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Comparison between Widal Test and Stool Culture in the Diagnosis of Typhoid Fever in the Clinical Set-Up in Niger Delta Region of Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Authors OAO and EBB designed the study. Authors OAO and EBB performed the experiments. All authors analyzed the data and drafted the manuscript. All the authors read and approved the final manuscript.

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ABSTRACT

Among Enterobacteriacae are causative agents of typhoid fever, Salmonella typhi and Salmonella paratyphi , a common infection among the inhabitants of Bayelsa State and in Nigeria at large. The aim of this study was to compare the widal test and stool culture for adopting better technique in the diagnosis of Salmonella species and its antibiotic susceptibility evaluation in the clinical setup in Bayelsa. A total of four hundred 400 {stool 200} {blood 200} samples collected from Diete Koki Memorial Hospital, Niger Delta University clinic and Federal Medical Centre, Bayelsa State were immediately taken to the medical microbiology laboratory, Niger Delta University for culture and serological test using Deoxycholate Citrate agar, Selenite-F broth and Smart Diagnostic Kit-Ref F-Kit8X5C.LOT:521-14 respectively. The standardized pure isolates to 0.5 McFarland turbidity

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standard were subjected to gram staining, biochemical tests and susceptibility testing using Kirby Beur agar diffusion method on Muller Hinton agar. Findings showed that out of two hundred (200) stool samples analyzed, one hundred and twenty-four 124(62%) Salmonella species were isolated 44(22%) Proteus and 32(16%) Escherichia Coli. Of one hundred and twenty-four Salmonella species isolated, 44(35.5%) were Salmonella typhi, 52(42%) Salmonella paratyphi A, while 28(22.5%) were of other Salmonella Species. Serological test on 200 blood samples analyzed revealed eighty-eight 88(44%) Salmonella typhi, eight 8(4%) Salmonella paratyphi A, 12(6%) Salmonella paratyphi B and zero (0%) Salmonella paratyphi C. Serological testing revealed eighty-four 84(42%) negative titre level and some patients with positive serological test had positive stool culture, while some negative but had positive growth of Proteus specie and Escherichia Coli. Antimicrobial susceptibility testing revealed ciprofloxacin as the most potent antibiotic with 100%, followed by ofloxacin 85% for salmonella infection and there was no significant difference at p=0.5 in their effectiveness. One hundred and twenty-four (124) patients were positive to cultural method and eighty-four 84 to serological method. Serological test detects early infection of Salmonella species while stool culture detects late infection (3weeks of incubation). These study has x-ray that both techniques (serological and cultural) should be adopted for effective diagnosis of Salmonella species in the health facilities. In conclusion serological test is Sensitive while stool culture is specific.

Keywords: Serological test; stool culture; salmonella species; Proteus sp.; Echerichia coli; antimicrobial susceptibility testing.

1. INTRODUCTION

Salmonella species are of two major types *Salmonella typhi* and *Salmonella paratyphi*, the contaminants of water and food products respectively. These microorganisms have two major antigenic determinants; these are flagellate and somatic antigens. *Salmonella typhi* and *paratyphi* belong to the class enterobacteriacea, non-coliform, rod shaped gram negative, lactose fermenting organism [1]. The organisms have the ability to cause typhoid fever when ingested from contaminated water or food products especially in immunocompromised and immunosuppressive individuals as a result of immune systems impairments. Salmonella species dwell in faecally contaminated environments. World Health Organization has recorded the prevalence of 600,00 death worldwide especially in the south and Central America, Africa, India and China [2], [3].

Typhoid disease is accompanied by headache fatigue, nausea, joint pains anaemia, lack of appetite for food and drinks, passing of watery and greenish stools as a result of shedding the Salmonella pigments. This infection is fatal if not treated on time, it causes dehydration and coma especially in immunosuppressive individuals such as HIV, Tuberculosis, Diabetes, broncho pneumonia. Unhygienic practices such as lack of adequate sewage disposal and water treatment can trigger and aid in the spread of typhoid fever, migration from rural area to urban centers, leading to rapid population growth, lack of pipe borne water that can provide safe drinking water, infrastructure and health challenges, lack of proper handling of ready to eat foods by food vendors. Diagnosis of Salmonella species is very crucial in the clinical set up, clinical samples that are mostly used in the diagnosis include blood, stool, urine, body fluid, wound aspirates, cerebrospinal fluid. The typhoid fever itself has a great challenge on the socio and cultural economic affairs of the country and the world at large, hence proper and immediate diagnosis of the infected patient could be highly beneficial to the growth of the society and economy [4], [5]. There are various laboratory techniques of diagnosis; these include widal test, stool culture, urine and blood culture and the skilled Medical Laboratory Scientist expertise is a prerequisite to effective diagnosis of this microorganism. These methods are believed to be effective in the diagnosis by the medical laboratory experts and also felt the cure to this infection is not challenging as the organism only targets human host and its sources are faecally contaminated food or water. A century ago, there are various antibiotics that were used in the cure of typhoid fever, these include Chloramphenicol, Ampicillin and Trimethoprim – Sulphamethozazone; meanwhile there was an emergence of multidrug resistance among these causative agents and there was a combination therapy shifting by the physicians to cure this infection, some antibiotics employed in this case include Cephalosporin examples are Ceftriaxone, Ciprofloxacin in Fluoroquinolone class of antibiotics. Generally, majority of the hospitals in Nigeria believes that

widal test is simple and cheap to perform in the laboratory, but false widal test result could be attributed to ineffective therapy or blood collected in early disease processes.

In Nigeria in-vitro susceptibility testing has been in display in the academic research and clinical treatment and the most potent drugs applied still pose difficult to treat the infection. The aim of this study is to compare stool culture and blood widal test and evaluate the more sensitive and specific diagnostic methods to be adopted in the clinical setup for the diagnosis of Salmonella infection in Nigeria.

2. MATERIALS AND METHODS

2.1 Study Area

This study was conducted in Federal Medical Centre, Dietei Koki Memorial Hospital and Niger Delta University Clinic in Bayelsa State; they are the largest public hospitals in Bayelsa State.

2.2 Study Population

This study was carried out to compare the performance of Widal test with stool culture in the diagnosis of *Salmonella* infection in 400 patients (Adult and Children).

2.3 Sample Collection

A total of four hundred (400) {200 stool and 200 blood} samples collected, 206(51.5%) males and 194(48.5%) females in this study with age group of 10 to 50years. Majority of the participants were 10-20 years age bracket. Participants from diete koki memorial Hospital were 5(10%), Federal Medical Centre 5(10%), and 38(80%) from Niger Delta University clinic. About 3 - 4ml of blood sample collected from the participants through venepuncture and the stool samples were immediately dispensed into plain tubes allowed to clot and further centrifuged to make it ready for Widal test and in the universal (plastic) disposable bottles with screw cap for culture respectively.

2.4 Laboratory Diagnosis

2.4.1 Widal agglutination test for *Salmonella* antibodies

Widal agglutination test was performed on each blood sample using the Widal agglutination kit (Biotech Lab, United States) according to manufacturer's instruction. *Salmonella paratyphi* A, B, and C antigens as well as *Salmonella typhi* O and H antigens were present in the reagents. A titre of greater than or equal to 1/160 indicates the presence of Salmonella infection, and both positive and negative controls were included. The reagents and samples were brought to room temperature and the antigens was shaken for proper mixing before dispensing. A drop of patient's serum to be tested was placed onto each of the required number of circles on the tile, and then one drop of Widal antigen suspension was added to the reaction circles containing patient serum. Using different mixing applicator sticks provided, the tile was rocked gently back and forth and observed for agglutination macroscopically for one minute.

2.4.2 Stool culture

A portion of stool was collected from the universal bottle using a heat fixing wire loop and streaked on deoxycholate agar, *Salmonella* and shigella agar, and was also inoculated into selenite f broth and incubated at 37^{o}C for 24 hours; following a subculture on deoxycholate citrate agar, *Salmonella* and shigella agar from overnight selenite F broth to obtain discrete colonies. Deoxycholate agar and *Salmonella* and shigella agar enhance the growth of *Salmonella*, and the presence of *Salmonella* is indicated by pink – red colonies, whereas hydrogen sulphide (H_2S) also produces red colonies with black centers [6].

2.4.3 Colonial characteristics of *Salmonella* species

On Deoxycholate agar, *Salmonella* produce non-lactose fermenting colorless colony with black centers except *S. paratyphi A* whose colonies do not have black centers. On *Salmonella* and shigella agar *Salmonella* produce non-lactose fermenting colonies with black centers except *S. paratyphi A* whose colonies do not have black centers.

3. RESULTS

Fig. 1. Percentage Distribution of Serological Test; Serological test using smart diagnostic kit. Result showed that S. *typhi* titre was 88(44%) *S.* para*typhi* A 8(4%), S. para*typhi* B 12(6%), *S.* para*typhi* C 0(0%) and Negative results were 92(46%).

Fig. 2 showed Percentage distribution of Salmonella isolates and other microorganisms

from the stool samples cultured in selenite-F broth, deoxycholate citrate agar, *Salmonella* and *Shigella* agar showed 124(62%) *Salmonella* specie, 44(22%) Proteus and 32(16%) *Escherichia Coli.*

Fig. 3. shows: Percentage frequency of Microbial isolates of S. *typhi* 44(35.5%), S. para*typhi* 52(42.0%) and other *Salmonella* species 28(22.6%) in stool culture

Fig. 4. Overall percentage distribution of Widal test and Stool culture

Fig. 5a. The result of antimicrobial susceptibility testing for Salmonella species revealed that Ciprofloxacin 12 (100%) and Ofloxacin 8 (85%) were most potent to Salmonella species

Fig. 5b. Antimicrobial Susceptibility testing for *Salmonella paratyphi* A revealing the effectiveness of Ciprofloxacin 85.7% potency against *Salmonella typhi* A.

Fig. 5c. Antimicrobial susceptibility testing for other *Salmonella paratyphi* which showed that only Ciprofloxacin was the only potent antibiotic suitable for the treatment of this isolate with percentage susceptibility testing of 100.

Table 1. Age distribution of widal test results.

Table 2. Age distribution of stool culture results.

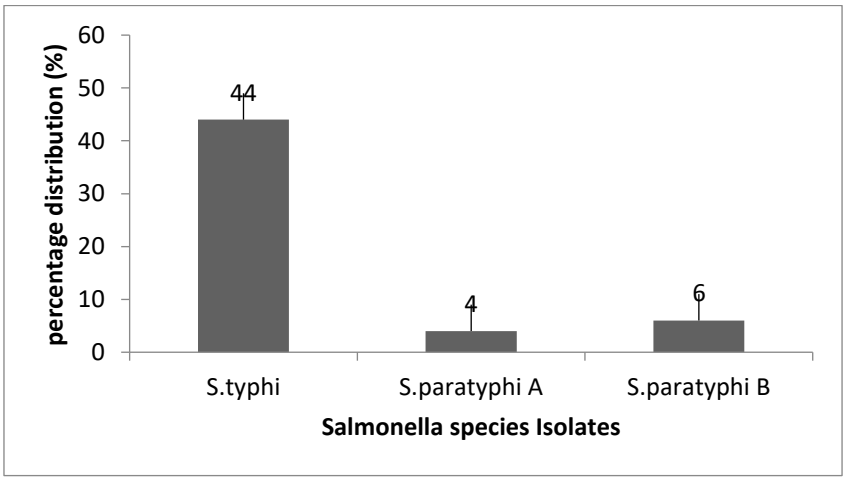

Fig. 1. Percentage distribution of *Salmonella* species in serological test

Keys:
1. *S.typhi: Salmonella typhi*
2. *S. paratyphi A: Salmonella paratyphi A*
3. *S. paratyphi B. Salmonella paratyphi B*

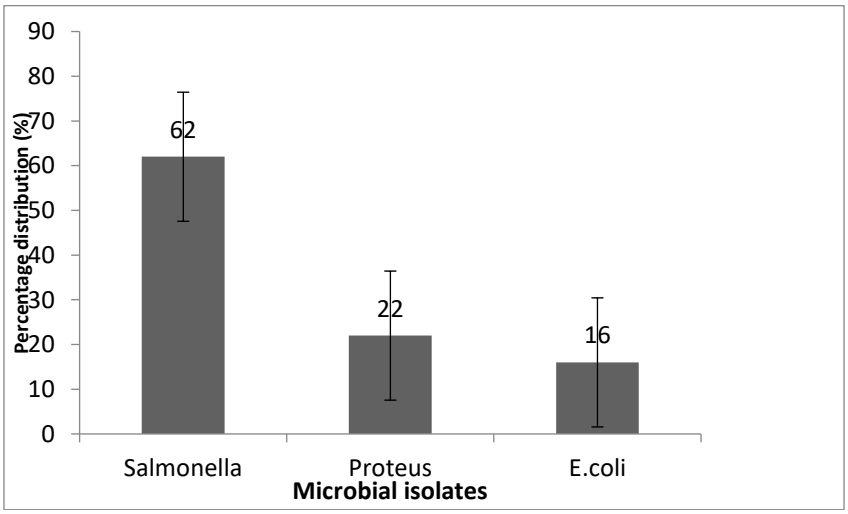

Fig. 2. Percentage distribution of *Salmonella* isolates and other microorganisms in stool culture

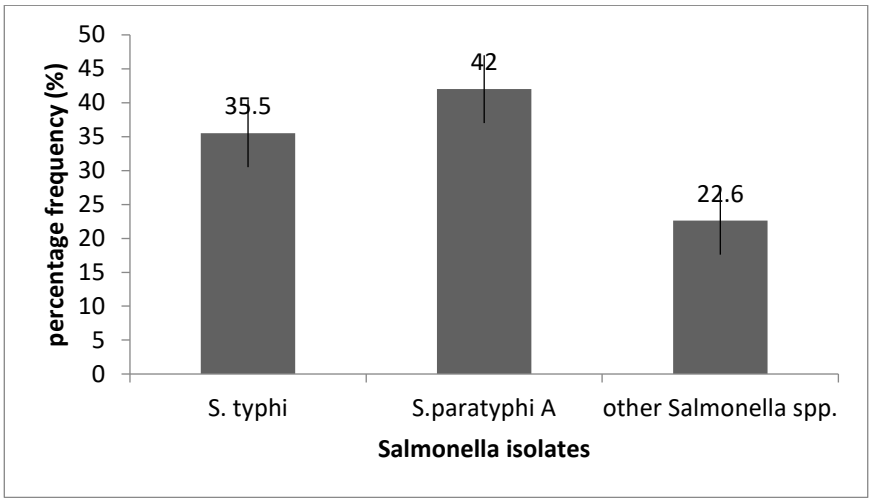

Fig. 3. Percentage frequency of microbial isolates of *S typhi* 44(35.5%), *S paratyphi* A 52 (42.0 %), and other *Salmonella* species 28 (22.6%) in stool culture

Keys:
S. typhi: Salmonella typhi 35.5%()
S. paratyphi A: Salmonella paratyphi A (42.0%)
Other Salmonella spp. Other Salmonella species (22.6%)

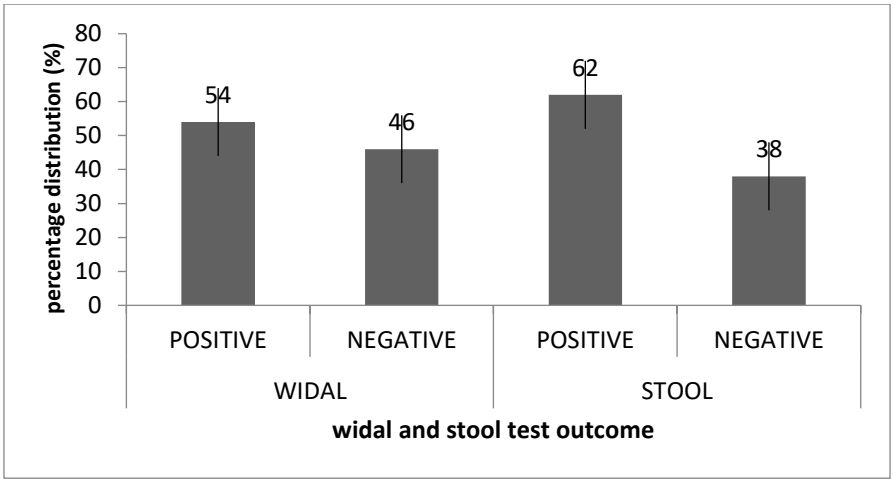

Fig. 4. Overall percentage distribution of widal test and stool culture

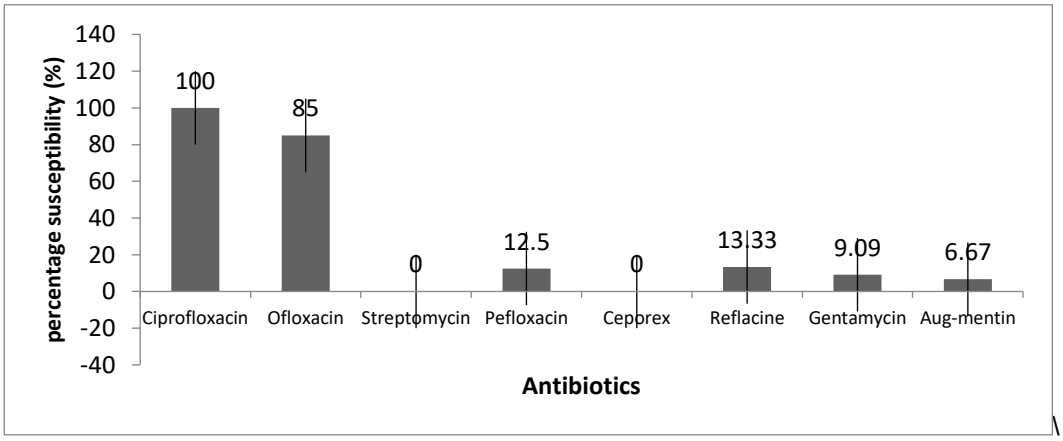

Fig. 5a. Showing antimicrobial susceptibility testing for *Salmonella typhi*

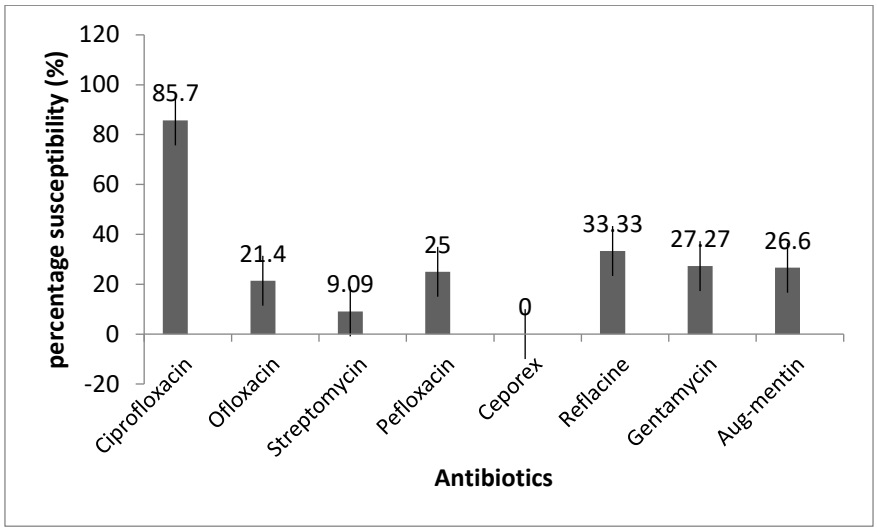

Fig. 5b. Showing antimicrobial susceptibility testing for *Salmonella paratyphi A*

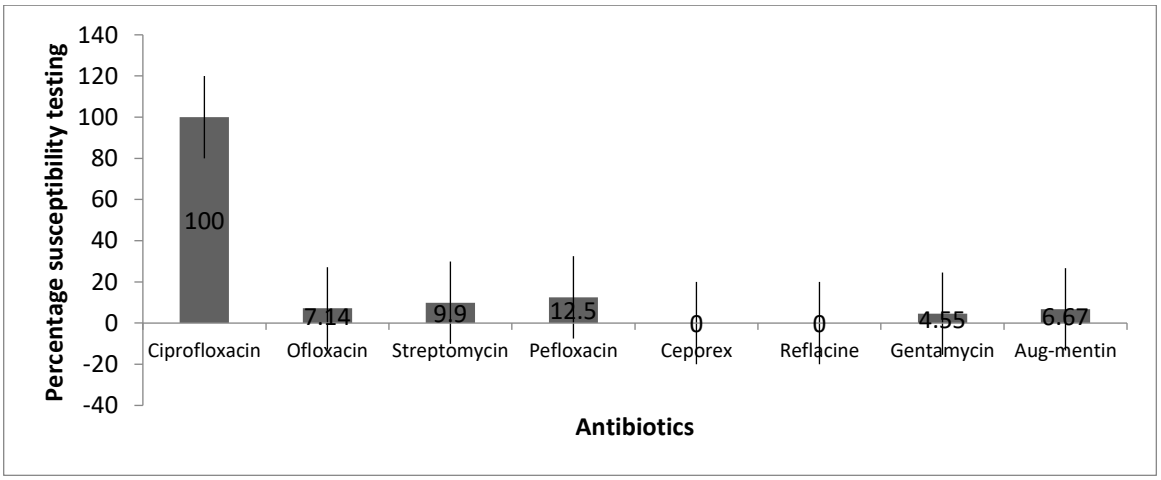

Fig. 5c. Showing antimicrobial susceptibility testing for other *Salmonella paratyphi*

Table 1. Age distribution of widal test results

	Parameter	
Age years	**+**	**-**
0 – 20 n=(82)	48(24%)	34(17%)
21 – 30 n=(78)	36(16%)	42(21%)
31 – 40 n=(27)	16(8%)	11(5.5%)
Above 50 n=(13)	8(4%)	5(2.5%)
Total	**108(54%)**	**92(46%)**

Table 2. Age distribution of stool culture results

	Parameter	
Age years	**+**	**-**
0 – 20 n=(82)	25(23.72%)	57((33.17%)
21 – 30 n=(78)	52(45.2%)	26(20%)
31 – 40 n=(27)	08(5.6%)	19(5%)
Above 50 n=(13)	9(16.1%)	4(4%)
Total	**94(62%)**	**106(54%)**

4. DISCUSSION

Due to the significant socioeconomic impact of typhoid fever, early diagnosis of the illness is crucial for both etiological diagnosis and the identification of potential carriers who could be in charge of an acute typhoid fever outbreak [4] [7]. The findings in this study showed that individuals of all age are susceptible to infection by *Salmonella* species ranging from 0 to 20 years, followed by 21-30 years; this is in agreement with the studies conducted in 2019 and 2013 by Wam et al [8] and Ramyil et al [9], respectively; both found 21-30 and 24-29 years more susceptible to *Salmonella* infection respectively. Wam et al. [8] then stated that this could be due to improper sanitation and hygiene. Adolescents, children and matured minded males and females could be infected with *Salmonella* infection through eating or swallowing food products (cooked or uncooked) and water that harbour salmonella species. Migration from one region to typhoid endemic region could pose a high risk of being infected [10]. With respect to gender of the febrile patients, males have the highest prevalence of typhoid fever with 56.5% and females 53.2% while 43.5% and 46.8% widal test and stool culture respectively. These findings also agree with that of Ramyil et al. [9] which reported that the males were more positive to widal test and stool culture than females. Also, Udeze et al. [11], stated that females are not usually exposed to the activities that are normally associated with the disease like sanitation, poor sewage disposal, swimming, farming, fishing or resting in a dirty environmental as their male counterparts.

However, this result is not in agreement with the findings of Wam et al. [8] and Ameya et al., [12] who both reported higher prevalence of typhoid fever in females than in males. The result showed that patients within the age range 0-20 had the highest incidence 48(24%) of widal test and 31-40 years has the highest incidence of typhoid fever 52(45%) in stool culture. On the basis of gender, male had the highest prevalence of typhoid fever of 88(65%) in stool culture while female account for 12(35%) for widal test. One hundred and eight 108 (54%) among the study patients were positive to widal test (serological test) were significant for salmonella while 124 (62%) were positive to culture test. Based on this results significance of antibodies titres detected in patients' serum by widal test is not specific for having *Salmonella* infection, but in conjunction with the stool culture. This study was similar to the finding from the study conducted by Mohammed and Thapa [13], which found that the widal test and other serological tools have limitations because of their non-Specificity [14]. Observation showed high number of *Salmonella* in stool than in serological tests. This is in correlation with the work done in 2010 by Malisa and Nyaki [15] in Cameroon who reported 74% for serological test and 91% of stool culture; the authors stated that widal test has low specificity and high sensitivity to *Salmonella* infection, there was a significant difference ($p<0.05$) between the serological test and stool culture. The efficacy, availability and cost are important criteria for the selection of first line antibiotics to be used in developing countries [16] [17]. This selection reviews the therapeutic guidelines for treatment of typhoid fever across all age groups, from this study, it was observed that ciprofloxacin 12(100%), Ofloxacin8(85%) were most potent drugs on *Salmonella* species. According to Chim et al [18] the fluoroquinones are widely regarded as optimal for the treatment of typhoid fever in adult and children, they are relatively inexpensive, well tolerated and more rapidly and reliably effective than the former first-line drugs, viz chloramphenicol, ampicillin, amoxicillin. The fluoroquinolone attains excellent tissue penetration, kill *Salmonella* species in its intracellular stationary stage. According to Gupta et al., [19] and Brown et al, [20], ciprofloxacin, ofloxacin, perfloxacin and fleroxacin have generally proved effective. There was no cogent research on the toxicity Ciprofloxacin administration had on the salmonella infected children during treatment according to Gupta et al. [19], the drugs could have significant effect on their bone marrow.

Also the study reveals that Augmentin, Cephalotin, Nalidixic acid, streptomycin were poorly potent to *Salmonella* species in this study. The study also revealed the presence of Proteus species and *Escherichia coli* from the biochemical tests on the plates isolates among the study patients who were positive to both serological and stool culture tests for salmonella species. This shows that these patients harbour other microorganisms aside the Salmonella species.

5. CONCLUSION

This study has X-rayed the authenticity of stool culture over serological test, therefore the medical laboratory scientist, medical personnels are advised to adopt both methods in carrying

out the diagnosis of suspected patients with typhoid fever.

6. RECOMMENDATION

1. Both stool culture and serological antibodies evaluation for typhoid fever are advised for laboratory diagnosis of salmonella species
2. Conventional antibiotics (fluoroquinolones such as ciprofloxacin and ofloxacin) should be considered for treating enteric fever.
3. Further research especially molecular research on resistance mechanism of *Salmonella* species against Ceftriaxone need to be conducted.
4. Potable pipe - borne water, education, Immunization should be provided to the people living in the areas.
5. General hygiene and environmental sanitation must be practiced as they are significant to reduce the proliferation of the salmonella species.

CONSENT AND ETHICAL APPROVAL

Ethical clearance was obtained from the Federal Medical Centre, Dietei Koki Memorial Hospital and Niger Delta University Clinic, Bayelsa State. 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 was obtained, and confidentiality of the result was ensured.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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