Antibiotics susceptibility patterns and characterization of clinical Salmonella serotypes in Katsina State, Nigeria

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Serotyping characterization and antimicrobial susceptibility of Salmonella clinical isolates were examined for a period of 10-months to study the most frequently encountered serovars in salmonellosis and their antimicrobial susceptibility patterns. Seven hundred and twenty (720) samples of both stool and blood specimens were collected from out patients attending three hospitals in Katsina State, Nigeria. The samples were collected from patients diagnosed by clinicians with either pyrexia, gastroenteritis or both. Samples were cultured; isolates were identified and antibiotic susceptibility test was performed using standard procedures. The total number of 108 (15%) of the 720 samples collected yielded positive for Salmonella strains. Out of the 108 isolates, 61 (56.5%) were responsible for typhoidal salmonellosis, while 47(43.5%) were responsible for non-typhoidal salmonellosis. Of the 108 cases of salmonellosis, 91(84.3%) were from children and 17(15.7%) from adults. S. Typhi (40.7%) was the most frequently encountered, followed by S. Enteritidis (26.9%) and least encountered was S. Arizonae (2.8%). There was no significant difference in the serotypes isolated from each of the hospitals with respect to the type of salmonellosis caused with their p values > 0.05. Of the total isolates, 94.2% were found to be resistant to ampicillin, 22.2% resistant to cefotaxime, 72.8% resistant to chloramphenicol, 31.8% resistant to co-trimoxazole and 4.9% resistant to nalidixic acid. However, resistance to ofloxacin and ciprofloxacin by the isolates were not found. Therefore, ofloxacin and ciprofloxacin remains the drug of choice for severe cases of salmonellosis, although caution should be exercised by clinicians in their prescriptions such that fluoroquinolones antibiotic therapy is used only in laboratory-proven cases of typhoid fever and Salmonella-associated bacteraemia to preserve its efficacy.

Key words: Antimicrobial, fluoroquinolones, Salmonella, salmonellosis, susceptibility, resistance.

INTRODUCTION

Salmonella is a bacterial genus, divided into two species, Salmonella bongori and Salmonella enterica. S. enterica itself is comprised of six subspecies: they are S. enterica subsp. enterica, S. Enteric subsp. salamae, S. enterica subsp. arizonae, S. Enteric subsp. diarizonae, S. enterica subsp. indica, and S. Enteric subsp. houtenae, 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 are 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) for the year 2009 registered 360 different serovars in human infections in the U.S. Approximately 50% of these infections were caused by only three Salmonella serovars, specifically Salmonella Typhimurium, Salmonella Enteritidis and Salmonella 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 preparing of foods, substandard water supply, inadequate sanitary measures and emergence of multidrug resistance in some Salmonella strains. 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 belongs) 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.

The collapse of primary healthcare system couple with the unavailability of drugs in hospitals in this country has resulted in most people resulting to purchasing drugs in some cases from road side sellers instead of going to the hospitals; thereby exposing themselves to the danger of acquiring multidrug resistant (MDR) microorganisms.

S. Typhi causes typhoid fever (enteric fevers) only in humans. Other serotypes, namely, nontyphoidal Salmonella serotypes, such as S. Typhimurium, S. Enteritidis and S. Choleraesius can cause a wide spectrum of diseases in human and animals, such as acute gastroenteritis, bacteremia and extraintestinal localised infections involving many organs (Lin-Hui et al., 2004). A syndrome similar to typhoid fever is caused by paratyphoidal serotypes of Salmonella. The paratyphoid serotypes (S. Paratyphi A, S. Paratyphi B and S. Paratyphi C) are isolated more 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). Fluoroquinolones have been found to be efficacious both in vitro and 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). The scope of this study include characterization and antimicrobial susceptibility patterns of Salmonella serovars implicated in typhoidal and non-typhoidal salmonellosis in some selected hospitals of Katsina, Nigeria.

MATERIALS AND METHODS

Patient population, case definition, samples collection and enrichment of specimen for isolation

This study was conducted on 720 samples of both blood and stool of patients attending General Hospital, Funtua, General Hospital, Katsina and Turai Umaru Yar’adua Maternity and Children Hospital, Katsina State, Nigeria. The samples were collected from patients diagnosed by clinicians with either pyrexia, gastroenteritis or both. Both blood and stool were collected from each patient. This study was conducted from April, 2010 to February, 2011. Important bio-data, history of vaccination, antimicrobial therapy, time of onset of illness, etc. of these patients were recorded. Three millimeters of blood and a loopful of fresh stool collected from each patient were directly inoculated onto 27 ml of tryptic soy broth (PRO-LAB Diagnostic, USA) and 9 ml of Selenite-F Broth (PRO-LAB Diagnostic, USA) contained in bijou bottle, respectively. The tubes containing the samples were transported to Biological Laboratory of Umar Musa Yar’adua University, Katsina, and incubated overnight at 37°C for bacteriological culture.

Isolation and identification of Salmonella spp. based on cultural and biochemical characteristics

A loopful of the culture in triptic 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 overnight at 37°C, and colonies growing on the plates were selected for biochemical confirmation based on morphological and biochemical properties exibited. Colonies were first subjected to biochemical test as described by Cowan and Steel (1993). The comercially-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).

Antibiotics susceptibility testing

Antibiotic susceptibility tests were carried out on the Salmonella serotypes. Seven antibiotics were screened using disc diffusion methods of WHO (2010) on Mueller-Hinton Agar. Three colonies were inoculated onto 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 min 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-Hinton Agar plate of 15 cm was streaked using the swab. Inoculation was completed 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-Hinton Agar surface. The discs were ensured to make complete contact with the agar surface by torching the top of the
Table 1. Number and percentages of Salmonella isolated with respect to the type of disease caused.

<table>
<thead>
<tr>
<th>Hospitals</th>
<th>No. of samples</th>
<th>Salmonella isolates</th>
<th>TS</th>
<th>NTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>GHK</td>
<td>240</td>
<td>38 (15.8)</td>
<td>24 (63.2)</td>
<td>14 (36.8)</td>
</tr>
<tr>
<td>GHF</td>
<td>240</td>
<td>29 (12.1)</td>
<td>19 (65.5)</td>
<td>10 (34.5)</td>
</tr>
<tr>
<td>TMC</td>
<td>240</td>
<td>41 (17.1)</td>
<td>18 (43.9)</td>
<td>23 (56.1)</td>
</tr>
<tr>
<td>Total</td>
<td>720</td>
<td>108 (15)</td>
<td>61 (56.5)</td>
<td>47 (43.5)</td>
</tr>
</tbody>
</table>

TS = Typhoidal salmonellosis; NTS = non-typhoidal salmonellosis; GHK = General Hospital Katsina; GHF = General Hospital Funtua; TMC = Turai Umaru Yar’adua Maternity and Children Hospital. Figures in parenthesis indicate percentages.

Table 2. Total number and percentages of Salmonella serotypes isolated from the three hospitals.

<table>
<thead>
<tr>
<th>n</th>
<th>Salmonella serotypes no. (%)</th>
<th>ND</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S. Typhi</td>
<td>S. Paratyphi A</td>
</tr>
<tr>
<td>108</td>
<td>44 (40.7)</td>
<td>14 (13)</td>
</tr>
</tbody>
</table>

n = Number of Isolates. Figures in parenthesis indicate percentages; ND = untypeable.

RESULTS

From the 720 samples of both blood and stool screened, 108 Salmonella were isolated, amounting to 15% incidence rate. Based on the symptoms, manifestations and information extracted from the questionnaire, sixty one Salmonella isolates were implicated in typhoidal salmonellosis and 47 were implicated in non-typhoidal salmonellosis. Therefore, of the 108 cases of salmonellosis, 56.5% (typhoidal salmonellosis) and 43.5% (non-typhoidal salmonellosis) were recorded (Table 1). Forty four S. Typhi were isolated, accounting for 40.7% of the total number of the isolates.

Fourteen (13%) S. Paratyphi A and 3 (2.8%) S. Arizonae were isolated. In the case of non-typhoidal salmonellae, 29 (26.9%) of S. Enteritidis and 14 (13%) of S. Typhimurium were isolated. However, 4 (3.7%) of the isolates were untypeable (Table 2). Age-group 0 to 5 years had the highest number of isolates, followed by age-group 6 to 15yrs. Age-group ≥46 years had the lowest number of isolate (Figure 1). Of the 44 S. Typhi isolated, 39 (88.6%) were implicated in typhoidal salmonellosis, while 5 (11.4%) were implicated in non-typhoidal salmonellosis. All the 14 and 3 S. Paratyphi A and S. Arizonae respectively isolated were implicated in typhoidal salmonellosis. Of the 29 S. Enteritidis isolated, 1 (3.4%) was implicated in typhoidal salmonellosis, while 28 (96.6%) were implicated in non-typhoidal salmonellosis. All the 14 S. Typhimurium isolated were implicated in non-typhoidal salmonellosis. The four untypeable isolates were all implicated in typhoidal salmonellosis (Table 3). Male accounted for 19 (59.3%) cases of salmonellosis, while female accounted for 10 (40.7%) cases (Figure 2).

In this study, of the total isolates (108), 94.2% were resistant to ampicillin, 72.8% were resistant to chloramphenicol, 31.8% were resistant to co-trimoxazole, 22.2% were resistant to cefotaxime and 4.9% were resistant to nalidixic acid. However, resistance to fluoroquinolones (ofloxacin and ciprofloxacin were not encountered (Table 4).

S. Typhi, S. Paratyphi A and S. Arizonae were 100% resistant to ampicillin, 95.5, 92.9 and 100% resistant to chloramphenicol respectively, while resistance to cotrimoxazole were 61.4, 42.9 and 0%, respectively. Resistance to ampicillin by S. Enteritidis and S. Typhimurium (non-typhoidal salmonellae) were 82.8 and 92.9% respectively, while resistance to chloramphenicol were 41.4 and 42.9%, respectively. The resistance of all the serotypes to fluoroquinolones (ciprofloxacin and ofloxacin) was not observed (Figure 3).

DISCUSSION

Out of the total samples (720) of both blood and stool collected in the three Hospitals, 108 Salmonella were isolated in this study, giving an incidence rate of 15% of salmonella-associated illness for the period of 11-months. Of the 108 cases of salmonellosis, 61 (56.5%) were typhoidal salmonellosis and 47 (43.5%) were non-
typhoidal salmonellosis. This showed that typhoidal salmonellosis is more prevalent than non-typhoidal salmonellosis and it is in agreement with the findings of Sood et al. (1999) on “Salmonellosis in developing countries”.

Threlfall and Ward (2001) reported a 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 Salmonellae. 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 Katsina belongs. The results of this study indicated that the frequency of isolation of 44 (40.7%) S. Typhi from patients with pyrexia and gastroenteritis was higher than 14 (13%) S. Paratyphi A and 3 (2.8%) S. Arizonae from patients with pyrexia only. The frequency of isolation of 29 (26.9%) S. Enteritidis from patients with gastroenteritis was higher than 14 (13%) of S. Typhimurium. The higher number of salmonella recorded against age-group 0 to 5 years and 6 to 15 years indicated that salmonellosis manifested more in children than adults. No significant relationship between the ages and the isolated serotypes implicated

Table 3. Number and percentages of serotypes implicated in salmonellosis.

<table>
<thead>
<tr>
<th>Serotype</th>
<th>No. Isolated</th>
<th>Salmonellosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>TS</td>
</tr>
<tr>
<td>S. Typhi</td>
<td>44</td>
<td>39</td>
</tr>
<tr>
<td>S. Paratyphi A</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>S. Arizonae</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>S. Enteritidis</td>
<td>29</td>
<td>1</td>
</tr>
<tr>
<td>S. Typhimurium</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td>ND</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>108</td>
<td>61</td>
</tr>
</tbody>
</table>

Numbers in parenthesis indicate percentages. ND = Untypeable.
in both typhoidal and non-typhoidal salmonellosis as \( p > 0.05 \). This trend appears in agreement with the results of similar studies from Saudi Arabia (Kambal, 1996) and Hong Kong (Ling et al., 1991). However, the present study shows a much higher than usual proportion of the isolates from children when compared with that of Saudi Arabia where the prevalence was 60 and 40% in children and adults, respectively (Kambal, 1996). S. Typhi was more frequently isolated in typhoidal Salmonellosis than S. Paratyphi A and S. Arizonae or any other serotype in this study. 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. S. Enteritidis was more frequently isolated in non-typhoidal salmonellosis than S. Typhimurium. There was no significant difference in S. Typhi, S. Paratyphi A, S. Enteritidis and S. Typhimurium isolated from each of the hospitals with respect to the type of salmonellosis caused with \( p > 0.05 \).

This finding conformed with the report of Akinyemi et al. (2007) which showed 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. Of the total cases of Salmonellosis, 59.3% were from male and 40.7% from female. This is in agreement with report of Kambal (1996) and it may be due to the fact that male are more exposed to infections because they tend to eat more of road side
Figure 3. Percentages of antibiotics resistant Salmonella serotypes.

and fast foods as compared to their female counterparts who are more reserved and for cultural reasons are unexposed to outdoor activities. Most reported cases of Salmonellosis are due to contaminated food and water (Threlfall and Ward, 2001).

Resistance to ampicillin, chloramphenicol, cefotaxime and co-trimoxazole was observed in this study. The resistance could be as a result of indiscriminate use of antibiotics. 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, 94.2, 72.8 and 31.8% 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 usually subsides without antibiotics treatment especially in immune competent patients. For this reason, the implicated organisms (non-typhoidal Salmonellae) are not usually exposed to these antibiotics as compared to S. Typhi and Paratyphi A. In the case of typhoidal Salmonellae, there was resistance to all the typhoidal salmonellosis (Singer et al., 2003). Out of the 108 isolates, 24 (22.27%) were resistant to cefotaxime. This is contrary to reports by Campos et al. (1990) and Sonstein and Burnham (1993) where high percentages of Salmonella isolates were sensitive to cefotaxime. 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 (4.9%) of the isolates were resistant to nalidixic acid, 100% of them were susceptible 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 antibiotics resistance of the non-typhoidal Salmonellae (S. Enteritidis and S. Typhimurium) to co-trimoxazol and cefotaxime was quit low as compare to typhoidal Salmonellae but in the case of ampicillin and chloramphenicol, the resistance was high. This may be as a result of the fact that non-typhoidal conventional antibiotics and the third generation cephalosporin. All the serotypes were susceptible to the fluoroquinolones. The high susceptibility of the serovars to fluoroquinolones recorded in this study may be connected to relatively high cost of ciprofloxacin and ofloxacin (Akinyemi et al., 2007). Therefore, fluoroquina-
nolones are not used indiscriminately because not many could afford them.

This is the first study on antimicrobial susceptibility pattern of *Salmonella* species in Katsina, Nigeria (to the best of our knowledge). There was no observation of reduced susceptibility of these organisms to fluoroquinolones as 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 geographical distinct areas may account for the difference in the observed susceptibility patterns.

In conclusion, majority of the *Salmonella* isolated were from children with S. Typhi accounting for the highest percentage of the isolates. This may indicate the non-portability of drinking water children are exposed to, because 80% of those who had typhoid fever got their drinking water from untreated surface water sources. Apart from contaminated water, faecal-oral route of transmission may also be a reason for the preponderance of salmonellosis in children. Majority of the *Salmonella* isolated were S. Typhi. This is the most commonly isolated serotypes in developing countries. It accounts for majority of the resistant isolates in this study. The resistance to first line antibiotics (ampicillin, chloramphenicol, and co-trimoxazole) was high. This may be as a result of misuse of these antibiotics in human infection treatment and animal feeds as growth promoters.

Though some *Salmonella* serotypes were found to be resistant to nalidixic acid, resistance to ofloxacin and ciprofloxacin were not recorded which may be due to less frequent use of fluoroquinolones in infection treatment because of their costs. Despite the resistance recorded against nalidixic acid, fluoroquinolones still remained the drug of choice but caution should be taken by clinicians to avoid resistance of *Salmonella* to fluoroquinolones (only in severe cases of salmonellosis should fluoroquinolones be prescribed). 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.

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REFERENCES


