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Distribution of mastitis pathogens and antibiotic resistance patterns in dairy animals

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Abstract
The current research was built using the culture sensitivity test data gathered from the Central Laboratory of College of Veterinary Science, LUVAS, Hisar (India). A one hundred culture sensitivity testing (CST) reports on milk samples of mastitis in cattle (n=23) and buffaloes (n=77) presented from across the Haryana state of India were accessed. The majority of the cases reported fell within the range of 5-8 years, in terms of age group. Additionally, it was found that an infectious agent was present in both sections of the udder in about 75% of the cases, as samples were drawn quarter wise. Moreover, it was discovered that *Staphylococcus, Streptococcus* or their mixed infections were present in the majority of cases. The majority of the antibiotics frequently used in field settings have either acquired total resistance or intermediate resistance, with the exception of a few antibiotics, the highest resistance was seen for oxytetracycline and the lowest for enrofloxacin. Given that the majority of the currently used antibiotics are starting to develop resistance and that no new antibiotics are being found, significant public health problems could arise in the near future.

Keywords: Antimicrobial resistance, clinical mastitis, dairy animals, subclinical, superbugs

Introduction
Bovine mastitis is the foremost endemic infectious disease of dairy cattle worldwide, as well as in India. Mastitis is responsible for major economic losses to dairy producers and the milk processing industry, resulting in reduced milk production, and alteration in milk composition, discarded milk, increased replacement costs, treatment costs, and veterinary services (Petrovski *et al*., 2006) [18]. Apart from the substantial economic losses associated with the disease, mastitis has serious zoonotic potential and has been associated with the increasing development and the rapid emergence of multidrug resistant strains globally (Oliver *et al*., 2011) [17]. The etiology of mastitis includes contagious microorganisms that survive and proliferate on the skin and teat wounds, as well as environmental microorganisms (Zeryehun and Abera, 2017) [22]. Previous studies have documented major pathogens of mastitis such as *Staphylococcus aureus*, *Streptococcus agalactiae*, and coliforms (Amer *et al*., 2018) [3].
The main treatment of mastitis is commonly administered by intramammary infusion or parenteral administration of antibiotics, such as streptomycin, ampicillin, cloxacillin, penicillin, and tetracycline (Bhosale *et al*., 2014) [6]. The emergence of drug resistance is a serious challenge for mastitis control, as resistance profiles are often herd specific (Silveira-Filho *et al*., 2014) [19]. Combining more than one synergistic antimicrobial agent may be more effective than using a single drug, and can achieve a high cure rate (Oliver *et al*., 2011) [17]. The spread of AMR is a significant global health threat, making it more challenging to treat common infections and increasing the risk of morbidity and mortality (WHO, 2015) [21]. The use of antimicrobial agents in livestock has been identified as a major driver of AMR (Wall *et al*., 1970) [20]. This has been demonstrated in numerous studies, which have found high levels of resistant bacteria in livestock and their environment (e.g., soil, water) (Aarestrup *et al*., 2010 [1]; McEachran *et al*., 2015) [16]. The present study was planned to explore the distribution of mastitis pathogens and antibiotic resistance patterns in dairy animals.

Material and Methods
The present study was based on the retrospective analysis of culture reports (n=100) generated by the central laboratory of the College of Veterinary Science, Lala Lajpat Rai University of Veterinary and Animal Sciences (LUVAS) in Hisar on milk samples of mastitis in cows and buffaloes brought in by farmers from various regions in Haryana, India.

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Following the methodology of Carter *et al*. (1995) [9], bacterial isolation was carried out from milk samples of various quarters on 5% defibrinated sheep blood agar and McConkey's lactose agar. The plates were kept in an incubator overnight at 37 °C. By looking at the colony form and Gram's staining, bacteria isolates were identified. Antimicrobial sensitivity testing was done in accordance with Bauer *et al*. (1966) [5] recommended technique. The percentage and frequency of distribution across the categories was calculated, interpreted and presented in an understandable form.

Results and Discussion
Out of 100 milk samples tested, majority (77%) of them were from buffaloes affected with mastitis followed by cows (23%). The similar findings were reported by Ali *et al*. (2021) [2], who reported that the overall prevalence of subclinical mastitis was more in buffaloes. The results of the study indicate that buffaloes were more susceptible to subclinical mastitis (SCM) than cows. Krishnamoorthy *et al*. (2021) [13] indicated a higher SCM and CM prevalence in buffaloes than the cattle. Mastitis can reduce milk yield and alter the composition of milk, making it unsuitable for human consumption with increased veterinary costs and decreased profitability for farmers (Awale *et al*., 2012) [4]. Therefore, it is important to implement appropriate management practices to prevent mastitis in buffaloes and cows. These practices may include proper hygiene during milking, regular screening of animals for mastitis, and appropriate use of antibiotics for treatment. Additionally, farmers should be educated about the importance of maintaining proper sanitation and hygiene in their farms to prevent the spread of mastitis.

Age Group and Quarters Affected
Majority of the infected animals (66%) were in the age group of 5-8 years, followed by 24% of animals less than 4 years and 10% of animals in the age group of more than 8 years. These results are consistent with the findings reported by Kurjogi and Kaliwal (2014) [14], who also found the highest prevalence of subclinical mastitis (SCM) in the cows of age group 7-10 years, followed by greater than 10 years, and the least in the age group of 3-6 years.

With regards to distribution of mastitis infection in the fore and hind-quarters of dairy animals, the left fore-teat was infected in 82% of the cases, while the right fore-teat had an infection in 75% of the cases. In contrast, the left and right hind teats were infected in almost equal frequency (73 & 74%) These findings highlight the asymmetrical distribution of mastitis infection in the udder of dairy cows (Breen *et al*., 2009) [8].

Presence of Infectious Agent
The distribution of different pathogens causing mastitis in the udder of dairy cows is presented in the Table 1. It shows that a large proportion of cases were of *Staphylococcus* and *Streptococcus* infection in different teats followed by mixed infection of both. In contrast, the infection with *Escherichia coli* and *Diplococci* was seen only in 7% and 3% cases, respectively. Moreover, no case of *Bacillus* and *Klebsiella* infection was found in the selected samples.

Table 1: Distribution of mastitis pathogens in milk samples (n=100)

Pathogens	Present	Absent
Staphylococcus	83	17
Streptococcus	76	24
E. coli	7	93
Bacillus	0	100
Klebsiella	0	100
Diplococci	3	97
Pseudomonas	0	100
Candida	3	97

The prevalence of Gram-positive bacteria, specifically *Staphylococcus* and *Streptococcus*, as the most commonly occurring pathogens causing mastitis in dairy cows has been highlighted in numerous previous studies. *Staphylococcus* and *Streptococcus* are ubiquitous bacteria that can easily enter the teat canal during milking and cause mastitis if proper milking procedures and hygiene practices are not followed (Bradley, 2002) [7].

In contrast, *Escherichia coli* and *Diplococci* are Gram-negative bacteria that are more commonly associated with environmental mastitis in dairy cows. *Bacillus* and *Klebsiella* are also less commonly associated with mastitis in dairy cows (Dohoo *et al*., 1984) [10]. The importance of identifying the specific pathogens causing mastitis in dairy cows to develop targeted treatment and prevention strategies has been emphasized in various studies (Lundberg *et al*., 2016) [15].

Antibiotic Resistance Pattern
As indicated in Table 2 Oxytetracycline was found resistant in 95% of the cases. It is consistent with previous study that has reported high levels of resistance to this antibiotic. As far as other antibiotics are concerned maximum resistance was seen against Amoxicillin (79%) followed by Ampicillin (78%), Penicillin G (77%), Cloxacillin (72%), Streptomycin (62%), Levofloxacin (58%), Chloramphenicol (45%), Gentamicin (44%), Amikacin (42%), Ceftriaxone (32%), Moxifloxacin (32%), Neomycin (27%) and Cefoperazone (24%). It was also found that Kenamycin was resistant in 5 out of the 7 cases of *E. coli* infection, *i.e.,* 71.4% resistance.

It was also found that Cefoperazone, Enrofloxacin, Ceftriaxone, Moxifloxacin, Chloramphenicol, Gentamicin, Levofloxacin, Streptomycin, Penicillin G, Amoxicillin, Amikacin and Cloxacillin showed intermediate resistance in varying cases against microbial infection. In mixed infection of *Staphylococcus* and *Streptococcus*, it was noticed that in majority of the cases there was either total resistance or intermediate resistance. The high rates of resistance observed in this study are concerning, as they suggest that the use of these antibiotics may not be effective in treating certain infections. The high resistance rates to commonly used antibiotics found are consistent with previous studies (Kamathewatta *et al*., 2020) [12]. The intermediate resistance

observed for some antibiotics, such as cefoperazone and gentamicin, may indicate the emergence of resistance and highlight the need for continued monitoring of antibiotic resistance patterns (Gilliver *et al*., 1999) [11].

Table 2: Distribution of Antibiotics according to sensitivity (n=100)

Antibiotic	Resistant	Intermediate Resistant	Sensitive
Enrofloxacin	15	12	73
Penicillin G	77	1	22
Streptomycin	62	3	35
Amoxicillin	79	2	19
Oxytetracycline	95	0	5
Chloramphenicol	45	6	49
Ceftriaxone	32	10	58
Moxifloxacin	32	10	58
Levofloxacin	58	5	37
Ampicillin	78	0	22
Gentamicin	44	7	49
Neomycin	27	5	68
Amikacin	42	6	52
Cloxacillin	72	2	26
Cefoperazone	24	13	63
Kanamycin	5	0	2

Conclusions
The study found that most samples brought to the laboratory for Culture Sensitivity Test were from buffaloes (77%) and cows (23%) aged 5-8 years, with infectious agents present in both quarters in 75% of cases. *Staphylococcus* and *Streptococcus* were the most commonly found infectious agents, either separately or in combination, while *E. coli* was present in only 7% of samples. Resistance to most commonly used antibiotics was high, with the highest resistance seen for Oxytetracycline and the lowest for Enrofloxacin. The increasing development of resistance against antibiotics, combined with the lack of new discoveries in the field, poses a significant threat to public health. There is a need to regulate the use of antibiotics in the livestock sector, as well as explore the potential of using ethno-medicines in conjunction with antibiotics. Raising awareness among stakeholders about the dangers of inappropriate antibiotic use, importance of milk testing to detect infections at an earlier stage, and clean milk production practices can be implemented to decrease the risk of environmental bacterial infections, and reducing the prevalence of antibiotic resistance and safeguarding public health.

Conflicts of Interest
The authors have no conflicts of interest.

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