Jpn. J. Infect. Dis., 70, 308-310, 2017

Short Communication

Bloodstream Infection Due to CTX-M-15 and TEM-1 Extended-Spectrum β-Lactamase-Producing *Salmonella enterica* serovar Virchow ST16

Jeong-Hwan Hwang[1,2,3†], Gee-Wook Shin[4†], Joo-Hee Hwang[1], and Chang-Seop Lee[1,2,3*]

[1]*Department of Internal Medicine;* [2]*Research Institute of Clinical Medicine; and*
[4]*Biosafety Reference Center and College of Veterinary Medicine,*
Chonbuk National University, Jeonju; and
[3]*Biomedical Research Institute of Chonbuk National University Hospital,*
Jeonju, Republic of Korea

SUMMARY: A 57-year-old man presented with high fever and diarrhea. A blood culture revealed the presence of a Group C nontyphoidal *Salmonella* (NTS) isolate. On *Salmonella* serotyping, the isolate was identified as *Salmonella enterica* serovar Virchow. Its sequence type was determined to be ST16 by sequence analysis of 7 different housekeeping genes. The $bla_{\text{CTX-M}}$ group 1 and bla_{TEM} genes were amplified using multiplex PCR assay for detecting extended-spectrum β-lactamases (ESBL) genes. Sequences of both amplicons were respectively identical to CTX-M-15- and TEM-1-encoding genes. Since NTS is a cause of foodborne illness outbreaks in communities and an important cause of community-acquired bloodstream infection, clinicians should consider ESBL- or AmpC-producing NTS species in the differential diagnosis.

Most nontyphoidal *Salmonella* (NTS) infections lead to self-limiting enterocolitis and do not require antimicrobial treatment (1). However, complications such as bloodstream infection are common in immunocompromised patients, infants, and the elderly (1). In these situations, fluoroquinolones and expanded-spectrum cephalosporins (ESCs) are the antibiotics of choice (2). However, resistance to fluoroquinolones and ESCs among NTS strains has been increasingly reported worldwide (2). Kim et al. commented that the rates of the ESC-resistant *Salmonella enterica* serovar Virchow in human stool specimens in Korea have risen noticeably, from 21.4% in 2011 to 82.3% in 2014 (3).

A 57-year-old man was admitted to our emergency room with fever and diarrhea. On physical examination, abdominal tenderness was observed in the right lower quadrant area. Other vital signs were normal except for a body temperature of 38.3°C. The patient had no underlying comorbidities. On the day before symptom development, the patient and 2 companions ate pupa food broiled with soy. The companions also presented with gastrointestinal symptoms but those resolved spontaneously within 1 to 2 days, whereas the symptoms in the patient seen in the emergency room had worsened. On laboratory testing, complete blood count was within normal range as were other chemical profiles except for the following: blood urea nitrogen, 56 mg/dL; creatinine, 4.11 mg/dL; albumin, 3.4 g/dL; and C-reactive protein, 158.77 mg/L. A methylene blue stain of the stool sample showed polymorphonuclear leukocytes, but the stool culture was negative.

Intravenous ciprofloxacin was empirically administered. A peripheral blood culture performed on admission day showed the presence of a Group C NTS isolate. The results of antimicrobial susceptibility testing of the present isolate (B14-9257) are given in Table 1. Con-

Table 1. Antibiotic susceptibilities of *Salmonella enterica* serovar Virchow B14 -9257, its transconjugant B14-9257 TC, and recipient strain *Escherichia coli* J53

Antimicrobial agent	MIC (μg/mL)		
	B14-9257	B14-9257 TC	*E. coli* J53
Amikacin	≤ 4	≤ 4	≤ 4
Gentamicin	≤ 1	≤ 1	≤ 1
Ampicillin	≥ 128	≥ 128	4
Cephalothin	≥ 128	≥ 128	4
Cefoxitin	≤ 1	≤ 1	≤ 1
Cefotaxime	≥ 8	≥ 8	≤ 1
Ceftriaxone	≥ 64	≥ 64	≤ 1
Imipenem	≤ 2	≤ 2	≤ 2
Nalidixic acid	≥ 256	≥ 256	4
Ciprofloxacin	1	0.25	≤ 0.12
Tetracycline	128	128	≤ 2
Trimethoprime/ sulfamethoxazole	≤ 1/19	≤ 1/19	≤ 1/19

Received April 1, 2016. Accepted September 9, 2016.
J-STAGE Advance Publication October 31, 2016.
DOI: 10.7883/yoken.JJID.2016.142

*Corresponding author: Mailing address: Department of Internal Medicine, Chonbuk National University Medical School, 567 Baekje-daero, Deokjin-gu, Jeonju-si, Jeollabuk-do 54907 Republic of Korea. Tel: +82-63-250-2391, Fax: +82-63-254-1609, E-mail: lcsmd@jbnu.ac.kr
†These authors coutributed equally to this work.

sidering the result of antimicrobial susceptibility testing had shown resistance to nalidixic acid and ESCs as well as the persistent fever in the patient, the antimicrobial agent was changed to intravenous meropenem (2.0 g every 8 h). Meropenem treatment was maintained for 3 weeks. His clinical course improved, and the patient was discharged. Two weeks after discharge, the patient was stable and symptom free.

The isolate was classified as *Salmonella enterica* serovar Virchow, according to the Kauffmann–White scheme. Its sequence type (ST) was determined to be ST16 by sequence analysis of 7 different housekeeping genes (*aroC*, *dnaN*, *hemD*, *hisD*, *purE*, *sucA*, and *thrA*) (4). *Salmonella enterica* serovar Virchow strains belong to the eBurst Group BG9, and the majority of BG9 strains are classified as ST16 in the multilocus sequence typing database (5). The $bla_{\text{CTX-M}}$ group 1 and bla_{TEM} genes were amplified using multiplex PCR assays for detecting extended-spectrum β-lactamases (ESBL) genes (6). Sequences of both amplicons were respectively identical to CTX-M-15- and TEM-1-encoding genes based on BLAST searches. However, the AmpC β-lactamase-encoding genes were not detected via PCR assays. ESBL-producing genes were transferred by conjugation from *Salmonella enterica* serovar Virchow to the azide-resistant *E. coli* J53 strain as the recipient. In addition, a 9.4- to 23.1-kb plasmid and its transconjugants (B14-9257 TC) was identified in the present isolate (Fig. 1).

NTS is a major cause of community-acquired bloodstream infection (7). Bacteremia develops in about 5% of patients with gastrointestinal illness caused by NTS (1,7). Bloodstream infection is a serious complication of salmonellosis resulting in sepsis, endarteritis, meningitis, septic arthritis, osteomyelitis, and death (1). In the case of invasive infections such as bacteremia by ESBL-producing NTS, clinicians may select inappropriate empirical antibiotics. The case-fatality rate of NTS bacteremia is greater than 20% and a dely in effective treatment of ESBL-producing bacteremia is associated with increased mortality (8,9). The initial empirical antibiotic prescribed in our case was inapproate.

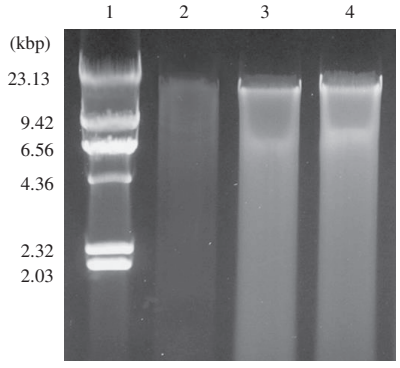

Fig. 1. Gel electrophoresis of plasmid from transconjugation assay. Lane 1. λ*Hin*dIII, DNA size marker, Lane 2, *E. coli* J53 (recipient strain), Lane 3, *Salmonella* Virchow (the present isolate as donor strain), Lane 4, Transconjugant of recipient strain from mating with the present *Salmonella* Virchow isolate).

NTS resistant to ESCs that produce ESBL or AmpC enzymes have been recognized since the late 1980s and have been reported worldwide (2). However, the prevalence of ESC-resistant *Salmonella* species from human specimens appears to be low: 1.6% in Belgium, 0.55% in England and Wales, and 0–7% in Southeast Asia (10,11). With the prevalence of ESBL-producing *Salmonella enterica* serovar Virchow markedly increasing in Korea since 2011, invasive *Salmonella enterica* serovar Virchow infections such as this bacteremia case may occur more frequently (3). Considering that NTS is a cause of foodborne illness outbreaks in communities and an important cause of community-acquired bloodstream infection, public health surveillance for ESBL- or AmpC-producing NTS species will continue to be needed (7,9).

Acknowledgments This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (1201000897) and the fund of Biomedical Research Institute, Chonbuk National University Hospital. The pathogen for this study was provided by the Chonbuk National University Hospital Culture Collection for Pathogens.

Conflict of interest None to declare.

REFERENCES

1. Hohmann EL. Nontyphoidal salmonellosis. Clin Infect Dis. 2001;32:263-9.
2. Miriagou V, Tassios PT, Legakis NJ, et al. Expanded-spectrum cephalosporin resistance in non-typhoid Salmonella. Int J Antimicrob Agents. 2004;23:547-55.
3. Kim JS, Yun YS, Kim SJ, et al. Rapid emergence and clonal dissemination of CTX-M-15-producing *Salmonella enterica* Serotype Virchow, South Korea. Emerg Infect Dis. 2016;22:68-70.
4. Harbottle H, White DG, McDermott PF, et al. Comparison of multilocus sequence typing, pulsed-field gel electrophoresis, and antimicrobial susceptibility typing for characterization of *Salmonella enterica* serotype Newport isolates. J Clin Microbiol. 2006;44:2449-57.
5. Achtman M, Wain J, Weill FX, et al. Multilocus sequence typing as a replacement for serotyping in *Salmonella enterica*. PLoS Pathog. 2012;8:e1002776.
6. Dallenne C, Da Costa A, Decre D, et al. Development of a set of multiplex PCR assays for the detection of genes encoding important beta-lactamases in Enterobacteriaceae. J Antimicrob Chemother. 2010;65:490-5.
7. Crump JA, Medalla FM, Joyce KW, et al. Antimicrobial resistance among invasive nontyphoidal *Salmonella enterica* isolates in the United States: National Antimicrobial Resistance Monitoring System, 1996 to 2007. Antimicrob Agents Chemother. 2011;55:1148-54.
8. Schwaber MJ, Carmeli Y. Mortality and delay in effective therapy associated with extended-spectrum beta-lactamase production in Enterobacteriaceae bacteraemia: a systematic review and meta-analysis. J Antimicrob Chemother. 2007;60:913-20.
9. Graham SM, English M. Non-typhoidal salmonellae: a management challenge for children with community-acquired invasive disease in tropical African countries. Lancet. 2009;373:267-9.

10. Ceyssens PJ, Mattheus W, Vanhoof R, et al. Trends in serotype distribution and antimicrobial susceptibility in *Salmonella enterica* isolates from humans in Belgium, 2009 to 2013. Antimicrob Agents Chemother. 2015;59:544-52.

11. Burke L, Hopkins KL, Meunier D, et al. Resistance to third-generation cephalosporins in human non-typhoidal *Salmonella enterica* isolates from England and Wales, 2010–12. J Antimicrob Chemother. 2014;69:977-81.