

Risk Factors of Non-typhoidal Salmonella Bacteremia versus Typhoidal Salmonella Bacteremia in Patients from a General Hospital in Karawaci, Tangerang, Indonesia: a five-year Review

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Abstract

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Salmonella infections including Non-typhoidal Salmonella (NTS) and enteric fever are important global public health problem, causing approximately 94 million human cases of gastroenteritis with 150,000 deaths annually around the globe. The aim of this study was to determine risk factors for NTS bacteremia patients compared to Typhoidal patients in Indonesia, area with high incidence of enteric fever. This retrospective descriptive study was conducted in Siloam Hospital in Karawaci, Indonesia, from January 2011 to December 2015. Logistic regression model was used to determine independent predictors of NTS bacteremia including demographic and epidemiologic characteristics, clinical presentations, and laboratory results. Out of 129 positive isolates for Salmonella with complete medical records, 18 (13.9%) were positive for NTS. Patients with NTS bacteremia were more likely to belong in the age group below 5 or above 60 year-old, more frequent to have anemia and abnormal leucocyte count. The susceptibility patterns against antimicrobial of NTS bacteremia and Typhoidal bacteremia were similar. In logistic regression analysis, age below 5 or above 60 year-old, hemoglobin level below 12 g/dL and leucocyte count below 4,000/ μ L or above 12,000/ μ L were independent risk factors for NTS bacteremia.

Introduction

Salmonella infections are important public health problem worldwide, particularly in developing countries, where they are the leading cause of morbidity and mortality.¹ In many regions Salmonella surveillance data is limited, but in South East Asia, it is estimated that there are approximately 22.8 million cases with 37,600 deaths annually.² Non-typhoidal Salmonella (NTS) is a group of *Salmonella enterica* spp. except for *S. enterica* serovar Typhi, Paratyphi A, Paratyphi B, and Paratyphi C. Out of 2500 serovars of *Salmonella enterica* that have been identified, human cases of NTS infection are caused by a limited number of serovars.³ In humans, NTS it is estimated that there are approximately 94 million cases of gastroenteritis with 150,000 deaths annually.² In Africa, NTS infection was one of the major causes of bacteremia,

especially in children and immunocompromised hosts such as HIV and malaria individuals.⁴ On the other hand, NTS infection in Asia is estimated to be much lower than in Africa. A surveillance study found only 6 cases of invasive NTS out of more than 20,000 blood cultures.⁵ In the settings of high incidence of *S. enterica* serovar Typhi infection in Indonesia, comparison between NTS bacteremia with Typhoidal bacteremia is important in diagnosing and managing both infection. The aim of this study was to compare demographic characteristics, epidemiologic characteristics including comorbidities, clinical presentations, laboratory results, and sensitivity pattern against antimicrobials of NTS bacteremia patients and of Typhoidal bacteremia patients admitted to a general hospital in Karawaci, Tangerang, Banten, Indonesia during the period of 2011-2015.

Materials and Methods

Study setting

This retrospective descriptive study was conducted in Siloam Hospital in Karawaci, Tangerang, Banten, Indonesia, which is a private teaching hospital affiliated to Faculty of Medicine, Pelita Harapan University. All patients admitted to the hospital from January 2011 to December 2015 with blood culture positive for *Salmonella* spp. were considered eligible. All patients were grouped as (1) NTS bacteremia group, which had positive blood culture for *Salmonella* spp. other than *S. typhi* and *S. paratyphi*, and (2) Typhoidal bacteremia group, which had positive blood culture for *S. typhi* and *S. paratyphi* as control group. Demographic characteristics (age and sex), and epidemiologic characteristics including comorbidities (diabetes mellitus and pneumonia), clinical presentations (fever, body temperature, pulse, diarrhea), laboratory results (hemoglobin, leucocyte, neutrophil, lymphocyte, eosinophil, platelet, and sensitivity pattern against antimicrobials) were collected from medical records. Fever was defined as axillary temperature above 37.8°C. Diarrhea was defined as loose or watery stools at least three times per day, or more frequently than normal for an individual.⁶ Clinical sepsis was defined as presence or suspected presence of infection, with any two of the following (1) hypo- (below 35.0°C) or hyperthermia (above 38.5°C), (2) abnormal age-adjusted leucocyte count below 4,000/ μ L, above 12,000/ μ L or above 10% bands, (3) tachycardia (defined as pulse rate above the upper normal limit according to age), (4) tachypnea (defined as respiratory rate above the upper normal limit according to age), and (5) abnormal cognition.⁷

Specimen, culture and identification

Venous blood collected from patients was inoculated into enriched soybean-casein digest broth with resins in BACTEC aerobic plus/F (Becton-Dickinson, New Jersey, USA) bottles. For patients with body weight less than 12.8 kg of weight, BACTEC Peds Plus/F bottles were used. When there was bacterial growth indicated by the BACTEC machine, blood culture bottles were sub-cultured onto a MacConkey agar plate.

Susceptibility testing

Susceptibility of *Salmonella* isolates against antimicrobials were detected using agar dilution method according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI). Minimal inhibitory concentrations (MICs) for each antibiotic was determined by VITEK 2 compact (bioMérieux, Marcy l'Etoile, France). Susceptibility interpretations were based on CLSI M100-S23 clinical breakpoints.⁸ The antibiotics used included carbapenems (meropenem, imipenem, ertapenem), penicillins (ampicillin, amoxicillin, amoxicillin clavulanate), cephalosporins (ceftriaxone, cefotaxime, ceftazidime), piperacilin tazobactam, fluoroquinolones (ciprofloxacin and levofloxacin), trimethoprim sulphamethoxazole, aminoglycosides (amikacin and gentamicin), and tigecycline. Results were included in the analysis only when the corresponding QC isolates tested were within the acceptable range according to CLSI guidelines. Chloramphenicol susceptibility for *Salmonella* was not tested according to the policy of the Indonesian Microbiology Association.

Statistics

The data were analyzed statistically using Statistical Package for Social Sciences (SPSS, version 24.0.0.0). Continuous data were presented as mean \pm SD or median (range), while categorical data as frequency (percentage). The student's t-test was used to compare groups of continuous data with normal distribution and the Mann-Whitney U test for groups of non-parametric data. Fisher's exact test was used to compare groups of categorical data when the expected cell value of 2 x 2 table was less than 5, and for all other cases Chi Squared test was used. Comparison between NTS and Typhoidal bacteremia group to determine independent predictors of NTS bacteremia using logistic regression model included demographic and epidemiologic characteristics, clinical presentations, and laboratory results variables with $p \leq 0.05$ from the univariate analysis.

Ethical considerations

This study was approved by the Research Review Committees of the Siloam General Hospital.

Results

In the period January 2011 to December 2015, a total of 1,679 positive blood cultures were collected from patients admitted to the Siloam Hospital in Karawaci, Tangerang, Banten, Indonesia. Of these, 29 were positive for NTS, 168 for *S. typhi* and *S. paratyphi A*. Out of these figures, 11 NTS, 57 *S. typhi* and *S. paratyphi A* patients were excluded due to unavailability of complete data in the medical records. None of the patients with NTS and Typhoidal bacteremia died in the period of admission due to infection or other causes. NTS bacteremia patients were more likely to be older and have lower mean hemoglobin level. Compared to patients with Typhoidal bacteremia, the patients with NTS bacteremia were more likely to belong in the age group below 5 year-old or above 60 year-old, and more frequent to have anemia and abnormal leucocyte count (Table 1). In logistic regression analysis, after adjusting for potential confounders, age group below 5 year-old or above 60 year-old, anemia, and abnormal leucocyte count were independent risk factors for NTS bacteremia (Table 2). The susceptibility patterns against antimicrobial of NTS bacteremia and Typhoidal bacteremia were similar (Table 3). The NTS bacteremia and Typhoidal bacteremia group had high susceptibility rate to most antimicrobials and high resistancy rate against amikacin and gentamicin.

Discussion

Invasive NTS infections causes significant morbidity and mortality worldwide, particularly associated with HIV, malnutrition, and malaria in sub-Saharan Africa. This study described the epidemiological and clinical characteristics along with laboratory results and antimicrobial susceptibility pattern of patients presenting with invasive NTS to a general hospital in Karawaci, Tangerang, Banten, Indonesia where HIV, malnutrition, and malaria rates were low.

This study found that NTS bacteremia occurrence was low, and no patients with NTS bacteremia and Typhoidal bacteremia died during hospital admission. NTS bacteremia is uncommon finding at Siloam Hospital, accounted for approximately 0.3% of positive blood cultures, compared to *S. typhi* which accounted for 1.7% of all positive blood

cultures. A systematic review has identified *S. typhi* as the most common community acquired blood stream infection in South and Southeast Asia among both adults and children.⁹ Studies in Asia and Africa found that NTS incidence ranged from 1.8 – 7.2 per 100,000 populations in Asia⁵ compared to 175 – 388 per 100,000 populations in Africa.¹⁰⁻¹² No fatalities occurred in patients with NTS and Typhoidal bacteremia died during admission of this study, in contrast with studies in China,⁴ Bangladesh,¹³ Sub-Saharan Africa,¹⁴ and Vietnam¹⁵ that found high fatality rate, ranged between 19.7% - 26%.

According to age distribution, 16.7% and 27.8% of patients with NTS bacteremia were infants and elderly, in contrast with Typhoidal bacteremia which found only 5.4% and 1.8% of patients were infants and elderly ($p = 0.000$). Studies have shown that infants and elderly were age groups known to be of highest risk for invasive NTS.^{5,7} Previous studies have reported differences in age distribution of patients with NTS and Typhoidal bacteremia.^{5,16}

Studies have shown NTS bacteremia to be more often invasive in immunocompromised patients than the healthy patients,¹⁷ and cytokines played important role in the susceptibility to NTS infection compared to Typhoidal bacteremia.¹⁸ Studies from South Asia and Africa suggested that malnutrition was a risk factor for NTS bacteremia compared with non-Salmonella bacteremia.¹⁹ Other study confirmed that malnutrition as risk factor remains when compared to age-matched Typhoidal bacteremia patients.¹³ It is hypothesized that malnutrition renders to immunocompromised state which was a predisposing factor for NTS bacteremia compared to Typhoidal bacteremia. Other condition that could contribute to immunocompromised state is chronic disease such as diabetes mellitus. In this study, no patient suffered from malnutrition. Proportion of diabetes in NTS and Typhoidal bacteremia patients was not significantly different.

A study found that higher proportion of NTS bacteremia patients had clinical signs of sepsis, acute kidney injury, and abnormal leucocyte count compared to Typhoidal bacteremia, which indicated more severe presentation.¹³ This study showed that median pulse rate, temperature, and leucocyte count of NTS

bacteremia patients did not differ significantly compared to Typhoidal bacteremia patients, although proportion of NTS bacteremia patients with leucocyte count below 4,000/ μ L or above 12,000/ μ L of was significantly higher compared to Typhoidal bacteremia patients. As clinical sepsis is the sum of the above variables, it could be predicted that proportion of NTS bacteremia patients with clinical signs of sepsis did not differ significantly compared to Typhoidal bacteremia patients.

Duration of fever and duration of diarrhea were not significantly different between NTS and Typhoidal bacteremia patients, which was in line with previous studies.^{13,20,21} A review article stated that NTS bacteremia disease presentation closely resembles Typhoidal bacteremia, in that patients had high fever, hepatosplenomegaly and respiratory complications.²²

Patients with NTS bacteremia had significantly lower mean hemoglobin level than Typhoidal patients. This is in contrast to findings of a study in Bangladesh,¹³ which found lower hemoglobin level in Typhoidal patients, as hematological changes in typhoid fever were caused by bone marrow suppression and hemophagocytosis.²³ The possible explanation for the higher proportion of anemia in this study would be the acute blood loss, especially in the gastrointestinal tract. Acute blood loss in the gastrointestinal tract could not be proven, because lack of data on feces analysis.

The susceptibility pattern of blood NTS isolates in this study shown high susceptibility against most antimicrobials except amikacin and gentamicin. The pattern is quite similar to a study in South East Asian countries, including Indonesia that found no resistance against ciprofloxacin and ceftriaxone.²⁴ The difference to the South East Asian study was resistance against sulphamethoxazole of 9.0% while in this study it was 5.6%. Other study in South Asia found NTS isolates resistance rate against ceftriaxone was 5.0%, trimethoprim sulphamethoxazole was 10.0% and no resistance against ciprofloxacin was.¹³ The situation was quite different in Africa where resistance rate were high, including against amoxicillin 55.6%, amoxicillin clavulanat 22.2%, trimethoprim sulphamethoxazole 31.8%, ciprofloxacin

30.4%.²⁵ Susceptibility pattern of Typhoidal isolates in this study was similar to NTS isolates, in contrast to other study which found different susceptibility pattern between NTS and Typhoidal isolates.¹³

There were several limitations of this study. The samples of this study were blood of patients admitted to the hospital, so the susceptibility pattern of *Salmonella* spp. representing the hospital and not the entire population of Banten province or Indonesia. The unavailability of data on chloramphenicol susceptibility because of the policy of the Indonesian Microbiology Association not to test chloramphenicol for *Salmonella* spp. anymore. Other antimicrobial not tested was nalidixic acid. In vitro nalidixic acid is more appropriate to test for in vivo fluoroquinolone resistance. The study used medical records to collect epidemiological, clinical characteristics, and laboratory results according to the retrospective nature of the design, thus data of some variables were missing due to incomplete medical records. Despite these limitations, this study is the first to analyze NTS bacteremia in Indonesia.

In conclusion, the study at a general hospital in Karawaci, Tangerang, Banten, Indonesia found age below 5 year-old and above 60 year-old, hemoglobin level below 12 g/dL and leucocyte count below 4,000/ μ L or above 12,000/ μ L were independent risk factors for NTS bacteremia. The high susceptibility pattern against antimicrobials of NTS isolates was similar to Typhoidal isolates. Further investigation is needed to describe epidemiology and antimicrobial susceptibility pattern of invasive NTS infections in Indonesia.

Competing Interests

The authors declare that there is no conflict of interest regarding the publication of this paper.

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Table 1. Demographic, clinical and laboratory characteristics of NTS bacteremia vs Typhoidal bacteremia

Variables	Non-typhoidal Salmonella Bacteremia (n=18)	Typhoidal bacteremia (n=111)	OR	95% CI	p value
Sex					
Male	9 (50.0%)	65 (58.6%)			
Female	9 (50.0%)	46 (41.4%)	0.71	0.26 – 1.92	0.671
Age	34 (1 – 77)	18 (4 – 80)			0.035*
Age group					
< 5 y.o	3 (16.7%)	6 (5.4%)			
5 – 60 y.o	10 (55.6%)	103 (92.8%)			0.000*
> 60 y.o	5 (27.8%)	2 (1.8%)			
Fever	15 (83.3%)	109 (98.2%)	0.09	0.01 – 0.59	0.019*
Duration of fever	1.5 (0 – 21)	5.0 (0 – 30)			0.111
Duration of fever ≥ 7 days	12 (66.7%)	68 (61.3%)	0.79	0.28 – 2.26	0.860
Duration of diarrhea ≥ 7 days	8 (44.4%)	37 (33.3%)	1.60	0.58 – 4.39	0.515
Pulse rate	90 (76 – 130)	90 (50 – 150)			0.362
Temperature	37.3 (36.0 – 38.5)	37.8 (35.8 – 40.2)			0.887
Hemoglobin	11.7 (1.96)	13.1 (1.73)			0.004*
Leucocyte	8,170 (3,250 – 23,730)	6,330 (1,910 – 81,160)			0.091
Neutrophil	60 (2 – 85)	61 (3 – 90)			0.174
Lymphocyte	24 (6 – 61)	25 (0 – 50)			0.968
Eosinophil	0.3 (0 – 2)	0.0 (0 – 5)			0.102
Platelet	189,295.5 (99,750,52)	207,380.27 (76,619.32)			0.471
Hemoglobin < 12.0 g/dL	9 (50.0%)	24 (21.6%)	3.63	1.29 – 10.14	0.018*
Leucocyte < 4,000/μL or > 12,000/ μL	9 (50.0%)	12 (10.8%)	0.89	0.27 – 2.93	0.000*
Clinical sepsis	4 (22.2%)	27 (24.3%)	1.57	0.17 – 14.93	1.000
Comorbidities					
Diabetes Mellitus	1 (5.6%)	4 (3.6%)	2.02	0.49 – 8.19	0.534
Pneumonia	3 (16.7%)	10 (9.0%)	8.25	2.74 – 24.81	0.391

Table 1. Logistic regression of NTS bacteremia vs. Salmonella typhi bacteremia

Variables	OR	95% CI	p value
Age group			0.004
< 5 y.o.	3.11	0.55 – 17.64	0.210
> 60 y.o.	28.31	3.70 – 216.51	0.001
Hemoglobin < 12.0 g/dL	4.39	1.19 – 16.24	0.026
Leucocyte < 4,000/μL or > 12,000/ μL	8.97	2.42 – 33.29	0.001

Table 2. Susceptibility pattern of NTS bacteriemia vs. Salmonella typhi typhoidal from blood culture.

Antimicrobial	Non-typhoidal Salmonella Bacteremia (n=18)	Typhoidal bacteremia (n=111)
Amikacin	2 (11.1%)	7 (6.3%)
Amoxicillin clavulanate	18 (100.0%)	111 (100.0%)
Amoxicillin	16 (88.9%)	109 (98.2%)
Ceftazidime	18 (100.0%)	111 (100.0%)
Ceftriaxone	18 (100.0%)	111 (100.0%)
Ciprofloxacin	18 (100.0%)	111 (100.0%)
Cefotaxim	18 (100.0%)	111 (100.0%)
Ertapenem	18 (100.0%)	111 (100.0%)
Gentamicin	1 (5.6%)	7 (6.3%)
Imipenem	18 (100.0%)	110 (99.1%)
Levofloxacin	18 (100.0%)	111 (100.0%)
Meropenem	18 (100.0%)	111 (100.0%)
Tigecycline	18 (100.0%)	111 (100.0%)
Trimethoprim sulphamethoxazole	17 (94.4%)	111 (100.0%)
Piperacillin tazobactam	17 (94.4%)	11 (99.1%)