SEROLOGICAL CHARACTERIZATION AND ANTIMICROBIAL SUSCEPTIBILITY PATTERNS OF CLINICAL ISOLATES OF SALMONELLA FROM PATIENTS ATTENDING GENERAL HOSPITAL, FUNTUA, NIGERIA

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INTRODUCTION

Salmonella is a genus of rod-shaped, Gram-negative, non-spore forming, predominantly motile bacteria that obtain their energy from oxidation and reduction reactions using organic sources. They are facultative anaerobes and most species produce hydrogen sulphide (Giannella, 1996).

The bacterial genus, Salmonella is divided into two species, Salmonella bongori and S. enterica. S. enterica itself comprised of six subspecies viz; S. enterica subsp. enterica, S. enterica subsp. salamae, S. enterica subsp. arizonae, S. enterica subsp. diarizonae, S. enterica subsp. indica, and S. enterica subsp. houtaeae, or I, II, IIIa, IIIb, IV, and VI, respectively (Porwollik et al., 2003). Of these six subspecies, only subspecies I is associated with disease in warm-blooded animals. To date, there exist over 2,300 serovars identified within subspecies I. However, only a small fraction of the thousands of described subspecies I serovars frequently cause disease in humans and domestic animals. For example, the annual report of the Centers for Disease Control and Prevention (CDC) registered 360 different serovars in human infections in the U.S. in 2009. Approximately 50% of these infections were caused by only three Salmonella serovars, specifically S. typhimurium, S. enteritidis and S. newport. The 12 most prevalent Salmonella serovars were responsible for >70% of all human Salmonella infections (McClelland et al., 2004).

Typhoidal salmonellosis and non-typhoidal salmonellosis are major health problems in developing countries in which Nigeria belongs. The problems, according to many findings are associated with unhygienic processing and preparation of foods, substandard water supply, inadequate sanitary measures and emergence of multidrug resistance in some Salmonella strains (Threlfall and Ward, 2001 and Wright et al., 2005). Contaminated food is the major source of transmission of salmonellosis (Guithrie, 1991). Frequency of occurrence is greatest in foods which are not cooked or have been incompletely cooked, such as roasted meat (in which Suya and Balangu belong) or roasted chicken. Eating of half cooked Suya or Balangu processed at road side under unhygienic condition is a common practice in Nigeria, there by exposing the consumers to Salmonella infections (Wright et al, 2007).
The collapse of primary healthcare system couple with the unavailability of drugs in hospitals in this country has resulted in most people resorting to purchasing drugs on counters and in some cases from road side sellers instead of going to the hospitals there by exposing them to the danger of acquiring multidrug resistant (MDR) microorganisms (Akinyemi et al, 2007).

S. typhi causes typhoid fever (enteric fevers) only in humans. Other serotypes, namely, non-typhoidal Salmonella serotypes, such as S. typhimurium, S. enteritidis and S. cholerasuis can cause a wide spectrum of diseases in human and animals, such as acute gastroenteritis, bacteremia, and extraintestinally localised infection involving many organs (Lin-Hui et al., 2004). A syndrome similar to typhoid fever is caused by paratyphoidal serotypes of Salmonella. The paratyphoid serotypes (i.e., S. paratyphi A, S. paratyphi B, and S. paratyphi C) are isolated less frequently than S. typhi (Mindy, 2003).

Like other developing countries, treatment of patients in Nigeria has been based on the use of first line (conventional) antibiotics, such as ampicillin, chloramphenicol and co-trimoxazole and the third generation cephalosporins. However, efficacies of some of these drugs have been doubtful, following the emergence of multidrug resistance in Salmonella strains (Akinyemi et al., 2000). Flibourquinolones have been found to be efficacious both in vitro and in vivo in the treatment of several Salmonella-associated illnesses, although strains with reduced susceptibility to ciprofloxacin among travellers have been reported in some parts of the globe (Hakanen et al., 2001). So far as we know there has been no reported data on characterization and distribution of Salmonella serotypes and their antimicrobial resistivity patterns from patients diagnosed to be suffering from Salmonella infection in General Hospital, Funtua. This form the basis for the serological characterization and antimicrobial susceptibility patterns of Salmonella isolates implicated in typhoidal and non-typhoidal salmonellosis in General hospital, Funtua, Katsina state of Nigeria.

MATERIALS AND METHODS

The samples were collected from patients diagnosed by clinicians of having either pyrexia, gastroenteritis or both. Both blood and stool were collected from each patient. Three millimetre of blood and a loopful of fresh stool collected from each patient were directly inoculated into 27ml of trypticase soy broth (PRO-LAB Diagnostic, USA) and 9ml of selenite-F broth (PRO-LAB Diagnostic, USA) contained in bijou bottle respectively. The tubes containing the samples were transported to Biological sciences Laboratory of Umaru Musa Yar’adua University, Katsina, and incubated for 24 hours at 37°C.

Isolation and identification of Salmonella spp.

A loopful of the culture in tripticase soy broth and selenite-F broth were streaked seperately on Xylose Lysine Deoxycholate (XLD) agar, Brilliant Green Agar (BGA), MacConkey (MC) agar, Deoxycholate Citrate (DC) agar and Salmonella-Shigella (SS) agar. Cultures were incubated for 24 hours at 37°C, and colonies grown on the plates were selected for biochemical characterization as described by Barrow and Feltham (1993). The commercially-available identification system-API 20E (bio Mereux, France) was used.

Serotyping of identified Salmonella species

Colonies considered to be of Salmonella spp. were further tested for somatic (O) and flagella (H) antigens with polyvalent antisera (Serotype-Lab, Thailand).

Antibiotic susceptibility testing

Antibiotic susceptibility tests were carried out on the Salmonella serotypes. Seven antibiotics were screened using disc diffusion methods on Mueller-Hilton Agar. Three colonies were inoculated into a tube containing tryptic soy broth (PRO-LAB Diagnostic, USA ) and incubated at 37°C. Standardization of the inocula was performed. The turbidity of the inocula was adjusted to match that of 0.5 McFarland standard. Within 15 minutes of preparing the adjusted inocula, a sterile cotton swab was dipped into the inocula. The swab was rotated several times and pressed firmly on the inside wall of the tube above the fluid level to remove excess inocula from the swab. The surface of the Mueller-Hilton Agar plate was inoculated by running the swab around the rim of the Agar. Sterile forceps was used to dispense the single discs (PRO-LAB Diagnostic, USA) onto the Mueller-Hilton Agar surface. The standard discs used for susceptibility testing include ofloxacin (20µg), nalidixic acid (30µg), ciprofloxacin(20µg), co-trimoxazole (25µg), ampicillin (25 µg), chloramphenicol (30µg) and third generation ceftazidime(25µ). After 24 hours of incubation at 37°C, zones of inhibition were measured and recorded with the help of vernier caliper in millimeters (mm). The standard zones of inhibition interpretation chart by WHO (2010) was used to interpret the zones of inhibition. Escherichia coli ATCC 25922 was used as control.

RESULTS

Out of the 240 samples of both blood and stool screened, 29 Salmonella were isolated, amounting to 12.1% incidence rate. Based on the symptoms manifestation and information extracted from the questionnaire, nineteen Salmonella isolates were implicated in typhoidal salmonellosis and ten were implicated in non-typhoidal salmonellosis. Therefore, of the 29 cases of salmonellosis, 65.5% typhoidal salmonellosis and 34.5% of non-typhoidal salmonellosis were recorded. Sixteen S. typhi were isolated, accounting for 55.2% of the total number of the isolates. Of the 16 S. typhi isolates, 8(50%) were recovered from blood only, 2(12.5%) from stool only while 6(37.5%) from both blood and stool of the patients. All the 3 S. paratyphi A isolated were recovered from blood. Both S. enteritidis and S. typhimurium were recovered from stool only (Table.1).
The *S. typhi* were recovered from patient belonging to age-groups 0-5, 6-15, 16-30 and ≥46 yrs. Three (10.3%) *S. paratyphi A* were isolated and all were implicated in typhoidal salmonellosis. The *S. paratyphi A* were found among age-group 6-15yrs. Seven (24.1%) *S. enteritidis* were isolated and all were implicated in non-typhoidal salmonellosis, distributed among age-groups 0-5 and 6-15yrs. Three (10.3%) *S. typhimurium* were isolated and implicated in non-typhoidal salmonellosis which were isolated among age-group 0-5 years. Twelve (41.4%) of the total isolates were recovered from each of the age-group 0-5yrs and 6-15yrs. Therefore, 82.8% of the total cases were associated with children while 17.2% cases were associated with adults (Table 2). Males accounted for 19(65.5%) cases of salmonellosis while females accounted for 10(34.5%) cases (Fig. 1). In this study, *S. typhi* and *S. paratyphi A* were 100% resistant to ampicillin, 93.8% and 66.7% respectively resistant to chloramphenicol while resistance to cotrimoxazole were 37.5% and 66.7% respectively. The resistance of both serotypes to fluoroquinolones (ciprofloxacin and ofloxacin) was not observed. Resistances to Ampicillin by *S. enteritidis* and *S. typhimurium* (non-typhoidal salmonellae) were 57.1% and 100% respectively while that of both the non-typhoidal salmonellae to Fluoroquinolones was not observed (Figure 2).

**Table 1. Cases of Salmonellosis and their Implicated Serotypes.**

<table>
<thead>
<tr>
<th>Serotype</th>
<th>No. of Isolates</th>
<th>% of Isolates from Samples</th>
<th>% of Salmonellosis cases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>bld</td>
<td>stl</td>
<td>bl/st</td>
</tr>
<tr>
<td><em>S. typhi</em></td>
<td>16</td>
<td>8(50)</td>
<td>2(12.5)</td>
</tr>
<tr>
<td><em>S. paratyphi A</em></td>
<td>3</td>
<td>3(100)</td>
<td>0(0)</td>
</tr>
<tr>
<td><em>S. enteritidis</em></td>
<td>7</td>
<td>0(0)</td>
<td>7(100)</td>
</tr>
<tr>
<td><em>S. typhimurium</em></td>
<td>3</td>
<td>0(0)</td>
<td>3(100)</td>
</tr>
<tr>
<td>Total</td>
<td>29(12.1)</td>
<td>11(37.9)</td>
<td>12(41.4)</td>
</tr>
</tbody>
</table>

**Keys:** TS=Typhoidal salmonellosis. NTS= Non-typhoidal salmonellosis. bld= Blood. stl= Stool. bl/st= Blood/Stool. Figures in parentheses indicate percentages.

**Table 2. Distribution of Salmonella Serotypes in relation to Age Groups of patients**

<table>
<thead>
<tr>
<th>Age-group (Yrs)</th>
<th><em>S. typhi</em> TS</th>
<th>NTS</th>
<th><em>S. paratyphi</em> A TS</th>
<th>NTS</th>
<th><em>S. enteritidis</em> TS</th>
<th>NTS</th>
<th><em>S. typhimurium</em> TS</th>
<th>NTS</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHILDREN</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-5</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>6-15</td>
<td>7</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>24</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>3</td>
<td></td>
<td>12(41.4)</td>
</tr>
<tr>
<td>ADULTS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16-30</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>31-45</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>≥ 46</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>5</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>7</td>
<td>0</td>
<td>3</td>
<td></td>
<td>5(17.2)</td>
</tr>
</tbody>
</table>

**Keys:** TS= Typhoidal Salmonellosis; NTS= Non-Typhoidal Salmonellosis; Yrs= Years; n=Number of cases; Figures in parentheses indicate percentages.
Figure 1. Cases of Salmonellosis with Respect to Sex

DISCUSSION
Twenty nine Salmonella strains, which include 11(37.9%) from blood and 6(20.7%) from both blood/stool of patients with pyrexia and 12(41.4%) from stool samples of patients with gastroenteritis, were isolated in this study, giving an incidence of 12.1% of Salmonella-associated illness for the period of 8-months. The results of the study indicated that the frequency of isolation of *S. typhi* (55.2%) from patients with pyrexia was higher than that of *S. paratyphi A* (10.3%). The frequency of isolation of *S. enteritidis* (24.1%) from patients with gastroenteritis was higher than that of *S. typhimurium* (10.3%). Therefore, *S. typhi* was more frequently isolated in typhoidal salmonellosis than *S. paratyphi A* and *S. enteritidis* was more frequently isolated in non-typhoidal salmonellosis than *S. typhimurium*. This finding conformed with the report of Akinyemi *et al.* (2007) who reported higher frequency of *S. typhi* and *S. enteritidis* in typhoidal and non-typhoidal salmonellosis respectively in Lagos. Mindy, (2003), also reported that paratyphi association in typhoidal salmonellosis is less frequent than *S. typhi*. This may be as a result of the fact that *S. typhi* possesses capsular polysaccharides (Vi antigen) (Boyd *et al.*, 1993), which make it to be more virulent than other serotypes implicated in typhoidal- salmonellosis.
This study also showed that typhoidal salmonellosis 19(65.5\%) cases, are more prevalent than non-typhoidal salmonellosis 10(34.5\%) cases. This is in agreement with the findings of Sood et al., (1999) on "Salmonellosis in developing countries". Thr elfall and Ward, (2001), reported decrease in cases of typhoidal salmonellosis in developed countries (due to adequate sanitary measures). However, in these countries, non-typhoidal salmonellosis is more common and most of these cases are associated with food contaminated by Salmonella (Wright et al., 2007). The differences in the pattern of salmonellosis in developed and developing countries may be as a result of unavailability of portable drinking waters and cultural habits of eating overcooked food in Africa in which Funtua belongs. Out of the 29 cases of salmonellosis in Funtua, children accounted for 24(82.8\%) while adults accounted for 5(17.2\%). This trend appears in agreement with the results of similar studies from Hong Kong(Ling et al., 1991) and Saudi Arabia(Kambal, 1996). However, the present study showed a much higher than usual proportion of isolates from children compared with that of Saudi Arabia where the prevalence is 60\% and 40\% in children and adults respectively (Kambal, 1996). Of the total cases of Salmonellosis, 65.5\% were from male and 34.5\% from female. This is in agreement with report of Kambal (1996) and it may be due to the fact that male (children) are more exposed to infections because they tend to eat more of road side and fast foods compared to their female counterparts who are more reserved and for cultural reason are unexposed to outdoor activities. Most reported cases of Salmonellosis are due to contaminated food and water (Threlfall and Ward, 2001 and Wright et al., 2005).

Resistence to ampicillin, chloramphenicol, cefotaxime and co-trimoxazole was observed in this study which could be as a result of indiscriminate use of these drugs in poultry and humans. The inclusion of preventive doses of antimicrobial agents in poultry feed as growth promoters is often associated with the development of resistance in enteric bacterial flora of poultry. These resistant bacteria contribute to the reservoir of resistant bacteria found in the human intestinal tract including resistant Salmonella (Dupont and Steele, 1987). During this study, 89.7\%, 82.8\% and 31.0\% of the total Salmonella isolates were resistant to ampicillin, chloramphenicol and co-trimoxazole respectively. This is not surprising because these are most commonly used drugs in human and poultry(Threlfall et al., 2001). The use of antimicrobials for growth-promotion, prophylaxis and treatment of animal’s food increases the prevalence of resistance in human pathogens, particularly non-typhoidal salmonellosis (Singer et al., 2003). Out of the 29 isolates, 6(20.7\%) were resistant to cefotaxime. This is in contrary to reports by Campos et al. (1990) and, Sonstein and Burnham (1993) where high percentage of Salmonella isolates were sensitive to cefotaxime. This may be as a result of indiscriminate use of this antibiotics. This trend is of particular concern because the extended spectrum cephalosporins are the antibiotics of choice for children(Weill et al., 2004). High susceptibility of Salmonella spp. was observed against fluoroquinolones (ofloxacin and ciprofloxacin). Despite the fact that 2(6.9\%) of the isolates were resistant to nalidixic acid, 100\% of them were sensitive to ofloxacin and ciprofloxacin. This finding is similar to a report by Campos et al. (1990) and Sonstein and Burnham (1993). However, in Lagos, Nigeria, Akinyemi et al. (2007) reported 18\% reduced susceptibility of Salmonella spp. to ofloxacin and ciprofloxacin. The high susceptibility of Salmonella spp. to fluoroquinolones recorded in this study may be connected to relatively expensive cost of ciprofloxacin and ofloxacin. Therefore, fluoroquinolones are not used indiscriminately because not many could afford them. This is the first study on antimicrobial susceptibility pattern of Salmonella species in Funtua, Nigeria (to the best of our knowledge). There was no observation of reduced susceptibility of these organisms to fluoroquinolones compared to Asia and United Kingdom and some other Europeans countries where resistance to fluoroquinolones have been recorded most likely due to approval of these drugs for use in animals (WHO, 1998). Though there was report of reduced susceptibility of Salmonella spp. to fluoroquinolones in Lagos, by Akinyemi et al. (2007), differences in drug use patterns in the two geographically distinct areas may account for the differences in the observed susceptibility patterns.

CONCLUSION
In conclusion, majority of the Salmonella isolated were from children with S. typhi accounting for the highest percentage of the isolates.

Recommendations
More importantly, there is need for the Government to enforce the existing laws that prohibit smuggling of all kinds of animal products and the sale of drugs by unauthorized people.

Acknowledgment
The authors acknowledge and appreciate the moral and financial support given by Umaru Musa Yar'adua University, Katsina.

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Bajopas Volume 5 Number 1 June, 2012


