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# Antimicrobial Resistance Phenotypes of *Escherichia coli* Isolated from Tropical Free Range Chickens

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Abstract: ***Antimicrobial resistance is one of the major problems facing the world and it is increasing in high rates in developing countries. In this study, antimicrobial phenotypes of resistant Escherichia coli isolates from tropical free range chickens were investigated using Kirby-Bauer disc diffusion method. A total of 77E. coli isolated from cloaca swabs were examined for susceptibility to antimicrobials of veterinary and human importance. Sixty seven out of 77 isolates showed resistance to at least one antibiotic, while 54 out of 67 isolates were multidrug resistant. The highest rate of resistance was against tetracycline (75%) followed byampicillin (63.63%), ofloxacin (54%), co-trimoxazole (53%) and cefotaxime (29%). The lowest resistance was against chloramphenicol (5%), and cefoxitin(6%).To the best of our knowledge, this is the first study in Tanzania to investigate antimicrobial resistance in E. coli isolates from tropical free range chickens. Notably, we report for the first time the occurrence of third generation cephalosporins resistant E. coli strains. These are essential first line drugs used to treat severe enteric bacterial infections in human medicine. Since sampled chickens did not receive any medication or vaccination, it is evident that the environment is the likely source of multidrug resistant bacteria. There is a risk of resistant bacteria transmission to humans through contaminated poultry products and the environment.***

**Keywords:** Escherichia coli, antibiotic resistance, tropical free range chicken, resistance phenotypes

## 1. Introduction

The increasing prevalence of antimicrobial resistance is one of the major problems facing the world; hence it is important to study the emerging resistance trends so as to establish control strategies [1, 2]. Antimicrobial resistance was initially viewed as only being a human medical problem in hospital-acquired infections, nevertheless, the antimicrobial resistance phenomena have spread to the point that the general population is considered to be at risk, bringing about an era where many common bacterial infections are becoming increasingly difficult to treat [3]. One of the factors leading to the spread of antibiotic resistance is widespread use of antibiotics in livestock production. In developing countries, unregulated use of antibiotics counts as one of the factors for increasing resistance [4].Other factors are misuse and overuse of antibiotics [5,6].

In Africa, more than 80% of chickens are kept in free range system [7]. Local chickens are the famous birds kept by most of the households in Tanzania, as a source of food and limited income generation and also are an important component of food security for the rural poor [8]. It is estimated that Tanzania had about 34 million chickens by2011, out of which almost 95% are local chickens and the rest are exotic breeds. Most of households keep free range chickens since they do not require much input in terms of resources and investments due to the fact that they are scavengers almost taking care of themselves [8].Most of the farmers manage them under the extensive system, and are usually neither vaccinated nor given any antibiotic medication. The main feeds of these chickens are the kitchen remains, grasses and other waste which are dumped in our environment. These dumping sites have been used for among other things, improper disposal of pharmaceuticals such as expired antibiotics which pauses a threat to the microbiota in these areas to develop antimicrobial resistance [9]. Chickens are the most domesticated birds in developing countries and are among the domestic animals threatened by being exposed to this environment with the high risk of picking bacteria containing antibiotic resistance genes.

Escherichia coli is one of the pathogenic bacteria which can cause effect to chicken production and which are the ones that are picked up by chickens during scavenging[10].Besides, E.coliis mostly found in the intestinal tract of healthy animals and humans, and these zoonotic bacteria can be transferred from animals to humans. Humans may become infected with resistant E.coli from chicken by direct contact with infected chicken or faeces but the most important source of human infections is infected food products of chicken origin[11].This study aims at determining antimicrobial resistance phenotypes in E. coli isolates from tropical free range chickens in northern Tanzania.

## 2. Materials and Methods

### 2.1 Study Site and Households Selection

The study was done in Arusha region located in northern Tanzania. Two villages were randomly selected from Arusha periurban areas. Preliminary field study was done for eight consecutive days whereby a detailed discussion about research was done with smallholder chicken keepers. Following the interview 45 households were randomly selected and sampled.

### 2.2 Sample collection, isolation and identification of bacteria

A total of 235 samples of cloacae swabs were collected from free range local chickens in the study area. Sampling was carried out systematically at 7-day intervals from 14 March

2014 to 14 May 2014. All samples were taken using sterile swabs, placed insterile falcon tubes containing 2ml of 0.9% normal saline solution and kept in cool box for transporting to the laboratory for further analysis. The samples were cultured on the same day upon arrival at the laboratory.

Mac Conkey (Himedia, Mumbai, India)agar was prepared according to the manufacturer's instructions one day before sample collection and incubated overnight at 370C to confirm that there was not contamination during the process of preparation. The collected samples were inoculated on media immediately after arriving in the laboratory and incubated at 370C for 48h to allow bacterial growth. The presumptive pink colonies were picked and sub cultured to get pure colony. Identification of bacterial strain was performed by classical identification methods using API 20 E System (bioMerieux, France).

### 2.3 Antibiotic Susceptibility Testing

Seventy seven isolates were screened for antimicrobial resistance using the Kirby Bauer disc diffusion method according to the Clinical Laboratory Standard Institute standards (formerly National Committee for Clinical Laboratory Standards Guidelines). This was done by streaking the surface of Muller Hinton Agar (Oxoid Ltd, Basingstoke, Hampshire, England)plates uniformly with the organisms adjusted with sterile broth to match the 0.5 McFarland turbidity standards and thereafter exposing them to the discs (Oxoid Ltd). Commercial antibiotics discs used in the study were ampicillin (AMP, 10µg), tetracycline (TET, 30µg), cefotaxime (CTX, 30 µg), ofloxacin (OFL, 5 µg), co-trimoxazole (SXT, sulfamethoxazole/trimethoprim, 25 µg), cefoxitin(FOX, 30µg), and chloramphenicol (CHL, 50µg). The inhibition zone sizes were interpreted by using the standard recommendations of the CLSI (2013).Escherichia coli strain K12 was used as a control organism.

### 2.4 Interpretations and data treatment

Susceptibility data were recorded quantitatively by measuring the diameters to the nearest whole millimeter using digital sliding calipers. Following the interpretative chat of the Kirby-Bauer sensitivity test method, the zones were interpreted as resistant, intermediate or sensitive. Furthermore, proportions of isolates resistant to individual antibiotics were computed as averages and percentages.

## 3. Results

### 3.1 Antimicrobial resistance profiles of E. coliisolates

The overall mean antibiotic resistance of E. coli strains isolated is shown in Table 1. The most commonly resistances observed were against tetracycline (75.32%), ampicillin (63.63%), ofloxacin (54.54%), trimethoprim/sulfamethoxazole (53.25%), and cefotaxime (29.87%) while the least commonly resistance observed was against cefoxitin (6.49%), and chloramphenicol(5.19%).

**Table 1:**.Antimicrobial resistance phenotypes in Escherichia coli fromtropical free range chickens

| *Antimicrobial agent* | *Antimicrobial class* | *No. of isolates(n = 77)* |
|---|---|---|
| Tetracycline | Tetracycline | 58 |
| Ampicillin | Penicillin | 49 |
| Ofloxacin | Fluoroquinolone | 42 |
| Trimethoprim/sulfamethoxazole (Co-trimoxazole) | Dihydrofolate reductase inhibitor/Sulfonamide | 41 |
| Cefotaxime | Third generation cephalosporin | 23 |
| Cefoxitin | Cephamycin | 5 |
| Chloramphenicol | Phenicol | 4 |

Analysis of multidrug resistance phenotypes indicated several patterns as depicted in Table 2. The most commonly resistances patterns observed were AMP, TET, and SXT (14 isolates), AMP, TET, OFL, SXT, and CTX (8 isolates), AMP, TET, and SXT (5 isolates), TET, OFL, and SXT (4 isolates), AMP, TET, and OFL (3 isolates), AMP, TET, OFL, and CTX (3 isolates) and AMP, TET, FOX, OFL, and CTX (3 isolates). All isolates resistant against third generation cephalosporins and cephamycin showed multidrug resistance to at least four classes of antimicrobial agents tested.

**Table 2:** Multiple antimicrobial resistance patterns of Escherichia coli isolates

| *No. of antibiotics* | *Resistance pattern* | *No. of isolates* |
|---|---|---|
| 2 | AMP, TET | 1 |
| | TET, CTX | 2 |
| | CHL, FOX | 1 |
| | TET,OFL | 1 |
| 3 | AMP, TET, SXT | 5 |
| | AMP, TET, OFL | 3 |
| | TET, OFL, SXT | 4 |
| | AMP, TET, CTX | 1 |
| | AMP, OFL, SXT | 1 |
| 4 | AMP, TET, CHL, SXT | 1 |
| | AMP, TET, OFL, SXT | 14 |
| | AMP, TET, OFL, CTX | 3 |
| | AMP, TET, SXT, CTX | 2 |
| 5 | AMP, TET, FOX, OFL, CTX | 3 |
| | AMP, TET, FOX, OFL, SXT | 1 |
| | AMP, TET, OFL, SXT, CTX | 8 |
| | TET, FOX, OFL, SXT, CTX | 1 |
| 6 | AMP, TET, FOX, OFL, SXT, CTX | 1 |
| | AMP, TET, CHL, OFL, SXT, CTX | 1 |
| Total | | 54 |

Key: AMP, ampicillin; TET, tetracycline; CTX, cefotaxime; CHL, chloramphenicol; FOX, cefoxitin; OFL, ofloxacin; SXT, sulfamethoxazole/trimethoprim

## 4. Discussion

This is the first study in Tanzania to investigate antimicrobial resistance phenotypes in E. coli isolates from tropical free range poultry. In the present study, we focused on E. coli strains and their sensitivity patterns to different classes of antibiotics which are commonly administered to combat bacterial infections in human medicine. Antimicrobial resistance in E. coli is an important indicator of the emergence of resistant microbes in the community. The isolates were recovered from chickens that normally do

not receive any medication or vaccination according to the information obtained through questionnaire survey involving randomly selected(n = 45) smallholder poultry keepers in two villages. From this study 64 out of 77 recovered isolates showed resistance to at least one antimicrobial agent, while 54 out of the 64 isolates were multidrug resistant. Our major finding is the detection, for the first time, of resistance against third generation cephalosporins. Drugs belonging to this class of antibiotics are essential first line medicines prescribed in the treatment of severe enteric infections in humans.

There is scanty information describing antimicrobial resistance phenotypes from food-producing animals in developing countries. Limited information reported occurrence of resistance to antimicrobial agents such as ampicillin, tetracycline, trimethoprim, sulfamethoxazole, chloramphenicol and fluoroquinolones in local chickens in Nigeria [12,13]. However, there are no reports so far describing the emergence of resistance to third generation cephalosporins may be attributed to exposure to contaminated environment where chickens pick up bacteria harboring resistance traits when scavenging. Similar observation has been previously described in India [14].

A majority of isolates exhibited multidrug resistance to three or more drugs belonging to unrelated classes of antibiotics. It has been described that high prevalence of antimicrobial resistance in bacterial isolates from chickens could be attributed to misuse and abuse of antibiotics and poor understanding of the poultry farmers [15]. Also, increased use of these drugs in veterinary medicine may select for antimicrobial resistant zoonotic bacterial pathogens [16]. The findings of our study raise concerns about the indirect evidence of occurrence of antimicrobial resistance in the environment, as seen through free range chickens which serve as a source of meat for human consumption mostly in rural, periurban and urban areas of developing countries.

Previously, it was reported that antibiotic resistant E. coli occur frequently in commercial broiler chickens which are frequently administered with antimicrobials for the prevention and treatment of infectious diseases and/or for growth promotion purposes [17,18].However, our findings in the present study provide evidence that antibiotic resistant E. coli isolates are also frequent in free range local chickens which normally do not receive antimicrobials for the prevention and treatment of infectious diseases or for growth promotion purposes. Thus, free range poultry may serve as one of the potential reservoirs of resistant zoonotic bacteria which may be transferred to humans through the food chain.

## 5. Conclusion

Prevalence of antimicrobial resistance E.coli isolates is significantly high in free range local chickens which do not normally receive vaccinations or medications. Notably, occurrence of multidrug resistant E. coli exhibiting resistance to third generation cephalosporins poses threat to the public health. In the near future, there is an urgent need to conduct a detailed longitudinal investigation to elucidate the role played by the environment in the maintenance and dissemination of antimicrobial resistance determinants in animals and humans.

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