Republic of Iraq Ministry of Higher Education and Scientific Research University of Diyala College of Medicine



Insilico bactericidal potential Synthesized silver nanoparticles of clinical *Staphylococcus aureus* and *Escherichia coli* Isolated

A Thesis

Submitted to Council College of Medicine - University of Diyala in Partial Fulfillment of the Requirements for the master's degree of Sciences in Medical Microbiology

By

Sarah Mutasher Hateem

B.Sc. College of Science Diyala University (2013)

Supervised by

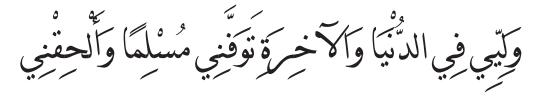
Professor Dr. Alyaa Saad Abed Karkosh Professor Dr. Amer D.Majeed

2021 A.D.

1442 A.H.











يوسف (101)

Acknowledgments

At the outset, thanks and praise be to God Almighty for the above, so all credit is attributed to him for completing this work.

And I turn to my professor, Dr. Alia - the supervisor of my message - with thanks and appreciation that no words have fulfilled his right. If it were not for his great support, this work would have been done.

From which doors of praise, I will go to the one who overwhelmed me with her love. I will thank my mother who drank the cup of misery bitterly to give me the nectar of happiness .. To the one who sacrificed a lot for the sake of being alive .. and whatever your recipe, I will not be able to complete, not complacency, but something deeper than that.

My thanks and appreciation go to my father who gave everything he could for us year after year to see us today before him, after we grew up, no matter how much you thank, I feel inadequate towards you. May God keep you as a treasure and he attributed to us.

I extend my sincere thanks and appreciation to my brothers (Maha, Mustafa, Bakr, Abdul Rahman and Hiba) for their favor and support for me throughout my studies, as they overwhelmed me with their love and help ... You have all my love and affection.

Out of loyalty, sincerity and respect, I would like to thank those who have given me a favor with all the fine and kind my teachers: (Mr. Ahmed Rahim Al Mousawi, Mr. Hussein Jassem Al Zuhairi, Dr. Ibtihal Hamid Mohsen, Alia Mahmoud Abdullah and Mustafa Salam Abdulkhaleq)

To the unknown fighter who helped me and stood next to me and took my hand to the highest ranks to Mr. Mounir

Sarah

Dedication

To whoever illuminated with his knowledge a mind of jealousy.. To whom God has bestowed upon the great creation before the messeng) (Prophet Muhammad, peace be upon him)

From you we learned that success has value and meaning ... and from you we learned how dedication and sincerity in work can be ... and from you we believe that there is no impossible in the way of creativity and advancement ... "my honorable teacher Prof. Dr.Aliaa Saad

To those who bought us at night our security with their pure blood .. To souls in the sky who are blessed with God "The martyrs of Iraq"

To a sun that melted the rigidity of my heart and blew up the springs of hope ... Oh moon, the darkness of my mind lit up a path in life ... To whom I planted the love of God in my heart "My dear mother"

To the gentle smile that splashed the beauty of its light in the darkness of my heart, wiping all of my worries ... To that tender touch that blew in my heart a fountain of hope in this cruel life ... "My brothers"

To you for the splendor of your morals, your remarkable distinction, your upscale presence and the everlasting smile ... "my colleagues"

Sarah

Supervisor Certification

We, certify that this thesis entitled (Insilico bactericidal potential Synthesized silver nanoparticles of clinical *Staphylococcus aureus* and *Escherichia coli* Isolated), has been conducted under our supervision at College of Medicine, University of Diyala, as a partial fulfillment of the requirements for the Master Degree of Science in Medical Microbiology.

Professor Dr. Alyaa Saad Abed Karko Ph.D. In Genetic engineering and molecular genetics Professor Dr. Amer D.Majeed Ph.D. In Biomedical physics

In view of available recommendation, I forward this thesis for debate by the examining committee.

Signature

Assistant Professor Dr. Luma Taha Ahmed

Head of Microbiology Department

College of Medicine - University of Diyala

Examination Committee Certification

We certify that we have read this thesis entitled (Insilico bactericidal potential Synthesized silver nanoparticles of clinical *Staphylococcus aureus* and *Escherichia coli* Isolated) and as the examination committee examined the student (Rawa Faris Hussein Al-Saeedi) on its content and in our opinion it is adequate as a thesis for the Degree of the M.Sc.In Medical Microbiology

Signature

Name: Prof.Dr. Eman Mohammad Jarallah

Scientific degree: Prof.Dr

Date: / /2021

Signature

Signature

Name: Prof.Dr. Ahmed M.Athab

Scientific degree:lecturer.Dr

Name: lecturer Dr. Taif Majid Abdulhussein

Scientific degree: Prof.Dr

Date: / /2021

Signature

Data: / / 2021

Name: Alyaa Saad Abed Karko

Scientific degree : Prof.Dr

Date: / /2021

Signature

Name: Amer D.Majeed Scientific degree : Prof.Dr

Date://2021

Approved by the Council of the College of Medicine – University of Diyala.

Signature:

Professor Dr. Ismail Ibrahim Latif

Dean College of Medicine-University of Diyala

Date: / /2021

SUMMARY

The study included collecting 100 samples of UTI for women, including 20 samples of Escherichia coli bacteria and 10 samples of staphylococcus bacteria in Al-Batoul Hospital for the period from 1/7/2020 to 1/9/2020

The resulte showed positive growth in appropriate culture media. Maconkey agar, between blood agar, amid Eocin blue methyl solid. Isolates were diagnosed using bacteriological and chemical tests. It was found that Escherichia coli bacteria were among the main causes of inflammation..Urinary tract and vaginal infections. The diagnosis was confirmed and a sensitivity tested.

Silver nitrate nanoparticles are manufactured in laboratories Department of Physics at the Faculty of Science, Diyala University, the minute sizes were at 45. The inhibitory activity of metal nitrate nanoparticles Ag was detected alone. The inhibitory activity of silver nanoparticles was estimated by the Agar well diffusion method, wherein the silver nanoparticles with a concentration of $3\mu g / ml$ recorded the highest inhibition diameters. While the silver nanoparticles were recorded at a concentration200 $\mu g / ml$, the lowest rate inhibition

The molecular docking approach can be used to model the interaction between a small molecule and a protein at the atomic level, which allow us to characterize the behavior of small molecules in the binding site of target proteins as well as to elucidate fundamental biochemical processes. The docking process involves two basic steps: prediction of the ligand conformation as well as its position and orientation within these sites (usually referred to as pose) and assessment of the binding affinity. These two steps are related to sampling methods and scoring schemes, respectively, which will be discussed in the theory section.

Content List

Subject	Page No.
Dedication	
Acknowledgment	
Abstract	Ι
Table of Contents	III
List of Tables	VII
List of Figures	VIII
List of Abbreviations	X

Table of Contents

Items	Subject	Page No.
	Chapter One: Introduction	
1-1	Introduction	1
1-2	Aims of study	2
	Chapter Two : Literatures review	
2.1	Escherichia coli	3
2.1.1	Morphology and General Characteristic	4
2.1.2	Epidemology	6
2.1.3	Pathogenicity	10
2.1.4	Symptoms	12
2.1.5	Antibiotic resistance of E. coli	14
2.2	Staphylococcus aures	15
2.2.1	Morphology and general characteristics	17
2.2.2	Characterization of Staphylococcus aures	19
2.2.3	Pathogenicity of Staphylococcus aures	
2.2.4	Virulence factors	
2.2.4.1	Plasma Coagulase	
2.2.4.2	Hemolysine	

2.2.5	Antibiotic resistance of <i>staphlococcus</i> . <i>aureus</i>	
2.3	Nanotechnology	
2.3.1	Sliver nanoparticles	
2.3.2	Silver nanoparticles mode of action on microb	
2.4	Insilico study	
2.5	Docking methodologies	
2.5.1	Rigid ligand and rigid receptor docking	
2.5.2	Flexible ligand and rigid receptor docking	
2.5.3	Flexible ligand and flexible receptor docking	
	Chapter Three : Patients , Materials and Methods	
3-1	Patients	1
3.1.1	Collection of urine samples	2
3.1.2	Isolation of bacteria	3
3.2	materials.	4
3.2.1	Laboratory equipment's.	6
3.2.2	Chemical and solution	10
3.2.3	Culture media	12
3.2.4	Antibiotic	14
3.3	Culture media preparation of bacteria <i>E.coli</i>	15
3.4	Pationt,s specimen,s collection	17
3.4.1	Isolation of <i>E.coli</i> isolates	19
3.4.2	Identification of <i>E.coli</i> isolates	
3.5	Antimicrobial susceptibility test (Disk diffusion test)	
3.6	S Staphylococcus aureus	
3.6.1	Sterilization	
3.7	Preparation of reagents and solutions used in the diagnosis of bacteria	
3.8	preparation of culture media	
1		1

3.9	Collection of Samples Identification of Bacteria Isolates	
3.9.1	Identification of bacterial isolates	
3.9.2	Preservation and maintenance of bacterial isolates	
3.10	Examine the sensitivity of isolates to antibiotics using the tablet method	
3.11	Preparation of silver nanoparticles	
3.12	Computational Study	
	Chapter four : Result	
4.1	Isolation and Identification of <i>E.coli</i>	1
4.1.1	Microscopy Characteristics	2
4.1.2	Biochemical Tests	3
4.1.3	Antibacterial susceptibility test	4
4.2	Isolation and identification of Staphylococcus. aureus	6
4.2.1	Microscopic examination	10
4.2.2	Biochemical tests	12
4.3	Antibacterial Susceptibility test for S. aureus isolates	14
4.4	Treatment by silver nanoparticles	15
4.5	In silico Study	17
4.5.1	Determination of target:	19
4.5.2	Active Site	
4.5.3	Molecular Docking	
Chapter FIVE : DISCUSSION		
5.1	Diagnostic tests for isolates <i>E.coli</i>	1
5.1.1	Diagnostic cultivar characteristics of isolates	2
5.1.2	Antibiotic sensitivity test for <i>E. coli</i> bacteria	3
5.2	Antibiotic sensitivity test of S. aureus isolates	4
5.3	Treatment by silver nanoparticles.	6
5.4	In silico Study	10

List of Table

Table Number	Title	Page No.
3.1	Equipment's and Apparatus used in this study	
3.2	Chemical using in this study	
3.3	Culture media used in this study	
3.4	antibiotics used in the current study.	
4.1	The total number and percentage of <i>E. coli</i> isolates	
	from UTI	
4.2	Biochemical test of <i>E.coli</i> bacteria	
4.3	The total number and percentage of <i>Staphylococcus</i> .	
	aureus isolates from UTI	
4.4	Biochemical test of S. aureus bacteria	
4.5	Antibiotics used in the current study	
4.6	showing the types of bacteria and the concentrations	
	of nanoparticles used in the study	
4.7	Docking Result that Indicate Minimum Binding	
	Free Energy of the AgNP Molecule	

List of titles of Figures

Figure number	Title	The page
	Growth of S. aureus bacteria on selective mannitol	
4.1	saline growing at an incubation of 37 ° C. for 18	
	hours	
4.2	showing powder nanomaterial	
4.3	Diameters of inhibition zones for nanoparticles on	
4.3	bacteria <i>E.coli</i>	
4.4	The effect of silver nanoparticles on bacteria	
4.4	Staph.aureas	
	DNA gyrase 3D protein modeling by pyMOL	
4.5	A: protein model of staphylococcus aureus.	
	B: protein model of <i>E. coli</i>	
	The Best Predicted Active Site :-	
4.6	A- Active site of staphylococcus aureus B- Active	
	site of <i>E. coli</i>	
4.7	Molecular Docking of AgNP Molecule of	
4./	staphylococcus aureus and E. coli	

List of Abbreviations

Abbreviate	Key
DEC	Diarrheagenic
NPs	Nanoparticles
EMB	Eosin Methylene Blue
TSI	Triple Sugar iron
GUD	Glucoronidase
LT	Heat-labile toxin
ST	Heat-stable toxin
LPS	Lipopolysaccharides
UTI	Urinary tract infection
OMPs	Outer-membrane proteins
EAST	Enteroaggregative heat-stable toxin
EAEC	Heat-stable enterotoxin
ETEC	Heat-labile enterotoxin
Pet	Plasmid-encoded toxin
VAT	Vacuolating autotransporter toxin
MDR	Multi-drug resistant
PDR	Pan-Drug Resistant
DNA	Deoxyribonucleic acid
CF	cystic fibrosis
ROS	Reactive oxygen species
AFM	Aomic force microscope
AgNO3	Silver nitrate
H2O2	Hydrogen peroxide reagent
Dnase	A deoxyribonuclease
DEG	Database of Essential Genes
PBPS	Penicillin Binding Proteins
KEGG	The Kyoto Encyclopedia of Genes and Genomes
SHV	Extended-spectrum of hydrolyzing activity

Chapter One Introduction

1.1Introduction

Escherichia coli is a Gram-negative, facultative anaerobic, rod-shaped, coliform bacterium of the genus Escherichia that is commonly found in the lower intestine of warm-blooded organisms (endotherms) Most *E.coli* strains are harmless, but some serotypes (Tenaillon *et al*, 2010).

Some strains of this bacterium acquire virulence genes that give it the ability to cause disease. These pathogenic strains are classified as dangerous inside and outside the intestine. These pathogenic strains are classified as pathogenic *Escherichia coli* For the intestines that cause diarrhea (DEC) Diarrheagenic *E. coli*, and pathogenic Escherichia coli ExPEC (Kaper *et al.*, 2014).

Extraintestinal pathogenic *E. coli.* Each group includes strains of diseases that share similar virulence factors and cause similar diseases. UpEC belongs to ExPEC and is characterized by its ability to be present in the gut without causing disease, but when it is available. Adequate conditions infect other parts of the host's body, including the blood and nervous system Central and urinary tract, causing a number of serious diseases, UPEC bacteria is the main cause For community-acquired urinary tract infections (90-80%) (Foxman, 2014).

It is Staphylococci, considered a coexisting bacterium in a large percentage of people and is dangerous as it has the ability to infect any tissue of the human body and among these diseases, the most important of which are sepsis, osteitis, carditis and pneumonia, and constitute 22% of the bacteria that cause bloodstream infections and 23% of the bacteria Which causes pneumonia, while infections of the skin and soft tissues.

Chapter one

Constitute 39%. Bacteria infect most sites of the human body, and the front openings are the main environmental places for them. Within a healthy adult population, 20% are persistent nasal carriers intermittent carriers, and 50% noncarriers20% Persistent nasal carriers have an increased risk of *S. aureus* infection compared with intermittent carriers and noncarriers Higher levels of some antistaphylococcal antibodies were observed in persistent carriers than in others, and recently (Basak *et al*, 2016).

It grows anaerobically compulsively or anaerobically in certain conditions, it grows in all agricultural areas, its colonies are colorless in the middle of the component, and are not fermented carbohydrates, especially lactose sugar, they are colored with bluish green, yellowish green and reddish brown pigments in other agricultural media. (Forbes *et al*,2002)

It grows at a temperature of 37 and resists drought and heat at 50 degrees for 30 minutes. It is considered one of the types of natural flora of the skin and the digestive system as it appears naturally on the skin and mucous membranes of the human being (Jawetz *et al*,2004).

The technique of large-scale particles demonstrated Due to their wide physical and chemical properties, realistically studies have shown that nanoparticles are You face a problem you are facing a problem facing challenges Go back to the use of nanomaterials For new satisfying drinks, beverages, beverages, beverages, beverages, beverages, beverages, beverages, beverages, beverages, beverages Difficult cells, continuous delivery and inhibition of intracellular pathogens and their sterilizing capabilities J Antimicrobial mechanisms NPs include destruction of cell membranes Enzyme pathways, induction

Chapter one

of events in microbial cell wall fabrication, and operation of manufacturing pathways Microbial DNA (Galdiero *et al.*,2011).

Silver nanoparticles are one of the most good antibacterials as nanomaterials Inorganic, which makes it included in the composition of medicines for treating bacterial diseases and that these Particulate matter is one of the factors used for effective treatment of microbes in general, and they have both an internal and an external effect, And according to the preparation methods used, the size and shape of the silver nanoparticles depend on the time, the temperature of the reaction and the concentration of the reactants.(Moghimi *et al*,2001).

1.2. Aim of study

This study aimes to bactericidal Potential of silver nanoparticales synthesized of *S.aureus* and *E.coli*