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Synergistic effect of antibiotics and silver nanoparticles on *Mycobacterium bovis* organism: preliminary study

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Abstract

Bovine tuberculosis is becoming more widely acknowledged as a hazard to public health. The advent of drug-resistant strains that compromise the efficacy of existing treatment plans poses a challenge to the control of BTB. A promising technique involves the use of silver nanoparticles (AgNPs) with traditional anti-TB therapies, which synergistically augment antimycotic efficacy both extracellularly and intracellularly. During the period from March 2023 to August 2024, a single intradermal tuberculin test was performed on 3500 animals, we found 84(2.4%) tuberculin positive animals and samples collected from tuberculin positive animals were cultured on Lowenstein-Jensen (LJ) media then molecularly examined by RT-PCR. Sixty out of 84 cases (71.43%) demonstrated growth on culture media and positive for the IS6110 PCR primer. By calculating the MIC, the in vitro activity of the streptomycin and streptomycin conjugated with AgNPs against mycobacterial isolates ranging from 1 - 0.25 mg/ml and 94 - 2.93 µg/ml respectively. Finally, it is clear that AgNPs enhance the antibacterial action of streptomycin.

Introduction

Bovine Tuberculosis is a chronic infectious deblitating disease of large ruminants attributed to bacteria from the Mycobacterium tuberculosis complex (MtbC), primarily *M. bovis*, and to a lesser extent by infections with other mycobacteria such as *M. avium*. Tuberculosis (TB) is the greatest cause of mortality from a single infectious agent and is among the top ten causes of death globally. Tuberculosis accounted for 1.23 million fatalities in 2024, while 10.7 million individuals contracted the

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disease during that year **(WHO, 2025).** TB is a zoonotic and anthropozoonotic illness characterized by a complex pathophysiology, caused mostly by bacteria from the MtbC, chiefly *M. tuberculosis,* with lower contributions from other mycobacteria such as *M. bovis*. The precise role of recently included members of MtbC, such as *M. mungi*, in human tuberculosis remains inadequately understood. MtbC bacteria belong to the Actinomycetales order and are non motile, non sporulated bacilli with a noticeably thick, lipid-rich cell wall. Drug-resistant forms of MtbC have emerged in recent decades, posing new difficulties for anti-TB prevention and control initiatives (**Saravanan *et al.,* 2018**). Bovine tuberculosis (TB) is a dangerous infectious illness that affects both humans and animals. BTB is a zoonotic illness that mainly affects cattle, but it can also infect humans when they come into intimate contact to infected animals or eat unpasteurized dairy products. Countries with middle and low incomes are disproportionately affected by zoonotic tuberculosis, which is closely linked to deprivation and poor sanitation. In poorer nations, BTB is becoming more widely acknowledged as a hazard to public health. However, correctly estimating the exact burden of this disease is hampered by the absence of efficient monitoring systems in several of these nations. Additionally, the introduction of resistant strains that compromise the efficacy of existing treatment protocols poses a danger to the management of BTB. **(Kasir *et al*., 2023).** The minimal inhibitory concentration (MIC) values are frequently established to assess a new compound's antibacterial properties or to validate results from tests for borderline qualitative susceptibility. The percentage approach, the resistance ratio method, or the absolute concentration method are typically used to interpret DST data for M. tuberculosis **(Mitchison, 2005).** Low patient compliance to treatment may result from the lengthy course of tuberculosis treatment and the severity of anti tuberculosis medication side effects, such as ototoxicity and renal failure. A serious challenge to TB control and an expanding public health issue, particularly in developing nations, are the rising incidence of MDR M. tuberculosis and M. bovis. **(Selim *et al.,* 2018).** Recent advances in nanotechnology and nanoscience have led to the development of a wide spectrum of nano materials for the treatment of infectious diseases. Due to their special optical, chemical, and physical characteristics as well as the potential to control their shape and composition, metal nanoparticles have recently drawn a lot of attention as potential antibacterial agents. Silver (Ag) nanoparticles, among other metal nanoparticles, have become a potent therapeutic technique to fight antimicrobial resistance. Because Ag is less harmful to human cells, it has long been recognized as a good antiseptic and antibacterial agent. Therefore, the creation of a novel class of antimicrobials using Ag nanoparticles is highly desirable. Ag nanoparticles have been shown by our lab to damage bacterial cell membranes and walls, resulting in harmful structural alterations.

Additionally, it has been shown that Ag ions prevent bacteria from producing selenium-based antioxidant enzymes, which ultimately causes oxidative stress and cell death. Additionally, it is known that Ag ions attach to vital bacterial macromolecules and prevent the bacterium from growing **(Shruthi *et al*., 2018).**

This study's goals were to ascertain the MIC of streptomycin and streptomycin conjugated with silver nanoparticles (strept-AgNPs) against *M. bovis* isolates.

Material and Methods

Tuberculin testing

Between March 2023 and August 2024, a single intradermal tuberculin test was administered to 3500 animals. During the national screening program, these animals underwent testing. The general organization of Veterinary Services of Egypt conducted a single intradermal tuberculin test using an intradermal injection of bovine purified protein derivative (PPD), and testing and interpretation were carried out in accordance with WOAH recommendations **(WOAH, 2024).**

Collection of the samples

Positive single intradermal tuberculin test animals were slaughtered according to Egyptian law. We targeted a total of 84 slaughtered animals at the abattoirs at different governorates in Egypt . The average age of the slaughtered females was 5-7 years old while that of males

was 2-4 years old. All animals were subjected to clinical and PM examination. followed by postmortem examination. Different samples were collected as left and right bronchial, retropharyngeal (lateral and medial), mesenteric , mediastinal and hepatic lymph nodes. Also lungs, intestines, spleen and liver samples were examined and collected. Animals were classified as either visible-lesion or non-visible-lesion based on whether a lesion was visible following dissection and visual examination of target tissues. During the grinding process, the samples from each case were pooled into a single collective sample, which was subsequently purified and condensed using Petroff's approach to culture and isolate Mycobacterium species. **(Mackie and McCartney, 1989).**

Identification of BTB

All samples (lesions from different organs of the affected slaughtered animal) were transferred on ice to TB unit, Bacteriology Department, AHRI, Giza (Egypt) for examination by molecular (RT-PCR) and culturing on Lowenstein-Jensen (LJ) media and Middle brook 7H11 agar.

***M. bovis* isolation and identification**

Each sample was cultivated in two tubes of Lowenstein-Jensen (LJ) media (purchased from Thermo Fisher Scientific, Waltham, MA, USA): one tube contained sodium pyruvate, and another tube contained glycerol (purchased from Thermo Fisher Scientific, Waltham, MA, USA) (ABID AG, Austria). Each sample was incubated in two tubes at 37 °C. Every day, we looked at grown tubes and identified acid-fast bacilli using Ziehl-Neelsen staining **(Romano *et al.*, 2005).**

Molecular validation of isolates and DNA extraction

The QIAamp DNA extraction was used to carry out the DNA extraction.

According to the manufacturer's guidelines for Gram-positive bacteria, use the Miniprep Kit (Qiagen, Germany). A 2:1 mixture of methanol and chloroform that was bought from CLN GmbH (Germany) was used to kill the M. bovis isolates. After adding 200 µL of Tris EDTA (TE) to a few colonies in 1.5 mL Eppendorf tubes, the tubes were refrigerated for the whole night. 200 µL of newly made methanol: chloroform was added after freezing. Before decanting the supernatant, the tubes underwent a centrifuge for 20 minutes at 2,000 × g. After centrifuging the tubes for five minutes at 6,000 × g, any leftover supernatant was disposed of. The tubes were then frozen until DNA extraction could start after being air dried for one hour. Chemical, physical, and enzyme-based approaches were combined to extract DNA **(Warren *et al.*, 2006).**

Verification of IS6110 and RD-based *M. bovis* differentiation:

Target gene	Primer sequences (5′-3′)	Accession number	Reference
IS6110	5’-AGTTTGGTCATCAGCCGTTC-3’ 5’-CGAACTCAAGGAGCACATCA-3’	NC000962.3	**(Thacker *et al*., 2011)**

Silver nanoparticles loaded with streptomycin preparation using the chemical reduction method

Prepare Silver Nitrate Solution: Dissolve silver nitrate (AgNO3) in deionized water to obtain a solution of a specific concentration (e.g., 1 mM to 10 mM).

Prepare Streptomycin Solution: Dissolve streptomycin sulfate (or other streptomycin salt) in deionized water. The concentration needs to be optimized. Start with a range (e.g., 0.1 mg/mL to 1 mg/mL) and adjust based on results.

Mix Reactants: Slowly add the streptomycin solution to the silver nitrate solution under continuous stirring. The ratio of AgNO3 to streptomycin is a critical parameter. Experiment with different ratios. Some protocols suggest adding streptomycin solution dropwise to the silver nitrate solution **(Guzmán *et al.*, 2009).**

Determination of minimum inhibitory concentration (MIC)

Proportion method using **Middlebrook** 7H11 agar media according to Mycobacterium tuberculosis complex control recommendations for livestock **(WOAH 2024).**

To homogenize the sample, colonies from the solid medium of each isolate were scraped onto an appropriately labeled tube that contained 5–6 mL of 7H9 broth with albumin-dextrose-catalase (ADC) supplement, 0.05% Tween 80, and 3 mm glass beads. The tube was then vigorously vortexed for about a minute., After letting the tube stand for 30 minutes to allow big bacterial clumps to settle, two milliliters of each isolate's upper suspension were removed and transferred separately to a sterile tube. The suspension's turbidity was then adjusted to 106 CFU/ml using a McFarland number 1 standard. Streptomycin (Sigma-Aldrich A1774-250MG, UK) drug diluted in distilled water to reach concentration 1 mg/mL then we made serial twofold dilutions of streptomycin antibiotic.

Streptomycin conjugated silver nanoparticles (strept-AgNCs) with concentration 94 µl/ml (prepared in faculty of pharmacy, Sina University) then we made serial twofold dilutions of (strept-AgNCs).

Middle brook 7H11 agar media was prepared according to the manual of the media and each drug dilution was added to the agar media seperately after addition of OADC supplement then mixed well and soldified at room temperature.

Middle brook 7H11 agar plates were divided into four groups (one group containing streptomycin drug, second group containing strept-AgNCs drug, group three free from any drug for control positive and the group four free from any drug for control negative).

First, second and third group were inoculated with 0.1ml of colonies suspension, the fourth group inoculated with 0.1ml sterile distilled water. Then incubated at 37°C for 3-4 weeks.

By comparing the number of colonies on the equally inoculated mediums with and without drug, the drug resistance ratio was computed and the presence of drug resistance was ascertained. The percentage of resistance is calculated as follows: (number of colonies growing on the drug-containing medium)/(number of colonies growing on the control medium) times 100%. The examined microorganisms are deemed resistant to the anti tuberculosis medication if the percentage of drug resistance is more than 1%. In order to see and document the colony count on the lab log, the culture medium was examined. The number more than 20 for colonies growing on the drug-containing medium can be identified as drug resistance based on the assumption of robust colony growth on the control medium **(Yu-mei Zhu. *et al.*, 2024).**

Results

Results of tuberculin and isolation of Mycobacterium

In many Egyptian governorates, 84 out of 3500 (2.4%) tuberculin-positive animals were slaughtered and their lymph nodes and organ tissues checked for granulomas. On Lowenstein-Jensen (LJ) medium containing sodium pyruvate, isolates grew in 60 out of 84 cases (71.43%), but not in Lowenstein-Jensen (LJ) medium containing glycerol. Of the 84 instances, 24 (28.57%) had no development at all in the LJ medium.

Molecular verification of the isolates obtained

By testing 100% positive for the IS6110 PCR primer, all 60 isolates were verified to be

MTBC.

Effects of streptomycin and streptomycin conjugated with silver nanoparticles on mycobacterial isolates

By calculating the minimum inhibitory concentration (MIC), the in vitro efficacy of streptomycin antibiotic against mycobacterial isolates at various concentrations, ranging from 1 mg/ml to 0.25 mg/ml, was evaluated.

By calculating the minimum inhibitory concentration (MIC), the in vitro activity of streptomycin conjugated with AgNPs against mycobacterial isolates at varying concentrations, ranging from 94 µg/ml to 2.93 µg/ml, was evaluated.

The negative control plates showed no growth of colony.

The positive control plates (without any drug) showed uncountable number of colonies.

Discussion

The primary source of infection with *M. bovis*-caused bovine tuberculosis for humans is cattle, which is regarded as a zoonotic organism. **(WOAH, 2024).** Ten to twenty percent of human tuberculosis cases in Africa and other developing nations are caused by *M. bovis* **(Wernery and Kinne, 2012).** Since gastrointestinal illness is a common clinical symptom and *M. bovis* is usually obtained by oral consumption, the development of extrapulmonary tuberculosis is anticipated to increase the global prevalence of *M. bovis* infection **(Elsohaby *et al.,* 2020).** The frequency of human TB cases linked to *M. bovis* has dramatically declined in developed nations due to milk pasteurization and eradication attempts. Humans can still get *M. bovis*, though, if they come into contact with sick animals. **(Cosivi *et al.,* 1998).**

The study's tuberculin test result was 2.4%, which was lower than both a recent tuberculin report from Egypt that showed a 2.7% positivity rate using the single intradermal skin tuberculin test and a report from Egypt that showed a 3.4% positive result rate applying the single intradermal comparative skin tuberculin test for dairy cattle **(Abdelaal *et al.,* 2019)** and less than recent tuberculin report from Egypt which indicated a 2.7% using the single intradermal skin tuberculin test **(Elsayed *et al*.,2025)** . This decrease was attributed to the Egyptian Agriculture Ministry objectives to prevent animal infection with *M. bovis* and its possible human transmission, as well as the strict implementation of the General Authority of Veterinary Services' test and kill policy **(El-Gedawy et al., 2021).** Another factor contributing to this alteration may be linked to inaccuracies in the tuberculin testing methods employed during the execution of the national control program, potentially leading to overlooked cases of infection. **(Elsayed *et al.,* 2016).**

Antibacterial agents play a crucial role in various sectors, including the textile industry, water disinfection, pharmaceuticals, and food packaging. Nanoparticles are promising candidates for use as antimicrobial agents due to their ability to attach to DNA and prevent its unwinding, leading to cell death in the end. The integrity of bacterial membranes is compromised when silver nanoparticles interact with them, while silver ions attach to sulfur, oxygen, and nitrogen found in vital biological molecules, thereby inhibiting bacterial proliferation **(Selim *et.al.,* 2018).**

Nanoparticle-based systems have undergone testing for the prevention, diagnosis, and treatment of tuberculosis (TB). These systems could potentially address several issues, including: drug bioavailability, the necessity for frequent dosages, the hydrophobic characteristics of the cell wall, which hinder the binding and penetration of anti-TB medications, thus diminishing their bactericidal effectiveness, and the emergence of multi-drug resistance. These pose serious obstacles to controlling tuberculosis outbreaks. By specifically targeting phagocytic cells affected by intracellular pathogens and acting directly on the cell wall, nanoparticle-based technologies may offer answers **(Praba *et.al.,* 2013).**

This study revealed that the minimal inhibitory concentration (MIC) of streptomycin and streptomycin conjugated with silver nanoparticles (strept-AgNPs) against *M. bovis* isolates 1 mg/ml to 0.25mg/ml and 94 µg/ml to 2.93 µg/ml respectively, the result of this study agree with the result of **(Salar *et al*. 2015)** who mentioned that the antibacterial efficacy of streptomycin, both alone and in combination with AgNPs, was evaluated, revealing an enhancement in antibacterial activity between 2.02% and 57.98%. It is evident that AgNPs increase

the antibacterial properties of streptomycin, the AgNPs possess a wide range of antibacterial effectiveness against human pathogens and further synergistically boost the antibacterial action of streptomycin. And also agree with **(Bhabatush 2026)** who explained the physical behavior of functionalized AgNCs with INH in addition to their antimicrobial and biocompatible properties and stated that the antimicrobial efficiency of isoniazide nanocarriers (INH-AgNCs) was almost 2.7 times higher than that of free INH based on antimicrobial testing.

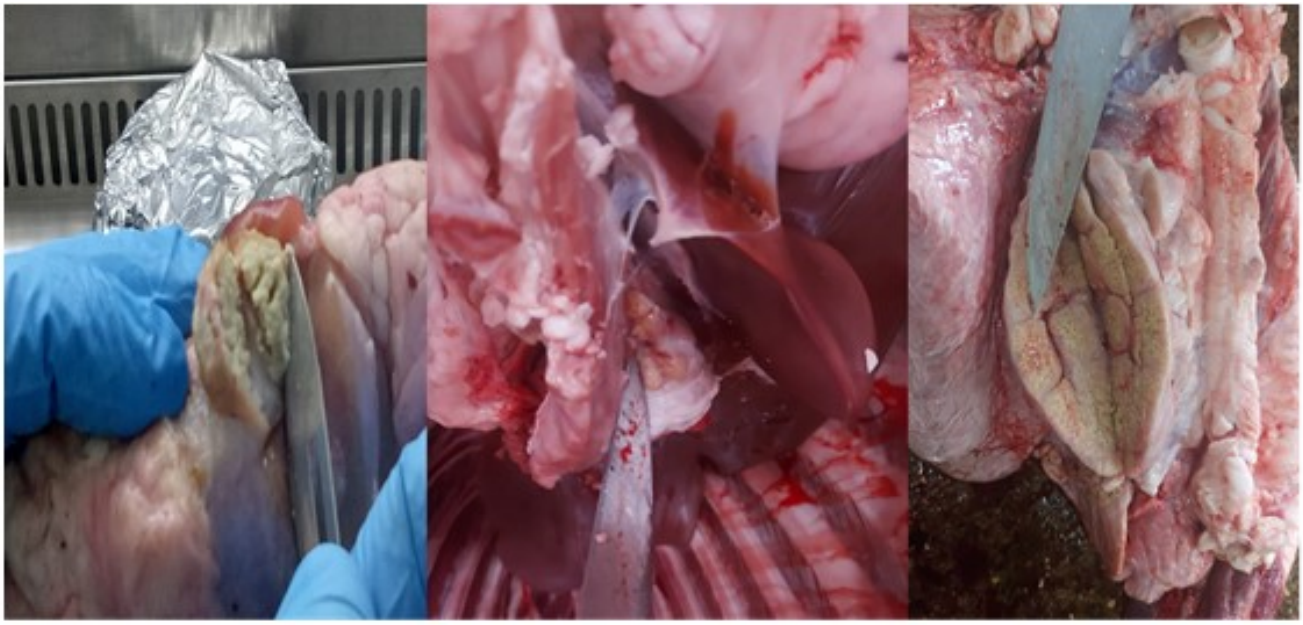

Fig. (1). Lymph nodes (Retropharyngeal, Mesentric and bronchial) showing suspected Tuberculosis lesions with calcification from slaughtered tuberculin reactor cattle

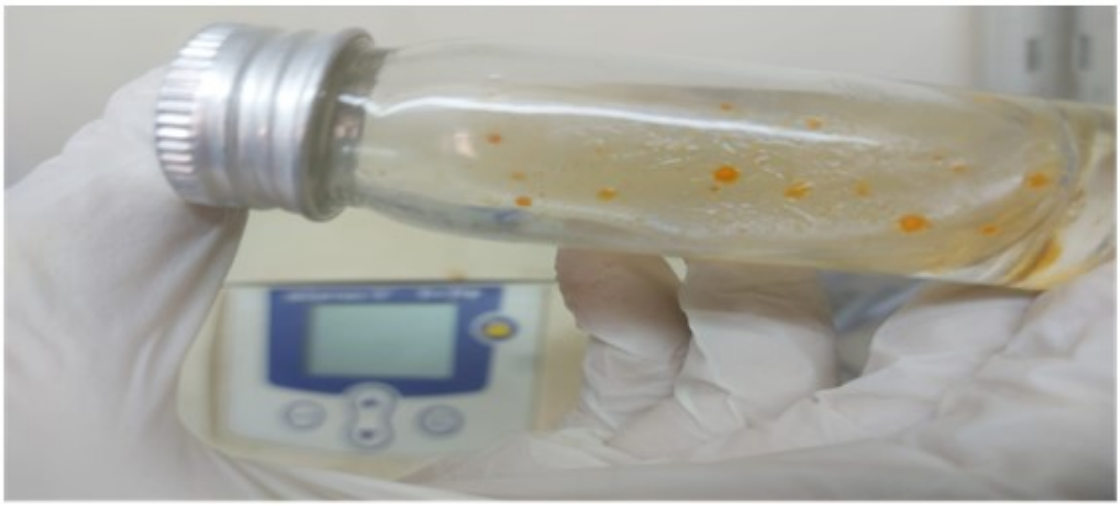

Fig. (2). Macroscopic apperance of Mycobacterium colonies on middle brook 7H10

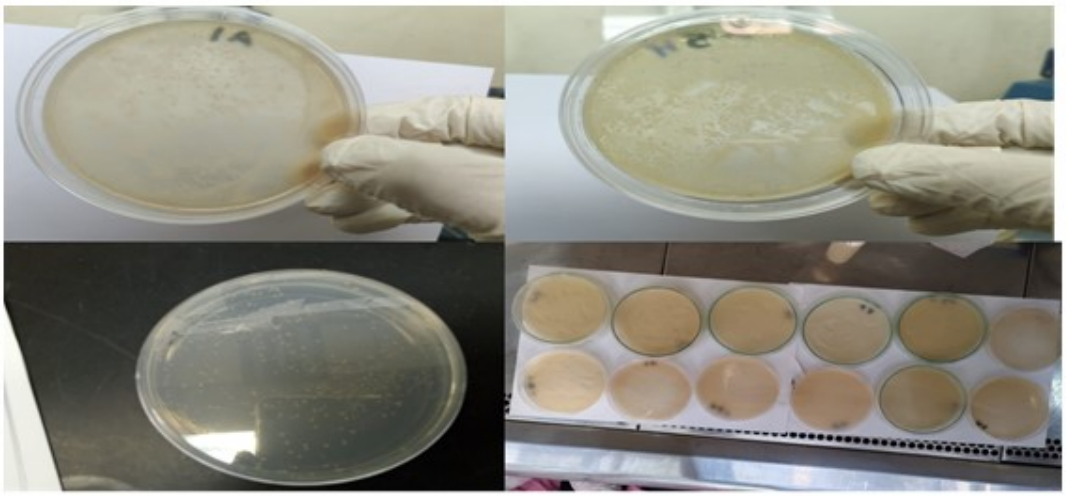

Fig. (3). Minimum inhibitory concentration (MIC) proportion method using Middlebrook 7H10 agar media of streptomycin and strep-AgNCs

References

Abdelaal, H.F.M.; Spalink, D.; Amer, A.; Steinberg, H.; Hashish, E.A. and Nasr, E. A. (2019). Genomic polymorphism associated with the emergence of virulent isolates of *Mycobacterium bovis* in the Nile Delta. Sci. Rep. 9, 11657. doi: 10.1038/s41598-019-48106-3, PMID: [DOI] [PMC free article] [PubMed] [Google Scholar]

Bhabatush, Biswas; Bandyopadhyay, T.K. and Bhowmick, T.K. (2026). Functionalization of silver nanocarrier with anti-tubercular drug for effective antimycobacterial activity, Applied Surface Science, Volume 715, 2026, 164590, ISSN 0169-4332, https: //doi. org/10. 1016/j.apsusc.2025.164590.

Cosivi, O.; Grange, J.M.; Daborn, C.J.; Raviglione, M.C.; Fujikura, T. and Cousins, D. (1998). Zoonotic tuberculosis due to *Mycobacterium bovis* in developing countries. Emerg. Infect. Dis. 4, 59–70. doi: 10. 3201/eid0401.980108

El-Gedawy, A.A.; Abdelghafar, A.E.; El-Shazly, Y.A.; Elkady, M.A.; Abdelrahman, M.A. and Shalaby, M. (2024). Highlighting on *Mycobacterium Bovis* in cattle, focusing on its antimicrobial resistance. Egy. J. Anim Health 4, 134–140. doi: 10. 21608/ejah.2024.327059

Elsayed, M.S.A.E. and Amer, A. (2019). The rapid detection and differentiation of Mycobacterium tuberculosis complex members from cattle and water buffaloes in the delta area of Egypt, using a combination of real-time and conventional PCR. Mol. Biol. Rep. 46, 3909–3919. doi: 10.1007/s11033-019-04834-3

Elsayed, M.S.A.; Elkerdasy, A.F.; Akeila, M.A. and Elsayed, A.A. (2016). Comparison between immunological and molecular based methods for diagnosis of mycobacterium infections in cattle, buffaloes and human in Egypt. Cell Mol. Biol. 62,125. doi: 10.4172/1165-158X.1000125.

Elsayed, M.S.A.; Alqaim, Z.H.; Fayed, A.M.; Eldsouky, S.M.; Basiouny, M.S.; Metwally, A.M.; Abdelbadee, A.; Hasan, A.S, ElAlfy AKEM, Nasr MA, Wahdan SME, Elsayed RA, Anwer MM, El-Bahy AM, Salah A. Genetic diversity and antibiotic resistance of *Mycobacterium bovis* in bovines in the Delta area of Egypt. Front Cell Infect Microbiol. 2025 Sep 12;15:1600225. doi: 10.3389/fcimb.2025.1600225. PMID: 41017912; PMCID: PMC12463954.

Elsohaby, I.; Ahmed, H.A.; El-Diasty, M.M.; Elgedawy, A.A.; Mahrous, E. and El Hofy, F.I. (2020). Serological and molecular evidence of *Mycobacterium bovis* in dairy cattle and dairy farm workers under the intensive dairy production system in Egypt. J.Appl. Microbiol. 129, 1207–1219. doi: 10.1111/jam.14734

Guzmán, M. G., Dille, J., Godet, S. (2009): Synthesis of silver nanoparticles by chemical reduction method and their antibacterial activity. International Journal of Chemical and Biomolecular Engineering 2:3 2009.

https://www.omnis-mg.com/Radovi/4bak-4siz-peru.pdf

Kasir, D.; Osman, N.; Awik, A.; El Ratel, I.; Rafei, R.; Al Kassaa, I.; El Safadi, D.; Salma, R.; El Omari, K. and Cummings, K.J. (2023). Zoonotic Tuberculosis: A Neglected Disease in the Middle East and North Africa (MENA) Region. Diseases 2023, 11, 39. https://doi.org/10.3390/diseases11010039

Mackie, A.M. and McCartney, P. (1989). Practical medical microbiology (Edinburgh: Churchill Livingstone). 13th edition.

Mitchison, D.A. (2005). Drug resistance in tuberculosis. Eur Respir J. 2005 Feb;25(2):376-9. doi: 10. 1183/ 09031936. 05.00075704. PMID: 15684305.

Praba, V.L.; Kathirvel, M.; Vallayyachari, K.; Surendar, K.; Muthuraj M., Jesuraj P.J., Govindarajan S. & Raman K.V. (2013). Bactericidal effect of silver nanoparticles against *Mycobacterium tuberculosis*. J. Bionanosci., 7 (3), 282-287. doi: 10. 1166/ jbns.2013.1138.

Romano, M.I.; Amadio, A.; Bigi, F.; Klepp, L.; Etchechoury, I. and Llana, M.N. (2005). Further analysis of VNTR and MIRU in the genome of *Mycobacterium avium* complex, and application to molecular epidemiology of isolates from South America. Vet. Microbiol. 110, 221–237. doi: 10. 1016/ j.vetmic.2005.07.009

Salar, Raj Kumar; Pooja, Sharma and Naresh, Kumar (2015). Enhanced antibacterial activity of streptomycin against some humanpathogens using green synthesized silver nanoparticles Resource-Efficient Technologies, Volume 1, Issue 2, 2015, Pages 106-115, ISSN 2405-6537, https:// doi. org/ 10.1016/j.reffit.2015.11.004.

Saravanan, M.; Niguse, S. and Abdulkader, M. (2018). Review on emergence of drug-resistant tuberculosis (MDR & XDR-TB) and its molecular diagnosis in Ethiopia. Mi crob Pathog. 2018;117:237 – 242. doi: 10. 1016/ j. micpath.

Selim, A.; Elhaig, M.M.; Taha, S.A. and Nasr, E.A. (2018). Antibacterial Activity of nanoparticles against field and reference strains of *Mycobacterium tuberculosis*, *Mycobacterium bovis* and multiple-drug-resistant tuberculosis strains. Rev.Sci. Tech. Int,. Epi., 2018,37(3).

Shruthi, T.S.; Meghana, M.R.; Medha, M.U.; Sanjana, S.; Navya, P.N. and Hemant, K.D. (2018). Streptomycin functionalization on silver nanoparticles for improved antibacterial activity, Materials Today: Proceedings, (10), 2019,8-15,ISSN 2214-7853, https://doi.org/10.1016/j.matpr.2019.02.181.

Thacker, T.C.; Harris, B.; Palmer, M.V. and Waters, W.R. (2011). Improved specificity for detection of *Mycobacterium bovis* in fresh tissues using IS6110 real-time PCR. BMC Vet. Res. 7, 50. doi: 10.1186/1746-6148-7-50.

Warren, R.M.; Gey van Pittius, N.C.; Barnard, M.; Hesseling, A.; Engelke, E. and de Kock, M. (2006). Differentiation of *Mycobacterium tuberculosis* complex by PCR amplification of genomic regions of difference. Int. J. Tuberc. Lung Dis. 10, 818–822.

Wernery, U. and Kinne, J. (2012). Tuberculosis in camelids: a review. Rev. Sci. Tech. 31, 899–906. doi: 10.20506/rst.31.3.2161

World Organisation for Animal Health (WOAH) (2024). "Guidelines for the control of *Mycobacterium tuberculosis* complex in livestock," in Beyond test and slaughter. (Paris: WOAH), 37. doi: 10.20506/woah.3538.

World Organisation for Animal Health (WOAH) (2024). Technical manual for culture-based drug susceptibility testing of antituberculosis drugs used in the treatment of tuberculosis. In: WHO operational handbook on tuberculosis. Module 3: diagnosis - rapid diagnostics for tuberculosis detection, third edition. Geneva: World Health Organization; 2024. Licence: CC BY-NC-SA 3.0 IGO.

World Organisation for Animal Health (WOAH) (2025). Global Tuberculosis report 2025.

Yu-mei Zhu; Liu, Hm.; Wang, F.; Shan, Ws.; Yin, Q. and Lu, Kz. (2024). Laboratory Examination for Drug-Resistant Tubercle Bacillus. In: Lu, PX., Lu, Hz., Yi, Yx. (eds) Diagnostic Imaging of Drug Resistant Pulmonary Tuberculosis. Springer, Singapore. https://doi.org/10.1007/978-981-99-8339-1_3