
FREQUENCY OF *Shigella flexneri* SEROTYPES ISOLATED IN THE STATE OF PARÁ, BRAZIL (1979-2009)

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ABSTRACT

The genus *Shigella* has been one of the most common causes of diarrhea in underdeveloped countries, being responsible for the mortality rate and morbidity in high risk populations such as children under five and the elderly. The aim of this study, developed at the Evandro Chagas Institute, Pará, Brazil, from 1979 to 2009, was to evaluate the frequency of species and serotypes of 122 isolates of *Shigella* spp. isolated from patients with acute diarrhea. The isolates were identified by bacterial culture media with selective indicators and biochemical tests, and serogroups and serotypes were determined by the slide agglutination test. The serogroups most frequently found were *S. flexneri* (66.4%) and *S. sonnei* (32.8%). The serotype 2a of *S. flexneri* was the most frequent (54.3%) followed by 1b (17.2%). Most patients were aged between 0-5 years (44.6%), of which 38.2% cases had *S. flexneri* and 47.5% *S. sonnei*. Patients older than 18 years accounted for 39.2% of infections, with 37.0% *S. flexneri* cases, 32.5% *S. sonnei*, and 100% *S. boydii*. These results emphasize the need for continuous surveillance in the State of Pará.

KEY WORDS: *Shigella*. Serotypes. State of Pará.

RESUMO

Frequência de sorotipos de *Shigella flexneri* isoladas no estado do Pará, Brasil (1979-2009)

O gênero *Shigella* tem sido uma das causas mais comuns de diarréia em países pouco desenvolvidos, sendo responsável pela mortalidade e / ou morbidade em população de alto risco, como crianças menores de cinco anos e idosos. Este estudo, realizado no Instituto Evandro Chagas (IEC), Estado do Pará, Brasil, no período de 1979 a 2009, teve como objetivo avaliar a frequência de espécies e

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sorotipos de 122 isolados de *Shigella* spp. provenientes de pacientes com diarreia aguda. Os isolados bacterianos foram identificados por meios de culturas seletivos e indicadores e testes bioquímicos. Todos os isolados foram identificados quanto ao sorogrupo e sorotipo por meio da soroaglutinação em lâmina. Os sorogrupos mais frequentemente encontrados foram *S. flexneri* (66,4%) e *S. sonnei* (32,8%). O sorotipo de *S. flexneri* mais frequente foi o 2a (54,3%) seguido de 1b (17,2%). A maioria dos pacientes tinham idade entre 0 a 5 anos (44,6%), dos quais 38,2% apresentaram *S. flexneri* e 47,5% *S. sonnei*. Pacientes com idade superior a 18 anos representaram 39,2% das infecções, sendo 37,0% casos de *S. flexneri*, 32,5% de *S. sonnei* e um isolado de *S. boydii*. Esses resultados reforçam a necessidade de uma vigilância epidemiológica contínua no Estado do Pará.

DESCRITORES: *Shigella*. Sorogrupos. Sorotipos. Estado do Pará.

INTRODUCTION

The genus *Shigella*, belongs to the family Enterobacteriaceae. It is a small, non-capsulated, non-motile Gram negative, nonsporulating, facultative anaerobic bacilli. It is an intracellular pathogen that shows specificity for the human host and, exceptionally for other primates (monkeys and chimpanzees), causing epidemic dysentery (4). *Shigella* presents some clinical and epidemiological interesting properties such as low infectious dose (10^1 a 10^3 bacteria), especially with the Shiga bacillus (*Shigella dysenteriae* serotype 1), and being extremely easy to spread from person to person. It is usually spread through hands contaminated by food and drinking water with feces of patients and/or carriers. Another interesting feature is related to the diversity of virulence factors implicated in the pathogenesis of the disease (6).

This enteropathogen is a common cause of diarrhea that affect populations in developing countries or those lacking hygiene and sanitation, resulting in high rates of morbidity and mortality, particularly in the extreme groups of age (17). Worldwide, it is estimated that approximately 150 million individuals are infected each year, including four million in developed countries (20).

The frequency of *Shigella* infections increases with children's age, in our country the prevalence of these bacteria is between 8 to 10% in children under one year of age and 15 to 18% in those aged 2 to 5 (13). In studies conducted in São Paulo and Rio de Janeiro, 12% and 7.6% of isolates of *Shigella* spp., occurred respectively in these age groups (2, 8).

The genus is composed of four species, phenotypically characterized as: *S. dysenteriae* (serogroup A), *S. flexneri* (serogroup B), *S. boydii* (serogroup C), and *S. sonnei* (serogroup D) (7). *S. flexneri* is the most commonly isolated in developing countries, followed by *S. sonnei* (21).

On the other hand, *S. dysenteriae* type 1 is responsible for epidemic and endemic outbreaks in developed countries and *S. sonnei* causes sporadic outbreaks mainly. The species *S. boydii* is rarely isolated (19). Records of studies undertaken in Brazil show that the *Shigella* species most frequently isolated in the country are *S. flexneri* and *S. sonnei*, isolated mainly from children aged under 5 (13).

Each species, except *S. sonnei*, contains multiple serotypes defined by specific somatic antigenic fractions. Thus, 49 serotypes or serovars of *Shigella* were identified, from which 15 belong to the species *S. flexneri* (3, 7, 24), with the predominant serotypes cosmopolitan 2a and 1b (12).

Given the issues described and given its regional importance, the aim of the study was to analyze the frequency of species and serotypes of *Shigella* spp. isolated from different patients with diarrhea in Pará State, Brazil, in the period from 1979 to 2009.

MATERIAL AND METHODS

Bacterial Strains

From 1979 to 2009, 400 isolates of *Shigella* spp. were obtained at the Evandro Chagas Institute from stool samples of different patients with diarrhea residing in Pará State. The isolates were stored in stock agar for the study; 122 were randomly selected for this study (Table 1). In order to recognize the purity of the culture and isolate only the smooth colonies, reactivation by passage in nutrient broth and nutrient agar plates was performed and the samples proved to be viable.

Bacterial Isolation

The isolation and identification of *Shigella* spp. were performed according to a method described previously (Ewing 1986). In addition to conventional procedures, the automated system (Vitek 2 Compact – bioMérieux™) was included.

Determination of serogroups and serotypes

The samples identified through biochemical profile compatible with *Shigella* species were grouped in the species through the slide agglutination test (Ewing 1986) with polyvalent somatic antiserum (Bio-Rad Laboratories, USA) for the species *S. dysenteriae* (serological group A), *S. flexneri* (serological group B), *S. boydii* (serological group C), and *S. sonnei* (serological group D). After defining the serogroups, the technique of rapid agglutination test (Ewing 1986), using the somatic monovalent antiserum (Enterobacteria Laboratory of the Oswaldo Cruz Institute, FIOCRUZ, RJ) was performed to identify the serotypes of *S. flexneri*.

RESULTS

The serogroup prevalence of the 122 *Shigella* isolates was the following: *S. flexneri*, n=81 (66.4%), *S. sonnei*, n=40 (32.8%), and *S. boydii*, n=1(0.81%). From the nine geographical origins of patients, 86 (70.5%) originated from residents

in the city of Belém, of those 51 (59.3%) were carriers of *S. flexneri*, 39 (39.5%) of *S. sonnei* and 1.1% of *S. boydii*, distributed in the three decades studied. In the 13 (11.9%) isolates from patients of Ananindeua city, *S. flexneri* (84.6%) predominated followed by *S. sonnei* (15.3%) (Table 1).

Table 1. Geographical distribution of *Shigella* species isolated in the State of Pará, between 1979 and 2009.

Municipalities	Decades	Species		
		<i>S. flexneri</i>	<i>S. sonnei</i>	<i>S. boydii</i>
Belém n = 86 (70.5%)	1979-1989	24	11	1
	1990-1999	18	13	
	2000-2009	9	10	
Ananindeua n = 13 (10.7%)	1979-1989	2	1	
	1990-1999	5	1	
	2000-2009	4		
Outros* n = 10 (8.2%)	1979-1989	1	1	
	1990-1999	3	1	
	2000-2009	4		
Sem dado n = 13 (10.6%)	1979-1989	4		
	1990-1999	6	1	
	2000-2009	1	1	
Total		81	40	1

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The most frequent serotypes identified for *S. flexneri* were 2a (54.3%) and 1b (17.3%). *S. boydii* showed only one isolate. Table 2 shows the distribution of *Shigella flexneri* serotypes.

Table 2. Distribution and frequency of *Shigella flexneri* serotypes detected in cases of diarrhea, occurred in Pará State (1979-2009).

<i>S. flexneri</i> Serotypes	Total N (%)
1a	2 (2.5)
1b	14 (17.3)
2a	44 (54.3)
2b	1 (1.2)
3a	2 (2.5)
3b	2 (2.5)
4a	1 (1.2)
4c	6 (7.4)
6	9 (11.1)
Total	81

Gender information was available for 116 patients, a slight prevalence of shigellosis in male patients (51.7%) over female ones (48.3%) was observed. In

relation to age, the age group 0 to 5 showed 44.6% (50/112) of infections, of which 38.2% (31/81) cases were *S. flexneri* and 47.5% (19/40) *S. sonnei*. In contrast, in patients aged over 18 years, there was a large number of isolates of *S. flexneri* (37%) followed by 32.5% of *S. sonnei* (Table 3).

Table 3. Distribution of isolates of *S. flexneri*, *S. boydii* and *S. sonnei* according to the age of the 122 patients with acute diarrhea in Pará State (1979-2009).

Species	Total (%)				Sem Registro
	0-5	6-12	13-18	>18	
<i>S. flexneri</i> (n=81)	31 (38.3)	8 (9.8)	4 (5.0)	30 (37.0)	8 (9.9)
<i>S. boydii</i> (n=1)	0 (0.0)	0 (0)	0 (0)	1 (100)	0 (0.0)
<i>S. sonnei</i> (n=40)	19 (47.5)	3 (7.5)	3 (7.5)	13 (32.5)	2 (5.0)
Total	50 (41.0)	11 (9.0)	7 (5.8)	44 (36.0)	10 (8.2)

DISCUSSION

The frequency of studies in the medical literature addressing the problem of diarrhea is a suggestive indication of its importance for both clinicians and microbiologists. Nevertheless, most studies are limited to the isolation of certain enteropathogens or indirect tests such as those performed in serum. However, it is essential to further analysis of these microorganisms, to support the clinical findings, including treatment, as well as its epidemiology. The World Health Organization (WHO) has stressed the need to broaden the knowledge on shigellosis in developing countries, linking bacteriological data with clinical and epidemiological aspects (27).

The bacteriological sampling conducted in *Shigella* spp. isolated in the state of Pará, procedure that covered three decades, revealed that the species *S. flexneri*, serogroup B was markedly predominant (66.4%), with over 32.8% of isolates of *S. sonnei*, serogroup D and serogroup C, and *S. boydii* in only 0.81% of the isolates. In 122 selected isolates serogroup A, *S. dysenteriae* was not detected. These data are consistent with those obtained in other regions of the country and Latin America (1, 9, 11, 13, 18, 23), except to those in Ribeirão Preto, São Paulo (17) and Salvador, Bahia, (5) which marked the predominance of *S. sonnei* over other species. Such variations of a particular species or serotype are recognized in different parts of the world and in the U.S *S. sonnei* (72%) on *S. flexneri* (19%) prevail markedly. In India, *S. dysenteriae* serotype 1 (Shiga bacillus) is responsible for the classic dysenteriform syndrome (19). Such variations may be a result of the levels of sanitation offered to populations, in conjunction with the improvement of the concepts of personal hygiene and food; and these factors are obviously associated with socio-economic ones (19).

This study point also refers to the first observations on gastroenteritis agents in the Amazon region, Santarém, Pará, made by Maroja & Lowery in 1956 (16). The problem of shigellosis is still of public health relevance, as shown in the

most recent studies by Linhares et al. (1983) and Loureiro et al. (2010) (14, 15). These authors, unlike Maroja & Lowery, detailed the species involved in diarrhea in children and adults in the cities of Belém and Juruti (16). In both cases, the proportions of isolates of *S. flexneri* surpassed those of *S. sonnei*.

The serotypes 2a (54.3%) and 1b (17.2%) were the most commonly observed in the *S. flexneri* serogroup, according to reports in other regions of the country and the world (10, 12, 21, 25), with small variations in the prevalence of serotypes. It is worth mentioning the detection of serotypes 6 (11.1%) and 4c (7.4%) as an event that deserves further studies, mainly of epidemiological nature.

Regarding patients' age, the group aged 0-5 had 44.6% (50/112) of infections, of which 38.2% (31/81) were cases of *S. flexneri* and 47.5% (19/40) of *S. sonnei*. The group older than 18 years had 39.2% (44/112) of infections, with 37.0% (30/81) cases of *S. flexneri*, 32.5% (13/40) of *S. sonnei* and 100% (1/1) of *S. boydii*. These results are compatible with other studies in developed countries (4, 22, 26).

CONCLUSION

The data presented in this study are very important for the knowledge of the epidemiology of this enteropathogen in our region, considering the paucity of information about the etiology and epidemiology of shigellosis in Pará. Therefore, it is extremely important to implement a laboratory system of continuous monitoring of diarrhea resulting from enteropathogenic bacteria in particular of *Shigella* in the Amazon region, aiming to offer subsidies to the adoption of epidemiology and control measures.

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