

Enteric Fever in a 6-Year-Old Traveler Caused by *Salmonella enterica* Serotypes Typhi and Paratyphi A: Laboratory Detection Strategies and Treatment Options[∇]

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We report the first pediatric case of enteric fever caused by *Salmonella enterica* serotypes Typhi and Paratyphi A. Mixed infections are infrequently reported, potentially because detection of two different *Salmonella* serotypes in blood cultures is technically challenging. We suggest laboratory strategies to aid in the recognition of mixed infections.

CASE REPORT

A previously healthy 6-year-old boy was admitted with a history of intermittent fever and constipation for 7 days which progressed to vomiting, neck pain, myalgias, and headache for 2 days. Three weeks prior to admission, the patient had returned from a vacation to Pakistan with his family, at which point the patient had had 5 days of loose green stools which seemed to resolve. Two days prior to admission, the patient was given two doses of azithromycin by his family doctor. Of note, the patient had several sick contacts in Pakistan, including two cousins who had similar symptoms of vomiting and diarrhea, who were diagnosed with typhoid fever. Upon returning from Pakistan, the patient's father also had symptoms of nausea, indigestion, and low-grade fevers, but the father's urine, blood, and stool cultures were all negative for *Salmonella*. The patient's mother and 3-year-old brother were asymptomatic. Negative stool and urine cultures were obtained from the brother; the mother was not tested.

Blood cultures obtained upon admission turned positive for Gram-negative bacilli after 12 h of incubation in the BacT/Alert system (bioMérieux, Durham, NC). According to our laboratory's standard protocol, broth taken directly from the blood culture bottle was inoculated to a triple sugar iron (TSI) slant and MacConkey and 5% sheep blood agar plates. When the patient's physician phoned the laboratory to indicate a high suspicion of enteric fever in this patient, a drop of broth from the patient's blood culture bottle was inoculated into 3 ml of Mueller-Hinton broth and incubated at 37°C until the culture reached a turbidity equivalent to that of a 0.5 McFarland standard. This was then streaked onto a Mueller-Hinton agar plate, and a preliminary disk diffusion antibiotic susceptibility test was performed (32). An acid-over-alkaline reaction with

weak H₂S production was noted on the TSI slant, and the disk diffusion susceptibility test results appeared mixed, with growth inside the zone of inhibition for both the ampicillin and trimethoprim-sulfamethoxazole (SXT) disks. No obvious differences in colony morphology were noted on the blood or MacConkey agar subculture plates. A single colony from the blood agar was identified by API 20E (bioMérieux, Marcy l'Etoile, France) as *Salmonella enterica* serotype Paratyphi A, an organism that does not usually produce H₂S. No H₂S production was similarly noted on the API 20E performed on this colony; the organism was also negative for citrate and lysine decarboxylase, both indicative of *S. enterica* serotype Paratyphi A (17). The identification was further supported by serogrouping, using Wellcolex latex agglutination reagents (Thermo Fisher Scientific-Remel Products, Lenexa, KS), into group A. Multiple TSI reactions were repeated from several single isolated colonies from the original blood agar subculture plate, all of which also did not produce H₂S. Organisms recovered from the inner zones of ampicillin and SXT inhibition on the disk diffusion plate were subcultured, and a single colony from this process was used for API 20E testing and serogrouping by Wellcolex latex agglutination reagents. Biochemically, the organism resembled *S. enterica* serotype Typhi; in particular, negative citrate, arginine, ornithine, arabinose, and rhamnose reactions, which distinguish *S. enterica* serotype Typhi from other *Salmonella* serotypes, were noted on the API 20E. Wellcolex latex agglutination (Thermo Fisher Scientific-Remel Products, Lenexa, KS) revealed reactions with both group D and Vi antigens, also suggestive of *S. enterica* serotype Typhi. The identity of both organisms was confirmed by full serotype determination, using the Kauffman-White scheme, by the Los Angeles County Department of Public Health. The *S. enterica* serotype Paratyphi A isolate was assigned the antigenic formula I 2,12:a:1, whereas the *S. enterica* serotype Typhi isolate was assigned the antigenic formula I 9,12[Vi]:d:–. *S. enterica* serotype Typhi was the sole pathogen present in stool cultures obtained from the patient upon the resolution of constipation (2 days following admission); however, these cultures were not scrutinized for the presence of *S. enterica* serotype Paratyphi

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TABLE 1. Select antimicrobial susceptibility results

Susceptibility test and antibiotic	MIC ($\mu\text{g/ml}$) for:	
	Paratyphi A isolate	Typhi isolate
Broth microdilution		
Ampicillin	2	>32
Ceftriaxone	<0.5	<0.5
Ciprofloxacin	0.5	<0.25
Levofloxacin	<2	<2
Trimethoprim-sulfamethoxazole	<1/20	>4/80
Chloramphenicol	>32	>32
Azithromycin	16	16
Etest, azithromycin		
In inner zone of inhibition	4	4
In outer zone of inhibition	1	1
80% inhibition	2	2

A, in that only one representative of each lactose-negative colony morphotype was worked up.

Susceptibility tests were performed on each isolate using either the Clinical and Laboratory Standards Institute (CLSI) reference broth microdilution method and in-house-prepared panels or Etest (bioMerieux, Marcy l'Etoile, France). In addition, a nalidixic acid (NAL) disk diffusion test was performed to detect low-level fluoroquinolone (FQ) resistance. Susceptibility data are presented in Table 1. The *S. enterica* serotype Paratyphi A isolate was susceptible to all antibiotics tested, with the exception of NAL, for which no zone of inhibition was noted. The *S. enterica* serotype Typhi isolate was resistant to ampicillin, ampicillin-sulbactam, chloramphenicol, and SXT but susceptible to ceftriaxone. The azithromycin MIC was determined for the *S. enterica* serotype Typhi isolate using a custom reference broth microdilution method prepared in-house and by Etest (bioMerieux, Marcy l'Etoile, France). No CLSI criteria exist for azithromycin and *Salmonella*; however, the European Committee on Antimicrobial Susceptibility Testing (EUCAST) suggests a susceptibility breakpoint of $\leq 16 \mu\text{g/ml}$. The MIC by broth microdilution was $16 \mu\text{g/ml}$ for both organisms. On Etest, two zones of inhibition were noted; the MICs were read as $4 \mu\text{g/ml}$ for the inner zone of reduced growth and $1 \mu\text{g/ml}$ for the outer zone. The *S. enterica* serotype Typhi ciprofloxacin MIC was elevated, $0.5 \mu\text{g/ml}$ (Table 1), and there was no zone of inhibition surrounding the NAL disk.

The patient was initially treated with 1 g ceftriaxone (48 mg/kg of body weight/dose) once daily intravenously (i.v.) but remained febrile for 11 days. Due to persistent fevers, the ceftriaxone dose was increased to 1 g (48 mg/kg/dose) i.v. twice daily while further testing was performed to investigate for focal areas of salmonellosis. Echocardiogram results were normal; however, magnetic resonance imaging (MRI) revealed a focal area of increased signal intensity in the posteromedial aspect of the left distal femoral metaphysis which appeared to be consistent with osteomyelitis. The patient was discharged home following placement of a peripheral intravenous central catheter line for 4 weeks of extended treatment with ceftriaxone. Following the fourth week of treatment, the patient's inflammatory markers had normalized, whereupon the patient was transitioned to oral cefpodoxime, 200 mg twice a

day, for 2 more weeks. A repeat MRI revealed no evidence of osteomyelitis.

We report herein the first case of enteric fever caused by both *S. enterica* serotype Typhi and serotype Paratyphi A in a pediatric patient or in North America. Given the high prevalence of both serotype Typhi and serotype Paratyphi A in Pakistan and India (15, 19), clinicians and laboratories need to be mindful of the possibility of a mixed infection in patients who have recently visited these countries, where these pathogens are endemic. In our laboratory, setting a disk diffusion plate directly from the positive blood culture was integral to the recognition of a mixed infection, particularly as our isolates were identical in colony morphology on both blood and MacConkey agars. This procedure not only allowed the identification of a mixed culture but also gave a preliminary indication of significant antimicrobial resistance. While susceptibility results obtained by disk diffusion performed directly from blood culture broth generally agree with those obtained by testing isolated bacterial colonies from subculture (8, 27), certain organism/antimicrobial combinations may display false resistance or susceptibility when tested by this method (25, 31), and these results must be confirmed using standardized approaches.

Multidrug-resistant (MDR) *S. enterica* serotype Typhi isolates, defined as resistant to ampicillin, SXT, and chloramphenicol (16), are common in underdeveloped countries and are particularly prevalent in Pakistan, where the incidence of MDR *S. enterica* serotype Typhi isolates is 65% (19) and that of serotype Paratyphi A isolates is 44% (1). Treatment options for MDR *Salmonella* include expanded-spectrum cephalosporins, azithromycin (7), or an FQ in adults. The addition of an FQ was considered for our patient, given the length of time to respond to therapy. FQ-susceptible strains that test resistant to NAL may be associated with clinical failure or delayed response (4, 14, 21, 26, 30). Nearly all NAL-resistant *Salmonella* strains have elevated ciprofloxacin MICs (0.125 to $1.0 \mu\text{g/ml}$). It is thought that these elevated MICs are due to mutations in *gyrA*, where a single mutation is sufficient to confer resistance to NAL and reduced susceptibility to FQ. A second mutation in *gyrA* is thought to lead to high-level FQ resistance. For this reason, CLSI suggests that laboratories screen extraintestinal *Salmonella* isolates for NAL resistance as a predictor of ciprofloxacin response. However, NAL resistance may no longer be a reliable marker for decreased ciprofloxacin susceptibility. Reports of NAL-susceptible isolates with elevated ciprofloxacin MICs (2, 20) and, conversely, NAL resistance in the absence of elevated ciprofloxacin MICs (24) have arisen. Some have suggested changing the ciprofloxacin resistance breakpoint to $\geq 0.125 \mu\text{g/ml}$ to address this issue (29).

Oral azithromycin was recently found to perform significantly better than ceftriaxone for the treatment of typhoid fever (9) and was associated with fewer relapses than ceftriaxone. No CLSI interpretative criteria have been determined for the *Enterobacteriaceae*, but some suggest a breakpoint of $\leq 16 \mu\text{g/ml}$ as susceptible (3). There are also no CLSI recommendations for testing azithromycin in Gram-negative bacteria. Difficulties in interpreting azithromycin susceptibility tests per-

formed by diffusion methods (disk and Etest) have been reported (10), as dual zones of growth inhibition are observed. Furthermore, azithromycin MICs performed by Etest are generally lower than those measured by dilution methods (3), leaving the laboratory with the dilemma of how to best test and report these results. In the absence of clinical outcome data, it is unknown which method provides the most accurate predictor of patient response to treatment, a caveat that is important for the laboratory to communicate to clinicians.

The time to clear fever following the initiation of appropriate therapy is generally lengthy in cases of enteric fever, ranging from 96 to 145 h in children infected with *S. enterica* serotype Typhi (7, 20). This makes patient management difficult, as clinicians may question their choice of appropriate antibiotic therapy in the absence of clinical response. While the persistence of fever is not an adequate measure of antibiotic efficacy (28), our case clearly demonstrates the possibility of mixed infections by organisms with different antibiograms. The low reported incidence of these mixed infections (5, 6, 13, 23) and the apparently accidental laboratory findings that have led to their discovery highlight how easily this phenomenon can be missed. Laboratories and clinicians therefore need to be cognizant of this possibility and investigate any clues, from the laboratory tests or the patient's status, that a mixed infection may be present. Our patient was febrile for an excessively long period of time (265 h) in spite of i.v. ceftriaxone therapy. As resistance to ceftriaxone is exceedingly rare (18), we sought a possible focus for the infection. This led to our discovery of osteomyelitis of the distal femur; increasing the patient's ceftriaxone dose to 1.0 g twice daily resulted in successful treatment.

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