Case Report

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First case report of blood and urine cultures positive bacteraemia by *Salmonella enterica* serotype Choleraesuis from India

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Introduction: Non-typhoidal *Salmonella* (NTS) are commonly implicated in causing bacteraemia in infants, the elderly and immunosuppressed individuals in sub-Saharan African countries. However, NTS bacteraemia in otherwise healthy adults from India has been rarely reported. Here, we report a case of bacteraemia caused by *Salmonella enterica* serovar Choleraesuis (S. Choleraesuis), isolated simultaneously from the blood and urine of an adult febrile patient from Kolkata, India.

Case Presentation: A middle-aged man was admitted to a private hospital in Kolkata with clinical suspicion of acute enteric fever on 25 October 2013. His blood report showed neutropenia and mild thrombocytopenia, with an elevated C-reactive protein level. The Widal test was negative. S. Choleraesuis isolates were grown simultaneously by microbiological culture of blood and urine samples. The patient recovered without complications following antibiotic therapy. On further characterization, both of the S. Choleraesuis isolates showed identical antibiotic-susceptibility patterns and virulence-gene, plasmid and PFGE profiles, confirming their clonality (100 % similarity).

Conclusion: This is the first report of S. Choleraesuis bacteraemia associated with a human infection in India. The identification and reporting of uncommon *Salmonella* serovars from various countries are important for understanding the global epidemiology of salmonellosis.

Keywords: bacteraemia; ciprofloxacin; enteric disease; parenteral ceftriaxone; *Salmonella* Choleraesuis; salmonellosis.

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Introduction

Salmonella is one of the most common bacterial food- and water-borne pathogens. It primarily has four different clinical manifestations: enteric fever, gastroenteritis, bacteraemia and an asymptomatic carrier state (Coburn *et al.*, 2007). Although systemic infections such as enteric fever caused by serovars Typhi and Paratyphi are common in developing countries such as India (Ochiai *et al.*, 2008), invasive salmonellosis caused by non-typhoidal *Salmonella* (NTS) species has been more frequently reported from sub-Saharan African countries (Gordon *et al.*, 2008; Mtove *et al.*, 2010) and south-eastern Asian countries such as Taiwan (Chen *et al.*, 2007; Chiu *et al.*, 2004; Jean *et al.*, 2006). While a longer duration of fever and younger age (school age) are associated with typhoid fever, invasive NTS infections are more common in patients with malaria, anaemia, jaundice, hypoglycaemia, malnutrition, HIV infection and other immunosuppressive conditions

(Chen *et al.*, 2007; Chiu *et al.*, 2004; Gordon *et al.*, 2008; Mtove *et al.*, 2010).

Among more than 2500 serotypes of *Salmonella enterica*, certain serotypes such as Typhimurium and Enteritidis have a broad host range, and some animal-adapted serotypes such as Choleraesuis (swine) and Dublin (cattle) show a much higher predilection for causing invasive disease in humans (Chiu *et al.*, 2004; Coburn *et al.*, 2007). Although there have been occasional reports of NTS septicemia from India (Patil *et al.*, 2006; Randhawa *et al.*, 2006), this is the first report of S. *enterica* serovar Choleraesuis (S. Choleraesuis) bacteraemia from India where the organism was simultaneously isolated from the blood and urine samples of a hospitalized, middle-aged, febrile patient in Kolkata.

Case report

A 55-year-old male was admitted to the Advanced Medical Research Institute Hospital in the eastern part of Kolkata

Abbreviation: NTS, non-typhoidal *Salmonella*.

on 25 October 2013, complaining of diarrhoea and fever for 5 days. Clinically, he was suspected of suffering from enteric fever. On physical examination the patient was found to be febrile, with a body temperature of 38.8 °C (102 °F), blood pressure of 110/80 mmHg and relative bradycardia (62 bpm pulse rate). There was no cardiac murmur. The patient's spleen was palpable without tenderness and his skin was normal, with no rash. On admission, blood was drawn for routine examination, culture sensitivity, serology and liver function tests. A mid-stream clean-catch urine sample was also taken for routine examination and culture sensitivity to exclude a concurrent urinary tract infection. A stool sample was taken for routine tests and microbiological culture to isolate any enteric pathogens.

Liver function tests were normal. The total white blood cell count was 8400 cu mm⁻¹. The differential white blood cell count suggested neutropenia (50 %), and mild thrombocytopenia (120,000 cu mm⁻¹) was recorded. The C-reactive protein level was 14.8 mg l⁻¹ and serological tests for typhoid and paratyphoid fever (Widal), dengue fever (ELISA) and malarial parasites (quantitative buffy-coat method) were negative. Wet (saline and iodine) mount of stool samples showed few pus cells, occasional cysts of *Entamoeba histolytica* and absence of ova or trophozoites. Microscopic examination of urine was negative for the presence of casts, crystals and red blood cells. However, pus cells (5–7 cells per high-power field) and motile bacteria were seen. The stool culture was negative for potential pathogens.

Blood and urine cultures yielded non-lactose-fermenting colonies on MacConkey agar within 24 h. Gram-stained smears of pure colonies from both samples showed the presence of Gram-negative rods. Based on results of biochemical tests such as triple-sugar iron agar, lysine iron agar, mannitol motility, indole and Simmons citrate agar, organisms from both samples were provisionally identified as *Salmonella* species following standard procedures. The isolates were sent to the National Institute of Cholera and Enteric Diseases, Kolkata, for confirmation of their identification and further serotyping.

The isolates were tested at the Advanced Medical Research Institute for their antimicrobial susceptibility on Mueller–Hinton agar using the Kirby–Bauer disk-diffusion method against a panel of the following antimicrobials: ampicillin (10 µg), chloramphenicol (30 µg), tetracycline (30 µg), co-trimoxazole (25 µg), nalidixic acid (30 µg), ciprofloxacin (5 µg), norfloxacin (10 µg), ofloxacin (5 µg), cefotaxime (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), aztreonam (30 µg), amoxiclav (30 µg) and azithromycin (15 µg) (BD BBL™ Maryland, USA). The results were interpreted according to Clinical and Laboratory Standards Institute guidelines (CLSI, 2012) and *Escherichia coli* ATCC 25922 was used as a control. The isolates were pan-susceptible to all of the antimicrobials tested. At the National Institute of Cholera and Enteric Diseases, both of the isolates were

identified as *S. Choleraesuis* (6,7: c:1,5) by slide and tube agglutination using *Salmonella* poly- and monovalent O and H antisera (Denka Seiken, Tokyo, Japan). The results were interpreted according to the Kauffmann–White scheme.

Following sample collection, the patient was immediately given empirical treatment with parenteral ceftriaxone (2 g twice daily) in anticipation that the patient was suffering from typhoid fever; most of the recent *S. Typhi* Kolkata isolates had shown decreased susceptibility to ciprofloxacin (Dutta *et al.*, 2014). When the culture-susceptibility results became available, the clinician added ciprofloxacin (200 mg twice daily) for 10 days to prevent relapse, which is common with *S. Choleraesuis* infection (Wang *et al.*, 2006). The patient showed clinical improvement (subsidence of fever and other symptoms) with antibiotic therapy and recovered without complications. He was discharged after 10 days of treatment, when blood and urine cultures became sterile.

Simultaneous isolation of *S. Choleraesuis* from the blood and urine samples of a single patient prompted us to investigate whether the isolates were identical with respect to their genetic makeup. DNA fingerprinting was performed by PFGE of *Xba*I-digested DNA from both isolates using the CHEF-DR III system (Bio-Rad), following the standard PulseNet 1-day protocol and using *Salmonella* serotype Braenderup H9812 as the control (CDC, 2013). The PFGE profiles were analysed using FP Quest software, version 4.5 (Bio-Rad). The extent of homology was determined using the Dice coefficient, and clustering was based on the unweighted pair group method with arithmetic mean. The isolates had identical PFGE patterns (Dice coefficient of similarity 100 %) and were described as genetically indistinguishable (Fig. 1).

Fig. 1. PFGE patterns of *Xba*I-digested DNA from *S. Choleraesuis* Kolkata isolates. Lane 1, *S. Choleraesuis* (blood isolate); 2, *S. Choleraesuis* (urine isolate); M, *S. Braenderup* H9812 (PFGE standard strain). Strains in lanes 1 and 2 were clonal.

Fig. 2. Plasmid profiles of *S. Choleraesuis* Kolkata isolates. Lane 1, *Shigella flexneri* YSH6000 (plasmid molecular-weight marker for heavy plasmids); 2, *E. coli* V517 (plasmid molecular-weight marker for smaller plasmids); 3, *S. Choleraesuis* (blood isolate); 4, *S. Choleraesuis* (urine isolate). Strains in lanes 3 and 4 showed identical plasmid profiles.

In addition, the isolates were also studied for their plasmid profiles following plasmid extraction by the Kado and Liu method (Kado & Liu, 1981) and electrophoresing on 0.8 % agarose at 70 V for 3 h. *E. coli* V517 and *Shigella flexneri* YSH6000 were used as plasmid molecular-weight markers. Both of the isolates harboured three identical plasmids of sizes 50, 5.1 and 4.6 kb (Fig. 2). The plasmids were of incompatibility types IncFIIS and IncFIB. PCR detection of virulence genes showed the presence of *spvB* and *spvC* (virulence plasmid), *pef* (plasmid-encoded fimbriae), *invA* (invasion protein) and *stn* (enterotoxin) genes in both isolates. All PCRs were carried out using suitable published primers and including suitable positive and negative controls (Soto *et al.*, 2006; Villa *et al.*, 2010).

Discussion

S. Choleraesuis is a swine-adapted serotype of *S. enterica* that causes swine paratyphoid and is highly pathogenic to humans. *S. Choleraesuis* is of particular concern in Taiwan, where it is the second most common serotype to cause human infections (after *S. Typhimurium*) (Chen *et al.*, 2007; Chiu *et al.*, 2004). It is usually associated with bacteraemia and extraintestinal focal infections, including mycotic aneurysms, osteomyelitis and pleuropulmonary infections in both children and adults, with little or no involvement of the gastrointestinal tract (Chen *et al.*, 2007; Chiu *et al.*, 2004; Jean *et al.*, 2006).

The global epidemiology of NTS serotypes causing human bloodstream infections varies greatly in different countries. In sub-Saharan African countries, *S. Typhimurium* followed by *S. Enteritidis* are the two most common serovars associated with bacteraemia (Gordon *et al.*, 2008). In India, only a few reports have documented the isolation of NTS from blood, with no predominant serotype. *S. Typhimurium* and *S. Virchow* have been isolated from paediatric sepsis cases in

Chennai and New Delhi, respectively (Karthiyeekan *et al.*, 2011; Randhawa *et al.*, 2006). Another report has documented the isolation of *S. Worthington* and *S. Weltevreden* from neonatal sepsis cases from Pune and South India, respectively (Muley *et al.*, 2004; Patil *et al.*, 2006). In Pondicherry, *S. Agona* and *S. Typhimurium* have been isolated from adult patients complaining of diarrhoea and fever (Menezes *et al.*, 2010). So far, to the best of our knowledge, this is the first report from India of human bacteraemia caused by *S. Choleraesuis*, in which isolates were recovered both from blood and urine samples.

A healthy individual might suffer from bacteraemia caused by NTS isolates (Chiu *et al.*, 2004; Coburn *et al.*, 2007; Jean *et al.*, 2006). The study patient had no underlying disease, was not diabetic and was not taking any medication. He did not have a history of foreign travel in the past year. The patient was of middle socioeconomic status. He resided in the city and owned two cattle farms on the outskirts of Kolkata. Because he was involved in cattle-rearing activities, he had frequent contact with farm animals (e.g. cows, goats), which might have been the source of his infection.

Both of the study isolates were pan-susceptible to all drugs tested, which is in contrast to earlier reports from Asian countries such as Taiwan and Thailand where the organisms were multidrug resistant (Chiu *et al.*, 2004; Jean *et al.*, 2006; Kulwichit *et al.*, 2007; Su *et al.*, 2011), indicating a different origin of the Indian isolates. In Taiwan, *S. Choleraesuis* resistant to first-line drugs (e.g. ampicillin, chloramphenicol and co-trimoxazole) has been reported since 2000, since when increasing numbers of isolates have shown resistance to ciprofloxacin and ceftriaxone (Chiu *et al.*, 2004; Jean *et al.*, 2006; Kulwichit *et al.*, 2007; Su *et al.*, 2011).

The previously reported presence of a 50-kb virulence plasmid of incompatibility types IncFIIS and IncFIB in *S. Choleraesuis* (Chiu *et al.*, 2004; Jean *et al.*, 2006) aligned with the findings of the current study. The presence of virulence markers such as the *spvB*, *spvC*, *pef*, *invA* and *stn* genes suggests a potentially virulent nature of the study isolates, although the expression of these genes was not investigated. Thus, in NTS infection in humans, virulence plasmids might play an important role in the pathogenesis of invasive salmonellosis.

The identical (100 % similarity) PFGE profiles of *S. Choleraesuis* blood and urine isolates indicates the presence of the same isolate in both samples. This observation is noteworthy with respect to the diagnosis of enteric fever. Since urine collection is non-invasive, urine culture might be a better option than blood culture for diagnosing salmonellosis. Although the sensitivity of urine culture has been reported to be very low in the case of typhoid fever (Kumar *et al.*, 2012), sensitivity was found to be higher for urine as compared with blood and stool samples when a molecular-based method (nested PCR for the *fliC* gene) was used to diagnose typhoid fever (Kumar *et al.*, 2012).

Furthermore, ELISA-based detection of the Vi polysaccharide antigen of *S. Typhi* in urine was found to be more sensitive and advantageous when compared with conventional blood culture, because the Vi antigen can be detected in urine even 10 days following antibiotic treatment (Fadeel *et al.*, 2004). Because the aetiology of *Salmonella* bacteraemia might not be restricted to *S. Typhi* or *S. Paratyphi*, it is recommended that molecular or immunological-based diagnostics should target genes or antigens conserved in most of the *Salmonella* serovars commonly associated with human infections.

Prompt diagnosis followed by treatment of invasive salmonellosis is necessary because of its associated high morbidity and complications such as endocarditis and septic arthritis, among others. The identification and reporting of uncommon *Salmonella* serovars from various countries causing human infection are important for understanding the global epidemiology of salmonellosis.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

This study was reviewed and approved by the Institutional Ethical Committee of the National Institute of Cholera and Enteric Diseases, Kolkata. This report has no personally identifiable information, and informed consent was obtained from the patient. This study was supported by the Indian Council of Medical Research (ICMR), New-Delhi intramural fund. ICMR senior research fellowships to P. Jain and S. Das are gratefully acknowledged.

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