Comparative Study on the Use of Widal Test and Stool Culture in the Laboratory Diagnosis of Salmonella Infection in Adult and Children in Jos Metropolis, Plateau State, Nigeria

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Abstract: Background-Salmonella infection is a common bacteria disease that causes enteric fever in humans which are shed in faeces. Presently, Widal test, Stool culture, blood culture and urine culture are the most diagnostic means of confirming salmonella infection in humans since they are based on the isolation, identification, detection and demonstration of the presence of antibodies in the serum. However, the stool and serum sample of an infected patient against the O (somatic) and H (flagella) antigents of the bacteria requires thorough laboratory analysis. This study aimed to compare the diagnostic performance of Widal test and Stool culture in the laboratory diagnosis of salmonella infection in children (0-14 yrs) and Adult (18 yrs and above). Methods: A random selection of Adult and Children with symptoms clinically suspected to be typhoid visiting the Hospital (Bingham University Teaching Hospital) at the period of the study were recruited. Informed consent of volunteers and guardians were obtained and in addition confidentiality of the result was ensured. A total of 91 patients samples were tested and cultured including Adults and Children. The bacteria isolate d were identified by gram’s reaction and biochemical characteristics. Evaluation of sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of both adult and children were calculated. Results: Of the 91 patients among whom were 48 adults and 43 children. The total number of adults positive for stool culture was 12 (25%) among which were 10 (31.2%) males, 2 (12.5%) females, while the total number of children positive to culture were 9 (20.9%) among which were 7 (26.9%) males and 2 (11.7%) females respectively. The Widal test shows that 30 (62.5%) were positive among which were 17 (53.1%) males and 13 (81.2%) females, while for children, out of 13 (30.2%) who were positive, 8 (30.7%) were males and 5 (29.4%) were females respectively. Adults stool culture were found to be sensitive to Offloxacin, Ciprofloxacin, Ceftaxole and Cefuroxime while children stool culture were also found to be sensitive to Ciprofloxacin, Amoxicillin/Clavulinate, Ampicillin, Cefazidimine and Cloxacillin making them the drugs of choice. Conclusions: From the results obtained, adults were more affected than children; and the males in general were affected more than the females. The Widal test therefore was found to be more sensitive while the stool culture more specific in adults and children, but salmonella infection could still occur irrespective of age or gender. Hence, Health care personnel should not totally depend on the widal test alone for the diagnosis of enteric fever, but should use other diagnostic methods to differentiate salmonella infection from other infections.

Keywords: Salmonella infection, Widal test, Stool culture and Laboratory diagnosis of enteric fever

1. Introduction

Typhoid fever is a systemic disease caused by salmonella typhi and is the major cause of morbidity and mortality worldwide [1]. It emerges as an important infectious disease in the early 19th century. The illness begins with mounting fever, headache, vague, abdominal pain and constipation which may be followed by appearance of rashes during the third week, the patient reaches a state of prolonged apathy, toxemia, delirium, disorientation or coma, followed by diarrhoea which if left untreated can lead to complications affecting various organ of the body [2]. Infection occurs in all age group with higher incidence and more variable clinical presentation in children [3]. Human beings are the only reservoir and host for typhoid fever, the disease is transmitted by feacally contaminated water and food in endemic areas, especially by carriers handling food [2],[3]. WHO estimate for annual global incidence of salmonella infection are about 20 million cases with greater than six hundred thousand (> 600, 000) deaths. It is encountered in tropical countries including India, South and Central America and Africa, where they constitute serious source of morbidity and mortality with rapid population growth, increased urbanization, limited safe water, and infrastructure and health problems [4], [6] - [7]. Population based studies indicated that typhoid fever is about 10 – 100/100,000 per year with annual peak in August. However, the definitive diagnosis of typhoid fever depends on the isolation of salmonella typhi from blood, stool, urine and other body fluids [1]. Typhoid fever has an important socio-economic impact, so accurate diagnosis of the disease at an early stage is important not for etiological diagnosis but also for identifying individuals that may serve as potential carrier who may be responsible for acute typhoid fever outbreak [5], [6].
The Widal test, stool culture, blood culture and urine culture are the most reliable diagnostic means of confirming salmonella infection. However, it requires laboratory equipment and technical training. Scientists now believe that the isolation of the bacteria from clinical and laboratory samples can play a major role in the diagnosis of the infection and can also increase the sensitivity of confirming typhoid fever [1], [6]. Theoretically, it is possible to eliminate the salmonella causing enteric fever since they can only survive in human host and are spread by contaminated food and water. However, given the high prevalence of the disease in developing countries that lack adequate sewage disposal and water treatments, elimination is totally unrealistic [3] – [7].

Since the late 1940s, typhoid fever was successfully treated with one of the several antibiotics (i.e. Chloramphenicol, Ampicillin and Trimethoprim-sulphamethazine). However, from 1990, multidrugs resistant strain (MRD) to previously useful antibiotics have emerged and the treatment for such strain requires those of more expensive Quinolone antibiotics such as oral Ciprofloxacin of third generation Cephalosprin such as Ceftriaxone [7] – [9]. Hence, the specific purpose of this study is to find some clue on some of the debates concerning Widal test and stool culture in the diagnosis of salmonella infection in both adults and children.

1.1 Statement of Problems

Laboratory diagnosis of salmonella infection requires the isolation and identification of salmonella enterica in many areas where the disease is endemic, laboratory capacity is limited [1], [8]. The role of Widal test has been to increase the index of suspicions for the presence of typhoid fever by demonstrating a positive agglutination; unfortunately, in most developing countries including Nigeria the situation is quite different as the Widal test appears to be the only laboratory means employed in the diagnosis of typhoid fever among suspected patients and the test continues to suffer from serious cross-reactivity with other infectious agents that produces false-positive results. Clinically, it is obvious that a single Widal test has no diagnostic relevance whatsoever the results of such has no diagnostic sign [8]. However, in endemic regions, due to difficulty in establishing a steady state or baseline titre of Widal agglutination, that limits the usefulness of the test (6); the signs and symptoms of uncomplicated typhoid fever is non-specific [9], [10]. Therefore, diagnosis on clinical ground alone is difficult as many other infections have the same clinical presentations [8], [11].

1.2 Aim and Objective

1.2.1 Overall Goal

The main objective of this study is to compare the diagnostic performance of Widal test and stool culture in the laboratory diagnosis of Salmonella infection in children (0-14yrs) and adults (18yrs and above); to isolate, identify and determine the presence of salmonella specie from stool and serum of adults and children; and to determine the sensitivity, specificity, positive predictive value and negative predictive value of adults and children.

2. Signs and Symptoms of Salmonella Infection

Classically, the cause of untreated typhoid fever is divided into four individual stages each lasting approximately one week. In the first week, the temperature rises slowly and fever fluctuations are seen with malaise, headache, cough, bloody nose (seen sometimes in a quarter of cases) and abdominal pain, normally blood culture in this week is positive with a positive reaction for salmonella typhi. The classic Widal test is negative in the first week. In the second week of the infection, the patient is stroked down with high fever (e.g. in Plateau around 40°C) and Bradycardia, delirium gives typhoid the nickname “Nervous Fever”. The abdomen becomes distended and painful, diarrhoea can occur in this stage with 6 – 8 times greenish stool in a day with a characteristic smell. However, constipation is frequent. The spleen and liver are enlarged (Hepatosplenomegaly), tender and elevation of liver Transaminases. At this stage, the Widal test is strongly positive with anti – D and nti – 4 antibodies, the major symptoms of this fever is that it usually ruses in the afternoon up to the last second week. In the third week, a number of complications can occur. This includes; intestinal haemorrhage due to bleeding in congested Payers’ patches which can be serious but not fatal, intestinal perforation can be serious and fatal, Metallic abscesses and endocarditis. The fever is still very high and oscillates very little over 24 hours. Dehydration ensures and the patient becomes delirious (typhoid stage). By the end of the third week, the fever starts subsiding; this carries on into the fourth week and final week [11] – [13]. Factors that may increase risk of salmonella infection includes activities that brings one into closer contact with salmonella bacteria by eating raw or undercooked meat, poultry or egg product and contaminated fruits and vegetables [6], [9], [10], [15].

2.1 Significance of this Study

Typhoid fever remains a major public health problem in the developing world with very poor estimates of the number of cases of deaths annually, information across sub-Saharan Africa is very scarce and the issue clearly require urgent and rapid action particularly Africa including Nigeria which seems to have a high burden of typhoid fever [16]. Many slides of salmonellosis conducted by different investigators in Ethiopia have shown the wide spread of salmonella isolation in the community [17]. Lack of research capacity, funding, support and institutional infrastructure are problems to promoting and strengthening typhoid fever and other infectious disease research in these regions, so true progress in controlling the diseases have not yet occurred [16].

2.2 Test and Diagnosis of Salmonella Infection

The basis for diagnosis of salmonella infection (typhoid fever) is a positive result from blood culture, Widal test, stool culture and urine culture. Many developing countries lack adequate infrastructure to perform cultures and many depends on cheaper serological test results [18]. The interpretation of the Widal test remains a challenge till today with a great number of articles reporting different cut offs and the test has lost some popularity in recent years as antigenic determinant of typhoid and non-typhoid salmonella organisms are now characterized [8]. In many
The study was conducted to compare the diagnostic performance of Widal test with stool culture in the diagnosis of salmonella infection in patients (Adults and children) at the Bingham University Teaching Hospital Jos, Plateau state. A random selection of adults and children with symptoms clinically suspected to be typhoid visiting the hospital at the period of the study were recruited.

3. Materials and Methods

3.1 Study Design and Study Population

The study was conducted to compare the diagnostic performance of Widal test with stool culture in the diagnosis of salmonella infection in patients (Adults and children) at the Bingham University Teaching Hospital Jos, Plateau state. A random selection of adults and children with symptoms clinically suspected to be typhoid visiting the hospital at the period of the study were recruited.

3.2 Ethical Clearance

Ethical clearance was obtained from the Bingham University Teaching Hospital Management. In the course of this research, patients were lectured on the study process and its importance in designing an intervention strategy against the infection. Informed consent of volunteers and guardians who were obtained, and in addition confidentiality of the result was ensured. A questionnaire designed for patients in which they could be enlightened on the causes of the infection and measures taken to control or avoid it.

3.3 Sample Collection

About 3 – 4mls of blood sample were collected from the 91 patients through venepuncture and immediately dispensed into plain tubes allowed to clot and further centrifuged to make it ready for Widal test. Stool samples on the other hand was collected from the same patient in universal (plastic) disposable bottles with screw cap and preserved in the refrigerator for culture.

3.4 Widal Test (slide agglutination) Method

The swemed diagnostic Widal test kit, produced in Chunchunagatta cross, Yalechenahali, Bangalore India, was used to perform Widal test (slide agglutination) and was carried out in accordance to manufacturer’s instruction. The reagents contained salmonella typhi O and H antigens and salmonella paratyphi A, B, and C antigens. Positive and Negative controls were included and a titre of greater than 1/80 indicates salmonella infection. The reagents and samples were brought to room temperature and the antigens were shaken properly to mix well before dispensing. A drop of patient’s serum to be tested was placed onto each of the required number of circles on the tile; one drop of Widal antigen suspension was added to the reaction circles containing patient’s serum using a capillary pipette. The contents in each circle were mixed using different mixing applicator sticks provided; the tile was rocked back and forth and observed for agglutination macroscopically for one minute.

3.5 Stool Culture

The already prepared media (i.e. Xylose Lysine Deoxycholate agar [XLD] and Deoxycholate citrate agar [DCA]) were incubated at 37°C for 24 hours to ensure sterility for quality control of the media. A piece of stool was quickly collected from the universal bottle using a heat sterilized applicator sticks provided; the tile was rocked back and forth and observed for agglutination macroscopically for one minute. Even when medical attention for salmonella infection is not needed, one still needs to take care not to dehydrate a common concern with diarrhoea and jaundice. Adults should drink water or suck-on-ice chips, children can use oral rehydration tablets or solution [6]. Salmonella infection is contagious; therefore some precautions need to be taken to prevent the spread of the disease [6].

3.6 Gram Staining and Reactions

Gram staining, Biochemical reaction (Indole test, Citrate test, Catalase test, Urase test and Mortility test) were performed to confirm and determine the ability of the organism to split indole from amino acid and tryptophan, to utilise citrate and production of gas bubbles in the presence of salmonella specie.

4. Results

Results for the test were obtained, tabulated, analysed and compared based on gender, the sensitivity, specificity, positive predictive value and negative predictive value of stool culture and Widal test were all tabulated and evaluated. Of the 91 patients among whom were 48 adults and 43 children, the total number of adults positive for stool culture was 12 (25%) among which were 10 (31.2%) males, 2 (12.5%) females; while the total number of children positive to culture were 9 (20.9%) among which were 7 (26.9%) males and 2 (11.7%) females respectively. The Widal test shows that 30 (62.5%) were positive among which were 17 (53.1%) males and 13 (81.2%) females, while for children, out of 13 (30.2%) who were positive, 8 (30.7%) were males.
and 5 (29.4%) were females respectively. Adults stool culture were found to be sensitive to Offloxacin, Ciprofloxacin, Ceftriaxole and Cefuroxime while children stool culture were also found to be sensitive to Ciprofloxacin, Amoxicillin/Clavulinate, Ampicillin, Cefazidimine and Clozacinill making them the drugs of choice.

Table1: Frequency of adults and children affected and those that were not affected.

<table>
<thead>
<tr>
<th>Age</th>
<th>Children</th>
<th>Adults</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N=48</td>
<td>N=48</td>
</tr>
<tr>
<td></td>
<td>AFFECTED</td>
<td>NON-AFFECTED</td>
</tr>
<tr>
<td>0-2</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>3-5</td>
<td>3</td>
<td>11</td>
</tr>
<tr>
<td>6-8</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>9-11</td>
<td>7</td>
<td>17</td>
</tr>
<tr>
<td>12-14</td>
<td>7</td>
<td>13</td>
</tr>
</tbody>
</table>

Table 2: Positive results of Widal test and stool culture in Adults based on gender

<table>
<thead>
<tr>
<th>Adults</th>
<th>Widal Test</th>
<th>stool Test</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>N=48</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>17 (53.1%)</td>
<td>10 (31.2%)</td>
<td>27</td>
</tr>
<tr>
<td>Female</td>
<td>13 (81.2%)</td>
<td>2 (12.5%)</td>
<td>15</td>
</tr>
<tr>
<td>Total</td>
<td>30 (62.5%)</td>
<td>12 (25%)</td>
<td>42 (87.5%)</td>
</tr>
</tbody>
</table>

Table 3: Positive results of Widal test and stool culture in Children based on gender

<table>
<thead>
<tr>
<th>Adults</th>
<th>Widal Test</th>
<th>stool Test</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>N=43</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>8 (30.7%)</td>
<td>7 (26.9%)</td>
<td>15</td>
</tr>
<tr>
<td>Female</td>
<td>5 (29.4%)</td>
<td>2 (11.7%)</td>
<td>7</td>
</tr>
<tr>
<td>Total</td>
<td>13 (59.1%)</td>
<td>9 (40.9%)</td>
<td>22 (100%)</td>
</tr>
</tbody>
</table>

Table 4: Shows Comparison of Widal test and stool culture of Adults

<table>
<thead>
<tr>
<th>Stool Culture</th>
<th>Pos. Widal test</th>
<th>Neg. Widal test</th>
<th>PPV</th>
<th>NPV</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Widal test Pos.</td>
<td>30</td>
<td>6</td>
<td>83.3%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Widal test Neg.</td>
<td>12</td>
<td>12</td>
<td>50.0%</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Sensitivity, Specificity, Positive predictive value and Negative predictive value of adults determined.

Table 5: Shows Comparison of Widal test and stool culture of Children

<table>
<thead>
<tr>
<th>Stool Culture</th>
<th>Pos. Widal test</th>
<th>Neg. Widal test</th>
<th>PPV</th>
<th>NPV</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Widal test Pos.</td>
<td>13</td>
<td>2</td>
<td>86.7%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Widal test Neg.</td>
<td>9</td>
<td>32</td>
<td>78.0%</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Sensitivity, Specificity, Positive predictive value and Negative predictive value of children determined.

5. Discussion

Currently, the laboratory diagnosis of salmonella infection is dependent upon either the isolation of salmonella enterica serotype typhi from stool culture or the detection of raised titre of agglutinating serum antibodies against the lipopolysaccharide (Lps) [O] or flagella antigens of serotype typhi (the Widal test). In this study, a total of 91 patients (Adults within the ages of 18 years and above and Children within the ages of 0-14 years) visiting the Bingham University Teaching Hospital at the period of this study were randomly selected; therefore, the total number of adults were 48 while that of Children were 43.

In table 2, out of 48 adult patients whose blood samples were tested, 32 were males and 16 female. Of the 32 males, 17 (53.1%) responded positive to Widal test, out of the 16 females, 13 (81.2%) also responded to Widal test. The number of males positive to Widal test was more than those of the females. Also, out of the 32 male adults whose stool samples were cultured, 10 (31.2%) showed a positive culture and 2 (12.5%) out of the 16 females showed a positive culture respectively. It was still observed that the number of male adults whose stool were affected were more than the females. In a research conducted by Chalya in 2012, the study representing 8.7% typhoid fever cases, He observed that males were more affected two times than the females [5]; The Food and Agriculture Organization in also stated that several studies indicated that men seem to be more affected by this disease than the females which is in line with the observed finding in our study [23]. WHO also found out that a male–female ratio of 1:1 has been reported on several occasions but the study conducted in Nigeria by Udeze indicates more significant titre in females than in males, however, in others studies, females stool culture
samples showed more positive than males within the ages of 20 and 72 years [23], [24]. But the fact still remain that the disease occurs irrespective of gender [10].

In table 3, out of the 43 Children whose serums were tested, 26 were males and 17 were females. Out of the 26 males, 8 (30.7%) tested positive and out of the 17 females, 5 (29.4%) tested positive. It was observed that males showed more positive results to Widal test than females; out of the 26 male children whose stool samples were cultured, 7 (26.9%) showed positive stool culture reported and 2 (11.7%). Out of 17 females also showed a positive stool culture. It could be as a result of the differences in food handling practice and hygiene mostly in children, together with the quantity of food consumed, in the sense that children (mostly male children) could pick food directly from the floor and it could also be their eating habit wherever they find themselves. These could contribute to the apparent gender differences [10].

In summary of tables 2 and 3, the number of adults whose blood samples tested positive for Widal test was 30 (62.5%), out of a total of 48 adults who were tested; 13 (30.2%) of children also tested positive to Widal test out of a total of 43 children whose blood samples were tested. It was observe that the adults who responded positively to Widal test were more than the children who also responded positively. This result was compared with the research findings of Parry that the numbers of agglutinins were correlated with age, with greater number in children than in adults [25]. The result is consistent with numerous researchers who documented the greatest incidence of salmonella infection among children which differs from the observed results [26] – [30].

Comparing Widal test and stool culture generally, the stool culture remains the Gold standard method for the detection of salmonella infection because of its high level of specificity; however, it could be useful in resource limited areas where laboratory capacity is limited alongside with authentic clinical investigations [7], [31]. From the observed results, the Widal test positive report seems to be more than the positive stool culture. This could be that salmonella possesses peritricou flagella that while in the blood stream, enable it to move and multiply freely as a result of favourable condition thereby circulating its toxins causing salmonella infection; this may not really be so in stool because salmonella might not gain as much easy access in stool as in blood. Salmonella in stool occurs only when one becomes a potential carrier of the infection [7].

Table 4 and 5 shows that 13 children were positive to both Widal test and stool culture, 9 were positive stool culture and negative for Widal test while 2 were found to be positive to Widal test and negative to stool culture. From the table it was observed that the test were more sensitive in adults than in children and more specific in children than in adults. This differs from Parry’s findings that his test was more sensitive in children than in adults and more specific in adults than in children [25], [32]. He concluded that the increased sensitivity in adults could be due to the duration of the illness; this is not so with children as there is a significant relationship between the test results and the history of prior antibiotic. The Widal test though specific, but cannot be reliable; but the stool culture is sensitive and should be the gold standard for the diagnosis of salmonella infection [9], [33]. The sensitivity tells how likely the stool culture will be positive with the Widal test confirms positive with 71.4% sensitivity for adults and 59.1% for children. Also the specificity tells how likely the culture will be negative when the Widal test confirms negative with 66.7% specificity for adults and 94.1% for children. Thus, the sensitivity test for adults confirmed that there are chances that the stool culture will confirm positive when Widal test is positive, while children confirmed equal chances which means that it is likely the stool culture will confirm a positive result or negative result. The positive predictive value of adults and children shows that a positive stool culture could indicate the presence of salmonella infection, while the negative predictive value for children are more than those of adult, but still indicating that a negative stool culture can still indicate the presence or absent of salmonella infection.

In table 6, isolates of salmonella specie from 12 adults stool culture were found to be more sensitive to Ofloxacin, Ciprofloxacin, Ceftriaxole and Cefuroxime making drug of choice for treatment of these adults. However, they were more resistant to Augmentin, Amplicillin, Cefazidime and Cloxicillin. In table 7, isolates of salmonella specie from 9 children were more sensitive to Ciprfloxacin, Augmentin, Ceftriaxole and Ofloxacin making them the drug of choice for these children. However, they were also more resistant to Cefazidime and Cloxicillin. This interrelates with the findings of WHO that the treatment of salmonella infection requires the use of more expensive Quinolones antibiotics such as oral Ciprofloxacin or third generation Cephalosporin’s such as Ceftrixaxole [9], [34].

5.1 Conclusion

From the study, Widal test is more sensitive than stool culture and stool culture more specific than the Widal test. In the results of the diagnosis of salmonella infection, the males (adults and children) were more positive to the Widal test and stool culture than the females, different from previous studies. Hence it could be explained or noted that salmonella infection occurs irrespective of age or gender; one only needs to take appropriate precautions to avoid recurrences of the infection or completely prevent it.

5.2 Recommendation

Laboratory personnel should not totally depend on the Widal test alone for the diagnosis of salmonella infection but should also use other diagnostic method to differentiate salmonella infection from other infections. The screening (slide agglutination test) and the tube agglutination (confirmatory) should be started to reduce the rate of false positive results. Regarding drug resistance, salmonella showed more resistance, some drugs showed resistance to some commonly used drugs. Therefore sensitivity test based on prescription is recommended to prevent continuous drugs resistance development.
Health care personnel poses a challenge to be explored. Hence, the widely depended use of Widal test by salmonella specie from stool and serum of adults and Salmonella infection in children (0-14yrs) and adults (18yrs and above); to identify, determine the presence of salmonella specie from stool and serum of adults and children. Hence, the widely depended use of Widal test by Health care personnel poses a challenge to be explored.

6. Competing Interests
No conflict of interest.

7. The Future Scope of Study

Typhoid fever remains a major public health problem in the developing world with very poor estimates of the number of cases of deaths annually, information across sub-Saharan Africa is very scarce and the issue clearly require urgent and rapid action particularly Africa including Nigeria which seems to have a high burden of typhoid fever. Many slides of salmonellosis conducted by different investigators in Ethiopia have shown the wide spread of salmonna isolation in the community. Lack of research capacity, funding, support and institutional infrastructure are problems to promoting and strengthening typhoid fever and other infectious disease research in these regions, so true progress in controlling the diseases have not yet occurred. However, this study aims to compare the diagnostic performance of Widal test and stool culture in the laboratory diagnosis of Salmonella infection in children (0-14yrs) and adults (18yrs and above); to identify, and determine the presence of salmonella specie from stool and serum of adults and children. Hence, the widely depended use of Widal test by Health care personnel poses a challenge to be explored.

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Author Profile

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Papers presented by Mr. MS Ramyil at international and National conferences: