

**Salmonella enterica serovars Panama and Arechavaleta:
Risk Factors for Invasive Non-Typhoidal Salmonella
Disease in Guadeloupe, French West Indies**

Stéphanie Guyomard-Rabenirina, Blandine Muanza, Sylvaine Bastian, Edith Malpote, Pauline Jestin, Meggie Guerin, Antoine Talarmin, François-Xavier Weill, Arnaud Legrand, Sebastien Breurec

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Title

Salmonella enterica serovars Panama and Arechavaleta, risk factors for invasive non-typhoidal *Salmonella* disease in Guadeloupe, French West Indies

Authors

Stephanie Guyomard-Rabenirina,¹ Blandine Muanza,² Sylvaine Bastian,³ Edith Malpote,³ Pauline Jestin,³ Meggie Guerrin,³ Antoine Talarmin,¹ François-Xavier Weill,⁴ Arnaud Legrand,⁵ Sebastien Breurec^{1, 4, 6*}

Authors' institutions

¹ Unité Environnement et Santé, Institut Pasteur de Guadeloupe, Pointe-à-Pitre, France

² Service de Pédiatrie, Centre Hospitalier Universitaire de Pointe-à-Pitre/les Abymes, Pointe-à-Pitre, France

³ Laboratoire de Microbiologie clinique et environnementale, Centre Hospitalier Universitaire de Pointe-à-Pitre/les Abymes, Pointe-à-Pitre, France

⁴ Unité des Bactéries pathogènes entériques, Centre National de Référence des *Escherichia coli*, *Shigella* et *Salmonella*, Institut Pasteur, Paris, France

⁵ Direction de la recherche clinique et de l'innovation, Centre Hospitalier Universitaire de Nantes, Nantes, France

⁶ Faculté de Médecine Hyacinthe Bastaraud, Université des Antilles, Pointe-à-Pitre, France

*Corresponding author:

Sébastien Breurec, Centre Hospitalier Universitaire de Pointe-à-Pitre/les Abymes, Laboratoire de Microbiologie clinique et environnementale, Route de Chauvel, Pointe-à-Pitre, France. Tel. (+590) 590 89 17 30. E-mail: sebastien.breurec@chu-guadeloupe.fr

Running title

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Abstract

A retrospective study was conducted to identify the risk factors associated with *Salmonella enterica* bacteremia in infants and children in Guadeloupe, French West Indies. The 171 patients with *S. enterica* infection seen between 2010 and 2014 included 155 (90.6%) with acute gastroenteritis, of whom 42 (27.1%) had concomitant bacteremia, and 16 (9.4%) with primary bacteraemia. Most cases (97.7%) were in infants and children with no underlying health condition. Two subspecies were recovered: *enterica* (n=161, 94.2%) and *houtenae* (n=10, 5.8%). All but one (serovar Typhi) were non-typhoidal *Salmonella*. The most common serovars were Panama (n=57, 33.3% of isolates) and Arechavaleta (n=28, 16.4%). Univariate analysis showed a strong association only between age > 6 months and infection with the Panama or Arechavaleta serovar ($P = 0.002$). The rate of resistance to all classes of antibiotics during the study period was low (< 15%); however, the detection of one extended-spectrum beta-lactamase-producing *S. enterica* strain highlights the need for continued monitoring of antimicrobial drug susceptibility. Infection with Panama ($P < 0.001$) or Arechavaleta ($P < 0.001$) serovar was significantly associated with bacteremia in a multivariate analysis. These serovars are probably poorly adapted to humans or are more virulent. A delay between onset of symptoms and hospital admission > 5 days ($P = 0.01$), vomiting ($P = 0.001$) and increased respiratory rate ($P = 0.001$) contributed independently to bacteremia in the multivariate analysis. Thus, if non-typhoidal infection is suspected, blood should be cultured and antibiotic treatment initiated in all patients who meet these criteria.

Introduction

Salmonella enterica serotypes Typhi, Paratyphi A, Paratyphi B, and Paratyphi C are grouped as typhoidal *Salmonella*, and other serovars are described as non-typhoidal *Salmonella* (NTS). Typhoidal *Salmonella* are human host-restricted bacteria that cause typhoid fever, a systemic disease, and paratyphoid fever. NTS strains may be host-generalists, capable of infecting or colonizing a broad range of vertebrate animals species, or host-specialists, adapted or restricted to particular non-human animal species.¹ Although most salmonellosis due to NTS results in self-limited acute gastroenteritis, NTS have emerged as an important cause of bloodstream infection.² In 2010, they were estimated to have caused approximately 3.4 million invasive infections and 681 000 deaths worldwide. Europe (102 cases/100 000 population) and the Americas (23 cases/100 000 population) had the second and third most cases of invasive NTS, respectively, but the numbers were substantially lower than that for Africa (227 cases/100 000 population). Young children, the elderly, malaria-infected and malnourished children, and immunocompromised people are at particular risk for invasive disease, which explains at least partially the discrepancies in incidence among regions.³

Guadeloupe, a French overseas territory located in the Caribbean, is a very high resource country according to the Human Development Index in 2013. Although few data are available on the epidemiology of *Salmonella* in humans in the Caribbean, it appears to be specific. In Martinique and Guadeloupe, *S. enterica* Panama was a major serovar, representing 35% of all isolates between 1990 and 1994 (first rank)⁴ and 15% between 1992 and 1995 (second rank).⁵ Surprisingly, this serovar has been rarely encountered in metropolitan France or in other regions of the world.⁶ At the University Hospital of Pointe-à-Pitre, around 35% (268/805) of the *Salmonella* isolates from humans belonged to serovars Panama and Arechavaleta during the period 2005–2014 (personal communication). The Panama serovar appears to have a propensity

to cause bloodstream infection and severe human disease.^{4, 5, 7} We therefore conducted a retrospective study to identify the risk factors associated with NTS bacteraemia in infants and children in Guadeloupe and to determine the pathogenicity of *Salmonella* serovars by analysing their contribution to *Salmonella* bacteremia. Antimicrobial susceptibility was also evaluated.

Material and methods

Population

Between January 2010 and December 2014, 171 infants and children aged ≤ 15 years admitted to the emergency room at the University Hospital in Pointe-à-Pitre with *Salmonella* infection confirmed by stool and blood culture were included. A specific, standard anonymized medical questionnaire was completed to collect demographic, clinical, and biological data and information on associated pathologies. Empirical antibiotic therapy was considered appropriate if the treatment regimen included at least one antibiotic that was active *in vitro* against the infecting microorganisms. The study protocols were approved by the French Advisory Committee on Information Processing in Material Research in the Field of Health (CCTIRS 11–40).

Microbiological analysis and serotyping

Strains were serotyped on the basis of somatic O and both phase 1 and phase 2 flagellar antigens by agglutination tests with antisera (Bio-Rad, Marnes-La-Coquette, France), as specified in the White-Kauffmann-Le Minor scheme.⁸ Antibiotic susceptibility to amoxicillin, amoxicillin-clavulanic acid, ticarcillin, cefalotin, cefoxitin, cefotaxime, ceftazidime, amikacin, tobramycin, gentamicin, nalidixic acid, ciprofloxacin, chloramphenicol, sulfonamides, cotrimoxazole, and tetracycline was determined by the disk diffusion method on Mueller-Hinton agar (Bio-Rad) according to the 2017 guidelines of the French Society for Microbiology/EUCAST (<http://www.sfm-microbiologie.org>). The minimum inhibitory

concentrations (MICs) for ceftriaxone and ceftazidime, and for ciprofloxacin for strains resistant to this antibiotic, were determined by the disk diffusion method on E-test strips (BioMerieux, Marcy L'Etoile, France). *Salmonella* strains that showed resistance to cefotaxime and/or ceftazidime were tested for the presence for extended-spectrum beta-lactamase (ESBL) by the double disk synergy method.

If more than one isolate with the same serotype and antimicrobial resistance phenotype was recovered from the same patient, only the first was retained for the analysis.

Statistical analysis

Children and infants were divided into those with bacteremia with or without gastroenteritis and those without bacteremia. Acute gastroenteritis was defined as diarrhea with a stool culture positive for *Salmonella*. Bacteremia was established when *Salmonella* was isolated from a blood culture.

Microsoft Access 2003 was used for data entry and Stata Version 10 for statistical analysis. In univariate analyses, the chi-squared test (or Fisher's exact test when appropriate) and Student's *t* test were used to compare categorical and continuous variables, respectively. Factors with *P* values < 0.20 in the univariate analysis were retained for the multivariate analysis. We considered *P* values < 0.05 to be significant.

RESULTS

Patient characteristics

A total of 171 patients with *S. enterica* infection were included retrospectively over the 36-month study period. The sex ratio (M/F) was 0.94, and the median age was 18.3 months (mean, 30.3 months; 25th percentile, 6.4 months; 75th percentile, 43.0 months). No specific medical history was reported, except for sickle-cell anemia in three children (one SS homozygous, one

AS heterozygote, and one S/β heterozygote) and a brain tumor in one. A total of 155 (90.6%) patients presented with acute gastroenteritis, of whom 42 (27.1%) had concomitant bacteremia and 16 (9.4%) primary bacteremia. Of the 171 cases, 151 (88.3%) were hospitalized: 56 (96.6%) with bacteremia and 95 (84.0%) without ($P = 0.016$).

The characteristics of the 171 patients are presented in Table 1. Five children were in septic shock, but no deaths were reported.

Serotypes, antibiotic susceptibility, and antibiotic treatment

All 171 isolates were analyzed. Two subspecies were recovered: *enterica* (n=161, 94.2%) and *houtenae* (n=10, 5.8%). All but one (serovar Typhi) were NTS. The most common serotypes were Panama (n=57, 33.3% of isolates) and Arechavaleta (n=28, 16.4%). The other major serotypes were Enteritidis (n=23, 13.5%), Typhimurium monophasic variant 4,[5],12:i:- (n=15, 8.8%), and 43:z4,z32:-:- (*houtenae* subspecies) (n=9, 5.3%). Among the *Salmonella* blood isolates (n=58), all but one (serovar Typhi) were NTS, of which 48 (82.8%) were assigned to Panama (n=31, 53.4%) or Arechavaleta (n=17, 29.3%) (Table 2).

Overall, the 171 *Salmonella* isolates showed a low level of resistance to all antibiotics: amoxicillin (n=15, 11.4%), amoxicillin-clavulanic acid (n=1, 0.8%), ticarcillin (n=15, 11.4%), cefalotin (n=2, 1.5%), cefoxitin (0%), cefotaxime (n=1, 0.8%), ceftazidime (n=1, 0.8%), imipenem (0%), amikacin (0%), tobramycin (0%), gentamicin (0%), nalidixic acid (n=3, 2.3%), ciprofloxacin (n=2, 1.5%), and cotrimoxazole (n=2, 1.5%). No increase in the prevalence of resistance to antibiotics was observed during the study period. The only serovar Typhi strain, isolated in 2014, was resistant to ciprofloxacin (MIC, 1.5 mg/L), and an isolate belonging to serovar Typhimurium (2010) was resistant to all beta-lactams (MIC ceftazidime, 32 mg/L, MIC ceftriaxone, > 256 mg/L) except cefoxitin and imipenem and was susceptible to other antibiotic families. The double-disk synergy test was positive, indicating production of an ESBL.

Only four patients were admitted to the emergency room with a probabilistic antibiotic (amoxicillin). Most of patients received antibiotic treatment on admission (86.0%, 147/171), consisting mainly of third-generation cephalosporins (76.6%, 131/171). Other commonly prescribed antibiotics were amoxicillin (n=8), amoxicillin-clavulanic (n=7), and cotrimoxazole (n=1). The treatment was adequate in all cases except one (third-generation cephalosporin for a strain resistant to this antibiotic). Antibiotic treatment was prescribed significantly more to patients with bacteremia than to those without (95.0% vs 81.4%, $P < 0.016$).

Risk factors for *Salmonella* bacteraemia

A delay between onset of symptoms and hospital admission > 5 days ($P = 0.002$), age > 6 months ($P = 0.002$), infection with Panama or Arechavaleta serovar ($P < 0.001$), vomiting ($P = 0.005$), and increased respiratory rate ($P = 0.004$) were significantly associated with bacteremia in the univariate analysis. A delay between onset of symptoms and hospital admission > 5 days, infection with Panama or Arechavaleta serovar, vomiting and increased respiratory rate were the main independent contributors to bacteremia in the multivariate analysis (Table 1).

Univariate analysis showed that only age > 6 months was associated with infection with Panama or Arechavaleta serovar ($P = 0.002$).

Discussion

The serovar most often recovered in our study was Panama, as found in Martinique (38.5% of all strains investigated) and French Guiana (11.7%).^{4,6} Panama was also the major serovar in humans in Colombia and Chile.^{9,10} This high prevalence is in contrast to that in other regions of the world, including mainland France (0.5% in 2011),¹¹ highlighting the specific epidemiology of these regions. In industrialized countries, NTS is transmitted predominantly

through commercially produced food contaminated with animal feces.¹ The reservoir appears to be different for the Panama serovar, as suggested by the four recently described cases of *Salmonella* Panama meningitis in exclusively breastfed infants in French Guiana.¹² In addition, no *Salmonella* Panama isolate was found among 275 *Salmonella* spp. isolated from 1636 samples from bovine, porcine, and avian food products and from poultry and the poultry environment between 2010 and 2014 at the Food Testing Laboratory at the Institut Pasteur in Guadeloupe (unpublished data). Contact with animals, such as reptiles, is an important non-food source of NTS infection.^{13, 14} In Guadeloupe, wild reptiles and amphibians (e.g. lizards, geckos, frogs) are commonly found in and around houses. Thus, a reptilian reservoir for the Panama serovar is plausible, as it is found in frogs, toads, turtles, lizards, and snakes,^{6, 15, 16} and the host range might be much larger due to its presence in wild birds, swine, poultry, and Indian mongooses.^{17, 18, 19, 20, 21} In our study, Arechavaleta was the second most important serovar. To the best of our knowledge, Arechavaleta has been identified only in cane toads, dogs, and Indian mongooses.^{21, 22, 23} The major serovar 43:z4,z32:- (*houtenae* subspecies) in our study is also exotic. It was recovered in one case of osteomyelitis in a Taylor's cantil pit viper²⁴ but never in humans. The other serovars have commonly been associated with human infections. Further investigations are needed to identify the reservoir of the exotic Panama, Arechavaleta, and 43:z4,z32:- *Salmonella* serovars in Guadeloupe, and a reptilian source of contamination should be investigated.

Although we had a small sample, we detected a tangible trend of association between age > 6 months and the Panama and Arechavaleta serovars in our study. Guadeloupe has a high rate of breastfeeding (around 90% in 2013),²⁵ which protects neonates against infection through specific and nonspecific immune factors,²⁶ and exclusive breastfeeding also avoids exposure to contaminated water or food.

NTS *Salmonella* bacteremia usually occurs in 6–11% of children with gastroenteritis,^{7, 27,}
²⁸ which is less than the 27% observed in our study. Several factors are known to be associated with an increased risk for invasive disease among children with *Salmonella* infection, including HIV infection, sickle cell disease SS, and specific serovars of *Salmonella*. HIV status was not systematically investigated in our study, but none of the parents spontaneously declared their child to be seropositive, and we assumed that HIV infection was low in our population. Only 4 (2.3%) of the 171 patients with NTS bacteremia had underlying disease, three with sickle-cell anemia, indicating that most of the cases of *Salmonella* bacteremia occurred in children with no underlying health condition.

Infection with Panama and Arechavaleta serovars was significantly associated with the occurrence of bacteremia. The Panama serovar was first isolated during an investigation of food poisoning among United States soldiers stationed in Panama in 1934,²⁹ and this serovar has repeatedly been described as causing invasive disease, such as bacteremia and meningitis, in children.^{30, 31, 32, 33} To the best of our knowledge, the Arechavaleta serovar has not been reported to cause severe infections. The way in which these two serovars cause invasive disease is unknown; however, both evolutionary theory and empirical comparisons predict that chronic pathogens such as *Helicobacter pylori* will become less virulent over time because of coevolution with their hosts.³⁴ The disruption of coevolved hosts and Panama and Arechavaleta serovars might explain the severity of infection, as these serovars are probably poorly adapted to humans, as indicated by the fact that reptile-related salmonellosis has been associated with young age, a high rate of hospitalization, and invasive disease.^{35, 36} The greater virulence of these serovars might also be an explanation. Further studies are needed to clarify their pathogenicity.

Patients whose clinical symptoms persisted for > 5 days and who had an increased respiratory rate on admission were significantly more likely to have bacteremia, in agreement with previous reports.^{37, 38} The association between vomiting and bacteremia has not been

described, but vomiting was found to be a predictive factor for NTS bacteremia in children < 5 years of age also infected with *Plasmodium falciparum* at a low parasite count.³⁹ No deaths were reported in our study, indicating prompt, effective management. In contrast to the worldwide situation,⁴⁰ the rates of resistance to all classes of antibiotics were low, and there was no increase in the prevalence of resistance to first-line antibiotics during the study period, which accounts for the high rate of adequate antibiotic treatment. One therapeutic failure due to the only ESBL-producing *Salmonella* isolate was observed. Although the rate of ESBL-producing *Salmonella* isolates in our study was low, consistent with studies elsewhere in the world (0–2.4%),^{8, 41, 42, 43} it is important to continue monitoring antimicrobial susceptibility in *Salmonella* isolates from humans, as effective antimicrobial therapy reduces mortality and complications and shortens the illness.

In conclusion, the data reported here add to understanding of the epidemiology of *Salmonella* in the Caribbean. NTS bacteraemia should be recognized in healthy infants and children of all ages. Panama and Arechavaleta were the two serovars most often recovered in our study, with a propensity to cause bloodstream infection. If NTS infection is suspected, blood should be cultured and antibiotics initiated in all infants and children ill enough to be admitted to hospital with clinical symptoms for > 5 days, vomiting, or an increased respiratory rate.

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Disclosures regarding conflicts of interest

The authors have no conflict of interest to declare.

Authors' current addresses

Stephanie Guyomard-Rabenirina, Institut Pasteur de Guadeloupe, Unité Environnement et Santé, BP 484, Morne Jolivière, 97183 Abymes, France. E-mail: sguyomard@pasteur-guadeloupe.fr

Blandine Muanza, Centre Hospitalier Régional, Service de Pédiatrie, Route de Chauvel, 97139 Abymes, France. E-mail: blandise.muanza@chu-guadeloupe.fr

Sylvaine Bastian, Centre Hospitalier Régional, Laboratoire de Microbiologie clinique et environnementale, Route de Chauvel, 97139 Abymes, France. E-mail: sylvaine.bastian@chu-guadeloupe.fr

Edith Malpote, Centre Hospitalier Régional, Route de Chauvel, Laboratoire de Microbiologie clinique et environnementale, 97139 Abymes, France. E-mail: edith.malpote@chu-guadeloupe.fr

Pauline Jestin, GH de La Rochelle-Ré-Aunis, Laboratoire de Biologie médicale, rue du Docteur Schweitzer, 17019 La Rochelle Cedex. E-mail: pauline.jestin@ch-larochelle.fr

Meggie Guerrin, Centre Hospitalier Andrée Rosemon, Laboratoire Hospitalier et Universitaire de Parasitologie-Mycologie, Avenue des Flamboyants, 97306 Cayenne. E-mail: meggie.guerin@gmail.com

Antoine Talarmin, Institut Pasteur de Guadeloupe, Unité Environnement et Santé, BP 484, Morne Jolivière, 97183 Abymes, France. E-mail: Antoine Talarmin, atarmin@pasteur-guadeloupe.fr

François-Xavier Weill, Institut Pasteur, Centre National de Référence des Escherichia coli, Shigella et *Salmonella*, Unité des Bactéries Pathogènes Entériques, 28 rue du docteur Roux, 75724 Paris cedex 15. Tel: (+33) 1 45 68 83 45. E-mail: fxweill@pasteur.fr

Arnaud Legrand, Centre Hospitalier Universitaire de Nantes, DRCI, 1 place Alexis Ricordeau, 44000 Nantes, France. E-mail: arnaud.legrand@chu-nantes.fr

Sebastien Breurec, Centre Hospitalier Universitaire de Pointe-à-Pitre/les Abymes, Laboratoire de Microbiologie clinique et environnementale, Route de Chauvel, Pointe-à-Pitre, France. E-mail: sebastien.breurec@chu-guadeloupe.fr

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