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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 bacteraemia. 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].

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.

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].

3. Materials and Methods

3.1 Sample collection

Feacal 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 both selective and differential media for bacterial identification [10][9], most colonies were able to grow within 24 hours of incubation on the differential media. Characterization of the isolates or serotyping was done through the immunological reactivity of two surface structures polysaccharide O antigen; flageline protein and the VI antigen which are specific to *Salmonella* this separated the unconfirmed cases of *Salmonella* from other enteric bacteria [3].

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), Oflofloxaxin (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.* Para*typhi* A, meaning *S.* Typhimurium demonstrated sensitivity to most of the antibiotics being tested, unlike *S.* Para*typhi* A.

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

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

Key	
AMC	Amox-Clav
AMP	Ampicillin
CTR	Ceftriaxone
D	Doxycycline
S	Streptomycin
C	Chloramphenical
OFX	Oflofloxacin
NA	Nalidixic acid
CIP	Ciproflaxin
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*

Profile	Isolate
AMC	*S.* Enteritidis
AMC, AMP	*S.* Typhimurium
AMC, AMP, SXT, CTR, S, C	*S.* Para*typhi* A
AMC, AMP, SXT, CTR, S,C, D	*S.* Para*typhi* B

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 Amoxicillin-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 Oflofloxacin, Ciproflaxin 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 Ciproflaxin and Ceftriaxone. In this study, resistance was observed in Ceftriaxone. *S.* Para*typhi* A and *S.* Paratyphi B were observed to be resistant to Ceftriaxone, this is in agreement with [6][11]. This indicates that Ciproflaxin 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.* Para*typhi* 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.* Para*typhi* A had the lowest (Figure 1). A significant variation in inhibition zone sizes of *Salmonella* under the commonly used drugs was observed, $F=19.48$, $P \leq 0.05$. Thus significant difference in their effectiveness, Ciproflaxacin (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. Ciproflacin, 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

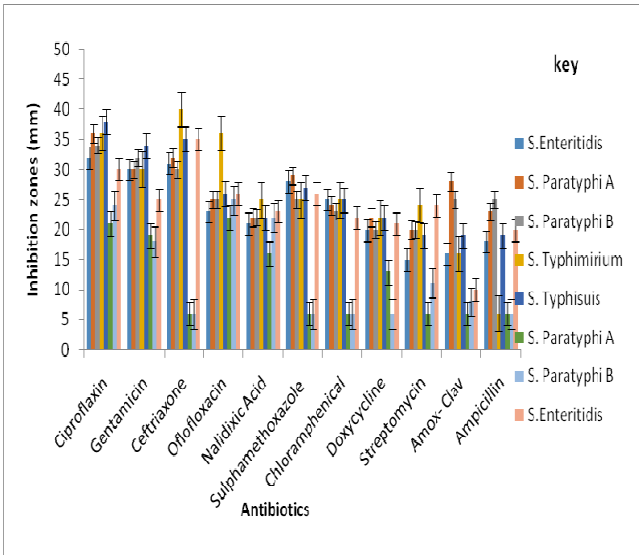

Figure 1: Average inhibition zone sizes of different antibiotics to isolated *Salmonella*

A significant variation in inhibition zone sizes of *salmonella* under the commonly used drugs was observed $F=19.48$ while $p \leq 0.05$, thus significant difference in their effectiveness. Ciproflaxacin (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 3: Comparison of antibiotic efficacy through their means

Antibiotic	n	Mean*
Ciprofloxacin	8	31.375^{a}
Gentamicin	8	27.250^{a}
Ceftriaxone	8	26.875^{ab}
Ofloflaxin	8	26.000^{bc}
Nalidixic acid	8	21.625^{cd}
Sulfamethoxazole-Trimethoprim	8	21.500^{cd}
Chloramphenical	8	19.500^{de}
Doxycycline	8	18.250^{de}
Streptomycin	8	17.375^{de}
Amox-Clav	8	16.000^{e}
Ampicillin	8	15.375^{e}

*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.

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