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Sub-acute mastitis associated with Methicillin Resistant *Staphylococcus aureus* in a cow: A case report

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ABSTRACT

A 5-year old Holstein Friesian cross breed cow was presented to Madras Veterinary College Teaching Hospital with the history of reduced milk yield. Clinical examination of udder revealed normal milk color and soft udder. The milk pH was 7.0, with California Mastitis Test score 3+, Electrical Conductivity 270U, and Somatic Cell Count as 328,000. Isolation and identification of causative agent revealed Methicillin Resistant *Staphylococcus aureus* (MRSA) from the sub-acute mastitis sample. Agar disc diffusion method for antimicrobial susceptibility revealed that the MRSA was sensitive to Enrofloxacin, Gentamicin, Oxytetracycline and Amoxicillin+Sulbactam. On the other hand, the isolate was resistance to Amoxicillin, Penicillin G, Ceftriaxone and Methicillin. The isolate was positive for β-lactamase resistance by Nitrocefin test. The MRSA was confirmed for the presence of *mec*A and *bla*Z target genes by polymerase chain reaction (PCR). The cow was treated with Enrofloxacin, Vitamin E and inorganic Selenium, and was recovered after 5 days of post-treatment.

Keywords

Beta lactamase, *bla*Z, Bovine mastitis, *mec*A, PCR, *Staphylococcus aureus*

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CASE HISTORY AND CLINICAL OBSERVATIONS

A 5-year old Holstein Friesian cross bred cow was brought to Madras Veterinary College Teaching Hospital Large Animal Medicine Ward with the history of reduced milk yield, and calved 2 months ago. Clinical examination of udder revealed normal milk color, soft udder and all other parameters were normal. Milk pH was 7.0, California Mastitis Test (CMT) score was 3+ , Electrical Conductivity (EC) was 270U (DRAMINSKI mastitis detector), and Somatic Cell Count (SSC) was 328,000 (DeLaval Somatic Cell counter, Sweden). Based on these observations, the animal was suspected to be infected with sub-clinical mastitis. Milk sample was collected in sterile culture tube for culture and antibiotic susceptibility test. The collected milk sample was cultured on 5% ovine blood agar plates. The isolate was characterized by their growth on blood agar and mannitol salt agar, and the results for catalase and coagulase.

Antimicrobial susceptibility against commonly used antibiotics was determined by agar disc diffusion method on Muller-Hinton agar plates (CLSI, 2008). Production of β-lactamase was tested using Nitrocefin (chromogenic cephalosporin) discs as recommended by manufacturer (Sigma Aldrich). There was a change in color of disc from yellowish to red, and hence was considered as positive.

CONFIRMATION OF MRSA

The MRSA commercial kit (MRSA Alert™ Kit, HiMedia, India) was used for confirmation of Methicillin Resistant *Staphylococcus aureus* (MRSA) following the instructions of the manufacturer, and presence of a clot and color change in the medium from red to yellow after 24 h (Selepak and Witebsky, 1985; Begum et al., 2007). Further, the *Staphylococcus* isolate was re-confirmed as MRSA by amplifying the MRSA specific genes (*mec*A and *bla*Z) using the gene specific primers, as described by Lee (2003) and Martineau et al. (2000), respectively (**Figure 1, 2**).

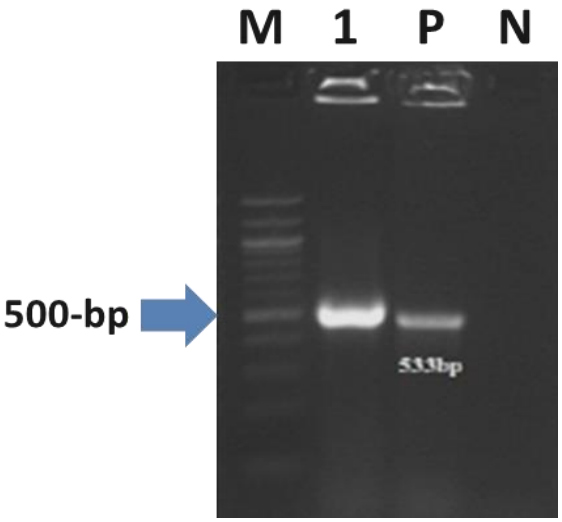

Figure 1. PCR amplification of *mec*A gene. Expected amplicon size 533-bp. M- 100-bp DNA ladder, Lane 1- Test sample, Lane P- Positive control (*mec*A gene), Lane N- Negative control.

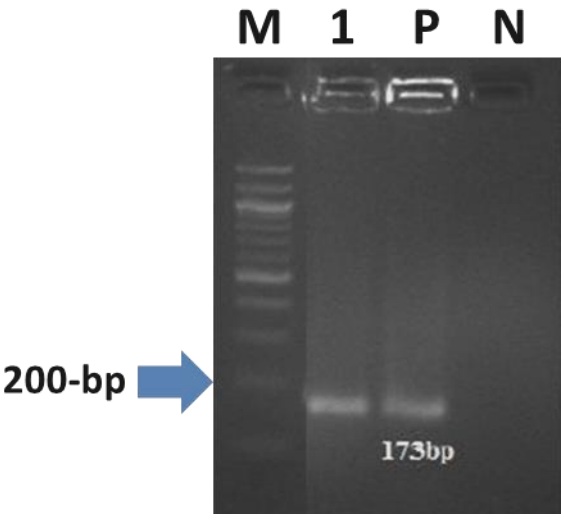

Figure 2. PCR amplification of *bla*Z gene. Expected amplicon size 173-bp. Lane M- 100bp DNA ladder, Lane 1- Test sample, Lane P- Positive control (*bla*Z gene), Lane N- Negative control.

TREATMENT AND MANAGEMENT

Based on antibiotic sensitivity test, the cow was treated with Enrofloxacin (dosed at 5 mg/kg b.wt., through intramuscular route, once for 5 days), Vitamin E (dosed at 500 IU/day orally for 5 days), and inorganic Selenium (dosed at 6 mg/day orally for 5 days). Post-treatment assessment was carried out after 5 days based on milk pH, EC and SSC.

DISCUSSION

In the present study, the milk pH was 7.0, CMT result was 3+, EC was 270U, and SSC was 328,000; these were suggestive for being the case as sub-clinical mastitis. The isolate was characterized by their growth on mannitol salt agar and positive for catalase and coagulase tests. The colonies of hemolytic *Staphylococci* were smooth, circular, moist, 1-2 mm in diameter, pinhead, raised, convex having entire margins, and golden-yellow in color, as described by Islam et al. (2014).

The antibiotic susceptibility profile for MRSA isolate showed sensitivity to Enrofloxacin, Gentamicin, Oxytetracycline and Amoxicillin+Sulbactam. In contrast, the isolate showed resistance to Amoxicillin, Penicillin G, Ceftriaxone and Methicillin. The high resistance of Penicillin G, Ceftriaxone, Amoxicillin and Oxacillin in MRSA mastitis in the present study could be attributed to the indiscriminate use of these drugs and intramammary preparations (Cloxacillin) used by the owner without the prescription of any veterinarian.

In the present study, change in color of Nitrocefin disc from yellowish to red was noticed. Nitrocefin discs were used for the detection of *mec*A-mediated resistance of MRSA or β-lactamase production of *Staphylococci* (Pitkala et al., 2007). The present observation was in agreement with (Kumar et al., 2010) who reported that MRSA strains were multi-drug resistant which might be due to production of β-lactamase and penicillin binding protein (PBP) 2a.

Selepak and Witebsky (1985) reported that MRSA Alert kit test was useful in the detection of Methicillin and β-lactamase resistant *S. aureus*. Loeffler and Lloyd (2010) opined that detection of *mec*A and *bla*Z genes by PCR was gold standard tests for confirmation of Methicillin resistance.

Post-treatment values of milk pH, EC and SCC were 6.7, 305U and 68,000, respectively. Post-treatment pH and SCC significantly decreased as compared to pre-treatment value, and post-treatment EC significantly

increased as compared to pre-treatment values, indicating that the treatment was effective in controlling the inflammation and infection. This might be due to effects of immunomodulation, and antibiotic used.

In the present study, the Enrofloxacin was found to be most effective against the MRSA, as reported by Kenar et al. (2012). On the other hand, Sharma (2007) found that dietary supplementation of Vitamin E (500 IU/day) and Selenium (6 mg/day) during early lactation in the cows suffering from subclinical mastitis caused 40% reduction in the infection rate. Supplementation of Vitamin E and Selenium enhance the chemotaxis process by macrophages, which are stimulated by opsonized *S. aureus*, and subsequently reduces the severity and duration of mastitis (Spears and Weiss, 2008).

CONCLUSION

The MRSA was isolated and identified from a case of sub-acute bovine mastitis using conventional and PCR based approach. The isolated MRSA was found to be sensitive to Enrofloxcain, Gentamicin, Amoxicillin+ Sulbactam, Ceftriaxone, and resistant to Penicillin G, Amoxicillin, Oxytetracycline and Oxacillin. The cow was successfully treated using Enrofloxacin, Vitamin E, and Selenium, and was recovered after 5 days.

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