

Characterization of Invasive *Salmonella* Serogroup C1 Infections in Mali

Fabien J. Fuche,^{1,2} Sunil Sen,^{1,2} Jennifer A. Jones,^{1,2} Joseph Nkeze,^{1,2} Jasnehta Permala-Booth,^{1,2} Milagritos D. Tapia,^{1,3} Samba O. Sow,⁴ Boubou Tamboura,⁴ Aliou Touré,⁴ Uma Onwuchekwa,⁴ Mamadou Sylla,⁴ Karen L. Kotloff,^{1,3} and Sharon M. Tennant^{1,2*}

¹Center for Vaccine Development and Institute for Global Health, University of Maryland School of Medicine, Baltimore, Maryland, ²Department of Medicine, University of Maryland School of Medicine, Baltimore, Maryland, ³Department of Pediatrics, University of Maryland School of Medicine, Baltimore, Maryland; ⁴Centre pour le Développement des Vaccins, Mali, Bamako, Mali

Abstract. Nontyphoidal *Salmonella* (NTS) are the leading cause of foodborne infections worldwide and a major cause of bloodstream infections in infants and HIV-infected adults in sub-Saharan Africa (SSA). *Salmonella* Typhimurium (serogroup B) and *Salmonella* Enteritidis (serogroup D) are the most common serovars in this region. However, data describing rarer invasive NTS serovars, particularly those belonging to serogroups C1 and C2, circulating in SSA are lacking. We previously conducted systematic blood culture surveillance on pediatric patients in Bamako, Mali, from 2002 to 2014, and the results showed that serovars Typhimurium and Enteritidis accounted for 32% and 36% of isolates, respectively. Here, we present data on 27 *Salmonella* serogroup C1 strains that were isolated during this previous study. The strains were typed by serum agglutination and multilocus sequence typing (MLST). Sixteen strains were *Salmonella* Paratyphi C, four were *Salmonella* Colindale, and two were *Salmonella* Virchow. Interestingly, five strains were identified as the very rare *Salmonella* Brazzaville using a combination of serum agglutination and flagellin gene typing. Phenotypic characterization showed that *Salmonella* Brazzaville produced biofilm and exhibited catalase activity, which were not statistically different from the gastroenteritis-associated *Salmonella* Typhimurium sequence type (ST) 19. All tested *Salmonella* Paratyphi C strains were poor biofilm producers and showed significantly less catalase activity than *Salmonella* Typhimurium ST19. Overall, our study provides insight into the *Salmonella* serogroup C1 serovars that cause invasive disease in infants in Mali. In addition, we show that MLST and flagellin gene sequencing, in association with traditional serum agglutination, are invaluable tools to help identify rare *Salmonella* serovars.

INTRODUCTION

Nontyphoidal *Salmonella* (NTS) are the leading cause of foodborne infections worldwide and a major cause of gastroenteritis and bloodstream infections. Of the 94 million cases and 155,000 deaths attributed to NTS every year worldwide, sub-Saharan Africa (SSA) bears the highest burden with 193–338 disability-adjusted life years per 100,000 individuals, compared with 50 and 67 for Europe and North America, respectively.¹ In particular, invasive NTS (iNTS) disease, in which bacteria invade the bloodstream and cause life-threatening disseminated infections, is a leading cause of morbidity and mortality among infants and HIV-positive adults in SSA, with up to a 30% mortality rate.^{2,3}

We recently emphasized the underestimated burden of NTS infections caused by *Salmonella enterica* serogroups C1 (O:6,7) and C2 (O:6,8).⁴ Although *Salmonella* serovars Typhimurium (serogroup B; O:4) and Enteritidis (serogroup D; O:9) have been extensively recognized as the major serovars responsible for iNTS infections in SSA, little is known about serovars from serogroup C1 or C2. Yet the prevalence of these two serogroups has been increasing worldwide (from 22.5% to 34.7% of NTS in the United States and from 5% to 8.6% in Europe, between 1995 and 2012), including in Africa where they now represent 19.5% of all reported NTS cases. Large studies that describe the *Salmonella* serovar distribution in Africa are needed to better understand the burden of iNTS disease.

We have been conducting systematic blood culture surveillance at l'Hôpital Gabriel Touré, the main teaching

hospital in Bamako, Mali, since 2002.⁵ This work has provided valuable information about the burden of invasive bacterial pathogens in pediatric patients, such as *Haemophilus influenzae* type b and *Streptococcus pneumoniae*, as well as *S. enterica*.^{6–9} In the period of 2002–2014, 687 NTS isolates were obtained from blood samples of 667 children.⁵ *Salmonella* Enteritidis (36%), *Salmonella* Typhimurium (32%), *Salmonella* I 4,[5],12:i:- (6%), and *Salmonella* Dublin (13%) were the most common serovars. In addition, 27 serogroup C1 or C2 strains were isolated, but were not serotyped further. Here, we describe the identification of *Salmonella* serovars that previously agglutinated with O:6 antisera by biochemical and molecular analyses and also report phenotypic characteristics of these strains.

MATERIALS AND METHODS

Ethics statement. The clinical research protocol and consent form were reviewed by the Ethics Committee of the Faculté de Médecine, de Pharmacie et d'Odonto-Stomatologie, University of Mali and by the Institutional Review Board of the University of Maryland, Baltimore. Consent was obtained from parents/guardians of every child and assent of the child was also required for enrollment of participants > 13 years of age.

Bacterial strains. Twenty-seven *Salmonella* strains that agglutinated with O:6 antisera were previously isolated from pediatric patients who presented with fever or clinical symptoms compatible with invasive bacterial disease at l'Hôpital Gabriel Touré in Bamako, Mali, between 2002 and 2014.⁵ Control strains for phenotypic assays included *S. Typhimurium* I77 (ST19) and D65 (ST313), *S. Typhi* Ty2, and *S. Paratyphi* A ATCC 9150 (American Type Culture Collection, Manassas, VA).^{5,10}

* Address correspondence to Sharon M. Tennant, Center for Vaccine Development, University of Maryland School of Medicine, 685 W. Baltimore St., HSF Room 480, Baltimore, MD 21201. E-mail: stennant@som.umaryland.edu

Bacterial growth conditions. Unless otherwise specified, all bacterial strains were grown in Hy-Soy medium (10 g/L soytone [Teknova, Hollister, CA], 5 g/L hy-yeast [Kerry Bio-Science, Norwich, NY], and 5 g/L sodium chloride [AmericanBio, Natick, MA]). When needed, agar (AmericanBio) was added at 15 g/L.

Clinical microbiology. NTS strains were agglutinated with O grouping and H typing antisera (Denka Seiken Co. Ltd, Tokyo, Japan and Difco, BD Diagnostics, Franklin Lakes, NJ), and serovars were identified according to the White–Kauffmann–Le Minor typing scheme.¹¹

The ability to ferment dulcitol and mucate was assessed using dulcitol-containing phenol red broth (BD Diagnostics) and Remel mucate medium (Thermo Fisher Scientific, Lenexa, KS), and hydrogen sulfide production was determined by precipitation of sodium thiosulfate and ferric ammonium citrate after inoculation of triple sugar iron medium (EMD Chemicals, Billerica, MA). These biochemical characteristics then allowed us to discriminate between *Salmonella* serovars Paratyphi C (dulcitol-positive, H₂S-positive, and mucate-negative), *Choleraesuis sensu stricto* (dulcitol-negative, H₂S-negative, and mucate-negative), *Choleraesuis* var. *Kunzendorf* (dulcitol-negative, H₂S-positive, and mucate-negative), and *Choleraesuis* var. *Decatur* (dulcitol-positive, H₂S-positive, and mucate-positive).

Antimicrobial susceptibility was determined using the Kirby–Bauer disk diffusion method, as previously described.⁵ Antimicrobials tested were amikacin (30 µg), ampicillin (10 µg), aztreonam (30 µg), cefazolin (30 µg), cefepime (30 µg), streptomycin (10 µg), ciprofloxacin (5 µg), nalidixic acid (30 µg), tigecycline (15 µg), and sulfamethoxazole/trimethoprim (cotrimoxazole, 25 µg).

Detection of *viaB* by polymerase chain reaction (PCR). The presence of Vi antigen in *S. Paratyphi C* isolates was evaluated by PCR using 0.3 µM of each primer JN07-Vi_F (5'-GCACCGTTTAACCAACATCAAG-3') and JN08-Vi_R (5'-TGTACCTGCGCTGATGATCTG-3'), 5 U of Green Taq polymerase (GenScript, Piscataway, NJ), and 0.3 mM stabilized dNTP (GenScript) in a 50 µL reaction under the following cycling conditions: initial denaturation at 95°C for 3 minutes, 30 cycles of amplification (95°C for 30 seconds, 60°C for 45 seconds, and 70°C for 5 seconds), and a final elongation step at 95°C for 5 minutes.

Multilocus Sequence Typing (MLST). MLST was performed according to Achtman et al.¹² Previously described primers were used to amplify seven loci (*aroC*, *dnaN*, *hemD*, *hisD*, *purE*, *sucA*, and *thrA*) from freshly extracted genomic DNA (GenElute Kit; Sigma-Aldrich, St. Louis, MO).¹³ Correct amplification was confirmed by agarose gel electrophoresis, and PCR fragments were gel-purified using a QIAquick gel extraction kit (Qiagen, Hilden, Germany). Sequencing data were obtained from Genewiz (South Plainfield, NJ) using described sequencing primers and applied to the MLST database search engine available at <http://mlst.warwick.ac.uk/mlst/dbs/Senterica> for allele and sequence type (ST) identification.

Flagellin typing by PCR. When needed, flagellar H-type was determined by amplifying the *fljC* gene using primers FLIC-F (5'-CAAGTCATTAATACAAACAGC-3') and FLIC-R (5'-TTAACGCAGTAAAGAGGAC-3') described by Weill et al.¹⁴ and the *fljB* gene using primers fljB-fw (5'-ATCAGGAT CCTCCAAAAGGAAA-3') and fljB-rev (5'-TCAGCTAGCTG

AATAAACGAAA-3') designed in this study. PCR assays were performed using 0.8 µM of each primer, 1 U of Vent DNA polymerase (New England Biolabs, Ipswich, MA), and 0.4 mM stabilized dNTP (GenScript) in a 50 µL reaction under the following cycling conditions: initial denaturation at 95°C for 2 minutes, 30 cycles of amplification (95°C for 30 seconds, 60°C for 30 seconds, and 72°C for 90 seconds), and a final elongation step at 95°C for 5 minutes. PCR products were verified and gel-purified as described earlier, and sequencing data obtained from Genewiz using the same primers that were used for PCR amplification. Sequences were then aligned against flagellin sequences whose antigenic type is known using the National Center for Biotechnology Information nucleotide collection database (<https://blast.ncbi.nlm.nih.gov>).

Biofilm quantification. We used the crystal violet assay to quantify the amount of biofilm synthesized by *Salmonella* strains.¹⁵ Briefly, overnight cultures were used to inoculate Hy-Soy lacking NaCl in quadruplicate wells of a 96-well plate. The plates were then incubated with shaking at 37°C for 48 hours. The wells were washed twice with PBS, and 100 µL of crystal violet staining solution (Sigma-Aldrich) was added into each well. The plates were incubated for 15 minutes at room temperature, before being washed five times with deionized water. The adherent material in the wells was destained by adding 200 µL of 70% ethanol into each well. Optical density at 600 nm was recorded using a plate reader (Molecular Devices, Sunnyvale, CA) and corrected by the optical density of the blank (medium only wells).

Catalase assay. We used the catalase assay developed by lwase et al.¹⁶ with slight modifications. Bacterial strains were grown overnight in 1% soytone at 37°C with shaking. Optical density at 600 nm (OD₆₀₀) was recorded to estimate bacterial concentration and 2 mL of culture (6–8 × 10⁹ bacteria) was centrifuged; the supernatant was removed; and the cell pellet was resuspended in 100 µL of normal saline (9 g/L sodium chloride; Quality Biological, Gaithersburg, MD) and transferred into 13 × 100 mm borosilicate glass tubes. One-hundred microliters of 1% Triton X-100 and 100 µL of 35% stabilized hydrogen peroxide were added. The tubes were left untouched at room temperature until the foaming stopped (about 2 minutes). The height of the foam in the tube was then recorded. A standard curve was also created using dilutions of commercially available bovine catalase (Sigma-Aldrich; 3,000 units/mg), and the final results were expressed as units of catalase (U) per 10¹⁰ bacterial cells, assuming an OD₆₀₀ of 1.25 corresponds to 10⁹ bacteria/mL of culture for all strains tested.

RESULTS

Of the 27 *Salmonella* strains that agglutinated with O:6 antisera and which were isolated from the blood of infants and children at l'Hôpital Gabriel Touré, Bamako, Mali, between 2002 and 2014, all were determined to be O:6,7 (serogroup C1) by agglutination with antisera and by MLST (Table 1). Sixteen isolates were identified as *S. Paratyphi C* (O:6,7;c;1,5), and all were Vi-positive. MLST revealed that although two strains belonged to ST146, the majority (14/16) belonged to the less common ST114.¹² Five isolates were serovar Brazzaville (O:6,7;b;1,2) based on O- and H-agglutination. The H-type was confirmed by sequencing the *fljC* and *fljB* genes. We were not able to determine the ST as there was no entry for this

TABLE 1

Distribution of 27 *Salmonella* serogroup C1 strains isolated from the blood of febrile pediatric patients in Bamako, Mali, from 2002 to 2014

Serovar	O*	H*	ST†	No. of isolates
Paratyphi C	6,7	c;1,5	114 146	14 2
Brazzaville	6,7	b;1,2	No assigned ST	5
Colindale	6,7	r;1,7	584	4
Virchow	6,7	r;1,2	121 755	1 1

ST = sequence type.

* Determined by agglutination with antisera against O polysaccharide and H flagella antigens.

† Determined by amplifying and sequencing seven housekeeping genes according to Achtman et al.¹²

combination of allele types in the *Salmonella* MLST database. The combination of alleles for all seven loci tested was identical for all five *S. Brazzaville* strains (Table 2), indicating that all five isolates of this serovar belonged to the same ST. Finally, four isolates were identified as *S. Colindale* (ST584) and two as *S. Virchow* (ST121 and ST755).

Of note, the five strains of *S. Brazzaville* were isolated within 10 months (April 2004 to February 2005; Figure 1), but were found in patients from four different communes (areas 2, 3, 5, and 6). In addition, *S. Paratyphi C* was isolated every year from 2002 to 2008 (except 2007).

Overall, children between 13 and 24 months of age had the most *Salmonella* serogroup C1 of all age groups (Figure 2A). *S. Paratyphi C* isolates were obtained from infants and children less than 6 years old, with a median age of 24 months (Figure 2B). *S. Brazzaville* strains were isolated from significantly younger children (median age of 15 months), with one strain isolated from a month-old newborn. *Salmonella* Colindale and *S. Virchow* infected children of a broader age range (10 months to 13 years). Clinical symptoms, diagnosis, and outcome for each patient are listed in Supplementary Table 1. Symptoms included fever, diarrhea, and chest indrawing, among others. The patients were mostly diagnosed with meningitis, septicemia, enteric fever, pneumonia, and malaria and there was one case of osteomyelitis. One patient, a 15-month-old child infected with *S. Brazzaville* and presenting with meningitis, died, whereas the rest of the patients recovered after hospitalization.

All 27 serogroup C1 isolates were sensitive to the 10 antibiotics we tested (amikacin, ampicillin, aztreonam, cefazolin, cefepime, streptomycin, ciprofloxacin, nalidixic acid, tigecycline, and sulfamethoxazole/trimethoprim).

The ability of *Salmonella* and other pathogenic bacteria to form biofilms has been linked to better persistence in the environment and increased resistance to environmental stresses and disinfection strategies.^{17–21} We compared biofilm-forming

TABLE 2

Allele type of each locus from the five *S. Brazzaville* strains

Strain	Locus						
	<i>thrA</i>	<i>purE</i>	<i>sucA</i>	<i>hisD</i>	<i>aroC</i>	<i>hemD</i>	<i>dnaN</i>
J40	65	138	151	156	19	8	102
J58	65	138	151	156	19	8	102
J93	65	138	151	156	19	8	102
J94	65	138	151	156	19	8	102
J98	65	138	151	156	19	8	102

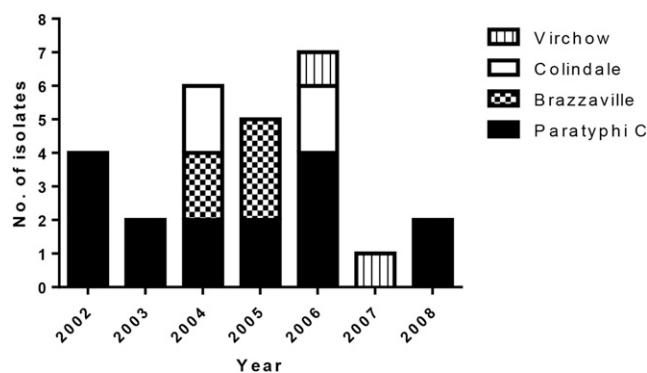


FIGURE 1. Number of isolates of each *Salmonella* serovar by year. Isolates were recovered from the blood of patients and assigned to a serovar by serum agglutination, multilocus sequence typing, and flagellin gene sequencing.

abilities of some of the *Salmonella* serogroup C1 strains isolated from Mali with two strains of *S. Typhimurium* also isolated from Mali: strain I77, which belongs to the most common ST of *S. Typhimurium* throughout the world, ST19; and strain D65, of ST313, which is circulating in sub-Saharan Africa.^{22,23} *Salmonella* Typhimurium I77 produced a strong biofilm, whereas strain D65 did not, as we have previously shown.²⁴ None of the four tested *S. Paratyphi C* strains (two for each ST) produced biofilms under our experimental conditions (Figure 3). Strains of *S. Virchow* and *S. Colindale* produced less biofilms compared with *S. Typhimurium* I77. *Salmonella* Brazzaville produced more biofilms than the other tested serogroup C1 strains and was not statistically different from that of *S. Typhimurium* I77.

Recently, reduced catalase activity has been reported for *S. Typhimurium* ST313, common in sub-Saharan Africa, compared with *S. Typhimurium* ST19, which is found worldwide.²⁵ We analyzed the serogroup C1 strains isolated from Mali for their ability to degrade hydrogen peroxide and observed that all tested *S. Paratyphi C* strains had significantly lower catalase activity (between 12 and 15 U per 10^{10} bacteria) than *S. Typhimurium* I77 (ST19; 45 U per 10^{10} bacteria), regardless of the ST (ST114 or ST146) of the strains (Figure 4). That level was, however, higher in Paratyphi C than Typhi or Paratyphi A ($P = 0.0014$). By contrast, serovars Virchow, Colindale, and Brazzaville had very high catalase activity and were not statistically significantly different from *S. Typhimurium* ST19 (45 U per 10^{10} bacteria). Highly human host-adapted serovars Typhi and Paratyphi A had no detectable activity. As previously reported for ST313 strains, *S. Typhimurium* strain D65 had very low activity (2 U per 10^{10} bacteria).²⁶

DISCUSSION

The data presented here provide insight into the *Salmonella* serogroup C1 serovars that cause invasive disease in Mali. The predominance of *S. Paratyphi C* is particularly intriguing, as this serovar is rarely reported, either in African countries or in industrialized countries such as the United States where only 13 cases were reported between 1995 and 2012. Here, we described the identification of 16 isolates of this serovar from four different areas within Bamako, Mali, over a period of seven years (from 2002 to 2008), suggesting a widespread

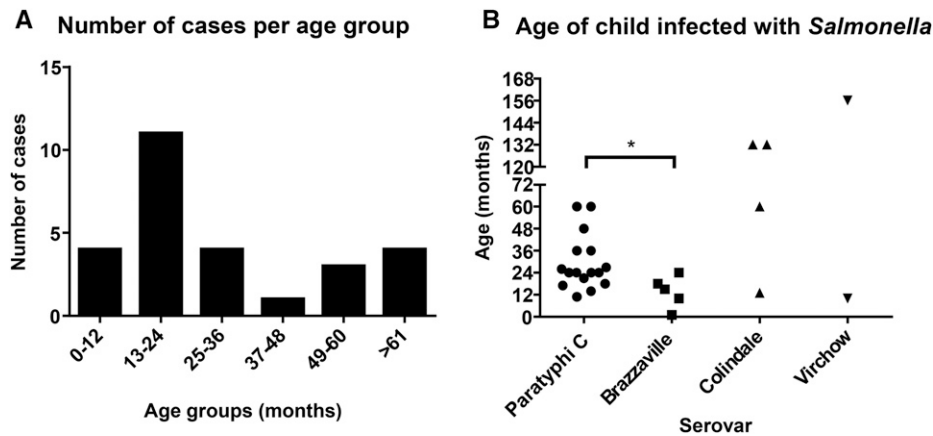


FIGURE 2. Distribution of *Salmonella* serogroup C1 nontyphoidal *Salmonella* cases by age (A) and serovar (B). * $P = 0.03$ (two-tailed t test).

presence rather than a transient, localized outbreak. Historically, *S. Paratyphi C* has been associated with metastatic, suppurative infections. However, of the 16 *S. Paratyphi C* cases, only one had osteomyelitis. We hypothesize that the patients had atypical clinical presentations of *S. Paratyphi C* because of their immunocompromised status and other comorbidities. Children who are admitted to l'Hôpital Gabriel Touré can have malaria (highly prevalent), HIV (low prevalence), and/or are malnourished (very common). Six patients had malaria and one of these patients also had HIV. Interestingly, the most common ST was ST114, which differs from what Achtman et al. reported after analyzing 51 isolates of *S. Paratyphi C* from around the world: 34 were ST146 and only 3 were ST114.¹² Similarly, the MLST database Enterobase shows that the majority of *S. Paratyphi C* strains belong to ST146 (92 strains of 165 isolates deposited in the database).²⁷ The situation in Mali is therefore unusual, both because of the number of cases in the surveillance period and in the distribution of the STs.

In addition, the presence of the very rare serovar *S. Brazzaville*, first identified 60 years ago in what is now the Democratic Republic of Congo, is surprising.^{28,29} It has historically been isolated from stool samples of patients with diarrhea as well as from pus of children with osteomyelitis.³⁰ In the United States between 1995 and 2012, only 10 cases out of 698,763 NTS infections reported to the U.S. Centers for Disease Control and Prevention have been attributed to *S. Brazzaville*. Comorbidities could explain the higher-than-expected frequency of these otherwise rare serovars, as coinfections with HIV or malaria have been associated with a higher risk of contracting iNTS as well as higher mortality.^{3,31,32} Moreover, our previous study identified 21 isolates of the rare serovar *S. Stanleyville* (serogroup B) during the same surveillance period, suggesting that the particular environment in Mali allows for otherwise rare serovars to cause invasive disease in pediatric patients.⁵

Biofilm formation on gallstones has been linked to the persistence of *Salmonella* Typhi in the gallbladder of infected patients, leading to a chronic carrier stage.³³ This has also been observed with *S. Typhimurium* in a murine gallbladder infection model.³⁴ In a previous study, we observed that invasive *S. Typhimurium* ST313 strains isolated from the blood of Malian infants were poor biofilm producers.²⁴ The amount of biofilm formed by ST313 strains was low and comparable

to that of *S. Typhi* and *S. Paratyphi A*. This lack of the *rdar* (red, dry and rough; a hallmark of extracellular matrix components production) morphotype has also been documented for other serovars, such as *Choleraesuis*, and it was suggested to correlate with a more invasive disease phenotype.³⁵ Our findings suggest that *S. Paratyphi C* is more similar to invasive *Salmonella* serovars than gastroenteritis-associated *Salmonella* in terms of biofilm production.

Salmonella Brazzaville showed no significant difference in biofilm production compared with *S. Typhimurium* I77 (ST19), suggesting that like I77, *S. Brazzaville* produces a biofilm and can survive desiccation.²⁴ Moreover, these data suggest that this serovar may not be particularly invasive. None of the tested *S. Paratyphi C* strains were able to produce biofilms, which is consistent with previous observations that typhoidal (*Typhi*, *Paratyphi A*) and highly invasive nontyphoidal

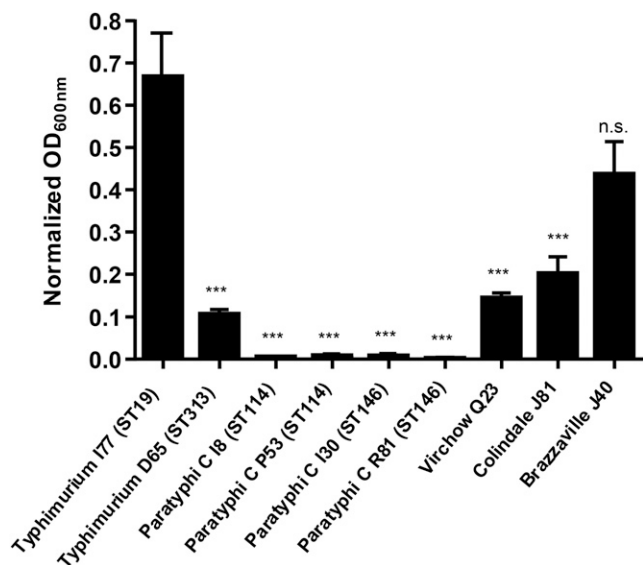


FIGURE 3. Biofilm formation in microtiter plates. The amount of biofilm formation was evaluated by the crystal violet binding assay and quantified by absorbance at 600 nm. Error bars indicate standard error of the mean, from at least four independent experiments. Asterisks indicate significant difference with *S. Typhimurium* I77. *** $P < 0.001$, n.s. = not significant (two-tailed t test).

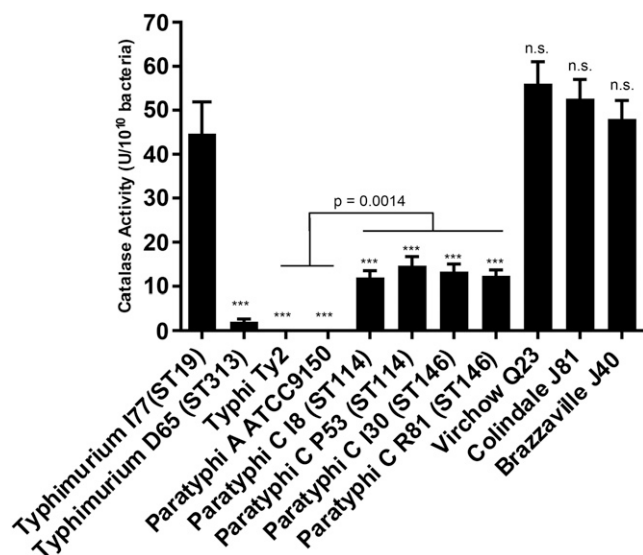


FIGURE 4. Catalase activity of various *Salmonella* strains. Catalase activity was evaluated for each strain in three independent experiments. Error bars indicate standard error of the mean. Asterisks indicate significant difference with *S. Typhimurium* I77. *** $P < 0.001$, n.s. = not significant (two-tailed t test).

(*Typhimurium* ST313, *Typhimurium* var. Copenhagen, *Choleraesuis*) *Salmonella* are poor biofilm producers.³⁵

Catalase activity has been linked to enhanced persistence in the environment, most likely by increasing survival during environmental stress. In addition, it has been suggested that catalase is required when bacteria are exposed to high concentrations of hydrogen peroxide, at high bacterial densities.^{36,37} Within *Salmonella*, loss of the ability to detoxify hydrogen peroxide is thought to be a marker of human host adaptation. Even though decreased catalase activity has been linked to increased susceptibility to oxidative stress, many articles report that human-adapted, invasive serovars tend to have lower or no catalase activity.²⁵ Moreover, catalase-deficient mutants of *Salmonella Typhimurium* were shown to be as virulent as the wild-type strain in mice.³⁶ We confirmed the low catalase activity of human host-adapted serovars, such as *S. Typhi* and *S. Paratyphi*, A. *S. Colindale*, *S. Virchow*, and *S. Brazzaville* displayed very high catalase activities that were not significantly different from *S. Typhimurium* I77, suggesting that they are poorly adapted to humans. *Salmonella Paratyphi* C showed significantly less catalase production than *S. Typhimurium* I77, but not as low as *S. Typhi* or *S. Paratyphi* A, suggesting that it may not be as human host-adapted as these typhoidal serovars.

In a previous study, we showed that most *S. Typhimurium* (serogroup B) and *S. Enteritidis* (serogroup D) strains isolated from Mali were highly resistant to several relevant antibiotics (ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole).⁵ Here, among 27 serogroup C1 strains isolated during the same surveillance period, none of these isolates were resistant to any of the 10 tested antimicrobials, suggesting that existing antibiotic treatments are currently sufficient to treat an NTS infection due to serogroup C1. However, several highly resistant serogroup C1 and C2 isolates, such as *S. Newport* and *S. Kentucky*, have been reported in other African countries, associated with both human and animal burden.^{4,38}

This study emphasizes the need for broad surveillance of iNTS in Africa. Moreover, given the high diversity of serovars of human interest within serogroups C1 and C2 *Salmonella*, MLST can be used to help identify these serovars.⁴ In the case of the very rare *S. Brazzaville*, however, MLST was not useful as *S. Brazzaville* is absent from the *Salmonella* MLST database. Instead, a combination of methods including flagellin gene sequencing, biochemical testing, and traditional serum agglutination was required to identify this serovar.

Received June 26, 2017. Accepted for publication November 1, 2017.

Published online December 26, 2017.

Note: Supplemental table appears at www.ajtmh.org.

Acknowledgments: We thank Dr. Patricia Fields of the U.S. Centers for Disease Control and Prevention for useful discussions about serotyping *Salmonella* groups C1 and C2. We are also grateful to the many children and parents who consented to participate in our ongoing surveillance activities at Hôpital Gabriel Touré in Bamako, Mali.

Financial support: This work was funded by Project 4: Vaccine strategy for broad spectrum protection against nontyphoidal *Salmonella* (project leaders: Sharon M. Tennant and Raphael Simon) NIH/NIAID Centers for Excellence in Translation Research grant U19 AI109776-01 awarded to Myron M. Levine.

Authors' addresses: Fabien J. Fuche, Sunil Sen, Jennifer A. Jones, Joseph Nkeze, Jasnehta Permala-Booth, Milagritos D. Tapia, Karen L. Kotloff, and Sharon M. Tennant, Center for Vaccine Development and Department of Medicine, University of Maryland School of Medicine, Baltimore, MD, E-mails: fuche@som.umaryland.edu, ssen@som.umaryland.edu, jjones@som.umaryland.edu, jonkeze@som.umaryland.edu, jpermalabooth@som.umaryland.edu, mtapia@som.umaryland.edu, kkotloff@som.umaryland.edu, and stennant@som.umaryland.edu. Samba O. Sow, Boubou Tamboura, Aliou Touré, Uma Onwuchekwa, and Mamadou Sylla, Centre pour le Développement des Vaccins, Mali, CVD-MALI CNAM, Ex-Institut Marchoux, Djicoroni Para BP:251, Bamako, Mali, E-mails: ssow@som.umaryland.edu, btamboura@som.umaryland.edu, alloutoure@yahoo.fr, uonwuche@som.umaryland.edu, and mbsylla1@yahoo.fr.

This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

REFERENCES

- Havelaar AH et al., 2015. World Health Organization global estimates and regional comparisons of the burden of foodborne disease in 2010. *PLoS Med* 12: e1001923.
- Feasey NA, Dougan G, Kingsley RA, Heyderman RS, Gordon MA, 2012. Invasive non-typhoidal *Salmonella* disease: an emerging and neglected tropical disease in Africa. *Lancet* 379: 2489–2499.
- Reddy EA, Shaw AV, Crump JA, 2010. Community-acquired bloodstream infections in Africa: a systematic review and meta-analysis. *Lancet Infect Dis* 10: 417–432.
- Fuche FJ, Sow O, Simon R, Tennant SM, 2016. *Salmonella* Serogroup C: current status of vaccines and why they are needed. *Clin Vaccine Immunol* 23: 737–745.
- Tapia MD et al., 2015. Invasive nontyphoidal *Salmonella* infections among children in Mali, 2002–2014: microbiological and epidemiologic features guide vaccine development. *Clin Infect Dis* 61 (Suppl 4): S332–S338.
- Tennant SM et al., 2010. Identification by PCR of non-typhoidal *Salmonella enterica* serovars associated with invasive infections among febrile patients in Mali. *PLoS Negl Trop Dis* 4: e621.
- Sow SO, Diallo S, Campbell JD, Tapia MD, Keita T, Keita MM, Murray P, Kotloff KL, Levine MM, 2005. Burden of invasive disease caused by *Haemophilus influenzae* type b in Bamako, Mali: impetus for routine infant immunization with conjugate vaccine. *Pediatr Infect Dis J* 24: 533–537.

8. Sow SO, Tapia MD, Diallo S, Keita MM, Sylla M, Onwuchekwa U, Pasetti MF, Kotloff KL, Levine MM, 2009. *Haemophilus influenzae* type b conjugate vaccine introduction in Mali: impact on disease burden and serologic correlate of protection. *Am J Trop Med Hyg* 80: 1033–1038.
9. Campbell JD, Kotloff KL, Sow SO, Tapia M, Keita MM, Keita T, Diallo S, Hormazabal JC, Murray P, Levine MM, 2004. Invasive pneumococcal infections among hospitalized children in Bamako, Mali. *Pediatr Infect Dis J* 23: 642–649.
10. Felix A, Pitt RM, 1951. The pathogenic and immunogenic activities of *Salmonella typhi* in relation to its antigenic constituents. *J Hyg (Lond)* 49: 92–110.
11. Grimont PA, Weill F-X, 2007. *Antigenic Formulae of the Salmonella Serovars*. Paris, France: WHO Collaborating Center for Reference and Research on Salmonella, Institut Pasteur. Available at: <http://www.scacm.org/free/Antigenic%20Formulae%20of%20the%20Salmonella%20Serovars%202007%209th%20edition.pdf>. Accessed September 29, 2015.
12. Achtman M et al., 2012. Multilocus sequence typing as a replacement for serotyping in *Salmonella enterica*. *PLoS Pathog* 8: e1002776.
13. O'Farrell B, Haase JK, Velayudhan V, Murphy RA, Achtman M, 2012. Transforming microbial genotyping: a robotic pipeline for genotyping bacterial strains. *PLoS One* 7: e48022.
14. Weill F-X, Lailler R, Praud K, Kérouanton A, Fabre L, Brisabois A, Grimont PAD, Cloeckert A, 2004. Emergence of extended-spectrum- β -lactamase (CTX-M-9)-producing multiresistant strains of *Salmonella enterica* serotype Virchow in poultry and humans in France. *J Clin Microbiol* 42: 5767–5773.
15. Merritt JH, Kadouri DE, O'Toole GA, 2005. Growing and analyzing static biofilms. *Curr Protoc Microbiol*. 00: B:1B.1:1B.1.1–1B.1.17.
16. Iwase T, Tajima A, Sugimoto S, Okuda K, Hironaka I, Kamata Y, Takada K, Mizunoe Y, 2013. A simple assay for measuring catalase activity: a visual approach. *Sci Rep* 3: 3081.
17. Joseph B, Otta SK, Karunasagar I, Karunasagar I, 2001. Biofilm formation by *Salmonella* spp. on food contact surfaces and their sensitivity to sanitizers. *Int J Food Microbiol* 64: 367–372.
18. Robijns SCA, De Pauw B, Loosen B, Marchand A, Chaltin P, De Keersmaecker SCJ, Vanderleyden J, Steenackers HPL, 2012. Identification and characterization of 4-[4-(3-phenyl-2-propen-1-yl)-1-piperazinyl]-5H-pyrimido[5,4-b]indole derivatives as *Salmonella* biofilm inhibitors. *FEMS Immunol Med Microbiol* 65: 390–394.
19. Giaouris E et al., 2015. Intra- and inter-species interactions within biofilms of important foodborne bacterial pathogens. *Front Microbiol* 6: 841.
20. Pande VV, McWhorter AR, Chousalkar KK, 2016. *Salmonella enterica* isolates from layer farm environments are able to form biofilm on eggshell surfaces. *Biofouling* 32: 699–710.
21. Bridier A, Sanchez-Vizueté P, Guillaud M, Piard J-C, Naïtali M, Briandet R, 2015. Biofilm-associated persistence of foodborne pathogens. *Food Microbiol* 45: 167–178.
22. Okoro CK et al., 2012. Intra-continental spread of human invasive *Salmonella* Typhimurium pathovariants in sub-Saharan Africa. *Nat Genet* 44: 1215–1221.
23. Kingsley RA et al., 2009. Epidemic multiple drug resistant *Salmonella* Typhimurium causing invasive disease in sub-Saharan Africa have a distinct genotype. *Genome Res* 19: 2279–2287.
24. Ramachandran G, Aheto K, Shirtliff ME, Tennant SM, 2016. Poor biofilm-forming ability and long-term survival of invasive *Salmonella* Typhimurium ST313. *Pathog Dis* 74: ftw049.
25. Singletary LA et al., 2016. Loss of multicellular behavior in epidemic African nontyphoidal *Salmonella enterica* serovar Typhimurium ST313 strain D23580. *MBio* 7: e02265.
26. Yang J, Barrila J, Roland KL, Kilbourne J, Ott CM, Forsyth RJ, Nickerson CA, 2015. Characterization of the invasive, multidrug resistant non-typhoidal *Salmonella* strain D23580 in a murine model of infection. *PLoS Negl Trop Dis* 9: e0003839.
27. Enterobase, 2017. Enterobase. Available at: <http://enterobase.warwick.ac.uk>. Accessed January 13, 2017.
28. Krubwa F, Van Oye E, Gatti F, Vandepitte J, 1970. Epidemiologie de la Salmonellose a Kinshasa: role des porteurs sains et des aliments. *Ann Soc Belg Med Trop* 50: 319–338.
29. Bernardo FMA, Brandao CFSN, 1996. Enquete epidemiologique preliminaire sur les prevaelences des *Salmonella* spp. a l'abattoir de Bissau (Guinee-Bissau). *Rev Elev Méd Vét Pays Trop* 49: 102–106.
30. Vassiliadis P, 1960. *Salmonellae* of the Belgian Congo (8th report). *Ann Soc Belg Med Trop* 40: 423–428.
31. Mahon BE, Fields PI, 2016. Invasive Infections with nontyphoidal *Salmonella* in sub-Saharan Africa. *Microbiol Spectr* 4: 341–357.
32. Falay D et al., 2016. Microbiological, clinical and molecular findings of non-typhoidal *Salmonella* bloodstream infections associated with malaria, Oriental Province, Democratic Republic of the Congo. *BMC Infect Dis* 16: 271.
33. Crawford RW, Rosales-Reyes R, Ramírez-Aguilar M de la L, Chapa-Azuela O, Alpuche-Aranda C, Gunn JS, 2010. Gallstones play a significant role in *Salmonella* spp. gallbladder colonization and carriage. *Proc Natl Acad Sci USA* 107: 4353–4358.
34. Adcox HE, Vasicek EM, Dwivedi V, Hoang KV, Turner J, Gunn JS, 2016. *Salmonella* extracellular matrix components influence biofilm formation and gallbladder colonization. *Infect Immun* 84: 3243–3251.
35. Römling U, Bokranz W, Rabsch W, Zogaj X, Nimtz M, Tschäpe H, 2003. Occurrence and regulation of the multicellular morphology in *Salmonella* serovars important in human disease. *Int J Med Microbiol* 293: 273–285.
36. Buchmeier NA, Libby SJ, Xu Y, Loewen PC, Switala J, Guiney DG, Fang FC, 1995. DNA repair is more important than catalase for *Salmonella* virulence in mice. *J Clin Invest* 95: 1047–1053.
37. Day WA, Sajecki JL, Pitts TM, Joens LA, 2000. Role of catalase in *Campylobacter jejuni* intracellular survival. *Infect Immun* 68: 6337–6345.
38. Afema JA, Byarugaba DK, Shah DH, Atukwase E, Nambi M, Sischo WM, 2016. Potential sources and transmission of *Salmonella* and antimicrobial resistance in Kampala, Uganda. *PLoS One* 11: e0152130.