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Salmonella Typhi stool shedding by enteric fever patients and asymptomatic chronic carriers in an endemic urban setting

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Abstract:

The burden of *Salmonella* Typhi shedding in stool and its contribution to transmission in endemic settings is unknown. During passive surveillance *S*. Typhi shedding was seen during convalescence in 332 bacteremic typhoid patients although none persisted at one-year follow-up. Anti-Vi-IgG titres were measured in age-stratified cohort of serosurveillance participants. Systematic stool sampling of 303 participants with high anti-Vi-IgG titres identified one asymptomatic carrier shedding. These findings suggest ongoing *S*. Typhi transmission in this setting is more likely to occur from acute convalescent cases although better approaches are needed to identify true chronic carriers in the community to enable typhoid elimination.

Key words: Typhoid fever, stool shedding, chronic carriers, transmission of *S*. Typhi

Background:

Humans are the only natural host and reservoir of *Salmonella enterica* serotype Typhi (*S*. Typhi), the causative organism of typhoid fever. The multiplication of *S*. Typhi outside the human host is not robust, although some ability to survive in the environment for an extended time is theoretically required for transmission [1]. Typhoid is transmitted by consumption of food and water contaminated by bacteria shed by acute cases or chronic carriers [2]. After recovery from acute disease, approximately 2–5% patients fail to clear the infections [3, 4]. To maintain this carriage state, *S*. Typhi colonizes the gallbladder to form dense bacterial biofilms on gallstones and other surfaces from where organisms are shed intermittently and are excreted in stool [5].

While implementing preventive measures such as vaccination will lead to elimination of typhoid from endemic regions, additional interventions are needed to stop the ongoing transmission of *S*. Typhi from chronic carriers. This requires tools to accurately identify and appropriately treat individuals shedding *S*. Typhi. Detection of chronic carriers is challenging, however, as the majority are asymptomatic and up to 25% do not recall symptoms or an episode compatible with acute typhoid fever [4]. Microbiological culture of bile, gallstones, or gallbladder tissue from individuals undergoing cholecystectomy are gold-standard approaches to the accurate, but often coincidental, diagnosis of chronic carriage. Methods to obtain this material (surgical or string-test) are not feasible to collect at large, community or population scales. Culture of serially collected stool specimens is not easy to perform and may only have a limited increase in diagnostic yield [6]. Some studies have demonstrated that high IgG isotype antibody titres to the Vi (virulence) capsule of *S*. Typhi (anti-Vi-IgG) can allow the identification of asymptomatic carriers as the apparent source of infection in

outbreak settings, although this approach has not been evaluated prospectively at the population level [5].

The rate of stool shedding and contribution to transmission from short-term, convalescent shedding and longer-term, temporary or chronic carriage is unknown in endemic settings. Bangladesh is an area of high endemicity for typhoid fever and therefore a well-placed setting to explore these unknowns. A comprehensive population-based study was carried out in a densely-populated area of Dhaka, Bangladesh, under the umbrella of the Strategic Typhoid Alliance across Africa and Asia (STRATAA) to detect the occurrence and duration of *S*. Typhi shedding from both acutely infected patients and from asymptomatic chronic carriers detected by serosurveillance.

Methods:

Enumeration of population and passive surveillance

Passive surveillance for acute typhoid fever was performed in wards 3 and 5 of the Mirpur area in Dhaka city, Bangladesh [7]. A baseline census and 6-monthly updates were carried out to enumerate the population and to collect baseline characteristics.

In passive surveillance, patients presenting with history of fever for ≥72 hours or axillary temperature ≥38.5^{o}C were enrolled. In a subsequent amendment to the study protocol the inclusion criteria were changed to include fever ≥48 hours or axillary temperature ≥38.0^{o}C. At enrollment (day 0), blood and stool specimens were collected for culture to confirm acute typhoid cases. Study participants diagnosed with typhoid by blood culture and whose households were enrolled in the census survey were identified as 'index case'. Index cases were treated with antibiotics determined by bacterial susceptibility. Stool specimens were collected on days 30 and 180 to identify prolonged shedding. A further stool sample was

collected in those found to be shedding at these timepoints one year following study enrollment. The participants who were blood culture negative but stool culture positive, were undergone the same schedule of follow up and household contact screening. They were also identified as 'index case' if their households were enrolled in the census.

Immediately after confirming the index cases, all household members were invited to participate in the study and stool specimens were collected on days 0 and 30 from the household contacts who have provided consent. A further stool specimen was collected on day 180 when culture results were positive at day 0 and/or day 30. A follow-up stool specimen was recommended at 1 year depending on the culture result at day 180 in order to confirm continued shedding.

Serosurvey

Age stratified (0-4 years, 5-9 years, 10-14 years and >14 years) apparently healthy participants were randomly selected from the census population and enrolled to a serosurveillance component. Recruitment of selected participants and collection of specimens was carried out throughout the year, in 3 monthly blocks. Blood specimens were collected from the participants on days 0 and 90 after enrollment and anti-Vi-IgG responses were measured. A Vi-IgG threshold value was calculated using the first 1000 specimens collected, based on the 97th centile of titres measured in participants <15 years, or the 95th centile of participants ≥15 years of age. This provided thresholds of 96.2 EU/ml and 57.1 EU/ml anti-Vi-IgG, respectively.

Participants with anti-Vi-IgG titres over these thresholds (seropositive) were followed-up on day 180 and two stool specimens were collected 48 hours apart to detect *S*. Typhi. Further

follow-up of those participants identified as possible asymptomatic carriers was performed (defined as a seropositive participant with stool culture positive for *S*. Typhi).

Laboratory procedure

Informed written consent was obtained from guardians of young participants (1–17 years) while adult participants (18–59 years) provided their own consent. Stool cultures were performed at the icddr,b laboratory. Inoculation and emulsification of stool in Selenite F broth media for parallel enrichment and direct inoculation to MacConkey and *Salmonella-Shigella* selective agar plates was performed. Incubation of all cultures were carried out at 37°C for 18-24 hours. Colonies of *S*. Typhi were identified by phenotypic morphology, standard biochemical tests and slide agglutination to *Salmonella*-specific antisera. Antimicrobial susceptibility testing was performed using standard disk diffusion methods [8].

Plasma was separated from participant blood by centrifugation and anti-Vi-IgG titres were measured using a commercial assay (the VaccZyme ELISA, The Binding Site, Birmingham, UK) following the manufacturer's guidelines. [9]

Treatment of chronic carriers

Antibiotic treatment was recommended for index cases and their household contacts as per a pre-agreed management plan, if shedding continued for up to 1 year after enrollment. Similarly, antibiotic treatment was also planned for any asymptomatic chronic carriers detected through serosurveillance [7]. After completion of antibiotic therapy 3 consecutive stool specimens collected at least one month apart were required to confirm curative treatment.

Statistical analysis

Chi-Square test and Mann Whitney U Test were used to evaluate the differences among groups

and results were considered statistically significant if $p<0.05$.

Results:

Passive surveillance

The total number of individuals included in the study census was 111,695, among whom 4,509 patients met the inclusion criteria and were enrolled to the passive surveillance study between August 2016 and January 2019. Of these, 332/4,509 (7%) were confirmed as typhoid cases by blood culture and formed the index case population and 109/332 (33%) were also stool culture positive at enrollment (day 0). In total, stool specimens were collected from 4,216 of the 4509 participants enrollment, of which 33/4216 (0.8%) patients were stool culture positive but negative by blood culture.

Among the 332 blood culture-confirmed index cases, stool culture was again positive for 15/281 (5%) and 1/270 (0.4%) patients on days 30 and 180 respectively (Table 1). None of these patients was found to shed *S*. Typhi one year later. In patients with only positive stool cultures, 1 out of 9 was positive on day 30 and none were positive at later time points.

For household contacts of blood-culture confirmed index cases (n=332), 530 household contacts from 217 households (2.4 contacts per household) were enrolled in the study. Stool specimens were obtained from 530 and 466 household contacts on days 0 and 30 and tested for culture. The median age of household contacts was 29 years (range from 5 months to 75 years) and the male-female ratio of the contacts was 1:1.5. Among them,

5/530 (0.9%) and 4/466 (0.7%) participants were *S*. Typhi positive in stool on days 0 and 30, respectively (Table 2). No shedding was revealed on day 180 among seven contacts from whom stool specimens were obtained and no stool was therefore collected at one year.

Of 332 blood culture-confirmed index cases, 16 patients from 8 households (2 patients per household) had typhoid fever episode at the same time within their households. Among them, one pair of index cases of the same household were also shedding the bacterium at the same time.

For stool culture-only positive index cases (n=33), 9 household contacts from 5 different households (1.8 contacts per household) were enrolled in the study. The median age of the contacts was 29 years (range from 8 years to 48 years) and the male-female ratio was 1:2. Stool specimens were collected from them and tested for culture on days 0 and 30 and none were found to shed *S*. Typhi at these time points (Table 2).

Exploratory analyses of baseline characteristics (age, temperature and duration of fever) demonstrated no significant differences between patients who were positive for both blood and stool culture and patients who were positive only by blood culture. However, the proportion of prior antibiotic usage for the both blood and stool culture positive patients (24/109 [22%]) was significantly lower (P=<0.05) than the patients that were blood culture positive only (90/223 (40%]).

Serosurvey component

Paired blood specimens were collected from 8,261 and 7,043 participants days 0 and 90 respectively. High anti-Vi-IgG titres were measured in 303 participants on days 0 and/or 90, among whom 136 had persistently high Vi for both time points. Two stool specimens 48 hours apart were collected from 192 of 303 participants on day 180.

Only one female aged 36 years was *S*. Typhi positive by stool culture and therefore identified as an asymptomatic chronic (probable) carrier and was treated with ciprofloxacin (750 mg BD for 28 days). No *S*. Typhi shedding was detected in 3 consecutive stool specimens collected after completion of antibiotic treatment. Ultrasonography of the hepatobiliary system revealed gallstones and further advice was provided regarding consideration for elective cholecystectomy.

Discussion:

The study results demonstrate the high proportion of typhoid fever patients who shed *S*. Typhi in their stool during the acute stages of typhoid fever. This is a potential high-risk period for human-to-human transmission to occur given the likely debilitation of the patient and their care needs. Those at greatest risk would include household members and care-givers. In contrast, very low levels of continued shedding was seen during treatment and convalescence stages, likely a reflection of high levels of effectiveness of modern antimicrobial therapy. No study participants developed continued shedding into the chronic carriage stage. Although no difference was found in duration of fever for the patients of blood and stool culture positive and the patients of only blood culture positive, but an association of early *S*. Typhi shedding and development of typhoid fever has also been described in the human challenge model study performed in Oxford [10]. History of prior

antibiotic intake was observed in more blood culture positive patients who were also shedding *S.* Typhi than the only blood culture positive patients. Similarly, after initiation of antibiotic in human challenge model no shedding was seen [10].

Participants who were stool culture positive but blood culture negative, indicating either recent infection with short term shedding or a falsely negative blood culture, have the potential to impact for continued maintenance of the organism within human population. Hence, they were followed-up in the same way of blood culture positive index cases and household contact screening. However, stool specimen collection and retesting on day 30 was possible for 9 out of 33 (27%) stool culture positive index cases and one of which was positive for *S*. Typhi. Therefore, it is difficult to make an inference from this study result regarding the rates and duration of bacterial shedding and the impact of only stool culture positive patients in spreading the organism.

The household contact study revealed that some household members of the acute patients shed *S.* Typhi in the absence of typhoid fever symptoms and during the active disease period of the index cases. In addition, some blood culture positive index cases had typhoid fever episode within a household at the same time among whom two index cases also had bacterial shedding in their stool. In support of our findings, many epidemiological studies have reported that *S*. Typhi is transmitted from convalescent carriers to close contacts of patients within their household [11, 12].

One person was confirmed as an asymptomatic carrier identified by serosurveillance. Increased age, female gender, gallbladder disease are risk factors for developing chronic carriage after recovery from acute typhoid disease [3], and were present in the individual identified.

There are several important points to consider in interpreting the low rates of carriage found in this study. First, the chronic carriage prevalence may be low and asymptomatic may not make any major contribution to on-going transmission in the community. Second, *S*. Typhi are excreted in feces in low numbers and intermittently, which makes the detection of carriers difficult, and thus true prevalence may have been missed. Third, anti-Vi-IgG may not be the most appropriate biomarker for serological identification of chronic carriers in endemic settings. However, measurement of anti-Vi-IgG is widely considered as potentially useful approach for detecting chronic carriers since increased levels of anti-Vi-IgG are highly prevalent in apparently healthy individuals of typhoid endemic areas [14]. Therefore, we decided to use this biomarker to increase the probability in detecting stool shedding in asymptomatic carriers in this setting. Its use is also recommended for investigating the outbreaks in non-endemic settings [15].

The study finding highlights that a) acute cases are the highest risk of shedding and therefore transmission (even in pre-symptomatic stages), b) use of anti-Vi-IgG is not helpful c) putting resources into finding cases within households has a low pickup rate and so should be considered carefully in terms of prioritization.

In conclusion, it is noteworthy accurate diagnosis and appropriate treatment of acute typhoid patients as well as short-term convalescent carriers are urgently needed to prevent the outbreaks and to eliminate typhoid fever from endemic countries. Similarly, better approaches are needed for surveillance and population level detection of carriers in endemic settings, as vaccine is rolled out and carriage becomes more of an issue/barrier to eradication [5].

Notes

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Potential conflicts of interest.

All authors: No reported conflicts.

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References:

1. Mitscherlich, E. and E.H. Marth, *Microbial survival in the environment: bacteria and rickettsiae important in human and animal health*. 2012: Springer Science & Business Media.
2. Gonzalez-Escobedo, G., J.M. Marshall, and J.S. Gunn, *Chronic and acute infection of the gall bladder by Salmonella Typhi: understanding the carrier state.* Nat Rev Microbiol, 2011. **9**(1): p. 9-14.
3. Levine, M.M., R.E. Black, and C. Lanata, *Precise estimation of the numbers of chronic carriers of Salmonella typhi in Santiago, Chile, an endemic area.* J Infect Dis, 1982. **146**(6): p. 724-6.
4. Parry, C.M., et al., *Typhoid fever.* N Engl J Med, 2002. **347**(22): p. 1770-82.
5. Gunn, J.S., et al., *Salmonella chronic carriage: epidemiology, diagnosis, and gallbladder persistence.* Trends Microbiol, 2014. **22**(11): p. 648-55.
6. Bokkenheuser, V., *DETECTION OF TYPHOID CARRIERS.* Am J Public Health Nations Health, 1964. **54**(3): p. 477-86.
7. Darton, T.C., et al., *The STRATAA study protocol: a programme to assess the burden of enteric fever in Bangladesh, Malawi and Nepal using prospective population census, passive surveillance, serological studies and healthcare utilisation surveys.* BMJ Open, 2017. **7**(6): p. e016283.
8. Wayne, P., *Clinical and laboratory standards institute. Performance standards for antimicrobial susceptibility testing.* 2011.
9. Mohan, V.K., et al., *Safety and immunogenicity of a Vi polysaccharide-tetanus toxoid conjugate vaccine (Typbar-TCV) in healthy infants, children, and adults in typhoid endemic areas: a multicenter, 2-cohort, open-label, double-blind, randomized controlled phase 3 study.* Clin Infect Dis, 2015. **61**(3): p. 393-402.
10. Waddington, C.S., et al., *An outpatient, ambulant-design, controlled human infection model using escalating doses of Salmonella Typhi challenge delivered in sodium bicarbonate solution.* Clin Infect Dis, 2014. **58**(9): p. 1230-40.
11. Karkey, A., et al., *Differential epidemiology of Salmonella Typhi and Paratyphi A in Kathmandu, Nepal: a matched case control investigation in a highly endemic enteric fever setting.* PLoS Negl Trop Dis, 2013. **7**(8): p. e2391.
12. Dongol, S., et al., *The microbiological and clinical characteristics of invasive salmonella in gallbladders from cholecystectomy patients in kathmandu, Nepal.* PLoS One, 2012. **7**(10): p. e47342.
13. Balasegaram, S., et al., *Guidelines for the public health management of typhoid and paratyphoid in England: practice guidelines from the National Typhoid and Paratyphoid Reference Group.* J Infect, 2012. **65**(3): p. 197-213.
14. Gupta, A., et al., *Evaluation of community-based serologic screening for identification of chronic Salmonella typhi carriers in Vietnam.* Int J Infect Dis, 2006. **10**(4): p. 309-14.
15. Olsen, S.J., et al., *Outbreaks of typhoid fever in the United States, 1960-99.* Epidemiol Infect, 2003. **130**(1): p. 13-21.

Table 1: Isolation of *S*. Typhi in stool specimens of blood culture and stool culture positive patients at different time points

	***S*. Typhi BC +ve index cases (N=332)**			**SC +ve index cases (N=33)**		
Days	**Stool collected for culture**	**SC +ve for *S*. Typhi**	**% of isolation**	**Stool collected for culture**	**SC +ve for *S*. Typhi**	**% of isolation**
Day 30	281	15	5	9	1	11
Day 180	270	1	0.4	9	0	0
Day 365*	15	0	0	1	0	0

Abbreviations: BC, blood culture; SC, stool culture

*Stool collected on day 365- if stool culture was positive for *S*. Typhi on day 30 and/or day 180

Table 2: Isolation of *S*. Typhi in stool specimens of household contacts of blood and stool culture positive patients at different time points

	HH contacts of BC +ve index cases			HH contacts of SC +ve index cases		
Days	**Stool collected for culture**	**SC +ve for *S*. Typhi**	**% of isolation**	**Stool collected for culture**	**SC +ve for *S*. Typhi**	**% of isolation**
Day 0	530	5	0.9	9	0	0
Day 30	466	4	0.7	9	0	0
Day 180*	7	0	0	0	0	0

Abbreviations: BC, blood culture; SC, stool culture; HH, household

*Stool collected on day 180- if stool culture was positive for *S*. Typhi on day 0 and/or day 30