Microbial Diversity and Antimicrobial-Resistant Profiles of Bacterial Communities in Goats and Sheep

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Committee Page

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We are submitting a thesis by Ashesh Basnet entitled *Microbial Diversity and Antimicrobial-Resistant Profiles of Bacterial Communities in Goat and Sheep Farms*. We recommend that it be accepted in partial fulfillment of the requirements for the degree, Master of Science in Agricultural Sciences, with a concentration in Food and Animal Sciences.

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Dedication

I am profoundly grateful for the unwavering support and encouragement my parents have provided throughout my academic journey. Their love, guidance, and sacrifices have been the foundation of my success, and I am proud to dedicate this thesis to them. Their belief in my abilities and their constant motivation have been a source of inspiration, and this dedication is a small token of my appreciation for their immeasurable contributions to my education and personal growth. Thank you, Mom, and Dad, for being my pillars of strength and for believing in my dreams. This thesis is as much yours as it is mine.

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Abstract

Animal production farms are significant sources of antimicrobial-resistant pathogens and genes, but there's a lack of understanding regarding antimicrobial resistance in small-scale farms. This study gathered 137 fecal samples from goat and sheep farms to investigate antimicrobial resistance and microbial diversity. Using a culture-dependent approach, the study identified prevalent bacteria such as *E. coli* (94.9%), *S. aureus* (91.3%), *S. saprophyticus* (81.0%), *Shigella* spp. (35.0%), and *Salmonella* spp. (3.0%). High resistance was observed against ampicillin (79.4%) and cephalothin (70.6%). Culture-independent results revealed that the dominant phyla in the fecal samples were Firmicutes, Bacteroidetes, Proteobacteria, and Spirochaetes. The α-diversity indices indicated similar microbial diversity regardless of sample type or farm location. However, β-diversity analysis demonstrated significant differences in microbial diversity by sample type and farm location, highlighting substantial variation in microbial community composition. The study underscores the need to explore further the prevalent microbes and resistant genes in these animal communities and their environments. Understanding the extent of resistant bacteria and microbial diversity in goat and sheep populations is vital for informed decision-making in livestock management, disease control, and sustainable agriculture. This knowledge is essential for enhancing the health, productivity, and well-being of these animals and ensuring the safety of food products derived from them.

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Chapter 1: Introduction

Small-scale food animal production is popular throughout the world (Okeno et al., 2012). In the United States of America (USA), goat and sheep are reared for milk, and meat production (Mazinani & Rude, 2020). Small-scale animal farming plays a crucial role in shaping regional food systems through the direct sale of meat and dairy products to local consumers, while also catering to niche markets like organic and natural milk and meat (Rahman et al., 2022). Small-scale animal production, particularly goats and sheep, constitutes a substantial portion of the nation's agricultural landscape.

The potential of animal food sources to combat the concerns of nutrient deficiencies in the world is well recognized (Melse-Boonstra, 2020). Foods of animal origin are dense sources of energy and are acknowledged as a major source of protein, minerals (Fe, Zn, and Ca), vitamins (vitamin B-12 and riboflavin), and fats (Obeid et al., 2019; Tardy et al., 2020). Small-scale food animal production is a key to prevailing malnutrition and food deficiency throughout the world. Various studies buttress the fact that small-scale food production is one of the major means of household income (*FAO - News Article*; *USDA ERS - Farming and Farm Income*; Nesheim et al., 2015) and requires less investment and effort. Such animal farming is also established as a source of side income. Although income is crucial, many small-scale farmers and ranchers consider other factors, such as the enjoyment of the farming or ranching lifestyle, to be equally or more important (Daghagh Yazd et al., 2019; *Small Farms, Big Differences*).

Despite multiple existing benefits of small-scale animal food production, small-scale farmers go through various challenges. Lack of capital, access to veterinary services and knowledge of emerging diseases are major challenges faced by the producers (Arvidsson et al., 2022; Espinosa et al., 2020; Lem, 2019). Infections and illnesses caused by bacteria and parasites can result in severe production and economic losses. In the USA, antibiotics are given to animals

for various reasons including treatment of liver and respiratory infection, and mastitis (Feeds, 1980; Landers et al., 2012a; Phillips et al., 2004). Hence, antimicrobials are widely used in small-scale animal production to combat infections and enhance the survival of animals. In meat-producing animals, especially in developing countries, antibiotics are used as growth promoters. However, the USA (in 2017) and other developed countries (Denmark, UK, France, Sweden) have banned the use of antibiotics as growth promoters (Wallinga et al., 2022). Sweden was the first country to ban the use of antibiotics as growth promoters in 1986 (Begemann et al., 2018; Maron et al., 2013). Restrictions on use of antibiotics as growth promoters are still not practiced in developing countries.

The use of antimicrobials in small-scale food production to prevent and reduce disease has contributed to the emergence of antimicrobial-resistant pathogenic and commensal bacteria (Ikhimiukor et al., 2022). In the United States, antimicrobial-resistant infections account for over 2.8 million infections and over 35,000 deaths each year (CDC, 2022). *Staphylococcus aureus, Pseudomonas enterocolitis, Escherichia coli,* and *Salmonella* spp. are some of the major resistant bacteria of concern in small-scale goat and sheep production (C. Manyi-Loh et al., 2018a; Marshall & Levy, 2011).

The primary sources of zoonotic bacteria transmission to humans are meat and dairy products. The main zoonotic bacterial pathogens that cause food-borne disease and mortality worldwide linked to the consumption of infected animal products include *S. aureus, Salmonella* spp., *Campylobacter* spp., *L. monocytogenes*, and *E. coli* (Abebe et al., 2016; Ehuwa et al., 2021a; Swoveland et al., 2019). The pathogenesis of these bacteria is brought on by the production of toxins and structural pathogenic elements (Heredia & García, 2018a). The prevalence of infections with *E. coli* significantly rose between 2018 and 2019 (Klima et al., 2020).

Antibiotics are administered to ruminants for disease and infection control (A. Abdalhamed et al., 2021). Amoxicillin, ampicillin, ceftiofur, enrofloxacin, erythromycin, lincomycin, oxytetracycline, sulfonamides, penicillin G, trimethoprim, and sulfonamide combination, tylosin, and tilmicosin are some of the antimicrobials routinely used in sheep and goat farming. However, tilmicosin, in the case of goats, is provided through the oral route as subcutaneous delivery has caused death in goats. When given per oral administration, ampicillin, erythromycin, lincomycin, trimethoprim, and sulfonamides, as well as some sulfonamides (e.g., sulfathiazole), can dramatically change the rumen microbial flora and, in some circumstances, cause mortality (Clark, 2013). Except for some sulfonamides and tetracyclines, which may be absorbed well by the rumen, it is preferred to administer antimicrobials through the subcutaneous route than the oral route (feed or water) in adult small ruminants (Hao et al., 2014).

Most foodborne microbial infections are associated with animal-based foods such as milk, meat, and poultry. Infectious pathogens can transfer to humans via direct or indirect contact (*Principles of Epidemiology | Lesson 1 - Section 10*, 2021). Consumption of contaminated milk, and meat and coming in direct or indirect contact with infected animals can transmit resistant pathogens from animals to humans. Due to the abuse of antimicrobial medications in animal feed, the prevalence of multidrug-resistant (MDR) microorganisms in foods is becoming a growing public health problem globally (Pérez-Rodríguez & Mercanoglu Taban, 2019). MDR infections have the potential to penetrate the food chain, posing a serious risk to both animals and humans. MDR pathogens are a challenge due to their resistance to a variety of antibiotics, particularly cephalosporins and carbapenems, as well as their capacity to produce extended-spectrum beta-lactamase (ESBL) (Meletis, 2016a). Furthermore, animal-derived commodities and food-related settings are potential vectors for the dissemination of multi-drug resistance genes, hastening the spread of worldwide antimicrobial resistance

(Ayukekbong et al., 2017). Infections caused by antimicrobial-resistant bacteria pose a grave challenge to healthcare and human resources. They not only have detrimental effects on health but also lead to significant economic losses in the efforts to manage and treat these infections. It is predicted that if the rising rate of AMR is not addressed urgently, by 2050, 10 million people will die each year (O'Neill, 2016). To effectively control infections worldwide AMR epidemic demands novel multidisciplinary solutions such as education on antimicrobial stewardship, awareness campaigns, the use of vaccines, a ban on the use of antibiotics as growth promoters all over the world, and application of government regulations.

The number of antimicrobials used, as well as the length and frequency of exposure, are expected to influence the pace of AMR bacteria formation in ecosystems including the human or animal gut (Kraemer et al., 2019). Assessing the relative importance of factors driving AMR onset and transmission in animal production is a severe issue that will likely endure for some time due to the interconnectedness and interdependence of epidemiological channels between people, animals, and the environment (Collignon & McEwen, 2019). AMR bacteria are becoming more common in meat-producing animals, particularly ruminants, posing a huge concern for human and veterinary health, as well as increasing patient morbidity and death (Ma et al., 2021). The presence of antimicrobial-resistant bacteria in sheep and goats has implications for human infection since their meat can transmit infections and illnesses either during the preparation process or by consumption by consumers (Ma et al., 2021). Antimicrobial-resistant bacteria in animal feces can infiltrate the food chain by fecal contamination of meat and milk with intestinal contents after slaughtering (C. Manyi-Loh et al., 2018). Direct and indirect animal-human contact, as well as human-to-human contact; both have a role in illness transmission (Fong, 2017). Therefore, introducing AMR mitigation strategies on farms in crucial to address the growing concern of antibiotic resistance in goat and sheep farms.

Antibiotic resistant bacteria from goats and sheep can transfer to the farms and to human through goats and sheep fecal deposits. Studying the fecal microbiome of goats and sheep is crucial for enhancing livestock health and productivity (Chen et al., 2021). By deciphering the composition and function of these animals' gut microbiota, their digestive efficiency and overall well-being can be enhanced, leading to increased meat and wool production (Q. Xu et al., 2021). Furthermore, research on goat and sheep feces helps in disease prevention, reducing the need for antibiotics, and promoting sustainable farming practices. Therefore, in this study we focus on understanding the resistance profile of various pathogenic and commensal bacteria while also studying and comparing the fecal microbiome of goat and sheep fecal samples by sample type (goat and sheep) and farm location (West TN, Middle TN, East TN, and Georgia).

Justification of the study

A healthy animal population is a pre-requisite for effective livestock production. Infectious diseases and illnesses associated with poor husbandry result in significant mortality and have a detrimental influence on growth rates, food production and, reproductive efficiency(Hopker et al., 2020; Perry & Grace, 2009). Management of sick animals requires capital, time, and knowledge from animal producers. There are also need for veterinarian relationship with producers, however, not all farmers can afford the veterinarian visit and cost. Lack of awareness regarding the urgency of AMR, limited reach to veterinarian services, limited knowledge, and economic scarcity are some of the major challenges faced by small-scale food animal producers. Veterinarians are vital resources for animal producers, contributing to the productivity of small-scale businesses and the safety of the US food supply; yet certain rural regions may lack a relationship with veterinarians (Enticott et al., 2011; *Tackling the U.S. Food Animal Veterinarian Shortage*). The demand for goat and sheep meat in Tennessee and Georgia has seen an increase in recent years, partly due to changing demographics, including immigration

(Sang, 2016). Small scale goat and sheep producers in Tennessee and Georgia have recognized the increasing demand and therefore, started catering to these niche markets (Denkins, 2015). For goats and sheep producers to be competitive in food animal commerce, they need support services on disease prevention and animal health management skills. It is important to provide these producers with the needed tools and training on the prudential use of antibiotics, prevention, and treatment of diseases. Antimicrobial-resistant bacterial profiles and microbial diversity distinctins data gaps exist in small-scale goats and sheep farming environments in the United States, hence, highlighting this study.

The hypotheses of this study are as follows:

Hypothesis 1: Small-scale goat and sheep production systems represent an important reservoir of antimicrobial-resistant bacteria.

Hypothesis 2: Fecal microbiome of goat and sheep vary by farm type and farm location.

This research project aims to (i) determine the antimicrobial resistance profiles of bacteria and compare the microbial diversity in small-scale goat and sheep farms and (ii) raise awareness on the emergence of resistant bacteria and establish better biosecurity measures among small-scale goat and sheep producers.

The specific objectives of this study are to (i) determine and compare microbial diversity in goat and sheep feces by sample type (goat and sheep) and farm location (East Tennessee, West Tennessee, Middle Tennessee, and Georgia), (ii) determine the resistance profile in bacteria in goat and sheep feces in Tennessee and Georgia, and (iii) develop educational booklets on best management practices and prudential use of antibiotics tailored for small-scale goat and sheep producers.

Chapter 2: Review of Literature

Small-Scale Farms: Economic Perspective

Small-scale animal farming plays an important role in impoverished farmers' efforts to evade poverty. These animals represent valuable financial assets that can be liquidated to address immediate cash needs and cover essential household expenses. Small animals are commonluy favored in small-farming over larger ones because they demand fewer resources (such as land, feed, and time), are more prolific in breeding, and offers a faster turnover rate (Hao et al., 2014; *The Small Livestock Advantage*). Small livestock is frequently used to accumulate assets; farmers may have a small herd/flock, but as the herd/flock grows, they may sell the animals to buy more costly animals that they would not have been able to afford otherwise (Sneeringer et al., 2017). Processed and added-value items such as yogurt, cheese, and processed meats can also provide livelihoods or income; these processed foods serve to bridge food availability, especially during lean seasons.

Tennessee and Georgia Small Scales Farms

Tennessee is a significant producer of a variety of agricultural products. The agricultural output provides more than $3 billion in agricultural cash revenues each year, excluding forest products (*USDA/NASS 2021 State Agriculture Overview for Tennessee*). Cattle and calves, broilers, dairy products, eggs, farm chickens, goats, and sheep are among Tennessee's major agricultural commodities. Tennessee is ranked second in the national meat goat inventory and the milk goat inventory is rapidly increasing as well. The sheep industry in Tennessee is growing rapidly as well. (Tennessee Department of Agriculture). There is high influx of immigrants in Tennessee from regions where goats and sheep meat is a dietary staple such as Middle East, North America, South Asia, and Latin America (Joassart-Marcelli, 2022). As they settle, they bring their culinary traditions and preferences, driving up the demand for goat and sheep meats.

The diversity has also resulted in broader range of food options and increased access to traditional goat and sheep dishes for immigrant communities and others who like to explore international cuisine (Tourigny, 2020).

Animal agriculture is Georgia's largest agricultural industry, accounting for about $5.8 billion in farm gate value (*Farm Gate Value | Georgia Data*). According to the census, there are more than 42,000 farms in operation across the state, covering 9.9 million acres. More than 17,000 of those farms bred beef or dairy cows. Broilers, eggs, beef, and dairy are among the top 10 commodities of this state (*About Georgia Agriculture*). Georgia is also popular for sheep production and dairy and meat goat production is one of the top producers in the nation (*Meat Goat Production in Georgia*).

Common Pathogens in Animal Farming Systems and Zoonotic Diseases

Food of animal origin are the main reservoirs for many foodborne pathogens including *Listeria monocytogenes, Salmonella typhimurium, Salmonella enterica* non-Typhi serotypes, *Escherichia coli* strains (produce Shiga toxin), and *Campylobacter* species (Heredia & García, 2018).

Escherichia coli is a motile, rod-shaped, gram-negative, anaerobic bacterium that exists as a normal flora in the intestinal tract of healthy animals and humans (Titilawo et al., 2015). It serves as a reliable indicator of fecal contamination and indicates the likelihood of enteric pathogens and/or toxins present in food and water, thereby posing a risk to community health. *Escherichia coli* O157:H7 found in cattle manure has been reported to be the most notorious pathogen that produces potent toxins that can cause severe infections in humans (Manyi-Loh et al., 2016). This strain may be referred to as verocytotoxic (VTEC) or enterohaemorrhagic (EHEC) or Shigatoxin-producing (STEC) *E. coli*. The presence of *E. coli* in goats and sheep has been the subject of considerable interest within the area of veterinary and agricultural research.

Goats and sheep naturally harbor various strains of *E. coli* in their gastrointestinal tracts, where they play vital roles in digestion and gut health (Reuben et al., 2022). However, pathogenic *E. coli* strains can pose significant health risks to goats and sheep leading to diarrhea, septicemia, and urinary tract infections (Gambushe et al., 2022). Furthermore, the zoonotic potential of certain *E. coli* strains in goats and sheep emphasizes the importance of this research, highlighting the need for comprehensive strategies to mitigate the risks to both animal and human health.

Salmonella species belong to the family Enterobacteriaceae and are gram-negative, short, rod-shaped, non-spore-forming, motile, non-upturned, aerobic, and anaerobic bacilli (Cox et al., 1983; Oludairo et al., 2022). These pathogens can be found in a wide range of animals including dogs, birds, cats, cattle, pigs, and humans and are responsible for an infection known as salmonellosis. *Salmonella* infections in goats and sheep are a significant concern in farming and veterinary medicine. Goats and sheep are susceptible to various strains of *Salmonella*, which can lead to a range of clinical manifestations (Abdalhamed et al., 2021). Infection may result in gastroenteritis, characterized by diarrhea, fever, lethargy, and loss of appetite (Mondal et al., 2008). In severe cases, *Salmonella* can lead to septicemia, posing a grave threat to animal health and well-being. Moreover, some *Salmonella* strains affecting goats and sheep can be zoonotic, potentially infecting humans and thus necessitating rigorous biosecurity and hygiene measures for those who come into contact with these animals (Libera et al., 2022). Preventative strategies, such as maintaining clean living conditions, providing uncontaminated feed and water, and vigilant surveillance, are vital for minimizing the risk of *Salmonella* outbreaks within herds and flocks. Timely diagnosis and treatment, guided by veterinary expertise, play crucial roles in managing and mitigating *Salmonella* infections in livestock (de Mesquita Souza Saraiva et al., 2022).

Similar to gram negative bacteria, gram-positive (*Staphylococcus aureus, S. saprpohyticus Streptococcus pyogenes, Strep. pneumoniae,* etc*.)* bacteria can also cause zoonoses (Rahman et al., 2020). *Staphylococcus aureus* and *Staphylococcus saprophyticus* are two significant bacterial species that can affect the health of goats and sheep (Andrade et al., 2021). *Staphylococcus aureus* is a common cause of intramammary infections in dairy goats and sheep. These infections can lead to mastitis, a painful inflammation of the udder, resulting in decreased milk production and compromised animal well-being (Al-Rasheed et al., 2022). Additionally, *S. aureus* can be zoonotic, posing a risk to human handlers. On the other hand, *Staphylococcus saprophyticus* is generally considered a less common pathogen in goats and sheep but has been associated with infections such as joint and soft tissue infections, especially in lambs and young animals (Lawal et al., 2021). While both these staphylococcal species have the potential to cause health issues in these ruminants, proper hygiene, and management practices, including regular health monitoring, are essential to minimize the risk of infection and maintain the overall health and productivity of the animals.

Different infectious illnesses arise and spread because of interactions between people, animals, and the environment. Animals are the main source of pathogens that infect people (Thompson & Kutz, 2019). According to the "Asia Pacific strategy for emerging diseases: 2010" study, more than 70% of these pathogens originated from animal species and account for over 60% of human illnesses (A. Li & Kasai, 2011). In recent decades, there have been emergence of novel illnesses in human, often associated with consumption of food products from animal sources (Leroy et al., 2022; Slingenbergh, 2013).

Zoonoses, which pose a serious threat to human health and can potentially be fatal, have had a significant impact. The 13 most common zoonotic diseases alone have caused 2.4 billion cases of illnesses and 2.7 million human deaths each year. These effects are particularly

burdensome for impoverished livestock workers in low and middle-income nations (Grace et al., 2012; Salyer et al., 2017). Moreover, most of the diseases not only harm human health but also have detrimental effect on animal health and livestock productivity.

Antimicrobial Resistance

Antimicrobial resistance (AMR) is a phenomenon where micro-organisms adapt through evolution to survive anti-microbial drugs that are active against a wide range of infections caused by bacteria (antibiotics), viruses (antivirals), fungi (antifungals), and parasites (antimalarials) (Reygaert, 2018). Emergence of the antimicrobial resistance to different classes of antimicrobials are presumed to lead to the emergence of 'superbugs' with the genuine fear that the world would be heading into a post-antibiotic era if alternative drugs are not discovered immediately (Dhingra et al., 2020).

Multidrug resistant (MDR) bacteria have become one of the most present threats to public health. According to Magiorakos et al., 2012 and Wolfensberger et al., 2019, MDR is defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories. Extensively drug-resistant (XDR)is defined as non-susceptibility to at least one agent in all, but two or fewer antimicrobial categories. Meanwhile, pan-drug-resistant (PDR) is defined as non-susceptibility to all agents in all antimicrobial categories (Basak et al., 2016).

Antimicrobial-resistant infections have a significant influence on mastitis and abortions in small ruminants (Pascu et al., 2022). The two pathogens of major concern that causes mastitis and abortion are *Staphylococcus spp*. and *Campylobacter jejuni* (Chlebicz & Śliżewska, 2018). Antimicrobial-resistant infections may be more challenging to treat and necessitating several rounds of therapy with different antimicrobial medications (Fair & Tor, 2014). Poor health has a detrimental impact on animals' well-being and cause farmers to incur large financial losses by lowering the quality and output of milk, meat, and fiber (Sinclair et al., 2019).

Naturally, antimicrobial resistance occurs through genetic alterations over time (Munita & Arias, 2016). However, the abuse, misuse, and overuse of antimicrobials are accelerating the emergence and spread of antimicrobial resistance (WHO, 2021). Antimicrobial-resistant microorganisms can spread between people and animals, including from food of animal origin (Woolhouse, Ward, van Bunnik, and Farrar, 2015). Poor infection control, inadequate sanitary conditions, and inappropriate food handling encourage the spread of antimicrobial resistance.

AMR has been cited as one of the most serious silent pandemic problems of increasing importance. The US Center for Disease Control and Prevention (CDC) estimated that in the US alone, more than two million people are affected by antimicrobial-resistant infections every year, with at least 23,000 deaths as a result of that infection and unless global attention is paid to the problem of AMR it could further aggravate (Zaman *et al*., 2017).

A dramatic increase over the past decade both in the proportion and absolute number of bacterial pathogens showing multiple resistance to essential drugs discovered to date is of serious concern. It is quite challenging to quantify the influence of antimicrobial resistance in terms of morbidity, mortality, and public health cost, however, few studies have been done to address this issue (Dadgostar, 2019; Jit et al., 2020; Naylor et al., 2018). WHO declared that “combat drug resistance: no action today, no cure tomorrow” in 2011. In the first decade of this century, strains of multidrug-resistant organisms have quadrupled worldwide (Magiorakos et al., 2012) and the emergence of antimicrobial resistance could lead to an “apocalyptic threat”. Serious concerns have arisen because not only multidrug resistant (MDR) but extensively drug-resistant (XDR) and pan drug-resistant (PDR) bacteria have been categorized and defined using standardized international terminology established by the European Center for Disease Control (ECDC) and Center for Disease Control and Prevention (CDC) (Saha & Sarkar, 2021).

Antimicrobial resistance (AMR) is a critical concern in goat and sheep farming, reflecting the broader global challenge of drug-resistant bacteria (Haulisah et al., 2021). Goats and sheep often receive antibiotics for various reasons, including treating bacterial infections, enhancing growth, and safeguarding against disease outbreaks (Lianou & Fthenakis, 2022). The presence of AMR in goats and sheep carries a zoonotic risk, as resistant bacteria may be transmitted to humans through direct contact or via the consumption of contaminated meat or dairy products. Therefore, a comprehensive One Health approach, involving collaboration between veterinary professionals, farmers, human health experts, and policymakers, is crucial to address AMR in goats and sheep. This approach includes promoting responsible antibiotic use, improving farm hygiene and biosecurity, monitoring, and surveillance of resistance patterns, and exploring alternatives to antibiotics. Ultimately, mitigating AMR in goats and sheep is vital for both animal and human health.

Nature and Scope of Antibiotics Use in Food Animals

Animal agriculture's usage of antibiotics increases the risk of drug resistance (H. M. Scott et al., 2019a). Although there is a paucity of comprehensive information about antibiotic usage in animals, data that is currently available indicates that around 70% of all medically significant antibiotics supplied in the United States are intended for agricultural use (Patel et al., 2020a). To track and evaluate the use of antibiotics in American animal agriculture, this is the only information that is currently accessible, but it is insufficient. In food animals, antibiotics are used to treat clinical illness, prevent, and manage typical disease occurrences, and promote animal development (Economou & Gousia, 2015). Antibiotics can be used in a variety of ways in food animals, including therapeutic, prophylactic, and subtherapeutic uses (Gelband et al., 2015). A single animal with a clinical condition or many animals can be treated with antibiotics. As per the new VFD, all antibiotic use in livestock requires a veterinary prescription (Medicine, 2023),