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Research Article

COMPARISON OF PERFORMANCE OF TWO WIDAL TEST TECHNIQUES WITH STOOL CULTURE IN THE DIAGNOSIS OF TYPHOID FEVER AT THE DSCHANG DISTRICT HOSPITAL, WEST CAMEROON

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ABSTRACT

Objective: This study is aimed to compare the performance of two Widal test techniques with stool culture in the diagnosis of typhoid fever.

Methods: A cross-sectional study was performed at the Dschang District Hospital in patients clinically suspected to have typhoid fever, whose stool was collected for stool culture. Furthermore, venous blood was collected and the serum was tested by both Widal slide agglutination test and Widal tube titration test.

Results: The results showed that out of 750 participants include in the study, 325 (43.33%) were positive for Widal slide agglutination test, 174 (23.20%) for Widal tube titration test, and 159 (21.20%) for stool culture. The sensitivity, specificity, positive predictive value, and negative predictive value of Widal slide agglutination test with respect to stool culture were 97.48%, 71.23%, 47.69%, and 99.22%, respectively, but 100%, 97.46%, 91.37%, and 100% for the Widal tube titration test. With the stool culture, Widal slide agglutination test had a moderate agreement (kappa = 0.47), but Widal tube titration test had an absolute agreement (kappa = 0.94).

Conclusion: Widal tube titration test should be used in place of Widal slide agglutination test in the diagnosis of typhoid fever in the case of limited access to stool culture test.

Keywords: Typhoid fever, Widal slide agglutination test, Widal tube titration test, stool culture.

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INTRODUCTION

Typhoid fever is an acute systemic infection caused by *Salmonella enterica* serotype Typhi. It is a worldwide public health problem and an important cause of morbidity and mortality with approximately 11–20 million people affected and 128,000–161,000 deaths each year [1]. This number is reduced by 1–4% in patients who received appropriate treatment. Laboratory results are very important to confirm the clinical diagnosis of this disease for effective treatment [2-4]. In the case of suspected typhoid fever, effective diagnosis requires the isolation and the identification of *S. enterica* serotype Typhi in many areas where the disease is endemic [5]. However, the situation is different in most developing countries as the Widal test (agglutination test) seems to be the most commonly used laboratory method for diagnosing the disease because it is relatively less expensive [6]. There are two types of agglutination technique, namely, slide agglutination test and the tube titration test. These serological tests are based on the detection of secreted circulating antibodies against *Salmonella typhi* O and H antigens. Notwithstanding the inconsistent results of Widal test in endemic areas, the two agglutination technics are still considered useful for typhoid fever screening [7]. The best diagnosis of typhoid fever is by the isolation of *S. typhi* from blood, bone marrow, or a specific anatomical lesion [7]. Advantageously, the causative agent of the typhoid fever can be cultured from the stool throughout the duration of the disease; although this is not as specific as blood culture. Nevertheless, feces taken from acute patients may be positive when the blood culture is negative. Widal test is simple to perform but has a low sensitivity and specificity that vary from one study to another [8-11]. However, Widal test continues to be accepted as an adjunct to the clinical diagnosis and isolation method. In Cameroon, there is very little information on typhoid fever and comparisons of diagnostics techniques in medical literature. Thus, this study aimed to compare the performance of Widal agglutinations tests commonly used at the Dschang District Hospital with stool culture in typhoid fever diagnosis.

METHODS

Type and study area

This was a comparative cross-sectional study, conducted at the Dschang District Hospital, West Cameroon. A total of 750 participants were included in the study.

Inclusion criteria

Patients of all ages and both sexes whom typhoid fever diagnostic test was prescribed by the doctor following clinical diagnosis and who voluntarily agreed to participate were included in the study.

Exclusion criteria

Patients on antibiotics, patients who received typhoid vaccine, and patients who refused to give their consent were excluded from the study.

Ethics approval and consent to participate

This study was preceded by authorizations obtained from the academic and medical authorities as well as structures responsible for the ethical framework of research. This was a research certificate issued by the Head of Department of Biochemistry of the University of Dschang, an authorization from the Director of Dschang District Hospital, an ethical clearance obtained from the National Committee of Ethical Research in Human Health of Cameroon under reference ethical number 2019/03/07/CE/CNERSH/SP of March 14, 2019. Informed consent was obtained from each participant before sample collection.

Collection of samples

Approximately 20–30 g of stools were collected in wide-mouthed plastic container from each participant included in the study for stool culture. A 8–10 ml of venous blood were collected in the dry tubes from participants and centrifuged at 5000 rpm for 10 min to obtain sera that will be used for the Widal tests.

Diagnosis tests

Widal slide agglutination test

The test was performed according to the manufacturer's instructions (Omega Diagnostic Ltd., 2015). Thus, one drop (50 µl) of each undiluted serum was placed on the circles of slides provided in the kit along with positive control serum followed by the addition of one drop (50 µl) of antigen solutions TO, AO, BO, CO, TH, AH, BH, and CH. The contents were mixed with separate applicator stick, and the slide was rocked gently for 1 min. If no agglutination was observed, the test was considered negative. If agglutination was visible within 1 min, the test was considered as positive.

Widal tube titration test

All the samples were subjected to Widal tube agglutination method to confirm the results of slide agglutination test. Following the manufacturer's instructions (MEDIFF, ONE FOR ALL, France), two groups of four rows containing six tubes in each row were set for each serum sample to be tested. Doubling dilutions of the test serum, i.e., 1:25, 1:50, 1:100, 1:200, 1:400, and 1:800 were prepared in all rows so that each tube contained 0.5 ml of the diluted serum. Then, in each dilution, 0.5 ml of the antigen solutions (TO, AO, BO, and CO in the first group of tubes; TH, AH, BH, and CH in the second group) were subsequently added. Hence, the final serum dilutions obtained for each antigen were 1:50, 1:100, 1:200, 1:400, 1:800, and 1:1600. Appropriate control tubes containing, for one, physiological saline and for the others, each antigen suspension were included to rule out auto agglutination of the reagent. All tubes were homogenized and incubated at 37°C for 24 h. By flicking on the bottom of each tube, the results were read as a granular appearance for the agglutinin O-positive reaction and as a fluffy appearance for the agglutinin H-positive reaction. The titer of the samples tested was for the last dilution showing a still clearly positive result. Titers between 50 and 100 were not taken into account.

Stool culture

Approximately 25 g of stools collected from each participant were diluted in 5 ml of NaCl solution to obtain the inoculum. A portion of each inoculum was used for microscopic examination (gram stain) to evaluate the percentage of Gram-positive and Gram-negative bacteria. Another part was seeded on Salmonella-Shigella (SS) medium and 1 ml was introduced into 9 ml of selenite broth for an enrichment culture. Both preparations were incubated at 37°C for 24 h. After 24 h, the incubated SS medium was observed. In case of the presence of bacterial growth (presence of colonies), subculture of the previously enriched selenite + stool mixture was made on a new SS medium and reincubated at 37°C for 24 h. After observation, colonies suspected to be *Salmonella* (black center colonies) were biochemically characterized using the Api 20E gallery to identify the type of *Salmonella.*

Analysis of collected data

Epi Info version 7.0 was used to analyze data collected. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated to compare Widal test with stool culture. Kappa test was used to assess the agreement between these diagnostic tests. The different calculation formulas were: Sensitivity (Se) = (true positives/true positives + false negatives) × 100, Specificity (SP) = (true negatives/true negatives + false positives) × 100, PPV = (true positives/true positives + false positives) × 100, NPV = (true negatives/true negatives + false negatives) × 100, and Kappa = Pa-po/1-Pa.

RESULTS

Of the 750 participants, 325 (43.33%) were positive and 425 (56.66%) were negative in Widal slide agglutination test while Widal tube titration test yielded positive results in 174 (23.20%) samples and negative results in 576 (76.80%) samples. In stool culture, of the 750 participants, 159 (21.20%) samples tested positive and 591 (78.80%) samples tested negative (Table 1).

Of the 325 positives in the Widal slide agglutination test, only 155 were positive for stool culture. Of the 425 negative results in the Widal slide agglutination test, 4 were positive with stool culture (Table 2).

Compared to the stool culture, sensitivity, specificity, PPV, and NPV of the Widal slide agglutination test were 97.48%, 71.23%, 47.69%, and 99.22%, respectively (Table 3). The agreement between these two tests was analyzed by the kappa test. However, moderate agreement was obtained between the Widal slide agglutination test and the stool culture (kappa = 0.49).

We also compared the Widal tube titration test with the stool culture. Among 174 positive results of the Widal tube titration tests, 159 were positive for the stool culture. All negative results (576) of the Widal tube titration test were also negative with the stool culture (Table 4). The overall sensitivity, specificity, PPV, and NPV of tube titration test were 100%, 97.46%, 91.37%, and 100%, respectively (Table 3). With respect to the concordance test, almost absolute agreement was obtained between the Widal tube titration test and the stool culture (kappa = 0.94).

DISCUSSION

In this study, 43.33%, 23.2%, and 21.2% of cases of typhoid fever were diagnosed by Widal slide agglutination test, Widal tube titration test, and stool culture, respectively. This indicates that there was a large variation among the tests. This very high positive result obtained in the case of Widal slide agglutination test compared to the other test shows the abundance of false positives for this test, which can be attributed to the endemicity of the disease in our area of study and probably the presence of cross-reactivity with antibodies from non-bacterial infections such as dengue fever, malaria, and hepatitis A [12,13]. Four cases out of the 425 negatives of the Widal slide agglutination test were positive with the stool culture. The result of the false-negative Widal slide agglutination test may be due to an early infection of the disease before the production of detectable antibodies or to a typhoid carrier [14].

Sensitivity of the Widal slide agglutination test and Widal tube titration test was 97.48% and 100%, respectively. This result shows the ability of the Widal test to detect truly positive results compared to the stool culture. Similar results were observed in a study conducted in India (91.2%) [15]. However, Widal sensitivity was lower in the study conducted by Olsen *et al.* (64%) [10] and Noorbakhsh *et al.* (2003) (75.86%) [11]. Specificity of the Widal test found in this study was

Table 1: Results of different diagnosis tests used

Diagnosis tests	Positive		Negative	
	n (%)		n (%)	
Widal slide agglutination test	325	43.33	425	56.66
Widal tube titration test	174	23.20	576	76.80
Stool culture	159	21.20	591	78.80

Table 2: Comparison of the Widal slide agglutination test with the stool culture on typhoid fever suspected cases

Widal slide agglutination test	Stool culture		Total
	Positive	Negative	
Positive	155	170	325
Negative	4	421	425
Total	159	591	750

Table 3: Sensitivity, specificity, PPV, NPV, and kappa test of the Widal slide agglutination and the Widal tube titration tests compared to the stool culture

Test methods	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	Kappa test
Widal slide agglutination test	97.48	71.23	47.69	99.22	0.49
Widal tube titration test	100	97.46	91.37	100	0.94

PPV: Positive predictive value, NPV: Negative predictive value

Table 4: Comparison of the Widal tube titration test with the stool culture on typhoid fever suspected cases

Widal tube titration test	Stool culture		Total
	Positive	Negative	
Positive	159	15	174
Negative	0	576	576
Total	159	591	750

71.23% for slide agglutination test and 97.46% for tube titration. These results were similar to the research conducted in Tanzania (98%) [8]. In contrast, other studies conducted in Nigeria on adults [16], Malaysia [9], Ethiopia [17], and Vietnamese hospitals showed that the Widal test has low specificity [10]. This may be due to geographical variations. In fact, the Widal test varies with geographical location, endemicity of typhoid fever, prevalence of non-typhoid *Salmonella* infection, and other infections that cross-react with antigen *Salmonella* [18].

PPV has been shown to be the most important variable in a clinical diagnostic method, especially since it represents the proportion of patients with positive outcomes who are well diagnosed [19]. In this study, the PPV of Widal slide agglutination test was low (47.69%) but very high for Widal tube titration test (91.37%). This is the case of Gaikwad and Rajurkar works [20] and Jahan *et al.* (2016) [15] who also recorded a low PPV for Widal slide agglutination test, 28.91% and 53.8%, respectively. In addition, PNV value of Widal slide agglutination test was 99.22% and 100% for Widal tube titration test. This indicates that negative result in Widal test has a good predictive value for the absence of the disease, but positive result would have a low predictive value for the presence of typhoid fever, especially in the case of Widal slide agglutination test [16]. This clearly proves that although Widal slide agglutination test is very effective in the detection of negative samples, expressed positivity is not always useful in the correct diagnosis of the disease.

Concerning the agreement between Widal slide agglutination test and stool culture or Widal tube titration test and stool culture, there was an absolute agreement (kappa = 0.94) between Widal tube titration test and stool culture, and a moderate agreement (kappa = 0.49) between Widal slide agglutination test and the stool culture. This indicates that the result obtained from the Widal slide agglutination test in the diagnosis of typhoid fever was less reliable as compared to the stool culture. Thus, Widal slide agglutination test cannot be used alone in the diagnosis of typhoid fever because a good number of patients will be falsely diagnosed positive and the doctor will submit them to antimicrobial treatment against typhoid fever without these patients suffering from the disease. This may consequently result in the development of resistance by these bacteria to antibiotics [20].

CONCLUSION

Widal slide agglutination test is the routine test used at the Dschang District Hospital for typhoid fever diagnosis because it is simple, easy to achieve, fast in implementation, and less expensive. In this study, Widal tube titration test has good sensitivity, good specificity, good PPV, and good PNV with stool culture. In contrast, Widal slide agglutination test has low sensitivity and low PPV. Because of the abundance of false positives for this test, it cannot be used alone in the diagnosis of typhoid fever. With stool culture, Widal slide agglutination test has a moderate agreement while Widal tube titration test has absolute agreement. Considering this finding, doctors should not only use Widal slide agglutination test for typhoid fever diagnosis in endemic areas where it is the only diagnostic method. If there is no access for culture, it is better to use Widal tube titration test than Widal slide agglutination test.

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AUTHORS' CONTRIBUTIONS

CKN and BPT designed the study; CKN collected data. CKN and BPT write and approved the final version of the manuscript.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

1. World Health Organization. Typhoid Fever. Geneva: World Health Organization; 2018.
2. Khan S, Miah MR, Haque S, Naheen CR. Comparison of results obtained by widal agglutination test and polymerase chain reaction among clinically suspected typhoid fever cases. Bangladesh J Physiol Pharmacol 2014;30:46-50.
3. Crump JA, Luby SP, Mintz ED. The global burden of typhoid fever. Bull World Health Organ 2004;82:346-53.
4. Ismail A. New advances in the diagnosis of typhoid and detection of typhoid carriers. Malaysian J Med Sci 2000;7:3-8.
5. Wain J, Diep TS, Bay PV, Walsh AL, Vinh H, Duong NM, *et al*. Specimens and culture media for the laboratory diagnosis of typhoid fever. J Infect Dev Ctries 2008;2:469-74.
6. Wijedoru L, Mallett S, Cm P. Rapid diagnostic tests for typhoid and paratyphoid (enteric) fever (review). Cochrane Libr 2017;2017:CD008892.
7. Bhutta ZA. Current concepts in the diagnosis and treatment of typhoid fever. BMJ 2006;333:78-82.
8. Ley B, Mtove G, Thriemer K, Amos B, Seidlein L Von, Hendriksen I, *et al*. Evaluation of the widal tube agglutination test for the diagnosis of typhoid fever among children admitted to a rural hdospital in Tanzania and a comparison with previous studies. BioMed Cent Infect Dis 2010;10:180.
9. Lim P, Tam FC, Cheong Y. One-step 2-minute test to detect typhoid-specific antibodies based on particle separation in tubes one-step 2-minute test to detect typhoid-specific antibodies based on particle separation in tubes. J Clin Microbiol 1998;36:2271-8.
10. Olsen SJ, Pruckler J, Bibb W, Thi N, Thanh M, Trinh TM, *et al*. Evaluation of rapid diagnostic tests for typhoid fever. J Clin Microbiol 2004;42:1885-9.
11. Noorbakhsh S, Rimaz S, Mamishi S. Interpretation of the widal test in infected children. Iran J Public Health 2003;32:35-7.
12. Itah AY, Akpan CJ. Correlation studies on widal agglutination reaction and diagnosis of typhoid fever. Southeast Asian J Trop Med Public Health 2004;35:88-91.
13. Ibadin MO, Ogbimi A. Antityphoid agglutinins in African school aged children with malaria. West Afr J Med 2004;23:276-9.
14. Olopoenia LA, King AL. Widal agglutination test-100 years later : Still plagued by controversy. Postgrad Med J 2000;76:80-4.

15. Jahan N, Khatoon R, Mehrotra S, Kumar S. Comparative evaluation of slide agglutination and widal tube agglutination test in detecting enteric fever among patients attending a tertiary care hospital in North India. Int J Res Med Sci 2016;4:4290-6.
16. Ramyil M, Ihuoma O, Ogundeko TO, Ameh JM, Olorundare F, Adeniyi OG, *et al*. 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. Int J Sci Res 2013;2:435- 41.
17. Ameya G, Atalel E, Kebede B, Yohannes B. Comparative study of widal test against stool culture for typhoid fever suspected cases in Southern Ethiopia. Pathol Lab Med Int 2017;2017:1-7.
18. Tankeshwar A. Widal Test: Principle, Procedure, Results, Interpretation, Significance and Limitation-learn Microbiology; 2015. Available from: https://www.microbeonline.com/widal-test-principle-procedure-results. [Last accessed on 2020 Feb 22].
19. Altman DG, Bland JM. Diagnostic tests 2: Predictive values. Br Med J 1994;309:102.
20. Gaikwad UN, Rajurkar M. Diagnostic efficacy of widal slide agglutination test against widal tube agglutination test in enteric fever. Int J Med Public Health 2014;4:6-9.