Detection of typhoid carriers by duodenal fluid culture in a tertiary care hospital, Karachi: A cross-sectional study

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Abstract
We aimed to detect typhoid carriers by performing duodenal fluid culture in patients in a tertiary care hospital in Pakistan. A cross-sectional study was conducted during 2017 at the Aga Khan University Hospital, Karachi. Patients who underwent upper gastrointestinal endoscopy were included. Participants were interviewed, and duodenal fluid samples were taken for culture to detect *Salmonella typhi* (S. typhi) and *paratyphi*. A polymerase chain reaction on 100 randomly selected sub-samples was also conducted. A total of 477 participants were enrolled. The mean age was 42.4±15.5 years. History of typhoid fever was present in 73 (15.3%) participants. Out of the 477 duodenal fluid cultures tested for various micro-organisms, 250 (52.4%) were positive. Neither *S. typhi* nor *paratyphi* were isolated. *S. typhi* was also not detected by PCR. To better detect *S. typhi* carriage in general population, future studies should target people with gall bladder diseases and screen them using culture and PCR based methods.

Keywords: Typhoid fever; *Salmonella* species; Typhoid carrier

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Introduction
Globally the estimated incidence of typhoid fever ranges between 12 to 27 million and mortality ranges from 129,000 to 223,000 deaths each year. Typhoid fever has a faeco-oral mode of transmission and is, hence, common in regions with poor water supply and sanitation, particularly among the young population. The case-fatality rate of typhoid fever is estimated to be 1% in those who receive appropriate antibiotics compared to >10% in those who receive inappropriate treatment.1

Despite the high incidence of typhoid, the prevalence and epidemiological risk factors for becoming a chronic carrier have not yet been widely explored. Studies that were conducted on typhoid carriers had limitations, such as epidemiological rigor, inefficient diagnostic methods, or small sample size and have been performed on a selected population—predominantly food handlers and people with biliary diseases.5 At present, typhoid fever is one of the leading public health problems due to the emergence of resistant *S. typhi* strains.6 With the recent advances in the development of novel vaccines and human being the only reservoir for *S. typhi*, regional eradication of typhoid fever should be promising. Yet, spread of pathogen by chronic carriers obscures the elimination of typhoid fever. Thus, it is imperative to identify this challenging population prospectively. Here, we aimed to detect *salmonella* carriers by duodenal fluid culture among the Pakistani population.

Methods and Results
We conducted a cross-sectional study between January-August 2017 at Aga Khan University Hospital (AKUH) in Karachi, Pakistan. All individuals ≥1 year of age, who underwent upper gastrointestinal (GI) endoscopy, were included. Those who had a history of cholecystectomy or did not consent were excluded. Typhoid carrier was defined as an individual whose duodenal fluid, obtained during upper GI endoscopy, was positive for *S. typhi* or *S. paratyphi*. 

References

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on culture. Information on history of typhoid fever and its treatment, socioeconomic status, and source of drinking water was collected from all subjects. Safe water was defined as the use of boiled or bottled (filtered) water for drinking. All other sources of water were deemed unsafe. A confirmed case of typhoid fever was defined as an individual whose blood culture showed growth of *S. typhi* or *S. paratyphi* at the time of past illness. Eligible participants were interviewed after taking informed written consent from participants/legal guardian. Assent was obtained for children age ≥7 years. Duodenal fluid samples were collected prospectively. Prospectively, ~2-10 ml of duodenal fluid was collected in labelled mucus traps through endoscopy. Normal saline flush was used in patients in whom it was difficult to collect the adequate amount of duodenal fluid. The samples were then transferred to the hospital’s research laboratory to perform the culture. Polymerase chain reaction (PCR) was also performed on 100 randomly selected sub-samples of archived duodenal fluid specimens which were stored at -80°C. Ethical approval was taken from the Ethical Review Committee of AKUH (4526-Ped-ERC-16). All participants’ related information was kept confidential. Statistical analysis was carried out using standard statistical software. For socioeconomic status, factor analysis was performed for constructing a wealth index. The crowding index was reported as ‘not crowded’ if there were ≤2 persons/room and considered as ‘crowded’ if there were >2 persons/room. For visualising the study participants’ geographical location, GIS mapping was done using Landsat satellite. Patients residing in the regions outside Karachi were identified and categorised according to the city. However, participants residing in Karachi were mapped according to their residential addresses.

![Figure: Geospatial map showing the geographical location of the participants as black and red dots. There was a high concentration of participants in Karachi; however, the distribution of participants was widespread representing from all over Pakistan including our neighbouring country Afghanistan. (Sindh 74.5%, Balochistan 13.6%, Punjab 3.4%, Khyber Pakhtunkhwa (KPK) 3.4%, and Afghanistan 5.0%). Geospatial map source: Landsat satellite software.](image-url)
A total of 801 patients were approached, out of whom 141 (17.6%) were ineligible, 43 (5.4%) refused to participate, 78 (9.7%) developed complications, 45 (5.6%) patients were non-co-operative, endoscopy was cancelled in nine patients, duodenum was not visualised or obstructed due to mass in eight patients each, five patients developed vomiting, and three had gastrointestinal bleeding, and in 62 (7.7%), the doctor refused to or forgot to aspirate. We were left with a sample size of 477. The mean age of the participants was 42.4±15.5 years. Children ≤18 years accounted for 17/477 (3.6%) of the sample size. The majority, 287 (60.2%), of participants were males. Gastrointestinal illness was the most common underlying reason for endoscopy, (389; 82%) (Table). On the GIS map, our study participants were widely dispersed, representing around 356 (74.5%) from Sindh (54% from Karachi) (Figure).

### Table: Descriptive characteristics of study participants undergoing GI endoscopy at a tertiary care hospital, Karachi (n=477).

<table>
<thead>
<tr>
<th>S.No</th>
<th>Demographic Variables of study participants</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Age (years)</td>
<td>≤18</td>
</tr>
<tr>
<td>2.</td>
<td>Gender</td>
<td>Male</td>
</tr>
<tr>
<td>3.</td>
<td>Race</td>
<td>Sindhi</td>
</tr>
<tr>
<td>4.</td>
<td>Socioeconomic status</td>
<td>Low</td>
</tr>
<tr>
<td>5.</td>
<td>Reason of endoscopy</td>
<td>GI illness</td>
</tr>
<tr>
<td>6.</td>
<td>Hand hygiene present in participants</td>
<td>Yes</td>
</tr>
<tr>
<td>7.</td>
<td>Unsafe Drinking water</td>
<td>Yes</td>
</tr>
<tr>
<td>8.</td>
<td>Antibiotic use at the time of typhoid fever illness</td>
<td>Yes</td>
</tr>
<tr>
<td>9.</td>
<td>Duration of antibiotic use at the time of typhoid fever illness</td>
<td>&lt;7 days</td>
</tr>
<tr>
<td>10.</td>
<td>Crowding Index (persons per room)</td>
<td>≤2</td>
</tr>
<tr>
<td>11.</td>
<td>History of Typhoid Fever</td>
<td>Yes</td>
</tr>
<tr>
<td>12.</td>
<td>Typhoid disease status</td>
<td>Confirmed cases</td>
</tr>
<tr>
<td>13.</td>
<td>Antibiotic use at the time of typhoid fever illness</td>
<td>Yes</td>
</tr>
<tr>
<td>14.</td>
<td>Duration of antibiotic use at the time of typhoid fever illness</td>
<td>&lt;14 days</td>
</tr>
<tr>
<td>15.</td>
<td>Antibiotic Use in the last two weeks</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Out of 477 duodenal fluids samples tested for various microorganisms, 250 (52.4%) were positive. Among the 250 species isolated on duodenal fluid culture, none had growth of *S. typhi* or *S. paratyphi*. The various other microorganisms isolated were *E. coli* in 68 (27.2%), followed by *Pseudomonas spp.* in 58 (23.6%), *K. pneumoniae* in 35 (14%), *P. aeruginosa* in 17 (6.8%), *Staphylococcus spp.* in 11 (4.4%), *Streptococcus spp.* in 10 (4%), and *Enterobacter species* in 9 (3.6%) among others. Of the 250 positive duodenal fluid cultures, 22 (8.8%) showed growth of more than one species. We also failed to detect any *S. typhi* or *S. paratyphi* on the PCR performed.

### Discussion

Controlling typhoid fever is challenging for many developing countries due to poor health care facilities and the interdependent nature of WASH (water, sanitation and hygiene) factors. This issue is further compounded by the presence of silent typhoid carriers. In order to identify chronic *S. typhi* carriers, screening through anti-Vi serology by ELISA (Enzyme-Linked Immunosorbent Assay) is recommended before culture. However, serological techniques are only useful when the titres are interpreted with regards to the background antibody levels in the local population, something which is not possible in developing countries. Hence, we studied culture and PCR techniques on duodenal fluid collected from patients during routine upper GI endoscopy.

In our study, *Escherichia coli* (*E. coli*) and *Pseudomonas spp.* were the most frequently isolated bacteria; this is consistent with past literature. This observation may suggest that most of the bacteria in bile originate from the intestinal tract. The non-isolation of *S. typhi* or *S. paratyphi* in our study may be attributed to our selected study population. We might have had a better yield in detecting *S. typhi* in the duodenal fluid if we had included patients with gall bladder diseases because of the role of *Salmonella* carriage in the pathophysiology of cholangiocarcinoma and cholelethiasis. In addition, it might be because the method used to collect bile from duodenal fluid is not ideal. We suspect that collecting bile and gallbladder tissue directly by more invasive techniques may yield better detection rates. Robert H. G. et al used string capsule for isolating *S. typhi* from patients with acute typhoid fever. Other methods include direct puncture of gallbladder, through endoscopic retrograde cholangiography or during cholecystectomy. Dongol et al found 24 out of 1,377 (1.7%) *S. typhi* carriers in bile from cholecystectomy patients in Nepal. Similarly, Vaishnavi C. et al found 21 out of 445 (4.7%) *S. typhi* from all bile samples.

Furthermore, reports from past studies have shown that...
isolating Salmonella from culture is difficult as compared to molecular (PCR) based methods. Lucilia L. et al analysed the presence of S. typhi in gallbladder obtained from 99 adult corpses by culture and PCR. They reported 10 out of 99 (10.1%) S.typhi carriers. However, they observed that only one sample was positive on culture while nine samples were positive by PCR and confirmed by DNA sequencing. However, we failed to detect typhoid carriers by PCR on 100 randomly selected duodenal fluid specimens. This could be because we tested only limited number of samples.

Our study design was the major limitation and it was difficult to overcome given the diagnostic concerns of identification of typhoid carrier status at the outset. Literature shows that the sensitivity of salmonella detection from duodenal fluid samples is ~50% which is inconsistent with our study; therefore, further studies are needed to investigate this discrepancy by studying patients with gall bladder diseases.

Conclusion

Even though we did not identify salmonella carriage on duodenal fluid culture and PCR in our study, we still assume that there is a high prevalence of typhoid carriers considering the burden of typhoid fever in Pakistan. We understand that UGI endoscopy is not feasible for identification of typhoid carrier status at a public health level and the yield is low. We recommend development of serological tests that can identify salmonella carriage and have the ability to distinguish between acute illness from chronic carriage.

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References