

Use of stool culture as a determinant parameter of enteric fever in adults attending Bingham university teaching hospital Jos, Nigeria

Ramyil, Mamzhi-crown Seljul¹, Ogundeko, Timothy Olugbenga², Idyu, Iorkyase Isaiah³, Ameh, Joshua Momoh⁴

¹Department of Medical Microbiology and Parasitology, College of Health Sciences Bingham University Teaching Hospital, Jos, Nigeria

²Department of Pharmacology and Therapeutics, College of Health Sciences Bingham University Teaching Hospital, Jos, Nigeria

³Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmaceutical Sciences, University of Jos, Nigeria

⁴Department of Laboratory Services and Histopathology, Bingham University Teaching Hospital, Jos, Nigeria

Email address:

crownramyil@yahoo.com (Ramyil, MS), tim_ogundeko@yahoo.com (Ogundeko, TO), idyui@unijos.edu.ng (Idyu, II), Jameh43@yahoo.com (Ameh, JM)

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Abstract: Background: Enteric fever caused by salmonella typhi is an endemic disease in the tropics and sub-tropics; and has become a major public health problem in developing countries of the world. Presently, cultures of Stool and blood are the most diagnostic means of confirming salmonellosis in humans. However, the stool and serum sample of an infected patient against the somatic (O) and flagella (H) antigens of the bacteria requires thorough laboratory analysis. This study was biased on the use of stool culture as a confirmatory analysis of typhoid fever indices in the region of research particularly among adolescents (18 yrs and above). Methods: 60 adults attending Bingham University Teaching Hospital with symptoms clinically suspected to be enteric fever were randomly selected. Informed consent of volunteers was obtained in 48 patients and stool specimens collected and were cultured. Stool specimens were processed using isolation method and biochemical characteristics of susceptibility testing of typhoid fever from the individual patient. A significant mean difference of male and female that were affected by enteric fever was determined. Results: Results obtained from a total number of 48 patients (32 male and 16 female) indicated 12 positive stool cultures among which 10 were males and 2 were females (31.25% and 12.5%) respectively. Salmonella was found to be susceptible to Ofloxacin, Ciprofloxacin, Ceftazidime and Cefuroxime respectively; thus constituting the choice drugs in the treatment of enteric fever. Conclusion: Result showed that a significant mean difference between the number of affected patients and those not affected ($P.v = 0.0521$) authenticates stool culture as confirmatory test for enteric fever as against clinical diagnosis. *Salmonella enterica* showed more resistance to some commonly used drugs. Therefore, sensitivity testing based on prescription is recommended to prevent continuous drug resistance development. Results further showed that men were more affected than women; however, a suggested area to explore in the study of enteric fever infections.

Keywords: Enteric Fever, Stool Culture, Salmonellosis, Nigeria

1. Introduction

Enteric fever is a systemic infection caused by the human adapted pathogens *Salmonella enterica* serotype Typhi (*S. Typhi*) and is the major cause of morbidity and mortality worldwide [1]. Enteric fever caused an estimated 21.7 million illnesses with greater than six hundred thousand (> 600,000) deaths [2]. Salmonellosis is an

endemic disease in the tropics and sub-tropics; and has become a major public health problem in developing countries of the world including Nigeria with an estimated annual incidence of 540 per 100,000 [2 - 6]. However, most enteric fever occurs in low- and middle-income countries where blood cultures are often unavailable, unaffordable, or inconsistently applied [3, 7, 8]. In Nigeria, enteric fevers caused by salmonella typhi and salmonella

paratyphi are not only endemic but constitute a great socio-medical problem, being responsible for many cases of pyrexia of unknown origin, high morbidity and mortality [6, 9 – 15]. Although enteric fever remains a public health problem in the region, it does provide a model for what can be accomplished for countries with a high incidence of the disease [3]. Despite the limitations of currently available epidemiologic data, a number of recent trends in enteric disease epidemiology have emerged in the African, Asian, and Latin American regions [2, 6].

2. Literature Review

In sub-Saharan Africa, where the burden of enteric fever is the least well characterized, hospital based studies indicate that non-Typhi serotypes of *Salmonella*, particularly *S. enterica* serotype Enteritidis and *S. enterica* serotype Typhimurium, greatly outnumber *S. Typhi* and *S. Paratyphi* as causes of bloodstream infection [6, 16, 17]. Nonetheless, outbreaks of typhoid fever are frequently reported from sub – Saharan Africa, often with large numbers of patients presenting with intestinal perforations leaving open important questions about the epidemiology of enteric fever in the region [18, 19].

2.1. Clinical Manifestation

Salmonella organisms are responsible for a broad spectrum of clinical syndromes that include asymptomatic carriage, self-limited gastroenteritis, bacteremia, enteric fever and metastatic focal infections [20, 21]. Several distinct clinical syndromes can develop on adult and children infected with *Salmonella*, depending on both host factors and the specific serotype involved in Enteric fever [16, 17]. The dose of organisms ingested is an important determinant of the incubation period, symptoms, and severity of acute salmonellosis. Gastric acidity is an important barrier to infection. Impaired reticuloendothelial or cellular immune response (which occurs on persons with chronic granulomatous disease, transplantation, hemoglobinopathy, malaria, AIDS, Cancer, and SLE) raises the risk for severe, complicated infection of enteric fever [1, 15, 20, 21].

Signs and symptoms of typhoid fever include a persistently high fever, headache, malaise, lethargy, and skin rash, loss of appetite, hepatosplenomegaly, and bradycardia. Older children and adults usually become constipated; younger children may have diarrhea. Not all patients experience classic symptoms, however, so stool and blood cultures should be performed on patients with persistent high fever who have recently traveled to a developing country [12, 14, 22].

Isolation of *Salmonella* organisms from cultures of stool, blood, urine and material from foci of infection is diagnostic; Gastroenteritis is best diagnosed through cultures of stool specimens rather than rectal swab specimens [20, 21]. It is not positive as often as blood culture. However, it is a valuable diagnostic test. Contrary

to the usual teaching, it is often positive before the third week of illness, and may be positive at any stage of the disease [23, 24].

2.2. Antimicrobial Resistance and Patient Management

Antimicrobial resistance is a major public health problem in both *S. Typhi* and *S. Paratyphi*, and timely treatment with appropriate antimicrobial agents is important for reducing the mortality associated with enteric fever [25]. Optimal antimicrobial treatment of patients with enteric fever depends on an understanding of local patterns of antimicrobial resistance and is enhanced by the results of antimicrobial susceptibility testing of the *Salmonella* isolated from the individual patient. Ciprofloxacin continues to be widely used, but clinicians need to be aware that patients infected with *Salmonella* with decreased ciprofloxacin susceptibility may not respond adequately [26]. However, the cost and route of administration make ceftriaxone less suitable for patient treatment in some low- and middle-income countries, and the oral third-generation cephalosporin cefixime appears to be inferior to other oral agents both in terms of fever clearance time and treatment failure [27].

2.3. Statement of the Problem

Laboratory diagnosis of enteric fever requires the isolation and identification of *salmonella enterica* in many areas where the disease is endemic, laboratory capacity is limited [28]. However, Enteric fever remains a major public health problem in the developing world with very poor estimates of the number of cases of deaths annually, and 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 enteric fever [29, 30].

2.4. Aim and Objective

The main objective of this study is to isolate and identify enteric fever from stool samples as a confirmatory analysis of same among adolescents (18 yrs and above); and to ascertain the specific choice drug for treatment through authentic sensitivity test.

3. Methodology

3.1. Study Design, Area and Population

A cross sectional study was conducted at the Bingham University Teaching Hospital, Jos from September to December 2012. 60 adults attending same Hospital with symptoms clinically suspected to be enteric fever were randomly selected. In the course of the research, patients were lectured on the study protocol and its relevance in designing an intervention strategy against the infection. Informed consent of volunteers was obtained in 48 patients and stool specimens collected and were cultured. Stool

specimens were processed using isolation method and biochemical characteristics of susceptibility testing of typhoid fever from the individual patient. A significant mean difference of male and female that were affected by enteric fever was subsequently determined.

3.2. Ethical Clearance

Ethical clearance was obtained from the ethical research committee of the Bingham University Teaching Hospital (BHUTH), Jos, Nigeria.

3.3. Diagnostic Tests/Stool Culture

Stool samples were collected in plastic disposable bottles with screw caps and processed using cultural isolation method. 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 their sterility. A piece of stool was quickly collected from the universal bottle using a heat-fixed wire loop and streaked on the Deoxycholate citrate agar (DCA) and incubated at 37°C for 24 hours, following a subculture on XLD agar to obtain a discrete isolate. Xylose Lysine Deoxycholate agar enhances the growth of salmonella and the presence of salmonella is indicated by pink-red colonies; whereas, Hydrogen sulphide (H₂S) producing salmonella also produces red colonies with black centers. Growth showing central sheen, which indicated *Salmonella* organism, was sub cultured into nutrient agar for further characterization and susceptibility.

3.4. Biochemical Reaction

Presumptive analysis of salmonella colonies was tested. Gram staining was done by preparing a heat-fixed bacteria smear on a free glass slide, salmonella specie found were gram (pink in appearance) negative bacteria possessing flagella that makes them motile causing enteric fever. Indole, Citrate, Catalase and Urease test were also carried out and results showed positive.

4. Results

Results obtained from a total number of 48 patients (32 male and 16 female) indicated 12 positive stool cultures among which 10 were males and 2 were females (31.25% and 12.5%) respectively. *Salmonella enterica* was found to be susceptible to Ofloxacin, Ciprofloxacin, Ceftriaxone and Cefuroxime respectively; thus constituting the choice drugs in the treatment of enteric fever.

Table 1. Frequency of adults affected and those that were not affected.

Age	N = 48	
	affected	not affected
18-23	4	0
24-29	9	9
30-35	5	7
36-41	7	0
42-47	7	0

Table 2. Positive results of stool culture (based on percentage (%)).

Stool culture	
Sex	N = 48
Male	10 (31.25%)
Female	2 (12.5%)
Total	12 (25.0%)

Table 3. Determination of mean difference of male and female subjects affected by *Salmonella* infection based on gender.

Gender	Positive	Negative	Total	P.v= 0.0521
Male	10	22	32	
Female	2	14	16	
Total	12	36	48	

Table 4. frequency of susceptibility of salmonella isolates from Adults.

Antibiotics	Number of Isolates	% occurrence of sensitivity	% occurrence of resistance
Ciprofloxacin	12	8 (66.6)	4 (33.3)
Ofloxacin	12	9 (75.0)	3 (25.0)
Augmentin	12	2 (16.6)	10 (83.3)
Nitrofurantoin	12	5 (41.6)	7 (58.3)
Ceftriaxone	12	7 (58.3)	5 (41.6)
Ceftazidime	12	3 (25.0)	9 (75.1)
Cefuroxime	12	7 (58.3)	5 (51.6)
Gentamycin	12	5 (41.6)	7 (58.3)
Ampicillin	12	2 (16.6)	10 (83.3)
Cloxacillin	12	4 (33.3)	8 (66.6)

5. Discussion

Currently, the laboratory diagnosis of enteric fever is dependent upon either the isolation of salmonella enterica serotype typhi from stool culture or blood culture. In this study, a total of 48 patients (Adults within the ages of 18 years and above) visiting the Bingham University Teaching Hospital at the period of this study were randomly selected; consisting 32 male and 16 female adults.

Tables 1 signifies an inverse relationship in the sense that if there is a decrease or increase in the number of affected males there will also be a very small corresponding increase or decrease in the number of female affected.

In table 2, out of 48 adult patients whose stool specimens were cultured, 32 were males and 16 female. Of the 32 males, 10 (31.25%) showed a positive culture and 2 (12.5%) out of the 16 females showed a positive culture respectively. It was observed that the number of male adults whose stool were affected were more than the females. In a research conducted by Charles in 2012, the study representing 8.7% typhoid fever cases, He observed that males were more affected two times than the females [31]; The Food and Agriculture Organization 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 [32]. A study conducted by Butler in 1991 showed that infection rate in stool is slightly higher in male

Adults reflecting greater exposure of the male adults to contaminated food and water outside the home [33]. However, *Salmonella* in stool occurs only when one becomes a potential carrier of the infection [34]. In other studies, females stool culture specimens showed more positive report than males mostly within the ages of 20 and 72 years [32]. In a research findings also made by Muhammad in 2012 in Pakistan, 50% males positive to stool culture and 49% females were also positive to stool culture. These differences could be so as a result of the proportion of male and female patients of volunteers who were tested, but the fact still remains that the disease occurs irrespective of gender [35].

In table 3, study indicates a significant mean difference between the number of affected patients and those that were not affected with P. value of 0.0521[36].

In table 4, 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, Ampicillin, Ceftazidime and Cloxacillin [36], [37].

6. Conclusions

The fact that a significant mean difference between the number of affected patients and those not affected ($P.v = 0.0521$) as indicated in table 3 clearly authenticates stool culture as confirmatory test for enteric fever as against clinical diagnosis. Drug resistance, *salmonella* showed more resistance to some commonly used drugs. Therefore, sensitivity testing based on prescription is recommended to prevent continuous drug resistance development.

Results further showed that men were more affected than women; thus, suggested area to explore in study of enteric fever infections.

Conflicting Interests

No conflict of interest.

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