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A Retrospective Audit of Widal Testing For Enteric Fever in the City Of Ahmedabad

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ABSTRACT

Introduction: Widal test has been used extensively for the sero-diagnosis of Enteric fever in India, however, its accuracy and reliability are debatable. We studied widal testing and widal positivity rates in the entire city of Ahmedabad for the diagnosis of Enteric Fever. **Methods** We screened all 1700 possible diagnostic laboratory facilities, in Ahmedabad, in the public and private sector. We performed telephonic surveys for the initial filtering of facilities that could be conducting widal testing. It was followed by physical visits to probable facilities to confirm testing methods and preservation of reports of widal testing. We followed a systematic process for screening and selection of 23 laboratories, which conducted widal tests and had reliable data. While 14 laboratories refused to share data, data provided by three of them were inappropriate and couldn't be used. We finally analyzed data from four large public hospitals, one private trust hospital and one corporate laboratory for variable periods in a span of 15 years (2000 – 2015). **Result:** The Widal testing rate was found to be 8.7% and widal positivity as 12.5% in a sample of 1.2 million clinically suspected in-patients. In 15 years, the private hospital had admitted 1/10th as many cases as all the public hospitals together. However, the widal testing and positivity rates were similar in both. We observed a lower proportion of widal positivity among children below 12 years and a disproportionate, but insignificant, gender distribution of widal positivity. **Conclusion:** This study indicates that the widal test, which is meant to be an initial screening test, is widely used in the city. We propose linkage of testing and reporting of widal with other more reliable and accurate tests such as Typhidot and blood culture in order to strengthen our knowledge of enteric fever epidemiology in India.

Key words: *Enteric Fever, Widal test, Salmonella Typhi, Typhoid, Ahmedabad, Typhidot*

Enteric fever is a highly endemic disease and a public health priority in India and many south Asian countries. Enteric fever is a systemic infection caused by human adapted pathogens Salmonella enterica serotype Typhi (S. Typhi) and S. Paratyphi A, B, and C [1]. Early and accurate diagnosis of enteric fever is essential for the treatment of cases as well as to prevent potential carriers [2]. However, the early diagnosis of a patient with enteric fever is often not feasible clinically as symptoms vary in patients and mimic those of other febrile illnesses, particularly in endemic regions [3]. An accurate diagnosis is an additional challenge in such countries [4]. A true positive diagnosis of enteric fever necessitates isolation of the causative organism (Salmonella typhi or paratyphi) from the patient, but lack of adequate facilities for microbiological culture, its cost, and waiting times for blood culture results, have resulted in the quicker widal test gaining wide popularity. Patients with positive widal

reports are treated as confirmed cases of enteric fever and are given antibiotics [3,5].

Studies have noted that the sensitivity and specificity of widal testing are of moderate to low reliability. The result of such a single test has no diagnostic significance in an endemic region due to difficulty in establishing a steady-state or baseline titer of widal agglutination test, as repeated exposures to Salmonella typhi in endemic regions is a common occurrence [6,7]. In India, the widal test has been used extensively for the serodiagnosis of enteric fever and it remains the only practical test available. Published literature from India is ambivalent regarding the accuracy of the widal test; while some authors recommend widal as a suitable test for our settings, others, with similar sensitivity and specificity values find it unreliable [8–10]. Although a new rapid test with better sensitivity and specificity, Typhidot, is now available in the market, it is not much in use in Ahmedabad due to its higher cost and inconsistent availability [4,11,12].

Furthermore, due to the costs and the lack of adequate laboratories for culturing Salmonella, the actual practice in the country is to diagnose enteric fever by just a single semi-quantitative slide widal test. A titre higher than a cut-off mark of 1:80 for both 'O' and 'H' agglutinins, is commonly considered as diagnostic for enteric fever. Establishing rising titres in order to diagnose enteric fever is extremely rare, even in public facilities [2,3,11,13]. The fact that slide widal is employed in very large scale for the diagnosis (rather than screening), makes such a large collection of widal reports valuable. A collection of a large number of widal test reports over a long period from a given area may be treated as indicative of the trend of clinical suspicion of enteric fever and of the institution of antibiotics for enteric fever in the area.

MATERIALS & METHODS

We conducted a retrospective analysis of Widal testing data from Ahmedabad city in Gujarat. Diagnostic laboratory services in the city are provided by public and private sectors. There are five large public hospitals with bed sizes ranging from 500 to 2000 beds. Four shared data with us (PH1 to 4). Small public sector clinics do not conduct widal tests. Ethical approval for this study was obtained from the Institutional Ethics Committee of Indian Institute of Public Health, Gandhinagar (TRC-IEC No: 5/2015).

Private diagnostic services in Indian cities of three types -- healthcare institution attached (HAL), standalone corporate and standalone non-corporate (NC) laboratories [15]. We obtained a record of 1437 private hospitals with their telephone numbers registered by the AMC in 2015-16. We identified five standalone corporate laboratories in the city based on our personal knowledge and through internet searches. The AMC provided us a list of 254 standalone non-corporate laboratories, which had been prepared in 2013. We found that all corporate laboratories were NABL certified, but only four of the non-corporate labs were certified [16].

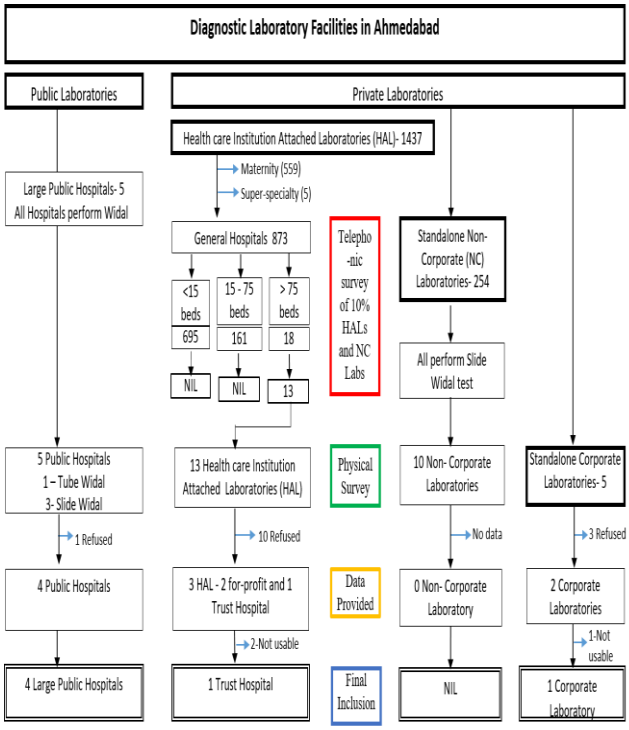

Figure 1- Identification of laboratories equipped with Widal testing in Ahmedabad city

We sorted the list of 1437 private facilities by specialties and range of bed sizes. One-third (559) were purely maternity, and five were purely super-specialty hospitals which did not carry out investigations for *S. typhi*. The remaining 879 facilities had mean and median bed sizes of 13.3 and 10 (Range: 1 to 310 beds; Interquartile range: 5 to 15 beds). We used an arbitrary cut-off bed-size of 15 and 75 beds to create three groups of private general facilities. We telephonically surveyed 10% of randomly sampled facilities from the first 2 groups and all the 18 facilities with more than 75 beds in the third group. All sampled facilities from the first two groups

reported that they did not possess any laboratory facilities. Of 18 facilities in the third group, 13 were conducting widal tests.

Similarly, we randomly sampled 10% (25 laboratories) of the 254 standalone non-corporate (NC) laboratories for a telephonic survey regarding enteric fever testing offered by them. All of them reported that they performed slide widal tests. We visited 10 of these laboratories to request for the data. Data was mostly not maintained and wherever it was maintained, it was only for the amount charged from patients for tests (not the results of tests) and was definitely discarded every year. Therefore, this data, although large, was not available for our analysis. In spite of numerous follow-ups visits, only one trust and two for-profit hospitals shared data with us, out of which the data provided by the trust hospital was usable Also, two standalone corporate laboratories shared data with us, of which one was usable. Data from one for-profit hospital was for less than one year and from the other was not usable.

In the final tally, we were able to include data from four large public hospitals, one private trust hospital and one corporate laboratory for our analysis. All four public hospitals and the standalone corporate laboratory were accredited by NABL, while the trust hospital included in our analysis was not NABL accredited [16].

We received data for variable periods; two public hospitals shared continuous data of five years (PH1 and PH2) and another discontinuous data of six years (PH4). One public hospital provided us data of eight years (PH3), and a private trust hospital provided us with continuous data for 15 years. The data was collected in the form of pictures of registers, from two of the public hospitals, and were manually transferred into excel sheets; whereas, others shared excel sheets of their records. Only two public hospitals provided data separately for out-patients (OP) and in-patients (IP) by gender and age groups for the period of 2011- 2015. However, there was a lot of missing data. Both hospitals provided gender and age data only for 65% of the widal positives and only one of them provided gender distribution of the denominator IP numbers. One public hospital and the corporate laboratory conducted widal tube test. All others conducted only slide agglutination test. However, reports from all of them uniformly reported only positive and negative widal results; none of them mentioned agglutinin titer.

RESULTS

We obtained widal test reports for approximately 1.2 million OP and IP samples over variable numbers of facility-months, spanning over 14 years, from 2001 until 2015. These yielded approximately 15000 positive tests (12.5%). More than 1 million patients had been admitted to the five of the studied facilities during 467 facility-months. Widal testing had been advised to 7.9% of these in-patients, and 13.2% of those tested were reported widal positive. Widal testing and positivity rates in the indoor patients of the private hospital did not vary from that in public hospitals (Table 1).

Although, the private hospital report was for a continuous span of 15 years, it reported 1/10th as many IP as the public hospitals. The private hospital had conducted approximately 40 widal tests per month, while the public hospitals had collectively conducted 286 widal tests per month. Interestingly, widal testing rate and positivity rate was very similar in both settings (Fig. 2).

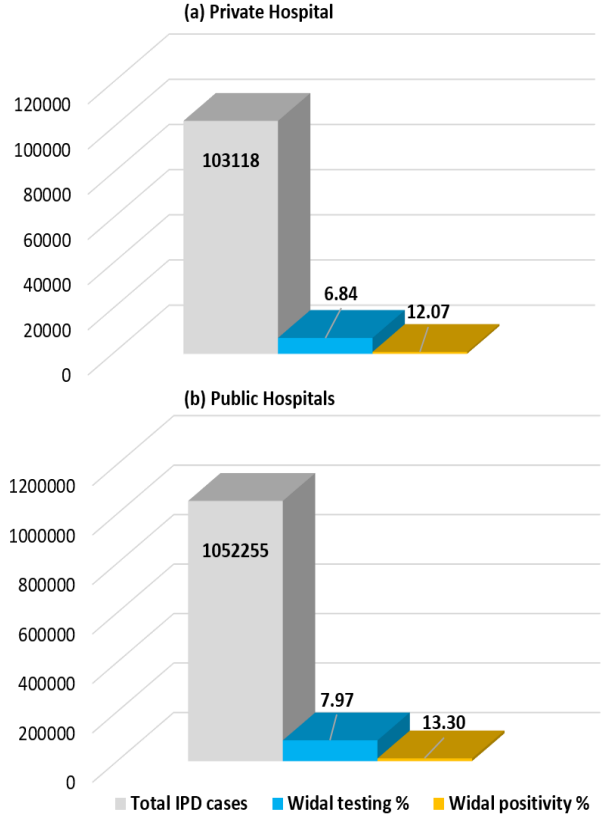

Figure 2: Total in-patients, Widal testing rates, and Widal positivity rates in (a) Private hospital and (b) Public hospitals of Ahmedabad city.

Table 1- Distribution of widal testing and positivity rate among in- patients and out- patients in Ahmedabad

	Health care Institution Attached (HAL) Laboratories						Standalone Corporate Laboratories
Hospitals	PH1	PH 2	PH 3	PH 4	Private	Total	
Periods of data	57	56	108	72	174	467	24
Facility-Months	Apr 2011 - Dec 2015	May 2011 - Oct 2015	Jan 2002 -Dec 2010	Apr2009-Mar 2010, Jan2011-Dec2015	July 2001-Dec 2015		
TOTAL IN-PATIENTS	216863	446758	226383	162251	103118	1155373	NA
Number of Widal tests done	17622	25818	12417	28051	7058	90966	NA
Totals Widals positive	2384	5385	1698	1692	852	12011	NA
Widal testing rate	8.1	5.7	5.4	2.5	6.8	7.9	NA
Widals positivity rate	13.5	20.9	13.7	6.0	12.1	13.2	NA
TOTAL OUT-PATIENTS	845997	4240326	3755913	924795	390342	-	NP
Number of Widal tests done	6983	11671	7569	NP	NP	26223	2945
Total Widals positive	951	1968	NP	NP	NP	2919	80
Widal testing rate	0.8	0.3	0.2	NP	NP	-	NP
Widals positivity rate	13.6	16.9	NP	NP	NP	-	2.7
NP – Not Provided; NA- Not Available							

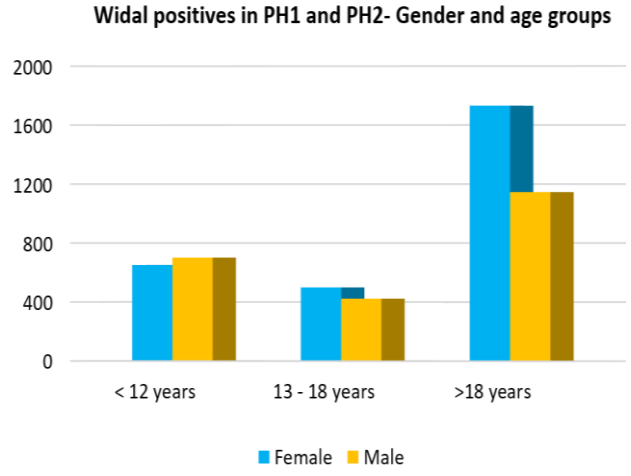

Figure 3: Distribution of Widal positives among IPD patients of PH1 and PH2 by gender and age groups

One quarter of all widal positives among IP were children below 12 years of age. (Fig. 3) A larger proportion of widal positives were females (56%) in the latter two age groups; this may be due to a higher number of female patients reporting to these facilities. We were unable to test these age and gender proportions for significance due to lack of denominator data. However, PH1 had provided gender distribution of 17622 IP and based on this we found that 8.9% of females and 8.4% males had been reported widal positive. This difference in the gender distribution of widal positives in PH1 was not significant ($p > 0.05$).

DISCUSSION

Enteric fever is a life threatening systemic infection occurring in developing countries and continues to be a major public health concern. Widal test has been used in the diagnosis of enteric fever, since long in India, but it has low sensitivity and specificity [12]. Our study showed 12.5% widal positivity in a sample of 1.2 million suspected patients of enteric fever during 467 facility-months from 2001 to 2015 in the city of Ahmedabad. However, due to the refusal to share data by a few large laboratories and poor quality and maintenance of data in

all small laboratories in the city, our report is lacking in completeness.

In this study, widal positivity was in concordance with a study from a rural hospital in Maharashtra with a small sample size of 83 cases, which had also shown a positivity of 14.4% [17]. Other studies from India have shown much higher widal positivity rates with 79.6%, 45.7%, and 57.8% in Delhi, Mysore, and Varanasi hospitals, respectively, among clinically suspected enteric fever cases [3,11,18]. This may be because the criteria for clinical suspicion in a prospective research setting would usually be more stringent than in routine hospital practice, which was the setting for our retrospective prevalence study from secondary hospital data. In a global context too, only one case-control study from Pakistan showed comparable widal positivity of 11.45% with a sample size of 733 clinically suspected patients [19]. Research studies from Vietnam and Nigeria reported a higher widal positivity of 30.8% and 24.5% of clinically suspected patients [6,20].

Public hospitals, in our study, reported a much higher volume of patients than the private trust hospital. However, widal testing rate was almost similar in both, which may indicate a similarity in the clinical practice of recognizing a probable case of enteric fever to be referred for widal testing in both settings. Interestingly, widal positivity also was very similar in both settings.

We found that widal positivity was lower among younger age-groups, which is contradictory to other studies [21,22].We also found higher, though insignificant, widal positivity among females than males. A similar finding was reported by a cross-sectional study in Northwest Ethiopia where, typhoid fever was higher though insignificant in females (22.5%) than males (16.7%) [23]. Conversely, a study in the highly endemic region of Dhaka suggested that enteric fever is more prevalent in males than females [22]. However, the age and gender distribution of the disease in the city, as reported in this study, is not reliable since the data lacks completeness.

The major limitation of our study is the splintered and incomplete nature of our data and the lack of corroboration with culture for Salmonella. However, since our study has reported on 1.2 million widal tests, after a systematic survey of numerous facilities in the city, our observation that widal testing records are very poorly maintained across all types of hospitals and laboratories is our most significant finding. This, despite the fact that widal testing is a very popular test at all levels of health facilities. We found that even microbiologists from large public hospitals and standalone corporate laboratories do not maintain records linking widal testing with culture for Salmonella. This probably indicates a level of tiredness with the ubiquitous nature of Salmonella organism and despair for the lack of an overall strategy to put an end to enteric fever in our settings.

Developing countries like ours rely heavily on widal testing for the diagnosis of Enteric Fever.. Furthermore, it has been established that blood culture is unable to consistently capture Salmonella typhi in all cases [4,24]. Our study is further proof that in spite of its low diagnostic value, single slide widal tests are being conducted in large volumes in the country. In fact, we can safely assume that due to lack of facilities and lengthier turnaround times for the more accurate blood culture, we will continue to use slide widal test as the single diagnostic test for Enteric Fever in the near future [11,13]. We are desperately in need of a good point-of-care test for Enteric fever [25].

An audit of 100 consecutive pediatric subjects, who were advised widal test in a tertiary hospital in Delhi, showed that only around half of them were widal positive and only seven were culture positive. However, all were administered antibiotics prior to test results. Since salmonellae are obligate human parasites, antibiotic consumption is usually the pathway through which these organisms are exposed to and develop antibiotic resistance [3]. This routine practice of early initiation of antibiotics in India, given the high endemicity of Salmonella, increases the probability of creating antibiotic resistant Salmonella hotspots.

The latest National Health Profile report of the Central Bureau of Health Intelligence (CBHI) for the two-year period from 2016-17 shows that the state of Gujarat reported approximately 100,000 cases of enteric fever, and India reported approximately 4.4 million cases [26]. Assuming that these cases were diagnosed through a slide widal test, at a minimal cost of Rs 200 (3 USD) per test, Gujarat spent INR 20 million (290,000 USD) and India spent INR 880 million (12.75 million USD) on widal tests in the past two years [27,28]. This is a highly conservative estimate of the number of tests conducted in the country

since it includes only the positive cases. The actual numbers may be hundreds of times more than this, as has been noted for Dengue testing [29]. Despite the huge expenditure on Widal tests, we lack information on the true burden of enteric fever in the country.

CONCLUSION

Enteric fever is a major public health concern in our country. Even today, a large number of cases are confirmed through a single semi-quantitative Slide Widal test, inspite of its known low diagnostic value. This is leading to false diagnoses and initiation of inappropriate antibiotic treatment, which may cause increased antibiotic resistance in Salmonella. It is impossible to change this firmly entrenched, century-old clinical practice in the near future. Instead, we could extract more valuable information from this ongoing practice by improving our existing systems of testing and record maintenance in small and large diagnostic laboratories.

It is essential that we understand the epidemiology of enteric fever in the country before we can set about reducing its burden. For this, a first step is to improve the diagnostic accuracy of commonly used tests in our country. A uniform system for maintaining and reporting Widal test reports by large and small laboratories, and linking these reports with better quality kit-based tests and blood cultures needs to be established. We could achieve this by involving both manufacturers of screening tests, and small and large private laboratories in a systematic manner in the local and regional surveillance systems.

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