

Does *Salmonella* Typhi primarily reside in the liver of chronic typhoid carriers?

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Chronic carriage of *Salmonella enterica* subspecies *enterica* serovar Typhi (*S. Typhi*), usually in the gallbladder, is considered far more common than in any other site. The chronic typhoid carrier state may develop either as a sequel to typhoid fever or after subclinical infection with the bacterium. The incidence of subclinical typhoid infection has been predicted to be five times higher than that of acute cases, and about 10% of the apparently healthy population have been found to be chronic typhoid carriers based on Vi serology in endemic areas [1]. There are several reports suggesting a definite association of carcinoma of the gallbladder (Ca GB) and chronic typhoid carriage [2-4]. About one third of gallbladder cancer (Ca GB) cases have been reported to exhibit significant titers of antibody against the Vi antigen (ViAb) of *S. Typhi* [3], but the rate of *S. Typhi* culture isolation from gall bladder tissue, stone, and bile specimens from Ca GB patients has been found to be quite low, i.e. only 10% [4].

The low isolation rate of *S. Typhi* may be attributed to either one or more of the following: i) the bacterium might be residing somewhere other than gall bladder; ii) it may be present in the biliary system in a viable but non culturable state; or iii) it may be present in sparse numbers. Alternatively, the low isolation rate of *S. Typhi* may be the result of inefficient isolation techniques. We have already reported on the efficiency of PCR-based amplification of the flagellin (*fliC*) gene of *S. Typhi* in acute typhoid fever cases [5]. To overcome the above-mentioned problems in *S. Typhi* isolation and to ascertain the probable niche of the bacterium, we

investigated the presence of the bacterium using an extremely sensitive and specific nested PCR technique in specimens of the gallbladder and liver.

In this study, a total of 424 cadavers were included. They were victims of unnatural deaths brought to the Centre for Advanced Studies, Department of Forensic Medicine, Banaras Hindu University, for post mortem examination during the period of January 2007 to December 2007. Approximately one mL of bile (available from 322 cadavers), one gram of gallbladder tissue (which included mucosal surface), and one gram of liver tissue were collected from each of the cadavers. These specimens were subjected within one hour to culture isolation of *S. Typhi* by enrichment in Selenite F broth and subsequent subculture on MacConkey agar and deoxycholate citrate agar. DNA was extracted following the standard phenol chloroform method within 24 hours of specimen collection.

A 100 ng quantity of DNA from each of the specimens was subjected to PCR using primers specific to the flagellin (*fliC*) gene of *S. Typhi* following the method described by Frankel [6]. The primers for the first round PCR, forward- ST1 5'-TAT GCC GCT ACA TAT GAT GAG-3' and reverse- ST2 5'-TTA ACG CAG TAA AGA GAG-3', were used to amplify a 495 bp fragment. For the nested PCR, these forward- ST3 5'-ACT CGT AAA ACC ACT ACT-3' and reverse -ST4 5'-TGG AGA CTT CGG TCG CGT AG-3' primers were used to amplify a 364 bp fragment. All the necessary precautions were taken to avoid laboratory

Figure 1

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|----------------------------|--|
| Lane 1 & 19: | Molecular marker (100 bp DNA ladder) |
| Lane 36: | Negative control |
| Lane 2: | Positive control |
| Lane 3-15, 17, 18 & 20-29: | Positive samples of secondary amplicon (364bp) |
| Lane 16, 30-35 | Negative samples of secondary amplicon |

contamination. Known positive (DNA from a reference strain of *S. Typhi*, MTCC 3216) and negative (distilled water) controls were used to detect contamination. Five randomly selected amplicons (two each from liver and gallbladder tissues and one from bile) from different specimens were sent for sequencing to Bangalore Genei, India. The sequences were analyzed using BLAST N (<http://www.ncbi.nlm.nih.gov/BLAST/>) to verify their identity as *S. Typhi*.

A total of 35 (8.2%) corpses were found to be positive for the presence of *S. Typhi*-specific DNA sequences. Liver tissues exclusively were positive in 57.1% (20/35) of the cases. However, liver tissue, gallbladder and bile were all positive in 28.6% (10/35) while gallbladder and bile were positive in 14.3% (5/35). Thus total liver tissue positivity reached 85.7% (30/35). Every amplification protocol consisted of negative as well as positive controls and the negative control was always negative for the specific amplicon. Furthermore, many of the specimens (liver, bile, gallbladder tissues) processed for DNA isolation and amplification simultaneously were also found to be negative for *S. Typhi* specific amplification, which rules out the possibility of contamination. These observations suggest that in chronic carriers, the liver is the major organ where *S. Typhi* persists and from where it is excreted intermittently into the gallbladder. Involvement of the liver during acute typhoid fever can be predicted due to the occurrence of hepatomegaly, jaundice and

raised serum enzymes and bilirubin levels. Frequent failure of cholecystectomy to eradicate the carrier state also supports the view that the gallbladder is not the sole niche of the bacterium in chronic typhoid carriers [7]. Bile is released at intervals with food ingestion but for the most part it is simply stored in the gall bladder, serving as a reservoir that provides an opportunity for bacterial multiplication.

As the gall bladder concentrates bile, the bacteria themselves and the mutagens produced by bacterial metabolic activity are also concentrated; thus the main brunt of these chemicals is borne by the gallbladder, resulting in malignant and benign gall bladder diseases. Detailed investigations such as derangement in liver function tests and immunohistochemistry are recommended to ascertain the presence of *S. Typhi* in the liver of individuals in a chronic typhoid carrier state. The goal of such precautions in the treatment of the chronic carrier state would be to protect the individual from gall bladder cancer and to help in global eradication of typhoid fever.

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