Monitoring of Antimicrobial Resistance in diagnostic Veterinary laboratory in Dar es Salaam, Tanzania

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SUMMARY

Antimicrobial resistance (AMR) is a global one health issue threatening our ability to treat bacterial infections in humans and animals. Surveillance of AMR is important in order to estimate the size of the problem, to identify targets for measures and to evaluate the effect of implemented measures. The study was conducted to determine how microbiological samples were collected, results interpreted and the number of samples collected for bacterial culture and sensitivity testing; to identify bacteria frequently isolated from milk and avian samples; and to determine the trend of samples submitted for bacterial culture, sensitivity testing and AMR prevalence. A retrospective study was conducted to collect AMR data by extracting information from laboratory logbooks and laboratory information system from 2010-2017. About 90% of samples were submitted by farmers, and then registered into laboratory registration systems at the reception. A total of 4157 samples were collected for bacterial culture, which included 3571 milk samples collected from cows, 555 samples obtained from live or dead chicken, and 31 samples collected from other animal species. Four hundred and thirty (430) samples requested bacterial culture and sensitivity testing, of which 346 (80.5%) were from cow milk samples, 53 (12.3%) from avian samples, and 31 (7.2%) from other animal species. The common bacterial isolates were *Micrococcus, E. coli, Salmonella, Staphylococcus, Enterobacter,* and *Bacillus species*. The use of diagnostics and detection of drug susceptibility is important to support rational use of antibiotics and tracking of AMR.

Key words: *Bacteria, Antimicrobial resistance, Antimicrobial susceptibility.*

INTRODUCTION

Antimicrobial resistance is an increasing problem of humans and animal health worldwide. But there is still a need for an efficient livestock production, which requires healthy animals. In many countries, this has led to extensive and inappropriate use of antimicrobials, which may contribute to increasing antimicrobial resistance (AMR).

Antimicrobial drugs have helped dramatically in curing animals and humans suffering from bacterial infections; this miracle can be reversed by emergence of AMR. The overuse and misuse of antimicrobials and their inappropriate disposal have led to widespread of antimicrobials in the environment resulting into disproportionate rise in antimicrobial resistant pathogens in humans and animals (MLF, 2018). Resistant organisms can reach people through the food chain, the environment, or contact with affected humans and animals. AMR causes loss of drugs efficacy and their effectiveness for infectious disease treatment become less or even useless (FAO, 2016).

Antimicrobial resistance is an enormous problem in Tanzania and there are high levels of inappropriate use of antimicrobials in the livestock sector due to weak regulation on antimicrobial use in livestock; data on AMR including those generated from the veterinary laboratories in the country are scarce (MLF, 2018). The scarcity of data may be contributed by use

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of drugs without prescription or prescribing drugs without laboratory test to identify bacterial pathogens and their susceptibility to antibiotics. AMR is also a problem in human and veterinary healthcare, due to significant impacts in socio-economic development, people's livelihoods, and food security.

In Tanzania, farmers use antimicrobials in animals to compensate for poor farm management practices, lack of formal veterinary services, lack of regulatory capability and because of the high prevalent animal diseases (Kimera *et al*, 2015; Mdegela *et al*., 2004; Mmbando, 2004; Nonga *et al*., 2010). Commercial chicken and cattle production account for most of antimicrobial use in Tanzania.

The most common antimicrobials used are oxytetracycline, amprolium, sulphonamides, chlortetracyclines, doxycycline, flumequine, penicillin, neoxyvital, trimazine and tylosin (Karimuribo *et al*., 2005; Nonga *et al*., 2010; Mubito *et al*., 2014).

In dairy production, mastitis is the most common disease; the present data shows a number of multidrug resistant bacteria known to cause mastitis in lactating cows (Mdegela *et al.,* 2009).

High levels of resistance have been reported to penicillin G, chloramphenicol, streptomycin and oxytetracycline among *Staphylococcus hyicus, Staphylococcus intermedius and Staphylococcus aureus* from cattle with mastitis. In commercial chicken production, Salmonellosis, Colibacillosis, Mycoplasmosis, Infectious coryza and Coccidiosis are commonly reported infections, and resistance to amoxicillin and clavulanic acid combination, sulpha-methoxazole and neomycin have been found in poultry products contaminated with *E. coli* isolates (Nonga *et al*, 2009).

Surveillance and monitoring of resistant bacteria is an important and critical component of the response to AMR, and it can be a useful tool to quickly assess the current situation, and over time the efforts can be scaled up and act as inputs to national surveillance.

To meet this, laboratories are important surveillance sites where AMR data can be collected, analyzed and generated based on antimicrobial susceptibility of pathogens that have potential to develop resistance which is of concern to human, animal and environmental health (MLF, 2018).

Through these sites, the emergence of AMR can be detected and monitored, the prevalence of AMR and the driving factors for its spread can be determined, and therefore, planning for intervention, guiding animal and human treatments, and assessing the impact of AMR intervention (WHO, 2015).

The study was conducted to determine the number of samples collected for bacterial culture and sensitivity testing; to identify bacteria frequently isolated from milk and avian samples; and to determine the trends of samples submitted for bacterial culture, sensitivity testing and AMR prevalence between 2010 and 2017.

MATERIALS AND METHODS

Study area

The study was conducted at the Central Veterinary Laboratory (CVL) in Dar es Salaam which serves as a Zonal laboratory of Tanzania Veterinary Laboratory Agency and the National Veterinary Reference Laboratory in Tanzania. The laboratory has the capacity to provide advanced diagnostic services including detection of antimicrobial resistant bacteria through bacterial culture and antimicrobial sensitivity tests.

Study design

A retrospective study was conducted by collecting the daily routine recorded data at CVL in Dar es Salaam, and the AMR information was obtained from the laboratory records mainly soft and hard

copy records recorded between 2010 and 2017.

Collection of Microbiological Samples

Information of microbiological samples was obtained from the laboratory logbooks, sample registration forms and Laboratory Information System (LIS) at the receptions of the Central Veterinary Laboratory.

The information collected from sample registration systems included clinical histories of sick animals, animal species, kind of samples registered (milk, pooled organs, dead or live animals, swabs, water, fish) at the reception, and how samples were collected for bacterial culture and sensitivity test between 2010 and 2017.

Collection, analysis and interpretation of AMR data

AMR data were collected from the laboratory logbooks and laboratory information system. The information recorded in both hard and soft copies included contact address of the sample owner, type of a sample submitted, test requested and the sections to which the sample was distributed, and the sample tracking number.

The collected AMR information obtained from laboratory records at the CVL were transferred into MS Excel 2007, where descriptive analysis was used to analyze the prevalence of AMR and then interpreted based on the trends of AMR between 2010 and 2017.

RESULTS

The study has found that most of microbiological samples were collected at the reception of the Central Veterinary Laboratory submitted by individual famers after experiencing symptoms of disease infections in animals.

When farmers submitted their specimens/samples for laboratory analysis, they first registered their personal details and their sample/specimen in the laboratory registration systems (hard and soft copies).

Other information included in the registration of samples included sample track number and testing laboratory section for bacterial culture and sensitivity testing.

The live and dead chicken specimens were distributed to pathology section for necropsy examination where pooled organ samples for bacterial culture were taken and redistributed to bacteriology section in a labeled clean closed container.

Milk samples were collected by farmers themselves from animals with a clinical mastitis that has responded poorly to antibiotic treatment.

To collect milk samples, dairy smallholder farmers visited the laboratory to get clean sampling containers and instructions for collection, storage and transporting of samples to the laboratory.

Swab samples were collected by qualified laboratory technician at farms or hatchery units.

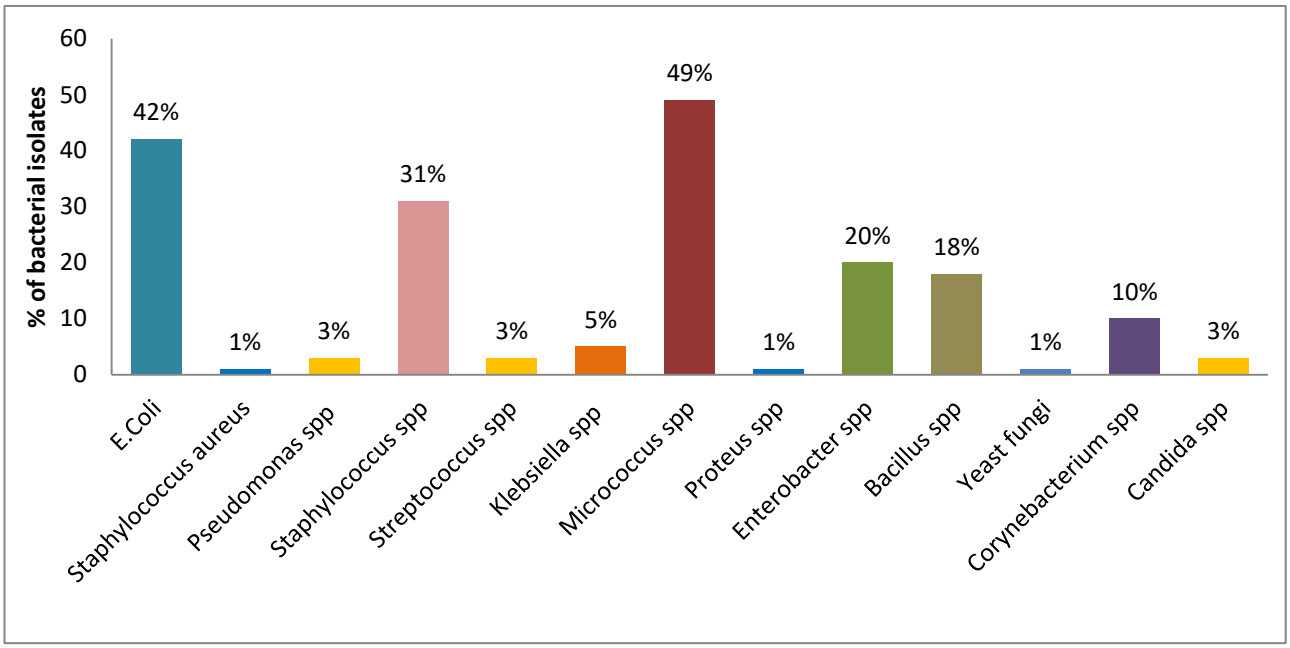

Figure 1: Bacteria isolated from dairy cow milk samples

To obtain bacterial colonies for suspected bacteria from samples, blood and MacConkey agar media were used to grow bacteria and morphologically differentiated based on the size, texture and shape of the colony, the hemolytic and lactose fermentation properties on blood and MacConkey agar.

Biochemical identification of bacteria was carried out using Indole, Methyl-Red, Voges-Proskauer test, and Citrate test (IMVIC) method.

The isolates from pure bacterial culture were inoculated into the Müeller-Hinton Agar media; antibiotic discs of tested antibiotics were placed on agar and incubated for overnight at 37ºC. The resistance patterns were determined by measuring the Zone of Inhibition on the Müeller-Hinton Agar (MHA) plate using a ruler (in millimeters).

By using Kirby-Bauer disc concentration chart, the pattern of resistance was interpreted based on the inhibition zones caused by antibiotic disc inhibited the growth of bacteria in the media.

Antimicrobial susceptibility information were recorded and interpreted as Resistance (R), Intermediate (I), and Susceptible (S) according to Clinical Laboratory Standards Institute guidelines (CLSI) (Wayne, 2012).

Number of samples submitted for bacterial culture and sensitivity test in 2010 – 2017

A total of 4157samples were submitted for bacterial culture during 2010-2017. Of these samples, 3571 were milk from cows with mastitis, 555 avian samples from live or dead chicken, and 31 samples from pigs, canine, rabbit, fish, water and tortoises.

Out of 4157 samples, only 430 samples requested both bacterial culture and sensitivity test in which isolates were obtained for antibiotic susceptibility test. Of these samples, 346 (81%) were milk, 53 (12%) live or dead chickens, and 31 (7%) samples from pigs, canine, rabbit, fish, water, and tortoise.

Milk and samples from avian species were 93% of all the samples submitted and analyzed by Central Veterinary Laboratory from 2010 to 2017, this can be contributed by larger number of cows and chickens in the city and on the other hand, the lower number of farmers keeping pigs, rabbit and fish may be rarely utilized the laboratory probably due to lack of awareness, hence few samples were captured in the study period. The clinical history of samples

collected from animals and submitted at CVL in the studied period as it was revealed in the laboratory information system and sample submission form indicated that, most of samples were obtained from sick animals which have undergone prolonged treatment and demonstrated treatment failure.

This information was captured in the submission form where it requested the sample owner to indicate the type of drugs attempted to treat the animals.

Frequently isolated bacteria from milk and avian samples

In milk samples collected from dairy cows, the most frequently isolated bacteria were *Micrococcus spp* (49%), *E. coli* (42%), *Staphylococcus spp* (31%), *Enterobacter spp* (20%), and *Bacillus spp* (18%) (Figure1). The proportion of non-pathogenic *Micrococcus spp* isolates was higher compared to other bacterial species isolated from milk samples. *E. coli* (71%), *Salmonella spp* (34%) and *Micrococcus spp* (18%) were frequently isolated from avian samples (Figure 2).

Furthermore, the study identified that the laboratory has no capacity to characterize bacteria to species levels that becomes a challenge to identify the bacteria of pathogenic importance and none pathogenic ones.

The unavailability of important reagents such as anti-sera for typing of pathogenic *E. coli* and *Salmonella spp* limits characterization of these bacteria.

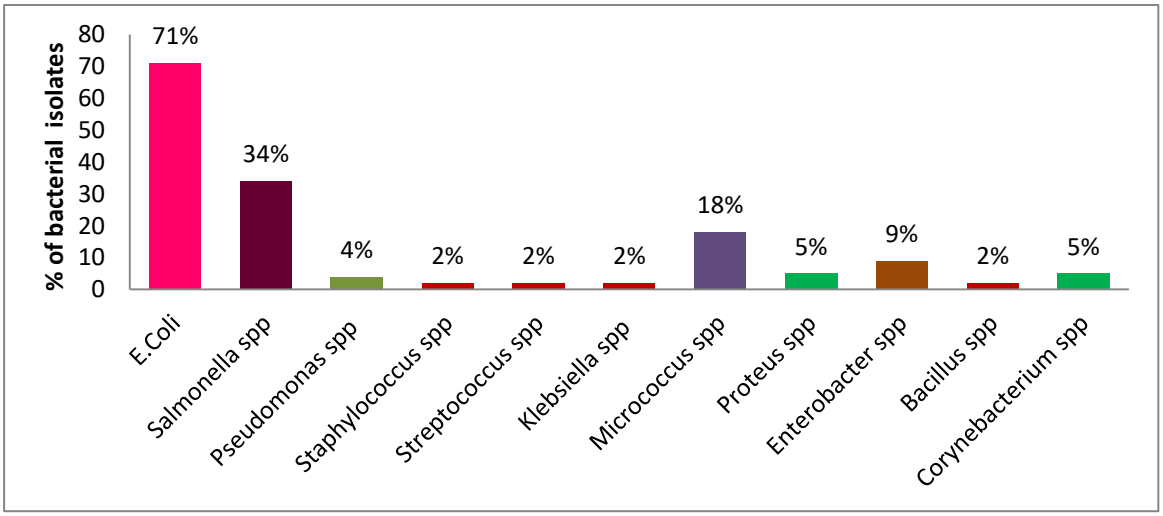

Figure 2: Bacteria isolated from avian samples

Trend of samples submitted for Bacterial Culture and Sensitivity testing in 2010 – 2017

The trend of samples submitted at CVL for bacterial culture over years showed progressive decrease for samples requested bacterial culture only and those requested both bacterial culture and sensitivity testing over 1,216 and 119 for bacterial culture respectively in 2010 down to just around 309 samples that requested bacterial culture in 2013 and 18 in 2016 (Figure 3). Similar progressive decrease was observed in milk and avian samples submitted for bacterial culture and sensitivity testing between 2010 and 2017, but the trend of samples from pigs, dogs, rabbit, fish and water remained lower in the studied period. However, the number of milk samples rapidly decreased from 1106 samples in 2010 to 296 in 2013 and it remained higher to over 300 samples compared to samples from avian and other animal species (Figure 4).

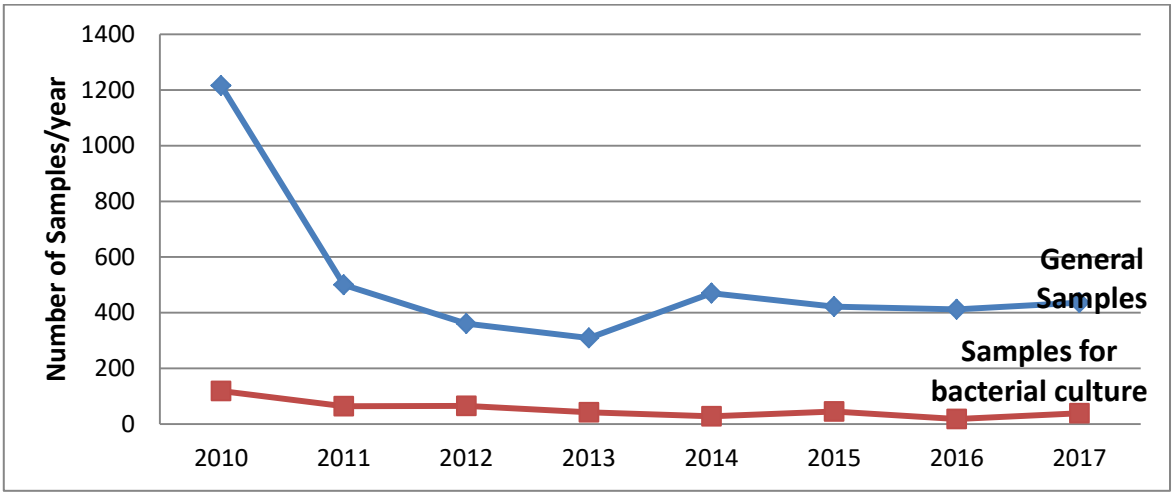

Figure 3: Samples submitted for bacterial culture and antimicrobial susceptibility test

Trends of Antimicrobial Resistance Prevalence between 2010 and 2017

The data collected and analyzed to determine prevalence of AMR based on antimicrobial susceptibility test indicated that, the prevalence of AMR rapidly increased from 52% in 2010 to 62% in 2011 and decreased to 40% in 2013. Similar trend of increase of AMR prevalence was noted in 2014 and the decrease was noted between 2015 and 2017 (Figure 5). The unsteady decrease of the AMR prevalence was associated with the progressive decrease of samples submitted for bacterial culture and sensitivity testing between 2010 and 2017. This explains that the more samples are collected and screened for AMR, the susceptibility of bacteria to antimicrobials can be easily evaluated and prevalence of AMR can reflect the real situation of the tested antimicrobials.

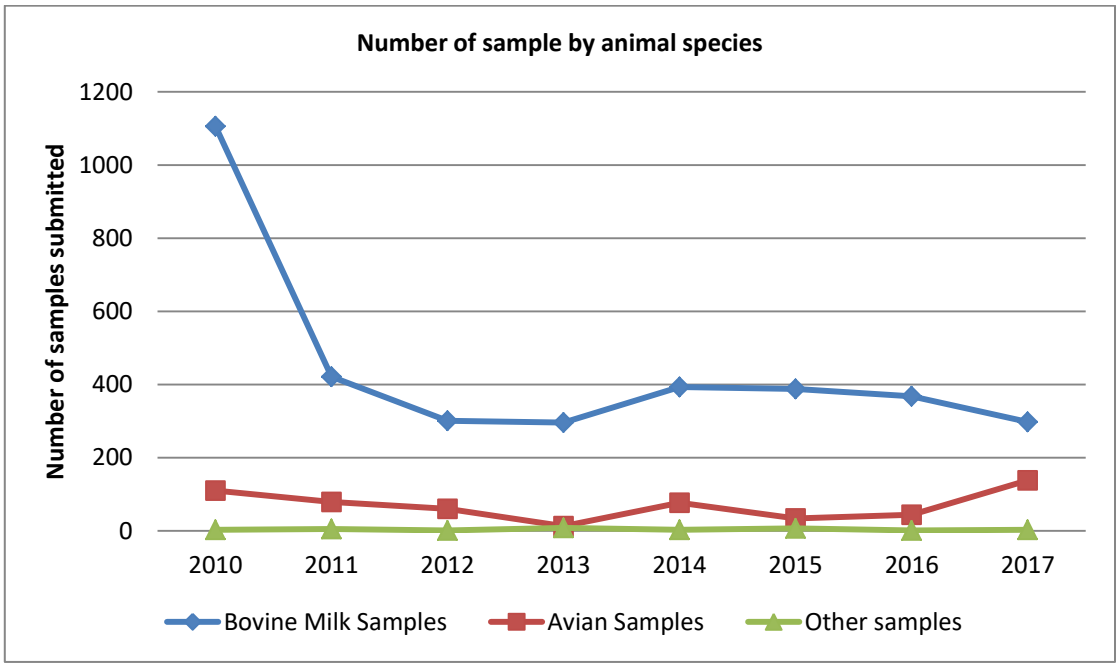

Figure 4: Trends and number of samples by animal species submitted for bacterial culture

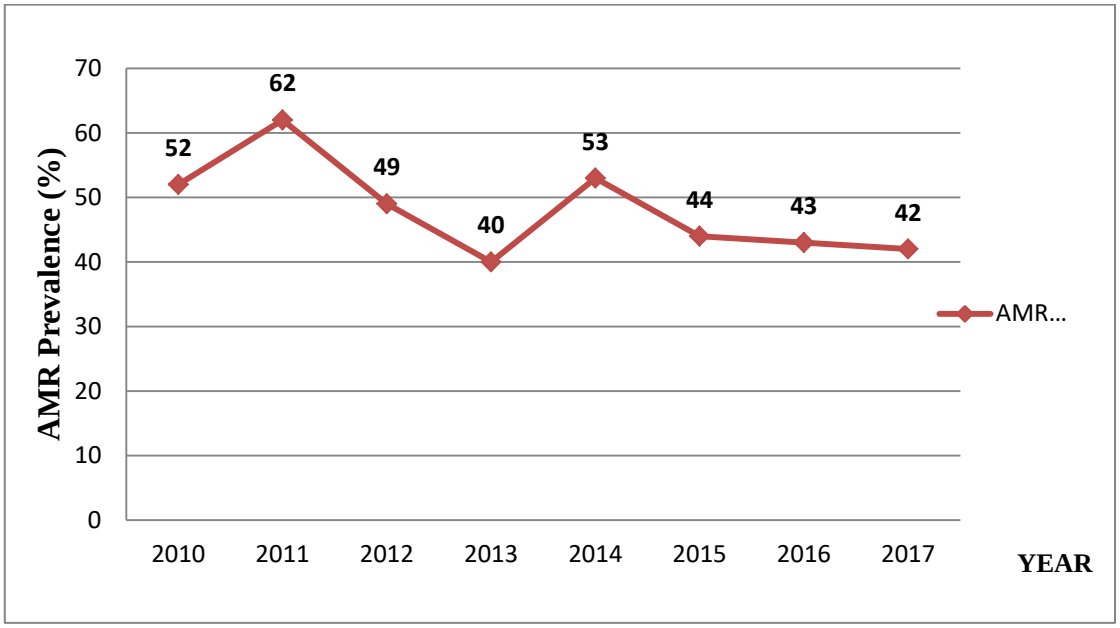

Figure 5: Trends of antimicrobial resistance prevalence

DISCUSSION

The present study has found very few AMR data due to lower number of samples submitted for bacterial culture and isolates for antimicrobial susceptibility testing. Samples were received and recorded both electronically and in hard copy that could be easily retrieved and compared.

AMR data were manually recorded in the laboratory logbook in a way that data collection and analysis became very difficult.

The most encountered resistant bacteria were *Micrococcus spp*, *E. coli*, *Salmonella spp*, *Staphylococcus spp*, *Enterobacter spp*, *Bacillus spp*, and *Corynebacterium spp*, and showed resistance to Tetracycline, Penicillin, Norfloxacin, Ampicillin, Amoxyllin, Streptomycin, Neomycin, Cloxycyllin, Gentamycin, Penicillin, Vancomycin, and Chloramphenicol.

The study has found very low number of samples submitted for bacterial disease diagnosis and as well as screening of resistant bacteria at the laboratory in domestic animals. The lower number of samples may have been contributed by many cases to be reported to veterinary vendors and managed without laboratory diagnosis to identify the likely agents causing disease symptoms. There was a clearly observation from the laboratory sample submission form which were filled by veterinarians when receiving samples that most dairy and poultry smallholder farmers submitted their samples after experiencing treatment failure. The clinical history in the submission form mentioned the duration of symptoms and the drugs that have been used to treat sick animals.

Similar observation was seen on avian samples that where there was progressive decrease of sample submission at the laboratory from 2010 to 2017. The increase of the number of avian samples in 2010 was associated with export of wild birds that were screened for Salmonella infection as requirement from the importing countries.

The records from the laboratory showed that the samples from wild birds requested only bacterial culture and there was no advanced test conducted to determine the presence of resistant bacteria. The trends of AMR prevalence progressively decreased as the number of samples for bacterial culture were decreasing. The highest AMR prevalence was detected to be 62% in 2011 and suddenly decreased to 40% in 2013. The decreasing pattern of AMR prevalence was associated with the decrease of samples

submitted for bacterial culture and sensitivity testing. Another study reported that, AMR prevalence in livestock sector is higher due to high imprudence use of antimicrobials in animals (Mubito, *et al*., 2014).

The lowest number of samples submitted for bacterial culture and sensitivity test illustrates the lack of awareness in most of dairy and poultry farmers on the importance of utilizing laboratory diagnosis to enhance animal disease treatment. A study in Dar-es-Salaam reported that, up to 93% of farmers treated their flocks themselves after receiving advice from veterinary drug vendors (Mubito, *et al*., 2014).

The lack of enforcement of legislation bodies to govern antibiotic use in farm animals as well as monitoring and control of their residues as described by other studies in Tanzania (Nonga *et al*., 2009) may have increased the access of veterinary drugs over the counter, and this can explain why samples are inadequately submitted at the laboratory because farmers can easily access antibiotics from veterinary drug vendors and treat their animals by themselves based on the observed clinical symptoms shown by sick animals.

The higher number of mastitis cases in dairy cows illustrates poor management practices such as poor hygiene and udder health. The most bacterial isolates obtained from dairy cows with history of clinical mastitis were *Micrococcus spp, E. coli, Staphylococcus spp, Enterobacter spp, Bacillus spp,* and *Corynebacterium spp*, and however, *E. coli, Salmonella spp* and *Micrococcus spp* were the most isolates detected from chickens.

The study has also found that *E. coli, Staphylococcus spp, Enterobacter spp*, and *Bacillus spp* isolates from cows with mastitis showed more resistance to at least one of the tested antibiotics, and the situation worsened by fungal infection. The most resistance was noted against tetracycline, ampicillin, pen streptomycin and cloxacyclin, the resistance of bacteria to antibiotics ranged between 40 - 62% respectively. Another study reported that, *Staphylococcus hyicus, Staphylococcus intermedius and Staphylococcus aureus* isolated from dairy cattle with mastitis have high levels of resistance to penicillin G, chloramphenicol, streptomycin and oxytetracycline (Mdegela *et al.,* 2009).

In samples obtained from commercial chickens, *E. coli* and *salmonella spp* showed high resistance to amoxicillin, neomycin, streptomycin, ampicillin and cloxacylin (Nonga *et al.,* 2009). Other studies reported an increasing trend in the incidence of antimicrobial resistance with significant increase in multidrug resistant *E. coli, Klebsiella pneumoniae, Staphylococcus aureus* and *Salmonella spp* in food animals, and also indicated an increase in methicillin resistant *Staphylococcus aureus* (MRSA) and extended-spectrum beta-lactamase (ESBL) in the food animals in Tanzania (Mshana *et al*., 2013).

Similarly, high prevalence of Antimicrobial resistance of *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 (Mshana *et al*., 2013). These findings indicates that antimicrobial resistance in food animals is high and surveillance of resistant pathogens is urgently needed in order to implement effective and sustainable control strategies to lower AMR development and spread.

The use of diagnostic tests for detection of bacterial diseases and drug susceptibility is critical to support rational clinical decisions for effective animal disease treatment and sustainable use of antimicrobials. This study suggest; (1) installation and application of WHONET, Afya Data or any other electronic system in the laboratory in order to manage AMR data for improved future data analyses, reporting and sharing to national and international stakeholders; (2) use of advanced biochemical methods for identifying and typing of bacteria so that bacterial species can be typed reliably to determine their susceptibility to antimicrobials at a time; (3) building capacity of dairy farmers on sampling and sample handling techniques to reduce cross contamination of samples; (4) Creating

awareness to smallholder farmers, animal health practitioners, veterinary pharmacies, animal feed millers, and hatcheries on the importance of laboratory diagnosis for effective animal treatment.

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