Epidemiological characteristics and molecular typing of *Salmonella enterica* serovar Typhi during a waterborne outbreak in Eastern Anatolia

Y. BAYRAM*, H. GÜDÜCÜOĞLU†, B. OTLU‡, C. AYPAK§, N. C. GÜRSOY‡, H. ULUÇ¶ and M. BERKTAŞ†

*Van Education and Research Hospital, Microbiology Laboratory, Van, Turkey

†Department of Medical Microbiology, Medical School, Yüzüncü Yıl University, Van, Turkey

‡Department of Medical Microbiology, Medical School, İnönü University, Malatya, Turkey

§Department of Family Medicine, Van Gevaş Hospital, Van, Turkey

¶Van Health Directorates, Department of Infectious Diseases, Van, Turkey

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In this study, we aimed to study the molecular and epidemiological characteristics of *Salmonella enterica* serovar Typhi (*S. typhi*) outbreak in Eastern Anatolia.

Six hundred and thirty-seven patients from the same county with clinical diagnosis of typhoid fever were investigated with conventional methods from stool, urine and blood specimens. Antibiotic susceptibility tests and identifications were performed for positive specimens. Clonal relationships between the isolates were investigated using pulsed field gel electrophoresis (PFGE) method. A questionnaire was completed for the water consumption habits of patients.

Of 91 culture positive specimens, 76 were blood, 13 were stool and 2 were urine. The isolates were resistant to ampicillin, ampicillin/sulbactam, chloramphenicol, cefuroxime, amikacin, gentamicin and trimethoprim-sulfamethoxazole. Although there was a single band difference in some isolates, PFGE results indicated that this was an outbreak caused by single strain according to the Tenover criteria.

This outbreak thought to be associated with the consumption of tap water contaminated with sewage represents a breakdown of the basic public health and civil engineering infrastructure. Appropriate public health measures should be taken in order to avoid such outbreaks in the future.

INTRODUCTION

Typhoid fever is an acute febrile disease caused by *Salmonella enterica* serovar typhi (*S. typhi*) and may be food and waterborne (O'Reilly *et al.*, 2007; Bhunia *et al.*, 2009). Its incubation period varies between 3 days and 1 month. The early symptoms are fever, headache, abdominal discomfort, lack of appetite, constipation following diarrhea, unproductive cough, bradycardia, fatigue and rash. Mortality associated with typhoid fever is seen

in <1% of the patients that receive treatment and in 10% of untreated patients. Because the transmission is by food and water contaminated with the urine and stool from the patients and carriers, the prevention is only possible with consumption of safe water and hygienic food (Bhunja *et al.*, 2009).

The diagnosis of typhoid fever is based on the culture evaluation of blood, bone marrow, stool, urine and bile specimens. Blood and stool cultures should be repeated a few times. The chance of growth in the blood cultures is higher in the first week. In the stool cultures, bacteria can be detected starting from the second week. Although bacteria are detected in urine after the

Reprint requests to: Cenk Aypak, Department of Family Medicine, Van Gevaş Hospital, 65110 Gevaş, Van, Turkey. E-mail: cenkaypak@yahoo.com.

second week, the isolation rate is very low (Crump *et al.*, 2004).

In addition to the identification of etiological microorganism in an epidemic, it is very important to determine the source and clonal relationships between the microorganisms. Thereby, the source of epidemic can be determined and transmission routes can be identified. This will facilitate presentation of epidemiological data and take measures to prevent current and future epidemics. Pulsed field gel electrophoresis (PFGE), a molecular method used in typology of microorganisms and investigations of clonal relationships, is a highly valuable method for epidemiological analysis of epidemics caused by food or water-borne pathogens (Kubota *et al.*, 2005).

In this study, the patients who were admitted to two state hospitals in Van from the vicinity of Erçek town during a typhoid fever outbreak thought to be associated with the consumption of tap water contaminated with sewage were conventionally evaluated for isolation of the etiological agent, antibiotic sensitivity, and the *Salmonella* strains isolated were evaluated for clonal relationships through molecular epidemiological methods.

MATERIALS AND METHODS

Epidemiological Assessment

On 15 October 2008, the medical staff of Van Women's and Children's Health Hospital informed the local government health center about a patient with clinical diagnosis of typhoid fever from Erçek town which is located 60 km north of Van in Eastern Anatolia, Turkey. There was not another case with typhoid fever diagnosis from this area before this date. After the index case, a sudden increase in the number of patients from the same town (population of 4700) with the similar diagnosis was observed. All the cases (totally 637) from the vicinity of Erçek town who were admitted to two state hospitals in Van which had features of typhoid fever enrolled into the study. A

case that met the following criteria was taken to be a clinically diagnosed typhoid fever: a stepladder pattern of fever, headache, malaise with loss of appetite, usually with gastrointestinal symptoms and having two or more of the following symptoms/signs: toxic look; relative bradycardia; rose spots on the trunk; splenic enlargement; or non-productive cough. A confirmed case was a case that was confirmed by positive stool or blood culture for *S. typhi*. Daily numbers of patients at two hospitals' emergency and outpatient departments were recorded by the medical records officer.

Having alerted about outbreak of typhoid fever in Erçek, Epidemic Investigation Team (EIT) from local government public health authorities reviewed all the cases, a detailed clinical and epidemiological history was taken. At the same time, a medical field team visited each home at town and interviewed with the patients and healthy inhabitants by a structured questionnaire about environmental risks including water consumption habits. A spot map of the town showing water supplies (Fig. 1) was obtained from city authorities. All patients with clinical diagnosis of typhoid fever and culture conformed cases were marked on the spot map. Field team also collected serial samples from water network of town for bacteriological analysis.

Isolation, Identification and Antimicrobial Susceptibility

At Van Women's and Children's Health Hospital the urine and stool samples were inoculated into *Salmonella Shigella* agar (Oxoid, Basingstoke, UK) and eosin methylene blue agar (Oxoid) plates to isolate *Salmonella* and *Shigella* species. For blood culture, 0.5–5 ml blood was obtained under aseptic conditions and inoculated into BacT/ALERT (Biomérieux, Craponne, France) culture tubes. The blood samples with growth in 2–4 days were transferred into brain–heart infusion agar (Oxoid) and eosin

FIG. 1. Spot map of water network for distribution of cases.

methylene blue agar. The lactose negative and H_2S reaction positive colonies were suspected to be *Salmonella* species and thus, species and thus, additional biochemical tests were performed. The isolates that were compatible with *Salmonella* species were evaluated for H and O antigens with anti-serums (Becton Dickinson, Franklin Lakes, NJ, USA). To identify the agents in the culture positive samples and their antimicrobial susceptibility to Ampicillin (AMP), Ampicillin-Sulbactam (SAM), Cefuroxime (CXM), Ceftazidime (CAZ), Ceftriaxone (CRO), Cefepime (FEP), Aztreonam (AZT), Imipenem (IPM), Amikacin (AN), Gentamicin (GM), Ciprofloxacin (CIP), Levofloxacin (LVX), Piperacillin-Tazobactam (TZP) and Trimethoprim-Sulfamethoxazole (SXT),

Vitek-II (Biomérieux) automatic microbiology system was used.

The same microbiological tests were applied on patients who were admitted to Van Education and Research Hospital. The blood cultures obtained under aseptic conditions were incubated in Bactec (Becton Dickinson), an automatic blood culture device. The species isolated were identified and evaluated for antimicrobial sensitivity for the same antimicrobials with Phoenix system (Becton Dickinson). The susceptibility test for chloramphenicol (CAP) was performed by Kirby-Bauer disk diffusion method (30 μ g) in two laboratories.

The antimicrobial susceptibilities were determined according to the Clinical and Laboratory Standards Institute (CLSI)

breakpoints in both laboratories (CLSI, 2007).

PFGE

Ninety-one strains isolated by culture tests were subjected to PFGE. Bacterial isolates were inoculated onto blood agar overnight at 37°C. The colonies were suspended with 1 ml cell suspension [100 mM Tris-HCl and 100 mM EDTA (pH: 8.0)], and their optic densities were adjusted to 3 ($\lambda = 610$). The bacteria embedded into agarous blocks were broken and subjected to dialysis (Durmaz *et al.*, 2009). The genomic DNA in the plates was sliced (30 U) in water bath at 37°C for 2 hours with XbaI (Fermantas Corporation, Glen Burnie, MD, USA) enzyme. DNA sections were advanced on 0.8% pulse field certified agarous gel (Bio-Rad Laboratories, Nazareth, Belgium) in $\times 0.5$ Tris-borate-EDTA buffer [44.5 mM Tris, 44.5 mM boric acid and 1 mM EDTA (pH: 8.6)] with CHEF-DR II (Bio-Rad Laboratories) device and were broken into pieces. The parameters used for electrophoresis were set as follows: initial switch time, 0.1 second; final switch time, 90.0 seconds; 12°C, 6 V/cm², and the runtime was 22 hours (Murchan *et al.*, 2003). After the electrophoresis procedure, the gel was stained with ethidium bromide (5 µg/ml) and photographed under UV ray. The band difference was

interpreted using the method of Tenover *et al.* (1995).

RESULTS

Epidemiological Assessment

A total of 637 residents of the town were admitted to the emergency department and outpatient clinics of two state hospitals as typhoid fever cases. The weekly epidemic curve is depicted in Fig. 2. *S. typhi* was isolated from the samples of 91 patients (total $n=637$, 14.3%). These isolates were from 76 blood samples (83.5%), 13 stool samples (14.3%) and 2 urine samples (2.2%). Fifty-one of the patients were female and forty were male. The mean age of 91 patients was 19.16 years (range 2–86 years).

The two storages that supply tap water to the town are shown in Fig. 1. Seventy-eight patients that were hospitalized throughout the outbreak were interviewed for the water consumption habits by EIT. Of the patients, 48 (61.5%) reported consuming both tap water and storage water; 22 (28.2%), only tap water; and 8 (10.3%), only storage water. It indicates that 90% of the patients consumed tap water from the south storage. In the investigations conducted by EIT, it was noted that the contaminated water from the bathrooms and kitchens of some of the homes in the outbreak area was discharged

FIG. 2. Epidemic curve.

directly into the streets and sideways. Moreover, the stables were adjacent to the homes and the excretions from these stables were scattered in front of the houses and on the streets and avenues. Because of lack of a sewage system in the town, the human wastes are directly pumped into the ground via wells, which may have contaminated underground water sources and water system of the town through a leak in the pipes. Investigations revealed that a few days before the outbreak a sewage well near the water distribution reserve of the south part of the town where the outbreak occurred had over flown and during repairs, one of the pipes within the water distribution system had burst. This was supported by the fact that the outbreak had started among the people residing in the area where the water reserve was contaminated (Fig. 1). The presumptive coliform tests on the water samples from south part of town on 17 October 2008 were found to be unsatisfactory with coliform counts >300/ml. After super chlorination of the reserve that provided water to south part of town on 18 October (Fig. 2), the contamination in the tap water was eradicated. The municipality was instructed to keep chlorination incessantly for 24 hours and told that otherwise, the contamination would reoccur. Recommendations to supply a fully automatic chlorination device were made.

Medical field team visited each home at town. The residents were provided with information on general hygiene and water sanitation. Pamphlets on sanitation methods were handed out, and chlorine tablets were provided for 57 homes with water wells. They were also warned not to use water from the wells and be sure to have the water chlorinated if they had to consume the water. The members of each household were recommended to build a leak proof sewage well, and the officials of the municipality were informed on the importance of building the sewage infrastructure.

Isolation, Identification and Antimicrobial Susceptibility

The antimicrobial sensitivity tests showed that all the strains isolated were resistant to AMP [minimal inhibitory concentration (MIC) ≥ 32 $\mu\text{g/ml}$], SAM (MIC ≥ 32 $\mu\text{g/ml}$), CXM (MIC > 4 $\mu\text{g/ml}$), SXT (MIC ≥ 320 $\mu\text{g/ml}$), AN (MIC ≥ 2 $\mu\text{g/ml}$), GM (MIC ≥ 1 $\mu\text{g/ml}$) and CAP (MIC ≥ 256 $\mu\text{g/ml}$) and susceptible to CAZ (MIC ≤ 1 $\mu\text{g/ml}$), CRO (MIC ≤ 1 $\mu\text{g/ml}$), FEP (MIC ≤ 1 $\mu\text{g/ml}$), AZT (MIC ≤ 1 $\mu\text{g/ml}$), IMP (MIC ≤ 1 $\mu\text{g/ml}$) and TZP (MIC ≤ 4 $\mu\text{g/ml}$) and had decreased susceptibility to CIP (MIC < 1 $\mu\text{g/ml}$) and LVX (MIC < 1 $\mu\text{g/ml}$) according to CLSI breakpoints.

PFGE

In the PFGE, all the strains had 10 or more bands. The PFGE data indicated that the strains are closely related to each other (Fig. 3). Thus, all strains were considered to be the same strain according to Tenover criteria (Tenover *et al.*, 1995).

DISCUSSION

Salmonella enteric fever is one of the important bacterial infections detected in humans that can cause waterborne epidemics. Although it is not common in industrial countries, it is considered a serious public health problem in developing countries. Nevertheless, even in countries with no hygiene problems, outbreaks occur due to insufficient water networks. In countries with uncompleted infrastructure, this kind of outbreaks are frequent (O'Reilly *et al.*, 2007; Kanungo *et al.*, 2008).

Major epidemics associated with *Salmonella* occur due to consumption of contaminated water in the public water systems (Hunter, 2003; Lewis *et al.*, 2005).

This kind of epidemics affect all the people living in one residential area and cause disease among all (Bhunja *et al.*, 2009). In this study, although no *S. typhi*

FIG. 3. The results of pulse field gel electrophoresis (PFGE) typology of 28 *Salmonella typhi* (The relationships of the strains selected for demonstrative purposes out of 91 strains were as follows: the typified strains had 10 bands required for evaluation of PFGE results. No band differences were found on lines 1–16, 21, 23 and 26–28, and these were found to be the same isolates. However, there were band differences on lines 17–20, 22, 24 and 25, and these were determined to be the same isolates according to Tenover criteria).

was grown in the water samples collected from various locations of the waterworks of the town by public health officials, based on the information provided by the people affected by the outbreak, tap water was considered the probable source of the outbreak. The epidemic curve shows a sudden peak and abrupt fall suggestive of common point source outbreak. Most of cases were among the residents of south part of town. A few sporadic cases occur in other parts of town. These few cases were due to the visit of these inhabitants to the household members of contaminated zone and drinking tap water during these visits. This spatial clustering was also suggestive of waterborne outbreak. There was no foodborne transmission or information on any community gathering involving large number of people and food consumption as in a community dinner, which supports our hypothesis. Moreover, PFGE results for *S. typhi* strain confirmed an outbreak of a single strain.

There are few studies from Turkey on waterborne *S. typhi*-associated outbreaks. One of these studies reports an outbreak which was considered to be caused by consumption of raw vegetables and fruit washed with contaminated water in the water tank of a university hospital (Demirdal *et al.*, 2007), another has reported an outbreak due to sewage leak into the waterworks of

Ahmetli village in the vicinity of Diyarbakır-Ergani (Ceylan *et al.*, 2003) and the last one is on a waterborne outbreak affecting 10 people in Battalgazi town in the vicinity of Malatya (Iseri *et al.*, 2009). The outbreak reported in this study is important because it affected many people in Van-Erçek vicinity, and *S. typhi* bacterium isolated from the affected people provides epidemiological information.

S. typhi isolates resistant to the old first line antimicrobials, AMP, CAP and SXT, are usually described as multidrug resistant (MDR). MDR typhoid outbreaks have been increasing in Asia (Parry and Threlfall, 2008). Although in another study it was shown that all *S. typhi* isolates ($n=78$) from eastern Anatolia region were susceptible to AMP, CAP and SXT (Hosoglu *et al.*, 2003), *S. typhi* isolates in this outbreak were MDR. This is the first typhoid fever outbreak caused by MDR *S. typhi* in the region and indicates emerging problem in *S. typhi* in Turkey, particularly in MDR strains.

The high susceptibility rates to ofloxacin and ciprofloxacin are important as these antimicrobials provide alternatives in the treatment of resistant cases (Akinyemi *et al.*, 2005). Our study isolates were intermediately susceptible to CIP (MIC < 1 µg/ml) and in another study during this outbreak, it was shown that CIP and CRO are

appropriate alternatives for the treatment of MDR typhoid fever (Aypak *et al.*, 2010).

The evaluations suggested that the outbreak occurred probably due to the leakage of the sewage from the overflowed sewage well into the water reserve through a burst in the pipes. Unless the substructure problems are corrected, such outbreaks are likely to occur and threaten public health. Since waterborne outbreaks can affect many people, main water systems from where the public receives water or where the water is stored should be frequently analyzed by local authorities, and old systems should be reconstructed to meet the contemporary standards.

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