

ISOLATION AND ANTIBIOGRAM OF BETA HAEMOLYTIC STAPHYLOCOCCUS AUREUS ASSOCIATED WITH BOVINE CLINICAL MASTITIS

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ABSTRACT

The present study was conducted to detect beta haemolytic *Staphylococcus aureus* associated with clinical mastitis in bovines. A total of 112 milk samples were collected from all the four quarters of each of 28 animals (13 cattle and 15 buffaloes) suffering from clinical mastitis. Forty milk samples originating from these animals showed the presence of *Staphylococci*. Isolates were confirmed to be *S. aureus* by a combination of various tests i.e., catalase, coagulase and growth on mannitol salt agar plate. Beta haemolytic activity of 10 field isolates was determined by 'Hot-Cold' lysis on 5% sheep blood agar plate and titres in culture supernatant of these isolates were determined by microtitre plate method. Beta haemolytic titres ranged from 16 to 256 indicating significant variation in the ability of these isolates to produce toxin. All the isolates were found sensitive to methicillin, chloramphenicol and ampicillin/sulbactam.

Key words: *Staphylococcus aureus*, beta haemolysin titres, antibiogram, bovine mastitis

Staphylococcus aureus, a Gram-positive bacterium, is found most frequently associated with bovine mastitis; an economically the top-ranking infectious disease of dairy industry (Sordillo and Streicher, 2002; Zecconi *et al.*, 2003). Due to production of toxins and antibiotic resistance, these infections represent a serious public health threat. Majority of isolates of *S. aureus* associated with bovine mastitis are beta-haemolytic (h1b). A survey by Aarestrup *et al.* (1999) found that h1b was produced in 72% of bovine mastitis isolates.

H1b has Mg⁺⁺ dependent sphingomyelinase C activity to degrade sphingomyelin in cell membranes of erythrocytes, leukocytes, neurons and other tissue cells (Gow and Robinson, 1969; Bernheimer *et al.*, 1974). *S. aureus* h1b causes classical 'hot-cold' haemolysis of sheep erythrocytes, i.e., it binds at 37°C to sphingomyelin in sheep erythrocyte membranes and then degrades it for causing membrane dissolution at 4°C within a few minutes. The present study was undertaken to detect the presence of h1b *S. aureus* in clinical mastitis cases in bovines.

MATERIALS AND METHODS

Sampling and Isolation: Milk samples were collected from 112 quarters of 28 bovines (13 cattle and 15 buffaloes) suffering from clinical mastitis at village Dhansu,

district Hisar and at the Teaching Veterinary Clinical Complex, LUVAS, Hisar. After disinfecting the teats with 70% alcohol and discarding first stripping, milk samples were collected in 15 ml sterile disposable centrifuge tubes and immediately transported to the laboratory for examination. The milk samples were inoculated on 5% defibrinated sheep blood agar plates on the same day or after storing at -20°C overnight. Suspected isolates of *Staphylococci* were identified by Gram staining and catalase test.

Mannitol Fermentation Test: All the isolates of *Staphylococci* were inoculated on mannitol salt agar plate as a selective media for staphylococci and incubated overnight at 37°C. The isolates which changed the colour of the agar medium from red to yellow as a result of acid production were considered mannitol fermenters.

Beta Haemolytic Activity: *S. aureus* isolated from milk samples were streaked on blood agar plate and incubated at 37°C overnight. The plate showing bacterial colonies was transferred to refrigerator at 4°C and observed for clear zones of haemolysis around the colonies.

Titration of *S. aureus* h1b in Culture Supernatant: H1b in the culture supernatant was titrated according to the method of Singh *et al.* (2006). Briefly, 1% sheep erythrocyte (1% SRBC) suspension was made in toxin diluent buffer, after washing the peripheral whole blood

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thrice in SRBC wash buffer. Culture supernatant containing *S. aureus* hlb (SA-hlb) was serially diluted two-fold, from neat to 1:4096 in the toxin diluent buffer, in a volume of 50 µl/well in a 96-well U-bottom microtitre plate. SRBC suspension was added 50 µl/well in all wells and mixed by tapping the plate gently.

The microtitre plate was incubated at 37°C for one hr. The SRBCs settled in the wells were resuspended and the microtitre plate was transferred to refrigerator at 4°C and incubated for about two hrs before observing for ‘hot-cold’ haemolysis (i.e., β-haemolysis occurring only when the plate is shifted from 37°C to 4°C) in the wells. The highest dilution of the culture supernatant showing complete haemolysis was recorded as the end-point dilution and its reciprocal taken as SA-hlb titre.

Antibiotic Sensitivity Test: All the isolates of *S. aureus* were subjected to *in vitro* drug sensitivity by the disc-diffusion method of Bauer *et al.* (1966). Results were scored as sensitive (S), intermediate (I) and resistant (R) to the antimicrobial based on zone size diameter using the manufacturer’s table for interpretation (Himedia, Mumbai).

RESULTS AND DISCUSSION

Isolation and Characterization of Beta-Haemolytic *S. aureus*: Forty milk samples showed the presence of *Staphylococci*. Only ten isolates representing 10 animals were used in further investigations. All the ten isolates had typical grape-like clusters of Gram-positive cocci when examined by Gram’s staining. A typical β-haemolytic pattern was exhibited by each isolate on blood agar plate. All the isolates were catalase positive and fermented mannitol. Coagulase gene was also detected in all the isolates by polymerase chain reaction (data not shown).

Hlb titres in culture supernatant of 10 field isolates as determined by ‘hot-cold’ lysis of sheep RBCs are presented in Fig. 1. Hlb titres ranged from 16 to 256. In spite of identical culture conditions, variations in hlb titres in culture supernatants of the 10 isolates were noticed possibly because of the isolates differed in their physiology for secretion of hlb in combination with various other exoproteins. Several different exotoxins and other proteins are produced by virulent *S. aureus* isolates/strains, but individual isolate do not secrete the entire set of such proteins (Nakano *et al.*, 2002). Depending on the number of different proteins produced, relative proportions of individual exotoxins would have differed in these isolates.

Nakano *et al.* (2002) have reported the proportion of hlb to be about 5.5% of total exoproteins secreted by an *S. aureus* strain 2932 from human. Beta toxin strongly stimulates biofilm formation *in vivo* as demonstrated by Huseby *et al.* (2010) as a role in causation of infectious endocarditis in a rabbit model. Role of hlb in biofilms forming *S. aureus* in chronic recalcitrant mastitis cases is therefore speculated.

Sensitivity Pattern: Sensitivity pattern exhibited by *S. aureus* isolates to 14 different antimicrobials is shown in Fig. 2. All the isolates were found 100% sensitive to methicillin, chloramphenicol and ampicillin/sulbactam. Methicillin-resistant *S. aureus* (MRSA) and multi-resistant *S. aureus* strains have also been reported in some cases in veterinary medicine (Lee, 2003; Baptiste *et al.*, 2005)

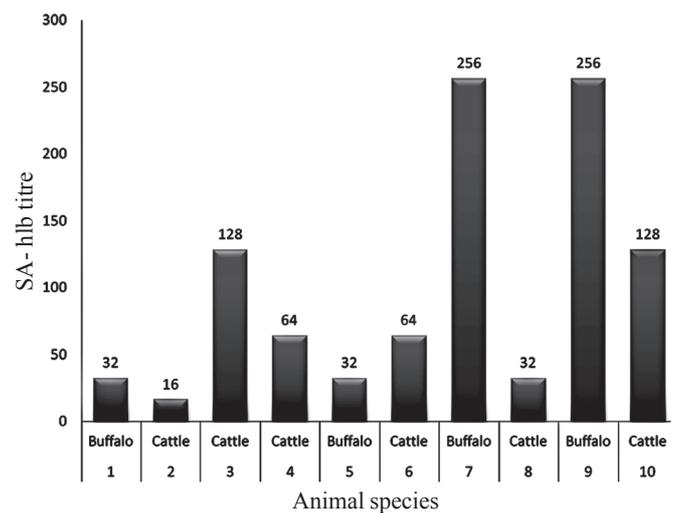


Fig 1. Beta-haemolysin titres of culture supernatants of the field isolates of *Staphylococcus aureus*

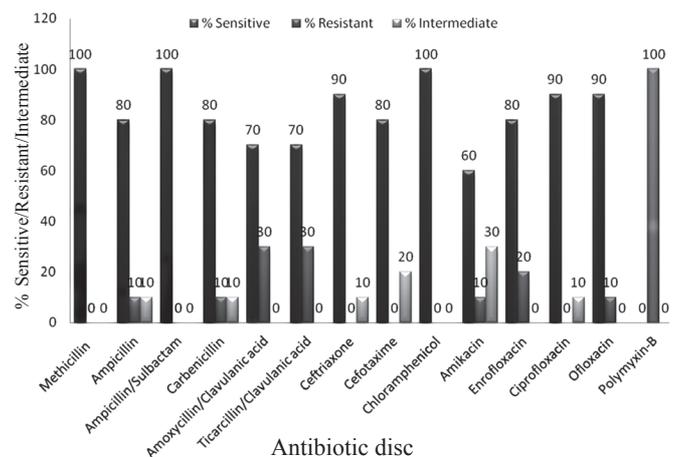


Fig 2. Antibiotic sensitivity patterns of *Staphylococcus aureus* field isolates

but we did not encounter any isolate that is methicillin resistant fortunately.

All the isolates were resistant to polymyxin-B. Various isolates were resistant to antibiotics commonly used in the field i.e. amoxicillin/clavulanic acid, ceftriaxone, enrofloxacin and ofloxacin. It has been observed that amoxicillin alone or in combination with β -lactamase inhibitors is potentially useful for the treatment of mastitis caused by pathogenic organisms (Wilson *et al.* 1999; De-Oliveira *et al.* 2000). Resistant bacteria involved in mastitis cases make disease difficult and costly to treat and are of major concern.

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