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Outbreak of *Shigella sonnei* in a rural hotel in La Gomera, Canary Islands, Spain

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Summary. *Shigella sonnei* is a significant cause of gastroenteritis in both developing and industrialized countries. Knowledge of the diversity and antimicrobial susceptibility of the bacterium may be helpful in the management of both individual cases and outbreaks. This study was undertaken to evaluate the molecular epidemiology of an outbreak of diarrhea due to *S. sonnei*. The outbreak involved 14 of 28 (50%) tourists in a small rural hotel in La Gomera, Canary Islands, Spain. All of the *S. sonnei* isolates recovered had the same antimicrobial susceptibility and pulsed-field gel electrophoresis patterns, suggesting that the outbreak was produced by a single strain. [**Int Microbiol** 2005; 8(2):133-136]

Key words: *Shigella sonnei* · pulsed-field gel electrophoresis (PFGE) patterns · outbreaks

Introduction

The ingestion of food contaminated with infectious or toxigenic microorganisms is a major cause of morbidity and a very significant cause of death throughout the world [2,21]. Many of the cases of acute diarrheal disease in both developing and industrialized countries are due to infection by *Shigella* species [4,19,20]. The estimated incidence of shigellosis is 164.7 million cases annually, of which 163.2 million occur in developing countries, resulting in 1.1 million deaths. Moreover, 69% of all episodes of *Shigella* infection and 61% of all *Shigella*-related deaths involve children younger than 5 years old [1]. *Shigella dysenteriae* and *Shigella sonnei* are the predominant species in the tropics, while *S. sonnei* is the predominant species in industrialized countries [14]. Although usually confined to the colonic mucosa, shigellosis may also result in extraintestinal complications. Recent publications have shed light on the clinical implications of *Shigella*-induced bacteremia, surgical complications, urogenital symptoms, and neurologic manifestations, and on the unique manifestations of shigellosis in the neonatal period [1]. The spread of *S. sonnei* is particularly problematic in institutional and other crowded settings, such as day-care centers, prisons, and military facilities [6,7]. The *Shigella* is frequently spread directly by person-to-person transmission via the fecal-oral route, but also indirectly by fecal contamination of food or water [20].

Most *S. sonnei* infections are usually mild, self-limiting. However, antibiotic treatment may be useful in some cases to manage infection and reduce fecal excretion of the bacterium in order to prevent further transmission. Antimicrobial resistance patterns are valuable as a guide to empirical therapy, as a typing method, and as an indicator of dissemination of antimicrobial resistance determinants. *S. sonnei*, unlike most enteric pathogens, has no natural reservoir other than humans; therefore, antimicrobial resistance in *S. sonnei* most likely reflects gene transfer and selective pressures in the human gastrointestinal tract.

Outbreaks of *Shigella* infection are difficult to control because of the relatively low infectious inocula, the ease of transmission, and a progressive increase in resistance to multiple antibiotics [2,16]. Although multi-antibiotic-resistant *S. sonnei* is still relatively uncommon, this species is the infectious agent in more than 50% of shigellosis cases; thus, the

development of antibiotic resistance by this species would pose an even greater risk to human health [5,18].

The Canary Islands are one of the most frequently visited European tourist destinations due to their unique geography, in close contact with the African continent. Since the Canary Islands therefore provide a permanent bridge for intercontinental transmission of microorganisms and vectors, the control of bacterial dissemination and infectious disease outbursts is crucial. A comparative analysis of different epidemiological markers is therefore important in order to know which is the best for tracing the source of infection during an outbreak. Unfortunately, the application of molecular techniques for the analysis and control of infection has not constituted part of recent health-care measures.

The aim of the present study was to evaluate the molecular epidemiology of an outbreak of multi-antibiotic-resistant *S. sonnei* in a rural hotel of La Gomera, Canary Islands, by comparing pulsed-field gel electrophoresis (PFGE) patterns of the isolated strains and by determining their antimicrobial susceptibility.

Materials and methods

The outbreak. All *Shigella* isolates were recovered from tourists staying at an 80-bed rural hotel on the island of La Gomera, one of the seven Canary Islands, Spain. Between March 11th and March 13th, 2004, 14 people, 7 of whom were registered at the hotel, were affected. The 6 males and 8 females ranged in age from 14 to 58 years old. Stool samples from 13 of these 14 affected persons (93%) were examined in the Microbiology Department of the Ntra. Sra. de Candelaria University Hospital, Sta. Cruz de Tenerife, Canary Islands, Spain. All 14 of the affected individuals had abdominal cramps and diarrhea, 5 of them also vomited, and 2 had fever. One of the 14 patients had renal failure as a consequence of dehydration.

Bacterial isolates. Bacterial isolates were obtained from 12 of the 13 clinical samples (92%). For recovery and identification, samples were grown on several different types of media according to recommended guidelines [15], including Columbia agar plates containing 5% lamb's blood, *Salmonella-Shigella* (SS) agar plates, MacConkey (MCK) agar plates, *Yersinia* CIN-agar (YER), and Campylosel (CAM) and selenite broth. This collection of media was chosen for the isolation of *Salmonella* spp., *Shigella* spp., *Campylobacter* spp., *Yersinia* spp., and *Aeromonas* spp. Non-fermenting lactose colonies were first isolated on MCK agar plates and then on Kligler medium to test for glucose and sucrose fermentation, sulfide production, and gas production. Urease activity was then tested using Christensen medium. [6,7]. Species were identified using API 32GN (bioMérieux, Lyon, France) and the automated Vitek 2 system (ID-GNB) (bioMérieux), and were confirmed by slide agglutination assay using specific antiserum against O groups A, B, C, and D (performed by the Centro Nacional de Microbiología, Instituto de Salud Carlos III, Majadahonda, Madrid, Spain).

Antimicrobial susceptibility testing. Antibiotyping was performed using API ATBGN (bioMérieux) and the automated Vitek 2 system (Card AST-N020,) (bioMérieux), recording both susceptibility [susceptible (S), intermediate (I), and resistant (R)] and the MICs. Ampicillin, ciprofloxacin, and cotrimoxazole resistances were confirmed using the standard disk diffusion method on Mueller-Hinton agar plates with disks containing 10 µg, 1 µg and 25 µg, respectively. Inhibition of growth was interpreted according to the National Committee for Clinical Laboratory Standards (NCCLS) [13], currently the Clinical and Laboratory Standards Institute (CLSI), Wayne, PA.

DNA agarose blocks preparation and PFGE. After phenotypic identification, all *S. sonnei* isolates were characterized by macrorestriction analysis of *Xba*I-digested genomic DNA and PFGE to elucidate a putative epidemiological linkage [10,11]. Genomic DNAs were prepared in agarose blocks using the CHEF Bacterial Genomic DNA Plug kit (Bio-Rad Laboratories, Richmond, CA) with minor modifications of the manufacturer's instructions. The DNA in the plugs was digested overnight with *Xba*I at 37ºC according to the manufacturer's instructions (Promega, Madison, WI). After digestion, restriction fragments were resolved by PFGE with a CHEF-DRIII (contour-clamped homogeneous electric field) apparatus (Bio-Rad) on a 1% (w/v) Seakem Gold agarose gel (FMC, Rockland, ME) in 0.5× TBE buffer at 11.3ºC. The CHEF-DRIII apparatus was programmed at 200 V (6 V/cm) for 28.5 h, with switching times ramped from 0.5 to 35.0 s. An included angle of 120º was used. Following electrophoresis, the gels were stained with ethidium bromide (0.5 µg/ml), visualized under UV illumination, and photographed with the Gel Doc 2000 system (Bio-Rad). Digital images were stored electronically as TIFF files. After visual inspection of the banding patterns obtained by PFGE, computer analyses were carried out using the Diversity Database fingerpringting software package, version 2.2 (Bio-Rad). The PFGE banding patterns of all *S. sonnei* clinical isolates were normalized by lambda concatemers (Sigma, St. Louis, MO). A tolerance of 1% in the band position was applied during the comparison of PFGE fingerprinting patterns. PFGE patterns were interpreted using the criteria established by Tenover et al. [17]. For cluster analyses, the Dice coefficients were calculated to compute the similarity matrix, and transformed into an agglomerative cluster using the unweighted pair group method with arithmetic averages (UPGMA).

Results and Discussion

Cultures of stool specimens from all 12 patients grew *S. sonnei* and all isolates showed the same antibiogram (resistance to amikacin, cefaclor, cefalotin, cefuroxime, cefuroxime axetil, gentamicin, tobramycin and trimethoprim/sulfamethoxazole, intermediate resistance to nitrofurantoin, and susceptibility to amoxicillin/clavulanic acid, ampicillin, cefepime, cefotaxime, cefoxitin, cefpodoxime, ceftazidime, ciprofloxacin, meropenem, norfloxacin, ofloxacin, piperacillin, piperacillin/tazobactam). The 12 *S. sonnei* isolates presented identical *Xba*I PFGE restriction patterns (Fig. 1), which clearly indicated the clonal nature of the outbreak.

Food contamination with *Shigella* could not be documented retrospectively, despite comprehensive bacteriological examinations. This was not unexpected, however, since the isolation of *Shigella* spp. from food and feces is generally considered to be difficult [20]. The bacterium competes poorly with other enteric flora and is easily overgrown. In addition, current laboratory detection methods are relatively insensitive [20]. Nonetheless, the presence of even a small number of *Shigella* may be epidemiologically significant because of the low infective dose of these organisms. Unlike most other enteropathogenic bacteria, the ingestion of only of 10^1–10^2 *Shigella* cells is known to cause infection in adults.

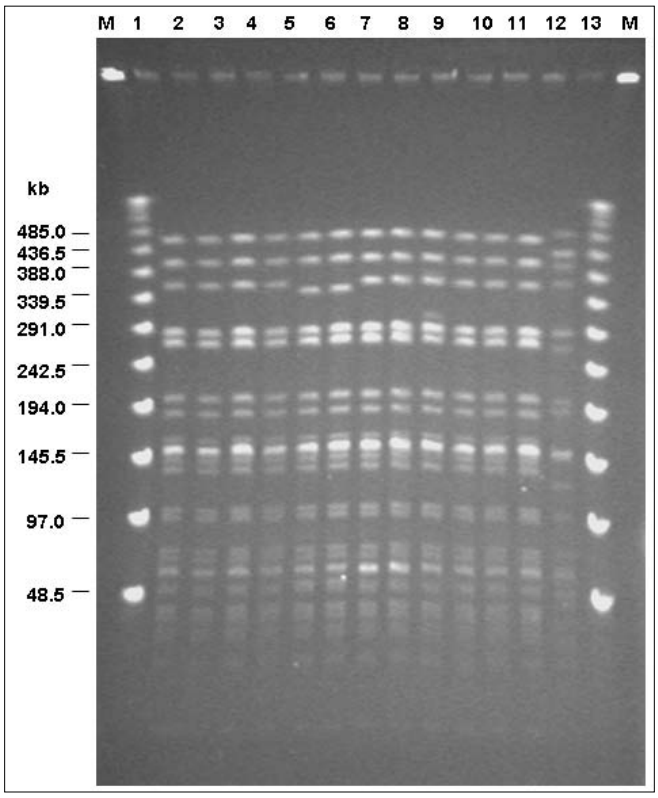

Fig. 1. Molecular fingerprinting of *Shigella sonnei* isolates by pulsed-field gel electrophoresis. *Lane M*, lambda DNA concatemers size markers; *lanes 1–12*, patterns of *Xba*I-digested DNA of the 12 *S. sonnei* isolates, all showing the same profile; *lane 13*, *S. sonnei* clinical isolate processed in the microbiology laboratory before the outbreak. Numbers on the left correspond to the weights of the lambda bands in kilobases (kb).

The first patient to be infected was admitted to the hospital with renal failure as a consequence of dehydration after diarrhea and vomiting. Two days later, 13 people were simultaneously affected. These data suggest a common source for all the affected individuals and two different exposure times during the outbreak [3,8,9,12]. A preliminary investigation was conducted by the Spanish regional epidemiology services. Patients identified as suspect cases were interviewed using a specifically designed questionnaire addressing relevant clinical features (when and how they began, stool characteristics, associated symptoms and their frequency and characteristics) and possible risk factors (consumption of unsafe foods, swimming in or drinking untreated fresh water, contact with other ill patients, recent or regular medication, underlying medical condition).

After detection of the outbreak, general environmental risk factors were evaluated and control policies were applied at the affected hotel. The single risk factor common to all infected individuals was the consumption of uncooked vegetables that had not been previously washed in peroxide. However, samples of food prepared before the date of onset of symptoms of the first case were not available; therefore specific action to prevent the reoccurrence of infection could not be taken. General control measures included checking of the hotel facilities, determination of the chlorine level in the water, monitoring of the hygienic policies regarding all stages of meal preparation, temperature control, and washing all uncooked food in peroxide. The outbreak of a *Shigella* infection highlights that it is essential to train food handlers to maintain high standards of food hygiene. After implementation of the above measures, no further acquired cases of shigellosis were documented.

The outbreak described in this study is the first *Shigella* spp. outbreak reported in the Canary Islands since the 1980s, when strict water-quality control policies were instituted. Since *S. sonnei* outbreaks occur infrequently in Spain, detection of the outbreak described here has critical significance for Spanish health-care programs [15]. The findings of the present study indicate that the risk of *S. sonnei* infections has not decreased, even though control policies have been strongly enforced [4]. The results described herein demonstrate the importance of regular application of molecular techniques as part of health-care control protocols.

Foodborne outbreaks of shigellosis still occur in both developed and developing countries. It is difficult to investigate outbreaks among tourists; since, by the time such outbreaks have been identified, the tourists have often departed and may be difficult to trace. More resources and greater collaboration among regional and national health-care departments are needed if outbreaks of food poisoning in tourist settings are to be investigated thoroughly.

The risk of infectious disease outbreaks could be reduced in tourism-dependent regions like Canary Island by regular inspection and monitoring of drinking-water supplies and waste-water systems, by ensuring the chlorination of supplemental drinking-water supplies, and by establishing food-safety initiatives. The prompt detection and efficient management of gastroenteritis outbreaks in tourists also requires a set of international guidelines, drawn up by authorities in the countries of destination and origin, for the management of foodborne and waterborne outbreaks at holiday resorts and other popular sites visited by tourists. Protocols for cross-national investigations of outbreaks in Europe should list the specific objectives in investigating an outbreak, outline the roles and responsibilities of investigators and control agencies, and require the formal reporting of the outcome of an investigation.

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Brote de *Shigella sonnei* en un hotel rural de La Gomera (Islas Canarias, España)

Resumen. *Shigella sonnei* es una causa significativa de gastroenteritis, tanto en países en desarrollo como industrializados. El conocimiento de la diversidad de esa bacteria y de su sensibilidad a los antimicrobianos puede ser una ayuda en el tratamiento de casos individuales y de brotes infecciosos. Este estudio se realizó para evaluar la epidemiología molecular de un brote de diarrea debido a *S. sonnei*. El brote afectó a 14 de los 28 (50%) turistas en un pequeño hotel rural en La Gomera, Islas Canarias, en España. Todos los aislados de *S. sonnei* recuperados presentaron el mismo patrón de sensibilidad a los antimicrobianos y el mismo patrón de electroforesis en gel de campo pulsado, lo cual indica que el brote fue causado por una sola cepa. [**Int Microbiol** 2005; 8(2):133-136]

Palabras clave: *Shigella sonnei* · electroforesis en gel de campo pulsado (PFGE) · brotes

Broto de *Shigella sonnei* em um hotel rural da Gomera (as Ilhas Canárias, Espanha)

Resumo *Shigella sonnei* é uma causa significativa de gastrenterite, tanto em países em desenvolvimento como industrializados. O conhecimento da diversidade dessa bactéria e de sua sensibilidade aos antimicrobianos pode ser uma ajuda no tratamento de casos individuais e de surtos infecciosos. Este estudo se realizou para avaliar a epidemiologia molecular de um surto de diarréia devido a *S. sonnei*. O surto afetou 14 dos 28 (50%) turistas em um pequeno hotel rural na Gomera, as Ilhas Canárias, na Espanha. Todos os isolados de *S. sonnei* recuperados apresentaram o mesmo patrão de sensibilidade aos antimicrobianos e o mesmo patrão de electroforesis em gel de campo pulsado, o qual indica que o surto foi causado por uma só cepa. [**Int Microbiol** 2005; 8(2):133-136]

Palavras chave: *Shigella sonnei* · eletroforese em gel de campo pulsado (PFGE) · surtos