

# Changing prevalence and antibiotic susceptibility patterns of different shigella species in Tehran, Iran

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## Abstract

During the 3 years of study period (April 2002 to April 2005) 220 strains of shigella isolated from fecal samples of patients having acute diarrhea. *Shigella sonnei* with 157 (78.5%) isolates had the highest frequency of isolation. Resistance to Ampicillin and Trimethoprim/sulphamethoxazole was observed in 88.5% and 98% isolates respectively. 11.5% of isolates were resistant to nalidixic acid and 5.5% to ceftriaxone. Resistance to Chloramphenicol and Ciprofloxacin was 2.5% and 1% respectively.

## INTRODUCTION

Shigelosis is an acute gastroenteritis caused by *Shigella* species, including *Shigella dysenteriae*, *Shigella flexneri*, *Shigella boydii* and *Shigella sonnei*. It is one of the most common causes of morbidity and mortality in children with diarrhea in developing countries. Worldwide, approximately 165 million cases of shigellosis occurs and 1,100,000 death are caused by the disease per year, which two-third of the patients are children under 5 years of age. Epidemic usually occur in area with crowding and poor sanitary conditions, where transmission from person to person common, or when food or water are contaminated by organism (1,2). Emergence of multiple drug resistance to cost effective antibiotics against shigella is a matter of concern for the health authorities in developing countries.(3) Outbreak of shigellosis have been reported from different parts of world. Two studies from Bangladesh showed an increasing frequency of shigella strains with multiple resistance to ampicillin, Trimethoprim –sulfamethoxazole (TMP-SMZ) and nalidixic acid. outbreaks of shigellosis caused by strains that were resistant to ampicillin and TMP-SMZ, or both drugs have been reported in other countries in Asia, Africa, central America and Europe. . However, reports regarding various serogroups of shigella. In our country there are only a few documented studies regarding the prevalence and susceptibility pattern of shigelosis. The present study was therefore undertaken-(i) to study the incidence and serogroup prevalence of shigella isolated from cases of dysentery during three year period (ii) to determine drug resistance pattern and (iii) to compare the results of present study with that of previous years regard that

serogroup and antimicrobial sensitivity of shigella spp isolated.

## MATERIAL AND METHODS

All patients admitted to the Milad hospital in Tehran during 3 years from 2003 to 2005 with history of diarrhea of less than 7 days duration were included in this study. A total 4351 patients were admitted. Individual case records of these patients were scrutinized. A special Performa was designed to collect information regarding epidemiological variables such as age, sex, date of admission; other information such as the result of faecal cultures were also included. Incomplete records were excluded. for enteric pathogens. Samples of faeces were collected in sterile wide mouth containers and rectal swabs were transported in Cary- Blair transport medium and were processed within 2 hours of collection. The samples were examined microscopically for pus cells, RBCs, leukocyte, cysts and ova of parasites. The samples were inoculated directly on the MacConky agar, Xylose-lysine desoxycholate (XLD), Hekton Enteric agar (HE) and, Salmonella Shigella agar (SS). Enrichment was done on selenite F broth and incubated for 6 hours. After the 6 hours subculture was done on SS agar. All plates incubated at 35°C for overnight. All specimens were also processed for other enteropathogens by using standard laboratory procedures. For isolation of *Yersinia enterocolitica* samples, were inoculated on Cefsulodin –irgasin Novobiocin agar (CIN agar) and incubated at 20-25°C for 24 hours. For isolation of *Campylobacter jejuni* we used Campy- Blood Agar plate (Campy-BAP) medium and after inoculation of samples plates incubated at microaerophilic condition in

42°C for 48 hours. Finally, sorbitol MacConkey agar was used for isolation of Enterohemorrhagic E. coli.

All isolated enteric pathogens were identified by conventional bacteriological methods (4,5). Colonies resembling to shigella species were identified by biochemical reaction and confirmed by slide agglutination test using polyvalent and monovalent antisera (Bahar Afshan, Tehran Iran). Susceptibility testing for shigella spp isolates were performed by disk diffusion methods as recommended by NCCLS. (6) Commercially manufactured disks of antimicrobial agents and their concentration were as follows. Ampicillin 10 µg, ceftriaxone 30 µg, chloramphenicol 30 µg, nalidixic acid 30 µg, ciprofloxacin 5 µg, trimethoprim-sulfamethoxazole 1.25/237.5 µg

## RESULTS

4351 stool samples were sent to microbiology laboratory of Milad hospital for culture and susceptibility testing. In total 464 (10.15%) Enteropathogens were isolated. The rate of isolation was: Shigella spp 220 (46.5%) isolates salmonella spp 120 (26.3%), Enteropathogenic E. coli 100 (26.3%), enterohemorrhagic E. coli 16 (3.5%), Campylobacter jejuni 7 (1.5%). In our study shigella spp had the highest isolation rate. Of 212 shigella strains isolated 157 (78.5%) were S. sonnei followed by S. flexneri 44 (20%), S. boydii 16 (7.2%) and S. dysenteriae only with 2 isolates (1%). The majority of patients with shigellosis were children under 12 years old. The mean age of patients were 9.8 years (SD ±16.3). Microscopically examination of stool specimens showed Leukocyte and red blood cells in more than 90% of dysenteric specimens.

Result of susceptibility testing of Shigella spp to various antibiotics were as follows: Resistance to Ampicillin and Trimethoprim / sulphamethoxazole was observed in 88.5% and 98% isolates respectively. 11.5% of isolates were resistant to nalidixic acid and 5.5% to ceftriaxone. Resistance to Chloramphenicol and Ciprofloxacin were 2.5% and 1% respectively.

## Figure 1

Table 1: Frequency of different enteropathogens isolated from stool specimens during 2002-2005 in Milad hospital of Tehran.

Pathogen	frequency (%)
Shigella spp	220 (46.5)
Salmonella spp	120 (26.3)
Enteropathogenic E. coli	100 (21.9)
Enterohemorrhagic E. coli	16 (3.5)
Campylobacter jejuni	7 (1.5)
Yersinia enterocolitica	1 (0.2)
Total	464

## Figure 2

Table 2: Serogroups of Shigella isolates from 2002-2005 in Milad hospital of Tehran

Shigella spp	2003	2004	2005
Shigella sonnei	41	66	50
Shigella flexneri	22	18	4
Shigella boydii	4	5	7
Shigella dysenteriae	3	0	0

## DISCUSSION

The isolation rate of Shigella species from stool specimens in our study was 5%. Among enteropathogenic isolated bacteria, Shigella species accounted 46.5% of isolation, and Shigella sonnei had the highest isolation rate.

The result of present study were compared with that previous studies in Tehran. In a study that carried out by Moez-Ardalan Shigella flexneri was the predominant serogroup. (7) Another study by Nikkah showed that of 230 shigella isolates 61.2% were S. flexneri. (8) However in recent years there are changes in frequency of Shigella serogroups isolates in our country and S. sonnei has become predominant species. The present study reveals that S. sonnei with 157 (71.3%) isolates had the predominant serogroup. Ranjbar also showed that of 302 shigella isolates in Tehran during 2002-2003, 178 (59%) isolates were S. sonnei. (9) The data are also in keeping with those from other countries. Reports from Saudi Arabia by Panhotra BR showed that 80% of shigella isolates were S. sonnei. (3) Studies in our neighbor country Turkey are also revealed that 75-78% of shigella isolates are S. sonnei. Serogroup. (10,11).

S. sonnei serotype is believed predominant and endemic in industrialized countries and known to cause milder self-limiting disease and to be less resistant to antimicrobial agents. However recently S. sonnei has become the prevalent serotype in developing countries and has developed multidrug resistance and is responsible for outbreaks of clinically severe disease

Antimicrobial therapy is recommended for shigellosis because it can shorten the severity and duration of illness, reduce shedding of the organism, and prevent secondary complication and death. However antimicrobial resistance occurred among shigella spp, since the 1940s, when sulfonamide resistance among shigella organism was first recognized in Japan<sup>(12)</sup>. In our study more than 88.5% of isolates were multiple drug resistant. The previous studies also showed that 87.8% of shigella multiple resistant. Resistance to nalidixic acid as a first line drug is going to increase in our country, In a study by Nikkah et al during 1984-1985 in Tehran, all isolates of shigella were susceptible to nalidixic acid<sup>(8)</sup> and in a report by Moez Ardalan in 2002-2003 only 1% of shigella spp were resistant to nalidixic acid.<sup>(7)</sup> In our study this figure was 11.5%. In other countries like India resistance of *S. sonnei* to nalidixic acid between 2001-2002 was 94-100%<sup>(13,14)</sup> In a study which carried out by Aysev in 1993-1996 in Turkey the resistance of *Shigella* spp to TMP-SMZ, Ampicillin, and chloramphenicol was 55.7%, 27.7% and 19.7% respectively. There was not any resistance to nalidixic acid and ciprofloxacin in their study<sup>(10)</sup> In another of our neighbor countries Pakistan in a study in Karachi by Zafar et al all isolates of shigella were susceptible to ceftriaxone, but a high rate of resistance was observed to Cotrimoxazole (87.7%) and ampicillin (55.5%) in their study and emerging resistance against nalidixic acid (39%) were observed. Jeong et al in Korea between 1980-2000 showed that 30 and 86% shigella isolates were resistant to Ampicillin and nalidixic acid.<sup>(15)</sup> In a study by Mache et al in Ethiopia highest resistance shigella was encountered to tetracycline (63.6%) ampicillin (70.1%) respectively. Resistance to Trimethoprim-sulfamethoxazole and nalidixic acid was 32.5% and 6.5%.

The relative antimicrobial susceptibility of different *Shigella* spp may vary geographically. It may be due to pattern of antibiotic using for treatment of shigellosis. Further studies on the antibiotic resistance mechanism and genetic relatedness of isolates are required to understand the progression of antibiotic resistance in shigella.

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