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SUPPLEMENT ARTICLE

Prevalence of *Salmonella* Excretion in Stool: A Community Survey in 2 Sites, Guinea-Bissau and Senegal

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Background. Chronic and convalescent carriers play an important role in the transmission and endemicity of many communicable diseases. A high incidence of *Salmonella enterica* serovar Typhi and invasive nontyphoidal *Salmonella* (NTS) infection has been reported in parts of sub-Saharan Africa, yet the prevalence of *Salmonella* excretion in the general population is unknown.

Methods. Stool specimens were collected from a random sample of households in 2 populations in West Africa: Bissau, Guinea-Bissau, and Dakar, Senegal. Stool was cultured to detect presence of *Salmonella*, and antimicrobial susceptibility testing was performed on the isolated organisms.

Results. Stool was cultured from 1077 and 1359 individuals from Guinea-Bissau and Senegal, respectively. *Salmonella* Typhi was not isolated from stool samples at either site. Prevalence of NTS in stool samples was 24.1 (95% confidence interval [CI], 16.5–35.1; n = 26/1077) per 1000 population in Guinea-Bissau and 10.3 (95% CI, 6.1–17.2; n = 14/1359) per 1000 population in Senegal.

Conclusions. Evidence of NTS excretion in stool in both study populations indicates a possible NTS transmission route in these settings.

Keywords. *Salmonella*; stool culture; carrier; typhoid; NTS.

Salmonella is a genus of gram-negative bacteria consisting of 2 species. *Salmonella enterica* can cause human illness spread via fecal–oral transmission through contaminated food or water. *Salmonella enterica* serovars Typhi and Paratyphi A, B, and C are restricted to human hosts, whereas nontyphoidal *Salmonella* (NTS) serotypes have zoonotic reservoirs, although long-term asymptomatic carriage in humans has been reported [1, 2]. Patients with acute *Salmonella* infection shed the organism in their stool and occasionally urine, and may continue to excrete bacteria following symptom resolution during convalescent or temporary carriage [3–7]. An ill-defined proportion of convalescent carriers may progress to become chronic carriers, defined as individuals experiencing asymptomatic shedding for >1 year following acute infection [6–8]. Additionally, up to 25% of chronic carriers of *S.* Typhi report no history of disease [9]. In many developing countries, open defecation can lead to contamination of water systems, resulting in an increased risk of *Salmonella* transmission through the ingestion of bacteria in water used for drinking, washing, or irrigating produce [10–12]. Factors related to water source and storage, sanitation practices, and street food consumption have also been associated with increased risk of infection [13].

In humans, NTS causes a self-limiting diarrheal disease, although some NTS serotypes are more frequently implicated in severe bloodstream infections. Infection with *S.* Typhi results in typhoid fever, an invasive infection characterized by high fever. Untreated infections can lead to severe complications, including intestinal perforation, septicemia, and death [14]. Whereas historically, invasive NTS (iNTS) bloodstream infections have been well described in Europe and the Americas, iNTS has recently been recognized as an important cause of febrile illness in sub-Saharan Africa alongside typhoid fever. These 2 infections are now among the most common causes of bacteremia in sub-Saharan Africa [15, 16]. In children <15 years of age, there were estimated to be up to 698 cases of typhoid fever and 577 cases of iNTS per 100 000 person-years in study populations included in the Typhoid Fever Surveillance in Africa Program (TSAP) (Marks et al, unpublished data). Despite high incidence rates in multiple sites, little information exists on the prevalence of *Salmonella* excreters in sub-Saharan Africa and the extent to which they contribute to transmission. This study attempted to estimate the prevalence of *Salmonella* excreters in 2 TSAP sites in sub-Saharan Africa, Guinea-Bissau and Senegal.

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MATERIALS AND METHODS

Study Design

The study was designed as a household-level randomized stool culture survey of the general population in Dakar, Senegal and Bissau, Guinea-Bissau. A simple random sample of households from each site was selected, and all individuals living in the dwellings were recruited. Participants were not asked about prior infection or current symptoms at time of enrollment. As such, health status was not used to determine eligibility for participation in the study. Informed consent was obtained from participants, and households were visited the day following recruitment for stool collection. Fresh stool samples were collected in containers and transported in a cooler box at ambient temperature to the site laboratory within 6 hours of collection. At the laboratory, stool was suspended in Selenite broth for enrichment and incubated for 24 hours at 36°C. Samples were then cultured on xylose lysine deoxycholate agar for selective growth of *Salmonella* and *Shigella* species [17]. Excreters are defined as individuals with *Salmonella* isolated from stool culture. Isolates were initially identified by biochemical reactions and then confirmed using polymerase chain reaction (PCR). Antimicrobial susceptibility testing was performed by the agar diffusion method (Kirby–Bauer) and assessed by Clinical and Laboratory Standards Institute M100-S23 criteria at the Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany. A multiplex PCR kit (Qiagen, Hilden, Germany) and targeted selected primers were used for typing and identification of *S. enterica* subspecies *enterica* and, specifically, serotypes Typhi, Enteritidis, Typhimurium, and Dublin.

Study Participants

The study sites in Bissau, Guinea-Bissau and Dakar, Senegal are both located in West Africa and participated in the TSAP surveillance program [18]. Both sites were classified as urban settings and have established laboratories performing bacterial stool culture. In Guinea-Bissau, the study area included 6 districts from a region of the capital, Bissau, with a combined population of 100 000 in 2011 (Table 1). In Senegal, study participants were recruited from Pikine, a suburb of Dakar. The population of the study area was 360 000 in 2012 and included districts comprised of temporary urban settlements. All individuals living in the household, regardless of age or sex, were eligible for enrollment.

Sample Size Calculation and Statistical Analysis

A sample size for this study was calculated as 1049 and 1053 for Guinea-Bissau and Senegal, respectively, according to the Henderson and Sundaresan [19] standard formula applying finite population correction factor and conservative design effect estimate of 2 to account for within-household correlation. An estimate of 2.0% for the true proportion of carriers was used, and power was set at 90% with a precision of 1.0%. A z test of proportions was conducted to examine the difference in overall prevalence of excreters between sites as well as between females and males stratified by age group. Within-site comparison of age-stratified prevalence risk was also examined using a z test of proportions. Confidence intervals (CIs) for prevalence estimates were calculated using the Wilson score method without continuity correction.

Ethical Approval

The Internal Review Board of the International Vaccine Institute and the local ethical review boards in Guinea-Bissau and the Senegal approved the study protocol. Written informed consent was received from adult participants and adult caregivers of children and adolescents.

Table 1. Descriptive Overview of Study Sites

Characteristic	Guinea-Bissau	Senegal
City	Bissau	Dakar
Site	Bandim	Pikine
Study period	June 2012–September 2013	January 2013–June 2014
Population size	100 153	342 178
Households enrolled, No.	164	211
Household size, median (IQR)	9 (6–14)	7 (4–13)
Total individuals	1299	1869
Total individuals with stool specimen cultured, No. (%)	1077 (82.9)	1359 (72.7)
Female sex, No. (%)	624 (57.9)	857 (63.1)
Stool cultures performed, by age group, No. (%)		
<5 y	199 (18.5)	220 (16.2)
5–14 y	238 (22.1)	371 (27.3)
15–34 y	468 (43.5)	440 (32.4)
≥35 y	172 (16.0)	328 (24.1)
All	1077	1359

Abbreviation: IQR, interquartile range.

Table 2. Estimates of the Prevalence of Nontyphoidal *Salmonella* Excretion in Stool in Guinea-Bissau and Senegal

	Bissau, Guinea-Bissau		Dakar, Senegal	
Age Group, y	Culture Positive for NTS, no./No. (%)	Prevalence, per 1000 (95% CI)	Culture Positive for NTS, no./No. (%)	Prevalence, per 1000 (95% CI)
Male and female sex				
<5	3/199 (1.5)	15.1 (5.1–43.4)	2/220 (0.9)	9.1 (2.5–32.5)
5–14	10/238 (4.2)	42.0 (23.0–75.6)	4/371 (1.0)	10.8 (4.2–27.4)
15–34	11/468 (2.3)	23.5 (13.2–41.6)	6/440 (1.3)	13.6 (6.3–29.4)
≥35	2/172 (1.1)	11.6 (3.2–41.4)	2/328 (0.6)	6.1 (1.7–22.0)
All	26/1077 (2.4)	24.1 (16.5–35.1)	14/1359 (1.0)	10.3 (6.1–17.2)
Male sex				
<5	1/108 (0.9)	9.3 (1.6–50.6)	0/117 (0)	. . .
5–14	5/104 (4.8)	48.1 (20.7–107.6)	3/183 (1.6)	16.4 (5.6–47.1)
15–34	4/181 (2.2)	22.1 (8.6–55.4)	2/119 (1.6)	16.8 (4.6–59.2)
≥35	0/60 (0)	. . .	0/83 (0)	. . .
All	10/453 (2.2)	22.1 (12.0–40.2)	5/502 (1.0)	10.0 (4.3–23.1)
Female sex				
<5	2/91 (2.2)	22.0 (6.0–76.6)	2/103 (1.9)	19.4 (5.3–68.1)
5–14	5/134 (2.7)	27.3 (16.0–84.4)	1/188 (0.5)	5.3 (0.9–29.5)
15–34	7/287 (2.4)	24.4 (11.9–49.5)	4/321 (1.2)	12.5 (4.9–31.6)
≥35	2/112 (1.7)	17.9 (4.9–62.8)	2/245 (0.8)	8.2 (2.2–29.3)
All	16/624 (2.5)	25.6 (15.8–41.2)	9/857 (1.0)	10.5 (5.5–19.8)

Abbreviations: CI, confidence interval; NTS, nontyphoidal *Salmonella*.

RESULTS

In Guinea-Bissau, participants from 164 households were enrolled (57.9% female; median age, 9 years; interquartile range [IQR], 6–14 years), and 1077 stool specimens were collected for culture (Table 1). In Senegal, participants from 211 households were enrolled (63.1% female; median age, 7 years; IQR, 4–13 years), and 1359 stool specimens were collected for culture. Infants and children aged <5 years accounted for 18.5% and 16.2% of the study population in Guinea-Bissau and Senegal, respectively. The majority of stool cultures were collected from people aged 15–34 years at both sites.

Salmonella Typhi was not isolated in any of the stool specimens collected at both sites; however, NTS was isolated in all age groups at both sites. All NTS isolates were typed as other *Salmonella* serotypes by multiplex PCR. The overall prevalence of *Salmonella* excreters in the sampled population was higher ($P = .004$) in Guinea-Bissau, at 24.1 per 1000 population (95% CI, 16.5–35.1; n = 26/1077), compared with Senegal, at 10.3 per 1000 population (95% CI, 6.1–17.2; n = 14/1359) (Table 2). In 5- to 14-year-olds, the prevalence of excreters was 4 times higher in Guinea-Bissau than in Senegal ($P = .006$); however, in other age groups there was no significant difference in prevalence between sites. The detection of *Shigella* species was very low; *Shigella* species was found in 1 isolate in Guinea-Bissau, and none in Senegal.

The median age of the 26 positive NTS excreters from Guinea-Bissau was 15 years (range, 0–38 years; 62% female). The median age of the 14 positive NTS excreters in Senegal was 16 years (range, 2–59 years; 64% female). There was no evidence to suggest higher risk of excretion in females (16/624 [2.6%]) compared with males (10/453 [2.2%]) in Guinea-Bissau ($P = .352$). The same was true in Senegal, where 9 of 857 (1.1%) females and 5 of 502 (1.0%) males were identified as excreters ($P = .460$).

In Guinea-Bissau, 7 of 22 *Salmonella* isolates exhibited resistance to ampicillin. All isolates were susceptible to trimethoprim-sulfamethoxazole (cotrimoxazole), chloramphenicol, and ciprofloxacin. Antimicrobial susceptibility tests were not conducted on 4 isolates. In Senegal, 3 of 14 isolates were resistant to cotrimoxazole, 1 of which was additionally resistant against ampicillin and 1 additionally resistant against ciprofloxacin. All isolates were susceptible to chloramphenicol.

DISCUSSION

Few previous studies have examined the prevalence of *Salmonella* excreters in the general population of sub-Saharan Africa; however, studies of food handlers and various subpopulations in Togo, Ghana, and Nigeria detected a prevalence of NTS excretion in stool ranging from 8 to 75 per 1000 population [20–25]. The overall prevalence of *Salmonella* excreters estimated in the general population in urban Guinea-Bissau and Senegal falls in the lower bound of this range. The detection rate of NTS excreters in Guinea-Bissau was more than twice that in Senegal; however, no *S.* Typhi was isolated by stool culture in either setting. The study was powered to detect a prevalence of 20 per 1000, which may have been an overestimation in both settings.

These findings are supported by the low incidence of typhoid fever (9 per 100 000 person-years) compared with iNTS (32 per 100 000 person-years) in febrile patients in Guinea-Bissau during TSAP. Adjusted incidence rates for bloodstream infection could not be calculated for TSAP surveillance in Senegal. The results presented are also comparable to the estimates of past studies in neighboring countries, where the prevalence of *S.* Typhi excretion has been reported to be <1% of the sampled population [20, 21, 23, 24]. Notably, patients with an uncomplicated NTS infection are reported to develop convalescent carriage more frequently (25%–100% of cases) than those with typhoid fever (0%–13.3%) [6, 7, 26–36]. This factor may contribute to the overall higher prevalence of NTS compared with *S.* Typhi excreters reported here and in other West African countries, where the incidence of NTS is higher than that of typhoid fever. However, this is weakened by the shorter maximum duration of excretion reported for NTS (1 year) compared with *S.* Typhi (lifelong carriage). Given the mounting evidence for the emergence of multidrug-resistant *Salmonella* strains in sub-Saharan Africa with resistance to chloramphenicol, ampicillin, and cotrimoxazole and often to streptomycin sulfonamides and tetracyclines [37–42], the isolation of *Salmonella* in Senegal with multiple resistance against first-line treatment options should be carefully monitored.

The biological mechanisms of *S.* Typhi colonization and persistence in the hepatobiliary or genitourinary tract are well documented [43]. However, fewer data have been reported on the potential for NTS persistence and carriage, particularly following invasive infections. It is generally assumed that the risk of transmission is similar between acutely infectious individuals and chronic carriers. Therefore, determining the prevalence of excreters, including asymptomatic carriers, in any population is important for modeling transmission dynamics and the potential impact of vaccination for this disease [44, 45]. The role of *S.* Typhi carriers in the persistence of typhoid fever in endemic regions has been observed in parts of Chile [46] and Vietnam [47], and the impact of carriers on incident typhoid fever cases is consistent with output from stochastic modeling of typhoid fever transmission [48]. Less information has been documented on the factors that contribute to iNTS transmission. The epidemiology of *S.* Typhi and iNTS are different; as such, further investigation into iNTS infection in humans must be conducted before transmission dynamics can be modeled from existing data.

The multiplex PCR technique used by the reference laboratory could only identify 4 common *Salmonella* serotypes, none of which were isolated in this study. During surveillance of invasive *Salmonella* infections that was conducted during the same period at these study sites, 3 of 7 (43%) of iNTS isolates in Guinea-Bissau and 2 of 3 (67%) of iNTS isolates in Senegal were also identified as other *Salmonella* serotypes [49]. Further serotyping and genetic analysis of *Salmonella* excreters in the general population could allow for inference to incident iNTS cases as well as infections causing less-severe diarrheal disease, although this was beyond the scope of the present study.

Potential for selection bias due to nonparticipation exists in both sites. Differential participation rates by age, sex, and other risk factors in each site may affect estimates of *Salmonella* excretion prevalence. Overall, nonparticipation was mainly attributed to participant absence during household visits and failure to collect a stool sample after 3 attempts by the field worker. The underrepresentation of adult males in both sites may be important, as working-age males, particularly farmers, fishermen, and food handlers, have increased exposure to environmental bacterial reservoirs, which may increase their risk of infection. There was a strong correlation in household prevalence of excretion in Guinea-Bissau; 20 of 26 isolates were isolated from households where at least 1 other positive excreter was identified. Only 3 *Salmonella* excreters in Senegal were found in the same household. Household clustering may introduce bias due to within-household correlation of the risk of *Salmonella* infection and subsequent excretion, although we attempted to mitigate this by including intrahousehold correlation in the sample size estimate. Further robust statistical analysis accounting for correlated data is required.

Importantly, clinical symptoms were not assessed upon enrollment, and therefore participants cannot be classified as asymptomatic. Additionally, chronic carriage has been defined as persistent excretion for 1 year after infection onset [50]. As this study does not follow up individuals for this duration, chronic carrier status was not determined. Interpretation of results is limited by the absence of information on human immunodeficiency virus (HIV) infection in the study population, as HIV infection increases the risk of NTS infection [51]. Additionally, excretion may follow seasonal patterns of infection that are not captured in a single point survey. Information collected on recent gastrointestinal illness, medical treatment, hospitalization, and antimicrobial use would aid in the interpretation of the estimated prevalence risk, as patients with acute NTS gastroenteritis treated with antimicrobials have been observed to shed *Salmonella* for a longer period compared with untreated individuals [27, 52–55].

The sensitivity of *Salmonella* detection from stool samples is low [56]. Other studies have demonstrated that shedding among both convalescent and chronic carriers can be intermittent, and multiple stool samples taken over the course of several days are recommended [57–59]. As only 1 sample was collected to determine positivity at the time of recruitment, prevalence estimates presented here likely underestimate the true prevalence of excreters at both sites. Future studies should include follow-up of positive excreters to determine the length of carriage and possibility of chronic carrier status. In addition, more-exhaustive serotyping methods are necessary to describe the distribution of NTS serotypes in the general population at these sites.

This, along with genetic analysis, can assist in elaborating the transmission dynamics between community excreters and incident invasive *Salmonella* cases, as well as identifying strains of NTS or *S.* Typhi that carry a higher risk of chronic carriage.

CONCLUSIONS

Salmonella Typhi was not identified in stool cultures taken from members of the community in Guinea-Bissau or Senegal. The prevalence of NTS excretion in stool was higher in Guinea-Bissau than in Senegal, particularly in 5- to 14-year-olds, and there was no difference in prevalence of excretion by sex in either country. Future studies should incorporate follow-up periods to determine the prevalence of chronic carriers in the community, and include more exhaustive serotyping and genetic analysis methods to evaluate excreters' role in the transmission of invasive *Salmonella* infections.

Notes

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