Original Article

An Outbreak of Shigellosis due to *Shigella flexneri* Serotype 3a in a Prison in Iran

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Abstract

Background: On June 16 and 17, 2007, the medical clinic of a prison in Isfahan, Iran received multiple reports of gastrointestinal illness among prisoners. A cross-sectional study was therefore undertaken to determine the extent, causative agent and possible source of the outbreak.

Methods: A case-patient was defined and patient information was collected with a standardized questionnaire. Stool samples were collected from the patients and restaurant employees, and analyzed for the presence of enteric bacteria by routine bacteriological methods. *Shigella* isolates were identified and serotyped by commercially available antisera. The relationship between the strains was determined using antimicrobial drug resistance pattern analysis and enterobacterial repetitive intergenic consensus polymerase chain reaction (ERIC-PCR).

Results: Seven hundred one inmates experienced gastrointestinal illness and severe diarrhea. The attack rate was 14.02%. Rectal swabs and stool cultures recovered from patients tested positive for *Shigella flexneri* serotype 3a. All tested isolates had a similar antibiotic resistance and ERIC-PCR pattern. Our findings demonstrated that raw vegetables were more likely to be the causative agent of this outbreak.

Conclusion: The results indicated that a single clone of *S. flexneri* was responsible for this outbreak. Although we could not trace the exact origin of the organism, the consumption of raw vegetables one day prior to the onset of illness was strongly associated with an increased risk of *S. flexneri* infection. This study emphasizes the need for accurate monitoring and surveillance of food and vegetables consumed in prisons.

Keywords: outbreak, prisoners, *Shigella flexneri*, Shigellosis

Introduction

Infections caused by *Shigella* species remain a major cause of diarrheal disease in developing countries such as Iran.[1–3] The *Shigella* species is readily spread by person-to-person transmission via the oral-fecal route; however, contaminated water, food and vegetables have also been implicated as vehicles of transmission.[4] Many foodborne and waterborne outbreaks of shigellosis have been reported. Some studies have shown that the ingestion of raw or fresh vegetables is an effective and potential source of infection caused by *Shigella* organisms.[5,6]

On June16 and 17, 2007, the medical clinic of a prison in Isfahan, Iran received multiple reports of gastrointestinal illness among prisoners. We conducted a cross-sectional study to determine the extent, causative agent, and possible source of the outbreak.

Materials and Methods

A case-patient was defined as an individual with acute gastroenteritis and diarrhea with at least three loose stools per 24 hr and/or vomiting and/or abdominal pain. A standardized questionnaire was used to interview case-patients about age, gender, and type of food consumed during the previous two days, timing, symptoms, possible sources, and presence of illness in close contacts. Restaurant employ-

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ees of the prison were also interviewed and required to submit two consecutive stool specimens, collected no less than 24 hr apart.

Stool samples or rectal swabs were obtained from case-patients. Tap and well water samples were also collected for bacteriological analysis; however, food and vegetables consumed 24 – 48 hr before the outbreak day were no longer available in the restaurant for inspection and assessment with microbiological assays.

Stool samples were inoculated into Carry-Blair transport medium and processed within 2 – 4 hr. The samples were analyzed for the presence of *Salmonella*, *Shigella*, *Yersinia*, *Campylobacter*, *Aeromonas,* and *Plesiomonas* species by routine bacteriological methods.[7] For isolation of *Shigella* spp. the specimens were cultured on Shigella-Salmonella (SS), Hektoen enteric, Xylose lysine deoxycholate, and MacConkey agars (Difco, Detroit, MI, USA). Suspected colonies were selected after incubation for 24 hr at 35°C. *Shigella* spp. were preliminarily identified by colony morphology, gram stain, and motility, as well as by results of general biochemical tests. [7]

For serological typing, *Shigella* strains were subcultured on trypticase soy agar and tested for agglutination on glass slides with the use of a commercially-available antisera via standard methods.[7] The relationship between the strains was determined using antimicrobial drug resistance pattern analysis and enterobacterial repetitive intergenic consensus polymerase chain reaction (ERIC-PCR).

Clonal investigation was conducted with outbreak-associated isolates, one sporadic isolate and one standard strain (ATCC 12022/NCTC 12698). Antimicrobial susceptibility testing of *Shigella* spp. to 15 different antibiotics (Oxoid Limited, Hampshire, England) was determined according to standard methods as outlined by the National Committee for Clinical Laboratory Standards.[8]

The following primers were used for ERIC-PCR: ERIC 1R (5'-ATG TAA GCT CCT GGG GAT TCA C-3') and ERIC 2 (5'-AAG TAA GTG ACT GGG GTG AGC G-3'). ERIC-PCR was performed as described previously[9] with some modifications in temperature profile: 1 initial cycle at 94°C for 4 min, 35 cycles of denaturation at 94°C for 1 min, annealing at 52°C for 1 min, and extension at 65°C for 8 min, with a single final extension step at 65°C for 15 min.

Results

The outbreak occurred from June 16 to 17, 2007. Out of 5000 inmates, 701 experienced gastrointestinal illness and severe diarrhea. The attack rate was 14.02%.

A total of 327 out of 701 suspected cases completed the questionnaire and met the study definitions for case-patients. More than 98% of the case-patients were male. The ages ranged from 18 to 50 years, however the majority of cases were aged 40 – 50 (39%). Symptoms reported included: diarrhea (100%), headache (100%), fever (100%), nausea (99%), abdominal cramping (97%), vomiting (95%), and bloody stools (51%). Our records showed that all prisoners consumed canned fish, bean conserve, sausage, and raw vegetables 48 hr prior to the onset of the outbreak.

Restaurant employees were asymptomatic and their stool cultures were negative for *Shigella*. No bacterial growth was also detected in any of the water samples. Rectal swabs and stool cultures obtained from some randomly selected case-patients were positive for *S.flexneri* serotype 3a. All tested isolates were uniformly susceptible to amikacin, ciprofloxacin, gentamycin, ceftizoxime, ceftriaxone, ceftazidime, cephalothin, tobramycin, chloramphenicol, nalidixic acid, and kanamycin; but were resistant to ampicillin, trimethoprim-sulfamethoxazole, streptomycin, and tetracyclin. ERIC-PCR produced only a single pattern with 13 DNA band fragments in all selected *S.flexneri* strains associated with the outbreak (Figure 1).

Discussion

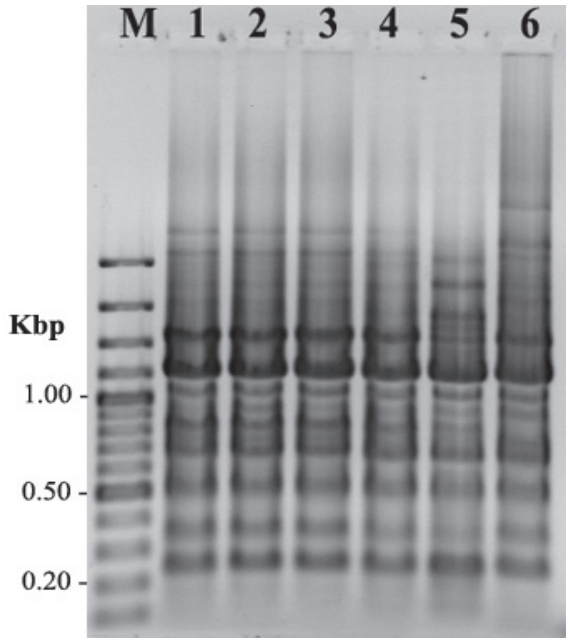
Gastroenteritis and inflammatory diarrhea, as important public health problems, are common.[10] Shigellosis, one of the most common bacterial diarrheal diseases, is the most important cause of morbidity in developing countries such as Iran.[1–3, 11–13]

In several studies the source of many other shigellosis outbreaks have been traced to the ingestion of raw or fresh vegetables. The contamination of vegetables by *Shigella* spp. is due to washing the vegetables with contaminated water, or handling of vegetables by infected workers or vendors.[14] The low infective dose of *Shigella* spp. and the lack of lethal treatment during production, processing

or preparation make vegetables a likely source for shigellosis. An outbreak of *S. flexneri* reported from Michigan was linked to a tossed salad in 1992.[5] In another study, iceberg lettuce was the incriminated food in a 1994 outbreak of *S. sonnei* in a few European countries.[6]

In the United States, outbreak investigations linked *Shigella* spp. to lettuce and fresh green onions in 1995.[15,16] In 2005, Reller and colleagues reported a large, multiple-restaurant outbreak of infection with *S. flexneri* serotype 2a which was traced to tomatoes.[17]

We found that the symptoms of diarrhea, headache, fever, nausea, abdominal cramping, vomiting, and bloody stools were observed in the majority of patients. These findings suggested a possible occurrence of shigellosis. Clonal assays indicated that a single clone of *S.flexneri* serotype 3a was responsible for this outbreak since all tested isolates were uniformly susceptible to 11 antibiotics and resistant to four other antibiotics. ERIC-PCR confirmed this assumption with the production of only a single pattern with 13 DNA band fragments in all *S.flexneri* strains associated with the outbreak (Figure 1).



Legends to figure

Figure 1. Genomic typing of *S. flexneri* by ERIC-PCR fingerprinting. Lanes 1 – 4 are identical patterns of four representative outbreak strains. Lanes 5 and 6 are patterns of a non-outbreak and standard strain (ATCC 12022 (NCTC 12698) of *S. flexneri,* respectively. Lane M is a molecular size marker (100 bp)

The possibility of cross-transmission between the cases was considered to be low since all case-patients presented with the symptoms of the disease within the same time period. In addition, all restaurant employees were asymptomatic and their stool cultures yielded no growth of *Shigella* spp. No bacterial growth was also detected in any water samples. These findings indicate occurrence of a point source infection that was more likely due to contaminated food consumed 24 – 48 hr prior to the outbreak. Our records showed that the food eaten 48 hr before the onset of the outbreak included canned fish, bean conserve, sausage, and raw vegetables. Unfortunately these consumed food and vegetables were no longer available in the restaurant to be inspected and assessed by microbiological assays.

We could not trace the exact origin of the organisms; however, raw vegetables were considered more likely than other types of consumed foods to become contaminated with the causative agent of this outbreak.

These shigellosis outbreaks illustrate the potential for disease transmission when contaminated or untreated salads or raw vegetables are consumed and emphasize the need for accurate monitoring and surveillance for food or water borne illnesses in crowded living conditions such as prisons. The implementation of sanitary protocols, especially for food and vegetable preparation is highly recommended. In addition, increased education of restaurant staff about the potential for spreading vegetable-associated infections is crucial to decreasing the occurrence of shigellosis in a prison population.

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