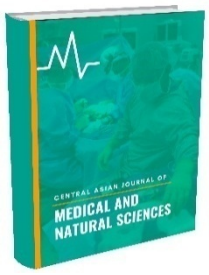
CENTRAL ASIAN JOURNAL OF MEDICAL AND NATURAL SCIENCES



Volume: 04 Issue: 03 | May-Jun 2023 ISSN: 2660-4159

http://cajmns.centralasianstudies.org

Effect of Silver and Zinc Oxide Nanoparticles on Gene Expression of Some Swarming Genes in *Proteus Mirabilis*

1. **Ibraheem M. AL-Dulaimy**
2. **Hadi R. Rasheed Al-Taai**
3. **Ali Jaffar Saleem**

Received 22nd Mar 2023,
Accepted 27th Apr 2023,
Online 6th May 2023

[1,2,3] Department of Biology, College of Education Pure Science, University of Diyala, Iraq

Key words: Proteus mirabilis; wosA, rsbA gene; gene expression , nanoparticle

Abstract:

Background: Due to the emergence of multiple antibiotic resistance by P. mirabilis bacteria, it was necessary to search for alternatives other than antibiotics to eliminate these bacteria, and this is what prompted scientists to use nanoparticles, which are nano-size materials that have a significant impact on the components of the bacterial cell. Among these nanomaterials are zinc oxides, titanium oxides, copper, cobalt, silver and gold.

Objectives: The study of effect of nanoparticles on the gene expression of some swarming genes in Proteus mirabilis bacteria.

Methods: A total of 30 samples collected from urinary tract infections and wounds in sterile containers in different hospitals in Baquba city (Baquba Teaching Hospital and Al-Batool Hospital) were subjected to Molecular detection about *wos*A and *rsb*A genes and Detecting the effect of nanoparticles on gene expression of gene *wos*A and *rsb*A **.**

Results: Twenty isolates were obtained, all of which had *wos*A and *rsb*A genes . Two isolates of urine and burns were selected to determine the effect of nanomaterials on gene expression of the wosA and rsbA genes encoding for swarming. The results of the test of the effect of silver and zinc oxide nanoparticles on the expression of the *rsb*A and *wos*A genes responsible for the phenomenon of swarming showed a decrease in gene expression for isolates after treatment compared to pre-treatment gene expression and by 1 before treatment and 0.44 after treatment for the *rsb*A gene, while for *wos*A gene, the gene expression of isolates decreased, as the expression before treatment 1 decreased to 0.76 after treatment with nanomaterial.

Conclusion: Isolates of *Proteus mirabilis* bacteria, which have virulence factors, it causes many diseases and susceptible to infection through their ability to move, transfer, adhere, and form a biofilm.

Introduction

Proteus spp. causes many diseases in humans such as urinary tract infections, wound and burn injuries, ear infection and diarrhea as well as one of the causes of meningitis in children and osteomyelitis, which is an opportunistic pathogen Opportunistic Pathogens .Infection with these bacteria occurs through burns as a result of the destruction of the skin and the loss of its layers and damage to the skin tissues and dermis as a result of exposure to heat or electric current or incendiary chemicals, which leads to contamination of the cells of the dermis and subdermis and may spread to different areas of the body and because of the tissue crash and the exudation of plasma to the area for the growth of bacteria and their reproduction affected become a good plant (1). Gram-negative bacteria may be transmitted from the natural flora found in the gastrointestinal gut, including P. mirabilis bacteria, to the area affected by burns, causing the surface of the damaged tissue, especially in the first days after infection (2).Where exposed wounds are susceptible to infection with various types of bacteria and after the injury occurs cause suppuration of the affected area, which in turn leads to the formation of wound abscesses (3) (Wounds Abscess), serious wound injuries lead to death or morbidity on a global scale, as double bacterial infections and settlement with more than one type of bacteria can cause damage to the affected tissues, and the main problem in wound injuries is the constant increase in the resistance of bacteria to antibiotics, especially bacteria. P.mirabilis (4). These bacteria largely infect immunodeficiency people as they are the main cause of nosocomial infections (5). Bacteria occur fluctuations urinary tract infections and there are two ways to cause infection is the bloody road Haematogenous route and the ascending route, and the ascending route is the most common as bacteria enter the urethra and then the bladder bladder and ureter until the kidney reaches Kidneys (6) . As a result of the ability of bacteria to resist antibiotics and the developments in their form, scientists have been pushing to search for alternative solutions, one of the promising strategies in this field was the use of nanotechnology, as well as following some of the mechanisms available to overcome antibiotic resistance by reducing the excessive and indiscriminate use of antibiotics and developing new treatments.. Due to their shape- and size-dependent adjustable properties, metal nanoparticles have become a major focus of many biomedical applications including antimicrobials, and metal nanoparticles such as silver, zinc, copper, titanium and iron can be used against multidrug resistant microorganisms (MDR) due to their antibacterial nature. (7). More importantly, nanoparticles are mainly used in antimicrobial applications due to their long-term stability and biocompatibility, and the mechanisms behind the antidotes effect of these nanoparticles are oxidative stress, metal ion release and non-oxidizing stress that occurs simultaneously (8). One of the nanoparticles that are used to inhibit bacteria is silver particles (AgNPs), which are characterized as a non-toxic inorganic antibacterial health agent, as they have been used for centuries ago as a result of their ability to kill various types of pathogenic microorganisms, and one of the most important applications of silver nanoparticles (AgNPs), especially in the medical aspect, as therapeutic ointments to prevent infection in burn injuries and wounds) (9) . Point out that one of the main mechanisms by which silver nanoparticles (AgNPs) acquire their antibacterial properties is their ability to adhere and penetrate the cell membrane of bacterial cells. According the zinc oxide is generally considered a safe, non-toxic substance, as applications of ZnONPs depend on shape, size, surface state, crystal structure and permeability. (10) ZnONPs are synthesized with many different techniques including mechanical and sedimentation methods and from different parts of plants such as leaves, roots, fruits and flowers (11).The antimicrobial mechanism by ZnONPs is carried out through the synthesis of hydrogen peroxide H2O2 and the production of zinc ion Zn+2 as it damages the cell membrane and interacts with components within the cell, and it has been shown that ZnONPs produce reactive oxygen species

(H2O2, OH) that cause proteins and nucleic acids to leak from the ros membrane by promoting the presence of oxygen peroxide on the ZnO membrane and thus inhibiting bacterial cell growth (12) .

The study aims Molecular investigation of some virulence genes of Proteus mirabilis and study of the effect of nanoparticles on the gene expression of some anthelial genes.

Materials and Methods

The study was approved by the Ethics Board of the university of Diyala and informed written consent was taken from each participant in the study. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Isolation and Identifaction of *proteus mirabilis*

A total of 50 samples were collected from different clinical sources (urinary tract infections, wounds) in sterile containers in different hospitals in Baquba city (Baquba Teaching Hospital and Al-Batool Hospital). During sample collection from 1/11/2021 to 12/2/2022.The samples were cultured on MacConkey`s agar and blood agar plates, and then incubated for 24 hours at 37◦C. The isolates were identified depending on the morphological and biochemical tests and compared to the scheme described by [13]. Morphological and Microscopic . Colonies were isolated and stained using Gram stain. In order to determine their form and length, they were ultimately viewed under a microscope. Testing biochemical reactions using indole and oxidase [14] .

Molecular detection of virulence genes

Genomic DNA was extracted by DNA extraction kit (Promega, USA) and stored at -20 °C. PCR approach was used to detect the presence of virulence genes including (*wosA* and *rsb*A). The primers of this study were specifically designed and synthesized by NCBI – Genbank using a program Primer 3 plus design. Using agarose gel at a concentration of 2% with a potential difference of 100 volts for 60 minutes. The primer sequences, their annealing temperatures and product sizes are given in Table 1.

Table 1: list of primers which were used in this study

primer sequence (5 -3)	**Target genes**	**Size(bp)**	**Tem** °C	Reference
F/ ATG CTC TAG AAC CAA TGA CTT TTA CCA GTT AC **R/ GGT ACT CGA GCG ACA TCG GAA AAT GTT TGA TG**	***wos* A**	404	**95C** **95C** **55C** **72C** **72C**	(15)
F/TTG AAG GAC GCG ATC AGA CC **R/ACT CTG CTG TCC TGT GGG TA**	*rsb* A	467	**95C** **95C** **58C** **72C** **72C**	(16)

Detection of swarming gene expression by quantitative RT-PCR of *rsb*A *and wos*A

To ascertain the highest and lowest value of the resistant isolates forming the swarming by measuring the gene expression determined on the basis of comparing the difference in the average CT value of the rsbA and wosA genes from finding the value of Folding=2^(-ΔΔCT) when CTΔ is equal to the difference between the CT of the target gene and the reference gene, which was obtained from the interaction of the quantitative real time-PCR sequence of the rsbA genes, wosA (Target gen) and the gene (Reference gene) 16SrRNA per isolate.

$\Delta CT = CT_{taget\ gene} - CT_{reference\ gene}$

CT is the value used in gene amplification (Reo et al. 2013) Several instructions were followed for extracting RNA qPCR Master Mix, GoTaq (1- step RT-qPCR TRIzol) Isolation (11) isolated from diuresis and isolation (12) isolated from burns was selected for the purpose of detecting gene expression for the rsbA gene and wosA gene and Table (11-3) Components of qRT-PCR™ interaction**.**

Table 2: Components of RT-PCR Interaction

Volume for one sample	Stock content ratio	Solution	T
5	2X	q PCR Master Mix	1
0.25	50X	R T mix	2
0.25		$MgCl_2$	3
0.5	10pmol/µl	Forward primer	4
0.5	10pmol/µl	Reverse primer	5
2.5		Nuclease free water	6
1	ng/µl	RNA	7
10µl		Total Volume	

Effect ZnONPs and AgNPs on gene expression of swarmming *P. mirabilis* for *rsb*A *and wos*A gene

The dilution method of liquid nutrient medium was used to estimate and know the inhibitory effect of nanoparticles processed by Sky Spring Nano Materials according to (Saginur et al., 2006) as follows:

Results

Isolation of *P. mirabilis*

This study included 100 samples of different clinical sources (urinary tract infections, wounds), the rate of male patients was 40% and female patients 60% their age ranged between (1-≥30) years. It was obtained twenty isolates of *Proteus spp*. were recovered included 15 isolates of *P. mirabilis* and 5 isolates of *P. vulgaris*.

Molecular Detection of Virulence Genes

The results of the present study are show that 20(100%) of Proteus mirabilis isolates give positive result at 467bp, this result was shown in Figure (1).

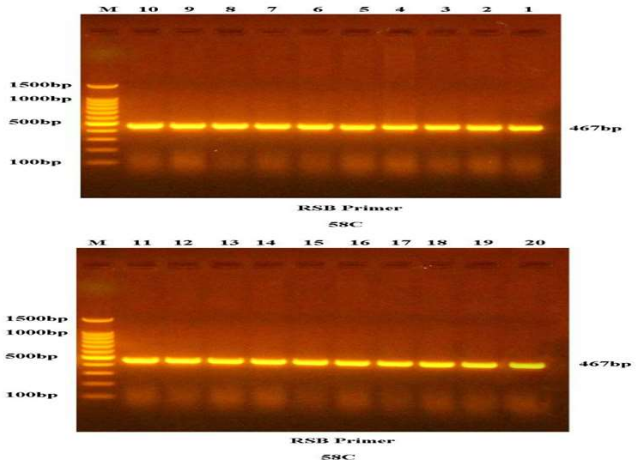


Figure1. Electrophoresis for the *rsb*A gene where the result showed appearance of the gene in all isolates. M: Ladder with(1500bp) and agarose 2% at a voltage difference of 100 volts for 60 minutes.

The results of PCR amplification to specific *wos* A primers indicated that 20(100%) . The result of electrophoresis showed of the present of a segment of length 404bp belonging to the *wosA* gene, this result was shown in Figure (2) .

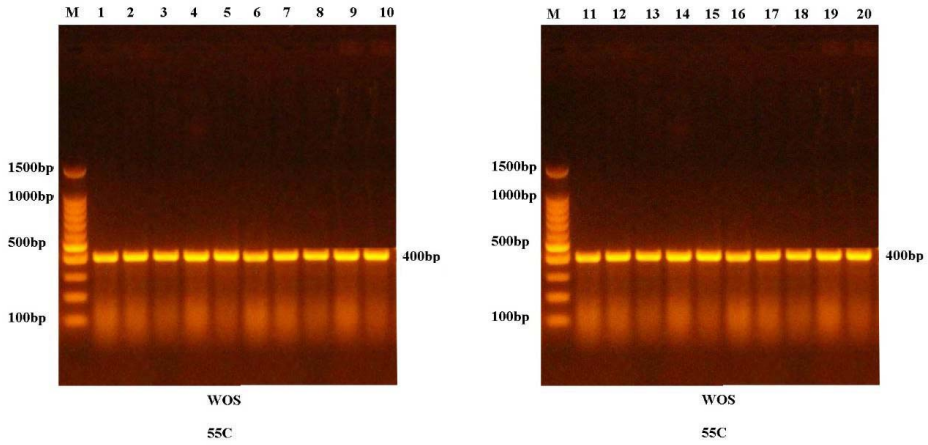


Figure2. Showed the electrophoresis of the *wos*A gene and the appearance of the gene in all isolates. M: Ladder with (1500bp) and agarose 2% at a voltage difference of 100 volts for 60 minutes.

Determine of the minimum inhibitory concentration of Oxid zinc and Silver nanoparticle

The selected P. mirabilis isolates (12, 11) showed a different response towards the zinc oxide nanomaterial, as the value of the minimum inhibitory concentration MIC for zinc oxide nanoparticles (ZnO) ranged between (15000 – 5200) µg / ml, the isolation showed (11) the value of the minimum inhibitory concentration MIC as it reached (15000) µg / ml while the value of MIC sub (10000) µg / ml, The isolation (12) showed the value of the minimum inhibitory concentration (MIC) (12000) µg/ml while the value of MIC sub (10000) µg/ml, and the value of MIC sub (10000) µg/ml.

As for the silver nanoparticles, the value of the minimum inhibitory concentration MIC ranged between (5200-10000) µg/ml, where the MIC reached (15000) µg/ml for isolation (11) and (10000) µg/ml for isolation (12) and the subMIC for isolation (11) and isolation (12) (7000) µg/ml and Table (11-4) shows the minimum inhibitory concentration MIC and sub MIC for zinc oxide nanoparticles and silver nanoparticles

Table (11-4) Results of the minimum inhibitory concentration MIC and Sub MIC of zinc oxide nanoparticles and silver nanoparticles.

AgN		MIC(zinc oxide) µg/ml		source		Number
SubMIC	MIC	subMIC	MIC			
7000	15000	10000	15000	Urin	11	
7000	10000	10000	12000	Burn	12	

The gene expression of *rsb*A after treated with zinc oxid nanoparticles and silver nanoparticles

The gene expression of the rsbA gene was measured for two isolates of Proteuse mirabilis bacteria, which is isolation (11) from diuresis and isolation (12) isolated from burns, and after treating it with zinc oxide nanoparticles at a concentration of (10000) µg / ml and silver nanoparticles at a concentration of (7000) µg / ml, the results of the study of gene expression of this gene using RT -

PCR technique showed a decrease in the gene expression of this gene in the presence of the nanoinhibitor material, which is zinc oxide nanoparticles ZnONPs and silver nanoparticles AgN.

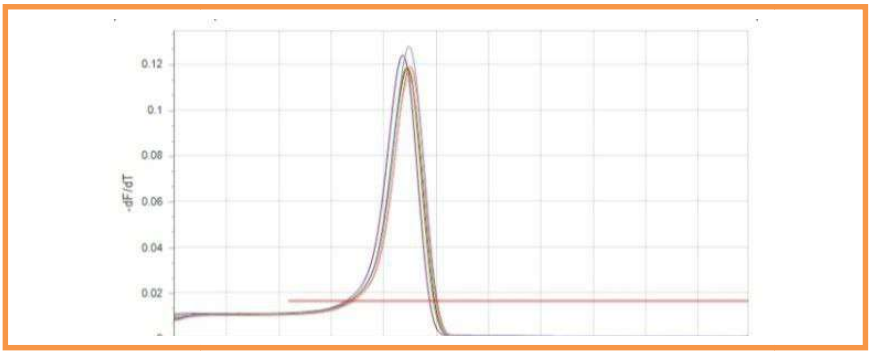


Figure (14-4) shows the measurement of the gene expression of the rsbA gene for the two isolates (11,12).

The test was performed twice for each isolate and the average of the two values was the amount of gene expression for that isolate The results of this study showed that the gene expression of the rsbA gene compared with the reference gene (16SrRNA) was reduced after the isolates were treated with the nanoinhibitory material. The gene expression was calculated from the folding value after calculating from ΔΔCT by subtracting the ΔCT of the sample from the ΔCT of the reference gene (16SrRNA)

Table (4-14) Gene Expression of rsbA Gene and Reference Gene (16SrRNA) of P. mirabilis after nanoinhibitor treatment

Average Of Folding	Folfing	ΔΔCT	ΔCT	*rsbA*	H.K 16SrRNA	isolate number	Totals
1	1.00	0.00	-0.77	12.95	13.72	11	**Before**
	1.00	0.00	0.41	16.18	15.77	12	
	0.34	1.57	0.81	14.23	13.42	11	**After**
0.44	0.55	0.84	0.08	14.42	14.34	12	

The gene expression of *wos*A after treated with zinc oxid nanoparticles and Ag nanoparticles

The gene expression of the wosA gene was measured for two isolates of P. mirabilis bacteria, which is isolation (11) from diuresis and isolation (12) isolated from burns, and after being treated with zinc oxide nanoparticles at a concentration of (10000) µg / ml and silver nanoparticles at a concentration of (7000) µg / ml, the results of the study of gene expression for this gene using RT - PCR technique showed a decrease in the gene expression of this gene in the presence of the nanoinhibitor material, which is ZnONPs and silver nanoparticles. Figure (17-4) shows the measurement of gene expression of the wosA gene for the two isolates (11,12).

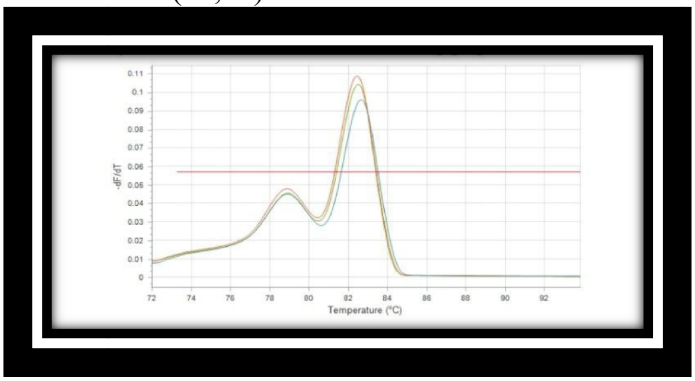


Figure (4-15) Gene expression of the wosA gene of P. mirabilis by RT-PCR technology

The results of this study showed that the gene expression of the wosA gene that was compared with the reference gene gene (16SrRNA) decreased after the treatment of the isolates with the nanoinhibitor material.The gene expression was calculated from the folding value after calculating it from the ΔΔCT by subtracting the CT Δ of the sample from the CT Δ of the reference gene (16SrRNA).

Table (4-14) Gene Expression of rsbA Gene and Reference Gene (16SrRNA) of P. mirabilis after nanoinhibitor treatment

Average Of Folding	Folfing	ΔΔCT	ΔCT	*wosA*	16SrRNA	Isolate number	totals
1	1.00	0.00	7.35	21;07	13.72	11	**before**
	1.00	0.00	7.49	23.26	15.77	12	
0.78	0.61	0.71	8.06	21.48	13.42	11	**After**
	0.95	0.08	7.34	21.77	14.34	12	

Discussions

The study showed that proteus bacteria have genes wosA and rsbA . In the present study, 100% of strains had the rsbA gene,and *wos*A. The results which agreed with [20,21] who found that 100% , 94.3% respectively . The phosphotransfer intermediate rsbA is encoded by the rsbA gene, which is part of the phosphorelay system. The rsbA gene and wosA gene which regulates swarming behavior, has been identified as a swarming repressor. The possession of 100% of the isolates indicates that these bacteria are able to migrate on hard surfaces and move to new sites that help them in the colonization process and the formation of the biofilm and thus enable them to survive with other bacterial species.

CONCLUSIONS

1- Possession of *P. mirabilis* isolates Many virulence factors that increase its pathogenicity.

2- Reduced gene expression of wosA, rsbA genes regulating the phenomenon of anthial using zinc oxide nanoinhibitors and silver nanoparticles

REFERENCES

1. Schaffer , J. N.; and Pearson, M. M. (2017). Proteus mirabilis and urinary tract infections. Urinary Tract Infections: Molecular Pathogenesis and Clinical Management, 383-433. https://www.bing.com/search?q=Proteus+mirabilis+and+urinary+tract+infections&form=ANSPH1&refig=c1b900f885154bf0b93d66f816f45218&pc=U531
2. Church, D., Elsayed, S., Reid, O.,Winston, B., & Lindsay, R. (2006). Burn wound infections. Clinical microbiology reviews, 19(2), 403-434 pphttps://www.bing.com/search?pglt=2083&q=Burn+wound+infections&cvid=162286308ed04b26a030fffaf7858044&aqs=edge..69i57j0l8.5695j0j1&FORM=ANNTA1&PC=U531
3. Hamoshi, R. M. D. (2004). Inhibitory Effect of some medicinal plants on Staphylococcus aureus isolated from different cutaneous infections. Thesis Submitted. Mosul University. https://www.bing.com/search?q=Inhibitory+Effect+of+some+medicinal+plants+on+Staphylococcus+aureus+isolated+from+different+cutaneous+infections&qs=n&form=QBRE&sp=-1&lq=1&pq=inhibitory+effect+of+some+medicinal+plants+on+staphylococcus+aureus+isolated+from+different+cutaneous+infections&sc=1-112&sk=&cvid=E304279B8D6540F491151AB74FFA20E7&ghsh=0&ghacc=0&ghpl=

4. Zafar, U., Taj, M. K., Nawaz, I., Zafar, A., & Taj, I. (2019). Characterization of Proteus mirabilis Isolated from Patient Wounds at Bolan Medical Complex Hospital, Quetta. Jundishapur Journal of Microbiology, (In Press).https://www.bing.com/search?q=Characterization+of+Proteus+mirabilis+Isolated+from+Patient+Wounds+at+Bolan+Medical+Complex+Hospital%2C+Quetta&qs=n&form=QBRE&sp=-1&lq=1&pq=characterization+of+proteus+mirabilis+isolated+from+patient+wounds+at+bolan+medical+complex+hospital%2C+quetta&sc=0-108&sk=&cvid=E503DD40A9A04E0586242F4186C494E2&ghsh=0&ghacc=0&ghpl=

5. Filimon, R. and Iacob, E. (2007). Incidence of nosocomial infections at the recovery clinic of Iasi Hospital,in 2004-2005.Revista Medico-Chirurgicala aSocietatii de Medici si Naturalistai din lasi. 111(1): 255-257.https://www.bing.com/search?q=Incidence+of+nosocomial+infections+at+the+recovery+clinic+of+Iasi+Hospital%2Cin+2004-2005&qs=n&form=QBRE&sp=-1&lq=1&pq=incidence+of+nosocomial+infections+at+the+recovery+clinic+of+iasi+hospital%2Cin+2004-2005&sc=0-87&sk=&cvid=7DD90BF499C343E78CF8481F8724C7F4&ghsh=0&ghacc=0&ghpl=

6. Zain-alabidin, S. S. and Ahmed, B. H. (2015). Effect of hot water extract of pomegranate peel on swarming and hemolysin production in multiple antibiotics resistance Proteus mirabilis. Kirkuk university journal/scientific studies (KUJSS).10(2):91-106pp.https://www.bing.com/search?q=Effect+of+hot+water+extract+of+pomegranate+peel+on+swarming+and+hemolysin+production+in+multiple+antibiotics+resistance+Proteus+mirabilis&qs=n&form=QBRE&sp=-1&lq=1&pq=effect+of+hot+water+extract+of+pomegranate+peel+on+swarming+and+hemolysin+production+in+multiple+antibiotics+resistance+proteus+mirabilis&sc=0-137&sk=&cvid=B33FD9D9A9F74D77A055DBA61D4FF71A&ghsh=0&ghacc=0&ghpl=

7. Huh , A.J. and Kown , Y.J. (2011) . Anew paradigm for treating infectious diseases using Nano materials in the antibiotics resistant era . J. control . Release : off , J. control . Release . Soc . 156 : 128 – 145 .https://www.bing.com/search?q=Anew+paradigm+for+treating+infectious+diseases+using+Nano+materials+in+the+antibiotics+resistant+era&qs=n&form=QBRE&sp=-1&lq=1&pq=anew+paradigm+for+treating+infectious+diseases+using+nano+materials+in+the+antibiotics+resistant+era&sc=0-100&sk=&cvid=99318B3B62E74CDDB4F9FD3D8A922719&ghsh=0&ghacc=0&ghpl=

8. Zaidi , S. ; Misba , L. ; Khan , A.U. (2017). Nano – therapeutics : A revolution in infection control in post antibiotic era . Nanomed . Nanotechnol . Biol . Med . 13 : 2281 – 2301 .in+infection+control+in+post+antibiotic+era+&qs=n&form=QBRE&sp=-1&ghc=1&lq=1&pq=nano+–+therapeutics+%3A+a+revolution+in+infection+control+in+post+antibiotic+era+&sc=1-79&sk=&cvid=D395B24FF08245238A1E277C1CBC966E&

9. Radif, H. M., & Al-Bahrani, R. M. (2019). Green synthesized of silver nanoparticles and study their properties and applications. Jornal of Biotechnology Research Center, 13(2), 5-9pp.https://www.bing.com/search?q=Green+synthesized+of+silver+nanoparticles+and+study+their+properties+and+applications&qs=n&form=QBRE&sp=-

1&lq=1&pq=green+synthesized+of+silver+nanoparticles+and+study+their+properties+and+applications&sc=1-85&sk=&cvid=AF75B2CD2F184228B1EBF5B7E6125C2F&ghsh=0&ghacc=0&ghpl=

10. Xie.L.X.;Hsieh.E.J.;Watanabe,S.;Allan,C.M.;Chen,J.Y.;Tran,U.:Clake,C.F (2011). Expression of the human a typical Kinase ADK3 rescues coenzy me Q biosyn thesis and phosphorylation of Coq polypeptides in Yeast co98 mutants. Biochim. Biophys Acta.1811(5):348-360.https://www.bing.com/search?q=Expression+of+the+human+a+typical+Kinase+ADK3+rescues+coenzy+me+Q+biosyn+thesis+and+phosphorylation+of+Coq+polypeptides+in+Yeast+co98+mutants&qs=n&form=QBRE&sp=-1&lq=1&pq=expression+of+the+human+a+typical+kinase+adk3+rescues+coenzy+me+q+biosyn+thesis+and+phosphorylation+of+coq+polypeptides+in+yeast+co98+mutants&sc=0-141&sk=&cvid=DCF25495742447DEA0F4E29882892905&ghsh=0&ghacc=0&ghpl=

11. Ahmed , S. ; Annu , Chaudhry , S.A. ; Ikram , S. (2017) . A review on biogenic synthesis of ZnO Nanoparticles using plant extracts and Microbes : A prospect towards green chemistry .https://www.bing.com/search?q=A+review+on+biogenic+synthesis+of+ZnO+Nanoparticles+using+plant+extracts+and+Microbes+&qs=n&form=QBRE&sp=-1&ghc=1&lq=1&pq=a+review+on+biogenic+synthesis+of+zno+nanoparticles+using+plant+extracts+and+microbes+&sc=1-86&sk=&cvid=04B3AC3EAB094FB393D11B215D46DFCF&ghsh=0&ghacc=0&ghpl=

12. Yamamoto , O . (2011) . Influence of Particle size on the antibacterial activity of zinc oxide . Inter.J. Inorganic Materials , 3 (7) : 643 – 646 .https://www.sciencedirect.com/science/article/pii/S1466604901001…

13. Holt J, Kreig N, Sheath P *et al.* (1994): Bergey's Manual of Determinative Bacteriology. https://agris.fao.org/agris-search/search.do?recordID=XF2015028636

14. Procop G, Church D, Hall G *et al.* (2016): Koneman's color atlas and textbook of diagnostic microbiology. https://books.google.com/books?hl=ar&lr=&id=HF3sDwAAQBAJ&oi=fnd&pg=PP1&dq=14-+Procop++G,+Church+D,+Hall+G++et+al.++(2016):+Koneman%27s+color+atlas+and+textbook+of+diagnostic+microbiology.+7thed.+Lippincott+Williams+and+Willkins.+Philadelphia.+Baltimore.+New+York.+London.&ots=rE9soZxDw9&sig=xZP681Op8JWib5K8FO5yO6PoR20

15. Sosa V, Schlapp G, Zunino P (2006). Proteus mirabilis isolates of different origins do not show correlation with virulence attributes and can colonize the urinary tract of mice. Microbiology, 152(7): 2149-2157.

16. Badi S, Nowroozi J, Sepahi A (2014): Detection luxS, qseC and rsbA genes' bands in Proteus mirabilis and Escherichia coli isolated from urinary tract infections. Pajoohandeh Journal, 19(3): 142- 147.

17. Mirzaei, A, Habibi M, Bouzari S *et al.* (2019): Characterization of antibiotic-susceptibility patterns, virulence factor profiles and clonal relatedness in Proteus mirabilis isolates from patients with urinary tract infection in Iran. Infection and drug resistance. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6938180/

18. Gupta A, Mumtaz S, Li C *et al.* (2019): Combatting antibiotic-resistant bacteria using nanomaterials. Chemical Society Reviews, 48(2): 415-427.

19. Al-Hamdani H, Al-Hashimy A (2020): Molecular Detection Of urec, hpma, rsba and mrpa genes of Proteus mirabilis Urinary Tract Infection In Patient With Rheumatoid Arthritis.The Iraqi Journal of Agricultural Science, 51: 245-251.

20. Al–Atbi A (2020): Molecular study for one gene responsible for biofilm formation and the effect of some biofilm inhibitors in proteus mirabilis isolated from Burns and Wounds . MS thesis. college of science. Baghdad University.

21. Singla P, Sangwan J, Garg S *et al.* (2015): Prevalence and Antibiogram of Multidrug resistant Uropathogenic Isolates of *Proteus mirabilis* in a Teaching Tertiary Care Hospital. International Journal of Current Microbiology Applied Sciences. 4(12): 675-682.

22. Al-Bassam W, Al-Kazaz A (2013): The isolation and characterization of Proteus mirabilisfrom different clinical samples. Journal of Biotechnology Research Center. 7(2):24-30

23. Latif B, Zayn S, Ahmed I (2017): A Comparative Bacteriological and Molecular Study on Some Virulence Factor of *Proteus* spp Isolated from Clinical and Environment Specimen. International Journal of Current Research and Academic Review. 5(5): 26-33.

24. Wasfi R, Hamed S, Abd Allah N *et al.* (2020): Proteus mirabilis biofilm: Development and therapeutic strategies. Frontiers in cellular and infection microbiology, 10, 414.

25. Al-Jumaily E, Zgaer S (2016): Multidrug resistant Proteus mirabilis isolated from urinary tract infection from different hospitals in Baghdad City. Int. J. Curr. Microbiol. App. Sci, 5(9): 390-399.

26. Wang J, Chen P, Chang S *et al.* (2014): Antimicrobial susceptibilities of Proteus mirabilis: a longitudinal nationwide study from the Taiwan surveillance of antimicrobial resistance (TSAR) program. BMC infectious diseases., 14(1): 486.

27. Kadhim A, AL-Mathkury H, Obaid H (2014): Role of Proteus mirabilis DNA in Comparison to Candida albicans DNA in Rats' Joints Infection. Iraqi Journal of Science. 55(3B): 1170-1182.

28. AL- Dulaimi M(2019): Detection of Some Virulence Genes of *Proteus mirabilis* Isolated From Different Infectious in Baquba City .MS thesis college of science. Diyala University .

29. AL-Oqaili N, AL-Shebli M, Almousawi A (2017): Antimicrobial susceptibility and molecular characterization for some virulence factors of Proteus Mirabilis isolated from patients in AlQadisiyah Province/Iraq. Al-Qadisiyah Journal of Veterinary Medicine Sciences, 16(2): 1-9

30. Al-Mayahi F (2017). Phenotypic and Molecular detection of Virulence factors in Proteus mirabilis isolated from different clinical sources. Bas J Vet Res, 16(1): 369-88.

31. Sosa V, Schlapp G, Zunino P (2006): Proteus mirabilis isolates of different origins do not show correlation with virulence attributes and can colonize the urinary tract of mice. Microbiology, 152(7): 2149-2157.

32. Mirzaei A, Habibi M, Bouzari S *et al.* (2019): Characterization of antibiotic-susceptibility patterns, virulence factor profiles and clonal relatedness in Proteus mirabilis isolates from patients with urinary tract infection in Iran. Infection and drug resistance, 12: 3967