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Research Article

Incidence of Typhoid Fever Infection among Febrile Patients attending FUDMA Clinic

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ABSTRACT

Typhoid fever remains a public health concern in Nigeria, with its incidence poising substantial challenges to health care systems. Widal agglutination test is largely the only used diagnostic test method for the laboratory diagnosis of Typhoid infection which has been proven to be neither sensitive nor specific. This paper delves in to the incidence, contributory factors as well as sensitivity and specificity of commonly used diagnostic tests of typhoid fever infection among febrile patients attending FUDMA clinic. A total of 55 Blood and stool samples were analyzed using standard procedures for slide agglutination test (Widal) and stool culture, prior to culturing, the samples were first inoculated in enrichment medium (Selenite-F broth). After 4hours of pre-enrichment, the samples were inoculated in to Salmonella Shigella Agar and Mackonkey agar, while catalase, TSI, citrate utilization test, Indole test and urease tests served to identify the organism biochemically. Socio-demographic data revealed that Out of the 55 Participants, 52.8% were males, 72.7% were students and 80% were singles. Slide agglutination test recorded 90.9% incidence rate, stool culture had an incidence rate of 18.18%. Among the age groups that enrolled in this study, 44.1% incidence rate was recorded among the age Group 20-26 years. Stool culture was to found have performed better diagnostically with a sensitivity of 60% as well as specificity of 40% .Conclusively, A high Incidence of typhoid fever was observed in this study. This Therefore suggests that there is an urgent need for improved personal hygiene, and more accessibility to portable drinking water.

Keywords: Agglutination; Widal; Febrile; SSA; TSI; Diagnostic tools; Typhoid fever; *Salmonella typhi*

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INTRODUCTION

Salmonella typhi infection is tagged as enteric fever, this infection occurs in all communities worldwide, but highly endemic in the developing countries which is mainly transmitted by contaminated food and drinks. (Salama and Said, 2019). In Nigeria, infection with *S.typhi* is mostly affecting children (Opara, 2021). *Salmonella typhi* is transmitted through food or water contaminated with faeces from infected persons, or from chronic asymptomatic carriers who handle food. Humans are the only host for *S*. *typhi* and there are no known environmental reservoirs (Adedokun *et al*., 2020). The clinical manifestations of typhoid fever varies from a mild illness with low-grade fever, malaise, and slight dry cough, diarrhea to a severe clinical Symptoms with abdominal discomfort and multiple complications. (Badri *et al*., 2019)

Prompt diagnosis and identification of *S. typhi* in the laboratory coupled with timely institution of appropriate therapy can play significant roles in decreasing morbidity and mortality rates (Murugalatha, 2018).

Although, the incidence of *S. typhi* infection has fallen in developed countries to a level below 10/100,000 population/year, this is greatly attributed to improved standards of both personal and environment hygiene as well as modernized waste disposal channels. Whereas it is >100/100,000 population/year in developing countries. Nevertheless, Mortality rates due to Enteric Fever and its complications have fallen considerably due to availability of effective antibiotics. It is unfortunate

that antibiotic resistance is now threatening the efficacy of the treatment regimen. (Udayakumar *et al.*, 2017) Furthermore, accurate diagnosis of typhoid fever is problematic LMICs, several diagnostic approaches are commonly used, including microbiologic culturing of blood, and serologic assays such as the Widal or antigen-specific assays. All of these approaches suffer from poor sensitivity and/or poor specificity, especially in endemic areas (Andrews & Ryan, 2015). This paper is aimed at assessing the incidence of typhoid fever infection among febrile patients attending FUDMA clinic, as well as assessing the functionality, sensitivity as well as specificity of the common diagnostic test of typhoid fever. The specific objectives achieved include the determination of incidence of Typhoid fever among the study participants and to assess the sensitivity and specificity of both widal and stool culture tests in Typhoid fever diagnosis.

MATERIALS AND METHOD

Study Area

This study was conducted in FUDMA clinic at Dutsin-Ma. Katsina state is situated in the Northwest region of Nigeria. Participants of this study cut across all age groups and from both gender.

Selection Criteria

Inclusion Criteria include febrile male and female patients attending FUDMA clinic and Exclusion Criteria are Non-febrile Patients attending FUDMA Clinic.

Approval Letter

A copy of an introductory letter duly signed and stamped was obtained from the head of the department and submitted to the Federal University Health Service Director for acknowledgement.

Collection of Samples

Five milliliters of blood was collected from each subject by venipuncture, Samples were collected using sterile disposable syringes and poured into blood bottles containing anticoagulant (EDTA). The samples were then aseptically transported to the Microbiology Laboratory of the Federal University Dutsin Ma, for analysis**.** Universal (plane) bottle was used for collection of stool samples with proper orientation given to the patient. The freshly collected stool samples were immediately transferred to the laboratory for analysis.

Widal Slide Agglutination Test

Widal slide agglutination test was done using Salmonella antigen kits (Biotech widal test kit), the test was done while adhering to the manufacturer's guidelines. This test was used to determine the presence of anti O and H antibodies in patient's sera. The collected blood was centrifuged using a bacteriological laboratory centrifuge at 5000 rpm for 5minutes. Thereafter, a drop of *Salmonella typhi* O and H antigens were added on a drop of serum on the slide card. After rocking the slide back and forth for 1 min, the mixture was observed for macroscopic agglutination. If there was agglutination within 1 min it was reported as reactive, otherwise, non- reactive.

Isolation and Identification of *Salmonella typhi*

Stool samples were enriched with selenite F broth and incubated at 37°c for 4 hours. Subcultures from the selenite F were made onto Salmonella-Shigella Agar (SSA) as well as MacConkey agar, then, incubated at 37°C overnight. Cultures that yielded non-lactose fermenting colonies were gram stained and subjected to biochemical tests for proper identification. The biochemical tests conducted include indole test, triple sugar iron test, and citrate tests.

Gram Staining and Biochemical Identification

Gram's staining

A smear was fixed at the center of a grease-free slide and allowed to dry, then heat fixed. The fixed smear was flooded with crystal violet stain for 60 seconds, then washed off with clean water. The smear was flooded with Lugol's iodine for 30 seconds after which it was washed off with clean water. Acetone was then added and immediately washed off. Neutral red was added for about 1 minute, and then washed off with clean water. The back of the slide was wiped, and then the glass drained and allowed to air dry, after which it was viewed under the oil immersion lenses (Cheesbrough, 2009)

Triple sugar iron test

A Sterile inoculating needle was used to streak the slant as well as stab the butt of TSI agar tube. The agar was incubated at 37^0C for 24hrs. *S*. *typhi* produced red slant and yellow butt with Hydrogen sulphide (H_2S) production which was observed by blackening of the butt of the agar (Cheesbrough, 2009)

Catalase Test: 2ml of hydrogen peroxide solution was poured into a test tube, isolated colonies of the bacteria were immersed into a hydrogen peroxide solution and observed for immediate bubbling ortherwise (Cheesbrough, 2009).

Indole Test: 4ml of tryptophan broth was poured in a sterile test tube and the isolated colonies of the bacteria were inoculated and incubated at 37^0C for 24h. 0.5ml of kovac's reagent was added to the broth culture and the presence or absence of ring was observed. Red ring were considered as (Positive) and Yellow ring (Negative) (Cheesbrough, 2009).

Citrate Utilization Test

A colony was picked from the overnight culture and inoculated into Simon's citrate agar slant which were then incubated at 37°C. A change in colour of the medium from green to blue was considered positive (Cheesbrough, 2009).

Data Analysis

Data were analyzed using Microsoft excel version 2016. Categorical data were analyzed as frequency and percentage. All statistical analyses were performed using excel analysis tool pack using One way Analysis of Variance (ANOVA).

RESULTS

Table 1 above shows the socio-demographic characteristics of the study participants in relation with typhoid fever infection. Key parameters such as gender, occupation and marital status were assessed as possible contributory factors in this research.

Table 2 above gives a brief highlight of the incidence of *Salmonella typhi* infection. The incidence recorded according age brackets and the percentages were as highlighted above. The omission of age group 0-15 was intentional because students mostly form the bulk of the patients attending the university clinic and that age bracket rarely falls within the range of university student's age.

Table 3 above presents the incidence of *Salmonella typhi* using two different diagnostic tools; Widal agglutination and stool culture, the positive stool culture results obtained above could be attributed to the pre inoculation of the stool in an enrichment media before subsequent inoculation unto a selective media.

Table 4 compares the specificity and sensitivity of the two diagnostics parameters (Widal and stool test). Widal as a rapid test was found to bear high sensitivity with poor specificity against the stool cultured.

Table 1: Socio-demographic data of the study participants

Characteristics	Number (n=55)	Frequency (%)
Genders		
Male	29	52.8%
Females	26	47.2%
Occupation		
Student	40	72.7%
Non-student	15	27.27%
Marital status		
Single	44	80%
Married	11	20%

Table 2: Incidence of *S. typhi* according to age group

Age group	No of samples examined	No positive	No negative
15-20	12(21.81%)	5 (13.88%)	7 (36.84%)
20-26	17 (30.90%)	15 (41.66%)	2 (10.5%)
26-30	14 (25.5%)	10 (27.77%)	4 (21.05%)
31-35	7 (12.72%)	4 (11.11%)	3 (15.78%)
35-40	5 (9.09 %)	2 (5.55%)	3 (15.78%)
Total	55 (100%)	36 (65.45%)	19 (34.54%)

Table 3: Incidence of *S. typhi* using Serological and cultural test parameters

	Widal Test		Stool Culture	
	Frequency	**Percentages**	**Frequency**	**Percentage**
Positive	50	90.9%	10	18.18%
Negative	5	9.09%	45	81.81%
Total	55	100%	55	100%

Table 4: Comparison of Sensitivity and specificity of Widal test against Stool Culture

Test parameter	Outcome		Sensitivity	Specificity
	Positive	**Negative**		
Widal agglutination	50 (90.9%)	5 (9.09%)	90.0%	10.0%
Stool culture	10 (18.18%)	45 (81.81%)	60.0%	40.0%

DISCUSSION

The socio-demographic data generated in this study revealed that majority of the respondents were males, accounting for 52.8% while females accounted for 47.2% .This finding is in agreement with a study which found a higher incidence rate in the widal test conducted in male participants than that of females (Mujahid *et al*., 2022). Students who were not married also constituted a greater percentage of the study participants this is due to the fact that FUDMA clinic serves to offer the health needs of the students more than that of the non-students, Adeshina *et al*. (2009) also recorded a similar pattern in their research.

Out of the 55 patients presenting with signs and symptoms of typhoid fever examined in this study. Age group (20-25yrs) recorded the highest number of participants 17 (30.90 %.) This same age bracket yielded the highest percentage of positive result 15 (41.6%) as well. The least age bracket observed in this study was between age (30-40yrs) bearing a percentage no of participants of 5(9.09%) yielding a percentage positive of 2 participants accounting for (5.55%) of the study population. The high number of participants in the age bracket of (20-25yrs) can be attributed to the fact that, students form the bulk number of patients seeking medical care in FUDMA clinic. More so, poor personal/environmental hygiene of some food handlers/eateries as well as water distributors might poise as another predisposing factor in this age bracket because most students patronize eateries or road side snacks for their food. This finding is however found to be in contrast to the findings of Amsalu *et al.*,(2021) that reported consumption of street food bears no significant impact in acquiring typhoid fever infection but a significant association was however reported in Indonesia.

Selenite F media was used to enrich as well as encourage the growth of suspected *Salmonella* typhi in the stool sample. As such about 18.1% of the samples were found to be positive for Salmonella typhi. However, Ohanu *et al.,* (2019) reported that stools samples are considered to be less specific than blood for the isolation of pathogens, while encouraging the adoption of simultaneous blood, urine and stool cultures so as to increase the probability of detection of the pathogen in diagnosis of Typhoid fever.

The sensitivity and specificity values of both widal test and stool culture were also measured and as such, Widal test recorded 90.0% and 10.0% respectively by either H or O antigen. While the sensitivity and specificity value of the stool culture was pegged at 60.0%, 40.0% respectively, This result shows great similarities with the study conducted by Mujahid *et al*, (2022*)* on Comparative Study on the Use of widal test to stool Culture in the laboratory diagnosis of typhoid fever, in which the results demonstrated that, Widal test had a low sensitivity and high specificity of 40.9% and 32.4% respectively. A study conducted in Tanzania also reported the sensitivity of blood/stool culture to range from 40-97% (Mawazo *et al*., 2019). Consistently (Sultana et al., 2016) also emphasized that, cultural isolation of the *Salmonella* typhi remains the most effective diagnostic procedure in suspected typhoid fever infection.

Akinyemi *et al*. (2018) suggested that blood culture–positive typhoidal Salmonella remains the pivotal determinant to estimate true burden of typhoid fever. Unfortunately, only few hospitals, specifically, referral hospitals, perform blood culture for diagnosing typhoid cases in Nigeria.

This therefore clearly indicates that cultural procedure is more reliable as a diagnostic tool than serology. The low sensitivity recorded by widal test might also be due to certain conditions such as non-adherence to storage guidelines of the test reagents, or might be due to the fact that widal test is still read with human-eye visualizing and interpreting, this means that any defect on the sight of the researcher/scientist might lead to a false –positive result or an inflated or deflated titer values.

CONCLUSION

The incidence of typhoid fever was found to be very high among patients attending FUDMA clinic which could be largely attributed to lack of adequate hygiene practices amongst the study subjects, as well as exposure to the risk of infection by infected food-handlers and or contaminated food/water sources because students mostly eat out. More so, Male subjects were found to have been affected more with *S*. *typhi* than female subjects, the age range that recorded the highest infection rate was found to be 20-26 years which forms the bulk of average age of university students. This study also indicated that stool culture positivity among suspected typhoid fever patients is more likely achievable if diagnostic laboratories would adopt the largely abandoned pre-enrichment stage of stool inoculation procedures, because, as such enrichment with Selenite F broth should be mandated as an SOP in all diagnostic laboratories as it has proven to increase more positivity outcomes when culturing stool. This enrichment step that has largely been abandoned by most diagnostic laboratories could be the reason why most stool culture results are reported as no *Salmonella* or *Shigella* Isolated even when the patient harbours a high positive Widal test result.

Conflict of Interest
The authors declare no conflict of interest

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