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Characterization of antimicrobial resistance of samples of *Shigella* spp. isolated in Belém, Pará State, Brazil (1990-2000)

Caracterização da resistência antimicrobiana de amostras de *Shigella* spp. isoladas em Belém, Estado do Pará, Brasil (1990-2000)

Caracterización de la resistencia antimicrobiana de muestras de *Shigella* spp. aisladas en Belém, Estado de Pará, Brasil (1990-2000)

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ABSTRACT

We evaluated the antimicrobial resistance of *Shigella* spp. samples isolated from inhabitants of the City of Belém, Pará State, Brazil, aged between 6 months and 81 years, from 1990 to 2000. We analyzed 50 samples of *Shigella* spp. that had been identified in the Laboratory of Enterobacterial Infections at the Bacteriology and Mycology Section of the Instituto Evandro Chagas and stored in its bacteria bank. After the phenotypic characterization, 32 (64%) isolates were identified as *S. flexneri* and 18 (36%) as *S. sonnei*. Resistance to cephalothin, cefazolin, cefuroxime, cefuroxime axetil and tobramycin was observed in 100% of the samples. However, they presented 100% susceptibility to cefpodoxime, ceftriaxone, levofloxacin and norfloxacin. Of the samples, 2% showed resistance to ticarcillin/clavulanic acid, 8% to cefoxitin and 44% to ticarcillin. The results showed a marked resistance to various antibiotics in these enterobacteria that cause serious infections in humans.

Keywords: Diarrhea; *Shigella*; Drug Resistance, Microbial.

INTRODUCTION

Shigellosis is recognized by the World Health Organization (WHO) as a major global public health problem. This disease is caused by four species of the genus *Shigella* and is among the leading causes of diarrhea in children. Shigellosis occurs mainly in developing countries but is also responsible for diarrhea in developed countries. Moreover, its transmission mode is associated with poor sanitation and/or poor personal hygiene[1,2].

It is estimated that *Shigella* is responsible for approximately 500,000 cases of diarrhea in the United States annually, causing about 6,231 cases of hospitalizations and 70 deaths. The genus *Shigella* was ranked third among foodborne pathogens in 2004, according to the Foodborne Diseases Active Surveillance Network (Foodnet) of the Centers for Disease Control and Prevention[3].

In addition to several other studies performed in Brazil, Peirnano et al[4] analyzed *Shigella* isolates from the period of 1999 to 2004 and reported the prevalence of *S. flexneri* (52.7%) over *S. sonnei* (4.2%). The highest rates of isolation of this enteric pathogen were observed in the southeastern region (39%), followed by the

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northeastern region (34%) and a slight occurrence in the Southern region (3%) of the country.

The uncontrolled use of antibiotics allows for the selection of resistant microorganisms, thereby reducing the effectiveness of the medications. The establishment of a permanent system of laboratory analysis that shows infectious processes and examines the level of bacterial resistance of samples isolated from inpatients and outpatients is an essential measure towards resistance control[5].

The analysis of antibiotic resistance has been widely applied and combined with other methods that aim to characterize several pathogens, including the genus *Shigella*. Such characterization has been carried out in Brazil[2] and other countries, such as Chile[1], South Africa[6], Bangladesh[7] and Belgium[8].

In Belém, Pará State, little information is known about the *Shigella* spp. resistance to antibiotics. In this study, we characterize the resistance of *Shigella* spp. samples isolated from patients with acute diarrhea between 1990 and 2000.

MATERIALS AND METHODS

This study analyzed a total of 50 samples (viables) of *Shigella* spp. isolated between 1990 and 2000. All of the samples were isolated from stool cultures from patients with clinically acute diarrhea, including males and females with ages ranging from 0 to 81 years.

The isolation of enteropathogens was performed by seeding the suspension of fecal material on selective media indicators (MacConkey agar – MC, *Salmonella Shigella* agar – SS) (Difco™) and in enrichment medium (Selenite Cystine Broth) (Difco™). The suspected colonies were then inoculated in screening medium (Triple sugar iron – TSI) (Difco™). Afterwards, the biochemical characterization was performed in an automated system (Vitek 2 Compact - bioMérieux™) using the GN Test card. The serological identification followed the recommendations of Kauffmann[9] and Ewing[10] using the antiserum (Bio-Rad Laboratories) relative to *Shigella flexneri* (Group A), *Shigella boydii* (Group B), *Shigella dysenteriae* (Group C) and *Shigella sonnei* (Group D).

To characterize the resistance of the *Shigella* spp. samples, the automated system Vitek 2 Compact - bioMérieux™ and the AST-GN05 card were used.

RESULTS

Among the 50 samples of *Shigella* that were isolated, 32 (64%) were identified as *S. flexneri* and 18 (36%) as *S. sonnei*, regardless of the isolation period and the age of the patients. The *S. dysenteriae* and *S. boydii* species were not detected in the samples. *S. flexneri* prevailed in 1991 (46.8%), and its occurrence was reduced in the following years. *S. sonnei* showed similar rates throughout the period of ten years (Table 1).

Table 1 – Distribution of species of Shigella isolated in Pará State from 1990 to 2000

Year	Species (%)	
	S. flexneri (n = 32)	*S. sonnei* (n = 18)
1990	3 (9.3)	–
1991	15 (46.8)	2 (11.1)
1992	1 (3.2)	1 (5.6)
1993	1 (3.2)	1 (5.6)
1994	1 (3.2)	–
1995	1 (3.2)	–
1996	3 (9.3)	3 (16.6)
1997	3 (9.3)	6 (33.3)
1998	3 (9.3)	1 (5.6)
1999	1 (3.2)	2 (11.1)
2000	–	2 (11.1)

Conventional sign used: - Numerical data are equal to zero and not due to rounding of numbers.

The resistance of *Shigella* spp. samples to the antibiotics tested is shown in table 2. All of the samples showed resistance to cephalothin, cefazolin, cefuroxime, cefuroxime axetil and tobramycin. However, 100% of the strains were also susceptible to cefpodoxime, ceftriaxone, levofloxacin and norfloxacin. All of the strains showed sensitivity to all of the antibiotics tested.

Table 2 – Antibiotic resistance of species of *Shigella* isolated in Belém, Pará State, Brazil (1990-2000)

Antimicrobial agents	Number of samples and resistance (%)		
	Total (n = 50)	*S. flexneri* (32)	*S. sonnei* (18)
Cephalothin	50 (100)	32 (100)	18 (100)
Cefazolin	50 (100)	32 (100)	18 (100)
Cefoxitin	4 (8.0)	–	4 (22.2)
Cefuroxime	50 (100)	32 (100)	18 (100)
Cefuroxime axetil	50 (100)	32 (100)	18 (100)
Cefpodoxime	–	–	–
Ceftriaxone	–	–	–
Levofloxacin	–	–	–
Norfloxacin	–	–	–
Ticarcillin	22 (44.0)	21 (66.6)	1 (5.5)
Ticarcillin/ Clavulanic acid	1 (2.0)	–	1 (5.5)
Tobramycin	50 (100)	32 (100)	18 (100)

Conventional sign used: - Numerical data are equal to zero and not due to rounding of numbers.

Considering all of the samples analyzed, 22 (44%) samples were resistant to ticarcillin: 21 were *S. flexneri*, and one was *S. sonnei*. With respect to the antibiotic ticarcillin/clavulanic acid, only one sample (2%; *S. sonnei*) was resistant, and only four (8%) samples (*S. sonnei*) showed resistance to cefoxitin.

DISCUSSION

Shigella spp. is one of the enterobacteria most commonly isolated from the enteric system all over Brazil[11,12,13], including the northern region.

Regarding the epidemiologic studies of shigellosis in the northern region , it is important to evaluate the performance of antimicrobial susceptibility through permanent analyses in addition to considering the bacteriological aspects in the characterization of prevalent species[14,15,16].

The analysis of the resistance of the *Shigella* spp. samples indicated that these enterobacteria are more difficult to treat with cephalothin, cefazolin, cefuroxime and cefuroxime axetil because all of the strains were resistant to these antibiotics. In contrast, the results showed promise for other options for treatment, such as cefpodoxime, ceftriaxone, levofloxacin and norfloxacin, because resistance to these antibiotics was not detected. Other treatment options were also suggested, such as using cefoxitin and ticarcillin because of low resistance (8% and 44%, respectively; Table 1).

The data shown in this study are consistent with data shown for other states of Brazil[4,13,17,18] and other countries from Central America, North America, Africa, Middle East and India as described by Drews et al., 2010[19].

CONCLUSION

The results of this study have demonstrated that the continuous evaluation of antibiotic resistance of *Shigella* spp. samples is extremely important for determining the appropriate treatment for shigellosis, a disease that has great importance in public health.

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Caracterização da resistência antimicrobiana de amostras de Shigella spp. isoladas em Belém, Estado do Pará, Brasil (1990-2000)

RESUMO

Avaliou-se a resistência antimicrobiana de amostras de *Shigella* spp. isoladas de membros da população com faixa etárias de 6 meses a 81 anos, habitantes da Cidade de Belém, Estado do Pará, no período de 1990 a 2000. Foram analisadas 50 amostras de *Shigella* spp. identificadas no Laboratório de Enteroinfecções Bacterianas da Seção de Bacteriologia e Micologia do Instituto Evandro Chagas e mantidas na bacterioteca da referida seção. Na caracterização fenotípica, 32 (64%) dos isolados foram identificados como *S. flexneri* e 18 (36%) como *S. sonnei*. Resistência a cefalotina, cefazolina, cefuroxima, cefuroxima axetil e tobramicina foi observada em 100% das amostras, embora elas apresentassem 100% de sensibilidade a cefpodoxima, ceftriaxona, levofloxacina e norfloxacina. Das amostras, 2% apresentaram resistência a ticarcilina/ácido clavulânico, 8% a cefoxitina e 44% a ticarcilina. Os resultados obtidos evidenciaram a acentuada resistência a diversos antimicrobianos desta enterobactéria causadora de graves infecções em humanos.

Palavras-chave: Diarreia; *Shigella*; Resistência Microbiana a Medicamentos.

Caracterización de la resistencia antimicrobiana de muestras de *Shigella* spp. aisladas en la Ciudad de Belém, Estado de Pará, Brasil (1990-2000)

RESUMEN

Se evaluó la resistencia antimicrobiana de muestras de *Shigella* spp. aisladas de miembros de la población en una franja etaria de 6 meses a 81 años, habitantes de la Ciudad de Belém, Estado de Pará, Brasil, en el período de 1990 a 2000. Fueron analizadas 50 muestras de *Shigella* spp. identificadas en el Laboratorio de Enteroinfecciones Bacterianas de la Sección de Bacteriología y Micología del Instituto Evandro Chagas y mantenidas en la bacterioteca de la referida sección. En la caracterización fenotípica, 32 (64%) de los aislados fueron identificados como *S. flexneri* y 18 (36%) como *S. sonnei*. Se observó resistencia a la cefalotina, cefazolina, cefuroxima, cefuroxima axetil y tobramicina en 100% de las muestras, aunque las mismas presentaran 100% de sensibilidad a la cefpodoxima, ceftriaxona, levofloxacina y norfloxacina. De las muestras, 2% presentaron resistencia a la ticarcilina/ácido clavulánico, 8% a la cefoxitina y 44% a la ticarcilina. Los resultados obtenidos evidenciaron la acentuada resistencia a diversos antimicrobianos de esta enterobacteria causadora de graves infecciones en humanos.

Palabras clave: Diarrea; *Shigella*; Farmacorresistencia Microbiana.

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