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Antimicrobial effect of colloidal argentum colloid on ampicillin resistant *Staphylococcus aureus*

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Abstract
Mastitis is one of the main diseases affecting the dairy cattle around the world. It causes huge economical losses to dairy farmers worldwide. *Staphylococcus aureus* is one of the pathogens implicated in bovine mastitis. The *S. aureus* is a coagulase positive, facultative anaerobic bacterium and can be microscopically characterized as single, pairs or cluster of Gram-positive cocci. In current study 50 milk samples from a dairy farm were screened for *Staphylococcus aureus*. positive samples were checked for antibiotic sensitivity with ampicillin (5mcg). Isolates resistant to ampicillin were selected for treatment with silver nanoparticles. Silver nanoparticles were used at a concentration of 8 µg/ml and zone of inhibition was measured using ABST scale. Silver nanoparticles showed a zone of inhibition comparable to zone shown by *Staphylococcus aureus* sensitive to ampicillin.

Keywords: Colloidal argentum colloid, ampicillin resistant, *Staphylococcus aureus*

Introduction
Mastitis is one of the main diseases affecting the dairy cattle around the world. Mastitis destroys the milk-secreting cells. Scar or connective tissue replaces the milk secreting tissue, resulting in a permanent loss of productive ability. *Staphylococcus aureus* is one of the pathogens implicated in bovine mastitis.

The *S. aureus* is a coagulase positive, facultative anaerobic bacterium and can be microscopically characterized as single, pairs or cluster of Gram positive cocci (Deresinski, 2005) [4]. The *S. aureus* is non-sporulating, non-motile, oxidase-negative which can be differentiated from Streptococci and other gram-positive bacteria due to the production of catalase (Kloos and Schliefer, 1986). *S. aureus* ferment glucose and produce lactic acid. *S. aureus* is coagulase positive. Coagulase is a collagen binding protein encoded by *coa* gene and has been demonstrated to be directly related with bovine mastitis (Momtaz *et al*., 2010). The cocci commonly form irregular clusters with a grape like appearance under the microscope. Macroscopically it is a facultatively anaerobic bacterium which grows rapidly on blood agar and nonselective solid media nutrient agar under both aerobic and anaerobic conditions (Yu and Washington, 1985) [12]. Colonies appear smooth convex and sharply defined on blood agar plates when grown at room temperature (Lowy, 1998) [8]. The colonies are gold pigmented due to carotenoids (Waldvogel, 2000). They cause beta-hemolysis on sheep, horse and human blood agar plates.

Drug resistance in microorganisms is a predictable and perhaps inescapable response to the use of antimicrobial agent. The organisms exhibit remarkable versatility in their behavior towards antibiotics (Grassi, 1988) [5], with some strains having overcome most commonly used drugs. Exposure to new antibiotics often results in further selection of homologous resistant strains (Haley, *et al.*, 1982) [6], a phenomenon particularly favored by irrational antibiotic administration. Infection with such resistant strains is likely to be more severe.

Materials and Methods
Collection of samples
A total of 50 milk samples were collected from cows from a dairy farm in Rohtak Haryana. The samples were collected in sterile sample tubes and processed immediately.

Confirmation of samples for mastitis

Bromothymol blue test was used for confirming the samples for mastitis. A drop of BTB was spread on a blotting paper followed by drop of milk. Change in color was noticed.

Isolation and identification of bacteria

BTB positive samples were inoculated into nutrient broth and incubated overnight at 37°C. From broth the samples were inoculated on to nutrient agar and colonies were observed with the help of magnifying glass. Sub-culturing of samples was done till a pure culture was obtained. Selected colonies from nutrient agar were inoculated onto Mannitol salt agar and Baird Parker agar. *S. aureus* colonies were also inoculated on blood agar plates and observed for hemolysis.

Catalase test

A loopful of culture was mixedwith few drops of 3% hydrogen peroxide on a glass slide. Appearance of bubbles or effervescence within first few seconds was observed and considered positive. Absence of bubbles was considered negative.

Oxidase test

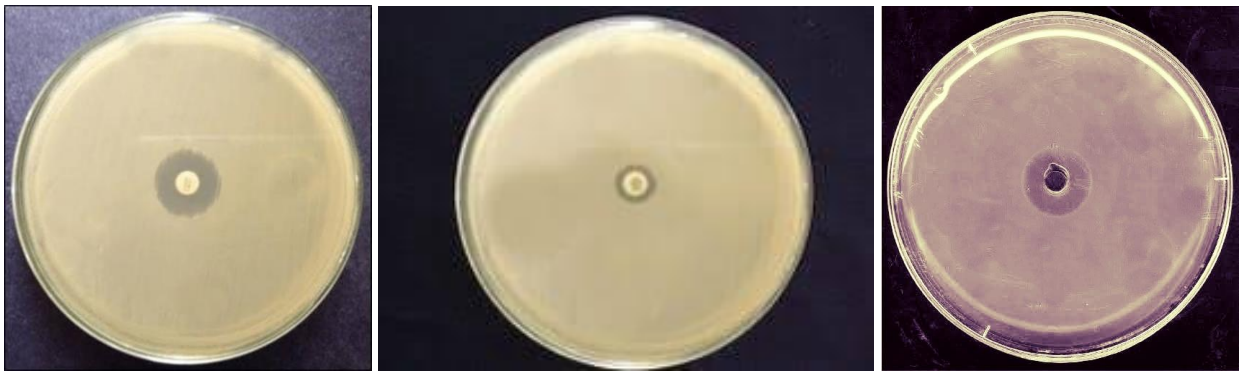
A loopful of culture was spread onto oxidase discs and change of color from white to purple was observed and considered positive. Absence of color change or delay in color change was considered negative.

Coagulase test

To a tube containing 500 µl of rabbit plasma few drops of culture of staphylococcus was added and mixed well by rotating the tube. Clotting of plasma within 2-5 hours indicates a positive test.

Antibiotic Sensitivity test

Confirmed *S. aureus* isolates were checked for susceptibility to ampicillin (5 µg) by disc diffusion method (Bauer *et al*., 1966). The bacteria from overnight broth culture was inoculated on Muller Hinton agar and spread evenly by using a spreader. Plates were left to dry and ampicillin discs were placed with the help of forceps. The plates were then incubated for 24 hours and zone of inhibition was measured with ABST scale. The ampicillin resistant isolates were selected for AgNP treatment.



A. B. C.

Fig 1: Antibiotic sensitivity test of *S. aureus* (A) Sensitive to Ampicillin and (B) Resistant to Ampicillin (C) Zone of inhibition of Argentium Nanoparticles against ampicillin resistant *Staphylococcus aureus*

Silver Nanoparticles treatment of ampicillin resistant isolates of *S. aureus*

Silver nanoparticles were synthesized by sally D Solomon method.

From overnight broth cultures of ampicillin resistant isolates of *S. aureus* 100 µl was spread on MH agar plates and was allowed to dry for 5 minutes. A well was cut in the middle of plate with the help of gel cutter and was loaded with 100 µl of silver nanoparticle solution of concentration 8 µg/ml and was incubated overnight at 37 ºC. The zone of inhibition was measured with the help of ABST scale.

Results and Discussion

Out of 50 milk samples 37 showed positive bromothymol test. On the basis of morphological characteristics and biochemical profile 21 out of 37 BTB positive samples were positive for *S. aureus* (Ali *et al*., 2008) [1]. Out of 21 *S. aureus* isolates 6 were resistant to ampicillin and were used in the study (Ranjan *et al*., 2011) [10]

Morphological characterization

On nutrient agar*S. aureus*produced golden yellow colonies. Gram stained slides of these selected colonies under microscope showed positive cocci which were in the form of bunch of grapes.

Biochemical characterization

S. aureus isolates were catalase positive, oxidase negative and coagulase positive.

Table 1: Biochemical tests for *S. aureus*

Test	Result
Catalase	Positive
Oxidase	Negative
Coagulase	Positive

These results were in confirmation with results for *S. aureus***.**

Antibiotic sensitivity Test and treatment with

*S. aureus*isolates with zone of inhibition >14 mm was considered susceptible, with 10-13 mm intermediately susceptible and <9 mm as resistant (Botrel *et al.*, 2010) [3]. Isolates showed a zone of inhibition as:

Table 2: Zone of inhibition observed in Antibiotic Sensitivity Test of *S.aureus*

Treatment	Average diameter of zone of inhibition
Susceptible *S. aureus* with ampicillin	46 mm
Resistant *S. aureus* with ampicillin	08 mm
Resistant *S. aureus* with AgNP	35 mm

Conclusion

S. aureus is one of the main causes of mastitis in dairy cattle. Due to indiscriminate use of antibiotics ampicillin resistant strains of S.*aureus* have evolved, which make the treatment and progression of disease even more difficult. In current study milk samples were screened for ampicillin resistant *S. aureus.* The resistant isolates were checked for inhibition by AgNPs and the results obtained were comparable to inhibition shown by ampicillin in non-resistant isolates of *S. aureus.*

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