FOCAL NON-TYPHOIDAL *SALMONELLA* INFECTIONS FROM A SINGLE CENTER IN MALAYSIA

WS Lee[1], A Hafeez[2], H Hassan[2], NS Raja[2] and SD Puthucheary[2]

Departments of [1]Pediatrics, [2]Medical Microbiology, University of Malaya Medical Center, Kuala Lumpur, Malaysia

Abstract. A retrospective review of patients with focal non-typhoidal *Salmonella* (NTS) infection was performed to determine its features and outcome. All patients with focal NTS infection admitted to the University of Malaya Medical Center, Malaysia, from 1993 to 2002 were studied. More than half (58%) of the 35 cases (54% male, median age 39 years, range 1.5 months to 79 years) were immunocompromised or had chronic medical conditions. One-third of the patients (34%) had superficial infections (lymphadenitis or subcutaneous tissue infection) and all recovered with antimicrobial therapy alone. Deep infections (66%) noted were: meningitis (9%), osteomyelitis or arthritis (26%), abscesses of the gastrointestinal tract or adjacent organs (20%), and others (11%). Deep infections were more likely to occur in the extremes of age (<6 months or >60 years, $p < 0.04$), associated with adverse outcomes with an overall mortality rate of 9%, or required major surgery (15%).

INTRODUCTION

Non-typhoidal *Samonella* (NTS) infection in humans can be classified according to the clinical syndromes caused by the organism: gastroenteritis, bacteremia, localized disease, and chronic carrier state (Saphra and Winter 1957). Bacteremia and localized infections have been termed extra-intestinal infections. Ramos *et al* (1996) further classified extraintestinal infection according to the presence of underlying disease, outcome and localization of the infection, into 4 groups: primary or secondary bacteremia (associated with enteritis) and digestive or non-digestive focal infection. Patients with non-digestive focal infections were older, more likely to be severely immunosuppressed, and had associated bacteremia more than the other groups (Ramos *et al*, 1996).

Meningitis was the most common form of focal NTS infection in young infants who were previously well and carries significant morbidity and mortality (Lee *et al*, 1999). Beyond infancy, risk factors of developing focal extraintestinal NTS infection were immunosuppression and chronic medical conditions, such as diabetes (Lepage *et al*, 1986; Brown and Eykyn 2000; Ispahani and Slack 2000; Lee *et al*, 2000). However, reports of focal NTS infections have either included patients with other forms of salmonellosis, such as typhoid fever (Ispahani and Slack, 2000), or bacteremia (Brown and Eykyn, 2000; Lee *et al*, 2000). We reviewed patients with focal extraintestinal NTS infections seen at a single center in Kuala Lumpur, Malaysia, to ascertain the epidemiology, sites of infection, risk factors of developing focal infections and outcomes.

MATERIALS AND METHODS

The University of Malaya Medical Center (UMMC), Kuala Lumpur, Malaysia, is a 900-bed, multi-disciplinary teaching hospital serving the urban area of Kuala Lumpur and its surrounding region. It is also a tertiary referral center in Malaysia for many disciplines, including oncology. The bacteriology records of the Medical Microbiology Department, UMMC, during a 10-year period (1993 to 2002, inclusive) were screened for positive isolates of NTS. Positive isolates from a focal site, such as cerebrospinal fluid, aspirates of synovial fluid or abscesses, and tissue biopsies were identified. The case notes of these patients were retrieved for review. Basic demo-

Correspondence: Dr WS Lee, Department of Pediatrics, University of Malaya Medical Center, 59100 Kuala Lumpur, Malaysia.
Tel: +603-7950-2065; Fax: +603-7955-6114
E-mail: leews@um.edu.my

graphic data, associated medical conditions, antibiotic treatment, outcomes and complications were all noted.

Definitions

Focal infection was defined as infection caused by NTS which was localized in nature, with or without concomitant bacteremia. The site of infection was classified as follows: superficial, such as skin and subcutaneous tissue infections, and lymphadenitis, or deep tissue infections, including (a) central nervous system (CNS, such as meningitis or cerebral abscess), (b) musculoskeletal system, including osteomyelitis and arthritis, (c) gastrointestinal tract (GIT) or adjacent organs, including oral and peritoneal cavities, and (d) others, such as cardiovascular and respiratory systems.

Underlying medical conditions were classified as follows: (a) primary or acquired immunodeficiency states, such as human immunodeficiency virus infection / acquired immunodeficiency syndrome (HIV / AIDS), or severe clinical immunosuppression, such as chemotherapy for malignancy, or chronic high-dose corticosteroids therapy for more than one month, and (b) chronic medical conditions which could potentially compromise immunocompetence, such as diabetes mellitus, alcoholism, or chronic debilitating states.

Bacteriology

Isolates were identified as NTS by standard biochemical reactions, as previously described (Lee *et al,* 2000, 2003). NTS was differentiated from *Salmonella enterica* serovar Typhi by its ability to produce gas on sugar fermentation. The identification of serotypes was performed at the Institute of Medical Research, Kuala Lumpur, the National Reference Laboratory for serotyping *Salmonella* species. Antimicrobial susceptibility testing was carried out using standard disc diffusion procedures.

Statistics

The data were analyzed and compared using descriptive statistics, analysis of variance, the Student's *t*-test and the χ^2 test with Yates correction or Fisher's exact test, where appropriate.

RESULTS

During the 10-year study period, a total of 36 cases of focal NTS infections were identified from the records of the Department of Medical Microbiology, UMMC. The case notes of one patient could not be retrieved and were excluded from analysis. The remaining 35 cases were studied.

Demography

Fifty-four percent of the patients were males (19 males and 16 females). The median age was 39 years (range: 1.5 months - 79 years). Nine patients (26%) were children (<15 years, Table 1). Approximately one third of the patients (31%, n=11) belonged to the extreme age groups: 3 were infants <6 months (all had meningitis), while 8 patients were aged >60 years. Four of the 6 remaining children had either malignancies or were immunodeficient.

Underlying medical conditions

Overall, 57% of the patients (n=20) had an increased susceptibility to infection: (a) 8 (23%)

Table 1
Distribution of focal non-typhoidal *Salmonella* infection according to age group.

	Total (%) (n=35)	<15 year (n=9)	>15 to <60 year (n=18)	>60 years (n=8)
Lymph nodes and subcutaneous tissue	12 (34)	3	8	1
Central nervous system[a]	3 (9)	3		
Musculoskeletal system	9 (26)	2[b]	3	4
Gastrointestinal tract or adjacent organs	7 (20)	1	5	1
Others sites[c]	4 (11)		2	2

a: All patients in this category were infants with meningitis; b: One infant had meningitis and septic arthritis.
c: pneumonia with pleural effusion, thyroid abscess, aneurysm of thoracic aorta and left internal iliac artery.

were immunocompromized (1 each for primary immunodeficiency and HIV/AIDS); 4 were undergoing chemotherapy for malignancies: Langerhan's cell histiocytosis, abdominal neuroblastoma, acute lymphoblastic leukemia, and non-Hodgkin's lymphoma (NHL); 2 had systemic lupus erythematosus (SLE) and were on steroids; and (b) 12 (34%) had chronic medical conditions (diabetes mellitus, chronic alcoholism, intravenous drug use, and melioidiosis with splenic abscess).

Sites of infection (Table 1)

Only one patient had a positive isolate from more than one site, while the remaining 34 had single site infections. Group 1: one third (n=12, 34%) had infections of subcutaneous tissue or lymphadenitis (5 had cervical lymphadenitis). Group 2: Two thirds (n=23, 66%) had deep infections: (a) 3 (9%) had meningitis, one was complicated by cerebral abscess and septic arthritis; (b) 9 (26%) had osteomyelitis or arthritis (osteomyelitis of the mandible, humerus, tibia, and femur, arthritis of the shoulder joint, and sacroilitis); (c) 7 (20%) had infections of the gastrointestinal tract or adjacent organs (parapharyngeal abscess, tubo-ovarian abscesses, parotid abscess, splenic abscess, and peritoneal cyst); and (d) 4 (11%) infections of other sites (pneumonia with pleural effusion, thyroid abscess, aneurysm of the thoracic aorta and left internal iliac artery).

The age of the patient and presence of associated medical conditions influenced the sites of infection. Meningitis was seen only in young infants. Of the remaining 20 patients with deep infections, 14 (70%) had immunodeficiency or chronic medical conditions. On statistical analysis, only extremes of age (<6 months or >60 years) were found to be a significant risk factor for acquiring deep infection (43% vs 8%, p=0.04), while having immunodeficiency or a chronic medical condition were not significant risk factors (p= 0.51).

Microbiology

Blood cultures were positive in 7 (20%) cases. Serotyping was performed in 12 cases, 4 of which were *S. enteritica* serovar Enteritidis. The remaining isolates were: *S. enteritica* serovar Haifa, Bareily, Blockley, Typhimurium, and Matopeni. Two others belonged to Kauffmann and White group D2 and C2.

Antimicrobials

Antimicrobials were prescribed for all the patients. They were started as soon as there was evidence of an infection. The choice of antimicrobials depended upon the sites and severity of infection, underlying medical conditions and the preference of the clinician-in-charge. They were adjusted according to the results of antimicrobial sensitivity patterns.

Ampicillin with or without salbactum (n=18), cloxacillin (n=16), second and third generation cephalosporins (n=15) were the main antimicrobials used. Others included ciprofloxacin, gentamicin and chloramphenicol. Generally patients with superficial infection or lymphadenitis were given ampicillin for 7 to 10 days. Patients with osteomyelitis were given cloxacillin for 3 to 4 weeks. Patients with other deep-seated infections or who were immunocompromised were given third generation cephalosporins or a quinolone. The two infants with meningitis were given a combination of ampicillin and chlormaphenicol, while the third was given ceftriaxone.

Morbidity and outcome

Ten of the patients with superficial infections required incision and drainage of abscesses, and all recovered uneventfully. The remaining patients with superficial infection recovered with antimicrobial therapy alone. Patients with osteomyelitis, arthritis and focal abscesses (thyroid, parotid, and parapharyngeal) recovered with antimicrobial therapy. No recurrences were noted in any of these patients.

Five patients required major surgery: two had salphingo-oophorectomy for tubo-ovarian abscess; the patient with splenic abscess had splenectomy, another two patients with aneurysms of the thoracic aorta and iliac artery had repair of the aneurysms.

There were 3 deaths (overall mortality: 9%) in patients with deep-seated infections, including two infants with meningitis, and a 44-year-old man with stage IV NHL who had NTS pneumonia complicated by a pleural effusion. The third infant with meningitis developed a cerebral

abscess requiring surgical drainage. This child survived with major neurological disability.

DISCUSSION

Focal infection caused by NTS may be either adjacent to the gastrointestinal tract or non-gastrointestinal, such as in the urinary tract, respiratory, musculoskeletal, central nervous or cardiovascular systems (Ramos *et al*, 1996; Ispahani and Slack, 2000). This review on focal NTS infection was performed at a big hospital in Kuala Lumpur over a 10-year period, with a total of 35 cases, representing an average of 3 to 4 cases per year. The actual incidence of focal NTS infection may be higher, as this study did not include focal infection manifesting as focal symptoms and signs with a positive blood or urine culture.

Risk factors for developing bacteremia following NTS gastroenteritis included young age, and the presence of immunosuppression or chronic medical conditions (Sirinavin *et al*, 1988; Chiu *et al*, 1999; Lee *et al*, 2000). However, risk factors leading to focal suppurative infections in certain patients with NTS bacteremia are not entirely clear. In a large study of extraintestinal NTS infections comprising 183 patients, Ramos *et al* (1996) noted that among patients who had focal extraintestinal NTS infections, elderly patients and those who were severely immunocompromised were more likely to develop focal non-digestive infections rather than focal digestive infections. The authors, however, did not address the issue of risk factors for developing focal infections following NTS bacteremia.

A few clinical patterns of focal extraintestinal NTS infection can be recognized from this study. Firstly, *Salmonella* meningitis is an unusual complication of NTS sepsis and occurred almost exclusively in infants and young children (Lee *et al* 1999). All 3 of the cases of meningitis described in this study were young infants who were all previously healthy. NTS meningitis in adults is mostly confined to patients who are severely immunocompromised, such as with HIV / AIDS (Leonard *et al*, 2002). It has rarely been reported in those who were previously healthy (Karim and Islam, 2002). The reason for this pro-

pensity for young infants is not entirely clear, but decreased opsonization, complement levels, phagocytosis and chemotaxis in young infants are considered important factors contributing to their susceptibility to gram-negative bacteria (Pabst and Kreth, 1980).

A wide variety of immunocompromised individuals, including patients with malignancy, HIV/AIDS, or diabetes, and those on steroid therapy or treatment with immunosuppressive agents have an increased susceptibility (Brown and Eykyn, 2000; Lee *et al*, 2000; Tsao *et al*, 2002). The precise mechanism responsible for the increased susceptibility remains unclear. The "virulence" of the bacteria, the infectious dose, the genetic makeup, and the immunological status of the host are important factors (Mastroeni, 2002). The virulence of any microorganism involves a complex interaction between the microorganism and the host's ability to limit infection. In *Salmonella* infection, intact cell-mediated immunity is vital in controlling this intracellular pathogen. In the early phase of infection, the control of bacterial growth in the reticuloendothelial system (RES) relies on the NADPH oxidase-dependent anti-microbial functions of resident phagocytes and is controlled by the innate resistance gene Nramp1. This early phase is followed by the suppression of *Salmonella* growth in the RES due to the onset of an adaptive host response. This response relies on the concerted action of a number of cytokines (TNFalpha, IFNgamma, IL12, IL18, and IL15), on the recruitment of inflammatory phagocytes in the tissues and on the activation of the recruited cells (Mastreoni 2002). Patients with deficiency in the interleukin-12 receptor are extremely susceptible to *Salmonella* infections, because interleukin-12 induces type 1 helper T-cell responses and interferon-type 1 helper T-cell responses and interferon-γ production. This innate immune pathway is necessary for resistance to salmonellosis (de Jong *et al*, 1998).

Lymphadenitis and abscesses are the commonest form of focal infection, and usually carry a good prognosis. The most severe form of focal NTS infection is vascular infection, arterial aneurysm (Benonson *et al*, 2001). It has been postulated that most patients with arteritis due

to NTS infection have preexisting atherosclerotic disease at the site of the infected aneurysm (Benonson *et al*, 2001). The two cases of arterial aneurysm in this study were successfully treated with surgical repair of the arterial wall in addition to antimicrobial therapy. Meningitis caused by NTS is associated with high mortality and morbidity (Huang *et al* 1997; Lee *et al* 1999). Antimicrobials preferred in the treatment of NTS meningitis or osteomyelitis are third generation cephalosporins or quinolones, rather than beta-lactams or chloramphenicol. This is because beta-lactam antibiotics are inferior, compared to intracellularly active preparations, like quinolones, and chloramphenicol is bacteriostatic, not bactericidal (Huang *et al*, 1997).

The duration of therapy is also an important consideration with focal NTS infection. For NTS meningitis, 4 to 6 weeks of antimicrobial therapy is generally considered necessary (Huang *et al*, 1997; Lee *et al*, 1999), while for NTS osteomyelitis, 4 weeks of therapy is sufficient (Sucato and Gillespie, 1997).

In conclusion, underlying immunocompromized states or chronic medical conditions were common among patients with focal extrainestinal NTS infections, with two-thirds of patients developing deep infections. Deep infections were more likely to occur in the extremes of age (<6 months or >60 years, p<0.04), associated with adverse outcomes, or required major surgery.

REFERENCES

Benonson S, Raveh D, Schlesinger Y, *et al*. The risk of vascular infection in adult patients with nontyphi *Salmonella* bacteremia. *Am J Med* 2001; 110: 60-3.

Brown M, Eykyn SJ. Non-typhoidal *Salmonella* bacteraemia without gastroenteritis: a marker of underlying immunosuppression. Review of cases at St Thomas' Hospital 1970-1999. *J Infect* 2000; 41: 256-9.

Chiu CH, Lin TY, Ou JT. Predictors for extraintestinal infection of non-typhoidal *Salmomella* in patients without AIDS. *Int J Clin Prac* 1999; 53: 161-4.

de Jong R, Altrare F, Haagen IA, *et al*. Severe mycobacterial and *Salmonella* infections in interleukin-12 receptor deficient patients. *Science* 1998; 280: 1435-8.

Huang LT, Ko SF, Lui CC. *Salmonella* meningitis: clinical experience with third-generation cephalosporins. *Acta Paediatr* 1997; 86: 1056-8.

Ispahani P, Slack RC. Enteric fever and other extraintestinal salmonellosis in University Hospital, Nottingham, UK, between 1980 and 1997. *Eur J Clin Microbiol Infect Dis* 2000; 19: 679-87.

Karim M, Islam N. *Salmonella* meningitis: report of three cases in adults and literature review. *Infection* 2002; 30: 104-8.

Lee WS, Puthucheary SD, Omar A. *Salmonella* meningitis and its complications in infants. *J Paediatr Child Health* 1999; 35: 379-82.

Lee WS, Puthucheary SD, Parasakthi N. Extra-intestinal non-typhoidal *Salmonella* infections in children. *Ann Trop Paediatr* 2000; 20: 125-9.

Lee WS, Puthucheary SD, Parasakthi N, Choo KE. Antimicrobial susceptibility and distribution of non-typhoidal *Salmonella* serovars isolated in Malaysian children. *J Trop Pediatr* 2003; 49: 37-41.

Leonard MK, Murrow JR, Jurado R, Gaynes R. *Salmonella* meningitis in adults infected with HIV: case report and review of the literature. *Am J Med Sci* 2002; 323: 266-8.

Lepage P, Bogaerts J, Nsengumuremyi F, *et al*. Metastatic focal infections due to multiresistant *Salmonella typhimurium* in children: a 34 month experience in Rwanda. *Eur J Epidemiol* 1986; 2: 99-103.

Mastroeni P. Immunity to systemic *Salmonella* infections. *Curr Mol Med* 2002; 2: 393-406.

Pabst HF, Kreth HW. Ontogeny of the immune responses as a basis of childhood disease. *J Pediatr* 1980; 97: 519-34.

Ramos JM, Garcia-Corbeira P, Aguado JM, Ales JM, Soriano F. Classifying extraintestinal non-typhoid *Salmonella* infections. *QJM* 1996; 89: 123-6.

Saphra I, Winter J. Clinical manifestations of salmonellosis in man. An evaluation of 7779 human infections identified at the New York *Salmonella* Center. *N Engl J Med* 1957; 256: 1128-34.

Sucato DJ, Gillespie R. *Salmonella* pelvic osteomyelitis in normal children: report of two cases and a review of the literature. *J Pediatr Orthop* 1997; 17: 463-6.

Sirinavin S, Jayanetra P, Lolekha S, Layangkul T. Predictors for extraintestinal infection in *Salmonella* enteritis in Thailand. *Pediatr Infect Dis J* 1988; 7: 44-8.

Tsao CH, Chen CY, Ou LS, Huang JL. Risk factors of mortality for *Salmonella* infection in systemic lupus erythematosus. *J Rheumatol* 2002; 29: 1214-8.