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

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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, Ceftriaxone 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
carryage, 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 Offloxacin, Ciprofloxacin, Ceftriaxone and Ceftazidime respectively; thus constituting the choice drugs in the treatment of enteric fever.

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

<table>
<thead>
<tr>
<th>Age</th>
<th>N = 48 affected</th>
<th>not affected</th>
</tr>
</thead>
<tbody>
<tr>
<td>18-23</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>24-29</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>30-35</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>36-41</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>42-47</td>
<td>7</td>
<td>0</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Stool culture</th>
<th>Sex</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N = 48</td>
</tr>
<tr>
<td>Male</td>
<td>10 (31.25%)</td>
</tr>
<tr>
<td>Female</td>
<td>2 (12.5%)</td>
</tr>
<tr>
<td>Total</td>
<td>12 (25.0%)</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Gender</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>10</td>
<td>22</td>
<td>32</td>
</tr>
<tr>
<td>Female</td>
<td>2</td>
<td>14</td>
<td>16</td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
<td>36</td>
<td>48</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Number of Isolates</th>
<th>% occurrence of sensitivity</th>
<th>% occurrence of resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciprofloxacin</td>
<td>12</td>
<td>8 (66.6)</td>
<td>4 (33.3)</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>12</td>
<td>9 (75.0)</td>
<td>3 (25.0)</td>
</tr>
<tr>
<td>Augmentin</td>
<td>12</td>
<td>2 (16.6)</td>
<td>10 (83.3)</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>12</td>
<td>5 (41.6)</td>
<td>7 (58.3)</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>12</td>
<td>7 (58.3)</td>
<td>5 (41.6)</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>12</td>
<td>3 (25.0)</td>
<td>9 (75.1)</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>12</td>
<td>7 (58.3)</td>
<td>5 (51.6)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>12</td>
<td>5 (41.6)</td>
<td>7 (58.3)</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>12</td>
<td>2 (16.6)</td>
<td>10 (83.3)</td>
</tr>
<tr>
<td>Clocxicillin</td>
<td>12</td>
<td>4 (33.3)</td>
<td>8 (66.6)</td>
</tr>
</tbody>
</table>

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 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 Ofloxicin, Ciprofloxacin, Ceftriaxole and Cefuroxime making drug of choice for treatment of these adults. However, they were more resistant to Augmentin, Amplicillin, Cefazidine and Cloxicillin [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.

Acknowledgements

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