

Case Report



Phylogenetic Analysis for the Origin of Typhoid Fever Outbreak on Jeju Island, Korea, in 2017

Keun Hwa Lee ^{1,*}, Jeong Rae Yoo ^{2,*}, Young Ree Kim ³, and Sang Taek Heo ²

¹Department of Microbiology and Immunology, Hanyang University College of Medicine, Seoul, Korea

²Division of Infectious Disease, Department of Internal Medicine, Jeju National University School of Medicine, Jeju, Korea

³Department of Laboratory Medicine, Jeju National University School of Medicine, Jeju, Korea

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Corresponding Author:

Sang Taek Heo, MD, PhD

Department of Internal Medicine, Jeju National University School of Medicine, 15 Aran 13-gil, Jeju 63241, Korea.

Tel: +82-64-754-8151

Fax: +82-64-717-1131

E-mail: neosangtaek@naver.com

*Lee KH and Yoo JR equally contributed to this study.

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ORCID iDs

Keun Hwa Lee

<https://orcid.org/0000-0002-2259-2751>

Jeong Rae Yoo

<https://orcid.org/0000-0002-5488-7925>

Young Ree Kim

<https://orcid.org/0000-0003-2454-8815>

Sang Taek Heo

<https://orcid.org/0000-0001-8947-5069>

Conflict of Interest

No conflicts of interest.

ABSTRACT

In June 2017, on Jeju Island, six patients were diagnosed with typhoid fever. All six patients were admitted to our hospital with fever, all of them had been working at the same hotel. The *gyrA* and *gyrB* genes of four out of six *Salmonella typhi* cases were amplified by polymerase chain reaction. The phylogenetic tree of the genes showed that the *S. Typhi* isolates from Jeju Island in 2017 had the same genotype and were similar to isolates from the United Kingdom. The origin of the isolates of the outbreak was the same, and the disease strain may have been imported from outside of Korea.

Keywords: Phylogenetic analysis; Outbreak; Typhoid fever; Jeju Island; Korea

INTRODUCTION

Typhoid fever caused by *Salmonella enterica* serovar Typhi (*Salmonella typhi*) is a food- and water-borne disease associated with acute systemic febrile illness and is found only in humans [1]. Symptoms such as fever, loose stools, abdominal pain, and headache occur; they continue for 4 - 7 days and finally resolve without treatment, but some patients need to be admitted for management with antibiotics for critical conditions, such as severe dehydration or hypotension [2]. Older patients, infants and children, and immunosuppressed patients are especially likely to require treatment. *S. typhi* remains one of the important infectious diseases in the world.

The Korea Centres for Disease Control and Prevention (KCDC) report that approximately 120 cases have occurred annually in Korea since 2010 according to the Infectious Disease Surveillance Report [3, 4]. On Jeju Island, Korea, no cases of typhoid fever were reported from 2012 to 2014, and one case was reported from 2015 to 2016 [4].

In June 2017, six patients were diagnosed with typhoid fever in the southern region of Jeju Island, and we observed that the genotype of *S. typhi* in the outbreak was the same for all patients. The study protocol was approved by the Institutional Review Board of Jeju National University Hospital (JNUH 201706018), and informed consent was obtained from all participants.

Author Contributions

Conceptualization: JRY, STH. Data curation: KHL. Formal analysis: JRY, STH. Funding acquisition: STH. Investigation: JRY, YRK. Methodology: KHL. Resources: YRK, KHL. Supervision: STH. Writing - original draft: KHL, JRY. Writing - review & editing: STH, KHL.

CASE REPORT

1. Patients

Six patients were admitted to our hospital with fever between June and July 2017. All of them worked at the same hotel. One had been working as a cafeteria cook, one as a cleaner, one as a manager, one as an electric engineer, and two as maids. All patients were diagnosed with typhoid fever, and the hotel was closed for two months for an epidemiological investigation. **Table 1** shows the clinical features of the six patients. Patient 1 had complained of mild haematochezia for two months, and this symptom was aggravated seven days prior to presentation. The patient was suspected of being a carrier and was the index patient for this outbreak. She was a cook in the staff cafeteria, and other patients ate meals in this cafeteria. She did not have any history of international travel for the six months before symptom onset. She had experienced abdominal discomfort for two months. She underwent laparoscopic cholecystectomy on the ninth day of hospitalization. The result of biopsy after operation was chronic active cholecystitis.

S. typhi was identified in the blood of four patients and had the same susceptibility to antibiotic agents in all four cases. Patients 5 had clinical characteristics and laboratory results consistent with typhoid fever, although the infectious organism was not identified. All patients were treated with antibiotics such as quinolone or third generation cephalosporin and were discharged without any complications.

2. Molecular diagnosis and phylogenetic analysis of *S. Typhi*

For the molecular diagnosis of *S. typhi*, we extracted DNA from cultured bacteria using the bead beater extraction method and used it as the template for polymerase chain reaction (PCR) [5]. The *gyrA* and *gyrB* genes of *S. typhi* were amplified by PCR, and the PCR products were sequenced using the BigDye Terminator Cycle Sequencing Kit (Perkin Elmer Applied Biosystems, Warrington, UK) [6, 7]. After the PCR products were sequenced, the *gyrA* and *gyrB* sequences were compared with *S. typhi* in GenBank using the multiple-alignment algorithm in the MegAlign program (Windows version 3.12e; DNASTAR, Madison, WI, USA) and the ClustalX program, respectively. Based on the aligned sequences, phylogenetic analyses were conducted in MEGA6, and phylogenetic trees were constructed using the

Table 1. Clinical characteristics of six patients diagnosed with typhoid fever

Patient	Age/Sex	Onset of symptoms	Occupation	Symptoms	Pathogen identification	Splenomegaly	Abdominal LAP	Cholecystitis	Antibiotics
1	53/F	01-June-2017	Cook	Hematochezia ^a , nausea, fever	Yes	Yes	Yes	Yes	Cefotaxime/ciprofloxacin
2	48/F	01-June-2017	Cleaner	Fever, chills	No ^b	Yes	Yes	No	Ceftazidime/ciprofloxacin
3	46/F	09-June-2017	Maid	Fever, general weakness, myalgia, sweating	Yes	NA	NA	No	Ciprofloxacin
4	26/M	12-June-2017	Manager	Fever, headache	Yes	Yes	No	Yes	Ciprofloxacin/ceftriaxone
5	25/F	13-June-2017	Maid	Fever, myalgia, headache, chilling	No ^c	NA	NA	No	Ciprofloxacin/ceftriaxone
6	61/M	14-June-2017	Electric engineer	Fever, headache, myalgia, cold sweating, nausea, abdominal pain	Yes	Yes	Yes	No	Ciprofloxacin

^aHematochezia began two months prior to presentation and became aggravated 7 days prior to presentation.

^bShe was transferred to our hospital from local hospital. *Salmonella typhi* was identified at local hospital but not at our hospital.

^cShe was transferred to our hospital from local hospital. The pathogen was not identified at either hospital.

Case 1 and Case 4 underwent laparoscopic cholecystectomy, and the pathology of the gall bladder showed chronic active cholecystitis.

LAP, lymphadenopathy; F, female; M, male.

neighbor-joining method [8]. Neighbor-joining is a bottom-up (agglomerative) clustering method for the creation of phylogenetic trees [9]. It is usually used for trees based on DNA or protein sequence data, and the algorithm requires knowledge of the distance between each pair of taxa (*e.g.*, species or sequences) to form the tree.

DISCUSSION

This is the first reported outbreak of typhoid fever in a hotel on Jeju Island. We performed a phylogenetic analysis using *gyrA* and *gyrB* sequences after the amplification and sequencing of four *S. Typhi* isolates from the described patients, and these sequences were compared with those of Korean peninsula isolates and isolates from other countries [6, 7] (Fig. 1 and 2).

The phylogenetic tree of *gyrA* and *gyrB* showed that the *S. typhi* isolates from Jeju Island in 2017 had the same genotype and were similar to isolates from the UK. However, these isolates were different from isolates from the Korean peninsula in 2017, even though Jeju Island is affiliated with the Korean peninsula (Fig. 1 and 2).

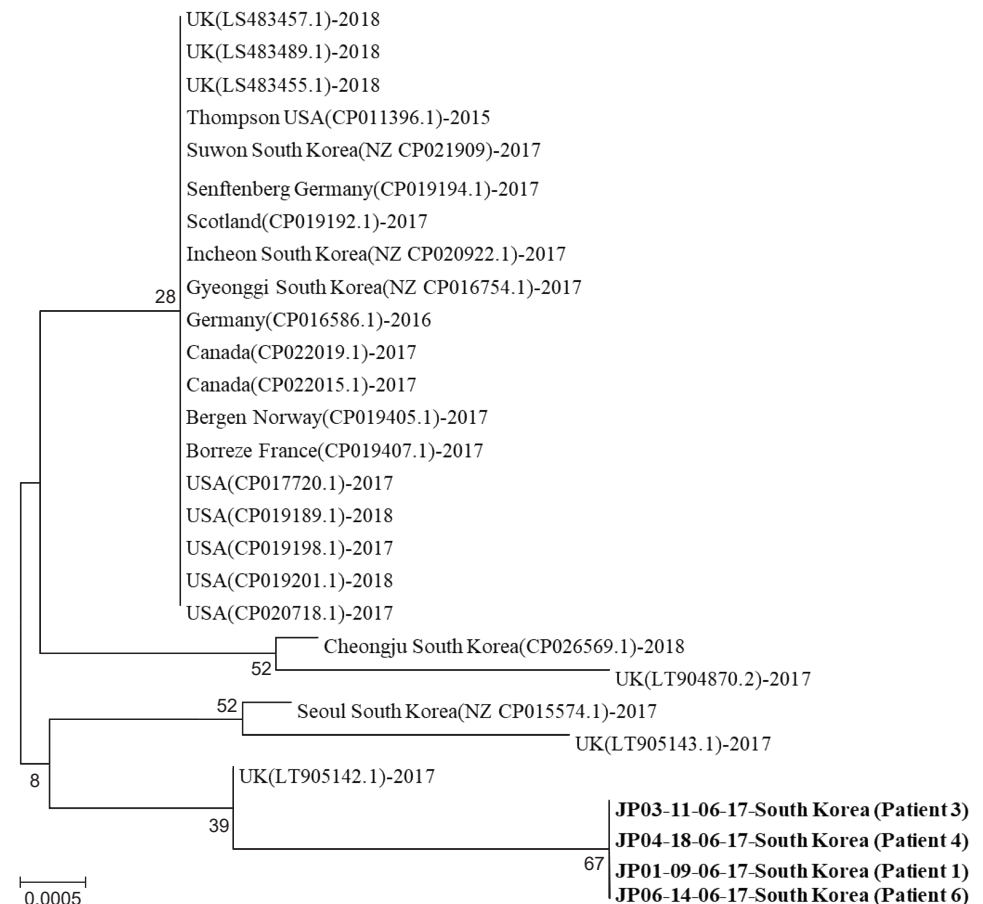


Figure 1. Phylogenetic tree constructed based on the partial *gyrA* gene. The tree was constructed using the neighbor-joining method with MEGA6. The partial *gyrA* gene from stored sera collected on Jeju Island in 2017 and analysed in this study is shown in bold. The partial *gyrA* gene data of *Salmonella typhi* identified in Korea and other countries were obtained from NCBI/BLAST. MEGA6, Molecular evolutionary genetic analysis 6; NCBI, National center for biotechnology information; BLAST, Basic local alignment search tool; UK: United Kingdom; JP, Japan patient.

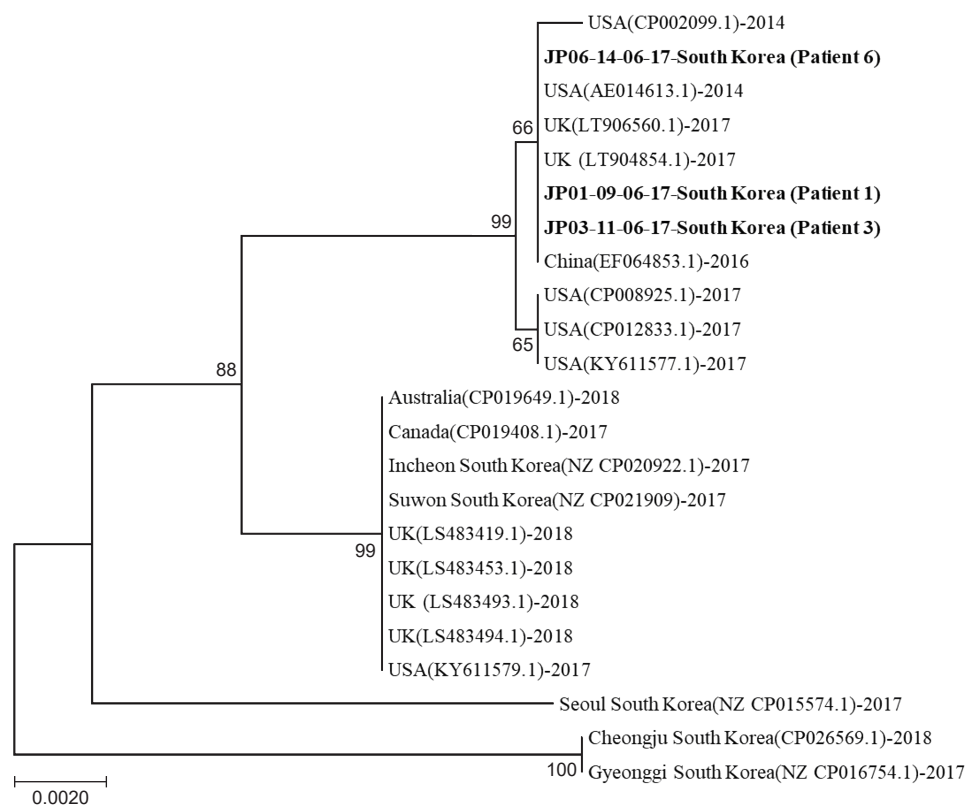


Figure 2. Phylogenetic tree constructed based on the partial *gyrB* gene. The tree was constructed using the neighbor-joining method with MEGA6. The partial *gyrB* gene from stored sera collected on Jeju Island in 2017 and analysed in this study is shown in bold. The partial *gyrA* gene data of *Salmonella typhi* identified in Korea and other countries were obtained from NCBI/BLAST. MEGA6, Molecular evolutionary genetic analysis 6; NCBI, National center for biotechnology information; BLAST, Basic local alignment search tool; UK: United Kingdom; JP, Japan patient.

Some foodborne outbreaks of non-typhoid fever on Jeju Island were previously identified and were caused by bacteria of different clonal origins [10]. However, no outbreak of typhoid fever had occurred on Jeju Island for ten years, to the best of our knowledge. We did not find an outbreak source, such as contaminated water, vegetables, or animal products, nor were we able to determine the course of transmission. We assume that the other patients were infected by patient 1 considering the duration of the patient's symptom or that the disease may have spread among the patients from the reservoir (Table 1). Finally, no additional patients developed typhoid fever due to the isolation and suspension of this hotel and the monitoring of employees by the KCDC and community health centres.

Regarding the molecular analysis, this study had a minor limitation in that phylogenetic analysis of the sequences of *gyrA* and *gyrB* associated with the development of antimicrobial resistance in *S. typhi* was performed. To obtain more comprehensive data, we should have performed whole-genome sequencing or pulsed field gel electrophoresis (PFGE). A review of the international literature shows that *S. typhi* strains have spread globally and that *S. typhi* H58 has become the predominant strain. Multidrug resistance is highly associated with the H58 haplotype [11, 12]. However, we could not look for H58 in our isolates. This study had a small number of isolates compared to similar outbreak studies of *S. typhi* [13-15]. Therefore, the data presented are not sufficient to claim that the outbreak may have been associated with other countries' isolates. Another limitation is that we cannot provide a detailed description

of surveillance efforts to control this *S. typhi* outbreak. The outbreak was controlled well by the Public Health Centre, and we did not participate in that surveillance.

Nevertheless, this is a meaningful study from a public health perspective that offers a distinct lesson in dealing with restaurant-associated typhoid fever outbreaks. Our results showed that the origin of the outbreaks on Jeju Island in 2017 was the same and that the disease may have been imported from outside of South Korea by the effects of globalization, such as increased international travel, trade, and transportation. Thus, further epidemiological research is needed to better understand the ecological transmission dynamics and geographic distribution of *S. typhi* [16].

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