Comparative evaluation of stool, urine and blood culture for isolation of *Salmonella* spp. from patients with clinical evidence of salmonellosis

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*Salmonella* infection remains a serious problem worldwide and cause substantial economic loss, resulting in mortality, morbidity and poor growth. A total of 150 samples (50 each from Stool, Urine and Blood) were collected from 50 patients from January to June 2015. The research was conducted in Microbiology laboratory of Kebbi State University of Science and Technology Aliero. The culture media used for this study were selenite broth, cysteine lactose electrolyte deficient agar, hektoen enteric agar, cooked meat agar, nutrient agar, chocolate agar and blood agar. The isolates were identified base on colonial appearance, gram staining reaction, microscopic morphology and series of biochemical tests. Blood sample yielded the highest number of *salmonella* isolates of 12 (24%), followed by stool sample with number of occurrence of 8 (16%), and urine sample yielded the lowest number of *Salmonella* spp isolated i.e. 2(4%). The results obtained also showed that 22 (21.8%) of the isolates were *Salmonella* species whereas 52 (51.5%), 25 (24.8%) and 2 (2.0%) were *Escherichia coli*, *Staphylococcus aureus*, and *Shigella* spp respectively. Out of 22 *Salmonella* species isolated, 13 (59.1%) were *Salmonella* typhi, 6 (27.3%) were *Salmonella paratyphi* and 3 (13.6%) were other *Salmonella* Species. *Salmonella typhi* and *paratyphi* are more prevalence compare to other non-typhoidal *Salmonella*. Simply because *S. typhi* and *paratyphi* A are species specific to humans and do not occur in other animals. From this research, it was concluded that blood culture is the best test samples for isolation of *Salmonella* followed by stool sample; this simply implies that typhoidal fever is higher than the nontyphoidal fever in this population.

**Keywords:** Blood, *Salmonella* spp., Stool, Urine.

**INTRODUCTION**

*Salmonella* are gram-negative motile bacilli. The genus *Salmonella*, which belongs to the family Enterobacteriaceae, was named after Daniel E. Salmon, an American veterinarian who first isolated *Salmonella choleraesuis* from pigs with hog cholera in 1884 Kim *et al.* (2004). *Salmonellae* are ubiquitous human and animal pathogens. They colonize virtually all animals including poultry, birds, livestock, reptiles, rodents, domesticated animals, and humans Subhash (2001). As with the closely related bacterium *Escherichia coli*, salmonellae are potential enteric pathogens and a leading cause of bacterial foodborne illness. In addition, *Salmonella* species have been implicated in a spectrum of other diseases, including enteric or typhoid fever (primarily *Salmonella typhi* and *Salmonella paratyphi*), bacteria, endovascular infections, focal infections (eg, osteomyelitis), and enterocolitis (typically *Salmonella typhimurium*, *Salmonella enteritidis*, and *Salmonella Heidelberg*) Kim *et al.* (2004).

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This result in substantial economic loss, mortality, morbidity, poor growth and represents a serious problem to food industries Khan (2007). Human spreads Salmonella mainly through the stool. Food and water borne transmission can occur when food and water are contaminated with stool or through direct fecal-oral route. Salmonellosis is a worldwide issue and People at risk of serious complications due to Salmonella food poisoning include: adults, pregnant women, infants, children, and people who have compromised immune systems Merchant and Packer, (1967). Symptoms usually include: headache nausea, vomiting, fatigue, gastroenteritis, abdominal cramps and bloody diarrhea with mucus and sometimes reactive arthritis (Reiters Syndrome) Dworkin et al. (2001).

The incidence of salmonellosis has markedly increased in many countries; however, a paucity of good surveillance data exists. In 2000, approximately 21.6 million worldwide cases of typhoid fever caused 216,500 deaths Crump et al. (2004). The incidence of typhoid fever in south-central Asia, Southeast Asia, and, possibly, southern Africa was high (>100 cases per 100,000 population per year). The rest of Asia, Africa, Latin America, and Oceania (except for Australia and New Zealand) typically see intermediate rates of typhoid fever (10-100 cases per 100,000 population), while the incidence is low in the other parts of the world (<10 cases per 100,000 population). In countries where typhoid fever is endemic, most cases of the disease occur in children aged 5-19 years and young adults Bhan et al. (2005). The incidence of Salmonella infections in the United States has been stable since 2004 but has decreased approximately 8% from 1996-1998 levels (8) In 2007, the reported annual incidence of salmonellosis was 14.9 cases per 100,000 population CDC (2007). The true annual burden of nontyphoidal Salmonella infection in the United States is calculated to be 520 cases per 100,000 population, compared with 13.4 laboratory-confirmed cases per 100,000 population per year. This reflects an estimate of approximately 38.6 cases of nontyphoidal Salmonella infection for each culture-confirmed case Voetsch et al. (2004).

Typhoid fever is among the major widespread diseases affecting the population in Nigeria and has been rated eighth among these common infections Odugbemi et al. (1994). Nigeria, like many other tropical and developing countries, has been described as an endemic zone for typhoid fever (Oboegbulam et al. (1995) and Idoko et al. (1988)) In Nigeria, transmission of typhoid fever occurs all year round but rates are slightly higher in April and July, coinciding with the height of the hot, dry season and the onset of the rainy season, respectively Oboegbulam et al. (1995) . The highest number of cases of typhoid fever are recorded during the rainy season in south-east Nigeria Odugbemi et al. (1994). Typhoid fever has been reported in all age groups and classes in Nigeria Rasaily et al. (1994). Owing to the irregular nature of bacterial shedding, several samples should be examined in order to identify carriers Smith et al. (2008). Active surveillance on salmonellosis in recent years is rare. Besides, there is a paucity of data on studies emphasizing on isolation and characterization of Salmonella species considering human stool, blood and urine in Nigeria. A lot will be achieved through the evaluation of these samples to ascertain which of them will be the best to isolate these bacteria in order to inform the clinicians on which sample to send for diagnosis. Therefore, this research was aimed at isolating and characterizing Salmonella species from patients attending Federal Medical Centre B/ Kebbi.

MATERIALS AND METHODS

Study population

The study population, were both male and female adult patients who attended the General out patients Department of Federal Medical Centre Birnin Kebbi, Nigeria, with the clinical evidence of Salmonellosis (i.e. headache nausea, vomiting, fatigue, gastroenteritis, abdominal cramps and bloody diarrhoea with mucus) Dworkin et al. (2001), as diagnosed by the attending physicians. These patients did not include those who were on antibiotics about two weeks prior to specimen’s collection.

Ethical clearance

Ethical approval was sought for from the Kebbi State Ministry of Health. Informed consents were also obtained from all the participants.

Sample collection

Three different samples (blood, stool, urine) were collected from 50 patients attending Federal Medical Center Birnin Kebbi with clinical evidence of salmonellosis i.e a total of 150 samples were obtained. All patients were instructed on how to collect appropriate specimens. Blood samples were collected from patients using blood sample bottles by the attending clinician while stool and urine specimens were collected using a sterile, clean, dry, disinfectant-free, wide-necked container with tight fitting lid. All the specimens were taken to the laboratory for analysis without delay.

Bacteriological investigation

Stool culture

The purulent or mucoid parts of the stool samples were picked using a sterile wire loop and inoculated into selenenite broth medium and then incubated at 37°C overnight. (18-24hrs). The broth culture was then subcultured into salmonella shigella medium agar, and hektoen enteric agar, the plates were then incubated at 37°C for 24hrs.
Urine culture

Using a sterile wire loop, a loop full of the urine sample was picked and inoculated into Bismuth sulfite agar, McConkey agar, Blood Agar, XLD (xylose, lysine deoxycholate agar). The plates were then incubated at 37°C for 24hrs.

Blood culture

Using a sterile syringe and needle, 4ml of whole blood from the patient was dispensed into 20ml of cooked meat medium. The mixture was then incubated at 37°C for 24-48hrs before subculture into blood agar, CLED, hektoen enteric agar and salmonella shigella agar. The plates were then incubated at 37°C for 24hrs. The sub culturing was done three times before conclusion that there is no growth.

Identification of isolates

The suspected colonies were characterized morphologically using Gram’s staining method according to the method described by Merchant and Packer, (1967) . For carbohydrate fermentation tests, triple sugar iron agar (TSIA) slant reaction, methyl red-Voges-Proskauer (MR-VP) and Indole reaction tests were carried out for identification of suspected Salmonella according to the methods described by (Douglas et al. (1998) and OIE (2000)).

RESULTS

Table 1: Salmonella isolates obtained from three different samples

<table>
<thead>
<tr>
<th>Sample Source</th>
<th>No Tested</th>
<th>No. of Salmonella</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stool sample</td>
<td>50</td>
<td>8(16%)</td>
</tr>
<tr>
<td>Urine sample</td>
<td>50</td>
<td>2(4%)</td>
</tr>
<tr>
<td>Blood sample</td>
<td>50</td>
<td>12(24%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>150</td>
</tr>
</tbody>
</table>

Table 2: Prevalence of Salmonella species and other bacteria in three different samples

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Stool</th>
<th>Urine</th>
<th>Blood</th>
<th>Total</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. Coli</td>
<td>21</td>
<td>30</td>
<td>1</td>
<td>52</td>
<td>51.5%</td>
</tr>
<tr>
<td>Staph. aureus</td>
<td>0</td>
<td>10</td>
<td>15</td>
<td>25</td>
<td>24.80%</td>
</tr>
<tr>
<td>Shigella</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>2.0%</td>
</tr>
<tr>
<td>Salmonella</td>
<td>8</td>
<td>2</td>
<td>12</td>
<td>22</td>
<td>21.8%</td>
</tr>
<tr>
<td>Total</td>
<td>35</td>
<td>42</td>
<td>24</td>
<td>101</td>
<td>67.3%</td>
</tr>
</tbody>
</table>

Total number of salmonella isolates obtained each sample source.

Table 3: prevalence of Salmonella species in three different samples

<table>
<thead>
<tr>
<th>Salm. Species</th>
<th>Stool</th>
<th>Urine</th>
<th>Blood</th>
<th>Total</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. typhi</td>
<td>5(38.5%)</td>
<td>2(15.4%)</td>
<td>6(46.2%)</td>
<td>13</td>
<td>59.1%</td>
</tr>
<tr>
<td>S. paratyphi A</td>
<td>2(33.3%)</td>
<td>0(0%)</td>
<td>4(66.7%)</td>
<td>6</td>
<td>27.3%</td>
</tr>
<tr>
<td>Other salmonella SPP</td>
<td>2(66.7%)</td>
<td>0(0%)</td>
<td>1(33.3%)</td>
<td>3</td>
<td>13.6%</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td>22</td>
<td>100%</td>
</tr>
</tbody>
</table>

Figure 1: Pie chart showing the percentage of different Species of Salmonella
Salmonella causing human disease are divided into human-restricted typhoidal serovars (Typhi and Paratyphi) causing typhoid fever, and non-typhoidal Salmonella (NTS) serovars which have a broader host-range and are frequently zoonotic Han et al. (2011). This study verified that out of 150 specimens (50 each from blood, urine and stool) analysed, 22 were positive for salmonella spp giving the overall prevalence of 14.7%. It was also observed from this study that blood sample yielded the highest number of salmonella isolates of 24%, followed by stool sample, but it was documented from this study that urine sample yielded the lowest percentage of Salmonella spp isolated. This result is in conformity with the work of (Melita (2011) and Kabir et al. (2007)) who also reported high positive results of salmonella from blood samples. From the result outlined above, it is clear that incidences of typhoid fever are relatively high, although less; when compared to some instances reported in Nigeria (Melita (2011) and Kabir et al. (2007)).

The results of the study indicated that the frequency of isolation of S. Typhi were 6 (46.2%) from blood sample, 5 (38.5%) were from stool sample and 2 (15.4%) were from urine samples. S. paratyphi A has the frequency of 4 (66.7%) from blood samples, 2 (33.3%) were from stool samples and none of the salmonella paratyphi A were isolated from urine samples. This result is in conformity with the study conducted by Okonko et al. (2010) where salmonella typhi and Salmonella paratyphi A has the highest occurrence from blood samples. This study also agrees with the work of Okonko et al. (2010) where they documented only few number of salmonella spp from stool samples than the blood samples.

The importance of Salmonellosis in public health sector is a growing concern day by day throughout the world and over the last several decades there have been significant shift in predominant Salmonella serovars associated with human infections Isa et al. (2013). Salmonellosis in the past has caused tremendous loss to society in many countries around the world. Two to four million of cases have been reported annually and yet a significant number of cases have been unreported worldwide. Non-typhoidal Salmonella is the leading cause of food borne illness and its increasing antimicrobial resistance is associated with higher risks of hospitalization Steven et al. (2011).

Out of the 22 patients Salmonella species isolated, 8 of them were actually having typhoid fever because the organisms (Salmonella) were isolated from both, the blood and stool sample of these patients, since their blood culture were positive. Isolation of Salmonella from blood is definitive proof of septicemia Han et al. (2011). The remaining four patients who are positive to Salmonella from their stool sample but negative to their blood culture samples may be carriers since the isolation of the organisms from faeces is not as valuable as blood culture in diagnosis Han et al. (2011).

CONCLUSION

From the above results of this study, it was concluded that blood samples yielded highest number of Salmonella spp when compare to stool and urine samples, this simply implies that, most of the patients are suffering from septicaemia caused by Salmonella spp. The results obtained also showed that out of 22 Salmonella species...
isolated, *Salmonella typhi* has the highest number of occurrence of 13 (59.1%), followed by *Salmonella paratyphi* and other *Salmonella* spp with the number of occurrence of 6 (27.3%) and (13.6%) respectively. Therefore, blood culture should be of priority for the diagnosis of the disease cause by *Salmonella* spp in this population.

RECOMMENDATION

1. The clinician (Medical Doctors) should be educated on the importance of confirmation of *Salmonella* infection before prescription of antibiotic in order to avoid the emergent of resistance strain to available sensitive drugs.
2. Further studies should increase the number of patients for the research and more samples apart from the one used should be included in future studies.
3. PCR and other modern identification procedures should be used for proper identification of the species and strain of *Salmonella*.

REFERENCES


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