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A STUDY ON COMPARATIVE EFFECTIVENESS OF WIDAL TEST AND STOOL CULTURE IN DIAGNOSIS OF *SALMONELLA* INFECTION AMONG INHABITANT OF ULI, IHIALA LOCAL GOVERNMENT AREA ANAMBRA STATE, NIGERIA

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ABSTRACT

The study was carried out to know the comparative effectiveness of widal test and stool culture, in diagnosis of salmonella infection among inhabitant of Uli, Ihiala local Government Area. Between August to September 2014.A total of 100 specimens both widal and stool were screened randomly.11% tested positive to widal and stool culture.14% tested positive to widal test and negative to stool culture.49% tested positive to stool culture and negative to widal test.26% tested negative to both widal test and stool culture. In age prevalence, the age bracket 21-25 years (28%) have the highest infection while 31-35 years(5%) have the least infection. Among the sex prevalence, male (16.5%) were more infected than the female(5%) both in widal and stool culture. The result shows that stool culture is the gold standard in diagnosis is of salmonella infection and salmonella occurs irrespective of age or gender. It is recommended that healthcare personnel should not totally depend on widal test alone for diagnosis of enteric fever but should use other diagnostic method. It is also recommended that, Government should mount community campaign scheme, educating people about this infection salmonella.

INTRODUCTION

Typhoid fever is a systemic disease caused by *salmonella typhi* and is the major cause of morbidity and mortality worldwide. (Bulter, 2000).It emerges as an important infectious disease in the 19th century. The illness begins with fever, headache, abdominal pain and constipation. Infection occurs in all age group with higher incidence and more variable clinical presentation in children (Asgbar *et al;* 2008). Human beings are the only reservoir host for typhoid fever, the disease is transmitted by feacally contaminated water and food in endemic areas, especially by carriers handing food (Asghar *et al.,* 2002). Highest incidence usually occurs where water supplies serving large populations are contaminated by feacal matters. In Nigeria, this illness causes thousands of severe cases on a yearly basis (Blasser *et al.*, 2005).

Salmonella infection perforates through the bacterium then alters its structure so as to resist destruction and allow to exist within the macrophage. The organism is then spread through the lymphatic white cells inside the macrophage whereby giving them access to human system and different organs in the body. (Beyene *et al.*, 2008). The widal test, stool culture, blood culture and urine culture are the most reliable diagnostic means of confirming salmonella infections.

Since, late 1940s typhoid fever was successful treated with one of the several antibiotic that is chloramphenicol, Ampicilin and Trimethoptrim-sulphamethazone. However, from 1990 multi drugs resistant strain 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 (Dimitrov *et al.*, 2007).

Typhoid fever is not sex-related but age related, as it affects the young, old and children (Faucis *et al*., 2008). A major outbreak could cause thousands of severe cases which could lead to death. Its incidence is highest in children and young adults (Le Bacq *et al.*, 2001).

MATERIALS AND METHODS

Study Area

This study was carried out from August to September, 2014 in the Uli Community in Ihiala Local Government Area of Anambra State, South Eastern Nigeria. The climate is tropical and the vegetation characteristic is predominantly the rainforest with an average annual rainfall of 1932mm and average daily atmospheric temperature of 27^0C. There are two distinct seasons, the wet and the dry season, the former takes place between April to October, while the later occurs from November to March. The area is transverse by a number of streams which constitute the major source of water supply in the locality. Basic amenities such as good roads, pipe-borne water, proper sewage disposal system are greatly lacking except for some areas of the community occupied by students where they have been improvements in sewage disposal systems as well as borehole water supply. Farming and trading are the major economic activities. Educational status of most of the inhabitants is termed a little below average.

Study Population

The study population composed 100 inhabitants of Uli community. The area of Uli that houses the Anambra State University was taken as the sample area.

Sample Collection

Blood samples were collected from 100 patients. Venous blood collections were used. The samples were labeled with the patient's name, age, and gender. The sample was transported to Dezan Medical Laboratory at Amajeke Owerri, where it was analyzed.

Stool sample on the other hand was collected from the same patient in sterile universal disposal bottles with screw cap. The sample container was also labeled with the patient's name, age and gender, transported to Dezan medical laboratory where it was analyzed too.

Widal Test (Slide Agglutination) Method

This is a serological test carried out to check for the presence of salmonella antibodies in a patient's blood serum. It is also for diagnosing a patient for typhoid and paratyphoid in several cases. In the confirmatory test for typhoid, the serum sample from a patient is tested for O and H antibodies (agglutinations) against the following antigen suspension. *Salmonella typhi* O antigen is red in colour, while *salmonella typhi* H antigen is blue in colour.

Procedure

One drop of antigen solution was collected from a specified bottle, dropped on the tile in the right pattern. The O-antigen solution was dropped firstly on the top portion of the tile while the H-antigen solution on the bottom portion of the tile. A drop of serum sample was dropped against each antigen solution using a sterile dropper, all in the right pattern. The bottom of a clean dropper was used to emulsify each solution which is been done with caution, in the sense that the bottom is cleaned with cotton wool before each use. A rocking action was applied by hand rocking the tile and watched for any visible agglutination.

Significance of the test result

a. Negative results: When there is no visible agglutination.
b. Positive results: A visible agglutination is present significantly in raised H or O agglutination or both. For example, titres greater than 1 in 180 or 1 in 160 or 1 in 360. Shows a positive reaction.

Stool Culture

c. This is mainly used to diagnose *salmonella typh*i and serial infection in patient with severe and unknown illness.

d. The already prepared media (salmonella shigella agar) was incubated at 37^0C for 24 hours to ensure sterility for quality control of the media. A fresh loopful of stool was quickly collected from the universal bottle using a heat fixing wire loop and streaked on SS agar, incubated at 37^0C for 24hours. It was subcultured on another media to obtain a specific isolate. *Salmonella Shigella* agar enhances the growth of salmonella, *Escherichia coli* and *shigella*. Presence of *salmonella* on SS agar media is indicated by black colonies and identified by its cultural shape, colour, texture and ability to ferment lactose.

RESULTS

A total number of 100 patients were screened for salmonella infection among inhabitant of Uli in Ihiala Local Government Area, Anambra State.

In comparison of widal test and stool culture among the inhabitant. 11 patients were positive to both widal test and stool culture, 49 patients were positive to stool culture and negative for widal test while 14 patients were found to be positive to widal test and negative to stool culture, 26 patients were negative to both widal test and stool culture.

From the result below, stool culture is the gold standard in diagnosis of salmonella infection.

Prevalence of salmonella infection by sex was shown on table 2. Out of the 100 respondent sampled both widal and stool 53 were females while 47 were males. In male, 33(16.5%) tested positive to widal test and 14(7.0%) negative to widal while in stool culture 31(15.5%) tested positive and 16(8.0%) tested negative. In females, 30(15.0%) tested positive to widal and 23(11.5%) negative to widal while in stool culture 30(15.0%) tested positive and 23(11.5%) tested negative. From the result it was observed that males positive to both widal and stool culture were more than the females.

Prevalence of salmonella by age was shown on table 3, highest prevalence of the infection was in age range of 21-25(25%) and least infection was in age range of 31-35(5%).

Table 1: Shows the comparison of widal test and stool culture among inhabitant of Uli, in Ihiala L.G.A

	Stool Culture positive %	Stool Culture negative %	Total
Widal test positive	11(11%)	14(14%)	25(25%)
Widal test negative	49(49%)	26(26%)	75(75%)
Total	60%	40%	100%

Table 2: Prevalence of salmonella infection by sex.

$N = n_1 + n_2 = 200$

Sex	Total no. examined (%)	Widal test no. positive (%)	Widal test no. negative (%)	Stool culture no. positive (%)	Stool culture no. negative (%)	Total (%)
Male	47(47%)	33(16.5%)	14(7.0%)	31(15.5%)	16(8.0%)	94(47%)
Female	53(53%)	30(15.0%)	23(11.5%)	30(15.0%)	23(11.5%)	106(53%)
Total	100	63(31.5%)	37(18.5%)	61(30.5%)	39(19.5%)	200(100%)

KEY:

n_1 = Number of widal test (100)

n_2 = Number of stool culture (100)

N = Total number of $n_1 + n_2$

Table 3: prevalence of salmonella infection

Age	Total no. examined	Widal test affected	Widal test non-affected	Stool culture affected	Stool culture non-affected	Total
11-15	22	7(3.5%)	15(7.5%)	9(4.5%)	13(6.5%)	44(22.0%)
16-20	24	12(6.0%)	12(6.0%)	10(5.0%)	14(7.0%)	48(24.0%)
21-25	28	22(11.0%)	6(3.0%)	20(10.0%)	8(4.0%)	56(28.0%)
26-30	21	18(9.0%)	3(1.5%)	18(9.0%)	3(1.5%)	42(21.0%)
31-35	5	4(2.0%)	1(0.5%)	4(2.0%)	1(0.5%)	10(5.0%)
Total	100	63(31.5%)	37(18.5%)	61(30.5%)	39(19.5%)	200(100%)

DISCUSSION

Currently, the laboratory diagnosis of salmonella infection is dependent upon either the isolation of *salmonella enteric* serotype typhi from stool culture or detection of raised titre of agglutinating serum antibodies against lipopolysaccharide or flagella antigens of serotype typhi (widal test). This study have shown that many patient tested positive to stool culture than widal test, which records an overall infection rate of 49%. This result is almost in line with 46% infection rate recorded by Fauci *et al.,* (2008) among students at metropolitan secondary school Jos, Nigerian. It was disagreed with studies conducted in Nigeria by Udeze *et al.,* (2010) with infection rate of 20%. This could be related to factor that various laboratory personnel use different diagnostic technique for detection of the salmonella infection.

This study also showed that male tested positive more than female both in widal and stool culture with infection rate of 16.5%. This results corresponds with 16% infection rate recorded by Charles *et al.,* (2012) at Singapore. FAO (2012) also in their research conducted in Nigeria reported that males were more infected than female with 12.5% infection rate. This could be related to several factors including standard of personal hygiene and social habit.

Age group distribution showed that salmonella infection was more amongst age group 21-25 years (28%) and less in age group 31-35 years (5%). This agreed with the report of Bulter *et al.,* (2000) at Italy with infection rate of (30%) 21-25 years and (10%) 31-35 years. Also agreed with Beyene *et al.,* (2008) at Ethiopia with infection rate of (35%) 21-25 years and (08%) 31-35 years. This disagree with the report of Onyekwere (2007) at Nigeria with infection rate of (15%) 21-25 years and (40%) 31-35 years. This could be attributed to their exposure as a result of outdoor activities they usually indulge in like parties, travelling and so on. As a result of this they eat poorly cooked food and untreated water. It also result as improper personal hygiene, that is washing of hands before and after eating. This infection is spread by handshake in churches, place of work and school because it is an oral feacal route transmission.

CONCLUSION

In conclusion, from the study stool culture is more reliable than widal test in diagnosis of salmonella infection with infection rate of 49%. Therefore, stool culture is gold standard for diagnosis of salmonella infection. The study also shown that males (16.5%) were more infected than females (15%). It was also observed in the study that the highest prevalence of the infection occur in the age group of 21-25 years (28%) and least infection in age group of 31-35 years (5%). Hence, salmonella infection occurs irrespective of age and gender.

RECOMMENDATION

Laboratory personnel should not totally depend on widal test alone for diagnosis of salmonella infection but should also use other diagnostic method to differentiate salmonella infection from other infections.

Government should mount a community Campaign scheme, educating people about this infection (salmonella). They should also provide well equipped laboratory for accurate diagnosis of infections.

Individuals should ensure proper hygiene like washing hands with soap and water or sanitizer after eating and going to toilet. They should always go for regular checkup to avoid prolonged stay of this infection. Individuals should always learn from the training given to them about the infection and abstain from the factors that attributes to the salmonella infection.

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