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ANTIBIOTIC RESISTANT *SALMONELLA* AND *ESCHERICHIA COLI* ISOLATED FROM INDIGENOUS *GALLUS DOMESTICUS* IN NAIROBI, KENYA
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ANTIBIOTIC RESISTANT *SALMONELLA* AND *ESCHERICHIA COLI* ISOLATED FROM INDIGENOUS *GALLUS DOMESTICUS* IN NAIROBI, KENYA

S. M. WESONGA, G.M. MULUVI, P.O. OKEMO and S. KARIUKI

ABSTRACT

Objective: **To characterise and investigate antimicrobial resistance of *Escherichia coli* and *salmonella* strains isolated from indigenous *Gallus gallus* in a leading slaughterhouse/ market outlet in Nairobi-Kenya.**
Design: **A repeated cross sectional study and based on random sampling was used.**
Setting: **The study was carried out in a leading market outlet in Nairobi, Kenya.**
Results: **A hundred and four indigenous chicken rectal swabs were analysed, of which 67.3% were contaminated with *Escherichia coli* and 12.5% with *Salmonella typhimurium*. Seventy *Escherichia coli* isolates showed resistance phenotypes to one, two or more antibiotics. The most common antimicrobial resistance pattern was the single resistance to Tet (21.43%), followed by Amp Cot Tet (14%), Aug Amp Cot Tet (4.29%), Aug Amp Cot Tet Kan Chl (2.86%), Amp Cot Tet Chl, Cot Tet (2.86%) and Crx Amp Cot Tet Chl, Crx Amp Cot Chi, Amp Cot, Aug Amp, (1.43%) respectively. The highest rate of resistance was against Tet (55.7%), followed by Cot (40%). Third in line of resistance was Amp 32.86%, followed by Aug (11.43%), low or moderate resistance was against Chl (8.57%), Kan (4.29%), and Crx (2.86%) (P<0.0002).** *Salmonella typhimurium* **recovered displayed single resistance pattern to Tet (16.67%), Gen Cot Tet (8.33%), Amp Cot Tet (8.33%), Aug Amp Cot Tet (8.33%) and Amp Cot Tet Chl (16.67%). The highest resistance was against Tet (58.3%), Cot (41.7%), Amp (33.3%), Chl (16.7%), Aug and Gen (8.3%) respectively (P<0.0001). 3.0kb and 5.6kb plasmids isolated were not transferable by conjugation.**
Conclusion: **Routine surveillance at slaughter/market outlets of *Escherichia coli* and *Salmonella enterica* should be done to identify infected flocks as a regulatory procedure for food safety and security programme.**

INTRODUCTION

Food animals harbour food borne pathogens and act as a source of contamination, which is important in the spread of *Salmonella* and *Escherichia coli* in human (1-3). For example, chickens can serve as reservoirs of *E. coli*, *Salmonella spp*., *Campylobacter spp* and antibiotic resistance genes from these bacteria may be co-transferred to humans (4,5). The shedding of pathogens by asymptomatic animals is increasing concern as a source, distribution of food borne diseases (FBDs) and antibiotic resistance (6-8). The process of evisceration during slaughter of food animals is regarded as one of the most important sources of carcass and organ contamination with pathogens (9). Food items such as poultry products are regarded as the common source of food borne *Salmonella* and *E. coli (*9,10).

Antibiotics are widely used for the treatment of infectious diseases in the poultry industry, humans and other animals (1) that are believed to contribute toward the development of antibiotic resistance in both the pathogens and normal micro-flora of poultry (8,7). Zoonotic pathogens could acquire antibiotic resistance while inhabiting the gastrointestinal tracts of food animals and could then transfer this resistance to humans via the food chain (8,9,12). Such practices often lead to the excretion of (7) and sometimes illness (1) due to, drug-resistant bacteria in animals and humans. The emergence and widespread of

antimicrobial-resistant E. *coli*. and *Salmonella* strains in chickens and humans may be associated with the indiscriminate use of antimicrobials both in animal and human treatments (13). Antibiotic resistance in these bacteria is often mediated by plasmids, some of which are self-transmissible (7,9,11,14,15), whereas others may be cotransferred by conjugative plasmids (14,15). As part of food safety policy, it is important to determine local patterns of antimicrobial resistance in food borne pathogens on a regular basis.

MATERIALS AND METHODS

Sample collection: Chicken rectal swabs samples were sourced from indigenous chicken in a leading slaughterhouse/market outlet in Nairobi, Kenya.

Bacterial isolates: Chicken rectal swabs were pre-enriched in buffered peptone water (BPW) at 37°C for 24 hours.

Escherichia coli strains: Each of the specimens were cultured onto MacConkey agar (OXOlD) and incubated at 37°C overnight. Colonies suspected to be E. *coli* were being isolated from the MacConkey agar plates and identified using biochemical tests, confirmed by API 20E strips (Biomerieux, Basingstroke UK). The isolates were stored at -70°C in microvials.

Salmonella enterica serovars: The specimens were inoculated onto Xylose Lysine decarboxylase agar (XLD). Cotton tipped swabs were used to spread rectal swabs on a plate. Using aflame-sterilised wire loop, the inoculum were streaked into four quadrants of the plates with flaming after each quadrant had been streaked, to obtain discrete colonies after overnight incubation at 37°C. The inoculated plates and bottles were incubated aerobically at 37°C for between 18-24 hours. The XLD agar plates were removed from the incubator and examined for non-lactose fermenting black centred colonies with clear edges and identified using biochemical tests. Confirmation of *Salmonella* and E. *coli* isolates were done using API 20E strips and stored in 15% glycerol at -80°C.

Antimicrobial susceptibility testing: The antimicrobial resistance tests of *Salmonella* strains were carried out with the agar diffusion method as recommended by the National Committee for Clinical Laboratory Standards (NCCLS), (16). Briefly, the strains were grown to 0.5-1.0 McFarland density in Mueller Hinton (MH) broth (Difco.) and replica plated onto MH agar plates (Difco.) containing antimicrobials at the concentration as follows: Augumentin (Aug) 30μg; Ampicillin (Amp) 10μg; Cefuroxime (Cfx) 30μg; Norfloxacin (Nor) 10μg; Chloramphenicol (Chl) 30μg: Gentamicin (Gen) 10μg; Kanamycin (Kan) 30μg; Nalidixic acid (Nal) 30μg; Tetracycline (Tet) 30μg; Cotrimoxazole (Cot) 25μg. An isolate was defined as resistant if it was resistant to one or more of the antimicrobial agents tested whereas multiple resistances was defined as resistance to two or more antimicrobial agents. Standard reference strains of E. *coli* (ATCC 25922) were used and interpretation of the strains as susceptible, intermediate or resistant was made following the recommendations of the NCCLS (16).

Plasmid isolation: Plasmids were isolated using the method of Kado and Liu (17). Samples were analysed by electrophoresis on horizontal 1% agarose gel in 0.5xTBE buffer at 125V for 2 hours. Plasmid sizes were determined by coelectrophoresis with plasmids of known sizes for *E. coli* strains V517 (53.7. 7.2, 5.6, 3.9, 3.0, 2.7, 2.1kb) and 39R861 (147.63, 43.5, 6.9kb). DNA bands were visualised with ultraviolet transilluminator (UVPinc.) after staining with 0.05% ethidium bromide.

In-vitro conjugation experiment: To determine mobility of antibiotic-resistance genes, *in-vitro* conjugation was done as described by Yamamoto and Yokota (18) using E. *coli* K-12 (Nalidixic acid resistant) (30μg/ml) and Ampicillin (30μg/ml). To determine the transferable antibiotic resistance, transconjugants were tested for susceptibility to the battery of antibiotics previously used for isolates.

Statistical analysis: The isolation and identification of organisms were entered as plus (+) for presence and negative (-) for absence of organisms. Response to antibiotics was recorded as either Susceptible (S). Intermediate (I), or Resistant (R). Plasmid fingerprinting was entered as bands. All the data was entered into the computer and subsequently analysed using SPSS 11.5 (2000) and MS Excel 2000 package for windows at confidence interval of 95% to determine significance. The overall trends were computed using descriptive and quantitative analysis. These were further subjected to analysis of variance (ANOVA).

RESULTS

During this study, 104 samples were collected from indigenous chicken (*Gallus gallus*) rectal swabs. The samples collected were from four districts namely Bomet, Kericho, Kitui and Murang'a that supply indigenous chicken to the leading slaughterhouse/market outlet in Nairobi. In the study, eighty three isolates of gram-negative bacteria were obtained from rectal swabs from indigenous chicken. *Salmonella*

enterica was present at a frequency of 15.7% and *Escherichia coli* 84.3%. Murang'a district had the highest number of *E. coli* isolates, followed by Kitui, Bomet and Kericho. On the other hand, Bomet district had the highest isolates of *Salmonella typhimurium* (four isolates), while Kericho, Murang'a and Kitui had three isolates each (Table 1).

Table 1
Distribution of bacterial isolates from indigenous chicken rectal swabs

District	Isolates		Totals
	E. coli	S. *typhimurium*	
Murang'a	19	3	22
Kitui	18	3	21
Bomet	17	4	21
Kericho	16	3	19
Total	70	13	83

Table 2
Levels of Escherichia coli in indigenous chicken rectal swabs (n=17)

Source	Range	Mean (n=17)
Bomet	$2.0x10^2$------ $1.64xl0^4$	$4.48x\ 10^3$cfu/g
Kericho	$1.0x10^2$------$1.01 x\ 10^4$	$3.45x\ 10^3$cfu/g
Kitui	$1.0x\ 10^2$-------$1.15x10^4$	$3.62x\ 10^3$cfu/g
Murang'a	$1.0x\ 10^3$ ------ $1.50x\ I\ 0^4$	$5.15x\ 10^3$cfu/g

Table 3
Levels of Salmonella typhimurium in indigenous chicken rectal swabs (n= 17)

Source	Range	Mean (n=17)
Bomet	$1.0x10^1$---$5.0x\ 10^1$	$2.67x10^1$cfu/g
Kericho	$1.0x10^1$---$4.0x10^1$	$2.67x10^1$cfu/g
Kitui	$1.0x10^1$---$2.0x\ 10^1$	$1.33x10^1$cfu/g
Murang'a	$1.0x10^1$----$2.0x\ 10^1$	$1.33x10^1$cfu/g

Table 4
Serotype of Salmonella enterica isolated from indigenous chicken rectal swabs

Serotype					Totals
	Bomet	Murang'a	Kitui	Kericho	
Salmonella Typhimurium	4	3	3	3	13

Table 5
Serotypes of E. coli strains isolated from indigenous chicken rectal swabs

	Isolates				
Serotype	Bomet	Kericho	Murang'a	Kitui	Total
ETEC	16	10	14	13	53
EPEC	1	6	5	5	17

Table 6
Antimicrobial susceptibility of E. coli isolates from indigenous chicken rectal swabs

Antimicrobial agent	Susceptibility of strains					
	Resistant		Intermediate		Susceptible	
	No.	(%)	No.	(%)	No.	(%)
Ampicillin	23	32.86	0	0	47	67.14
Amoxicillin-clavulanic acid	8	11.43	4	5.71	58	82.86
Cefuroxime	2	2.86	66	94.29	2	2.86
Chloromphenicol	6	8.57	0	0	64	91.43
Cotrimoxazole	28	40	2	2.86	40	57.14
Gentamicin	0	0	1	1.43	69	98.57
Kanamycin	3	4.29	21	30	46	65.71
Nalidixic acid	0	0	2	2.86	68	97.14
Norfloxacin	0	0	0	0	70	100
Tetracycline	39	55.71	1	1.43	30	42.86

Table 7
Antibiogram patterns of E. coli isolates from indigenous chicken rectal swabs

Resistance patterns	No. of strains	Resistance patterns	No. of strains
Tet	15	Amp Cot Tet Chl	2
Cot	3	Crx Amp Cot Tet	1
Aug Amp	1	Aug Amp Cot Tet Kan	1
Amp Cot	1	Aug Amp Cot Tet Chl	1
Cot Tet	2	Crx Amp Cot Tet Chl	1
Amp Cot Tet	10	Aug Amp Cot Tet Kan Chl	2
Aug Amp Cot Tet	3		

Table 8
Antimicrobial susceptibility of Salmonella typhimurium isolatesfrom indigenous chicken rectal swabs

Antimicrobial agent	Susceptibility of strains					
	Resistant		Intermediate		Susceptible	
	No.	(%)	No.	(%)	No.	(%)
Ampicillin	4	30.77	0	0	9	69.23
Amoxicillin-clavulanic acid	1	7.69	2	15.39	10	76.92
Cefuroxime	0	0	10	76.92	3	23.08
Chloromphenicol	2	15.39	0	0	11	84.61
Cotrimoxazole	5	46.15	0	0	8	53.85
Gentamicin	1	7.69	0	0	12	92.31
Kanamycin	0	0	5	46.15	8	53.85
Nalidixic acid	0	0	0	0	13	100
Norfloxacin	0	0	0	0	13	100
Tetracycline	7	53.85	0	0	6	46.15

Table 9
Antimibiogram patterns of Salmonella enterica Typhimurium isolates from indigenous chicken rectal swabs

Resistance patterns	No. of strains
Tet	2
Gen Cot Tet	1
Amp Cot Tet	1
Amp Cot Tet Chl	2
Aug Amp Cot Tet	1

DISCUSSION

The study showed a dramatic appearance of multi-drug enteric pathogens (*Salmonella typhimurium* and *E. coli*) in apparently healthy indigenous chicken sold in the slaughterhouse cum market outlet in Nairobi, Kenya. The microbes may easily contaminate chicken carcasses during evisceration (8), leading to a possibility of cross contamination directly from raw chicken or indirectly via contaminated surfaces or niches. The result of this study clearly shows a high isolation rate of *E. coli* and *Salmonella typhimurium* (79.8%) from indigenous chicken rectal swabs. *E. coli* in this study was 55.7% resistant to tetracycline, Cot (40%), Amp (32.86%), Aug (11.43%), Chl (8.57%), Kan (4.29%) and Crx (2.86%). The *E. coli* strains were susceptible to Nor (100%), Gen (98.57%), Nal (97.14%), Chl (92.43%), Aug (82.86%), Amp (67.14%), Kan (65.71%), Cot (57.4%), Tet (42.86%) and Crx (2.86%) respectively.

Salmonella typhimurium isolates in the study were 53.85% resistant to tetracycline, Cot (46.15%), Amp (30.77%), Chl (15.39%). Aug and Gen (7.69%) respectively. On the other hand, *Salmonella typhimurium* was fully susceptible to Nor and Nal (100%). Gen (92.31%), Chl (84.61%), Aug (76.92%). Amp (69.23%), Cot and Kan (53.85%), Tet (46.15%), and Crx (23.08%) respectively.

Indigenous chicken poke and scratch anything they find in the quest for food, thus they may pick up pathogens and other materials such as drug residues from the environment. This is in line with findings done by Appanjalati *et al* (2). At the same time indigenous chicken (*Gallus gallus*) are reared together with other monogatric animals (2,19), this may lead to zoonotic cross transmission of pathogens.

In a previous study, thirty seven strains of *E. Coli* recovered from cases of septicaemia in chicken in Kenya showed resistance to Trimethoprim-Sulphamethoxazole (100%), Kanamycin (13.5%) and Gentamycin (2.7%) (11). In another study, high resistance rates were observed in the chicken *E. coli* isolates as Tetracycline (99.1%), Cotrimoxazole (92.2%), Gentamicin (89.7%), Ampicillin (88.7%) and Chloramphenicol (57.0%) in Saudi Arabia (20,21).

Avian E. *coli* from faeces has also been shown to display multidrug resistance in Iran (14) while in Spain up to 67% to Cotrimoxazole and Fluoroquinolones (20). It is now evident in this study that there is an upward trend in the numbers of antibiotics to which E. *coli* strains and *Salmonella typhimurium* are resistant.

The isolation of multidrug R-types of E. *coli* and *Salmonella typhimurium* in indigenous chicken in particular, resistance to Augumentin as displayed in the resistance patterns as follows (Aug Amp), (Aug Amp Cot Tet), (Aug Amp Cot Tet Kan). (Aug Amp Cot Tet Chl), (Aug Amp Cot Tel Kan Chl) of *E. coli* isolates (Table 7) and *Salmonella enterica* Typhimurium (Aug Amp Cot Tet) (Table 9), the drug of first choice for extra intestinal and serious intestinal infections in adults, may reduce the efficacy of early empirical treatment, the consequence being treatment failure.

In Kenya the unregulated over-the-counter sale of these antibiotics due to self-treatment of suspected infection in humans, and to a lesser extent for use in animals without prescription contribute to emergence and rapid dissemination of resistance (15, 22). This has exacerbated the problem of controlling microbes in a disease setting and has caused a resurgence of many bacterial diseases. The high level of resistance to Tetracycline, Cotrimoxazole and Ampicillin is of concern as these drugs form the mainstay antibiotics used in human medicine.

Two E. *coli* isolates were resistant to Crx. Cefuroxime is widely used in the treatment of certain human infections, bovine mastitis, feline and canine upper respiratory tract infections (13). This should be explained from the view that indigenous poultry are usually raised together with other domestic animals (e.g. monogastric species such as pigs and rabbits, small and large ruminants) and in some cases with fish (19). The isolates were from Bomet where we have intense rearing of domestic animals.

In Kenya, *Salmonella typhimurium* is a major cause of illness and high mortality in children below three years (15). *Salmonella enterica* typhimurium presents as diarrhoeal disease acquired as food poisoning with several foods being implicated as transmitting vehicles of salmonellosis to human as poultry, beef, pork, eggs, milk, vegetables, fresh fruits and juices in the food chain (15,23).

In this study, the presence of S. *enterica* serotype typhimurium in indigenous chicken demonstrates the potential for food contamination during handling and processing. The prevalence of multidrug resistant *Salmonella typhimurium* in indigenous chicken retail outlet reflects a reservoir of resistanee in poultry (24,25), that can be transmitted to humans (26).

None of *Salmonella typhimurium* were resistant to Norfloxacin, Kanamycin, Nalidixic acid and the third generation Cephalosporin, Cefuroxime. A study by Zahraei *et al* (27) showed, Salmonella isolates from chicken, showed resistance to Kanamycin (34.6%), Tet, Amp. Trim, Nal (20.7%) (19,27). Most of the latter antibiotics are commonly used in Kenya both in the public health and veterinary.

Scavenging indigenous *Gallus gallus* sold in leading slaughterhouse cum market outlet in Nairobi, intended for food shed resistant *Salmonella typhimurium* and *E. coli* pathogens which may enter the food chain. The poultry litter also may find its way to surroundings such as aquatic environment due run-off leading to potential reservoirs of bacterial drug resistance. This is in line with the high prevalence of seasonal intestinal infections as noted by Kariuki *et al* (15) in tropical Africa during rainy seasons. Thus, animal litter is now considered as a route of human exposure to antimicrobials used in food producing animals. *Escherichia coli* 0157:H7 and *Salmonella typhimurium* have been seen to survive in cow manure, slurry (28), swine manure and environment (29).

In conclusion, these data confirm that indeed there is significant of drug resistance in strains of E. *coli* ($P<0.0002$) and *Salmonella typhimurium* ($P<0.0001$) in indigenous *Gallus gallus*. The problem of resistance in enteric pathogens will remain an ongoing threat in food industry in Kenya.

Thus routine systematic surveillance and timely reporting of antibiotic resistance patterns among enteric pathogens should become a high priority to establish possible sources of bacterial resistance and provide data that can be used to select appropriate treatment. Norfloxacin, Nalidixic acid and Gentamicin are the most effective antibiotics against E. *coli*. While in Norfloxacin, Nalidixic acid, Cefuroxime and Kanamycin are the most effective against *Salmonella typhimurium* in indigenous *Gallus gallus*.

To diminish E. *coli* and *Salmonella typhimurium* contamination rates in retail indigenous chicken, it is critical that risk reduction strategies are used throughout the food chain. These strategies include on-farm practices that reduce pathogen carriage, regulated movement, increased hygiene at slaughter and poultry meat processing, consumer-education efforts to protect public health and continued implementation of HACCP systems. This will minimise indigenous chicken contamination with these pathogens that can occur at multiple steps along the food chain, including production, processing, distribution, retail marketing and handling or preparation.

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