Research Journal of Animal, Veterinary and Fishery Sciences _____ISSN 2320 – 6535
Vol. **12(2),** 14-17, November **(2024)** *Res. J. Animal, Veterinary and Fishery Sci.*

Determination of Multidrug-resistant Livestock associated Bacteria from Goats, Cows, and Buffaloes in Pokhara Kaski, Nepal

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Available online at: www.isca.in, www.isca.me
Received 28th June 2024, revised 8th September 2024, accepted 24th October 2024

Abstract

Misuse of antibiotics in people and animals resulted in bacteria becoming resistant to several drugs. Antibiotic abuse is likely rampant among goats, cows, and buffaloes in order to boost growth and reduce production losses. The aim of this study is to determine the multidrug-resistant (MDR) bacteria in goats, cows, and buffaloes. Out of 68 samples that were examined, S. aureus, Bacillus spp., E. coli, Shigella spp., Klebsiella spp., S. epidremidis, and Salmonella spp. were isolated. S. aureus was the most isolated bacteria (91.17%), followed by Bacillus spp. (61.76%), E. coli (48.52%), Shigella spp. (22.05%), Klebsiella spp. (17.64%), S. epidermidis (13.23%), and Salmonella spp. (7.35%). Multidrug resistance was demonstrated by Salmonella spp. and E. coli to at least four antibiotics (Amoxicillin, Tetracycline, Piperacillin, and Ciprofloxacin) and to at least three antibiotics (Amoxicillin, Tetracycline, and Nalidic Acid). The highest-resistant bacteria, Salmonella *spp., showed (57.14%)* E. coli *and* Bacillus *spp. showed (42.85%),* S. aureus, S. epidermidis, *and* Shigella *spp. showed (28.57%), and* Klebsiella *spp. showed (14.28%). This study demonstrated that healthy farm animals like goats, cows, and buffaloes harbor large concentrations of antibiotic-resistant bacteria that are resistant to tetracycline, amoxicillin, and penicillin. This will probably mean fewer options for antibiotic therapy—for both humans and animals. Therefore, there needs to be tight regulation over the usage, distribution, storage, and sale of antibiotics in veterinary offices.*

Keywords: Multidrug resistance (MDR), multidrug resistance bacteria, susceptibility testing, bacterial infections.

Introduction

Nepal is a landlocked agricultural country in South Asia between India and China, where agriculture is the primary source of national income, accounting for around 32% of national GDP and employing 65% of the economically active population. Domestication is well recognized not only for its sluggish progression but also for its drastic technological and economic changes, from hunting and gathering to food production. Many key aspects influence it, including a strong demographic transition, the development and husbandry of valued domesticates, as well as profound social and spiritual transformations. Cattle are huge, domesticated animals with cloven hooves. Livestock production is a major employer and source of food worldwide. It is one of the agricultural subsectors that is expanding the quickest. In order to maintain global food security in the future and satisfy the growing demand for foods derived from animals, the livestock sector will be essential. The Nepalese agricultural sector includes an important subsector called livestock, which makes up around 25% of the country's agricultural GDP. Buffalo, cattle, goats, sheep, pigs, and poultry serve as the primary agricultural animals in Nepal's domestic livestock industry[1].

Antimicrobial resistance displayed by a type of microbe to at least one antimicrobial drug in three or more antimicrobial categories is known as multidrug resistance (MDR). Antimicrobial drugs are categorized according to their unique target species and manner of action. The MDR types that present the biggest risks to public health are those that are resistant to many antibiotics.

Globally, antibiotic resistance is a serious problem that affects both human and animal health. The fact that certain germs are resistant to many antibiotics ranks among the top ten hazards to global health[2]. Antibiotics are probably used excessively to stimulate growth and reduce production losses in goats, cows, and buffaloes. With technical assistance from Bangladesh, the National Public Health Laboratory, which serves as the focal point for antimicrobial resistance surveillance, started in Nepal in 1999. In order to promote growth and avoid disease, sub-therapeutic doses are likely to create resistant bacteria that can spread to people. There is still a lack of safe and responsible antibiotic usage on dairy farms, and residues in milk and meat pose a serious threat to public health[3]. Nowadays, there is an increasing need for high-quality animal protein in emerging nations. When livestock is allowed to roam freely, they occasionally come into contact with tainted food, water, and sick people[4]. Farmers and animals have been using human-designed antibodies to treat or prevent certain diseases, and this has been linked to the rise of pathogens that are resistant to several drugs in both humans and animals. The primary cause of

the rise in multidrug-resistant bacteria is the widespread use of inadequate antibody levels in animal farming, which includes the raising of goats, cows, and buffaloes. The common commensal opportunistic pathogen *Staphylococcus aureus* has a detrimental effect on the health of humans and animals, as well as the cattle industry[5]. This bacterium is widely acknowledged as a significant pathogen that affects breast-feeding cows, buffaloes, and goats, producing mastitis. Though the udder is considered the principal site of infection in dairy farms, the nares are likely sources of contamination for the udder and milk. *Staphylococus aureus* isolation from the nares of agricultural animals has recently been widely documented[6]. In veterinary medicine, anthrax is thought of as a single illness that quickly proceeds to death without any particular clinical signs, making this classification less relevant. It is frequently characterized by a large septicemia where the lethal toxin (LeTx) is primarily blamed for hypotension, shock, and abrupt death. *Escherichia coli* is one of the most common causes of mastitis, usually associated with acute symptoms such as diarrhea and cool extremities. *Shigella occurs* in impoverished nations where the immune systems of the impacted populace are weakened by malnutrition and underlying diseases, resulting in a high morbidity rate from schigellosis. It is known as a zoonotic infection and is caused by the bacteria Salmonella. Salmonellosis is a prominent cause of bacterial enteric sickness in both humans and animals. *Salmonella* is the organism that causes this illness[7]. Certain species of *Klebsiella* can develop into serious bacterial infections that can cause meningitis, pneumonia, bloodstream infections, wound infections, and urinary tract infections. Both humans and other animals have *Staphylococcus epidermidis* in their nasal flora, and in some situations, it can lead to infection. The inflammatory process in the current investigation was most likely caused by *Staphylococcus epidermidis* infection and colonization.

Many of the antimicrobial substances used in human medicine are also used in livestock production and treatment, including Gentamycin, Erythromycin, Tetracycline, Amoxicillin, Piperacillin, Norfloxacin, Nalidic Acid, Ciprofloxacin, Penicillin, Chloramphenicol, and Vancomycin. According to the World Health Organization for Animal Health (OIE) Guidelines for Antimicrobials, these medications are classified as "veterinary critically important antimicrobials," highlighting their importance for both human and animal therapies[8].

Materials and Methods

A descriptive cross-sectional study was conducted from April to June 2023 at the Microbiology Laboratory of Prithvi Narayan Campus, Pokhara. A total of 68 nasal swab samples, comprising goats (18), cows (20), and buffaloes (30), were collected, and laboratory analysis and the isolation of bacteria were performed using the aseptic technique. In accordance with the Clinical and Laboratory Standards Institutes (CLSI, 2021), the antibiotic resistance was ascertained by the disk diffusion method (adapted by Kirby-Baeur). The purpose of the antibiotic susceptibility testing was to evaluate the antibiotics' efficacy against bacterial isolates and ascertain the patterns of susceptibility.

Results and Discussion

68 samples in all were taken from buffaloes (n = 30), cattle (n = 20), and goats (n = 18). Every single one of the 68 (100%) samples had bacteria. Nostril swab samples yielded the isolation of seven categories of bacteria. *Staphylococcus aureus* (91.17%), *Bacillus* spp. (61.76%), *Escherichia coli* (48.52%), and *Shigella* spp. (22.05%) were the most common bacterial isolates found in the samples. Conversely, *Salmonella* spp. (7.35%), *Staphylococcus epidermidis* (13.23%), and *Klebsiella* spp. (17.64%) were the least common bacterial isolates. Six distinct types of bacteria were present in the buffalo, five different types were in the cow, and four different types were in the goat. In *Klebsiella* and *Salmonella*, antibiotic resistance ranged from 14.28% to 57.14%. *Salmonella* (57.14%), *E. coli* and *Bacillus* spp. (42.85%), and *S. aureus*, *S. epidermidis*, and *Shigella* spp. (28.57%) showed the highest levels of resistance. The isolates were extremely resistant to Amoxicillin, Penicillin, Tetracycline, Nalidic Acid, Piperacillin, and Ciprofloxacin, among other antibiotics. Amoxicillin and penicillin resistance was more widespread in goats, cows, and buffaloes, according to the resistance pattern.

Discussion: This study was conducted among 68 goats (n = 18), cows (n = 20), and buffaloes (n = 30) samples collected from different farms and animals in Pokhara Valley with the objective of determining multidrug-resistant livestock-associated bacteria in goats, cows, and buffaloes. To accomplish this, the samples were subjected to the isolation and identification of bacteria, gram staining, enzymatic testing, and biochemical testing. Furthermore, testing for antibiotic susceptibility was done using the Kirby-Bauer disk diffusion method. These tests facilitate the assessment of the samples' sensitivity or resistance to different antibiotics. In nasal swab samples, the presence of *S. aureus* is a great concern due to their association with nasal secretion fluids, and they pose a health risk to animals as well as humans. These bacteria can cause a range of diseases in farm animals, including mastitis in dairy cows, which can result in the loss of milk production and even death in extreme cases. These bacteria can also infect humans who come into contact with farm animals or their products, causing skin infections or more serious conditions such as sepsis[9].

In total, 178 bacterial isolates of *S. aureus* were the most prevalent, accounting for 91.17% of the total isolates. The next most frequently identified species was *Bacillus* spp., comprising 61.76% of the total isolates, followed by *E. coli* (48.52%), *Shigella* spp. (22.05%), *Klebsiella* spp. (17.64%), and *S. epidermidis* (13.23%), while *Salmonella* spp. was the least commonly found bacteria, representing 7.35% of the total isolates. This finding aligns with several other studies[10]. It's

critical to establish appropriate sanitation and hygiene procedures on the farm in order to reduce bacterial growth and stop the emergence of antibiotic resistance. This includes the regular cleaning and disinfection of animal housing areas, equipment, and utensils used in milk production. Additionally, prevention measures such as vaccination or medication can prove to be effective in reducing the prevalence of *S. aureus* in farm animals. Proper use of antibiotics, limiting their use to only when necessary and under the guidance of a veterinarian, can also help prevent antibiotic resistance[11].

Using microbiological and epidemiological techniques, the epidemiology and antimicrobial susceptibility of several species of bacteria isolated from goats, cows, and buffaloes were described in this study. The idea that different bacteria commonly infect farm animals utilized for food production is supported by the predominance of bacteria found in the described samples. There were differences in the location and identification of the isolated bacteria in the samples in this investigation. *S. aureus* and *S. epidermidis* showed high and low prevalent populations, respectively. From different farm animals, seven types of bacteria were isolated. Buffaloes had a high prevalence of some types of bacteria (85.71%), whereas cows had (71.42%) and goats had (57.14%). The types of bacteria described here give an idea of the likelihood that these buffaloes and other farm animals will come into contact with bacteria. In reality, the findings suggest that the causes may be complex because it was clear from a visual inspection of the animals and feeds that the level of sanitation was subpar. For instance, there were different animals and unclean surfaces. Bacterial contamination was associated with similar criteria, such as animal density and scrapings, manure storage, water, and pest control, among others.

Table-1: Distribution of Bacteria Isolates from Goats, Cows and Buffaloes.

Isolated bacteria	Prevalence in different sample			Total (n=68)
	Goat (n=18)	Cow (n=20)	Buffalo (n=30)	
Bacillus spp.	12 (66.7%)	12 (60%)	18 (60%)	42 (61.76%)
S.aureus	18 (100%)	14 (70%)	30 (100%)	62 (91.17%)
S.epidermidis	-	9 (45%)	-	9 (13.23%)
E. coli	9 (50%)	10 (50%)	14 (46.67%)	33 (48.52%)
Klebsiella spp.	6 (33.3%)	5 (25%)	1 (3.3%)	12(17.64%)
Salmonella spp.	-	-	5 (16.67%)	5 (7.35%)
Shigella spp.	-	-	15 (50%)	15 (22.05%)

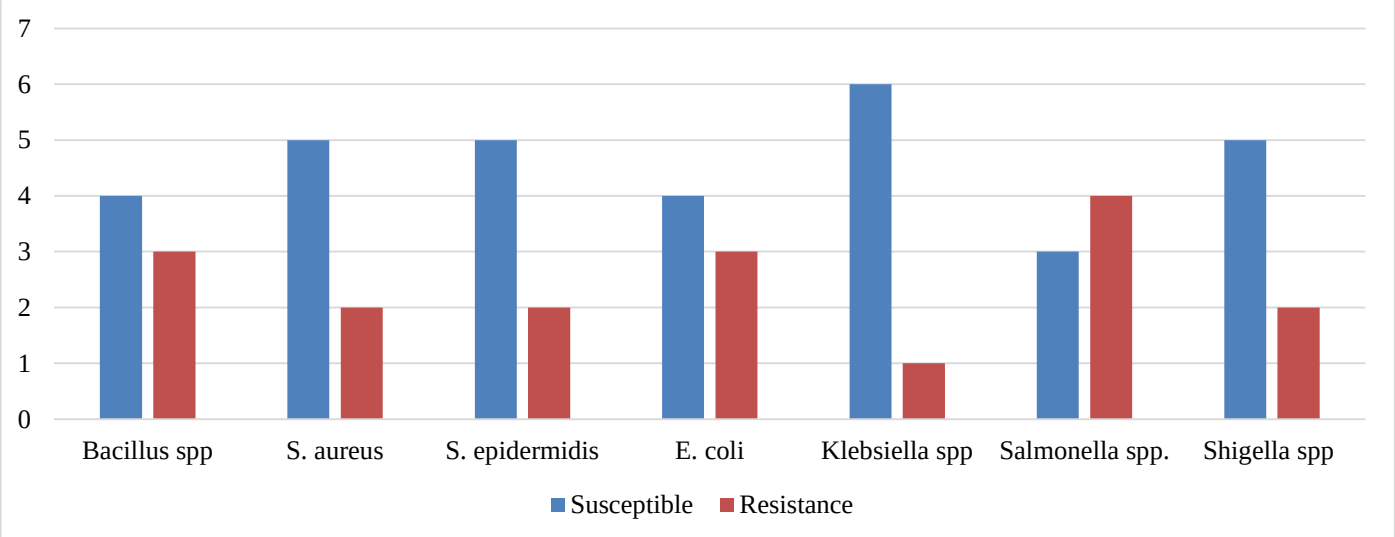

Figure-1: Antimicrobial Susceptibility Profile of Isolated Bacteria.

This study discovered a unique bacterial profile that was resistant to antibiotics in goats, cows, and buffaloes. *Salmonella* spp. and *E. coli* showed high multidrug resistance profiles to routinely used antibiotics in goats, cows, and buffaloes. For example, these bacteria have consistently demonstrated resistance to Ciprofloxacin, Penicillin, and Amoxycillin. Given that farmers have openly acknowledged using antibiotics to treat their animals, it seems plausible that inappropriate antibiotic usage on farms is the cause of the multidrug resistance issue. Others had mentioned doing so independently of veterinary authorities. According to this, farmers are not using antibiotics in a way that complies with WHO recommendations for animal production. Another reason could be that the owners let their animals roam freely, particularly after harvesting crops, and that the animals were also exposed to other chemicals, such as those that resembled antibiotics and could promote cross-resistance.

Not only in Nepal but also on other continents where agriculture has been booming, the resistant strains are widely spread. In samples taken from dairy farm animals in Ethiopia, comparable patterns of resistance for *Salmonella* spp., *E. coli,* and *S. aureus* have been observed. However, the current study was conducted on physically healthy animals; the results are unique. According to the study's findings, even though farm animals may appear physically healthy, they may harbor bacteria that have genes for resistance, much like humans. This environment affects food security and safety significantly since humans can be exposed to it. This rise in resistance to commonly used antibiotics in local hospitals poses a severe threat to low- and middle-income countries with limited access to antibiotics. This should act as a warning to the Ministry of Agriculture and all farmers to push for consumer-protecting regulations, like careful surveillance of antibiotic resistance in agriculture and appropriate use of antibiotics. Additionally, it is highly recommended that Nepal and other developing nations properly oversee and control the handling, distribution, storage, and retail of antibiotics. These precautions can safeguard citizens who are unaware of the health concerns linked to these bacterial strains while helping to minimize the emergence and spread of resistant variants.

Conclusion

This study gives information on the profiles of strains of antimicrobial resistance that were found in goats, cows, and buffaloes in Nepal. The most commonly isolated bacteria, according to the study, are *Salmonella* spp., *E. coli, Shigella* spp., *Klebsiella* spp., *S. aureus*, and *Bacillus* spp. The most common types of resistance found in the samples were to tetracycline, amoxicillin, and penicillin; however, multidrug-resistant strains of *Salmonella* spp. *and E. coli* were the most prevalent. The data presented here shows that mitigation actions are needed to stop the spread of resistant bacterial strains in farm animals in order to address the issue of antibiotic resistance in Nepal and around the world.

Acknowledgements

The Microbiology Laboratory in Pokhara is appreciated by the authors for allowing them to carry out the study. We are especially grateful to the owners of the different cow farms who let us do the research.

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