Antibiotic susceptibility in *Salmonella* species isolated from asymptomatic food handlers in Westlands, Nairobi, Kenya

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Abstract: *Salmonella* cause a wide range of diseases in diverse hosts ranging from gastroenteritis, enteric fever and bacteremia. These infections can lead to a convalescent lifetime carrier status/asymptomatic carriers. The occurrence of emerging resistant *Salmonella* strains is evident. The aim of this study was to determine the antibiotic susceptibility of *Salmonella*. Among the asymptomatic carriers who work within the hotel industry in Westlands, Nairobi. A cross sectional laboratory based design was employed. 400 stool samples were used. Xylose Lysine Deoxycholate agar (XLD) and Mac Conkey agar both selective and differential media for isolation of *Salmonella* sp were used. Serotyping and biochemical test were conducted for the confirmation of strains. The Kirby-Bauer disc diffusion method was employed to test susceptibility of the isolated *Salmonella* to antibiotics commonly used in animal and human health. Data analysis was by analysis of variance (ANOVA) and descriptive statistical methods. A total of 6 serotypes were isolated. A significant variation in inhibition zone sizes of salmonella under the commonly used drugs was observed. F=19.48 while p≤0.05, thus significant difference in their effectiveness. A drug susceptibility of 81.8% was observed. Multi-drug resistance of 37.5% was observed with four (4) different antimicrobial resistance profiles. Results from Ampicillin and Amox-clav showed more resistance among the *Salmonella* isolates.

Keywords: *Salmonella*, antibiotics, drug resistance, food handlers, asymptomatic.

1. Introduction

There are two types of *Salmonella* associated with the human host, non-typhoid *Salmonella* and typhoid *Salmonella*. Non-typhoid *Salmonella* is more frequently experienced in developing countries as compared to the developed countries, it can affect as many as 21.5 million [14] individuals each year, especially where unhygienic conditions are likely to occur. Salmonellosis caused by non-typhoid *Salmonella* is considered as one of the most widespread and common food borne diseases. There is broad scientific consensus that the use of antibiotics in food animals on some occasions has detrimental effects on human health [4]. Food animals exposed to additives such as the antibiotics used for growth promotion may serve as a reservoir of resistant bacteria and resistance genes that may spread to the human population, thereby limiting the medical value of antimicrobial drugs [1].

The antimicrobials currently widely regarded as most favourable for the management of *Salmonella* infections in adults is the group of fluoroquinolone. In young children the third generation cephalosporin which are given by injection are widely used for severe infections. Chloramphenicol, Ampicillin and Amoxicillin and Trimethoprim-Sulfamethoxazole is occasionally used as alternative drugs [14].

Resistance by *Salmonella* to fluoroquinolones has emerged in several countries as a result of using antibiotics for human treatment in the treatment of animals which are later used as a food source [6]. In addition, under dosage and misuse of antibiotics in the treatment of human infections has led to mutations in the bacterial genome, enabling the *Salmonella* to gain resistance to antibiotics that were once effective, posing a public health concern. In some cases, multi-drug resistance by bacteria is transferred through one coherent piece of DNA, referred to as a plasmid [14]. Multi-drug resistant (MDR) strains of *Salmonella* are now encountered frequently and the rates of multi-drug resistance have increased considerably in recent years [14]. A recent example is the global spread of a multi-drug resistant *S. typhimurium* phage type DT104 in animals and humans [5].

2. Literature Review

Although salmonellosis is a self-limiting infection, antibiotics is used to cut short the length of illness [12]. Millions of human incidents are reported worldwide to cause thousands of deaths each year [13] especially among the young, immuno-compromised persons and the elderly whose immune systems are weak. Typhoid fever is known to be spread via faecal oral route through the contamination of water sources or food by faecal material of an infected person [8]. Therefore, asymptomatic food handlers and employees in catering departments of institutions can pose great danger of infecting their clientele.
3. Materials and Methods

3.1 Sample collection

Fecal samples were obtained from asymptomatic food handlers in Westlands division, Narobi. The study employed a cross sectional laboratory based study design involving stratified random sampling. The study population was identified and listed according to the defined strata; the required sample size was determined by fisher et al, formula.

3.2 Isolation of Salmonella by enrichment and differential method

The populations of Salmonella in a stool sample maybe too low for the samples to be routinely cultured, necessitating subjection to enrichment culture was used to enrich the samples. Samples were then isolated on Mac Conkey agar and Xylose Lysine Deoxycholate (XLD) agar, which are selective and differential media for bacterial identification [10][9], most colonies were able to grow on both selective and differential media for bacterial identification. Samples were then isolated on Mac Conkey agar subjection to enrichment culture was used to enrich the samples to be routinely cultured, necessitating sample size was determined by fisher et al, formula.

3.3 Antimicrobial Susceptibility testing

The Kirby-Bauer disc diffusion method [2] was used to test susceptibility of the isolated Salmonella to the commonly used antimicrobials on disks containing; Amox-Clav (10 mg), Oflofloxacin (20mg), Ceftriaxone (30mg), Doxycycline (30 mg), Chloramphenicol (30 mg), Sulphamethoxazole/Trimethoprin SXT (30:2 mg), Gentamicin (10 mg), Streptomycin (10 mg), Nalidixic acid (30 mg) Ciprofloxacin (5 mg) and Ampicillin.

4. Results

4.1 Identification of the isolates

Of the 400 samples collected from the asymptomatic food handlers, 8 (2%) turned out to be positive for Salmonella while 392 (98%) turned out to be negative for Salmonella.

4.2 Antibiotic sensitivity reactions by the isolates

4.2.1 Susceptibility of the isolates to the test drugs

The antibiotics used in this research are considered the most common or readily available for the population in the Kenyan market. The Disk susceptibility data were interpreted according to criteria set by the Clinical and Laboratory Standards Institute. Classified as either sensitive (S), intermediate (I) or resistant (R) (Table 1). Multi-drug resistance (MDR) was defined as resistance to at least two of the antimicrobials tested (Table 2). The largest zones of inhibition was noted in S. Typhimurium and the least in S. Paratyphi A, meaning S. Typhimurium demonstrated sensitivity to most of the antibiotics being tested, unlike S. Paratyphi A.

Table 1: Antimicrobial susceptibility pattern of Salmonella isolates from food handlers

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>S</th>
<th>I</th>
<th>R</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMC</td>
<td>3 (37.5%)</td>
<td>1 (12.5%)</td>
<td>4 (50%)</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>OFX</td>
<td>8 (100%)</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>CTR</td>
<td>6 (75%)</td>
<td>0</td>
<td>2 (25%)</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>NA</td>
<td>7 (87.5%)</td>
<td>1 (12.5%)</td>
<td>0</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>6 (75%)</td>
<td>1 (12.5%)</td>
<td>1 (12.5%)</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>CIP</td>
<td>8 (100%)</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>GM</td>
<td>8 (100%)</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>6 (75%)</td>
<td>0</td>
<td>2 (25%)</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>6 (75%)</td>
<td>0</td>
<td>2 (25%)</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Sxt</td>
<td>6 (75%)</td>
<td>0</td>
<td>2 (25%)</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>AMP</td>
<td>5 (62.5%)</td>
<td>0</td>
<td>3 (37.5%)</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>69 (78.4%)</td>
<td>16 (18.2%)</td>
<td>88</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Key
- AMC: Amox-Clav
- AMP: Ampicillin
- CTR: Ceftriaxone
- D: Doxycycline
- S: Streptomycin
- C: Chloramphenical
- OFX: Oflofloxacin
- NA: Nalidixic acid
- CIP: Ciprofloxacin
- GM: Gentamicin
- SXT: Sulphamethoxazole Trimethoprin

Table 1 indicates the number of isolates a drug was observed to be sensitive (S), intermediate (I) and resistant (R) to. Example; of all Salmonella isolates, 4 were sensitive, 1 was intermediate and 3 were resistant to Amox-Clav. In total 69 S, 3 I and 16 R results were observed. Measurement was taken in mini meters (mm).

Table 2: Antimicrobial and multi-drug resistance profiles of isolated Salmonella

<table>
<thead>
<tr>
<th>Profile</th>
<th>Isolate</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMC</td>
<td>S. Enteritidis</td>
</tr>
<tr>
<td>AMC, AMP</td>
<td>S. Typhimurium</td>
</tr>
<tr>
<td>AMC, AMP, SXT, CTR, S, C</td>
<td>S. Paratyphi A</td>
</tr>
<tr>
<td>AMC, AMP, SXT, CTR, S, C, D</td>
<td>S. Paratyphi B</td>
</tr>
</tbody>
</table>

Key
- AMC: Amox-Clav
- AMP: Ampicillin
- CTR: Ceftriaxone
- D: Doxycycline
- S: Streptomycin
- C: Chloramphenical
- SXT: Sulphamethoxazole Trimethoprin

Table 4.6 indicates the antimicrobials groupings that resistance by several isolates was observed, their frequencies of occurrences and percentages.

Multi-drug resistance (MDR) was observed in 3 of the 8 Salmonella isolates which represented 37.5% of all isolates tested. A total of 4 antimicrobial resistance profiles were observed in this test based on the types of antibiotics and isolate. The most frequent antibiotic that the isolate demonstrated resistance to was Ampicillin and Amoxicilllin-Clavulanic acid. Among the Salmonella isolated, only three of the isolates were sensitive to all the drugs tested.
representing 37.5% of the total isolates, which is slightly higher compared to a similar research by [7], with 23.4% isolates from asymptomatic food handlers sensitive to all the tested antibiotics this could be due to the geographical difference and types of antibiotics that were used in these two research.

5. Discussion

5.1 Antibiotic sensitivity reactions by the isolates

5.1.1 Susceptibility of the isolates to the test drugs

Most isolates were observed to be sensitive to the drugs under test, with 69 sensitive reactions observed. The most efficient drugs observed were Ofloxacin, Ciprofloxacin, and Gentamicin. These were sensitive in all the Salmonella serotype isolates. This agrees with the finding by [6] that all the NTS isolates were found to be susceptible to Ciprofloxacin and Ceftriaxone. In this study, resistance was observed in Ceftriaxone. S. Paratyphi A and S. Paratyphi B were observed to be resistant to Ceftriaxone, this is in agreement with [6][11]. This indicates that Ciprofloxacin has maintained its efficacy to NTS and is an effective choice of remedy unlike Ceftriaxone.

5.1.2 Variations in inhibition zones of the different serotypes to test antibiotics

Intermediate resistance of 3.4% was observed in Amoxi-Clav by S. Enteritidis, Doxycycline and Nalidixic acid by S. Paratyphi A, indicating the presence of antibiotic resistance among Salmonella isolates from the asymptomatic food workers. This was observed to indicate reduced potency of the antibiotic drugs to the isolates.

5.1.3 Observed variation in sizes of inhibition zones under different drugs

Elevated levels of antibiotic resistance by the Salmonella isolates were observed in Ampicillin and Amoxicillin-Clavulanic acid. This corresponds to an outcome in a study by [6] and [11] who noted increased resistance by Salmonella isolates to Ampicillin. There was observed variation in the response to the different antibiotics used in the research by the isolates. S. Typhimurium isolate had the highest average zones of inhibition while S. Paratyphi A had the lowest (Figure 1). A significant variation in inhibition zone sizes of Salmonella under the commonly used drugs was observed, F=19.48, P≤0.05. Thus significant difference in their effectiveness, Ciprofloxacin (31.375) had the largest mean inhibition zones. These variations were grouped into five categories of similar significance in efficiency through comparison of the antibiotic average means of inhibition zones. Ciprofloxacin, Gentamicin and Ceftriaxone were observed to be more susceptible (Table 3) among the isolates with average means of 26.875-31.375 compared to the rest of antibiotics tested.

Variations in inhibition zones of the different serotypes to test antibiotics

![Figure 1: Average inhibition zone sizes of different antibiotics to isolated Salmonella](image)

A significant variation in inhibition zone sizes of salmonella under the commonly used drugs was observed F=19.48 while P<0.05, thus significant difference in their effectiveness. Ciprofloxacin (31.375) had the largest mean inhibition zones while Amox-Clav (16.00) and Ampicillin (15.375) had the smallest recorded mean inhibition zones.

5.1.4 Observed variation in sizes of inhibition zones

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>n</th>
<th>Mean*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciprofloxacin</td>
<td>8</td>
<td>31.375</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>8</td>
<td>27.250</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>8</td>
<td>26.875</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>8</td>
<td>26.000</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>8</td>
<td>21.625</td>
</tr>
<tr>
<td>Sulfamethoxazole-Trimethoprim</td>
<td>8</td>
<td>21.500</td>
</tr>
<tr>
<td>Chloramphenical</td>
<td>8</td>
<td>19.500</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>8</td>
<td>18.250</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>8</td>
<td>17.375</td>
</tr>
<tr>
<td>Amox-Clav</td>
<td>8</td>
<td>16.000</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>8</td>
<td>15.375</td>
</tr>
</tbody>
</table>

*Means with the same letter are not significantly different

6. Conclusion

Drug and MDR serotype are present among the isolates from the asymptomatic food workers. This indicates the presence of risk in the spread of Salmonella, especially MDR Salmonella in most urban populations who consume food prepared by the asymptomatic food handlers from the food outlets.

7. Scope for Future Study

Frequency of mandatory screening of food handlers by regulatory authorities should be standardized. Advisory services to all handlers, with appropriate treatment of carriers should be routine.
References


Author Profile

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