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SILVER NANOPARTICLES PREPARED BY SOL-GEL METHOD EFFECT ON ESCHERICHIA COLI AS ANTIMICROBIAL

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Abstract

In last decades, the awareness bacterial resistance against antibiotics a raised. These concerns leads to develop nanomaterials another resource could be healthial and commercial source such as Nanoparticles (NPs). Nanotechnologies reports consider silver one of the best candidate because its available, and nanotechnology-enabled products. Therefore, present study has been design to evaluate the sol-gel methods in preparation NPs of Silver, and evaluation effectiveness on the Escherichia coli. Present results showed increasing inhibition effect for Ag Nanoparticles on bacterial growth. AgNPs preparation by sol-gel method that result in sol-gel as a good method to preparation nano solution as antibacterial.

Present study Focused on virulence of bacteria depends on its capacity to form biofilms. Biofilm is an important feature for chronic colonization of inflamed bacteria. Samples from patients foot ulcer has been collected then E. coli isolated, after diagnosing by bacterial method. E. coli using as example from many kind of isolated that was isolated and studied in Iraqi hospital, E coli could be as a chronic colonization in foot ulcer. In environmental study E. coli consider as biological indicator for fecal contamination in assessment of water quality Highly effect for present AgNPs against E.coli growth that make it an optimistic procedure to produce alter faster antibacterial treatment from bacterial superficial effect of some classical antibiotic.

Keywords: E.coli , MIC, Antibacterial, Nanoparticles, Silver.

Introduction

The 21st century beginning cast down a spotlight on using Nanoparticles (NPs) to use them in fundamental building blocks of diverse and versatile biological processes Consequently in medical and environmental and agricultural purpose, because of NPs can be used in applications that are different from those of their bulk materials. Among these, interest is growing for NPs from Silver due to the attractive physical and chemical properties. Specifically, there are different types of Silver NPs that can be

synthesized via multiple methods: Electrochemical method, Thermal disintegration and pulsed laser Ablation. [1]. However, several studies have provided direct evidence that the widespread use of antibiotics has led to creates a multidrug-resistant bacterial strains. These super-bacteria, which are resistant to nearly all antibiotics, needs super antimicrobial agents, NPs has this abilit. bacteria with biofilm is one of super bacteria therefore prevention of biofilms could achieved by NPs. NPs has a remarkable effect on biofilm destruction. These Ag NPs exhibited activity against bacterial biofilms. However, several studies have provided direct evidence that the widespread use of antibiotics has led to the emergence of multidrug-resistant bacterial strains. In fact, super-bacteria, which are resistant to nearly all antibiotics, have recently developed due to abuse of antibiotics [2].

Silver nanoparticles have received unlimited attention due to their exceptional properties such as chemical stability, catalytic activity and excellent conductivity, most importantly as antimicrobial and antifungal. Silver is also known to be non-toxic and harmless to the human body at low concentrations, unlike other metallic nanoparticles [3].

According to previous papers nanoparticles has a deletion effect on DNA of bacteria which made it as promising alternative antibacterial materials [4,5]. In contrast to antibiotics many antimicrobial elements are generated recently instead of antibiotics such us of different types of nanoparticles, in light of the microbial possibilities for developing of resistance against antibiotics [6]. Biofilms are organized colonies of bacteria, forming heterogeneous entities on biotic or abiotic surfaces by secreting extracellular polymeric substances produce biofilm [3].

The removal and inactivation of pathogenic microorganisms are the major step in the treatment of water. The presence of E.coli in drinking water would indicate faecal contamination of the water. It is paramount to protect water resources in our country from any source of contamination and to construct water disinfection and delivery systems, as well as sewage treatment facilities that can remove these microorganisms [7].

Experimental Work

Specimens collection and Isolation of E. coli

A study conducted from February to June 2020. A total of 60 specimens were collected from the burn, wound of the patients, from Al-Imam Ali hospital in Baghdad. Swab specimens were aseptically transferred under cooling conditions to the laboratory for analysis. According to McFadden [7], swabs were taken carefully from the site of infection and placed in tubes containing transferred medium to maintain

the swab wet during transferring to laboratory. Each specimen was inoculated on MacConkey agar and Eosin methylene blue (EMB) agar. All plates were incubated aerobically in incubator at 37 0C for 24 hrs. Morphological characteristics of colonies were studied on both agar. Color; size and edge of colonies were recorded after 24 hrs of incubation at 37˚C. then, a single colony of each isolate were tested by gram stain

Biochemical Tests

Suspected isolates were identified by traditional biochemical test as mentioned by McFadden [8], and vitek 2 system. Biochemical tests Catalase test, oxidase test and IMVC test.
Microtitre plate biofilm assay: The procedure to conduct microtitre plate biofilm assay was adapted from [9].

Antibiotic Susceptibility Testing

Antibiotic susceptibility tests were done on Mueller-Hinton agar using Agar Well Diffusion Method as described by [10]. Antibiotic for burn and wound used as a treatment in the Iraqi hospital are: Ampicilin, Ceftriaxone, Cefoxitin, Levofloxacin, Ciprofloxacin, Oflaxacin, Gentamicin, Netimicin, Refampicin. Bacterial isolates were prepared to match 0.5 McFarland standards. Using the micropipette, 100 µl of organisms was spread over the surface of an agar plate. Using a sterile glass pipette, five holes with a diameter of 6 to 8 mm were punched in each of the culture plates. One of the holes was punched in the center of the plate where 20-100 µl of D.W was added as a negative control; 20-100 µl of each antibiotics were put in the remaining four holes. The culture plates were then incubated at 37°C for 24 h. The clear zone of inhibition around the antibiotic was measured in mm.

The Silver Nanoparticles Preparation by Sol-Gel Method

In material science, the sol-gel process is a method for producing solid materials for small molecules. In this chemical procedure, a "sol"(a colloidal solution) is formed that then gradually evolves towards the formation of a gel- like diphasic system containing both a liquid phase solid phase. The method is used for the fabrication of metal oxides; the process involves conversion of monomers in to a colloidal solution(sol) that acts as the precursor for an integrated network (or gel) of either discrete particle [11].
After the weight of Silver nitrate [$AgNO_3$] and citric acid [$C_6H_8O_7.H_2O$] were dissolved in de- ionized water with a molar ratio of 1:1. The solution was stirred with a magnetic stirred 90°C. stirring continued until gel formation approximately 1 hour.

Afterwards, the gel was allowed to burn at 100°C. Alight fluffy mass was obtained as a result of combustion, which was further annealed for 2 hours at varying temperature 300 °C to obtain highly crystalline AgNPs.

This sol gel method tested by XRD analysis was used to confirm the structure. It was scanned in the range of 2θ (20°-80°). The AgNPs chart by XRD type (Shimadzu 6000), the measurement conditions were as follows: Target: Cu Kα, wavelength=1.5406 A°, voltage=40KV, current=30 mA, and scanning speed=5(degree/min). Moreover, it tested by Transmission electron microscopy (TEM) used for the measurement of AgNPs.

The Minimum inhibitory concentration MICs of AgNPs were prepared by using tube broth dilution method according to the CLSI guidelines [12]. The centration range was (50-100-200-400-800-1000) μg/ml.

Extraction of DNA

In accordance with the manufacturer's instructions, was performed using DNA extraction kit provides by Promega. Primers specific these were provided by Macrogen/Korea provided them. Forward primer 5'- GCAGAGCACGGTGTTGTC-3', Reverse primer 5'- CCACATAATTGATCGTTTGCTGG-3'. Primers used for the amplification bdm gene E. coli by a researcher, Size amplification about 115 bp. DNA concentration and purity was estimated by Nano drop equipment.

Preparation and Conditions PCR Mixture

Polymerase chain reactions were performed using a reaction mixture consisting of 12.5μL of Go Tag Green Master Mix/ Promega, 2 μL of each primer, 5.5 μL of D.W and 5 μL of DNA, with final volume 25 μL. Amplification conditions were for final thermocycling program was as follows: the initial denaturation was single cycle for 95 °C at 2 min , 30 cycles each one includes denaturation at 94 °C for 30s, annealing at 59°C for 30s and extensions at 72 °C for 30s, then final cycle extension at 72 °C for 4 min. The size of PCR product is 115bp.

Result

The suspected isolates were tested by gram stain and colonial morphology of E. coli on Media used for bacterial isolation and identification are ordinary media such as MacConkey agar, and Eosin methylene blue agar. Isolates were identified as E. coli by biochemical tests like then confirmed the results by API 20 EN biochemical kit (bioMérieux, France) .

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Table 1- Biochemical test for detection samples

Type of test	Gram stain	Catalase	Oxidase	IMVC test			
				Indole	Methyl-red	Vogas-Proskauer	Citrate utilization
Results	-	+	-	+	+	-	-

Determination of minimum inhibitor concentration (MIC) according to CLSI with some modifications [12]. the final results from one hundred samples there were only 52 isolates were detected as E.coli , after that all E. coli isolates tested for the ability to formation Biofilm by Microtiter Plate Method (MTP) in table 2. There were 38 isolates producing biofilm, while 14 E. coli isolates non-producing biofilm.

Table 2- Ratio of isolates producing biofilm for E. coli

Producing of Biofilm	Number of isolates E. coli with ratio			Total Percentage
positive isolates producing of biofilm	38	Strong (+++)	22(15 %)	73.1%
		Moderate (++)	10(6.8 %)	
		Weak(+)	04(2.7 %)	
Non-producing isolates of biofilm	14			26.9%
Total	52			100 %

Structural Properties AgNPs

The AgNPs pepared then tested by X-ray diffraction analysis of AgNPs solution, which was prepared by the sol-gel method at a temperature 300°C. Fig 1- shows the result of X-ray diffraction. The angle of diffraction (2θ) is (87) and the corresponding is (111), where the crystallite size of the nanometer is amounted about (20 nm) was calculated using Scherrer equation [4]. Its noticed that the average of crystallite size of Ag in nanostructure range and the patterns indicate that all of the nanoparticles have a polycrystalline and exhibited monoclinic phase according to (JCPDS) card no. (04-0783).

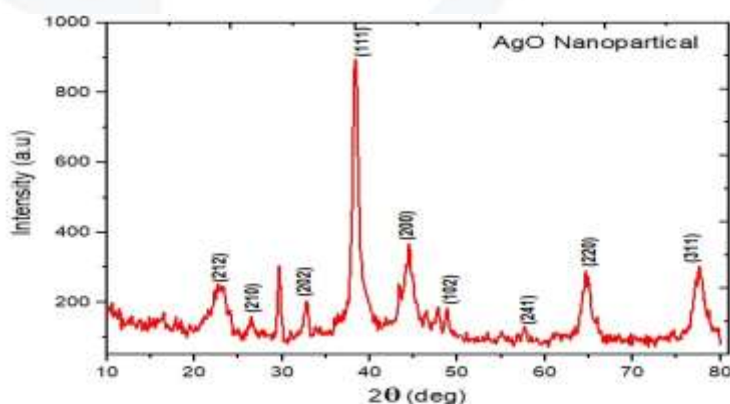

Figure 1: XRD patterns of AgNPs obtained by sol gel method and Ag standard

Agar Well Diffusion Method

MIC, and antibacterial activity of AgNPs against producing biofilm isolates were studied using the agar well diffusion method to show a clear inhibition zone around wells loaded with bacteria-induced AgNPs culture plates.

Results of the present study showed different concentrations similar to those used in the dilution method of tube broth of AgNPs synthesis when determining the MIC. The result was compared with that before using the method. In general, the inhibition of concentration observed as a result of using this method was significantly higher than other concentrations.

This result shows the highest antibacterial effect against studied isolates at concentrations (100 and 50). MIC of AgNPs was provided for antibacterial activities in the fig. 2.

This means that the lowest concentration gave an inhibition diameter of 135nm is 100 μg/ml, which is the same for the MIC broth dilution method. The antibacterial activity of AgNPs was against clinical bacterial isolates when using well diffusion agar. Therefore, the concentration 100 μg/ml of AgNPs was the best use to inhibit the growth of isolates under study, the use of single dose in biofilm experiments and detection of the effect of nanoparticles in the genes of the present study.

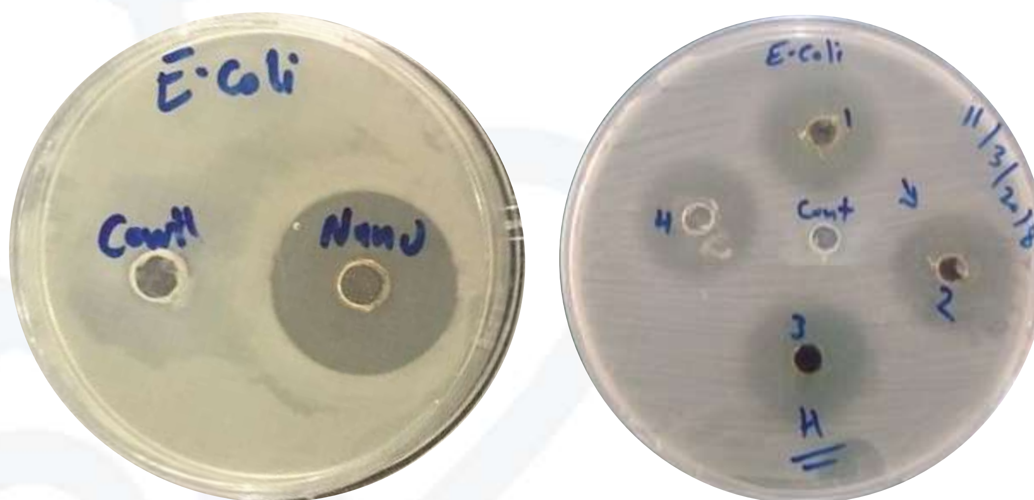

Figure 2. MIC and Antibacterial Activity of (AgNPs) against E.coli isolates by Using Agar Well Diffusion method, -, Ceftriaxone=1, Levofloxacin=2, Gentamicin=3, Refampicin=4, cont=control, Nano=AgNAPs

*(Ceftriaxone): Antibiotic for foot ulcers used as a treatment in the Iraqi hospital

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Table 3- Comparision the Antimicrobial susceptibility testing with Nanoparticles According to European Committee on Antimicrobial susceptibility testing as standard.

Antibiotic types groups	Antibiotics	Inhibition Zone diameter (standard) Diameter mM **(R <)**	Inhibition Zone diameter over night	Inhibition Zone diameter After 12 h.
Pencilin	Amoxicillin	<18	14	11
	Piperacillin	<18	14	11
	Ampicilin	<28	26	13
Cephalosporins	Ceftriaxone	<17	17	10
	Cefoxitin	<22	19	10
Fluroroquinolones	Levofloxacin	<22	18	09
	Ciprofloxacin	<21	20	11
	Oflaxacin	< 20	20	13
Aminoglycosides	Gentamicin	< 22	17	11
	Netimicin	< 18	17	10
	Refampicin	<23	21	12
Nanoparticles	AgNPs	No standard	28	24

R:- Resistance .

In last table number 3 there are comparison among different types of antibiotics with AgNPs by using inhibition zone (mM), Antibiotic test in this experiment showed inhibition zone range from 17Mm to 26 Mm, while the AgNPs showed the largest inhibition zone among them, 28mM.

Detection of Bdm Gene

Extraction genomic DNA for the isolates E. coli produced by the biofilm that was confirmed as bands by gel electrophoresis. In order to detect the presence of bdm gene

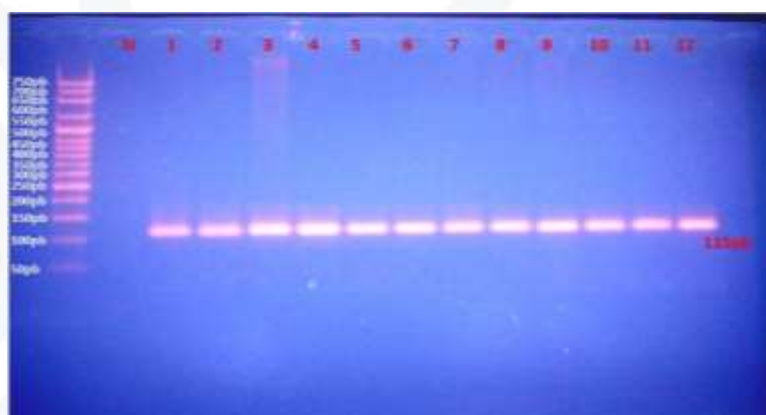

Figure 3. PCR product of the bdm gene, Electrophoresis on 1.5 % agarose at **90** volt. For 1:20 hours.

Determine the Sequences of Nitrogen Bases of the Bdm Gene

sequences of the nitrogen bases were determined for isolates of E. coli, after reading the DNA sequences of the forward and reverse strands, recollecting the two strands, deleting the anomalies in either of them, analysis them and matching them to National Center Biotechnology Information (NCBI) online at (http://www.ncbi.nlm.nih.gov). And Geneious Software has shown that these sequences are very similar with bdm gene sequences 100 %. By comparing the observed DNA sequences of these samples with their stored reference sequences (Gen Bank: CP087110.1). By analyzing the results of the sequencing of the bdm gene according to the Table, 4 the data showed that There are four mutations that ranged from Transition to Transversion.

Table 4- Changes in the nitrogen bases of sequences of isolates of E. coli Gen Bank: CP087110.1

No. of sample	Wild type	Mutant type	Location	Change in amino acid	Type of mutation	Effect	Type of substitution
E1	AAT	GAT	2189023	Asn> Asp	Substitution	Missense	Transition
E5	CGG	CGC	2188980	Arg > Arg	Substitution	Silent	Transversion
E2	GCA	GCG	2188983	Ala >Ala	Substitution	Silent	Transversion
E3	CAC	AAC	2188998	His>His	Substitution	Silent	Transversion

Discussion

Present study represent a new scientific trial to deal with E. coli according to its biofilm to resist different type of antibiotic and create antibacterial material that could be usfule in treat its nfectious. In order to make an overall measure of the whole biofilm in present work, the Crystal violet staining consider an essential dye known to bind with negatively charged molecules on the cell surface, as well as nucleic acid and polysaccharides. [13]. The hydrophobicity of bacterial surface played an important role in the attachment to diverse polymers, such as polystyrene. Moreover, It is related to bacterial adherence to the plastic surface, as is the case with catheters and prostheses [14]. The primary number of cells that might be successful for adherence or differences of the quality and quantity of auto-inducers quorum sensing signaling molecules system produced from each isolate [15]. That depending on biofilm surface, which related with Quorum sensing among the biofilm forming organisms plays significant role in the formation of biofilm. The formation of biofilm plays an important role in the pathogenesis of pathogens [16].

Silver effect on abiotic surfaces causes destruction of plasmid and DNA, leads to prevent the spread of infections and gene transfer. many repots emphasized the antimicrobial potential of Silver, its nanoparticles consist of a group of toxic

antibacterial for the cell membrane of Gram-negative and Gram-Positive bacteria [17].

During preparation steps for NPs, thermo reaction by burning to more 250 C° is very important to obtain AgNPs with good features [18], therefore in present work the 300 C°dependent, which gave us a good result in figure that shows the result of X-ray diffraction.

The absorption of spherical nanoparticles of Silver with sizes ranging from (30- 80) nm lies in the visible range and gives rise to a narrow peak, in present study the crystallite size of the nanometer is amounted about (20 nm) was calculated using Scherrer equation which acceptable with crystalline surface comparing with other paper depend Ag mean average size was 20 nm [2]. At the same time the present correspond to the (111) confirm that with present process steps, can obtain pure AgNPs which agree with another procedure [19]. This report results goes with result that documented as favorite features for AgNPs by Bamal and her Colleagues (2021) who has reported that Transmission electron microscopy analysis showed that these NPs are all monodispersed, spherical in nature, and well segregated without any agglomeration and with an average size of 20 nm. X-ray powder diffraction showed a polycrystalline nature and face centered cubic lattice and revealed characteristic diffraction peaks indicating the formation of AgNPs [3].

Present method in preparing Nano solution look as a good way as a synthesis method depend on the high reactivity of AgNPs, concentration and agitation speed during reaction. All these preparation factors will determine the features of Particles as well as make it as a good Nanoparticles because NPs have acceptable size with the larger the energy gap.

The lowest concentration gave an inhibition diameter is 100 µg/ml, which is the same for the MIC broth dilution method. the concentration 100 µg/ml of AgNPs was the best use to inhibit the growth of studied isolates. While the higher concentration as 200 µg/ml and highest caused a total inhibition of E.coli this result May not agree with other studies when higher concentrations are used of AgNPs for totally inhibition[20].

In present work antibiotic test showed inhibition zone range from 11Mm to 26 Mm, less than largest inhibition zone for AgNPs, 28mM, which make it more effect on E.coli growth as inhibition materials compering with antibiotic. AgNPs create inhibition zone more than any other Antibiotics in present study which make it highly effective as antibacterial, this result goes with previous findings that AgNPs able to eliminate 99.9%of pathogenic bacteria in hours by damage bacteria membrane within minutes [21]. Moreover, present results go with Agarwala and his collegeas who

recorded that efficiency of AgNPs to inhibit biofilm formation at sub-MIC concentrations was evaluated against MRSA and E. coli [22].

AgNPs have shown higher toxicity in microorganism comparing with human cells depending on their size by raped losing of membrane integrity and cell death moreover [23]. AgNPs induce the catalase reaction by producing superoxide dismutase, and formation intracellular ROS with oxidative DNA [24]. The current work were a greement with previously conducted approach on bacteria. Alzubadiy and coworkers in their reports showed the effectiveness of the use of copper nanoparticles against P. aeruginosa [5].

In addition, NPs disrupt bacterial membranes, effect on biofilm formation, the unique composition and structure of bacterial biofilms provide shelter or protection to the embedded microorganisms, helping them to escape from most antibiotics. In addition, bacterial biofilms are “a breeding ground” for frequent resistance mutations and the exchange and alteration of these mutations among different bacterial cells. [23], So that to overcome these bacterial ability, the NPs seems the best candidate could destruction membrane resistance and biofilm.

Toxicity of Silver to the pathogens is exerted by several parallel mechanisms, which lead to the death of pathogens. The first target site of Silver that it damages bacterial envelope. Silver ions also damage nucleic acids, by specific binding to DNA, repeated cyclic redox reactions generate several OH radicals near the binding site causing multiple damage to the nucleic acids. Besides, Silver oxidative damage to the genetic material may occur through Fenton mechanism [25].

The cytoplasm is then degraded and disappears, leading finally to cell death. The antibacterial mechanism is attributed mainly to the strong adsorption of Silver ions to bacterial cells, which imparts antibacterial efficacy in a concentration-dependent manner. Nanoparticles have a large surface-to-volume ratio, which enhances their bioactivity and makes them effective bactericidal agents [26], that goes in the same line with present study when the effect of nanoparticles occur after 12 h. so the inhibition zone was at optimum level, this result agree with finding emphasized by Wu and coworkers who illuserated that the effect of AgNPs were obsereved within the cells after 12to 24 hours [27].

NPs can attack bacteria cell through the formation of ROS leading to membrane, protein, and DNA damage; direct interaction occurs with cell membrane because some metal-based NPs can generate metal ion via dissolving, for example, inhibition of electron transport chain; and the regulation of bacterial metabolic processes. Earlier reports demonstrated that NPs interfere with biofilm integrity by interacting with EPSs. Ag NPs inhibit the production of as (extracellular polymeric substances)

EPSs, which further leads to action against the biofilms of drug-resistant strains of E. coli and Klebsiella pneumonia [28]., the AgNPs involved in several mechanisms could be a reason to explain its speed high effect within 12h.

Even that present results need more investigation on tissue culture and clinically to improve the effect of AgNPs as promising antimicrobial agents

Conclusion

A good method to preparation antibacterial nano solution from AgNPs Nano particles by sol-gel method because it submitted a good result in present research work. AgNPs showed a noticed effect on E.coli growth that make it an optimistic procedure to produce alter faster antibacterial treatment from bacterial superficial effect of some classical antibiotic because its effect on biofilm which is one of the virulent mechanisms in bacteria.

References

1. Sunil T. Galatage, Aditya S. Hebalkar, Shradhey V. Dhobale, Omkar R. Mali, Pranav S. Kumbhar, Supriya V. Nikade and Suresh G. Killedar (2021). Silver Nanoparticles: Properties, Synthesis, Characterization, Applications and Future Trends, DOI: 10.5772/intechopen.99173.
2. Linlin Wang1,Chen Hu, Longquan Shao.(2017).The antimicrobial activity of nanoparticles: present situation and prospects for the future. International Journal of Nanomedicine:12 1227–1249.
3. Bamal, D.; Singh, A.; Chaudhary, G.; Kumar, M.; Singh, M.; Rani, N.; Mundlia, P.; Sehrawat, A.R. (2021). Silver Nanoparticles Biosynthesis, Characterization, Antimicrobial Activities, Applications, Cytotoxicity and Safety Issues: An Updated Review. Nanomaterials. https://doi.org/10.3390/ nano11082086.
4. Ziad T. Khodair, Mohanad W. Mahdi Alzubaidy, Asmaa M. Salih Almohaidi, Ammar Ahmed Sultan, Sana M. H. AL-Shimmary, and Sarah S. Albusultan.(2019). Synthesis of copper oxide nanoparticles (CuO-NPs) and its evaluation of antibacterial activity against P. aeruginosa biofilm gene’s. Technologies and Materials for Renewable Energy, Environment and Sustainability AIP Conf. Proc. 2190, 020006-1–020006-7; https://doi.org/10.1063/1.5138492 Published by AIP Publishing. 978-0-7354-1937-7/.
5. Mohanad W. Mahdi Alzubaidy, Asmaa M. Salih Almohaidi, Ammar Ahmed Sultan, and Sana M. H. ALShimmary.(2019).Virulence gene of Pseudomonas aeruginosa with nanoparticle. AIP Conf. Proc. 2123, 020039-1–020039-9;

https://doi.org/10.1063/1.5116966 Published by AIP Publishing. 978-0-7354-1863-9/.

6. Gómez-Núñez MF, Castillo-López M, Sevilla-Castillo F, Roque-Reyes OJ, Romero-Lechuga F, Medina-Santos DI, Martínez-Daniel R and Peón AN. (2020). Nanoparticle-Based Devices in the Control of Antibiotic Resistant Bacteria. Front. Microbiol. 11:563821. doi: 10.3389/fmicb.2020.563821.
7. Ndebele, N.; Edokpayi, J.N.; Odiyo, J.O.; Smith, J.A.(2021). Field Investigation and Economic Benefit of a Novel Method of Silver Application to Ceramic Water Filters for Point-Of-Use Water Treatment in Low-Income Settings. Water. 13, 285. https://doi.org/10.3390/w13030285.
8. MacFadden, J.F. (2000). Biochemical tests for Identification of Medical Bacteria 3rd Ed. The Williams & Wilkins Co., USA: 689 – 691.
9. Lee, H.Y., L.C. Chai, C.F. Pui, S. Mustafa, Y.K. Cheah, M. Nishibuchi and S. Radu, (2013). Formation of biofilm by Listeria monocytogenes ATCC 19112 at different incubation temperatures and concentrations of sodium chloride. Braz. J. Microbiol., 44: 51-55.
10. Magaldi S., Mata-Essayag S., Hartung de Capriles C. (2004). Well diffusion for antifungal susceptibility testing. Int. J. Infect. Dis; 8:39–45. [PubMed] [Google Scholar].
11. Bouafia, A.; Laouini, S.E.; Ahmed, A.S.A.; Soldatov, A.V.; Algarni, H.; Feng Chong, K.; Ali, G.A.M. (2021). The Recent Progress on Silver Nanoparticles: Synthesis and Electronic Applications. Nanomaterials 11, 2318. https://doi.org/10.3390/nano11092318.
12. CLSI, Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Third Informational Supplement. l.33 (1); M100-S23.2013.
13. Matz, C.; McDougald, D.; Moreno, A.M.; Yung, P. Y.; Yildiz, F. H. and Kjelleberg, S. (2005). Biofilm formation and phenotypic variation enhance predation-driven persistence of Vibrio cholerae. Proc. Natl. Acad. Sci. USA. 102: 16819-16824
14. Magnusson, K. E. (1982). Hydrophobic interaction: a mechanism of bacteria binding. Scand J Infect Dis 33: 32-36.
15. Adina Schulze, Fabian Mitterer, Joao P. Pombo and Stefan Schild (2021). Biofilms by bacterial human pathogens: Clinical relevance - development, composition and regulation - therapeutical strategies. Microbial Cell 8(2): 28-56. doi: 10.15698/mic2021.02.741.
16. Muhammad, M. H., Idris, A. L., Fan, X., Guo, Y., Yu, Y., Jin, X., ... Huang, T. (2020). Beyond Risk: Bacterial Biofilms and Their Regulating Approaches.

Frontiers in Microbiology, 11. doi:10.3389/fmicb.2020.00928 (https://doi.org/10.3389/fmicb.2020.00928).

17. Prasher, P.; Singh, M.; Mudila, H.(2018). Oligodynamic Effect of Silver Nanoparticles: A Review. BioNanoScience . 8, 951–962.
18. Salleh, A.; Naomi, R.; Utami, N.D.; Mohammad, A.W.; Mahmoudi, E.; Mustafa, N.; Fauzi, M.B. (2020). The Potential of Silver Nanoparticles for Antiviral and Antibacterial Applications: A Mechanism of Action. Nanomaterials 10, 1566.
19. Sharmin, S.; Rahaman, M.; Sarkar, C.; Atolani, O.; Islam, M.T.; Adeyemi, O.S.(2021). Nanoparticles as antimicrobial and antiviral agents:A literature-based perspective study. Heliyon 7, e06456.
20. Osonga, F.J.; Akgul, A.; Yazgan, I.; Akgul, A.; Ontman, R.; Kariuki, V.M.; Eshun, G.B.; Sadik, O.A. (2018). Flavonoid-Derived Anisotropic Silver Nanoparticles Inhibit Growth and Change the Expression of Virulence Genes in Escherichia coli SM10. RSC Adv. 8, 4649–4661. [Google Scholar] [CrossRef].
21. Yuan, Y. G., Peng, Q. L., & Gurunathan, S. (2017). Effects of Silver Nanoparticles on Multiple Drug-Resistant Strains of Staphylococcus aureus and Pseudomonas aeruginosa from Mastitis-Infected Goats: An Alternative Approach for Antimicrobial Therapy. International journal of molecular sciences, 18(3), 569. https://doi.org/10.3390/ijms18030569.
22. Thammawithan, Saengrawee, Pawinee Siritongsuk, Sawinee Nasompag, Sakda Daduang, Sompong Klaynongsruang, Nuvee Prapasarakul, and Rina Patramanon. (2021). "A Biological Study of Anisotropic Silver Nanoparticles and Their Antimicrobial Application for Topical Use" Veterinary Sciences 8, no. 9: 177. https://doi.org/10.3390/vetsci8090177 .
23. Kim, S.-H.; Lee, H.-S.; Ryu, D.-S.; Choi, S.-J.; Lee, D.-S. (2011). Antibacterial Activity of Silver-Nanoparticles against Staphylococcus Aureus and Escherichia Coli. Microbiol. Biotechnol. Lett. 39, 77–85. [Google Scholar].
24. Liao, C.; Li, Y.; Tjong, S.C. (2019). Bactericidal and Cytotoxic Properties of Silver Nanoparticles. Int. J. Mol. Sci. 20, 449. [CrossRef].
25. Khameneh B, Diab R, Ghazvini K, Fazly Bazzaz BS.(2016). Breakthroughs in bacterial resistance mechanisms and the potential ways to combat them. Microb Pathog.95:32–42.
26. Dakal, T. C., Kumar, A., Majumdar, R. S., & Yadav, V. (2016). Mechanistic Basis of Antimicrobial Actions of Silver Nanoparticles. Frontiers in Microbiology, 7. doi:10.3389/fmicb.2016.01831 (https://doi.org/10.3389/fmicb.2016.01831) .

27. Wu, M., Guo, H., Liu, L., Liu, Y., & Xie, L. (2019). Size-dependent cellular uptake and localization profiles of silver nanoparticles. International Journal of Nanomedicine, Volume 14, 4247–4259.
28. doi:10.2147/IJN.S201107 (https://doi.org/10.2147/IJN.S201107)
29. Ansari MA, Khan HM, Khan AA, Sultan A, Azam A.(2019). Synthesis and characterization of the antibacterial potential of ZnO nanoparticles against extended-spectrum β-lactamases-producing Escherichia coli and Klebsiella pneumoniae isolated from a tertiary care hospital of north India. Appl Microbiol Biotechnol. 94(2):467–477.