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Research Article

Antimicrobial Resistance Profiling of *Escherichia coli* Isolated from Chickens in Northern Province of Rwanda

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ABSTRACT

Antimicrobial resistance (AMR) is a global public health concern due to inappropriate antimicrobial-use in humans and animals including poultry. *Escherichia coli* have been proposed as one of the pathogens to be used for AMR surveillance. The goal of current study was to determine the antimicrobial resistance of *E. coli* in chickens in Northern Province of Rwanda. A cross-sectional study was conducted between June and August 2021; the chickens were randomly selected in each of the twenty farms that were included in the study. The samples were collected from cloaca and from farm environment. *E. coli* was isolated and identified and antimicrobial susceptibility testing was done using disc diffusion method. The results were defined as resistance(R), susceptible(S) and intermediate (I). After Data processing, they were entered in Microsoft Excel for analysis. The data were presented as frequencies and percentages. In total, 384 samples were collected (139 in Gakenke, 114 in Rulindo and 131 in Musanze Districts). *E. coli* was isolated from 162(42.18%) among which 40 (24.7%) were from Musanze District, 57(35.2%) from Gakenke District and 65 (40.1%) were from Rulindo District. The highest resistance was observed for tetracycline (69.8%) followed by cotrimoxazole (39.5%). The highest susceptibility was observed for gentamycin (100%) followed by ciprofloxacin (96.9%) and amoxicillin (66%). This study indicated the presence of *E. coli* in chickens of Northern Province of Rwanda and a large number of antibiotic resistant *E. coli* were isolated. Therefore, the government should set policies to control the use of antibiotics on farms.

Keywords: Antimicrobial Resistance, *Escherichia coli,* Chickens, Rwanda

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INTRODUCTION

Rwanda is one of developing countries and experiences different problems of food insecurity, small household income and high prevalence of animal and human diseases. Poultry sector and global meat production industry is growing rapidly in less developed countries due to high population growth, rising urbanization and increase in people's income (high purchasing power) (Mbuza, 2017). Livestock production in Rwanda plays a great role in Agricultural households because it is a source of food income. Rwanda is a country in East Africa which has been grown over the past five years in the poultry sector (Henry, 2019). In developing countries like Rwanda, chickens are the most domesticated birds and the most threatened by environmental risk factors by picking bacteria containing antibiotic resistance genes (Hamisi et al., 2014). The contribution of indigenous chickens is now less competitive due to their low productivity compared to exotic breeds (Mahoro et al., 2010).

The occurrence of *E. coli* in food products shows direct or indirect fecal contamination due to deficits in hygiene during product preparation. In Rwanda, there is a poor regulation in the use of antibiotics and this can lead to resistance in the poultry farms (Manishimwe et al., 2017). Global poultry industry is affected by different bacterial pathogens including avian pathogenic *Escherichia coli* (APEC).

Initially, antimicrobial resistance was only considered as a human being issue which is not true. The increase of antimicrobial resistance is the overuse of antibiotics in livestock and this lead to the difficult treatment of many common bacterial infections (Hamisi et al., 2014). Antibiotics are used worldwide to treat colibacillosis but due to their enormous use in the treatment of poultry and other farm animals; the resistance has become a big problem in common bacteria isolated from farm animals (Amara *et al*. 1995). In human and veterinary medicine, Antimicrobials have been used for more than 60 years. Refer to World Health

Organization; the usage of antimicrobials in agriculture has positive impact as they are vital medicines in treating bacterial infections in humans and animals, used in livestock production sustainability and used in control of animal bacterial infections that could be transmitted to humans (Economou and Gousia, 2015). The reduction of efficacy of antimicrobials in human medicine is also observed in *E. coli* isolated from chickens; this resistance is mainly observed in third-generation cephalosporins and fluoroquinolones (Vounba et al., 2018). The drug misuse can lead to an increase antibiotic selection pressure on chicken gut which can cause emergence antibiotic resistance. Thus, *E. coli* is used as indicator of resistance of other organisms in the gastrointestinal tract of chickens. In animals like poultry, pigs and calves, antibiotics are not only used as therapeutic, they are also used as growth promoters and bacterial infection prevention and this can increase the level of resistance of pathogenic and commensal bacteria (Adelaide et al., 2008).

Resistant bacteria and virulence gene may be transported between animals and humans via food of animal due to contamination of carcasses by faecal flora during slaughtering (BUIE, 1947). The application and the improper use of antibiotics as growth promoters, prevention of infectious disease and in treatment have risen the consumption of antibiotics and resistance among bacteria in animal habitat. Transmission of resistance gene to human directly or indirectly may be acquired through indirect or direct consumption of contaminated food (Economou and Gousia, 2015). Therefore, the present study investigated the antimicrobial resistance of *E. coli* isolated from cloacae of chickens in Northern Province of Rwanda.

MATERIALS AND METHODS

Study area and sampling method: The cross-sectional study was conducted to determine the antibiotic resistance of *Escherichia coli* in farm chickens in three Districts of Northern Province of Rwanda. In each district, four sectors near the main road were selected. Farmers with more than ten chickens were targeted.

Sample collection: In total, 384 cloaca samples (139 in Gakenke, 114 in Rulindo and 131 in Musanze Districts) were collected using sterile swabs from chicken in Northern Province of Rwanda and its environments. A dry sterile swab was aseptically inserted into cloaca to ensure the collection of cloacal material. The samples were properly labelled and were transported immediately to the laboratory with ice packs for microbiological analysis.

Isolation and identification of Escherichia coli: On the arrival in laboratory, the samples were inoculated into tubes of nine milliliters of fresh prepared buffer peptone water for enrichment and incubated at 37^0C for 24hrs. The incubated broth culture was streaked into MacConkey Agar using sterile cotton swabs and incubated at 37^0C for 24hrs. The pink colored colonies were picked using sterile loop and streaked into Eosin Methylene Blue Agar and incubated at 37^0C for 24hrs. The colonies with Greenish Metallic Sheen on Eosin Methylene Blue were sub-cultured to obtain pure colonies. Rose pink colonies on MacConkey agar plates (Putative *E. coli*) that have showed greenish metallic sheen colonies on Eosin Methylene Blue agar were selected for biochemical identification tests. After an initial study of the colonial morphology, microscopy following Gram staining, oxidase and catalase reactions, indole tests, urease tests, substrate utilization tests and carbohydrates fermentation tests. (Barrow and Feltham, 2005).

Antimicrobial susceptibility testing: Isolates that were identified as *E. coli* were tested for susceptibility for antimicrobial agents. Susceptibility was determined by Kirby-Bauer disk diffusion method. A single colony of the isolate under test was inoculated unto 9ml of 0.9%Nacl until it achieved the 0.5 McFarland turbidity standards. A sterile cotton swab was dipped into the broth culture and inoculated unto the Mueller Hinton Agar (MHA) by swabbing entire surface of the plate of Mueller-Hinton agar. The plates were then allowed to dry. Antibiotics discs were then applied to the surface of the inoculated plates. Each disc was pressed down into the agar to ensure complete contact and the antibiotic discs (Amoxicillin (AMC,25mg), Cotrimoxazole (COT,250mg), Tetracycline (TET, 3Omg), Gentamycin (GEN,10mg) and Ciprofloxacin (CIP,5mg) were applied and incubated under aerobic condition for 18hrs at 37^0C. The results of inhibition zone were interpreted in accordance with the recommendation of Clinical and Laboratory Standard Institute (CLSI, 2019).

Interpretations of antibiotic susceptibility results: Susceptibility testing were interpreted by measuring the diameter of zones of inhibition of each antibiotics and the results were interpreted as recommended by Clinical and Laboratory Standard Institute, 2018 (CLSI). The results were interpreted as Resistant(R), Intermediate (I), Susceptible(S). The resistant isolates were computed as frequencies and percentages.

Data Analysis After Data processing, they were entered in Microsoft Excel for analysis. The data were presented as frequencies and percentages.

RESULTS

Prevalence of Escherichia coli in the chicken cloacae and the farm environments: *Escherichia coli* was isolated from 162 (42.2%) out of 384 cloacae swab samples among which 57(35.2%) were from Gakenke, 65(40.1%) from Rulindo and 40(24.7%) were from Musanze. In 162 isolates, 21(12.96%) were from farm environments and 141(87. 03) were from cloacae (Table 1).

Table 1.
The results of isolation rates in three Districts of Northern Province

Districts	Sampled chickens	Isolation rates
Gakenke	139	57(35.2%)
Rulindo	114	65(40.1%)
Musanze	131	40(24.7%)
Total	**384**	**162**

Table 2:
Antimicrobial Resistance profiling of *E. coli* isolated in three Districts of Northern Province:

Antimicrobial Agent	Disc potency	Number of isolates	Susceptible N (%)	Intermediate N (%)	Resistance N (%)
Cotrimoxazole	250	162	95(58.6%)	3(1.9%)	64 (39.5%)
Gentamycin	10	162	162 (100. %)	0%	0%
Tetracycline	30	162	46 (28.4%)	3 (1.9%)	113(69.8%)
Ciprofloxacin	5	162	157(96.9%)	0%	5 (3.1%)
Amoxicillin	30	162	107(66.0%)	0%	55 (34.0%)

Antimicrobial resistance in *Escherichia coli*: *Escherichia coli* isolates showed resistance to tetracycline (69.8%), cotrimoxazole (39.5%), amoxicillin (34.0%), ciprofloxacin (3.1%) and gentamycin (0%) Table 2. Thirty- one (19.1%) out of 162 isolates were resistance to at least three antimicrobial from different classes Fig. 1.

Multi drug resistance in E. coli isolates: This study indicated Multi Drug Resistance patterns to *E. coli* isolates. 30 isolates has showed the resistance to three antibiotics namely cotrimoxazole, tetracycline and amoxicillin and one isolate showed the resistance to four antibiotics(cotrimoxazole, tetracycline, amoxicillin and ciproflaxacin).

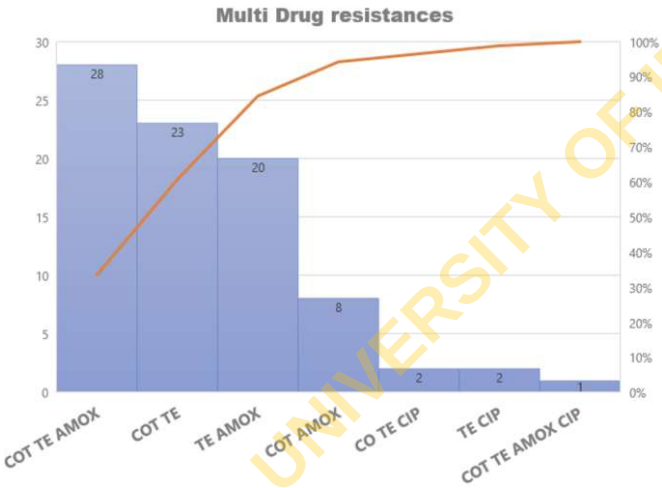

Figure 1:
Showing multi drug resistance of *E.coli* isolates of chickens in Northern province of Rwanda.
COT- Cotrimoxazole; TE-- Tetracycline; AMOX- Amoxicillin
CIP- Ciprofloxacin

DISCUSSION

Escherichia coli was isolated from 162 (42.18%) of 384 samples collected in three districts of the Northern Province. In Gakenke District, the positive samples were 35.2 % (57/162), Rulindo 40.1 %(65/162) and Musanze District 24.7 % (40/162). This difference in isolation rate may be due to management practices which can be different in these three Districts.

A study conducted in Kenya in 2019 showed that *E.coli* isolates in chicken droppings were 57% (Langata et al., 2019). Another study conducted in Kenya showed the prevalence of 58% (Ngai et al., 2021). These isolation rates are higher compared to this study; this may be due different management practices. The resistance patterns to antimicrobial agents used including cotrimoxazole, amoxicillin, ciprofloxacin, tetracycline and gentamycin revealed a high resistance were to tetracycline (69.8%), cotrimoxazole (39.5%) and amoxicillin (34.0%) and the low resistance was observed to gentamycin (100%) and ciprofloxacin (96.9%). None of the isolates was resistant to gentamycin. A study conducted in Rwanda by (Manishimwe et al., 2021) observed the following antibiotic resistance pattern in this order tetracycline (35.5%), ampicillin (19.6%) and streptomycin (16.5%) which is similar to this study on the same antibiotic used (Tetracycline). A study done in Kenya at Tigoni poultry processing plant revealed a resistance pattern in this order; tetracycline (75%), cotrimoxazole (72.4%) and ampicillin (39%) (Adelaide et al., 2008). This resistance is similar to one in this study; this may be due to the high use of tetracycline and sulfonamides in these countries. Another study conducted in Kenya revealed the resistance patterns to amoxicillin, cotrimoxazole, tetracycline, streptomycin and low resistance to ciprofloxacin and chloramphenicol (Langata et al., 2019). Aminoglycosides, quinolones and phenols are not commonly used in poultry industry.

In this present study, the higher percentage of intermediate isolates was observed for cotrimoxazole and tetracycline. A study conducted in Uganda in chickens indicated a high resistance of *E.coli* to tetracycline (86%), ciprofloxacin (22%), nalidixic acid (21%) and chloramphenicol (8%) (Kabiswa et al., 2018). Another study conducted in Uganda showed a high resistance to ampicillin, tetracycline and cotrimoxazole (Majalija et al., 2010). In Ethiopia, resistance was observed for 12 of the 17antimicrobials tested in *E. coli* isolates from chickens. The high resistance pattern was observed to oxacillin, cefuroxime and amoxicillin followed by cefotaxime (92.7%), tetracycline (46.3%), and all *E.coli* isolates were susceptible (100%) to ciprofloxacin, sulfamethoxazole-trimethoprim and norfloxacin followed by gentamicin (89%) (Sarba et al., 2019). A study conducted in Tanzania showed the highest resistance pattern in this order: Tetracycline (91.9%), sulfamethoxazole-trimethoprim (80.5%), ampicillin (70.9%) and ciprofloxacin (40.2%) (Mgaya *et al*., 2021) which has similarities and some differences when compared to this present study. May be these antibiotics are more used in Tanzania compared to their use in Rwanda.

These studies indicate that the drugs (Tetracycline, Ampicillin, Cotrimoxazole and Amoxicillin) have a high

resistance pattern in different Eastern Africa countries; this may indicate that these antibiotics are commonly used in the region. So, there is need to implement measures against misuse of antimicrobial drugs in animals especially poultry. The study conducted in Egypt on Broiler chicken at Beni-Suef University showed only high susceptibility to colistin sulphate (70%) and high resistance to amoxicillin (97.5%), cefotaxime sodium and florfenicol (95% for each), apramycin, ciprofloxacin and gentamicin (92.5% for each), streptomycin (90%), enrofloxacin (87.5%), trimethoprim-sulphamethoxazol and doxycycline HCl (77.5% for each) (Radwan et al, 2020) .This is different from this study, and may be due to over use of antimicrobials in broiler chickens compared to others birds. The study carried in Zambia indicated the resistance to tetracycline (100%) while the isolates were susceptible to gentamycin (77%) (Mtonga et al., 2021) which is similar to this study. This can be an indication of overuse of tetracycline and the low use of Gentamycin in poultry. In conclusion, resistance of avian pathogenic bacteria to commonly use antimicrobial agents in poultry may lead to loss due to bacterial infections that are resistant to antimicrobial therapy. Resistance to avian pathogens is also of public health implication as the resistant bacterial strains could be transmitted to humans. *Escherichia coli* is among the major zoonotic bacteria implicated in human food borne infection (Mead et al., 1999).

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