*Research Article*

Antimicrobial Resistance Profiles of *E. coli* Isolated from Pooled Samples of Sick, Farm, and Market Chickens in Nairobi

County, Kenya

ABSTRACT

Background: Bacterial antimicrobial resistance (AMR) is a global threat to both human and animal health. This is mainly because the same antimicrobial molecules are used for the treatment and prophylaxis of bacterial diseases in both cases, and about 60% of human pathogens are shared with animals. For effective control of AMR in any country,effective periodic surveillance exercises are recommended. In Kenya, there is scanty data on the prevailing AMR situation, especially in animals. Aim: Method: This paper reports on AMR profiles of 54 *E. coli* strains isolated from chickens in a cross-sectional study. Results: out of which 36/54 (72%) were from clinically ill chickens, 11/54 (22%) were from farm chickens, and 7/54 (9.7%) were from slaughtered chicken, respectively. All 54 isolates exhibited varying antimicrobial resistance profiles with the majority showing resistance to Ampicillin (85.22%), Tetracycline (66.7%), Co-trimoxazole (57.4%), and Streptomycin (40.7%). Very few isolates were resistant to Amoxicillin and Gentamicin (each at 3.7%), Ampicillin (11.1%), and Nalidixic acid (24.1%). A total of 44/54 (81.5%) showed multiple resistance to up to 6 antimicrobial agents. Conclusion: This information will augment current data on the AMR status of bacteria harbored by chickens in Kenya. It will also inform policymakers in their fight against AMR.

**Keywords:** antimicrobial resistance; chickens; *E. coli*; Kenya; multidrug resistance

# Introduction

Antimicrobials are essential for human and animal health [(https://www.reactgroup.org)](https://www.reactgroup.org/) but need to be used with caution. Livestock health (including that of poultry and fish) is important for human welfare in two ways: (1) It improves animal welfare, which translates to improved productivity and economic status of stakeholders in the value chain and contributes towards food security. (2) It ensures food safety since it is estimated that about 60% of bacteria that are pathogenic to humans are from animals/animal products [[1].](#_bookmark3) The major problem, concerning the development of

antimicrobial resistance, is the fact that some drugs are used in both humans and animals for treatment or prophylaxis of disease [[1–5],](#_bookmark6) and a large percentage of bacteria, both pathogenic or nonpathogenic, are shared between the two groups. Prudent use of antimicrobials in animals is, therefore, important as possible transfer of antimicrobial resistant genes through horizontal gene transfer between animals and humans have been documented [[6].](#_bookmark7) Indiscriminate usage of antimicrobials, for example, as growth promoters in veterinary medicine contributes directly to the emergence and spread of resistance [[7–](#_bookmark8)[10]](#_bookmark11) Globally, it is estimated that 66% of all antimicrobials are used for farm animals and as growth promoters or as pro- phylaxis [[11–14].](#_bookmark14) This has increasingly influenced antimicrobial resistance, causing resultant food-borne or animal-acquired illness in humans with less responsive treatment options to antimicrobial drug(s).

Since the fight against antimicrobial resistance is of global magnitude [[15](#_bookmark15)–[17],](#_bookmark17) each country needs to establish and continuously monitor its current status, to harness data for action. In Kenya, as in most developing countries, it is difficult to get a complete picture of the AMR situation, especially in animals, because antimicrobial susceptibility testing is not done routinely in diagnostic laboratories (it is only done on specific requests and in specific, although few researches). It is appreciated that several studies on antimicrobial resistance in animals have been carried out in Kenya [[18](#_bookmark18)–[24]](#_bookmark21) and there are also comprehensive reviews and individual researchers’ re- ports on the situation analysis of AMR in both animals and humans in Kenya [[25](#_bookmark22)–[27].](#_bookmark23) This study therefore determined the extent of antimicrobial resistance in *Escherichia coli* isolated from three groups of chickens from various sources. Chickens were used because they are kept and consumed by many Kenyans and there is also a high tendency to use antimicrobials when the chickens are kept under an intensive farming system. *Escherichia coli* was used because it is a common bacterium and also because it is easy to grow and characterize.

# Methods

* 1. *Study Design, Area, and Sample Chickens:* This was a cross-sectional study carried out in Nairobi County, Kenya. It comprised chickens purposively selected from three sources: (1) veterinary poultry clinic at the Department of Veterinary Pathology, Microbiology, and Parasitology, University of Nairobi (a total of 50 sick chickens that were brought to the Poultry clinic for disease diagnosis (post- mortem examination; regardless of their disease condition),

(2) a poultry commercial farm in Nairobi (50 chickens), and

(3) a poultry market/slaughterhouse in Nairobi (72 chickens).

|  |  |  |
| --- | --- | --- |
| S/N | Sample Source | Total  |
| 1 | Veterinary poultry clinic at the Department of Veterinary Pathology, Microbiology, and Parasitology, University of Nairobi | 50 |
| 2 | Chicken from commercial farm in Nairobi | 50 |
| 3 | Chicken from market/slaughterhouse in Nairobi | 72 |
| Total | 172 |

The clinical cases included broilers, layers, and In- digenous chickens from various farms, suffering from var- ious disease conditions (not necessarily caused by *E. coli*) including septicemia, pneumonia, coccidiosis, New castle disease, Gambaro disease, fowl pox, leucosis, nutritional deficiency, aflatoxicosis, yolk-sac infection, helminthiasis, ectoparasites, and trauma (e.g., liver rupture). Like healthy chickens, the clinical cases carry *E. coli* in their guts, as commensals; the isolated *E*. *coli* were, therefore, taken as representatives of strains present in other/healthy chickens in the respective farms. Market birds were mainly of in- digenous type and spent layers, brought to the slaughter- house from various parts of Kenya. The farmed chickens were from a farm that was keeping layers under a slatted

floor (wire mesh) system.

* 1. *Sample Collection, Handling, and Transport:* Cloacal swabs were taken from the selected test chickens. They were then placed in Stuart’s transport medium (Oxoid, Ltd) and

transported to the Microbiology laboratory of the De- partment of Veterinary Pathology, Microbiology, and Par- asitology, University of Nairobi, for bacterial isolation and identification.

* 1. *Isolation and Identification of E. coli:* Isolation of *E. coli* was done by swab-inoculation onto MacConkey agar (Oxoid Ltd), followed by incubation at 37°C overnight. Organisms from lactose-fermenting (pink) colonies were phenotyped and confirmed as *E. coli* through Gram-staining, growth on Eosin Methylene Blue agar, and testing for motility and biochemical reactions, including Indole, Methyl red, Voges Proskauer, Citrate, Urease, and interpretation done using the criteria given in Bergey’s Manual of systemic bacteriology [[28].](#_bookmark24)
	2. *Antimicrobial Susceptibility Testing of the E. coli Isolates:* Antimicrobial susceptibility testing was done by Agar Disk Diffusion method using Mueller Hinton agar (Oxoid Ltd), as previously described by Bauer *et al*. [[29]](#_bookmark25) and recommended by the Clinical and Laboratory Standards Institute (CLSI) [[30].](#_bookmark26) The *E. coli* isolates were tested for susceptibility against eight antimicrobials that are currently used for treating bacterial infections in both humans and animals in Kenya; including Ampicillin (AMP; 25 *μ*g), Tetracycline (TE; 25 *μ*g), Co-trimoxazole (COT; 25 *μ*g), Streptomycin (S; 10 *μ*g), Nalidixic acid (NA; 30 *μ*g), Amoxicillin (AMC; 30 *μ*g), Gentamicin (GEN; 10 *μ*g), Chloramphenicol (C; 30 *μ*g) (Oxoid, Basingstoke, United Kingdom). After incubation at 37°C overnight, the diameters of the growth-inhibition zones around the discs were measured. *E. coli*, ATCC 25922 [31], was used as the reference strain. Interpretation of the AMR data was done as per CLSI Guidelines [[30].](#_bookmark26)

# Results

* 1. *Escherichia coli Isolated from Chickens. Escherichia coli* organisms were isolated from a total of 54 chickens (31.4%), 36 of them being from the 50 clinical cases (72%); 11 from

the 50 farm chickens (22%); and 7 from the 72 market/

slaughtered chickens (9.7%).

* 1. *Determination of the Antimicrobial Susceptibility Profile of E. coli Isolates:* Antimicrobial susceptibility test results of the 54 *E. coli* isolates are shown in Table [3.](#_bookmark0) Figure [1](#_bookmark1) gives a graphical representation of antimicrobial resistance rates for the test isolates.

The organisms showed the highest resistance to Am- picillin (85.2%), followed by Tetracycline (66.7%); Co- trimoxazole (57.4%) and Streptomycin (40.7%). Low resistances were demonstrated for Nalidixic acid (24.1%) and Chloramphenicol [14.8% (1 isolate)]; while 96.3% susceptibility was observed agaisnt Amoxicillin and Gentamicin. Six [6 (11.1%)] isolates were resistant to one antimicrobial (Ampicillin) only, and 5 (9.3%) were susceptible to all the eight antimicrobials tested, while the rest showed variable resistances ranging from two to six antimicrobials.

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Table 3: Multidrug resistant patterns demonstrated by the test isolates.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Number of antimicrobials Tested** | **Number of isolates resistant** | **Antimicrobial Resistant Parttern** | **Classification of Resistance**  |  |  |
|  | 1 | COT-TE | MDR |  |  |
|  | 1 | S-TE | MDR |  |  |
| 2 | 1 | COT-AMP | MDR |  |  |
|  | 1 | S-AMP | MDR |  |  |
|  | 6 | TE-AMP | MDR |  |  |
|  | 1 | NA-S-TE | MDR |  |  |
|  | 1 | C-TE-AMP | MDR |  |  |
| 3 | 1 | C-S-AMP | MDR |  |  |
|  | 4 | COT-S-AMP | MDR |  |  |
|  | 8 | COT-TE-AMP | MDR |  |  |
|  | 1 | NA-S-TE-AMP | MDR |  |  |
| 4 | 2 | COT-NA-TE-AMP | MDR |  |  |
|  | 5 | COT-S-TE-AMP | MDR |  |  |
|  | 1 | COT-NA-GEN-TE-AMP | MDR |  |  |
| 5 | 2 | COT-NA-S-TE-AMP | MDR |  |  |
|  | 2 | COT-C-S-TE-AMP | MDR |  |  |
|  | 1 | COT-C-NA-GEN-TE-AMP | MDR |  |  |
| 6 | 1 | COT-NA-S-AMC-TE-AMP | MDR |  |  |
|  | 3 | COT-C-NA-S-TE-AMP | MDR |  |  |

Abbreviations: AMC = Amoxicillin; AMP = Ampicillin; COT, Co-trimoxazole; C, Chloramphenicol; GEN, Gentamicin; NA, Nalidixic acid; S, Streptomycin; TE, Tetracycline.

120

100

80

60

Percentage

40

20

0

Ampicillin (Amp)

Tetracycline (TE)

Co-trimoxazole (COT)

Streptomycin (S)

Amoxicillin (AMC)

Gentamicin (GEN)

Chloramphenical (C)

Nalidixic acid (NA)

Antimicrobials

Susceptible (%) Resistant (%)

Figure 1: Antimicrobial susceptibility/resistance patterns of *E. coli* isolates (*n* � 54).

* 1. *Multidrug Resistance in the E. coli Isolates.* Forty-four out of the 54 (81.5%) *E. coli* isolates showed multidrug resistance (resistance to two or more antimicrobials). Figure [2](#_bookmark2) presents

several organisms resistant to a respective number of an- timicrobials; antimicrobial combinations resistant-to are given in Table [1;](#_bookmark0) while Figure [2](#_bookmark2) shows the number of times

4

*E. coli* isolates patterns of resistance

16

14

Number of isolates resistant

12

10

8

6

4

2

0 2 3 4 5 6

Number of antimicrobials

AMR results of this study showed that *E. coli* isolates from the screened chickens were resistant, though at varying levels, to some of the commonly used antimicrobials, predictably because they are cheap and, therefore, affordable to the inhabitants of the study area. The antimicrobial resistance rates were as follows: Ampicillin (85.2%), Tetra- cycline (66.7%), Co-trimoxazole (57.4%), and Streptomycin (40.7%) (Table [1).](#_bookmark0) The resistance may have developed as a result of high or indiscriminate usage of the antimicrobials in the area; either on the humans or their animals; it may also be as a result of environmental contamination through human/animal movement across the area, through fecal contamination, spitting, or other excrements, or through

Figure 2: Number of *E. coli* isolates with multidrug resistant Profile

an antimicrobial was involved in cases of multidrug resistance among the *E. coli* isolates. Ten (22.7%) of the multidrug-resistant isolates were resistant to two antimicrobials; 15 (34.1%) were resistant to 3 antimicrobials; 8

(18.2%) to 4, while 5 (11.4%) each were resistant to 5 and 6 antimicrobials, respectively (Figure [2).](#_bookmark2) Of the 152 times that the test antimicrobials were included in multidrug combi- nations, the antimicrobial included most was Ampicillin at 26.3% (40/151); followed by Tetracycline at 23.7% (36/152); Co-trimoxazole at 20.3% (31/152); Streptomycin at 14.5% (22/152); Nalidixic acid at 7.9% (12/152); Chloramphenicol at 5.3% (8/152); Gentamicin at 1.3% (2/152); and lastly Amoxicillin at 0.7% (1/152) (Figure [2).](#_bookmark2)

# Discussion

This study was carried out to determine the current anti- microbial resistance profiles of *E. coli* organisms isolated from chicken cloacae, from selected study sites in Nairobi. There was a low *E. coli* recovery of 31.4% (54/172). This was contrary to what was expected since *E. coli* is one of the most common commensals in the intestinal tracts of both humans and animals; it is also the most commonly isolated bacterium (coprobacterium) from feces [[32].](#_bookmark28) However, this less-than- 100%-recovery using the cloacal swab method has been observed in other studies. Ibrahim *et al*. [[33],](#_bookmark29) isolated *E. coli* at 53.4% (269/504); Bebora [[34]](#_bookmark30) isolated the organism from 4 lots of chickens at 51.1% (97/133), 46% (98/176), 66% (66/ 100), and 88% (22/25). This may be due to intermittent shedding of the organisms in feces, as previously documented [[35–38].](#_bookmark33) Shedding is influenced by stress: muscular fatigue, cold, wetness, limitation of food and water, and concurrent infection [[36].](#_bookmark32) Working on *Salmonella typhimurium*, Brownell *et al*. [[36]](#_bookmark32) found that cloacal excretion of the organisms occurred during the first 5 days of infection, after which the excretion dropped considerably. Williams and Whittemore [[39]](#_bookmark34) had similar findings; they also concluded that the cloacal swab method was inadequate for the isolation of *Salmonella typhimurium*. The amount of fecal material in the cloacal swab is much less than in the intestinal swab, so there is a higher chance of not picking the organism, even though it is present.

careless disposal of medicines. This trend of resistance has also been reported in other studies [[22,](#_bookmark20) [40–44].](#_bookmark39) In this study, it was encouraging to find that some *E. coli* strains were still susceptible to the commonly-used antimicrobials; for ex- ample, 5 (9.3%) of the isolates were susceptible to all the 8 antimicrobials tested. High susceptibilities were also observed to Amoxicillin and Gentamicin (each at 96.3%), Chloramphenicol (85.2%), and Nalidixic acid (75.9%).

Antimicrobial-resistant bacteria could also have origi- nated from dogs and rats which are normally seen every- where in human dwellings (especially in informal settlements), in markets, and in farms [[45–49].](#_bookmark41) There is documentation on the presence of zoonotic antimicrobial- resistant bacteria in dogs [[44,](#_bookmark39) [50]](#_bookmark42) and rats [[22];](#_bookmark20) hence, these animals can easily and widely disseminate them. Allor- echtova *et al*. [[44]](#_bookmark39) specifically looked for ESBL-producing

*E. coli* strains in Northern Kenya and demonstrated their presence in humans, dogs, and, to a lesser extent, cats. Comparing genetic profiles of the ESBL-producing *E. coli* isolates, eight isolates from dogs and two isolates from humans gave identical profiles; while a close relationship (> 95%) was found in one human isolate and one cat isolate. This suggests that the spread of resistant bacteria between humans and dogs is a common occurrence; some of these organisms were found to be multidrug resistant. Most farmers practice mixed animal-raising; that is: they keep many types of animals; there is also a close relationship between humans/farmers and their animals; so, resistant bacteria can easily be transferred across the animals and to/ from humans.

Many classes of antimicrobials have been used to treat

both humans and livestock [[4].](#_bookmark5) They include: *β*-lactams (Penicillins and Cephalosporins); Sulphonamides with or without Trimethoprim; Tetracyclines; Macrolides, Lincosa- mides, and Streptogramins; and Quinolones including Fluoroquinolones [51]. Classes most used to treat livestock are Penicillin derivatives, such as Ampicillin and Cloxacillin; Sulphonamide, e.g., Tyrosine, used for the treatment of metritis and acute mastitis in cattle, sheep, and goats, enteritis, pneumonia, erysipelas, infectious arthritis in swine, and chronic respiratory disease in chickens [[9].](#_bookmark10) Tetracycline and Co-trimoxazole (containing sulfamethoxazole and tri- methoprim) are two most-used antimicrobials for pro- phylaxis and as growth promoters in livestock rearing, to increase productivity [[9].](#_bookmark10) Most of these are also used in Kenya. Resistance, particularly to the commonly available

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antimicrobials, poses a major health concern, as alternative therapeutic choices are either unavailable or too expensive to be affordable for most patients.

A high percentage (81.5%; 44/54) of the *E. coli* isolates, in this study, showed multidrug resistance; 10 of them (22.7%) were resistant to two antimicrobials; 15 (34.1%) were resistant to three antimicrobials; 8 (18.2%) to 4, while 5 (11.4%) each were resistant to five and six antimicrobials, respectively (Figure [2).](#_bookmark2) Of the 152 times that the test anti- microbials were included in multidrug combinations, the antimicrobial included most was Ampicillin at 26.3% (40/ 151); followed by Tetracycline at 23.7% (36/152); Co- trimoxazole at 20.3% (31/152); Streptomycin at 14.5% (22/152); Nalidixic acid at 7.9% (12/152); Chloramphenicol at 5.3% (8/152); Gentamicin at 1.3% (2/152); and lastly Amoxicillin at 0.7% (1/152) (Figure [2).](#_bookmark2) This further demonstrated the resistance pattern as being towards the cheap commonly-used antimicrobials; echoing the worldwide worry towards antimicrobial resistance [[2,](#_bookmark4) [15,](#_bookmark15) [16].](#_bookmark16) Multi- drug resistance has been reported by several researchers in Kenya; in animals–Bebora [[40],](#_bookmark35) Ombui, Kimotho, and Nduhiu [41], Mapeney *et al*. [[42],](#_bookmark37) Gakuya *et al*. [[22],](#_bookmark20) Kikuvi *et al*. [[43],](#_bookmark38) Allorechtova *et al*. [[44],](#_bookmark39) Igizeneza *et al*. [[52],](#_bookmark44) Wanja *et al*. [[18];](#_bookmark18) in environment–Wambugu *et al*. [[53],](#_bookmark45) Kutto [21]; in humans–Kariuki *et al*. [[54,](#_bookmark46) [55],](#_bookmark47) Bururia [[56],](#_bookmark48) Oundo *et al*. [[57].](#_bookmark49) It has also been reported by many re- searchers outside Kenya (Van den Bogaard *et al*. [[58];](#_bookmark50) Ryu *et al*. [[59];](#_bookmark51) Adzikey, Huda, and Ali [[60];](#_bookmark52) Nys *et al*. [61]; Kennedy and Collington [[62];](#_bookmark54) Ulstad *et al*. [[63];](#_bookmark55) GEN [[2].](#_bookmark4) Increased use of antimicrobials mainly for prophylaxis and as growth promoters in animals in Kenya is encouraged by the increased demand for milk, meat, and eggs, due to increased population and popularization of the products [[13,](#_bookmark13) [58].](#_bookmark50) Most of the antimicrobials are used in intensively kept chickens and pigs, while in other livestock, more antimicrobials are used in the treatment and prevention of mastitis. The Ministry of Agriculture, Livestock, Fisheries, and Irrigation animal census (2017) gave the chicken population to be 48,123,577 (broilers 3,819,515; layers 4,237,188; indigenous 40,067,874). Imprudent use of anti- microbials in chickens in Kenya; coupled with lay administration of the drugs to chickens, facilitated by easy access to over the counter drugs [[64],](#_bookmark56) is common practice in Kenya (personal observation) mostly by unprofessional drug administrators (farmers, etc.), whom are most times deprived of instructions and, hence tend to do so incorrectly, or purchase the wrong drug [[65].](#_bookmark57) The situation is made worse by human doctors and veterinarians who tend to use antimicrobials as a cover for any secondary bacterial infection; they use the assurance that: “if it is broad-spectrum, it can shoot better” [[66].](#_bookmark58) This is coupled with the increased use of antimicrobials in humans, mainly to treat respiratory, enteric, and hospital-acquired infections [[56,](#_bookmark48) [57,](#_bookmark49) [67,](#_bookmark59) [68].](#_bookmark60) There is also increased usage of antimicrobials, especially Tetracycline and Co-trimoxazole, in HIV-AIDS patients, to treat infections related to Acquired Immunodeficiency Syndrome (AIDS) in humans [[8].](#_bookmark9) The number of people living with HIV/AIDS in Kenya is estimated to be 1.6 million (UNAIDS, 2017 Data Book; National Aids Control Council report 2018). The wide, sometimes

unjustified use of antimicrobials in humans and animals in Kenya may explain the high occurrence of antimicrobial resistance in the *E. coli* strains tested in this study.

# Conclusion

This study has demonstrated the expression of antimicrobial- resistant by *E. coli* in Kenyan chicken; most of them showing multidrug resistances ranging from two to six antimicrobials. Data from this study is expected to augment the AMR baseline data already collected for Kenya, it will also inform policymakers in their fight against AMR.

# Data Availability Statement

The data used and analysed in this study are obtainable from the corresponding author on rational demand.

# Ethics Statement

This research was conducted after obtaining ethical approval from the Biosafety, Animal Use and Ethics Committee with reference number REF: FVM BAUEC/2016/104, Faculty of Veterinary Medicine, University of Nairobi, Kenya. This research was prepared under the Code of Ethics of the World Medical Association (Declaration of Helsinki) for Experiments in Animals.

# Consent

All authors declare consent for publication of the research.

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