The Brief Case: *Salmonella enterica* Serovar Typhi in a Central American Refugee

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**CASE**

An unvaccinated teenage male with no known past medical history presented to the emergency department (ED) with complaints of headache, back and neck pain, and fever with chills for 2 days before presentation. In addition, he reported constipation (bowel movement only every 3 days), rhinorrhea, and congestion. He denied abdominal pain, shortness of breath, or photophobia. Social history revealed that he had traveled alone from Central America to the United States and had spent 7 days in the Sonoran Desert of Mexico and southern Arizona with only crackers, apples, and small amounts of water (source unknown). The patient had been in United States Border Patrol custody for 2 weeks before presenting to the ED.

The patient’s vital signs at presentation revealed a temperature of 37.1°C, heart rate of 71 beats/minute, respiratory rate of 19 breaths/minute, blood pressure of 114/68 mm Hg, and an oxygen saturation of 95%. A thorough physical exam was unremarkable. His complete blood count, including white blood cells, was normal; and his electrolyte panel showed hyponatremia of 126 mmol/liter, hypochloremia of 90 mmol/liter, and hypocalcemia of 8.4 mg/dl. Additionally, the patient’s transaminases were elevated (aspartate transaminase 144 IU/liter, alanine aminotransferase 73 IU/liter, and alkaline phosphatase 83 IU/liter). The patient was admitted for management of hyponatremia and additional workup.

On the evening of the 1st day of admission, the patient had a fever of 40.5°C and heart rate of 82 beats/minute, as well as new diarrhea and continuing headache and chills. The patient’s first set of blood cultures was drawn during this febrile episode. Treatment with cefepime (2 g every 8 h) was initiated on the 2nd day of admission, when both the aerobic and anaerobic blood culture bottles were flagged positive by the BD Bactec FX system. Gram stain of the positive blood culture broth revealed Gram-negative rods. Molecular testing of the positive blood culture broth via the Verigene Gram-negative blood culture panel (Luminex Corporation, Austin, TX) did not identify the pathogen. After a report of the patient’s positive blood culture with Gram-negative rods and because of the patient’s new-onset diarrhea, a BioFire FilmArray gastrointestinal pathogen panel (BioFire Diagnostics, LLC, Salt Lake City, UT) was ordered on a stool specimen, with *Salmonella* spp. and *Shigella/enteroinvasive Escherichia coli* isolates detected.

Subculture of the positive blood culture broth revealed gray nonhemolytic colonies on 5% sheep’s blood agar and lactose-nonfermenting colonies on both MacConkey II agar (clear colonies) and Hektoen enteric agar (green colonies with slight H2S production [Fig. 1A]). The isolate tested spot indole and oxidase negative. Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) identification of the isolate via Vitek MS (bioMérieux, Durham, NC) identified *Salmonella enterica*...
**FIG 1** Growth and biochemical characteristics of *Salmonella enterica* subsp. *enterica* serovar Typhi. (A) Growth on Hektoen enteric agar (non-lactose fermenter, slight H₂S production). (B) Serological reactivity to Remel Wellcolex Color *Salmonella* rapid latex agglutination test reagents 1 (top: green agglutination with red background = serogroup D) and 2 (bottom: red agglutination with blue background = Vi antigen), consistent with *S. Typhi*. (C) Growth on a triple sugar iron agar slant (K/A with slight H₂S production).

*subsp. enterica* (99%). Confirmatory serological typing of the isolate using the Wellcolex Color *Salmonella* rapid latex agglutination test kit (Remel, Kent, UK) resulted in green agglutination with a red background for reagent 1 and red agglutination with a blue/turquoise background for reagent 2 (Fig. 1B). This result was positive for serogroup D (reagent 1) and the Vi antigen (reagent 2), consistent with *S. enterica* subsp. *enterica* serovar Typhi (*S. Typhi*) (1). Triple sugar iron agar testing revealed alkaline over acid (K/A) with light hydrogen sulfide (H₂S) production (Fig. 1C), also consistent with *S. Typhi*. Antimicrobial susceptibility testing showed susceptibility to ampicillin (≤2 μg/ml) and ceftriaxone (≤1 μg/ml) using the bioMérieux Vitek 2 GN81 card and to ciprofloxacin (0.06 μg/ml) using the bioMérieux Etest method. The isolate was forwarded to the Arizona Department of Health Services Public Health Microbiology Laboratory.

After the initial identification of *S. enterica* subsp. *enterica*, the patient was empirically transitioned to ceftriaxone (2 g every 12 h) but continued to be febrile for ~5 days. His headache and diarrhea resolved after several days of treatment. The patient was transitioned to oral ciprofloxacin (500 mg twice daily) on the 8th hospital day and, with improving laboratory values (down-trending transaminitis and improving electrolyte levels) and improved symptoms, was discharged after a 9-day hospital admission.

**DISCUSSION**

*S. Typhi*, a member of the order *Enterobacterales*, contains Vi antigen (shared with some strains of *S. enterica* subsp. *enterica* serovars and *Citrobacter freundii*), protein flagellar antigen H₆d, and lipopolysaccharide antigens O9 (serogroup D) and O12 (1–3). *S. Typhi* is restricted to humans, and f eccal-oral transmission through contaminated food and water is the most common mode of transmission (4).

The variable incubation period (1 to 6 weeks) and the constellation of potential presenting signs and symptoms make *S. Typhi* difficult to recognize and diagnose during history taking. Patients often present with headache, a gradually rising and sustained fever, and abdominal pain but often do not complain of diarrhea (5). Gastrointestinal symptoms (including constipation) may progress to bacteremia as the infection progresses. A pulse-temperature dissociation has been reported, but tachycardia may be more common (4, 5). Contrary to many infections, white blood cell counts are often normal or reduced, as are hemoglobin and platelet levels (4). Up to 30% of patients exhibit blanching erythematous maculopapular lesions (2 to 4 mm in diameter, known as rose spots) typically located on the chest and abdomen and rarely observed on the extremities (4, 5). Complications of infection include gastrointestinal bleeding, intestinal perforation, and encephalopathy and are most commonly seen after 2 weeks of illness (4).
Several factors contribute to the pathogenicity of *S. Typhi*, including its mode of entry, latency and dissemination, and resistance patterns. The organism is thought to enter the gut wall through microfold cells (M cells) and are then phagocytosed by macrophages in the gut wall. Its downregulation of flagellar protein synthesis averts a TLR5 response, and Vi capsular synthesis offers a mask to its lipopolysaccharide components, adding to its ability to evade detection by the immune system (4, 5).

Laboratory diagnosis of *S. Typhi* in the United States is predominantly culture based. Historically, bone marrow aspirate cultures have been shown to have excellent sensitivity, especially after initiation of antimicrobial treatment. A study comparing bone marrow with blood specimens for the recovery of *S. Typhi* found ~10-fold higher numbers of CFU per milliliter of specimen in bone marrow than in blood. Additionally, this study found that lysis of host cells (red and white blood cells) in the specimen further increased the recoverable CFU counts in bone marrow specimens (6). This suggests that use of a lytic system, such as the Wampole isolator system, would be ideal for the recovery of *S. Typhi* from bone marrow culture. However, with the improvement of blood culture media and blood culture monitoring systems over the past few decades, blood cultures likely have similar sensitivity (5). Cerebrospinal fluid may be positive in very young children. Rose spots, while often culture positive, are not always present for sampling (5). Testing of multiple stool specimens can improve the sensitivity of stool culture from 10% to 30%. Positive cultures of urine, feces, or bile (duodenal string capsule) specimens should be interpreted with caution, because positive results may reflect a carrier status and not an active infection (5).

*S. Typhi* grows well in aerobic conditions at 35°C. These colonies are gray and non-hemolytic on blood agar plates and lactose nonfermenters on MacConkey agar. Hektoen enteric agar will show green (colorless) colonies and can display central black areas representing H₂S production, although *S. Typhi* is generally a weak producer of H₂S compared with other *S. enterica* subsp. *enterica* serovars. Spot indole and oxidase tests are negative. Triple sugar iron agar slants show K/A, as glucose is fermented and lactose is not, as well as weak H₂S production (sometimes described as a “wisp” or “mustache” of H₂S). Additional biochemical tests that can aid in identification of *S. Typhi* include citrate and Ornithine decarboxylase (both negative) and can help to distinguish it from other nontyphoidal *S. enterica* subsp. *enterica* serovars (both positive) and *S. enterica* subsp. *enterica* serovar Paratyphi (citrate negative, ornithine decarboxylase positive) (7).

Wild-type strains of *S. Typhi* are susceptible to chloramphenicol (rarely used in the United States because of bone marrow aplasia), amoxicillin or ampicillin, and trimethoprim-sulfamethoxazole. However, *S. Typhi* isolates continue to show increasing levels of resistance, with reduced susceptibility to ciprofloxacin (7). Historically, nalidixic acid was used as a surrogate marker for reduced fluoroquinolone susceptibility (7), although an update to antimicrobial susceptibility testing guidelines in 2013 removed that recommendation. In these revised guidelines, lower MIC breakpoints for ciprofloxacin (susceptible, ≤0.06; intermediate, 0.12 to 0.5; resistant, ≥1 µg/ml) were introduced for *Salmonella* spp. to account for decreased fluoroquinolone susceptibility mediated by quinolone resistance genes and reported treatment failures in these isolates (8). Some automated susceptibility testing systems do not test MICs low enough to account for these revised breakpoints. In these circumstances, an alternative MIC method, such as broth microdilution or gradient diffusion, is often used to verify the fluoroquinolone result. It is important to note that in addition to MIC-generating antimicrobial susceptibility testing methods, disk diffusion interpretive criteria are available for all of the recommended first-line agents for treatment of *S. Typhi* infection, including ampicillin, ciprofloxacin, trimethoprim-sulfamethoxazole, and ceftriaxone (or other third-generation cephalosporin) (9). Aminoglycosides, first- and second-generation cephalosporins, and cephamycins should not be reported as susceptible, despite how they test *in vitro*, because they are not effective clinically (7, 9).

Adequate sanitation in food preparation and strategies for providing safe drinking
water are the primary strategies for municipal prevention of S. Typhi (10, 11). Households in areas of endemicity should take precautions with hand washing before food preparation, and people should avoid eating raw or uncooked foods. Local disinfection of unsafe drinking water is also necessary to avoid transmission. Although <25% of travelers infected with S. Typhi report obtaining a vaccination (11), two effective typhoid fever vaccines are available (4, 5).

SELF-ASSESSMENT QUESTIONS

1. Which of the following statements is true regarding culture for diagnosis of S. Typhi using current methods?
   a. Stool culture is the most sensitive.
   b. Bone marrow culture is the least sensitive.
   c. Blood culture is a sensitive method.
   d. Urine culture is a sensitive method.

2. Which of the following is true regarding the laboratory workup of S. Typhi?
   a. Hemolysis is often present on 5% sheep’s blood agar.
   b. MacConkey II agar shows no lactose fermentation.
   c. Triple sugar iron agar slant shows abundant H2S production.
   d. Spot oxidase and indole tests are positive.

3. Which of the following is true regarding the presentation and course of S. Typhi infection?
   a. The white blood cell count is often normal.
   b. Incubation time is 1 to 6 days.
   c. Rose spots are commonly seen on the extremities.
   d. Transmission from respiratory droplets is common.

REFERENCES