Antimicrobial resistance patterns and risk factors for cloacal *Escherichia coli* in chickens in central Tanzania

C.A. Newberry*[1], J.K. Lane[1], B.A. Byrne[1], M. Mwanzalila[2], T. Hamza[3], J. Okachu[4], R.R. Kazwala[2], W.A. Smith[1]

[1]*School of Veterinary Medicine, University of California, Davis, CA, USA*
[2]*Sokoine University of Agriculture, Morogoro, Tanzania*
[3]*Iringa Municipal Council, Veterinary Investigation Center, Iringa, Tanzania*
[4]*Tanzania Veterinary Laboratory Agency, Iringa, Tanzania*

[*]*Corresponding Author: Email: canewberry@ucdavis.edu*

Article timeline

Submitted: 26-05-2021, Revised: 11-08-2021, Accepted: 14-08-2021, Published:28-08-2021

Antimicrobial resistance patterns and risk factors for cloacal *Escherichia coli* in chickens in central Tanzania

C.A. Newberry*[1], J.K. Lane[1], B.A. Byrne[1], M. Mwanzalila[2], T. Hamza[3], J. Okachu[4], R.R. Kazwala[2], W.A. Smith[1]

[1]*School of Veterinary Medicine, University of California, Davis, CA, USA*
[2]*Sokoine University of Agriculture, Morogoro, Tanzania*
[3]*Iringa Municipal Council, Veterinary Investigation Center, Iringa, Tanzania*
[4]*Tanzania Veterinary Laboratory Agency, Iringa, Tanzania*

[*]*Corresponding Author: Email: canewberry@ucdavis.edu*

SUMMARY

Antimicrobial resistance is a serious and escalating threat to global health. This study established a baseline antimicrobial resistance profile and prevalence for cloacal *Escherichia coli* in chickens in central Tanzania. Animal husbandry practices, flock size, and chicken breed were recorded for risk factor evaluation to elucidate potential drivers of resistance across populations. Cloacal samples were collected from poultry in Iringa, Tanzania, and *E. coli* isolates cultured were then tested for susceptibility to seven medically important antimicrobial drugs: ampicillin, amoxicillin-clavulanate, cefoxitin, enrofloxacin, gentamicin, sulfamethoxazole-trimethoprim, and tetracycline using the Kirby-Bauer disk diffusion method. Over 94% of the 59 *E. coli* isolates tested were resistant to at least one antimicrobial drug, and over 61% of isolates were resistant to three or more classes of antimicrobial drugs. The highest prevalence of resistance found was to tetracycline and sulfamethoxazole-trimethoprim (88% and 86%, respectively), which were used regularly on many sampled chickens. Previous antimicrobial usage emerged as the only significant risk factor associated with increased detection of multi-drug resistant *E. coli*. Further surveillance and educational outreach about antimicrobial resistance and stewardship is recommended to reduce antimicrobial drug use and to limit the potential spread of resistance to antimicrobial drugs in Tanzania.

Key words: Antimicrobial resistance, antimicrobial stewardship, chicken production

INTRODUCTION

Antimicrobial resistance (AMR) is a worldwide threat to human and livestock health as well as livelihoods (WHO, 2018). Defined as the ability of a microorganism to resist the effects of antimicrobial medicine typically used against it, AMR often results in resistant infections with increased mortality, morbidity, and cost of health care (WHO, 2018). The World Health Organization, Food and Agriculture Organization of the United Nations, and World Organisation for Animal Health have all recognized the threat of AMR and the need for a One Health multidisciplinary approach to limit the emergence and spread of AMR (FAO, 2016; OIE, 2016; WHO, 2018). In Tanzania, as with many places around the world, easy access to and inappropriate use of antimicrobial drugs contribute to selection of resistant bacterial strains in both humans and animals (Katakweba *et al*., 2018; Kimera *et al*., 2020; Mdegela *et al*., 2021). Resistant infections are especially concerning in livestock as they not only threaten producer's livelihoods with their increased livestock mortality rates and treatment costs, but also may spread to human consumers (Mdegela *et al*., 2021). Though AMR is a growing global threat, there still remains a shortage of data on its levels and trends in Tanzania (Katakweba *et al*., 2018). Notably, a recent study in Arusha, Tanzania found that intensively-raised commercial chickens have higher prevalence of resistant *E. coli* than free-ranging village chickens

(Rugamisa *et al*., 2016). In contrast, a separate study in Morogoro and Dar-Es-salaam, Tanzania found that free-ranging chickens had higher numbers of resistant *E. coli* than commercial chickens (Katakweba *et al.,* 2018). While these two studies had some differences in sampling and laboratory methods, the discrepancy between their findings highlights the importance of further research on AMR trends in Tanzania and across different production systems.

Furthermore, in northern Tanzania, researchers identified a high prevalence of antimicrobial resistance in bacterial isolates from livestock, people, and water sources; this study identified livelihood factors that promote general transmission of bacteria to be associated with increased prevalence of antimicrobial resistance (Subbiah *et al*., 2020). The need to increase antimicrobial resistance surveillance in poultry is further supported by the priorities identified in Tanzania's National Action Plan on Antimicrobial Resistance (The United Republic of Tanzania, 2017). This study established a baseline prevalence and profile of antimicrobial resistant *E. coli* in chickens in Iringa, Tanzania by working with local stakeholders and veterinarians involved with the Health for Animals and Livelihood Improvement project. Risk factors for AMR, such as husbandry and poultry-keeping practices, were also evaluated to elucidate possible reasons for differences in resistance across chicken populations.

All surveillance activities were conducted in collaboration with, and with permission from relevant regional authorities, as well as approved through formal ethical review committee approval and support letters. All animal sampling activities were conducted under permission of the Institutional Animal Care and Use Committee at the University of California, Davis (Protocol 21042) and in collaboration with SUA and TVLA, Iringa Region under the HALI permit number 2019-340-NA-2006-79.

RESULTS AND DISCUSSION

A total of 12 farms were enrolled and 120 chickens were sampled. Of these samples, 107 isolates were grown, 89 of which were confirmed to be *E. coli*. Fifty-nine pure *E. coli* isolates were successfully tested for susceptibility to all seven antimicrobial drugs, ampicillin (AM), amoxicillin-clavulanate (AMC), cefoxitin (FOX), enrofloxacin (ENO), gentamicin (GM), sulfamethoxazole-trimethoprim (SXT), and tetracycline (TE), with 41 of those isolates coming from commercial chickens and 18 from indigenous chickens (Table 1). Of these 59 isolates, 95% were resistant to at least one antimicrobial drug. Only 3 of the 59 isolates were susceptible to all drugs. All pan-susceptible isolates had been sampled from chickens on one small-scale farm of indigenous chickens. The prevalence of resistance was determined as the number of isolates resistant to a given antimicrobial drug divided by the number of isolates tested for resistance to that drug and is shown in Figure 1. The antimicrobial drugs with the highest prevalence of resistance were TE and SXT, with 88% and 86% of isolates resistant to TE and SXT respectively. Just over 81% of isolates were resistant to both TE and SXT. AM was the antimicrobial drug with the third highest resistance, with 48% of isolates being resistant. Multi-drug resistance (MDR), defined as resistance to drugs in 3 or more classes of antimicrobials, was seen in 61% of isolates. Enrolled farms included a range of husbandry practices and flock demographics. Breed, age, sex, diet, flock size, and housing near other livestock species were all examined as risk factors in the logistic regression model and showed no significant association with the outcome of MDR. To verify no other risk factor impacting MDR was missed due to small sample size, prevalence of resistance was examined across each risk factor individually. Usage of antimicrobial drugs on farms was identified as a statistically significant risk factor for MDR (n=59, p=0.018). Sulfonamides and tetracycline were reported as being used on 9 of the 12 farms surveyed, and those farms were found to have higher prevalence of resistance to every antimicrobial drug tested when compared to farms not using antimicrobials.

Table 1: Flock characteristics of chickens tested for antimicrobial resistant *E. coli**

	Sex		Antibiotic usage		Farm size		Feed		Housing		Other livestock on farm		Commercial purpose	
	Female	Male	Yes	No	<500 chickens	>500 chickens	Homemade	Commercial	Indoor only	Indoor/outdoor	Yes	No	Broiler	Layer
Commercial chickens	35	6	31	10	12	29	22	19	41	0	21	20	18	23
Indigenous chickens	12	6	14	4	17	1	17	1	8	10	17	1		
Total	47	12	45	14	29	30	39	20	49	10	38	21	18	23

*A total of 59 chickens from farms and households Iringa, Tanzania were enrolled and successfully tested for antimicrobial resistant *E. coli* in June 2019. Information about each flock and its husbandry practices was gathered using a farm enrollment form. Each chicken was given a brief physical exam and sampled via a sterile cloacal swab (BD Diagnostics, Sparks, MD). One *E. coli* isolate was obtained from each cloacal swab and confirmed to the species level using indole, oxidase, and API Rapid 20E identification tests strips (bioMérieux, Marcy-l'Etoile). A total of 59 isolates were tested for antimicrobial susceptibility by the Kirby-Bauer disk diffusion method according to CLSI (2018a) guidelines. The following antimicrobial drug discs were tested: AM (10 µg), AMC (20:10 µg), FOX (30 µg), ENO (5 µg), GM (10 µg), SXT (1.25:23.75 µg), and TE (30 µg) (BD Diagnostics, Sparks, MD). Isolates were identified as susceptible, intermediate, or resistant using human breakpoints for AM, AMC, FOX, GM, SXT, and TE and using poultry breakpoints for ENO (CLSI 2018b; CLSI 2020). Reference strains *E. coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, and *Pseudomonas aeruginosa* ATCC 27853 were used for quality control (Microbiologics Inc, St. Cloud, MN). To assess each risk factor's association with MDR, a mixed effects logistical regression of MDR was run. Significance was defined as P values ≤ 0.05. Risk factor statistical analysis was conducted in Stata/IC 16.0.

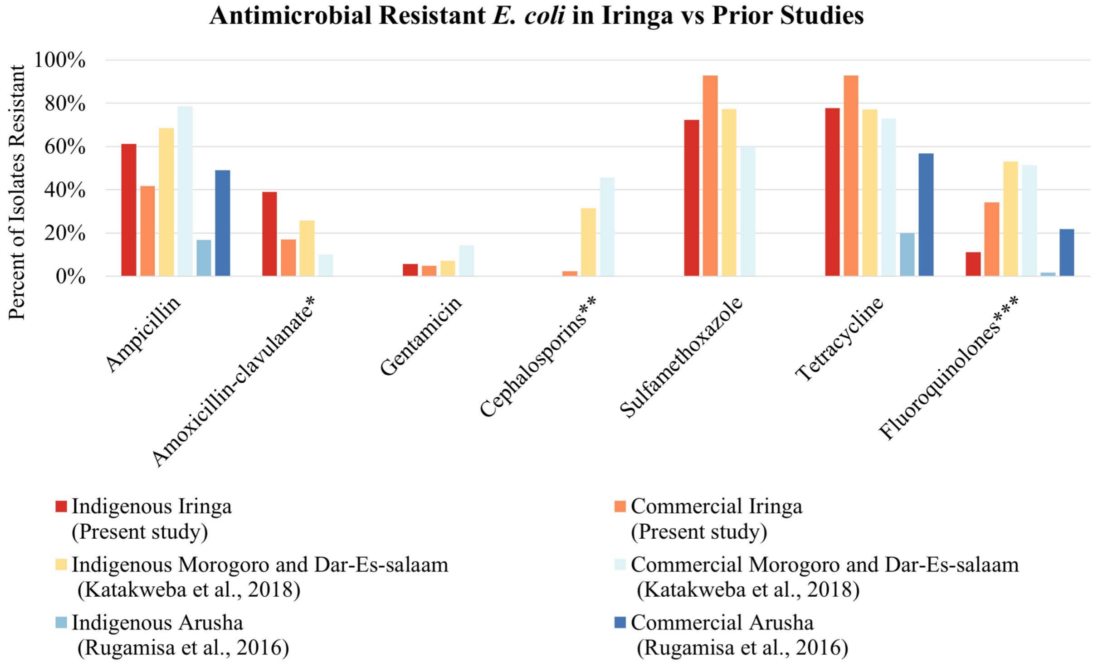

Figure 1: Prevalence of resistant *E. coli* isolates from indigenous and commercial chickens, in the present study and two prior studies. The Dar-Es-salaam and Morogoro study tested 140 *E. coli* isolates, 70 from indigenous chickens and 70 from exotic chickens, for antimicrobial resistance between 2011-2013 (Katakweba *et al*., 2018). The Arusha study sampled 75 chickens, 25 scavenging local chickens and 50 penned broiler chickens, and tested 1,800 *E. coli* isolates for antimicrobial resistance in 2016 (Rugamisa *et al*., 2016). *Amoxicillin-clavulanate and sulfamethoxazole-trimethoprim were not tested in Arusha (Rugamisa *et al*., 2016).
**Cephalosporins: resistance to cefotaxime, a third generation cephalosporin, was tested in Dar-Es-salaam, Morogoro, and Arusha (Katakweba *et al*., 2018; Rugamisa *et al*., 2016). In Iringa, resistance to cefoxitin, a second generation cephalosporin, was tested. ***Fluoroquinolones: resistance to ciprofloxacin was tested in Dar-Es-salaam, Morogoro, and Arusha. In Iringa, resistance to enrofloxacin was tested (Katakweba *et al*., 2018; Rugamisa *et al*., 2016).

This study was among the first to characterize antimicrobial resistance in *E. coli* from chickens in Iringa, Tanzania. High prevalences of resistance to TE, SXT, and AM were found, which is consistent with reported trends of *E. coli* resistance both internationally and within Tanzania (Lei *et al*., 2010; Rugamisa *et al*., 2016; Katakweba *et al*., 2018; Subbiah *et al*., 2020).

When compared to prior studies on antimicrobial resistant *E. coli* in chickens in other areas of Tanzania, this study found a higher prevalence of resistance to TE and SXT (Figure 1) (Rugamisa *et al*., 2016; Katakweba *et al*., 2018). However, different sample sizes and sampling methods may influence the differences between studies.

These two antimicrobial drugs were commonly used on poultry farms in Iringa (used in 75% of farms in our study), and are considered inexpensive and readily available without prescription in the region (Rugamisa et al., 2016). Additionally, this study identified prior usage of antimicrobial drugs as a significant risk factor for increased prevalence of multi-drug resistant *E. coli*. Given the known link between antimicrobial use and the development of resistance (WHO, 2018), this suggests the use of these antimicrobial drugs may play a role in selecting for resistance. However, as demonstrated in a recent study in northern Tanzania, the driving factors of antimicrobial resistance are complex and also include livelihood factors that promote the general transmission of bacteria, such as shared water sources (Subbiah *et al.*, 2020). Notably, antimicrobial usage had a stronger association with multi-drug resistance than breed of chicken (i.e. commercial breed or local indigenous breed), and that was unexpected. While the indigenous chicken farms were generally smaller scale and produced no other livestock species, these farms reported using antimicrobials and vaccines as often as commercial farms.

This contradicts the prior belief that classically free-ranging indigenous chickens are rarely exposed to antimicrobial drugs but may explain the similarity in resistance profiles between indigenous and commercial chickens. Chickens not treated with antimicrobial drugs had a lower prevalence of resistance across all antimicrobial drugs, yet some were still found to carry resistant *E. coli*. Previous studies have shown the environment is a vast reservoir of resistant organisms and their associated genes (Kimera *et al.*, 2020; Subbiah *et al.*, 2020), therefore chickens may be exposed to antimicrobial drugs, antimicrobial residues, or resistant bacteria in their daily environments.

While this study established use of antimicrobial drugs as a significant contributing factor to AMR, there were several limitations to consider. Due to challenges with specialized laboratory media, only 59 *E. coli* isolates yielded interpretable susceptibility results. This limited the sample size from its expected 120 isolates to 59 isolates, leading to an overrepresentation of commercial chickens as compared to indigenous, as well as a decrease in power due to sample size. Furthermore, inclusion of more than 1 isolate per chicken sample may have improved study power. This study's initial findings were shared with the community and partner organizations through presentations held in Iringa, Tanzania. These meetings fostered discussion and interest in potential steps to combat AMR. However, there is limited research around antimicrobial stewardship interventions in Africa (Frumence *et al*., 2021). Of the many strategies existing, persuasive interventions using education, audits, and guidelines have been successful at reducing antimicrobial drug usage in human health care in Africa (Akpan, 2020), suggesting similar educational outreach for livestock producers may be efficacious.

Additionally, future projects may focus on improving infectious disease control by promoting vaccination, safe water, sanitation, and farm biosecurity practices to limit the need for antimicrobial drug use (Mdegela *et al*., 2021; Subbiah *et al.*, 2020). Lastly, increasing infrastructure and policies that support rational antimicrobial use and continued AMR surveillance is critical to both monitor the extent of the AMR crisis and to design targeted intervention strategies (Kimera *et al.,* 2020; Frumence *et al*., 2021; Mdegela *et al*., 2021).

It is the authors' hope that this pilot study will lead to further AMR surveillance and research into the drivers of AMR in poultry across Tanzania. Additionally, molecular characterization of these isolates would increase knowledge of the genetic elements responsible for AMR and contribute to the national database of resistant strains of *E. coli* in Tanzania. Longitudinal studies may follow up on antimicrobial stewardship interventions for livestock producers to determine if this outreach is effective in lowering usage of antimicrobial drugs, and if that in turn impacts the prevalence of AMR.

This study found high levels of antimicrobial resistant *E. coli* in chickens across production systems in Iringa. Prevalence of resistance was highest to the two antimicrobial drugs commonly used in chickens. Additionally, prior antimicrobial usage was identified as a significant risk factor for multi-drug resistant *E. coli*. This suggests current antimicrobial drug usage may be driving the selection of resistance. These findings highlight the importance of improving antimicrobial stewardship and continuing AMR surveillance in these farming communities to combat the potential spread of resistance to antimicrobial drugs.

ACKNOWLEDGMENTS

Financial support for this project was provided by the UC Davis School of Veterinary Medicine Students Training in Advanced Research program, the UC Davis Office for Global Programs, and the Foundation for Food and Agriculture Research.

The authors would like to thank the Tanzania Veterinary Laboratory Agency, Iringa Municipal Council Veterinary Investigation Center, the Health for Animals and Livelihood Improvement Project, and the Foundation for Food and Agriculture Research.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

REFERENCES

Akpan MR, Isemin NU, Udoh AE, and Ashiru-Oredope D. Implementation of antimicrobial stewardship programmes in African countries: a systematic literature review. *Journal of Global Antimicrobial Resistance* 22: 317-324, 2020.

CLSI. Performance Standards for Antimicrobial Disk Susceptibility Tests. In: 13th ed. CLSI Standard M02. Clinical and Laboratory Standards Institute, Wayne, PA, 2018a.

CLSI. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals, 5th edition. In: CLSI standard VET01. Clinical and Laboratory Standards Institute, Wayne, PA, 2018b.

CLSI. Performance Standards for Antimicrobial Susceptibility Testing, 30th ed. In: CLSI supplement M100. Clinical and Laboratory Standards Institute, Wayne, PA, 2020.

FAO. FAO action plan on AMR in food and agriculture. Retrieved 23 July 2020 from http://www.fao.org/antimicrobial-resistance/background/what-is-it/en, 2016.

Frumence G, Mboera LEG, Sindato C, Katale BZ, Kimera S, Metta E, Durrance-Bagale A, Jung AS, Mshana SE, Clark TG, Rweyemamu M, Legido-Quigley H, and Matee MIN. The governance and implementation of the National Action Plan on Antimicrobial Resistance in Tanzania: a qualitative study. *Antibiotics* 10(3):273, 2021.

Katakweba AA, Muhairwa AP, Lupindu AM, Damborg P, Rosenkrantz JT, Minga UM, Mtambo MM, and Olsen JE. First report on a randomized investigation of antimicrobial resistance in fecal indicator bacteria from livestock, poultry, and humans in Tanzania. *Microbial Drug Resistance* 24(3): 260-286, 2018.

Kimera ZI, Frumence G, Mboera LEG, Rweyemamu M, Mshana SE, and Matee MIN. Assessment of drivers of antimicrobial use and resistance in poultry and domestic pig farming in the Msimbazi River Basin in Tanzania. *Antibiotics* 9(12): 838, 2020.

Lei T, Tian W, He L, Huang XH, Sun YX, Deng YT, Sun Y, Lv DH, Wu CM, Huang LZ, Shen JZ, and Liu JH. Antimicrobial resistance in *Escherichia coli* isolates from food animals, animal food products and companion animals in China. *Veterinary Microbiology* 146(1-2): 85-89, 2010.

Mdegela RH, Mwakapeje ER, Rubegwa B, Gebeyehu DT, Niyigena S, Msambichaka V, Nonga HE, Antoine-Moussiaux N, and Fasina FO. Antimicrobial use, residues, resistance and governance in the food and agriculture sectors, Tanzania. *Antibiotics* 10(4): 454, 2021.

OIE. The OIE strategy on antimicrobial resistance and the prudent use of antimicrobials. World Organisation for Animal Health. Retrieved 23 July 2020 from https://www.oie.int/en/for-the-media/amr/international-collaboration, 2016.

Rugumisa BT, Call DR, Mwanyika GO, Mrutu RI, Luanda CM, Lyimo BM, Subiah M, and Buza JJ. Prevalence of antibiotic-resistant fecal *Escherichia coli* isolates from penned broiler and scavenging local chickens in Arusha, Tanzania. *Journal of Food Protection*, 79(8): 1424-1429, 2016.

Subbiah M, Caudell MA, Mair C, Davis MA, Matthews L, Quinlan RJ, Quinlan MB, Lyimo B, Buza J, Keyyu J, and Call DR. Antimicrobial resistant enteric bacteria are widely distributed amongst people, animals and the environment in Tanzania. *Nature Communications*, 11(1), 2020.

The United Republic of Tanzania, Ministry of Health Community Development Gender Elderly and Children. The National Action Plan on Antimicrobial Resistance 2017 - 2022. Retrieved on 23 July 2020 from https://www.flemingfund.org/wp-content/uploads/8b8fc897c422e11504c8c2ba126fac02.pdf, 2017.

WHO. Antimicrobial Resistance. Retrieved on 12 January 2019 from https://www.who.int/en/news-room/fact-sheets/detail/antimicrobial-resistance, 2018.