

## Mixed Salmonella infection: a case series from Pakistan

Binish Arif Sultan, Naima Fasih, Saba Qaiser, Erum Khan, Afia Zafar, Seema Irfan

### Abstract

Enteric fever remains a major health problem in the developing world, including Pakistan. Poor sanitation and hygienic conditions are the major predisposing factors. Salmonella infection with different strains in the same patient has rarely been reported previously. We are reporting two cases of bacteraemia with simultaneous detection of two strains of Salmonella in a single episode of infection. In both the cases, 2 different serotypes of Salmonella were causing bacteraemia leading to fever. In highly endemic area, one must be aware of mixed Salmonella infections as inappropriate diagnosis of such infections may lead to treatment failure.

**Keyword:** Mixed Salmonella infection.

### Introduction

Enteric fever is a global health problem responsible for 21 million cases and 21,000 deaths annually. Of these, 80% of cases occur in Asia alone. Pakistan is a high-burden country with an annual incidence of 413/100,000 person/year.<sup>1</sup> Inadequate sanitary conditions and lack of personal hygiene are the key factors responsible for its prevalence in this part of the world.

Mixed Salmonella infection with different strains in the same patient has rarely been reported previously even from a highly endemic country like Pakistan. We are reporting two cases of bacteraemia with simultaneous detection of two strains of Salmonella in a single episode of infection.

### Case Report

A five-year-old female child was brought to a general practitioner with complaints of high-grade fever and vomiting for five days. On examination, the only positive finding was fever of 39°C. Investigations revealed haemoglobin level of 8.7g/dl, platelets 265 and total leukocyte count (TLC) of 7900, with 39.9% neutrophils, 50.2% lymphocytes and 9.9% monocytes.

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Department of Pathology Microbiology, Aga Khan University Hospital, Karachi, Pakistan.

**Correspondence:** Naima Fasih. Email: naima.fasih@aku.edu

Serology was negative for Salmonella Typhi IgG and IgM (TyphoidIgG/IgM; CTK Biotech, Inc; USA). Blood culture was also requested by the physician. It was processed using automated system (Bactec 9240). Blood culture came positive in 24 hours of incubation and on Gram stain showed Gram-negative rods. Sub-culture from blood culture bottle was performed on chocolate and MacConkey agar plates. Direct susceptibility testing of unknown Gram-negative rod was also set simultaneously using Mueller Hinton Agar (MHA) plates. After 24 hours of 37°C aerobic incubation, MacConkey agar plates showed growth of single morphotype of non-lactose fermenting colonies. However, the direct antimicrobial susceptibility plates revealed dual zones of inhibition around nalidixic acid. Identification and sensitivity were repeated from isolated colony grown on MacConkey agar which turned out to be Salmonella Paratyphi A, susceptible to nalidixic acid, ciprofloxacin, ceftriaxone and cefixime. The colonies growing around nalidixic acid on primary susceptibility plate were isolated, and identification and drug susceptibility were performed. That isolate was identified as Salmonella Typhi sensitive to ceftriaxone and cefixime, but resistant to nalidixic acid, and had higher minimum inhibitory concentration (MIC) (0.5µg/ml) of ciprofloxacin. Both strains were resistant to first-line antibiotics i.e. ampicillin, chloramphenicol and cotrimoxazole. To make sure that both isolates belonged to the same patient, repeat sub-culture and primary sensitivities were performed from the blood culture bottle, which showed the same results. The patient was treated with cefixime 20mg/kg divided 12 hourly for 14 days, and she soon improved.

In the other case, a three-year-old boy presented with continuous high-grade fever and weakness for 5 days to a general practitioner. The boy was a traveller from Canada, visiting Karachi in summer vacations. His general and systemic examinations were unremarkable except fever of 39°C. He had no history of having unhygienic food and water. He had a TLC of 6000 with 40% neutrophils and 55% lymphocytes. Rapid diagnostic test for malaria (Malaria Antigen p.f/Pan, Standard Diagnostics, INC; Korea) was negative. Blood culture was also sent. Done by Bactec 9240 system, it

Table-1: Strains and their antibiogram.

Strain type	AMP (10µg)	C (30µg)	SXT (25µg)	CRO (30µg)	CFM (5µg)	Nalidixic acid(30µg)	CIP (5µg)
<b>Case 1</b>							
Salmonella Para Typhi A	R	R	R	S	S	S	S
Salmonella Typhi	R	R	R	S	S	R	R
<b>Case 2</b>							
Salmonella species	R	S	R	R	R	S	S
Salmonella species	R	S	R	S	S	R	R

AMP: Ampicillin; C: Chloramphenicol; SXT: Co-trimoxazole; CRO: Ceftriaxone; CFM: Cefixime; CIP: Ciprofloxacin; R: Resistant; S: Sensitive.

signalled positive in 24 hours of incubation, showing Gram-negative rod on Gram stain. Sub-culture on chocolate and MacConkey agar plates and direct susceptibility on MHA were performed. Two different types of non-lactose fermenting oxidase-negative colonies were observed on chocolate agar plate and mixed susceptibility pattern was observed on MHA. Two different strains of Salmonella species were identified with Analytical Profile Index (API) 20E, each with different antibiotic susceptibility pattern (Table). One strain was resistant to ciprofloxacin and co-trimoxazole, and the second one was resistant to third-generation cephalosporin. He was empirically prescribed intravenous ceftriaxone by the general practitioner, but was lost to follow-up.

## Discussion

In contrast to the two cases with mixed Salmonella infection that this paper has reported, a literature search showed paucity of such reported cases even from endemic areas. To the best of our knowledge, this is the first case series of mixed Salmonella infection reported from Pakistan.

Both of our cases belonged to the paediatric age group. The reason behind this could be higher prevalence of Salmonella infection in this age group. However, other reported cases in literature have related to different age groups. For example, one reported case of mixed Salmonella Typhi and Paratyphi A infection related to a 20-year-old man.<sup>2</sup> Another was reported in an 81-year-old woman.<sup>3</sup> Like our cases, a case reported mixed Salmonella Typhi and Paratyphi A infection in a six-year-old.<sup>4</sup> Most of the cases referred above are from the developed world with history of travel to endemic areas. However, very sparse published data is available from high-burden countries like Pakistan. Possible reasons for this discrepancy include empirical use of antibiotics for febrile illnesses, use of serological test to get quicker information, and the non-availability of standard laboratories for performing blood culture in

developing countries.

Serological diagnosis was initially misleading in our first case, but was picked up by blood culture detection. Similar misleading experience was also encountered previously in a study that showed lack of sensitivity of serological test.<sup>2</sup> In our second case, serological testing for Typhoid was not performed and only blood culture was done along with baseline investigations.

The common finding in both of our cases was the growth of second strain within the susceptibility zones of the other strain. In the first case, suspicion was raised because double zone of inhibition was identified on the susceptibility plate. In our laboratory, this is a routine practice to set Kirby Bauer disc diffusion test directly from all positive blood culture bottles. This gives early preliminary antimicrobial resistance and helps in tailoring the antibiotic therapy. In most of the cases, there is an agreement between primary susceptibility results with standardised susceptibility testing (those obtained by isolated colony testing).<sup>5,6</sup> Here, in both our cases, discrepancy between the primary susceptibility and standardised susceptibility testing helped to raise suspicion for the possibility of more than one type of strain in culture.

Most of the reported cases showed mixed infection with Salmonella Typhi and Paratyphi A, as in our first case. However, the second case revealed two different Salmonella species. Non-typhoidal Salmonella infections are usually related to immuno-compromised status, but in our scenario we failed to evaluate the immunity status of the patient, his further course of infection and response to treatment. We found only a single case report in literature of mixed non-typhoidal Salmonella infection which related to a 21-year-old male, known case of haemophilia A, infected with dual strains. Salmonella Typhimurium was isolated from blood culture and Salmonella group C2 from the stool. Salmonella Typhimurium was pan-sensitive, but Salmonella group C2 was resistant to ampicillin and

chloramphenicol.<sup>7</sup>

### Conclusion

There is a need to put emphasis on awareness of mixed infection among clinical microbiologists and general practitioners. Because of differences in drug susceptibility, failure to diagnose mixed infection could result in inadequate antibiotic treatment. This shows the importance of diagnostic capabilities of laboratories, particularly in an era where antimicrobial resistance is on a rising trend.

### Acknowledgement

We would like to thank all the staff of Clinical Microbiology Laboratory of Aga Khan University, Karachi, for providing due assistance.

### References

1. Ochiai RL, Acosta CJ, Danovaro-Holliday MC, Baiqing D, Bhattacharya SK, Agtini MD, et al. A study of typhoid fever in five Asian countries: disease burden and implications for controls. *Bull World Health Organ* 2008; 86: 260-8.
  2. Joshi S, Wattal C, Sharma A, Prasad KJ. Mixed Salmonella infection — a case report. *Indian J Med Microbiol* 2002; 20: 113-4.
  3. Perera N, Geary C, Wiselka M, Rajakumar K, Andrew Swann R. Mixed Salmonella infection: case report and review of the literature. *J Travel Med* 2007; 14: 134-5.
  4. Humphries RM, Yeganeh N, Ward KW, Lewinski MA, Ching N. Enteric fever in a 6-year-old traveler caused by Salmonella enterica serotypes Typhi and Paratyphi A: laboratory detection strategies and treatment options. *J Clin Microbiol*; 2011; 49: 452-4.
  5. Edelmann A, Pietzcker T, Wellinghausen N. Comparison of direct disk diffusion and standard microtitre broth dilution susceptibility testing of blood culture isolates. *J Med Microbiol* 2007; 56: 202-7.
  6. Tan TY, Ng LS, Kwang LL. Evaluation of disc susceptibility tests performed directly from positive blood cultures. *J Clin Pathol* 2008; 61: 343-6.
  7. Papasian CJ, Bartholomew WR, Neter E, Amsterdam D. Recovery of Salmonella group B from blood and Salmonella group C2 from feces and serological evidence of dual infection in one patient. *J Clin Microbiol* 1984; 20: 584-5.
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