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Antimicrobial susceptibility patterns of *Escherichia coli* isolated from poultry samples at a laboratory in Kenya

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Abstract
Bacteria of the genus *Escherichia coli*, cause Colibacillosis in poultry and are transmitted to humans through handling and/or consumption of contaminated products raising concern over food safety and spread of resistant bacteria. We determined proportion of pathological samples where *E.coli* was isolated and its antimicrobial susceptibility patterns. From 106 birds and148 organs, *Escherichia coli* was isolated in 65% organs with 20% susceptible to Ampicillin, 30% to Tetracycline, 10% to Cotrimoxazole, 20% to Streptomycin, 40% to Kanamycin, 60% to Gentamycin and 10% to Sulphamethoxazole. High proportions of *E.coli* in samples including resistance to common antibiotics was detected. A holistic approach for prevention, control and treatment of avian Colibacillosis embracing good management, prudent use of antibiotics and utilization of laboratory services are important in controlling infections and overuse of antibiotics reducing resistance development. Enhanced food safety and handling practices are important in preventing spread of the drug resistant genes to human populations.

Keywords: *Escherichia coli*, colibacillosis, antimicrobial resistance, poultry

1. Introduction
Bacteria of the genus *Escherichia coli* (*E.coli*) are gram negative flagellated rods which can be found in water, soil and vegetation and the gastrointestinal tracts of birds and mammals where they live as commensals with most strains causing no pathological illness (Jordan, 1990) [7]. Occasionally, these bacteria which are found in large numbers in the small intestines and caeca of birds can cause high morbidity and mortality in affected flocks. *Escherichia coli* infection in poultry is manifested in the form of Colispeticemia, egg peritonitis, yolk sac infection among other infections broadly termed as Colibacillosis. Acute cases of *E.coli* infection present with septicemia with congestion of abdominal organs visible on post-mortem and high mortality in affected poultry flocks with cases of a chronic nature presenting post-mortem findings of fibrinous pericarditis, perihepatitis, air sacculitis and peritonitis (Jordan, 1990) [7].
Colibacillosis is associated with high economic losses in poultry farming due to decreased productivity, high mortaly and high prophylaxis and treatment costs (Luftur, 2010). Control of avian Colibacillosis is mainly aimed at management interventions, infection control and vaccination strategies (Luftur, 2010). High stocking rates, poor ventilation in poultry houses, poor hygiene practices, prophylactic use of antibiotics and use of antibiotics as growth promoters have over-time led to excessive and inappropriate use of antimicrobials. This overuse of antibiotics has led to development of antimicrobial resistance and the emergence of multidrug resistant bacteria. Resistance of *E.coli* to commonly available drugs both in the human and the Veterinary field is a common phenomenon in both developed and developing countries. Antimicrobial resistance poses a big threat to the poultry production industry and has resulted to increased concerns about the safety of poultry meat and eggs and the spread of multidrug resistant bacteria (Ibrahim *et al*., 2019) [6].
Escherichia coli of animal origin has been found experimentally to spread rapidly and to colonize the intestinal tract of humans and of other animals (B. Marshall, D. Petrowski, and S. B. Levy 2019) [1]. The bacteria can be transmitted to humans through handling and/or consumption of contaminated poultry products like poultry meat and eggs (Stromberg *et al*., 2017). Epidemiological studies and molecular typing, show that food-producing animals are a source of bacteria capable of causing human extra-intestinal pathogenic infections (Be "Langer *et al*., 2011).

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Antimicrobial resistant poultry pathogens can be a source of resistant bacteria/genes (including zoonotic bacteria) that may represent a risk to human health (Nhung *et al*., 2010) [12]. Antimicrobials spread from food producing animals have been found to cause alteration of intestinal microflora in consumers and appearance of antibiotic residues in food products (Miles TD 2006) [10].

The poultry industry in Kenya is composed of both large scale and small scale farmers. Kenya has an estimated poultry population of 31 million birds. Of these, 75% consist of indigenous chicken, 22% of broilers and layers and 1% of breeding stock (The Poultry, Meat and Processing Trade Mission to Kenya 2015) [15].

2. Materials and Methods

2.1 Site of study

The study was conducted at the Regional Veterinary Laboratory in Karatina, Kenya.

2.2 Collection of samples

Birds submitted to the laboratory for post-mortem examination were dipped in chlorinated water before post-mortem and organs with gross pathological lesions were collected aseptically.

2.3 Duration of study

The study was conducted between January and September, 2019.

2.4 Sampling technique

All birds submitted for post-mortem and all organs collected during pathology procedures during this period were analyzed as a purposive sample.

2.5 Laboratory procedures

Specimens collected in pathology were cultured in MacConkey agar and Blood agar and incubated at aerobic atmosphere at 37°C for 24 hours. From pure cultures obtained, *Escherichia coli* were identified through the gram staining procedure and other standard laboratory biochemical tests (Indole, Methyl Red, Voges Proskeur, Citrate, Catalase, Oxidase) for the conformation of the isolated organisms was done according to the standard microbiological methods. Antimicrobial susceptibility testing of the isolates was done using the Kirby-Bauer disc diffusion method as recommended by Clinical and Laboratory Standards Institute (CLSI 2014) [4]. Susceptibility to 7 commonly used antimicrobials on disks containing; Ampicillin (AMP 25 µg), Tetracycline (TE 25 µg), Cotrimoxazole (COT 25 µg), Streptomycin (S 10 µg), Kanamycin (K 30 µg), Gentamicin (GEN 10 µg) and Sulphamethoxazole (SX 200 µg) was tested. The Zone of inhibition was measured by a ruler using millimeters. Susceptibility and resistance data were interpreted according to Clinical and laboratory Standards Institute (CLSI) guidelines. Pure cultures of reference strains of *Escherichia coli* ATCC 25922 were used as controls.

2.6 Data interpretation

Proportions and percentages were calculated for microbial isolates from samples analyzed and for drug susceptibility patterns of the bacteria isolated.

3. Results

A total of 106 carcasses or live birds that were sacrificed for post-mortem were submitted to the laboratory for analysis between January and September 2019. One hundred and forty eight samples were collected for bacteriology analysis. Sixty one (42%) of the samples were lung samples, 44(30%) were liver samples, 17 (11.5%) were long bone samples while the other 26 (17.6%) was from other organs. Bacteria of the species *E. coli* were isolated from 96 of 148 organs either as pure cultures (58%) or mixed with other bacterial isolates (7%). There were no isolates recovered from 11% of specimens analyzed, while bacteria of other species were isolated from 24% of the organs submitted. These bacteria included *Staphylococcus aureus, Staphylococcus epidermidis, Klebsiella pneumoniae, Listeria monocytogenes, and Corynebacterium, Pseudomonas, Acinetobacter* and *Salmonella* species. The bacterial isolate profile is as shown in figure 2 below.

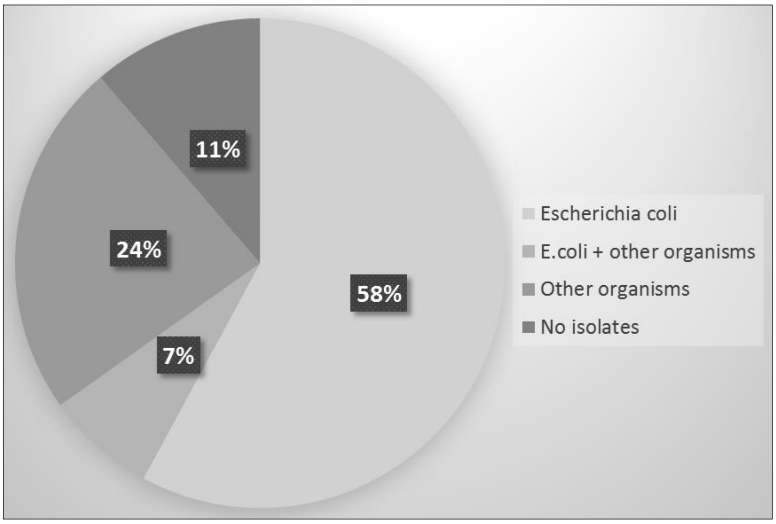

Fig 2: Microbial isolates from poultry specimens at RVIL, Karatina 2019

From the antimicrobial susceptibility results, 29 out of 148(20%) of *E. coli* isolated were susceptible to Ampicillin, 45(30%) to Tetracycline, 15(10%) to Cotrimoxazole, 29(20%) to Streptomycin, 57(38.5%) to Kanamycin, 89(60%) to Gentamycin and 15(10%) to Sulphamethoxazole. Four out of the 96 samples (4.1%) where *E.coli* was isolated were resistant to all antimicrobials tested for. Figure 3 below shows the results of antimicrobial susceptibility testing.

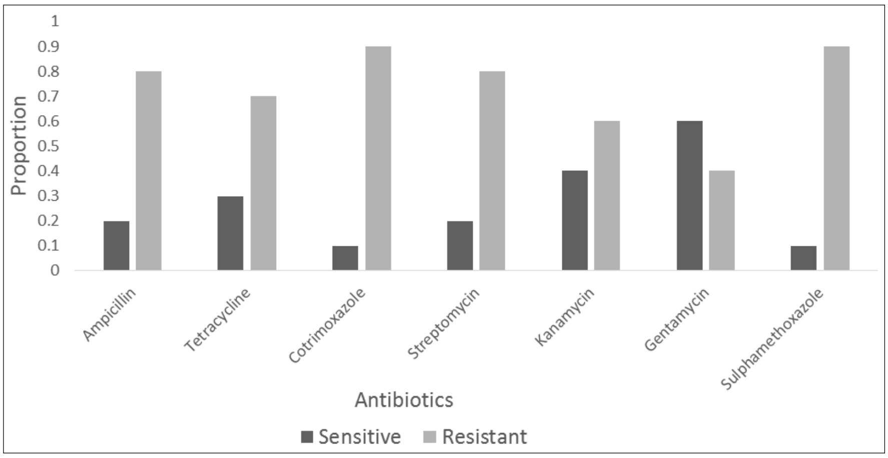

Fig 3: Drug susceptibility patterns of *E.coli* isolated from poultry specimens at RVIL, Karatina, 2019

4. Discussion
Our results indicate that sixty five percent of bacteria isolated from bacteriological samples from poultry specimens had *E.coli* as the only isolate (58%) or combined with other organisms (7%). These was slightly lower than that obtained by Odwar *et al,* 2017 who obtained a percentage of 78% of *E.coli* from samples of raw poultry meat sold in Nairobi (Odwar, 2014) [13]. This shows that *Escherichia coli* is an important and common poultry pathogen and can be found in birds that are considered to be fit for human consumption and as well can cause morbidity and mortalities in birds when it colonizes various other organs other than the gastro-intestinal tract.

This study shows growing resistance of *E. coli* towards most common antibiotics used in the poultry industry in Kenya. Drug susceptibility results showed increased development of resistance with Cotrimoxazole and Sulphamethoxazole being at 90% resistance, Streptomycin and Ampicillin at 80%, Tetracycline at 70%. Kanamycin at 60% and Gentamycin at 40%. Four percent of the isolates were resistant to all antimicrobials tested. This is a worrying trend considering that infections can thrive in poultry flocks despite treatment with antibiotics and that bacteria are capable of transmitting resistant genes even to human consumers (Mora, Azucena & Viso, Susana & López, Cecilia & Alonso, María & García-Garrote, Fernando & Dabhi *et al*. 2013) [11]. A study done to examine antimicrobial resistance in commensal *E.coli* isolates from healthy layer chickens from central and eastern Uganda showed more than 70% antibiotic resistance thereby indicating growing levels of resistance (Kabiswa *et al.*, 2018) [8]. The high prevalence of *Escherichia coli* in poultry samples coupled with high resistance to common antibiotics is of great concern due to possible transmission of antibiotic resistant food borne bacteria to consumers (Bester LA, Essack SY 2010) [3].

5. Recommendations
A holistic approach is required for the prevention, control and treatment of avian Colibacillosis and can be achieved with the active involvement of farmers, drug marketers, animal health service providers and government regulatory agencies.

Good management practices could reduce incidences of Colibacillosis in poultry flocks. These should include proper feeding, adequate hygiene and biosecurity practices and ensuring proper ventilation in poultry houses. Vaccinations which prevent against common poultry diseases (i.e. Newcastle, Gumboro, Infectious bronchitis, should be emphasized as they help prevent against secondary *E.coli* infections). Vaccines against Avian pathogenic *Escherichia coli* (e.g. Poulvac® *E.coli*), administered through sprays have been used in several countries in the developed world and have shown benefits against morbidity and mortality in poultry (Cookson *et al.*, 2013) [5]. Vaccination of birds against *Escherichia coli* helps improve health by lowering mortality, increasing weight gain and feed conversion (van Biljon, 2013) [16].

Prudent use of antibiotics should be embraced by Veterinary practitioners and farmers. Approaches that could control or eliminate the routine or excessive use of antibiotics can reduce chances of development of resistance and spread of resistant bacteria from poultry to humans and the environment. These include controlled use of antibiotics as feed additives and for prophylaxis including use of laboratory facilities for establishing correct antibiotics to use in infection control. Gentamycin has been found to have higher susceptibility than other antimicrobials to *Escherichia coli*. Unfortunately, Gentamycin preparations available in the Country are in injectable form and are not suitable for use in large poultry flocks. The pharmaceutical industry could explore ways of providing Gentamycin preparations for poultry use.

More studies on serotyping and gene characterization of isolates from poultry to assess resistance genes and the associated risk factors are important so as to draw attention to relevant control points including identifying which poultry systems are more likely to be the source of resistant genes and their zoonotic transmission.

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