

# Antimicrobial Susceptibility Profile of Salmonella Enterica Serovars from Meru Teaching and Referral Hospital

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## ABSTRACT

Salmonellosis causes substantial morbidity and mortality in Sub Saharan Africa. This warranted the appraisal of antibiotic susceptibility patterns of clinical *Salmonella* isolates from the study catchment area. The study aimed to determine *in vitro* antibiotic susceptibility profile of *Salmonella* species isolated from stool samples. Kirby Bauer and MIC results indicated significant resistance to antimicrobial agents ( $p < 0.001$ ). One isolate exhibited resistance to ten antibiograms tested and the resistance phenotype was; Ampicillin, tetracycline, cotrimoxazole, streptomycin, kanamycin, gentamycin, sulfamethoxazole, chloramphenicol, nalidixic acid and ciprofloxacin (AmpTCotSt KGmSxCNaCip). Routine surveillance of local system is vital to monitor emerging resistance trends in study area.

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## Introduction

Salmonella is one of the four key global causes of diarrhoeal diseases [1]. The burden of Salmonellosis, a foodborne infection is substantial, approximately 550 million people fall ill including 220 million children under the age of 5 years and 33 million of healthy life years are lost yearly [1]. Non typhoidal Salmonella predominantly cause enteric self-limiting illness in developed countries, however, NTS is responsible for a significant burden of life threatening bloodstream infections in sub Saharan Africa [2], [3]. Invasive NTS infection occurs when the organism spreads beyond the gastrointestinal mucosa to infect normal sterile sites, such as the bloodstream, the meninges, bone, and joint spaces. Non Typhoidal Salmonella causes bacteremia in 33 out of 54 African countries and 39% of community acquired bacteremia in sub Saharan Africa [3].

Routine antimicrobial therapy is not recommended for mild or moderate cases in healthy individuals. However, antimicrobial treatment is required in severe cases occurring in susceptible host population or to combat invasive infections. Antimicrobial resistance in *S. enterica* is a cause of serious concern in clinical management of adult and pediatric infections. Many of the recent isolates obtained worldwide are resistant to a number of antibiotics [4], [5]. Resistance to first line therapy for enteric fever and other NTS infections is common in Kenya and other countries in sub Saharan Africa [6].

Multi Drug resistance (MDR) to more than three antimicrobials tested (chloramphenicol, trimethoprim-sulfamethoxazole and ampicillin) in western Kenya was reported to be 76% [7]. In a related study, [8] documented resistance to most commonly used antimicrobials in addition to development of ceftriaxone resistance in Siaya District hospital in the same region.

In a related study in Kisumu and Kapsabet County hospitals, 70% of *Salmonella* isolates were MDR and resistance phenotype was ACSSuT [9].

Surveillance study carried out in Igembe district hospital in Meru county reported resistance at 95% [10]

This shows the fluidity in resistance patterns of *Salmonella* worldwide and regionally hence the need for strategies to maintain spectrum of activity of existing antibiotics especially in resource limited settings. Antibiotic resistance in bacteria can develop by accumulation of point mutation or by horizontal exchange of DNA between bacteria of same or different species [11], [12].

Exposure of bacteria pathogen to antibiotics enhances resistance by selecting for those cells that are able to tolerate them [13], [12]. This creates positive correlation between level of antibiotic use and prevalence of antibiotic resistance in bacteria in the same human population both at national and regional levels [14]. The rate of emergence of resistance is also related to total antibiotic use, including widespread prescription of antibiotics for respiratory viral infection [13], [12] in addition to use of antibiotics in low doses as growth promoters in livestock farming [15], [16].

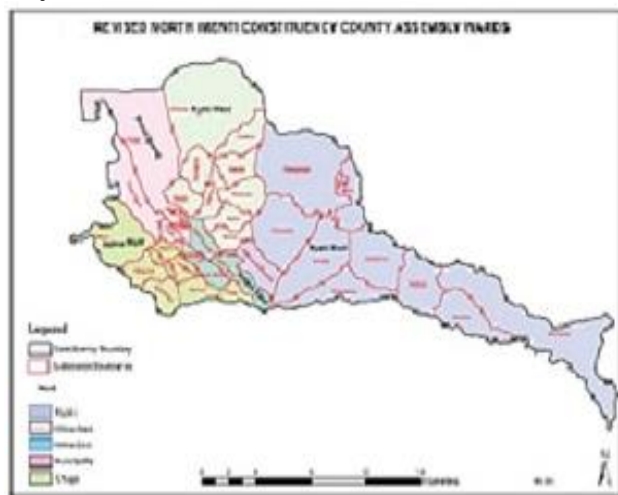
Because of the global increase of antimicrobial resistance, treatment guidelines should be reviewed on a regular basis taking into account the resistance pattern of the bacteria based on the local surveillance system [1]. Hence, characterization of resistance phenotypes in recovered isolates is significant for routine surveillance of spread of antibiotic resistance and epidemiologically important for clinicians to keep abreast on treatment options in the study catchment area.

## Study Design

This was a cross sectional descriptive study involving clinical *Salmonella* isolates obtained from patients treated for

## Materials and Methods

### Study site



fever, defined as  $\geq 38^{\circ}\text{C}$  and diarrhea (defined as presence or absence of visible blood in stool;  $\geq 3$  bowel movements within 24hrs period during the preceding 5 days) at Meru Teaching and Referral hospital in Kenya between July 2015 and June 2016.

### Patients

On arrival to hospital, both children and adult patients were examined by a medical physician and those found to have fever defined as  $\geq 38^{\circ}\text{C}$ , without acute respiratory illness, irrespective of malaria blood smear results and regardless of bloody diarrhea (defined as presence of visible blood in stool;  $\geq 3$  bowel movements within 24 hrs period during the preceding 5 days) were included in study.

Both whole stool and rectal swabs from patients enrolled for the study were received at the laboratory reception area and immediately placed in Cary- Blaire transport medium by laboratory personnel and transported within 6 to 12 hrs in iced cool box at  $8^{\circ}\text{C}$  for culture and isolation of Salmonella. Only the first three (3) patients who met the above criterion per day were enrolled for the study. For children less than 15 years old, parents or guardian gave consent to permit their participation. Consent to carry out the study in hospital was sort from hospital administration authority and KEMRI Ethical Review Committee.

### Culture and Isolation of Salmonella species

Stool samples were aerobically cultured at  $37^{\circ}\text{C}$  in selenite F broth (Himedia Laboratories Pvt Ltd, Mumbai, India) for 18-24 hrs and sub- cultured when orange onto plates of Salmonella Shigella agar, incubated for 18- 24 hrs at  $37^{\circ}\text{C}$ .

Colonies suspected to be Salmonella were then sub cultured onto plates of Xylose Lysine Deoxycholate (XLD) plate, incubated for 18-24 hrs at  $37^{\circ}\text{C}$ . Bacterial isolates were identified by biochemical tests using Salmonella API 20E Strips (Biomérieux, Marcy L'etoli, and France) and serotyped using agglutinating antisera (Murex Diagnostics, Datford, United Kingdom). Salmonella isolates were stored at  $-20^{\circ}\text{C}$  in Tryptic Soy Broth in 15% glycerol and stored in 2ml eppendorff tubes (Sarstedt Ltd, Germany) until they were analyzed.

### Antimicrobial Susceptibility Testing

Antimicrobial susceptibility of the isolates was done by Kirby Bauer disk diffusion technique. The antibiotic disks (all from Himedia Laboratories, Pvt Ltd Mumbai, India) contained:

Ampicillin (AMP)(25mcg), Gentamicin (GM)(10mcg), Streptomycin(ST)(10mcg) Kanamycin(K)(30mcg), Chloramphenicol(C)(30mcg), Tetracycline(TET)(25mcg), Sulfamethoxazole (SX) (200mcg), Nalidixic acid(Na)(30mcg), Ciprofloxacin (CIP) (30mcg) and Ceftriaxone (CTR)(30mcg). Fresh Salmonella colonies were inoculated in 0.85% NaCl suspension to turbidity equivalent to 0.5 Mac Farland standard which is equivalent to  $1.0 \times 10^8$  corresponding to approximate density of bacteria/ml. The culture was swabbed onto a Muller- Hinton agar from Himedia. Antibiotic discs were applied using sterile forceps onto the bacterial lawn in the plates after drying for 5mins under lamina airflow chamber, the plates were incubated at  $37^{\circ}\text{C}$  for 24 hrs. The MICs of these antibiotics were determined by using E-test strips (AB Biodisk, Solna, Sweden) according to manufacturer's instructions. *E.coli* ATCC 25922 was used as a control for potency of antibiotic discs. Disk sensitivity tests and MICs were interpreted according to guidelines provided by Clinical Laboratories Standard Institute (CLSI). Isolates resistant to two or more of antimicrobials tested were categorized as multidrug resistant. Isolates of intermediate values were considered susceptible for the purpose of this study so as not to overestimate the extent of resistance.

### Results

All the isolates were resistant to three or more antimicrobials tested. The highest resistance was displayed by ampicillin ( $\text{MIC}_{90}=250 \mu\text{g/ml}$ ) and kanamycin ( $\text{MIC}_{90}=214 \mu\text{g/ml}$ ) at 84%. This was followed by resistance to tetracycline ( $\text{MIC}_{90}=200 \mu\text{g/ml}$ ) and cotrimoxazole ( $\text{MIC}_{90}=32 \mu\text{g/ml}$ ) at 61%. Lowest resistance was observed in gentamicin ( $\text{MIC}_{90}=215 \mu\text{g/ml}$ ) at 16% followed by streptomycin ( $\text{MIC}_{90}=252 \mu\text{g/ml}$ ) at 18%. Resistance to ciproflaxin ( $\text{MIC}_{90}=$ ) was 45% followed by nalidixic acid at 32% among the quinolones.

**Table 1. Antimicrobial susceptibility profile of non typhoidal Salmonella isolates by disc diffusion (n=38).**

| Antimicrobial Agent tested | Minimum Inhibition Concentration ( $\text{MIC}_{90}$ ) | Zone of diameter in mm |       |           | % Resistance | % Intermediate | % Sensitive |
|----------------------------|--------------------------------------------------------|------------------------|-------|-----------|--------------|----------------|-------------|
|                            |                                                        | R                      | I     | S         |              |                |             |
| Ampicilin                  | 0.25->256                                              | $\leq 13$              | 14-16 | $\geq 17$ | 84           | 5              | 11          |
| Tetracycline               | 0.064 ->256                                            | $\leq 11$              | 12-14 | $\geq 15$ | 61           | 31             | 8           |
| Cotrimoxazole              | 0.064 ->256                                            | $\leq 14$              | 10-15 | $\geq 15$ | 61           | 0              | 39          |
| Streptomycin               | 0.038 ->256                                            | $\leq 11$              | 12-14 | $\geq 15$ | 18           | 53             | 29          |
| Kanamycin                  | 0.06 ->256                                             | $\leq 13$              | 14-17 | $\geq 18$ | 84           | 16             | 0           |
| Gentamycin                 | 0.06 ->256                                             | $\leq 12$              | 13-14 | $\geq 15$ | 16           | 11             | 71          |
| Sulfamethoxazole           | 0.032-32                                               | $\leq 10$              | 11-15 | $\geq 16$ | 53           | 10             | 37          |
| Chloramphenicol            | 0.19-> 256                                             | $\leq 12$              | 13-17 | $\geq 18$ | 55           | 3              | 42          |
| Nalidixic acid             | 0.5-32                                                 | $\leq 13$              | 14-18 | $\geq 19$ | 32           | 58             | 10          |
| Ciproflaxin                | 0.015- 4                                               | $\leq 15$              | 16-20 | $\geq 21$ | 45           | 18             | 37          |
| Cefriaxone                 | 0.25- 64                                               | $\leq 19$              | 20-22 | $\geq 23$ | 26           | 42             | 32          |

**Table 2. Antimicrobial Resistance analysis of NTS isolates.**

| Antimicrobial Class    | % of resistant strains (n=38) |
|------------------------|-------------------------------|
| <b>Penicillins</b>     |                               |
| Ampicillin             | 32(84%)                       |
| <b>Aminoglycosides</b> |                               |
| Gentamicin             | 6(16%)                        |
| Streptomycin           | 7(18%)                        |
| Kanamycin              | 32(84%)                       |
| <b>Phenicol</b>        |                               |
| Chloramphenicol        | 21(55%)                       |
| <b>Tetracycline</b>    |                               |
| Tetracycline           | 23(61%)                       |
| <b>Sulfonamides</b>    |                               |
| Sulfamethoxazole       | 20(53%)                       |
| Co-trimoxazole         | 23(61%)                       |
| <b>Quinolones</b>      |                               |
| Nalidixic acid         | 12(32%)                       |
| Ciprofloxacin          | 17(45%)                       |
| <b>Cephalosporins</b>  |                               |
| Ceftriaxone            | 10(26%)                       |

**Table 3. Antibigrams of NTS isolates from Meru teaching and referral hospital.**

| Antibiogram tested (N=11) | No.in the category | No. of Resistant antibiogram (%) | Category |
|---------------------------|--------------------|----------------------------------|----------|
| AmpTK                     | 1                  | 3(27)                            | A        |
| AmpKCtr                   | 1                  | 3(27)                            | B        |
| AmpKGm                    | 1                  | 3(27)                            | C        |
| AmpKGmCip                 | 1                  | 4(36.4)                          | D        |
| AmpTKCtr                  | 1                  | 4(36.4)                          | E        |
| AmpCotKC                  | 1                  | 4(36.4)                          | F        |
| Cot KSxCip                | 1                  | 4(36.4)                          | G        |
| AmpKNaCipCtr              | 1                  | 5(45.5)                          | H        |
| AmpStKCNaCip              | 1                  | 6(54.5)                          | I        |
| AmpTCotKSxC               | 5                  | 6(54.5)                          | J        |
| AmpTCotKSxCCTR            | 1                  | 7(63.6)                          | K        |
| AmpTCotKSxCcCip           | 3                  | 7(63.6)                          | L        |
| AmpTCotStKGmSxC           | 1                  | 8(72.7)                          | M        |
| AmpTCotKSxCNaCtr          | 1                  | 8(72.7)                          | N        |
| AmpTCotStKSxCNa           | 1                  | 8(72.7)                          | O        |
| AmpTCotStKNaCipCtr        | 1                  | 8(72.7)                          | P        |
| AmpTCotKGmSxCcCip         | 1                  | 8(72.7)                          | Q        |
| AmpTCotKSxCNaCip          | 1                  | 8(72.7)                          | R        |
| AmpTCotKSxCNaCipCtr       | 1                  | 9(81.8)                          | S        |
| AmpTCotStKSxCNaCipCtr     | 1                  | 9(81.8)                          | T        |
| AmpTCotStKSxCNaCip        | 1                  | 9(81.8)                          | U        |
| AmpTCotKGmSxCNaCip        | 1                  | 9(81.8)                          | V        |
| AmpTCotKGmCNaCipCtr       | 1                  | 9(81.8)                          | W        |
| AmpTCotStKGmSxCNaCip      | 1                  | 10(90.9)                         | X        |

**Amp:**Ampicillin, **Gm:** Gentamicin, **St:** Streptomycin, **K:** Kanamycin, **C:** chloramphenicol, **T:**Tetracycline, **Sx:** Sulfamethoxazole, **Na:** Nalidixic acid, **Ctr:** Ceftriaxone, **Cot:** Cotrimoxazole

Category J, had five isolates exhibiting resistance to ampicillin, tetracycline, cotrimoxazole, kanamycin, sulfamethoxazole and chloramphenicol (55%). Category L, had three isolates resistant to ampicillin, tetracycline, cotrimoxazole, kanamycin, sulfamethoxazole, chloramphenicol, ciproflaxin (64%).Category X, had one isolate exhibiting resistance to ten antimicrobials tested (91%) and the resistance phenotype was; AmpTCotStKGmSxCNaCip.Categories S,T,U,V,W exhibited resistance to nine antibiograms tested (Table 3)

## Discussion

Antimicrobial susceptibility profiles of 38 NTS isolates were determined. The isolates were recovered from patients in all age groups from infants to adults. Out of these, 100% (n=38) of the isolates were resistant to three or more antimicrobials. Resistance to first line drugs, gentamycin and streptomycin was low at 16% and 18% (Table 2). This shows a decline in resistance to aminoglycoside group of antibiotics. This may be attributed to change in prescription preference for other antibiotics for infections reducing their circulation within the population. Although the study observed reduction in resistance to other first line antibiotics compared to related studies[7], [10], [9] resistance to these antibiotics is still clinically significant. The highest resistance was observed in ampicillin and kanamycin at 84% followed by tetracycline and cotrimoxazole at 61%, chloramphenicol and streptomycin exhibited the lowest resistance at 55% and 53% respectively. Fluoroquinolones (ciprofloxacin, nalidixic acid) and third generation cephalosporins (ceftriaxone) are drugs of choice for treatment of severe Salmonella infections; however these isolates exhibited decreased susceptibility to these antimicrobial agents. Resistance to ceftriaxone was 26% (n=10), Nalidixic acid was 32% (n=12) and Ciprofloxacin was 45% (n=17). Though the frequency of resistance is relatively low, resistance to these extended spectrum antimicrobial agents is clinically significant since they are recommended for the management of invasive non typhoidal infections[6]. Resistance to quinolones (nalidixic acid, ciprofloxacin) and cephalosporins (ceftriaxone) was indicative of mutations in the quinolones resistant determining regions of DNA genes, active efflux (AcrAB efflux) and decreased outer permeability strains as documented by other authors [17], [16].

Resistance to ciprofloxacin 45% (n=17) was higher than nalidixic acid 32% (n= 12). These findings are in line with other related studies [5] that reported increased percentage of Salmonella isolates that exhibited decreased susceptibility to ciprofloxacin but displayed susceptibility to nalidixic acid. This indicated plasmid mediated quinolone resistance. However, resistant determinant genes and genetic elements were not studied due to restricted funding.

The isolates exhibited specific co-resistance phenotypes. The most common resistant phenotype was; ampicillin, tetracycline,cotrimoxazole, kanamycin, sulfamethoxazole, chloramphenicol (AmpTCotKSxC); representing six CLSI classes, followed by ampicillin, tetracycline,cotrimoxazole, kanamycin, sulfamethoxazole, chloramphenicol,ciproflaxin (AmpTCotKSxCcCip). Five isolates were resistant to nine antimicrobial agents and the phenotypes included; ampicillin,tetracycline,cotrimoxazole,kanamycin,gentamicin, chloramphenicol, nalidixic acid, ciproflaxin, ceftriaxone (AmpTCotKGmCNaCipCtr)andampicillin,tetracycline,cotrim oxazole,kanamycin,gentamicin,sulfamethoxazole,chloramphenicol,nalidixic acid,ciproflaxin AmpTCotKGmSxCNaCip while one isolate was resistant to ten antimicrobials and the resistantphenotypewas; ampicillin,tetracycline,cotrimoxazole, streptomycin,,kanamycin,gentamicin,sulfamethoxazole,chloramphenicol,nalidixicacid,ciproflaxin(AmpTCotStKGmSxCNaCip). These results imply that isolates exhibited varied co-resistance phenotypes. These findings indicates emergence of new patterns of resistance (Table.3), this could be linked to changes in resistance within serotypes. Previous studies of non typhoidal Salmonella co- resistance phenotypes reported. Resistance to Ampicilin, chloramphenicol, streptomycin,

sulfonamide and tetracycline (ACSSuT); representing at least five CLSI classes[5]. Widespread use of antibiotics in human, veterinary and food production in addition to mobile genetic elements have been implicated as the cause of antimicrobial resistance. Given the generic nature by which different microbial strains acquire resistance determinant genes and in order to better understand antimicrobial resistance in the study area, future studies should focus on the genetic basis of antimicrobial resistance. The need for routine surveillance to detect emerging resistance trends and to provide interventions within animal and public health is important. Training in personal hygiene, sanitation and provision of quality water cannot be overemphasized.

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