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Molecular detection and antibiogram of Methicillin resistant *Staphylococcus aureus* isolated from the nares of cattle in Aizawl, Mizoram

Lalnunfela[1] • Blessa Sailo[2] • C. Lalremruata[3] • Joy Lalmuanpuia[1*]

[1] Dept. of Veterinary Public Health and Epidemiology, College of Veterinary Sciences and Animal Husbandry, CAU (I), Selesih, Mizoram-796015

[2] ICAR- Manipur Centre, Lamphelpat, Imphal, Manipur-795004

[3]KVK, North Vanlaiphai, Serchhip district, Mizoram-796184

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ABSTRACT

A total of 160 nasal swabs were collected from cattle and processed for *Staphylococcus aureus* isolation. All samples were initially screened for the presence of *S. aureus*. All the isolates were confirmed by PCR using a species-specific primer (*nuc* gene), yielding an overall prevalence rate of 8.125%. The positive isolates were then screened for MRSA targeting the *mecA* gene, which had a 5.0% prevalence rate. The study's findings on MRSA's antimicrobial sensitivity pattern indicate a significant prevalence of resistance to oxacillin and ampicillin.

1. Introduction

Gram-positive commensal *Staphylococcus aureus* is a significant opportunistic pathogen that affects both humans and animals and is responsible for a wide range of illnesses. *S. aureus* is recognised globally as a major pathogen that causes mastitis in lactating cows, sheep, and goats (Rahimi *et al.,* 2015). In humans, it is considered to be a major human pathogen in both community and medical settings, causing a variety of diseases ranging from mild skin infections to potentially fatal diseases such as toxic shock syndrome, pneumonia, and endocarditis (Chai *et al.,* 2021). Animal nares are likely source of the contamination for the udder and milk in dairy farms, however the udder is thought to be the primary source of infection (Vautor *et al.,* 2005). *S. aureus* isolation from farm animal nares has recently been widely documented (Nemeghaire *et al.,* 2014; Mork *et al.,* 2012). Presence of *S. aureus* has a significant influence on both the animal sector and public health (Peton *et al.,* 2014).

Major infections that infect and/or colonise both people and animals include methicillin-resistant *S. aureus* strains (MRSA) (Nemeghaire *et al.,* 2014). Nasal carriage has been demonstrated to be an infection risk factor by acting as a reservoir for the pathogen (Wertheim *et al.,* 2005). The acquisition of the *mec*A gene (Mourabit *et al.,* 2020), which encodes for a low affinity Penicillin-Binding Protein (PBP), or its counterpart *mec*C (Garcia-Alvarez *et al.,* 2011) is associated with the formation of MRSA. Early in the 1960s, MRSA made its initial appearance in a hospital (Jevons *et al.,* 1963). Since then, it has spread throughout the world as a pathogen that is endemic to hospitals (Hospital Acquired-MRSA, or HA-MRSA), and as Community-Acquired-MRSA, or CA-MRSA, which causes human infections in the community (Vandenesch *et al.,* 2003). In reports of MRSA colonisation and infection have been documented for a range of animal species in addition to humans (Cuny *et al.,* 2010). These strains, also known as Livestock associated MRSA (LA-MRSA) strains, were primarily clonal complex CC39 isolates and had a genetic origin distinct from that of previously documented human infections.

Methicillin-resistant *S. aureus* (MRSA), for example, has recently emerged as a public health hazard in both humans and animals due to the rise of antimicrobial resistance (AMR) bacteria. Numerous studies have shown that the spread of *S. aureus*, antibiotic-resistant bacteria, among farm animals, farm surroundings, and farmers can result in life-threatening sickness (Kanagarajah *et al.,* 2017). It has already been established that the widespread presence of multidrug-resistant pathogenic bacteria or their resistance genes in poultry, meat, and dairy products leads to persistent infections, a higher incidence of complications, and an

*Corresponding author: joympvet@gmail.com

increase in morbidity and mortality in both humans and animals (Miranda *et al.,* 2021; Algammal *et al.,* 2020). This could be a risk factor for zoonotic illnesses that are hard to treat or that colonise humans. The aim of the present study was to detect the MRSA by Polymerase Chain Reaction (PCR) and to study the antibiotic susceptibility profile of *S. aureus* isolated from the nares of cattle.

2. Materials and Methods

Sample collection

A total of 160 nasal swab samples of cattle were collected from different places of Aizawl, Mizoram, using sterile cotton swabs. The collected swab samples were kept in transport media under 4°C and transported to Laboratory for further analysis.

Isolation and identification of S. aureus

The swab samples were homogenized with 9 ml sterile 0.1% peptone water enrichment broth and was centrifuged at 8000 rpm for 2-3 minutes for extraction of the sample in the broth and incubated for 24 hours at 37°C (Cowan and Steele, 1993). A loopful of the broth was then streaked onto Baird Parker agar (BPA) and was incubated at 37°C for 45± 2 hours. Typical suspected *Staphylococci* colonies appeared as jet black colonies surrounded by a halo zone on BPA. A typical colony from Baird Parker agar was streaked on Mannitol Salt agar (MSA) plate and incubated for 48 h at 37°C and golden yellow, smooth, circular, convex, and moist was observed for confirmation. The suspected *S. aureus* colonies were then identified by standard biochemical tests including Gram staining, catalase reaction, oxidase reaction, mannitol fermentation and coagulase production (Quinn *et al*., 1999).

DNA extraction

All the phenotypically positive *S. aureus* isolates were processed for extraction of bacterial DNA lysate using boiling and snap chilling method. Bacterial isolates were inoculated into one ml of Luria Bertani (LB) broth and incubated at 37°C overnight. After overnight incubation at 37°C, cells were pelleted by centrifugation at 8000 rpm for 15 minutes at 4°C. Then the pellet was washed three times with sterile normal saline solution (0.85%) and finally re-suspended in 300µl of *nuc*lease free sterile distilled water and boiled in a water bath for 10 minutes followed by immediate chilling. The lysate was centrifuged again at 5000 rpm for 5 minutes. The supernatant was used as template for PCR assay.

Genotypic Determination of Staphylococcus aureus

PCR was carried out to screen the presence of the *nuc* gene (Brakstad *et al.,*1992) of *S.aureus*. The positive samples were then further screened for *mec*A gene (McClure *et al.,* 2006). The primer detail and cycling conditions for the various genes used in the study are detailed in Table 1 and 2. Amplified products were analysed by agarose gel (1%) electrophoresis and documented (Figure 1).

Antibiogram

In vitro antimicrobial susceptibility pattern of *mec*A positive samples were determined as per disk diffusion method (Bauer *et al.,* 1966) using 10 commonly used antimicrobial agents. The antimicrobial discs were amikacin (30 mcg), ampicillin (1mcg), cefoxitin (30 mcg), ceftriaxone (30 mcg), chloramphenicol (30 mcg), ciprofloxacin (5 mcg), erythromycin (5 mcg), gentamicin (10 mcg), oxacillin (1 mcg), tetracycline (10 mcg) according to Clinical and Laboratory Standard Institute (CSLI) guidelines (2019). The results were noted after 24 h of incubation at 37°C by measuring the diameter of zone of inhibition and interpretation as sensitive, intermediate and resistant were made as per manufacturer's instruction (Table 3).

3. Results and Discussion

Sixty isolates were found to be positive by the cultural and biochemical methods out of 160 nasal swabs from the cattle. Only 13 of the 60 staphylococci isolates that had been culturally and biochemically described so far had their species-specific gene (*nuc* gene) amplified by PCR. Overall, 8.125% of cattle had *S. aureus* in their nasal canals. Rahimi *et al.* (2014) also showed 5.06% prevalence of *S. aureus* from nasal samples of cattle from various regions of Iran, which was similar to the current study. The prevalence of *S. aureus* nasal carriage was likewise found to be low in animals (9.97%), but extremely high among breeders (60%) (Mourabit *et al.,* 2020). The variations in sample size, location, and geographic area could be the cause of the discrepancies in *S. aureus* prevalence.

Extracellular thermostable nuclease, or TNase, is a protein that was produced by *S. aureus*. The *nuc* gene has the potential to be utilised for the quick diagnosis of *S. aureus* because it is an endonuclease gene that breaks down both DNA and RNA. It was specifically cloned and sequenced for *S. aureus* and can be used for the final confirmation of the species (Diab *et al.,* 2021).

The prevalence rate from the cattle nares was 5.0%, and 8 of the 13 positive isolates carried the *mec*A virulence gene. MRSA prevalence was similarly determined to be 5.59% by Liu *et al.,* (2018). Weese *et al.* (2010) did not isolate any MRSA from cattle nasal swab samples, in contrast to our investigation. The presence of the chromosomally localised *mec*A genes, which encode for penicillin-binding proteins, is what causes MRSA to be resistant to -lactam antibiotics (PBP 2a). The gene is found on the staphylococcal cassette chromosome mec (SCCmec), a mobile genetic

element (Zambrano-Mila *et al.,* 2020). While the majority of MRSA were once thought to have a specific host, more recent research has revealed that some strains lose their host specificity and are easily transmitted from animals to humans or humans to animals (Kalayu *et al.,* 2020).

All of the MRSA-positive samples in the current investigation displayed 100% resistance to oxacillin and ampicillin. Similar results against gentamicin and chloramphenicol showed 100% and 87.5% sensitivity, respectively. The failure of treatment and control is being caused by the growth of multidrug-resistance (MDR) in MRSA. MRSA is a microbe that is dangerous to both humans and animals because it has evolved new, efficient defences against contemporary antibiotics (Algammal *et al.,* 2020). The repeated therapeutic or indiscriminate use of antibiotics leads to the development of antimicrobial resistance in bacteria almost always (Ariffin *et al.,* 2019). Antimicrobial resistance genes are frequently transferred when bacteria become resistant to antibiotics (Nicolaou and Rigol, 2018). As a result, it might be able to predict that *S. aureus* isolates resistant to widely used antibiotics like oxacillin and ampicillin include genes for resistance that produce protective enzymes, efflux pumps, or ribosome protection mechanisms (Foster, 2017). Gentamicin and chloramphenicol, however, were both effective against all MDR isolates, indicating that recurrent *S. aureus* infections in cattle may be treated with these two antibiotics. The existence of a resistance gene in the nasal *S. aureus* isolates from cattle used in this investigation suggests that these species can infect humans.

4. Conclusion

In conclusion, MRSA in ruminant nares may serve as a reservoir for *S. aureus* that is resistant to antibiotics. A hazard to the public's health is the widespread presence of MRSA in the neighbourhood. The greatest challenge in treating MRSA infections is the emergence of multi-drug resistance. To stop the MRSA infection from spreading, public health experts and veterinary officials must work together. This study may be the first of its kind in the North East region of the country, to the best of our knowledge.

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6. References

Algammal AM, El-Sayed ME, Youssef FM, Saad SA, Elhaig MM, Batiha GE, Hozzein WN and Ghobashy MO (2020). Prevalence, the antibiogram and the frequency of virulence genes of the most predominant bacterial pathogens incriminated in calf pneumonia. AMB Express. 10: 1-8. doi: 10.1186/s13568-020-01037-z.

Ariffin SM, Hasmadi N, Syawari NM, Sukiman MZ, Ariffin MFT, Chai MH and Ghazali MF (2019). Prevalence and antibiotic susceptibility pattern of *Staphylococcus aureus*, *Streptococcus agalactiae* and *Escherichia coli* in dairy goats with clinical and subclinical mastitis. Journal of Animal Health and Production. 7: 32-37. https://doi.org/ 10.17582/journal.jahp/2019/7.1.32.37.

Bauer AW, Kirby WMM, Sherris JC and Truck M (1966). Antibiotic susceptibility testing by a standardized single disc method. American Journal of Clinical Pathology 45: 493–496.

Brakstad OG, Aasbakk K and Maeland JA (1992). Detection of *Staphylococcus aureus* by polymerase chain reaction amplification of the nuc gene. Journal of Clinical Microbiology. 30(7): 1654-1660.

Chai MH, Sukiman MZ, Liew YW, Shapawi MS, Roslan, FS, Hashim SN, Mohamad NM, Ariffin SMZ and Ghazali MF (2021). Detection, molecular characterization, and antibiogram of multi-drug resistant and methicillin-resistant Staphylococcus aureus (MRSA) isolated from pets and pet owners in Malaysia. Iranian Journal of Veterinary Research 22(4): 277-287.

Clinical and Laboratory Standards Institute (2019). Performance Standards for Antimicrobial Susceptibility testing. Vol. 39, 29th edn. CLSI supplement M100. Wayne, USA, Clinical and Laboratory Stanadards Institute

Cowan ST and Steele KJ (1993). Cowan and Steel's manual for the identification of medical bacteria 3rd edition. Barrow and Feltham, Cambridge University Press, Cambridge, pp.15-52

Cuny C, Friedrich A, Kozytska S, Layer F, Nübel U, Ohlsen K, Strommenger B, Walther B, Wieler L and Witte W (2010). Emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) in different animal species. International Journal of Medical Microbiology. 300(2-3):109-17. doi: 10.1016/j.ijmm.2009.11.002. Epub 2009 Dec 16. PMID: 20005777.

Diab MS, Ibrahim NA, Elnaker YF, Zidan SA and Saad MA (2021). Molecular detection of *Staphylococcus aureus* enterotoxin genes isolated from mastitic milk and humans in El-Behira, Egypt. International Journal of One Health 7(1): 70-77.

Foster TJ (2017). Antibiotic resistance in Staphylococcus aureus. Current status and future prospects. FEMS Microbiology Review. 41: 430-449. https://doi.org/10.1093/ femsre/fux007.

García-Álvarez L, Holden MT, Lindsay H, Webb CR, Brown DF, Curran MD, Walpole E, Brooks K, Pickard DJ, Teale C, Parkhill J, Bentley SD, Edwards GF, Girvan EK, Kearns AM, Pichon B, Hill RL, Larsen AR, Skov RL, Peacock SJ, Maskell DJ and Holmes MA (2011). Methicillin-resistant *Staphylococcus aureus* with a novel mecA homologue in human and bovine populations in the UK and Denmark: a descriptive study. Lancet Infectious Diseases. 1(8):595-603. doi: 10.1016/S1473-3099(11)70126-8. PMID: 21641281; PMCID: PMC3829197.

Jevons MP, Coe AW and Parker MT (1963). Methicillin resistance in staphylococci. Lancet. 1:904–7

Kalayu AA, Woldetsadik DA, Woldeamanuel Y, Wang SH, Gebreyes WA and Teferi T (2020). Burden and antimicrobial resistance of *S. aureus* in dairy farms in Mekelle, Northern Ethiopia. BMC Veterinary Research. 16(1), 20.

Kanagarajah RR, Lee DCW, Lee DZF, Yusoff K, Paramasivam SJ, Low WY, Jeevaratnam K and Lim SHE (2017). Antibiotic profiling of Methicillin-Resistant *Staphylococcus aureus* (MRSA) isolates in stray canines and felines. Cogent Biology. 3: 1-10. https://doi.org/10.1080/ 23312025.2017.1412280.

Liu B, Sun H, Pan Y, Zhai Y, Cai T, Yuan X and Hu G (2018). Prevalence, resistance pattern, and molecular characterization of *Staphylococcus aureus* isolates from healthy animals and sick populations in Henan Province, China. Gut Pathogens. 10(1): 31.

McClure JA, Conly JM, Lau V, Elsayed S, Louie T, Hutchins W and Zhang K (2006). Novel multiplex PCR assay for detection of the staphylococcal virulence marker Panton-Valentine leukocidin genes and simultaneous discrimination of methicillin-susceptible from resistant *Staphylococci*. Journal of Clinical Microbiology. 44: 1141-1144.

Miranda C, Silva V, Igrejas G and Poeta P (2021). Impact of European pet antibiotic use on enterococci and staphylococci antimicrobial resistance and human health. Future Microbiology. 16: 185-203.

Mork T, Kvitle B and Jorgensen HJ (2012). Reservoirs of *Staphylococcus aureus* in meat sheep and dairy cattle. Veterinary Microbiology. 155(1):81–7.

Mourabit N, Arakrak A, Bakkali M, Zian Z, Bakkach J and Laglaoui A (2020). Nasal carriage of *Staphylococcus aureus* in farm animals and breeders in north of Morocco. BMC Infectious Diseases. 20(1):602. doi: 10.1186/s12879-020-05329-4. PMID: 32799799; PMCID: PMC7429463.

Nemeghaire S, Argudin MA, Haesebrouck F and Butaye P (2014). Epidemiology and molecular characterization of methicillin-resistant *Staphylococcus aureus* nasal carriage isolates from bovines. BMC Veterinary Research. 10:153.

Nicolaou KC and Rigol S (2018). A brief history of antibiotics and select advances in their synthesis. The Journal of Antibiotics. 71: 153-184. https://doi.org/10.1038/ja.2017.62.

Peton V and Le Loir Y (2014). *Staphylococcus aureus* in veterinary medicine. Infection, Genetics and Evolution. 21:602–15

Quinn PJ, Carter ME, Markey B and Carter GR (1999). Clinical Veterinary Microbiology. Vol. 118. Moss by International Limited, Spain, pp. 209-242.

Rahimi H, Saei HD and Ahmadi M (2015). Nasal Carriage of *Staphylococcus aureus*: Frequency and Antibiotic Resistance in Healthy Ruminants. Jundishapur Journal of Microbiology 8(10): e22413

Vandenesch F, Naimi T, Enright MC, Lina G, Nimmo GR, Heffernan H, Liassine N, Bes M, Greenland T, Reverdy ME and Etienne J (2003). Community-acquired methicillin-resistant *Staphylococcus aureus* carrying Panton-Valentine leukocidin genes: worldwide emergence. Emerging Infectious Diseases. 9(8):978-84. doi: 10.3201/eid0908.030089. PMID: 12967497; PMCID: PMC3020611.

Vautor E, Abadie G, Guibert JM, Chevalier N and Pepin M (2005). Nasal carriage of *Staphylococcus aureus* in dairy sheep. Veterinary Microbiology 106(3-4):235–9

Weese JS, Hannon SJ, Booker CW, Gow S, Avery BP and Reid-Smith RJ (2012). The Prevalence of Methicillin-Resistant *Staphylococcus aureus* Colonization in Feedlot Cattle. Zoonoses and public health. 59(2), 144-147.

Wertheim HF, Melles DC, Vos MC, van Leeuwen W, van Belkum A, Verbrugh HA and Nouwen JL (2005). The role of nasal carriage in *Staphylococcus aureus* infections. Lancet Infectious Diseases. 5(12):751-62. doi: 10.1016/S1473-3099(05)70295-4. PMID: 16310147.

Zambrano-Mila M, Rodriguez AS, Rivera-Olivero IA, Salas-Rueda M, Caceres-Orellana MV, de Waard JH and Garcia-Bereguiain MA (2020). Methicillin resistant *Staphylococcus aureus* carriage among guinea pigs raised as livestock in Ecuador. One Health. *9*, 100118.