Risk Factors for Nontyphi *Salmonella* Bacteremia Over 10 Years in Fort-de-France, Martinique, West Indies

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Abstract. Nontyphoidal *Salmonella* infections can result in bacteremia. This study was undertaken to determine the predictive factors for bacteremia in children aged less than 16 years. Medical data were collected for every child with positive nontyphoidal *Salmonella* cultures in blood or stools at the University hospital of Martinique, French West Indies, between January 2005 and December 2015. Among 454 patients, 333 were included; 156 cases had confirmed bacteremia, and 177 were included as control group with nontyphoidal *Salmonella* only isolated in stools. Age at diagnosis, delay before consulting, prematurity, immunosuppression, or hyperthermic seizures were not significantly associated with bacteremia. C-reactive protein was higher in cases of bacteremia ($P = 0.01$); however, after adjusting to the threshold of 30 mg/L, there was no longer any difference. There were also significant relations for electrolytes such as hyponatremia (odds ratio (OR) = 2.08 [95% CI = 1.31–3.95]; $P < 0.01$), high urea level (OR = 0.53 [95% CI = 0.32–0.88], $P < 0.01$). The infecting serotype was the most discriminant risk factor ($P < 10^{-4}$). Among 28 serotypes isolated between 2005 and 2015, *Salmonella panama* was the most common serotype: 122 strains (78.2%) were isolated from bacteremic patients versus 60 (33.9%) from nonbacteremic patients ($P < 10^{-4}$). *Salmonella panama* was the most important risk factor for bacteremia (OR = 7.37 [95% CI = 3.18–17.1], $P < 10^{-4}$) even after multivariate analysis (OR = 13.09 [95% CI = 5.42–31.59], $P < 10^{-4}$). After adjusting for bacteremia, *S. panama* was associated with a significantly higher body temperature than other *Salmonella*: 39°C (standard deviation [SD] = 0.92) versus 38.2°C [SD = 1.1], linear regression $P < 10^{-3}$. Children with *Salmonella* serotype panama infection were at higher risk of bacteremia than children infected with other *Salmonella* serotypes.

INTRODUCTION

In 2010, minor *Salmonella* also known as *Salmonella* nontyphi (NTS) were considered responsible for about 3.4 million infections worldwide and 681,000 deaths¹; the majority (57%) occurred in Africa.² In western countries, most of the time these infections are mild.³ The most frequent presentation is acute diarrhea, often associated with high fever. Most of the time, patients do not need specific treatment to get cured. However, in 6% of the cases, NTS infections are disseminated with severe clinical presentations^{4,5} (bacteremia, meningitis, or bone infections).^{6,7} In otherwise healthy individuals, systematic antibiotic therapy is not yet recommended against mild-to-moderate forms, but should be administered if the infection spreads beyond the intestine.⁸ There is currently no consensus on the indications for antibiotic treatment.

The epidemiology of NTS in the West Indies today remains unknown, and there are no data to predict the severity of NTS infections. However, NTS infections are frequently responsible for blood disseminations and for hospitalizations in Martinique, French West Indies. Thus, there may be specific factors responsible for more severe *Salmonella* infections.

The aim of this study was to define the clinical and biological characteristics of children presenting at the University Hospital of Martinique, French West Indies, with NTS bacteremia, and to compare them with those of children with nonbacteremic NTS gastroenteritis.

MATERIALS AND METHODS

Patients. Children evaluated in the University Hospital, Fort-de-France from January 1, 2005 through December 31, 2015, with documented NTS infection (positive blood culture or positive stool culture) were identified from logs maintained by the microbiology laboratory. All medical records were reviewed. The initial visit was defined as the day when the first positive blood culture was taken. Clinical data obtained from the initial visit (or the day the sample was drawn if the patient was already hospitalized) included the patient's age and sex, presence of any immunodeficiency or chronic illness, vital signs, presence of blood in the stools, laboratory results, antibiotic therapy, and length of hospital stay.

Patients were separated in two groups: bacteremic patients (cases) consisting of patients with a positive blood culture for NTS regardless of the result of the stool culture versus nonbacteremic patients (control group) consisting of patients with a positive stool culture for NTS and a negative blood culture or an isolated stool culture if there was no blood culture. Patients without a blood sample were considered with no demonstrated bacteremia and belonged to the nonbacteremic patients, which was the maximum bias hypothesis.

Bacteriology. We used aero-anaerobic blood culture bottles and grew cultures with BACTEC 9050 incubator for a maximum of 7 days. Bacteria were identified by a combination of morphological and biochemical tests. *Salmonella* strains were identified using a biochemical identification panel from Microscan (Beckman Coulter) which is the standard laboratory method used at the hospital laboratory. Every bacterial strain isolated in the laboratory of microbiology were stocked and sent to the National Reference Center for *Escherichia coli*,

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Shigella, and *Salmonella* (Pasteur Institute, Paris) to identify the corresponding serotype.

Biological normal values. For the C-reactive protein (CRP), a value of 30 mg/L was chosen according to the medical literature.¹⁰ It has been shown as the threshold value with the best positive predictive value for bacteremia in children in a context of acute gastroenteritis.

Other biological data categories used laboratory standards based on the patients' age.

Data analysis. Statistical analyses were conducted with the Statistical Stata (STATA® Version 12 for Windows). The two groups were compared starting with a bivariate analysis, followed by a multivariate analysis. Chi-squared and student tests were used to compare qualitative and quantitative variables between groups. Medians and interquartile ranges (25th to 75th percentile) were used to describe non-Gaussian data. Odds ratio (OR) was calculated with 95% confidence intervals. For qualitative variables, Dummy variables were created. The reference group corresponded to the Dummy variable not included in the logistic model. For multivariate analysis, the model included significant variables in the bivariate analysis. Statistical significance was based on an alpha risk equal to 5%.

Data protection and privacy ethical management. The anonymized monocentric data issued from medical records was analyzed which is authorized according to the regulatory authorities (Commission Nationale Informatique et Liberte's number 1906276v0, November 23, 2016).

RESULTS

Among the 454 patients with a positive sample for NTS between 2005 and 2015, 109 were older than 16 years of age at the time of diagnosis; 36 patients had another type of sample than blood or stool samples (such as cerebrospinal fluid or urine sample), and five serotypes appeared to be *Salmonella typhi* after identification. Among patients

older than 16 years of age, 29 had a second exclusion criterion.

Finally, 333 patients were included in our study; 156 belonged to bacteremic patients and 177 to nonbacteremic patients (Figure 1).

For those patients, the medical records were exhaustive in 124 patients in bacteremic patients and 136 in nonbacteremic patients. *Salmonella* serotype was known for 267 patients (132 in bacteremic patients and 135 in nonbacteremic patients).

The two populations were comparable in terms of gender and age (Table 1), and there was no difference regarding history of prematurity.

There was no significant difference regarding associated comorbidities, including immunosuppression ($P = 0.944$).

For the clinical variables, only body temperature on admission was significantly associated with bacteremia: 39.1°C (standard deviation [SD] = 0.85) for bacteremic patients versus 38.2 [SD = 1.06] for control patients ($P < 0.001$). There was no significant difference for consultation delay, heart rate on admission or for the presence of bloody-mucoid stools. However, for 73 patients (32 in bacteremic patients and 41 in nonbacteremic patients, clinical examinations were not included in the medical record and we only had access to constants taken at the emergency unit). The results were not different when the subgroup of individuals lacking clinical exams was included within the analysis.

Table 2 shows the comparisons regarding biological variables. The biological values on admission showed that natremia under the normal value were associated with risk of bacteremia: OR = 2.08 [1.31–3.95] ($P = 0.005$). In patients without bacteremia, serum urea was significantly higher than in patients with bacteremia. There was a difference for CRP level, potassium level, and hematocrit between groups in absolute value, but this association was no longer found after adjustment on age at diagnosis according to laboratory references.

FIGURE 1. Flow diagram for the case/control recruitment.

TABLE 1
Characteristics of the population with a positive culture for nontyphi *Salmonella*

Characteristics	Cases: bacteremic patients	Control: nonbacteremic patients	P* †
Male, n (%)	74 (47.44)	88 (49.72)	P = 0.678*
Female, n (%)	82 (52.56)	89 (50.28)	
Age (years), mean [SD]	3.42 [3.42]	3.42 [3.72]	P = 0.997†
Delay between the first symptoms and diagnosis (days), mean [SD]	4.26 [2.46]	3.96 [4.84]	P = 0.549†
Heart beat when admitted (bpm), mean [SD]	137.58 [24.90]	131.83 [26.02]	P = 0.147†
Body temperature on admission (°C), mean [SD]	39.09 [0.85]	38.15 [1.06]	P < 0.001†
Patient with mucoid-bloody stools, n (%), NA, n	39/124 (31.45) NA = 32	53/136 (38.97) NA = 41	P = 0.205*
Prematurity, n (%), NA, n	4/125 (3.20) NA = 31	6/138 (4.35) NA = 39	P = 0.627*
Hyperthermic seizure, n (%), NA, n	5/125 (4.00) NA = 31	3/138 (2.17) NA = 39	P = 0.389*
Septic shock, n (%)	3/124 (2.42)	0/136 (0)	–
Immunosuppression, n (%)	1/124 (0.8)	1/136 (0.74)	
Death, n (%), NA, n	0/124 (0) NA = 32	0/136 (0) NA = 41	

bpm = beats per minute, NA = not available, n (%) = number of patient (associated percentage).

* χ^2 = chi-squared test value.

†t = student test value.

Salmonella panama was isolated in 122 samples (78.21%) in patients with bacteremia versus only 60 samples (33.90%) in the control group. The OR associated with *S. panama* was 7.37 [95% CI = 3.18–17.1], ($P < 10^{-4}$) which was the risk factor with the highest OR among the studied covariables.

Only two *Salmonella* (*Salmonella enteritidis* [1.28%]) identified among the cases belonged to one serotype subject to French veterinary legislation for a screening (*Salmonella typhimurium*, *S. enteritidis*, *Salmonella infantis*, *Salmonella hadar*, *Salmonella virchow*) versus 46 in the control group (18 *S. typhimurium* [10.17%], 21 *S. enteritidis* [11.86%], 6 *S. infantis* [3.39%], and 1 *S. hadar* [0.56%]).

Eight cases (5.13%) and 29 controls (16.38%) had other *Salmonella* serotypes than *S. typhimurium*, *S. enteritidis*, *S. infantis*, *S. hadar*, *S. virchow*, or *S. panama*. In total, 28 different serotypes were isolated between 2005 and 2015.

Sixty-six serotypes were not found in medical records (24 in bacteremic patients [15.38%] and 42 in nonbacteremic patients [23.73%]; $P = 0.06$).

After multivariate analysis of the significant biological variables, the infecting serotype remained the main risk factor for having a bacteremia or not: the infecting serotype seems to be the more influent factor predicting a blood spreading evolution.

Among all the patients (bacteremic and nonbacteremic), those with *S. panama* had a higher temperature than other *Salmonella*: 39°C [SD = 0.92] for *S. panama*-infected patients versus 38.2°C [SD = 1.10] for patients with other serotypes, ($P < 10^{-4}$). This remained significant despite adjusting for the confounding effect of bacteremia (linear regression $P < 10^{-3}$). There was no other significant difference for other variables.

DISCUSSION

As shown in other studies, it is difficult to use clinical data as a predictive factor for blood dissemination in the case of NTS infections.^{11–15} Indeed, even if the fever at the time of admission seems to be a reliable risk factor, it was not specific of bacteremia. For biological variables, notably the

dehydration-related variables, such as sodium, potassium, and hematocrit, it has been shown elsewhere⁷ that patients susceptible to have a bacteremic evolution often show less important dehydration signs than patients with infections circumscribed within the intestinal sphere. Patients without bacteremia had significantly higher urea level than patients with bacteremia. We have not found similar results in the medical literature, and we could not explain this with the collected data.

Although other studies^{12,16–18} show that NTS bacteremia occurs more often in younger patients, especially before the age of 36 months, there was no significant difference in our population. Similarly, the length between the first symptoms and the positive sample was not a discriminant factor. These differences with other studies on clinical presentation could be explained by the important age range of our patients (from birth to 16-year old) and the physiological variations associated with the population. Another explanation could be the relatively easy access to health care in the area of Fort-de-France, which could explain short delays before medical consultation.

Among the possible biases was the retrospective nature of this study with missing data and incomplete patient charts. The patients were selected in a public university hospital when a biological sample seemed to be needed. This situation may have led to an overestimation of the severity of cases, which could explain the important proportion of patients with bacteremia in our study. Indeed, only 40% of the referenced strain of *Salmonella* at the National Reference Center of *E. coli*, *Shigella* and *Salmonella* are sent by public hospital laboratories.¹⁹

The study of serotypes in contrast serves to emphasize the high pathogenicity of the *panama* serovar. Although many studies worldwide involve the *virchow* serotype, *typhimurium* or recently *heidelberg*,^{11,12,18,20} *S. panama* remains poorly studied in the medical literature. *Salmonella panama* was strongly associated with bacteremia in Martinique even after multivariate analysis. The same conclusion was reported in Taiwan in 2002²¹ and 2007²² but only on a small number of patients. Our study confirmed this trend. Such a high

TABLE 2
Biological data for bacteremic and nonbacteremic patients between 2005 and 2015

Characteristics	Bacteremic patients	Nonbacteremic patients	Univariate analysis		Multivariate analysis*	
	N = 166	N = 177	Crude odds ratio [95% CI]	P†‡	Adjusted odds ratio [95% CI]	P§
White blood cells (G/L), mean [SD]	9.75 [4.10]	9.56 [4.21]	–	P = 0.726†	–	–
C-reactive protein (mg/L)	75.4 [61.21]	56.4 [51.92]	–	P = 0.007†	–	–
< 30 mg/L,¶ N (%)	32 (20.51)	52 (29.38)	Ref.	P = 0.063‡	Ref.	–
≥ 30 mg/L,¶ N (%)	124 (79.59)	125 (70.62)	1.61 [0.95–2.77]	–	1.04 [0.51–2.1]	P = 0.923
Sodium (mmol/L), mean [SD]	133.0 [3.07]	134.7 [3.87]	–	P < 10 ^{−3} †	–	–
Lower than normal value, N (%)	81 (51.92)	59 (33.33)	2.08 [1.31–3.95]	P = 0.003‡	1.04 [0.53–2.03]	P = 0.910
Normal interval value, N (%)	43 (27.56)	65 (36.72)	Ref.	–	Ref.	–
Higher than normal value, N (%)	32 (20.51)	53 (29.94)	0.91 [0.51–1.64]	–	0.62 [0.1–3.95]	P = 0.612
Potassium (mmol/L), mean [SD]	4.1 [0.52]	4.3 [0.51]	–	P = 0.014†	–	–
Lower than normal value, N (%)	4 (2.56)	3 (1.69)	1.59 [0.34–7.31]	P = 0.811‡	–	–
Normal interval value, N (%)	74 (74.44)	88 (49.72)	Ref.	–	–	–
Higher than normal value, N (%)	78 (50)	86 (48.59)	1.08 [0.70–1.67]	–	–	–
Urea (mmol/L), mean [SD]	2.9 [1.07]	3.5 [1.81]	–	P = 0.001†	–	–
Lower or within normal value, N (%)	117 (75)	109 (61.58)	Ref.	P = 0.009‡	Ref.	–
Higher than normal value, N (%)	39 (25)	68 (38.42)	0.53 [0.32–0.88]	–	0.47 [0.17–1.34]	P = 0.159
Bicarbonate (mmol/L), mean [SD]	21.3 [3.66]	20.1 [3.25]	–	P = 0.051†	–	–
Lower than normal value, N (%)	15 (9.62)	23 (12.99)	0.63 [0.29–1.34]	P = 0.464‡	–	–
Normal interval value, N (%)	48 (30.77)	46 (25.99)	Ref.	–	–	–
Higher than normal value, N (%)	93 (59.62)	108 (61.02)	0.83 [0.51–1.35]	–	–	–
Hematocrit (%), mean [SD]	33.7 [2.66]	34.9 [3.67]	–	P = 0.021†	–	–
Lower than normal value, N (%)	2 (1.28)	7 (3.95)	0.27 [0.05–1.35]	P = 0.138‡	Ref.	–
Normal interval value, N (%)	74 (47.44)	70 (39.55)	Ref.	–	0.54 [0.27–1.08]	P = 0.081
Higher than normal value, N (%)	80 (51.28)	100 (56.5)	0.76 [0.49–1.17]	–	–	–
Serotype, n (%)						
<i>Salmonella typhimurium</i>	0 (0)	18 (10.17)	–	–	–	–
<i>Salmonella enteritidis</i>	2 (1.28)	21 (11.86)	0.35 [0.07–1.79]	–	–	–
<i>Salmonella infantis</i>	0 (0)	6 (3.39)	–	–	–	–
<i>Salmonella hadar</i>	0 (0)	1 (0.56)	–	P < 10 ^{−4} ‡	–	–
<i>Salmonella virchow</i>	0 (0)	0 (0)	–	–	–	–
<i>Salmonella panama</i>	122 (78.21)	60 (33.90)	7.37 [3.18–17.1]	–	13.09 [5.42–31.59]	P < 10 ^{−4}
Other serotypes	8 (5.13)	29 (16.38)	Ref.	–	Ref.	–
Unknown serotype	24 (15.38)	42 (23.73)	2.07 [0.82–5.25]	–	4.18 [1.45–12.06]	P = 0.008

n (%) = number of patient (associated percentage); Ref. = reference value; SD = standard deviation; 95% CI = 95% confidence interval.

* Multivariate analysis according to a multivariate regression model.

† t = student test value.

‡ LR = likelihood ratio test value.

§ Wald test value.

¶ Best value for true bacteremia in children in a context of acute gastroenteritis according to Shaoul et al.¹⁰

|| Normal values adjusted on age on diagnosis according to laboratory references.

frequency of *S. panama* in Martinique is very unusual as this serotype ranks tenth in order of frequency in 2014 in France.¹⁹ Among the serotypes associated with higher risk for bacteremia, most are not yet included in the french panel for systematic veterinary and environmental screening. This result suggests that this panel is not optimized yet for tropical areas. There are reports of *S. panama* being responsible for severe infections,^{6,23,24} but the pathophysiology of clinical virulence remains under investigation. *Salmonella panama* belongs to the group D *Salmonella* species with *S. typhi*, but also *S. enteritidis* or *Salmonella Dublin*. Regardless of the bacteremic status, *S. panama* was associated with higher body temperature but without tachycardia as seen with *S. typhi* infections. *Salmonella panama* is presumably more virulent than other NTS.

In the multivariate analysis, unknown serotypes were associated with bacteremia. Among unknown serotypes, *S. panama* may have partially explained this result. It is likely that some of the unknown serotypes are of type *S. panama*, which could explain an association with bacteremia, but it is impossible to confirm this and other assumptions could explain this association.

In our study, three children were admitted in intensive care for septic shock. We cannot interpret this result as a reflection of serotype virulence in the absence of data on antibiotic therapy in these three children. Currently, there is no consensus about

the systematic antibiotic therapy in salmonellosis, including with positive blood cultures. The exhaustive list of treatments and their durations were not clearly listed in patients' medical records, so we were not able to analyze the antibiotic data among the period studied. However, because of the risk of developing resistance, it seems interesting to study the modalities of antibiotic treatment of salmonellosis in Martinique. When collecting data from the study, the antibiotic treatment modalities were often incomplete, and a new data collection is being studied to explore them.

Salmonella panama is currently not part of the *Salmonella* considered as having a high infectivity risk for humans in the French legislation²⁵; our study suggested that it would be advantageous to integrate *S. panama* in Martinique in a specific panel for veterinary and environmental screening. Early identification of the infecting serotype in the stools as *S. panama* could also be an indication for an early empirical antibiotic therapy before spreading in the bloodstream.

Our initial hypothesis was that specific risk factors are responsible for bacteremic salmonellosis in Martinique and thus that guidelines may need to be adapted. The results of this retrospective analysis confirm this hypothesis. Indeed, *S. panama*, currently a poorly studied serotype, appears to be specific to the

Caribbean region and appears as a major risk factor for bacteremia in children.

CONCLUSION

In Martinique, between 2005 and 2015, the most important risk factor for NTS bacteremia infection was being infected with the serotype *S. panama*. *Salmonella panama* was also the most frequent cause of NTS infection. *Salmonella panama* infections are a public health problem in Martinique.

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