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ORIGINAL ARTICLE

Fatality due to shigellosis with special reference to molecular analysis of *Shigella sonnei* strains isolated from the fatal cases

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ABSTRACT

Background: Shigellosis as a global human health problem is more severe than other forms of gastroenteritis and causes over a million deaths in developing countries worldwide annually. Fatality due to shigellosis is usually due to dehydration and two-third of fatalities are seen among children. The aim of current study was to describe fatal cases of shigellosis due to infection with *Shigella sonnei* and *S. flexneri.*

Patients and methods: We investigated the fatal cases of shigellosis among all children with acute diarrhea admitted to Children's Medical Center, Tehran, Iran. Bacterial isolation and identification was achieved according to standard bacteriological methods. Antibiotic susceptibility tests, plasmid profiling and ribotyping were performed to investigate the clonal relationship among the isolates.

Results: Among 1200 children with acute diarrhea, 140(12.7%) cases had shigellosis. Of these, three patients died. No signs of severe dehydration were observed among the fatal cases. The symptoms were not improved following antibiotic therapy and all three cases died after 24 h of hospitalization despite receiving intensive treatments. Stool cultures yielded *S. flexneri* and *S. sonnei* for one and two cases, respectively. The isolates were resistant to streptomycin, ampicillin, and sulfamethoxazole-trimethoprim. *S. sonnei* strains were further studied and showed a single pattern of antibiotic susceptibility and ribotyping.

Conclusion: Mortality due to species other than *S. dysenteriae* is rare, however, in current study we found *S. sonnei* and *S. flexneri* as the cause of fatality among pediatric patients during the study.

Keywords: *Shigellosis, Mortality rate, Shigella sonnei, Shigella flexneri*.
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INTRODUCTION

Shigellosis as a global human health problem is more severe than other forms of gastroenteritis. Endemic *Shigella* is responsible for approximately 10% of all diarrheal episodes among children younger than five years living in developing countries and up to 75% of diarrheal death (1). Death due to shigellosis is rare in developed countries; however, it causes over a million deaths

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in developing countries worldwide annually (2). Children and the elderly are at greatest fatal risk from shigellosis.

Shigellosis is one of the major causes of morbidity in children with diarrhea in Iran (3-7), but the reports about the mortality caused by *Shigella* are scanty. Recently we have investigated the distribution of *Shigella* species in Tehran, Iran, and found *S. sonnei* as the most prevalent *Shigella* species (5,6). In current study, we described three fatal cases of shigellosis among pediatric patients with gastrointestinal disease admitted to a major children hospital in Tehran.

PATIENTS and METHODS

We investigated the fatal cases of shigellosis among all 1200 patients with acute diarrhea admitted to Children's Medical Center in Tehran. One hundred-forty (12.7%) cases have been diagnosed as having shigellosis. Of all shigellosis-diagnosed patients, dead cases were further studied. Before death, these patients were transferred to intensive care unit (ICU) and intubated immediately. Since they were not able to take oral antibiotics, treatment with intravenous therapy with ceftriaxone was immediately commenced.

Bacterial isolation and identification: The rectal swab specimens were collected from patients on the day of hospital admission before antibiotic treatment. Single specimen was obtained from each patient. The isolates were identified as *Shigella* by the conventional methods (8) and were serotyped using slide agglutination with specific antisera (MAST Group LTD, Merseyside, UK).

Antibiotic susceptibility testing and molecular analysis: Antibiotic susceptibility tests were achieved according to the standard guideline of the Clinical and Laboratory Standards Institute (9) using the following 11 antibiotics: ampicillin (AM, 10µg), cefotaxime (CTX, 30µg), ceftazidime (CAZ, 30µg), ceftizoxime (CT, 30µg), ceftriaxone (CRO, 30µg), cephalothin (CF, 30µg), ciprofloxacin (CP, 5µg), gentamicin (GM, 10µg), kanamycin (K, 30µg), streptomycin (S, 10µg), and sulfamethoxazole-trimethoprim (SXT 23.75/1.25µg). *Escherichia coli* ATCC 25922 was used as a quality control strain. Plasmid profiling and ribotyping were performed according to the previous report (4) to investigate the clonal relationship among the *S. sonnei* isolates.

RESULTS

Of 140 patients diagnosed as having shigellosis, 3 died. Before death, these patients had been admitted with a history of watery diarrhea, vomiting, and generalized tonic colonic seizures. No signs of severe dehydration were observed among the cases. The symptoms in patients were not improved after antibiotic therapy and all three cases died after 24 h of hospitalization despite receiving intensive treatments.

Patient 1 was an eight-year-old boy from Karaj city whose stool culture yielded a growth of *S. sonnei* that was resistant to streptomycin, ampicillin, and sulfamethoxazole-trimethoprim but susceptible to kanamycin, gentamicin, ceftriaxone,

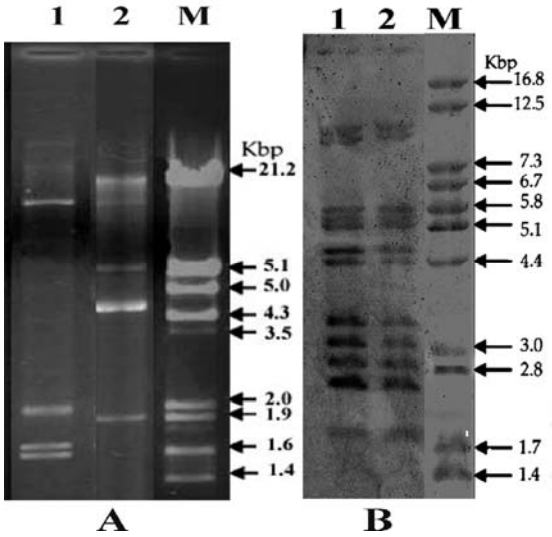

Figure 1. The patterns obtained from plasmid profiling (A) and *Pvu*II restriction analysis of DNA (ribotyping) (B) of *S. sonnei* strains. Lanes 1 and 2 are the patterns of strains isolated from dead patients. M: molecular size marker.

ceftizoxime, ceftazidime, cephalothin, cefotaxime and ciprofloxacin.

Patient 2 was a two and-a-half-year-old girl for whom *S. flexneri* was isolated from her stool culture. The isolate had additional resistance to amoxicillin compared to resistance pattern of the isolate which was recovered from case 1.

Patient 3 was a two-year-old girl from Tehran whose culture yielded a growth of *S. sonnei* that showed similar antibiotic resistance pattern with the isolate recovered from case 1. As shown in figure 1 two *S. sonnei* isolates had similar ribotype pattern, however they showed different plasmid profiles.

DISCUSSION

Bacillary dysentery is regarded as one of the leading causes of diarrhea-associated morbidity and mortality among children aged <5 years (10). Among children, the risk of death from shigellosis is greatest in infants and those who are severely malnourished (11,12). Most episodes of shigellosis in otherwise healthy individuals are self-limited and resolve within 5 to 7 days without sequel. Acute, life-threatening complications are most often seen in malnourished infants and young children living in developing countries. These include metabolic derangements, such as dehydration, hyponatremia, and hypoglycemia, intestinal complications such as toxic megacolon, rectal prolapse, intestinal perforation, and rarely sepsis (2).

Almost all fatal cases of shigellosis occur in developing countries (13). Many studies have shown that mortality due to shigellosis may be as high as 10-15% with some strains, however, some other regional reports have shown that more than 50% of all diarrhea deaths can be attributed to bacillary dysentery in some settings (10).

For example, this disease is known to be one of the major causes of persistent diarrhea and malnutrition in the Eastern Mediterranean Region. The latest regional estimates are a million total *shigella* cases annually with approximately 40000 deaths (an average 4% case fatality rate) (10).

In addition to endemic shigellosis, large epidemics of dysentery due to *S. dysenteriae* type 1 have been reported from some countries in Eastern Mediterranean Region (such as the Republic of Yemen) which is characterized by a particularly high case fatality rate (10).

In current study, of 1200 pediatric patients with acute diarrhea, 12.7% were diagnosed as having shigellosis and three patients died as a result of infection with *S. sonnei* (two cases) and *S. flexneri* (one case). Previous regional studies have also shown that high case fatality rates (≥5%) may be related with any of the four *Shigella* species including *S. dysenteriae* type 1, *S. flexneri*, *S. boydii* and *S. sonnei*, however, very high case fatality rates have been observed only in epidemics caused by *S. dysenteriae* type 1 (10).

Attack rates during epidemics of *S. dysenteriae* type 1ranged from 1% to 33%, and case-fatality rates ranged from 1% to 7% (13).

The majority of deaths from shigellosis are known, however, to result from endemic disease, especially that caused by *S. flexneri* (13). In Bangladesh, one another Asian country, shigellae dysentery has been reported to be as a cause of 75000 deaths annually among children younger than five years during peak epidemic years and an estimated 35000 deaths in non-epidemic years (13). For example, in Matlab, a rural district in this country, most diarrhea-related deaths and approximately 25% of all deaths among children aged 1-4 years were attributable to dysentery. As reported by Dhaka Treatment Centre of the International Centre for Diarrheal Disease Research, the fatality rate for 970 in patients with shigellosis was 11%, with most deaths occurring among malnourished children who were infected with *S. flexneri* (13).

Several typing methods such as serotyping, drug resistance pattern, plasmid analysis and ribotyping

have been frequently used for subtyping of *Shigella* in epidemiological investigations (3-5,14). Therefore, we used these techniques to study the genetic relatedness between our *Shigella* strains. Antibiotic susceptibility testing and ribotyping showed a single pattern for *S. sonnei* strains. The results indicated that a single cluster of *S. sonnei* might be responsible for two deaths during the study period. We also concluded that *Shigella* is still one of the most important causes of diarrhea-associated mortality in pediatrics patients in Iran.

The prevention of *Shigella* infections in children is an essential approach to control the mortality of shigellosis in our country.

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