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Antimicrobial resistance against fluoroquinolones and cephalosporins of *Escherichia coli* and *Klebsiella pnuemoniae* in chickens entering the food chain in Mwanza, Tanzania

Abstract

The use of antimicrobials in poultry production accelerated the emergency and spread of antimicrobial resistance. AMR data of commensal resistant bacteria in chicken to antibiotics considered essential in animals and humans is lacking in poor countries. This study was conducted to determine the resistance patterns of *E. coli, K. pnuemoniae,* and ESBL producing bacteria to fluoroquinolones and cephalosporins in healthy chickens, and to assess the risk that can be posed by resistant bacteria to humans. A cross-sectional and time series study was conducted to obtain faeces from healthy chickens from biosecurity level 1 and 2 poultry farms between May and September 2021. Bacterial isolates were identified by biochemical test. Disc diffusion method was used to test susceptibility of *E. coli* and *K. pneumoniae* isolates to ciprofloxacin, ceftriaxone and cefepime according to CLSI standard. A total of 200 pooled fresh faecal samples were collected; 189 samples were from biosecurity level 1 and 11 were from level 2. Similarly, 104 samples were collected from layers and 96 from broiler chickens. In total, 150 strains were isolated: 80 were from broiler samples; and 70 strains were from layer chicken samples. Overall, the prevalence of *E. coli* was 75%; and no *K. pneumoniae* was isolated. The resistance of *E. coli* was 63.3% against ciprofloxacin, 0.7% against ceftriaxone, and 0% against cefepime. No ESBL-producing *E. coli* was detected. This study revealed that resistance to fluoroquinolones is high and that of cephalosporins is emerging in poultry production. The risk associated with high prevalence of commensal *E. coli* is significant due to transmission of AMR to human via food and environmental contamination.

Keywords: antimicrobial resistance, antimicrobials, chickens, *E. coli, Klebsiella pnuemoniae*

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Introduction

The emergence, spread, and persistence of antimicrobial resistance (AMR) continue to be an issues of global concern affecting the health of human and animals. Animal husbandry, in particular poultry, protracts a substantial portion of the global antimicrobial use.[1] Antimicrobials are used in animals to control pathogens and thereby curbing production losses. Empirical use of antimicrobials on a long term invokes antimicrobial resistance in bacteria. Emergence of antimicrobial resistance subsides the efficacy of antimicrobials on bacteria dominate, causing higher morbidity and mortality in animals due to untreatable bacterial infections. Production losses and expenditure on managing resistant infections in animals affect the livelihoods of farmers and other stakeholders in food production systems.[2]

Poultry production is among the key activities in the fight against hunger and poverty especially in developing countries. To increase productivity, a lot of antibiotics are employed in poultry production; raising the safety issue of such products due to an increasing possibility of emergency and spread of microbial resistance.[3] In Tanzania, chickens are the most farmed species with an estimated 72 million chickens being kept in different farming systems.[4] Diverse classes of antimicrobials are used to raise poultry; and the same classes of antimicrobials are considered to be essential in human medicine. The indiscriminate use of such essential antimicrobials in animal production is likely to increase the development of resistant pathogens, as well as resistance in commensal organisms. This would result in treatment failures, economic losses, and impaired food security; it could also act as a source of resistant gene pool for transmission to humans via infected animal by-products.

Consumption of animal products has been up-trending in the previous years, leading to an increased demand. Intensive production, based on high input (including uptake of antimicrobials), high output is being adopted as a coping mechanism to meet these increasing demands for animal proteins. Animal production is linked to AMR in complex ways. On one hand, animals are may be given antimicrobials without adhering to withdraw period whose residues resulting from metabolic breakdown can be contained in foods. This increases the likelihood of foodborne diseases and consumption of contaminated food with drug residues especially chicken meat and eggs. In addition, some groups of antimicrobials given to animals for treatment are essential for human health as a 'last resort' treatment.[5] Fluoroquinolones and cephalosporins are one of the priority antibiotics included in the Tanzania National Action (NAP) and AMR Surveillance Framework for AMR surveillance programs because of their critical importance in the promoting and improving human and animal health in the country.[6] In the livestock sector, bacteria resistant to fluoroquinolones and cephalosporins are considered a food safety hazard as they might become zoonotic pathogens or contribute to the horizontal spread of resistance genes contaminated food and environment.[7] Commensal

intestinal bacteria (e.g. *E. coli* and *K. pneumonia*) in healthy chickens are a potential reservoir for antimicrobial resistance genes. Given that *E. coli* is prevalent at >90% in healthy chickens,[8] there is a risk that resistant genes to these critically important antibiotics will be transferred to human pathogenic organisms.[3,9]

Given that no major new types of antibiotics have been produced in past three decades, and numerous antibiotics classes may increase losing their efficacy against pathogenic bacteria due to frequently use in animals and humans that leads to acquisition or development of resistant genes in bacteria, efforts have been directed towards controlling antimicrobial use in humans, animals, and plants.[10] Therefore, to inform the AMR and antibiotic control policy that is governed by NAP in Tanzania, evidence on the trends of fluoroquinolones and cephalosporins resistance in pathogens such as *E. coli* and *K. pneumoniae* in animals is urgently needed. This information is limited in Tanzania. The purpose of this study was to generate, and share on antibiotic resistance patterns in healthy chickens, and to provide initial data as evidence to support AMR control program and policy development. The objective of the study was to determine the prevalence of *E. coli* and *K.* pneumonia in chicken entering the food chain, resistance patterns of these bacteria to fluoroquinolones (ciprofloxacin) and cephalosporins (ceftriaxone and Cefepime), and recommend the risk posed by the prevalent resistant commensal bacteria to human and the environment in Tanzania. Testing healthy animals (chickens) that are entering the food chain over time is of paramount because it can help to identify resistant commensal bacteria, which can transmit antimicrobial resistant genes to humans leading to complication of conditions such as urinary tract infection as well as contaminating the environment. This information allows targeted strategies to mitigate AMR and can reflect the effectiveness of strategies applied to tackle AMR.[6]

Material and methods

Study design

This is a cross-sectional and time series study design to assess resistance of priority bacteria according NAP (*E. coli* and *K. pneumoniae)* to fluoroquinolones and cephalosporins isolated from healthy chicken feaces. The study was conducted over six-month period, between April and September 2021.

Study area

The study was conducted in two districts forming Mwanza city. Geographically, Mwanza city is located on the spectacular southern shores of Lake Victoria in Northwest Tanzania. The city is the second-largest business center after Dar-es-Salaam with more than 700,000 inhabitants.[11] The increasing population has attracted urban agriculture including commercial poultry farming. Medium-scale (400-1000 birds) poultry farms are mainly found in the peri-urban areas, including Ilemela and Nyamagana districts that form Mwanza City, due to availability of space that allows livestock farming. This study was conducted in the peri-urban areas of Ilemela and Nyamagana districts as shown in the map. In Mwanza, Tanzania Veterinary Laboratory Agency (TVLA) has a Zonal veterinary laboratory with the capacity to perform bacterial culture and characterization using the established laboratory procedures, including biochemical tests; and provides animal disease diagnostic services to livestock producers in Lake Zone.

Study Population

Inclusion and exclusion criteria: Broilers that reached the slaughter weight and culled layer hens that had reached the end of their laying period and being considered to be sold for human consumption were considered for sampling. The commercial broiler and layer production systems were targeted to capture resistance patterns in these two types of production systems. Sick chickens, and broilers and layers that had been treated in the previous seven days, were excluded from the study as their sample could not represent the status of resistance in bacteria carried by chickens that enter the food chain.

Selection of sampling farms: Poultry farms in Ilemela and Nyamagana districts were mapped and classified according to the Food and Agricultural Organization of the United Nations (FAO) biosecurity levels (level I: with improved hygiene, II - with medium hygiene, and III – farms with poor housing and hygiene), production types, and production cycle; and farms having more than 100 broilers and/or layer chickens kept for commercial purposes were included in the study. To cover as much areas as possible, farms at a radius of more than 100 - 150 meters apart were considered for a study.

Sample size and sampling procedure

Sample size: The study identified 10 commercial poultry farms with more than 100 healthy chickens that did undergo treatment for the past seven days was considered for sample collection. From each farm, 4 pooled samples were collected to make a sample size of 40 pooled samples collected monthly *E. coli* was chosen as the indicator pathogen because it's prevalent in more than 90% in healthy chickens,[8] and it's a non-fastidious organism which makes it easy to culture, store and transport.

Sampling procedure: As stated above, poultry farms in Ilemela and Nyamagana districts were mapped and classified according to the commercial poultry production system (level 1) with low to moderate bio-security level, and the small-scale production system (level 2) with a low biosecurity level.[12] Then, a total of 10 poultry farms in each of biosecurity levels 1-2 were intentionally selected as surveillance sites based on geographical representation, willingness to participate, and year-to-year production cycle. From each commercial poultry farm, a shed with the oldest chicken was selected and faecal sample was collected from healthy broiler and layer chicken monthly for identification and determination of susceptibility to antibiotics of *E. coli* and *K. pnuemoniae*. Where many sheds existed, one chicken shed was selected randomly and a pooled sample, containing 10 fresh chicken droppings from a selected chicken shed, was obtained. Each of the farm contributed four pooled samples, to make a sample size of 40 (4 samples x 10 farms) samples every month for six months.

Sample collection procedures: The designated farms were visited every month to collect samples after contacting the farmer by phone to ask if the chickens on the farm were symptomatically healthy and have not administered antibiotics in the past seven days. Feacal samples were collected, packed, and transported as described by procedure for sampling and handling of the test items (TVLA_SOP_PR 7.3, 2020) developed by Tanzania Veterinary Laboratory Agency (TVLA). Briefly, after the collection shed was divided into 4 quadrants, 10 fresh faecal droppings were collected from each quadrant by scooping on top with spatula. A total of 10 faecal samples from each quadrant were pooled to make one sample per quadrant, for a total of 4 pooled samples from each shed. Samples were labelled and transported using transport media under cold chain to the zonal diagnostic veterinary laboratory in Mwanza for bacterial culture and biochemical identification. At the laboratory, pooled samples were inoculated into specific media and incubated overnight at 37°C. Reference *E. coli* and *K. pneumoniae* isolates were used to compare with the isolates

from the field samples as detailed in TVLA procedures for bacterial culture. Then the isolates were transported to the national reference laboratory at the Central Veterinary Laboratory (CVL) in Dar es Salaam for confirmation, and antimicrobial susceptibility testing (AST). *E. coli* and *K. pneumoniae* were tested for susceptibility to fluoroquinolones (ciprofloxacin) and cephalosporins (ceftriaxone and cefepime) as indicator drugs. Macfarland standards (0.5) and Clinical and Laboratory Standard Institute (CLSI) guidelines (reading zone of inhibition and cut off points) was used to produce reliable and quality AST results. Extended spectrum β-lactam (ESBL) was determined based on the disc approximation method where ceftriaxone, ceftazidime and Amoxicillin/Clavulanic acid were placed 20mm away from each other in the same Muller Hinton Agar.[6,13]

Data management and analysis

The AMR data was formatted in WHONET (version 2020) and exported to Microsoft Excel and STATA (version 13) software for analysis as appropriate. The WHONET was formatted in the laboratory to capture all clinical, demographic, and microbiology variables on the request form. The analysis and display of resistance data was based on CLSI - M39-A2 consensus document (Hindler and Stelling, 2007). Frequencies and percentages were used to summarize the microbiology data. The proportion of *E. coli, K. pneumoniae* or ESBL isolates recovered from faecal samples were calculated separately. Isolates with intermediary susceptibility or complete resistance were considered non-susceptible; and the proportion (with 95% CI) of non-susceptible *K. pneumoniae* and *E. coli* against, ciprofloxacin, ceftriaxone and cefepime was calculated (the denominator was the number of isolates and/or the number of samples).

Table 1 Antibiotic susceptibility pattern

Antibiotic name	Number of isolates Tested	%R	%I	%S
Ceftriaxone	150	0.7	0	99.3
Cefepime	150	0	0	100
Ciprofloxacin	150	63.3	17.3	19.3

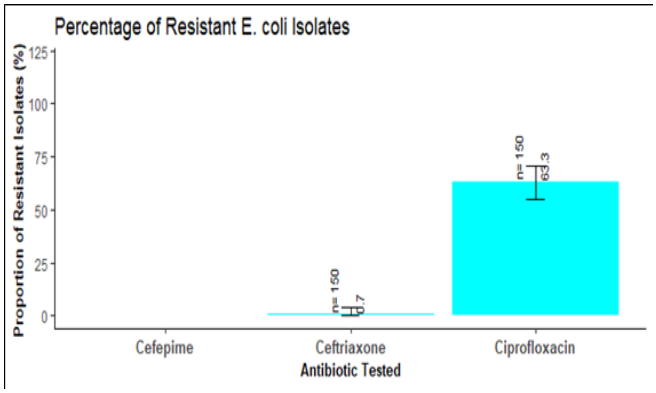

Figure 1 Prevalence of resistant *E. coli* to 3rd and 4th generation cephalosporins and fluoloquinolones.

Trend of antibiotic resistance

It was found that the trends of resistant *E. coli* against ciprofloxacin was consistently high between May and September with an average resistance of 63.18% (Figure 2).

Ethical clearance

Ethical clearance to conduct the study was obtained from the National Institutional of Medical Research (NIMR) with the permission number NIMR/HQ/R.8a/Vol.IX/3554. In addition, a written permission to conduct the study was provided by the Ministry of Health, Ministry of Livestock and Fisheries, Regional and District Councils and signed consent form by a farmer. The poultry farmer's information was kept confidential and stored in a password-protected computer.

Results

From a total of 200 feacal samples collected from poultry farms between April and September 2021, 150 (75%) were positive for *E. coli.* No *K. pnuemoniae* was isolated from any of the samples analyzed.

The *E. coli* isolates were further tested for AST against three antibiotics (cefepime, ceftriaxone and ciprofloxacin) by subjecting the pure culture to Muller Hinton Agar and measured the zone of inhibition expressed by antibiotic disc. Overall, the highest resistance was observed against ciprofloxacin (63.3%); whereas, the lowest resistance of 0% and 0.7% was recorded against cefepime and ceftriaxone respectively (Figure 1). The study further revealed that none of the screened isolates belonged to ESBL producing *E. coli*. Antibiotic susceptibility pattern of *E. coli* isolated from feacal samples collected from healthy chickens against cephalosporins and fluoroquinolones indicated high susceptibility against cefepime (100%) and ceftriaxone (99.7%). the resistance of *E. coli* against ciproflaxicin was detected to be 63.3%, 17.3% intermediate, and 19.3% was susceptible (Table 1).

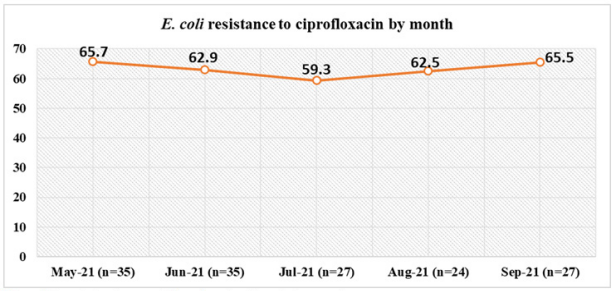

Figure 2 Trend of resistance of *E. coli* to ciprofloxacin by month.

Discussion

In veterinary medicine, fluoroquinolones have been effective therapeutics for treating enteric and respiratory infections in food-producing and companion animals.[14] Their antimicrobial activity against a broad spectrum of pathogenic bacteria, low toxicity, and other beneficial pharmacokinetics, make them attractive for use in farmed animals (Globbel *et al.,*[15] Frye and Jackson,[16] However, a public health concern is long lasting impact of these drugs in livestock that can be transmitted into the food chain and pose risks to humans due to long time withdraw period in treated food producing animals. For instance, the transmission of ciprofloxacin-resistant *E. coli* and *Salmonella* from food animals to humans has been demonstrated even though ciprofloxacin is rarely used in animal husbandry practices.[17] This can be explained by the cross-resistance between fluoroquinolones because enrofloxacin, a drug commonly used in animals, is partially metabolized to ciprofloxacin.[18]

The *E. coli* isolates confirmed by biochemical test from the pure culture indicated significant differences in their resistance against the three tested antibiotics in the study. As shown in Table 1, *E. coli* isolated from healthy chicken feacal samples in the studied districts exhibited resistance of 63.3% against ciprofloxacin. However, resistance against 4th generation cephalosporin (cefepime) and 3rd generation cephalosporin (ceftriaxone) was very low in poultry production (Figure 2). This might be due to low exposure of *E. coli* to these group of antibiotics because they are not commonly used in livestock production. A study conducted in Ghana also identified low level of resistance of *E. coli* to ceftriaxone (1.34%) and cefotaxime (0.67%).[19] The alarming prevalence of resistance genes against ciprofloxacin reported in this study could have been contributed by the use of other antibiotics found in the class of fluoroquinolones such as enrofloxacin and norfloxacin which are frequently used in poultry farms for treatment of enteric and respiratory diseases. Nonetheless, the high rate of resistance of *E. coli* against ciprofloxacin could be associated with easy access to enrofloxacin and norfloxacin, which are available over the counter at minimal cost. A high resistance of 68.6% of *E. coli* against ciprofloxacin which is similar to the resistance found in the present study was reported in a study conducted in Mwanza and Arusha.[20] Recently, it has been shown that the application of enrofloxacin induced development of resistant commensal *E. coli* in the intestines of poultry.[21,22]

The lowest emergence of *E. coli* isolates resistant to ceftriaxone as an antibiotic representing 3rd generation cephalosporins found in the study should not be ignored because studies have demonstrated that pigs and poultry could carry *E. coli* isolates with decreased susceptibility to 3rd generation cephalosporins.[23,24] This observation is a testament that a commensal *E. coli,* from healthy chickens, that is resistant against cephalosporins is rapidly emerging and is entering the food chain through chicken products such as meat. Thus, what we have seen so far could be the tip of the iceberg as the information on resistance is still incomplete and trends are difficult to assess. In this study, the trend of resistance of *E. coli* to ciprofloxacin was observed to be consistently high ranging between 59.3 – 65.7% in the study period (Figure 3).

Increased use and misuse of antimicrobials and other microbial stressors, such as environmental pollution, create favourable conditions for microorganisms to develop resistance both in humans and the environment.[25] Bacteria in water, soil and air, for example, in the chicken meat processing facilities, can acquire resistance gene by either horizontal transfer following contact with resistant bacteria including commensals in the environemnt or vertical transfer. Human exposure to AMR in the environment can occur by contact with contaminated food and environment that contain antimicrobial resistant microorganisms. Most chicken informal slaughter slabs have been found to have improper handling of chicken carcasses and unregulated waste disposal from slaughter poultry slabs including blood, feces and wastewater disposed into municipal drains without either monitoring or treatment.[26] Contaminated chicken products pose higher risk to human and the enviroment and resistance genes can be spread to bacterial community in the enviroment through interaction of bacterial community in the nevironment. The high prevalent of resistance to fluoquinolones reported in the present study suggests possible risk of transmiting antimicrobial resistance genes to human and to the environment via the food chain. In Tanzania especially in Mwanza city, chicken are slaughtered at the market with poor hygienic practice leading to food and environmental contamination. The challenge that was noticed is that the market chain for poultry products is largely informal. The study presupposes that chickens habouring resistant *E. coli* to fluoquinolones are likely to contaminate the environment during slaughtering, and the resistant genes can be transmitted to human via the contaminated food and environment. People with immunosuppression are at higher risk to be infected with commesal *E. coli* which may lead to opprtunistic infection leading to severe illness.

Conclusion

The emergence of cephalosporin resistance has been documented in poultry production systems where no cephalosporins and other drugs with synergistic effects are authorized for use.[27] This study revealed that resistance to 3rd and 4th generation cephalosporins is emerging in animal populations in Tanzania. The indiscriminate use of unrelated classes of antimicrobials in animal populations may engender resistant microbes against fluoroquinolones, and 3rd and 4th generation cephalosporins. Measures to counter a further increase and spread of drug resistant microbes in animals should be considered under one health approach to integrate AMR microbes in animals, humans and the environment, as multidrug resistant bacteria such as *E. coli* may be transmitted from animals to humans via food chain and contaminated environment.[28–30]

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Conflicts of interest

Authors declare that there is no conflict of interest

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