

Molecular and Phenotypic Characteristics of *Salmonella enterica* Serovar *Typhi* Isolated from Asymptomatic Carrier

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Background: *Salmonella enterica* serovar *typhi* (*S. typhi*) the cause of the acute febrile disease typhoid fever is the major public health problem in developing countries. Asymptomatic carriers are the main sources of typhoid. The aim of this study was to investigate methods for isolation and identification of *S. typhi* in asymptomatic carriers.

Materials and Methods: Two hundred stool samples were collected from foodstuff workers and distributors. Then culture characterization, biochemical tests, and nested-PCR were done.

Results: One hundred and seventy-one (85%) of the total cases were male and the mean age of cases was 35 years. Stool culture yielded bacterial colonies consistent with fecal flora but did not yield *S. typhi*. In nested PCR technique just one of the 200 samples (0.5%) was positive for the *S. typhi* capsular gene (*vi* gene).

Conclusion: Due to the improvement in the health status of the country and the low typhoid carriers, it is recommended that efforts be focused on other hygienic issues.

Keywords: *Salmonella typhi*, Carrier state, *vi* gene

1. Background

Salmonella enterica serovar *typhi* is the major cause of enteric fever that is considered as a public health problem in the World. (1, 2). As estimated, 16 to 22 million cases and about 21,600 deaths occur annually due to typhoid, most of which are reported in Asia. Typhoid is one of the widest spread bacterial disease in the world. An individual can asymptotically carry the typhoid bacteria for days to years without manifesting any of the symptoms related to typhoid fever. Typhoid is transmitted by fecal - oral route from an infected person who is an acute or chronic carrier (3).

Since enteric fever caused by *Salmonella* has no environmental reservoir, therefore, chronic asymptomatic carriers of the bacteria are the key factors in its stability in the human population. Chronic carriers transmit infection to others through direct contact or by food or water contaminated with their feces. They are an important reservoir of the infection in endemic areas. *Salmonella typhi* is also passed in the urine of infected individuals (2-4). Being a chronic carrier is a risk factor for gallbladder and biliary system cancers (4, 5).

Studies have reported that people involved in food distribution can be potentially a source of infections attributed to pathogens such as *S. typhi* (5,7). Therefore, identifying typhoid carriers is important for disease prevention (8). The method of gold standard to identify chronic carriers of *S. typhi* is aspiration of biliary or duodenal secretions; however, these methods are invasive and practically not feasible. Bacteriological methods such as culturing carriers also require several turns of stool culture or a culture of bile or duodenal fluid (2). Since gallbladder carrier state is often transient, and dose of *S. typhi* in specimens is small, to reach to a valuable diagnosis, several tests are needed (9,10). In addition, a variety

of bacteriological tests are required to obtain an acceptable detection value (8, 9,11). These tests are expensive and time consuming.

To identify the carriers of *S. typhi*, researchers have tried a number of methods. Culture of various samples such as urine and feces, alone or in combination with different screening techniques of antigen or antibody detection, has been exploited. Nath and colleagues investigated the Flagellin gene of *Salmonella enterica* subspecies enterica serotype *typhi* for chronic typhoid carriers. Also, to detect the carriers, Chandrasekaran and colleagues collected the bile samples and performed identification of the bacterium based on the cultural characteristics (8, 12, and 14). Approximately 1-4% of patients continue to carry *S. typhi* in their intestinal tract and gallbladder for months or years, which are known as asymptomatic carriers (15).

Unlike most other serovars of *Salmonella enterica*, *S. Typhi* can express a carbohydrate capsule commonly known as Vi-CPS antigen. The expression of this antigen related to environmental signals, is important for extra-cellular survival and protection against the oxidative burst of neutrophils. It also reduces the response of TNF-alpha in human macrophages after uptake. The current view is that Vi-CPS is involved in immune evasion during infection in the human host, and therefore, is of vital importance during infection (16-18).

Studies have reported that specific nested-PCR amplification of the *S. typhi* genes such as flagellar or capsular genes is more sensitive than assaying anti-Vi antibodies titers in serum for detection of *S. typhi* carriage (1, 12, and 8). Because all pathogenic strains of *Salmonella* have capsular antigen (1), we also targeted the *vi* gene for nested-PCR.

2. Objectives

The aim of this study was to identify carriers of typhoid by phenotypic and molecular methods among foodstuffs workers and distributors in Tehran.

3. Materials and Methods

3.1. Sample collection

In this cross-sectional study, 200 foodstuffs workers and distributors in Tehran were enrolled from October 2013 to December 2014. The cases were referred to Taleghani Laboratory at Shemiraniat Clinic to issue health card. Written informed consent was obtained from all the cases. The study was approved by review board of the local ethics committee.

3.2. Cultural characterization

A loop of stool samples was inoculated into the 10 mL Selenit F broth (QueLAB) to enhance the growth of the organism and then was incubated at 37 °C for 24hr. After the growth was observed in the enrichment media, the supernatant was streaked into the MacConkey agar and Salmonella – Shigella agar (Merck) and incubated at 37 °C for 24hr. IMVIC test (Indole production, Methyl Red test, Vogues-Proskauer test, Citrate test), Urease test, and Triple Sugar Iron agar test were done.

3.3. DNA Extraction

Nested PCR was performed on DNA extracted from stool samples (Bioneer- Korea). These samples were stored at 70 °C until PCR was performed.

3.4. Amplification of *vi* gene by nested PCR

R1 and R2 primers were used for first and second PCRs, respectively (Gen Bank accession no D14156). R1 (5'-GTTATITCAGCATAAGGAG-3') and (5'-ACTTGCCGTG-TTTTACTC-3') and R2 (5'-GTGAACCTAAATCGCTACAG-3') and (5'-CTTCCATACCCTTCCG-3') amplified 599 bp (nucleotides 745 to 1343) and 307 bp (nucleotides 877 to 1183) of DNA, respectively.

The PCR was carried out in a Palm cycler (Australia). The temperature cycling was as follows: 35 cycles were done at 94 °C for 5 min, 49 °C for 30 s, and 72 °C for 30 s. The temperature was maintained at 94 °C for 3 min at the start of the first cycle and at 72 °C for 5 min at the end of the last cycle. The second PCR, with R2 primers, was performed as follows: 5 µl of the first PCR product amplified with R1 primers was transferred to the second reaction mixture. The temperature cycling of the second PCR was at 56 °C. The amplified PCR products were separated on a 2% agarose gel (invirogen - 16500500) stained with ethidium bromide and visualized by UV transillumination.

The amplification was carried out in 25 µL reaction volume containing 12 µL of 2X PCR master mix (Amplicon, Denmark), 2 µM of primers (*viaB*-F and *viaB*-R), 6 µL DDW, and 5 µL DNA Template. *S. enterica* serovar *typhi* ATCC 19430 was used as positive control.

4. Results

Two hundred food stuff workers and distributors (171 males and 29 females) were included in the study. The mean of patients' age was 35.

4.1. Isolation and identification

A visible growth was detectable in SF broth after 24 hour. All isolates with colorless colonies and black center on SS

agar were characterized by biochemical –based bacteriological investigation. All of the stool cultures yielded bacterial colonies consistent with fecal flora; none of them yielded *S. typhi*.

4.2. Detection of virulence gene by polymerase chain reaction

In nested- PCR assay amplification of virulence gene with the primers of *viaB* gene, among 200 isolates tested, only one isolate generated fragments of the predicted size at 307 bp (Figure1).

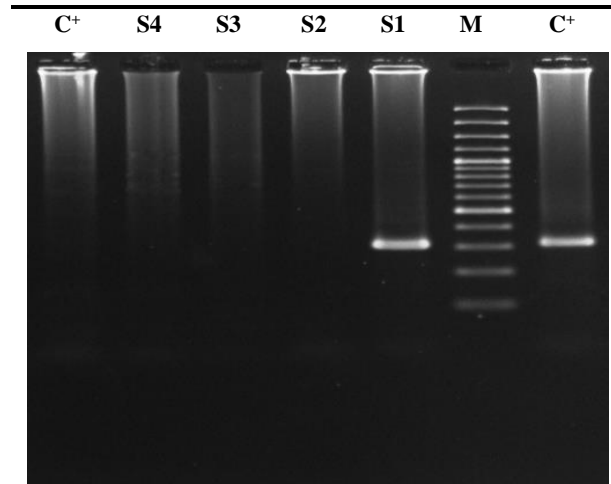


Figure 1. Detection of *viaB* gene (307 bp) by nested-PCR. The figure demonstrates the positive results for detection of *viaB* gene in one of the samples assessed. C⁺: positive control, M: Mid-range DNA size marker; S1-S4: samples, C⁻: negative control.

5. Discussion

Typhoid carriers are regarded as disease reservoirs especially those involved in food production and distribution. Overall, female to male ratio among typhoid carriers is 3:1. Poor hygiene can serve as a potential source for transmission of *S. typhi*. The incidence of subclinical typhoid infection has been estimated to be five times higher than that of acute cases (8, 19).

The present study is the first report in Iran on molecular investigation of fecal specimen taken from people involved in food production and distribution to detect typhoid carriers. Stool culture results were negative for *S. typhi* since shedding is typically low level and intermittent. Disabling of *vi* gene in some patients is another reason for the negative result of typhoid carriers (20). Our findings are consistent with other studies that reported *S. typhi* culture results. Andargie and colleagues (7) and Hamze and colleagues (21) reported no positive results for typhoid carriers. The first author aimed to determine the prevalence of intestinal bacterial infection and parasitic infestation among food handlers by taking specimens from hand fingernail contents and stool. They cultured the specimens and exploited standard bacteriological procedures to identify the intended germs. Hamze and colleagues performed a similar method to identify the microorganisms.

Other reports from Iran demonstrated typhoid carrier prevalence of 0.94% in Hamedan (22) and 0.6% in Sanandaj (23) respectively. They used bacteriological and serological methods for the detection of asymptomatic carriers of *S. typhi* among food handlers.

In order to quickly identify carriers, which is important in eradicating typhoid, molecular techniques to detect antigens or

antibodies have been used in recent years. Since all pathogenic *S. typhi* have the Vi capsular antigen, we used *vi* gene to identify carriers by nested-PCR. Only one sample of 200 specimens was positive for the *vi* gene. Nath and colleagues reported a carrier rate of 13.1% in India using nested-PCR (12). Typhoid is endemic in India, which could justify the findings.

Birhaneslassie and colleagues reported positive results in one out of 107 stool specimens taken from food handlers; they used bacteriological characteristics, biochemical and serologic methods to spot the organism (24). In another study, Pratap and colleagues designed a new nested-PCR primer methodology to target the *staA* gene, which is a member of the fimbrial gene family specific to *S. typhi* only (25).

The prevalence of carriers in some countries like India was reportedly as high as 17.4% to 79% (26, 27). In the report of Senthil Kumar and colleagues the authors collected stool samples from the suspected food handlers and used culture characteristics, biochemical tests, antibiotic sensitivity test (disc diffusion), agarose gel electrophoresis, and conjugation protocols to spot the intended organism. Women constituted 20% (4 of 6) of the positive cases reported in the study (26). In another study, Francis and colleagues collected smear swabs and nail cuts of hotel workers to screen for *S. typhi* carriers. They used bacteriological characteristics as well (27).

In another report from typhoid endemic area, the Ethiopia, a rate of 1.6%, in which 78% of carriers were of women, was found (28). Charles and colleagues applied an immune-screening technique called in vivo-induced antigen technology (IVIAT) to identify potential biomarkers unique to carrier of *S. typhi* in the biliary tract. Thirteen *S. typhi* antigens that were immunoreactive in carriers were identified (29).

Periodic shedding of the organism and low doses of bacteria content as well as inactivation of *vi* gene could justify low rate of typhoid carriers in our study. On the other hand, the predominance of men in our included cases can be another cause.

6. Conclusion

The low rate of typhoid carrier state detected in this study requires further studies with more sensitive diagnostic methods on a larger sample size to have better evaluation of their presence.

Conflict of interests

Authors declare they have no conflict of interests.

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Authors' Contribution

Fatemeh Fallah designed the study and performed the laboratory tests. Hossein Godarzi analysed the data and contributes to laboratory tests and review literature. Fariba Lahoopour accomplished the review Literature and gathered data. Latif Gachkar entered the data and contributed to writing of paper. Mojgan Bandehpour designed the molecular assay. Saeed Soleyman-Jahi contributed to writing the paper and to

data gathering. All of the authors read and revised the manuscript and approved its final version

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