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Clinical characteristics and antibiotic resistance of Shigella gastroenteritis in Ankara, Turkey between 2003 and 2009, and comparison with previous reports

Elif Nursel Özmert [a], Osman Tolga İnce [a,*], Emel Örün [b], Songül Yalçın [a], Kadriye Yurdakök [a], Deniz Gür [c]

[a] Department of Pediatrics, Social Pediatrics Unit, Hacettepe University Faculty of Medicine, 06100 Ankara, Turkey
[b] Department of Pediatrics, Fatih University Faculty of Medicine, Ankara, Turkey
[c] Clinical Microbiology Laboratory, İhsan Doğramacı Children's Hospital, Hacettepe University, Ankara, Turkey

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SUMMARY

Objectives: The aim of this study was to define the epidemiological, clinical, and antibiotic susceptibility patterns of Shigella gastroenteritis cases occurring during the years 2003–2009 and to compare results with those of the years 1987–2002.

Methods: A hospital-based study was conducted over a 22-year period. All 238 Shigella strains isolated between 2003 and 2009 were compared to 618 isolates from the period 1987–1994 and 218 Shigella strains isolated during 1995–2002 with regard to antimicrobial resistance patterns and patient clinical characteristics.

Results: The predominant species during all periods was *Shigella sonnei*, with an increasing predominance across the periods (64.0%, 71.5%, and 87.8%, respectively; $p < 0.001$). Neither the prevalence of bloody diarrhea nor other clinical characteristics changed across the study periods, except for the prevalence of dehydration, which increased (11.0%, 20.6%, and 28.6%, respectively; $p < 0.001$). During the period 2003–2009, 69.9% of Shigella were resistant to trimethoprim/sulfamethoxazole, 35.8% to ampicillin, and 4.7% to nalidixic acid. No case resistant to ciprofloxacin was detected. Multidrug resistance was also found to be similar in the last two periods (24.0% vs. 28.1%, respectively).

Conclusions: There was both a microbiological and a clinical change in childhood Shigella gastroenteritis cases over the 22 years. The antibiotic resistance pattern appears to have remained stable over the last two periods. There is a need to re-examine the criteria and clinical management guidelines for suspected shigellosis cases.

1. Introduction

Bloody diarrhea in young children is usually a sign of an invasive enteric infection that carries a substantial risk of serious morbidity and death.[1,2] Shigella is the most frequently isolated pathogen from the stools of young children with bloody diarrhea in developing countries.[3] Bloody diarrhea is treated empirically as shigellosis unless proven otherwise, and it is one of the limited indications for antibiotic treatment as recommended by the Diarrheal Diseases Control Program of the World Health Organization (WHO).[4] Appropriate antimicrobial treatment for shigellosis will reduce the duration and severity of dysentery and may also prevent lethal complications.[5]

Four species of Shigella are pathogenic in humans. *Shigella sonnei* and *Shigella boydii* usually cause a relatively mild illness in which diarrhea may be watery or bloody.[6] *Shigella flexneri* is the chief cause of endemic shigellosis in developing countries. Although *Shigella dysenteriae* type 1 is associated with the highest case fatality rates, the majority of deaths from shigellosis worldwide result from endemic disease, especially that caused by *S. flexneri*.[7]

Changes in Shigella epidemiology may change the clinical presentation as well as the antibiotic resistance patterns. Recognition of these changes is necessary for appropriate clinical management.

Resistance to ampicillin and trimethoprim/sulfamethoxazole (TMP–SMX), formerly the drugs of choice, is now widespread.[8–11] Reporting changes in resistance patterns helps maintain effective empirical antibiotic choices. In this study we report changes in clinical characteristics and antibiotic resistance patterns of Shigella cases over time.

2. Materials and methods

This study was conducted among the culture-proven Shigella cases admitted to Hacettepe University Ihsan Doğramacı Children's Hospital Diarrheal Diseases Training and Treatment Unit,

* Corresponding author. Tel.: +90 312 3051133; fax: +90 312 3243284.
E-mail address: oti1974@yahoo.com (O.T. İnce).

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where cases are managed according to WHO criteria.[12] Stool cultures were obtained from all cases and inoculated in selenite F broth, Salmonella–Shigella (SS) agar, and eosin–methylene blue (EMB) agar, and were incubated overnight at 35–37 °C in ambient air. The following day, subcultures were taken from selenite F broth and inoculated on SS agar and EMB agar. Lactose-negative colonies suggesting Shigella species were further tested by routine biochemical tests and confirmed by slide agglutination tests with Shigella antisera (Denka, Seiken Co. Ltd, Tokyo, Japan) and BBL Crystal E/NF identification panel (Becton Dickinson, Sparks, MD, USA).[13]

In vitro susceptibility to TMP–SMX, ampicillin, nalidixic acid, and ciprofloxacin was determined by the Kirby–Bauer disk diffusion method following the Clinical and Laboratory Standards Institute (CLSI) guidelines.[14] Multidrug resistance was defined as strains resistant to two or more different classes of antibiotics.

Patient clinical characteristics were obtained from hospital files. Bloody diarrhea was defined as any diarrheal episode in which the loose or watery stools contain visible red blood observed macroscopically by the clinicians and/or related by the parents. Dehydration was evaluated and treated according to WHO guidelines.[12] In this study, 238 Shigella strains isolated between 2003 and 2009 were compared to those isolated and reported during two previous periods (618 Shigella isolates from the period 1987–1994[15] and 218 Shigella isolates from the period 1995–2002[16]) for antimicrobial resistance patterns, clinical characteristics, and physician antibiotic prescription patterns.

Statistical calculations were performed with SPSS statistical software v. 11.5 (SPSS, Chicago, IL, USA). The Chi-square test, Mann–Whitney *U*-test, and Student *t*-test were used for statistical comparisons, as appropriate.

3. Results

Between the years 2003 and 2009, 238 Shigella cases were identified (136 male, 102 female). The overall isolation rate of *Shigella sp* was 1.6% (238/14 803) during this period. Among the isolates, 209 (87.8%) were *S. sonnei*, 25 (10.5%) were *S. flexneri*, and four (1.7%) were *S. boydii*. The comparative distributions of *Shigella sp* within the three periods are shown in Figure 1. During the study period, as in the previous periods, most of the Shigella cases (65.9%; $p < 0.001$) were identified in the dry and hot season (between July and October).

Table 1
Comparison of the clinical characteristics of Shigella gastroenteritis cases across the three study periods

Clinical characteristic	1987–1994 (n = 618)	1995–2002 (n = 218)	2003–2009 (n = 161)	p-Value
Age group				
<1 year	11.0	5.6	2.1	
1–5 years	59.0	48.6	29.4	
>5 years	30.0	45.8	68.5	<0.001
Bloody diarrhea	37.0	35.8	31.1	0.38
Fever	80.0	83.5	74.5	0.09
Vomiting	53.0	54.6	52.2	0.88
Convulsions	3.0	0.9	3.7	0.16
Dehydration	11.0	20.6	28.6	<0.001
Hospitalization	1.0	2.3	3.1	0.10
Death	0	0	0	

Values are % of isolates.

The clinical characteristics of the cases and comparison with the previous periods are shown in Table 1. In our study the mean age of the cases was 93 ± 48 months (range 5–202 months). *S. flexneri* was isolated from younger patients compared with *S. sonnei* (72.4 ± 68.5 months, 96.9 ± 44.8 months, respectively; $p < 0.05$), but during the previous period *S. sonnei* was isolated from younger patients compared with *S. flexneri* (52.8 ± 39.8 months, 70.2 ± 46.6 months, respectively; $p < 0.001$). Except for convulsions ($p = 0.04$), none of the clinical characteristics differed significantly among the different species during the study period (Table 2).

When the cases were analyzed according to blood in stool, it was found that, although not statistically significant, the Shigella cases who were admitted with bloody diarrhea were older (92.6 ± 43.2 months vs. 90.9 ± 46.6 months; $p = 0.85$), had a higher daily stool output frequency (8.2 ± 5.5/day vs. 6.9 ± 5.1/day; $p = 0.17$), and were hospitalized more frequently (6.0% and 1.8%; $p = 0.15$) than the cases without bloody stool.

Approximately 38.5% (62/161) of the cases received antibiotics on the basis of a clinical suspicion of shigellosis; 40.0% received antibiotics during 1995–2002 and 43.0% received antibiotics during 1987–1994. During the study period, 52% (26/50) of the patients with bloody diarrhea and 32.4% (36/111) of cases with

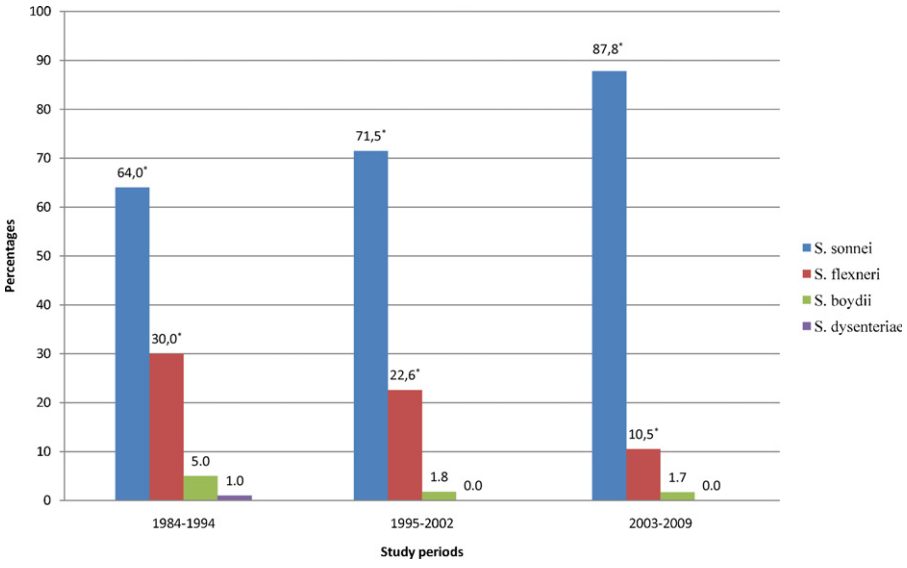

Figure 1. Shigella species during the different study periods (*$p < 0.001$).

Table 2
Comparison of clinical characteristics according to Shigella species, 2003–2009

Clinical characteristic	*Shigella sonnei* ($n = 144$)	*Shigella flexneri* ($n = 15$)	p-Value
Age group			
<1 year	1.0	12.0	
1–5 years	26.3	56.0	
>5 years	72.7	32.0	<0.001
Bloody diarrhea	31.3	33.3	0.87
Fever	75.7	60.0	0.18
Vomiting	54.2	40.0	0.44
Abdominal pain	46.5	26.7	0.23
Convulsions	2.8	13.3	0.04
Dehydration	27.1	46.7	0.11
Hospitalization	2.8	6.7	0.41
Death	0	0	

Values are % of isolates.

non-bloody diarrhea received antibiotics on admission ($p < 0.05$). The most frequently prescribed antibiotic was cefixime (46/62), and there was a sharp increase in the prescription rate of cefixime (16.3% to 74.2%). As in the previous period, ciprofloxacin (6/62) was the second most commonly prescribed antibiotic during the study period; the third was ceftriaxone (3/62). TMP–SMX, the most frequently used antibiotic of the previous periods, was not prescribed for any patient during the years 2003–2009. Only four patients' initial antibiotic therapies (6.6%) were changed to another antibiotic (all were ciprofloxacin) after the results of stool cultures. Antibiotic therapy was initiated in 21 cases after obtaining the results of stool cultures, and among these the most frequently prescribed antibiotic was ciprofloxacin (14/21).

The antibiotic resistance patterns as well as multidrug resistance rates of each Shigella species during the study period are displayed in Table 3. The most common multidrug resistance pattern was to TMP–SMX and ampicillin (58 cases), followed by TMP–SMX and nalidixic acid (6 cases), TMP–SMX, ampicillin, and nalidixic acid (2 cases), and ampicillin and nalidixic acid (1 case). Among the multi-resistant species, 66 (98.5%) were resistant to TMP–SMX, similar to the 1995–2002 period. Comparison of the antibiotic resistance patterns between the three periods is shown in Figure 2. Multidrug resistance increased for *S. sonnei* (6.3% to 26.8%; $p < 0.001$) and decreased for *S. flexneri* (71.1% to 44.0%; $p = 0.03$) from 1995–2002 to 2003–2009.

Table 3
Antibiotic resistance pattern between the years 2003 and 2009 according to the Shigella species

Antibiotic	*Shigella sonnei*, *n* (%)	*Shigella flexneri*, *n* (%)	Total, *n* (%)
TMP–SMX	116/162 (71.6)	14/24 (58.3)	130/186 (69.9)
Nalidixic acid	6/173 (3.5)	3/19 (15.8)[a]	9/192 (4.7)
Ampicillin	65/207 (31.4)	18/25 (72)[b]	83/232 (35.8)
Ciprofloxacin	0/208 (0)	0/25 (0)	0/233 (0)
MDR	56/209 (26.8)	11/25 (44)	67/234 (28.6)

TMP–SMX, trimethoprim/sulfamethoxazole; MDR, multidrug resistance.
[a] $p < 0.05$.
[b] $p < 0.001$.

4. Discussion

Between the years 1987 and 1994 the Shigella isolation rate at our center was 3.1%. The isolation rate decreased to 1.6% in the 1995–2002 period and had not changed during the study period (2003–2009). Our isolation rate is lower than others reported in our region; for example in Tehran, Iran between the years 2001 and 2006, the Shigella isolation rate was found to be 4.5%.[17]

Before 1987, *S. flexneri* was the most common Shigella species isolated in Turkey.[18,19] A similar significant species shift has also been reported in Vietnam and Iran.[10,17] However, there are some countries from our region still reporting *S. flexneri* as the predominant species.[20,21]

Most endemic shigellosis occurs in children aged between 6 months and 3 years.[3] At our center, while the number of patients under 1 year of age has decreased over the years, the number of patients over 5 years of age has increased. The low isolation rate of Shigella in those under 1 year of age and the shift of cases to older age groups may be due to the protective effect of breastfeeding. Breastfeeding reduces the severity of Shigella infections and shifts the spectrum of Shigella infections from severe to non-severe illness.[22,23] According to the Turkey Demographic and Health Survey, 2008, 69% of infants are exclusively breastfed in the first 2 months of life. The median duration of breastfeeding for all children is 16 months.[24] However the trends in increased age of cases cannot be explained solely by prolonged breastfeeding. This change could be related to changes in referral to our hospital or to improved hygiene and/or socioeconomic status decreasing the intensity of enteric pathogen transmission in Ankara, Turkey.

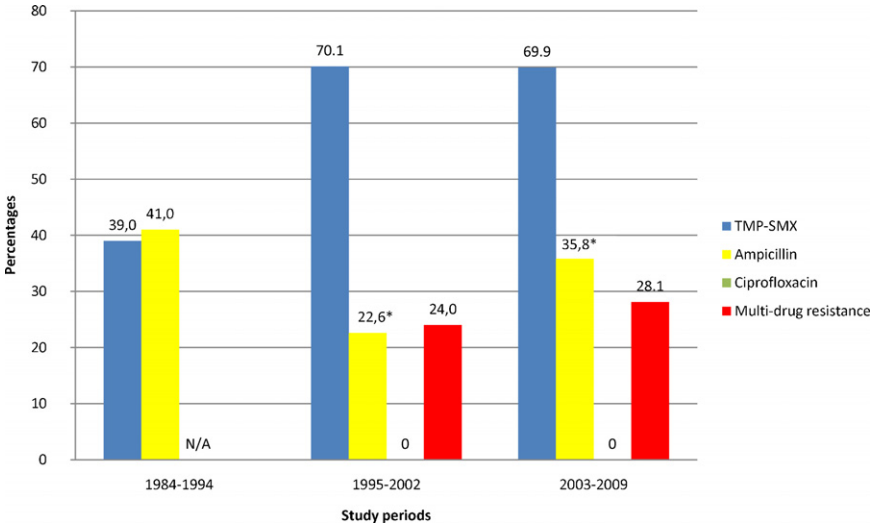

Figure 2. Antibiotic resistance patterns during the different study periods(*$p < 0.001$; N/A: not applicable).

The shift of cases to older ages and the predominance of *S. sonnei* may explain the mild clinical course, low hospitalization rate, and absence of death among our cases. On the other hand, *S. flexneri* cases were found to be younger and more often had bloody diarrhea, which may be the reason for more frequent antibiotic use and hospitalization. Dehydration seems to be the most important emerging complication in our patients, but oral rehydration therapy appears to be adequate for these cases. However among 238 cases, only 161 files were available for the determination of clinical characteristics. We believe that this may be a drawback of our study in terms of evaluating the clinical characteristics of our patients.

The first choice of antibiotic at our center has changed from TMP–SMX to cefixime. This may be mostly related to the high resistance rate to TMP–SMX. High TMP–SMX resistance rates of between 47.4% and 96.5% are being reported from other regions.[10,11,17,20,25,26] Ampicillin, the second most commonly used antibiotic during the 1987–1994 period, was not prescribed for any patient during the years 1995–2002 and was prescribed for only one patient during the years 2003–2009. The resistance rate to ampicillin has generally increased since the 1995–2002 period at our center.[16] However another recent article from Turkey[18] reported that most strains of Shigella were susceptible to ampicillin (86.4%). Vinh et al.[10] reported a sequential decrease in resistance to ampicillin, which is now rarely used in Vietnam to treat gastrointestinal infections. Other reports from our region and all over the world also indicate high resistance rates (58.4% to 86%) to ampicillin.[17,19,25–27] On the other hand, resistance rates for ampicillin differ significantly among Shigella species. *S. flexneri* was found to be highly resistant during all periods (69.0%, 72.9%, and 72.0%), but the resistance rate of *S. sonnei* decreased to 5.7% in the 1995–2002 period and after that increased to 31.4% in the 2003–2009 period. Interestingly Karacan et al.[18] reported that although they found an overall high ampicillin susceptibility rate, *S. flexneri* was highly resistant to ampicillin (60.0%). Studies from Pakistan[26] and Vietnam[10] have also reported high species-specific (*S. flexneri*) resistance rates to ampicillin, which is also the case in our country. On the other hand results from the USA[28] have shown similar high resistance rates for both *S. sonnei* and *S. flexneri*.

In the 1990s, quinolones emerged as the preferred agents for the treatment of Shigella. Most authorities recommend an oral quinolone for proven or suspected shigellosis. Nalidixic acid and ciprofloxacin are highly effective treatment alternatives, despite reservations about their use in children. However Shigella resistance to ciprofloxacin is increasingly common in India and in travelers returning from India.[29] A recent report from Bangalore, India, described an increase of resistance to ciprofloxacin from 0 to 48% over a 5-year period between 2002 and 2007.[30] On the other hand in both periods of our study there was no Shigella species resistant to ciprofloxacin. Nalidixic acid resistance at our center was found to be only 4.7%, however nalidixic acid is not available in Turkey, so is not prescribed.

Multidrug resistance increased slightly at our center between the last two periods. Multidrug resistance was more common among *S. flexneri* cases. This is also in accordance with other reports.[21,28]

In conclusion, there was a change in the clinical characteristics of childhood Shigella gastroenteritis at our center over the 22 years, mostly related to the species shift. The antibiotic resistance pattern appears to have remained stable over the last two periods. Clinical management guidelines covering the increasing cases of non-dysenteric shigellosis on admission may be helpful in decreasing the transmission of this disease in society.

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Conflict of interest: The authors declare no conflicts of interest, real or perceived, financial or non-financial.

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