Brief Original Article

Analysis of a temporal cluster of *Shigella boydii* isolates in Mpumalanga, South Africa, November to December 2007

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Abstract

Background: Shigellosis is a global human health problem. The disease is most prevalent in developing countries with poor access to safe potable water and sanitation. *Shigella boydii* is of particular epidemiological importance in developing nations such as African and Asian countries. In the present study, we report on the analysis of a temporal cluster of 29 *S. boydii* serotype 2 strains, isolated in the Mpumalanga Province of South Africa (SA) over the period of November to December 2007.
Methodology: Bacteria were identified as *S. boydii* using standard microbiological identification techniques and serotyped using commercially available antisera. Susceptibility testing to antimicrobial agents was determined by the Etest. Genotypic relatedness of strains was investigated by pulsed-field gel electrophoresis (PFGE) analysis of digested genomic DNA.
Results: The cluster of 29 isolates revealed comparable antimicrobial susceptibility profiles, while dendrogram analysis of PFGE patterns showed that the cluster of isolates grouped together and could clearly be differentiated from a random selection of unrelated *S. boydii* serotype 2 strains. Our data has strongly suggested that this cluster of isolates may share a common ancestry. However, this cannot be substantiated by epidemiological data because a detailed epidemiological investigation was not conducted.
Conclusions: We have documented the first cluster of *S. boydii* infection in SA. Due to the lack of adequate epidemiological investigation, we cannot emphatically state that an outbreak had occurred. However, we do hypothesis that this was an outbreak for which a waterborne source cannot be excluded. This study has highlighted the urgent need for timely and appropriate systems of epidemiological investigation of all suspected outbreaks of disease in developing countries.

Introduction

Shigellosis is a global human health problem [1]. The disease is most prevalent in developing countries with poor access to safe potable water and sanitation. *Shigella sonnei* is of particular epidemiological importance in developed nations (USA and Europe), while *Shigella flexneri* and *Shigella boydii* are of particular epidemiological importance in developing nations such as African and Asian countries [2-4]. *Shigella* infection is commonly food-borne and can also easily be transmitted from person to person via the faecal-oral route [5]. In addition, *Shigella* is one of five species of microorganisms most commonly associated with water-borne disease [6]. Notably, *Shigella* has been classified as a potential agent for biological terrorism due to its low infectious dose, route of infection, and environmental stability [7]. Recent data regarding food-borne and water-borne outbreaks of *Shigella* demonstrate that *S. sonnei* is the most commonly reported *Shigella* species associated with such outbreaks [8-11]. In contrast, outbreaks of *S. boydii* appear to be an infrequent occurrence and rarely reported. A search of published data (English language) found that the last report of an outbreak involving *S. boydii* occurred in Chicago, USA, in 1998 and involved bean salad infected with a serotype 18 strain, where it was alleged that parsley and cilantro ingredients introduced the contamination into the bean salad [12]. For South Africa (SA), very little data exists for outbreaks of *Shigella* infection, irrespective of serotype, so the frequency of such outbreaks in this country is unknown. In the present study, we report on the analysis of a temporal cluster of 29 *S. boydii* serotype 2 strains, isolated in the Mpumalanga Province of SA over the period of November to December 2007, henceforth referred to as the Mpumalanga cluster.

Materials and Methods

The cluster of 29 strains were isolated from patients ranging in age from 1 year to 62 years (median age = 22 years); all patients presented with symptoms of diarrhoea. No patient deaths were reported. Patient specimens included stools or rectal swabs. Specimens were collected from patients living in the towns of Mashishing (n=22), Standerton (n=5), Delmas (n=1) and Ba-Mokgoko (n=1). Figure 1 shows the location of towns in the province. The first specimen was collected on 7

Figure 1. Map of the Mpumalanga Province in South Africa showing the location of major towns. Δ, indicates the towns associated with the cluster of S. boydii isolates.
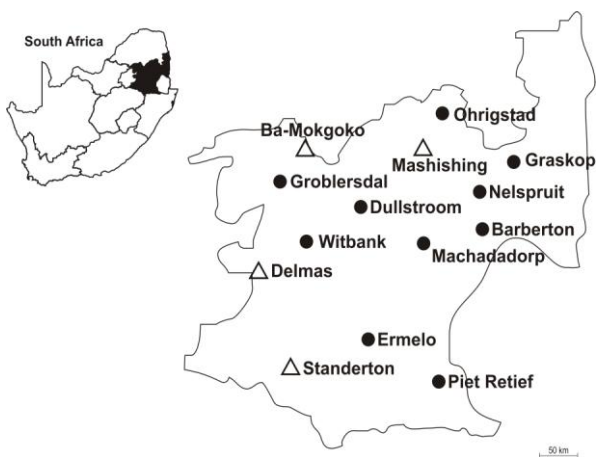
November 2007 from Delmas and the last specimen was collected on 25 December 2007 from Standerton. For comparative purposes, we included an additional 24 *S. boydii* serotype 2 strains which were randomly selected from a larger group and unrelated to the Mpumalanga cluster with regards to province of isolation or date of isolation. Bacteria were identified as *S. boydii* using standard microbiological identification techniques [13] and serotyped using commercially available antisera (Mast Assure, Mast Group Ltd, Merseyside, UK) as instructed by the manufacturer. Susceptibility testing to antimicrobial agents (ampicillin, augmentin, trimethoprim, sulfamethoxazole, chloramphenicol, nalidixic acid, ciprofloxacin, tetracycline, kanamycin, streptomycin, imipenem, ceftriaxone and ceftazidime) was determined by the Etest (AB BIODISK, Solna, Sweden), as

instructed by the manufacturer. The genotypic relatedness of strains was investigated by pulsed-field gel electrophoresis (PFGE) analysis of digested genomic DNA using a PulseNet protocol incorporating separate analysis with *Xba*I and *Not*I restriction enzymes [14]. The CHEF-DR III system (Bio-Rad Laboratories Inc., Hercules, CA, USA) was used for PFGE analysis and programmed (block 1) as follows: electrophoresis gradient, 6 V/cm; included angle, 120°; initial switch time, 2.2 seconds; final switch time, 63.8 seconds; run time, 22 hours.

Results and Discussion

The Enteric Diseases Reference Unit (EDRU) of the National Institute for Communicable Diseases (NICD) is a reference centre in SA for various diarrhoeal/enteric pathogens including *Salmonella* species, *Shigella* species, diarrhoeagenic *Escherichia coli* and *Vibrio cholerae*. The EDRU participates in national laboratory-based surveillance for these pathogens. Isolates are voluntarily submitted to the EDRU from ~120 clinical microbiology laboratories covering all provinces across SA. Surveillance data shows that in SA, *S. flexneri* is the most commonly isolated *Shigella* species in humans. For the years 2005 and 2006, *S. flexneri* accounted for 79% (1756/2217) of all *Shigella* isolates (K. H. Keddy, unpublished data). In contrast, *S. boydii* is an infrequent isolate. For the years 2005 and 2006, only 38 strains of *S. boydii* were isolated in SA, of which 23 strains were *S. boydii* serotype 2. For this period, in the Mpumalanga Province of SA, only a single *S. boydii* strain was isolated, a *S. boydii* serotype 2 strain isolated in February 2005. It is within this context that the sudden emergence of a cluster of 29 isolates of *S. boydii* serotype 2 in Mpumalanga over the period November to December 2007 suggested that an outbreak was occurring. The increased numbers of infections due to this serotype in the year 2007 (53/1230, 4%) versus the years 2003 to 2006 (45/3832, 1%) meant that *S. boydii* infection was 3.8 times more likely in the year 2007 than in the years 2003 to 2006 (P=0.0001 [95% CI=2.48-5.80]). Table 1 shows the number of *S. boydii* serotype 2 infections in the nine provinces of SA for the years 2005 to 2007. There were very few isolates annually followed by the sudden emergence of 29 isolates in Mpumalanga over the period November to December 2007.

Table 1. Number of *S. boydii* serotype 2 infections in the 9 provinces of South Africa for the years 2005 to 2007 (the value in parenthesis shows the number of *S. boydii* serotype 2 infections as a percentage of the total number of *Shigella* infections for that year).

|		Province								
		Eastern Cape	Western Cape	Northern Cape	Gauteng	Free State	KwaZulu-Natal	Limpopo	North West	Mpumalanga
Year	2005	0 (0%)	5 (0.47%)	0 (0%)	3 (0.28%)	1 (0.09%)	2 (0.19%)	0 (0%)	1 (0.09%)	1 (0.09%)
	2006	1 (0.09%)	3 (0.26%)	0 (0%)	3 (0.26%)	0 (0%)	1 (0.09%)	0 (0%)	2 (0.18%)	0 (0%)
	2007	0 (0%)	4 (0.33%)	0 (0%)	12 (0.98%)	2 (0.16%)	4 (0.33%)	1 (0.08%)	1 (0.08%)	29 (2.36%)

These 29 isolates were identified during the investigation of several outbreaks of watery diarrhoea occurring in several districts of the province, which overwhelmed available resources for epidemiological investigation in this country. The unusual predominance of this serotype, in one area in particular, initiated a genotypic analysis of this cluster of strains to determine their clonal relatedness.

The cluster of 29 *S. boydii* isolates revealed comparable antimicrobial susceptibility profiles. All isolates (except the Delmas isolate) showed resistance to ampicillin [minimum inhibitory concentration (MIC), 256 µg/ml], trimethoprim (MIC, 32 µg/ml), sulfamethoxazole (MIC, 1024 µg/ml) and streptomycin (MICs, 192-512 µg/ml). Susceptibility was shown to augmentin (MICs, 4-8 µg/ml), chloramphenicol (MICs, 1.5-2 µg/ml), nalidixic acid (MICs, 1.5-2 µg/ml), ciprofloxacin (MICs, 0.008-0.064 µg/ml), tetracycline (MICs, 1.5-4 µg/ml), kanamycin (MICs, 3-6 µg/ml), imipenem (MICs, 0.19-0.38 µg/ml), ceftriaxone (MICs, 0.032-0.064 µg/ml) and ceftazidime (MICs, 0.047-0.125 µg/ml). The Delmas isolate showed slight differences in its susceptibility profile in that it was susceptible to sulfamethoxazole (MIC, 4 µg/ml) and streptomycin (MICs, 8 µg/ml). Within the enteric group of bacteria, resistance genes coding for sulfamethoxazole and streptomycin are commonly located on mobile genetic elements (integrons, transposons and plasmids) of a promiscuous nature. The loss of such a mobile element in the Delmas isolate could explain the loss of resistance to sulfamethoxazole and streptomycin. Dendrogram analysis of PFGE patterns (*Xba*I digestion) showed that the Mpumalanga cluster of

29 strains grouped together on the dendrogram and could clearly be differentiated from a random selection of unrelated serotype 2 strains (Figure 2). The Mpumalanga cluster was represented by five unique (but similar) PFGE patterns. Four of these patterns were each represented by an individual isolate, while the fifth pattern was represented by 25 isolates [for these 25 isolates, PFGE analysis with a second restriction enzyme (*Not*I) also revealed an indistinguishable pattern (data not shown)]. Following this Mpumalanga cluster, another two strains of serotype 2 were isolated from the same area from specimens taken on 29 January 2008 and on 14 February 2008; however, PFGE patterns (*Xba*I digestion) showed them to be unrelated to the Mpumalanga cluster strain. To verify that PFGE analysis (*Xba*I digestion) is a valid genotypic method to differentiate SA strains of *S. boydii* serotype 2, we also performed PFGE analysis on 24 sporadic isolates of *S. boydii* serotype 2; these were randomly selected from a larger group and unrelated to the Mpumalanga cluster with regards to province of isolation or date of isolation. Dendrogram analysis of PFGE patterns showed that these 24 sporadic isolates could clearly be differentiated from our Mpumalanga cluster (Figure 2). These 24 sporadic isolates were differentiated into 22 unique PFGE patterns proving that this method is able to differentiate SA strains of *S. boydii* serotype 2. PFGE analysis incorporating *Xba*I digestion has previously been shown to be an appropriate method for subtyping strains of *S. boydii* [15,16] and our current study corroborates this.

Our laboratory investigation has strongly suggested that the current Mpumalanga cluster of *S. boydii* strains may share a common ancestry. However, this cannot be substantiated by epidemiological data because a detailed epidemiological investigation

Figure 2. Dendrogram of PFGE fingerprint patterns (XbaI digestion) for *S. boydii* isolates showing the Mpumalanga cluster of isolates as compared to sporadic unrelated isolates.
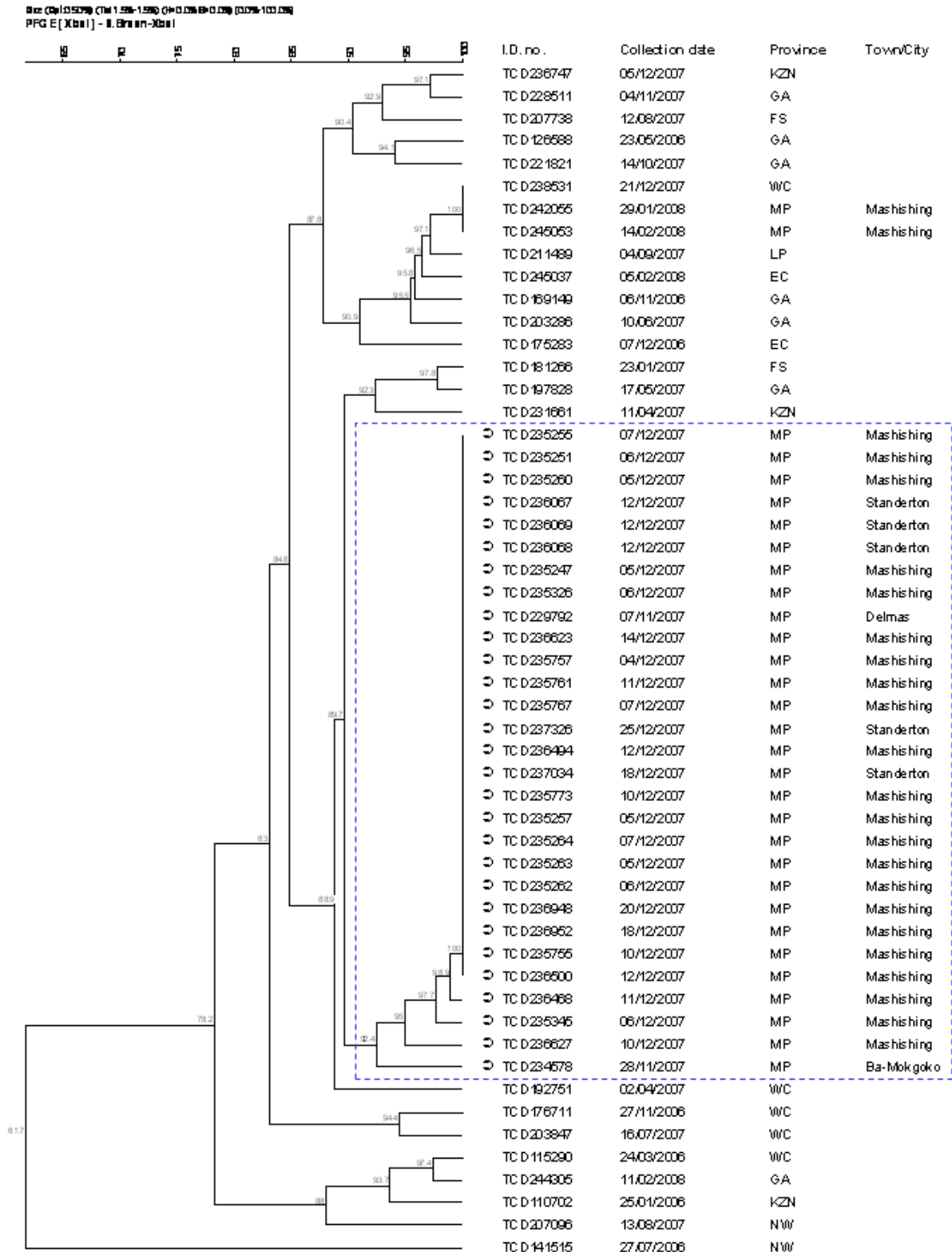
was not conducted, although statistical data support this premise. The minimal patient data collected were insufficient to provide further support for our laboratory findings. No data exist for travel history or other exposures for that time period and we were therefore unable to formulate a hypothesis regarding a likely source of infection. However, other diarrhoeal disease outbreaks occurring in surrounding areas were believed to be water-borne. We have no data on quality of household water amongst these cases but they are resident in rural areas of a province where safe potable water may not be available. This province has been plagued by regular outbreaks of water-borne disease, such as the outbreaks of typhoid fever in Delmas in 1993 [17] and 2005 (K. H. Keddy, unpublished data). Also, from 14 October 2007 to 9 December 2007, there were 1,301 cases of diarrhoea reported from Delmas. Stool and rectal swabs from these patients detected a diverse group of pathogens including *Shigella*, *Salmonella*, diarrhoeagenic *E. coli*, adenovirus, astrovirus, norovirus, rotavirus, *Schistosoma mansoni*, *Gardia lamblia* and *Isospora belli* (G. M. de Jong, personal communication). This extremely diverse array of pathogens provides strong evidence for a water-borne source.

In conclusion, we have documented the first cluster of *S. boydii* infection in SA. No detailed epidemiological investigation was conducted, so we cannot emphatically state that an outbreak had occurred. However, we do hypothesize that this was an outbreak for which a water-borne source cannot be excluded. This study has highlighted the implications of the lack of adequate epidemiological investigation of a suspected outbreak. Without a proper epidemiological investigation, we can never say for certain that an outbreak has or has not occurred, nor can we accurately speculate on the source of the outbreak. The major reason for the lack of epidemiological investigations is the lack of resources. This is a major problem in SA and most other developing countries, including most African countries. SA is still in its infancy when it comes to investigation of outbreaks. Timely and appropriate systems for epidemiological investigation of all suspected outbreaks of disease in SA need to be prioritized and urgently put in place.

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