

## ORIGINAL ARTICLE

# Screening of Enteric Fever in the Human Population of District Khairpur, Sindh, Pakistan

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## ABSTRACT

Typhoid fever, commonly known as enteric fever, is a bacterial infection that can affect many organs and spread throughout the body. Bacteria known as *Salmonella typhi* are the most common cause of Typhoid/ Enteric fever; the same bacteria are also most commonly involved in food poisoning. Typhoid fever is more common in areas with contaminated water and food and poor sanitation. In crowded and unsanitary places, typhoid fever is one of the most common causes of death and morbidity. Headaches and stomach pain are frequently associated with this fever. A course of antibiotics, vaccine prophylaxis, various care, surgery, and sanitation prevention are usually effective treatments for typhoid fever. Antibiotics are exclusively effective against typhoid fever, and the most prescribed antibiotics are Ciprofloxacin and Ceftriaxone. The primary goal of the present study was to diagnose typhoid fever using a Widal test, estimate the percentage prevalence of typhoid-affected patients according to their age and gender, and compare the percentage of typhoid-affected patients in different parts of Khairpur Mir's district. During data collection, 143 tests of typhoid fever were performed with the human population of different areas of District Khairpur Mirs. Results of this study showed a total of 83 (58.04%) of positive cases in males, and 60 (41.95%) were positive in females; among them, 73 (51.04%) patients were above the age of 25, and 70 (48.95%) were below the age of 25, 70 (48.04%) patients were diagnosed with typhoid fever while others were not affected. According to the area, Lukman contains 27 (18.88%) of the positive cases; mall road contains 28 (19.58%) cases, Punjhuti contains 29(20.27%) cases, Bhens colony contain 30 (20.97%) cases and Milk colony 29 (20.27%) patient of typhoid fever. Present work will be helpful for the spread of awareness of typhoid fever at District Khairpur Mirs.

**Keywords:** Human population. Prevalence. Typhoid fever, Widal test. Khairpur mirs.

## INTRODUCTION

Typhoid fever is a bacterial infection caused by *Salmonella enteric* subspecies enteric serovar Typhi, a Gram-Negative bacterium (*S.Typhi*). Typhoid fever is caused by ingesting food or drink contaminated with faecal or urine carries excreting *S.Typhi*. (Parry CM, Hien TT et al., 2002(a)). High fever is the most common infection symptom, although additional symptoms include nausea, abdominal pain, and abnormal irregular bowel movements. (Crupm JA, Sjolund-Karlsson M et al., 2015). Improvements in the availability of clean water and sewerage system have resulted in a remarkable reduction in the prevalence of Typhoid fever, with the disease now mainly affecting low-and middle-income nations with inadequate sanitary conditions. (Parry et al., 2002(b)). Typhoid fever is mostly a travel-related disease in developed countries (Connor, Schwartz et al., 2005(a)). The travelling population in endemic areas, such as military personnel, temporary employees, tourists, and travelers visiting friends or relatives (VFR) or at risk, varies according to the geographical location. (Connor BA, Schwartz et al., 2005(b)). Ekdahl K, de jong B et al., 2005). Each year, up to 21 million people develop typhoid after consuming faeces-contaminated food or water, with up to 161 000 people dying. (Ahmad M et al., 2018(a)). In South Asia, which includes Pakistan, 3-6 cases of typhoid are expected to occur per 100 000 people each year. The Punjab and Sindh provinces of Pakistan have the highest risk of contracting typhoid among the 16 Asian countries where

the disease is widespread. (Antillon M et al., 2017). Typhoid fever is endemic in South Asia and is the most frequent bacteremic infection among children, most affected under the age of 15. (Ahmad M et al., 2018(b)). The combination of drinking water and sewage was the primary cause of the outbreak. Ahmad et al., (c)). It should be clinical to approach typhoid patients. Patients with febrile sickness for more than three days, gastrointestinal manifestations living in areas with poor sanitation or impure drinking water, or a travel history from endemic areas are highly suspicious in the first week. However, the diagnosis is complicated by a variety of laboratory tests. (Bhutta et al., 2006). Typhoid fever is a significant public health concern. The Issue around the world. The bacteria live in the human intestine and bloodstream, and they are spread among individuals via direct contact with an infected person's excrement, causing the disease to spread to animals, resulting in constant human to human transmission. (*S.typhi*) means enters the body through the mouth and stays in the intestine for 1 to 3 weeks. After that, the infection spreads to other tissue and organs via the gut wall and the bloodstream. The immune system of the host cell do little to fight back because *S.typhi* can live within the host cell, safe from the immune system. (Newman, 2017). Currently, typhoid fever laboratory diagnosis is based on the isolation of *Salmonella enteric* subspecies enterica serotype, although blood culture is still the most common approach to confirm a typhoid fever diagnosis, and it is neither expensive nor technically tricky. (J Park et al.,

2016). In the first week, stool culture is less effective, but it is diagnostic in the second and third weeks. (P Hornick et al., 1975).

In order to diagnose typhoid, a bone marrow culture is required. (Matee MIN et al., 2019 (a)) The Widal test for enteric fever is a serological test that looks for antibodies to the antigens O (Surface) and H (flagellar). A cut of level for predicting recent typhoid fever infection in an endemic area is an antibody titer of greater than 1:160 and greater than 1:80 for anti-H antigens and anti-O antigens, respectively. (Matee MIN et al., 2019 (b)). Enzyme-linked immunosorbent Assay (ELISA) identifies antibodies to capsular polysaccharide Vi antigens are a type of antigen that can be useful in identifying carriers, but they are rarely beneficial in cases of acute sickness. (Sotomayor et al., 1983). Although urine and duodenal content cultures in string capsules are not routinely performed, they can help identify *Salmonella typhi*. (Woodward TE et al., 1988). Antibiotics therapy, prophylaxis for the vaccine, miscellaneous care, surgery, and sanitation prevention. Antibiotics are the only effective treatment for typhoid; the most prescribed antibiotics or Ciprofloxacin (for non-pregnant adults) and Ceftriaxone. In addition to medication, enough water consumption is required for rehydration. Surgery may be required in more serious cases where the bowel has been perforated. (Newman, 2017). Some studies have shown that *Salmonella Typhimurium* resistance rates are about 35%. (Newman, 2017).

The Widal agglutination test invented by F Widal in 1896 to diagnose typhoid fever uses a suspension of killed *Salmonella typhi* as antigen to diagnose serum typhoid fever from suspected *S typhoid*-infected patients with the febrile disease. Widal agglutination was developed as a serologic producer to aid in diagnosing typhoid disease. The test was based on the proof of the presence of agglutinin (antibody) against the H (flagellar) and O (somatic) antigens of *Salmonella typhi* in the serum of the infected patient. Reynolds et al. concluded that diagnosis of typhoid fever based on serology (Widal agglutination) alone is frequently inaccurate. (Olopona and King et al., 2000). The disease is considered a serious public health issue, and the WHO has suggested that high-risk locations where typhoid fever is a concern be vaccinated with the existing Vi polysaccharide vaccine. (Weekly Epidemiol et al., 2008). In 2019, Pakistan became the first country to introduce the WHO-recommended typhoid conjugate vaccine (TCV) into its routine immunization programme. Sindh, Pakistan's second-most population province, has seen a surge in blood-culture proven Typhoid fever (TF) patients resistant to routine treatment. Akram J, et al., 2020). In Khairpur, Sindh, Pakistan, typhoid disease is still endemic. The current investigation was conducted to look into the likelihood of antibiotics resistance as a cause of this endemicity. A standard microbiological procedure was used to isolate (*S. Typhi*) strain from clinical blood samples. Although it is a prevalent type of fever that causes a huge loss of economy and poses a problem for humanity around the globe, much work has been done in the world. However, some or little work has been done in Pakistan, especially in Sindh, so, therefore, this study is aimed to work on the diagnosis and prevalence of typhoid fever at District Khairpur Mirs Sindh Pakistan.

## MATERIAL AND METHODS

This research was carried out at the Department of Zoology, Shah Abdul Latif University Khairpur Mir's. The subjects who participated in the study were indoor and outdoor patients who arrived at Civil hospital District Khairpur. The research period started from August to November 2021. In this study, we distribute the subjects according to age-wise, years (1-5), years (6-10), years (11-15), years (16-20), years (21-25), years (26-30), and years (31-35). Ethical permission was taken from the Department of Zoology, Shah Abdul Latif University of Khairpur. Furthermore, the benefits, purpose, and risks were properly informed to all the volunteers from which samples were collected. Serum samples were used to perform the Widal test from all seven groups of patients and controls. *Salmonella* tissue was isolated from blood samples for the diagnosis of typhoid fever. The control group was comprised of 2 subgroups. The clinical evolution and accompanying a laboratory investigation were used to diagnose each of these patients. This study does not include those children who were small in the age with minor complications. We followed the instructions for performing the Widal test. After that, blood samples were taken to the hospital's laboratory, and blood serum was separated from a venous blood sample (2-3mL) was collected aseptically using 70% alcohol in a sterile test container and centrifuged at 2500 rpm for 5 minutes. The manufacture instruction was followed by performing the Widal test (slide) with *S. typhi* O and H antigens. SPINREACT reagent Ltd provided the antigen suspension, commercially available in a 5mL amount. The agglutination ability of sera was determined qualitatively using a direct slide agglutination approach in this investigation. The test was performed by combining one drop of serum with one drop of O and H antigens on a slide individually. The mixture was examined for macroscopic agglutination after shaking the slide back and forth for one minute. If agglutination occurred within 1 minute, it was classified as a reactant; otherwise, the non-reactive agglutination test was performed on every serum using traditional agglutination methods, utilizing antigens that are commercially accessible. 0.4 ml of two folds serially diluted patient's sera in 0.9% normal saline (diluted range: 1:20 to 1:320) were tested by adding an equal volume of antigen. Each batch of the test includes a negative saline control. The patient's symptoms of Typhoid were elicited by questionnaires, which included weakness, stomach pain, discomfort, headache, diarrhea, constipation, cough, loss of appetite, and abdominal pain.

## RESULTS

Seventy patients were found positive Typhoid and seventy-three were negative cases in a Civil hospital, Khairpur Mir's Sindh, Pakistan, as shown under table no. 1. Our results show the frequency of typhoid fever in 58.04% of males and 41.96% of females, as described in table 2. According to the age-wise impact ratio, 1-5 years had four positives and eleven negative cases, 6-10 years had seven positive and eleven negative cases, 11-15 years had eleven positives and ten negative cases, 16-20 years had nine positives and seven negative cases, 21-25 years had ten positive and nine negative cases, 26-30 years had

seventeen positive and nine negative cases and 31-35 years had twelve positives and sixteen negative cases as given in table 1. Our prevalence of typhoid fever was 48.95% were positive cases, and 52.04% were negative cases got from the different selected areas of Khairpur Mir's Sindh, Pakistan, as shown in table 03.

Table 1: Age wise impact ratio of Typhoid in district Khairpur Mirs

Age (Group)	Positive +ve case	Negative -ve case	Total
1 - 5 years	4+ve	11-ve	15
6 - 10 years	7+ve	11-ve	18
11 - 15 years	11+ve	10-ve	21
16 - 20 years	9+ve	7-ve	16
21 - 25 years	10+ve	9-ve	19
26 - 30 years	17+ve	9-ve	26
31 - 35 years	12+ve	16-ve	28

**Table 1:** Shows the age wise group with the distribution of S.typhi test in which the positive cases were 70 and 73 were negative.

Table 2: Gender wise Frequency of Male and Female patients with Typhoid in Khairpur Mirs

Gender	Frequency	Percentage
Male	83	58.04%
Female	60	41.96%
Total	143	100%

**Table2:** This table shows the frequency and percentage of male and female in the Civil hospital and other areas.

Table 3: Area wise impact of Typhoid in Khairpur mirs

Area name	Positive +ve cases	Negative-ve case	Total
Luqman	12+ve	15-ve	27
Mall Road	15+ve	13-ve	28
Punjhuthi	13+ve	16-ve	29
Bhens colony	17+ve	13-ve	30
Milk colony	13+ve	16-ve	29

**Table3:** These tables show the area wise impact of S.typhi in different areas of Khair Pur mir is out of 143 subjects, Luqman had 12+ve and 15-ve cases of typhoid, Mall Road had 15+ve and 13-ve cases, Punjhuthi has 13+ve and 16-ve cases, Bhens colony had 17+ve and 13-ve cases while Milk colony had 13+ve cases and 16-ve cases of S.typhi. 48.95% positive cases and 52.04% negative cases got from selected areas of Khairpur mirs.

## DISCUSSION

The Widal is the test to detect any bacterial or viral infection in any serum (blood sample without fibrinogen). This test was given by F.Widal in 1896. Typhoid is a bacterial disease caused by S.typhi bacteria that are tainted with infected faecal matter and propagated by food and drinking water. If polluted water is used, washing fruits and vegetables will disperse it. Some individuals are asymptomatic typhoid carriers, which means they carry the bacteria but experience ill effects. After their symptoms are gone, some continue to harbour the bacteria often; the disease may occur again; people who test positive as carriers will not be able to work with children or order

people until medical testing indicates that they are safe. (Newman,2017) Typhoid fever continues to be a significant public health issue around the world. The Ratio of paratyphoid to typhoid fever cases could be higher due to urbanization and growing reliance; there has been a shift on the street vendor-purchased food.

Consequently, S-typhi is difficult to isolate from blood and faeces in developing countries or other bodily fluids. Diagnostic O and H widal agglutination titre the use of agglutinins in a diagnostic will be considered our surrounding of typhoid fever. (Taiwo et al., 2007). In an area where malaria, bacterial septicaemia, and dengue fever are significant causes of admission with fever, evaluating the Widal test presents several challenges. The number of agglutinins detected in a non-infected population varies significantly. It also depends on the severity of infection from different Salmonellae with cross-reacting antigens and the choice of the suitable gold standard for diagnosis. As a result, it is crucial to assess widal tests in the context they will be employed (Schroeder, 1968). This research is being carried out in the several Talukas of Khairpur Mirs to see how the impact of typhoid has changed over time in different patients based on their age and gender. This research will aid future scholars in learning more about typhoid in the district of Khairpur Mirs.

## CONCLUSION

From this research, it was concluded that the ratio of typhoid fever in various areas of Khairpur mirs from age 1 to 5 ( 4+ve, 11-ve) from the age of 6 to 10 (7+ve, 11-ve) from 11 to 15 (11+ve, 10-ve) from 16 to 2 (9+ve, 7-ve) from 21 to 25 (10+ve, 9-ve) from 26 to 30 (17+ve, 9-ve) and age 31 to 35 (12+ve, 16-ve). It is also concluded that Typhoid fever is more prevalent in males 58. % Positive and less common in females 48% positives. Present work will be helpful for the spread of awareness of typhoid fever at District Khairpur Mirs.

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## REFERENCES

1. Ahmad, Muhammad Aurangzeb, Carly Eckert, and Ankur Teredesai. "Interpretable machine learning in healthcare." In Proceedings of the 2018 ACM international conference on bioinformatics, computational biology, and health informatics, pp. 559-560. 2018.
2. Andoh, L.A., Ahmed, S., Olsen, J.E., Obiri-Danso, K., Newman, M.J., Opintan, J.A., Barco, L. and Dalsgaard, A., 2017. Prevalence and characterization of Salmonella among humans in Ghana. *Tropical medicine and health*, 45(1), pp.1-11.
3. Antillón, M., Warren, J.L., Crawford, F.W., Weinberger, D.M., Kürüm, E., Pak, G.D., Marks, F. and Pitzer, V.E., 2017. The burden of typhoid fever in low-and middle-income countries: a meta-regression approach. *PLoS neglected tropical diseases*, 11(2), p.e0005376.
4. Bhutta, Z. A. (2006). Current concepts in the diagnosis and treatment of typhoid fever. *Bmj*, 333(7558), 78-82.
5. Chinh, N.T., Solomon, T., Thong, M.X., Ly, N.T., Hoa, N.T.T., Wain, J., Diep, T.S., Smith, M.D., Day, N.P., Phi, L.T. and Parry, C., 1997. Short courses of ofloxacin for the treatment of enteric fever. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 91(3), pp.347-349.

6. Connor, B.A. and Schwartz, E., 2005. Typhoid and paratyphoid fever in travellers. *The Lancet infectious diseases*, 5(10), pp.623-628.
7. Cruickshank, R., J. P. Duguid, B. P. Marmion, and R. H. Swain. "Medical microbiology: the practice of medical microbiology." In *Medical microbiology: the practice of medical microbiology*, pp. 587-587. 1975.
8. Crump, J.A., Sjölund-Karlsson, M., Gordon, M.A. and Parry, C.M., 2015. Epidemiology, clinical presentation, laboratory diagnosis, antimicrobial resistance, and antimicrobial management of invasive *Salmonella* infections. *Clinical microbiology reviews*, 28(4), pp.901-937.
9. Ekdahl, K., De Jong, B., Wollin, R. and Andersson, Y., 2005. Travel-associated non-typhoidal salmonellosis: geographical and seasonal differences and serotype distribution. *Clinical Microbiology and Infection*, 11(2), pp.138-144.
10. Gilman, R., Terminel, M., Levine, M., Hernandez-Mendoza, P. and Hornick, R., 1975. Relative efficacy of blood, urine, rectal swab, bone-marrow, and rose-spot cultures for recovery of *Salmonella typhi* in typhoid fever. *The Lancet*, 305(7918), pp.1211-1213.
11. Lanata, C., Ristori, C., Jimenez, L., Garcia, J., Levine, M., Black, R., Salcedo, M. and Sotomayor, V., 1983. Vi serology in detection of chronic *Salmonella typhi* carriers in an endemic area. *The Lancet*, 322(8347), pp.441-443.
12. Luxemburger, C., Duc, C.M., Lanh, M.N., Wain, J., Hien, T.T., Simpson, J.A., Kam, L.H., Tu Thuy, N.T., White, N.J. and Farrar, J.J., 2001. Risk factors for typhoid fever in the Mekong delta, southern Viet Nam: a case-control study. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 95(1), pp.19-23.
13. Mawazo, A., Bwire, G.M. and Matee, M.I., 2019. Performance of Widal test and stool culture in the diagnosis of typhoid fever among suspected patients in Dar es Salaam, Tanzania. *BMC Research Notes*, 12(1), pp.1-5.
14. Mogasale, V., Ramani, E., Mogasale, V. V., & Park, J. Y. (2016). What proportion of *Salmonella Typhi* cases are detected by blood culture? A systematic literature review. *Annals of Clinical Microbiology and Antimicrobials*, 15(1), 2–3. neglected tropical diseases, 5(6), p.e1163.
15. Olopoenia, L. A., & King, A. L. (2000). Widal agglutination test– 100 years later: still plagued by controversy. *Postgraduate medical journal*, 76(892), 80-84.
16. Parry, C.M., Vinh, H., Chinh, N.T., Wain, J., Campbell, J.I., Hien, T.T., Farrar, J.J. and Baker, S., 2011. The influence of reduced susceptibility to fluoroquinolones in *Salmonella enterica* serovar *Typhi* on the clinical response to ofloxacin therapy. *PLoS*
17. Schroeder, S.A., 1968. Interpretation of serologic tests for typhoid fever. *Jama*, 206(4), pp.839-840.
18. Taiwo, S.S., Adesiji, Y.O. and Adekanle, D.A., 2007. Screening for syphilis during pregnancy in Nigeria: a practice that must continue. *Sexually transmitted infections*, 83(5), pp.357-358.
19. Waddington, C. S., Darton, T. C., Woodward, W. E., Angus, B., Levine, M. M., & Pollard, A. J. (2014). Advancing the management and control of typhoid fever: a 35 review of the historical role of human challenge studies. *Journal of Infection*, 68(5), 405-418