Original Article

Extra-intestinal salmonellosis in a tertiary care center in South India

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Abstract

Introduction: The present study is a retrospective analysis of a total of 36 cases of bacteriologically proven extra-intestinal salmonellosis, managed at Nizam's Institute of Medical Sciences, between 1987 and 2012 (25 years). The extra-intestinal sites involved were the skin, cerebrum, spleen, ovary, synovium, and the skeletal muscle.

Methodology: The extra-intestinal specimens were first processed using standard methods. Colonies suspected as *Salmonella* were identified by standard laboratory methods, initially by manual biochemical reactions and later by the API system (bioMerieux, Marcy l'Etoile- France) and the Vitek-2 system (bioMerieux). All the *Salmonella* isolates were sent to Central Research Institute, Kasauli, for serotyping.

Results: The predominant serotype isolated was *Salmonella* Typhi (*S.* Typhi) in 27 (75%) patients, followed by *Salmonella* Senftenberg (*S.* Senftenberg) in 5 (14%), *Salmonella* Paratyphi A (*S.* Paratyphi A) in 3 (8%), and *Salmonella* Typhimurium (*S.* Typhimurium) in 1 (3%). There was an increasing resistance to ampicillin, chloramphenicol, cephalosporins (third generation), and quinolones over the 25 years.

Conclusions: The diagnosis of extra-intestinal salmonellosis requires a high degree of clinical suspicion and should be included in the differential diagnosis in patients with deep-seated abscesses.

Key words: *Salmonella* Typhi; non-typhoidal *Salmonella*; deep-seated abscesses; endemicity.

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Introduction

Salmonella is an enteroinvasive Gram-negative bacterium and a primarily enteric pathogen. On ingestion, the organisms bypass gastric defenses, multiply, and penetrate the intestinal mucosa. They survive within the macrophages of the reticuloendothelial system and disseminate via systemic circulation, causing infection [1]. Salmonella infection of humans can be broadly divided into five clinical groups: enteric fever, septicemia without localization, focal disease (with or without associated bacteremia), gastroenteritis, and the carrier state [2-4].

The clinical significance of these extra-intestinal manifestations of *Salmonella* is not often appreciated. There are very few documented reports on the actual prevalence of these infections, especially from an endemic region such as India.

Therefore, a retrospective analysis of all the bacteriologically proven cases of extra-intestinal salmonellosis managed at our institute over the past 25 years was performed to document the prevalence of these highly invasive infections.

Methodology

The microbiology laboratory records of the extra-intestinal specimens with a clinical suspicion of focal infections that were culture-positive for *Salmonella*, documented between 1987 through 2012 (25 years), were included in this retrospective study. The corresponding medical records of the patients were further reviewed for site of infection and evidence of any underlying or predisposing illnesses.

The extra-intestinal specimens were purulent aspirates, including wound aspirates, body fluids (excluding blood and urine), stool, and bone marrow aspirates. They were first processed using standard methods, on 5% sheep blood agar and MacConkey agar or chromogenic agar (CPS ID, bioMerieux, Marcy l'Etoile, France).

Colonies suspected as *Salmonella* were identified by standard laboratory methods, initially by manual biochemical reactions and later (from 2001 onwards) by the API system and by the Vitek-2 system (since 2005) in the laboratory and then sent to Central Research Institute (CRI), Kasauli, Himachal Pradesh, India, for serotyping. Antimicrobial susceptibility testing was performed by Kirby Bauer disk diffusion

until 2001 and later by the API and Vitek-2. Nalidixic acid susceptibility testing (NAST) was done using the API system for all isolates obtained after 2001.

Results

Over the 25-year study period, 36 patients were diagnosed with extra-intestinal salmonellosis. There was no history of enteric fever in these patients. Blood cultures were negative and Widal tests were non-reactive in all the cases. The average number of cases per year was 1.4 (Figure 1).

The *Salmonella* culture-positive specimens included purulent aspirates (n = 24) from deep-seated abscesses, aspirated body fluids (n = 6) excluding blood and urine, catheter tips (n = 3), bronchial wash (n = 1), ascitic fluid (n = 1), and tracheal aspirate (n = 1) (Table 1). Abscesses were rarely found in ovaries. *S.* Senftenberg was isolated from ascitic fluid, bronchial wash, tracheal aspirate, and catheter tips.

The age and sex distribution of these cases are shown in Table 2.

The predominant serotype isolated was *Salmonella* Typhi (*S*. Typhi) in 27 (75%) patients, followed by *Salmonella* Senftenberg (*S*. Senftenberg) in 5 (14%), *Salmonella* Paratyphi A (*S.* Paratyphi A) in 3 (8%), and *Salmonella* Typhimurium (*S.* Typhimurium) in 1 (3%).

All the *Salmonella* isolates were sent to CRI, Kasauli, for serotyping. The antigenic structure of the *S.*Typhi was 9,12,Vi:d:-, *S.* Senftenberg was 1,3,19:g,s,t:-, *S* .Paratyphi A was 1,2,12:, and *S.* Typhimurium was 4,5,12:I:1,2.

There was an increasing trend in antibiotic resistance among the *S*. Typhi isolates over the 25 years, from 0% to 63% for ampicillin, 0% to 58% for chloramphenicol, 0% to 68% for trimethoprim-sulfamethoxazole, 0% to 50% for ciprofloxacin and ceftriaxone, while non-typhoidal *Salmonella* (NTS) was resistant to all the antibiotics (Figures 2 and 3).

Discussion

Salmonella species are true pathogens, capable of causing both intestinal and extra-intestinal infections in humans. However, the inherent virulence of each serotype and host resistance are the deciding factors for the different types of manifestations. An increased susceptibility to invasive disease may result from an alteration of locally protective mechanisms (*e.g.*, gastric acid), presence of diseased tissue (*e.g.*, bone infarcts), or impairment of the immune system (*e.g.*, human immunodeficiency virus infection) [4].

Extra-intestinal focal infections, though less frequent, may affect different sites in the body, causing different disorders. These infections frequently occur during or after *Salmonella* bacteremia but may also occur concomitantly with other syndromes.

Ours is a tertiary care super specialty and referral hospital in South India. Per the records of the past 25 years, 36 patients had bacteriologically proven extra-intestinal salmonellosis. The incidence of these infections has been steadily increasing, with two distinct peaks in 1997 and 2007, the significance of which cannot be explained.

There are few case reports from India of extra-intestinal salmonellosis due to *S*. Typhi and NTS (Table 3).

S. Typhi was the predominant serotype, isolated from 27 (75%) cases in our study. This is in contrast to studies from other countries where NTS was more frequent [4-8]. In recent years, *S.* Enteritidis has replaced *S.* Typhimurium as the most frequent NTS species producing extra-intestinal infections [6]. The predominance of *S*. Typhi in our study may be attributed to the endemicitiy of the organism in India.

Figure 1. Yearly distribution of extra-intestinal salmonellosis

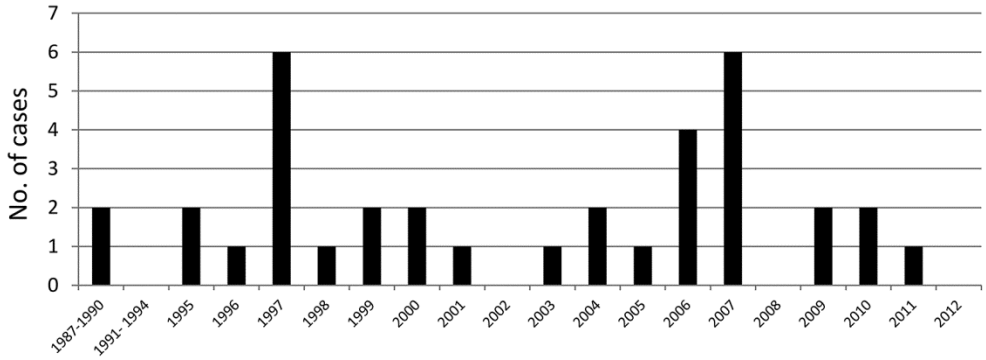


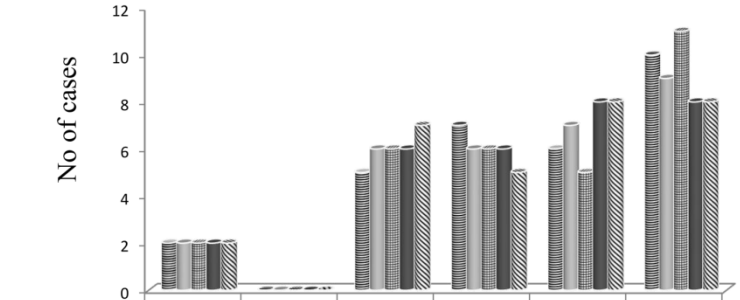
Table 1. Clinical spectrum of extra-intestinal salmonellosis

Sample	Diagnosis	No. of cases	Organism	Predisposing factors
Purulent aspirate from ovary	Ovarian abscess	2	*S.* Typhi	PID
Purulent aspirate from central nervous system	Brain abscess	1	*S.* Typhi	Septicemia
	Myelitis	1	*S.* Typhi	None
Purulent aspirate from skin and soft tissue	Intramuscular abscess	3	*S.* Typhi	Systemic sclerosis in one Others-none
	Osteomyelitis	5	*S.* Typhi	None
	Psoas abscess	2	*S.* Typhi	None
	Gluteal abscess	2	*S.* Typhi	None
	Septic arthritis	1	*S.* Typhi	None
	Cellulitis	1	*S.* Typhi	Diabetes
	Fracture leg	1	*S.* Typhi	None
	Raw area leg	1	*S.* Typhi	None
	Necrotizing fasciitis	1	*S.* Paratyphi A	Renal failure
Purulent aspirate from spleen	Splenic abscess	2	*S.* Typhi *S.* Paratyphi A	None
Purulent aspirate from liver	Liver abscess with prerenal azotemia	1	*S.* Paratyphi A	Pre-renal azotemia
Synovial fluid	Septic arthritis	2	*S.* Typhi *S.* Typhimurium	Dermatomyositis Renal failure
Peritoneal fluid	Pelvic abscess	1	*S.* Typhi	None
Cerebrospinal fluid	SLE with chronic meningitis	1	*S.* Typhi	SLE
Pleural fluid	Empyema	1	*S.* Typhi	None
Pericardial fluid	Pericarditis	1	*S.* Typhi	None
Cavafix tip	Vasculitis and digital gangrene	1	*S.* Typhi	Liver dysfunction

Table 2. Demographic characteristics of extra-intestinal salmonellosis

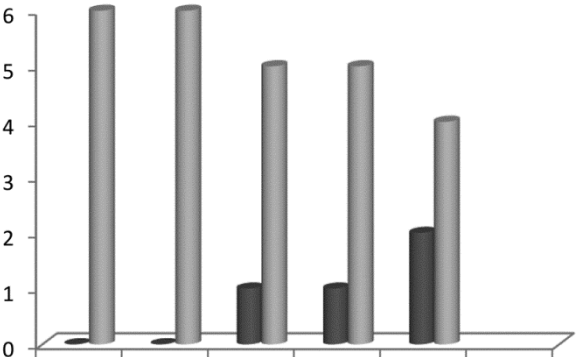
Age in years	No. of Males	No. of Females
0-10	0	1
10-20	4	3
20-30	4	5
30-40	3	4
40-50	4	3
>50	4	1

Figure 2. Antibiotic susceptibility of *S.* Typhi from cases of extra-intestinal salmonellosis



	1987-1994		1995-2002		2003-2012	
Ampicillin	2	0	5	7	6	10
Chloramphenicol	2	0	6	6	7	9
Cotrimoxazole	2	0	6	6	5	11
Ciprofloxacin	2	0	6	6	8	8
Ceftriaxone	2	0	7	5	8	8

Figure 3. Antibiotic susceptibility of NTS from cases of sxtra-intestinal salmonellosis



	Ampicillin	Chloramphenicol	Cotrimoxazole	Ciproflox acin	Ceftriaxone	
Sensitive	0	0	1	1	2	
Resistant	6	6	5	5	4	

Table 3. Case reports of extra-intestinal salmonellosis from India

S. No.	Clinical spectrum	*Salmonella* serotype	Journal & Year
1	Post-operative wound infection in chronic osteomyelitis [38]	*Salmonella* Typhi	Indian J Med Sci (2000) 54: 149-150
2	Pleural empyema [39]	Group B *Salmonella*	Indian Pediatr (2001) 38: 186-189
3	Meningitis [40]	*Salmonella* Enteritidis	Indian J Med Microbiol (2001) 19: 151-152
4	Splenic abscess [41]	*Salmonella* Enterica Serotype Worthington	Braz J Infect Dis (2002) 6: 88-90
5	Meningitis [42]	*Salmonella* Typhimurium	Kuwait Med J (2003) 36: 45-46
6	Pleural effusion [43]	*Salmonella* Paratyphi A	Indian Pediatr (2003) 40: 252-254
7	Osteomyelitis [20]	*Salmonella* Typhi.	Joint Bone Spine (2003) 75: 67-69
8	Hepatic abscess [44]	*Salmonella* Typhi	Indian Pediatr (2006) 43: 81-82
9	Hemorrhagic pleural effusion [45]	*Salmonella* Typhimurium	J Clin Diag Res (2007) 4: 299-302
10	Pericardial effusion [46]	Group B *Salmonella*	Cases J (2008) 1: 375
11	Brain abscess [47]	*Salmonella* Typhimurium	Indian J Pathol Microbiol (2009) 52: 269-270.
12	Empyema thoracis [48]	*Salmonella* Typhi	J Lab Physicians (2012) 4: 45-47
13	Septic arthritis [49]	*Salmonella* Typhi	J Biosci Med (2012) 2: 2-4
14	Meningitis [50]	*Salmonella* Enteritidis	Indian J Med Microbiol (2012) 30: 474-476

The extra-intestinal manifestations of *Salmonella* included abscesses in the skin and soft tissue and deep-seated abscesses within solid organs, bones, and the synovium. The NTS isolates in our study were *S.* Typhimurium and *S.* Senftenberg. According to a literature review, *S.* Senftenberg is considered predominantly to be a nosocomial pathogen [7].

Salmonella spp. is a rare cause of ovarian abscess. Ovaries may be infected by a hematogenous route in most cases or by direct propagation from an inflamed bowel wall or by an ascending route [9]. In females, though the most common serotype was *S*. Typhi [10] as in both the cases in our study, ovarian abscesses due to NTS have also been reported [9,11,12].

Two young patients presented with ovarian abscesses. One had a bilateral ovarian abscess and secondary infertility with underlying pelvic inflammatory disease. The abscess ruptured, leading to pelvic peritonitis. Exploratory laparotomy was done. Pus yielded growth of *S*. Typhi. She was treated with intravenous ceftriaxone and metrogyl. During the hospital stay, she developed septicemia and succumbed to the infection

The other patient had a right-sided ovarian abscess. She was successfully treated with salpingo-oopherectomy and intravenous ampicillin and metrogyl. Pus yielded growth of *S*. Typhi.

Gokul *et al*. reported 27 cases of *Salmonella* infections of the central nervous system [13]. Brain abscesses may develop following *Salmonella* infection elsewhere in the body, or after recovery from typhoid fever without any previous history of *Salmonella* infection [14,15], as in our case. *S*. Typhi was isolated from a brain abscess in a case of septicemia. The patient was successfully managed with surgical drainage of the abscess and with antibiotics.

Although acute transverse myelitis due to *Salmonella* is rare, there are case reports of infection with *S*. Paratyphi B and NTS [16], while our case was due to *S.* Typhi.

In the present study, *S*. Typhi was also isolated from a splenic abscess (n = 1) and from intramuscular abscesses (n = 7 [right arm, left thigh, left forearm, glute, and psoas]). Percutaneous drainage was done for two patients with splenic abscesses, followed by treatment with intravenous ceftriaxone and ciprofloxacin, respectively. The patient with an intramuscular abscess over the left forearm had an underlying systemic sclerosis. Drainage of the skin and soft tissue abscesses were done, followed by treatment with intravenous ampicillin and ciprofloxacin.

Soft tissue infections are rare, accounting for 6%–12% of all *Salmonella* infections [1,17]. Most of the infections are caused by NTS [17]. Psoas abscesses are rare [18,19,20].

Salmonella spp. rarely cause osteomyelitis, accounting for 0.8% of all *Salmonella* infections and 0.45% of all types of osteomyelitis. The three most common serotypes causing osteomyelitis are *S.* Typhimurium, *S.* Typhi, and *S.* Enteritidis, with *S.* Typhi being the only serotype to be transmitted between humans [21]. We report five cases of osteomyelitis due to *S*. Typhi. One patient was a three-month-old baby who was treated with oral trimethoprim-sulfamethoxazole. Two patients were treated with intravenous ampicillin, and the other two patients were treated with intravenous ceftriaxone. After discharge all patients, except the three months old baby, were advised to continue oral ciprofloxacin for one week.

In the present study*, S.* Paratyphi A was isolated from necrotizing fasciitis of the right lower limb with renal failure (n = 1), splenic abscess (n = 1), and liver abscess (n = 1). Debridement was done in the patient with necrotizing fasciitis, and he was successfully treated with intravenous ceftriaxone and sodium bicarbonate, followed by grafting. *Salmonella* splenic abscesses often result from a systemic bacteremia due to infective endocarditis or secondary infection in an infarcted or traumatized spleen [22]. Approximately 20% of splenic abscesses are caused by *Salmonella,* with serotype Typhi being most commonly reported [23].

Liver abscess due to *Salmonella* spp. [23] is rare and usually associated with a liver cyst [25] and HIV infection [26]. Our patient had a *Salmonella* liver abscess with pre-renal azotemia.

Aspiration of the liver abscess was done and the patient was given intravenous cefotaxime for two weeks and then discharged.

S. Typhimurium was isolated from a case of septic arthritis of the left hip; the patient was treated with intravenous ceftriaxone for three weeks, followed by oral therapy with ofloxacin for one week. *S.* Typhi was isolated from a case of dermatomyositis with septic arthritis of the right knee. The patient was on steroids for dermatomyositis and was given intravenous ceftriaxone. The two patients were successfully treated and discharged. *Salmonella* is a very rare cause of septic arthritis and, if associated, it was previously reported to be a metastatic extension of NTS infection from elsewhere [27,28].

In adults, *Salmonella* meningitis is most commonly seen in patients with an intercurrent illness [29,30], particularly HIV-related immunosuppression [31,32]. It occurs mostly in the pediatric age group and rarely in adults [33]. Our patient was a 20-year-old female who was diagnosed with systemic lupus erythematosus (SLE) with meningitis and was on immunosuppressant drugs.

Salmonella empyema, though reported in 85 cases by Saphra *et al*., is a rarely reported entity [31,34]. We reported one case of *S.* Typhi pleural empyema.

Salmonella pericarditis, also a rare presentation, was first recorded in association with typhoid fever in 1844, with few subsequent reports [35].

One of the very rare sites of infection with *S.* Typhi, in our study, was from a Cavafix tip in a case of vasculitis with digital gangrene. The patient had toxic encephalopathy, adult respiratory distress syndrome, and disseminated intravascular coagulation with septic shock. The patient was successfully managed with rehydration, ionotrophic support, and antibiotics.

S. Senftenberg was isolated for the first time in India from sewage samples in 1963. Recently, its increasing rate of isolation from human sources, especially from infants and neonates in hospital environments, has made it an important pathogen [7]. *S.* Senftenberg is mostly associated with indwelling devices, such as IV cannulas, tracheal tubes, bronchial lavage, and drains. In our study, *S.* Senftenberg was isolated from ascitic fluid, bronchial wash, tracheal aspirate, and catheter tips.

Antibiotics

In addition to ampicillin, quinolones, and trimethoprim-sulfamethoxazole for intestinal isolates, third-generation chloramphenicol and cephalosporin are recommended for extra-intestinal salmonellosis [36]. The present study demonstrates that there has been an increasing trend in antibiotic resistance over the years in *S.* Typhi and NTS. Antibiotic resistance is higher in NTS.

Of therapeutic importance in this situation is the increasingly frequent association of multiple drug resistance with NTS.

Conclusions

Infections with *S.* Typhi can involve any organ or system. Localization is usually associated with severe septicemia. Abscesses may occur as a late complication in almost any *Salmonella* infection [37]. The diagnosis of extra-intestinal salmonellosis requires a high degree of clinical suspicion and should be included in the differential diagnosis in patients with deep-seated abscesses.

The availability of automated and semi-automated instruments in our laboratory helped us to isolate the organism with its antibiotic susceptibility rapidly, which helped the clinicians of our institute ensure prompt initiation of appropriate antibiotic therapy against these isolates. Hence, the outcome of these patients was generally positive.

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