

Article

Antimicrobial Use, Residues, Resistance and Governance in the food and agriculture sectors, Tanzania

Robinson H. Mdegela¹, Elibariki R. Mwakapeje^{2*}, Bachana Rubegwa^{2†}, Daniel T. Gebeyehu^{3,4}, Solange Niyigena³, Victoria Msambichaka², Hezron E. Nonga⁵, Nicolas Antoine-Moussiaux^{3*}, Folorunso O. Fasina^{2,6*}

- ¹ Department of Veterinary Medicine and Public Health, Sokoine University of Agriculture, Morogoro, United Republic of Tanzania; rmdegela2012@gmail.com.
 - ² Emergency Centre for Transboundary Animal Diseases (ECTAD), Food and Agriculture Organization of the United Nations (FAO), Dar es Salaam, United Republic of Tanzania; elibariki.mwakapeje@fao.org; BRu-begwa@path.org; msambichakavictoria@yahoo.com; Folorunso.fasina@fao.org.
 - ³ Faculty of Veterinary Medicine, University of Liège, Belgium; dteshome11@gmail.com; niso20052000@yahoofr; nantoine@uliege.be.
 - ⁴ Department of Veterinary Medicine, School of Veterinary Medicine, Wollo University, Dessie, Ethiopia
 - ⁵ Directorate of Veterinary Services, Ministry of Livestock and Fisheries, Dodoma, United Republic of Tanzania; nongahezron@yahoo.co.uk.
 - ⁶ Department of Veterinary Tropical Diseases, University of Pretoria, South Africa; daydupe2003@yahooco.uk
- [†] Current address: PATH - Infectious Disease Detection and Surveillance (IDDS), Tanzania
- * Correspondence: folorunso.fasina@fao.org; Tel.: (+255686132852, FOF); elibariki.mwakapeje@fao.org; Tel.: (255784342389, ERM); nantoine@uliege.be; Tel.: (+32495282914, NA-M).

Abstract: All infections are potentially curable as long as the etiological agents are susceptible to antimicrobials. The increased rate at which antimicrobials are becoming ineffective is a global health risk of increasing concern that threatens withdrawal of beneficial antimicrobials for disease control. Increased demand for food of animal origin, in particular eggs, meat and milk has led to intensification and commercial production systems where excessive use and misuse of antimicrobials may prevail. Antimicrobials, handled and used by farmers and animal attendants with no formal education may predispose to incorrect dosages, misuse, incorrect applications and non-adherence to withdrawal periods. A multimethod approach (desk review, field study and interviews) was used. Relevant establishments were also visited. High levels of resistance to penicillin G, chloramphenicol, streptomycin and oxytetracycline have been reported especially for *Actinobacter pyogenes*, *Staphylococcus hyicus*, *Staphylococcus intermedius* and *Staphylococcus aureus* from dairy cattle with mastitis and in humans. Similar trends were found in poultry where eggs and meat are contaminated with *Escherichia coli* strains resistant to amoxicillin + clavulanate, sulphamethoxazole and neomycin. An increasing trend of emerging multidrug resistant *E. coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Salmonella* was also found in food animals. An increase in methicillin resistant *Staphylococcus aureus* (MRSA) and extended-spectrum beta-lactamase (ESBL) in the livestock sector in Tanzania have been reported. Specific antimicrobials resistant to were ampicillin, augmentin, gentamicin, cotrimoxazole, tetracycline, amoxicillin, ampicillin, gentamicin, streptomycin, erythromycin, tetracycline, ciprofloxacin, nalidixic acid, azithromycin, chloramphenicol, tylosin, erythromycin, cefuroxime, norfloxacin and ciprofloxacin. An increased usage of antimicrobials for prophylaxis, anaphylaxis and therapeutics against pathogens and for growth promotion in livestock, aquaculture and crops production were observed. One Health strategic approach is advocated to combat AMR in the food and agriculture sectors in Tanzania. Practical recommendations include a) legislation review and implementation, b) AMU, AMR and AR awareness and advocacy among stakeholders along the value chain, c) strengthening of surveillance and monitoring programs for AMU, AMR and AR, d) enhance development and use of rapid and innovative diagnostic tests and promotion biosecurity principles and e) good husbandry practices. The utilization of this information to improve public health policies and reduce the burden of AMR will be beneficial.

Keywords: Antimicrobial use, antimicrobial residues, antimicrobial resistance, food and agriculture sectors, Tanzania.

Abbreviations: AMR = Antimicrobial resistance; AMU = Antimicrobial use; AR = Antimicrobial residue; FDG = focus group discussions; GARP-TWG = Global Antibiotic Resistance Partnership—Tanzania Technical Working Group; GDP = Gross domestic product; KII = key informant interview; MoLF = Ministry of Livestock and Fisheries; TLMI = Tanzania Livestock Modernization Initiative; TLMP = Tanzania Livestock Master Plan; TFDA = Tanzania Food and Drugs Authority, now referred to as the Tanzania Medicines & Medical Devices Authority (TMDA); VCT = Veterinary Council of Tanzania; ZIS = Zoosanitary Inspectorate Services

Contact the editorial office of the journal or support@mdpi.com.

1. Introduction

Agriculture is Tanzania's economy mainstay that by 2014 it was contributing nearly to 30% of the country's GDP and 67% of total employment. Tanzania has the third largest livestock population on the African continent. The 2012/13 National Panel survey revealed that 50% of households keep livestock (4.6 million households), 62% of which are rural and 23% urban based. The ownership patterns in terms of dominance put chickens as the highest (86% of the households), goats (48%), cattle (35%), pigs (9%) and other livestock (10%). Meat production in Tanzania is at about 563,086t, as indicated in the 2014–2015 Annual Report of the Tanzania's Ministry of Agriculture, and Food Security [1].

In 2015, Tanzania's Ministry of Livestock and Fisheries launched the Tanzania Livestock Modernization Initiative (TLMI) and recently, in 2019, the Tanzania Livestock Master Plan (TLMP) was launched to transform the traditional livestock sub-sector into a modern, responsive, sustainable and environmentally-friendly engine for rural development.

However, livestock diseases hinder the development of this sector and expose producers to high livelihood risks and uncertainties [2]. The intensification of livestock farming, large number of wildlife and close interaction of human and animal aggravate the circulation of zoonotic and infectious diseases in animal and human population and equally affects the food chain in Tanzania [3]. In addition to emerging and zoonotic diseases challenge, more intensification and mass production of animals also expels the poor rural farmers from the market [4].

Antimicrobials are agents that kill microorganisms or suppresses their multiplication or growth [5], and the failure of antimicrobial's effect against the growth and multiplication of microorganism is called antimicrobial resistance (AMR). AMR happens when microorganisms (such as bacteria, fungi, viruses, and parasites) change the typical response patterns when they are exposed to antimicrobials (such as antibiotics, antifungals, antivirals, antimalarial, and anthelmintic) [6].

Although different factors may aggravate AMR, over-use and misuse of antimicrobials in food and agriculture production play a major role in the emergence and spread of AMR. Indeed, AMR emergence and diffusion correspond to a selective process which allows bacterial populations to adapt to their environment. Hence, AMR is inextricably tied to antimicrobial use (AMU) and will be favored when this use is sub-optimal, abused or widespread. In particular, avoidable practices that are recognized as key contributors to AMR are AMU in animal production for growth promotion, prophylaxis and metaphylaxis, AMU without professional oversight or after poor diagnostic techniques [7].

Drug-resistant bacteria can circulate in population of humans and animals, through food, water and the environment [6]. Transmission of AMR is facilitated by trade, travel and both human and animal migration. Resistant bacteria can be found in food animals and food products destined for consumption by humans [8]. Since the need for protein of

animal origin is increasing based on the increased human population, the antimicrobials use in food animals as growth promoters, for infection prevention and treatment is approximately 4 times than the use of antimicrobials in humans [6].

Farmers, particularly broiler producers utilize much antimicrobials by mixing with feed and/or water with resultant high prevalence of AMR in broiler meat compared with other animal products [9]. Using of antimicrobials in animal production sector results in AMR and drug residue in the public and animal health sectors [10]. Infections caused by antimicrobial-resistant bacteria become more difficult to treat hence patients are exposed to: longer period of stay in hospital, non-affordable and toxic last-resort drugs and sometimes unsuccessful surgical operations [11].

Aquaculture in Tanzania has a vast but yet untapped potential. The industry is dominated by freshwater fish farming in which small-scale farmers practice both extensive and semi-intensive fish farming. In Tanzania, Aquaculture was largely regarded as a part-time activity involving about 17,100 fish farmers (14,100 involved in freshwater fish farming and about 3,000 in seaweed farming). Aquaculture is one of the fast growing production sectors in Tanzania, where the current production stands at 11,000 MT per year. With lessons learned from Norway, it is feasible to increase productivity in aquaculture through vaccination programs and sound bio-security measures without the use of antimicrobial agents (Figure 1).

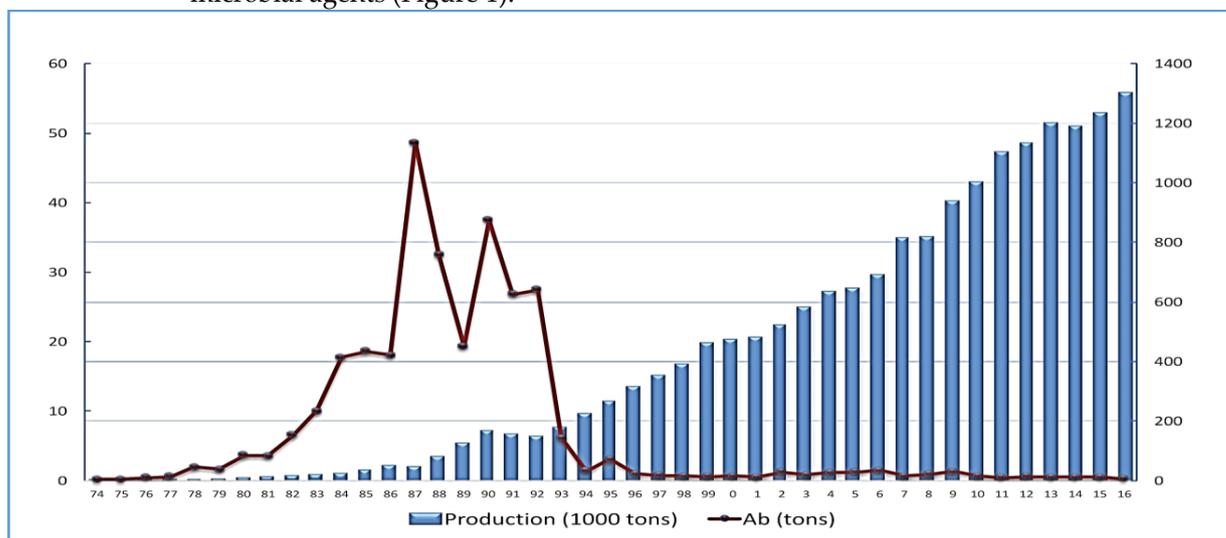


Figure 1: Use of antibiotics (line) and production volume (bars) of Atlantic salmon in Norway between 1980 and 2016 (adapted from Aly and Albutti, [12])

The Codex Alimentarius [13], has indicated that AMU governance that minimizes the development and transmission of AMR along the food chain is vital and it encourages the food control authorities to tackle AMR through the introduction of a range of standards related to AMR, veterinary drugs and their residues, food hygiene and animal feed [13]. Similarly, the tripartite collaboration of WHO, FAO and OIE have set action plans to minimize AMU along the food chain (FAO-OIE-WHO, 2010) and these have guided countries to set their own AMU governance legislations. As part of the AMU governance systems promoting good practices like proper waste management, animal husbandry, alternative health managements (vaccination and biosecurity) are strongly recommended [14]. Because the assessment of the governance of AMU along the food chain is the foundation for the determination of necessary actions for the AMR mitigation, and because previous situation analysis study in Tanzania has indicated that the use of antimicrobials in the broiler sector is high, this sector was purposively selected for this study. The target stakeholders were broiler value chain actors (feed manufacturers, farms and slaughterhouses), input suppliers (veterinary pharmacies) and antimicrobial use regulators (higher and medium level antimicrobial use regulatory institutions). The assessment focused on both the

vertical and horizontal interactions of the broiler value chain actors, input suppliers and antimicrobial use regulators.

Crop production accounts for 55% of agricultural GDP and the main export crops are sugar, coffee, cotton, tobacco, and tea. While the most common staple crops in Tanzania include maize, cassava, rice, sorghum, and millet, cash crops are maize, cassava, sweet potatoes, bananas, sorghum, and sugar cane. Multiple factors influence the farmer's choice of crops, including: i) physical factors, such as soil quality and water availability; ii) economic factors, such as marketability and seed prices; iii) personal preferences of the household; iv) crop profiles, including crop yield and pest resistance; and v) resource availability such as machinery and fertilizer. It should be noted that Tanzania developed a roadmap for mitigation of AMR with seven strategic milestones covering the situation analysis up to the implementation of national action plan on AMR and to date, progress in this regard is monitored closely and reported regularly to the governance structure of AMR in Tanzania, the Multisectoral Coordinating Committee (MCC).

The general objective of this review was to assess the governance of AMU, establish the pattern and extent of AMU, antimicrobial residues (AR), and AMR in food animals and crop agriculture value chains, and relate to strategies in place for combating the emergence of AMR in Tanzania. Specifically, the current study is meant to i) determine the extent of AMR in isolates from food animals and crop agriculture; ii) establish the status of AR in food of animal and plant origins and iii) assess the regulatory oversights related to AMU, AR and AMR in Tanzania.

2. Results

Farmers buy antimicrobial (AM) agents for treating animals without prescription of animal health experts and AM management is characterized by incorrect dosages, misuse, incorrect applications and non-adherence to withdrawal periods.

Antimicrobial agents are generally used in all domestic animals, and to a limited extent in the wildlife and aquaculture sectors. The most commonly used classes of antimicrobial agents in animals in Tanzania are Tetracycline, Sulphonamides, Penicillin, Macrolides and others including antiprotozoal agents (Table 1). During this review, it was reported that animals bought for slaughter from the auction markets were stabilized with antibiotics during trucking to destination. Animals are injected with Oxytetracycline (OTC) in order to prevent transit fever caused by *Pasteurella multocida*. After application of this drug, withdrawal periods were not observed at all.

Table 1. Imported and registered antimicrobials for use in animal health and crop production in Tanzania from 2015-2017

<i>Class of antimicrobial</i>	Type of antibiotic/ common name
<i>Tetracyclines</i>	Oxytetracycline
	Doxycycline + Colistine
	Oxytetracycline + Vitamins
	Chlortetracycline
	Tylosin + Doxycycline
	Oxytetracycline + Colistine
	Oxytetracycline Hydrochloride

<i>Penicillins</i>	Cloxacillin Ampicillin + Cloxacillin Procaine Benzyl Penicillin Penicillin G + Neomycin Penicillin G + Dihydrostreptomycin Amoxicillin trihydrate
<i>Sulfonamides and antiinfectives</i>	Diaveridine + Sulfadimidine Sulfamethaxazole + Trimethoprim Trimethoprim + Sulphadiazine Sulfamethazine + Trimethoprim
<i>Macrolides</i>	Tylosin
<i>Quinolones</i>	Norfloxacin Enrofloxacin Toltrazuri Kanamycin + Prednisolone + Procaine Benzyl Penicillin Diclazuril
<i>Antiprotozoal</i>	Amprolium HCl + Sulfaquinoxaline + Vitamin K3 Diminazine Diacetate IP + Cyanocobalamine BP + Antipyrine Diminazine Diacetate IP + Antipyrine Isomedium Homidium chloride Parvaquone Diminazine + Phenazone Imidocarb Amprolium HCl Buparvaquone Diminazine

The primary reason why farmers preferably use antimicrobials is for prevention (60%), growth promotion (26%) and treatment (14%). Farmers usually use antimicrobials (61%) and they sometimes use other diseases prevention techniques like biosecurity and vaccination (39%). The farmers confirm that the poor biosecurity system of their farm exposes the broiler to diseases. As a result they repeatedly use antimicrobials to avoid the risk of mortality. The manure originating from the broiler operations was used for land fertilization for vegetable farming.

The farmers contact the animal health professionals only if they cannot solve the problem through 'trial and error' self-medication and they don't ask professional advice regularly, rather they prefer to interact with drug seller and suppliers directly. If the farmers need to administer antimicrobials they can purchase it from the nearby veterinary or human pharmacy, drug store or clinic without prescription from the veterinary experts. All members of the farmers group agreed that they individually administer antimicrobials by mixing with feed and water in the dose range of the drug sellers' recommendation.

The farmers administered antimicrobials when it is necessary regardless of the withdrawal period, but using antimicrobial like oxytetracycline (20%) challenges their market, since the color and smell of the broiler meat resembles the drug and it is not accepted by

consumer. According to the farmers, the animal health professionals themselves were prescribing antimicrobials for prevention and growth promotion purposes. The farmers complain that expired antimicrobials and fake expired date of the antimicrobials were a huge challenge for them. It was also reported that animals bought for slaughter from the auction markets are stabilized with antibiotics during tracking to destination. Animals are injected with OTC in order to prevent transit fever caused by *Pasteurella multocida* and after application of this drug, withdrawal periods are not observed.

With growing trends of aquaculture production in Tanzania, and in the absence of vaccines and formal biosecurity principles and protocols, use of antimicrobial agents meant for poultry in the treatment of diseases in fish are increasingly been observed. Diseases and infections including broken head syndrome in African sharp tooth catfish, bacterial haemorrhagic septicaemia due to *Aeromonas hydrophila* infection, *Pseudomonas* infections, *Edwardsiella tarda* infections, as well as *Streptococcus iniae*, *S. agalactiae* and *Lactococcus* spp infections attract use of AM. Fertilization of ponds using cattle and poultry manure from treated animals, is regarded as an indirect source of antimicrobial agents and their metabolites in aquaculture [15]. No study has established extent of wash offs, run offs and leaching from animal and crop farms into aquaculture farms/ ponds and therefore were not established in this study due to dearth of information.

In aquaculture, there is a variable extent of resistance of about 70% and the resistance observed was due to environmental contamination. The origin of the pathogens is anthropogenic through human and animal faeces and urine with resistant pathogens or drugs and their residues. Prominent genes encoding resistance to tetracycline, trimethoprim, amoxicillin, streptomycin, chloramphenicol, and erythromycin were identified in integrated fish farming systems in the country.

In addition antimicrobial agents are used in feed formulations, commonly used ones include Tetracycline (Chlortetracycline and Oxytetracycline), Colistine and Neomycin. Furthermore, additional supplementation with drugs such as Neoxychick through water is also a common practice in the country. In Tanzania, the Grazing Land and Animal Feed Resources Act No.13 of 2010; and its Regulations of 2013, gives power to the Director responsible for grazing-lands utilization and animal feed resources, the "Competent Authority" to regulate and control use of feed additives. In addition, during this study, formulation of animal feeds by farmers' themselves or unregistered feed formulators was observed as a common practice. The complexity of the matter is due to the fact that, the feed additives including coccidiostats and antibiotics when used in feeds do not require prescription from Veterinarians as stated in the Tanzania Food and Drugs Authority (TFDA; now referred to as the Tanzania Medicines & Medical Devices Authority (TMDA)) guideline for Registration of Premises for Essential Veterinary drugs of 2015. Since through feeds, the coccidiostats and antibiotics, though in low concentrations, are used for longer durations, they are considered as more risky than those used in high doses for disease therapy. Over 90% of drugs and animal feeds sold and used in Zanzibar are imported from Tanzania mainland, hence effective preventive and control measures in the mainland will also have a positive impact in Zanzibar.

However, regulatory bodies in Tanzania have not yet set standards for regulate the use of growth promoters in the livestock production systems. This is compounded by lack of One Health monitoring frameworks in the rational use of antimicrobials in the human, livestock, agriculture and environmental sectors.

Table 2: The Withdrawal period (in days) for some popularly accessible antimicrobial agents found in the market in Morogoro Municipality, Tanzania, July 2017

Antimicrobial agent	Withdrawal period in days indicated in the labels and leaflets						
	Beef	Liver /Kidney	Milk	Broiler	Eggs	Pork	Cattle, Sheep & goats
Penistrep	10						
Tylosin (20%)	28		3				

Gentamycin	7	45	2	
Sulphadimidine + Diaveridine				12 12
Trimethoprim + Sulphadiazine				12 Do not administer
Oxytetracycline (50%)				7 0
Sulfachloropyramine				7 3
Doxycycline + Colistine				7 8 14
Oxytetracycline (20%) injectable	8			6
Amprolium Hydrochloride 20% with Vitamin K3				14 14
Chlotetracycline (20%)				7 9 10
Doxycycline + Tylosin				7 8 14
Sulphadimidine sodium 400 mg + Sulphaquinoxaline sodium 150 mg				7 8 14
Doxycycline				21 6 10
Flumesol Flumequine				3 8
Trimethoprim + Sulphadiazine				12 10
Sulphaclozime (30%)				
Oxytetracycline + Neomycin +Chloramphenicol				12
Amoxicillin + Colistine				7
Doxycycline + Gentamycin				14
Amprolium				3 3
Triple Sulpha		7		10 14
Oxytetracycline Hydrochloride 20%				7
Doxycycline hydrate + Colistine				7
Ammonium chloride + Magnesium sulphate + Sodium sulphate + Sorbitol				Nil
Tylosin Tartrate 20%				7
sulphamezathine (sulphadimethylpyrimidine) + sulphadiazine (sulphapyrimidine)				14
Oxytetracycline 40%				6 8 8
Trimethoprim + Sulfadiazine				12
Toltrazuril				12 9

3. Discussion

In 2010, the global consumption of antimicrobials in food animal production system was estimated at 63,151 MT and was projected to rise by 67%, to 105,596 MT by 2030 [16]. Apart from their utilization for treatment of animal diseases, worldwide, antimicrobials are also used at sub-therapeutic levels as growth promoters, a practice that contributes significantly to emergence of AMR.

A recent study in Morogoro revealed high usage of antimicrobials for therapy and animal feed additive particularly in poultry feeds. The study findings indicated that Oxytetracycline, Penicillin and Streptomycin combination (Penstrep), Tylosin and Sulphonamides were by far the most commonly used antimicrobial agents in cattle in the study area [17]. Furthermore, some of the prohibited antimicrobial agents for animal use such as furazolidone and chloramphenicol were found in some veterinary stores and in poultry farms. A significant proportion of Tanzanian poultry farmers treat their chicken by themselves [18-20].

In 2009, 90% of broiler farmers interviewed in Morogoro reported high and frequent use of Tetracycline, Amprolium, Sulphonamides, Trimethoprim, Neomycine and Flumequine in their chicken flocks and 100% of them slaughtered their broilers before the end of the withdrawal period [21]. Most of the chicken farmers involved in the study in Morogoro used antimicrobial agents for prevention and for treatment of common chicken diseases namely fowl typhoid, infectious bursa disease (Gumboro), infectious coryza, colibacillosis, coccidiosis, Newcastle disease, helminthosis and fowl pox. Antibiotics accounted for 85% of the drugs used in the farms by volume (Nonga et al., 2010). Another study by Katakweba et al. [22], reported that 40% of Tanzanian small-scale livestock keepers did not know if antimicrobial agents used in animals could pose any risk to human health.

Conventional antimicrobial agents for prophylactic treatment of bacterial diseases of plants are limited in availability, use and efficacy; and the therapeutic use is largely ineffective. Where studies have been conducted, the most applications have been by spray treatment using tetracycline and streptomycin. Use of antimicrobial agents in particular against viral diseases is recorded in horticultural crops including vegetables [19,23], and in cashew nuts. Sulphur dust is widely used for controlling powdery mildew disease caused by *Oidium anacardii* Noack. Increasingly, there is also high use of low quality water from waste water treatment plants in urban areas for horticulture. This is an important route that predispose consumers to the risk of exposure to antimicrobial agents and the associated residues [24,25].

The available data from limited studies show a number of multidrug resistant bacteria that cause mastitis in lactating cows [26]. High levels of resistance have been reported to penicillin G, chloramphenicol, streptomycin and oxytetracycline among *Actinobacter pyogenes*, *Staph. hyicus*, *Staph. intermedius* and *Staph. aureus* from cattle with mastitis. Similar resistance results to amoxicillin + clavulanate, sulphamethoxazole and neomycin have been found in poultry products contaminated with *E. coli* isolates [27]. Moreover, there is an increasing trend in the incidence of antimicrobial resistance with significant increase in multi drug resistant *E. coli*, *Klebsiella pneumoniae*, *Staph. aureus*, and *Salmonella* in food animals in Tanzania [28]. The same authors indicated an increase in methicillin resistant *Staph. aureus* (MRSA) and extended-spectrum beta-lactamase (ESBL) in the food animal sector in Tanzania. Similarly, high prevalence of antimicrobial resistant *E. coli* and *Campylobacter* spp isolates from animals have been reported in Tanzania for ampicillin, augmentin, gentamicin co-trimoxazole, tetracycline, amoxicillin, erythromycin, cefuroxime, norfloxacin and ciprofloxacin [28].

Antimicrobial resistant *Campylobacter* in important food animals in Tanzania was reported in isolates from pigs, dairy, and beef cattle with specific resistance to ampicillin, gentamicin, streptomycin, erythromycin, tetracycline, ciprofloxacin, nalidixic acid, azithromycin, chloramphenicol, and tylosin [29]. Another study reported resistance of

Campylobacter isolates from ducks in Morogoro to cefuroxime sodium, tetracycline, Ampicillin, erythromycin, gentamicin, cloxacillin and amoxicillin [21]. In 2014, other studies revealed a high number of resistant *E. coli* and Enterococci isolates from wildlife and cattle in Tanzanian wildlife ecosystems [30]. Antimicrobial resistance has also been reported in isolates from fish and aquatic environment [31].

A study by Jabbar and Grace [32], revealed that Antimicrobial residues are present at high levels in foods of animal origin in Tanzania. In support of this observation, Nonga et al. [33] reported antimicrobial residues in 76.4% of 72 broiler meat samples from Zanzibar. In a similar study in Morogoro municipality it was found that all 70 egg samples from a commercial poultry farm were positive for antimicrobial residues [19]. In another study on broiler chickens, the same researchers revealed that 70% of sampled farms had antimicrobial residues in their chicken meat [21]. In 2006, Kivaria and colleagues [34] reported antimicrobial residues in raw milk marketed by smallholder dairy producers in Dar es Salaam [34].

Moreover, Kurwijila et al. [35] reported antimicrobial residues in about 36% of milk marketed across Tanzania, suggesting an average risk of about 11 exposures per month for a daily consumer of milk. In addition, Mdegela et al. [36] reported contamination of milk with antimicrobial residues in smallholder dairy farms in Mvomero and Njombe districts. Using liquid chromatography-mass spectrometry (LC-MS), a high proportion of beef samples in Dodoma had Oxytetracycline residues [37]. Oxytetracycline residues were reported at 78% in ready-to-eat beef, of which 25.7% had violative residue levels above the maximum residue limits recommended by the FAO and the WHO [38].

Similar observations were made in studies carried out by Kimera et al. [39] who reported Oxytetracycline residues in 71.1% of muscle, liver and kidney samples from slaughtered cattle, of which 68.3% had residues above acceptable regulatory levels. Another study by Bilashoboka et al. [40] demonstrated that 53% out of 137 muscle samples from cattle had violative levels of over 0.2 mg/kg. In the same study 65% out of 20 liver samples had levels above tolerable levels of 0.6 mg/kg; and 7.1% out of 14 kidney samples had levels above tolerable levels of 1.2 mg/Kg.

A number of studies reported that farmers in Tanzania do not observe withdrawal periods as directed by the Government policies and drug manufacturers' recommendations. This is due to ignorance on associated public health risks and perceived economic losses [17,21,22,39,41]. Caudell et al., [17] reported a high use and self-administration of antimicrobials in cattle herds in Northern Tanzania. The study also revealed low adherence to withdrawal periods from consumption of meat and milk during and following antimicrobial treatment especially among the Maasai, Arusha and Chagga herders. Similar observations were reported among smallholder poultry farmers in Morogoro where 85% of them were unaware of possible side effects of antimicrobial residues in humans [19]. The risks for consumption of contaminated food of animal origin with Antimicrobial Residues (AR) was found to be due to consumption of animal products directly from the farm as well as to non-adherence to withdrawal periods.

Farmers fail to observe and adhere to withdrawal periods due to perceived economic losses. For instance, a withdrawal period of 28 days for Tylosin (20%) and 45 days for Gentamycin seems impractical (Table 2) and findings by Kimera et al. [39] and Mgonja et al. [37] support the observation. Similarly, the withdrawal periods for different drugs for eggs and broiler meat seems rather difficult to observe from economic viewpoint, an observation which is supported by findings by Nonga et al. [19].

During this study, it was also reported that animals bought for slaughter from auction markets are stabilized with antibiotics during trucking to destination. Animals are injected with OTC in order to prevent transit fever caused by *Pasteurella multocida*. After application of this drug, withdrawal periods are not observed. This might be the reason for OTC that has been detected in beef in different studies [37-39].

The Tanzania Veterinary Laboratory Agency established in 2012, is mandated to undertake diagnosis of animal diseases, regulate veterinary laboratories, conduct research on animal diseases and vectors, develop and produce vaccines and other biologicals. The

agency offers laboratory diagnostics services throughout the country through its eight diagnostic centers. In addition to TVLA, diagnostic services for animal health are also provided by SUA through her teaching and research laboratories. During this study it was found that most of microbiology laboratories are poorly supplied with equipment and reagents for microbial identification and testing for resistance.

The supply chain of veterinary medicines from the sources to the end users is done by the private sector. The Government is mainly involved on regulatory and monitoring functions through the Tanzania Medicines and Medical Devices Authority (TMDA) and the Veterinary Council of Tanzania. The supply chain of Veterinary Medicines include the source (manufacturers and importers), distributors, and end users. Antimicrobial agents are imported from Europe, mainly Belgium, Netherlands, France and Turkey; as well as Asian countries such as China, India, and Indonesia. During 2009 – 2017, the major companies importing veterinary medicines were Twiga, Chemical Industries, Bytrade (T) co. Ltd, Norbrook, Farmers Centre, Farm Base, Anicrop Services, Tan Veterina, Ultravetis, Cooper Tanzania (TFDA, 2017) and Bajuta. Once imported, they are distributed to sellers and eventually to the end users of antimicrobial agents.

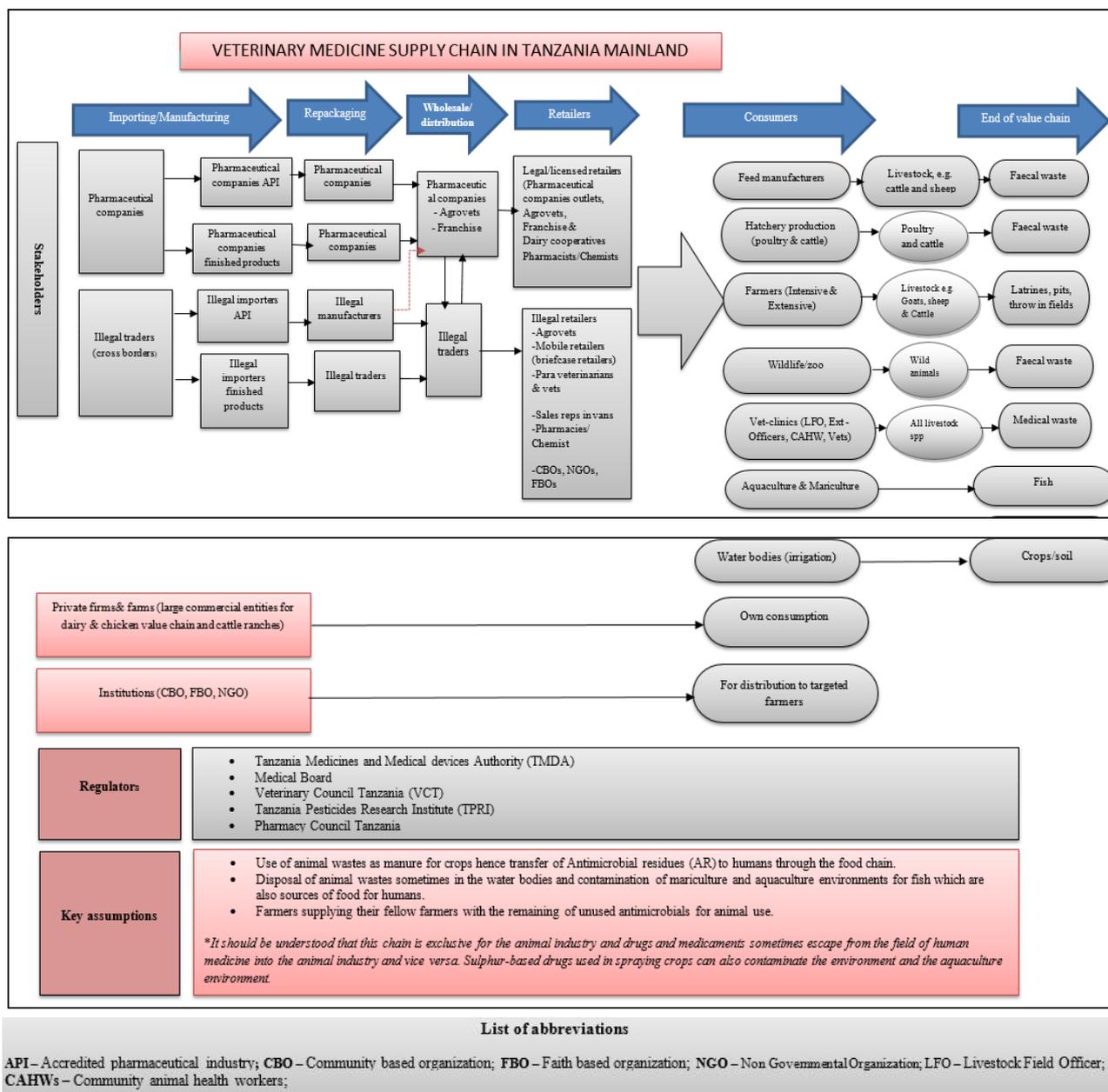


Figure 4: The supply chain covering different nodes in the pathways from manufactures to the clients as end users of Veterinary pharmaceuticals in Tanzania.

As demonstrated in Figure 4, the client can receive the antimicrobial agents through legal and illegal pathways. The access of antimicrobial agents through illegal pathway contribute significantly to misuse and associated residues in food of animal origin and the perpetuation of development and spread of antimicrobial resistance to animals and consequently to humans.

While the quality assessments are performed to the great extent on imported veterinary drugs at the port of entry, there is very weak post-market surveillance. The supply chain is driven by the private sector and thus it is not uncommon to find importers, distributors and wholesalers supplying drugs directly to consumers, a route which is considered illegal. With weak regulations, antimicrobial agents are commonly sold during auction markets for livestock by informal vendors, such as petty traders and livestock keepers. Antimicrobial agents found in markets like these are often unregistered, and therefore sold at cheap prices. The quality of these medicines are also difficult to determine because access to them is difficult.

The delivery of animal health services and dispensing of veterinary medicines to consumers is governed by the Veterinary Act No. 16 of 2003. In Tanzania, the Animal Health work-force responsible for delivery of animal health services include 700 degree-holding veterinarians, over 1500 veterinary paraprofessionals (diploma level) enrolled and 1340 veterinary paraprofessional assistants who hold certificates and enlisted by the Veterinary Council of Tanzania (VCT). Another 60% of veterinary paraprofessionals operate without being enrolled or enlisted. Public veterinary services also includes Zoosanitary Inspectorate Services (ZIS) established way back in 1996 and manages 36 border posts, 19 quarantine stations and 381 internal check points. Regulation of veterinary services delivery is provided for in the Animal Diseases Act, 2003 and Veterinary Act of 2003.

Tanzania is endowed with different capacities for research and monitoring the state and trends of antimicrobial resistance. Such capacities however are based primarily in Research and Training Institutions. The list of institutions with capacity and that are conducting research on AMR with focus on humans, animals and the environment are as listed in table 3.

Table 3: List of Training and Research Institutions currently involved in AMR research activities in Tanzania

Institution	Culture and Sensitivity Tests & Techniques	DNA based test & Techniques	Animal focus	Human focus	Environmental focus
Sokoine University of Agriculture	+	+	+	+	+
Nelson Mandela-African Institute of Science and Technology	+	+	+	+	+
Tanzania Veterinary Laboratory Agency	+	-	+	-	-
Muhimbili University of Health and Allied Sciences	+	-	-	+	-
Bugando Medical Centre	+	+	+	+	+
Kilimanjaro Christian Medical Centre	+	+	-	+	-
National Institute for Medical Research	+	+	-	+	-

The animal health care system in Tanzania is organized from the primary level (at farm or community level), the secondary level (district level) and tertiary level (with services provision by Sokoine University of Agriculture, the only University undertaking veterinary training in the country). Following privatization and decentralization of Veterinary services in 1990's, documentation and record keeping was rather disorganized. Thus, the information on AMU, and testing for resistance and residues is inexistent.

National Sample Survey indicate that only 20% of the farmers utilize extension services [1] and given the malpractice observed it is prudent that awareness creation of AMU and AMR to farmers, require a reliable extension service system.

Developed countries that banned use of antimicrobial agents as feed additives to prevent and control the emergence of AMR since 1980, in Sweden, for instance, enacted legislations on ban of antimicrobial use as growth promoters (AGPs) in 1986 and thus use of veterinary medicines in animals became restricted to prescription only by veterinarians. Moreover, in the European Union, a total ban on use of antibiotics as growth promoters was introduced in 2006. Such kind of interventions however are lacking in developing countries including Tanzania.

The Veterinary Act No. 16 of 2003 provides for regulation of veterinarians and paraprofessionals however the law also provides circumstances under which unqualified personnel may perform some of the duties. During the study it was observed that, exceptions provided are misused by unqualified personnel to deliver veterinary services such as disease diagnosis and treatment including self-medication done to animals by owners and other farm attendants.

The withdrawal period is defined as the interval between the last administration of a drug to the animal under normal condition of use and production of foodstuff from such animals. This is a requirement in food producing animals to ensure that such food stuffs do not contain residues in quantities exceeding the maximum accepted residue limits. Adherence to withdrawal periods is perceived negatively by farmers due to high economic losses from discarded milk during and after treatment of diseases such as mastitis in high producing animals. In poultry production for instance, there is high prevalence of fowl typhoid and other bacterial diseases as well as coccidiosis that could have been minimized if practical bio-security measures instead of antimicrobial agents were available during the entire production cycle. In addition, inadequate number veterinary service providers and inadequate regulation of service provision aggravate the challenge.

Application of animal manure which is regarded as a good source of nutrients is a common practice in crop agriculture as well as in aquaculture. Animal manure from intensive production system often contain antimicrobial agents resulting from extensive therapeutic and sub-therapeutic uses. Since, absorption of antimicrobial agents in the animal gut is never complete and that, substantial amounts are excreted through urine and faeces that end up in manure, this exposure scenario should be considered in the risk analysis.

Though scarcely studied in Tanzania, there is potential human health risks associated with consumption of fresh vegetables, water and fish contaminated with manure with antimicrobial agents. Such risks may be higher to consumers who are allergic to antimicrobial agents. The same pathway may also contribute and enhance development of antimicrobial resistance. The National strategy to reduce the threat of AMR in food animals and crop agriculture should consider prudent use of antimicrobial agents, improve capacity for disease diagnosis and detection of Antimicrobial residues, developing bio-security guideline fitting into current production systems, control of antimicrobial Residues. The National Action Plan on Antimicrobial Resistance 2017 - 2022, outlines key strategic objectives, interventions and activities to slow down the development and spread of AMR and improve patient outcomes.

4. Materials and Methods

Study Design, Sampling strategy, Study coverage and data collection

A multimethod approach was used including a desk review, focus group discussions (FGD), key informant interviews (KII) and field observations. Identification of all stakeholders in the governance structure was conducted through a rapid survey of the current documents and physical visits were made to relevant establishments located in Dodoma, Arusha, Morogoro and Dar es Salaam Regions (Figure 2). During the visits, a range of information was collected in order to map out the state, trends and magnitude of antimicrobial use in food animals and crop agriculture, antimicrobial resistance as well as antimicrobial residues. Analysis and interpretation of the data collected was referenced to FAO and OIE strategic objectives for combating AMR [14, 42]. The establishments/institutions visited were purposively selected due to their relevance to the subject matter. This review in the food and agriculture sector was meant to complement previous situational analyses and research conducted on AMR, AR and AMU in Tanzania, particularly in the human health sector [17,43,44]. The list of grey literatures were obtained from the legal documents archives and the legislation that directly or indirectly related with AMU and AMR. A total of eight documents from grey literature were reviewed and analyzed. The

national and sub-national implementation and governance structures have been elaborated in figure 3 below.

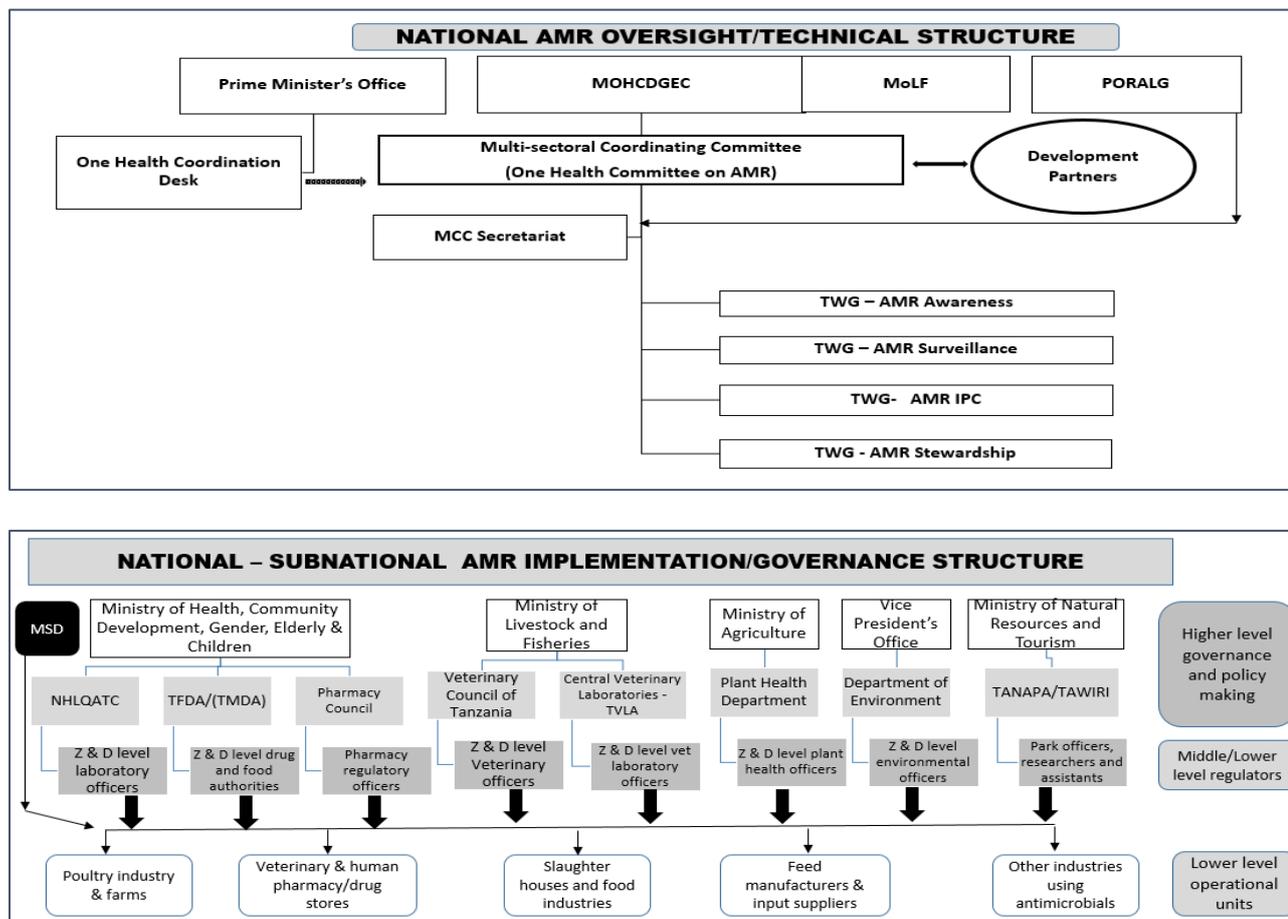


Figure 2: The antimicrobial related governance structure for the food and agriculture sector in Tanzania. MoHCDGEC = Ministry of Health, Community Development, Gender, Elderly and Children; MoLF = Ministry of Livestock and Fisheries; PORALG = President's Office Regional Administration and Local Government; TWG = Technical Working Group; MCC = Multi-sectoral Coordinating Committee; IPC = Infection Prevention and Control; MSD = Medical Stores Department; NHLQATC = National Health Laboratory Quality Assurance and Training Centre; TFDA/TMDA = Tanzania Food & Drugs Authority now called Tanzania Medicine & Medical Devices Authority; TVLA = Tanzania Veterinary Laboratory Agency; TANAPA = Tanzania National Parks Authority; TAWIRI = Tanzania Wildlife Research Institute; Z & D = Zonal and District (these are the secondary and tertiary level units of government in the United Republic of Tanzania).

It should be noted that while this structure under consideration relates primarily to the food and agriculture industries, the broader health industry has a Public Health System Referral Pyramid which also influences antimicrobial use and antimicrobial resistance in humans [45,46]. Details of other associated regulatory bodies that influence issues related to AMR in Tanzania are available in [47].

Secondly, a qualitative cross-sectional field study on the situation of AMU governance along the broiler value chain was conducted from April to June 2019. The components of this study were FGD with broiler farmers and KIIs with high and medium level AMU regulatory experts and further grey literature reviews of AMU and AMR legislations in Tanzania. Regulatory bodies of AMU was directly chosen for the key informant interviews. The responsible experts from each regulatory institutions were identified and invited for interview with the help of ECTAD-Tanzania. To double check the information, field observations of the broiler value chain were conducted to complement the FGD and KIIs. During the field observations, informal (non-structured) interviews were

conducted based on the AMU phenomena and driving factors for AMR. By using these methods, the knowledge, perception and practice of the broiler value chain actors, input supplier and antimicrobial use regulators about AMU and AMR were assessed and analyzed for AMU governance.

Using the updated AMU situational analysis in the food and agriculture sector, [10], study areas were chosen based on the following criteria: (i) high human population density, (ii) high number of broiler farms, (iii) existence of regulatory bodies and (iv) presence of broiler farm input suppliers. Specifically, Dar es Salaam, Dodoma, Morogoro and Arusha cities were purposively selected as the study areas. The study participants for the field study were chosen by snowball sampling method, a situation in which one food chain actor (broiler farmer, feed producer, slaughterhouse and veterinary pharmacy) interviewed referred the researchers to another actor. In this way the data collection was continued until information saturation point was reached. For the FGDs with the broiler farms participants were chosen randomly from the list provided by the broiler farmers association using the inclusion criteria, 'minimum of secondary level education' to avoid peer dominance during discussion and ease communication. In total, eight broiler farms, 11 veterinary pharmacies, seven slaughterhouses (three abattoirs and four open areas), six broiler feed manufacturers were visited to conduct one focus group discussion and 11 KIIs.

The situational analysis on the AMU, AR and AMR in food animals including fish farming (aquaculture) and crop production was carried out in 19 institutions in Tanzania Mainland. Regions and locations visited for data collection are Arusha, Dar es Salaam, Dodoma, and Morogoro together with Unguja in Zanzibar.

Focus group discussions and Key informant interviews

The FGD, which was conducted with a group of broiler farmers, lasted for 3 hours. This was designed to extract information using relational diagram, impact analysis and trend analysis. By using those methods participants were facilitated to model, quantify, estimate, compare and rank categories. Proportional piling was used to prioritize categories and parameterize. The respondents prioritize and rank the purpose of AMU and diseases prevention technique in their farms. During ranking the farmers were not agreed and they complained as they had different AMU practices. Farmers prioritized individually and the mean values were calculated for all quantities.

The regulatory bodies that directly or indirectly controls the use of antimicrobials in broiler value chain were chosen for the key informant interviews. For these interviews, checklists were used as a guiding tool for the interviewers. The specific organizations include (i) Prime Minister's Office – One Health Coordination Desk, (ii) Central Veterinary Reference Laboratory for Tanzania, (iii) Ministry of Livestock and Fisheries, (iv) Tanzanian Veterinary Laboratory Agency, (v) Ministry of Health, Community Development, Gender, Elderly and Children (vi) Ministry of Agriculture, (vii) Veterinary Council of Tanzania, (viii) both zonal and national Tanzanian Food and Drug Authority (now Tanzania Medicine & Medical Devices Authority) and (x) Dodoma Municipality Veterinary Officer. From each stakeholders, information about the available and planned legislations of AMU, activities for the governance of AMU and its challenge, knowledge and perception of AMU externalities and their recommendation about the mitigation of AMU were collected.

Field observations

Observation is the selection and recording of users' behavior in the working environment. The field observations were conducted in the broiler business operations including feed producing companies, broiler farms, broiler slaughterhouses (including open slaughter areas) and veterinary pharmacies. Field observation of the broiler food chain were conducted starting from animal feed to the final end product of broiler (meat). For instance, the hygiene of the production environment, the input used for production (addition of

antimicrobial), manure management, reason of AMU in each stage of the food chain, application procedure of antimicrobials and availability of alternatives (Biosecurity and Vaccination) were among the focus issues of the informal interviews. By using the five sense organs, valuable and valid information were collected and associated with the governance of AMU in that particular context. Before the field work the expected AMU and AMR creation and transmission model was explained as shown in Figure 3.

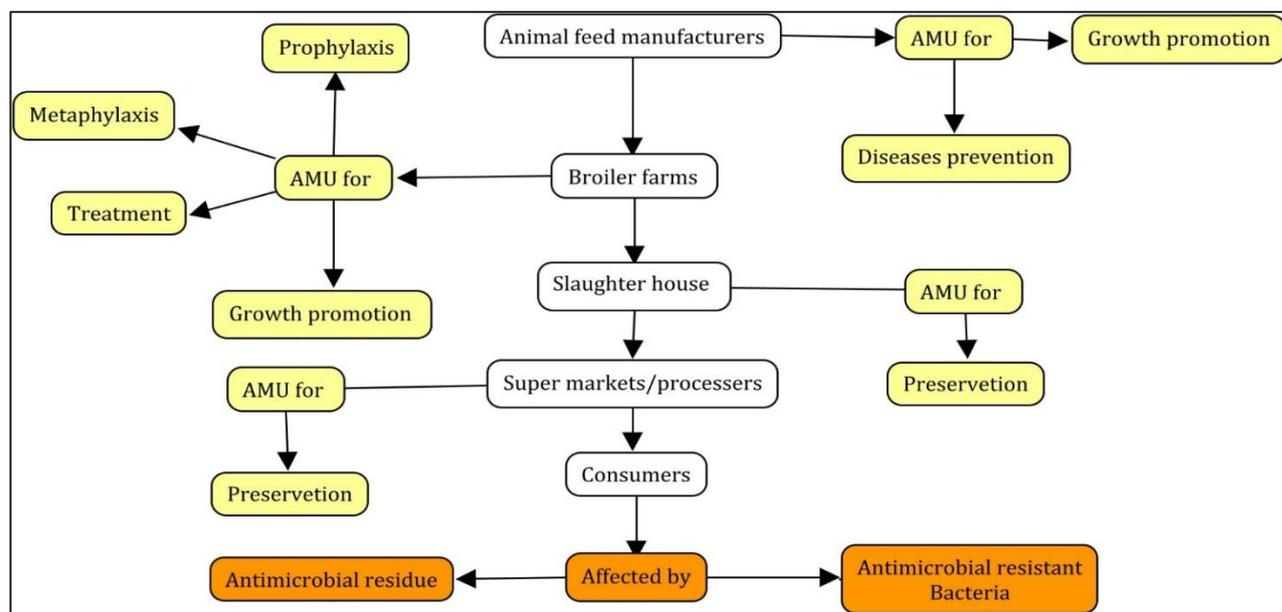


Figure 3: Potential antimicrobial use, development of AMR/residue and transmission model.

Grey Literatures that provide legislative tools guiding antimicrobial use in animals in Tanzania

Legislations like (i) Tanzania Foods, Drugs and Cosmetics Act of 2003, Part IV (2003), (ii) Tanzania Pharmacy Act (2011), (iii) Tanzania Animal Diseases Act 17 of 2003, Section 50 (2003), (iv) Tanzania Grazing Land and Animal Feed Resources Act (2010), (v) Tanzania Veterinary Act (2003), (vi) Tanzanian Water Supply and Sanitation Act (2009), (vii) Tanzania Animal Welfare Act (2008) and (viii) Tanzania Public Health Act (2009) were used as sources of information. Other legislative frameworks include the: (i) Fisheries Regulations of 2009, Section 33 (f); (ii) Fisheries Regulations of 2009, Section 33 (i); (iii) Fisheries Regulations of 2009; section 33 (j-1); (iv) Harmonized EAC SPS measures (under review) Section 2.8-(2.8.1), Section 3.7-(3.7.5), Section 3.7-(3.7.6) and Section 3.12 [46]. The focus of this grey literature review was to gathering information related to the available AMU and/or AMR legislation in each levels of the food chain and cross checking the implementations of these legislations in the lower broiler business operations.

Data analysis

Qualitative analysis was predominantly used and some information are expressed in numbers. Those numerical values were expressed in descriptive statistics (figures, tables and percentages). The data collected from the group discussions, field visit, key informant interview and grey literatures were interpreted in terms of antimicrobial use governance in the food chain. The information recorded in the forms of texts, photos and audios were textually and semi quantitatively analyzed towards AMU governance. For the textual analysis qualitative content analysis approach was used. The contents of grey literatures, recorded audios and visual pictures were analyzed in terms of AMU governance. The contents of grey literatures were categorized into direct and/or indirect based on the concept of their contribution to the governance of AMU and AMR along the food chain.

Some data like the result of proportional piling were collected in numerical form from the field and the quantity was directly taken for analysis. The frequency of repetition of the information from different participants were counted and analyzed quantitatively. The practice of visited farms were judged using 12 criteria of good AMU, each being classified along 3 categories (bad, moderate and good). The mean, minimum and maximum scores of these categories were calculated from the practice of the visited farms. The AMU nodes (introduction routes of antimicrobials) were ranked based on the AMU status (usage of antimicrobials or not) of broiler business operators (broiler feed manufacturers, broiler farms and broiler slaughter houses).

5. Conclusions

From this study, effective control of AMR in the food and agriculture sectors in Tanzania requires a thorough understanding of the governance structure on the issue of antimicrobial and concerted efforts of all stakeholders using the One Health approach. Delivery of plant/animal health services and dispensing of antimicrobial agents must be streamlined, regulated and done by professionals. Service delivery by unqualified personnel should be avoided. Overall, the practical recommendations based on the findings from the study include:

- a) Review of legislation governing AMU, AMR and AR in food and agriculture production systems;
- b) Awareness creation and education interventions to improve understanding of AMU, AMR and AR in food and agriculture and subsequent effects in humans and the environment;
- c) Establishment of and strengthening of the national surveillance and monitoring programs for AMU, AMR and AR in food and agriculture to enhance burden estimation, early detection of emerging resistant pathogens, as well as assessment of effectiveness of control measures;
- d) Development of and promotion of biosecurity principles (including good husbandry practices) for food animal production systems;
- e) Promotion of stewardship and legislations on AMU in food and agriculture sectors;
- f) Enhancement of the development and use of rapid and innovative diagnostic tests for “point-of-need” of antimicrobials as well as AR in food products; and
- g) Empowerment of consumers with knowledge so as to serve as pressure group and game changers by demanding high-quality products that are free from AR and antimicrobials resistant pathogens.

Supplementary Materials: No supplementary Figure, Table or Video is available for this work.

Author Contributions: Conceptualization, BR, FOF, NA-M; methodology, RHM, ERM, BR, DTG, SN, VM, NA-M, FOF; software, RHM, ERM, BR, DTG, SN, VM, NA-M, FOF; validation, HEN, ERM, BR, FOF, NA-M; formal analysis, RHM, DTG, SN, VM, FOF; investigation, RHM, DTG, SN, VM; resources, ERM, BR, NA-M, FOF, HEN; data curation, ERM, BR, FOF.; writing—original draft preparation, DTG, SN, VM, FOF; writing—review and editing, ERM, FOF; visualization, VM, DTG, FOF; supervision, ERM, BR, NA-M, FOF; project administration, FOF, ERM, BR; funding acquisition, FOF, ERM, BR. (For FAO). All authors have read and agreed to the published version of the manuscript.

Funding: This work and the publication was supported by partial funding from the UK Department of Health funded Fleming Fund 2 and USAID funded GHSA-ZDAH programme.

Institutional Review Board Statement: The information from the actors were collected based on verbal consent. Stakeholders were informed about the purpose of the information and they were requested to signify their agreement to participate in the discussion and interviews. Participation was based on individual availability and willingness to participate in the study, and everyone was

notified of his/her right to discontinue participation at any stage of the study in line with standardized protocol [48]. The researchers got an official letter of introduction from the FAO, Tanzania, backed by the permission to implement research on behalf of the Ministry of Livestock and Fisheries, the purpose of the study and the responsible re-searchers were expressed to all participants. For the sake of business privacy, the profile of each participants in group discussion, field visit and informal interviews was kept confidential.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study. Each subject was given the option of opting out of participation in the course of the interview.

Data Availability Statement: Data utilized in this study are publicly available and are accessible on request.

Acknowledgments: We want to thank Dr Charles Bebay, the Regional Manager, ECTAD East Africa for facilitating the linkages for FAO volunteer in 2019 and approval for the work. We thank Mr. Fred Kafeero, the FAOR, in United Republic of Tanzania for hosting the volunteers and interns in Tanzania. We thank the FAO-ECTAD staff for critical inputs in this work. We are grateful to the Ministry of Livestock and Fisheries for permission to carry out this work on its behalf and the field staff and individuals/stakeholders met in the course of the field investigations for their contributions. FAO-Tanzania and ARES provided the internship service and scholarship respectively. The University of Liege, Free University of Brussels (ULB) and University of Namur were the host institutions for the scholarship. Daniel T. Gebeyehu and Solange Niyigena were FAO AMR Volunteers in 2019 through the Specialized Master in Integrated Management of Health Risks in the Global South (IManHR) and Victoria Msambichaka was a 2020 FAO AMR Intern.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

1. National Bureau of Statistics (NBS). Country statistics of Tanzania, **2019**, available online: <http://tanzania.countrystat.org/search-and-visualize-data/en/>, (accessed 06 August 2019).
2. Matthew, M.; Mruttu, H.; Gebru, G. *Animal health strategy and vision for Tanzania*, **2016**, ILRI, Nairobi, Kenya & Tanzania Ministry of Agriculture, Livestock and Fisheries.
3. Centers for Disease Control and Prevention (CDC). *One Health Zoonotic Disease Prioritization for Multisectoral Engagement in Tanzania*, **2017**, Available online: <https://www.cdc.gov/onehealth/pdfs/tanzania-report-508.pdf>, (accessed 23 December 2020)
4. Food and Agriculture Organization of the United Nations (FAO). *Challenges of animal health information systems and surveillance for animal diseases and zoonoses*, **2011**, FAO Animal Production and Health Proceedings, No. 14. Rome, Italy, Available online: <http://www.fao.org/3/i2415e/i2415e00.pdf>, (accessed 23 December 2020)
5. American Veterinary Medical Association (AVMA). *Judicious therapeutic use of antimicrobials*, **2019**. Available online: <https://www.avma.org/resources-tools/avma-policies/judicious-therapeutic-use-antimicrobials>, (accessed 23 December 2020).
6. World Health Organization (WHO). *Antimicrobial Resistance*, **2017**. Available online: <https://www.who.int/health-topics/antimicrobial-resistance>, (accessed 23 December 2020).
7. Morar, A.; Sala, C.; Imre, K. Occurrence and antimicrobial susceptibility of Salmonella isolates from pig slaughter process in Romania. *J Infect Dev Countries* **2015**, *9*(1), 99–104.
8. World Health Organization (WHO). What to do about resistant bacteria in the food-chain? *Bull WHO*, **2015**, *93*, 217-218. doi: <http://dx.doi.org/10.2471/BLT.15.030415>.
9. Founou, L.L.; Founou, R.C.; Essack, S.Y. Antibiotic Resistance in the Food Chain: A Developing Country-Perspective. *Front Microbiol.*, **2016**, *23*, 7,1881. doi: 10.3389/fmicb.2016.01881.
10. Food and Agriculture Organization of the United Nations. *Situation Analysis of Antimicrobial Use and Resistance in the Food and Agriculture Sectors in Tanzania*, **2019**. Internal report of the FAO Representation, Tanzania.
11. Centers for Disease Control and Prevention (CDC). *Antibiotic/Antimicrobial Resistance*. Available online: <https://www.cdc.gov/drugresistance/about.html>, (accessed 23 December 2020).
12. Aly, S.M.; Albutti, A. Antimicrobials use in aquaculture and their public health impact. *J Aquaculture Res Dev.*, **2014**, *5*(4):1-6
13. Codex Alimentarius. *Code of practice to minimize and contain antimicrobial resistance*, **2005**, CAC/RCP 61-2005. Available online: <http://www.fao.org/fao-who-codexalimentarius/committees/ewg/detail/en/c/1026199/>, (accessed 23 December 2020)
14. Food and Agriculture Organization of the United Nations. *The FAO Action Plan on Antimicrobial Resistance, 2016-2020: Supporting the food and agriculture sectors in implementing the Global Action Plan on Antimicrobial Resistance to minimize the impact of antimicrobial resistance*, **2016**, Available online: <http://www.fao.org/3/a-i5996e.pdf>, (accessed 18 August 2020).
15. Minich, J.J.; Zhu, Q.; Xu, Z.Z.; Amir, A.; Ngochera, M.; Simwaka, M.; et al. Microbial effects of livestock manure fertilization on freshwater aquaculture ponds rearing tilapia (*Oreochromis shiranus*) and North African catfish (*Clarias gariepinus*). *Microbiol Open* **2018**, *7*:e716, <https://doi.org/10.1002/mbo3.716>.

16. Van Boeckel, T.P.; Brower, C.; Gilbert, M.; Grenfell, B.T.; Levin, S.A.; Robinson, T.P.; et al. Global trends in antimicrobial use in food animals. *Proc Natl Acad Sci U S A*, **2015**, 112(18):5649-54. doi: 10.1073/pnas.1503141112.
17. Caudell, M.A.; Quinlan, M.B.; Subbiah, M.; Call, D.R.; Roulette, C.J.; Roulette, J.W.; et al. Antimicrobial Use and Veterinary Care among Agro-Pastoralists in Northern Tanzania. *PLoS ONE*, **2017**, 12(1): e0170328.
18. Nonga, H.E.; Mariki, M.; Karimuribo, E.D.; Mdegela, R.H. Assessment of Antimicrobial Usage and Antimicrobial Residues in Broiler Chickens in Morogoro Municipality, Tanzania. *Pakistan J Nutrition*, **2009**, 8(3), 203-207.
19. Nonga, H.E.; Muhairwa, A.P. Prevalence and antibiotic susceptibility of thermophilic *Campylobacter* isolates from free-range domestic duck (*Cairina moschata*) in Morogoro municipality, Tanzania. *Trop Anim Health Pro*, **2010**, 42:165-17
20. Mubito, E.P.; Shahada, F.; Kimanya, M.E.; Buza, J. J. (2014). Antimicrobial use in the poultry industry in Dar-es-Salaam, Tanzania and public health implications. *Am J Res Comm.*, **2014**, 2(4): 51-63
21. Nonga, H.E.; Mariki, M.; Karimuribo, E.D.; Mdegela, R.H. Assessment of antimicrobial usage and antimicrobial residues in broiler chickens in Morogoro Municipality, Tanzania. *Pakistan J Nutrition*, **2009**, 8(3):203-207.
22. Katakweba, A.A.S.; Mtambo, M.M.A.; Olsen, J.E.; Muhairwa, A.P. Awareness of human health risks associated with the use of antibiotics among livestock keepers and factors that contribute to selection of antibiotic resistance bacteria within livestock in Tanzania. *LRRD* **2012**, 24 (10), <http://www.lrrd.org/lrrd24/10/kata24170.htm>.
23. Kilobe, B.M.; Mdegela, R.H.; Mtambo, M.M.A. Heavy metal concentration in tomatoes grown in wastewater irrigated sites in Dodoma District, Tanzania. *Int J Physical Soc Sci.*, **2013**, 3(6), 180-191
24. Agmas, B.; Adugna, M. Antimicrobial residue occurrence and its public health risk of beef meat in Debre Tabor and Bahir Dar, Northwest Ethiopia. *Vet World*, **2018**, 11(7):902-908. doi: 10.14202/vetworld.2018.902-908.
25. Mhongole, O.J.; Mdegela, R.H.; Kusiluka, L.J.M.; Dalsgaard, A. Occurrence of *Escherichia coli* in Brassica rapa L. chinensis irrigated with low quality water in urban areas of Morogoro, Tanzania. *African J Biotech*, **2016**, 15(49): 2772 - 2777, DOI: 10.5897/AJB2016.15671
26. Mdegela, R.H.; Kusiluka, L.J.M.; Kapaga, A.M.; Karimuribo, E.; Turuka, F.M.; Bundala, A.; et al. Prevalence and determinants of mastitis and milk borne zoonoses in smallholder dairy farming sector in Kibaha and Morogoro districts, Eastern Tanzania. *J Vet Med*. **2004**, B 51:123-128.
27. Nonga, H.E.; Simon, C.; Karimuribo, E.D.; Mdegela, R.H. Assessment of Antimicrobial Usage and Residues in Commercial Chicken Eggs from Smallholder Poultry Keepers in Morogoro Municipality, Tanzania. *Zoonoses Public Health*, **2010**, 57: 339-344
28. Mshana, S.E.; Matee, M.; Rweyemamu, M. Antimicrobial resistance in human and animal pathogens in Zambia, Democratic Republic of Congo, Mozambique and Tanzania: an urgent need of a sustainable surveillance system. *Annals Clin Microbiol Antimicrobials*, **2013**, 12:28
29. Kashoma, I.P.; Kasseem, I.I.; Kumar, A.; Kessy, B.M.; Gebreyes, W.; Kazwala, R.R.; Rajashekara, G. Antimicrobial Resistance and Genotypic Diversity of *Campylobacter* Isolated from Pigs, Dairy, and Beef Cattle in Tanzania. *Front. Microbiol*. **2015**, 6:1240.
30. Katakweba, A.A.S.; Møller, K.S.; Muumba, J.; Muhairwa, A.P.; Damborg, P.; Rosenkrantz, J.T.; et al. Antimicrobial resistance in faecal samples from buffalo, wildebeest and zebra grazing together with and without cattle in Tanzania. *J Applied Microbiol*. **2014**, 118: 966-975.
31. Shah, S.Q.A.; Colquhoun, D.J.; Nikuli, H.L.; Sørum, H. Prevalence of Antibiotic Resistance Genes in the Bacterial Flora of Integrated Fish Farming Environments of Pakistan and Tanzania. *Environ. Sci. Technol*, **2012**, 46, 8672-8679
32. Jabbar, M.A.; Grace, D. *Regulations for safety of animal source foods in selected Sub-Saharan African countries: Current status and their implications*, 2012. ILRI, Nairobi Kenya.
33. Nonga, H.E.; Sungura, K.H.; Ngowi, H.A. Assessment of veterinary drug use and determination of antimicrobial residues in broiler chicken meat in Urban district, Zanzibar, Tanzania. *Tanzania Vet J*, **2013**, 28(2), 26-36.
34. Kivaria, F. M.; Noordhuizen, J. P. T. M.; Kapaga, A. M. Evaluation of the Hygienic Quality and Associated Public Health Hazards of Raw Milk Marketed by Smallholder Dairy Producers in the Dar es Salaam Region, Tanzania. *Trop Anim Health Pro*, **2006**, 38:185-194.
35. Kurwijila, L. R.; Omore, A.; Staal, S.; Mdoe, N. S. Y. Investigation of the Risk of Exposure to Antimicrobial Residues Present in Marketed Milk in Tanzania. *J Food Protect*, **2006**, 69(10):2487-2492
36. Mdegela, R.H.; Ryoba, R.; Karimuribo, E.D.; Phiri, E.J.; Løken, T.; Reksen, O.; et al. Prevalence of clinical and subclinical mastitis and quality of milk on smallholder dairy farms in Tanzania. *J S. Afr. vet. Ass*, **2009**, 80(3): 163-168.
37. Mgonja, F.; Mosha, R.; Mabiki, F.; Choongo, K. (2017). Oxytetracycline residue levels in beef in Dodoma region, Tanzania. *African J Food Sci.*, **2017**, 11(2): 40-43.
38. Mgonja, F.; Mosha, R.; Mabiki, F.; Choongo, K. A simple and sensitive method for the detection of "Oxytetracycline" levels in ready-to-eat beef by liquid chromatography-mass spectrometry. *African J pharmacy pharmacol.*, **2016**, 10(28):571-578.
39. Kimera, Z.I.; Mdegela, R.H.; Mhaiki, C.J.N.; Karimuribo, E.D.; Mabiki, F.; Nonga, H.E.; et al. Determination of oxytetracycline residues in cattle meat marketed in the Kilosa district, Tanzania. *Onderstepoort J Vet Res.*, **2015**, 82(1), Art. #911
40. Bilashoboka, E.; Mudenda, B.; Munyinda, N.; Moshi, F. V.; Kambarage, D.M. Determination of oxytetracycline residue levels in edible tissues of slaughtered cattle. *Food Environment Safety*, **2018**, XVII, 404-412.
41. Mdegela, R.H. *Assessment of Knowledge, Attitude and Practices on Antibiotic Use, Resistance and Containment in Animal Keeping Households in Morogoro, Tanzania*. Presentation at the 2nd OHCEA International One Health Conference, Uganda **2015**, Available online: <http://www.ohcea.org/conference report>, (accessed, 15 April 2020).

42. World Organisation for Animal Health. *The OIE Strategy on Antimicrobial Resistance and the Prudent Use of Antimicrobials*, 2016. Available online: https://www.oie.int/fileadmin/Home/eng/Media_Center/docs/pdf/PortailAMR/EN_OIE-AMRstrategy.pdf, (accessed 18 August 2020).
43. Moremi, N.; Claus, H.; Mshana, S.E. Antimicrobial resistance pattern: a report of microbiological cultures at a tertiary hospital in Tanzania. *BMC Infect Dis.*, 2016, 16, 756, <https://doi.org/10.1186/s12879-016-2082-1>.
44. Subbiah, M.; Caudell, M.A.; Mair, C.; Davis, M.A.; Matthews, L.; Quinlan, R.J.; et al. Antimicrobial resistant enteric bacteria are widely distributed amongst people, animals and the environment in Tanzania. *Nature Comm.*, 2020, 11, 228 (2020). <https://doi.org/10.1038/s41467-019-13995-5>.
45. Strengthening health outcomes through the private sector (SHOPS). *Tanzania private health sector assessment*, 2013. Available online: <https://www.shopsplusproject.org/sites/default/files/resources/Tanzania%20Private%20Sector%20Assessment%202.pdf>, (accessed 06 August 2020).
46. Global Antibiotic Resistance Partnership—Tanzania Working Group (GARP-TWG). (2015). *Situation Analysis and Recommendations: Antibiotic Use and Resistance in Tanzania*, 2015, Center for Disease Dynamics, Economics & Policy, Washington, DC and New Delhi. Available online: https://cddep.org/wp-content/uploads/2017/06/garp-tz_situation_analysis-1.pdf, (accessed 05 August 2020).
47. Kurwijila, L.R.; Mwingira, J.; Karimuribo, E.; Shirima, G.; Lema, B.; Ryoba, R.; Kilima, B. *Safety of Animal Source Foods in Tanzania: A Situational Analysis*, 2011, 36p (internal report).
48. World Medical Association. World Medical Association Declaration of Helsinki. Ethical principles for medical research involving human subjects. *Bull. World Health Organ.*, 2001, 79, 373–374.