Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 13768 - 13789
Received 05 March 2021; Accepted 01 April 2021.

Green Synthesis of Silver Nanoparticles by *Enterobacter Aerogenes* Bacteria in Combination with Antibiotics Against Multidrug Resistance Streptococcus Mitis Isolated from Oral Cavity of Some Dental Caries Patients in Misan City.

Mohammed A. Abd Ali and Ali Aboud Shareef

Department of Biology, College of Science, University of Misan, Misan, Iraq.
Department of Biology, College of education for pure sciences, University of Basra, Basra, Iraq.

ABSTRACT

The present study aimed at the green, ecofriendly, cost-effective synthesis of silver nanoparticles (AgNPs) by using *Enterobacter aerogenes* bacterial isolated strains(S1, S2 and S3) from the oral cavity of dental caries patients in the center of Misan province, and also investigates their antibacterial effective combination with some traditional antibiotics against MDR *Streptococcus mitis* isolated from dental caries patients in the center of Misan province. The synthesized of (AgNPs) nanoparticles were characterized by process of UV-Visible spectrophotometer Transform Fourier (FT-IR). The Scanning Electron Microscopy (SEM) image refers to a spherical shape with the size range of the nanoparticles (AgNPs) synthesized was 47.22 – 105.00 nm. The X-ray diffraction (XRD) patterns revealed the crystalline structure of nanoparticles was AgNPs. Transform Fourier Infrared Spectroscopy (FTIR) analysis refers to a functional group that is implicated with the reduction of silver ion to AgNPs. All of the clinical bacterial strains isolated from the oral cavity of dental caries patients exhibited resistance against conventional antibiotics. The synergistic effect of antibiotics combined with (AgNPs) mentioned an increasing percentage of antibiotics. However, the maximum of synergistic effect was done only with Erythromycin E 30.7mm for the AgNPs (0.1 mg/ml), when in contrast Streptomycin 40mm were Streptomycin with the AgNPs 0.1 mg⁄ml recorded 6.0mm, were Tetracycline 15.6mm has been recorded only 6mm inhibition zone of when combination with AgNPs 0.1 mg⁄ml, that refer to antagonistic effects. Whereas optochin OP, penicillin P, and cephalexin CN antibiotics recorded in different effects. Finally, with this study report. It can be concluded that AgNPs prepared from *Enterobacter aerogenes* showed good antimicrobial activity when combined with antibiotics against Multidrug resistance *Streptococcus mitis* isolates. Thus these AgNPs can be used as a wide application to treat many oral cavity diseases in the dental field.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 13768 - 13789
Received 05 March 2021; Accepted 01 April 2021.

Keywords: Green synthesis of silver nanoparticles combination with antibiotics, *Enterobacter aerogenes*, MDR *Streptococcus mitis* oral cavity dental caries.

waleedabas22@uomisan.edu.iq

1. Introduction

At the beginning of 20th century the antibiotic penicillin was discovered , and since then humans have been working continuously to discover new antibiotics because bacteria at the same time, develop new mechanisms to resist these antibiotics. (Clatworthy *et al*.,2007) . although of these elevation a high accident rate to the rise of antibiotics resistance toward the traditional antibiotics is an important health care with globally serious impact (Mannaa *et al*.,2015) .In recent years, nanotechnology as developing field of nanscience with important applications , including synthesis of nanomaterials and applications in medicine , biology due to their unique shape and size based on their chemical , biological and physical properties (Mahasneh,2013) Nanotechnology is considered the fourth industrial revolution through the history of human civil evolution Parthasarathi *et al* (2011), it deals with greater part of material types including ceramics, metals, polymers, and biomaterials. It has a great deal in elaboration and improvement of biogenic products such as certain medications like, antibiotics , sunscreens and sunblocks Vijay *et al*,. (2015).The geen synthesis of nanoparticle metals has more favorable compared to chemical synthesis caused by use of eco-friendly deputy as reducing agents rather than hazardous of chemicals (Abdeen *et al*.,2014). Many studies refers to the evolution of nanoparticles products is distance presently , nanoparticles is establish in implant coatings, food containers , ointments and other items . US Food and Drug Administration have approval from it (Dunn &Edwards,2004). Different studies refers to the nanosilver bactericidal effective against gram positive and gram negative bacterial isolates (Morones *et al*.,2009).

2.Materials and Methods

2.1 Silver nitrate solutions

It's advised to prepare silver nitrate solution at real time. Silver nitrate was dissolved in double distilled water (DDW) in dark place to avoid phto-oxidation of silver nitrate. The solution was used as a substrate for silver nanoparticles biosynthesis (Chaudhari *et al*., 2012).

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 13768 - 13789
Received 05 March 2021; Accepted 01 April 2021.

2.2 Isolation and Identification of Bacteria:

Isolates samples were obtained from oral cavity of dental disease Dental Specialist Center of dental disease in Misan Governorate - Iraq. The isolates were diagnosed by Gram stain and biochemical tests. Finally by PCR with sequencing .These identified cultures were transferred to nutrient agar slant for preservation and then store in the refrigerator at 4°C.

2.3 Molecular Identification of Strains

One strain selected (S**2**) from three strains(S**1 ,** S**2 ,** S**3**) belonging to *Enterobacter aerogenes* bacterial isolated strains , were identified by 16S rDNA sequenced , extracted and DNA amplified using PCR, sequenced ,and aligned with other identified strains in Gene bank database by using BLAST tool an online to determine the similarity score .

2.4 Activation bacterial strains

Bacterial strains were activated in 10 ml of nutrient broth in serum tube by aseptic technique and incubated at 37°C overnight.

2.5 Biomass production

bacterial isolate (*Enterobacter aerogenes*) was inoculated in flask 500ml contained 6.5 gram⁄ml nutrient broth, incubated at 37°C in a shaking incubator at 200rpm for 48 h.

2.6 Harvesting the cell free medium

Supernatant of cell free was done through four steps: activation by inoculum , inoculation in the nutrient broth, culture of incubated for production biomass and centrifugation for cell excluding. Cell free supernatant was harvested by centrifugation of the broth culture pellet cells at bottom of the tubes leaving cell free supernatant. Supernatant then collected in a sterile flask for use in the production of silver nanoparticles (Singh *et al*., 2018).

2.7Biogenic synthesis of silver nanoparticles:

cellular synthesis of silver Nanoparticles was accomplished by mixing 250ml of cell free supernatant from 48 hour liquid culture of *Enterobacter aerogenes* strain (filtered through 0.2µm pore dimension) with 250ml of 1mM silver nitrate (AgNo**3**) solution. Then incubated in orbital shaker (200rpm) at 37°C in dark for 5 days. A flask with cell free supernatant without (AgNo3) utilized as control (Saifudin *et al*.2009).

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 13768 - 13789
Received 05 March 2021; Accepted 01 April 2021.

2.8 Characterization of biosynthesis(AgNPs) .

Biosynthesis characterization of (AgNPs) was performed by using UV-visible spectroscopy,The purified AgNPs were examined for the presence of biomolecules using FTIR spectrum (Thermo Scientific Nicolet 380 FT-IR SpectrometerX-ray diffraction(XRD)and scanning electron miucroscope (SEM)\ , particle sizes (Zhang *et al*.,2016).

2.9 Antimicrobial activity test

The antimicrobial activity test was evaluated by two (well and disk diffusion methods).
Johan *et al*. (2003).

2.10.1 Combination assay of antibiotics and AgNPs

Assays were performed in susceptibility of antibiotic with AgNPs it is Combination assay, the cells of bacterial were grown in nutrient broth .Disk-diffusion method was performed to assess the combination between antibiotics and AgNPs biologically synthesized with their antimicrobial activity against some bacterial isolates from dental caries and periodontitis. Nutrient broth were cultured with bacterial isolates *Streptococcus mitis* on Muller – Hinton lates (Johnson,2010).

2.10.2 MIC Determination.

The synergy effects between antibiotics drugs usually expressed by sum of Fractional Inhibitory Concentration (FIC) of two combinations . FIC is calculated by following for combination A , B:

$$FIC = \frac{\text{MIC of antibacterial A in combination}}{\text{MIC of antibacterial A alone}}$$

FIC was also calculated for combination B with same formula

Thus [FIC= FIC of antibacterial **A**+ FIC of antibacterial B

3.Results and Discussion

3.1 Morphological and biochemical characterization.

In the present study the clinical bacterial strains isolates from oral cavity of dental disease patients were obtained from center of Misan City- Iraq Identification of the isolate was done based upon exploring the role of bacterial strain (S2) *Enterobacter aerogenes* in

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 13768 - 13789
Received 05 March 2021; Accepted 01 April 2021.

synthesize AgNPs along with their biomedical application to controlling infectious bacteria , as shown in [Figure. 1,2].

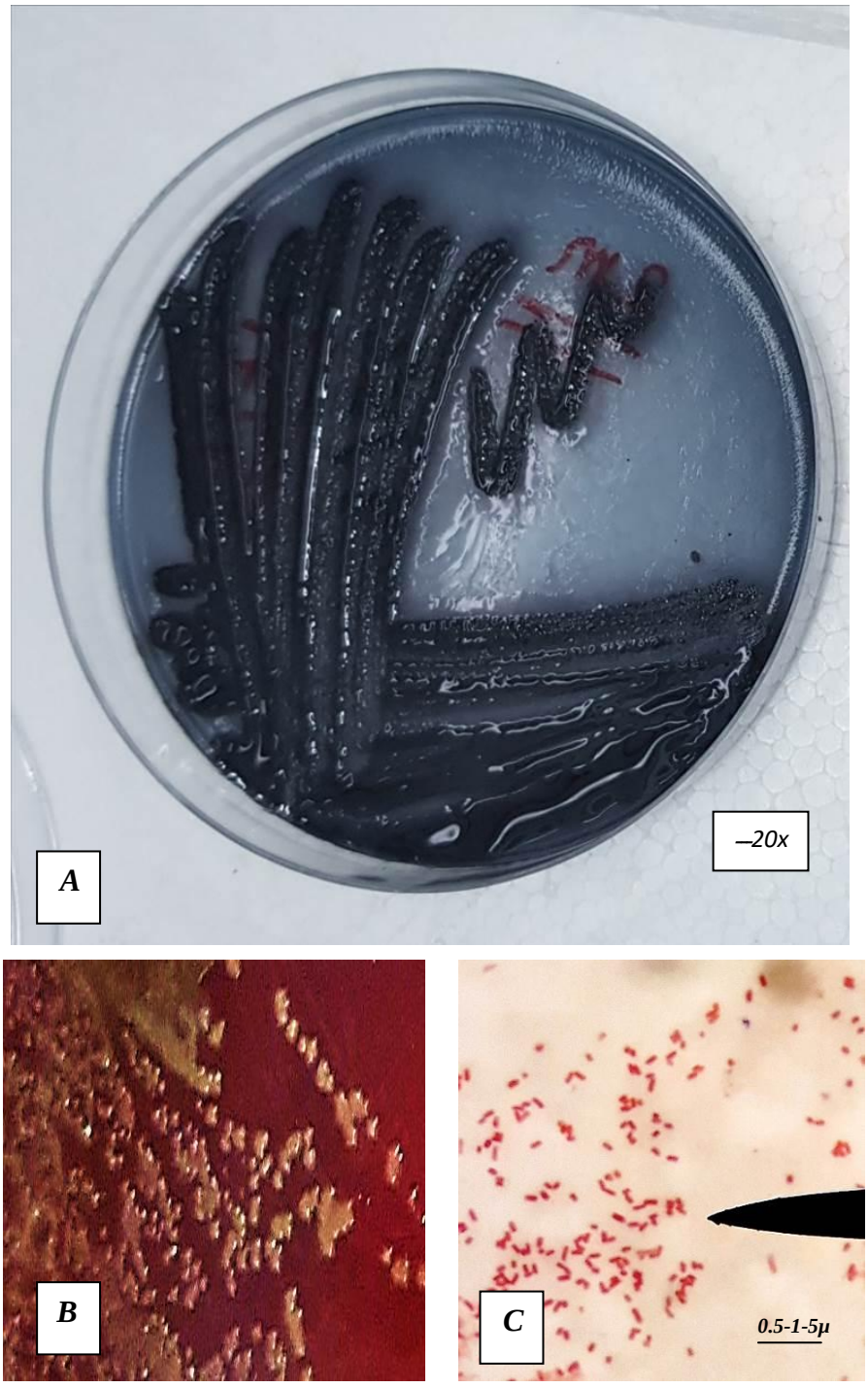
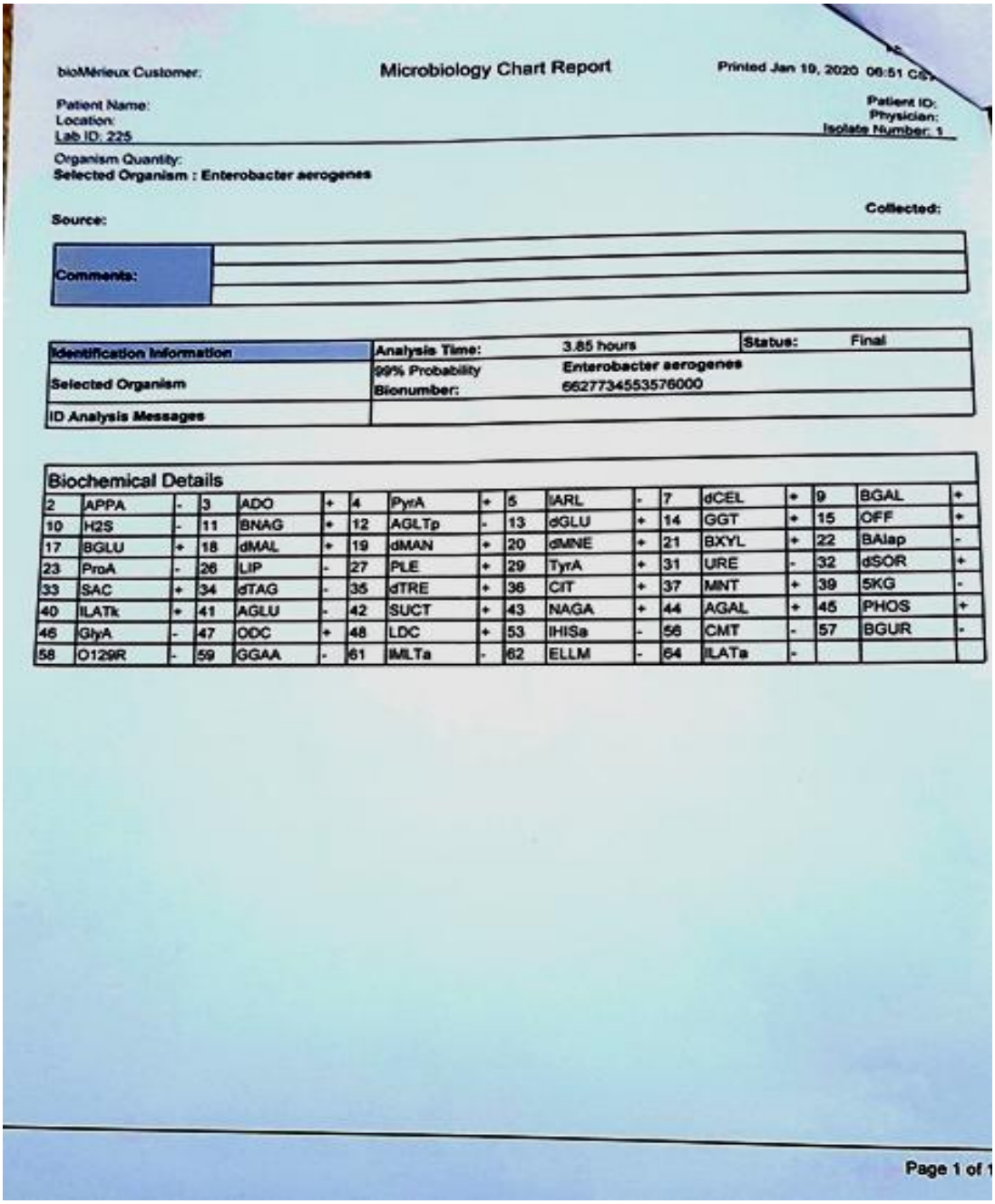


Figure (1): Shown bacterial strain of Enterobacter aerogenes cultured on (A) Mitis Saliverous Agar and(B) Blood Agar (C) Gram stain of bacterial isolate of Enterobacter aerogenes shown by microscopy 1250x

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 13768 - 13789
Received 05 March 2021; Accepted 01 April 2021.

Figure (2): Shown Vitek2 Chart Report Microbiology for Identification of *Enterobacter aerogenes*



bioMérieux Customer:

Microbiology Chart Report

Printed Jan 19, 2020 06:51 CST

Patient Name:
Location:
Lab ID: 225

Patient ID:
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Enterobacter aerogenes

Source:

Collected:

Comments:

Identification Information	Analysis Time:	3.85 hours	Status:	Final
Selected Organism	99% Probability Bionumber:	Enterobacter aerogenes 6627734553576000		
ID Analysis Messages				

Biochemical Details

2	APPA	-	3	ADO	+	4	PyrA	+	5	lARL	-	7	dCEL	+	9	BGAL	+
10	H2S	-	11	BNAG	+	12	AGLTp	-	13	dGLU	+	14	GGT	+	15	OFF	+
17	BGLU	+	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	+	22	BAlap	-
23	ProA	-	26	LIP	-	27	PLE	+	29	TyrA	+	31	URE	-	32	dSOR	+
33	SAC	+	34	dTAG	-	35	dTRE	+	36	CIT	+	37	MNT	+	39	5KG	-
40	ILATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	+	44	AGAL	+	45	PHOS	+
46	GlyA	-	47	ODC	+	48	LDC	+	53	IHISa	-	56	CMT	-	57	BGUR	-
58	O129R	-	59	GGAA	-	61	IMLTa	-	62	ELLM	-	64	ILATa	-			

Page 1 of 1

3.2 Molecular Identification

Characterization of AgNPs by bacterial strain.

Identification was done in addition to biochemical test, isolates was matching through 16SrRNA gene sequencing , molecular identification by extraction and amplification of gene

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 13768 - 13789
Received 05 March 2021; Accepted 01 April 2021.

.[Figure 3]. One strain selected (S**2**) from three strains(S**1 , S2 , S3**) belonging to *Enterobacter aerogenes* bacterial isolated strains , were identified by 16S rDNA sequenced of strain IHB B 6843 was 1290bp , extracted and DNA amplified using PCR, On the basis of analysis of the 16S rRNA strain IHB B 6843 showed the highest sequence similarity with NR_117547.1 *Enterobacter soli* ATCC BAA.2102 strain LF7.[Figure 4,Table1] sequenced ,and aligned with other identified strains in Gene bank database by using BLAST tool an online to determine the similarity score .

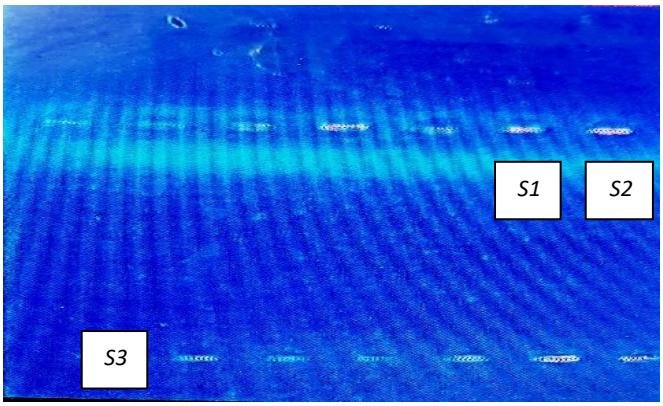


Figure (3): Agarose gel electrophoresis patterns show of genomic DNA extracted from the bacterial strains (S1,S2and S3) For *Enterobacter aerogenes* on 1% Agarose gel at 5vol𝓍cm for 1.15 hour. redsafe stained and documented UV gel viewer.

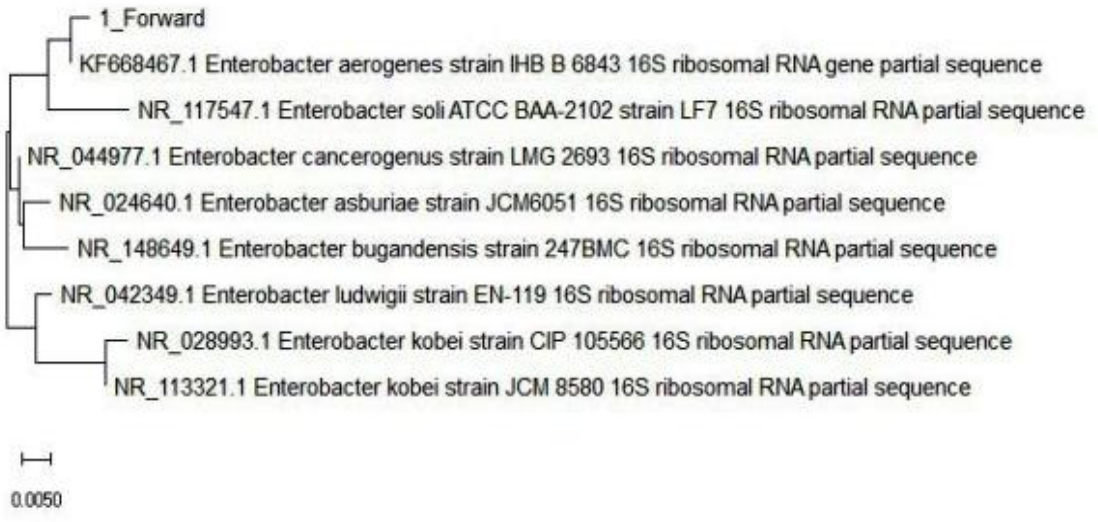


Figure (4): The phylogenetic tree relationships of isolated strain IHB B 6843 with related type strains. The scale par shows 0.005 substitutions per nucleotide position.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 13768 - 13789
Received 05 March 2021; Accepted 01 April 2021.

Table (1):Shown identical bacteria by 16SrRNA gene sequences , source and identical strains(%) .

Sequence description	Sequence	identification	Percent identity
1_Forward	ATGGATGCGGCAGCTACACATG CAAGTCGAGCGGTAACACAGAG AGCTTGCTCTCGGGTGACGAGC GGCGGACGGGTGAGTAATGTCT GGGAAACTGCCTGATGGAGGGG GATAACTACTGGAAACGGTAGC TAATACCGCATAACGTCGCAAG ACCAAAGTGGGGGACCTTCGGG CCTCATGCCATCAGATGTGCCCA GATGGGATTAGCTAGTAGGTGG GGTAATGGCTCACCTAGGCGAC GATCCCTAGCTGGTCTGAGAGG ATGACCAGCCACACTGGAACTG AGACACGGTCCAGACTCCTACG GGAGGCAGCAGTGGGGAATATT GCACAATGGGCGCAAGCCTGAT GCAGCCATGCCGCGTGTATGAA GAAGGCCTTCGGGTTGTAAAGT ACTTTCAGCGAGGAGGAAGGCG TTAAGGTTAATAACCTTGGTGAT TGACGTTACTCGCAGAAGAAGC ACCGGCTAACTCCGTGCCAGCA GCCGCGGTAATACGGAGGGTGC AAGCGTTAATCGGAATTACTGG GCGTAAAGCGCACGCAGGCGGT CTGTCAAGTCGGATGTGAAATC CCCGGGCTCAACCTGGGAACTG CATTCGAAACTGGCAGGCTAGA GTCTTGTAGAGGGGGGTAGAAT TCCAGGTGTAGCGGTGAAATGC GTAGAGATCTGGAGGAATACCG GTGGCGAAAGCGGCCCCCTGGA CAAAGACTGACGCTCAGGTGCG AAAGCGTGGGGAGCAAACAGGA TTAGATACCCTGGTAGTCCACGC CGTAAACGATGTCGACTTGGAG GTTGTGCCCTTGAGGCGTGGCTT CCGGAGCTAACGCGTTAAGTCG ACCGCCTGGGGAGTACGGCCGC AGGGTTAAAACTCAAATGAATT GACGGGGGCCCGCACAAGCGGT GGAGCATGTGGTTTAATTCGATG CAACGCGAAGAACCTTACCTAC TCTTGACATCCAGAGAACTTAGC	Enterobacter aerogenes strain IHBB 6843 16S ribosomal RNA gene, partial sequence	99.79

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 13768 - 13789
Received 05 March 2021; Accepted 01 April 2021.

	AGAGATGCTTTGGTGCCTTCGGG AACTCTTAGAACAGGTGCTGCA TGGCTGTCGTCCACCTCCGGTTG TGAAAAGTTGGGTTAATTCCCGC ACCCAGCGCCACCCCTTATTCCT TTGTGTGCCGCCGATTTTGTGCG GGAACCCCAAAGGGAAAATGCC CCTTTAAAAAACGGAAGAAAAG GGGGGGGGGGACGCTCCACTTT CCCTCGGCCC		

3.3 Characterization of AgNPs

Harvesting the cell free medium

Cell free supernatant was harvested by centrifugation of the broth culture pellet cells at bottom of the tubes leaving cell free supernatant. Supernatant then collected in a sterile flask for use in the production of silver nanoparticles (Singh *et al*., 2018). cellular synthesis of silver Nanoparticles was accomplished by mixing 250ml of cell free supernatant from 48 hour liquid culture of *Enterobacter aerogenes* strain (filtered through 0.2µm pore dimension) with 250ml of 1mM silver nitrate (AgNo**3**) solution. Then supernatant mixture incubated in orbital shaker (200rpm) at 37°C in dark for 5 days. A flask with cell free supernatant without (AgNo3) utilized as control (Saifudin *et al*.2009).

Because of increasing multi drug resistance (MDR) threat, we scope on search the efficient alternative sources to replace resisted antibiotics .Nanoparticles considered now as alternative to the traditional antibiotics .Recently , nanoparticles (AgNPs) were considered as attractive target for the fabrication of a new antibiotics generation (Rai *et al*.,2012 ;Singh *et al*.,2017) . The AgNPs of bacterial strain (S2) *Enterobacter aerogenes* showed effective antimicrobial activity against multi drug resistant bacterial isolate *Streptococcus mitis* [Figure 5 and 6],

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 13768 - 13789
Received 05 March 2021; Accepted 01 April 2021.

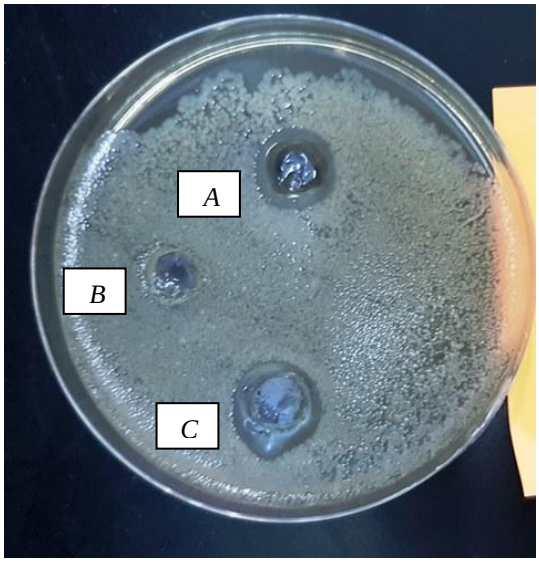


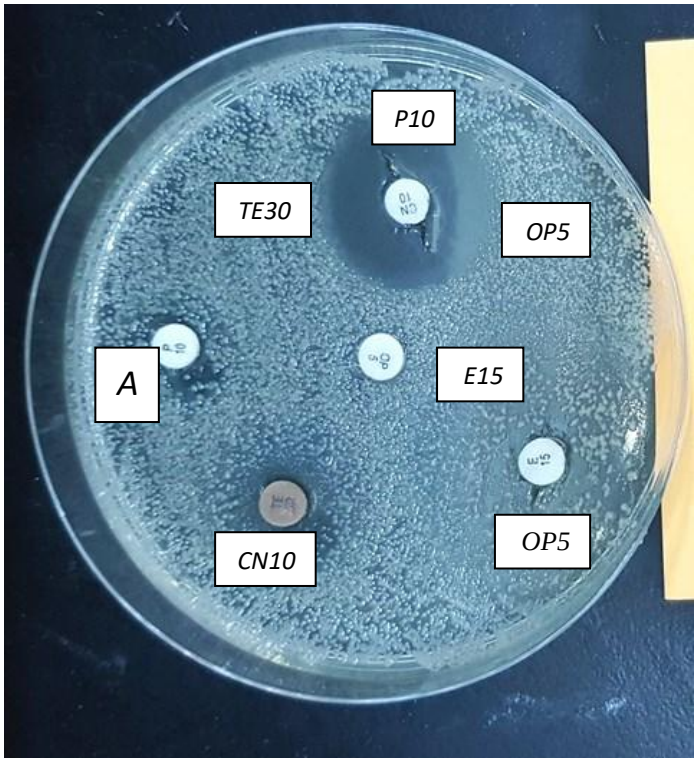
Figure (5): A. Effect of Nanoparticles on MDR *Streptococcus mitis* isolated from dental caries patients B. Control DDW C. Antibacterial activity of AgNo3.

3.4Antimicrobial activity:

Antimicrobial activity was done by disk diffusion method was used to evaluate Optochin OP , Penicillin P , Tetracycline TE ,Cefalexin CN , Streptomycin S antibiotics alone and in combination with Biogenic AgNPs synthesized by *Enterobacter aerogenes* have been evaluated for their antimicrobial activity against some pathogenic bacteria isolated from dental carries patients in misan province / Iraq , using agar well and disk diffusion method [Figure7 and 8] ,[Table1]. According to CLSI Standard , selective concentration of antibiotics .Selective concentration of AgNPs was 100µg. Its found that, at concentration of 100µg.The antibacterial activity of combined formulation of AgNPs through different discs of antibiotic , including optochin ,penicillin , tetracycline ,erythromycin ,cephalexin and streptomycin [Figure 8]. all of the clinical bacterial strains isolates from oral cavity of dental disease patients were obtained from center of Misan City-Iraq, exhibited resistance against conventional antibiotics such as Optochin OP ,Penicillin P, Erythromycin E , were bacterial isolate *Streptococcus mitis* showed more sensitively against Tetracycline TE ,Cefalexin CN , Streptomycin S. The results in present study showed only (Erythromycin E 30.7mm) as a synergistic effect of antibiotics , the combination with AgNPs mentioned increasing efficacies (percentage) of antibiotics. In similar with Fayaz et al (2010) the antimicrobial activities of synthesized of AgNPs were estimated with available antibiotics against Multi drug resistant *Streptococcus mitis* strains. However, maximum of synergistic

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 13768 - 13789
Received 05 March 2021; Accepted 01 April 2021.

effect was done only with Erythromycin E 30.7mm for the AgNPs(0.1mg/ ml) .When in contras Streptomycin S (Streptomycin 40mm were Streptomycin with AgNPs 6.0 mm) and Tetracycline TE (Tetracycline 15.6mm were were Tetracycline with AgNPs 6mm inhibition zone recorded) Antagonistic effects. Where Optochin OP , Penicillin P and Cefalexin CN antibiotics Indifferent effects [Figure 6 , Table2 and 3] .These resultsare in a similar with Birla *et al* (2009) the biologically synthesized AgNPs assessed with available antibiotics against both gram negative and gram positive bacteria. The antimicrobial activities of Erythromycin, ampicillin , kanamycin ,and chloramphenicol were extend in the presence of nanoparticles AgNPs. Finally, The minimum concentration of inhibition zone recorded with the concentration of the following (0.1 , 0.05 , 0.025 , 0.0125 and 0.0062 mg / ml) of the biosynthesized AgNPs detection for *Enterobacter aeruginose* [Figure 6 ,Table 4] .we findings that the action of mode of silver nanoparticles is same as, to that of silver ions, which interaction with groups of electron donor containing oxgen ,sufur or nitrogen atoms that are usually present as phosphates or thiols on amino acids and nucleic acids (McDonnell,2007).The(Kvitek et al.,2008) explain that AgNPs interaction with the surface of membrane of the bacterial cell by interacting with proteins continuing sulfur , that disrupting of the respiratory functions of the permeability of the cell membrane leading to cell death.



Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 13768 - 13789
Received 05 March 2021; Accepted 01 April 2021.

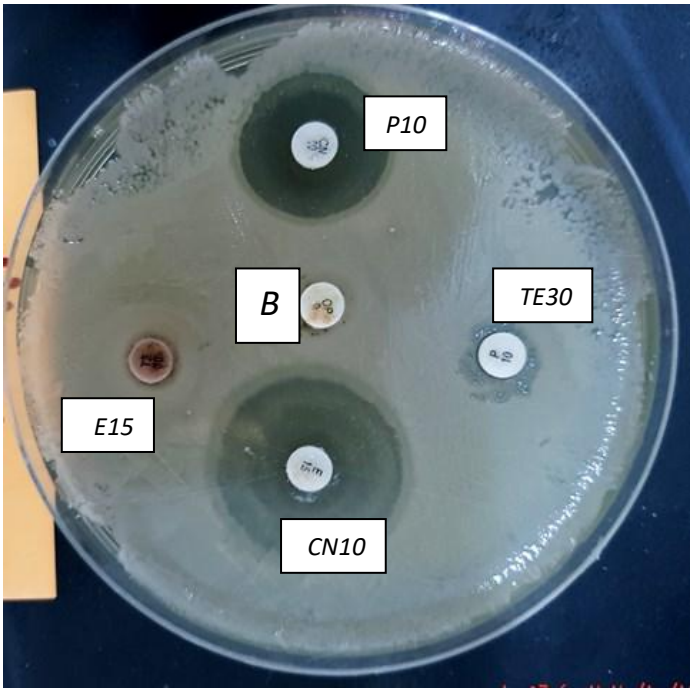


Table (2) : Percentage of inhibition zone by antibiotics alone and nanoparticles AgNPs combination with antibiotics against Streptococcus mitis

No	Antibiotics	Concentration µg⁄disk	Antibiotics Effects A	AntibioticsWith AgNPs Effects B	Increase in fold area
1	Optochin OP	5	6	7.3	21.66
2	Penicillin P	10	6.6	9.7	46.96
3	Tetracycline TE	30	15.6	6.0	-61.53
4	Erythromycin E	15	7.3	30.7	320
5	Cefalexin CN	30	26.3	20.6	-21.67
6	Streptomycin S	10	40.6	6.0	-85.22

+Mean of the inhibition zone (mm) for each antibiotic tested of the inhibition zone was done by mean diameter fold increases for diverse antibiotics studied as ((b-a) /a) 100 (Zarina &Nanda,2014).* Three replicates for each value.*

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 13768 - 13789
Received 05 March 2021; Accepted 01 April 2021.

No	AgNPs+Antibiotics	Interaction
1	Optochin + AgNPs	Indifferent
2	Penicillin + AgNPs	Synergistic
3	Tetracycline+ AgNPs	Antagonistic
4	Erythromycin+ AgNPs	Synergistic
5	Cefalexin+ AgNPs	Indifferent
6	Streptomycin+ AgNPs	Antagonistic

Table(3): Index of FIC to establishes the interaction between antibiotics agents

[FIC index to use for identify of th interaction between the two antibacterial combinations . The index is interpreted as follows : 1≥ synergistic , 0.2≤ antagonistic , 1=additive ; 1.1-2.0 indifferent (non-interactive) Habiba *et al*.,(2015).

Table (4): Shown minimum of inhibition zone concentration (MIC) of the biosynthesized AgNPs alone and with combination of six Antibiotics against Multi drug *Streptococcus mitis* bacterial isolated from oral cavity patients in misan city.

No	Multi drug Streptococcus miti MIC (mg/ml)	
1	NPs alone	0.009
2	Optochin + AgNPs	-
3	Penicillin + AgNPs	0.037
4	Tetracycline+ AgNPs	0.018
5	Erythromycin+ AgNPs	0.018
6	Cefalexin+ AgNPs	-
7	Streptomycin+ AgNPs	0.037

* Three replicates for each value

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 13768 - 13789
Received 05 March 2021; Accepted 01 April 2021.

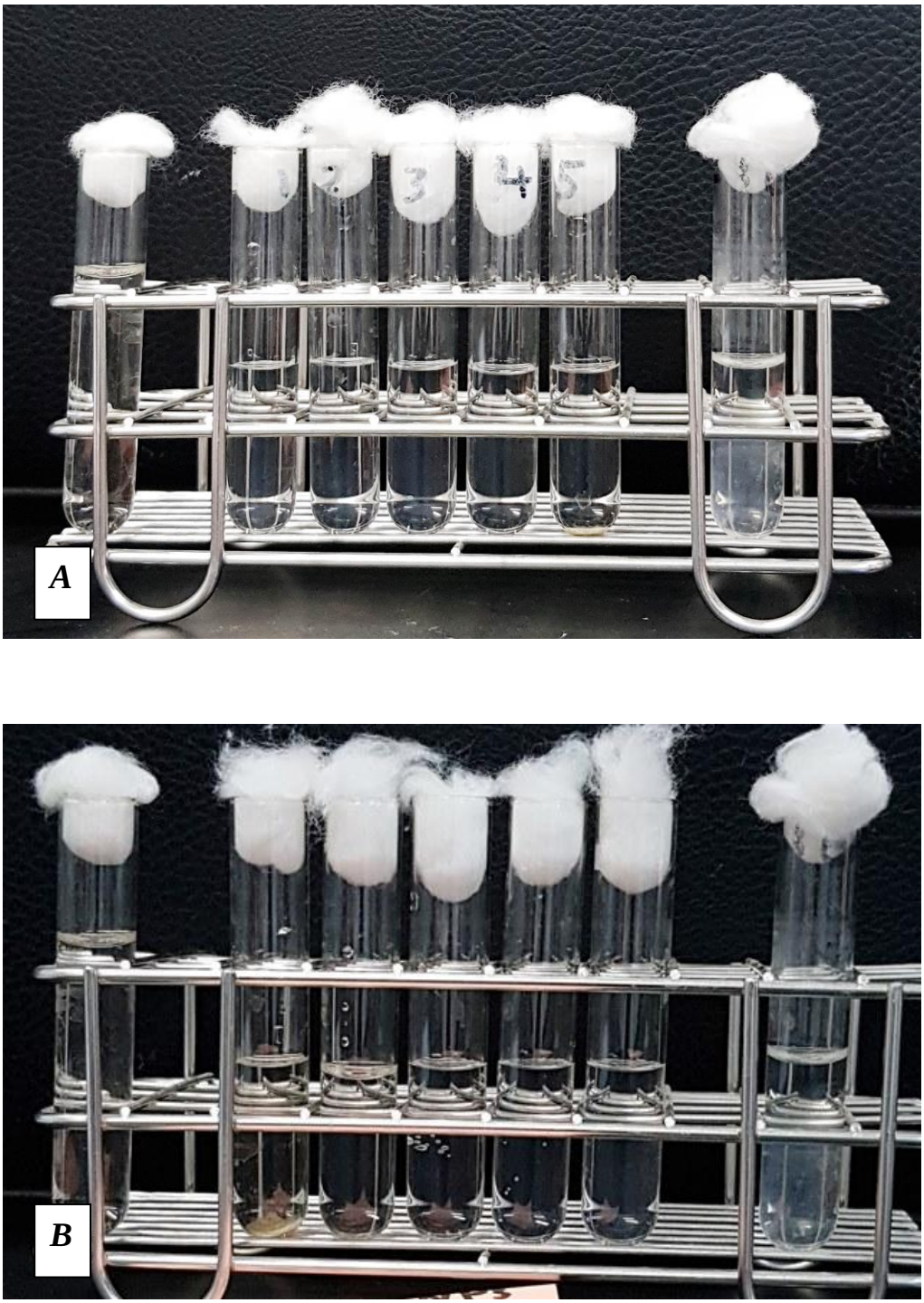


Figure (7): Shown Minimum inhibitory concentration (MIC µg ⁄ml) of NPs alone (A) , Minimum inhibitory concentration (MIC µg ⁄ml) of NPs with antibiotic (B).

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 13768 - 13789
Received 05 March 2021; Accepted 01 April 2021.

3.5 Physical characterization of synthesized nanoparticles AgNPs

Nanoparticle **AgNPs** s composed by (S2 strain) *Enterobacter aerogenes* was characterized visually by UV-Visible spectra analysis [Figure 14] , FT-IR

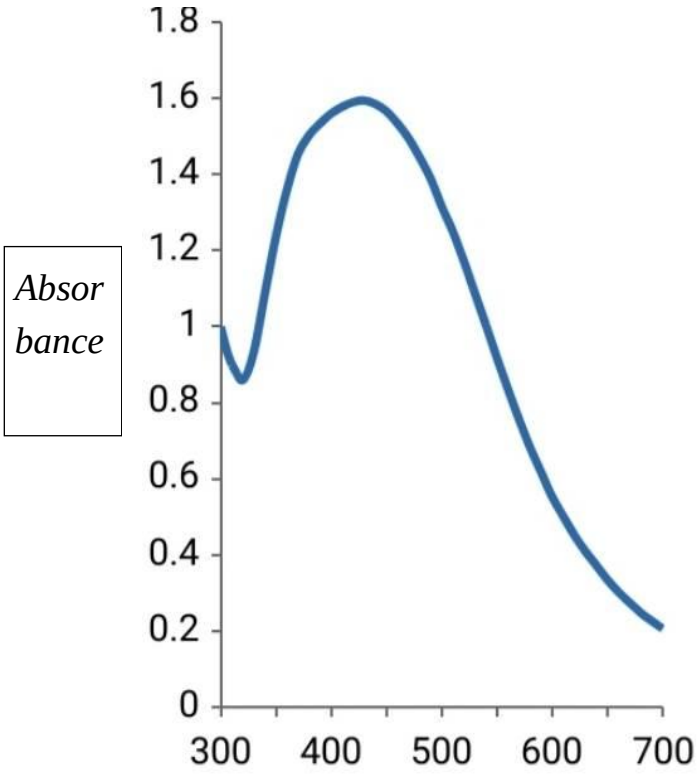
[Figure 15] and XRD assays [Figure16,Table 7] . The initial formation of the of silver nanoparticles **AgNPs** is mediated by the observation the colour change take place in the reaction solution from light yellowish brown to dark brown due to the excitation vibrations of surface plasmon of the incorporate AgNPs .The colour changed of solution can be considered clearly indication of silver nanoparticles formation (Kalimuthu *et al*.,2008 ; Luo *et al*.,2018).

Figure (8) : Extracellular of AgNPs synthesis at start of the reaction and after 72 hours (end of reaction). A. Positive control filtrate without AgNO3 B. Culture filtrate Enterobacter aerogenes with AgNO3 solution(0.1mM). C. After 96 hours extracellular of AgNPs synthesis ,dark brown solution that's detected the completion of reaction and D. shown Nanoparticales AgNPs after 24 h in ptridish.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 13768 - 13789
Received 05 March 2021; Accepted 01 April 2021.

This ranged in color also confirmed strength absorbance also by UV-Vis spectroscopy. The optical peaks absorption of the reaction mixture were nearly 420nm[figure6],this result supported by the researcher Basavaraja *et al*.,(2008) they were reported that the reduction of Ag+ to atomic Ag° in agreement with absorption at 420nm [Figure.14].

Figure (9): UV- Vis absorption spectrum of AgNPs synthesized by Enterobacter aerogenes isolated from dental caries patient .



The FT-IR measurements were carried out to reveal the possible biomolecules that responsible for the stabilization of the synthesized AgNPs (Ananthi ,2018).The FT-IR spectrum [Figure 15] illustrated peaks at 3448 , 3426, 2928, 2881 ,1651 ,1392 and1111,1045cm^{-}, the absorbance band at 3475-3383 cm^{-} assigned to the stretching vibrations of hydroxyl groups and amine groups, where N-H was recognized as stretching vibrations in primary and secondary amines of proteins , peptides and amino acids (Cheng *et al*.,2014).The peak of 2928 ,2958 cm^{-} indicated for secondary amine (Vigneshwaran *et al*., 2007) , while peak at 1647 cm^{-} shown to be responsible for the capping of silver ion and forming consequently covering protein around AgNPs to thereby stabilize medium with prevent agglomeration (Shanmuganathan *et al*.,2017).The peaks positioned at 1539,1384 and 1053 cm^{-} assigned to either function groups of sulfur or phosphorus , which attributed to

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 13768 - 13789
Received 05 March 2021; Accepted 01 April 2021.

possibly of stabilizing and capping process of AgNPs (Castro *et al*.,2013).The proteins can interactions with nanoparticles through cysteine or free amine groups residues in the proteins

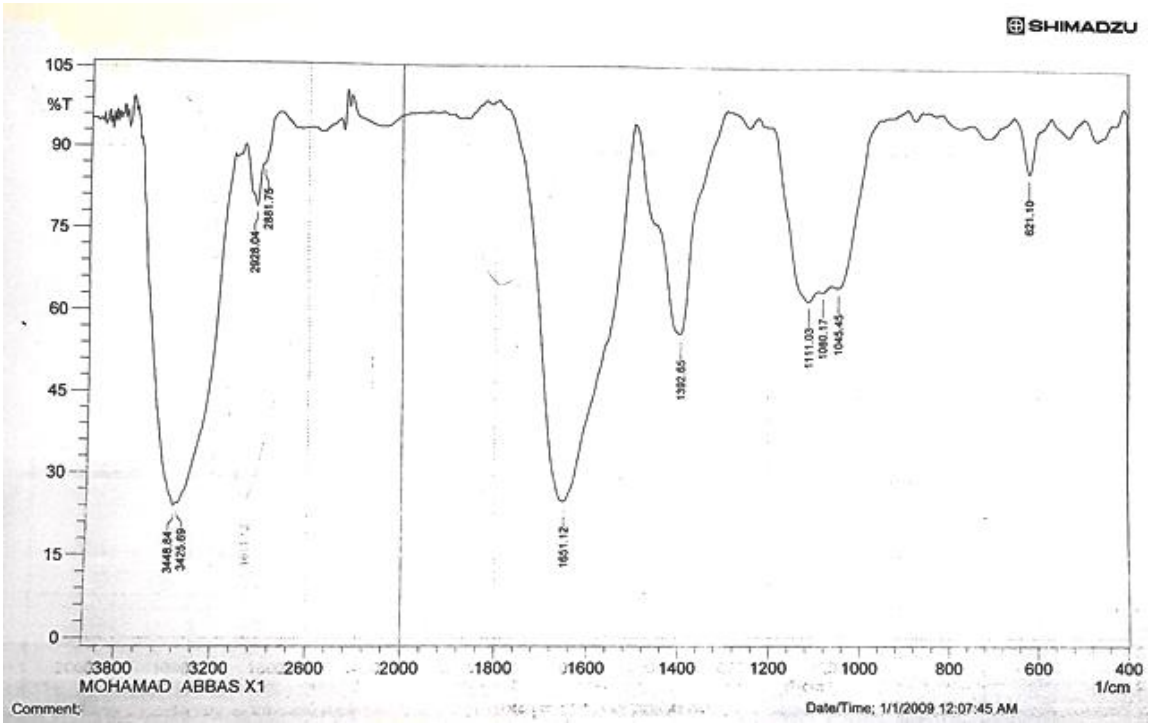


Figure (10): *FT- IR* spectrum of *AgNPs* synthesized by *Enterobacter aerogenes* isolated from dental caries patient .

(Mandal *et al*.,2013).

The Deby – Scherrer formula can be using the mean size of the AgNPs crystallized particles (Allafchian and Jalali,2015). XRD patterns refer to four major peaks in the entire spectrum of (2θ) 38.42 , 46,51, 67,76 and 77.09 . These values refer to the (111) , (200) , (220) and (311) planes of face –centered –cubic(FCC) sliver , with a network parameter of 4.08 °A, which is consistent with the Joint Committee Powder Diffraction Standards (JCPDS) Card No-087-0720 (Vanaja et al.,2014). Scanning electron microscope image showed various shapes of nanoparticles ; however , the shapes were spherical with ranged size 71.56 nm[Figure16 and Table 6 with7].

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 13768 - 13789
Received 05 March 2021; Accepted 01 April 2021.

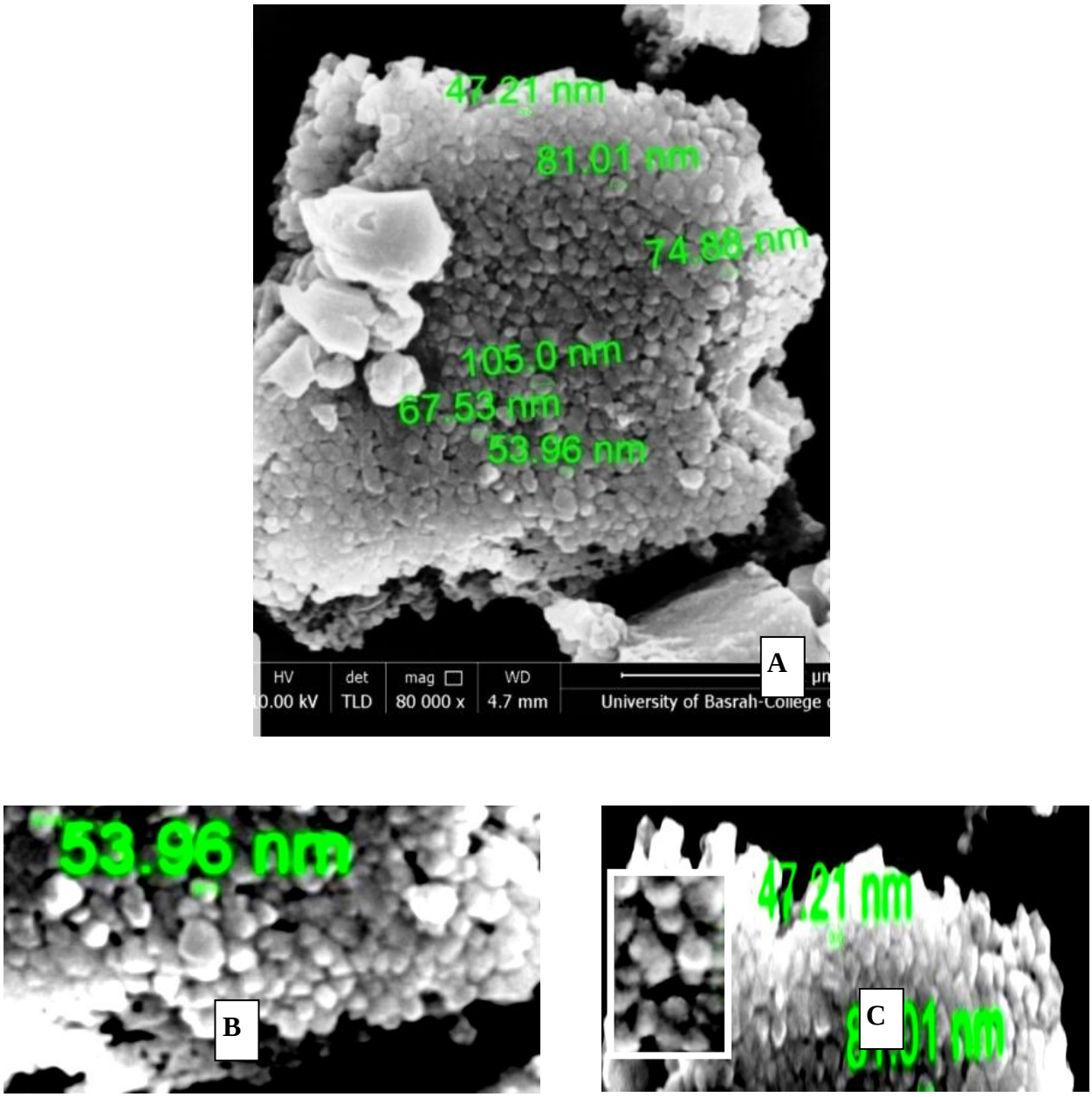


Figure (11): Chacterization of the biogenic AgNPs synthesized by Enterobacter aerogenes (A ,B,and C) Scanning electron microcopy .

Table (5):Lattice plane resulted AgNPs source by *Enterobacter aerogenes* as indexed to JCPDS data .

Source of AgNPs	h kl index			
	111	200	220	311

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 13768 - 13789
Received 05 March 2021; Accepted 01 April 2021.

Enterobacter aerogenes	38.4213	46.5167	67.7625	77.0906

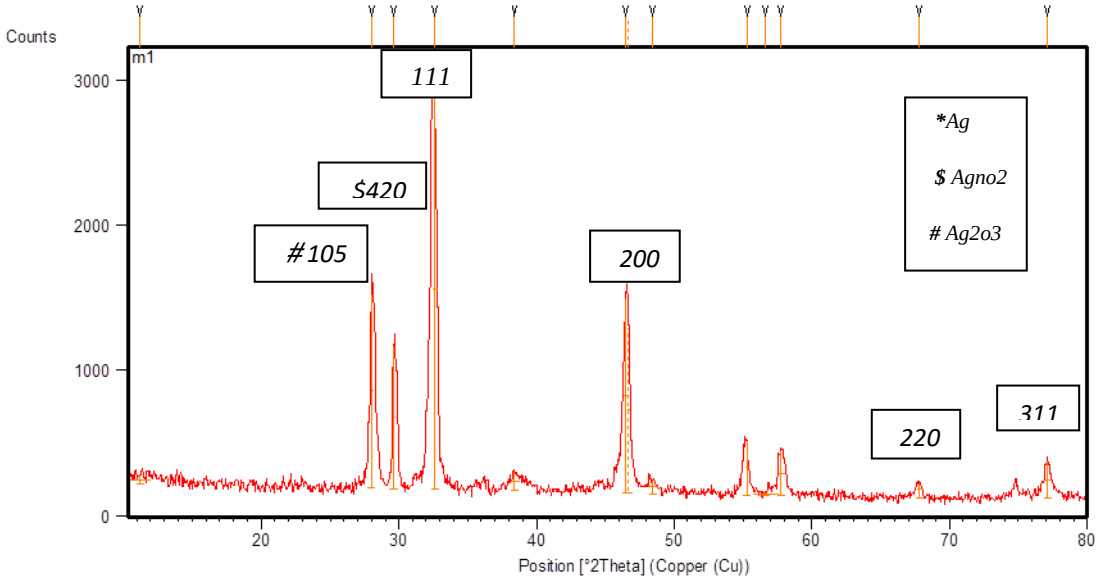


Figure (12): XRD report ,resulted by AgNPs fabricated on copper surface obtained from *Enterobacter aerogenes* isolated from oral cavity of dental caries patients in center of misan province .

Table (6).XRD results of silver nanoparticles

Pos. [°2Th.]	Height [cts]	FWHM Left [°2Th.]	d-spacing [Å]	Rel. Int. [%]	Tip Width
38.4213	132.40	0.5904	2.34297	4.80	0.7085
46.5167	1340.05	0.3000	1.95073	48.56	0.3600
67.7625	108.62	0.4800	1.38177	3.94	0.5760
77.0906	255.07	0.4800	1.23617	9.24	0.5760

Results of this study suggest that the nanoparticles AgNPs of *Enterobacter aerogenes* may be useful either alone or when combined with antibiotics against oral pathogens MDR *Streptococcus mitis* bacterial isolates , which can be used as a wide application to treat many of oral cavity diseases in dental field.

5.Conclusion

The results of present study showed synergistic effect of combination of Erythromycin antibiotic with AgNPs nanoparticles against Streptococcus mitis isolates from some dental

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 13768 - 13789
Received 05 March 2021; Accepted 01 April 2021.

caries patients . Some hypotheses were explain of these combinations mechanism between AgNPs nanoparticles with antibiotics. Finally, detailed is needed to evidence clarify mechanism of synergistic effect.

Acknowledgements

This paper is a part of PHD thesis . My gradeful thanks to the Dental specialist Center in misan governorate , medical staff of child hospital in the center of misan governorate ,The authors wish to thank department of biology College of Science in misan University. The authors wish to extend their gratitude to Department of Biology, College of Pure Sciences , University of Basra ,

References

1. Abdeen,S. ; Geo,S.; Sukanya.P. (2014).Nano Dimens. Int . J.(5):pp.155-162.
2. Allafchian, A.; Jalali ,S.(2015).Biosynthesis ,Characterization and therapeutic applications of plant –mediated sliver nanoparticles.J. Taiwan .Inst. Chem.Eng.(57):pp.154-155.
3. Al-Shammari ,A. ; Alshami,M. ; Umran ,M. (2015). Establishment and characterization of a receptor – negative hormone-nonresponsive breast cancer cell line from an Iraqi patient.J.Breast Cancer argets.Ther.,(7): pp. 223-230. DOI:10.2147⁄BCTT.S74509.
4. Amirjani,A. ; Marashi,P. ; Haghshennas ,D. (2014). The effects of physicochemical parameters on the synthesis of silver nanowires via polyol method. Int.Nano.Lctt.(4):pp.108-109.
5. Ananthi , V. (2018).Comparison biodiesel and antibacterial nano silver production by microalgal and yeast isolates .J.Photochem Photobiol B(186):pp.232-242.
6. Basavaraja,S.;Balaji ,D. Lagashetty,A.(2008).Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium semitectum* .DOI:10.1016/j.materresbull.2007.06.020.
7. Birla ,S. ; Tiwari , V. ; Gade ,A. (2009). Fabrication of silver nanoparticles by phoma glomerata and its combined effect against Escherichia coli , Pseudomonas aeruginosa and Staphylococcus aureus.J.Applied Microbiology .48(2):pp. 173 – 179.
8. Birla, S. ; Tiwari ,V. ; Gade ,A.(2009).Fabrication of silver nanoparticles by phoma glomerata and its combined effect against *Escherichia coli* , *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Applied Microbiology.J.48,(2):pp.173-179.
9. *Mezal, E. H., Yousif, A. F., Hanan, Z. K., Hanan, A. K., & Jalil, A. (2020). Isolation, Assessment of Antimicrobial Sensitivity of Bacterial Pathogens from Post-Cesarean section Infection of patients in Thi-Qar Province.* European Journal of Molecular & Clinical Medicine, 7*(3), 958-964.*
10. Chaudhari, P.R. ; Masurkar,S.A.; Shidore, V.B. and Kamble,S.P. (2012). Antimicrobial activity of extracellularly synthesized silver nanoparticles using

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 13768 - 13789
Received 05 March 2021; Accepted 01 April 2021.

Lactobacillus species obtained from vizylac capsule. Journal of Applied Pharmaceutical Science, 02 (03): 25-29.

11. Cheng, K ; Hung , Y ; Chen ,C.(2014).Green synthesis of chondroitin sulphate – capped silver nanoparticles : Characterization and surface modification. Carbohydr Polym
12. Clatworthy, A ;Pierson, and Hung ,D.(2007).Targeting virulence : a new paradigm for antimicrobial therapy .Nat.Chem.Biol,3(9): pp.541-8. Doi:10.1038⁄nchembio.
13. Clatworthy, A ;Pierson, and Hung ,D.(2007).Targeting virulence : a new paradigm for antimicrobial therapy .Nat.Chem.Biol,3(9): pp.541-8. Doi:10.1038⁄nchembio.
14. Dunn ,K. ; Edwards , V. ; Burns ,J.(2004). The role of Act coat with nanocrystalline silver in the management of burns. J.Burns.(30):pp. 51-59. https : // doi.org / 10.1016 /50305- 4179(04)90000-9.
15. Fayaz , M. ; Balaji ,K. ; Girilal ,M.(2010). Biogenic synthesis of nanoparticles and their synergistic effect with antibiotics :a study against gram-positive and gram – negative bacteria. DOI:10.1016/ j.nano.
16. Freshney ,R.(2010).Culture of animal cells a manual of basic technique and specialized applications sixth edition,Wily –Blackwell.P732.
17. *Mubark, N. N., Jalil, A. T., & Dilfi, S. H. (2020). DESCRIPTIVE STUDY OF HYDATIDIFORM MOLE ACCORDING TO TYPE AND AGE AMONG PATIENTS IN WASIT PROVINCE, IRAQ.* Global Journal of Public Health Medicine, 2*(1), 118-124.*
18. Johan, P.; Harley, I.; Prescott, M. (2003). Laboratory Exercise in Microbiology. McGraw-Hill. USA., (484): 149-1538.
19. Johnson ,C. ; Backlund ,J. (2010). Synergy of nitric oxide and silver sulfadiazine against gram-negative , gram- positive , and antibiotic – resistant pathogens. HHS Public Access.J.Mol Pharm.7(6):pp.2289-2296.
20. Kalimuthu,K. ; Babu ,R. ; Venkataraman ,D. (2008). Biosynthesis of silver nanocrystals by Bacillus licheniformis. Colloids Surf .B (65): pp. 150 – 153.
21. Kannan , R. ; Arumugam ,R. ;Ramya , D. (2013).Green synthesis of silver nanoparticles using marine macroalga Chaetomorpha linum.J.Applied Nanoscience.3(3):pp.229-233.
22. Kvitek , L. ; Panacek ,A. ; Soukupova , J. (2008).Biosynthesis , characterization and therapeutic applications of plant – mediated silver nanoparticles , J. Phys.Chem.(112): pp. 5825-5826.
23. Luo,K. ; Samuel, J. ; Park,H. (2018). Microbial biosynthesis of silver nanoparticles in different culture media . J .Agricultural and Food Chemistry . (4):pp. 66-69.
24. McDonnell ,G. (2007). Chemical disinfection. Book chapter ,pp 111-115.
25. Mahasneh ,A. ; Rababah ,T. ; Alhamad , N.(2017).J. Chemical functional and sensory properties of carob juice.J. Food quality.(36):pp 238- 244.
26. Manna ,P. ; Sushil , K.(2015).Obesity oxidative stress , adipose tissue dysfunction, and therapeutic strategies. J.Metabolic Synrome and Related Disorders.13(10):pp 423-444.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 13768 - 13789
Received 05 March 2021; Accepted 01 April 2021.

27. Morones , R. (2010). Roomtemperature synthesis of an optically and thermally responsive hybrid PNIPAM- gold nanoparticles, J. Nanoparticles Research.12 (4): pp.1401-1414.
28. Parthasarathi, V. and Thilagavathi G.(2011). "Synthesis and characterization ofzinc oxide nanoparticle and its application on fabrics for microbe resistant defense clothing".International Journal of Pharmacy and Pharmaceutical Sciences. ISSN-0975-1491.Vol: 3: Issue 4. 392-398.
29. Rai ,K.; Deshamukh ,S. ; Ingle ,A.(2012). Silver nanoparticles the powerful nanoweapon against multidrug- resistant bacteria. J. Applied Microbiology .112 (5):pp641`-852.
30. Saifudin ,N. ; Wong,W. ; Yasumira,A.(2009). Rapid biosynthesis of sliver nanoparticles using culture supernatant of bacteria with microwave irradiation . J.Chem.6(1):pp 61-70.\
31. Shanmuganathan , R. ; Davoodbasha ,M. ; Pugazhendhi ,A. (2018). An enhacement of antimicrobial efficacy of biogenic and ceftriaxone conjugated silver nanoparticles : green approach.J.Enviromental Science and Pollution Research (25):pp.10362- 10370.
32. *JALIL, A. T., DILFY, S. H., KAREVSKIY, A., & NAJAH, N. (2020). Viral Hepatitis in Dhi-Qar Province: Demographics and Hematological Characteristics of Patients.* International Journal of Pharmaceutical Research, 12*(1).*
33. Vanaja , M. ; Paulkumar , K. ; Gnanajobitha.(2014). Biosynthesis of silver nanocrystals by Bacillus licheniformis. Colloids Surf .B (65): pp. 150 – 153.
34. Zarina , A. and Nanda, A. (2014).Combined efficacy of antibiotics and biosynthesized silver nanoparticles from Streptomyces albaduncus.Int.J.Pharm.Tech.Res,(6):pp 1862-1869.
35. Zhang ,K. ; Cheng , S ; Zhang ,X. (2016). Effects of green synthesized silver nanoparticles on lung cancer cells in vitro and grown as xenograft tumors in vivo. International .journal. (11):pp. 1879-1887. ncbi.nlm.nih.gov.