Case report

Salmonella enterica var. Enteritidis osteomyelitis with pulmonary involvement in an immunocompetent young woman

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A B S T R A C T

Primary bacteremia due to non-typhoid Salmonella often occurs in immunocompromised individuals, but may also occur in immunocompetent individuals. Contrastingly, vertebral osteomyelitis with respiratory involvement caused by non-typhoid Salmonella in immunocompetent individuals is extremely rare. A 21-year-old healthy woman with histories of eating ready-to-eat roasted beef and a recent vertebral compression fracture developed high-grade fever and was diagnosed with bacteremia, complicated by vertebral osteomyelitis with pulmonary involvement characterized as an extra-intestinal infection. The pathogen was identified as Salmonella enterica var. Enteritidis using molecular and serotyping techniques. The appropriate antibiotic therapy and focal detection were based on antimicrobial susceptibility testing (including fluoroquinolone resistance), medical histories (eating ready-to-eat roasted beef and vertebral compression fracture), and diagnostic imaging. This case highlights the potential of vertebral osteomyelitis and pulmonary involvement caused by S. enterica var. Enteritidis in an immunocompetent individual, and misinterpretation of fluoroquinolone susceptibility with conventional methods.

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Introduction

The incidence of non-typhoid salmonellosis in 2017 was estimated to be 7.5 per 100,000 population worldwide [1]. Non-typhoid Salmonella causes infectious diseases of visceral organs, long bones, joints, muscles, the central nervous system, and large vessel walls [2]. Additionally, it can (rarely) cause vertebral osteomyelitis and affect the respiratory system in immunodeficient populations with conditions such as malignant tumors, human immunodeficiency virus (HIV) infection, immunosuppression (due to immunosuppressant use), and diabetes mellitus [2]. Mortality from non-typhoid Salmonella vertebral osteomyelitis is very low, while that from non-typhoid Salmonella pneumonia is 25–60 % despite the recommended empirical treatment, including third-generation cephalosporin and fluoroquinolone [3]. In the present case, bloodstream infection caused by Salmonella enterica var. Enteritidis with vertebral osteomyelitis and pulmonary involvement in an immunocompetent young patient was rapidly diagnosed using microbiological examinations and appropriately treated. This infection can be suspected based on a patient’s medical history (eating ready-to-eat roasted beef and vertebral compression fracture) and microbiological examinations; however, attention should be paid to fluoroquinolone-resistant strains.

Case

This study was approved by the institutional review board and ethics committee of Japanese Red Cross Ise Hospital (approval number: ER2020-24). Written informed consent was obtained from the patient for publication of this case report and accompanying images. A 21-year-old Japanese woman with no medical history presented to our hospital with lumbar pain after a fall during bouldering. She had an abrasion from the external wound; however, she was diagnosed with a vertebral compression fracture at the L1 level with lumbar X-ray by her previous doctor. Thereafter, she presented with high-grade fever, shaking chills,
and low back pain and was admitted to our hospital 9 days after trauma. She had a history of eating ready-to-eat roast beef for four consecutive days from the 15th to the 12th day before admission; however, she denied any gastrointestinal symptoms. She had no known allergies and had not been prescribed any medications recently.

On physical examination, she was alert, and her vital signs were: body temperature, 40.0 °C; blood pressure, 105/53 mmHg; heart rate, 109 beats/minute; respiratory rate, 30 breaths/minute; and percutaneous oxygen saturation, 90 % on room air. Her lung auscultation findings were normal. She had lumbago at the L1 level, worsened by percussion. Results of other examinations were unremarkable. Laboratory findings were as follows: total protein, 6.9 g/dL; albumin, 3.5 g/dL; alanine aminotransferase, 54 IU/L; aspartate aminotransferase, 31 IU/L; lactate dehydrogenase, 235 IU/L; blood urea nitrogen, 7 mg/dL; creatinine, 0.75 mg/dL; C-reactive protein, 8.95 mg/dL; white blood cell count, 6300/μL with 65.5 % neutrophils; hemoglobin, 12.8 g/dL; and platelet count, 15.5 × 10^9/μL. C-reactive protein, a recognized inflammatory marker, was elevated, in addition to slight liver dysfunction, and white blood cell count was within the normal range without left-shifted neutrophils.

Magnetic resonance imaging (MRI) showed a fresh compression fracture with an area of increased signal intensity area suspected to be a hematoma around the L1 vertebral body (Fig. 1; A1, A2). Chest computed tomography (CT) showed bilateral airspace opacities with thickened bronchial walls and interlobular septa predominantly in the posterior and lower fields and bilateral pleural fluid (Fig. 2). There was no evidence of infective endocarditis in transthoracic echocardiography, and no infected aeurysm was noted on contrast-enhanced CT. Blood, stool, and sputum cultures were taken, and intravenous ceftriaxone (2 g every 24 h) and a single 2-g oral dose of azithromycin were administered. Blood cultures results became positive after 1-day incubation, and gram-negative rods were seen (Fig. 3A). The MALDI biotyper (MBT) Sepsityper kit® (Bruker Daltonik GmbH, Bremen, Germany) revealed “Salmonella species” with a high score value (2.20). Moreover, Salmonella Shigella bromocresol purple (SSB) agar medium (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) showed black-pigmented colonies (Fig. 3B) and triple sugar iron (TSI) agar slant (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) revealed gas and blackening of the bottom due to H2S production (Fig. 3C). The VITEK® II system (bioMérieux, Marcy l’Étoile, France) indicated the Salmonella group, while S. enterica subsp. enterica was identified by 16S rRNA gene sequencing [4]. Isolate serotyping was performed by latex agglutination using mono- and poly-valent anti-sera for O and H antigens, according to the Kauffman–White scheme. The serovar was determined to be S. enterica var. Enteritidis (O9:g:m:-).

**Fig. 1.** Spinal MRI findings of the patient. (A1, A2) Spinal MRI on day 1 shows a fresh compression fracture (white arrow in A2) with an area of increased signal intensity area suspected to be a hematoma around the L1 vertebral body. (B1, B2) Spinal MRI on day 15 shows the development of the area (yellow arrowheads) suspected to be an infection around the T12 and L1 vertebral bodies. MRI, magnetic resonance imaging.
The patient developed dyspnea during 5 days of rest despite oxygen therapy. Thereafter, her dyspnea gradually resolved without oxygen therapy; however, her lumbago deteriorated, and low-grade fever persisted. The following MRI on day 15 revealed increased signal intensity area around the T12 and L1 vertebral bodies (Fig. 1; B1, B2) at the compression fracture site. She was diagnosed with bacteremia, secondary to vertebral osteomyelitis, with pulmonary involvement caused by *S. enterica* var. Enteritidis and treated continuously with intravenous ceftriaxone (2 g every 24 h). Repeated sputum and stool cultures were negative.

**Fig. 2.** Chest CT findings on admission. Chest CT on day 1 shows bilateral airspace opacities (white arrow) with thickened bronchial walls (red arrow) and interlobular septa (yellow arrow). Additionally, consolidation is identified in the posterior lower fields (red arrowhead) with bilateral pleural fluid (yellow arrowheads). CT, computed tomography.

**Fig. 3.** Microbiological findings of positive blood cultures. (A) Gram staining (>1000) shows large gram-negative rods (yellow arrow). (B) SSB agar medium shows black-pigmented colonies. (C) TSI agar reveals blackening of the bottom due to H2S production. (D) Antimicrobial susceptibility testing resulted in susceptibility to ciprofloxacin according to MIC ≤ 0.25 μg/mL by VITEK® II (bioMérieux). However, E-test® (bioMérieux) revealed 0.094 μg/mL as the MIC of ciprofloxacin, and the disk method showed resistance to ciprofloxacin, which were interpreted as non-susceptibility to ciprofloxacin in accordance with CLSI document. SSB, Salmonella Shigella bromcresol purple; TSI, triple sugar iron; MIC, minimum inhibitory concentration; CLSI, Clinical and Laboratory Standards Institute.

| Antimicrobial agents | MIC (μg/mL) | Interpretation of susceptibility |
|----------------------|------------|----------------------------------|
| 1 Amoxicillin         | ≤ 2        | S                                |
| 2 Cefazolin           | ≤ 4        | R                                |
| 3 Cefotiam            | ≤ 8        | R                                |
| 4 Cefmetazol          | ≤ 1        | R                                |
| 5 Ceftriacine         | ≤ 1        | S                                |
| 6 Cefadizime          | ≤ 1        | S                                |
| 7 Aztreonam           | ≤ 1        | S                                |
| 8 Cefepime            | ≤ 1        | S                                |
| 9 Imipenem            | ≤ 0.25     | S                                |
| 10 Meropenem          | ≤ 0.25     | S                                |
| 11 Azithromycin       |            | S*                               |
| 12 Ciprofloxacin      | 0.094**    | NS                               |

**MIC**, minimum inhibitory concentration; **S**, susceptible; **R**, resistant; **NS**, non-susceptible. *Interpreted according to disk method **Interpreted according to E-test®.
for *S. enterica* var. Enteritidis. In contrast, *S. enterica* var. Enteritidis from blood culture indicated decreased susceptibility to ciprofloxacin. Therefore, we continued with intravenous ceftriaxone for 28 days followed by oral sulfamethoxazole/trimethoprim 1600 mg/320 mg daily for 8 weeks according to susceptibility testing (Fig. 3D). Her lumbar was relieved after 12 weeks of therapy, and she remained disease-free with no recurrence and sequelae.

**Discussion**

This report highlights the potential of vertebral osteomyelitis and pulmonary involvement caused by *S. enterica* var. Enteritidis in immunocompetent individuals and the importance of timely clinical and microbiological diagnoses, paying attention to the misinterpretation of fluoroquinolone susceptibility with conventional automated methods.

*S. enterica* var. Dublin, Choleraesuis, Heidelberg, Virchow, Enteritidis, and Hadar are generally known as microorganisms that cause severe infection in adults [1,5]. However, *Salmonella* vertebral osteomyelitis with pulmonary involvement in immunocompetent individuals is extremely rare. We searched PubMed using Medical Subject Heading (MeSH) terms “Salmonella AND osteomyelitis NOT Salmonella typhi NOT Salmonella paratyphi” and identified 47 English-language cases or case series published since 2000. We eliminated 16 cases as they were related to immunocompromised individuals. Of the remaining 31 cases, six [6–11] were regarding *Salmonella* vertebral osteomyelitis in immunocompetent individuals, summarized in Table 1. Interestingly, four (67%) of the six reports were from Asia. There were no reports in English regarding non-typhoid *Salmonella* vertebral osteomyelitis with pulmonary involvement since 2000, despite an increase in reports of these infections. Although males are at a higher risk of *Salmonella* osteomyelitis [3], two (33%) of six cases were females. In all applicable five cases, patients had a high-grade fever, and their blood examinations showed elevated C-reactive protein. In contrast, white blood cell counts were <10,000/μL in four (80%) cases. Blood cultures were positive in four (67%) of six cases. Additionally, pus or bone marrow aspirate cultures were positive in two cases where blood cultures were negative for bacteria. In other words, all positive cultures were obtained from blood (including bone marrow aspirate) or pus in cases of non-typhoid *Salmonella* vertebral osteomyelitis in healthy individuals. Vertebral osteomyelitis was diagnosed using MRI in all applicable five cases. Therefore, blood and pus cultures and MRI may be plausible tools to diagnose non-typhoid *Salmonella* vertebral osteomyelitis without past medical history.

*Salmonella* pulmonary involvement, including pneumonia, empyema, and lung abscess, is rare. Radiological findings regarding *Salmonella* pneumonia are unclear due to its rarity. In our case, chest CT on day 1 showed diffuse airspace opacities with thick bronchial walls and interlobular septa. The finding was consistent with some sort of reaction, including acute respiratory distress syndrome or hypersensitivity pneumonia.

We could not diagnose the infection directly by flexible bronchoscopy due to the coronavirus disease (COVID-19) pandemic. However, the patient lived in a new apartment and did not have a history of working in a dusty environment. Additionally, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was not detected in the nasopharyngeal specimen via polymerase chain reaction (PCR) [12]. Thus, the findings were assumed to be due to *S. enterica* var. Enteritidis infection. Consequently, the patient was treated with appropriate antibiotics without artificial ventilation or corticosteroids.

Second, it is possible to suspect and identify *Salmonella* infections based on medical history and appropriate microbiological examinations. Old individuals and those with malignancy, diabetes mellitus, rheumatoid arthritis, achlorhydria, renal diseases, liver diseases, HIV infection, and malaria, and those who use immunosuppressants and antacid medication may be susceptible to non-typhoidal salmonellae (NTS) [5]. Additionally, *Salmonella* infections can be suspected in patients with a diet history of eggs, chicken, beef, and pork [13,14]. In our case, the patient had a history of eating ready-to-eat roast beef without past medical history. Thus, we suspected the involvement of *Salmonella* species. The predominant type of *Salmonella* outbreaks has shifted from roast to ground beef since the 2000s due to regulations that ensure proper cooking, including temperature, during processing [15]. It suggests that inadequate cooking temperature correlates with non-typhoid *Salmonella* infections. Ready-to-eat roast beef consumed by our patient may have been improperly cooked and saved. We speculated that she was a carrier of *Salmonella* species; however, repeated stool cultures were negative. We also speculated that she developed *Salmonella* bacteremia due to her wound during bouldering. However, she had no significant external wounds, which led to bacteremia, despite the vertebral compression fracture. The association between the vertebral compression fracture and *Salmonella* osteomyelitis is unclear; however, she had been healthy until she went bouldering. Additionally, MRI on day 1 showed the fresh compression fracture without increased signal intensity at the L1 vertebral body, consistent with secondary infection, as the hematoma around the L1 vertebral body via the bloodstream.

Blood culture showed gram-negative rods (Fig. 3A), identified as *S. enterica* var. Enteritidis by O and H antigen typing. Generally, serotyping H antigens is a complicated and time-consuming technique. In contrast, *S. typhi* and *S. paratyphi* A, which are life-threatening strains, scarcely produce Hs and blacken the bottom on TSI agar slant instead of non-typhoid *Salmonella* [16]. Moreover, *S. enterica* subsp. enterica, with positive O9 antigen, include *S. typhi*, which is the only microorganism that does not produce gas on sugar fermentation [3]. In our case, gas production and blackening the bottom in TSI agar helped us to suspect the possibility of non-typhoid *Salmonella* such as *S. enterica* var. Enteritidis. On these points, serotyping O antigen and the finding of TSI alar slant may indicate the possibility of non-typhoid *Salmonella*.

Antimicrobial susceptibility testing resulted in susceptibility to ciprofloxacin, as its minimum inhibitory concentration (MIC) was <0.25 μg/mL, as detected by VITEK® II (bioMérieux). However, E-test® (bioMérieux, Marcy l’Etoile, France) revealed a MIC value of ciprofloxacin as 0.094 μg/mL (<0.06 μg/mL), and the disk method showed resistance to nalidixic acid. The detection limit is 0.25 μg/mL for ciprofloxacin susceptibility of Enterobacteriaceae by VITEK® II (bioMérieux). In contrast, the ciprofloxacin breakpoint of *Salmonella* species is 0.06 μg/mL according to the Clinical and Laboratory Standards Institute (CLSI) document [17]. Additionally, E-test® (bioMérieux) can detect MICs less than 0.25 μg/mL. Thus, we considered the potential of low susceptibility to ciprofloxacin, according to the document (Fig. 3D), and treated the patient with intravenous ceftriaxone followed by oral sulfamethoxazole/trimethoprim. It is important not to misinterpret the susceptibility of non-typhoid *Salmonella* while using conventional methods with inappropriate limits of detection.

Non-typhoid *Salmonella* infections can cause various extra-intestinal infections without any preceding gastrointestinal symptoms. Clinicians should consider the potential of invasive non-typhoid salmonellosis, including vertebral osteomyelitis and pulmonary involvement, in immunocompetent individuals, paying attention to decreased susceptibility or fluoroquinolone resistance. Thus, appropriate history taking, microbiological techniques, and radiological examination are essential.
| Authors            | Age (years) | Sex | Location | Preceding symptoms          | Body temperature (°C) | Blood examination | Spinal MRI | Pathogen                             | Diagnosis                        | Complication                      | Antibiotic mainly used | Antibiotic duration |
|--------------------|-------------|-----|----------|------------------------------|------------------------|-------------------|------------|-------------------------------------|----------------------------------|------------------------------|---------------------|-------------------|
| Ikejiri et al [6]  | 22          | Male | Japan    | Fever, Altered mental status, Shaking chills, Fever, Lower back pain | 42.0                   | WBC 3.010/μL, CRP 11.19 mg/dL | Vertebral osteomyelitis | *Salmonella enterica* subsp. *enterica* var. *Enteritidis* | Vertebral osteomyelitis (L3/L4) | Bacterial meningitis | Ampicillin, Levofloxacin | 12w3d            |
| Cheng et al [7]    | 29          | Female | China    | Fever, Lower back pain       | 39.5                   | WBC 10,070/μL, CRP > 16 mg/dL | Vertebral osteomyelitis with paraspinal abscess | *Salmonella enterica* subsp. *enterica* var. *Potsdam* | Vertebral osteomyelitis (L4/L5) | None | Cefazidime, Levofloxacin | 6w               |
| Gill et al [8]     | 16          | Male | Mexico   | NA                           | NA                     | Positive            | NA         | *Salmonella enterica* subsp. *enterica* var. *Oranienburg* | Vertebral osteomyelitis (T10) | None | NA | NA |
| Chang et al [9]    | 60          | Male | Taiwan   | Right flank and lower back pain, Dark urine | 38.5                   | WBC 8,700/μL, CRP 38.5 mg/dL | Vertebral osteomyelitis with paraspinal abscess | *Salmonella enterica* subsp. *enterica* var. *Oranienburg* | Vertebral osteomyelitis (L4/L5) | None | Cefotaxime, Cefpodoxime, Ciprofloxacin | 8w3d            |
| Akiba et al [10]   | 8           | Female | Japan    | Lower back pain               | 39.1                   | WBC 6,000/μL, CRP 7.5 mg/dL | Vertebral osteomyelitis with paraspinal abscess | *Salmonella enterica* subsp. *enterica* var. *Choleraesuis* | Vertebral osteomyelitis (T11/T12) | None | Ampicillin, Cefazidime | 7w               |
| Skoutelis et al [11]| 50         | Male | Greece   | Fever, Shaking chills, Lower back pain | 40.5                   | WBC 2,370/μL | Vertebral osteomyelitis | *Salmonella enterica* subsp. *enterica* var. *Westerstede* | Vertebral osteomyelitis (L2/L3) | None | Ciprofloxacin | 12w            |
| This case          | 21          | Female | Japan    | Fever, Shaking chills, Lower back pain | 40.0                   | WBC 6,300/μL, CRP 8.95 mg/dL | Vertebral osteomyelitis with paraspinal abscess | *Salmonella enterica* subsp. *Enteritidis* | Vertebral osteomyelitis (T12/L1) | Respiratory involvement | Ceftriaxone, Sulfamethoxazole/Trimethoprim | 12w            |

CSF, cerebrospinal fluid; BMA, bone marrow aspirate; MRI, magnetic resonance imaging; NA, not applicable; WBC, white blood cells; CRP, C-reactive protein; w, week; d, day.
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Consent

Written informed consent was obtained from the patient for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal on request.

Ethical approval

This study was approved by the institutional review board and ethics committee of Japanese Red Cross Ise Hospital (approval number: ER2020–24).

CRediT authorship contribution statement

Hirokazu Toyoshima: Conceptualization, Methodology. Data curation, Writing – original draft, Writing – review & editing, Visualization. Naoto Masuda: Conceptualization, Methodology. Chiaki Ishiguro: Conceptualization, Methodology, Supervision. Motoaki Tanigawa: Supervision. Hiroyuki Tanaka: Methodology. Yuki Nakanishi: Methodology. Shigetoshi Sakabe: Supervision.

Declaration of Competing Interest

The authors report no declarations of interest.

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