Antimicrobial susceptibility pattern of *Salmonella* species isolated from blood and urine

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**Abstract:** This study was aimed at isolation, identification and antimicrobial susceptibility characterization of isolates of *Salmonella* species from blood and urine culture of outpatients attending hospital whose clinical history of fever fell within a period of first to fourth week of infections. A total of 24 (17.52%) isolates identified as *Salmonella* species by using cultural, biochemical and serological analysis were recovered from 137 samples (75 blood samples and 62 urine samples). Predominant isolates were *S. typhi* 16 (11.7%), followed by *S. paratyphi* A 6 (4.38%) and *S. paratyphi* C 2 (1.45%). Isolates were from all age groups studied, the median age being 30 years. There were 58 (42.3%) male and 79 (57.7%) female outpatients and the male to female ratio was 1:1.36. Infection was more prevalent in the age group 6-15years 6 (8.0%) followed by age group above 46 years 3 (5.30%) in blood cultures. Meanwhile, in urine cultures prevalence was highest in the age group above 46 years (3 (4.84%)) followed by age group 26-35 years (2 (3.20%)). Antimicrobial susceptibilities were determined by Kirby-Bauer disc diffusion method using 6 different antimicrobial agents suggested by Clinical & Laboratory Standards Institute (CLSI). The antimicrobial susceptibility testing showed that the 16 (100%) *Salmonella typhi* isolates were sensitive to Ciprofloxacin, 15 (93.8%) to Ceftriaxone, 14 (87.5%) to Chloramphenicol and 12 (75%) to Ampicillin. On the other hand, 10 (62.5%) were resistant to Tetracycline, and 12 (75%) resistant to Co-trimoxazole. Out of 6 *Salmonella paratyphi* Aisolates, all the 6 (100%) were susceptible to Ciprofloxacin and 5 (83.3%) susceptible to Ceftriaxone, 4 (66.7%) susceptible to Chloramphenicol while 4 (66.6%) were resistant to Tetracycline and Co-trimoxazole. Furthermore, 2 (100%) of *Salmonella paratyphi* C were susceptible to Ciprofloxacin and Ceftriaxone. The finding indicates that Ciprofloxacin can be used as a first line therapy for treatment and the need of blood culture, along with the urine culture and clinical history should be taken into consideration for an accurate diagnosis of enteric fever caused by *Salmonella* spp.

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**1. Introduction**

Salmonellae are Gram negative, non-lactose fermenting and non-sporing bacteria. With exception of *Salmonella pullorum-gallinarum*, all salmonellae are actively motile. They are also, non-capsulated with the exception of *Salmonella typhi* belonging to the family Enterobacteriaceae (Cheesbrough, 2002; Perilla et al., 2003). Salmonellae can be divided into two major groups of clinical importance: Group one, includes members of the genus that are involved as etiologic agents of enteric fever (typhoidal salmonellosis): *S. typhi* and *Salmonella paratyphi.* Group two, includes members of the genus that are involved as etiologic agents of food poisoning (non-typhoidal salmonellosis): *Salmonella typhimurium and Salmonella cholerae-suis*. Other members are *Salmonella enteritidis, Salmonella heidelberg, Salmonella agona, Salmonella newport, Salmonella hadar, Salmonella thompson, Salmonella virginia, Salmonella anatum,* and *Salmonella dublin* (Adkins and Santiago, 2006; Arora, 2006). Non-typhi Salmonellae (NTS) particularly *S. typhimurium* and less frequently *Salmonella enteritidis* are common causes of bacteraemia and septicaemia in young children in developing countries (Cheesbrough, 2002; Vieira et al., 2009). Gastroenteritis/salmonellosis is an infection caused by ingesting salmonellae (over 2,300 serovars/types other than *S. typhi*, and the Paratyphis) in food that is contaminated by faeces of animals or humans directly or indirectly.

It is a serious problem, of public health significance worldwide (Tabaraie et al., 1994) and causes substantial economic loss resulting from mortality, morbidity and poor growth, with the hazard of transmitting food poisoning with gastroenteritis to human. It also represents a serious problem for the food industry (Khan et al., 2007). Human stool acts as an important reservoir of *Salmonella* serovars that are the grouping of microorganisms based on their cell surface antigen.

Common sources of infection include poultry meat and meat products, eggs and egg products. Some of the symptoms of salmonellosis are diarrhoea, vomiting, fever and abdominal pain; these occur 12-36 hours after eating infected food. In acute infection, blood and mucous are present in faecal specimens (Al – jurayyan et al., 2004).

People most at risk for serious complications due to *Salmonella* food poisoning include older adults, pregnant women, infants, children, and people who have compromised immune systems. Salmonellosis is manifested clinically in all hosts by one of three major syndromes, per acute systemic infection, acute enteritis or chronic enteritis (Merchant and Packer, 1967).

Salmonellae can be isolated from blood, stool, urine, bone marrow, duodenal aspirates and rose spots. From blood, the organisms can usually be detected in 75-90% of patients during the first ten days of infection, and in about 30% of patients during the third week (Cheesbrough, 2002). The risk to salmonellosis is increased due to the following factors; absence of effective vaccines, modifying hand washing behaviour after defecating to control prolonged community out breaks and identifying high risk groups and targeting prevention measures (Perilla et al., 2003).

Increasing antimicrobial resistance is a worldwide concern. The prevalence of resistance in both out-patients and hospitalised patients with Salmonella infection is increasing, and it varies in accordance with geographical and region allocation. Moreover, recent studies indicate that the proportion of *S. paratyphi* is increasing over the years in the subcontinent (Mohanty et al., 2006; Padmapriya et al., 2003).Therefore, it is important to know the species of *Salmonella* that are prevalent in a country and the antibiotic susceptibility pattern of that prevalent *Salmonella* species. There has been increasing concern about the prevalence of multi-drug resistance *Salmonella typhi* and *Salmonella paratyphi* *A* strains in developing countries (Ahmed, 2006; Punjabi et al., 2007). There have also been several reports of multi-drug resistance *Salmonella typhi* with plasmid-mediated resistance to conventional antibiotics such as Chloramphenicol, Co-trimoxazole and Ampicillin in different parts of the world (Ahmed, 2006; Zhang et al., 2006). Multidrug resistance *Salmonella typhi* to antibiotic such as Chloramphenicol, Amoxicillin, Co-trimoxazole, and Fluoroquinolone have emerged as new challenges to the treatment of typhoid fever (Dutta et al., 2008; Zhang et al., 2006). Hence, there is a need for the study of incidence and susceptibility patterns of *Salmonella species* to commonly prescribed antibiotics in an attempt to understanding antibiotics susceptibility profile of the isolates and to prevent from the emergence of multi-drug resistance in our location.

In almost all cases, antimicrobial therapy is initiated empirically before the results of clinical samples culture are available. Keeping in mind the high mortality and morbidity associated with septicaemia, a right choice of empiric therapy is of utmost importance.

**1.1** **Objective of the study**

Therefore, the present study was undertaken to describe the antibiotic susceptibility and resistance pattern of blood and urine culture isolates as it may be a useful guide for clinicians initiating the empirical antibiotic therapy.

**2. Methodology**

**2.1 Sample Collection**

In this study, 75 blood and 62 urines samples were collected at Faith Foundation Hospital and St. Martins Catholic Hospital (both at Onitsha, Nigeria) during March 2014 to November 2014. The total of 137 blood and urine samples of suspected enteric fever outpatients of clinical history of first-fourth week of infection were all included. Blood and urine samples were collected from each patient by nursing personnel, male orderlies, or physicians, using strict aseptic precautions. Processing of samples was done in a specialist public health institution at St. Martins Catholic Hospital, Onitsha under strict biosafety precautions.

**2.2 Ethical consideration**

Consent for this study was obtained from the Chief Medical Director of the hospital, with subsequent approval of the work by the Ethical Committee. The anonymity of each patient was treated with confidentiality and for the purpose of this research.

**2.3 Laboratory methods**

**2.3.1 Culture of the samples**

Two loopfuls of each of the blood and urine samples were cultured onto separate plates of selective medium (*Salmonella-Shigella* Agar, Oxoid, UK) to allow the multiplication of bacteria and encourage growth of *Salmonella* over *Shigella* (if any); these were then subsequently sub-cultured onto a fresh SSA and blood agar. The plates were then incubated at 37oC for 24 h (Cheesbrough, 2002). Thus single pure colonies were obtained. These pure isolates obtained in this way were inoculated in Nutrient Agar (NA) (Oxoid, UK) slant and stored at 4 oC for further characterization and identification.

**2.3.2 Blood agar**

Two hundred and fifty ml of NA (7 g of powder; 250 ml distilled water) was prepared by autoclaving at 121oC for 15 minutes, and the medium was allowed to cool down to 50–55oC. When the agar has cooled to this temperature, 12.5ml (5% of NA) sterile defibrinated blood was added aseptically, gently mixed, and dispense into sterile Petri dishes. Bubble formations in the agar was avoided (Cheesbrough, 2002).

**2.3.3** **Morphological test for identification**

Routine Gram staining technique was employed as the preliminary identification screening and morphological characterization of isolates. We observed for Gram negative rods.

**2.3.4** **Motility test:**

When inoculating the medium, a stab was made with the wire loop, and the tube was stoppered, followed by incubation at 37°C overnight. Motility was shown by a spreading turbidity from the stab line or turbidity throughout the medium (compared with an uninoculated tube). With exception of *Salmonella pullorum-gallinarum,* all *Salmonella* species are motile (Cheesbrough, 2002).

**2.4 Biochemical tests for identification**

**2.4.1 Carbohydrate fermentation test**

The carbohydrate fermentation test was performed by inoculating a loopful of thick test bacterial culture into test tubes containing sterile peptone water and bromothymol-blue indicator solution. The individual tubes containing Durham tubes and 1 g of sugars like glucose, sucrose, lactose, xylose and arabinose were incubated at 37°C for 24-48 hrs. Acid production was indicated by the change of media from green to yellow color, while gas production was indicated by the appearance of gas bubbles in the inverted Durham fermentation tubes.

**2.4.2 Indole test**

The test organisms were cultured in a bijou bottle containing 3 ml of sterile tryptone water and incubate at 35-37ºC for 48 h. Half millilitre of Kovac’s reagent was added, mixed well by gently, and examined for a red color in the surface layer within 10min A red colour in the reagent indicated positive test. Most *Salmonella* culture gives negative test (lack of deep red color at surface broth).

**2.4.3 Triple sugar iron (TSI) agar test**

At least one of each colony type of the well-isolated colonies was selected on plate using a sterile straight wire loop. Inoculum was taken from the center of the colony. TSI medium (prepared as instructed by the manufacturer) were inoculated by stabbing the butt and streaking the slants. These were then incubated at 37 oC for 24 h. A yellow butt (acid) and red or pink (alkaline) slope indicates the fermenting of glucose only. Cracks and bubbles in the medium indicate gas production from glucose fermentation. A yellow (acid) butt indicates the fermentation of lactose. A red or pink (alkaline) slope and butt indicates no fermentation of glucose or lactose. Blackening along the stab line or throughout the slant indicates hydrogen sulphide (H2S) production. *Salmonella* forms a red slope (alkaline) and yellow (acid) butt with/out gas or H2S production (Cheesbrough, 2002).

**2.4.4 Urease test**

The entire surfaces of Urea agar (Oxoid, UK) of slants in Bijou bottles were heavily inoculated with isolate. The cap were loosened and then incubated at 37oC and observed for reaction after 6-24 hours. A urease-positive culture produced an alkaline reaction in the medium, evidenced by pinkish-red color of the medium. Urease-negative organisms do not change the colour of the medium, which is pale yellow-pink. *Salmonella* is always urease negative.

**2.4.5 Methyl-Red and Voges-Proskauer (MR-VP) test**

MR-VP medium (Oxoid, UK) was prepared according to manufacturer’s instructions. MR-VP offers two tests in one. It is a buffered peptone-glucose broth, used for the differentiation of microorganisms on the basis of acid or acetylmethylcarbinol (acetoin) production. The medium was dispensed into tubes and sterilized by autoclaving at 121 oC for 15 minutes and allowed to cool. The broth was inoculated with growth from a single colony, tubes stoppered with sterile cotton wool, and incubated at 37oC for 48h. After incubation, a sterile pipette was used to remove two aliquots (1ml each) and placed into two small tubes. To one of the small tubes, 0.2 ml of methyl red (MR) was added the result was read immediately. Mixing was avoided, and the tube observed for color change. To the other small tube, 0.6 ml of VP reagent A (5% α-naphtol) was added, and then 0.2ml of VP reagent B (40% KOH) was added. The content was gently mixed to aerate and observed for color change after 10 min. A positive methyl red test showed the appearance of bright red color at the surface, and indicated acidity, while a yellow or orange color was considered as negative, and development of pink-to-ruby red colour throughout the medium wass positive VP test. Most *salmonella* are VP negative, and MR positive.

**2.4.6 Citrate test**

Simon citrate agar (Oxoid, UK) was prepared according to manufacturer’s instructions, and dispensed into Petri dishes. Using a sterile straight wire loop, the medium was inoculated with a saline suspension of the test organism and incubated at 37 oC for 24- 48 h, and observed for a bright blue colour in the medium. Care was taken not to contaminate the medium with carbon particles, such as from a frequently flamed wire. Turbidity and blue color indicated positive for citrate utilization test and no color change indicated negative test. Most *salmonella* species are positive with the exception of *Salmonella typhi* and *Salmonella paratyphi* which are citrate negative (Cheesbrough, 2002).

**2.4.7 Serological identification of *Salmonella* species**

The principle of the serological identification of *Salmonella* involves mixing the suspected organism with antiserum containing specific *Salmonella* anti­bodies. The bacteria will agglutinate (clump) in the presence of homologous antiserum.

**2.4.8 Qualitative slide agglutination test**

Swemed Diagnostic Widal Test Kit (Chunchaghatta Cross, Yelachenahalli, Bangalore) was used for the serological identification of various *Salmonella* serotypes by slide agglutination method, following manufacturer’s instruction. The description and intended use of the *Salmonella* antisera for the agglutination of specific *Salmonella* O, H, and Vi antigens according to the Kauffmann-White classification as presented by Kauffmann and Edwards and Ewingwere strictly observed(Edwards and Ewing, 1972; Kauffmann, 1966).

A clean big glass slide was partitioned into ten parts with ten circles using a glass pencil. Eight drops of sterile 0.85% saline solution (saline) were singly placed onto the first eight circles. With an inoculation loop, pure isolate from a fresh agar plate or slope culture were emulsified into each of the first eight circles to produce a distinct and uniform turbidity and, a drop (0.05ml) each of positive control and Normal Saline in each of the last two circles respectively. One drop each of 'O', 'H', ‘AH’, ‘BH’, ‘AO’, ‘BO’, ‘CH’, and ‘CO’ antigens were added in the first eight circles respectively and one drop of any one antigen in the remaining two circles. The contents of each circle was separately mixed and spread in the entire circle by tilting the slide back and forth (rocking) for 60 seconds while viewing under indirect light against a dark background and observed for agglutination. A positive result was indicated by agglutination (in the test circles, also with positive control) and a negative result was indicated by no agglutination (also with Normal Saline).

**2.4.9 Antimicrobial susceptibility test**

*In vitro* susceptibility of the isolates to various antimicrobial agents was determined by the disc diffusion technique as adapted by Kirby-Bauer and procedure as recommended by the Clinical and Laboratory Standards Institute (CLSI) – formerly, National Committee for Clinical Laboratory Standards (NCCLS) (Bauer et al., 1966; CLSI, 2014). This method allowed for the rapid determination of the efficacy of a drug by measuring the diameter of the zone of inhibition that resulted from diffusion of the agent into the medium surrounding the disc.

This test was carried out using Mueller Hinton agar and were tested *in vitro* for susceptibility to six (6) different antimicrobial agents suggested by CLSI (i.e Ampicillin, Ceftriaxone, Co-trimoxazole (Sulphamethoxazole-trimethoprim), Chloramphericol, Tetracycline and Ciprofloxacin) (CLSI, 2014). The antibiotics discs (Oxoid, UK) used and concentration units were: Ampicillin (10 μg,) Ceftriaxone (30 μg), Tetracycline (30 μg), Ciprofloxacin (5 μg), Chloramphenicol (30 μg) and Co-trimoxazole (25 μg). The protocol followed were as outlined previously described, and the inhibition zone diameters for individual antimicrobial agents were translated into susceptible, intermediate and resistant categories by referring to an interpretation as previously described (CLSI, 2014).

The various reasons given by the farmers indicate proper understanding of the obvious advantages of the technology. These corroborated the objectives of the technology (Mutsaers *et al* 1995; Vandemeer, 1989). The reasons further laid credence to the need to consider the farmers’ socio–economic situations for technological design and dissemination.

**3. Results and discussion**

This study was aimed at isolation, identification and biochemical differentiation of *Salmonella* species from blood and urine samples of outpatients attending hospital with a history of fever during a period of first-fourth week of infection, and the antimicrobial susceptibility characterization of the isolates were also achieved.

Organisms isolated from the blood and urine samples under test, showed apparently morphological, cultural and biochemical properties resembling *Salmonella* species.

Serologic identification of *Salmonella* species was performed by slide agglutination test kit (Andrews et al., 2005; Cheesbrough, 2002). A Commercial kit was used to agglutinate and serogroup salmonellae by their antigens. When positive agglutination reaction was obtained in the antisera, the *Salmonella* subgroup was identified and no further testing with antisera needed to be conducted (Andrews et al., 2005).

A total of 24 isolates out of 137 samples were identified as *Salmonella* spp. by using cultural, biochemical and serological analysis. The results of cultural, morphological and motility characteristics of the isolates of *Salmonella* spp. are given in Table 1 and that of biochemical properties presented in Table 2. *Salmonella* spp. were isolated from 137 (17.5%) samples, of which 75 (22.7%) were of blood samples and 62 (11.3%) were of urine samples during the first-fourth week of infections of outpatients attending hospital with a history of fever within this duration. Predominant isolates were *S. typhi,* 16 (11.7%); others were *S. paratyphi* A, 6 (4.38%) and other *Salmonella paratyphi* C, 2 (1.45%), Table 3. Isolates were from all age group, the median age being 30 years. There were 58 (42.3%) male and 79 (57.7%) female outpatients and the male to female ratio was 1:1.36. Infection was more prevalent in the age group 6-15years, 6 (8.0%) followed by age group above 46 years, 3 (5.30%) in blood samples of outpatients during their first-fourth week of infection, Table 4.

Table 1. Cultural, morphological and motility characteristics of the isolates of *Salmonella* spp.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **No of isolates** | **Agars** | | | **Staining characteristics** | **Motility** |
|  | **SSA** | **BA** | **NA** |  |  |
| 24 | Translucent black smooth, small round colonies | grey-white non-haemolytic colonies | Translucent, opaque, smooth colonies | Pink short rod, gram negative bacteria arranged in single or pair | + |

SSA = *Salmonella-Shigella* agar; BA = Blood agar; NA = nutrient agar; + = Positive

Table 2. Biochemical properties of the isolates of *Salmonella* spp.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Sugar (carbohydrate) fermentation test** | | | | | **Citrate** | **Indole** | **Urease** | **TSI** | **MR** | **VP** |
| **G (gas)** | **A** | **X** | **S** | **L** |  |  |  |  |  |  |
| 16(-), 8(+) | 19(-), 5(+) | 18(+), 6(-) | all(-) | all(-) | 20(-)  4(+) | all(-) | all(-) | Black color colonies against a yellowish background | All (+) | All (-) |

G gas=glucose with gas production; A= arabinose; X = xylose; S = sucrose; L = lactose; Citrate = Simon citrate agar test; Indole = indole production test; Urease = urease production test; TSI = triple sugar iron agar test; MR = methyl red test; VP = Voges Proskauer test; (+) = Positive test; (-) = negative test.

Table 3. Distribution of *Salmonella* isolates from blood and urine during the first-fourth week of infection

|  |  |  |  |
| --- | --- | --- | --- |
| ***Salmonella* isolates** | **Sex (n% in bracket)** | | **Total** |
|  | **Male** | **Female** |  |
| *Salmonella typhi* | 4 (2.92) | 12 (8.76) | 16 (11.7) |
| *Salmonella paratyphi* A | 2 (1.45) | 4 (2.92) | 6 (4.38) |
| *Salmonella* *paratyphi* C | 1 (0.73) | 1 (0.73) | 2 (1.45) |
| Total | 7 (5.11) | 17 (12.41) | 24 (17.52) |

Table 4. Age & sex distribution of *Salmonella* spp. isolated from blood during the first-fourth week of infections

|  |  |  |  |
| --- | --- | --- | --- |
| **Age group (years)** | **Sex (n% in bracket)** | | **Total** |
|  | **Male** | **Female** |  |
| 0-5 | 0 (0.0) | 1 (1.33) | 1 (1.33) |
| 6-15 | 2 (2.67) | 4 (5.33) | 6 (8.00) |
| 16-25 | 0 (0.0) | 2 (2.67) | 2 (2.67) |
| 26-35 | 2 (2.67) | 1 (1.33) | 3 (4.00) |
| 36-45 | 0 (0.0) | 1 (1.33) | 1 (1.33) |
| ˃46 | 1 (1.33) | 3 (4.00) | 4 (5.30) |
| Total | 5 (6.67) | 12 (16.0) | 17 (22.7) |

Table 5. Age & sex distribution of *Salmonella* spp. isolated from urine during the first-fourth week of infections

|  |  |  |  |
| --- | --- | --- | --- |
| **Age group (years)** | **Sex (n% in bracket)** | | **Total** |
|  | **Male** | **Female** |  |
| 0-5 | - | - | - |
| 6-15 | - | 1 (1.61) | 1 (1.61) |
| 16-25 | - | 1 (1.61) | 1 (1.61) |
| 26-35 | 1 (1.61) | 1 (1.61) | 2 (3.20) |
| 36-45 | - | - | - |
| ˃46 | - | 3 (4.84) | 3 (4.84) |
| Total | 1 (1.61) | 6 (9.67) | 7 (11.30) |

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2

1

**Frequency of isolates (%)**

frequency

of

isolates

(%)

Weeks after infection in days

Blood samples

Urine samples

Fig. 1. Incidence pattern of *Salmonella* species in blood and urine during first-fourth week of infection. Blood cultures were found to be positive for *Salmonella* during early stages of infection (first-second week) while urine cultures become positive after second-fourth week of infection.

Meanwhile, in urine samples of outpatients during their first-fourth week of infection, prevalent was highest in the age group above 46 years 3 (4.84%) followed by age group 26-35 years 2 (3.20%), Table 5. The significant observation of this prevalence is represented in Fig. 1. It describes incidence pattern of *Salmonella* in blood and urine during the first-fourth week of infections. Blood cultures yield positive growth for *Salmonella* in the first-second week of infection while urine cultures become positive later in the clinical course between second and fourth weeks of illness. The observation could be explained by the pathogenesis of the organisms. Enteric fever mainly caused by *Salmonella enteric*a is an infection of lymphatic system and other tissues. It begins with the invasion of the mucosal epithelium and rapid movement of the pathogens to lymphoid tissue associated with the gastrointestinal tract. The invading pathogens multiply in the lymphoid tissue, move to the blood and spread through the body; blood cultures remain positive for only a short period as the pathogen become localized in various tissue.

The results of the antimicrobial susceptibility testing by disc diffusion method with six selected antimicrobial agents are given in Table 6. Out of 16 *Salmonella typhi* isolates, 16 (100%) were sensitive to Ciprofloxacin, 15 (93.8%) to Ceftriaxone, 14 (87.5%) to Chloramphenicol and 12 (75%) to Ampicillin. On the other hand, 10 (62.5%) were resistant to Tetracycline, and 12 (75%) resistant to Co-trimoxazole. Out of 6 *Salmonella paratyphi A* isolates, all the 6 (100%) were susceptible to Ciprofloxacin and 5 (83.3%) susceptible to Ceftriaxone, 4 (66.7%) susceptible to Chloramphenicol while 4 (66.6%) were resistant to two antibiotics. Furthermore, 2 (100%) other of *Salmonella paratyphi* C were susceptible to Ciprofloxacin and Ceftriaxone, Table 7.

Fluoroquinolone particularly ciprofloxacin was the most frequently used antibiotics in typhi and paratyphi cases and none of the isolates were resistance to this antibiotic this finding is similar to previous finding (Khanal et al., 2007).

Table 6. Antimicrobial susceptibility patterns of *Salmonella* isolates recovered from blood and urine during the first-fourth week of infection

|  |  |  |  |
| --- | --- | --- | --- |
| **Antibiotics potency (µg)** | **Susceptibility** | | |
|  | ***S. typhi*, 16 isolates, susceptible % in bracket** | ***S. paratyphi* A, 6 isolates, susceptible % in bracket** | ***S. paratyphi* C, 2 isolates, susceptible % in bracket** |
| Ampicillin (30) | 12 (75) | 3 (50) | 1 (50) |
| Ceftriaxone (30) | 15 (93.8) | 5 (83.3) | 2 (100) |
| Ciprofloxacin (5 | 16 (100) | 6 (100) | 2 (100) |
| Chloramphenicol (30) | 14 (87.5) | 4 (66.7) | 1 (50) |
| Tetracycline (30) | 6 (37.5) | 2 (33.3) | 1 (50) |
| Co-trimoxazole (25) | 4 (25) | 2 (33.3) | 1 (50) |

An *in vitro* antimicrobial sensitivity testing on isolates against six antimicrobial agents showed that Ciprofloxacin was the most effective agent with 24 (100%), followed by Ceftriaxone, Chloramphenicol and Ampicillin with 22 (91.7), 19 (79.2%), and 18 (66.7%) susceptibilities respectively.

Table 7. Resistance patterns of *Salmonella* isolates recovered from blood and urine during the first-fourth week of infection

|  |  |  |  |
| --- | --- | --- | --- |
| **Antibiotics potency (µg)** | **Resistant isolates** | | |
|  | ***S. typhi*, 16 isolates, susceptible % in bracket** | ***S. paratyphi* A, 6 isolates, susceptible % in bracket** | ***S. paratyphi* C, 2 isolates, susceptible % in bracket** |
| Ampicillin (30) | 4 (25) | 3 (50) | 1 (50) |
| Ceftriaxone (30) | 1 (6.3) | 1 (16.7) | - |
| Ciprofloxacin (5 | - | - | - |
| Chloramphenicol (30) | 2 (12.5) | 2 (33.3) | 1 (50) |
| Tetracycline (30) | 10 (62.5) | 4 (66.7) | 1 (50) |
| Co-trimoxazole (25) | 12 (75) | 4 (66.7) | 1 (50) |

**4．Conclusion and policy implications**

Prompt institution of appropriate antimicrobial therapy can reduce morbidity and mortality associated with enteric fever. Thus the sensitivity pattern of causative organisms must be studied as it contributes to knowledge of resistant patterns and emergence of drug resistance. This study concludes that an accurate diagnosis of enteric fever caused by *Salmonella* spp. may be achieved by identifying the pathogens from blood samples during the first week of infection and from urine after the second week if appropriate antimicrobial agents have not been administered. Infection caused by ingestion of the *Salmonella* spp.in contaminated food or water or directly from faeces or contaminated fingers can be prevented mainly by personal and domestic hygiene, provision of potable water supplies, safe disposal of feces and public education. Dedicated urban and rural agencies still need to develop more robust monitoring and/or hygiene standard inspection programs for local restaurants and fast joint to curtail the spread of infections.

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