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Antimicrobial resistant coliforms across four poultry production systems in Arusha and Moshi, Tanzania

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Abstract

Introduction: *resistance to antimicrobials poses a threat to human and animal health. This study aimed to determine the prevalence of resistant coliforms in poultry cloacal samples collected from different poultry systems in Arusha and Moshi districts, Tanzania.* ***Methods:*** *ten administrative wards were randomly chosen in Moshi and Arusha urban districts, with a random selection of one representative farm in each ward per production system (extensive, semi-intensive, intensive, and broiler systems). Per farm, 10 chickens were sampled using cloacal swabs. Samples were tested for the presence of coliforms using MacConkey agar without or with tetracycline, ciprofloxacin, ceftazidime, and Imipenem. R software was used for data analysis.* ***Results:*** *of the 80 farms targeted, samples were collected from 79 farms representing a total of 746 samples, of which 648 (86.8%) had coliforms corresponding to 74 of the 79 sampled farms. There was no significant difference in the overall prevalence of coliforms between Moshi (86%) and Arusha districts (87%) (p=0.81). The overall proportions of resistant coliforms in Arusha and Moshi varied depending on each antimicrobial type. The prevalence of coliforms resistant to tetracycline (95%) across all farm types in both districts was higher compared to ciprofloxacin (72%), imipenem (71%), and ceftazidime (84%) (p<0.0001). The median counts of coliform resistance (in log cfu) ranged from 4 to 10, with no significant distinctions between antimicrobial types.* ***Conclusion:*** *there is a widespread presence of antimicrobial resistant coliforms in poultry production systems. High tetracycline resistance was observed across all farm types in both districts.*

Introduction

Antimicrobial resistance (AMR) is regarded as one of the major public health problems of the 21st century, causing more than 700,000 deaths worldwide each year [1]. According to Centers for Disease Control (CDC), more than 2.8 million antimicrobial-resistant infections occur in the United States per year, resulting in more than 35,000 deaths [2]. There is strong evidence of acquisition of AMR through food, between animals and humans, although the directionality has not been clearly established [3]. Food animals are important reservoirs of antimicrobial-resistant bacteria due to regular use of antimicrobials in animal production. There are numerous ecological niches in the food production chain, including many bacteria that coexist and undergo selection pressures continuously [4].

These bacteria can be transmitted directly or indirectly to humans through food consumption, contact with colonised, infected animals, animal products, or excreta such as urine, faeces, and blood [5]. Selective pressure due to consistent use of antimicrobials in animal production may lead to the acquisition of AMR genes in commensals through horizontal or vertical gene transfer [6]. Poultry, particularly chickens, are the world's most common and numerous species of livestock [7,8]. Indigenous chickens are widely distributed in rural and peri- urban areas, where they play an important role in income generation, food production, and social interactions [8-10]. In Tanzania, chickens are reared under various production systems, including scavenging (free-range and semi-intensive systems) and intensive systems that constitute indigenous breeds and broilers that are imported from other countries for consumption as meat [11]. The widespread use of antimicrobials in poultry farming to increase production and control diseases in chickens, particularly in semi-intensive, intensive and broiler systems, may select for antimicrobial resistant commensal organisms in chickens. Faecal coliforms are often considered as good indicator for selective pressure imposed by antimicrobial use and widespread in the chicken intestine [12]. Few reports exist on AMR in poultry production in East Africa [13]. To date, there has been no study in Tanzania that has investigated the prevalence of AMR in coliforms across all four poultry production systems. Existing research in Tanzania has either investigated the prevalence of resistant bacteria in

a single type of poultry farm [14], such as, extensive poultry systems, or compared between two poultry systems such as broiler and extensive systems [15]. Comparison of resistance patterns and prevalence across multiple regions and production systems is pertinent to gaining a deeper understanding of whether geographical differences and intensification of poultry production may impact AMR patterns and prevalence. The aim of this study was therefore to determine the prevalence of AMR in coliforms isolated from chickens in four distinct farm types; extensive, semi-intensive, intensive and broiler farm types.

Methods

Study design: a prospective cross-sectional study was designed to determine whether different poultry husbandry practices on different farm types are linked to varying degrees of antibiotic resistance in poultry populations at a given point in time between September 2016 and December 2017. The target number of samples was 800, consisting of 10 cloacal swabs per farm type (n = 4), per ward (n = 10) and per district (n = 2), 746 were successfully collected".

Study location and farm types: the study was conducted in urban Arusha and Moshi districts, northern Tanzania, and involved four production farm type systems: extensive, semi-intensive, intensive, and broiler. The farm types were categorized based on the degree of confinement of the chickens; supplementation of feed; use of veterinary services; labour; flock size and the number of poultry houses. Extensive systems had a flock size ranging from 5 to 50 indigenous birds kept under free range conditions and obtained food through scavenging around the homestead. These systems involved little input in terms of time management, provision of water, feeding, housing, and disease control. The semi-intensive systems had 50 to 200 indigenous chickens enclosed in a facility but at some point, during the day the birds were released to scavenge. Commercial supplements were regularly incorporated into supplementary feed such as kitchen waste. Veterinary services were provided when necessary. Intensive systems were characterized as high-input urban and peri-urban commercial with 50 to 1000 birds reared for meat and egg production. The chickens were confined full time in constructed facilities and fed on feed and foundation stock from large-scale commercial poultry farms. The system involved full-time labour and the use of veterinary services for the prevention and management of the diseases. The broiler system focused on meat production by use of imported broiler breeds with flock sizes exceeding 200. The birds were confined full time in highly intensified units and involved the use of commercial feeds, supplements, and veterinary services."

Selection of wards, farms and chicken: we selected 18 wards from Arusha and 12 wards from Moshi containing all four production systems in a list of 25 and 21 administrative wards in Arusha and Moshi, respectively. Ten wards for each district were then randomly selected from these subsets. The selection was done by writing the name of each of the 18 wards in Arusha and 12 wards in Moshi on pieces of paper and folding to avoid disclosure and prevent bias during selection. Separately for Moshi and Arusha, the pieces of paper were randomized by tossing in a bowl. Then, five individuals each picked a piece of paper from the bowl without replacement. This procedure was done independently for Moshi and Arusha and repeated to generate a final list of 10 wards for each district. Four production systems were also randomly selected from a list of other similar farms within individual wards in a manner identical to the above-mentioned random selection process in each of the 10 wards. One farm was selected in each ward (randomly or purposively) per production system, followed by a convenience sampling of 10 birds in each farm. Random selection was carried out if a specific production system had more than 10 farms in a given ward. Each sampling day involved visiting one ward and sampling chickens through all four production systems. The selection of chicken in non-intensive production systems (i.e. extensive

and semi-intensive) was carried out without taking into account the age of the chicken, whereas in intensive production systems (i.e. intensive and broiler systems) the selection was based on how the chicken were sorted in their cages by age. The majority of the farms separated chicken with an age gap of two weeks into different cages. In multi-cage farms, we collected samples randomly from all the cages. For example, if a poultry farm belonging to a given system had three cages, three chickens would be picked out of each cage (making 9 samples) and the 10th chicken would be randomly selected from either cage.

Sample collection: of the 80 farms targeted for sample collection, only 79 farms were sampled. Consequently, 746 samples were collected. Cloacal swabs were collected using Amies transport media swabs (MML Diagnostics, Troutdale, OR) by gently swabbing the chicken cloaca mucosal wall. The swabs were transported to the Kilimanjaro Clinical Research Institute (KCRI) in ice-packed cool boxes and stored at -80 °C in a 1000ul mixture of 85% Brain Heart Infusion broth (Thermo Fisher Scientific Inc., Ottawa, ON, Canada) and 15% glycerol.

Isolation and enumeration of coliforms: the cloacal swabs were thawed at 2°C overnight, homogenized, and 50ul mixed with 450ul of maximum recover diluent (MRD) (Oxoid Thermo Fisher, Basingstoke, UK). The mixture was vortexed and 50ul plated using a spiral plater (Spiral System, Inc. Cincinnati, Ohio) on basic MacConkey agar and MacConkey agar supplemented with antimicrobials (tetracycline 16ug/ml, ciprofloxacin 4ug/ml, ceftazidime 8ug/ml, imipenem 4ug/ml), incubated for 24 hours at 37±3°C. Pink lactose fermenting colonies were isolated. Coliforms that grew on MacConkey agar with antimicrobials were considered resistant. Enumeration of coliforms was done using the spiral plater grid method on plain MacConkey agar and MacConkey with antimicrobials. A grid was placed on each plate, positioned on a level surface, and adjusted for the centre of each grid to match that of the plate on the viewer. The grid was divided into segments in which colonies were enumerated from the outer edge of the segment toward the centre allowing for an estimation of the corresponding microbial concentration (bacterial count/ml) on the entire plate according to standardised KCRI protocol.

Statistical analysis: data analysis was conducted using R (version 3.6.1). Chi-square test was used to determine any association between the presence of coliforms and district, farm type, and antimicrobial agents (tetracycline, ciprofloxacin, imipenem, and ceftazidime). The coefficient of determination (R2) was used to assess the strength of the relationship between counts of coliforms resistant to different pairs of antimicrobials. Kruskal-Wallis test was used to compare distributions of coliform counts between groups (farm type, district and antimicrobial type) and Mann-Whitney U test to compare medians.".

Results

Prevalence of coliforms: coliforms were present in 648 (86.8%) out of 746 samples collected (Table 1). There was no significant difference in the overall prevalence of coliforms between Moshi (86.4%) and Arusha districts (87.3%) ($p=0.81$). There was a difference between farm types in the prevalence of coliforms within the Arusha district ($p<0.001$) and within the Moshi district ($p<0.01$), but no consistent pattern in the prevalence across the farm types could be observed in either district. However, combining data across districts and across non-broiler farm types, showed that broiler farms had a significantly higher prevalence of coliforms (95.8%) than the other farm types combined (83.8%) ($p<0.0001$). Between districts, the extensive farm types showed the greatest difference (of almost 11.5%), but this difference was not statistically significant ($p=0.058$). Resistance to each of the four antimicrobial types was detected in every extensive farm, broiler farm, and in 18 of 19 semi-intensive and intensive farms. There was no consistently higher prevalence of resistance in either district. Similarly, there was no consistent increase or decrease in the prevalence of resistant coliforms with the intensification of farm

types (Table 2). The prevalence of tetracycline resistant coliforms across all farm types (95.0%) was higher compared to ciprofloxacin (71.5%), imipenem (70.81%), and ceftazidime (83.93%) ($p<0.0001$). The overall proportions of resistant coliforms in Arusha and Moshi varied depending on each antimicrobial type. In Arusha, there was a significant difference in the proportion of resistant coliforms between farm types for ciprofloxacin and imipenem ($p<0.001$) and ciprofloxacin, imipenem, and ceftazidime ($p<0.01$) in Moshi. There was evidence of interaction between farm type and district with the prevalence of ciprofloxacin-resistant coliforms; in Arusha, we noted a decline in the prevalence of resistant coliforms with the intensification of poultry production (with the exception of broiler farms) and an increase in prevalence with the intensification of poultry production in Moshi. The prevalence of imipenem-resistant coliforms in Moshi was higher, although only significant variation was noted in semi-intensive farms ($p<0.001$) while in both districts, no effect was observed on the prevalence of tetracycline, ciprofloxacin, and ceftazidime resistant coliforms ($p>0.05$).

The median counts for tetracycline, imipenem, and ceftazidime resistant colonies were similar across the antimicrobial agents in the semi-intensive, intensive, and broiler farm types, while median resistance in the extensive system was lower in this group of antimicrobials. Median counts for ciprofloxacin resistant colonies were found to be higher in semi-intensive compared to extensive, intensive, and broiler farmers (Figure 1). The median counts of coliform resistant antimicrobials (in log (cfu) ranged from 4 to 10, with no significant distinctions between antimicrobial agents. This pattern was also noted for individual antimicrobial types. Generally, there was a significant difference between districts for tetracycline, imipenem, and ceftazidime ($p<0.001$) and also in the distribution of coliform across all the four farm types ($p<0.001$). The distribution of total and resistant coliform counts was mostly bimodal and trimodal. In Arusha, for instance, bimodal distribution of coliform counts was observed in total coliforms and across all antimicrobial groups (i.e., tetracycline, ciprofloxacin, imipenem and ceftazidime resistant coliforms) in broiler farm types whereas in Moshi the distribution was predominantly trimodal across antimicrobial groups except for ciprofloxacin (Figure 2). Figure 3 illustrates the relationship between coliform counts (data transformed using log (cfu+1)) resistant to the four antimicrobial types. Variation was noted in the strength of relationship. Only 29% of the variation in coliform counts resistant to ciprofloxacin could be attributed to the change in the imipenem coliform counts (a weak association), whereas 55% of the variation in coliform counts resistant to ceftazidime could be explained by a change in the count of tetracycline resistant colonies (a strong association).

Discussion

The present study demonstrates that chickens are reservoirs of antimicrobial resistant coliforms. Resistance to at least one of the four antimicrobials was observed in each farm. These findings reflect observations documented in other studies supporting the existence of antimicrobial resistant bacteria in poultry [14-16]. Furthermore, as an ultimate selective pressure for resistance, the use of antimicrobials in this region has been reported to be quite common among different animal keepers including poultry farmers of different ethnic groups [17,18]. Intensification of poultry farms did not significantly affect the prevalence of resistance. We noted there was no increase or decrease of resistant coliform with intensification of poultry farms. These findings undermine the assumption that intensification of poultry production increases the likelihood of higher prevalence. This is contrary to the inference made by a previous study in this setting, in which a higher prevalence was observed in intensive systems compared to extensive systems, although comparison was only conducted between two types of farms; commercial layer and free range [15]. The latter observation is corroborated

by similar studies done in other parts of the world including Italy and Spain [19-23]. However, in concurrence to our findings, Obeng and colleagues in a similar study showed that there was no significant difference of resistant isolates between free range (extensive) and commercial chickens (intensive) [24].

Antimicrobials, in particular tetracycline, have been used in both districts for therapeutic and non-therapeutic purposes including prophylaxis and growth promotion [25]. Resistance to tetracycline was significantly higher in both districts for all farm types. This was anticipated as resistance to tetracycline in animals was found to be quite common in previous studies in the northern zone of Tanzania [11-18] and other parts of the world such as Egypt, the United States, Portugal, and Norway [16-29]. Extensive usage of tetracycline could be linked to its accessibility, low price, wide spectrum, and long shelf life. These factors, combined with the propensity of tetracycline resistance genes to co-select with other types of resistance genes, function to promote widespread tetracycline resistance [30,31].

Although the prevalence of resistance to other antimicrobials such as imipenem, ciprofloxacin, and 3rd generation cephalosporin was present, it was significantly lower than resistance to tetracycline. Resistance varied between farm types, and this may have been associated with the differential use of antimicrobials within those farm types. Resistance to imipenem and ceftazidime was not expected, as these antibiotics are not commonly used in poultry production [11]. Currently, there is no information on imipenem usage in poultry production documented in these districts to help illustrate the cause of this resistance. Nonetheless, this remains an area of concern to explore other potential driving factors besides antimicrobial use. Conversely, cephalosporin resistance reservoirs have been identified in water sources including tap and open water sources that are widely used in poultry production within these districts [32].

Our study found a strong association in a number of coliforms that were resistant to specific antimicrobial types; tetracycline versus that of ceftazidime; tetracycline versus imipenem; imipenem versus ceftazidime. Although such findings do not support the existence of numerous forms of resistance in a single isolate, a strong association suggests the likelihood of co-selective pressure between different antimicrobials. This potential is well known for tetracycline, often aided by co-transfer of tetracycline resistance genes along with genes responsible for conferring resistance to other forms of antimicrobials in the same genetic elements [30-33]. Tetracycline has been found present in large plasmids with several resistance genes in other studies, proposing that tetracycline has a high potential for co-selection with other genes.

Bimodal or trimodal distribution of bacterial populations have been linked to the presence of diverse resistance mechanisms [34,35]. In the present study, the distribution of coliform counts differed significantly between samples, and bimodal and trimodal distributions were observed [36,37]. Microbes can develop novel mechanisms when exposed to sub-therapeutic levels of antimicrobials, leading to lethal selection. Under sub-therapeutic conditions, microorganisms may accumulate multiple resistance mutations in a step-by-step process, contributing to heterogeneity in bacterial populations and the evolution of resistance with minimal effects [38]. Alternatively, in some bacterial populations, bimodal distributions may be prompted by phenotypic switching (often slow growth). In the event of ecological or antimicrobial resistance stress, it is presumed that there is a subset of bacterial species that defaults to slow growth, dividing bacterial populations into fast and slow growers. These slow-growing cells are referred to as persister cells and are thought to be capable of maintaining this phenotype for a long period of time [39-41]. Genetically, similar or different bacterial species may therefore respond heterogeneously to antimicrobial treatment, producing multimodal

distributions [39-41]. The major limitation in this study was that some farms had fewer than 10 chickens, and therefore, future research may consider using a large sample size.

Conclusion

There is a widespread presence of resistant coliforms in poultry production systems in the two districts in Northern Tanzania. In both Moshi and Arusha, high resistance to tetracycline was observed across the farm types. Additionally, no consistent antimicrobial resistance pattern changed with the gradation of the farm types. Additionally, no consistent antimicrobial resistance pattern changed with the gradation of the farm types. Considering imipenem resistance in poultry production systems, prospective studies need to examine all factors influencing resistance in the one health continuum. In order to reduce the development of antimicrobial resistant bacteria in animal production, poultry keepers should receive adequate antimicrobial resistance stewardship training in order to promote good practices and reduce antimicrobial overuse in poultry farms. Furthermore, continuous surveillance of antimicrobial resistance patterns in poultry should be conducted to detect emergent resistance patterns, especially those of last resort significance, such as third-generation cephalosporins and imipenem, which could have major implications for human medicine.

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What is known about this topic

- *Food animals are important reservoirs of antimicrobial-resistant bacteria due to regular use of antimicrobials in animal production;*
- *Indiscriminate use of antimicrobials in animal husbandry leads to increased prevalence of antimicrobial resistance."*

What this study adds

- *A high prevalence of tetracycline resistance was found in all four poultry productions;*
- *Resistance to antimicrobial agents was found in all four poultry production systems;*
- *There was no consistent increase or decrease in prevalence of antimicrobial resistant coliforms with intensification of farm types.*

Competing interests

The authors declare no competing interests.

Authors' contributions

RM, ES, GS and BTM were all involved in the project's conceptualization and design. RM and ES did sample collection, performed the laboratory experiments and analyzed the data. RM, VM and BM prepared the manuscript. All the authors read and approved the final manuscript.

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Tables and figures

Table 1: cloacal swab samples positive for coliforms (%) across four farm types in Arusha and Moshi districts

Table 2: distribution of antimicrobial resistant coliforms by antimicrobial and farm type in Arusha and Moshi districts

Figure 1: distribution of antimicrobial resistant coliforms by antimicrobial type within farm types across the two districts

Figure 2: density plots for the total and resistant coliform counts (in log cfu) in cloacal swabs across the four antimicrobials types

Figure 3: pair wise relationships between coliform counts (data transformed using log (cfu+1) resistant to the four antimicrobial types

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Table 1: cloacal swab samples positive for coliforms (%) across four farm types in Arusha and Moshi districts

District	Farm type typetypes	Number of samples		
		Coliforms absent	Coliforms present	Total
Arusha	Extensive	19 (22.6)	65 (77.4)	84
	Semi int.	90	78 (87.6)	89
	Intensive	90	74 (82.2)	
	Broiler	2 (2.2)	88 (97.8)	
	Total	48 (13.6)	305 (86.4)	353
Moshi	Extensive	11 (11.1)	88 (88.9)	99
	Semi int.	12 (12.1)	87 (87.9)	99
	Intensive	21 (21.9)	75 (78.1)	96
	Broiler	6 (6.1)	93 (93.9)	99
	Total	50 (12.7)	343 (87.3)	393
Combined	Extensive	30 (16.4)	153 (83.6)	183
	Semi Int.	23 (12.2)	165 (87.8)	188
	Intensive	37 (19.9)	149 (80.1)	186
	Broiler	8 (4.23)	181 (95.8)	189
	Total	98 (13.1)	648 (86.9)	746

Table 2: distribution of antimicrobial resistant coliforms by antimicrobial and farm type in Arusha and Moshi districts

Antimicrobial	District/farm type (%)							
	Arusha				Moshi			
	Exten (n=65)	Semi-Int (n=78)	Intens (n=74)	Broiler (n=88)	Exten (n=88)	Semi-Int. (n=87)	Intens. (n=75)	Broiler (n=93)
Tetracycline								
Resistant	59 (90.8)	73 (93.6)	72 (97.3)	87 (98.9)	82 (93.2)	84 (96.6)	69 (92.0)	90 (96.8)
Susceptible	6 (9.2)	5 (6.4)	2 (2.7)	1 (1.1)	6 (6.8)	3 (3.4)	6 (8.0)	3 (3.2)
Ciprofloxacin								
Resistant	40 (61.5)	38 (48.7)	55 (74.3)	80 (90.9)	41 (46.6)	75 (86.2)	53 (70.7)	82 (88.2)
Susceptible	25 (38.5)	40 (51.3)	19 (25.6)	8 (9.1)	47 (53.4)	12 (13.8)	22 (29.3)	11 (11.8)
Imipenem								
Resistant	39 (60.0)	54 (69.2)	63 (85.1)	60 (68.2)	39 (60.0)	54 (69.2)	63 (85.1)	60 (68.2)
Susceptible	26 (40.0)	24 (30.7)	11 (14.9)	28 (31.8)	26 (40.0)	24 (30.7)	11 (14.9)	28 (31.8)
Ceftazidime								
Resistant	50 (76.9)	63 (80.8)	65 (87.8)	78 (88.6)	50 (76.9)	63 (80.8)	65 (87.8)	78 (88.6)
Susceptible	15 (23.1)	15 (19.2)	9 (12.2)	10 (11.4)	15 (23.1)	15 (19.2)	9 (12.2)	10 (11.4)

Abbreviations: exten: extension; semi-int: semi-intensive; intens: intensive

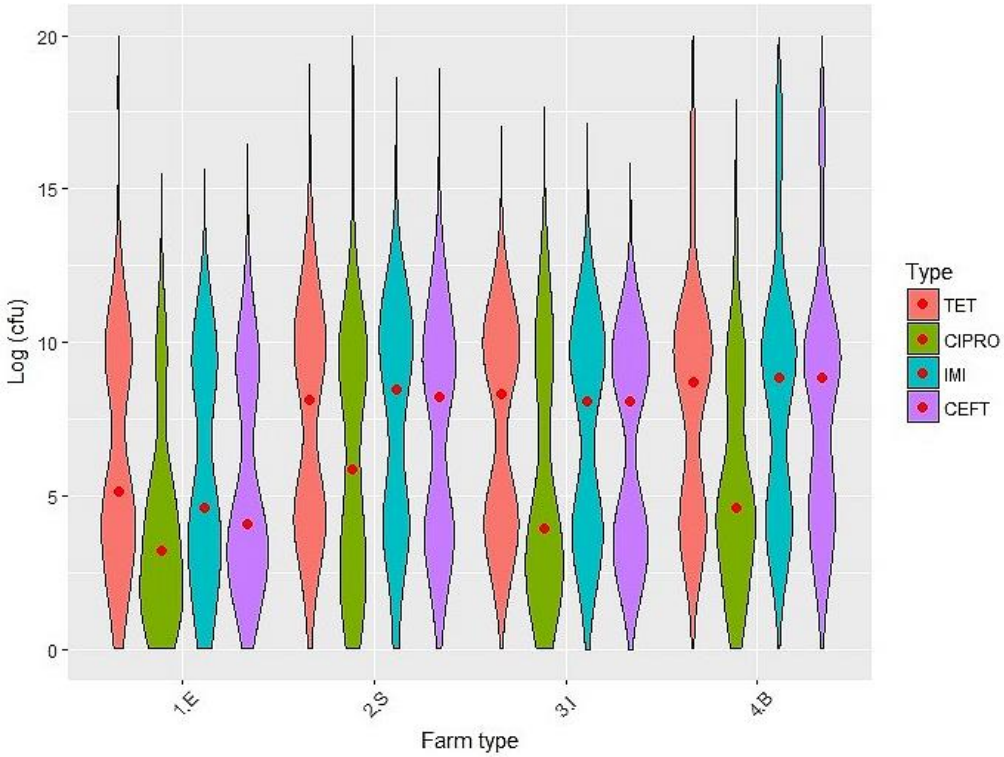

Figure 1: distribution of antimicrobial resistant coliforms by antimicrobial type within farm types across the two districts

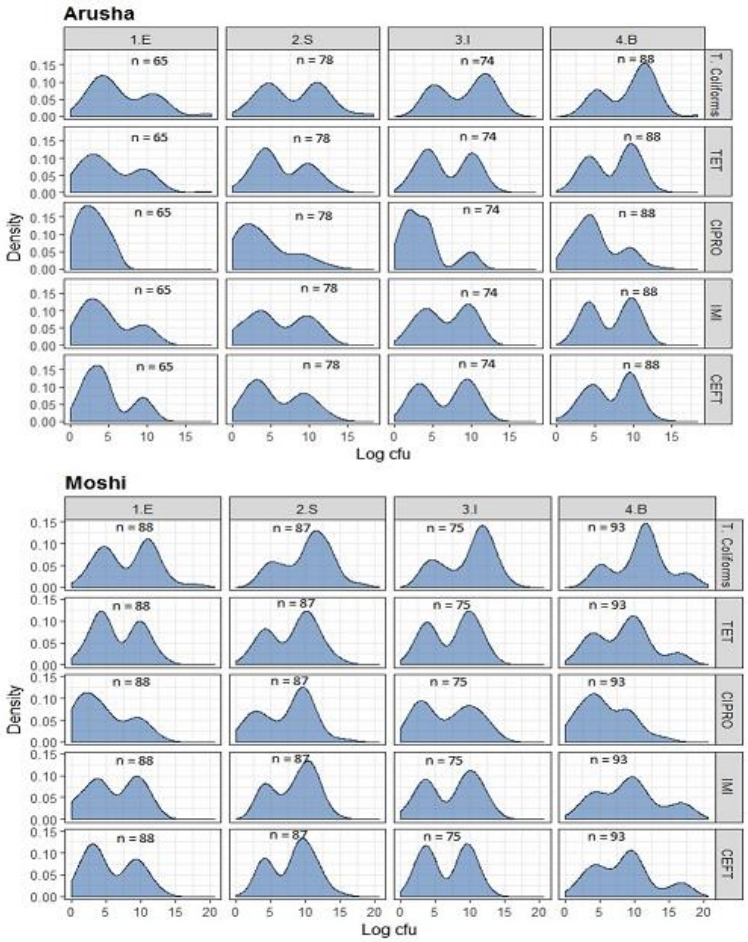

Figure 2: density plots for the total and resistant coliform counts (in log cfu) in cloacal swabs across the four antimicrobials types

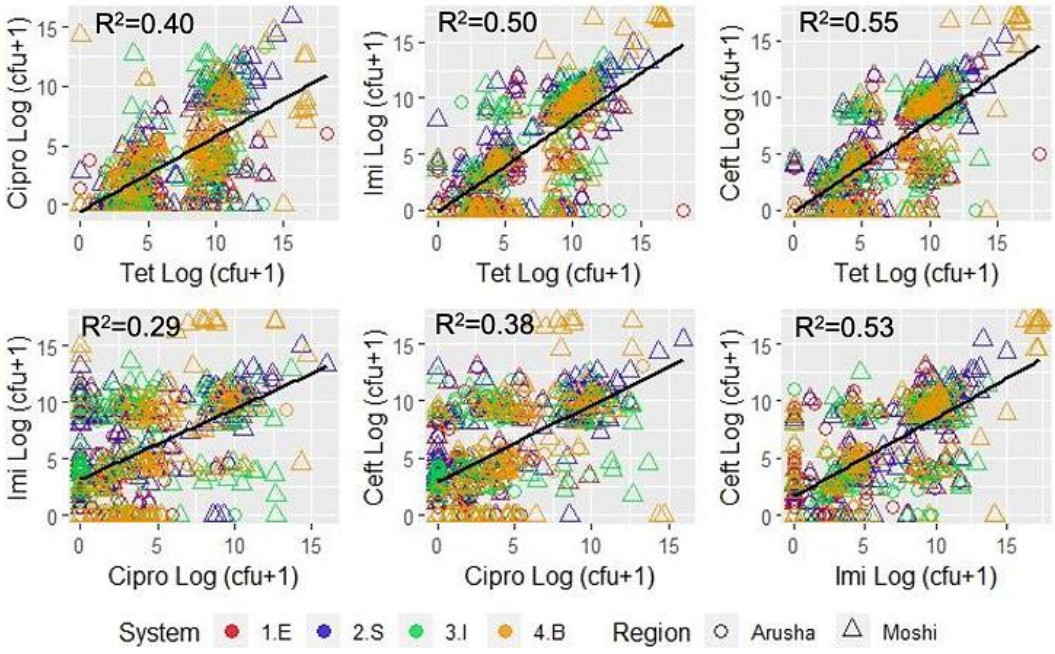

Figure 3: pair wise relationships between coliform counts (data transformed using log (cfu+1) resistant to the four antimicrobial types