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Antibacterial activity of silver nanoparticles synthesized by *Raoultella ornithinolytica* from dental root canal infections

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ABSTRACT

This study aimed to utilize AgNPs generated by *Raoultella ornithinolytica* bacteria as antibacterial activity against a *Streptococcus gordonii.* The production of nanomaterial and their application in biology and medicine exemplify the significant uses of the distinctive structural dimensions and configurations that have propelled nanotechnology's rapid ascent as a discipline of Nano science. Unlike eukaryotic cells, bacterial cells can swiftly create many physiologically active compounds due to their efficient metabolic pathways. This lets bacteria outperform eukaryotic cells. *R. ornithinolytica* collected from parsons with dental issues to produce silver nanoparticles in an eco-friendly and cost-effective manner. The nanoparticles have been tested against multidrug-resistant *Streptococcus gordonii* alone and in combination with other antibiotics. These bacteria were identified by morphology, biochemistry, and molecular approaches. The results were shown that several antibiotics, including Optochin OP, Tetracycline TE, Erythromycin E, and Streptomycin S., exhibited a synergistic impact on the suppression of *S. gordonii* bacteria when combined with silver nanoparticles (AgNPs). Conversely, streptomycin and tetracycline antibiotics exhibited contrasting effects. In summary, *S. gordonii* isolates were significantly suppressed by *R. ornithinolytica* (AgNPs); hence, (AgNPs) possess many applications in oral care. This study aimed to deliver a succinct summary of the various dental care applications of AgNPs. The study utilized AgNPs generated by *R. ornithinolytica* bacterial strains S1, S2, and S7 for individuals with oral issues. The antibacterial activity of (AgNPs) derived from *R. ornithinolytican* is demonstrated against a *S. gordonii* strain.

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Introduction

Even though bacteria continue to develop resistance to existing antibiotics, the search for new antibiotics has been ongoing since the discovery of penicillin in the early 1900s (Clatworthy et al., 2007). The emergence of antibiotic-resistant microorganisms and the associated threats to human health became a major issue soon after antibiotics were introduced. This danger affects both developed and developing countries alike (Clatworthy et al., 2007; Manna et al., 2015).

A significant global health concern today is the increasing resistance of bacteria and other microbes to conventional antibiotics, which raises the risk of treatment failure and complications (Parthasarathi & Thilagavathi, 2011). As a response, nanotechnology has emerged as a

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promising field, offering novel solutions in biology and medicine. Its rapid rise can be attributed to the unique size and structure of nanomaterials, which enable various biomedical applications (Raghavan et al., 2015). Often referred to as the "fourth industrial revolution," nanotechnology encompasses a broad range of materials, including biomaterials, metals, polymers, and ceramics (Abdeen et al., 2014).

Nanotechnology has contributed significantly to the advancement of biogenic products, including sunscreens, antibiotics, and other therapeutic agents (Dunn & Edwards-Jones, 2004). Among the many approaches in nanomaterial synthesis, green synthesis is particularly advantageous as it uses environmentally friendly and safer reducing agents to produce metal nanoparticles (Morones & Frey, 2010). Numerous studies have demonstrated that silver nanoparticles (AgNPs) possess strong antibacterial activity against both gram-positive and gram-negative bacteria. This has led to the approval of silver-based products by the U.S. Food and Drug Administration (Petersen 2007, Chopra et al. 2012, Abdel-Azeem et al. 2020, Gezaf et al. 2022, Mossa et al. 2024, Al-Ziadi et al. 2024, Lahmood et al. 2025).

The oral cavity hosts a distinct and complex microbial ecosystem (Marsh, 2005). Despite improvements in oral health in many countries, global challenges remain, especially among underserved and disadvantaged populations. Oral diseases such as dental caries and periodontal disease are prevalent and can lead to tooth loss when bacterial infection compromises the enamel, dentin, and cementum.

Dental caries is a significant public health issue, affecting 60–90% of school-aged children and the majority of adults in industrialized nations (Sutherland, 2001). These diseases are often caused by polymicrobial infections involving oral commensal bacteria. The oral microbiome, which includes over 600 identified microbial species, plays a key role in oral health and disease (Russell et al., 2009).

Biofilms—structured communities of microorganisms encased in a self-produced matrix—can form on any surface and are influenced by a variety of environmental factors (Angello et al., 2017; Topcuoglu et al., 2016). In the context of oral health, the degradation of the alveolar bone and gingiva supporting the teeth leads to periodontal disease, a condition associated with broader systemic health issues (Hajishengallis, 2015).

According to research by Pratik et al. (2012), silver nanoparticles (AgNPs) synthesized using the pathogenic *Raoultella ornithinolytica* bacterial isolate may be used in combination with antibiotics to treat *Streptococcus gordonii*-associated dental diseases. This suggests potential applications for AgNPs in the dental field as an alternative or complementary therapy.

Materials and Methods

Sampling

Swab samples were collected from the root canals of 50 patients by medical dentists (Prof. Dr. Sami Kh. Jabar) in the center of Misan Governorate. The swabs were placed in designated containers and sent to the laboratory for analysis. Samples were immediately cultured on brain heart infusion agar and tryptic soy agar. Morphological, biochemical, and molecular methods were used to diagnose and purify the cultures.

Molecular confirmation of isolated taxon

DNA was extracted from pure bacterial colonies and identified using the polymerase chain reaction (PCR) technique. To amplify the 16S rDNA gene, specific primer sequences were used: forward primer 5′-AGA GTT TGA TCC TGG CTC-3′ and reverse primer 5′-GGT TAC CTT GTT ACG ACT T-3′. The PCR process began with an initial denaturation step at 95°C for 5 minutes, followed by 30 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 1 minute. The resulting PCR products were analyzed by gel electrophoresis, stained with ethidium bromide, and visualized under UV light. After purification, the gene products were sent to Korea for sequencing. The obtained sequences were compared against the GenBank database using the BLAST tool to determine their similarity (Singh et al., 2018).

Biomass production

The most productive *R. ornithinolytica* strain was selected due to its ability to generate a high amount of biomass, producing enough colonies to prepare a bacterial solution. To begin, 100 mL of nutrient broth was poured into a conical flask and inoculated with the bacterial suspension. The flask was then placed in a shaking incubator and left to grow for 48 hours at 37 °C.

After incubation, the culture was centrifuged to separate the bacterial cells from the liquid. The supernatant—the liquid portion—was carefully poured into a sterile flask, passed through a 0.2 µm filter to remove any remaining cells, and then incubated at 40 °C to ensure sterility (Saifuddin et al., 2009). This filtered extract, now free of cells, was used in the next step to synthesize silver nanoparticles (Zhou et al., 2019).

Biogenic AgNP Synthesis

Silver nanoparticles (AgNPs) were produced using a bio-manufacturing approach. To do this, 250 mL of

bacterial extract was mixed with 250 mL of a 1 mM silver nitrate solution. The mixture was then placed in a shaking incubator at 40°C and left for 5 days. The formation of the nanoparticles was monitored regularly throughout the process until synthesis was complete. To analyze the composition of the AgNPs, the method outlined by Pratik et al. (2012) was followed.

Characterization of synthesized AgNPs

The presence of silver nanoparticles was confirmed by the shift in the mixture's reaction colour from light yellow to dark brown, which signifies their synthesis. The mixture was spun in a centrifuge to separate the filtrate from the precipitate, which stood for silver nanoparticles. Repeated washings with distilled water will eliminate any remaining bacterial extract. To confirm that these particles were synthesised, it was tested with a UV-Vis spectrophotometer operating at a wavelength of 330-800 nm. According to Singh et al. (2015), the UV spectra, scanning electron microscopy, and XRD techniques were used to characterise the AgNPs produced in this work.

Antibacterial Activity of AgNPs

Biosynthesized silver nanoparticles were investigated their antibacterial activity by agar well diffusion method against multi-drug-resistant (MDR) were isolated from Root canals of some dental patients. Briefly, the tested bacteria was cultured on the Muller hanten agar and adding 10 microliter of absolute AgNP solution in wells and leaved for 15 minute in room temperature, then incubated in 37 C for 24 hr. The inhibition zone around well was measured by millimeter (Abd Ali 2021).

Combination assay of antibiotics with AgNPs

The susceptibility of antibiogrem activity of AgNPs was tested using a combination assay against various bacterial isolates from dental diseases was evaluated using the disk-diffusion method. The antibiotics discs are Streptomycin Optochin, Tetracycline, Cefalexin, Penicillin, and Erythromycin. The inhibitory zone diameters surrounding the antibiotic discs were measured in millimeters to investigate the synergistic impact of AgNPs with antibiotics. According to Dewhirst et al. (2010), two approaches were used to determine the synergistic effect of antibiotics paired with AgNPs: The formula for the percentage fold increase area is (b − a)/a multiplied by 100.

MIC determination

The total fractional inhibitory concentration (FIC) of two antibiotic combinations is a common measure of their synergistic effects. To determine FIC for A and B, we use the following formula: Our goal is to learn more about the mechanism by which *R. ornithinolytica* produces AgNPs so that we might employ them to fight against harmful microbes. FIC = MIC of antibacterial A combination/ MIC of antibacterial B combination.

MIC of antibacterial A in combination FIC -MIC of antibacterial an alone FIC was also calculated for combination B with same formula Thus [FIC= FIC of antibacterial A+ FIC of antibacterial B.

Results

One hundred bacterial strains belonging to *Raoultella ornithinolytica* were obtained from (50 patients suffering from dental diseases and 50 healthy). Diagnosed using morphological characters and microscopically as in figure 1. Seven strains belonging to *R. ornithinolytica* named S1-S7 were given one clear band for 16SrRNA in gel electrophoreses after amplified by the PCR technique (Fig. 2). Macrogen Company in Korea received the PCR products and sent them there for sequencing. In order to create silver nanoparticles, the findings of the 16SrRNA sequencing were compared with the database provided in the NCBI blast GenBank (Fig. 3). Figure 4 shows the results of the genomic DNA extraction and PCR amplification, including the sequence that is most closely related to NR 114502.

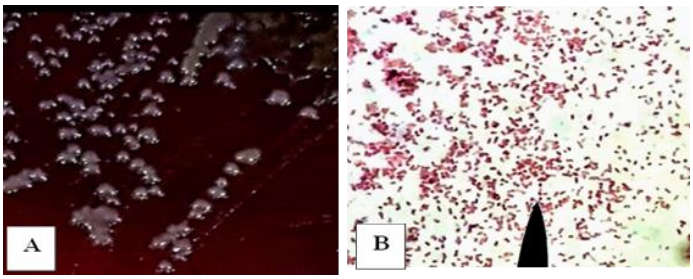

Fig 1. A: *Raoultella ornithinolytica* cultured on blood agar. B: Gram-stained *R. ornithinolytica* observed under a microscope at 125× magnification.

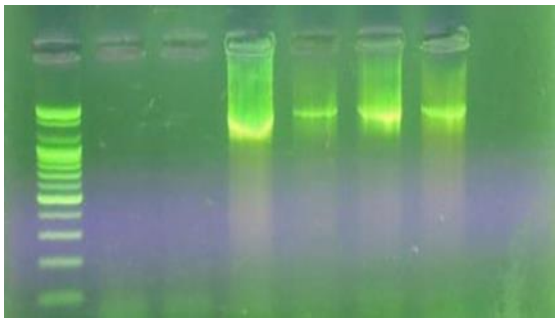

Fig 2. Gel electrophoresis patterns of genomic DNA extracted from *Raoultella ornithinolytica* on Agarose Gel 1% at 5 vol / cm for 1.15 hour and documented by UV.

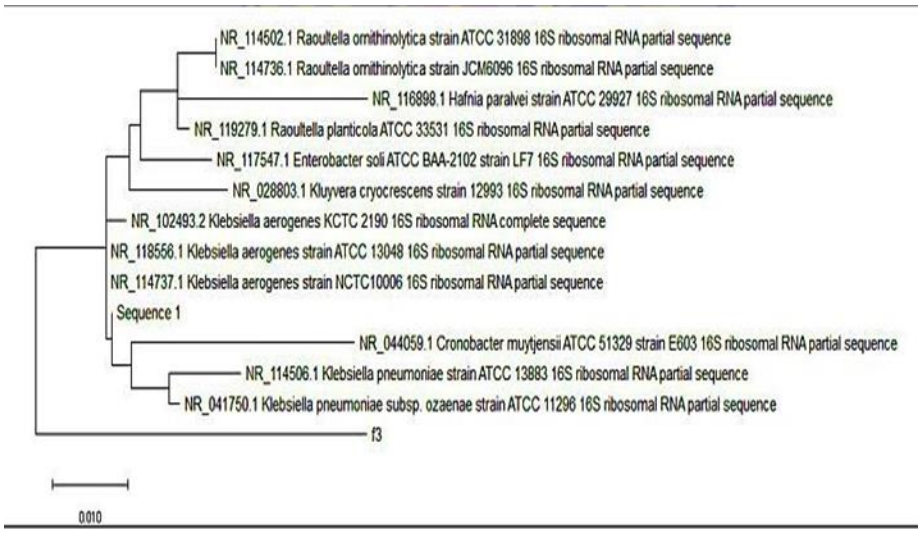

Fig. 3. Phylogenetic tree of *Raoultella ornithinolytica* showing its relationship to reference strains.

Biogenic synthesis of AgNPs

Figure 4 illustrates the ability of the *Raoultella ornithinolytica* strain (S2) to synthesize silver nanoparticles (AgNPs). At the beginning of the reaction (Figure 4A), the solution appeared clear. However, after 72 hours (Figure 4B), a noticeable color change to brown indicated the formation of AgNPs. By 96 hours (Figure 4C), the solution had turned a dark brown color, confirming the completion of extracellular nanoparticle synthesis. This visible color shift is a key indicator of successful AgNP production.

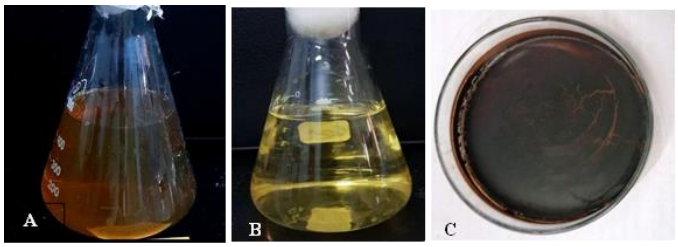

Fig. 4. Visual representation of AgNP synthesis by *Raoultella ornithinolytica* (S2). A: Initial reaction stage with a clear solution. B: After 72 hours, the solution begins turning brown, indicating nanoparticle formation. C: At 96 hours, the dark brown color confirms the completion of extracellular AgNP synthesis.

Antibacterial activity

Using *Streptococcus gordonii* bacteria obtained from dental patients in Misan Province, Iraq, the antimicrobial activity of Optochin (OP), Tetracycline (TE), Erythromycin (E), and Streptomycin (S) was tested (Table 1). Figure 5 shows the results of this evaluation in combination with biogenic AgNPs produced by *R. ornithinolytica*.

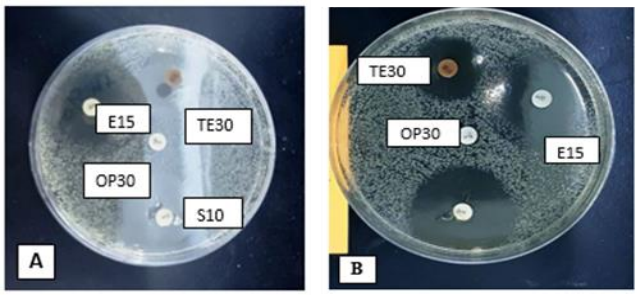

Fig. 5. A: Antibacterial effects of selected antibiotics on isolated *Streptococcus gordonii*. B: Enhanced antimicrobial activity of combined antibiotics and biogenic AgNPs against *S. gordonii* from dental infections.

Table 1 Index of fractional inhibitory concentration (FIC) to establishes the interaction between antibiotics agent

Antibiotics + AgNPs	Interaction
Optochin + AgNPs	Indifferent
Tetracycline + AgNPs	Antagonistic
Streptomycin + AgNPs	Antagonistic
Erythromycin + AgNPs	Synergistic

The index is showed as A and B are the minimum inhibition zone of combination effects. synergy ; FIC<1.0, antagonism ; FIC >1.0 , FIC= 1 additive ; FIC= 1,1- ,and 2 or more indifferent (non- interacttive) according to Birla et al.(2009) .

Table 2 shows the Minimum Inhibitory Concentration (MIC) values of silver nanoparticles (AgNPs) by themselves and when mixed with four antibiotics that are often used against Streptococcus gordonii, which was taken from patients with dental diseases. The findings unequivocally indicate that AgNPs possess significant antibacterial properties independently; however, their combination with antibiotics amplifies the inhibitory effect, implying a potential synergistic interaction. This synergism is especially important because it shows that lower amounts of both the nanoparticles and the antibiotics could be used to effectively kill bacteria. This could lower the risk of side effects and the development of antibiotic resistance. In general, the data show that AgNPs could be a useful addition to the treatment of dental bacterial infections.

Table 2 Minimum Inhibitory Concentration (MIC) of silver nanoparticles (AgNPs) alone and in combination with four antibiotics against *Streptococcus gordonii* isolated from patients with dental diseases.

Multi drug *Streptococcus gordonii* MIC (mg / ml)	
AgNPs alone	0.009
Optochin + AgNPs	-
Tetracycline + AgNPs	0.018
Streptomycin + AgNPs	0.037
Erythromycin + AgNPs	0.018

*For each value three replicates (Kurek et al., 2012)

Characterization of AgNPS

Physical characterization

UV-vis spectroscopy and XRD, were used to examine the AgNPs' optical properties. Infectious *Raoultella ornithinolytica* made up the bulk of them as shown in figure 6.

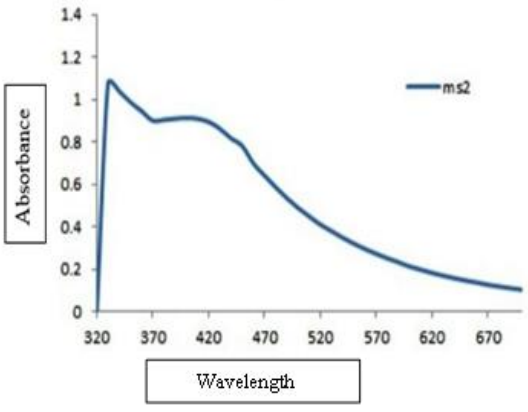

Fig 6. UV-V is absorption spectrum of AgNPs synthesized by *R. ornithinolytica.*

Analysis of (XRD) diffraction.

Patterns allude to four peaks whole spectrum of (2Ɵ) 77.09, 67.7, 46.5 and 38.4 showed in Figure 7.

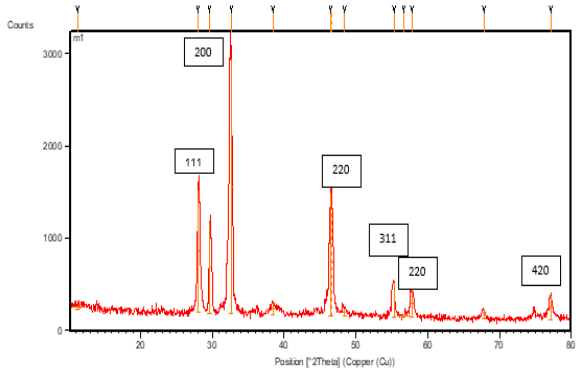

Fig 7. XRD report, resulted by AgNPs fabricated on copper surface obtained from *R. ornithinolytica* isolated from oral cavity of dental disease patients in center of Misan City.

Analysis of (SEM) Microscope.

Figure 8 shows a micrograph of SEM examined the crystalline shape and size of AgNPs nanoparticles made by *R. ornithinolytica*.

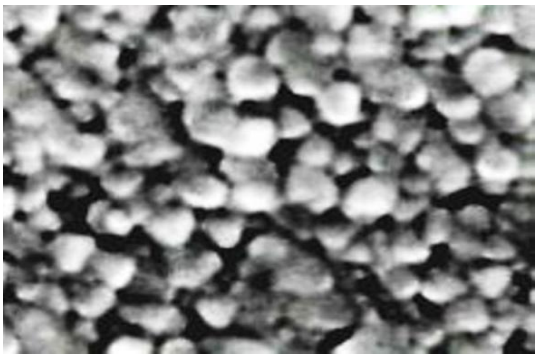

Fig 8. Characterizations of the biogenic AgNPs synthesized by *R. ornithinolytic*

The X-ray diffraction (XRD) analysis of the biosynthesized silver nanoparticles (AgNPs) is summarized in Table 3, which also includes specifics about the peak positions (2θ), peak height (intensity), full width at half maximum (FWHM), calculated d-spacing, relative intensity, and tip width. The produced nanoparticles' face-centered cubic (fcc) crystalline structure, which is typical of metallic silver, is evident from the diffraction pattern. The computed d-spacing values closely match standard JCPDS data for silver, and the peaks' sharpness and intensity indicate a high degree of crystallinity. These findings support the potential uses of AgNPs in antimicrobial and other nanotechnology-based fields by confirming that the biosynthesis method used yields well-defined, crystalline AgNPs.

Table 3. X-ray diffraction (XRD) analysis of biosynthesized silver nanoparticles. The table summarizes peak positions (2θ), peak height (intensity), full width at half maximum (FWHM), calculated d-spacing, relative intensity, and tip width. The observed diffraction peaks correspond to the face-centered cubic (fcc) crystalline structure of silver.

No.	P0s.[°2Th.]	Heigh[cts]	FWHM Left. [°2Th.]	d-spacing [°A]	Rel. Int. [%]	Tip Width
1	77.09	255.07	0.480	1.236	9.24	0.5760
2	67.76	108.62	0.480	1.381	3.94	0.57
3	46.51	134.00	0.300	1.950	48.56	0.36
4	38.42	132.40	0.590	2.342	4.80	0.7085

The lattice planes of silver nanoparticles (AgNPs) biosynthesised by Raoultella ornithinolytica are shown in Table 4, indexed using standard JCPDS data. At 38.42°, 46.52°, 67.76°, and 77.09°, respectively, the X-ray diffraction analysis showed distinctive peaks that corresponded to the (111), (200), (220), and (311) crystallographic planes. According to these findings, the produced AgNPs have a face-centered cubic (fcc) crystal structure, which is in line with what is predicted for metallic silver. The biosynthesis process produces well-defined, highly crystalline nanoparticles, as evidenced by the exact alignment of the observed diffraction peaks with JCPDS reference data. This shows how well *R. ornithinolytica* produces structurally stable AgNPs.

Table 4. Lattice planes of AgNPs biosynthesized by Raoultella ornithinolytica indexed according to JCPDS data.

Source of AgNPs	Hki index			
	111	200	220	311
R. ornithinolytica	38.4213	46.516	67.762	77.090

Discussion.

In this investigation, Raoultella ornithinolytica was isolated from root canal samples and verified through 16S rDNA gene sequencing and PCR. The potential of oral bacteria as biological factories for nanoparticle production is demonstrated by this strain's capacity to synthesize silver nanoparticles (AgNPs). In line with earlier findings, the observed color shift from yellow to dark brown during the reaction, along with UV-Vis spectroscopy and SEM analyses, validated the successful synthesis of nanoparticles (Singh et al., 2015; Zhou et al., 2019).

Multidrug-resistant (MDR) bacterial isolates from dental infections were significantly inhibited by the biosynthesized AgNPs. AgNPs by themselves produced inhibition zones that were similar to those found in previous research employing green-synthesised nanoparticles (Morones & Frey, 2010; Gezaf et al., 2022). This supports AgNPs' potential as substitute antimicrobials in applications related to oral health, especially in light of the rise in antibiotic resistance.

Streptococcus gordonii and other MDR strains were more effectively inhibited when the AgNPs were used in conjunction with traditional antibiotics like Erythromycin, Penicillin, and Cefalexin. The greatest synergistic response was generated by the combination of Erythromycin and AgNPs, as evidenced by the larger inhibition zones and lower minimum inhibitory concentrations (MIC). These findings are consistent with past research demonstrating that nanoparticles can improve the permeability of bacterial membranes, increasing the effectiveness of antibiotics (Pratik et al., 2012; Abdel-Azeem et al., 2020).

AgNPs' disruption of bacterial cell walls, the production of reactive oxygen species, and the increased absorption of antibiotics into bacterial cells are some of the possible mechanisms behind this synergy (Chopra et al., 2012; Hajishengallis, 2015). To elucidate the precise interactions between AgNPs and particular antibiotics, more molecular research is necessary.

Our results are particularly pertinent to dental infections, where treatment is complicated by biofilm formation and polymicrobial communities. The demonstrated effectiveness of AgNPs against biofilm-associated bacteria underscores their potential use in root canal therapy and the management of periodontal disease, as biofilms frequently exhibit resistance to conventional antibiotics (Angello et al., 2017; Topcuoglu et al., 2016).

Although the antibacterial and synergistic potential of biosynthesized AgNPs is supported by this study, it is important to acknowledge a number of limitations. The study was carried out in a lab setting, and in vivo research is required to assess the toxicity, safety, and therapeutic suitability of AgNPs in the oral cavity. Furthermore, it is still unknown how adding nanoparticles to the oral microbiome will affect the environment in the long run.

All things considered, the findings demonstrate that AgNPs produced with *R. ornithinolytica* not only possess potent inherent antibacterial qualities but also increase the efficacy of traditional antibiotics. This dual action encourages more research into clinical applications and may be a useful tactic against oral pathogens that are resistant to multiple drugs.

Conclusions

This study's results suggest that *Raoultella ornithinolytica* nanoparticles, either alone or in conjunction with antibiotics, could be useful in combating oral infections caused by multidrug-resistant *Streptococcus gordonii* bacterial isolates. The results of this study indicate that *Streptococcus gordonii* isolates from some patients with dental disorders were synergistically affected by the combination of the antibiotic Erythromycin and AgNP nanoparticles. The efficacy of these antibiotic-AgNP nanoparticle combinations has been the subject of much speculation. In conclusion, the mechanism of the synergistic influence has to be clarified with specific data.

Ethical approval

This study was achieved by the research ethics committee at the University of Misan. Every participant agreed to give blood samples to the researchers. Per the Declaration of Helsinki, each subject gave their informed approval. The present work was approved by the Ethics Committee of the Department of biology, College of Science, Misan, Misan, Iraq.

Conflict of interest

The author of this paper do not have any competing interests.

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