introduction: *Shigella* spp. are Gram-negative, nonsporulating, rod-shaped bacteria that belong to the family Enterobacteriaceae and are responsible for shigellosis or bacillary dysentery, an important cause of worldwide morbidity and mortality. **Methods:** We studied the antibiotic resistance profiles of 122 *Shigella* spp. strains (81 *S. flexneri*, 41 *S. sonnei*, 1 *S. boydii*) isolated from patients (female and male from 0 to 80 years of age) presenting diarrhea in different districts of the State of *Pará*, in the North of Brazil. The antibiotic resistance of the strains, isolated from human fecal samples, was determined by the diffusion disk method and by using the VITEK-2 system. **Results:** The highest resistance rate found was the resistance rate to tetracycline (93.8%), followed by the resistance rate to chloramphenicol (63.9%) and to trimethoprim/sulfamethoxazole (63.1%). Resistance to at least three drugs was more common among *S. flexneri* than *S. sonnei* (39.5% vs. 10%). Six (4.9%) strains were susceptible to all the antibiotics tested. All strains were susceptible to cefotaxime, ceftazidime, ciprofloxacin, nalidixic acid and nitrofurantoin. **Conclusions:** High rates of multidrug resistance in *Shigella* spp. are a serious public health concern in Brazil. It is extremely important to continuously monitor the antimicrobial resistances of *Shigella* spp. for effective therapy and control measures against shigellosis.

Keywords: *Shigella* spp. State of Pará. Antimicrobial resistance. Diarrhea.

RESUMO

Introdução: *Shigella* spp. são bactérias Gram-negativas, não esporuladas, em forma de bastonete, pertencentes a família Enterobacteriaceae responsáveis pela shigelose ou disenteria bacilar, uma importante causa de mortalidade e morbidade mundial. **Métodos:** Foi estudado o perfil de resistência a antimicrobianos de 122 amostras de *Shigella* spp. (81 *S. flexneri*, 41 *S. sonnei*, 1 *S. boydii*) isoladas de pacientes (sexo feminino e masculino com faixa etária de 0 a 80 anos) com distúrbios gastrointestinais em diferentes municípios no Estado do Pará, Brasil. A resistência antimicrobiana das amostras isoladas de coprocultura, foi determinada pelo método de difusão em disco e pelo sistema Vitek II. **Resultados:** A maior resistência foi observada em relação à tetraciclina (93,8%), seguida de cloranfenicol (63,9%), e trimetoprim-sulfametoxazol (63,1%). Multirresistência a pelo menos três antimicrobianos foi mais comum em *S. flexneri* comparada a *S. sonnei* (39,5% vs. 10%). Seis (4,9%) amostras foram sensíveis a todos antimicrobianos testados. Todas as amostras apresentaram sensibilidade a cefotaxima, ceftazidima, ciprofloxacina, ácido nalidixico e nitrofurantoína. **Conclusões:** As altas taxas de multirresistência de *Shigella* spp. são um sério problema de saúde pública no Brasil. Sendo assim, torna-se extremamente importante um monitoramento contínuo da resistência antimicrobiana de *Shigella* spp. para uma terapia efetiva e medidas de controle contra shigelose.

Palavras-chaves: *Shigella* spp. Estado do Pará. Resistência antimicrobiana. Diarréia.

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INTRODUCTION

Shigella spp. are Gram-negative, nonsporulating, rod-shaped bacteria that belong to the family Enterobacteriaceae. The bacteria are facultative intracellular pathogens that show a high specificity for primate hosts. *Shigella* spp., the human pathogens responsible for shigellosis, are highly infectious, even at low counts (10^2)[1]. Shigellosis is recognized by the World Health Organization as a major, global, public health concern[2]. It is responsible for morbidity and mortality in high risk populations, such as children under five years of age, senior citizens, toddlers in day-care centers, patients in custodial institutions, homosexual men, those affected by war and famine and patients with chronic disease (e.g., HIV), predominantly in developing countries[3]. Shigellosis is an acute intestinal infection the symptoms of which can range from mild, watery diarrhea to severe, inflammatory bacillary dysentery characterized by strong abdominal cramps, fever and stools containing blood and mucus[4]. The disease is usually self-limiting but may become life-threatening if patients are immunocompromised or adequate medical care is not available. A combination of oral rehydration and antibiotics leads to rapid resolution of infection. Unfortunately, *Shigella* spp. have become resistant to commonly used antibiotics, drastically reducing therapeutic possibilities, especially in children[5-6].

The genus *Shigella* includes four species: *S. dysenteriae* (serogroup A), *S. flexneri* (serogroup B), *S. boydii* (serogroup C), and *S. sonnei* (serogroup D). *S. flexneri* is the most common *Shigella* species in developing countries. In developed countries, *S. sonnei* is the most prevalent[7]. *S. dysenteriae* is implicated in epidemic disease outbreaks, the most severe form of dysentery and the majority of the fatal shigellosis cases[8].

This study was conducted to identify the antimicrobial resistance profile of 122 strains of *Shigella* spp. (81 *S. flexneri*, 40 *S. sonnei* and 1 *S. boydii*) isolated during 1979-2009 from patients (male and female, from 0 to 80 years of age) who presented with diarrhea and were assisted at the Evandro Chagas Institute (IEC) in the State of Pará, Brazil.

METHODS

Bacterial strains

From January 1979 to July 2009, many samples of 122 *Shigella* strains (81 *S. flexneri*, 40 *S. sonnei* and 1 *S. boydii*) were isolated from the clinical specimens of different patients (male and female from 0 to 80 years of age) presenting with diarrhea and assisted at the Bacteriology and Mycology Department of the IEC. The patients included in the study were from nine different districts in the State of *Pará: Abaetetuba, Ananideua, Belém, Benevides, Castanhal, Icoaraci, Marabá, Marituba* and *Moju*. The isolates, which belong to the bacterial collection of the IEC, were stored at -70ºC in Luria Bertani (LB) broth with 15% glycerol and at environmental conditions.

Determination of antibiotic resistance

Ampicilin (4, 8, and 32µg/mL), amoxilin (4/2, 16/8, and 32/16µg/mL), ceftazidime (1, 2, 8, and 32µg/mL), ciprofloxacin (0, 5, 2, and 4µg/mL), nalidixic acid (8, 16, and 32µg/mL), nitrofurantoin (16, 32, and 64µg/mL), and trimethoptim/sulfamethoxazole (0.5/9.5, 2/38, and 16/304µg/mL) were used to determine the resistance profiles of all *Shigella* strains studied using the VITEK-2 system (bioMérieux, Marcy I'Etoile, France). Thechloramphenicol (30µg), cefotaxime (30µg), gentamicin (10µg) and tetracycline (30µg) profiles of antibiotic resistance were determined by the diffusion disk method according to the Manual of the Clinical and Laboratory standards Institute (CLSI 2009)[9]. The standard reference strains of *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27,853 were used as controls throughout the study.

RESULTS

The resistances of the *Shigella* spp. strains to all the antimicrobials tested are shown in **Table 1**. A total of 115 (94.2%) were resistant strains. The highest resistance rate found was the resistance rate to tetracycline (93.4%), followed by the rate to chloramphenicol (63.9%) and to trimethoptim/sulfamethoxazole (63.1%). Sixty (49.2%) strains were multidrug-resistant (resistant to at least two antimicrobials).

A total of 7 (5.8%) strains were susceptible to all the antibiotics tested. All the strains were susceptible to cefotaxime, ceftazidime, ciprofloxacin, nalidixic acid and nitrofurantoin.

The antimicrobial resistance patterns of all the strains of *Shigella* spp. were compared to each other. Overall, 93.8% of the *S. flexneri* strains and 95% of *S. sonnei* strains were resistant to tetracycline. Seventy-five percent of the *S. flexneri* strains and 42.5% of the *S. sonnei* strains were resistant to chloramphenicol. Sixty-four percent of the *S. flexneri* strains and 62.5% of the *S. sonnei* strains were resistant to trimethoprim-sulfamethoxazole.

As shown in **Table 2**, only 39.5% of the *S. flexneri* strains were resistant to at least three of the antibiotics (AM, C, SXT) tested.

TABLE 1 - Antimicrobial resistance of *Shigella* spp. strains by species.

	Number resistant to antimicrobials/percent							
	total (n = 122)		*Shigella boydii* (n = 1)		*Shigella flexneri* (n = 81)		*Shigella sonnei* (n = 40)	
Antimicrobial agent	n	%	n	%	n	%	n	%
Ampicilina	53	43.4	0	0.0	46	56.8	7	17.5
Amoxilin	1	0.9	0	0.0	1	1.2	0	0.0
Cefotaxime	0	0.0	0	0.0	0	0.0	0	0.0
Ceftazidima	0	0.0	0	0.0	0	0.0	0	0.0
Chloramfenicol	78	63.9	0	0.0	61	75.3	17	42.5
Ciprofloxacin	0	0.0	0	0.0	0	0.0	0	0.0
Gentamicin	1	0.9	0	0.0	1	1.2	0	0.0
Nalidixic acid	0	0.0	0	0.0	0	0.0	0	0.0
Nitrofurantoin	0	0.0	0	0.0	0	0.0	0	0.0
Tetracycline	114	93.4	0	0.0	76	93.8	38	95.0
Trimethoprim/sulfametoxazole	77	63.1	0	0.0	52	64.2	25	62.5

TABLE 2 - Antimicrobial resistance patterns of *Shigella* spp. strains by species.

	Number resistant to antimicrobials/percent							
	total (n = 122)		*Shigella boydii* (n = 1)		*Shigella flexneri* (n = 81)		*Shigella sonnei* (n = 40)	
Resistance pattern	n	%	n	%	n	%	n	%
AM, AMC, C, SXT	1	0.9	0	0.0	1	1.2	0	0.0
AM, C, GN, SXT	1	0.9	0	0.0	1	1.2	0	0.0
AM, C, SXT	36	29.5	0	0.0	32	39.6	4	10.0
AM, C	8	6.5	0	0.0	8	9.9	0	0.0
AM, SXT	5	4.0	0	0.0	3	3.7	2	5.0
C, SXT	8	6.5	0	0.0	7	8.7	1	2.5
C, TE	1	0.9	0	0.0	1	1.2	0	0.0
AM	2	1.6	0	0.0	1	1.2	1	2.5
C	23	18.9	0	0.0	11	13.6	12	30.0
SXT	26	21.3	0	0.0	8	9.9	18	45.0
TE	4	3.2	0	0.0	4	4.9	0	0.0
No resistance	7	5.8	1	100.0	4	4.9	2	5.0

AM: ampicilin, **AMC:** amoxilin, **C:** chloramfenicol, **SXT:** trimethoprim/sulfametoxazole, **GN:** gentamicin, **TE:** tetracycline.

The most common antimicrobial resistance pattern observed was resistance to ampicillin, chloramphenicol and trimethoprim-sulfamethoxazole. Forty-five percent of the *S. sonnei* strains were resistant to only one antibiotic (trimethoprim-sulfamethozole). Resistance to at least three drugs (AM, C, SXT) was more common among *S. flexneri* than *S. sonnei* (39.5% vs. 10%).

DISCUSSION

It is well established that *S. flexneri* is the most commonly isolated species of the *Shigella* genus in developing countries, and its presence has been associated with inadequate sanitation; in contrast, *S. sonnei* predominates in developed countries but is predominantly involved in sporadic, common-source outbreaks. *S. boydii* was first detected in India, and up to now, it has rarely been found outside the Indian subcontinent[10]. The species of *Shigella* most frequently isolated in Brazil, mainly from children under 5 years of age, are *S. flexneri* and *S. sonnei*[7]. Research on the epidemiology and microbiological aspects of bacterial diarrhea in children from Salvador in the State of *Bahia*, Brazil, demonstrated that in the period from January of 2002 to December of 2003, 1,991 patients between 0 and 15 years of age had their stools cultured, of which 260 presented bacterial growth. *Shigella* spp. were the most common pathogens and were found in 141 (54.3%) cultures, of which 80.1% were identified as *S. sonnei* and 19.9% as *S. flexneri*[11]. The first study of gastroenteritis in the Amazonian area was accomplished in Santarém in the State of *Pará*, where 48% of the 320 patients with severe diarrhea were determined to have shigellosis[12]. In the period from 1979 to 1980, investigations were conducted at private clinics and in a public hospital in Belém, State of Pará, Brazil with the objective of establishing the role of enteropathogenic bacteria as the cause of acute diarrhea in children from 0 to 5 years of age. They found *Shigella* in 13% of the cases (*S. flexneri* [62%], *S. sonnei* [29%], *S. boydii* [6%] and *S. dysenteriae* [3%])[13]. In the present study, most of the strains identified were *S. flexneri* (66.4%) as well, followed by *S. sonnei* (32.8%) and *S. boydii* (0.8%).

The antibiotic resistance of *Shigella* spp. has been hindering the treatment of shigellosis, particularly in children[5-6]. A study conducted in the United States, involving 1,604 *Shigella* spp. strains isolated in the period from 1999 to 2002, revealed a high resistance (1,031/64%) to the antibiotics tested. The largest resistance (322/31%) was observed to the combination of ampicillin, estreptomicin and tetracycline, followed by ampicillin, estreptomicin and sulfametoxazol (149/14%) and ampicillin and estreptomicin (108/10%). Resistance to ampicillin in combination with cloranfenicol, estreptomicina, tetracycline and sulfametoxazole, was observed in 8% (85) of the strains[5].

From June 2006 to February 2009, all patients with shigellosis reported in New York City were interviewed. Their *Shigella* isolates were tested for antimicrobial susceptibility to examine the level of resistance and to identify risk factors for resistance. Analysis was conducted on two groups distinguished by a large outbreak that was documented during the data collection period. Of the 477 non-outbreak patients, 333 (70%) reported taking an antibiotic for shigellosis. Thirty-six (11%) patients were treated with an antibiotic to which their *Shigella* infection was resistant. Among this group, high levels of antimicrobial resistance were detected to ampicillin (68%) and trimethoprim-sulfamethoxazole (66%)[14].

Most of the *S. flexneri* strains isolated from cases of bacillary dysentery in Campinas, State of *São Paulo*, Brazil were resistant to more than one of the antibiotics tested, and 90% were resistant to at least three antibiotics. The antibiotics to which the bacteria were most commonly resistant were ampicillin (83.3%), chloramfenicol (70%), and tetracycline (80%). However, among the *S. sonnei* strains, resistance was observed to 2 (70%) or 3 (30%) antibiotics. Resistance to tetracycline was detected in 96.7% of the strains. Resistance to ampicillin (6.7%) was also detected among these strains[7].

A study conducted on strains of different species of *Shigella* from cases of diarrhea in the State of *Rondônia,* Brazil in the period from March of 2000 to March of 2002 found high resistance to trimethoprim/sulfametoxazole and ampicillin. *S. flexneri* was the only species that presented multidrug resistance[9].

Our study revealed that *Shigella* spp. isolated in Belém, State of Pará, Brazil were resistant mostly to tetracycline (93.4%), chloramfenicol (63.9%), trimethoprin/sulfametoxazole (63.1%) and ampicilin (43.4%). No resistance was found to cefotaxime, ceftazidima, ciprofloxacin or nalidixic acid.-

Because of increasing antimicrobial resistance in *Shigella* spp., empirical treatment options are decreasing. Antimicrobial therapy for shigellosis requires knowledge of the antimicrobial resistance patterns of the *Shigella* spp. strains circulating locally. Physicians should be aware of the high multidrug resistance rates of *Shigella* spp., especially resistance to tetracycline and chloramfenicol. *S. flexneri* is still the most prevalent species in many Brazilian cities, and its higher antimicrobial resistance rate compared to that of *S. sonnei* is a cause for concern.

It is extremely important to continuously monitor the antimicrobial resistances of *Shigella* spp. for effective therapy and control measures against shigellosis.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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