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Serogroup Distribution of *Shigella* in Tehran

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Abstract

In recent years, the importance of *Shigella* as an enteric pathogen with global impact has been increasingly recognized. In this study, serogroup distribution of *Shigella* isolated from clinically diagnosed cases of gastroenteritis and acute diarrhea in Tehran, capital of Iran was investigated between December 2002 and November 2003. Fecal specimens and rectal swabs were cultured for *Shigella* spp. using standard microbiological techniques. The isolates of Shigella were identified by biochemical assay and serological testing. From a total of 302 *Shigella* isolates, 178, 110, 10 and 4 strains were identified as *S.sonnei* (58.9%; 95% CI: 53.2-64.5), *S. flexneri* (36.4%; 95% CI: 31.0-42.2), *S.boydii* (3.3%), and *S. dysenteriae* (1.3%), respectively. The peak of infection occurred during summer. Overall, 167 patients (55.3%) were males and 135 (44.7%) were females.

Keywords: Shigella *spp, Epidemiology, Serogroup distribution, Iran*

Introduction

Enteric infections including acute diarrhoeal disease comprise the second commonest medical problem after respiratory infectious diseases and, in some populations reach hyper endemic proportions (1).

Several agents have been identified as the causes of diarrhea, among which are *Salmonella*, *Shigella*, *Yersinia*, *Campylobacter*, *Vibrio* and *Aeromonas* (2).

Shigellosis is an acute bacterial infection caused by the genus *Shigella* that produces an unspecific colitis affecting preferably the recto sigmoid colon (3, 4).

Shigella is a gram negative bacillus, motionless, belonging to Enterobactericeae family. The genus *Shigella* is divided into 4 major subgroups, which are divided into serologically sub types*: S. flexneri*, *S. sonnei*, *S. boydii* and *S. dysenteriae* (4).In recent years, the importance of *Shigella* as an enteric pathogen with the global impact has been increasingly recognized. Globally, about 1,100,000 deaths are caused by the disease annually, and two-thirds of the patients are children under 5 years of age (5). Bacillary dysentery is particularly common in younger children living in endemic areas. Epidemics occur most frequently in overcrowded populations with inadequate sanitation. Outbreak of infection due to *Shigella* spp. is difficult to control because of its low infective dose (6), ease of spread by person- to person transmission by fecal-oral route (7) and its ability to spread indirectly by fecal contamination of food and water (8).

The source of infection is the excreta of infected individuals or convalescent carries. Direct spread is by the fecal–oral route; indirect spread, by contaminated food and inanimate objects (4). In spite of the prevalence of shigellosis in Iran, there have been few epidemiological studies.

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The current study was undertaken to investigate the serogroup distribution of *Shigella* isolated from clinically diagnosed cases of gastroenteritis and acute diarrhea in Tehran, capital of Iran.

Materials and Methods

From December 2002 to November 2003, fecal specimens and rectal swabs were collected from patients diagnosed as having gastroenteritis and acute diarrhea, at the Children Medial Center and Mofid Children Hospital, two largest infantile hospitals, as well as three other main hospitals (Baghyatollah, Millad and Firozabadi), Thran, Iran.

Epidemiological data Epidemiological data were obtained from standardized case report forms filled in by hospital nurses following confirmation of shigellosis. In some cases, the patient or his/her parent was interviewed. The case report form included information about date of onset, symptoms, medical treatment, age, sex, travel history, and residency.

Isolation and identification Fecal specimens and rectal swabs were collected from individuals with gastroenteritis and acute diarrhea inoculated into Carry- Blair transport medium and was processed within 2-4 h. Specimens were cultured on *Shigella–Salmonella* (SS), Hektoen-Enteric (HE), Xylose Lysine Deoxycholate (XLD), and MacConkey (MC) agars (Difco, Detroit, MI, USA).Suspected colonies were picked after incubation for 24 h at 35° C. *Shigella* spp. were preliminarily identified by gram stain, colony morphology, lactose fermentation, motility, as well as by results of general biochemical tests (9).

Serological Typing Briefly, strains of *Shigella* were sub cultured on trypticase soy agar (Difco, Detroit, MI, USA) and tested for agglutination on glass slides. Strains were serogrouped by using commercially-available antisera from MAST Group LTD (Mast House, Derby Road, Bottle, Merseyside, L201EA, UK).Subsequently, all strains were stored at-70°C in Tryptic Soy Broth (Difco, Detroit, MI, USA) containing glycerol 15% for future experiments.

Results

Between December 2002 and November 2003, a total of 302 confirmed *Shigella* strains were isolated. Of these, 178 strains were identified as *S.sonnei* (58.9%; 95% CI: 53.2-64.5), 110 *S.flexneri* (36.4%; 95% CI: 31.0-42.2), 10 *S.boydii* (3.31%; 95% CI: 1.7-6.2), and 4 *S.dysenteria* (1.3%; 95% CI: 0.4-3.6). In this study, *Shigella sonnei* was the predominant species, followed by *Shigella flexneri*.

A marked variation as to number / season was demonstrated, as follows: winter, 32 cases; spring 40; summer 175; fall 55 ($P<0.001$). The typical seasonal increase in shigellosis occurred during the summer, with peak incidence in August. The month in which each strain was isolated is presented in Fig.1.

Of all patients, 167 (55.3%) were male and 135 (44.7%) were female. The ratio of males to females was 1.21(95% CI: 0.95-1.50). The familial transmission was observed on 3 occasions. *S. sonnei* was involved in the intrafamilial dispersion. In Children Medial Center, the rate of case fatality of shigellosis was 2.1%. From a total of 140 cases admitted to this hospital, 3 patients died who had been admitted with a history of watery diarrhea, vomiting, and generalized tonic clonic seizures. No signs of severe dehydration were observed. The clinical symptoms were not improved with treatment, and all of them were expired within 24 h of hospitalization. Of these, two patients were female and the other was male.The ages of the male and two female patients were 12, 2 and 3 years, respectively. Stool cultures were positive for *S. sonnei* in two cases and positive for *S. flexneri* in one case.

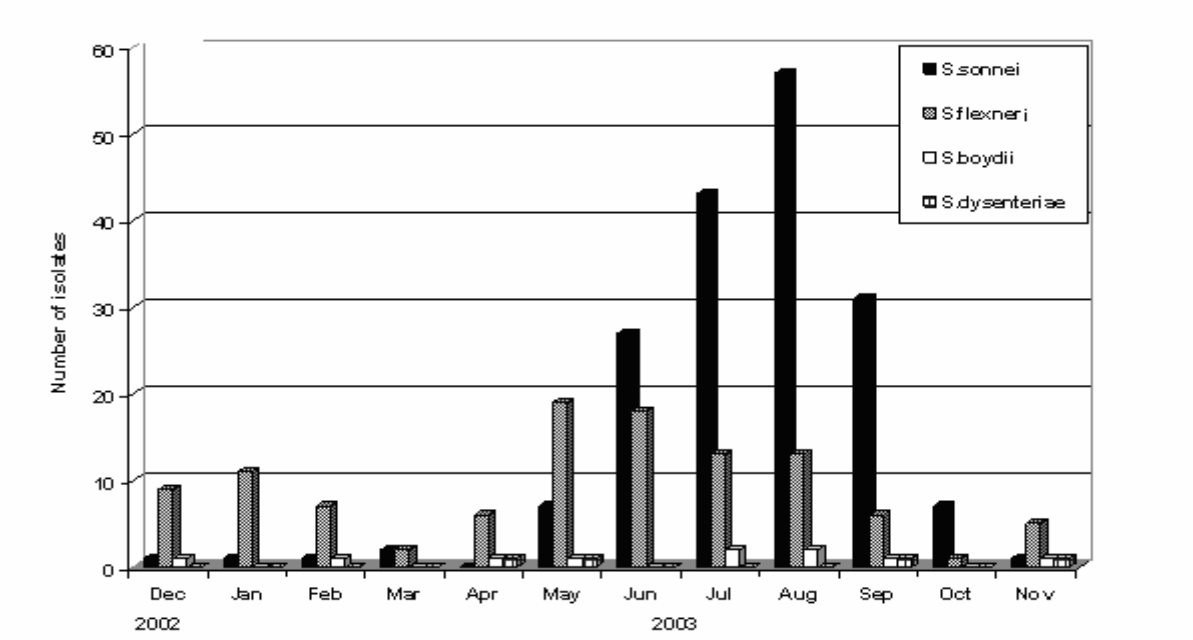

Fig.1: Numbers and serogroups of *Shigella* recovered monthly from Tehran during Dec 2002 to Nov 2003

Discussion

In this study, we investigated the serogroup distribution of *Shigella* in Tehran, the capital of Iran for 302 isolates obtained between December 2002 and November 2003. Shigellosis, also known as bacillary dysentery, is caused by four *Shigella* spp. including *S.dysenteria*, *S.flexneri*, *S.boydii*, and *S.sonnei.* Very large epidemics of *S.dysenteriae* with high morbidity and mortality were common before World War I. In 1920 *S. flexneri* became progressively the most common *Shigella.* After World War II, *S. sonnei* replaced *S.flexneri* in industrialized nations, while *S.flexneri* remained predominant in the developing world. At present time, in developing and oriental countries, *S.flexneri* is the most frequently isolated among the 4 *Shigella* spp, in Europe and the USA, however, *S.sonnei* is the most dominant (10-12).

In the previous studies in Iran (13, 14), *S.flexneri* had been reported as the most species, but in our study, *S.sonnei* was the predominant species. This was surprising since *S.sonnei* has, in spite of recent years, overtaken *S. flexneri* as the most frequently isolated *Shigella* spp in Iran. In a previous report in Tehran (13), among 230 strains isolated, *S.flexneri* was the species most commonly found (61.2%). Also in another study conducted during November 2001-October 2002 in Tehran, among 123 *Shigella* isolates, *S.flexneri* (55 strains) was dominant followed by *S.sonnei* (38 strains) (14). From 302 *Shigella* strains isolated in the present study, the proportion of *S.sonnei* isolated increased to 58.9 %, while that of *S. flexneri* fell to 36.4% ($P<0.001$).

The decrease in the proportion of *S. flexneri* strains in this study may reflect just hygiene improvements in the country in recent years.

In spite of the incidence of *S.flexneri*, *S.sonnei* infections were high during the warmer parts of year with peak incidence in August.

In healthy adults, shigellosis is usually a self-limiting illness ranging in duration from 3 - 10 days. The mortality rate in the United States, even in high-risk groups, is less than 1%. However, in developing countries, shigellosis often presents as an acute fulminate infection with

systemic complications and mortality rates as high as 10%. Deaths in Shigellosis are usually due to septicemia, toxic mega colon or acute renal failure (15). In this study, case fatality rate of shigellosis (only in Children Medial Center) was 2.1%. Whereas mortality caused by the other species than *S. dysenteriae* is rare, but in present study, *S.sonnei* and *S. flexneri* were causative agents of 3 deaths. In spite of the fact that the mortality by Shigellosis is low, the incidence and morbidity is high in pediatric ages; that is why a very deep knowledge of this disease is necessary, and also the reasons in order to treat it adequately, and mainly to prevent it.

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