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Original Research Article

Widal seropositivity in and around Akola, Maharashtra: unravelling the regional trends of enteric fever

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ABSTRACT

Background: Enteric fever remains an important public health concern in India. The Widal agglutination test is widely used as a presumptive diagnostic tool, particularly in resource-limited settings. However, its interpretation is influenced by background antibody titers, previous exposure, cross-reactivity and geographical variations. The objective is to determine the proportion of widal seropositivity among patients investigated for suspected enteric fever in and around Akola, Maharashtra, and to analyze the distribution of antibody types, age, gender and seasonal variation.
Methods: A study was conducted in the serology section, Department of Microbiology, Government Medical College, Akola, from January 2023 to July 2026. A total of 30,440 serum samples received from patients with suspected enteric fever were analyzed. Widal tube agglutination testing was performed using commercially available antigens. A titre of ≥1:80 for any of the tested antibodies (TO, TH, AH and BH) was considered significant. Demographic and temporal distributions of seropositive cases were analyzed.
Results: Out of 30,440 samples, 6,616 showed significant widal titers, giving an overall seropositivity of 21.73%. Among the positive cases, TO antibodies accounted for 4,216 (63.72%), followed by TH antibodies in 1,670 (25.24%), AH antibodies in 548 (8.29%) and BH antibodies in 182 (2.75%). Females constituted 64.55% (4,270) of positive cases, while males accounted for 35.45% (2,346). Adults represented 74.58% (4,934) and pediatric patients 25.42% (1,682) of positive cases. Seasonal analysis revealed maximum positivity during the rainy season, particularly in July (27%), followed by August (13%), June (11%) and September (9.4%).
Conclusions: Study demonstrated an overall widal seropositivity of 21.73% among patients investigated in and around Akola. Higher TO antibody positivity and increased seropositivity during the monsoon months were observed. These findings provide useful regional data regarding widal serological patterns. However, widal results should be interpreted in correlation with clinical findings, as single-sample testing without blood culture confirmation cannot establish active enteric fever.

Keywords: Enteric fever, Widal test, Seropositivity, *Salmonella* typhi, TO antibody, TH antibody, Seasonal variation

INTRODUCTION

Typhoid fever, also known as enteric fever, is a systemic bacterial infection caused primarily by *Salmonella enterica* serovar Typhi, whereas paratyphoid fever is caused by *Salmonella enterica* serovars Paratyphi A, B or C.[1] These infections remain important public health concerns in developing countries, including India, where enteric fever continues to contribute to the burden of febrile illness.[2] The clinical presentation of enteric fever is often nonspecific and may include prolonged fever, headache, malaise, chills and gastrointestinal symptoms, making laboratory investigations important for diagnosis.[1] The widal agglutination test is a conventional serological

method used for the presumptive diagnosis of enteric fever. It detects antibodies against the somatic (O) and flagellar (H) antigens of *Salmonella* Typhi and depending on the test system, the corresponding antibody of *Salmonella* Paratyphi.[3] Despite advances in diagnostic microbiology, the Widal test continues to be used in many healthcare settings because of its relatively low cost and accessibility.[3] However, interpretation of widal test results presents several challenges. Antibody titer may be influenced by previous infection, background exposure to *Salmonella*, vaccination history, cross-reacting antibodies, and the timing of sample collection.[3] consequently, a single elevated antibody titer cannot reliably distinguish active infection from previous exposure. Blood culture remains the preferred diagnostic method for confirming enteric fever, although its sensitivity may be affected by prior antibiotic administration and the volume of blood collected.[1,3]

Analysis of the data can provide useful information regarding the frequency and distribution of widal seropositivity among patients in specific geographical area. Previous systematic reviews have demonstrated geographical and methodological variation in reported enteric fever burden in India, highlighting the importance of interpreting local findings within their epidemiological context.[2] Analysis of laboratory data may also help identify patterns according to age, sex and seasonal distribution, provided these variables are available in the records.

Akola and its surrounding areas in Maharashtra provide a relevant setting for examining local patterns of widal seropositivity among patients undergoing routine diagnostic investigations. Local data on reactive widal tests and the distribution of anti-O and anti-H antibody titer may contribute to understanding serological trends in the region. However, such findings should be interpreted as serological reactivity and not as definitive evidence of confirmed enteric fever in the absence of appropriate confirmatory testing.[1,3]

The present study aims to analyse widal test results among patients investigated for suspected enteric fever. The study will determine the proportion of samples showing widal seropositivity, assess the distribution of antibody titers, and evaluate demographic and seasonal patterns in and around Akola. The findings may provide local baseline data on widal seropositivity and contribute to a more informed interpretation of serological results in routine diagnostic practice.

METHODS

The present study was conducted in the Serology section of Microbiology Department, Government Medical College Akola, after approval from institutional ethics committee. The study period was from January 2023 to July 2026. A total of 30,440 samples were received in serology laboratory from patients presenting with acute febrile illness, abdominal pain, and vomiting from outpatient, inpatient department. All the samples received in the laboratory for widal testing were included in study; Mismatched, unlabelled, hemolysed, icteric and lipemic blood samples were excluded from the study.

The widal tube agglutination test was performed on sera using commercially available antigens (biolab diagnostics). 0.5ml of two-fold serially diluted patients sera (1:20 to 1:320) in 0.5 ml 0.9% normal saline were tested by adding an equal volume of antigen. A negative control was included in each batch of the test. The tubes were incubated at 37ºC for 16 to 20 hrs and then examined for agglutination.[4] A diagnostic titer of 1:80 and above suggests positive reaction.

RESULTS

A total of 30,440 blood samples were analyzed during the study period, out of which 6616 samples had significant titer of ≥1:80 for O, H, AH and BH antibodies. So, the overall prevalence was found to be 21.73 % (Figure 1). Out of the 6616 positive samples, 4216(63.73%) were positive for O antibodies, 1670(25.24%) were positive for H antibodies, 548(8.29%) were positive for AH antibodies and 182(2.75%) were positive for BH antibodies (Figure 2). Out of the 6616 positive samples, 4270(64.55%) were female cases and 2346 (35.45%) were male cases of widal positive test (Figure 3).

Among Widal test positive cases, adult cases were 4934 (74.58%) and pediatric cases were 1682 (25.42%) respectively (Figure 4). There was Season wise variation seen in the seropositivity of the Widal test. The months from June to September showed the maximum number of widal positive cases, which corresponds to the rainy season here in Akola maximum cases of widal seropositivity were observed in July 1726(27%) followed by august 860(13%) followed by June 727(11%) followed by September 621(9.4%) (Figure 5).

Year wise widal seropositivity was also determined. It was 2106(31.83%) in 2023, 1591(24.04%) in 2024, 2276(34.41%) and 643(9.72%) upto July in 2026 as shown in Figure 6.

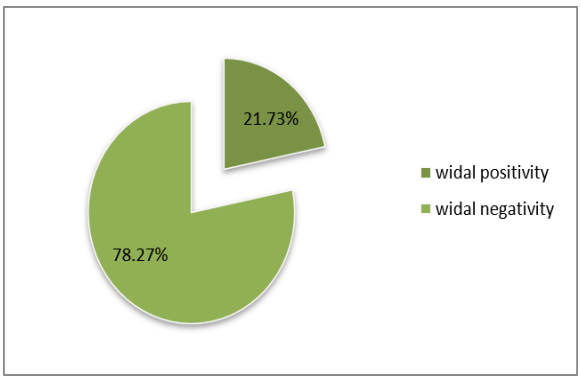


Figure 1: Prevalence of widal seropositivity.

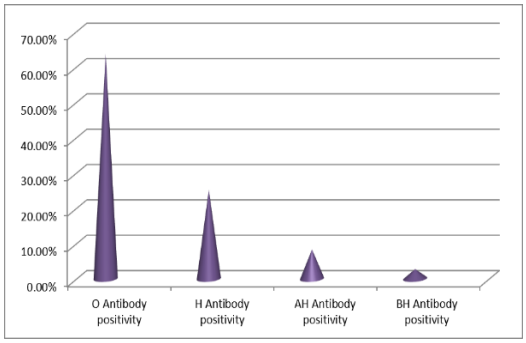


Figure 2: Antibody wise distribution of widal positive cases.

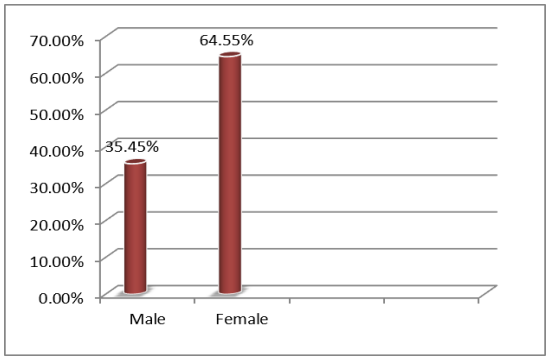


Figure 3: Prevalence of widal positivity in male and female population.

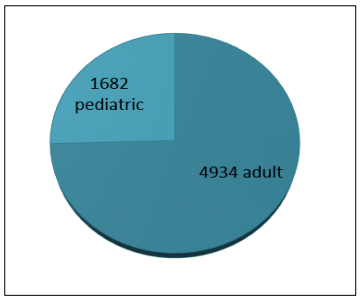


Figure 4: Prevalence of widal seropositivity in adult and pediatric population.

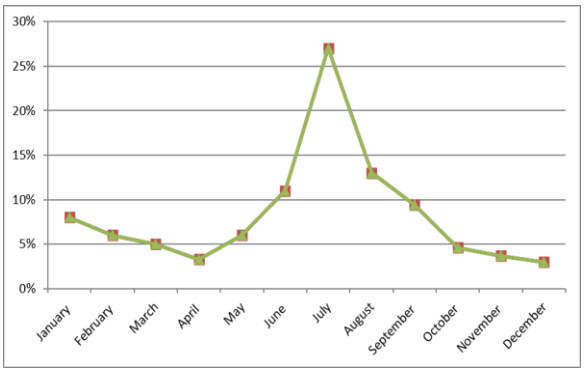


Figure 5: Seasonal variation in prevalence of widal seropositivity.

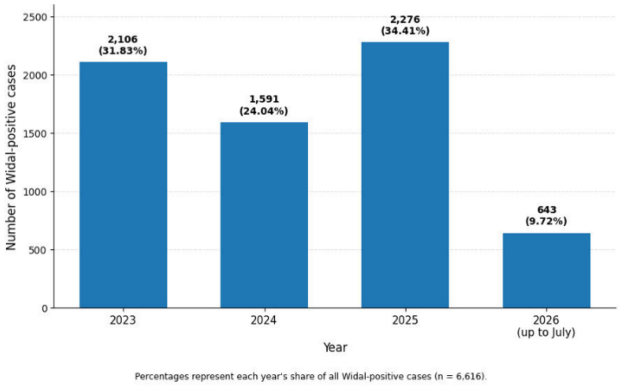


Figure 6: Year-wise distribution of widal-positive cases.

DISCUSSION

Typhoid fever is a systemic illness with a significant morbidity and mortality in developing countries.[5] Widal test has been used in the diagnosis of enteric fever, since long in India. During the treatment of typhoid fever, emergence of multidrug resistance among type strains is one of the chief causes of complications faced.[6] Delay in the diagnosis and beginning of effective treatment is associated with increased risk of complications during the course of the disease.[7]

A fourfold rise of antibody in paired sera is considered diagnostic of typhoid fever.[8] However, obtaining paired sera can be challenging, and specific chemotherapy often has to be initiated based on the results of a single widal test.[9,10] a single widal test, in association with relevant clinical findings, can still be used as a useful diagnostic tool for typhoid fever.[11,12]

Seropositivity of widal test

In the present study the prevalence of seropositivity was 21.73% which correlates with studies such as Bharadwaj et al reported 27.3 % and Isa et al reported 20.6% as the seroprevalence.[13,14]

In our study, percentage positivity of TO and TH antibody was 63.72% and 24.04% respectively. This correlates with Bijapur and Saha the present study demonstrated a higher prevalence of antibodies against the somatic O antigen (TO) of *Salmonella* Typhi compared with antibodies against the flagellar H antigen (TH).[15,16] This observation may be attributed to the relatively early appearance of anti-O antibodies during the course of acute enteric fever, whereas anti-H antibodies generally develop later. Furthermore, the somatic O antigen shares common antigenic determinants with other *Salmonella* species and certain members of the *Enterobacteriaceae* family, which may contribute to cross-reactivity and increased TO seropositivity. The endemic nature of enteric fever in India, along with geographical variations in background antibody titers, may also influence the observed pattern.

However, the higher frequency of TO positivity should not be considered definitive evidence of a higher incidence of acute typhoid fever, as widal test results are influenced by several immunological, epidemiological and technical factors." the positivity of AH and BH antibody was found to be 8.29% and 2.75% respectively. Similar findings were noted in two studies, namely Sharma et al and Lall et al.[17,18] In present studies, 1:80 or above is taken as diagnostic criteria for positive Widal test. Gunjal SP et al, Khorgade et al, Shyamala et al, Lall et al and Shende et al these studies also taken 1:80 and above as diagnostic criteria for positive widal test.[19,20,11,18,21]

Gender wise distribution

In our study maximum positive cases were from females (64.55%) followed by males (35.45%) which correlates with Bharadwaj et al, Kiran et al and Lall et al.[13,22,18] In the present study, a higher proportion of widal-positive cases were observed among females compared to males. Differences in healthcare-seeking behavior, socioeconomic conditions, environmental exposures and access to healthcare facilities may also influence the observed gender distribution. Study conducted by Sharma et al showed male predominance over female in seropositivity in Widal test.[17]

Age wise distribution

In present study, out of the 6616 positive samples, 4934 (74.58%) were adult cases and 1682 (25.42%) were pediatric cases of widal positive test. It might be due to the adult population has more and easy access to the healthcare facilities.

Month wise distribution

In our study maximum number of cases for widal seropositivity are observed in July (27%), August (13%), June (11%) and September (9.4%). Maximum widal seropositivity is seen during the rainy season. Similar findings were observed in Iyer et al, Abraham et al, Saad et al, Nilekar et al and Mangal et al.[23-27] This seasonal predominance may be attributed to increased risk of faeco-oral transmission of enteric fever during monsoon months. Heavy rainfall, flooding, overflowing drainage systems and sewage contamination may compromise the quality of drinking-water sources, thereby facilitating the transmission of *Salmonella* Typhi and *Salmonella* Paratyphi through contaminated water and food. Increased environmental humidity and changes in sanitation conditions may further contribute to seasonal variation in enteric fever transmission.

Limitations

The present study has certain limitations; like Widal test was performed using a single serum sample rather than paired serum samples. Comparison couldn't be done with blood culture and clot culture. The Widal-positive cases could not be microbiologically confirmed as active *Salmonella* Typhi or *Salmonella* Paratyphi infections. Furthermore, these are limitations related to background antibody titers in the healthy population, previous exposure to *Salmonella* organisms, vaccination, cross-reactivity and persistence of antibodies following previous infection. Hence, false-positive results cannot be excluded. The diagnostic cutoff of ≥1:80 adopted in this study may not have equal diagnostic significance for all four antigens and may vary according to geographical location and background antibody prevalence.

CONCLUSION

Widal test is a simple, inexpensive, and relatively non-invasive diagnostic tool, particularly useful in resource-limited settings where blood culture facilities are unavailable. Knowledge of local seropositivity rates helps clinicians interpret results alongside clinical findings and other investigations, while providing valuable epidemiological information for public health planning.

Early diagnosis and timely treatment of enteric fever are essential to prevent serious complications, thereby reducing morbidity and mortality. Despite its limitations, the widal test may serve as a supportive tool in the diagnosis of suspected enteric fever. Timely identification and appropriate management of suspected enteric fever are essential, as delays in treatment increase the risk of complications. Therefore, the widal test may play a supportive role in the early diagnostic evaluation of enteric fever and may indirectly help reduce morbidity and mortality when interpreted alongside clinical findings and other tests.

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