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Brief Original Article

Carriage prevalence of *Salmonella enterica* serotype Typhi in gallbladders of adult autopsy cases from Mozambique

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Abstract

Introduction: Typhoid fever is an important public health problem in many low-income countries where asymptomatic carriers play an important role in its dissemination. The bacterium causing typhoid fever can live in the gallstones of asymptomatic persons after the infection. These carriers are reservoirs of *S.* Typhi, are highly contagious, and spread the disease through the secretion of bacteria in feces and urine. The aim of this study was to determine the carrier rate in an area of Mozambique.

Methodology: The presence of *S.* Typhi was analyzed in gallbladder samples obtained from 99 adult corpses (in-hospital deaths) from Mozambique by gold-standard culture and polymerase chain reaction (PCR).

Results: Only one sample was positive with the culture. However, nine additional samples were positive by PCR and confirmed by DNA sequencing. Thus, the prevalence of *S.* Typhi was 10.1% (10/99).

Conclusions: We report a high prevalence of *S.* Typhi in gallbladders among adult autopsy cases from Mozambique.

Key words: *S.* Typhi; carriers; corpses.

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Introduction

Salmonella enterica serotype Typhi (*S.* Typhi) is the causative agent of typhoid fever. Typhoid fever is a major health problem in developing countries. It is acquired by contact with another patient or an asymptomatic carrier, or through contaminated food or water. The prevalence of *S.* Typhi cases is low in Europe, but much higher in low-income countries, with 20 million cases per year leading to over 200,000 deaths annually [1]. Children under five years of age are particularly susceptible to acquiring this infection, especially in Southeast and South Central Asia, Latin America, and Southern Africa [2,3]. The number of isolates resistant to treatment is rising, thereby considerably complicating the clinical management of this disease [4].

Salmonella spp. has developed mechanisms to survive and grow in the presence of bile. Thus, selective culture media, such as Hektoen agar and Rappaport-Vassiliadis, among others, are used to isolate this microorganism. Bile is a mixture of organic and inorganic compounds, bile acids, cholesterol, phospholipids, and biliverdin, the pigment that confers bile its colour [5]. Bile is sterile due to the secretion of IgA and mucus, thereby not allowing colonization by other bacteria [5].

The bacterium causing typhoid fever can live in the gallstones of asymptomatic persons after infection (carriers). It has been estimated that 3%–5% of individuals infected by this microorganism worldwide become carriers. This may explain how a disease causing fever, headache, nausea, loss of appetite, and diarrhea continues to spread despite not having a reservoir in the environment [6]. Carriers are the reservoir of *S.* Typhi, being highly contagious and spreading the disease through the secretion of bacteria in feces and urine [6]. Among carriers, cultivation in urine or feces may remain positive for one year or even decades after becoming infected. This prolonged *S.* Typhi-positive status is also associated with other

conditions such as gastrointestinal neoplasia and gallbladder abnormalities including cholelithiasis [7,8]. Indeed, it has been estimated that 90% of chronically infected carriers present gallstones [9]. It has been suggested that bacteria accumulate, forming biofilms in communities of microorganisms in the gallstones, leading to gallbladder colonization and persistence of the bacterium. Carriers are most often women [7].

No studies on the prevalence of *S.* Typhi have been reported in Mozambique. The aim of this investigation was to determine the presence of *S.* Typhi in the gallbladder of adult patients who died in a referral hospital and underwent autopsy by medical request to extrapolate an estimate of the carriage rate of *S.* Typhi among Mozambican adults.

Methodology

Samples

From November 2013 to May 2014, 2 mL of gallbladder bile (including a section of vesicular stone in cases with gallstones) were collected from 99 corpses by direct-vision puncture of the gallbladder.

S. Typhi isolation

One milliliter of each sample was cultured on Rappaport-Vassiliadis broth (Becton, Dickinson and Company, Heidelberg, Germany) specific for *Salmonella* selection and incubated at 42°C for 48 hours. Aliquots of this culture were plated on Hektoen agar (*Salmonella*-specific medium) and incubated at 37°C for 24–48 hours. Suspicious colonies were analyzed by matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF, Bruker, Barcelona, Spain) and by a *S.* Typhi-specific polymerase chain reaction (PCR) assay.

Another part of the sample was cultured on Luria Bertani broth (LB) (Becton, Dickinson and Company, Heidelberg, Germany) culture media at 37°C for 48 hours, plated on blood agar, and incubated for 24 hours at 37°C. The colonies obtained were analyzed by MALDI-TOF.

S. Typhi PCR detection

Additionally, DNA was extracted from each sample and an *S.* Typhi-specific PCR was performed using primers described elsewhere [10]. *S.* Typhi Ty2 strain was used as a positive control.

Results

Only one sample was positive with the culture. However, nine additional samples were positive by PCR and confirmed by DNA sequencing. Thus, the prevalence of *S.* Typhi was 10.1% (10/99). However, although these 10 individuals may have been all infected by *S.* Typhi, a full autopsy was only conclusive for one case as *S.* Typhi being the underlying cause of death. This patient was a 55-year-old male with a recent admission due to hepatic encephalopathy in the context of cirrhosis. He was HIV positive and receiving antiretroviral treatment, showing a CD4 count of 156. He was admitted due to a decreased state of consciousness and suspected clinical meningitis, and presented a herpetic scar injury on his side. He died 22 days after admission without having recovered from the symptoms. The autopsy confirmed cirrhosis, bilateral pneumonia, and meningitis. Microbiological analysis of autopsy samples demonstrated the presence of *Salmonella* spp. in blood, liver, brain, and lung.

The clinical records were reviewed for the other cases in which *S.* Typhi was detected in the gallbladder. A history of fever of variable duration (7–60 days) and/or gastrointestinal symptoms was present in six patients. Therefore, although the cause of death was determined to be different than typhoid fever infection, it is possible that some of these cases had a concomitant or past *S.* Typhi infection.

To determine the relationship between the presence of *S.* Typhi in the gallbladder and clinical and demographic variables, age, gender, HIV, hepatitis B or C status, and the cause of death were analyzed. Among the 10 *S.* Typhi-positive cases, 50% were women whose ages ranged from 21–76 years. Infection with HIV which, in the whole series, was highly prevalent (60/99; 60%), was not significantly associated with *S.* Typhi carriage (6/10, 60% in carriers versus 54/90, 60% in non-carriers; $p = 1.000$). No cases were associated with either hepatitis B virus or hepatitis C infection. The causes of death varied and were not related to *Salmonella* infection (data not shown).

Discussion

It has been demonstrated that *Salmonella* reaches the gallbladder and replicates within the epithelial cells of this tissue, provoking epithelial destruction and local neutrophil infiltrate [11]. This high carrier incidence is remarkable compared with the low frequency of *S.* Typhi recovered from blood cultures of children < 15 years in Manhiça district, a rural area 80 km of Maputo, where the autopsies were performed. Over a five-year period, only 3 isolates of *S.* Typhi were identified, while *S.* Typhimurium (263 isolates) represented the main etiology of bacteremia [12]. Further studies are needed to investigate this apparent discrepancy between the 10% prevalence of *S.* Typhi in the gallbladder and the

minimal number of typhoid fever cases, but the variable sensitivity of blood culture for *S.* Typhi recovery could be among the possible explanations.

Other studies have described lower rates of *S.* Typhi among feces collected from food handlers (one of the professional groups most frequently involved in the spread of *S.* Typhi), with rates ranging from 0.94% in Iran [13] to 2.7% in Nigeria [14]. A study carried out in Chile reported a prevalence of 11.2% of *S.* Typhi carriers in gallbladder samples collected after surgery for gallbladder infection [6]. In Kathmandu, Nepal, Dongol *et al*. [11] found a prevalence of *S.* Typhi carriers of 1.7% on analyzing bile samples from cholecystectomy patients. Gonzalez-Escobedo *et al*. [9] found that only 5% (5/103) of patients with cholelithiosis presented *S.* Typhi. These results are different from those found in the present study.

Although we did not find any relationship between HIV and the presence of *S.* Typhi, it could be speculated that HIV infection may increase the risk of becoming a carrier of *S.* Typhi [15].

Conclusions

We report a high prevalence of *S.* Typhi in gallbladders of adult autopsy cases from Mozambique. These results require further studies to thoroughly evaluate the prevalence of *S.* Typhi carriage among living individuals in this setting and the association with *S.* Typhi-related morbidity, using molecular methods that are more sensitive than culture-based methods (isolation rate of 10/99 versus 1/99, respectively, in our study).

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