Salmonella gastroenteritis in children (clinical characteristics and antibiotic susceptibility): comparison of the years 1995-2001 and 2002-2008

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We document herein the prevalence and serotype distribution among *Salmonella enterica* strains isolated from children treated for diarrhea over two seven-year periods spanning 14 years. Four hundred and eight (1.38%) *S. enterica* cases were isolated among 29,601 diarrheal admissions. Among the Salmonella isolates, 63.7% were *serogroup D* and 29.9% were *serogroup B*. Overall, 21.7% of cases were under one year of age, with 2.1% being younger than three months. Bloody diarrhea was found in 18.8% of the cases. The resistance rates were 25.8%, 18.2%, 7.0%, 4.7%, and 0.3%, to ampicillin, chloramphenicol, trimethoprim-sulfamethoxazole, ceftriaxone, and ciprofloxacin, respectively. In conclusion, our study has revealed that the predominance of *Salmonella serogroup D* continues. The clinical features of our patients were mostly mild, with no deaths or severe complications. While resistance to antimicrobial agents changes constantly, it is important to keep these strains under surveillance in order to formulate policies for the rational use of antimicrobial agents.

Key words: acute gastroenteritis, children, Salmonella enteritis.

Acute gastroenteritis is the second cause of death among all infectious diseases in children younger than five years of age worldwide. Up to 1.3 billion acute diarrhea cases with an average of 2-3 episodes per child are reported annually[1]. In developing countries, bacterial acute gastroenteritis continues to be an important cause of morbidity and mortality among young children. The knowledge of the etiology of diarrhea is important for epidemiological surveillance and, in several cases, for appropriate management.

Gram-negative bacteria of the species Salmonella are a major cause of food-borne illness in mostly developed countries. Children in developing countries become infected with a diverse group of bacterial (Salmonella, Shigella, and, most notably, the various diarrhea-producing *Escherichia coli* organisms are the most common pathogens), viral and parasitic pathogens. While there is little information on its epidemiology and the burden of Salmonella gastroenteritis from developing countries, Salmonella infections are recognized as one of the major causes of childhood diarrheal illness[1]. It is generally transmitted to humans through consumption of contaminated food of animal origin, mainly meat, poultry, eggs, and milk. In the United States of America (US), salmonellosis accounts for 30% of deaths resulting from food-borne illnesses[2]. In Germany, Salmonella was the most frequently detected bacteria found in patients with acute gastroenteritis[3]. Unfortunately, there is currently no national Salmonella reference center in Turkey, so no reliable statistical data are available on gastrointestinal infections caused by Salmonella. Epidemiologic data on Salmonella strains isolated in Turkey can be obtained only through the collective findings of individual studies. The aim of the present study was to document the prevalence and serotype

distribution among Salmonella enterica strains isolated from children treated for diarrhea in Ankara, Turkey, over two periods spanning 14 years, from 1995 to 2008.

Material and Methods

This study was conducted among the culture-proven *S. enterica* cases admitted to Hacettepe University İhsan Doğramacı Children's Hospital, Diarrheal Diseases Training and Treatment Unit[4]. Antibiotics are not generally recommended for the treatment of Salmonella gastroenteritis in our center. However, given the risk for bacteremia in infants (<3 months of age) and that of disseminated infection in high-risk groups with immune compromise (human immunodeficiency virus [HIV], malignancies, immunosuppressive therapy, immunodeficiency states), these children receive an appropriate antibiotic empirically until culture results are available[1,4]. Stool cultures were obtained from all cases and inoculated in Selenite F broth, Shigella-Salmonella agar and eosin-methylene blue (EMB) agar, and were incubated overnight at 37°C in ambient air. The next day, subcultures were taken from Selenite F broth and inoculated on Shigella-Salmonella and EMB media. Lactose-negative colonies suggesting Salmonella or Shigella species were further tested by routine biochemical tests. Salmonella isolates suspected by routine biochemical tests are further tested by the slide agglutination technique employing polyvalent and monospecific antisera for somatic (O) and flagellar (H) antigens to detect serogroups and serotypes (by using antisera from Difco Laboratories, Detroit, USA). Serotyping was performed on all isolates using standard Kauffmann-White method[5]. However, Salmonella serotypes were not routinely identified in all cases due to the unavailability of antisera for flagellar antigens. Antibiotic susceptibility testing was performed by Kirby-Bauer disk susceptibility method following Clinical and Laboratory Standards Institute (CLSI) guidelines[6]. Microbiological studies were performed at Hacettepe University İhsan Doğramacı Children's Hospital, Clinical Microbiology Laboratory.

Diarrhea is defined as a change in the child's normal stool pattern, characterized by an increases in the frequency (at least 3 discharges per day) and in the liquidity of fecal discharges. Persistent diarrhea is defined as any diarrhea, with or without blood, which begins acutely and last for 14 days or longer[4]. The patient's age, sex and clinical manifestations on admission and outcome of illness were obtained from hospital files. In this Unit, dehydration is evaluated according to the World Health Organization (WHO) guidelines, and all cases are called for a revisit 1-3 days after the first admission and at any time according to "seek advice criteria" defined in the WHO guidelines for diarrheal disease management[4]. Multi-drug resistant Salmonella species were defined as strain resistant to four or more different classes of antibiotics.

In this study, *S. enterica* cases isolated between the years 1995 and 2008 (divided into two 7-year periods) whose files were available were evaluated. The initial period (referred to as period A) was performed at the Hacettepe University İhsan Doğramacı Children's Hospital, Diarrheal Diseases Training and Treatment Unit, from January 1995 to December 2001. The second period (referred to as period B) was conducted at the same Unit, between January 2002 to December 2008, and all the management criteria were similar.

The Statistical Package for the Social Sciences software version 11.0 (SPSS Inc., USA) was used for statistical analysis. Chi-square, Mann-Whitney U or Student t-test was used for statistical comparisons where appropriate.

Results

Over the duration of the two periods, 408 (1.38%) *S. enterica* cases were isolated among 29,601 diarrheal admissions (period A: 1.71% (259/15126) cases and period B: 1.03% (149/14475) cases, $p<0.001$). There were variations in the rate of Salmonella isolates reported from year to year (Fig. 1). The lowest rate was reported in 2004 and the highest in 2005 (0.41% and 2.87%, respectively). Male-female ratio was 1.46 (242/166). During the study period, most of the Salmonella gastroenteritis cases were identified in the hot season (between June and September) (Fig. 2).

During the 14 years of the study period, among the Salmonella isolates, 260 (63.7%) were serogroup D, 122 (29.9%) were serogroup B, 22 (5.4%) were serogroup C, and 4 (1.0%)

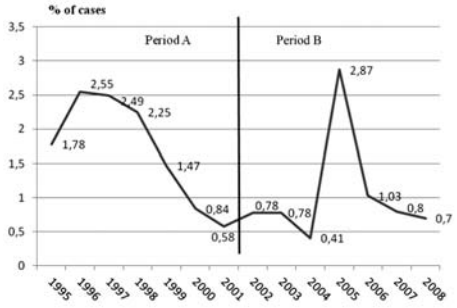

Fig. 1. Distribution of Salmonella gastroenteritis by year.

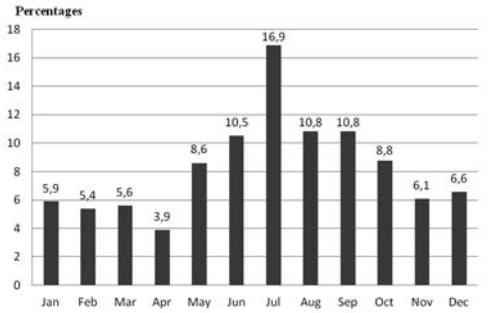

Fig. 2. Distribution of patients with Salmonella gastroenteritis by month

were *S. paratyphi* (Table I). In period A (1995-2001), Salmonella serogroup D was the most commonly isolated strain (58.7%), followed by serogroup B (36.7%). In period B (2002-2008), a significant increase in the serogroup D isolation rate (58.7% and 72.5%, respectively, $p<0.05$) and significant decrease in the serogroup B isolation rate were detected (36.7% and 18.1%, respectively, $p<0.001$). After 1996, no *S. paratyphi* case was isolated in our center.

Clinical information from files was available for 378 patients. During the study period, the mean age of the cases was 52±36 months (period A: 53±36 months, period B: 51±36 months, $p>0.05$). Overall, 21.7% of cases were under one year of age, 2.1% (8/378) being younger than three months (Table II). Compared to period A, while the percentage of other age groups was decreased, the percentage of 13-60 months of age patients was increased in period B (42.4% and 52.3%, respectively, $p>0.05$).

Table I. Distribution of Salmonella Serogroups by Periods

Salmonella serogroups	Periods		Total (n=408)
	1995-2001 (n=259)	2002-2008 (n=149)	
Serogroup B	36.7	18.1*	29.9
Serogroup C	3.1	9.4**	5.4
Serogroup D	58.7	72.5**	63.7
S. paratyphi	1.5	0	1.0

Values are % of isolates
* $p<0.001$
** $p<0.05$

The major presenting symptoms and signs are shown in Table II. Overall, duration of diarrhea on admission varied from 1-30 days (median: 2 days). In period A, the median duration of diarrhea on admission was 3 days (range: 1-30 days), whereas in period B, the median duration of diarrhea on admission had decreased to 2 days (range: 1-20 days) ($p<0.001$). Eight patients had persistent diarrhea on admission (5 cases in period A, 3 cases in period B). Mean frequency of stools before admission was 6.9±3.9 times per day, and no difference was found between the study periods. 9.7% of patients had daily stool output of >10. Bloody diarrhea was found in 18.8% of the cases (21.4% in period A, 14.8% in period B, $p>0.05$).

Compared to period A, in period B, there was a significant increase in the percentage of cases with fever (11.4% and 34.2%, respectively, $p<0.001$). The dehydration rate was increased from 6.6% to 13.4% of cases ($p=0.24$) between the study periods. The percentages of cases with vomiting and abdominal pain had also increased

Table II. Comparison of the Clinical Characteristics of Salmonella Gastroenteritis Cases According to the Two Study Periods

Clinical characteristics	Periods		
	1995-2001 (n=229)	2002-2008 (n=149)	Total (n=378)
Age groups (months)			
0-3	2.6	1.3	2.1
4-12	22.3	15.4	19.6
13-60	41.9	52.3	46.0
>60	33.2	30.9	32.3
Duration of diarrhea, median days (range), [mean ± SD]	3 (1-30) [3.6±3.5]	2 (1-20)* [2.7±3.1]	2 (1-30) [3.2±3.3]
Frequency of stools before admission, mean ± SD	6.9±3.9	6.8±3.9	6.9±3.9
Stool characteristics			
Bloody	21.4	14.8	18.8
Mucoid	38.4	37.6	38.1
Fever	11.4	34.2*	20.4
Vomiting	40.2	56.4**	46.6
Abdominal pain	21.2	57.7*	35.7
Convulsions	0.9	3.4	1.9
Dehydration status			
Mild	3.1	7.4	4.8
Moderate	3.5	5.4	4.2
Severe	0	0.7	0.3
Hospitalization	2.3	1.6	3.9
Antibiotic use	27.1	30.9	28.6

Values are % of isolates
* $p<0.001$
** $p<0.05$

significantly in period B. The hospitalization rates were 2.3% and 1.6%, respectively (Table II). No death was recorded between 1995 and 2008.

The clinical characteristics according to the Salmonella serogroups isolated between 1995 and 2008 are displayed in Table III. Salmonella serogroup D was isolated from older patients compared with Salmonella serogroup B (57.3±45.7 months and 41.6±42.8 months, respectively, $p<0.001$). Patients infected with serogroup B had more bloody diarrhea on admission than those infected with serogroup C or D, but the only significant difference was found between serogroups B and C (25.0% and 4.5%, respectively, $p<0.05$). Patients infected with serogroup B also had longer duration of diarrhea on admission than other patients ($p<0.05$). Abdominal pain was presented significantly more in patients infected with serogroup D than serogroup B (40.7% and 23.7%, respectively, $p<0.05$). Although more patients with serogroup C admitted with fever, vomiting and abdominal pain, none of these differences was statistically significant. The presence of fever, vomiting, dehydration, hospitalization, and antibiotic use did not differ significantly between the serogroups (Table III).

Table IV shows the antimicrobial resistance patterns of *S. enterica* serotypes over the 14-year study period. Salmonella serogroup B was significantly more resistant to ampicillin, amoxicillin-clavulanic acid, ceftriaxone, and chloramphenicol than the other serogroups.

Table III. Comparison of the Clinical Characteristics According to Salmonella

Clinical characteristics	Serogroups		
	Serogroup B (n=116)	Serogroup C (n=22)	Serogroup D (n=237)
Age, median months (range)	24 (2-204)	27.5 (2-168)	42 (1-192)*
Duration of diarrhea, median days (range)	3 (1-20)**	2 (1-7)	2 (1-30)
Frequency of stools before admission, mean ± SD	7.2 ± 4.1	5.6 ± 2.4	6.9 ± 3.9
Stool characteristics			
Bloody	25.0**	4.5	17.3
Mucoid	44.0	22.7	36.7
Fever	19.8	27.3	20.3
Vomiting	41.4	63.6	47.3
Abdominal pain	23.7	40.9	40.7**
Convulsions	0.9	0	2.5
Dehydration	7.8	4.5	10.5
Hospitalization	10.3	6.3	7.4
Antibiotic use	28.4	22.7	29.5

Values are % of isolates

* $p<0.001$

** $p<0.05$

Table IV. Resistance Patterns of Salmonella Serogroups

Antibiotic	Serogroups			Total	p
	Serogroup B	Serogroup C	Serogroup D		
Ampicillin	42/79 (53.2)[a]	3/19 (15.8)[b]	27/181 (14.9)[b]	72/279 (25.8)	<0.001
AMX-CLA	14/43 (32.6)[a]	1/7 (14.3)[ab]	3/58 (5.2)[b]	18/108 (16.7)	<0.001
TMP-SMX	9/87 (10.3)	3/20 (15.0)	8/179 (4.5)	20/286 (7.0)	>0.05
Ceftriaxone	6/51 (11.8)[a]	0/7 (0)[ab]	0/69 (0)[b]	6/127 (4.7)	<0.05
Cefotaxime	5/53 (9.4)	0/8 (0)	1/72 (1.4)	6/133 (4.5)	>0.05
Chloramphenicol	14/37 (37.8)[a]	1/7 (14.3)[ab]	5/66 (7.6)[b]	20/110 (18.2)	<0.001
Ciprofloxacin	0/91 (0)	0/21 (0)	1/199 (0.5)	1/311 (0.3)	
≥1 Antibiotic resistance	47/92 (51.1)[a]	4/21 (19.0)[b]	38/200 (19.0)[b]	89/313 (28.4)	<0.001
Multi-drug resistance	13/92 (14.1)[a]	1/21 (4.8)[a]	0/200 (0)[b]	14/313 (4.5)	<0.001

AMX-CLA: Amoxicillin-clavulanic acid. TMP-SMX: Trimethoprim-sulfamethoxazole.

Serogroup C was more resistant to trimethoprim/sulfamethoxazole (TMP-SMX) than the other serogroups, but this difference was not statistically significant ($p>0.05$). Multi-drug resistance was present in 4.5% (14/313) of the cases, of which 13 were among serogroup B. None of the cases with serogroup D was found to be multi-drug resistant. The changes in the antibiotic resistance pattern of *S. enterica* species between the study periods are shown in Table V. The antibiotic resistance changes between the study periods were not statistically significant. On the other hand, ≥1 antibiotic resistance ($p=0.002$) and multi-drug resistance ($p<0.001$) were decreased in the 2002-2008 period. No multi-drug resistant *S. enterica*

Table V. Changes in the Antibiotic Resistance Pattern of *Salmonella enterica* Species Between the Study Periods

Years	Antibiotic resistance, n (%)						
	Ampicillin	Ciprofloxacin	Ceftriaxone	Cefotaxime	TMP-SMX	≥1 Antibiotic resistance	Multi-resistance
1995-2001	46/151 (30.5)	0/162 (0)	6/125 (4.8)	5/124 (4.0)	13/153 (8.5)	60/166 (36.1)	15/166 (9.0)
2002-2008	26/128 (20.3)	1/148 (0.7)	0/2 (0)	1/9 (11.1)	7/133 (5.3)	30/149 (20.1)	0/149 (0)
Total	72/279 (25.8)	1/311 (0.3)	6/127 (4.7)	6/133 (4.5)	20/286 (7.0)	90/315 (28.6)	15/315 (4.8)

TMP-SMX: Trimethoprim-sulfamethoxazole.

species were detected in period B.

Discussion

Non-typhoidal Salmonella infections have a worldwide distribution with an incidence proportional to the standards of hygiene, sanitation, availability of safe water, and food preparation practices. In the developed world, the incidence of Salmonella infections and outbreaks has increased several-fold over the past few decades, which may be related to modern practices of mass food production and consumption that increase the potential for epidemics[1,2,7]. However, in our study, despite yearly variations, the overall *S. enterica* isolation rate has decreased from 1.71% in the 1995-2001 period to 1.03% in the 2002-2008 period ($p<0.001$). According to another study done in our center between 1987 and 1994, the overall Salmonella gastroenteritis isolation rate was 2.5%[8]. This finding is consistent with other reports from countries such as Greece, Iran and Palestine[7,9,10], which were done during the same period. On the other hand, in some countries such as India, the isolation rate was considerably higher (15%) than our result[11]. During the study period, most of the Salmonella gastroenteritis cases were identified in the hot season (June-September).

The availability of safe water is important for Salmonella gastroenteritis incidence as mentioned above; for the period of 1998-2004, *S. enterica* cases showed a steady decreasing trend to 0.4% among all childhood diarrheas in our center. However, following a drought period and water shortage in 2004 (the lowest total annual rainfall during the last 30 years in Ankara[12]), the prevalence sharply peaked to 2.9% in 2005 and then gradually decreased to 0.7% in 2008 (Fig. 3). We suggest that this might be due to great interruptions in the main water supply.

In our center, while the number of patients under one year of age has decreased over the study periods, the number of patients above one year of age has increased. During the 2002-2008 period, only 16.7% of the cases were under 12 months of age, compared with 24.9% during the 1995-2001 period. Overall, only 2.1% of cases were younger than three months of age, and incidence was greatest in the weaning period. In a previous study, 36.0% of

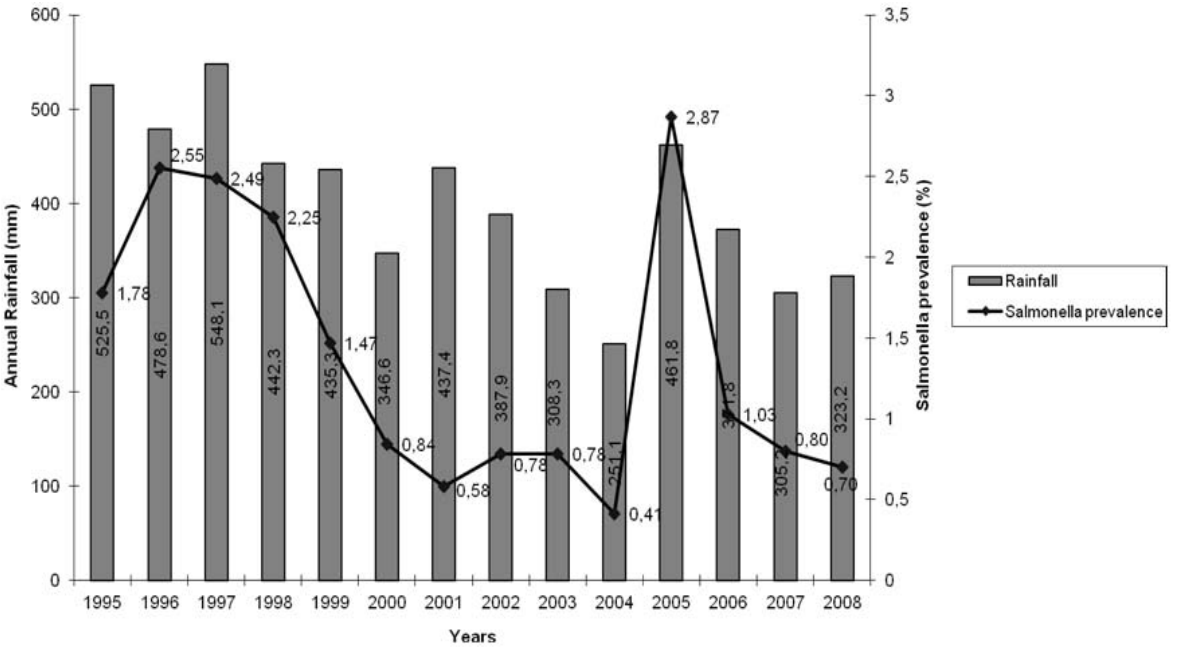

Fig. 3. Annual rainfall and Salmonella prevalence.

the patients were under 12 months of age, with 7.0% being younger than three months of age[8]. The low isolation rate of Salmonella cases under one year of age (especially under 3 months of age) and shift of cases to an older age may be due to the protective effect of breastfeeding, because case control studies in infants have strongly suggested that breastfeeding protects against acquisition of Salmonella infection in infancy[13,14]. In Turkey, breastfeeding is almost universal; 97% of all children are breastfed for some period of time, and in the first six months of life, the exclusive breastfeeding rate in Turkey increased from 10.7% to 41.6% between 1998 and 2008[15].

Since 1990, Salmonella serogroup D has emerged as the most common cause of Salmonella gastroenteritis in our center[8]. This was the case in our study, and there was a significant increase in the percentage of serogroup D isolation between period A and period B (58.7% and 72.5%, respectively, $p<0.05$). We were unable to identify the exposure route of cases due to the retrospective nature of our study. In other studies from Turkey, Salmonella serogroup D was found to be the most common cause of Salmonella gastroenteritis[16,17].

Resistance to antimicrobial agents has been described increasingly among Salmonella species worldwide. This resistance may be due to the use of antimicrobials both in humans and animal husbandry[18]. In Greece, the rates of resistance to ampicillin, amoxicillin clavulanic acid, gentamicin, chloramphenicol, tetracycline, nalidixic acid, and TMP-SMX were 8.5%, 7.0%, 0.7%, 5.4%, 14.2%, 2.7%, and 2.7%, respectively[7]. Another report from our region showed resistance rates of 36.4%, 15.5%, and 14.7% to TMP-SMX, ampicillin, and chloramphenicol, respectively[19]. High rates of antibiotic resistance in *S. enterica* species in Turkey have been reported previously[20-22]. In our center, the antibiotic resistance rates between the study periods were not significantly changed. The resistance rates of Salmonella species in our center were 25.8%, 18.2%, 7.0%, 4.7%, and 0.3%, to ampicillin, chloramphenicol, TMP-SMX, ceftriaxone, and ciprofloxacin, respectively. Although it also includes adult data, the Enter-net surveillance system showed similar results to those of our study (22.0%, 14.0%, 7.0%, and 0.5%, to ampicillin, chloramphenicol, TMP-SMX, and ciprofloxacin, respectively)[23]. Multi-drug resistance was found in 4.5% of *S. enterica* isolates in our study, and among these isolates, 28.4% were resistant to at least one antibiotic. Our results were similar to a Greek study[7], but lower than the other European studies[23,24]. One of the reasons for

low antibiotic and multi-drug resistance in our country may be the nationwide implementation of the diarrhea training and treatment program that endorses limited antibiotic use[1,4].

In conclusion, our study has revealed that the predominance of Salmonella serogroup D continues. The clinical features of our patients were mostly mild, with no deaths or severe complications. The shift of cases to older ages may explain the mild clinical course, low hospitalization rate and absence of death among our cases. Although most infections of children caused by *S. enterica* species are self-limiting and antimicrobial therapy is indicated only in selected cases, the high prevalence of *S. enterica* strains resistant to most of the commonly used antimicrobials is a major public health concern. While resistance to antimicrobial agents changes constantly, it is important to keep these strains under surveillance in order to monitor the local susceptibility and subsequently formulate policies for the rational use of antimicrobial agents. It is also important to limit the use of antibiotics both in children and farm animals in order to keep their therapeutic value.

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