

Diarrhea associated with *Shigella* in children and susceptibility to antimicrobials

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Abstract

Objective: To evaluate the distribution and susceptibility to antimicrobials of *Shigella* isolated from children with acute diarrhea and without diarrhea in Teresina, state of Piauí, Brazil.

Methods: Four hundred children aged up to 60 months were studied. Stools were collected from all the patients between January 2004 and August 2007. *Shigella* was identified by conventional methods and antibiogram and extended-spectrum β -lactamase (ESBL) were performed by agar diffusion.

Results: Shigellosis was only detected in children with acute diarrhea (26/250; 10.4%), especially in those aged from 6 to 24 months and in the rainy months. *Shigella* was susceptible to ceftriaxone, ciprofloxacin and nalidixic acid. More than half of the strains were resistant to sulphamethoxazole-trimethoprim and ampicillin. ESBL was not detected.

Conclusions: *S. flexneri* is common in Teresina. The resistance to ampicillin and sulphamethoxazole-trimethoprim gives cause for concern, as these drugs are widely used in practice and sulphamethoxazole-trimethoprim is also recommended for treating children suspected of having shigellosis.

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Introduction

Infectious enteritis is an important cause of morbidity and mortality, especially in the less developed regions of the planet.¹⁻³ Among others, *Shigella* is one of the main agents of the disease.² It is estimated that more than 160 million human beings are infected by the microorganism annually and that approximately 1.1 million die.² Although shigellosis, or bacillary dysentery, attacks individuals of

any age or socioeconomic class, more than 99% of the cases occur in children in developing countries aged under 5 years.^{2,4} Among the *Shigella*, *S. flexneri* predominates in developing countries and *S. sonnei* in industrialized countries.² Although the less severe cases of the disease can be cured by oral rehydration and regular feeding, it has been proposed that patients with symptoms suggesting

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shigellosis are treated with antimicrobials, aiming to reduce the duration and severity of the disease and the period taken to eliminate the bacterium.⁴⁻⁸ However, selecting an efficient therapy is rendered more difficult due to the alarming prevalence of resistant strains. Thus, the knowledge on the distribution and susceptibility to drugs of *Shigella* in different geographical settings is of paramount importance for the comprehension of the biology and epidemiology of shigellosis and, ultimately, for designing efficient strategies for preventing and controlling the disease.^{2,4,9}

This study aimed to evaluate the distribution and susceptibility to antimicrobials of *Shigella* isolated from children with acute diarrhea and without diarrhea in Teresina, state of Piauí, Brazil.

Patients and methods

This investigation is part of a prospective study on the etiology of diarrhea in children in Teresina. Clinical, demographic and epidemiological data were recorded on a separate sheet. Acute diarrhea was defined as the elimination of three or more loose or liquid stools per day, or more frequently than is normal for the individual, with an evolution of up to 7 days.⁵⁻⁷

Stools were obtained from 400 children aged up to 60 months from a low socioeconomic level, attended to in two public hospitals of Teresina, from January 2004 to August 2007: 250 with acute diarrhea and 150 without diarrhea in the 15 days prior to the appointment. Children with a history of hospitalization or antibiotic therapy in the 15 days preceding the diarrhea were not included.

Stools were obtained after spontaneous evacuation, transferred to sterile vials containing a glycerol and phosphate 0.033 M solution (1:1), pH 7.0, transported to the laboratory in an ice bath and processed within 1 hour. The specimens were cultivated onto MacConkey agar (Himedia, Mumbai, India) and SS agar (Becton, Dickinson and Co., Sparks, MD, USA). After incubation at 35 °C for 24 hours, five lactose-positive colonies and five lactose-negative colonies (whenever possible) were identified by means of conventional biochemical and physiological tests. The colonies identified as *Shigella* were submitted to agglutination reactions with specific antisera (Probac, São Paulo, Brazil) to determine the species.

A *Shigella* strain from each patient, randomly selected, was employed for testing the susceptibility of the bacterium to nalidixic acid, ampicillin, ceftriaxone, ciprofloxacin and sulphamethoxazole-trimethoprim and the production of extended-spectrum β -lactamase (ESBL) by agar diffusion.¹⁰

The Yates' chi-square or Fisher's exact tests were used and the differences were taken as significant when $p \leq 0.05$.

This project was approved by the Research Ethics Committees of the Universidade Federal de Minas Gerais and Universidade Federal do Piauí. The parents and/or those in charge agreed to the child taking part by signing the free informed term of consent.

Results

Shigella was isolated from 26 children, all with acute diarrhea (10.4%): *S. flexneri* from 21 (80.8%) and *S. sonnei* from 5 (19.2%).

Shigellosis was most common (19/73.1%) in children up to 24 months of age, not having been detected in those under 6 months or over 48 months of age. Approximately 70% of *S. flexneri* was isolated from children under 2 years old and *S. sonnei* only from those over 2 years old.

An association between shigellosis and the patient's sex was not detected.

Shigellosis was more common in 2004 (5/22, 22.7%) than in the remainder of the study period (6/96, 6.3% for 2005; 10/195, 5.1% for 2006; 5/87, 5.7% for 2007; $p = 0.012$; chi-square test). Concerning the two bacterium species, only *S. sonnei* was associated with the year of the investigation, being more common in 2004 (for *S. sonnei*: 3/5, 60.0% in 2004; 0/5, 0% in 2005; 1/5, 20.0% in 2006 and 1/5, 20.0% in 2007; for *S. flexneri*: 2/21, 9.5% in 2004; 6/21, 2.9% in 2005; 9/21, 42.9% in 2006; 4/21, 19.0% in 2007; $p < 0.001$; chi-square test).

Seasonality was observed for shigellosis and for infection by *S. flexneri* ($p = 0.007$ and $p = 0.022$), being more frequent in the rainy months ($n = 20$; 76.9% and $n = 16$; 76.2%, respectively).

An association was observed between shigellosis and bloody stools (16/26 – 61.5%; $p < 10^{-3}$; Fisher's exact test) and fever (23/26, 88.5%; $p = 0.023$; Fisher's exact test). There was no association between the disease and consistency of the stools and number of evacuations per day. Blood and mucous in the stools were more common in the infection by *S. flexneri* (14/21, 66.7% and 18/21, 85.7%, respectively) than in diarrhea by *S. sonnei* (4/5, 40.0% and 2/5, 40.0%, respectively) ($p = 0.008$ and $p = 0.005$, respectively; Fisher's exact test).

The results of the antibiograms of *Shigella* are shown in Table 1. ESBL was negative for all the samples.

Discussion

Shigellosis is an important cause of acute diarrhea all over the world, not only due to its high prevalence, but also because of the severity of the disease.^{1,2,4,8,10-12} As in other parts of the world, *Shigella* was responsible for more than 10% of the cases of acute diarrhea in Teresina. A similar frequency was observed in Trinidad¹¹ and Israel.¹²

Table 1 - Profile of susceptibility to antimicrobials of *Shigella* isolated from children aged up to 5 years old in Teresina, state of Piauí, Brazil, between January 2004 and August 2007

Antimicrobial	<i>S. flexneri</i> n (%)	<i>S. sonnei</i> n (%)	Total n (%)
Nalidixic acid	21 (100)	5 (100)	26 (100)
Ampicillin	7 (33.3)	5 (100)	12 (46.1)
Ceftriaxone	21 (100)	5 (100)	26 (100)
Ciprofloxacin	21 (100)	5 (100)	26 (100)
Sulphametoazole-trimethoprim	6 (28.6)	0 (0)	6 (23.1)

On the other hand, our findings show a higher frequency of shigellosis than that observed in the states of Paraíba (approximately 3%)¹³ and Rondônia (2.9%),¹⁴ Brazil. Among other reasons, these differences may result from diversity in the geographical distribution of the bacterium, characteristics of the population studied and the methodology of the investigations.

The temporal dominance of different *Shigella* species is still unclear. In recent decades, a drop in the global prevalence of *S. dysenteriae* and an increase in *S. flexneri* have been noted. Currently, especially in the industrialized regions, there is a greater prevalence of *S. sonnei*, a fact that has not yet been explained in a convincing way.² In agreement with data reported for other less developed regions,^{2,14,15} we observed that more than 80% of the cases of shigellosis are associated with *S. flexneri*.

In areas where shigellosis is endemic, the highest rates of the infection occur in the second year of life.² We noted similar data: most of the cases of shigellosis were identified in children aged from 6 to 24 months. The existence of several serotypes of the microorganism allows one to assume that, in the endemic regions, several episodes of the disease occur in childhood. In the first 6 months of life, children usually live in a more protected environment and receive protective factors conferred by breastfeeding. After this age, although still immunologically naïve, they come into contact with the microorganism more frequently. Consequently, they are more susceptible to the infection and develop the disease and progressive protection against the types of the microorganism circulating in that region. Thus, it is possible to explain the low frequency of shigellosis up to 6 months of age, its increase between 6 months and 2 years old and the drop after this age.

According to Naumova et al.,¹⁵ shigellosis is more common in the summer, owing to the greater recreational use of water and the precarious hygiene habits which

facilitate the transmission of diarrheagenic bacteria. The predominance of shigellosis in the rainy months, the hottest months in Brazil, can also be explained by the transmission of the microorganism by the rainwater.

Symptoms of shigellosis include acute abdominal pain, fever and bloody stools. Diarrhea may be initially watery and large in volume evolving into frequent small volume bloody mucoid stools.² In this study, fever and bloody stools were associated with the disease. Due to the more aggressive nature of the process, stools containing blood and mucous were more common in children infected by *S. flexneri*.

Less than 20% of the *Shigella* was susceptible to all the antimicrobial drugs tested. As with other bacteria, the sensitivity of *Shigella* to antibiotics has been changing and is affected by the habits of each population, explaining geographical differences,^{4,9,11} making the development of regional studies needed. Besides rehydration and regular feeding, treatment with antibiotics is advisable if shigellosis is suspected.⁴⁻⁶ In this study, all the samples of *Shigella* were susceptible to ceftriaxone, ciprofloxacin and nalidixic acid and more than 50% resistant to ampicillin and sulphametoazole-trimethoprim, similarly to data reported for other regions.^{4,9,14} Thus, sulphametoazole-trimethoprim and ampicillin are not recommended for treating diarrhea, aiming at eliminating *Shigella* in the region studied. Ceftriaxone is an expensive option and ciprofloxacin is to be used with caution in children.² Nalidixic acid is efficacious *in vitro* and would be an accessible choice. However, although it was the drug of choice for treating shigellosis between 1995 and 2005, its practical efficacy is considered to be low, even for samples of the bacterium considered to be sensitive *in vitro*.⁴

So, our data contribute to increasing knowledge about the distribution and the susceptibility profile of *Shigella* and, consequently, for designing strategies for preventing and controlling shigellosis.

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