Invasive nontyphoidal *Salmonella* disease in southern India: a 5-year experience from a tertiary care hospital

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Background/aim: The current study was carried out to describe the clinical presentation, antimicrobial susceptibility pattern, and outcome of invasive nontyphoidal *Salmonella* disease (iNTS) in a tertiary care center.

Materials and methods: A 5-year hospital-based retrospective study was carried out on blood culture-confirmed cases of iNTS. Medical records of patients were reviewed to obtain information on demography, clinical manifestations, comorbidities, complications, immune status, treatment, and clinical outcome.

Results: A total of 40 blood culture-confirmed cases of iNTS were diagnosed during the study period. Among these 40 isolates, 9 (22.5%) were identified as *Salmonella* Typhimurium. Fever (67.5%) with gastrointestinal disturbance (40%) was the most common clinical presentation. The majority of the patients were immunosuppressed (75%). All isolates were susceptible to all the antimicrobials tested. Ceftriaxone (92.5%) was the most common antimicrobial used in our setting. A total of 15% patients died during the hospital stay.

Conclusion: We conclude that iNTS disease is a severe infection prevailing in India with a high mortality rate. Anemia and diabetes were the two most common comorbidities. Though all NTS organisms isolated were sensitive to all the antimicrobials tested, we suggest that continued surveillance is necessary to monitor the presence of multidrug-resistant strains.

Key words: Nontyphoidal *Salmonella*, bacteremia, mortality, extraintestinal infection

1. Introduction

Nontyphoidal salmonellae (NTS) are an important cause of foodborne gastroenteritis worldwide (1), but bacteremia and other invasive diseases caused by these organisms have not been thoroughly explored. The increasing centralization and industrialization of our food supply have enhanced the spread of these organisms. Contamination of varied food products, including poultry, beef, pork, eggs, milk, cheese, fish, shellfish, vegetables, and fresh fruit and juice, is the main vehicle for transmitting the disease to humans (2). In high-income countries, *Salmonella* generally causes a self-limiting diarrheal disease in immunocompetent people; bloodstream or other invasive infections are rare and are mainly associated with specific underlying conditions (1). In low-income countries, such as in sub-Saharan Africa, NTS are consistently the most common bacterial bloodstream isolates in both adults

and children, specifically in patients with HIV infection (3–7). Bacteremia due to NTS was first noticed in 1987 in children suffering from malaria (1). Later, a variety of risk factors were identified in both children and adults, including infection with HIV, malaria, malnutrition, and anemia. The clinical presentation of invasive nontyphoidal *Salmonella* (iNTS) infection is typically febrile systemic illness resembling enteric fever. Fever with pneumonia, diarrhea, and hepatosplenomegaly are the most common clinical presentations found in African studies (3–5). The mortality rate is also very high (22%–47%) (8).

In Asia, the epidemiology and clinical manifestations of iNTS disease are not well documented. Diarrhea is the third leading cause of childhood mortality (13% of all deaths/year) in India (9). Due to its high endemicity, typhoid fever has gained attention, whereas NTS strains are always considered to be a causative agent of

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diarrheal diseases. The ability of NTS strains to cause invasive infections has mostly been neglected and poorly documented. Therefore, we aimed to investigate the clinical and microbiological manifestations of iNTS in a tertiary care hospital in southern India.

2. Materials and methods

A 5-year hospital-based retrospective study was carried out on the culture-confirmed cases of bacteremia caused by NTS at a tertiary care teaching hospital in southern India between January 2012 and December 2016. Medical records of patients were reviewed to obtain information on demography, clinical manifestations, predisposing factors, complications, immune status, antibiotic susceptibilities, treatment, and clinical outcome. Diagnosis of infection was done by blood culture using the BacT/ALERT 3D system (BioMérieux, India). Cultures from other sites were done according to the clinical requirements of patients. Organisms were presumptively identified as *Salmonella* spp. when they showed nonlactose fermenting colonies; were negative for oxidase, indole, and urease; were positive for glucose and mannitol fermentation; and showed lysine decarboxylase and characteristic features on triple sugar iron agar (glucose fermentation, gas, and H_2S). These were tested for agglutination with *Salmonella* polyvalent "O" (Poly A–I & Vi, Cat No. 222641) and polyvalent "H" (Poly −a−z, Cat No. 224061) antisera (Difco, Becton Dickinson). Furthermore, isolates that were confirmed as *Salmonella* spp. and negative for agglutination by *Salmonella* Typhi (*Salmonella* agglutinating serum 9-O and d-H) and *S.* Paratyphi A (*Salmonella* agglutinating serum 2-O and a-H) specific antisera, but + for gas production and ± citrate utilization, were tested with specific antisera for *Salmonella* Typhimurium (*Salmonella* agglutinating serum 4-O and i-H). Isolates that serologically tested negative for *S.* Typhimurium but were positive for the genus *Salmonella* were grouped as nontyphoidal *Salmonella*. Antimicrobial susceptibility testing was performed using the Kirby–Bauer disk diffusion susceptibility test following the standard CLSI guidelines (10). All isolates were tested with ampicillin, chloramphenicol, sulfamethoxazole–trimethoprim, ciprofloxacin, and ceftriaxone antimicrobial disks. Interpretative standards for *Salmonella* spp. were used for reading ciprofloxacin results.

2.1. Ethics statement

This study was approved by the institutional ethics committee of Kasturba Hospital, Manipal. This was a retrospective study with routine anonymous laboratory and clinical data; individual patient identities were not accessed and hence informed consent was not required.

2.2. Statistical analysis

All patient information was documented in a study proforma, which was further digitized, and data analyses were performed using SPSS 15.0 (SPSS Inc., USA). Quantitative variables are described as means with standard deviations and medians with interquartile ranges (IQRs). Clinical and laboratory data were compared between groups using Fisher's exact test or Mann–Whitney U tests for the categorical and continuous variables, respectively.

3. Results

During the study period, among 78,238 blood cultures, the incidence of isolation of *Salmonella* spp. was 0.5, 0.4, and 0.1 per 100 blood cultures for *S.* Typhi, *S.* Paratyphi A, and NTS, respectively. NTS represented 6% of all salmonellae from cases of bacteremia, while *S.* Typhi and *S.* Paratyphi A were attributed to 52% and 42%.

A total of 40 blood culture-confirmed cases of iNTS were diagnosed during the study period. The majority of the patients were male (31, 77.5%). Mean age of the patients was 47.3 ± 19 years. Table 1 describes the basic demographics, clinical features, and coinfections among patients diagnosed with iNTS. Fever (27, 67.5%) with gastrointestinal disturbances (16, 40%) was the most common clinical feature found among the patients. The duration of illness prior to hospital admission was a median of 3 days (IQR: 2–7 days). The majority of the patients were immunosuppressed (20/40, 50%) due to various causes; 14 out of 20 patients (70%) were on immunosuppressive therapy, including steroids, radiation therapy, and antiretroviral therapy (ART). An additional 25% of patients had diabetes mellitus. Detailed descriptions of the duration of hospital stay, time to diagnosis, comorbidity, complications, and laboratory findings are tabulated below (Table 2). Laboratory findings were mostly within the normal ranges. Liver enzymes were elevated in 11 (27.5%) patients. Abnormal renal function tests were observed among 17 (42.5%) patients.

The mean time to detection of the pathogen in blood culture was 2.15 days ± 0.92 days. Among 40 NTS isolates, only 9 were identified as *S.* Typhimurium using specific antisera due to limited availability of agglutinating sera at the local facility. Acute gastroenteritis was observed in 16 patients; among these, a stool specimen for culture was obtained for only 10 patients, but none yielded NTS. Furthermore, 4/40 (10%) patients were also diagnosed with LRTI; 2 *Klebsiella pneumoniae*, 1 *Escherichia coli*, and 1 *Enterobacter cloacae* were isolated from their respiratory specimens. All NTS isolates were susceptible to ampicillin, chloramphenicol, sulfamethoxazole–trimethoprim, ciprofloxacin, and ceftriaxone. Acute kidney injury (AKI) was the most common (25%) complication of NTS bacteremia observed in our study cohort. One among these cases, 10 had yielded NTS from urine in addition to bacteremia. Three of the patients with AKI had diabetes mellitus as a comorbidity.

Table 1. Basic demographics, clinical features, and coinfections among the patients diagnosed with invasive nontyphoidal *Salmonella*.

Variable	n (%)
Age group	
<18	3 (7.5)
18–30	4 (10)
31–60	22 (55)
>60	11 (27.5)
Sex	
Male	31 (77.5)
Female	9 (22.5)
Clinical presentations	
Fever	27 (67.5)
Acute gastroenteritis	16 (40)
Abdominal pain	10 (25)
Sepsis	10 (25)
Headache	6 (15)
Altered sensorium	5 (12.5)
Seizure	3 (7.5)
Encephalitis	3 (7.5)
Other neurological symptoms	8 (20)
Coinfections	
HIV infection	4 (10)
Pulmonary tuberculosis	3 (7.5)
Bacterial pneumonia	3 (7.5)
Urinary tract infection	3 (7.5)
Hepatitis C infection	1 (2.5)
Laboratory parameters	**Median (IQR)**
Total leucocyte count ($\times 10^3$/µL)	10.2 (4.8–15.4)
Hemoglobin (g/dL)	11.4 (8.8–13.8)
Aspartate amino transferase (IU/L)	35 (21–60.7)
Alanine transaminase (IU/L)	21 (16–45)
Alkaline phosphatase (U/L)	89 (60–151)

A total of 39 (97.5%) patients received empirical therapy (Table 3). Among them, 28 (71.8%) patients received at least one drug that is active against *Salmonella* spp. Median time to administration of first empirical therapy was 1 day (IQR: 0–1 day). The majority of iNTS patients received a disease-specific antimicrobial treatment (27/40; 67.5%) (Table 3). The most commonly prescribed antimicrobial was ceftriaxone; 25 (92.5%) patients received it as mono- or combination therapy. Ceftriaxone was given to 14 (35.9%) patients and it was continued after the diagnosis was made. Once the blood culture was reported, empirical therapy was changed to ceftriaxone for another 11 patients. Overall, 26/40 (65%) patients improved before hospital discharge, 8 (20%) patients were discharged against medical advice, and 6 (15%) patients died.

4. Discussion

Invasive NTS infections are a significant cause of morbidity and mortality worldwide, but iNTS cases are rarely reported from India. In this study, we identified 40 blood culture-confirmed cases of iNTS over a period of 5 years. A systematic review from sub-Saharan Africa showed that 39% of community-acquired bloodstream infections were due to NTS pathogens, with a case fatality rate of 19% (2,3). Due to the lack of reports, the true burden of iNTS infection in Asia is unclear. A study from southern Vietnam identified more than 100 cases of iNTS infection in their setting (11). In Africa, both children and adults are equally affected by invasive NTS infection (2,3). In our setting, the majority of our patients were adults and belonged to the age group of ≥50 years. Similar findings were observed in the study from Vietnam (11). The clinical presentation was more or less similar in all of the studies.

All NTS infections start with an episode of gastroenteritis and then enter the bloodstream after invading the intestinal barrier. The common risk factors for bacteremia by NTS are extremes of age, HIV infection, malignancy, diabetes, autoimmune disorders, and immunosuppressive therapy (11–18). Dysregulated cytokine production, depletion of CD4 T-cells and especially interleukin-17-producing T cells (Th17 cells), and impaired serum killing of NTS strains were three important defects found in HIV-infected individuals with invasive NTS infection (19–21). In children, malnutrition, anemia, and malaria were found to be important risk factors. Macrophage dysfunction and cytokine dysregulation in severe malaria and homozygosity for sickle-cell anemia were found to be important defects associated with invasive NTS infection (22,23).

In low- and middle-income countries, malnutrition, anemia, and HIV infection are the most common predisposing factors for iNTS infections (2–5). As in African countries, in Vietnam, HIV infection is the primary risk factor for iNTS in adults (11), while only 10% of our patients were infected with HIV. In our study, the important comorbidities were found to be anemia (30%) and type II diabetes mellitus (25%). AKI was the predominant complication observed here. Although the underlying mechanisms are unclear, AKI is a well-known complication of salmonellosis, as reported by many studies (24–31).

India is a well-known endemic country for enteric fever and gastroenteritis. Typhoidal serovars are known to cause systemic infections, and nontyphoidal serovars of *Salmonella* are strongly associated with gastroenteritis. Therefore, the ability of NTS to cause invasive infections is neglected in this country. The zero fecal-isolation rate of NTS in our study is speculated to be due to either delay

Table 2. Duration of hospital stay, time to diagnosis, comorbidity, complications, and laboratory findings among patients who improved or deteriorated during hospital stay.

Variable	Total n = 40	Outcome		P-value
		Improved n = 26 (65%)	Deteriorated n = 14 (35%)	
Median duration of hospital stay, days	6.5 (IQR: 4–13)	10 (IQR: 4–17)	3.5 (IQR: 1–5.25)	<0.001
Mean time to diagnosis, days	4.05 ± 1.8	4.04 ± 1.9	4.07 ± 1.5	0.937
Comorbidities				
Anemia	25 (62.5)	15 (57.7)	10 (71.4)	0.307
Hypertension	12 (30)	8 (30.8)	4 (28.6)	0.59
Type 2 diabetes mellitus	10 (25)	5 (19.2)	5 (35.7)	0.17
Malignancy	8 (20)	5 (19.2)	3 (21.4)	0.588
Chronic kidney disease	4 (10)	2 (7.7)	2 (14.3)	0.62
Chronic liver disease	4 (10)	1 (3.8)	3 (21.4)	0.17
HIV infection	4 (10)	4 (15.4)	0 (0)	0.164
Complications				
Acute kidney injury	10 (25)	6 (23)	4 (28.6)	0.492
Septic shock	6 (15)	5 (19.2)	1 (7.1)	0.115
Encephalopathy	8 (20)	5 (19.2)	3 (21.4)	0.65
Nephropathy	4 (10)	4 (15.4)	0 (0)	0.164
Pericarditis	1 (2.5)	1 (3.8)	0 (0)	0.65
Polyarthritis	1 (2.5)	0 (0)	1 (7.1)	0.65
Colitis	1 (2.5)	0 (0)	1 (7.1)	0.35
Pancreatitis	2 (5)	1 (3.8)	1 (7.1)	0.583
Massive gastrointestinal bleeding	1 (2.5)	1 (3.8)	0 (0)	0.35
Empirical treatment				
Ceftriaxone	14 (35)	12 (46.2)	2 (14.3)	
Piperacillin–tazobactam	10 (25)	4 (15.4)	6 (42.8)	
Ciprofloxacin/levofloxacin	5 (12.5)	4 (15.4)	1 (7.1)	
Amoxicillin–clavulanic acid	3 (7.5)	3 (11.5)	0 (0)	
Cefoperazone–sulbactam	3 (7.5)	1 (3.8)	2 (14.3)	
Meropenem	2 (5)	1 (3.8)	1 (7.1)	
Sulfamethoxazole–trimethoprim	1 (2.5)	1 (3.8)	0 (0)	
Ampicillin	1 (2.5)	0 (0)	1 (7.1)	

Table 3: Details of antimicrobial treatment given to iNTS patients

Disease specific treatment	No. of cases (%)	Median duration of treatment in days	Outcome
Ceftriaxone	19 (67.8)	7 (R: 4-15)	Improved
Ceftriaxone +Azithromycin	5 (46.2)	14 (R: 4-21)	Improved
Ceftriaxone + Sulfamethoxazole-Trimethoprim	1 (3.8)	7	Improved
Ciprofloxacin	1 (3.5)	7	Improved
Cefoperazone sulbactam	1 (3.5)	1	Died

in culture requests or early antimicrobial administration. All the patients who presented with gastroenteritis also had severe clinical presentations, which directed the attention of the practitioners towards the practice of blood culture and delay in obtaining fecal specimens. In limited resource settings, where identification of bacterial isolates depends on conventional biochemical tests, unavailability of specific antisera can lead to misdiagnosis of NTS. A few

case reports from various parts of India described NTS causing bacteremia in both immunocompromised and immunocompetent individuals (32–36). Bacteremia due to NTS is a significant cause of morbidity and mortality worldwide. We have observed a higher mortality rate (15%) among immunocompromised individuals than that observed in Vietnam.

S. Typhimurium is the most commonly identified serovar globally associated with iNTS infections, followed by *S*. Enteritidis. The limited number of *S*. Typhimurium infections reported here might be an underestimate, as antisera were not available for identification of all of the isolates. Fortunately, all our isolates were sensitive to all of the antibiotics tested. In other parts of India, a high percentage of resistance has been observed against commonly used antibiotics (36). In sub-Saharan countries, a large number of isolates were resistant to three or more antibiotics (37), and a multidrug-resistant (MDR) distinct genotype variant of *S*. Typhimurium (ST313) has also emerged (38). In Vietnam, the majority of *S*. Typhimurium strains showed high resistance against a variety of

antimicrobials, including ampicillin, chloramphenicol, sulfamethoxazole–trimethoprim, and ciprofloxacin (11). In the United States, NTS strains isolated from blood are most commonly resistant to tetracycline (39). Ceftriaxone-resistant isolates have also been detected in the United States and Bangladesh (39,40).

We acknowledge a few study limitations. Due to the retrospective nature of the study, we could not elicit histories of possible exposure. Furthermore, due to the limited number of cases, we could not identify the associated risk factors. Serological identification of all the isolates could not be done due to the limited availability of antisera.

In conclusion, iNTS is a potential cause of community-acquired bloodstream infection in southern India, with high morbidity and mortality. Although all NTS organisms isolated in this study were sensitive to all the antimicrobials tested, we suggest that continued surveillance is required to monitor for the presence or introduction of MDR strains. In this context, it is important to know the actual incidence of iNTS in India for the development of effective prevention and control strategies.

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