



## Case report

## Typhoid and paratyphoid fever co-infection in children from an urban slum of Delhi



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## ABSTRACT

We report two cases of co-infection with *Salmonella* Typhi and *Salmonella* Paratyphi A identified by blood culture and confirmed by serotyping from an ongoing fever surveillance cohort in an urban slum in New Delhi. Co-infections such as these have important implications on diagnosis, treatment options including choice of antimicrobial(s), disease outcome and strategy for prevention.

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## Introduction

Enteric fever, including typhoid and paratyphoid fevers, is a food and waterborne disease, known to be endemic in Indian settings. It is primarily transmitted through the fecal-oral route and is associated with poor water quality, sanitation and hygiene [1–3]. Typhoid is caused by *Salmonella enterica* serotype Typhi, whereas paratyphoid fever is caused by *Salmonella enterica* serotypes Paratyphi A, B or C. These organisms are human adapted pathogens having no animal reservoir, except *S. Paratyphi C* [1–3].

Co-infection with *S. Typhi* and *S. Paratyphi A* is known but has not been reported often [3,4]. The authors report two cases of *Salmonella* Typhi and Paratyphi A co-infection in a child from a community-based cohort in an urban slum of New Delhi where 6000 children are being followed-up for 24 months or till they reach 15 years of age. This cohort is part of a multicentric study to estimate the age-specific burden of culture-confirmed typhoid fever in the community in children aged 6 months to <15 years across India [5].

## Case report

The first case was a boy aged 8 years 5 months, belonging to a Hindu-nuclear family in Block B, Sangam Vihar, Delhi, residing in a pucca house with no overcrowding and a separate kitchen. The

child presented to the pediatric study fever clinic at Hakeem Abdul Hameed Centenary Hospital (HAHCH) with three days of fever associated with nausea, headache, sore throat, cough and abdominal pain. There was no history of rash, diarrhea, joint pains or blood in stools. On examination, an oral temperature of 98.0°F was noted. There was no contact with someone in the family with known tuberculosis and no travel history in the two weeks before the onset of illness. The child was previously vaccinated with a polysaccharide typhoid vaccine from a public health facility in March 2013 at two years of age, as documented in the immunization card. One day prior to presentation to the pediatric study fever clinic, the child was prescribed with antipyretics and amoxicillin (375 mg/kg) by a local practitioner. A provisional diagnosis of upper respiratory tract infection was made, and the antibacterial was continued for five days. Despite the medication provided, there were persisting fever spikes and the child revisited the clinic on the sixth day of fever onset with complaints of persisting high-grade fever. On examination, a temperature of 104.2°F was recorded and the child was suspected to have typhoid fever. Blood specimen of 5 mL was aseptically drawn into the Peds Plus bottle and sent to the study laboratory (Clinical and Research Laboratories-Society for Applied Studies, New Delhi) for blood culture to investigate typhoid fever.

Blood culture specimen was received in the Lab within two hours of collection and was processed into the Bactec Fx40 (Becton Dickinson, United States) which beeped positive within 24 h of incubation. Gram stained smear (K001, Hi-Media Laboratories, India) showed long and slender Gram-negative bacilli. Sub-culture was done on MacConkey Agar, Sheep Blood Agar and Nutrient Agar

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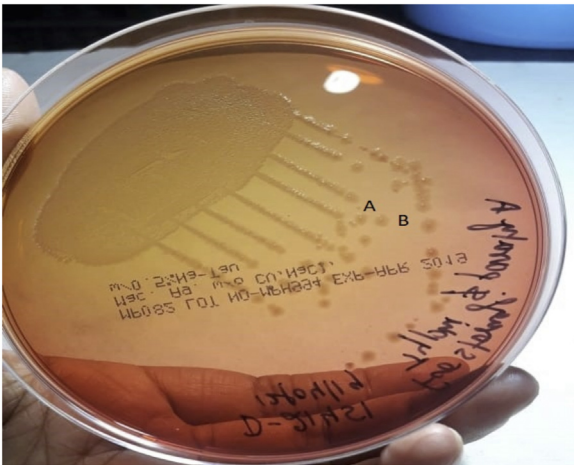
E-mail address: [drdeepak.more@yahoo.in](mailto:drdeepak.more@yahoo.in) (D. More).

plates (Pre-Prepared media plates, Hi-Media Laboratories, India) and incubated in the microbiological incubator overnight at 37 °C.

Colony morphology on MacConkey plate was small, moist, medium to large, non-lactose fermenting, further subjected to oxidase test resulting negative. Hanging drop motility showing actively motile bacilli under 40x immersion lens. Biochemical tests were performed from the growth plate and results were noted after 18–24 h of incubation in the TSI (Triple Sugar Iron) agar which showed both gas and speck of H<sub>2</sub>S. Suspecting a possible contamination, the biochemical tests were repeated and a fresh sub-culture was done. Repeat biochemical tests (two times) showed the same results.

A fresh sub-culture on the MacConkey agar plate from the blood culture bottle was done to relook at growth for a single or multiple colony types. We identified two distinct colonies; pure cultures of both the colonies were isolated and processed (Fig. 1).

Biochemical Identification- (A) Small, round colonies yielded alkaline/acid reaction with gas and no H<sub>2</sub>S, did not decarboxylate lysine (Fig. 3) and (B) Large, moist, irregular colonies yielded alkaline/acid reaction with speck of H<sub>2</sub>S and no gas, decarboxylated lysine (Fig. 2). Both the organisms were oxidase negative, motile, did not utilise citrate, indole negative and mannitol fermentative.



**Fig. 1.** Blood culture growth on MacConkey Agar showing non-lactose fermenting colonies with two different morphologies – small round (A) and large irregular (B).

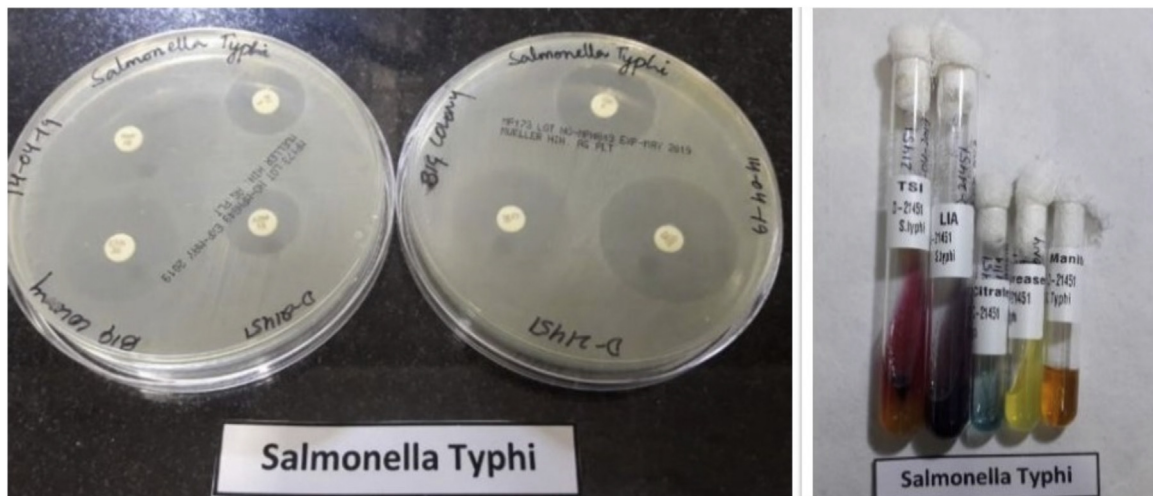
Serotype Identification- Larger colonies showed visible agglutination with 'O9' and 'd' antisera (procured from CRI, Kasauli, Himachal Pradesh) by slide agglutination method and were confirmed to be *Salmonella* Typhi. Smaller colonies showed visible agglutination with 'O2' and 'a' antisera (procured from CRI, Kasauli) by slide agglutination method and were confirmed to be *Salmonella* Paratyphi A.

Antibiotic susceptibility testing was performed for both the colonies separately, by Kirby-Bauer disc diffusion method. Both organisms showed similar antibiograms with intermediate susceptibility to ciprofloxacin, resistance to pefloxacin and susceptibility to ampicillin, azithromycin, chloramphenicol and cotrimoxazole (Figs. 2 and 3). Based on the lab findings, the child was diagnosed to be infected with both *Salmonella* Typhi and *Salmonella* Paratyphi A.

Based on the culture results, the child was treated with the combination therapy of azithromycin (20 mg/kg) and cefixime (10 mg/kg) for five days as per the advice of the pediatrician at our study fever clinic. The child recovered after five days of treatment without any complications. The total duration of the episode was 14 days. Repeated episodes of fever were observed in the child during the total follow-up period at 8 yr 10 m 23d, 8 yr 10 m 29d and 9 yr 1 m 11d ages. These fever episodes lasted for 11, 6 and 4 days, respectively. Blood culture revealed no growth in the episode that lasted for 11 days and the family did not agree for blood testing for the subsequent two episodes. All episodes subsided without any sequelae.

The child belonged to a four-membered family with highest education up to senior secondary level and monthly income of INR 6000/-. The younger sibling of the index child, a four-year-old female, also had an episode of culture-confirmed typhoid fever in six months after episode in the index case and was treated with cefpodoxime for four days and azithromycin for eight days. Child recovered without any hospitalization and further complications. There was no history of fever among the other family members.

A similar case of co-infection was reported in a girl aged four years, belonging to low socio-economic Hindu joint family in January 2020 from the same cohort. The child presented on the third day of fever with sore throat, cough, and abdominal pain. On physical examination in the pediatric study clinic, the child was found to be febrile with an oral temperature of 102.4°F and a provisional diagnosis of URTI was made. The child was prescribed antipyretics and azithromycin syrup 5 mL for five days, blood culture was performed. Blood culture showed Gram-negative bacilli. Sub-culture on MacConkey agar showed two types of



**Fig. 2.** Results of disc diffusion susceptibility testing (left) and biochemical reactions (right) of *Salmonella enterica* serovar Typhi.



Fig. 3. Results of disc diffusion susceptibility testing (left) and biochemical reactions (right) of *Salmonella enterica* serovar Paratyphi A.

colonies superimposed. A repeat sub-culture showed a pure culture of small, moist, regular edged non-lactose fermenting and large, moist, irregular edged non-lactose fermenting colonies. Biochemical identification and serotyping were performed for both colony types and identified to be as *Salmonella* Paratyphi A and *Salmonella* Typhi respectively.

The child was admitted to the Pediatric Intensive Care Unit (PICU) on the eighth day of illness due to persisting fever and complaints of two episodes of loose stools along with vomiting. The child was treated with IV ceftriaxone 700 mg for 10 days. The final diagnosis was complicated enteric fever with shock and moderate iron deficiency anemia. The child was further prescribed Syp Tonoferon (80 mg/5mL) for three months and Syp Beevon 5 mL for 15 days for anemia management and recovered without any further complications. Hospitalization duration was for 11 days; total duration of episode was 17 days. No other family members were diagnosed with enteric fever during the study period.

We captured household WASH practices in both the cases. In the first case, the main source of drinking water was piped water from Delhi Jal board and that in the second case was tube well water. Neither of the two households practiced any further treatment of water before drinking. Both used a pit latrine with slab which was not shared with other households. The frequency of buying ready-to-eat food from street vendors was around once a month and once a week in the first and second household, respectively.

## Discussion

*Salmonella* Typhi and *Salmonella* Paratyphi A co-infection is not uncommon and has implications on clinical management. While similar treatment strategies may work for both organisms, serotypes with different antibiograms can pose challenge to successful treatment in areas where multi drug-resistant strains are common. In India, the extensively drug-resistant H58 haplotype of *S. Typhi* has been reported, harboring a promiscuous plasmid conferring resistance to fluoroquinolones and third generation cephalosporins [6]. Multidrug-resistant *Salmonella* Paratyphi A harboring IncHI1 plasmids like those found in serovar Typhi have also been reported [7]. Such co-infections are likely to cause severe illness and may require hospitalization.

Infections caused by *S. Paratyphi* A have been increasing, particularly in Asia [1,8]. In a study by Pratap et al., 2014 [9] occurrence of co-infection with *S. Paratyphi* A was seen in >40% of the typhoid cases and chronic typhoid carriers by nested PCR.

Mixed infections with *S. Typhi* and *S. Paratyphi* have also been previously reported from hospital settings in India [10–13]. This co-infection may be more common than suspected and is often missed due to indistinguishable clinical features [14,15].

In a case of dual infection, both strains could have the same susceptibility profiles [10] or may differ in their antibiograms [11,12]. The strains isolated in the reported cases had the same antibiotic sensitivities. In our ongoing cohort, the cases of co-infection seem to be more severe in terms of duration of illness (14–17 days) as compared to infections with single organisms (median duration 9 days) and therefore require prolonged care [1]. The severity of fever was also higher in these cases (102–104°F).

Vigilant observation during lab testing is critical for diagnosis. During conventional processing of a clinical *Salmonella* isolate, presence of trace elements of hydrogen sulphide and gas should alert the microbiologist to the possibility of a co-infection. Further incubation of the culture plates could show two types of colonies (e.g. large and small). Going beyond blood cultures, novel multiplex PCR protocols can be useful for diagnosis of enteric fever infections [16].

WHO recommends programmatic use of typhoid vaccines to control typhoid fever, especially in countries with high burden of the disease and antimicrobial resistance [17]. New prevention strategies targeting both Typhi and Paratyphi together may be helpful in South Asia where co-infections are common. Polyvalent vaccines that protect against *S. Typhi* as well as *S. Paratyphi* A may be of greater relevance in these settings. Vaccines using Vi antigen as principal agent lacks ability to elicit cross protection against paratyphoid A. The approved typhoid vaccines (Vi Polysaccharide and live oral Ty21a) do not seem to be efficacious against *S. Paratyphi* A infection [18,19]. Increasing mixed infections with *S. Typhi* and *S. Paratyphi* A, may necessitate development of novel control strategies [18].

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## References

- [1] Crump JA, Mintz ED. Global trends in typhoid and paratyphoid fever. *Clin Infect Dis* 2010;50(January (2)):241–6.
- [2] Näsström E, Thieu NT, Dongol S, et al. *Salmonella* Typhi and *Salmonella* Paratyphi A elaborate distinct systemic metabolite signatures during enteric fever. *Elife* 2014;5(Jun (3)):e03100.
- [3] Arndt MB, Mosites EM, Tian M, et al. Estimating the burden of paratyphoid A in Asia and Africa. *PLoS Negl Trop Dis* 2014;8(6):e2925, doi:http://dx.doi.org/10.1371/journal.pntd.0002925.
- [4] Sztein MB, Salerno-Goncalves R, McArthur MA. Complex adaptive immunity to enteric fevers in humans: lessons learned and the path forward. *Front Immunol* 2014;(October (5)):516.
- [5] John J, Bavdekar A, Rongsen-Chandola T, et al. Estimating the incidence of enteric fever in children in India: a multi-site, active fever surveillance of pediatric cohorts. *BMC Public Health* 2018;18(December (1)):594.
- [6] Klemm EJ, Shakoor S, Page AJ, et al. Emergence of an extensively drug-resistant *Salmonella* enterica serovar Typhi clone harboring a promiscuous plasmid encoding resistance to fluoroquinolones and third-generation cephalosporins. *mBio* 2018;9(March (1)):e00105–18.
- [7] Holt KE, Thomson NR, Wain J, et al. Multidrug-resistant *Salmonella enterica* serovar Paratyphi A harbors IncHI1 plasmids similar to those found in serovar Typhi. *J Bacteriol* 2007;189(June (11)):4257–64.
- [8] Zhou Z, McCann A, Weill FX, et al. Transient Darwinian selection in *Salmonella enterica* serovar Paratyphi A during 450 years of global spread of enteric fever. *Proc Natl Acad Sci USA* 2014;111(August (33)):12199–204.
- [9] Pratap CB, Kumar G, Patel SK, et al. Mix-infection of *S. Typhi* and Paratyphi A in typhoid fever and chronic typhoid carriers: a nested PCR based study in North India. *J Clin Diagn Res* 2014;8(November (11)):DC09–14.
- [10] Debdas D, Joshi S. Mixed *Salmonella* infection. *Indian J Med Microbiol* 2008;26(July (3)):287.
- [11] Adhikary R, Joshi S. Dual *Salmonella typhi* infection. *Indian J Pathol Microbiol* 2011;54(October (4)):849.
- [12] Perera N, Geary C, Wiselka M, et al. Mixed *Salmonella* infection: case report and review of the literature. *J Travel Med* 2007;14(March (2)):134–5.
- [13] Arya SC, Agarwal N. Mixed *Salmonella* infection: case report and review of literature. *J Travel Med* 2007;14(September (5)):356.
- [14] Grema BA, Aliyu I, Michael GC, et al. Typhoid ileal perforation in a semi-urban tertiary health institution in north-eastern Nigeria. *S Afr Fam Pract* (2004) 2018;60(September (5)):168–73.
- [15] Chalya PL, Mabula JB, Koy M, et al. Typhoid intestinal perforations at a University teaching hospital in Northwestern Tanzania: a surgical experience of 104 cases in a resource-limited setting. *World J Emerg Surg* 2012;7(December (1)):4.
- [16] Ali A, Haque A, Haque A, et al. Multiplex PCR for differential diagnosis of emerging typhoidal pathogens directly from blood samples. *Epidemiol Infect* 2009;137(January (1)):102–7.
- [17] World Health Organization. Typhoid vaccines: WHO position paper, March 2018–recommendations. *Vaccine* 2019;37(January (2)):214–6.
- [18] Carbis R, Sahastrabudde S, Wierzbza TF, et al. Increasing rates of *Salmonella* Paratyphi A and the current status of its vaccine development. *Expert Rev Vaccines* 2013;12(9):1021–31, doi:http://dx.doi.org/10.1586/14760584.2013.825450.
- [19] Wahid R, Simon R, Zafar SJ, et al. Live oral Typhoid vaccine Ty21a induces cross-reactive humoral immune responses against *Salmonella enterica* serovar Paratyphi A and S. Paratyphi B in humans. *Clin Vaccine Immunol* 2012;19(June (6)):825–34, doi:http://dx.doi.org/10.1128/CVI.00058-12.