



## Antibiotics Resistance Trends of Clinical Bovine Mastitis in Cross Breed Cattles of Morang District, Nepal

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### Abstract

Mastitis is the inflammation of the mammary gland, the most frequent bacterial infection seen in dairy cattle causing reduced milk production, changes in milk composition and huge economic loss (almost 70%). Great technological advances and modernization have been made in veterinary field for improvement of disease treatment and prevention till today. However, mastitis continues to be a major economic issue to dairy producers for its handling, treatment and prevention. Nepal is one of the major contributors to the leading to Antibiotics Microbial Resistance (AMR) due to widespread irrational use of antibiotics along with poor health care systems, poor infection control and prevention measures. The antimicrobial resistance is also a growing concern for human health as the animal products are used by humans directly as food products and in via direct or indirect contact. Thus, this research was done to study antibiotic resistance trends of clinical bovine mastitis in cross breed cattle's i.e. Jersey cross and Holstein Friesian cross of Biratnagar, Morang, Nepal. A total of 106 milk samples were collected from cattle's. Of which, 80 samples were of Jersey cross and 26 samples were of Holstein Friesian cross cattle's. Five antibiotics i.e. Penicillin, Gentamycin, Ciprofloxacin, Amoxicillin and Tetracycline which are commonly used to treat mastitis were used for antibiotic sensitivity test. The samples were monitored for antibiotic resistance following disk diffusion method. The result revealed that the antimicrobial resistance is very high in case of Gentamycin, Oxytetracycline, Amoxicillin and Penicillin i.e. greater than 50%. The sensitivity of Ciprofloxacin (23.6%) was highest among five drugs however it is very low to be claimed as a proper drug to be used for treating mastitis for further years. It showed that the drugs which were frequently and indiscriminately used to treat mastitis are now on verge of complete resistance and ineffective against bacteria causing mastitis Ciprofloxacin had least resistivity as it was least frequent use in the study area in treatment of clinical mastitis. Thus, it is the most effective drugs that can be used to treat mastitis in Biratnagar area. The results of the present investigation demonstrated the development of very high level of resistance of drugs that are commonly used to treat mastitis in Biratnagar area. This research gave an alarming message to human, veterinary experts regarding misuse of antibiotics in field level, lack of implementation of laws about drug use by the government and less concern towards animal-human-health relationship caused by misuse of drugs in animal products.

**Keywords:** Mastitis; Resistance; Disk Diffusion; Antibiotics

### Introduction

Mastitis is the inflammation of the mammary gland, the most frequent bacterial infection seen in dairy cattle causing reduced milk production, changes in milk composition and huge economic loss (almost 70%) [1]. The disease is the infection of milk synthe-

sizing alveolar tissue leading to gross abnormality of milk associated with pathological changes of udder in the form of pain, edema, fibrosis, and milk damage, the extent depending on the severity of infection, etiological agent, physical condition of the animal, environment and so on [2]. Great technological advances and mod-

ernization have been made in veterinary field for improvement of disease treatment and prevention till today. However, mastitis continues to be a major economic issue to dairy producers for its handling, treatment and prevention [2]. Nepal is a developing country located between the two major emerging economies of China and India. Nepal's economy, where majority of peoples income is primarily based on agriculture and livestock. The livestock sector alone contributes around 13% to the national gross domestic products (GDP) and 27% to the agricultural gross domestic product (AGDP) [3]. The demand for livestock products has increased with increasing per capita income and hence livestock industry are also expanding exponentially. Livestock farming is growing at an annual rate of around 1.23% in Nepal [4]. With modernization, livestock production is shifting toward intensification and modernization whereas traditional subsistence farming is diminishing in an increasing rate. But with the advancement in technology and medicine, people are more concerned towards more production, profit and monetization rather than balance in health, management and safety of animals and human beings.

A survey on distributors of veterinary medicines and feed supplements in 2003 in six Nepali districts reported annual sales of USD 6.7 million [3]. Over 70% of veterinary drug sales were obtained from retailers, technicians and local professionals and not prescribed by veterinary professionals. There is no any strict law and policy for a limited use of antibiotics. So there is total misuse of the drugs from every level, technicians and doctors. Generally, Antibiotics are used to treat mastitis along with other needed medications in Nepal .Tetracycline, enrofloxacin, neomycin-doxycycline, levofloxacin, colistin, and tylosin are the top seven antibiotics consumed in Nepal with ampicillin, amoxicillin, ceftriaxone, and gentamicin being the most inappropriately prescribed medicines (DoDA. DDA: Drug Bulletin of Nepal. Kathmandu: Ministry of Health and Population). Nepal is one of the major contributors to the leading to Antibiotics Microbial Resistance(AMR) due to widespread irrational use of antibiotics along with poor health care systems, poor infection control and prevention measures [5,6]. Bacterial pathogens are becoming highly resistant to most first- and some second-line antibiotics as stated by many research conducted. The irrational and injudicious use of high doses of antibiotics for therapy and sub-optimal use of alternative medications, poor husbandry practices with inappropriate infection prevention and control (IPC) and lack of awareness on good management practices (GMP) are lead-

ing causes of Antimicrobial Resistance [5,7,8]. Most farmers have no good knowledge about antimicrobial residue and withdrawal periods of the drugs. Farmers themselves use antibiotics to compensate poor farm sanitation and hygiene. The lack of knowledge about drug withdrawal periods and its negative impact on animal and human health combined with long term use of drugs leads to animal products with residues above the permitted level which increases antimicrobial resistance [9].

The antimicrobial resistance is also a growing concern for human health as the animal products are used by humans directly as food products and in via direct or indirect contact [10]. Hence, we need to know the resistance level and trends of pathogens to allow appropriate prescription of antibiotics and ensuring long term efficacy of antibiotics. Studies have been done in different time, places and proportion but we need to be specific in selected population in selected geographical region to know about antibiotic resistance trend in that area as it is related to human health, animal health and veterinary medicine [11]. Various research have been conducted in Nepal regarding antibiotic resistance trends but it need to be updated time and again to know about the antibiotic sensitivity and resistivity against bacterial pathogens causing bovine mastitis.

Thus, this research was done to study antibiotic resistance trends of clinical bovine mastitis in cross breed cattle's i.e. Jersey cross and Holstein Friesian cross of Biratnagar, Morang, Nepal.

### Research objective

- To evaluate the antibiotics which are resistant to pathogens causing mastitis.
- To compare the sensitivity and resistivity of antibiotics which are generally used in veterinary practice to treat mastitis.
- To aware veterinary practitioners by demonstrating that antimicrobial resistance is developing rapidly than we expect.
- To connect the issue of misuse of antibiotics in animals, milk consumption and human health.

### Materials and Methods

#### Study area

This research was conducted in Regional Veterinary Laboratory (RVL) which is situated in sub-metropolitan city, Biratnagar-17, of eastern Nepal which was established in the fiscal year 1988/1989 AD. The major objectives of RVL, Biratnagar are:

- To provide prompt and efficient disease diagnostic services to the farmers of the region.
- To investigate and diagnose the epidemics in the region
- To assist and support DLSOs in disease diagnosis and epidemic control.
- To collect, analyze and predict the animal diseases prevailing in the region.
- To co-ordinate and support national animal disease control and eradication program.



**Figure 1**

<https://www.google.com/maps/place/Biratnagar>

### Data collection

A total of 106 milk samples were collected which were brought in Regional Veterinary Laboratory, Biratnagar as a suspect of mastitis. Among those samples, 80 samples were of Jersey cross and 26 samples were of Holstein Friesian cross cattle's. There was difference in age, parity and physical condition among the cattle's used in the research.

### Sampling

Equal amount of milk samples from all four quarters respectively were taken and was tested for mastitis using California Mastitis Test, also known as cow-side SCC test. In this test, equal amount of reagent is added with equal amount of milk in each four well. This reagent acts as a detergent with a pH indicator, meaning it will disrupt the cell wall of somatic cells present in the milk causing the cells to release their contents. The DNA released from the cells' nuclei will string together forming a gel, which is indicative of an increased somatic cell count [12]. Samples are differentiated as positive and negative based on formation of gels or absence of it respectively.

Positive milk samples were then collected aseptically in 10 ml quantities following standard methods. The samples were stored in ice box and processed accordingly.

### Sample processing methods

The samples were monitored for antibiotic resistance following disk diffusion method [1] according to Clinical and Laboratory Standard Institute (CLSI) guidelines.

- At first, milk samples were streaked using inoculation loop in Nutrient Agar and McConkey agar for the development of bacterial colony i.e. the bacteria which is the cause of mastitis.
- Then, these agar plates were incubated for 24 hours in 35°C in incubator.
- Then, the bacteria developed within 24 hours were emulsified aseptically in Nutrient broth for 4 hours until turbidity appears.
- After that, a sterile swab was inserted in broth culture of organism to collect the test organism.
- Then, the swab was streaked to the surface of a large Mueller-Hinton agar plate.
- Five antibiotics i.e. Penicillin, Gentamycin, Ciprofloxacin, Amoxicillin and Tetracycline which are commonly used to treat mastitis were used for antibiotic sensitivity test [5,13]. Thus, commercially prepared, fixed concentration, paper antibiotics disk of these five antibiotics were placed on the inoculated agar surface for 24 hours in the incubator at 35°C.
- At last, the sensitivity and resistance patterns were recorded by comparing the diameter of zone of growth inhibition with the zone diameter interpretation criteria published by Clinical and Laboratory Standard Institute (CLSI) [14].
- The diameter of the zone is related to the susceptibility of the isolate and to the diffusion rate of the drug through the agar medium.
- The result of the disk diffusion test are "qualitative" in which result is divided into three category of susceptibility (susceptible, intermediate or resistant).
- The obtained data were expressed in percentage [15].

## Result

For this research, total of 106 milk samples were collected for California Mastitis Test to diagnose mastitis. Of which, 80 samples belonged to Jersey cross cattle's and rest to HF cross cattle's. The mean age of Jx and HFx cattle's were 4.3 years and 4.8 years respectively. Similarly, the mean parity of Jx and HFx cattle's were 3 and 2.5 respectively.

The CMT test result had no any significant relationship with antibiotic resistance against the pathogens. However, it lead to a conclusion that mastitis may occur irrespective of the quarter the pathogens has entered.

This research was conducted to know the antibiogram trends in bovine mastitis of cross breed cattle's of Biratnagar, Morang as described in table 3. The result revealed that the antimicrobial resistance is very high in case of Gentamycin, Oxytetracycline, Amoxi-

cillin and Penicillin i.e. greater than 50%. Similarly, the resistivity of ciprofloxacin is also very alarming (37.7%). The resistivity of Penicillin (90.6%) is highest followed by Amoxicillin (86.8%), Gentamycin (71.7%) and Oxytetracycline (58.5%).

Breed	Jersey cross(Jx)	Holstein Friesian cross(HFx)
Total milk sample collected	80	26
Age(mean)	4.3 years	4.8 years
Parity(mean)	3	2.5

**Table 1:** General Information.

CMT Result	Left Rear	Left Hind	Right Rear	Right Hind
Positive	73.6%	61.3%	73.6%	59.4%
Negative	26.4%	38.4%	26.4%	40.6%

**Table 2:** California Mastitis Test Result.

Antibiotics	Sensitivity(S)	Intermediate(I)	Resistance(R)
Gentamycin(J)	0.9%	27.4%	71.7%
10mcq	(15 mm or more)	(13-14 mm)	(12 mm or less)
Ciprofloxacin(CL)	23.6%	38.7%	37.7%
5mcq	(21 mm or more)	(16-20 mm )	(15 mm or less)
Oxytetracycline(O)	10.4%	31.1%	58.5%
30mcq	(19 mm or more)	(15-18 mm )	(14 mm or less)
Amoxicillin(AM)	4.7%	8.5%	86.8%
10mcq	(18 mm or more)	(14-17 mm )	(13 mm or less)
Penicillin(P)	4.7%	4.7%	90.6%
10units	(28 mm or more)	( 20-27 mm)	(19 mm or less)

**Table 3:** Antibiogram Trends in bovine mastitis (Based on diameter of zone of inhibition).

\*Sensitivity(S): When bacterial strain is inhibited by a concentration of this drug that is associated with a high therapeutic success. Intermediate (I): When bacterial strain is inhibited by a concentration of this drug that is associated with an uncertain therapeutic effect. Resistant (R): When bacterial strain is inhibited by a concentration of this drug that is associated with a high therapeutic failure [19].

The sensitivity of Ciprofloxacin (23.6%) was highest among five drugs however it is very low to be claimed as a proper drug to be used for treating mastitis for further years. The sensitivity of Oxytetracycline, Amoxicillin, Penicillin and Gentamycin obtained was 10.4%, 4.7%, 4.7% and 0.9% respectively which is very low and similar to result obtained by [15]. These data were completely in contrast with data's obtained by [16-18].

Similarly, the five drugs used were also in intermediate state against the pathogens causing mastitis. The result showed Cipro-

floxacin is in highest intermediate level (38.7%) followed by Oxytetracycline (31.1%), Gentamycin (27.4%), Amoxicillin (8.5%) and Penicillin (4.7%).

## Discussion

The results of the present investigation demonstrated the development of very high level of resistance to frequently used drugs in bovine mastitis [15]. The antimicrobial resistance may be qualified poor when bacterial resistance increases above 75.00% and favorable when resistance remains below 25.00% [20]. The antibi-

otics used in this research were drugs that are frequently used in this area [15]. Penicillin, Oxytetracycline, Amoxicillin, Gentamycin and Ciprofloxacin were drugs that are commonly used to treat mastitis in Biratnagar area. Higher resistance was seen against Penicillin, Amoxicillin, Gentamycin and Oxytetracycline as they were most frequently and indiscriminately used drugs to treat mastitis. It showed that the drugs which were frequently and indiscriminately used to treat mastitis are now on verge of complete resistance and ineffective against bacteria causing mastitis [1,5,7,15]. Ciprofloxacin had least resistivity as it was least frequent use in the study area in treatment of clinical mastitis. Thus, it is the most effective drugs that can be used to treat mastitis in Biratnagar area. These data contrasted with [1,8,18] in their respective locations. Besides these drugs, other drugs may or may not be used as an effective drug to treat mastitis depending on their resistivity and sensitivity against the bacterial pathogens.

Antimicrobial resistance is caused due to improper biosecurity, lack of hygiene and more exposure to antibiotics that lead to burden of resistance in dairy farms [1]. In dairy farming, large amount of antibiotics as prophylactic and growth promoting agents are used. This non therapeutic application of antibiotics, their dosage, and withdrawal period needs to be re-evaluated and rationally defined [21]. Effective surveillance programs at multidisciplinary level will help to better understand and minimize the emergence of resistance. Establishment of a surveillance programme and a national plan following the National Antibiotics Treatment Guideline 2014 and generation of awareness among veterinarians, technicians, and medical physicians regarding use of antimicrobial drugs in Nepal could reduce the burden of Antimicrobial Resistance [5]. Besides, all countries need to be on the same side to tackle this global health threat, and in order to achieve this, efforts from each country have to be in line with the WHO's global action plan on AMR, which focuses on improving public awareness, strengthening knowledge on AMR through surveillance, reducing incidence of AMR infection, optimizing antimicrobial usage and developing sustainable investment for new AMR interventions [8].

## Conclusion

The results of the present investigation demonstrated the development of very high level of resistance of drugs that are commonly used to treat mastitis in Biratnagar area. This may be due to frequent and indiscriminate use of antimicrobial drugs without antibiotic sensitivity test prior to treatment and prevention of

mastitis. Since the research was done in a limited population in a limited area using limited drugs, it do not reflect a true picture of antimicrobial resistance. However, this research gave an alarming message to human, veterinary experts regarding misuse of antibiotics in field level, lack of implementation of laws about drug use by the government and less concern towards animal-human-health relationship caused by misuse of drugs in animal products. Thus, research and studies related to antimicrobial resistance should be done now and then to warn the people, experts, researchers, doctors and technicians about the detrimental effects caused by improper management and less concern of people for health of future generation.

## Recommendations

The conclusion of the research paper might present a simple and clear picture but it is what people are ignoring completely leading to detrimental effects of AMR for future generation. This research paper reported us about ongoing resistance regarding the drugs used for mastitis treatment and showed us the drugs which are effective to use against the pathogens causing mastitis. However, it recommends all the veterinary professionals to limit the use of antibiotics and avoid irresponsible use of antibiotics. It recommends the government to make strict laws regarding antibiotics use. It recommends the veterinary professions not to use antibiotics against any infection without proper Antibiotic Sensitive Test which is the main root cause of AMR in our country. It recommends farmers to contact veterinary doctors and professionals rather technicians while treating animals or using antibiotics. It recommends and aware people regarding the safety and hygiene they should maintain to create a balance between health, animal and human beings.

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