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jbpm@sbpc.org.br, adagmar.andriolo@gmail.com

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Conceição Moura Nunes, Maria do Rosário; Nogueira Mendes, Edilberto; Moura Nunes, João Maurício; Prazeres Magalhães, Paula; Penna, Francisco José
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Prevalence of *Salmonella enterica* in children aged less than 5 years with acute diarrhea and controls in Teresina-PI

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Prevalência de Salmonella enterica em crianças com idade inferior a 5 anos com diarreia aguda e controles em Teresina-PI

Maria do Rosário Conceição Moura Nunes¹; Edilberto Nogueira Mendes²;
João Maurício Moura Nunes³; Paula Prazeres Magalhães⁴; Francisco José Penna⁵

key words	abstract
<i>Salmonella enterica</i>	We investigated the prevalence of <i>Salmonella enterica</i> (SE) among children ($n = 400/250$ with diarrhea) in Teresina-PI from 2004 to 2007. SE Newport was isolated from two samples and O-C2-C3-ND, Enteritidis, and Muenchen serological variants were isolated from one sample each. SE infection was more prevalent among children aged less than six months. Increased fecal volume, 3-10 evacuations/day, vomit and fever were reported for all cases. Resistance to nalidixic acid (NAL) and sulphamethoxazole-trimethoprim (SXT) was ubiquitous. Our data substantiate the need for monitoring SE infections worldwide and the emergence of antimicrobial resistance.
Infectious diarrhea	
Antimicrobial susceptibility	
Clinical symptoms	
Epidemiology	

resumo	unitermos
Foi estudada a prevalência de <i>Salmonella enterica</i> (SE) em crianças ($n = 400; 250$ com diarreia) em Teresina-PI, entre 2004 e 2007. SE Newport foi isolada de duas crianças e os sorotipos O-C2-C3-ND, Enteritidis e Muenchen foram encontrados em uma criança cada. A infecção foi mais comum em crianças com até 6 meses de idade. Volume fecal aumentado, três a 10 evacuações/dia, vômitos e febre foram relatados para todas as crianças. Foi comum resistência a ácido nalidíxico (NAL) e sulfametoxazol-trimetoprima (SXT). Nossos dados demonstram a necessidade de monitoramento da magnitude das salmoneloses em diferentes regiões do mundo e da emergência de padrões de resistência.	<i>Salmonella enterica</i> <i>Diarreia infecciosa</i> <i>Suscetibilidade a antimicrobianos</i> <i>Manifestações clínicas</i> <i>Epidemiologia</i>

1. Doutora em Microbiologia; professora adjunta da Universidade Federal do Piauí (UFPI).

2. Pós-doutor em Microbiologia; professor titular da Universidade Federal de Minas Gerais (UFMG).

3. Médico do Instituto Nacional de Seguro Social (INSS) do Piauí.

4. Doutora em Microbiologia; professora adjunta da UFMG.

5. Doutor em Pediatria; professor titular da UFMG.

Introduction

Enteric infections are a leading cause of disease and death in children, particularly in the developing world^(1, 2). Estimates from World Health Organization (WHO) indicate that 1.6/2.1 million deaths occurred in children aged less than 5 years during 2000^(3, 9). Although mortality rates due to diarrhea have decreased, morbidity levels have not followed the same trend, especially in poor regions⁽⁸⁾. Furthermore, despite most diarrhea episodes are self-limited and dehydration can usually be controlled with oral rehydration, it would be desirable to prevent diarrhea or treat patients presenting with more severe disease⁽¹¹⁾. The number of pathogens that are responsible for diarrheal disease is large and increasing but the estimation of diarrhea episodes due to each pathogen is unclear. We conducted this study to better understand the distribution of SE diarrhea episodes in children aged less than 5 years in Teresina-PI, one of the poorest regions in Brazil. We also investigated clinical and epidemiological data associated with *Salmonella enterica* (SE) infection and the antimicrobial susceptibility of the isolates. To our knowledge this is the first systematic study on SE intestinal infection performed in this geographic area.

Patients and methods

This investigation was part of a prospective study of children's diarrhea and was conducted in Teresina. Fecal samples were collected from 400 children up to 60 months of age (mean 13.9 months; 56.7% male), all of them outpatients, from the low socioeconomic stratum (mean annual income ~ US\$4,700) seen at two public hospitals in Teresina from January 2004 to August 2007. Among them, 250 (mean age 14.6 months; 56% male) presented acute diarrhea and 150 (mean age 12.6 months; 58% male) had no diarrhea in the previous 15 days. No subject had history of hospitalization or antimicrobial therapy in the previous 15 days.

Fecal specimens were obtained and individually transferred to sterile vials containing a sterile solution of equal parts of glycerol and 0.033 M phosphate buffer pH 7. Specimens were transported to the Laboratório de Microbiologia of Departamento de Parasitologia e Microbiologia of the Universidade Federal do Piauí (UFPI) within an hour and streaked onto MacConkey agar and *Salmonella-Shigella* (SS) agar. After incubation at 35°C overnight around five lactose-positive, five lactose-negative

and five H₂S-positive colonies were picked up and further identified by microbiological methods. SE isolates were sent to a reference laboratory (Laboratório de Enterobactérias of Instituto Oswaldo Cruz, Rio de Janeiro, Brazil) for serotyping.

Antimicrobial susceptibilities of one selected SE isolate to ampicillin (AMP), ceftriaxone (CFX), chloranfenicol (CHL), ciprofloxacin (CIP), nalidixic acid (NAL), and sulfamethoxazole-trimethoprim (SXT) were assayed by disk diffusion according to Clinical and Laboratory Standards Institute (CLSI) 2005 guidelines⁽⁴⁾.

Data were analyzed using the χ^2 test with Yates' correction or Fisher's exact test as appropriate with the level of significance set at 5%.

The research protocol was approved by the Ethics Committee of the Universidade Federal de Minas Gerais (UFMG) and of the UFPI. The parents or guardians of all children gave written informed consent.

Results

SE was isolated from feces of five (5 out of 250; 2%) patients, all of them with diarrhea. Isolates were further identified as SE O-C2-C3-ND, SE serovar Enteritidis, SE serovar Newport ($n = 2$), and SE serovar Muenchen. The organism was not isolated from control patients. SE strains were evenly distributed in dry and rainy seasons. No association with sex, and presence of blood, pus and mucus in the feces was found. Increased fecal volume, three-10 bowel movements daily, vomits and fever (from 37.5 to 41°C) were reported for all SE infected children. SE infection was more common (three out of five) in patients aged less than 6 months; SE serovar Newport was identified in two out of these three patients. Association among SE serovars and clinical and epidemiological data is shown in **Table 1**.

The antimicrobial susceptibility profile of SE isolates is depicted in **Table 2**. No isolate was resistant to all antimicrobials tested. SE serovar O-C2-C3-ND was resistant to CIP, CHL, NAL, and SXT and SE serovar Enteritidis was resistant to NAL. All other strains were pansensitive.

Discussion

Salmonellosis is a worldwide health problem being the second leading cause of bacterial foodborne illness in the United States⁽⁶⁾. However, the actual magnitude of the problem in Brazil is unknown since no recent data on

Table 1 Association among *Salmonella enterica* serovar and clinical and epidemiologic data

<i>Salmonella enterica</i> serovar	Year	Season	Sex	Temperature ¹	Blood/pus/mucus	Age ²
O-C2-C3-ND	2005	Rainy	Female	37.5	Yes	22
Enteritidis	2006	Dry	Female	39	Yes	2
Newport	2006	Dry	Male	39.5	Yes	5
Newport	2006	Dry	Male	40	No	2
Muenchen	2007	Rainy	Female	39.5	Yes	48

¹: in Celsius degrees; ²: in months;**Table 2** Antimicrobial susceptibility of *Salmonella enterica* isolated from fecal specimens of 250 children with diarrhea

Antimicrobials	n – %
Ampicillin	5 – 100
Ceftriaxone	5 – 100
Chloranfenicol	4 – 80
Ciprofloxacin	4 – 80
Nalidixic acid	3 – 60
Sulphamethoxazole + thrimethoprim	4 – 80

human salmonellosis is available in specialized literature. We showed that SE infection is rare in children aged less than 5 years in Teresina, in the Brazilian Northeast, one of the poorest areas in the country. There are now over 2,500 identified serotypes of SE. Among them, a smaller number of serovars are significantly associated with human disease especially Typhimurium and Enteritidis⁽⁵⁻⁷⁾. In contrast to these data, serovar Newport was the most common detected in our population, a finding that demonstrates the uneven distribution of SE serotypes over the world. As expected by the fact that salmonellosis is a foodborne disease frequently associated with the consumption of naturally contaminated animal foods or derivatives, no seasonality was seen in the distribution of the disease.

SE can be associated with a number of different disease syndromes, the most common being enteritis, frequently

characterized by nausea, vomiting, diarrhea, and fever⁽⁶⁾. As expected, these clinical manifestations were reported by our patients. The disease is typically self-limited, but patients presenting with more severe manifestations should be treated aiming to eradicate the organism. Increasingly, SE isolates are being detected that demonstrate resistance to multiple antimicrobial agents, including third-generation cephalosporins, commonly recommended for treating severe infections⁽⁶⁾. Our results are similar to those reported by Vaz *et al.* ⁽¹⁰⁾ who found high resistance levels of SE Enteritidis to NAL and SXT, two antimicrobials frequently employed in our country for the treatment of patients supposed to have inflammatory diarrhea. Surprisingly, all SE we identified were susceptible to AMP, a drug largely employed in Brazil for treating patients with different kinds of bacterial infections.

In brief, SE infection is rare in children from the Brazilian Northeast. Our data support the need to establish monitoring programs to evaluate the magnitude of SE infections in different parts of the world and the emergence of potential resistance patterns in order to drive the empirically selected antimicrobial drugs for treating patients with more severe presentations of the disease.

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Mailing address

Edilberto Nogueira Mendes
 Av. Professor Alfredo Balena, 190, sala 423
 Santa Efigênia
 CEP: 30130-100 – Belo Horizonte-MG