Prevalence and Antimicrobial Resistance Profiles of *Salmonella* Species and *Escherichia coli* Isolates from Poultry Feeds in Ruiru Sub - County, Kenya

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Research note

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Abstract

Objectives: Contaminated poultry feeds can be a major source of *E. coli* and *Salmonella* infections in poultry. This study aimed at determining the microbial quality, prevalence and antimicrobial resistance and associated resistance genes to *Salmonella* and *E. coli* isolates from poultry feeds.

Results: A total of 150 samples of different poultry feed types were randomly collected from selected sites within Ruiru Sub-County. A microbial load was determined, *Salmonella* and *Escherichia coli* were isolated and antimicrobial susceptibility test accomplished. Antimicrobial resistance genes; *TEM*, *SHV*, *strB* and *Dfr* were established. Out of analyzed samples, 58% contained *Escherichia coli* and 28% *Salmonella*. Bacterial load ranged between 3.1 $x10^5$ cfug to 3.0 $x10^6$ cfu/g. The highest resistance was found with ampicillin (41%) for *Salmonella* and (62%) for *E. coli* isolates. All the Ampicilin resistant isolates carried *TEM* and *SHV* genes. In addition, *strB* and *Dfr* drug resistance genes associated with streptomycin and Cotri-moxazole were analysed. All isolates were susceptible to chloramphenicol and ciprofloxacin. The study reveals high bacterial contamination, presence of beta-lactamase, aminoglycoside and sulphonamide resistance genes across isolates from poultry feeds. Therefore, contaminated poultry feeds with bacteria are likely to lead to increase and spread of antimicrobial resistant strains across the community.

Introduction

Poultry feeds are contaminated with microbes during harvesting, preparation and sale of the produced feeds [1]. Poultry feed contamination has been associated with *Escherichia coli* and *Salmonella* species [2]. Antimicrobial resistance has a main concern with medical and veterinary sciences due to its effects on public health [3]. Indiscriminate use of antimicrobials may increase antibiotic resistant nonpathogenic and pathogenic bacteria considered threat to poultry and humans [4].

Antibiotics, enzymes, pigments and antifungals are non-nutritive additives in feed formulations to maintain health status of the poultry [5, 6]. High utilization of antibiotics in poultry production [7] has led to antibiotic resistance, hence a health threat worldwide [8]. Antibiotics in poultry promote growth, treat, and control and prevent infectious diseases [9, 10, and 5]. Antimicrobial resistance is a growing public health concern worldwide [11]. Different studies have been carried out to determine the existence of *Salmonella* and *E. coli* in domestic fowl feeds and antibiotic resistance patterns on isolates from poultry feed. [12, 13, 14]. However, investigation of associated resistant genes from poultry feed is minimal.

In Kenya increased poultry rearing has increased the demand for feeds leading to more feed companies. Microbiological health controls to avoid microbial poultry contamination are important in poultry feeds [15]. However, there are minimal reports on bacterial load, antimicrobial resistance, associated resistance genes and potential source of *Salmonella* and *E. coli* contamination in poultry feeds in Kenya.

This research determined the bacterial load, prevalence of *Salmonella sp*. and *Escherichia coli*, the antimicrobial resistance patterns and assessed presence of resistance genes from poultry feed in Ruiru

Sub-County Kenya.

Methods

Sampling

A cross-sectional study was carried out between January and April 2019. A total of 150 poultry feed samples was picked up randomly from selected outlets in Biashara, Gitothua, Gatong'ora, Kiuu and Mwihoko in Ruiru Sub County, Kenya. The samples consisted grower mash, layer mash, starter mash, finisher mash, kienyeji mash, chick mash, maize germ and sunflower. Estimated 400 g of the sample was collected in sterile khaki paper and taken to Kenyatta University Microbiology laboratory for analysis.

Before commencement of this study, permission was sort and granted from Commissions for Science Technology and Innovation and County Commissioner, Kiambu County.

Bacteriological analysis

One gram of feed sample was homogenized in 9 ml sterile deionized water, thoroughly mixed to form a ratio of 1:10 and a 4 fold serial dilutions were made. Aliquot of 0.1 ml of the serial dilution was drawn and inoculated into nutrient agar using the spread plate method. The inoculants were then incubated at 37 °C for 18-24 hours and colonies counted. Plate bacterial count was carried out using colony counter, recorded and total bacterial count calculated; CFU/g= level of Dilution plated x number of colonies counted/ amount plated [17]. Specific bacteria count considered ranged from 30 to 300 per plate and those above this categorized as numerous [18].

Isolation and Identification of *Salmonella sp*. and *Escherichia coli*

Samples were enriched in selenite F broth and incubated at 37 °C for 18 hours. Thereafter, the inoculants were sub cultured onto Xylose Lysine Deoxycholate (XLD) agar and Salmonella-Shigella (SS) agar for selection of *Salmonella sp*.

For isolation of *Escherichia coli*, samples were enriched in peptone water and incubated at 37 °C for 18 hours before sub culturing the inoculants onto sorbitol MacConkey agar. The inoculated cultures were then incubated at 37 °C for 24 hours and biochemical tests employed to confirm suspected *Salmonella sp*. and *Escherichia coli* as previously described [19, 20].

Antimicrobial susceptibility test

Kirby-Bauer disc diffusion technique was applied to establish the susceptibility of the isolates to antibiotics [21]. Briefly, antimicrobial agents; Ampicillin (10 µg), Ceftriaxone (30 µg), Co-trimoxazole (25 µg), Tetracycline (30 µg), Chloramphenicol (50 µg), Ciprofloxacin (30 µg) were evaluated using *Escherichia coli* ATCC 25922 as control organism. Antimicrobial susceptibility results were elucidated according to Clinical and Laboratory Standard Institute guidelines [22].

Extraction of Bacteria genomic DNA

Bacteria DNA extraction was done using boiling method as earlier described [23, 24]. One milliliter of overnight bacterial culture of the pooled 34 bacterial resistant isolates were suspended in 1000 µl of sterile distilled water and then boiled for 18 minutes at 100 °C. The resulting suspension was then centrifuged for 5 minutes at 14, 200 rpm to sediment the debris and the supernatant was stored at -20 °C for subsequent use as the DNA template.

Amplifications of drug associated resistance genes

Resistance genes encoding resistance to betalactams; *SHV* (sulphydryl variable enzyme), *TEM* (temoneira), sulpnomide; *Dfr* (dihydroflavonol 4-reductase enzyme), aminoglycoside; *strB* (streptomycin) genes were assessed as previously described [20, 21]. Polymerase Chain Reaction amplification product was validated by visualization using gel electrophoresis and the gel was stained using ethidium bromide as described [25].

Results

Microbial Quality of poultry feeds

A total of 150 samples was analyzed including layer mash, grower mash, chick mash, kienyeji mash, starter mash, finisher mash and maize germ/sunflower. The highest bacterial load was detected in layer mash (Table 1). Bacterial counts below 30 and above 300 excluded were 37. All the poultry feeds analyzed were contaminated with bacteria. According to Kenya Bureau of Standards, compounded poultry feeds should not contain afflatoxins, pathogenic bacteria or fungi. There was a significance difference in bacterial load for all poultry feeds ($p = 0.0001$) at 0.05 level of significance.

Table 1
Range of bacterial load in poultry feeds

Feed Type	Number of samples	Number of samples excluded	Bacterial load (cfu /g)	
			Lowest	Highest
Layer mash	29	5	3.2×10^5	3.0×10^6
Grower mash	27	7	3.5×10^5	2.04×10^6
Chick mash	30	2	3.2×10^5	2.55×10^6
Kienyeji mash	27	5	3.1×10^5	2.9×10^6
Starter mash	27	4	3.2×10^5	2.94×10^6
Finisher mash	6	2	7.0×10^5	1.37×10^6
Maize germ/sunflower	4	2	4.2×10^5	1.01×10^6
Total	**150**	**37**		

Prevalence of *Salmonella* and *E. coli*

Out of 150 samples 58% of the total isolates harbor *Escherichia coli* and 28% was detected with *Salmonella*. The prevalence of *Salmonella sp* in different poultry feeds was 38%, 37%, 19%, 17%, 19%, and 30% and *Escherichia coli* was 62%, 52%, 48%, 33%, 63%, and 63% in layer mash, grower mash, starter mash, finisher mash, kienyeji mash and chick mash respectively.

Antimicrobial susceptibility profiles for *Salmonella* and *Escherichia coli*

The *Salmonella sp*. showed diverse responses to different antibiotics used. *Salmonella sp*. were all susceptible to ciprofloxacin, chloramphenicol and streptomycin. However, in the tested isolates 41% were resistant to ampicillin, 2% to co-trimoxazole, 5% to ceftriaxone and tetracycline. Intermediate ranged between 0% and 19% (Table 2).

Table 2
Antimicrobial susceptibility profiles of *Salmonella* sp.

Antibiotic class	Antibiotic	Susceptible	Intermediate	Resistance
		n (%)	n (%)	n (%)
Betalactam	Ampicillin	17 (41%)	8 (19%)	17 (41%)
	Ceftriazone	37 (88%)	3 (7%)	2 (5%)
Sulphonamide	Co-trimoxazole	41 (98%)	0%	1 (2%)
Tetracycline	Tetracycline	39 (93%)	1 (2%)	2 (5%)
Phenicols	Chrolamphenicol	42 (100%)	0%	0%
Fluoroquinolones	Ciprofloxacin	35 (83%)	7 (17%)	0%
Aminoglycosides	Streptomycin	41 (98%)	1 (2%)	0%

Escherichia coli isolates showed highest resistance against ampicillin (71%). Resistance to other antibiotics ranged from 1% to 10% with no isolate showing resistance to ciprofloxacin. *Escherichia coli* isolates were 100% susceptible to ciprofloxacin, followed by chloramphenicol (98%), co-trimoxazole (89%), tetracycline and streptomycin (86%) and ceftriaxone (78%). The highest intermediate was observed in ampicillin with 21% (Table 3).

Table 3
Antimicrobial susceptibility profiles of *Escherichia coli*

Antibiotic class	Antibiotic	Susceptible	Intermediate	Resistance
		n (%)	n (%)	n (%)
Betalactam	Ampicillin	7 (8%)	18 (21%)	62 (71%)
	ceftriaxone	68 (78%)	13 (15%)	6 (7%)
Sulphonamide	Co-trimoxazole	77 (89%)	4 (5%)	6 (7%)
Tetracycline	Tetracycline	75 (86%)	3 (3%)	9 (10%)
Phenicols	Chrolamphenicol	85 (98%)	1 (1%)	1 (1%)
Fluoroquinolones	Ciprofloxacin	87 (100%)	0%	0%
Aminoglycosides	Streptomycin	75 (86%)	10 (11%)	2 (2%)

Antimicrobial resistance genes among the isolates

The total number of isolates screened for resistance genes were 34. Among the screened isolates *TEM*, *SHV*, *Dfr* and *strB* genes were detected (Additional files; Figure S1, figure S2, figure S3, figure S4). The *TEM* gene dominated with 24 %, followed by *Dfr* 21 %, *SHV* 12 % and *strB* 9 % (Additional file 1; table S1).

Discussion

A prevalence of 28% of *Salmonella* sp. in poultry feeds recorded was similar to a prevalence of 29% reported in Tanzania [26] and Bangladesh [27]. Nevertheless, studies in Africa have continued to show varying results. A prevalence of 38% and 31% was reported in Nigeria [16, 28] and a prevalence of 71%, 55% and 29% in Bangladesh [15, 20, and 27]. The prevalence of *Salmonella* sp. from different poultry feeds was 38%, 37%, 19% and 17% in layer mash, grower mash, starter mash and finisher mash, respectively, contrary to a previous study that recorded a prevalence of 20%, 0%, 40% and 25% in layer mash, grower mash, starter mash and finisher mash, respectively [14] and prevalence of 21%, 38%, 31% and 33% in layer mash, grower mash, starter mash and finisher mash respectively in Tanzania [26].

The disparity of *Salmonella* prevalence could be due to differences in sampling, testing methods and difficulties in *Salmonella* detection methods [26].

The prevalence of 58% of *Escherichia coli* obtained was similar to a related study carried out in Bangladesh with a prevalence of 57% [27]. Other related studies reported different prevalence of 16% in Iraq [1] and in Nigeria 10.6% and 11% respectively [29, 16]. The prevalence of bacteria varies considerably depending on nature of production, country and detection methods applied [30]. Microbial contamination of poultry feeds of plant and animal origin has also been associated with harvesting, manufacturing and climatic conditions encountered [12].

Therefore, it is important to reinforce hygienic handling of feeds and preventive control measures to minimize the danger of potential animal and human health hazards.

Varying results in this study could be attributed to methods of harvesting raw materials, different climatic conditions, food formulation, and storage and transportation technologies [16, 12]. The high bacteria count in layer mash could be due to use of fish wastes as animal proteins which harbors heavier bacterial growth [14]. The differences in bacterial load in different types of poultry feed could be as a result of mixed infections with other microbes, different environmental conditions and management [31]. Findings in this study indicates that contaminated poultry feed could be dangerous, cause infections and thus not fit for animal consumption [32, 33].

Different resistant patterns of *Salmonella sp*. and *E. coli* were similar to other related studies. Both isolates of *Salmonella* and *E. coli* registered the highest resistance for Ampicillin 41% and 71% respectively, and highest susceptibility to Ciprofloxacin, 83% and 100% respectively. The isolates of *E. coli* indicated highest resistance to ampicillin 71%, followed by tetracycline 10%, co-trimozaxole and ceftriazone 7% and 0% ciprofloxacin similar to a previous study in Bangladesh that recorded ciprofloxacin as most effective antibiotic against *E. coli* isolates from poultry feeds [34]. *Salmonella* sp showed highest

resistance to ampicillin 41%, tetracycline and ceftriaxone 5%, co-trimoxazole 2% and ciprofloxacin 0% contrary to a previous related study in Bangladesh that reported resistance of 30% to ciprofloxacin, 20% to gentamicin, 60% to nalidixic acid and 0% to ceftriaxone [35]. In Kenya, a study on antimicrobial resistance in *Salmonella* and *E. coli* isolates from poultry wastes reported high resistance to amoxicillin which is a beta-lactam, followed by tetracycline and co-trimoxazole [36]. High resistance to co-trimoxazole, beta-lactamst and etracycline among bacterial isolates from chicken in Kenya was also reported [37, 38]. This suggests possible transmission of antibiotic resistant bacteria through poultry feed to poultry.

The resistant isolates of *Salmonella sp* and *Escherichia coli* isolates carried *TEM* and *SHV* genes, contrary to a previous study that reported absence of major extended spectrum beta-lactamases in *Salmonella* isolates from poultry feeds in India [39].

The resistant isolates of *Salmonella sp* and *Escherichia coli* also carried (*Dfr* and (*strB)* genes. However, other related studies on poultry feeds did not look at resistance genes [27, 40, and 35].

Therefore, poultry feeds are potential source of antimicrobial resistant gene transfer to poultry and humans posing a health threat to the society.

Conclusion

This study found out that poultry feeds harbor bacteria and resistance genes, indicating potential danger to both humans and animals which is a public health concern. It's important for poultry feed to be assessed for microbial quality by manufacturers and health authorities to facilitate feed safety.

Limitation

This study never confirmed the relationship between resistance genes isolated from poultry feed and their sources. Its possible acquisition either from human prior or contaminated feeds with microorganisms or through processing of feed was never evaluated.

Abbreviations

SHV: sulphydryl variable enzyme*, Dfr*: Dihydrofolate reductase *TEM*: Temoneira *strB*: Streptomycin resistant gene

Declarations

Authors Contributions

Dorica Gakii Ngai was engaged in designing the study, sample collection, laboratory investigations, interpretation of the data and drafting the manuscript. Anthony Kebira Nyamache and Omwoyo Ombori

conceptualized the idea, helped in the planning of the experiment, and supervised the laboratory experiment, sample analysis, interpretation of the data and review of the article.

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Competing Interests

The authors declare that they have no competing interests

Availability of the data

Not applicable

Consent for publication

Not applicable

Ethical approval and consent to participate

Ethical clearance was not required. Poultry feeds were bought from the outlets. The study was approved by the Kenya National Commission for science, Technology and innovation and authorization given by County Commissioner, Kiambu County Kenya.

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Supplementary Files

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- Additionalfile1.FigureS1.TEMgene.docx