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Original Research

Prevalence and Antibiotic Resistance of Mastitis Causing *Staphylococcus aureus* in Bovine

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Abstract

The present study aimed to determine the prevalence of Staphylococcus aureus in mastitis milk from bovines. Mastitis milk samples from cattle (24) and buffaloes (26) were collected in and around Ludhiana. Out of 50 samples, 18 isolates of S. aureus (10 from buffaloes and 8 from cattle) were isolated and identified based on cultural, morphological, and biochemical characteristics. The prevalence of the isolates was higher in buffaloes (55.55%) than in cattle (44.44%). Antibiotic susceptibility testing revealed that 44.44% of the isolates were resistant to more than six antibiotics, and 83.33% showed multidrug resistance, i.e., resistance to three or more antibiotics.

Keywords: Antibiotic Sensitivity Test, Mastitis, Resistance, *Staphylococcus aureus*.

Introduction

Livestock plays a crucial role in providing livelihood to small and marginal farmers in India (Aatralarasi *et al*., 2021). However, infectious diseases, particularly mastitis, pose a significant challenge to the sustainability and growth of the dairy industry. Mastitis causes extensive financial losses for farmers, and it is notably the most important disease requiring attention of the dairy industry (Miller *et al*., 1993). Mastitis, whether caused by bacterial infections or injury, leads to decreased milk production as well as quality, higher veterinary expenses, and compromised animal welfare (Garcia, 2004). This inflammatory condition affects the mammary glands, causing tissue damage and changes in milk composition, often through infection via the teat canal. The udder provides an optimal environment for microbial growth due to favourable conditions such as temperature and nutrition. Among the microorganisms associated with mastitis, *Staphylococcus aureus* is one of the most important causative agents that causes both clinical and subclinical mastitis (Kour *et al*., 2020). *S. aureus* has been studied extensively because of its importance in all forms of mastitis in dairy cows as well as its development of antibiotic resistance (Kaszanyitzky *et al*., 2003). Mastitis control programs, based on hygiene and antibiotic therapy, recommend antibiotic treatment of clinical cases during lactation and of subclinical cases at drying off irrespective of the infectious status of the mammary gland (Oliver & Murinda, 2012). The extensive use or overuse of antibiotic treatment in bovine mastitis could represent a problem to public health, due to the potential emergence of resistant bacteria and their entrance into the food chain (White & McDermott, 2001). Therefore, there is a need to assess and gain insights into antibiotic resistance trends that can be used to identify challenging situations that require the establishment of strategic actions to ensure prudent use of antibiotics. Keeping in view of the above problem, the study has been designed to isolate *Staphylococcus* spp. from mastitis milk of cattle and buffaloes and study the antibiotic resistance pattern.

Material Methods

Isolation

For this study, fifty mastitic milk samples were collected from cattle and buffaloes in and around Ludhiana from November 2022 to March 2023. The mastitis was diagnosed based on the animal's clinical indications. Milk samples were subjected to the California mastitis test (CMT), and those positive were subjected to isolation of bacteria. All the samples were inoculated initially on Mannitol Salt Agar (MSA) at 37°C for 18-24 h, and agar plates showing golden-yellow colonies were Gram’s stained and those appearing positive further streaked on Baird Parker Agar (BPA) at 37°C for 18-24 h to observe typical colonies of *S. aureus*. Subsequently, the isolates were examined for hemolysis patterns by streaking them on Blood Agar (BA) at 37°C for 18-24 h. The positive isolates were identified using biochemical tests, which included oxidase, catalase, citrate, indole, urease, TSI, MR-VP, and further using HiStaph ™ Identification Kit as per the manufacturer’s instructions.

Antibiotic Sensitivity Test

The antibiotic sensitivity test was performed by the disc diffusion method according to the guidelines by Bauer *et al*., (1966). Bacterial culture grown in nutrient broth (equivalent to 0.5 McFarland standards) was spread evenly on Mueller-Hinton Agar. Various antibiotics such as penicillin-G (P10), ertapenem (ETP10), colistin (CL10), amoxyclav (AMC30), ceftriaxone (CTR3O), norfloxacin (NX10), oxytetracycline (O30), co-trimoxazole (COT25), tetracycline (TE30), gentamicin (GEN10), cefoxitin (CX30), erythromycin (E15), piperacillin (PI30), ceftazidime (CAZ10), nitrofurantoin (NIT200), aztreonam (AT30), trimethoprim (TR5), tylosin (TL15), ampicillin/sulbactam (A/S10/10), nalidixic acid (NA30), kanamycin (K30) were placed and gently pressed on the lawn of the bacteria using flame sterilized forceps at an equal distance of 25 mm and the plates were incubated at 37ºC for 18-24 h. Following incubation, the zone of inhibition was measured using a measuring scale, the isolates were marked as sensitive, intermediate, and resistant based on the zone of inhibition as per the standard CLSI (2020) guidelines.

Results and Discussion

Isolation and Identification

Out of the 50 suspected mastitis samples, a total of 18 samples showed growth on MSA. All the isolates exhibited mannitol fermentation on MSA, and upon staining, they appeared as gram-positive cocci arranged in clusters resembling bunches of grapes. Additionally, the isolates showed black colonies surrounded by an opaque halo on BPA and alpha hemolysis on BA. Based on biochemical tests, all 18 isolates were identified as *S. aureus*. Among all the isolates, 44.44% (8/18) were from cattle mastitis milk and 55.55% (10/18) were from buffalo mastitis milk samples. *S. aureus* was isolated and identified in 36.0% of the mastitis milk samples, which aligned with studies conducted by Arshad *et al*., (2006), Turutoglu *et al*., (2006), Bendahou *et al*., (2008) and Nirwan *et al*., (2022), where its prevalence was recorded to be observed at 25.0%. 48.0%, 43.09%, and 40.0%, respectively. In a similar study by Kour (2016), it was stated that the skin of the udder and the milk from the infected gland were identified as the primary reservoirs for mastitis. A total of 18 *S. aureus* isolates, 44.44% from cattle and 55.55% from buffalo, were isolated and identified from mastitis milk samples. This higher prevalence of *S. aureus* in buffalo milk samples was consistent with the earlier findings of Ali *et al*., (2021), reporting a high incidence of sub-clinical mastitis in buffaloes and *S. aureus* as the predominant pathogen. Conversely, Sharma *et al*., (2015) noted a higher occurrence of *S. aureus* in both clinical and sub-clinical mastitis cases in cattle compared to buffaloes. However, further studies are required to substantiate these findings.

Antibiotic Sensitivity Test

Antibiotic sensitivity test revealed resistance to aztreonam (100%), colistin (66.66%), nalidixic acid, and ceftazidime (61.11%). However, these findings do not align with the report of Khesar *et al*., (2008) in which resistance to colistin was observed to be 26.06%, whereas it was much higher in our case. Intermediate resistance was observed against tylosin and nalidixic acid (38.88%), ceftriaxone, erythromycin, ertapenem, ceftazidime (22.22%), gentamicin and kanamycin (16.66%), tetracycline and norfloxacin (5.55%). However, sensitivity was observed for amoxiclav, co-trimoxazole (100%), norfloxacin (94.44%) nitrofurantoin, oxytetracycline (88.88%), tetracycline, gentamicin, piperacillin (83.33%) and erythromycin and trimethoprim (77.77%) (Table 1) consistent with the findings of Stephan *et al*., (2001), Eswaran *et al*., (2011) and Hoque *et al*., (2022) reporting higher susceptibility to erythromycin. Looking into the data, it was also observed that 44.44% of the *S. aureus* isolates were resistant to more than six antibiotics tested, and multidrug resistance (resistance to three or more antibiotics) was observed in 83.33% of the isolates. Resistance to the highest number of antibiotics (11) was exhibited by a single isolate (S8), whereas resistance to seven antibiotics was exhibited simultaneously by 27.77% of the isolates.

Table 1 - *Staphylococcus aureus* isolates antibiotic sensitivity to different antibiotics

Antibiotics (Conc)	**Resistance Per cent (No. of isolates)**	**Sensitive Per cent (No. of isolates)**	**Intermediate Per cent (No. of isolates)**
Penicillin-G (P 10)	50(9)	50(9)	0(0)
Ceftriaxone (CTR 3O)	5.55(1)	72(13)	22(4)
Ertapenem (ETP 10)	80(9)	27.77(5)	22.22(4)
Colistin (CL10)	66.66(12)	33.33(6)	0(0)
Amoxyclav (AMC 30)	0(0)	100(18)	0(0)
Norfloxacin (NX10)	0(0)	94.44(17)	5.55(1)
Oxytetracycline (O30)	11.11(2)	88.88(16)	0(0)
Co-trimoxazole (COT 25)	0(0)	100(18)	0(0)
Tetracycline (TE 30)	11.11(2)	83.33(15)	5.55(1)
Gentamicin (GEN10)	0(0)	88.88(16)	11.11(2)
Cefoxitin (CX 30)	27.77(5)	72.22(13)	0(0)
Erythromycin (E15)	0(0)	77.77(14)	22.22(4)
Piperacillin (PI 30)	16.66(3)	83.33(15)	0(0)
Ceftazidime (CAZ 10)	61.11(11)	16.66(3)	22.22(4)
Nitrofurantoin (NIT 200)	0(0)	94.44(17)	5.55(1)
Aztreonam (AT 30)	100(18)	0(0)	0(0)
Trimethoprim (TR 5)	22.22(4)	77.77(14)	0(0)
Tylosin (TL15)	16.66(3)	44.44(8)	38.88(7)
Ampicillin/Sulbactam (A/S10/10)	50(9)	50(9)	0(0)
Nalidixic acid (NA 30)	61.11(11)	0(0)	38.88(7)
Kanamycin (K30)	38.88(7)	44.44(8)	16.66(3)

The isolates in the current study exhibited multidrug resistance. Dairy farms widely use antimicrobials, not only for treating diseases but also for preventive measures, such as blanket dry cow antibiotic therapy in mastitis control programs. This approach involves administering antibiotics to all cows regardless of the presence of intramammary infections, which can contribute to the selection and spread of resistant organisms (Saini *et al*., 2012).

Penicillin G and ertapenem showed a resistance rate of 50%, which is consistent with the observations of Bendahou *et al*., (2008), in which 50% of isolates were resistant to penicillin. Penicillin, along with other beta-lactams, has been extensively used in dairy farms for over 50 years, both as a therapeutic agent for lactating cows and as a therapeutic and prophylactic treatment for dry cows (Page & Gautier, 2012). Beta-lactam antibiotics are some of the most commonly used medications for dairy cattle globally, too (Stevens *et al*., 2016). Thus, the widespread use of penicillin and other beta-lactams for the prevention and treatment of mastitis could contribute to the development of resistance in *S. aureus* isolates.

Kanamycin was resistant in 38.88% isolates, which was similar to a study conducted by Zhang *et al*., (2016). While cefoxitin showed a resistance rate of 27.77%, which is almost comparable to the findings of Xavier *et al*., (2017), a study in which only 15.38% resistance was seen against cefoxitin. Tetracycline and oxytetracycline had a resistance rate of 11.11%, and this aligns with the resistance pattern noted in a study by Gianneechini *et al*., (2002) and Rabedo *et al*., (2005) against tetracycline. However, none of the isolates demonstrated resistance to gentamicin, a finding consistent with the report of Stephan *et al*., (2001) and Eswaran *et al*., (2011), in which all the isolates of *S. aureus* were susceptible to gentamicin. Maximum intermediate resistance was seen against tylosin and nalidixic acid (38.88%). This finding also supports the study conducted by Salauddin *et al*., (2020). Similarly, cefoxitin and ceftriaxone exhibited equal sensitivity rates of 72.22%. This finding also supports the earlier observations of Peles *et al*., (2007) and Hoque *et al*., (2022) in which the isolates were susceptible to cefoxitin. Thus, from the study, it was concluded that judicious use of antibiotics to prevent mastitis should be encouraged to prevent the development of antibiotic resistance, and continuous antibiotic sensitivity tests to be performed on mastitis-causing isolates to chalk out effective strategies to combat mastitis and help in the control of antibiotic resistance.

Conclusion

The study identified 18 *S. aureus* isolates from 50 mastitic milk samples based on colony morphology and biochemical test. Higher prevalence of *S. aureus* isolation was noted in buffaloes (55.55%) compared to cattle (44.44%). It was observed that many isolates demonstrated multidrug resistance to several commonly used antibiotics.

Contribution by Authors

All the authors contributed equally to writing the manuscript. The final manuscript was read by all authors and consented to publication.

Conflict of Interests

There is no conflict of interest.

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