International Journal of Veterinary Sciences and Animal Husbandry

ISSN: 2456-2912
VET 2023; SP-8(3): 43-46

www.veterinarypaper.com
Received: 23-05-2023
Accepted: 27-06-2023

A Shirisha
Ph.D. Scholar, Department of Veterinary Public Health and Epidemiology, Kamdhenu University, Gujarat, India

A Vijayakumar
Assistant Professor, Department of Veterinary Public Health and Epidemiology, PVNRTVU, Hyderabad, Telangana, India

Corresponding Author:
A Shirisha
Ph.D. Scholar, Department of Veterinary Public Health and Epidemiology, Kamdhenu University, Gujarat, India

Antibacterial activity of silver nanoparticles and selected antibiotics on streptococcus isolates and pure culture

A Shirisha and A Vijayakumar

DOI: https://doi.org/10.22271/veterinary.2023.v8.i3Sa.616

Abstract
The objective of the current study was to synthesize, characterize, and evaluate the antibacterial activity of silver nanoparticles (AgNPs) using an environmentally friendly approach against Streptococcus. A total of 100 milk samples were collected from various sources, and the culture-based method revealed that 27 samples (27%) tested positive for Streptococcus. The average zone of inhibition for tamarind fruit extract against Streptococcus was found to be 3 mm for pure cultures and 2.04±0.16 mm for milk isolates. Similarly, the mean zone of inhibition for moringa leaf extract was 2 mm for pure cultures and 1.00±0.14 mm for milk isolates. For both pure cultures and milk isolates, the average zone of inhibition of $AgNO_3$ solution against Streptococcus was 10 mm and 9.19±0.35 mm, respectively. The mean inhibition zone of AgNPs prepared with *Moringa oleifera* against Streptococcus was found to be 16 mm for pure cultures and 14.11±0.43 mm for milk isolates. On the other hand, AgNPs prepared with *Tamarindus indica* exhibited a mean inhibition zone of 14 mm for pure cultures and 12.19±0.46 mm for milk isolates against Streptococcus. The average inhibition zone of tetracycline against Streptococcus pure cultures and isolates was 6 mm and 4.04±0.38 mm, respectively. Ciprofloxacin displayed a mean inhibition zone of 12 mm for pure cultures and 10.30±0.40 mm for milk isolates, while Ampicillin's mean inhibition zone was 18 mm for pure cultures and 16.33±0.50 mm for milk isolates. Similarly, ceftriaxone exhibited a mean inhibition zone of 10 mm for pure cultures and 8.00±0.36 mm for milk isolates against Streptococcus.

Keywords: Streptococcus, anti-bacterial activity, silver nanoparticles, moringa, tamarind

Introduction
The bovine mammary gland is exposed to various microorganisms both during lactation and when the animal is not nursing. Mastitis is a condition that causes the death of milk-secreting cells (Barkema *et al.*, 2009) [1]. This leads to the replacement of milk-secreting tissue with scar or connective tissue, resulting in a lifelong reduction in productivity (Bradley, 2002) [2]. According to Sharma *et al.* (2010) [3], mastitis is a multi-etiological disease, with *Staphylococcus, Streptococcus, and Escherichia coli* (E. *coli*) being responsible for about 95% of reported cases. Despite extensive research, the aetiology of 20-35% of clinical cases remains unknown. Bacterial pathogens commonly found in mastitis milk are classified as infectious and environmental microorganisms (Gruet *et al.*, 2001) [4]. The infectious group includes Coagulase-Negative *Staphylococcus* species (CoNS) and *Streptococcus agalactiae*, while the environmental group includes *Staphylococcus aureus* and *Streptococcus dysgalactiae* (Riffon *et al.*, 2001) [5]. Streptococcus species are considered one of the significant bacteria causing mastitis [2]. Early diagnosis of streptococcal mastitis is crucial as it is often subclinical. Along with Staphylococcus species, the most frequently isolated Streptococcus species from mastitis cases are S. *agalactiae*, S. *Dysgalactiae*, and S. *Uberis*. The gold standard for identifying bacteria in milk has traditionally been conventional microbiological techniques. Standard culture methods are employed to identify mastitis-causing pathogens, followed by bio-typing of bacterial isolates.

Mastitis is a leading cause of the indiscriminate use of antibiotics in dairy animals, which leads to treatment failure, increased treatment costs, and the emergence of antibiotic resistance.

The use of Silver Nanoparticles (AgNPs) as an antibacterial agent is based on their effectiveness against harmful microorganisms at low concentrations and the advantages of environmentally friendly production methods (Vigneshwaran *et al.*, 2006) [6]. The aim of the current study was to determine the drug sensitivity pattern of streptococcus, one of the major agents causing mastitis. This research effort aims to combat the issue of antibiotic resistance and the economic losses associated with it.

Materials and Methods

Collection of milk samples

Milk samples were obtained from various dairy farms in and around Telangana state. Sterile test tubes were used to collect milk samples from cows diagnosed with mastitis. Five millilitres of milk were collected from each quarter. The collected milk samples were transported in an ice box to the Department of Veterinary Public Health and Epidemiology at the College of Veterinary Science, Rajendranagar.

Bacterial isolation

To isolate the bacteria, the milk samples were streaked on 5% Blood agar plates. The plates were then incubated aerobically at 37°C for 24 to 48 hours. The isolates were identified based on their cultural, morphological (Gram's-staining in Figure 01), and biochemical characteristics, following the method described by Barrow and Feltham (1993) [7]. Pure cultures of Streptococcus were obtained from Chandigarh at the Microbial Type Culture Collection and Gene Bank (MTCC). *In vitro* antibiotic sensitivity of the isolates was determined using selected antibiotic discs and AgNPs, following the technique described by Bauer *et al.* (1966) [8]. Blood agar plates were used for the susceptibility testing.

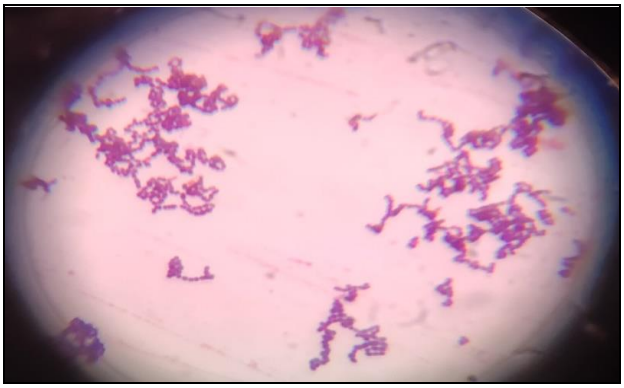


Fig 1: Gram staining (Chain like Streptococcus)

Antibiogram

The drug sensitivity test was conducted using a Disc Diffusion Assay, following the recommendations of CLSI [9]. Antibiotic discs including Tetracycline (30 µg), Ampicillin (10 µg), Ciprofloxacin (5 µg), and Ceftriaxone (30 µg) were aseptically placed on the surface of the Blood agar plates at equal distances from each other, as shown in Table 01. Additionally, 50 µl of each of the following substances: silver nitrate, Moringa leaf extract, Tamarind fruit extract, AgNPs prepared from Moringa leaf extract, and AgNPs prepared from Tamarind fruit extract, were added to the plates. The plates were then incubated at 37°C for 36 hours. The susceptibility of the organisms to different medications was determined by measuring the zone of inhibition around each disc [8].

Table 1: Antimicrobial discs with symbol, concentration

S. No	Antimicrobial material/ discs	Symbol	Concentration(µg/unit)
1.	Tetracycline	TE	30
2.	Ampicillin	AMP	10
3.	Ciprofloxacin	CIP	5
4.	Ceftriaxone	CTR	30

Results and Discussion

Incidence of Streptococcus in milk samples: Among the 100 milk samples obtained from various sources, 27 samples (27%) tested positive for Streptococcus using the cultural approach.

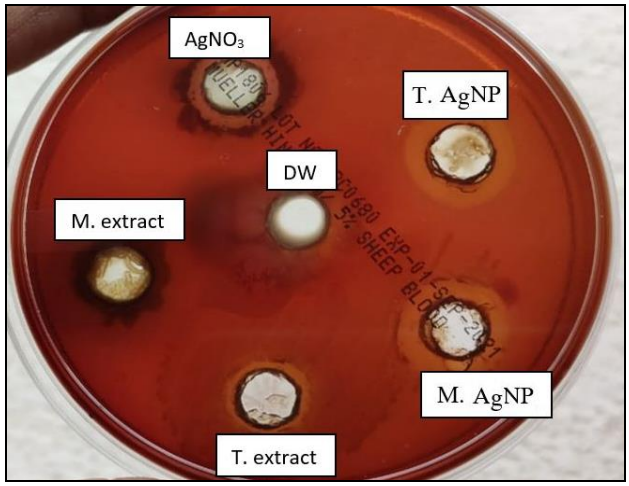
Antibiogram

The antibacterial activity of Moringa leaf extract, tamarind fruit extract, pure silver nitrate solution, Ag Nanoparticles (with Moringa leaf and Tamarind fruit extract), and antibiotics against Streptococcus pure culture and isolates are shown in Table 02 and Figures 02, 03, 04, and 05. Distilled water was used as a control, and no zone of inhibition against Streptococcus was observed.

For both pure cultures and milk isolates of Streptococcus, the mean zone of inhibition of Moringa leaf extract was 2 mm and 1.00±0.14 mm, respectively. Similarly, the mean zone of inhibition of tamarind fruit extract was 3 mm and 2.04±0.16 mm, respectively. The mean zone of inhibition of pure AgNO3 solution was 10 mm for pure cultures and 9.19±0.35 mm for milk isolates of Streptococcus.

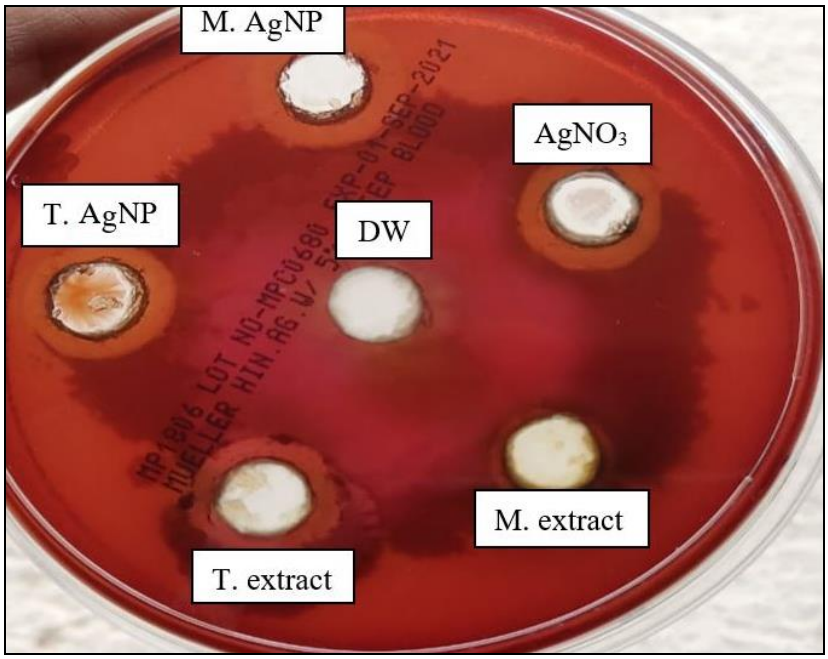
The mean zone of inhibition for Ag nanoparticles prepared using *Moringa oleifera* leaf extract was 16 mm for pure cultures and 14.11±0.43 mm for milk isolates. On the other hand, nanoparticles prepared using tamarind fruit extract exhibited a mean zone of inhibition of 14 mm for pure cultures and 12.19±0.46 mm for milk isolates.

In addition, the antibiotic discs showed the following mean zone of inhibition (ZOI) for pure cultures and milk isolates of Streptococcus: Tetracycline - 6 mm and 4.04±0.38 mm, Ciprofloxacin - 12 mm and 10.30±0.40 mm, Ampicillin - 18 mm and 16.33±0.50 mm, and Ceftriaxone - 10 mm and 8.00±0.36 mm.



M. AgNP: *Moringa oleifera* Silver Nanoparticles
T. AgNP: *Tamarindus indica* Silver Nanoparticles
$AgNO_3$: Silver Nitrate solution
DW: Distilled Water
T. Extract: Fruit extract of *Tamarindus indica*
M. extract: Leaf extract of *Moringa oleifera*

Fig 2: For *Streptococcus* pure culture ZOI of *Moringa oleifera* leaf extract, *Tamarindus indica* fruit extract, $AgNO_3$ solution, AgNPs.



M. AgNP: *Moringa oleifera* Silver Nanoparticles
T. AgNP: *Tamarindus indica* Silver Nanoparticles
$AgNO_3$: Silver Nitrate solution
DW: Distilled Water
T. extract: Fruit extract of *Tamarindus indica*
M. extract: Leaf extract of *Moringa oleifera*

Fig 3: For *Streptococcus* isolates ZOI of *Moringa oleifera* leaf extract, *Tamarindus indica* fruit extract, $AgNO_3$ solution, AgNPs.

Fig 4: ZOI of antibiotics for pure cultures of *Streptococcus*

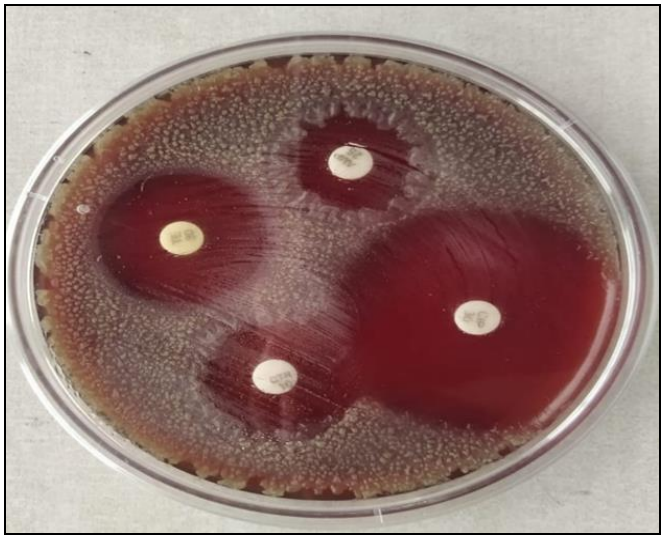


Fig 5: ZOI of antibiotics for isolates of *Streptococcus*

Table 2: The antimicrobial activity of silver nitrate solution, extracts, silver nanoparticles and antibiotics on pure cultures and isolates of *Streptococcus* from milk samples

S. No	Antimicrobial material/ discs	Zone of inhibition (mm) Mean ± SE	
		On pure cultures	On isolates
1	$AgNO_3$ Solution	10	9.19±0.35
2	*Moringa oleifera* leaf extract	2	1.00±0.14
3	*Tamarindus indica* fruit extract	3	2.01±0.16
4	*Moringa oleifera* AgNPs	16	14.11±0.43
5	*Tamarindus indica* AgNPs	14	12.19±0.46
6	Tetracycline	6	4.04±0.38
7	Ciprofloxacin	12	10.30±0.40
8	Ampicillin	18	16.33±0.50
9	Ceftriaxone	10	8.00±0.36

Comparison of antibacterial activity against *Streptococcus*

The mean zone of inhibition (ZOI) for $AgNO_3$ solution against pure cultures was 10 mm, and 9.19±0.35 mm against isolates from milk samples. In comparison, the mean ZOI for Ag nanoparticles prepared with *Moringa oleifera* was 16 mm and 14.11±0.43 mm, while for Ag nanoparticles prepared

with *Tamarindus indica* fruit extract, it was 14 mm and 12.19±0.46 mm for pure cultures and isolates from milk. The ZOI was lower for the pure $AgNO_3$ solution compared to Ag nanoparticles. When comparing Streptococcus-targeting nanoparticles made with *Moringa oleifera* leaf extract to those made with tamarind fruit extract, the ZOI was higher for the former.

The mean ZOI for Streptococcus with Ampicillin was the highest, at 18 mm and 16.33±0.50 mm for pure cultures and isolates from milk, respectively. Ciprofloxacin followed with a ZOI of 12 mm and 10.30±0.40 mm, ceftriaxone with 10 mm and 8.00±0.36 mm, and tetracycline had the least activity with a ZOI of 6 mm and 4.04±0.38 mm.

The mean ZOI by AgNPs made with Moringa was higher than the other three antibiotics employed in this study and nearly comparable to ampicillin. AgNPs made with tamarind fruit extract had a ZOI that was higher than ceftriaxone, tetracycline, and ciprofloxacin but lower than ampicillin. The mean zone of inhibition with Moringa leaf extract was 2 mm for pure cultures and 1.00±0.14 mm for isolates from milk samples of Streptococcus. However, Al-Kalifawi (2016) [10] observed no ZOI with Moringa leaf extract.

The mean zone of inhibition of Ag nanoparticles generated using *Moringa oleifera* leaf extract was 16 mm for pure cultures and 14.11±0.43 mm for isolates from milk samples. This was consistent with the findings of Al-Kalifawi (2016) [10] for AgNPs generated with *Moringa oleifera*. Gizachew *et al.* (2019) [11] reported higher ZOI values for ciprofloxacin, ceftriaxone, tetracycline, and ampicillin (24 mm, 26 mm, 18 mm, and 26 mm) against Streptococcus compared to the values obtained in the present study.

Conclusion

The study indicates that the AgNPs prepared with both *Moringa oleifera* and *Tamarindus indica* were almost equally efficient against Streptococcus, and their antibacterial activity is comparable to most of the antibiotics studied.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. Barkema HW, Green MJ, Bradley AJ, Zadoks RN. Invited review: The role of contagious disease in udder health. Journal of Dairy Science. 2009;92:4717-4729.
2. Bradley AJ. Bovine mastitis: an evolving disease. The veterinary journal. 2002;164(2):116-128.
3. Sharma N, Pandey V, Sudhan, NA. Comparison of some indirect screening tests for detection of subclinical mastitis in dairy cows. Bulgarian Journal of Veterinary Medicine. 2010;13:2.
4. Gruet P, Maincent P, Berthelot X, Kaltsatos V. Bovine mastitis and intra-mammary drug delivery: Review and perspectives. Advanced Drug Delivery Reviews. 2001;50:245-259.
5. Riffon R, Sayasith K, Khalil H, Dubreuil P, Drolet M, Lagace J. Development of a rapid and sensitive test for identification of major pathogens in bovine mastitis by PCR. Journal of Clinical Microbiology. 2001;39(7):2584-2589.
6. Vigneshwaran N, Kumar S, Kathe AA, Varadarajan PV, Prasad V. Functional finishing of cotton fabrics using zinc oxide–soluble starch Nanocomposites. Nanotechnology. 2006;17(20):5087.
7. Barrow GI, Feltham RKA. Cowan and Steel's Manual for the Identification of Medical Bacteria, 3rd ed., Cambridge University Press, Cambridge; U.K; c1993, p. 140.
8. Bauer AW, Kirby WM, Sherries JC, Turk M. Antibiotic susceptibility testing by a standardized single disc method. American Journal of Clinical Pathology. 1966;45:493-496.
9. CLSI, Clinical and Laboratory Standards Institute. Methods for Dilution Antimicrobial Susceptibility Tests of Bacteria Isolated from Aquatic Animal; Approved Guideline M49-A. CLSI, Waune, PA, USA; c2006.
10. Al-Kalifawi EJ. Green synthesis of silver nanoparticles using leaf extract of al-raw tree (*Moringa oleifera* Lamarck) cultivated in Iraq and efficacy the antimicrobial activity. Mesop Environ J Special Issue A; c2016. p. 39-48.
11. Gizachew M, Tiruneh M, Moges F, Tessema B. *Streptococcus agalactiae* maternal colonization, antibiotic resistance and serotype profiles in Africa: a meta-analysis. Annals of Clinical Microbiology and Antimicrobials. 2019;18:1-14.