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Dr. Ritu Mahala
PhD Scholar, Department of
Veterinary Medicine, College of
Veterinary and Animal
Science, Bikaner, Rajasthan,
India

Dr. Rahul Krishaniya
Assistant Professor,
Department of Veterinary
Physiology, Shekhawati
Veterinary College, Sikar,
Rajasthan, India

Dr. Amita
PhD Scholar, Department of
Veterinary Public Health and
Epidemiology, College of
Veterinary and Animal
Science, Bikaner, Rajasthan,
India

Dr. Ravindra Pal Mandar
Assistant Professor,
Department of Veterinary
Pharmacology and Toxicology,
Mahatma Jyotiba Fule College
of Veterinary and Animal
Science, Chomu, Jaipur,
Rajasthan, India

Corresponding Author:
Dr. Ritu Mahala
PhD Scholar, Department of
Veterinary Medicine, College of
Veterinary and Animal
Science, Bikaner, Rajasthan,
India

Antimicrobial resistance profiles on *Staphylococcus aureus* and *Escherichia coli* in mastitis affected lactating cattle

Dr. Ritu Mahala, Dr. Rahul Krishaniya, Dr. Amita and Dr. Ravindra Pal Mandar

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Abstract

The present study was conducted to isolate *Staphylococcus aureus* and *Escherichia coli* from mastitic milk samples and identify AMR pattern. A total of 250 cattle were screened, where 12 (4.80%) and 89 (35.60%) were affected with clinical and sub-clinical mastitis, respectively. The predominant causative pathogen was *S. aureus* followed by *E. coli*. The prevalence of *S. aureus* was 41.66% and 48.31% while, *E. coli* was 33.33% and 15.73% in clinical and sub-clinical mastitis affected cattle, respectively. More than, 70% of *S. aureus* showed resistance against ampicillin, oxacillin and tetracycline and more than 60% of *E. coli* exhibited resistance against ampicillin and sulfamethoxazole-trimethoprim.

Keywords: Mastitis, Antimicrobial resistance, *Staphylococcus aureus* and *E. coli*

1. Introduction

Mastitis in cattle is still a very complicated condition that affects productivity. It presents a significant economic challenge to the global dairy industry. Numerous pathogen groups, including fungi, bacteria, and mycoplasma, have been identified as its causative agents (Ashraf and Imran, 2020) [3]. Contamination source of these organism includes hands of milking people, contaminated towels, water, dust, air, skin of animals, glandular tissues etc. Therefore, consumption of raw or under-pasteurized milk and their products from dairy cows constitutes potential microbiological hazards and affects the human health. The main strategy to treat mastitis is through the use of antibiotics (penicillin, ampicillin, tetracycline, gentamicin, etc.) (Ruegg, 2018) [12]. Even though bacteria quickly develop resistance to β -lactam antibiotics, such as penicillin and cephalosporins, they are still the most often used antibiotics (Royster and Wagner, 2015) [11]. *Staphylococcus aureus* and *E. coli* are the most common causes of contagious and environmental clinical mastitis, respectively. Antimicrobial treatment is frequently used to prevent and control mastitis. Unfortunately, bacteriological cure failures occur frequently even with the best antimicrobial therapies available; this is especially prevalent for *S. aureus* mastitis and antimicrobial resistance (AMR), which is considered to be one of the main causes of low recovery rates (Barkema *et al.*, 2006) [4].

2. Materials and Method

2.1 Study population

The current investigation was carried out for a period of one year from November 2022 to December 2023, in the Department of Veterinary Medicine, College of Veterinary and Animal Science, Navania, Udaipur (RAJUVAS, Bikaner). Cattle of different age and breed were examined.

2.2 Milk sampling

After the confirmation of both clinical and subclinical mastitis, 70% ethyl alcohol was used to disinfect the cattle's teats. The first milk squirt was discarded before sampling, and around 5 mL of milk was aseptically collected into a sterile test tube for microbiological examination. Finally, all of the samples were moved to the lab by using an icebox to keep an

appropriate cooling chain going. Before being streaked onto the agar plate, all milk samples were stored at room temperature.

2.3 Diagnosis of clinical and sub-clinical mastitis

Clinical mastitis was diagnosed based on observable symptoms such as a firm and inflamed udder, sensitivity to touch, abnormal size and texture of the mammary gland, as well as the production of abnormal milk, including flakes, clots, watery consistency, pus, and the presence of blood-stained milk. Additionally taken into consideration are related general clinical symptoms such as fevers, depression and lethargy, appetite loss, and abruptly decreased milk production.

The California Mastitis Test (CMT) was conducted to screen for sub-clinical mastitis in each cattle. Following the procedure outlined by Rana *et al.* (2020) [10], 2 mL of milk was collected from each quarter of the cow's udder and placed into individual wells on CMT paddles. Equal amounts of CMT reagent were added, and the mixture was gently swirled for 30 seconds. Sub-clinical mastitis was diagnosed based on the CMT results, specifically the coagulation and viscosity of the mixture. The test outcomes were interpreted as negative (0 or trace), weakly positive (+), clearly positive (++), or strongly positive (+++) following the guidelines provided by Abebe *et al.* (2016) [12].

2.4 Isolation and identification of *S. aureus* and *E. coli* from mastitic milk

In general, 20 µL of milk was streaked from the appropriate samples onto a 5% bovine blood agar (Hi Media) plate. The plate was then incubated at 37 °C for up to 48 hours, and the plate was checked every 24 hours to ensure that the desired bacteria were growing as effectively as possible. *Staphylococcus* colonies have a distinct look that includes elevated, smooth, yellow, pigmented, and either full or partial hemolysis. Suspect colonies were further sub-cultured on MSA (Hi Media) or Mannitol Salt Agar (Hi Media) and incubated for 48 hours at 37 °C. Finally, bacterial colonies that fermented MSA were subjected to the

tube coagulase, catalase, and Gram's staining tests. Likewise, Eosin methylene blue (EMB) agar and MacConkey agar (Hi Media) were used to further sub-culture and incubate suspected *E. coli* colonies, which were spherical, thick, moist, smooth, and greyish white. Gram's staining, along with the Triple Sugar Iron (TSI) test, Indole, Methyl Red, Voges-Proskauer, and Citrate utilization (IMViC) tests, were conducted to biochemically confirm the presence of *E. coli*.

2.5 Antibiotic susceptibility pattern of *S. aureus* and *E. coli*

The Kirby-Bauer disk diffusion method was utilized to assess the antibiotic sensitivity of all *S. aureus* and *E. coli* isolates against a total of twelve antibiotics. Ampicillin, Ceftriaxone, Ciprofloxacin, Erythromycin, Gentamicin, Oxacillin, Penicillin-G, Streptomycin, Sulfamethoxazole-Trimethoprim, and Tetracycline were among these antibiotics. A pure culture of every identified strain of *S. aureus* and *E. coli* was eliminated from nutrient broth for the susceptibility test. The bacterium was then streaked along the surface of Muller-Hinton agar using a cotton swab, and the solution was allowed to dry for three to five minutes. Using clean, sterile forceps, antibiotic disks were then placed on the agar surface and gently squeezed to ensure attachment. The plates were then incubated aerobically for 24 hours at 37 °C. Furthermore, using the meter scale to measure the diameters of the zone of inhibition surrounding the discs to the nearest millimeter, the isolates were categorized as sensitive and resistant to the medications tested based on the manufacturer's chart.

3. Result and Discussion

3.1 Overall samples

In total, 250 cattle were screened for clinical and sub-clinical mastitis. Of them, 89 (35.60%) (89/250) cattle were found positive for the CMT test and identified as having sub-clinical mastitis. In addition, 12 out of 250 cattle (4.80%) (12/250) were found affected with clinical mastitis showing clinical signs and symptoms (Table 1).

Table 1: Prevalence of *S. aureus* and *E. coli* in clinical and sub-clinical mastitis affected cattle.

S. No.	Mastitis affected cows (%)	Overall prevalence (n=250)	<i>S. aureus</i>	<i>E. coli</i>
1.	Sub-clinical mastitis	35.60% (89/250)	48.31% (43/89)	15.73% (14/89)
2.	Clinical Mastitis	4.80% (12/250)	41.66% (5/12)	33.33% (4/12)

3.2 Grading of bovine clinical and sub-clinical mastitis based of CMT

Among 250 lactating cows, 149 animals were found CMT negative, 22 were identified as weakly positive (+) and 35 were distinctly positive (++) and 32 were diagnosed as strongly positive (+++) for sub-clinical mastitis. However, all of the 12 clinical mastitis cases were found to be strongly positive (+++) for the CMT.

3.3 Prevalence of *S. aureus* and *E. coli* in clinical and sub-clinical mastitis affected cattle

In total, 101 clinical and sub-clinical mastitic samples were cultured with *S. aureus* being the most prevalent pathogen in both cases, which were 5/12 (41.66%) and 43/89 (48.31%), respectively (Table 1). The prevalence of *E. coli* was found

to be 4/23 (33.33%) and 14/89 (15.73) in clinical and sub-clinical mastitis affected cows, respectively.

3.4 Antimicrobial susceptibility profiles

All the *S. aureus* and *E. coli* isolates were found to be multi-drug resistant (MDR). Among the *S. aureus* isolates, the highest resistance was observed for tetracycline (77.08%), oxacillin and ampicillin (70.83%) and gentamicin (64.58%) (Table 2). More than 58% of the isolates exhibited resistance against amoxicillin-clavulanic acid, erythromycin and streptomycin. In the case of *E. coli*, this exhibits the highest resistance against ampicillin and sulfamethoxazole-Trimethoprim (61.11%) antibiotics. More than 70% of *E. coli* isolates were sensitive to ceftriaxone, ciprofloxacin and erythromycin (Table 3).

Table 2: Results of antibiotic resistance profile of *Staphylococcus aureus* isolates (n=48).

S. No.	Name of Antibiotics	Sensitive	Resistance
1.	Amoxicillin-Clavulanic acid	20 (41.66%)	28 (58.33%)
2.	Ampicillin	14 (29.16%)	34 (70.83%)
3.	Ceftriaxone	34 (70.83%)	14 (29.16%)
4.	Cefoxitin	35 (72.91%)	13 (27.08%)
5.	Ciprofloxacin	31 (64.58%)	17 (35.41%)
6.	Erythromycin	20 (41.66%)	28 (58.33%)
7.	Gentamicin	17 (35.41%)	31 (64.58%)
8.	Oxacillin	14 (29.16%)	34 (70.83%)
9.	Penicillin-G	23 (47.91%)	25 (52.08%)
10.	Streptomycin	20 (41.66%)	28 (58.33%)
11.	Sulfamethoxazole-Trimethoprim	25 (52.08%)	23 (47.91%)
12.	Tetracycline	11 (22.91%)	37 (77.08%)

*All intermediately resistant isolates are considered as susceptible.

Table 3: Results of antibiotic resistance profile of *Escherichia coli* isolates (n=18)

S. No.	Name of Antibiotics	Sensitive	Resistance
1.	Amoxicillin-Clavulanic acid	10 (55.55%)	8 (44.44%)
2.	Ampicillin	7 (38.88%)	11 (61.11%)
3.	Ceftriaxone	13 (72.22%)	5 (27.77%)
4.	Cefoxitin	12 (66.66%)	6 (33.33%)
5.	Ciprofloxacin	13 (72.22%)	5 (27.77%)
6.	Erythromycin	14 (77.77%)	4 (22.22%)
7.	Gentamicin	12 (66.66%)	6 (33.33%)
8.	Oxacillin	11 (61.11%)	7 (38.88%)
9.	Penicillin-G	10 (55.55%)	8 (44.44%)
10.	Streptomycin	12 (66.66%)	6 (33.33%)
11.	Sulfamethoxazole-Trimethoprim	7 (38.88%)	11 (61.11%)
12.	Tetracycline	10 (55.55%)	8 (44.44%)

*All intermediately resistant isolates are considered as susceptible.

4. Discussion

In the present study, the overall prevalence of subclinical mastitis at cattle level was 35.60% and the smallest proportion (4.80%) was suffered from clinical mastitis. Finding of present investigation is in agreement with that of Rana *et al.* (2022) [9]. Zeryehun and Abera (2017) [14] showed comparatively higher prevalence of sub-clinical and clinical mastitis in Eastern Ethiopia, which was 51.8% and 12.5%, respectively. The occurrence of mastitis in lactating cattle varies due to the geographical location, breed, age, stages of lactation, status of the udder, number of parity, immunity, management, hygiene and milking practices in dairy farms (Ashraf *et al.*, 2018 and Rana *et al.*, 2022) [1, 9]. However, the predominance of sub-clinical mastitis in the current study area is a concerning issue that negatively impacts the human food chain in addition to causing economic losses due to decreased milk production. Worldwide, *S. aureus* and *E. coli* are the most common causative agents of clinical and subclinical mastitis in cattle (Haubert *et al.*, 2017) [6]. However, in both clinical and sub-clinical cases, the most frequently isolated organism was *S. aureus*, which represented 41.66% and 48.31%, respectively. Finding of present investigation is in agreement with that of Traversa *et al.* (2015) [13] in Italy and Rana *et al.* (2022) [9] in Bangladesh and slightly higher in Brazil (56%) (Gundogan *et al.*, 2014) [5]. Moreover, the incidence of environmental fatal mastitis caused by *Escherichia coli* is dependent on a number of parameters, including farm management, hygienic techniques, and the presence of virulence factors. Surprisingly, any type of mastitis in the mammary gland always first attempts to be treated with antimicrobials to reduce the fatal consequence, especially to control the contagious infections (Rana *et al.*, 2022) [9]. Since the

screening of antimicrobial resistance test, both *S. aureus* and *E. coli* isolates harboured multiple types of resistance to bacteria. According to different mastitis research, it is focused that β -lactams antimicrobials are most frequently used in both parental and intramammary routes in lactating cows for treatment of mastitis infection (Priyantha *et al.*, 2021) [8]. In the present study, high antimicrobial resistance frequency to the β -lactams penicillin (52.08%), amoxycillin-clavulanic acid (58.33%), ampicillin and oxacillin (70.83%) was observed, which is closely related by previous findings of Priyantha *et al.* (2021) [8], Haubert *et al.* (2017) [6] and Rana *et al.* (2022). According to previous findings, *S. aureus* and *E. coli* resistance to penicillin, ampicillin, amoxycillin, and oxacillin is the most common type of antimicrobial resistance among mastitis-causing bacteria (Rana *et al.*, 2020) [10]. Many earlier investigations have revealed the high resistance frequency of *E. coli* to sulfamethoxazole-trimethoprim (58.33%) (Priyantha *et al.*, 2021) [8]. It was observed that a significant percentage of *S. aureus* and *E. coli* strains were susceptible to ceftriaxone (70.83% and 72.22%) and ciprofloxacin (64.58% and 72.22%). These results compatible with those of earlier research studies (Rana *et al.*, 2022; Rana *et al.*, 2020) [9, 10]. The broad-spectrum nature of these antimicrobials combined with reduced exposure in dairy farm conditions may account for the high rate of sensitivity to ceftriaxone and ciprofloxacin.

5. Conclusion

It was concluded that, there was considerable, variations in the prevalence of clinical and sub clinical type mastitis in cattle. AMR prevalence was more common in bovine mastitis pathogens namely *S. aureus* and *E.coli*. Overall,

antimicrobial resistance to ampicillin, oxacillin and tetracycline was found in mastitis cases. The determination of AMR will facilitate the selection of an appropriate therapeutic approach.

6. References

1. Ashraf A, Imran M, Yung-Fu C. Antimicrobial resistance of *Escherichia coli* isolates from mastitic milk and its possible relationship with resistance and virulence genes. Pak J Zool. 2018;50(4):1435-41. doi:10.17582/journal.pjz/2018.50.4.
2. Abebe R, Hatiya H, Abera M. Bovine mastitis: prevalence, risk factors and isolation of *Staphylococcus aureus* in dairy herds at Hawassa milk shed, South Ethiopia. BMC Vet Res. 2016;12(1):1.
3. Ashraf A, Imran M. Causes, types, etiological agents, prevalence, diagnosis, treatment, prevention, effects on human health and future aspects of bovine mastitis. Anim Health Res Rev. 2020;21(1):36-49.
4. Barkema HW, Schukken YH, Zadoks RN. Invited review: The role of cow, pathogen, and treatment regimen in the therapeutic success of bovine *Staphylococcus aureus* mastitis. J Dairy Sci. 2006;89:1877-95.
5. Gundogan N, Avci E. Occurrence and antibiotic resistance of *Escherichia coli*, *Staphylococcus aureus* and *Bacillus cereus* in raw milk and dairy products in Turkey. Int J Dairy Technol. 2014;67(4):562-9.
6. Haubert L, Kroning IS, Iglesias MA. First report of the *Staphylococcus aureus* isolate from sub-clinical bovine mastitis in the South of Brazil harboring resistance gene *dfrrG* and transposon family Tn916-1545. Microb Pathog. 2017;113:242-7.
7. Oliver SP, Murinda SE. Antimicrobial resistance of mastitis pathogens. Vet Clin North Am Food Anim Pract. 2012;28:165-85.
8. Priyantha MA, Fernando PS, De Alwis PS. Emerging antimicrobial resistance in coagulase-positive *Staphylococci* and *E. coli* isolated from bovine clinical mastitis in Sri Lanka. Asian J Anim Vet Adv. 2021;28:29-35.
9. Rana EA, Fazal MA, Alim MA. Frequently used therapeutic antimicrobials and their resistance patterns on *Staphylococcus aureus* and *Escherichia coli* in mastitis affected lactating cows. Int J Vet Sci Med. 2022;10(1):1-10.
10. Rana EA, Das T, Dutta A. Coagulase-positive methicillin-resistant *Staphylococcus aureus* circulating in clinical mastitic goats in Bangladesh. Vet World. 2020;13(7):1303.
11. Royster E, Wagner S. Treatment of mastitis in cattle. Vet Clin North Am Food Anim Pract. 2015;31:17-46.
12. Ruegg PL. Making antibiotic treatment decisions for clinical mastitis. Vet Clin North Am Food Anim Pract. 2018;34:413-25.
13. Traversa A, Gariano GR, Gallina S. Methicillin resistance in *Staphylococcus aureus* strains isolated from food and wild animal carcasses in Italy. Food Microbiol. 2015;52:154-8.
14. Zeryehun T, Abera G. Prevalence and bacterial isolates of mastitis in dairy farms in selected districts of Eastern Harrarghe zone, Eastern Ethiopia. J Vet Med. 2017. doi:10.1155/2017/6498618.