



ISSN: 2456-2912

VET 2024; 9(4): 238-239

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www.veterinarypaper.com

Received: 21-05-2024

Accepted: 30-06-2024

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Bovine mastitis due to multidrug resistant Coagulase-negative staphylococci (CNS)

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DOI: <https://doi.org/10.22271/veterinary.2024.v9.i4d.1552>

Abstract

Infections that cause bovine intramammary (mastitis) pose a serious risk to the health of the animal, diminishing its hygienic value, altering its processing characteristics, altering the composition of milk, and causing dairy farmers to suffer large financial losses. A clinical sample from an infected animal receiving treatment at TVCC, Mannuthy, Thrissur, was used to investigate the study. The current study's main goal is to isolate and identify the Coagulase-negative staphylococci (CNS). Tests for sugar fermentation, biochemical analysis, and culture all support the presence of *Staphylococcus*. It was discovered that the results from several antibiotics like Methicillin, Amoxicillin and Penicillin were resistant and widely used in clinics, mainly for the treatment of highly infectious diseases caused by Gram-positive microorganisms.

Keywords: Bovine mastitis, Coagulase-negative staphylococci, multidrug resistance

1. Introduction

Subclinical mastitis is frequently caused by *Staphylococcus aureus* (*S. aureus*), which is significant economically for the dairy industry (Miles *et al.*, 1992) [10]. *S. aureus* can be found in milk and milk products, particularly when pasteurisation is inappropriate and raw milk is consumed. It appears that the contaminated quarter is the primary source of *S. aureus*. Molecular epidemiological examination of the *S. aureus* population in cows revealed that strains had a wide geographic spread and that a small number of clonal types caused most infections (Fitzgerald *et al.*, 1997; Kapur *et al.*, 1995; Salasia *et al.*, 2004) [6, 8, 13].

Upon the discovery of penicillin in the 1940s, *S. aureus* strains that produced penicillinase were identified. New β -lactam antibiotics that were resistant to the action of penicillinase, like methicillin, oxacillin, and cephalosporins, were subsequently created. The first case of methicillin-resistant *S. aureus* (MRSA) was documented in the UK shortly after these medications were introduced (Kalstone, 1989) [7]. The present study's focus is to isolation and identification of MRSA strains in dairy cattle.

2. Materials and Methods

A 4-year old cow with mastitis infection was presented to TVCC in Mannuthy, Thrissur during June 2024. Milk samples were taken for the investigation of the causative agents. The sample was cultured on brain-heart Infusion agar (BHIA), blood agar (BA) and Mannitol Salt Agar (MSA), then incubated at 37°C for 24-48 hrs. The colonies were identified by morphological, staining and biochemical techniques (Koneman *et al.*, 1983; Quinn *et al.*, 1994) [9, 12].

Antibiogram was performed with 25 antibiotics by disc diffusion method as per Bauer *et al.*, (1966) [1]. The following antibiotics were used: Gentamicin (G) 10 mcg, Chloramphenicol (C) 30 mcg, Levofloxacin (LE) 5 mcg, Cefotaxime (CTX) 5 mcg, Amikacin (AK) 30 mcg, Cotrimoxazole (COT) 16mcg, Ciprofloxacin (CIP) 5 mcg, Amoxicillin (AMX) 30 mcg, Penicillin G (P) 10unit, Ceftazidime (CAZ) 10 mcg, Cefepime (CPM) 30 mcg, Ampicillin (AMP) 10 mcg

Ceftriaxone sulbactam (CIS) 30 mcg, Imipenem (IPM) 10 mcg, Polymixin-B (PB) 50 units, Cephalexin (CN) 30 mcg, Erythromycin (E) 10 mcg, Tylosine (TL) 15 mcg, Methicillin

(MET) 10 mcg, Cefoperazone (CPZ) 75 mcg, Clarithromycin (CLR) 15 mcg, Azithromycin (AZM) 15 mcg, Amoxyclav (AMC) 30 mcg, Doxycycline (DO) 10 mcg, Moxifloxacin (MO) 5 mcg. The zones of inhibition were measured in mm and compared with CLSI (2020)^[3].

3. Results and Discussion

The organisms in BHIA showed flat, moist and shiny large, golden-yellow colonies. In BA with normal conditions, colony showed beta-haemolytic and anaerobic BA had mucoid, flat non-haemolytic colony. In MSA the Coagulase-negative staphylococci show red colonies that don't change the colour of the medium. On gram staining, the bacterial cell is identified as gram-positive with a tiny, spherical, chain coccus that is immobile. The biochemical and sugar fermentation data of the bacteria revealed that Coagulase-negative staphylococci (CNS). CNS in this instance produce tiny pink or red colonies without changing the colour of the medium, in contrast to the conformation used for selectively and differentially recovering isolates of *S. aureus*, which will appear yellow on MSA. However, in this present study, the antibiotics GEN, C, LE, CTX, AK, COT, CIP, CLR, and DO showed sensitive patterns and AZM, CIS, E, TL, P, AMX, CAZ, CPM, AMP, IPM, PB, CN, MET, CPZ, AMC, MO exhibits resistant pattern.

Subclinical mastitis is mainly caused by the central nervous system (CNS); while less pathogenic than *Staphylococcus aureus* (*S. aureus*), it can nevertheless lead to persistent clinical infections in the mammary gland. Bergonier *et al.* (2003)^[2], Contreras *et al.* (2003)^[4], and Contreras *et al.* (2007)^[5]. Bovine mastitis caused by *Staphylococcus* species is thought to be a source of enterotoxins that are harmful to human health (Contreras *et al.*, 2007; Podkowik *et al.*, 2013)^[5, 11]. While many staphylococci species are capable of producing these enterotoxins, little is known about how enterotoxic bacteria can affect the central nervous system (Podkowik *et al.*, 2013)^[11].

Antimicrobial resistance is highly prevalent and has various reasons. One of the main cause may arise from the misuse of antibiotics, which may lead to gene mutations resulting in highly resistant variants and the spread of resistant genes from one individual to another (Turnidge, 2003)^[14].

4. Conclusion

In this study, Coagulase-negative staphylococci (CNS) was isolated from Bovine mastitis. Biochemical tests and colony morphology were used for identification. The organism demonstrated resistance to several antibiotics. Gene mutation and high variant resistance may result from the improper use of antibiotics and the transfer of resistant genes from one person to another.

5. Acknowledgment

The authors thank the Dean, College of Veterinary and Animal Sciences, Mannuthy for providing necessary facilities to carry out this work.

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How to Cite This Article

Ajayan H, Anaswara AR, Smrithi A. Bovine mastitis due to multidrug resistant Coagulase-negative staphylococci (CNS). *International Journal of Veterinary Sciences and Animal Husbandry.* 2024;9(4):238-239.

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