Damanhour Journal of Veterinary Sciences 12 (2), (2024) 24-34

Damanhour Journal of Veterinary Sciences

Journal homepage: https://djvs.journals.ekb.eg/

E-ISSN 2636-3003 | ISSN 2636-3011

In Vitro Study on the Effect of Silver Nanoparticles Loaded Penicillin on Streptococcus Spp. Isolated From Mastitic Milk With Reference To Its Biofilm, Sequence, and Detection of Some Antibiotic Resistance Genes

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Abstract: Mastitis is one of the most prevalent diseases in dairy cattle diagnosed nationwide. Numerous mechanisms of antibiotic resistance have been identified in Streptococcus species, which are the major pathogenic bacteria causing mastitis. Streptococci are commonly obtained from dairy cow mastitis, but little is understood about the susceptibility of these organisms to antibiotics. Over time, antibiotics used to treat bacterial infections may lose their effectiveness. due to increase of antibiotic-resistant infections with the development of nanotechnology, silver nanoparticles (AgNPs) have drawn a lot of interest as a medicinal agent because of silver's well-known antibacterial property. Numerous physicochemical, biological, and practical features of silver nanoparticles (AgNPs) have been proved to be suitable for biomedical applications, such as their antibacterial and drug-carrying capabilities. Thus, our study's objective was to demonstrate an in vitro analysis of the effectiveness of some antibacterial drugs against streptococcus isolated from mastitis in dairy cattle and to illustrate the effect of AgNPs and AgNPs -loaded penicillin on that isolate. In the current study, Antibiotic resistance profiles in *streptococcus* spp. and resistance genes against penicillin and gentamycin was detected for *Pbp1A* target gene and *aac (6') aph (2")* target gene, respectively, penicillin was loaded onto AgNPs, thus enhancing penicillin antibacterial activity against *streptococci*. The prepared AgNPs and their conjugates were characterized by zeta potential measurement and transmission electron microscopy (TEM). AgNPs and their conjugates' minimum inhibitory concentrations (MIC) were also ascertained. The results explained that the lowermost concentration that prohibited streptococcus species growth. was 50 PPM for AgNPs, while 12.5 PPM for AgNPs loaded penicillin by agar plate diffusion technique. The findings indicated that, using the agar plate diffusion method, 50 PPM was the lowest concentration of AgNPs that prevented streptococcus species growth.but the lowest value for AgNPs loaded penicillin was 12.5 PPM. Furthermore, when compared to penicillin alone, the new nanocomposite, AgNPs-loaded penicillin, had the most antibacterial properties against the tested pathogenic bacteria, per the study's findings. For potential usage as an antibacterial combination, the preparation exhibits good efficiency.

Keywords: *streptococci;* penicillin*;* AgNPs; antibacterial effect

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P ISSN: 2636-3003
EISSN: 2636-2996
DOI: **10.21608/DJVS.2025.333726.1141**
Received: 04-11-2024
Accepted: 14-01-2025

1. Introduction

Mastitis is the most prevalent, expensive, and globally serious disease in dairy farming. (Ruegg, 2017). The economic costs of mastitis are considered 124€ (=147$) per cow annually, resulting in losses of 125 billion € worldwide (Cheng & Han, 2020). The consequent drop in milk production and quality premium losses (93 percent and 7% of SCM costs, respectively) were the two major cost components of Egypt's (21,933,258.6 LE) yearly economic loss from mastitis.(Azooz, El-Wakeel, & Yousef, 2020)

Traditionally, 30 to 50 percent of all cows worldwide suffer from bovine mastitis each year (Heringstad, Klemetsdal, & Ruane, 2000). Along with the monetary losses caused by reduced milk output and quality, there are additional expenses for staffing, medicine, and veterinary care, mastitis is a significant animal welfare concern and the primary cause of dairy cow disposal. Cows with mastitis may exhibit a variety of symptoms, including anorexia, lethargy, elevated body temperature, abnormally shaped milk, swelling, and soreness in the udder (Heringstad et al., 2000; Kibebew, 2017).

According to Bradley (2002), there are more than 130 pathogens linked to bovine mastitis, some of which are members of the Streptococcus genus. Gram-positive streptococci are spherical (0.5–2 µm) bacteria that classically arranged as chains or pairs. Many of them are non-pathogenic facultative anaerobes that are part of both human and animal commensal microbiota. Bovine mastitis one of the most hazardous infections and health issues that certain streptococci can cause. The species that are most pertinent in this case include S. uberis, S. dysgalactiae spp. dysgalactiae, and S.

agalactiae. Acute mastitis can develop into a chronic condition that reduces fertility if it is not well treated (Ruegg, 2017). Well-known pathogens that promote chronic mastitis include S. uberis and S. agalactiae (Jain, Tewari, Bhandari, & Jhala, 2012; Kabelitz, Aubry, van Vorst, Amon, & Fulde, 2021). The utmost mutual reasons for usage of antibiotics in dairy cows are to treat and prevent mastitis ("Distribution and Antimicrobial Resistance of Clinical and Subclinical Mastitis Pathogens in Dairy Cows in Rhône-Alpes, France," 2010; Kabelitz et al., 2021), that poses the potential of enhanced variety favoring antibiotic-resistant bacteria. (Bradley, 2002; Cheng & Han, 2020). One of the most important environmental risks is Strep. spp., which causes infectious mastitis in dairy farms and a number of human illnesses (Song et al., 2020). It has been established that Strep. agalactiae is a pathogen that causes either acute or chronic mastitis in cattle, which lowers milk output. This disease frequently causes herd epidemics, which are very important to the dairy industry (Ariffin et al., 2020). *Strep. spp.* is considered one of the most vital environmental hazards (Song et al., 2020), that result in several human diseases and infectious mastitis in dairy farms. *Strep. agalactiae* was recognized as a pathogen causing acute or chronic mastitis in cattle, which lead to decrease in milk production. Epidemics in herds are common by this pathogen with a major significance for the dairy industry (Ariffin et al., 2020). In accordance with Wilczewska, Niemirowicz, Markiewicz, and Car (2012), the misapplication of antibiotics causes the progress of microorganism resistance, which was initially classified by the progression of treatment resistance, decreased therapeutic indices, toxicity, side effects, non-specific effects, and incorrect dosage. During the resistance period, there has been a constant search for better-acting antibiotics due to the rise in antibiotic resistance, which is frequently produced by the extensive and inappropriate use of these medicinal drugs.

Since silver nanoparticles (AgNPs) were discovered to be an efficient antibacterial agent, research into the potential effectiveness of AgNPs in treating pathogenic infections has been ongoing. Accordingly, the feasibility of producing particles with distinct physical and chemical characteristics that variety in size from 1 to 100 nm has been successfully addressed by nanotechnology, a key tool in the development of nanomedicine. The components can act as delivery vehicles for many therapeutic compounds in various human organs due to their tiny size and higher ratio of surface area to volume.

In addition to enhanced antibacterial activity caused by increased loading, enhanced bioavailability, reduced toxicity, and a longer half-life of the pharmaceutical compound, nanoparticles (NPs)enable site-directed targeted delivery, precise release of the pharmaceutical agent(s), high drug-loading capacity with an augmented half-life, provisional stability, and safety (R. Sharma, S. Patel, & K. C. Pargaien, 2012).

More recently, the term "nano biotics" has emerged in medical science to replace "nano-scale antibiotics." When combined with specific antibiotics, It has been shown that AgNPs could eliminate about 650 harmful bacteria without causing any adverse consequences. (Deng et al., 2016). One of the major trials in treating antibiotic-resistant bacterial infections is the necessity to develop agents that can control the infection at the site of initiation that commonly occurs in parts of the body where water-soluble drugs classically have low access. However, the use of lipophilic medicines to combat such infections also has limited success because of problems with uptake and delivery caused on by low water solubility and biodistribution. (Turos et al., 2007); (Costerton, Stewart, & Greenberg, 1999). Novel drug delivery systems, like nanoparticles, provide a great means of enhancing the bioavailability and effectiveness of medicinal substances. (Turos et al., 2007).

The current study's objectives were to synthesize penicillin loaded on AgNP surfaces in order to increase its antibacterial activity and demonstrate its antibacterial efficiency against streptococcus-induced mastitis in vitro.

2. Materials and Methods

2.1 . Chemicals

2.1.1. Silver nanoparticles

2.1.1.1. Silver nanoparticle synthesis and combination

With a few adjustments, AgNPs were made using Turkevich's approach (Ventouratou, Taoukis, Gatt, & Valdramidis, 2019). SDS was employed as a covering mediator and trisodium citrate dihydrate as a reducing mediator. Using magnetic stirring, AgNO3 (4 Mm) was first dissolved in 100 mL of deionized water on a hot plate (80◦C). After that, a solution of SDS (0.5 mM) and trisodium citrate dehydrate (0.4 mM), both dissolved in 100 mL of deionized water, was dropped for 30 minutes while being constantly stirred. For two hours, the finished combination was maintained at 80°C and 350 rpm. The solution was allowed to cool to ambient temperature before being kept in the dark in the refrigerator once its color turned yellow, signifying the creation of AgNP.

2.1.1.2. Silver nanoparticles loaded penicillin.

As previously reported, AgNPs-penicillin were made (Ventouratou et al., 2019).To improve penicillin's interaction with the Nanomaterials 2022, 12, 2808 5 of 25 AgNPs, an aqueous solution of penicillin (0.001 M) was added to 100 mL of the previously produced AgNPs and ultrasonically agitated for two hours at room temperature. Successful AgNPs-penicillin conjugation was deemed to have been accomplished when the solution's tint changed from yellow to orange. Samples cooled to room temperature and then evaluated to ascertain their typical size, shape, stability in solution, and antibacterial qualities (Kaur, Preet, Kumar, Kumar, & Kumar, 2019).

2.1.1.3. Characterization of Silver Nanoparticles (Ag-NPs)

Transmission electron microscopy (TEM) was used to inspect the distribution and shap of the Ag-NPs arbitrated starch particles. A droplet of the solution was applied on carbon coated copper grids (CCG) and kept to dry at room temperature by allowing the water drain. The Regional Center for Mycology and Biotechnology (RCMB) at Al-Azhar University used a JEOL JEM-1010 transmission electron microscope set at 80 kV to make electron micrographs (Amin, Ahmed, El Gazzar, & Badawy, 2021). Using a Zeta sizer Nano ZS (Malvern Instruments Corp., Malvern, UK), the dynamic light scattering (DLS) method was used to determine the particle size (PS). Millipore-filtered deionized water was used to dilute each sample to the proper scattering intensity.

Animals and ethical statement.

150 dairy calves with mastitis, ages 4-6 on average, were used in the current study. They were sourced from private farms and individual dairy cow cases in the El-Beheira governorate. They were kept at a controlled temperature of 21 ± 2 °C and under a twelve-hour light-dark cycle. The Hygiene and Preventive Medicine research committee, Faculty of Veterinary Medicine, Damanhur University, Egypt, granted ethical approval for the animal investigations (DMU/VetMed-2023/020).

Experimental design and Sample collection

One hundred and fifty Mastitic milk samples were collected from numerous dairy farms in disinfected screw-capped tubes under hygienic settings. They were then labeled and sent to a laboratory for prompt chemical and bacteriological analysis.

2.3.1. Bacteriological examination of milk samples.

Samples of infected cases were centrifuged at 3000 rpm for 20 minutes. The sediment was streaked on Edwards media agar, while the cream and supernatant fluids were disposed of.

After 18 to 24 hours of aerobic incubation at 37°CThe inoculation plates were examined for the presence of bacteria. According to (Quinn et al., 2011; Workineh, Bayleyegn, Mekonnen, & Potgieter, 2002), biochemical identification was carried out.

2.3.2. Antibiotic resistance profiles in *streptococcus* spp. according to CLSI (Lynch, 2016)

The disc diffusion approach was applied to test the chosen isolates' susceptibility to ten antimicrobial drugs on Mueller-Hinton agar (Igbinosa, Ogofure, & Beshiru, 2022).

Table (1): Antimicrobial discs, concentration and clarification of their effect on the isolated STEC strains.

Antimicrobial agent	Sensitivity disc content (ug)	Resistant (mm)	Sensitive (mm)
Amikacin (AK)	**30**	≤ 12	**≥13**
Ampicillin (AM)	**10**	≤ 13	**≥14**
Ceftriaxone	**30**	≤ 17	**≥18**
Penicillin G	**5**	≤ 15	**≥15**
Gentamycin	**30**	≤ 14	**≥15**
Imipenem	**15**	≤ 13	**≥14**
Levofloxacin	**30**	≤ 13	**≥14**
Norfloxacin	**10**	≤ 20	**≥21**
Spiramycin	**25**	≤ 10	**≥11**
Vancomycin	30	**≤ 14**	**≥15**

2.3.3. Detection of resistance genes against penicillin and gentamycin in streptococcus strains by PCR.

DNA extraction.

The QIAamp DNA Mini kit (Qiagen, Germany, GmbH) was employed to extract DNA from samples with specific modifications in accordance with the manufacturer's guidelines.For ten minutes, 200 µl of the sample suspension was incubated at 56 OC Using 200 µl of lysis buffer and 10 µl of proteinase K. The lysate was incubated, and then 200 µl of 100% ethanol was included. Following the manufacturer's directions, the sample was centrifuged and cleaned. We used 100 µl of elution buffer to elute the nucleic acid. Metabion, located in Germany, provided the oligonucleotide primers that were utilized.

Table (2): Primers sequences, target genes, amplicon sizes and cycling conditions

Target gene	**Primers sequences**	**Amplified segment (bp)**	**Primary Denaturation**	**Amplification (35 cycles)**			**Final extension**	**Reference**
				Secondary denaturation	**Annealing**	**Extension**		
Pbp1A	AAACAAGGTCGGACTCAACC AGGTGC-TACAAATTGAGAGG	430	94˚C 5 min.	94˚C 30 sec.	57˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	(Mosleh, Kameli, Ranjbar, & Salamati, 2014)
Aac (6')aph (2'')	GAAGTACGCAGAAGAGA ACATGGCAAGCTCTAGGA	491	94˚C 5 min.	94˚C 30 sec.	54˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	(Duran, Ozer, Duran, Onlen, & Demir, 2012)

2.3.4. The antimicrobial properties of AgNPs and penicillin loaded with AgNPs

Strept suspensions and Mueller-Hinton agar medium were used to measure the antibacterial effecacy of Ag NPS and AgNPs loaded penicillin on the development of bacterial isolates. Suspensions at 1.5 × 108 colony-forming units (CFU) mL−1 were obtained using the McFarland standard. Following the swabbing of Mueller-Hinton agar medium plates with each bacterial suspension, two wells measuring 8 mm in diameter were punched out of each agar plate using a sterile well cutter. 80 µL of AgNPs and AgNPs-penicillin were added to the wells. The control was deionized water. To assess the antibacterial action, the inhibition zones surrounding each well were determinded (to the closest millimeter) after an incubation period of 24 hours
at 37°C.

2.3.5. Minimum inhibitory concentration (MIC) of the chemically synthesized AgNPs and AgNPs loaded penicillin against the isolated *strept* by Agar well diffusion method (Omara, Kiprop, & Kosgei, 2021).

Nanoparticles' bactericidal activity was assessed using a broth microdilution well diffusion test on 96-well microtiter plates. In each well, 50 µl of the AgNPs dilution was applied vertically, and 50 µl of the accustomed bacterial colony (5 × 105 CFU/ml) was added horizontally. Both sterility and growth control wells were included in each plate; the sterility control wells contained only a sterile MH broth to verify the sterility of the medium used, and the growth control wells contained MH broth medium with tested bacterial concentrations to verify the bacterial viability. To prevent the germs from drying out, The plates were incubated for 18 to 20 hours at 37°C after being loosely covered with cling film. The MIC was therefore defined as the lowest amount of each antimicrobial substance that prevented the development of bacteria (Parvekar, Palaskar, Metgud, Maria, & Dutta, 2020).

Results

3.1. Characterization of Silver Nanoparticles (Ag-NPs)

1-Transmission Electron Microscopy (TEM)

TEM was used to investigate the particle size of the solution, and the results showed that the nanoparticles had a consistently sphere-shaped. Silver nanoparticles **(Figure 1),** and silver nanoparticles loaded penicillin **(Figure 2)**

2- Zeta potential analysis

The particles' surface charge in the emulsions was 42.6 mV, according to zeta potential studies, indicating high particle stability.

Measured Data	
Zeta Potential	**42.6 mV**
Polarity	Positive
Automatic Mobility	3.33 um/s/V/cm
Conductivity	450 uS/cm
Field Strength (Req/Act)	10 / 9.7 kV/m SOP
Zeta Run Time	30 sec

3.2. Bacteriological examination of milk samples.

Table (3). Bacteriological examination of 150 milk samples showed that most of the isolates are *streptococci* 53.33 % while, 16.66% *staphylococci* and 10% *E. coli*.

Isolate	Cows	Buffaloes	%
Strept.	25	65	53.33
Staph.	15	10	16.66
E. coli	10	5	10

3.3. Antibiotic resistance profiles on *streptococcus* spp. and Antibacterial activity of *streptococcus* against different antibiotics.

Table (4) showed that the presented data clarified that *Strept* was sensitive to Levofloxacin, Ceftriaxone, Norfloxacin and Spiramycin, while it was resistant to Amikacin, Ampicillin, Penicillin g, Gentamycin and Vancomycin

:

***Strept.* sensitivity *Strept.* sensitivity** **Antibiotic**	**Resistant**	**Sensitive**
Amikacin (AK)	+	
Ampicillin (AM)	+	
Ceftriaxone		+
Penicillin G	+	
Gentamycin	+	
Ceftriaxone		+
Levofloxacin		+
Norfloxacin		+
Spiramycin		+
Vancomycin	+	

3.4. Detection of penicillin and aminoglycoside resistance genes:

Table (5) show that the strept isolates tested positive for genes that cause resistance of penicillin *(Pbp1A) at base pair 430* and for aminoglycosides *aac(6')aph (2'')at base pair 49.*

Sample	***aac(6')aph (2'')*(aminoglycoside resistance genes)**	***Pbp1A*(penicillin binding protein)**
1	+	+
2	+	+
3	+	+
4	+	+
5	+	+

Figure (3): showed that Resistance genes of penicillin *(Pbp1A)* were detected in the strept isolates *at base pair 430*

Figure (4): showed that strept isolates were positive for resistance genes of aminoglycosides *aac(6')aph (2'')at base pair 491*

3.5. Streptococci sensitivity against silver nanoparticles

Sensitivity of *streptococci* against silver nanoparticles were presented in Table (6). The table shows that 2 cases of strept were completely resistant to AgNPs, 2 cases were moderately sensitive, while 6 cases were highly sensitive

Strept Isolates	**Nano silver particles**		
	Resistant	**Moderately sensitive**	**Sensitive**
1	**+**	**-**	**-**
2	**-**	**+**	**-**
3	**-**	**-**	**+**
4	**-**	**-**	**+**
4	**-**	**+**	**-**
6	**+**	**-**	**-**
7	**-**	**-**	**+**
8	**-**	**-**	**+**
9	**-**	**-**	**+**
10	**-**	**-**	**+**

Figure (5). The figure show that 20 % of strept. isolates were completely resistant to AgNPs, 20 % were moderately sensitive and 60% cases were highly sensitive to AgNPs

3.6. Streptococci sensitivity against silver nanoparticles loaded penicillin

Sensitivity of *streptococci* against silver nanoparticles loaded penicillin were presented in the table (7). The table shows that no cases of strept were resistant to AgNPs loaded penicillin, 1case was moderately sensitive, while 9 cases were highly sensitive. This indicate the great effect of the cogugate of AgNPs and penicillin.

Strept Isolates	Sensitivity to Silver nano particles loaded Penicillin		
	Resistant	Moderately sensitive	Sensitive
1	-	-	+
2	-	-	+
3	-	-	+
4	-	-	+
4	-	-	+
6	-	-	+
7	-	-	+
8	-	-	+
9	-	+	-
10	-	-	+

Figure (6). The figure show that no strept isolates were resistant to AgNPs loaded penicillin, 10 % were moderately sensitive and 90% cases were highly sensitive to AgNPs loaded penicillin

3.7. Impact of nano silver and penicillin loaded nano silver on *strept.* growth using agar plate diffusion method

Table (8): Inhibitory impact of nano silver and penicillin loaded with nano silver on *strept. as* measured by plate diffusion method. According to the recorded results, The minimum concentration of nano silver that prevented strept development was 50 PPM, whereas the lowest concentration of Nano silver loaded penicillin that did the same was 12.5 PPM. using the diffusion method on an agar plate.

Concentration of Nano silver (PPM)			Nano silver loaded penicillin	
	Strept growth			
	Growth	No growth	Growth	No growth
3.125	+	-	+	-
6.25	+	-	+	-
12.5	+	-	-	+
25	+	-	-	+
50	-	+	-	+
100	-	+	-	+

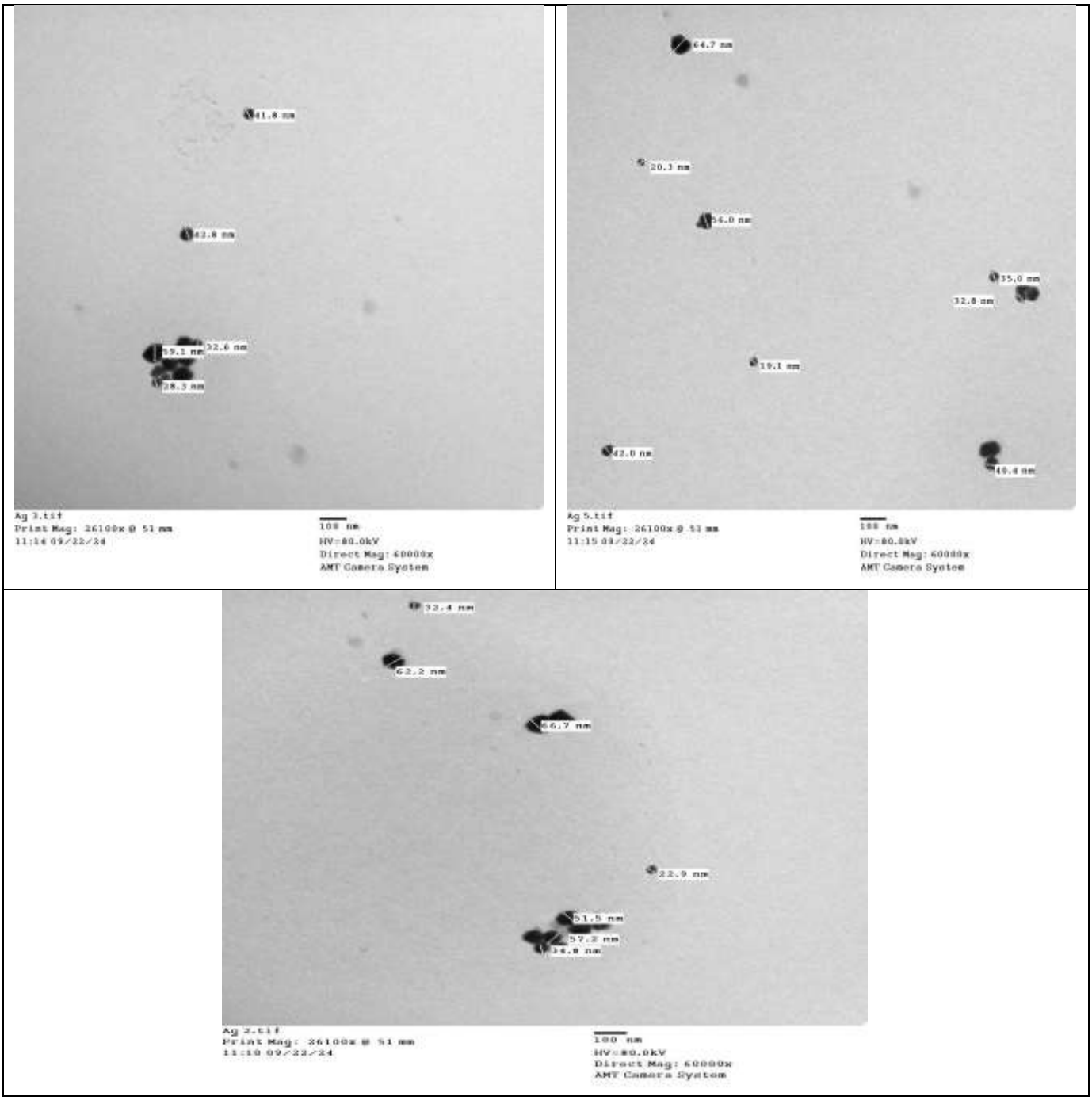

Figure 1: **Transmission electron microscopy of silver nanoparticles**

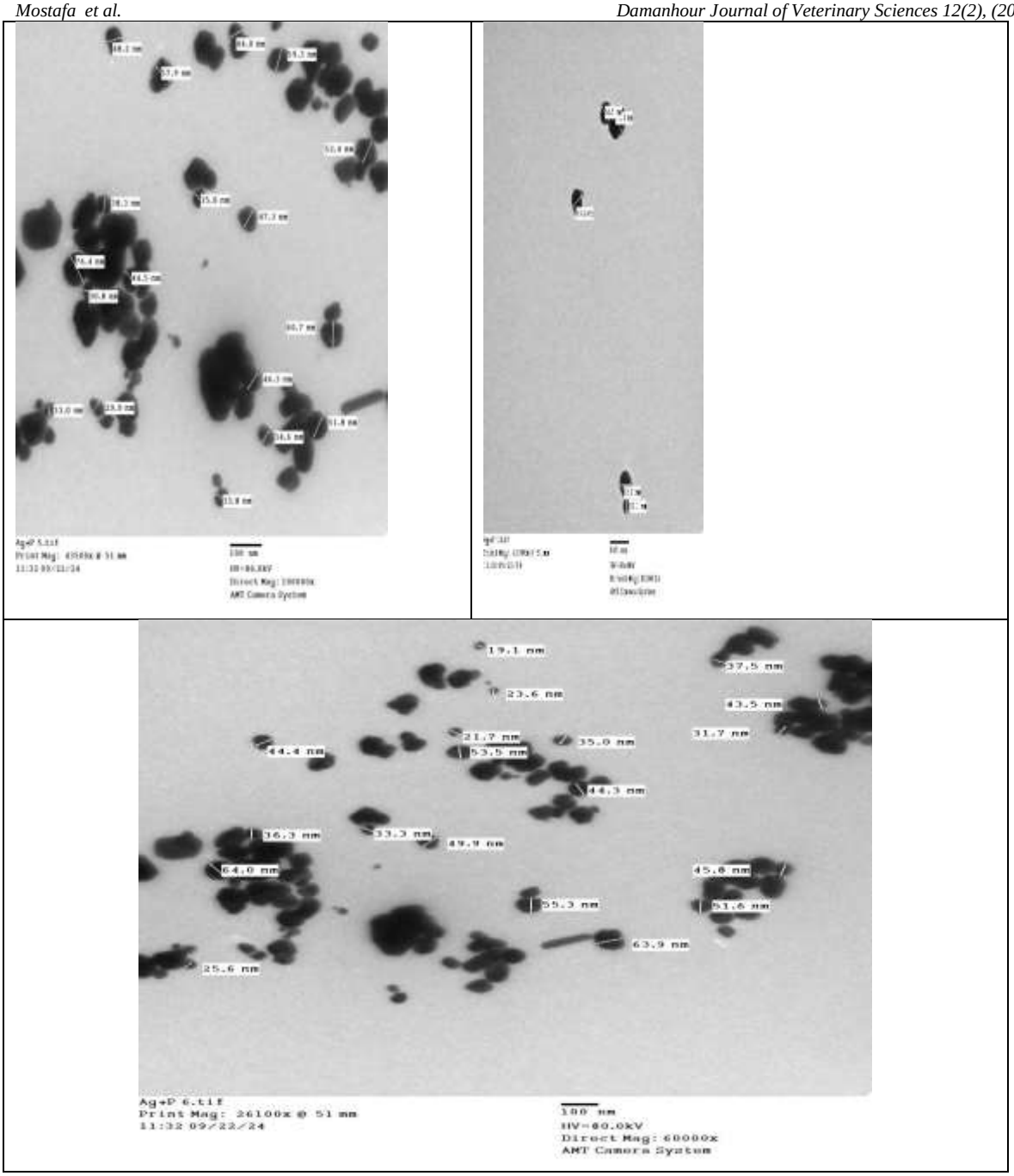

Figure 2: Transmission electron microscopy of silver nanoparticles loaded penicillin

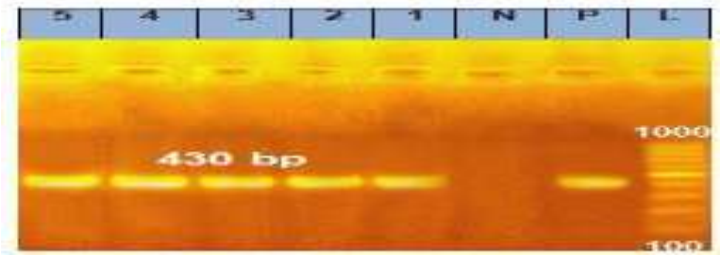

Figure 3 :The figure showed that strept isolates were positive for resistance genes of penicillin *(Pbp1A) at base pair 430*

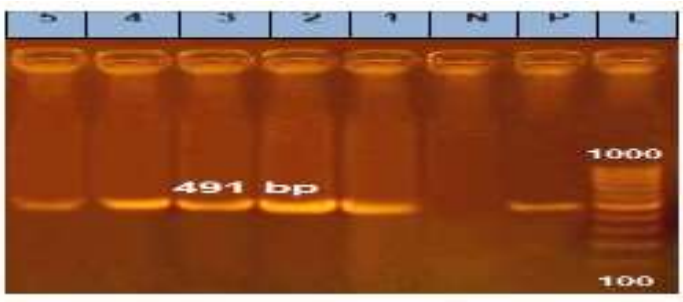

Figure 4 :The figure showed that strept isolates were positive for resistance genes of aminoglycosides *aac(6')aph (2'')at base pair 491*

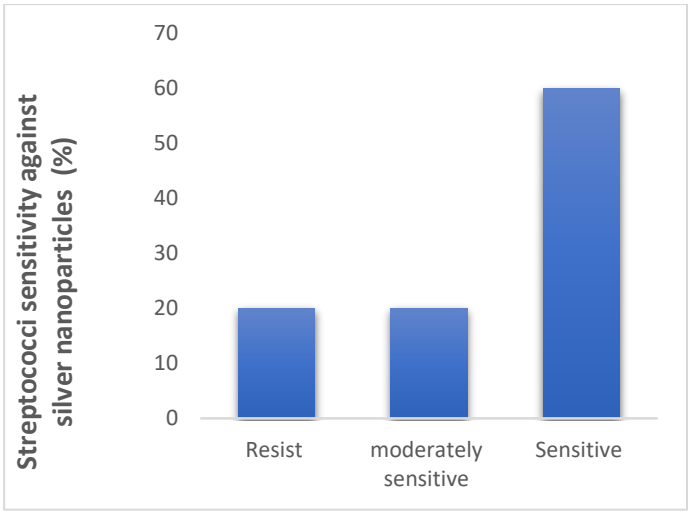

Figure 5: Streptococci sensitivity against silver nanoparticles

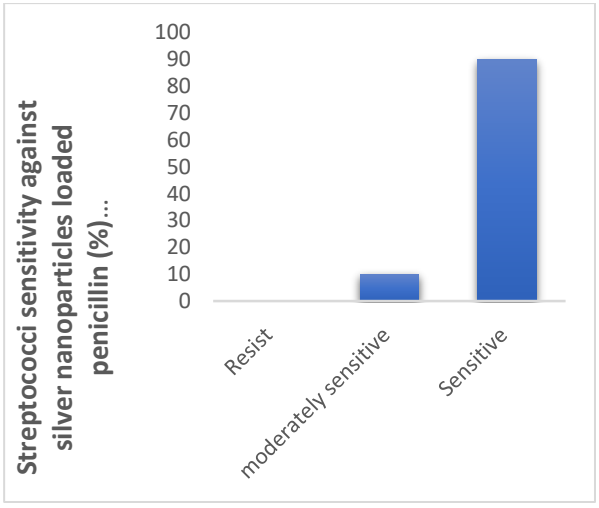

Figure 6 :Streptococci sensitivity against silver nanoparticles loaded penicillin

4. Discussion

Alongside coliform and Staphylococcus aureus, Streptococcus species are the most communal pathogen recognized from clinical mastitis and the primary cause of reinfection during the dry period (Bramley, 1984). The prevalence of Streptococcus dysgalactiae subsp. dysgalactiae has stayed constant over the same time period, whereas the prevalence of Streptococcus agalactiae mastitis was nearly eliminated by dry cow therapy and post-milking teat disinfection (Owens, Watts, Greene, & Ray, 1990; Watts et al., 1995).

Recently it have seen a numerous of papers on the antibiotic susceptibility of streptococci isolated from bovine mastitis. (Owens, Ray, Watts, & Yancey, 1997). The current study found that the bulk of isolates (53.33%) were streptococci, followed by 16.66% staphylococci and 10% E. coli, based on bacteriological investigation of milk samples.

According to Cobo-Angel et al. (2019), milk had a greater incidence of streptococcus mastitis; El-Jakee et al. (2013) and Elsherif & Elhabtey (2020) found findings that were almost identical. Since milk can become contaminated by dust, air, water, equipment, milkers, and handlers, the presence of Strep. spp. in raw milk may be related to the dairy farms under study's poor hygiene practices during milk production and handling (Alekish, Al-Qudah, & Al-Saleh, 2013). In particular, it was discovered in milk utensils, which had the greatest incidence (45.8%).

Streptococci isolates were shown to be very resistant to amikacin, ampicillin, penicillin G, gentamycin, and vancomycin, according to the findings of the antimicrobial resistance pattern. According to several writers (Camara, Dieng, & Boye, 2013; Elsherif & Elhabtey, 2020; Idrees & Saeed, 2013; Kia, Mehdi, & Keyvan, 2014; Rüegsegger et al., 2014), these results were consistent with each other. A public health concern is the multidrug resistance of Strep. species, which can be spread from animals to people through dairy products (Minst, Märtlbauer, Miller, & Meyer, 2012).

According to Kimura et al. (2008), penicillins are the first-choice medications for intrapartum antimicrobial prophylaxis. Reporting susceptibility data and keeping an eye on antibiotic resistance are crucial in veterinary medicine. S. uberis (Salmon, Watts, Aarestrup, Pankey, & Yancey, 1998), S. dysgalactiae subsp. dysgalactiae (Guérin-Faublée, Tardy, Bouveron, & Carret, 2002; Salmon et al., 1998), and S. agalactiae (Guérin-Faublée et al., 2002) isolates have been reported to be resistant or moderately susceptible to β-lactams, which is in line with the majority of authors. Because of the extensive use of antibiotics, some bacteria are able to express genes that make them resistant to the antimicrobial treatment, which leads to the spread of resistance. (Mayers, Lerner, Ouellette, & Sobel, 2009; Pelgrift & Friedman, 2013). *Streptococcus* species are thought to be the most significant environmental infections linked to issues in dairy farms and endanger the general public's health. The current investigation demonstrated that the strept. isolates tested positive for the resistance genes of aminoglycosides aac(6')aph (2")

at base pair 491 and penicillin (Pbp1A) at base pair 430 upon detection of these genes.

Since bacteria are less prone to develop nanomaterial resistance, a variety of metals, metal oxides, metal halides, and bimetals in nanoparticulate form have demonstrated antibacterial activity. (Sundara Baalaji, 2001; Wasef et al., 2020). These include NPs that contain superparamagnetic Fe, Ag, Au, Zn, Cu, Ti, Mg, Ni, Ce, Se, Al, Cd, Y, and Pd. When combined with X-rays, zerovalent bismuth-containing NPs have shown promise in treating diseases brought on by drug-resistant bacteria. (Hernandez-Delgadillo et al., 2012). The best nano weapon against bacterial illnesses is silver nanoparticles (Wahab, Hetherington, Iqbal, & Noshad, 2020).

Different bacteria have different levels of susceptibility to metal ions. By releasing Ag+ and Zn++ that interrupt the membrane, It has been demonstrated that Ag and ZnO NPs have antibacterial properties. (Dakal, Kumar, Majumdar, & Yadav, 2016; Durán et al., 2016; Sirelkhatim et al., 2015). Ag+ interacts with sulfhydryl groups in enzymes and further cellular constituents, making them active, revealing the antibacterial effect of Ag NPs. Metals kill bacteria by a variety of methods, including membrane disintegration, DNA and protein damage, and the creation of reactive oxygen species (Ahn et al., 2018). AgNPs are a very good substitute for antibiotic resistance. Numerous papers have proved the great effect of AgNPs against harmful bacteria (Jia, Duan, Wang, Zhang, & Wang, 2007). Since silver nanoparticles (AgNPs) were discovered to be an efficient anti-bacterial agent, research into AgNPs' potential efficacy in treating pathogenic illnesses has been ongoing (Ibraheem et al., 2022; Wasef et al., 2020). AgNPs display height bactericidal and antibacterial qualities against methicillin-resistant strains of bacteria as well as Gram-positive and Gram-negative bacteria (Wasef et al., 2020; Wahab, Khan, Adil, & Khan, 2021). High surface to volume ratios and nanoscale sizes are two of the many benefits of using nanoparticles as a novel infection treatment strategy (Kalishwaralal, BarathManiKanth, Pandian, Deepak, & Gurunathan, 2010). Increasing a nanomaterial's surface area facilitates interaction with bacteria and inhibits their growth (Bharde et al., 2008).

The influence of silver nanoparticles on streptococci isolates was assessed in this study, and the findings showed that 60% of cases were highly sensitive to AgNPs, 20% were moderately sensitive, and 20% of strept. isolates were totally resistant to AgNPs. Ag+ emitted by NPs has also been shown to bind with microbial DNA and prevent cell division and DNA replication. when free radicals are created, harming the DNA of bacteria (Lellouche, Friedman, Gedanken, & Banin, 2012). Numerous issues pertaining to antibiotic-resistant microorganisms have been resolved by nanobiotics. It has also been shown that conjugation with various NPs increases the antibiotic's efficacy against a variety of pathogen types due to their improved permeability (Kaur et al., 2019; Mohanta et al., 2018). The most mutual kind of nanoscale antibiotics that inhibit bacterial growth are silver nanoparticles. In addition to has a high capacity for binding
to the incoming medicinal item, the AgNPs are inert, chemically stable, and safe in biological environments. Additionally, they provide less harm (Aisida et al., 2019). Penicillin was loaded onto AgNPs in order to increase the antibiotic's effectiveness as a potent nano-antibiotic against the pathogenic bacteria under test. The current results, which confirmed that 90% of the isolates were very sensitive to AgNPs loaded penicillin, 10% were moderately sensitive, and no streptococci isolates were resistant to AgNPs loaded penicillin, approved the synergistic effects of the two substances. These results demonstrated the potent effect of penicillin on streptococci isolates following conjugation with AgNPs. That results are consistent with (Enan, Ashour, Basha, Felemban, & El-Rab, 2021; Hwang, Hwang, Choi, Kim, & Lee, 2012).

The inhibitory consequence of nano silver and nano silver loaded penicillin on streptococcal isolates was also assessed using the plate diffusion method, which revealed that the lowest amount of nano silver that prevented the isolates of streptococci growth was 50 PPM, while the lowest concentration of nano silver loaded penicillin that inhibited the growth of streptococcal isolates was 12.5 PPM. Comparing the growth of isolated streptococci to that of penicillin and AgNPs alone, these results clarified the synergistic antibacterial actions of AgNPs and penicillin. These results coincide with those of Hwang et al. (2012). The antibacterial effecacy of the penicillin-containing nanoparticles against streptococci actually showed that the bacterium's resistance gene has no effect whatsoever on the drug-bound nanoparticles' activity.

5. Conclusions

NPs' antibacterial properties have led to their consideration as a viable substitute for antibiotics. Antibiotics plus metallic nanoparticles reduce toxicity and the likelihood of resistance development when used to treat bacterial infections. The results of this investigation showed that, in contrast to molecularly free penicillin, loading penicillin onto chemically generated AgNPs produced an efficient formulation against streptococcus pathogenic bacterial isolates. Potent antimicrobial nano-conjugates were produced. In order to assess their usefulness and potential therapeutic uses, ongoing research is looking at their in vivo efficacy in animal infection models.

Conflict of Interest

No conflicts of interest are disclosed by the authors.

6. References

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