Foodborne-associated Shigella sonnei, India, 2009 and 2010

To the Editor: Infection with Shigella spp. is a major cause of foodborne diseases, which have increased considerably during the past decades, but only a small fraction of cases are reported (1). S. dysenteriae and S. flexneri are the predominant species in the tropics; clinically, S. dysenteriae serotype 1 is associated with severe disease, large outbreaks, or epidemics. S. sonnei occurs more frequently in industrialized than in developing countries and causes milder illness than S. dysenteriae and S. flexneri. However, occasional foodborne outbreaks by antimicrobial drug–resistant S. sonnei have been reported from the United States, Japan, and European countries, mostly among children (2–5). During recent years, in Thailand, Vietnam, and Sri Lanka, the predominant species has shifted from S. flexneri to S. sonnei, a phenomenon possibly linked with country’s level of development (6,7). As a result, S. sonnei outbreaks are also being reported from developing countries (8). In India, the scenario differed somewhat. Devastating outbreaks of dysentery by multidrug-resistant S. dysenteriae type 1, with high case-fatality rates, affected major parts of the country during 1984–1985 (9). After a gap of 18 years, during 2002–2003, S. dysenteriae type 1 with an altered antimicrobial drug resistance pattern (100% fluoroquinolone resistance) reemerged, causing several dysentery outbreaks in West Bengal (10). Although S. flexneri was the major species, since 2005, S. dysenteriae type 1 has not been isolated (9).

We report 2 foodborne outbreaks of S. sonnei in India, 1 each from Kerala (southern part) in February 2009 and Maharashtra (western part) in February 2010, which support extension of S. sonnei into India. The outbreak isolates were characterized by antimicrobial drug resistance and plasmid and pulsed-field gel electrophoresis profiles.

On February 1, 2009, >300 persons (age range 2–70 years) attended a marriage party at Thiruvananthapuram, Kerala, where they were served local food made of rice, lentils, milk, and water. Within 12 hours after eating, ≈60% of persons had onset of acute diarrhea, vomiting, and abdominal pain and were admitted to local village or district hospitals or the nearest government general hospital for treatment. Illness was more severe in children; because of clinical severity, 10 children (<10 years of age) were admitted to a referral hospital for children in Thiruvananthapuram. One child (7 years of age) was moved to the pediatric intensive care unit because of altered sensorium and drowsiness and was treated with intravenous ceftriaxone and metronidazole. Others were treated with oral cefotaxime until recovery and were discharged. Fecal samples from 15 patients were processed at the local public health laboratory for enteric pathogens; 9 (60%) of 15 samples yielded S. sonnei as the sole pathogen. On microscopic examination, 12 (80%) samples had erythrocytes.

The second outbreak occurred on February 11, 2010, at Kolhapur, Maharashtra, among day laborers and their family members who had eaten in 1 madrasa (religious place). Approximately 150 persons reported diarrhea, vomiting, abdominal pain, and fever. They were admitted to the Government Medical College, Kolhapur, and treated with intravenous fluid (lactated Ringer’s solution), oral rehydration solution, intravenous ceftriaxone, and metronidazole. All patients were discharged after complete recovery. S. sonnei was
isolated as the sole pathogen from 14 (70%) of 20 fecal samples or rectal swab samples from those patients. Six isolates from outbreak 1 and 11 isolates from outbreak 2 were sent to the National Institute of Cholera and Enteric Diseases (Kolkata, India) for confirmation. We characterized those isolates to determine whether the outbreak isolates of S. sonnei predominant in India were clonal in origin.

Antimicrobial drug resistance profiles differed in the 2 outbreaks (Figure) when drug susceptibility was tested by disk diffusion. MICs of antimicrobial drugs by Etest (AB Biodisk, Solna, Sweden) were >32 μg/mL for tetracycline and co-trimoxazole, >256 μg/mL for nalidixic acid, and ≈4 μg/mL for norfloxacain and ciprofloxacin. Plasmid profiles of the isolates showed absence of large plasmids (212 kb) and several smaller plasmids arranged in distinct patterns in each group (data not shown). Because the isolates caused invasive diarrhea (erythrocytes in feces), large plasmids might have been lost through repeated subculture. DNA fingerprinting was performed by pulsed-field gel electrophoresis in a CHEF-DRIII system (Bio-Rad Laboratories, Hercules, CA, USA) after DNA digestion by XbaI following standard PulseNet protocol and by using Salmonella enterica serovar Braenderup H9812 as control strain. A few sporadic S. sonnei isolates from patients of the Infectious Disease Hospital, Kolkata, were included for comparison. The patterns were analyzed by using Dice coefficient, and a dendrogram was generated by using FP Quest Software (Bio-Rad). The isolates with ≈90% similarity threshold were grouped under 1 cluster. Distinct clusters were observed for outbreak 1 (cluster A), outbreak 2 (cluster B), and sporadic 2009 (cluster C) isolates, and patterns in each cluster differed by only a few (1,2) smaller bands.

Therefore, our study supports emergence of S. sonnei outbreak clones in India during 2009–2010. These outbreaks may be the forerunners of many more S. sonnei outbreaks.

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Figure. Digested pulsed-field gel electrophoresis (PFGE) profiles of Shigella sonnei outbreak isolates, India (Thiruvananthapuram, Kerala; Kolhapur, Maharashtra), by cluster analysis and comparison with sporadic isolates (IDH). Thir, isolates from Thiruvananthapuram, Kerala; Kolh, isolates from Ispurli, Shiroli Taluk, Kolhapur district, Maharashtra; IDH, isolates from Kolkata, West Bengal; R, resistance; T, tetracycline (30 μg); Q, co-trimoxazole (25 μg); Na, nalidixic acid (30 μg); Nf, norfloxacain (10 μg); Ci, ciprofloxacin (5 μg); Of, ofloxacain (5 μg); S, streptomycin (10 μg).

References

Mosquito-associated Dengue Virus, Key West, Florida, USA, 2010

To the Editor: Except for sporadic cases along the Texas–Mexico border, local transmission of dengue virus (DENV) has not occurred in the contiguous United States since 1946. In 2009, DENV was diagnosed in a vacationer to Key West, Florida (1). Subsequently, 25 other cases were reported that year, transmission was confirmed by detection of DENV serotype 1 (DENV-1) in local mosquitoes, and a random serosurvey showed evidence of recent DENV infection in 5.4% of Key West residents (1). Transmission continued in 2010, and an additional 63 cases were confirmed (2). We used PCR amplification and sequence analysis of virus identified from mosquito collections during 2010 to identify the closest relatives, probable geographic origin, and divergence time of the Key West DENV.

A total of 1,178 pools of *Aedes aegypti* mosquitoes were collected in Monroe County, Florida, during January 27–December 17, 2010 (online Appendix Figure, panel A, wwwnc.cdc.gov/EID/article/17/11/11-0419-FA1.htm), by using BG-Sentinel (Biogens, Regensburg, Germany) or CDC (Clarke, Roselle, IL, USA) light traps, and stored at −80°C. Reverse transcription PCR was conducted on each pool by using primers designed to amplify all 4 DENV serotypes, followed by seminested PCR with serotype-specific primers (3). Results from 2 Key West mosquito pools collected on June 25 and 30 showed a positive first-round reverse transcription PCR and a positive second-round PCR specific for DENV-1. A third Key West pool collected on August 27 showed only a positive second-round PCR specific for DENV-1. No other DENV serotypes were detected.

DENV-1–specific primers (5′-GG GCCTTGAGACCCAGG-3′ and 5′-CCTCCC ATG CTTCCCAAT GCC-3′) were used to amplify products encompassing the envelope (E) gene region and parts of the premembrane and nonstructural 1 genes from the pools collected on June 25 and 30. PCR products were sequenced by using amplification and internal primers to provide double or triple coverage (Functional Biosciences, Madison, WI, USA). The sequences from these 2 pools were identical (GenBank accession no. JF519855). We used ClustalX (4) to align the Key West sequences with 175 nonredundant American DENV-1 sequences from the National Center for Biotechnology Information Virus Variation database (5) and 9 additional DENV-1 subgenomic E sequences from GenBank, which provided a comprehensive set of American DENV-1 sequences, including several isolates from Hawaii (USA) and Easter Island (Chile) that grouped with Asian DENV-1 clades as an outgroup. Maximum-likelihood phylogenetic analysis of the 1,484-nt E gene region was conducted by using SeaView software (6).

The analysis showed that American DENV-1 strains clustered by geography and by year of collection (online Appendix Figure, panel B). This clustering might be the result of lineage replacement that has been described in DENV-1 (7). Additionally, clustering might be influenced by serotype prevalence or sampling bias. For example, the sequence database contains few Caribbean isolates from after 2000 and few Central or South American isolates from before 2000. The Key West sequence grouped as a member of a large clade of recent viruses from Central America that was separated from Caribbean and South American viruses with a well-supported bootstrap value (86%) and relatively long branch length. The closest relatives were 2 strains isolated...