Ministry of Higher Education
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University of Al- Qadiysiah
College of Medicine
Department of Microbiology

Biosynthesis of Silver Nanoparticles Using *Agaricus bisporus* Extract and Its Antibacterial Activity against Multi- Drug Resistant Bacteria

A Thesis

Submitted to the Council of College of Medicine, University of Al- Qadiysiah in Partial Fulfillment of the requirements for the degree of Doctor of Philosophy in Medical Microbiology

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Supervisors' Certificate

I certify this thesis (**Biosynthesis of Silver Nanoparticles Using *Agaricus bisporus* Extract and Its Antibacterial Activity against Multi –Drug Resistant Bacteria**) that prepared under my supervision at the Department of Microbiology, College o f Medicine/ University of Al-Qadiysiah, as partial fulfillment of the requirements for the degree of Doctor of Philosophy Sciences in Medical Microbiology.

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Recommendation of Head of Microbiology Department

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

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Certification of Examining Committee

We, the members of examining committee, certify after reading this thesis **"Biosynthesized of Silver Nanoparticles Using *Agaricus bisporus* Extract and Its Antibacterial Activity Against Multi-Drug Resistant Bacteria"** and after examining the student in its contents, we found it is adequate for the award on the Degree of Doctor of Science in Medical Microbiology with excellent degree.

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List of Contents

List of Figures

List of tables

List of Abbreviations

Abbreviations	Key
Ag-NPs	**Silver nanoparticles**
AgNo3	**Silver nitrate**
TCP , TB	**Tissue culture method - Tube method respectively**
SEM,TEM	**Scanning and Transmission Electronic Microscope**
FTIR	**Fourier transmission infrared spectroscopy**
UV-Vis	**Ultraviolet Visible Spectroscopy**
MDR,XDR,PAN	**Multi dsug resistance , extensively drug resistance , pan drug résistance**
SSI	**Surgical site infection**
SMB	**Small multidrug resistance**
MFS	**Major facilitator super family**
RND	**Resistance nodulation division**
HA-MRSA	**Healthcare acquired- Methysiline Resistant *Staphylococcus aureus***
SNPs- NPs	**Sliver nanoparticles – Nanoparticles**

PBS	**Phosphate buffer solution**
ATP, ADP	**Adenosine triple phosphate ; Adenosine di-phosphate**
ESP	**Extra cellular substance**

Summery

The present study conducted the evaluate silver nanoparticles (Ag-NPs) biosynthesized by the edible mushroom (*Agaricus bisporus*), and there effect as inhibitory agents alone and in combination with antibiotics against some multi drug resistant bacteria.

A total of 250 clinical specimens were collected from patients (male and female) at Al-Diwaniyah Teaching of Maternity and Pediatrics Hospitals in Al-Diwaniyah city the period October 2016 to February 2017 in order to isolates multi drug resistant bacteria . Out of 150(60%) have shown positive bacterial cultures while 100(40%),were negative . According to specimens types ,the results revealed that bacterial isolates were distributed as 60(40 %) from urine ,48(32%) catheter, 26(17.3%)wound and 16(10.6%) boils and ear swabs .

Culture and biochemical tests identified that the isolated bacteria were as follows: 65(43%) *Escherichia coli* ,35(23%) *Pseudomonas aeruginosa*,30(20%) *Proteus mirabilis and* 20(13%) *Staphylococcus aureus* ,

Edible mushroom (*Agaricus bisporus)*was used as a bio- reductant of biosynthesis the silver nanoparticles Ag-NPs were visually detected by changing color from yellow to dark brown suspension containing cell free filtrate and silver nitrite.

The synthesized Ag-NPs have been characterized by UV/Vis spectroscopy, Fourier Transform Infrared (FTIR) and Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy(SEM) which revealed that Ag-NPs spherical or circular in shape, UV/Vis spectroscopy peak was in range of 430 nm and in rang of 5-35 nm by TEM and confirmed 5-50nm by SEM . FTIR analysis showed that the spectra of Ag-NPs between 250 to 4250 cm-1 of Ag-NPs that emphasized its contained: proteins, amino acids, aldehydes, alcohol and carboxylic acids responsible for the reduction, stabilization and capping of Ag-NPs.

Biosynthesis of Ag-NPs were found to possess remarkable antibacterial activity against tested pathogenic bacteria, in comparison with effects of antibiotics alone. 35 bacterial isolates of each bacterial species were selected with high resistance to antibiotics, as follow: Methicillin- resistance *S. aureus* (MRSA) *P. aeruginosa, E. coli* and *P. mirabilis.*

Antibacterial activity against tested pathogenic bacteria in comparison with effects of antibiotics, Ag-NPs were also evaluated for their efficacy to enhance the antibiotic activities of some important broad spectra commercial antibiotics , so the results revealed the increasing of growth inhibition of tested bacteria as follow: lowest zone inhibition in *E.coli* , *P. aeruginosa* and *P.mirabilis* were 10 mm while the highest zone inhibition were 19mm but MRSA ranged from 10 to 18mm. and increase fold-area spectra between 0.2-4. *E.coli,* 0.2-4.4 *P. aeruginosa and,*0.2-3.6 for MRSA *and P. mirabilis* .

On the other hand, the bacterial isolates were screened for biofilm production as a virulence factors using two different methods (Tissue culture plate method and Tube method). The results showed that all tested bacterial isolates were biofilm producer and the tissue culture plate method was the most sensitive method for MDR resistant bacteria . Additionally, the Ag-NPs in combination with antibiotics showed a remarkable reduction of biofilm production of tested bacteria in compared with the effect of antibiotic alone

Statistic analysis revealed a significant and non significant results P-value between < 0.05 .

The ability of Ag-NPs to inhibit ATPase was investigated using Tris, Triton X100 and NaN_3 inhibitors. To elucidate the antibacterial activity of Ag-NPs was associated with the altered membrane permeability ,the results showed that the highest level of inhibition the inhibitor Tris with Ag-NPs. In conclusion ,Ag-NPs may prove as a better candidate for drugs and can potentially eliminate the problem of chemical agents .

CHAPTER ONE

INTRODUCTION AND LITERATURES REVIEW

1.1Introduction

It is well-known that nanotechnology will be a standout amongst the greater part quickly developing application to science so Nano biotechnology is a multidisciplinary approach involves researches aid development of technology in various areas of science as biotechnology, nanotechnology, physics, chemistry, and material science this can be seen from the update literatures refers to that. So sliver NPs have highly effective as result of its good antimicrobial efficacy against bacteria, viruses and other eukaryotic micro-organisms .They are undoubtedly the most widely used nanomaterials among antimicrobial agents while some application of NPs in biomedicine include: creating fluorescent biological labels for important biological markers and molecules in research and diagnosis of diseases, Gene delivery systems in gene therapy or biological detection of disease causing organisms and diagnosis, detection of proteins(Ahmed *et al* ., 2010).

On the other hand , the advantages of using NPs as a drug delivery system include: the size and surface characteristics of NPs can be easily manipulated. This could be used for both passive and active drug targeting. The fungus basidiomycete, Mushrooms have been part of the normal human diet for thousands of years and in recent times, the amounts consumed have risen greatly, involving a large number of species. So that Mushroom was considered as a potent source of different antimicrobial agents to fighting such as tomb infections in addition Mushroom have many active biological components which causes health beneficial effects like immune system modulation, biological response modification(Foulongne-Orio *et al.,* 2013).

The problem with some of the chemical and physical methods of nanosilver production is that they are extremely expensive and involve toxic, dangerous

chemically, which may pose potential environmental and biological risks.

It is an unavoidable fact that the Ag-NPs synthesized have to be handled by humans and must be available at cheaper rates for their effective utilization there is a need for an eco-friendly and economically way to synthesize these NPs .The growing need to develop environmentally friendly and economically feasible technologies for material synthesis led to the search for biological methods of synthesis. Biofilm may be one of the leading causes for a shift from acute-phase diseases to chronic diseases. Most common diseases involving bacteria able to form biofilm The most common biofilm-forming bacteria associated with human infections are: *Escherichia coli*, *Staphylococcus. aureus*, *Proteus mirabilis* and *Pseudomonas aeruginosa* (Vardanyan *et al*.,2015) .

These bacteria lead to increasing hospital and community-acquired infections due to bacterial multidrug-resistant (MDR) pathogens for which current antibiotic therapies are not effective represent a growing problem . Antimicrobial resistance is one of the major threats to human health since it determines an increase of morbidity and mortality as a consequence of the most common bacterial diseases .Resistance genes have recently emerged favored by improper use of antibiotics hence, the first step in combating resistance envisions the reduction of antibiotic consumption (Cerceo *et al*.,2016).

ATPase is membrane-bound ion channels . triphosphate (ATP) synthase is an anabolic enzyme that harnesses the energy of a transmembrane proton gradient as an energy source for adding an inorganic phosphate group to a molecule of adenosine diphosphate (ADP) to form a molecule of adenosine ATP(Forrest *et al.*, 2014**).**

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The Aim of the study

The aim of the present study was to evaluate the use of Silver NPs (Ag-NPs) that bio-prepared by using edible mushroom (*Agaricus bisporus*) as bio-reductant of sliver ion and its effect against selected multi drug-resistant pathogens .To achieve this aim, the following objectives were conducted:

1. Isolation and identification of bacterial isolates from different clinical specimens(urine ,catheter ,wounds and ear swabs),using cultural and biochemical characteristics.

2. Conducting the antibiotics susceptibility test for bacterial isolates in order to determine of the multi drug resistant(MDR) by using disk diffusion method.

3. Bio reductant of Ag-NPs from Mushroom(Agaricus *bisporus*) by using standard methods and characterization of these NPs by using UV-visible spectronic, SEM,TEM and FTIR measurements.

4. Study the effect Ag-NPs alone in on the growth of standard and MDR isolate, with combination with conventional antibiotics using agar diffusion method.

5- Study the effect of Ag-NPs in biofilm production and ATPase activity as a virulence of the four tested bacteria .

1.2. Review of Literature

1.2.1. Nanotechnology:

Nanotechnology is one of the most rapidly growing areas in science. 'Nano' originates from the Greek meaning 'dwarf' and refers to the microscopic size that nanotechnology deals with Nanoparticles (NPs) structures usually range from 1-100 nm in size and . exhibit different shapes like spherical, triangular, rod (Danquah - Amoah and Morya,2017).

'Nano' means small, very small, But why is this special? There are various reasons why nanoscience and nanotechnologies are so promising in materials, engineering and related sciences. First, at the nanometer scale, the properties of matter, such as energy, change. This is a direct consequence of the small size of nanomaterials, physically explained as quantum effects

Nanotechnology can be termed as the synthesis ,design, manipulation of structure of particles with dimension smaller than 100nm , so dealing with various aspects of research and technology (Ahmad *et al.,* 2003).The consequence is that a material(e.g. a metal) when in a Nano-sized form can assume properties which are very different from those when the same material is in a bulk form. For instance, bulk silver is non-toxic, whereas silver NPs are capable of killing viruses upon contact. Properties like electrical conductivity, color, strength and weight change when the nanoscale level is reached: the same metal can become a semiconductor or an insulator at the nanoscale level. The second exceptional property of nanomaterials is that they can be fabricated atom by atom by a process called bottom up. The information for this fabrication process is embedded in the material building blocks so that these can self-assemble in the final product.Nanotechnology are particles between 1 and 100 nanometers in size (Filipponi and Sutherland, 2013).

In nanotechnology, a particle is defined as a small object that behaves as a whole unit with respect to its transport and properties. Particles are further classified according to diameter, fine particles are sized between 100 and 250 nanometers , and coarse particles cover a range between 250 and 1000 nanometers. A nanometer is one thousandth of a micrometer = 10^{9} meters illustrated in Figure 1-1 (Stephenson and Hubler ,2015). The unique properties of metal NPs are determined by their size (1-100nm) and shape such a triangle, hexagons, spheres, and rods (Gericke and Pinches ,2006).These particles have been widely used in many fields such as electronics, photochemical, biomedicine and chemistry (Di Guglielmo *et al*., 2010).

NPs have special and enhanced physical and chemical properties as compared to their bulk materials .These physic-chemical properties of NPs include size, different shape, composition and crystallinity, in addition to their large surface area than small volume ratio, homogeneity, and other features will provide valuable information of nanoscale systems (Ramezani *et al*.,2006).

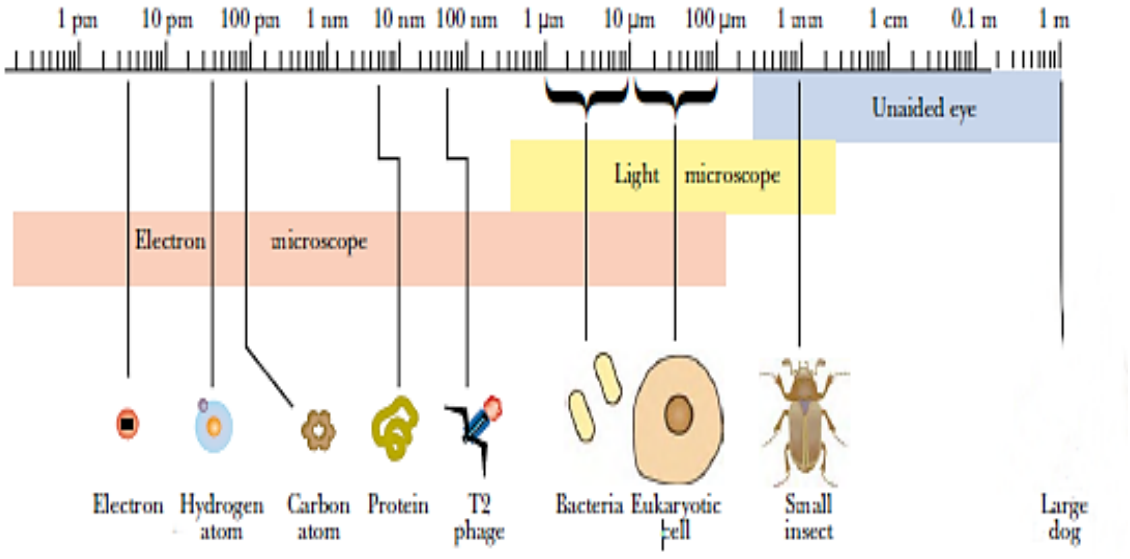


Figure (1-1) :Size Comparisons (Clark and Nanette ,2009).

NPs may be solid or hollow and are composed of a variety of materials, often in several discrete layers with separate functions. Typically there is a

central functional layer, a protective layer, and an outer layer allowing interaction with the biological world. The central functional layer usually displays some useful optical or magnetic behavior. Most popular is fluorescence. The protective layer shields the functional layer from chemical damage by air, water, or cell components and conversely shields the cell from any toxic properties of the chemicals composing the functional layer. The outer layer(s) allow NPs to be specific recognition (Clark and Nanette ,2009**)**.

1.2.2. Nanobiotechnology

Nanobiotechnology is a multidisciplinary field and involves researches aid development of technology in different fields of science like biotechnology, nanotechnology, physics, chemistry, and material science. Sliver nanoparticles (Ag-NPs) have proved to be most effective because of its good antimicrobial efficacy against bacteria (Hwang *et al*., 2012).

NPs exhibit new or improved properties based on specific characteristics such as size, distribution and morphology. New applications of NPs and nanomaterials are increasing rapidly. A new branch of nanotechnology is Nano biotechnology which combines biological principles with physical and chemical procedures to generate Nano-sized particles with specific functions and represents an economic alternative for chemical and physical methods of nanoparticles formation (Ahmad *et al*. 2003) Nanotechnology has provided the possibility of delivering drugs to specific cells using NPs (Ranganathan *et al*.,2012).Investigative methods of nanotechnology have made inroads into uncovering fundamental biological processes, including self-assembly, cellular processes, and systems biology (such as neural systems).

Key advances have been made in the ability to make measurements at the subcellular level and in understanding the cell as a highly organized, self-repairing, self-replicating, information-rich molecular machine

(Ishijima and Yanagida *et al* 2001). Some uses of NPs in biology and medicine include: Creating fluorescent biological labels for important biological markers and molecules in research and diagnosis of diseases, Drug delivery systems. Gene delivery systems in gene therapy or biological detection of disease causing organisms and diagnosis and Detection of proteins. NPs are being increasingly used in drug delivery systems. The advantages of using NPs as a drug delivery system include: The size and surface characteristics of NPs can be easily manipulated. This could be used for both passive and active drug (Hwang *et al*., 2012).

1.2.3. Classification of nanoparticles (NPs)

NPs are broadly classified in to three classifications :

• One dimension NPs: One dimensional system (thin fi lm or manufactured surfaces) has been used for decades. Thin films (sizes 1–100 nm) or monolayer is now common place in the field of solar cells offering, different technological applications, such as chemical and biological sensors, information storage systems, magneto-optic and optical device, fiber-optic systems.

• Two dimension NPs :Carbon nanotubes

• Three dimension NPs: Dendrimers, Quantum Dots, Fullerenes (Carbon 60), (QDs)(Hett ,2004).

Organic NPs include carbon NPs which defined as a solid particles consist of organic compounds like (lipids and polymeric).previously this type of organic NPs met a considerable expansion and a great investigation as result of wide potentialities of this type of NPs ranging from photonic, electronic ,conducting materials and medicine (Hwang *et al*., 2012).

Inorganic NPs are more important in modern technology, readily and inexpensive when synthesized and mass uses, so they can be easily integrated in different applications (Singhal *et al.,* 2011).They include magnetic NPs which capable to be manipulated by using so many fields like ferrites (iron oxide NPs).

Also they includes noble mate NPs as (gold and silver) and semiconductor NPs as zinc oxide and titanium dioxide (Kim *et al*., 2010).These materials can be synthesized with various chemical functional group that lead to conjugated with different legends , drugs and antibodies. This development opened wide applications in biotechnology, drug delivery magnetic separation and more diagnostic imagining (Cheon and Horace,2009). The recent developments of the biosynthesis of inorganic NPs including metallic NPs, oxide NPs, sulfide NPs, and other typical NPs. Different formation mechanisms of these NPs will be illustrated with the conditions to control the size/shape and stability of particles (Singhal *et al.*, 2011).

1.2.4. Silver nitrate ($AgNO_3$)

Silver nitrate is an inorganic compound with chemical formula $AgNO_3$ This compound is a versatile precursor to many other silver compounds, such as those used in photography. It is far less sensitive to light than the halides. It was once called *lunar caustic* because silver was called *luna* by the ancient alchemists, who believed that silver was associated with the moon. colourless rhombic crystals (molar mass): 169,870 g/mol , (melting point): 209,7 °C (decomposing temperature): 300 °C (Heyneman *et al*,. 2016). There are some physical and chemical methods available for Ag-NPs synthesis but these are so tedious, they consume lot of energy to maintain high pressure and temperature. Involvement of toxic chemicals in the synthesis process may be harmful to human beings (Chen *et al*., 2013).The use of silver for treating infections has regained importance. However, the use of ionic silver has one major flaw: it is easily inactivated by complexation and precipitation. As a result, the use of silver ions has been limited. (Heyneman *et al*,. 2016).

In 1881, silver nitrate eye drops was used by Carl S. F. Crede to cure ophthalmic neonatorum; later, B. Crede designed silver-impregnated dressings

for skin grafting (Sintubin *et al*., 2013).).In 1884, aqueous silver nitrate drops were used to prevent the transmission of Neisseria gonorrhoeae from infected mothers to children during childbirth .Early in the 19th century $AgNO_3$(0.5%) was used for the treatment and prevention of microbial infections such as Ophthalmia neonatorum (by German obstetrician Carl Crede) (Chen *et al*., 2013). The use of silver for creating infections has regained importance with the progress of Nano production of the nanoparticle possesses more surface atoms than micro particles, which greatly improves the particles physical and chemical characteristics. silver ion (Ag+)is bioactive and in sufficient concentration readily kills bacteria in vitro. Silver exhibits low toxicity in the human body, and minimal risk is expected due to clinical exposure by inhalation, ingestion, or dermal application.. Silver and silver NPs are used as an antimicrobial in a variety of industrial, healthcare and domestic applications. (Kim *et al*., 2010).

Silver NPs which are zero valent, can be a valuable alternative to ionic silver. Silver nitrate is the solid compound of silver and known by different names in different In the 19th century, silver nitrate was used to treat the burns, and it was believed that silver nitrate allows epithelization and promotes crust formation on the surface of wounds (Sintubin *et al*., 2013).

1.2.5. Application of NPs and silver NPs

Although the development and application of nanotechnology is primarily still in the research phase , the different fields that find potential performance of nanotechnology. Applying of nanotechnology for treatment , diagnosis, monitoring, and control of diseases has been referred to as nanomedicine . The most application of nanoparticle in medicine are drug delivery and cancer therapy. Nanomedicine, Nano biotechnology, Green nanotechnology, Energy

applications of nanotechnology , Industrial applications of nanotechnology Potential applications of carbon nanotubes and Nanoar (Sintubin *et al*., 2013).

Nano particles are used for site specific drug delivery. the required drug dose is used and side-effects are lowered significantly as the active agent is deposited in the morbid region only. The highly selective approach can reduce costs and pain to the patients (Lengke *et al.*,2007).

The strength of drug delivery systems is their ability to alter the pharmacokinetics and biological distribution of the drug. Nano particles are designed to avoid the body's defense mechanisms (Jha *et al*., 2009).

The development and optimization of drug delivery approaches based in NPs concerns the early detection of cancer cells or specific tumor biomarkers, and the enhancement of the efficacy of the treatments (Baptista, 2012).The immobilization of biomolecule–NP conjugates on surfaces provide general route for the development of optical or electronic biosensors .The advanced techniques of nanotechnology can help storage of energy, its conversion into other forms, ecofriendly manufacturing of materials and by better enhanced renewable energy. Nanotechnology can help in developing new ecofriendly and green technologies that can minimize undesirable pollution .

Biological methods can be used to synthesize AgNPs without the use of any harsh, toxic and expensive chemical substances (Ahmad *et al*., 2003). The use of microorganisms in the synthesis of NPs emerges as an ecofriendly and exciting approach, for production of NPs due to its low toxic, environmental permpatibility, reduced costs of manufacture, scalability, and nanoparticle stabilization compared with the chemical synthesis. Both bacteria and fungi make such an existing category of microorganisms having naturally bestowed property of reducing/oxidizing metal ions into metallic/oxide nanoparticle thereby functioning as mini nano factories (Jha *et al*., 2009). Both gram-positive

and gram-negative bacteria have been used to synthesize AgNPs (Sintubin *et al*., 2009).

The formation of extracellular and intracellular AgNPs by *E.coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* has been investigated (Lengke *et al.*,2007).The AgNPs were synthesized using a reduction of aqueous Ag^{+} ion with the culture supernatants of the fungus (Li *et al*.,2012).NPs biosynthesized when the microorganisms grab target ions from and then turn the metal ions into the element metal through enzymes their environment generated by the cell activities. It can be classified into intracellular and extracellular synthesis according to the location where NPs are formed. The intracellular method consists of transporting ions into the microbial cell to form NPs in the presence of enzymes. The extracellular synthesis of NPs involves trapping the metal ions on the surface of the cells and reducing ions in the presence of enzymes (Zhang *et al*., 2011).

Microbial source to produce the AgNPs shows the great interest towards the precipitation of NPs due to its metabolic activity. Nitrate reductase is an enzyme in the nitrogen cycle responsible for the conversion of nitrate to nitrite ,the reduction mediated by the presence of the enzyme in the organism has been found to be responsible for the synthesis (Rai *et al*., 2014). Ag-NPs are one of the most commonly used NPs both in everyday life, and in research laboratories. Ag-NPs are of interest because of the unique properties (e.g. size and shape depending optical, electrical, and magnetic properties) which can be incorporated into antimicrobial applications, biosensor materials, composite fibers, , cosmetic products, and electronic components (Jha *et al*., 2009).

Ag-NPs used as a selective coatings for solar energy absorption, as an intercalation material for electrical batteries, and as optical receptors for biolabeling (Bonsak *et al*,.2011). Ag-NPs are also commonly used in medical practice as an integral part of both surgical and nonsurgical equipment such as

wound dressings, bandages, catheters, etc.(Rai *et al.*, 2014).AgNPs have been well known for its strong inhibitory and bactericidal effects and can effectively use for the treatment of various infectious diseases (Afreen *et al*., 2011).

The application of AgNps as antifungal agents has become more common with advances of technology makes more economic ,one of the application is management of plant diseases (kim *et al*., 2009). Ag-NPs dose-dependent efficacy against *S. aureus* and *P. aeruginosa* biofilm was also demonstrated (Jena *et al*., 2012). AgNPs with an average size up to 50 nm at concentrations of 100 nM were able to inhibit the formation of biofilm s by Gram-negative and Gram-positive microorganisms almost equally (Kalishwaralal *et al*., 2010).

NPs can be use in biomedical applications to:Improve solubility - NPs can be used as carriers for hydrophobic drugs (e.g., Abraxane)

Give multifunctional capability- NPs with dual functionality can be used for diagnostic and therapeutic purposes (e.g., Fe_2O_3-Pt NPs)

Target tumors - NPs can be used to reduce toxicity of a therapeutic drug (e.g., Aurimune)

Gold NPs (AuNPs) are used in immunochemical studies for identification of protein interactions. They are used as lab tracer in DNA fingerprinting to detect presence of DNA in a sample. They are also used for detection of aminoglycoside antibiotics like streptomycin, gentamycin and neomycin. Gold nanorods are being used to detect cancer stem cells, beneficial for cancer diagnosis and for identification of different classes of bacteria (Wei *at el*.,2011) Alloy NPs exhibit structural properties that are different from their bulk samples Since Ag has the highest electrical conductivity among metal fillers and, unlike many other metals, their oxides have relatively better conductivity Ag flakes are most widely used .Bimetallic alloy NPs properties

are influenced by metals show more advantages over ordinary metallic NPs (Narayanan and Sakthivel**,** 2010).

Magnetic: Magnetic NPs like Fe3O4 (magnetite) and Fe2O3 (maghemite) are known to be biocompatible. They have been actively investigated for targeted cancer treatment (magnetic hyperthermia), stem cell sorting and manipulation, guided drug delivery and gene therapy (Narayanan and Sakthivel**,** 2010).

1.2.6. Methods of NPs synthesis

NPs can be synthesized by various methods such as physical ,chemical and biological method. The NPs can be synthesized using the top-down in physical approach and bottom-up in chemical and biological (Cao and Hu ,2009).The physical method can be useful as a nanoparticle generator for long term experiments for inhalation toxicity studies, and as a calibration device for nanoparticle measurement equipment. Evaporation-condensation and laser ablation are the most important physical approaches (Jung and Lee,2008).

Different types of physical and chemical methods are employed for the synthesis of NPs but the use of these methods requires both strong and weak chemical reducing agents and protective agents which are mostly toxic, flammable, cannot be easily disposed due to environmental issues and , a low production rate and elevated temperatures for synthesis process in addition these are capital intensive and are inefficient in materials (Rai *et al.*, 2008).The biological method for the synthesis of NPs employs use of biological agents are green algae, fungi and bacteria (Lengke *et al.*,2007). The biological method provides a wide range of resources for the synthesis of NPs. The biological agents secrete a large amount of enzymes, which are capable of hydrolyzing

metals and thus bring about enzymatic reduction of metals ions (Rai *et al*., 2014).

Despite the chemical and physical methods are able to produce large quantities of NPs with a defined size and shape in a relatively short time, they are complicated, outdated, costly, inefficient and produce hazardous toxic wastes that are harmful not only to the environment but also to human health The purpose to highlight on the biological synthesis of NPs, because of its easiness of rapid synthesis, controlled, controlling on size characteristics, reasonable, and ecofriendly approach (Danquah-Amoah and Morya,2017).

Biological method of NPs synthesis would help to remove harsh processing conditions by enabling the synthesis at physiological, temperature, pressure, and at the same time at lower cost. One of the options to achieve this goal is to use microorganisms to synthesize NPs (Ahamed *et al* 2010).

1.2.7. Biosynthesis of silver NPs by microorganisms

Bacterial and fungal synthesis of NPs is practical because bacteria and fungi are easy to handle and can be modified genetically with easiness. This provides a means to develop biomolecules that can synthesize Ag-NPs of varying shapes and sizes in high yield, which is at the forefront of current challenges in nanoparticle synthesis . Fungal strains such as Mushroom and bacterial strains such as *E .coli* can be used in the synthesis of silver NPs. When the fungus/bacteria is added to solution, protein biomass is released into the solution(Ahamed *et al* 2010).

Electron donating residues such as tryptophan and tyrosine reduce silver ions in solution contributed by silver nitrate. These methods have been found to effectively create stable monodisperse NPs without the use of harmful reducing agents (Gopinath *et al*., 2012). It was also found that this bacterium produced

the NPs with the smallest size distribution and the NPs were found mostly on the outside of the cells. It was also found that there was an increase in the pH increased the rate of which the NPs were produced and the amount of particles produced (Jha *et al*., 2009).

Biological methods can be used to synthesize Ag-NPs without the use of any harsh, toxic and expensive chemical substances (Ahmad *et al*., 2010).The use of microorganisms in the synthesis of NPs emerges as an ecofriendly and exciting approach, for production of NPs due to its low toxic, environmental compatibility, reduced costs of manufacture, scalability, and nanoparticle stabilization compared with the chemical synthesis (Sintubin *et al*., 2009).

Fungi are ideal candidates in the synthesis of metal NPs with different sizes, because of their ability to secrete large amount of enzymes This provides a means to develop bimolecular that can synthesize Ag-NPs of varying shapes and sizes in high yield, which is at the forefront of current challenges in nanoparticle synthesis. When the fungus/bacteria is added to solution, protein biomass is released into the solution. Electron donating residues such as tryptophan and tyrosine reduce silver ions in solution contributed by silver nitrate. These methods have been found to effectively create stable monodisperse NPs without the use of harmful reducing agents. (Absar *et al* .,2003) .

Both bacteria and fungi make such an existing category of microorganisms having naturally give property of reducing/oxidizing metal ions into metallic/oxide nanoparticle thereby functioning as mini Nano factories. Both gram-positive and gram-negative bacteria have been used to synthesize Ag-NPs (Sintubin *et al*., 2009). The formation of extracellular and intracellular Ag-NPs by bacteria *Escherichia coli, Pseudomonas aeruginosa*, and *Staphylococcus*

aureus (Lengke *et al.*,2007).The AgNps were synthesized using a reduction of aqueous Ag^{+} ion with the culture supernatants of *Aspergillus terreus* (Li *et al*.,2012).

1.2.8. Characterization of NPs

Nanoparticles have become an important branch of nanotechnology. A novel biosynthesis route for Silver Nanoparticles was attempted by using *Agaricus bisporus* in Iraq. Ag-NPs were spherical in shape and the average particle size was about 1-50 nm . The efficiency of mushroom for synthesis of silver Nanoparticles was found to be higher; also this method cost effective and easily scaled up for large scale synthesis. (Lee, 2007).

- electron microscopy including TEM and SEM
- atomic force microscopy (AFM)
- dynamic light scattering (DLS)
- x-ray photoelectron spectroscopy (XPS)
- powder X-ray diffraction (XRD)
- Fourier transform infrared spectroscopy (FTIR)
- matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF)
- ultraviolet-visible spectroscopy
- dual polarization interferometers
- nuclear magnetic resonance (NMR)
- Nanoparticle tracking analysis for tracking of the Brownian motion

The fastest and most popular techniques like photon-correlation spectroscopy (PCS) or dynamic light scattering (DLS), widely used to determine the size of Brownian nanoparticles in colloidal suspensions in the nano and submicron ranges. In this technique solution of spherical particles in

Brownian motion causes a Doppler shift when they are exposed against shining monochromatic light (laser) (DeAssis et al 2008).

1.2.8.1. Scanning Electron Microscopy (SEM)

This electron microscopy based technique determines the size, shape and surface

morphology with direct visualization of the nanoparticles. Therefore scanning electron microscopy offer several advantages in morphological and sizing analysis. However they provide limited information about the size distribution and true population average (Hall *et al*., 2007).

Size distribution and shape of nanomaterials can be directly acquired from SEM; however, the process of drying and contrasting samples may cause shrinkage of the specimen and alter the characteristics of the nanomaterials During the process of SEM characterization, solution of nanoparticles. This dry powder is then further mounted on a sample holder followed by coating with a conductive metal (e.g. gold) using a sputter coater. Whole sample is then analyzed by scanning with a focused fine beam of electrons (Jores *et al*., 2004).

1.2.8.2. Transmission Electron Microscope

Experimental difficulties in studying nanostructures stem from their small size,which limits the use of traditional techniques for measuring their physical properties. Transmission electron microscopy techniques can provide imaging, diffraction and spectroscopic information, either simultaneously or in a serial manner, of the specimen with an atomic or a sub-nanometer spatial resolution.. TEM imaging mode has certain benefits compared with the broad-beam illumination mode(Wang, 2001).

In the conventional TEM mode, an incident electron beam is transmitted through a very thin foil specimen, during which the incident electrons interacting with specimen are transformed to un scattered electrons, elastically scattered electrons or in elastically scattered electrons (Williams and Carter, 2009). The magnification of TEM is mainly determined by the ratio of the distance between objective lens and the specimen and the distance between objective lens and its image plane Overall, both TEM and SEM can reveal the size and shape heterogeneity of nanomaterials, as well as the degrees of aggregation and dispersion. TEM has advantages over SEM in providing (Williams and Carter, 2009).

1.2.8.3. Fourier Transform Infrared Spectrometry (FTIR)

Fourier Transform Infrared Spectrometry used in characterization of complex and specific samples it is not a trivial task to be fulfilled by chemists. The difficulty of FTIR characterization comes mainly from the high overlapping degree of the infrared absorption bands, making difficult the truthful ascription to certain functional groups, despite of the fact that up to date computer-searchable databases of spectra .

FTIR spectroscopy is widely used to study the nature of surface adsorbents in nanoparticles. Since the nanoparticles possess large surface area, the modification of the surface by a suitable adsorbate can generate different properties. The FTIR spectra of the nanoparticles, which contain some adsorbates, possess additional peaks in comparison with the FTIR pattern of a bare nanoparticle. So the property change with different adsorbates can easily be detected with FTIR spectroscopy. Due to the high surface to volume ratio, the activity at the surface of the nanoparticles would be significantly different from that of the bulk. From FTIR data it is possible to study the oxidation levels

of nanoparticles prepared at different partial oxygen pressures(Pakutinskiene *et al.,* 2007).

1.2.9. Factors influencing the bactericidal effect of Ag-NPs

1.2.9.1. Size of nanoparticles

Change in reactivity and properties of nanoparticles is attributable to their small size, compared with bulk matter. The smaller size is the larger surface-area-to volume ratio; hence, obviously the bactericidal activity of Inhibit cell wall.Ag-NPs showing multiple bactericidal actions. Activity of Ag-NPs against MDR The society for applied microbiology Ag-NPs is affected by the size of the nanoparticles. Depending on the size of the nanoparticles, large surface area comes in contact with the bacterial cells to provide a higher percentage of interaction than bigger particles (Pakutinskiene *et al.,* 2007).

Reactivity of nanoparticles is enhanced by the electronic effect produced by the interaction of nanoparticles with bacterial surface, and nanoparticles smaller than 10 nm have high percentage of interaction with bacteria. So, the bactericidal effect of Ag-NPs is size dependent .While the size dependency of bactericidal potential of nanoparticles 25 nm possessed highest antibacterial activity(Gopinath *et al.*,2010).

1.2.9.2. Shape of nanoparticles

Those bactericidal possibility about nanoparticles will be additionally impacted by their shapes, which may be indicated Eventually Tom's perusing mulling over the bacterial development restraint by differentially formed nanoparticles accounted for those impact for spherical, Pole furthermore triangular nanoparticles synthesized Eventually Tom's perusing citrate diminishment against *E.coli* at different focuses. It might have been found that round nanoparticles would that's only the tip of the iceberg animated over Pole

formed nanoparticles against *E.coli* something like that antibacterial exercises from claiming Ag-NPs need aid impacted by shape (Mallikarjuna *et al*.,2014).

1.2.9.3. Concentration of nanoparticles

Performed those study for bactericidal impact of Ag-NPs for extent 1–100 nm on Gram negative bacterium . They broke down those association about Ag-NPs with microscopic organisms toward developing those bacterial units dependent upon mid-log phase, measuring 595 nm, examined. The impact about distinctive focuses of silver ahead bacterial Growth What's more inferred that focus up to 50 µg /ml) might have been addition to bacterial growth at over that, might have no critical bacterial growth (Stephenson and Hubler *et al;*,2015).

1.2.10. Edible Mushroom (*Agaricus bisporus*)

Mushroom(s) have been promising source of nutrients and part of human diet. The carbohydrate content of mushrooms represents the bulk of fruiting bodies accounting for 50 to 65% on dry weight basis. Generally have more protein content than any other vegetable (Foulongne-Orio *et al*., 2013).

Agaricus bisporus (white button mushroom; WBM) contains high levels of dietary fibers and antioxidants including vitamin C, D, and B_{12}; folates; and polyphenols that may provide beneficial effects on cardiovascular and diabetic diseases (Muszynska *et al.*,2017) . Also *Agaricus bisporus* is an edible mushroom known for its nutritional and bio-medicinal properties. Chitin and chitosan are commonly used in the pharmaceutical industry Apart from their antimicrobial activity, are also used in wound dressings. Their action involves local pain relief (due to separating pain receptors from environmental exposure), wound healing enhancement and prevention of scaring .An important property of chitosan – its blood clotting ability – has been used in hemorrhaging wound

dressings. Chitosan can work without setting up a normal blood clotting cascade(De Castro *et al.*, 2012).

A. bisporus is a rich source of dietary fiber (chitin), essential and semi-essential amino acids and antioxidant substances (sterols, phenolic and indole compounds, ergothioneine, vitamins) (Foulongne-Orio *et al.*, 2013).

Indoles are important compounds due to their anti-cancer and anti-aging activity(Muszyńska *et al*.,2013) .*A. bisporus* a source for supplementation. The amino acids found in *A. bisporus* in the highest amounts are alanine, aspartic acid, glutamic acid, arginine, leucine, lysine, phenylalanine, serine, proline, tyrosine and threonine, They are antioxidant, antibacterial, antifungal, anti-infl ammatory, and gastric-secretion stimulatory actions(Muszyńska *et al*., 2013).

The oxidant cavity of *A. bisporus* methanol extract was also due to these bioactive compounds as most of them exhibited both antimicrobial and antioxidant activity. They exhibit a wide spectrum of biological activities which have been attributed to their strong antioxidant activity and ability to protect vital cellular structures, such as cell membranes, and also structural proteins, enzymes, membrane lipids or nucleic acids Flavonoid and phenolic compound are potent water soluble and free radical scavenger which prevent oxidative cell damage **(**Foulongne-Orio *et al.*, 2013).

Arginine pres ent in the *Agaricales* taxon should be given special attention because it is a component used in dietary supplements for patients with cancer. Arginine delays tumor growth and metastasis, and also has a benefi cial infl uence on the immunological system, body mass growth and the life-expectancy of oncological patients (Novaes *et al*., 2011)

1.2.11. Antibacterial properties

SNP have a broad antibacterial effect on a range of Gram-negative and Gram-positive bacteria Figure(1-2) (Sadeghi *et al* ;2015). Antimicrobial efficacy of depends on their size and concentration. Normally, a high

concentration leads to more effective antimicrobial activity, while particles of small sizes can kill bacteria at a lower concentration. Apart from size and concentration, shape also influences the antimicrobial efficiency of SNP (Sadeghi et al 2015), investigated the antimicrobial activity of different SNP shapes, which included silver Nano plates, silver Nano rods, and silver NPs, on *S. aureus* and *E. coli*. They found that silver Nano plates had the best antimicrobial activity(Shin and Ye, 2012).

It has also been reported that SNP combined with various antibiotics have better antimicrobial effects than SNP or antibiotics alone. (Li *et al*; 2012). for example, found a greater antibacterial effect on *E. coli* when amoxicillin and silver NPs were combined than when they were applied separately .The overall drug consumption and side-effects may be lowered significantly by depositing the active agent in the morbid region only and in no higher dose than needed(Chen *et al* .,2013). Targeted drug delivery is intended to reduce the side effects of drugs with concomitant decreases in consumption and treatment expenses. Drug delivery focuses on maximizing bioavailability both at specific places in the body and over a period of time. This can potentially be achieved by molecular targeting by Nano engineered devices. The NPs of metals like platinum, silver, and gold are widely applicable in diagnostic sensors, as antimicrobials, and as agents in drug and gene delivery (Bhowmik *et al*., 2010) .Currently, there is a growing demand for the devising of environmentally agreeable protocols for the synthesis of nanomaterials that would avoid the hazardous byproducts associated with current physicochemical processes. (Kumar *et al*., 2011) .

Although the antimicrobial effect of SNP has been widely studied, the exact mechanism of NSPs is still elusive. It is widely accepted. The formation of free radicals and subsequent free radical-induced membrane damage is another potential mechanism, it has also been found that SNP

can release silver ions and interact with the thiol groups of many vital enzymes and phosphorus-containing bases, thus inhibiting some functions in cells, such as preventing cell division and DNA replication. In addition, SNP may modulate signal transduction through changing the phosphotyrosine profile of bacterial peptides for the potential antibacterial mechanism (Blanco -Andujar *et al.*, 2014).AgNPs accumulation on the membrane cell creates gaps in the integrity of the bilayer which predisposes it to a permeability increase and finally bacterial cell death .

The antibacterial study of AgNPs was carried out on human pathogenic *E coli* by (Mahmood,2012). AgNPs have been shown to interact with bacterial membrane proteins, intracellular proteins, phosphate residues in DNA, and to interfere with cell division, leading to bacterial cell death (Sondi and Salopek-Sondi, 2004).The application of AgNPs as antifungal agents has become more common with advances of technology makes more economic ,one of the application is management of plant diseases (kim *et al*., 2009).

The synergistic effect of synthesized AgNPs as antifungal was evaluated and clearly revealed that AgNPs can be effectively used against various plant pathogenic fungi. (Mallmann *et al*.,2015).AgNPs with an average size and concentrations of 50 nM were able to inhibit the formation of biofilms by Gram-negative and Gram-positive microorganisms almost equally (Kalishwaralal *et al*., 2010).The antiviral effects of Ag-NPs, most publications have suggested that Ag-NPs could bind to outer proteins of viral particles, resulting in inhibition of binding and the replication of viral particles in cultured cells. Although the antiviral mechanism of Ag-NPs has not been fully known yet, Ag-NPs are still suggested as potential antiviral agents in the future (Galdiero *et al*., 2011).

Silver NPs (AgNps) are one of the most commonly used NPs both in everyday life, and in research laboratories. AgNps are of interest because of the unique properties (e.g. size and shape depending optical, electrical, and magnetic properties) which can be incorporated into antimicrobial applications, biosensor materials, composite fibers, , cosmetic products, and electronic components (Sondi and Salopek-Sondi, 2004).

AgNps used as a selective coatings for solar energy absorption, as an intercalation material for electrical batteries, and as optical receptors for biolabeling (Bonsak *et al*,.2011). AgNps are also commonly used in medical practice as an integral part of both surgical and nonsurgical equipment such as wound dressings, bandages, catheters, etc.(Singh *et al*., 2014).

The antibacterial study of AgNps was carried out on human pathogenic *E coli* by (Mahmood, 2012). AgNps have been shown to interact with bacterial membrane proteins, intracellular proteins, phosphate residues in DNA, and to interfere with cell division, leading to bacterial cell death (Sondi and Salopek-Sondi, 2004).

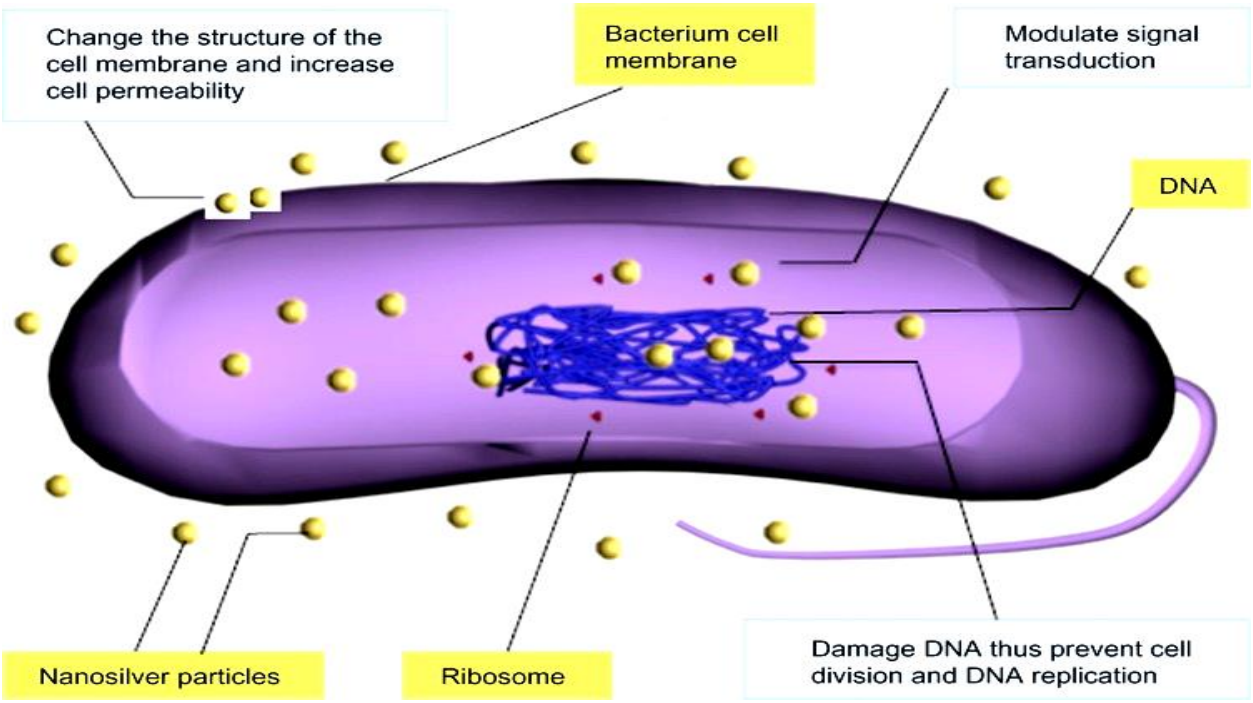


Figure (1.2)Various modes of action of silver nanoparticles on bacteria.

1.3. Multidrug Resistance

Multidrug Resistance MDR is antimicrobial resistance shown by a species of microorganism to multiple antimicrobial drugs, other types include MDR viruses, fungi, and parasites (resistant to multiple antifungal, antiviral, and anti- parasitic drugs(of a wide chemical variety). Recognizing different degrees of MDR, the terms extensively drug resistant (XDR) and pan drug-resistant (PDR) have been introduced. Infection of bacteria that pose a physical and rapid risk can cause patients in hospitals, especially patients in intensive care units. Infection caused by dendritic strains is associated with increased morbidity, mortality and prolonged hospitalization. Thus, these bacteria pose not only a threat to global public health, but also create a significant burden on health care systems. These bacteria pose a significant public health risk because of the limited treatment options available, as well as the lack of newly developed antimicrobial drugs(Cerceo *et al.*,2016).

In fact, some strains have become virtually resistant to all the factors normally available. A notorious condition is methicillin-resistant *S. aureus* (MRSA), a resistance Not only for methicillin (which was developed to fight against penicillinase-producing *S.aureus*, But also commonly to aminoglycosides, macrolides, tetracycline and chloramphenicol. These strains are also resistant to antiseptics, and MRSA can act as a key The source of acquired infections in hospitals. The old antibiotic, Vancomycin, was revived Treatment of urinary infections. However, the resistance to convert to Vancomycin is now quite Common in the intestinal tract finally found its way to MRSA in 2002, although such strains Still rare (de Lencastre *et al*.,2007).There are many factors which could be contributed to the existence and spread of MDR gram-negative bacteria such as the:

Overuse of existing antimicrobial agents, which has led to the development of adaptive resistance mechanisms by bacteria; a lack of responsible antimicrobial stewardship such that the use of multiple broad-spectrum agents has helped perpetuate the cycle of increasing resistance and a lack of good infection control practices Microorganisms can resist antibiotics through many defense mechanisms. These mechanisms can be expressed in the following (Cerceo *et al*.,2016):

1.3.1.Producing enzyme modification that breaks down antibiotics

Bacteria can produce an enzymes which most important clinically that are inactive antibiotics are called beta-lactamase. This enzymes can decompose the beta-lactam loop of β-lactamase. Other important enzymes can destroy chloramphenicol by producing chloramphenicol acetyl transferase . Beta-lactamases are enzymes that are major cause of bacterial resistance to the beta-lactam family of antibiotics such as penicillins, cephalosporins, cephamycins, and carbapenems. These enzymes catalyze the hydrolysis of the amide bond of four-membered beta-lactam ring and render the antibiotic

inactive against its original cellular target, the cell wall transpeptidase. On the basis of their primary structure, beta-lactamases are grouped into four classes A, B, C, and D enzymes (Eftekhar *et al* .,2012).

1.3.2.Changes in cell permeability

Gram-negative bacteria can resist antibiotic by developing a permeability barrier. The outer membrane contains special protein holes known as porins, are in the form of channels filled with water. They are non-specialized in exchange . The reduction of the number of holes in the outer membrane of some intestinal bacteria leads to a reduction in the flow of the antibody through these membranes (Wilson, 2013).

One of the antibiotic to anti-betalactamase in the gram-negative bacilli changes the proteins of the porine into the cell membrane causing reduced permeability.(There are five families of bacterial drug efflux pumps: the ATP-binding cassette (ABC) superfamily ,the major facilitator superfamily (MFS) the multidrug and toxic compound extrusion (MATE) family , the small multidrug resistance (SMR) family (a) subgroup of the drug/metabolite transporter superfamily(Kuroda *et al.,* 2009) and the resistance-nodulation-division (RND) superfamily (Jack *et al*.,2001).

1.3.3.Alteration in efflux mechanisms

Many organisms can show resistance to β-lactam, the aminoglycosides group and macrolides by this tetracycline resistance mechanism are usually mediated by Efflux. Efflux pumps naturally occur in bacterial cells. They are primarily concerned with waste removal, but changes in morphology can enable them to remove antimicrobial drugs. (Sun *et al* .,2014).

1.3.4.Alteration in the structure of target site

The target site of the antibiotic to betalactamase is a group of penicillin-binding protiens (PBPs) involved in the final phase of the peptidoglycan process), and the change in target site is a mechanism of resistance to Betelactam antibiotic. PBPs represent the site of association with anti-Betelactam (Atlas, 1995). Bacteria are capable of not only altering the enzyme targeted by antibiotics, but also by the use of enzymes to modify the antibiotic itself and thus neutralize it. Example for the target-altering pathogens are *S. aureus*, vancomycin – resistant , enterococci and macrolide-resistant *Streptococcus*, while examples of antibiotic-modifying microbes are *P. aeruginosa* and amino glycoside -resistant *Acinetobacter baumannii*. (Hussain.,2015).

1.3.5.By pass of metabolic pathway

Some antibiotics work on enzymes in metabolic pathways. Microbial cells can develop a novel metabolic pathway that bypasses the effect of the antimicrobial, so rendering it ineffective. Resistance to sulphonamides and trimethoprim is mediated by such metabolic bypass ,in this case bacteria tends to synthesis altered dihydropteroate synthetase and dihydrofolate reductase, then reduced susceptibility and affinity for sulphoamides and trimethoprim, respectively (Floyd *et al*.,2010).

1.4. Virulence factors

1.4.1 Biofilm Formation

A biofilm is any group of microorganisms in which cells stick to each other and often also to a surface. These adherent cells become embedded within a slimy extracellular matrix that is composed of extracellular polymeric substances (EPS). The EPS components are produced by the cells within the biofilm and are typically a polymeric conglomeration of

extracellular DNA, proteins, and polysaccharides. Because they have three-dimensional structure and represent a community lifestyle for microorganisms, biofilms are frequently described metaphorically as "cities for microbes. Biofilm(s) are resistant to physical forces such as the shear forces produced by blood flow and washing action of saliva. Organisms within biofilm can withstand nutrient deprivation, pH changes, oxygen radicals, disinfectants, and antibiotics better than plank tonic organisms. Biofilm is also resistant to phagocytosis and the phagocytes that attempt an assault on the biofilm may actually do more harm to surrounding tissues than to the biofilm itself(Jayaraman *et al*., 2008).

MRSA has an ability to form biofilm which is an important virulence mechanism that complicates infections, especially those involving foreign materials like catheters and prosthetic joints. One study found that between 2006 and 2007, 56 % of all device-related infections caused by S. aureus were MRSA infections in the US (Hassan *et al*., 2011).

Biofilm(s) have been defined as surface-attached communities of cells encased in an extracellular polymeric matrix that are more resistant to antibiotics. Biofilm accumulation was recently isolated in a strain of MRSA from a burn unit (Gurunathan *et al*., 2014).

MRSA transitions between planktonic and biofilm stages, defined as a multicellular response to coordinate expression of genes required for biofilm in a population density dependent manner (Bordi and de Bentzmann, 2011).

The most studied *P. mirabilis* biofilms are those formed when the organism is grown in urine, resulting in unique features including swarming cells and struvite and hydroxyapatite crystals upon growth in urine. Factors relevant to *P. mirabilis* biofilm formation include adhesion factors, proteins involved in LPS production, transporters, transcription factors, two component systems, communication factors and enzymes(Jayaraman *et al*., 2008).

P. mirabilis biofilm research will lead to a better understanding of the disease process and will subsequently lead to the development of new prevention, and treatment options. This step includes detachment of bacteria from the mature biofilm and their dispersal, that is the transmission of the bacteria to a planktonic state, which can lodge at distant site and form biofilm. (Sato *et al*. 2013). modulation of type IV bundle-forming pili which is a crucial surface structure in enter pathogenic *E. coli* and aggregative adherence fimbriae in enter aggregative *E. coli* result in the detachment of bacteria from the biofilm and surface (Gurunathan *et al*., 2014).

The *P. aeruginosa* growth within human body can be asymptomatic until the bacteria form a biofilm, which overwhelms the immune system. These biofilm s are found in the lungs of cystic fibrosis and primary ciliary dyskinesia, and can prove fatal.(Sato *et al*. 2013).Biofilms of *P. aeruginosa* can cause chronic opportunistic infections, which are a serious problem for medical care in industrialized societies, especially for immune compromised patients and the elderly.

They often cannot be treated effectively with traditional antibiotic therapy. Biofilms seem to protect these bacteria from adverse environmental factors. *P. aeruginosa* can cause nosocomial infections and is considered a model organism for the study of antibiotic-resistant bacteria. Recent studies have shown that the dispersed cells from *P. aeruginosa* biofilms have lower levels and different physiologies from those of planktonic and biofilm cells. Such dispersed cells are found to be highly virulent against macrophages, but highly sensitive towards iron stress, as compared with plank tonic cells. (Chua *et al*.,2015) **.**

The ability of bacteria to develop antibiotic resistance by forming biofilms is a major cause of medical implant-associated infections and results in prolonged hospitalization periods and patient mortality (Ansari *et al*., 2014). Biofilm

formation that result by the aggregation of microbial cells, which produce a matrix of polymeric compounds called Extracellular Polymeric. AgNps have been well known for its strong inhibitory and bactericidal effects and can effectively use for treatment of various infectious diseases (Afreen *et al*., 2011).

Due to the lack of effective anti-biofilm antibiotics, novel alternative compounds or strategies are urgently required. Nanotechnologies have become a promising tool for biofilm prevention and control (Sadekuzzaman *et al*., 2015). AgNPs dose-dependent efficacy against *S. aureus* and *P. aeruginosa* biofilm was also demonstrated (Jena *et al*., 2012).Catheters is another important virulence mechanism for MRSA and others pathogen biofilm formation especially on medical implants . Bacterial cells show much greater resistance to antibiotics than free living cells, biofilm also help micro-organisms evade host immune responses associated with human diseases as osteomyelitis chronic wound infections and cystic fibrosis (Afreen *et al*., 2011).

It is reported that nearly 65% of all nosocomial infections in USA are associated with biofilm. A recent study by Thiele and her coworkers(Gurunathan *et al* .,2014)shows the first systems biology approach to identifying candidate drug targets for treating *P. aeruginosa* in biofilm. Four potential incentives behind the formation of biofilms by bacteria during infection are considered: (1) defense, (2) colonization, (3) community, (4) biofilm as the default mode of growth (Kim *et al*.,2010). The number of infections associated with antibiotic-resistant bacteria are continuously increasing. Microorganisms growing in biofilm s cause many of these infections (Barraud *et al*.,2015). Bacteria able to form biofilm are biliary tract infections, cystic fibrosis, dental caries, endocarditis, otitis and periodontal diseases. Moreover, several infections may be associated with foreign body material such as contact lens, sutures, artificial heart valves, catheters and orthopedic prostheses(Donlan *et al.*, 2012).The sites of infections may be different but the

characteristics mechanism for biofilm formation and development of resistance causative agent are similar(Sasirekha *et al*., 2012) .

1.4.2. ATPase Inhibitors

F-ATPase, also known as F-Type ATPase (also called ATP synthase), is an ATPase found in bacterial plasma membranes, in mitochondrial inner membranes (in oxidative phosphorylation, where it is known as Complex V), and in chloroplast thylakoid membranes. It uses a proton gradient to drive ATP synthesis by allowing the passive flux of protons across the membrane down their electrochemical gradient and using the energy released by the transport reaction to release newly formed ATP from the active site of F-ATPase. In some bacteria, sodium ions may be used instead. F-ATPase consists of two domains: the Fo domain, which is integral in the membrane and the F1, which is peripheral (on the side of the membrane that the protons are moving into(Glavinas *et al.*,2008)

ATPases are enzymes which catalyze the hydrolysis of ATP to form ADP, releasing energy which can be used to drive other reactions and transmembrane ion transport. As a result, ATPase Inhibitors can have effects on a multitude of biological processes and metabolic pathways. ATPase, adenosine triphosphatase areaclassof enzymes that catalyze the decomposition of ATP into ADP and a free phosphate ion. This dephosphorylation reaction releases energy, which the enzyme (in most cases) harnesses to drive other chemical reactions that would not otherwise occur. This process is widely used in all known forms of life. (Jung & Lee, 2008).Transmembrane ATPase import many of the metabolites necessary for cell metabolism and export toxins, wastes, and solutes that can hinder cellular processes. An important example is the sodium-potassium exchanger (or Na^+/K^+ATPase) that maintains the cell membrane potential. And

another example is the hydrogen potassium ATPase (H^+/K^+ATPase or gastric proton pump) that acidifies the contents of the stomach.

This enzyme works when a proton moves down the concentration gradient, giving the enzyme a spinning motion. This unique spinning motion bonds ADP and P together to create ATP. ATP syntheses can also function in reverse, that is, use energy released by ATP hydrolysis to pump protons against their electrochemical gradient(Glavinas *et al.*,2008)

1.4.3 . Antibacterial activity with detergents and ATPase inhibitors.

To understand whether the antibacterial activity of nano-Ags was associated with altered membrane permeability or the action of an energy-dependent protective function including multidrug-resistant pumps or repair systems, the antibacterial susceptibility of nano-Ags was examined in the presence of detergents or ATPase-inhibiting agents. (Jung & Lee, 2008). NaN3 and N,N9-dicyclohexylcarbodiimide (DCCD) ,Tris and Trion X-100were used as inhibitors of ATPase (Linnett and Beechey, 1979).

CHAPTER TWO

MATERIALS

and

METHODS

2. Materials and Methods

2.1. Samples collection

This study was carried out during the period (October 2016 to February 2017).Samples were taken from 250 inpatients , their ages ranged between (20 -65 years) who admitted to the hospital of Maternity and Pediatric and women hospitals in AL-Diwaniyah city. The samples included urine ,catheters, wound ,ear and boils .The samples were collected in sterilized containers and sent immediately to the laboratory for further study.

2.2 Materials

2.2.1. Laboratory equipment's and instruments:

The equipments and instruments which used the study are listed in (table 2-1).

Table(2-1) Equipment and instruments used in the study:

Equipment and instrument	**Manufacturers company (country)**	
Autoclave	Hiclave	(Japan)
Digital camera	Sony	
Compound light microscope	Olympus	
UV-Visible Spectroscopy	Bruker,Tenso	
Sensitive balance	A& D co.	
Shaker incubator	Lab-line	(USA)
Vortex	Melrosee park	
Micropipette	Oxford	
Millipore filter (0.22μm)	Difco	
Laminair-flow cabinet,lab safely cabinet	GallenKamp	(UK)
Centrifuge	Hettich	(Germany)

High speed centrifuge- Hettich (Germany)		
Incubator	Memmert	
Incubator shaker		
Micro ELISA Auto Reader		
Plastic tissue culture plate(96-well flat bottom)		
pH-meter	Ino-lab.	
Screw capped bottles (30 ml).	Hirschmann	
Fourier transmission infrared (FTIR)	Karl kolb	
Water bath	Kottermann	
Distillator	LabTech	(Korea)
Transsmion electronic microscope	HRTEN	
Hot plate with magnetic stirrer	L.I.P	(England)
Scanning electronic microscope	Inspect S50/ FEl-	Netherland

2.2.2.Biological and Chemical materials

The chemical and biological materials used in this work with their remarks are listed in table (2-2)

Table (2-2) The biological and chemical materials used with their remarks:

Material	Manufacturers company (country)	
$AgNo_3$ (silver nitrate-purity 99.99%)	Sigma (Germany)	
Iodine	Mast Diagnostic	(USA)
Urea		
Normal saline	Difco	

Glucose ($C_6 H_{12} O_6$)		
Peptone		
Acetic acid	BDH (England)	
Dipotassium hydrogen phosphate (K_2HPO_4)		
Ethanol (96%)		
Potassium hydroxide (KOH)		
Sodium azide		
Kovac's reagent		
potassium hyDidrogen phosphate (KH_2PO_4)		
Sodium chloride (NaCl)		
Sodium citrate		
Sodium hydroxide (NaOH)		
Tris –methyle amine		
Glycerol ($C_3H_8O_3$)	Fluka (Switzerland)	
Gram Stain Kit		
Barium chloride ($BaCl_2$)		
N,N,N,N-tetramethyl paraphenylen diamine dihydrocholride		

2.2.3. Culture Media

The culture media used in this study with their remarks are listed in table (2-3)

Table (2-3): Culture media used in this study

Media	Manufacturer's company (country)
Blood agar Base	Oxoid (England)
Brain Heart Infusion broth	
MacConkey agar	
Muller Hinton agar	
Mannitol salt agar	
Nutrient agar	
Nutrient broth	
Tryptic soy agar	
Tryptic soy broth	
Peptone water broth	
Urea agar	

2.2.4. Commercial diagnostic kits

The diagnostic kits used in this study with their remarks are listed in table(2-4)

Table 2-4: Kits used in this study.

No.	Kits	Manufacturer's (country)
1	API 20 NE Kit	Biomerieux, France
2	API 20E kits	
3	API staph Kit	

2.2.5. Standard bacterial strains

Standard bacterial strains and their source used in this study are listed in table (2-5)

Table(2-5): Standard bacterial strains used in the characteristics study

Standard bacterial strains	Characteristic	Source
Mithcilline Resistant Staph aureus (ATCC 43300	**- Methicillin-resistant *Staphylococcus aureus* ; GAR-936; Linezolid; Quinupristin/dalfopristin**	American type culture collection
***Eschershia coli* (ATCC 28739)**	***Escherichia coli* Produces OXA-48 Quality control strain -Carbapenem-Resistant Enterobactericeae**	
***Pseudomonas aeruginosa* (ATCC 27853)**	***Pseudomonas aeruginosa* (*ATCC 27 853*)-Tobramycin has a narrow spectrum of activity and is active and Rifampicin resistant**	
***Proteus mirabilis* (ATCC 16404)**	**Sequencer or array type Sequencer; Roche 454- R.** Nitrofurantoin 100%	

2.2.6. Fungal isolate

The basidiomycete fungus which used in the study is Edible Mushroom *(Agaricus bisporus)* collected from Oriental market and brought into the laboratory for further process**.**

2.2.7 Antibiotic discs

Antibiotics discs used in this study with their remarks that used in this study in (table 2-6)

Table2-6 :Antibiotics discs used in this study with their remarks.

Antimicrobial Class	Antimicrobial Subclass	Agents Included Generic Names	Symbol	Disc Content	Manufactures company (country)
Penicillins	Penicillin	Penicillin G	**P G**	unit 10	**Hi Media (India)**
Penicillins	Aminopenicillin	Ampicillin	**AMP**	10 µg	
Penicillins	Ureidopenicillin	Piperacillin	Pip	100 µg	
Penicillins		Doxacyllin	Dox	30 µg	
β-lactam/ β-lactamase inhibitor combinations		amoxicillin-clavulanic acid	A/C	20/10 µg	
Cephems (parenteral)	Cephalosporin III	Cefotaxime	CTX	30 µg	
	Cephalosporin IV	Cfepime	FEP	30 µg	
	Cephamycin	Cefoxitin	FOX	30 µg	
monobactams		Aztreonam	ATM	30µg	
Penems	Carbapenem	Imipenem	IPM	10 µg	
Aminoglycosides		Gentamicin	GM	30 µg	

		Amikacin	Ak	30 µg	
Ansamycins		Rifampin	RA	5 µg	
Quinolones	Quinolone	Nalidixic acid	NA	30 µg	
	Fluoroquinolone	Ciprofloxacin	CIP	10 µg	
Folate pathway inhibitors		Co-trimethoprim	Cm	25 µg	
Phenicols		Chloramphenicol	C	30 µg	
		Nitrofurantoin	Nit	300 µg	

2.2.8. Antibiotic powders:

Antibiotics used in this study with the remarks are listed in table (2-8)

Table (2-7) Antibiotic powders used in this study

Antibiotic	Antibiotic Symbol	Manufacturer's company country)
Penicillin G	P G	Ajainta (India)
Ampicillin	AMP	Ajainta (India)
Cefipime	CPM	SDT, Iraq
Chloramphenicol	C	Ajainta (India)
Rifampicin	RV	Ajainta (India)
Piperacillin	PIP	A.P.m. (Jordan)

2.3. Methods

2.3.1. Sterilization Methods(Benson,2002).

A- Wet-heat sterilization

The Ready and synthetic culture media were sterilized in the Autoclave with a temperature of 121 ° C for 15 minutes.

B - Dry hot sterilization

The glasses used in the oven were heated at 120 ° C for an hour and a half.

C- Filtration sterilization

The heat-sensitive materials and solutions were sterilized by fine filters (Millipore diameter 0.22 μm) like antibiotics and 0.1 μm to filtrate mushroom (Benson, 2002).

2.3.2. Preparation of reagents, solutions and stains

2.3.2.1. Reagents

2.3.2.1.1. Catalase reagent

Hydrogen peroxide 3% was prepared from the stock solution and used for detection of ability of isolates to produce catalase enzyme (Forbes *et al*., 2007).

2.3.2.1.2. Oxidase reagent

The reagent was prepared freshly in a dark bottle by dissolving 0.1 gm of Tetramethyl p-phenyl diamine- dihydrochloride in 10 ml distilled water(Forbes *et al*., 2007).

2.3.2.1.3 Kovacs reagent

It was prepared by dissolving 5 gm of P-dimethyl-aminobenzylaldehyde in 75 ml of amyl alcohol. 25 ml of HCl is added to this mixture. The reagent was placed in dark bottle until use .This reagent was used for Detection of Indole ring (Forbes *et al*., 2007).

2.3.3. Preparation of buffers and solutions

2.3.3.1. Phosphate buffer solution (PBS)

This buffer consisted of 18 gm of NaCl, 0.34 gm of KH_2PO_4 and 1.12 gm of K2HPO4 were all dissolved in 90 ml of D.W, and then completed to 100 ml with D.W. solution was autoclaved at 121˚C for 15 min, and stored at 4˚C until used. (Forbes *et al*., 2007).

2.3.3.2 McFarland 0.5 Turbidity Standard:

It is composed from mixed of 0.05 ml of 1.175% $BaCl_2$ solution and 9.95 ml of 1% (0.36N) H_2SO_4 solution, the turbidity of a $BaSO_4$ solution was adjusted at 625 nm to be 0.08- 0.10, and stored in closely sold dark tube at room temperature. It is used to approximate the turbidity of bacterial suspension (CLSI, 2016).

2.3.3.3. Sodium Acetate Solutions 2%

This solution was prepared by dissolving 2 gm of **sodium acetate** in in 90 ml of D.W, and then completed to 100 ml with D.W. It was used for Detection of biofilm formation (Forbes *et al*., 2007).

2.3.3.4. Triton X-100

It was prepared 0.001%of Triton X-100 in 90 ml of D.W, and then completed to 100 ml with D.W. used for Detection of ATPase enzyme

(Hawng *et al*.,2012).

2.3.3.5. Sodium Azide(NaN3)

It was prepared 0.001%of NaN_3 in 90 ml of D.W, and then completed to 100 ml with D.W Its was used for Detection of ATPase enzyme (Hawng *et al*.,2012).

2.3.3.6.Tris buffer

This buffer was prepared by dissolving 30µM Tris-OH in 800 ml distilled water, the pH was adjusted to 8 with HCl and completed to one litter by distilled water then sterilized by autoclaving and stored at 4˚C until used . Itis was used for Detection of ATPase enzyme (Hawng *et al*.,2012).

2.3.4. Antibiotic Solutions

A stock solution and at a final concentration of (10mg/ml), for each by dissolving 0.1mg of antibiotics in 9 mL distilled water of for Ampicillin, and Penicillin G dissolving in PBS then supplemented to 10 ml of distilled water, with Cefipime in 9 ml of distilled water and then completed to 10 ml. Ethanol used to prepared chloramphenicol stock solution(10mg/ml) and Methanol to prepared Rifampicin stock solution(10mg/ml) . these solutions were sterilized using a 0.22 µm micro-filter and kept in the refrigerator until use and for as (CLSI, 2016) .

2.3.5. Stains:

(Gram Stain)

All the bacterial isolates were examined under light microscope after stained by Gram stain was prepared according to the method of stain and differentiate the bacteria based on their reaction to stain (MacFaddi, 2000).

2.3.6.Biochemical tests

2.3.6.1. Coagulase test (tube coagulase test)

This test was used to detect the ability of tested bacteria to produce the Coagulase which is an enzyme-like protein that clots oxalated or citrated plasma. The test was performed as the following:

Citrated human plasma were diluted 1:5 then mixed with an equal volume of an overnight bacterial broth culture, incubated at 37ºC. If clots form in 1-4 hours, the test is positive. A tube of plasma mixed with sterile broth was

included as control (Brooks *et al*., 2007).

2.3.6.2.Coagulase slide test

was used to detect the bound coagulase enzyme (clumping factor)by mixing one drop of sterile distilled water with one colony of bacteria on a glass slide then one drop of human plasma was added and mixed carefully and the result was read after 10-15 minutes. A positive and negative controls were prepared by the same way for comparison.

2.3.6.3. Oxidase test

A strip of filter paper (Whatman No.1) was soaked with a little freshly made 1% solution of tetramethyl-P-phenylene-diamine dihydrochloride, and then the colony to be tested was picked up with a sterile wooden stick and smeared over the filter paper. A positive result was indicated by an intense deep-purple color which appeared within 5-10 seconds. (MacFaddin, 2000).

2.3.6.4.Catalase test

Using sterile needle, a 24 hours-old bacterial colony was placed on clean glass slide and drop of 3% H2O2 solution was added to it. Immediate release of oxygen babbles indicated as appositive result (MacFaddin, 2000).

2.3.6.5. Indole Test:

Peptone water was inoculated with a young culture bacteria and incubated at 37 ˚C for 48 hr Kovacs reagent (0.5 ml) was added. A red color in the alcohol layer indicated a positive reaction (MacFaddin, 2000).

2.3.7. Ready-prepared culture Media

Media used in this study listed in table (2-3) were prepared accordance with the manufacturer's instructions fixed on their containers. All the above media were sterilized in the autoclave at 121˚C for 15 minutes.

2.3.8. Maintenance medium

The bacterial isolates were preserved on nutrient agar slant at 4˚C. The isolates were maintained monthly during the study by culturing on new culture media to preserve the bacterial isolates at -20˚C for long term storage a prepared of nutrient broth as a basal medium, supplemented with 15% glycerol. After autoclaving at 121˚C for 15 minutes, and cooling at 56˚C in water bath, it was distributed in 5 ml amounts in sterile tubes, and then kept at 4 ˚C until used. This medium was used (Forbes *et al*., 2007);(MacFaddin, 2000).

2.3.9. The identification of the bacterial isolates using system strips

2.3.9.1. Preparation of the inoculum.

A single isolated colony (from a pure culture) was transferred to the test tube which contained 5 ml of normal saline, mxed well using the mixer . The turbidity of bacterial suspension was compared with McFarland standard no. 0.5

2.3.9.2.Preparation of the strip

- An inoculum box (Tray and Lid) was prepared by distributing 5ml of sterile distilled water to the wells of the tray to provide the humidity for bacterial growth.
- The strip was removed from its package and placed in the tray.
- The strip was fill up (up to the brim) with the bacterial suspension by Pasteur pipette.
- the sterile oil was added into the ADH, LDC, ODC, H2S and URE compartments. Put some drops of water in the tray and put the API Test strip and close the tray.

- Mark the tray with identification number (Patient ID or Organism ID), date and your initials.
- Incubate the tray at 37^{o}C for 18 to 24 hours.
- **2.3.9.3.Addition of reagents**

After the incubation period was finished, the following reagents was Added to these specific compartments

VP test : One drop of reagent VP1 (40 % potassium hydroxide), then one drop of reagent VP2 (5% alfa- nephthol).

NIT test : One drop from NIT1 and NIT2 .

PAL test : One drop from ZYMA and ZYMB reagents .

1. TDA: Put one drop of Ferric Chloride
2. IND: Put one drop of Kovacs reagent
3. VP: Put one drop of 40 % KOH (VP reagent 1) & One drop of VP Reagent 2 (α-Naphthol) (you have to wait for 10 minutes before telling it negative).

4. NIT test : One drop from NIT1 and NIT2

2.3.9.4. Reading the results

- After 10 minutes from the addition of the reagents, the results were read according to the (Appendix-1) supplied by the Api Staph system .
- The results were recorded on the results sheets .
- after words the identity of test bacteria (Genus and Species) was interpreted using the analytical profile index supplied by the manufacturing company (Bio-Merieux).

2.3.10. Antibiotics susptibilty test :

The selection of antibiotic disks and interpretation of zones of inhibition were performed according to CLSI(2016).

- From an overnight culture plate, 4-5 colonies of bacterial isolate were picked up by sterilized inoculating loop and emulsified in 5ml of sterile

normal saline until the turbidity is approximately equivalent to that of the McFarland No. 0.5 turbidity standard .

- A sterile swab was dipped into the bacterial suspension, any excess fluid was expressed against the side of the tube.
- The surface of a Mueller-Hinton agar plate was inoculated by bacterial isolate as follows: The whole surface of the plate was streaked with the swab, then the plate was rotated through a 45º angle and streaked the whole surface again; finally the plate was rotated another 90º and streaked once more.
- By a sterile forceps the antimicrobial disc was picked up and placed on the surface of the inoculated plate. The disc was pressed gently into full contact with the agar.
- The plates were incubated at 35ºC for 18-24 hours. After overnight incubation clear. The diameter of growth inhibition zones were measured by using transparent ruler. The results were compared with the minimum inhibition diameter of the CLSI (2016).

2.3.11.Preparation of crude extract of edible mushroom(*Agaricus bisporus)*

Ag-NPs used were synthesized from edible mushroom fresh mushrooms *Agaricus bisporus* (white button mushrooms) were procured from commercial sources. About 20 gm. of the mushroom was weighted out and washed thoroughly with double distilled water. then crushed and transferred to a beaker containing 100ml of sterile distilled water. This mixture is stirred for about 2 hours and then filtered using Whatt man No.1 filter paper. The extract of mushroom can be preserved for further experiments by storing it at 40° C(Sudhakar *et al*.,2014).

2.3.12.Preparation of biosynthesized Silver Nanoparticles by using Edible Mushroom

Samples of different concentrations of mushroom extract and AgNO3 was prepared to derive the most efficient preparatory method for efficient and faster synthesis of silver nanoparticles. Sample no.**1** was prepared by using 50ml of mushroom extract which was added to 50 ml of 1mM AgNO3 aqueous solution. Sample no. **2** was prepared using 10 ml of mushroom extract was added to 40 ml of distilled water into which 1mM of AgNO3 (approximately 8.5 mg) was added. Sample no.**3** was prepared using 450 ml of distilled water was taken in a conical flask into which 50 ml of mushroom extract was added. The above mixture is stirred well and into it about 1mM of AgNO3 (approximately 8.5 mg) was added. Control sample was prepared by mixing 40 ml of 1mM AgNO3 (approximately 8.5 mg) directly to 10 ml of sterilized soil extract .

The formation and development of silver nanoparticles was indicated by change yellow color and the reduction is completed in 24hr.All above samples were incubate in shaker incubation at $37°C$ and 150 rp/min for 24 hr. The concentration of nanoparticles was examined under UV-visible spectrophotometer (Lee *et al*.,2007).

2.3.13. Characterization of Ag-NPs

So many properties of the biosynthesized of SNPs were investigated, optimization of physical and chemical conditions is important for determination the rate of synthesis and stability of SNPs, so that different parameter such as PH(8-11),temperature(20-50 C^0) ,culture media and AgNo3(1-5mM) concentration.(katetaa *et al*., 2014) .

2.3.13.1.Color changing and UV-Visible Spectroscopy

Synthesis of Ag-NPs using *Agaricus bisporus* extract was observed by the color change from yellow to dark brown within 24 hours. Further, it has been characterized by UV-Visible Spectroscopy. The process of reaction between AgNO3 and mushroom extract was monitored by UV-Visible spectra with resolution of 2.0 nm, between the wavelength 200 to 700 nm (Absar *et al*., 2003 ;Karwa *et al*., 2011).

2.3.13.2 .Scanning and Transmission Electron Microscopes (SEM,TEM)

Characterization the shape and size of biosynthesized(Ag-NPs) were done by analyzing with Scanning electron microscope (Inspect S50/ FEl-Netherland) at Al-Kufa university college of science / microscopy unit . The dark brown colored silver nanoparticles were obtained washed and centrifuged for 20 min at 10.000 g. The pellets thus obtained was washed many times to remove any residual silver nitrate. The pellets of Ag-NPs allowed to dry completely. Transmission electron microscope analysis was used measure the size of Ag-NPs in Iran at Isfahan university/ engeeneric college (HRTEN).The silver nanoparticles synthesized using mushroom extract was allowed to dry completely and grounded well to a powder .For TEM the specimen is normally required to be completely dry since the specimen is at high vacuum.

2.3.13.3. Fourier transmission infrared (FTIR) spectroscopy measurements

The residual solution of Ag-NPs by *Agaricus bisporus* extract after reaction was centrifuged at 10000 rpm for 15 min to remove the unwanted impurities and then supernatant is again centrifuged 10 time for 15 min the resulting solution was repeated. Pellets obtained were washed with deionized water to get the pure Ag-NPs. The sample was completely air dried at room temperature; the collected powdered Ag-NPs were taken to FTIR analysis in the range of 250 to 4250 cm-1 (Banu *et al*., 2011).

2.3.14.Antibacterial activity of Ag-NPs

Antibacterial activity of Ag-NPs using *Agaricus bisporus* extract were determined by agar well diffusion method (Sudhakar *et al*.,2014).volume of 50µl Ag-NPs and concentration (17 mg/ml ,10mg/ml, 8.5mg/ml) was investigated by agar well diffusion method to determine the better volume and concentration.

- From an overnight culture plate, 4-5 colonies of bacterial isolate were picked up by sterilized inoculating loop and emulsified in 5ml of sterile normal saline until the turbidity is approximately equivalent to that of the McFarland No. 0.5 turbidity standard
- Wells of **7**mm diameter were made on nutrient agar plates using gel puncture .
- A sterile swab was dipped into the bacterial suspension, any excess fluid was expressed against the side of the tube.
- The surface of a Mueller-Hinton agar plate was inoculated by bacterial isolate as follows: The whole surface of the plate was streaked with the swab, then the plate was rotated through a 45º angle and streaked the whole surface again; finally the plate was rotated another 90º and streaked once more.
- the wells were loaded with (20µl, 30µl 40µl and 50µl) Ag-NPs suspension .The plates were incubated at 37°C for 24 hours. After incubation, the plates were analyzed for the zones of inhibition. The activity was evaluated by calculating the increase in folded area.

2.3.15. Combination of antibiotics and Ag-NPs

The Combination between Ag-NPs 17mg/ml (50µl)and antibiotics against bacterial isolates were done by disc diffusion method. To determine the combination effect of antibiotics and Ag-NPs .The discs were impregnated

against indicated bacterial isolates with prepared Ag-NPs and then these discs were used for antibacterial activity assays the plates were incubated at 37°C for 24 hours. After incubation, the plates were analyzed for the zones of inhibition (Birla *et al.*, 2009). The activity was evaluated by calculating the increase in folded area.by $(B^2 - A^2)/A^2$, where A and B are the zone of inhibition for antibiotic and antibiotic with Ag-NPs, respectively.

2.3.16. Detection of some virulence factors:

2.3.16.1. Biofilm formation

2.3.16.1.1. Tube method

Trypticase soy broth with 1% glucose (TSB glu) media (10mL) was inoculated with loop full of bacterial colonies from culture plates and incubated for 24 hours at 37°C. The tubes were decanted and washed with phosphate buffer saline (pH 7.3) and the dried tubes were stained with crystal violet (0.1%). Excess stain was removed and tubes were washed with deionized water. Tubes were than dried in inverted position and observed for Biofilm formation. It was considered positive when a visible film lined the wall and bottom of the tube The absence of a film or the exclusive observation of a stained ring at the liquid-air interface should be considered negative result (-). Based on biofilm production, the positive results were recorded as strong (+++),moderate(++),weak (+) (Pramodhini *et al*., 2012).

2.3.16.1.2 Tissue culture plate (TCP) Method

Tissue culture plate assay described by (Christensen *et al*,1985; Hwang *et al.*, 2012) .To investigate the biofilm activity of Ag-NPs alone and in combination with antibiotics with minor modifications. This method was widely used and considered as standard test for detection of biofilm formation. This method was applied on bacterial isolates .These isolates selected according to

the previous tests (Tube Method) also the media was used to evaluate biofilm formation ; Trypticase soya broth (TSB), Individual wells of sterile, polystyrene, 96-wells TCPs were filled with 170 µL of the single populations of the bacterial species that equivalent to the McFarland No.0.5 at 10^5 CFU.

Tissue culture plates were incubated for 8 hours ,After overnight culture, Ag-NPs in combination with antibiotic was added with the final concentration30µl . Biofilm formed by adherent 'sessile' organisms in the plates were fixed with sodium acetate (2 %) and stained with crystal violet (0.1 %, w/v). Excess stain was rinsed off by thorough washing with deionized water and the plates were dried. After drying, 95% ethanol was added to the wells . The percentage of biofilm inhibition was calculated according to (OD) of stained adherent bacteria were determined with a micro ELISA auto reader at wavelength of 590 nm .These OD values were considered as an index of bacteria adhering to surface and forming biofilm. OD readings from sterile medium, fixative and dye were averaged and subtracted from all test values .

2.3.16.2 ATPase inhibitors

Antibacterial activity of Ag-NPs was associated with altered membrane permeability including multidrug-resistant pumps or repair systems, the antibacterial susceptibility of Ag-NPs examined in the presence of detergents or ATPase-inhibiting agents.

To increase the outer membrane permeability , concentration of Ag-NPs determined by combination assay with other therapeutic then added 30µl of 0.001% Triton X-100 and 30 mM Tris (Jung & Lee, 2008 agents, Individual wells of sterile, polystyrene, 96-wells TCPs were filled with 170 µL of bacterial suspension that equivalent to the McFarland No.0.5 at 10^5 CFU; Sun *et al.*,2014). 0.001% NaN3 were used as inhibitors of ATPase (Linnett & Beechey, 1979). TCPs were incubated for 18 h at 37°C .Experiments were performed in triplicate in a plate and in three\ independent assays

2.3.17 . Statistical analysis

Statistical analysis was performed by Social Science Statistics and the Statistical Package For Social Sciences version 19 for Windows Software and Microsoft Excel 2010. Continuous random variables that normally distributed are described by mean, SD (standard deviation) and the parametric statistical tests of significant. ANOVA test are used to analysis the statistical significance of difference in mean between more than 2 groups and when ANOVA model shows statistically significant differences, additional exploration of the statistical significance of difference in mean between each 2 groups was assessed by Bonferonni t-test. Descriptive analysis was done by calculating frequencies and approximate percentages (Sheskin, 2004).The statistical significance of the effect of antibacterial gents or nanoparticles on bacteria isolates was examined by the Fisher Exact Probability Test or Chi-Square test (χ^2)) that used to measure the strength of association between categorical variables. All these statistical tests considered that *P*- value less than the 0.05 level was statistically significant (Viera, 2008).

CHAPTER THREE

RESULTS

and

DISCUSSION

3.1. Bacterial isolation

3.1.1.Cultural characterization and identification

Out of 250 clinical specimens ,150(60%) have shown positive bacterial cultures were found of four types of pathogenic isolates of MRSA ;*E.coli; P. aeruginosa and P.mirabilis* Table (3-1), while other100 isolated were divided into 60 % growth of other than previous isolates and other 40% were no growth of pathogens. The result of 16% probably due to previous and repeated antibiotic therapy on the patients or no growth of pathogenic bacteria on an ordinary media Table (3-2). Positive bacterial isolates of cultures were isolated from various specimens which included; 60 (40%) urine, 48(32%) Catheter , 26(17.3%) wound and 16(10.7%) boils and ear swabs. Culture and biochemical tests revealed that most isolates belong to *Escherichia coli* 65 isolates (43.34%) followed by *Pseudomonas aeruginosa* 35 isolates (23.33%) *Proteus mirabilis* 30 isolates (20 %) and *Staphylococcus aureus* 20 isolates (13.33%) as shown in.

Table(3-1):Number and percentage of bacterial isolates according to the source of infection

Clinical specimens		Bacterial Isolates			
		S.aureus	***E .coli***	***P.aeroginosa***	***P.mirabilis***
Type	**N (%)**	**N(%)**	**N*(%)***	**N*(%)***	**N*(%)***
Urine	**60 (40)**	**8 (13.33)**	**30 (50)**	**17 (28.33)**	**11 (18.3)**
Catheters	**48 (32)**	**6 (12.5)**	**20 (41.66)**	**13 (27.1)**	**10 (20)**
Wounds swab	**26 (17.3)**	**4 (15.4)**	**15 (57.65)**	**3 (11.5)**	**9 (34.5)**
Ear swabs	**6 (9.33)**	**2 (12.6)**	**-**	**1 (16.6)**	**-**
Boil	**10(6,66)**	**1(1.3)**	**-**	**1(10)**	**-**
Total	**150**	**20 (13.33%)**	**65 (43.34%)**	**35 (23.33%)**	**30(20%)**

Table(3-2): Number and percentage of other bacterial isolates

Types		Clinical specimens				
Bacterial Isolates	**N0.**	**Urine (%)**	**Catheters (%)**	**Wounds (%)**	**Ear swabs (%)**	**Boil (%)**
Candida albicans	**18**	**7 (38.88)**	**3 (16.66)**	**-**	**8 (44.44)**	**-**
Enterococcus faecalis	**6**	**6 (100)**	**-**	**-**	**-**	**-**
Klebseilla pneumonia	**20**	**10 (50)**	**8 (40)**	**2 (10)**	**-**	**-**
Enterobacter aero genes	**11**	**7 (54.54)**	**4 (36.36)**	**-**	**-**	**-**
Streptococcus pyogenes	**5**	**-**	**-**	**-**	**5 (100)**	**-**
Total	**60**	**30 (50)**	**15 (25)**	**2 (3.33)**	**13 (21.66)**	**-**

3.1.2. Primary diagnosis of bacterial isolates using biochemical tests

Firstly ,Bacteria was recognized on MacConkey agar, their colonies appeared as a flat smooth and pink in color as a result of lactose fermentation while on blood agar it gave dark convex colonies these qualities often characterized by the genus *E. coli*(table 3-2). Gram negative rod shaped bacterium .Biochemical tests showed its ability to produce catalase enzyme when they tend to produce bubbles after H_2O_2 addition ,it is gave positive results for Indole by forming red ring after addition Kovac´sreagent and also it gave negative result to Urease tests (Forbes *et al,* ,2014).The selection of pale-colored colonies (non-fermented lactose sugar) on MacConkey agar, which produced the pyocyanin dye. *P. aeruginosa* a Gram negative rod shaped motile organism Table (3.2) .The results showed that it was positive for the Oxidase ,Catalase tests as an initial diagnosis, while the isolates exhibited a variation in their production of urease. the Indole test was negative(Forbes *et al*, ,2014).

Colonies of *P. mirabilis* bacteria were seemed to be small and colorless on MacConkey because its non-lactose fermenter. On blood agar its ability appeared to swarm which is the most distinguishable phenomenon for this kind ,the bacterium can distinguish form a rod shaped vegetative cell into an elongated (Mobley and Belase,1995).Biochemical tests for *P.mirabilis* bacteria revealed that it was able to produce Catalase ,Urease enzymes, but it was negative results for Oxidase(table 3.2).The remaining isolates grown on the blood agar with Beta hemolysis can be identified as *S.aureus* they developed yellow colonies on the mannitol salt as a result of fermentation of mannitol.the general morphological characteristics of the colonies appeared circular cream color to yellow. These isolates were initially identified as *Staphylococcus* species Table (3.3) .All isolates were able to coagulate a plasma and give positive result to Coagulase test (slide and tube).This gives the impression that it may be a *S. aureus* (Benson, 2001).

Table (3-3) The biochemical tests of bacterial isolates

Test	Bacterial species			
	E. coli	***P. aeruginosa***	***P. mirabilis***	***S.aureus***
Gram stain	-	-	-	+
Catalase	+	+	+	+
Oxidase	-	+	-	-
Indole	+	-	-	\
Urease	-	**V**	+	+
Coagulase	\	\	\	+
Lactose fermentation on MacConkey	+	-	-	\

(+) positive test , (-) negative test , V (variable),\ (not tested)

3.1.3. The identification of bacterial isolates was confirmed using API system strips :

API 20 E for *E. coli* , *P. mirabilis* , API 20N E system for diagnosis of *Ps. aeruginosa* and API Staph system for diagnosis *S. aureus.*

3.2. Antibiotic susceptibly testing of bacterial isolates

A total of 150 bacterial isolates of *E. coli* (65), *Ps. aeruginosa* (35) , *P. mirabilis* (30) and *S. aureus* (20) were tested for their antibiotic resistance against 21 antibiotics using disk diffusion method. In this study, all tested isolates were resistant to minimum of 3 classes of antibiotics which they were tested, so the isolates were considered multidrug resistant. MDR has been identified as a major threat to the public health of human being by the World Health Organization(Sun *et al*.,2014). These bacteria drastically reduced the efficacy of antibiotic , consequently, increasing the frequency of therapeutic failure and mortality. This rising antimicrobial resistance has been accompanied by a decline in new antibiotic discovery over the last few decades, which now poses a serious threat to public health (Kovač *et al*.,2015)

The results revealed that showed different degrees of resistance to antibiotics that used in the study. Table 3-4 showed that the percentage of resistant isolates of *E. coli* and *P. mirabilis* to antibiotics were as to Ampicillin, Amoxillin- clavulanic acid ,Azethronam, Cefotaxime , Cefepime , Trimethoprim, Nitrofurantoin , Chloramphenicol Nalidaxic acid Piperacillin and Gentamycin. while *E.coli* did not show any resistance to Imipenem and Doxcycline , but it gave less resistance for both Amikacin and Ciprofloxacin

The isolates of *P. aeruginosa* also resistant to the same groups of antibiotics resisted by *E. coli* and *P. mirabilis* have different degrees of resistance to Azethronam , Cefepime, Piperacillin, Gentamycin , Imipenem , Ciprofloxacin and Amikacin.

Table(3.4): Antibiotic resistant patterns of bacterial isolates against different antibio

Antibiotic	No. (%) of resistant of bacterial isolates		
	E. coli (n=65)	*P. aeruginosa* (n=35)	*P. mirabilis* (n=30)
Ampicillin	50 (76.9%)	/	26(86.6)
Amoxicillin-clv	50 (67.9%)	/	20(66.6)
Cefoxitin		/	/
Cefipime	30 (46.1)	25(71.4)	15(50)
Cefotaxime	32 (49.2)	-	20(66.6)
Amikacin	10 (15.3)	15(42.8)	2(6.6)
Gentamycin	40 (61.5)	15(42.8)	15(50)
Imipenem	(0)	1(2.8%)	0
Azethronam	50 (76.9)	35(100)	30(100)
Nalidixic acid	36 (55.3)	/	15(50)
Ciprofloxacin	5(7.9)	10(28.5)	8(26.6)
Doxcycline	(0)	/	10(33.3)
Chloramphenicol	26 (40 %)	/	22(73.3)
Trimethoprim	35(53.8)	/	20(66.6)
Nitrofurantoin	35(53.8)	/	20(66.6)
Piperacillin	45(69.2)	30(85.7)	28(93.3)

Cefoxitin were used in this test according to CLSI(2016) recommendations for detection of MRSA isolates. Results showed that out of 20 *S. aureus* isolates, 5 (25%) isolates were resistant to both of these antibiotics Table (3-5) .The isolates of *S. aureus* were tested against antibiotics under study

In this study all MRSA isolates exhibited resistance rate to antibiotics as Cefotaxime , Cefipime,Rifampicin ,Trimethoprim , Nitrofurantoin Chloramphenicol, Gentamycin ,Doxycycline , Ciprofloxacin , Penicillin G and Cefoxitin .

Table(3.5): Antibiotic resistant patterns of bacterial isolates against different antibiotic

Antibiotic	No. (%) of resistant of bacterial isolates
	***S. aureus* (n=20)**
Penicillin G	**20(100)**
Cefoxitin	**7(35)**
Cefipime	**15(75)**
Cefotaxime	**18(90)**
Amikacin	**12(60)**
Gentamycin	**10(50)**
Rifampicin	**8(40)**
Ciprofloxacin	**12(60)**
Doxcycline	**3(15)**
Chloramphenicol	**12(60)**
Trimethoprim	**13 (59.1)**
Nitrofurantoin	**8(40)**

In this study 30 isolates of *E. coli* , *P. aeruginosa* and *P.mirabilis* are considered MDR are resistant to representative of three or more classes of antimicrobial agents (Table 3.6).

Table(3.6): MDR and non MDR Bacterial isolates

Isolates	*E. coli*	*P. aeruginosa*	*P. mirabilis*	*MRSA*	Total No. MDR isolates
MDR	10	10	10	5	35
Non MDR	55	25	20	15	115
Total	65	35	30	20	150

3.3. Characterization of biosynthesized silver nanoparticles (Ag-NPs)

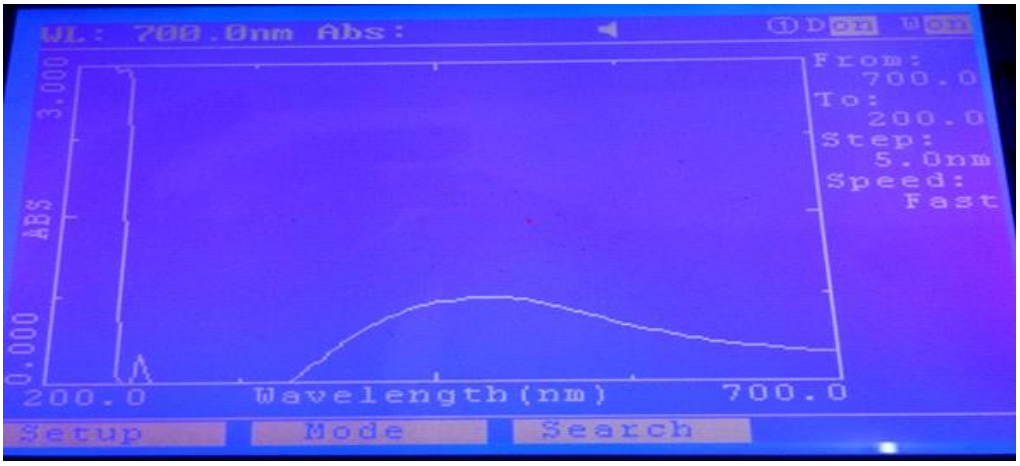
3.3.1. Visual detection of Ag-NPs

Ag-NPs were visually detected by changing color from yellow to dark brown of suspension. Figure3-1 containing cell free filtrate and silver nitrate .The reduction of silver ions to Ag-NPs (Ag^+ to Ag^0) lead to changing color from transparent or light yellow to brown(Sudhakar *et al.*,2014), which indicated the formation of Ag-NPs. This brown color was due to the excitation of the surface Plasmon vibrations in the metal nanoparticles, The control (soil)did not show any change in its initial color when incubated under the same conditions (Karwa *et al.*,2011) . Al- Ziadi *et al* ,(2015) from Iraq revealed that the approach of dark brown synthesis seems to be cost effective, eco-friendly and easy alternative to conventional methods of silver nanoparticles synthesis by *Trichoderma harizanum* .Ag-NPs release silver ions, generating an amplified biocidal effect, which is size- and dose-dependent (Marambio-Jones and Hoek 2010).

Figure (3-1): Color change results from adding AgNO3 to Mushroom(*Agaricus bisporus*) extracts at 37Ċ ,10,000 rp , shaker incubator 150 rp at mints ; (A)- before (B) after reaction time 24 hours.

3.3.2.UV/ Visible spectrophotometer

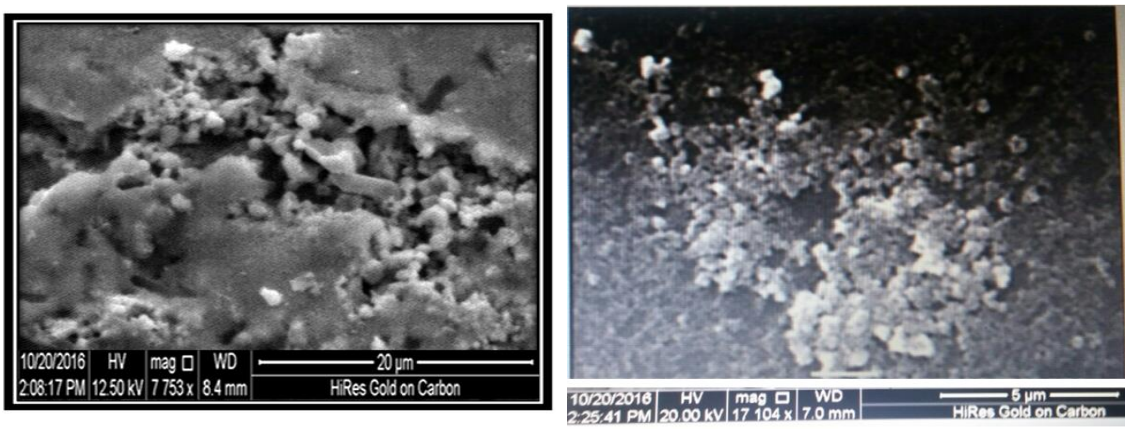
Figure (3-2) shows the UV-Vis spectrophotometer has also been used to detect the synthesis of Ag-NPs recorded at different wavelengths (200–700 nm). The results revealed that Ag-NPs of a peak in wavelength 430 nm which is considered the defined value of the Ag-NPs taken after every 24 hours for 3 days which resemble to the surface plasmon resonance of SNP so the bio reduction of the silver nitrate has taken place following incubation of the AgNO3 solution in the presence of the cell free extract. The production of Ag-NPs by *Agaricus bisporus* and their detection using UV-Vis spectrum was reported by several authors Haq *et al*.(2015) found that prepared nanoparticles ranging of 420-430 nm. Dhanasekaran *et al*,(2013) reported that the maximum absorption of the prepared nanoparticles were observed at 420–444nm for *A. bisporus* ,while Nithya & Ragunathan,(2009) reported that UV-Vis spectrum of solution of *Pleurotus sajor caju* showed absorbance band at 381 nm.



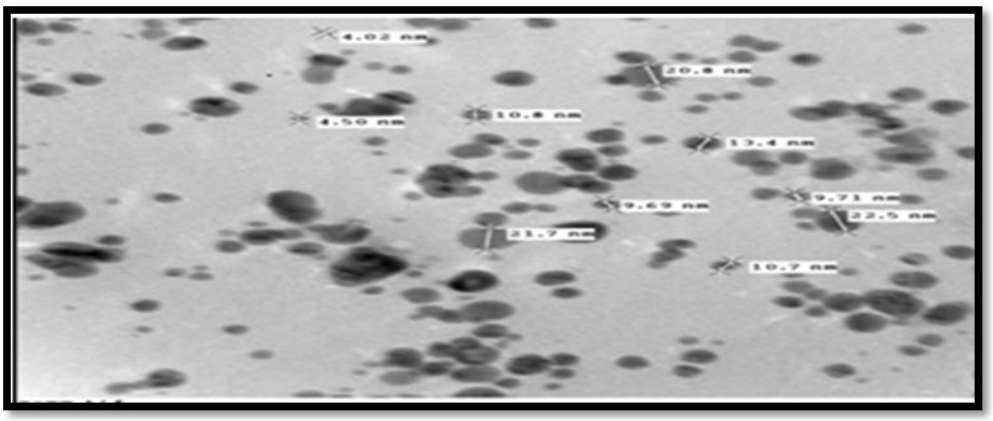
Figure(3.2): A Peak(430) of silver nanoparticles synthesized by *Agaricus bisporus* after (24, 48 and72 hours)by UV-visible spectroscopy .

3.3.3. Scanning and transmission electron microscopy analysis (SEM,TEM) .

Characterization of Ag-NPs were observed under SEM revealed that nanoparticles had varied shaped (spherical , irregular) with size in the range of 5-50 nm Figure(3-3A).Spherical silver nanoparticles aggregates were reported by Nithya and Ragunathan (2009) that synthesized silver nanoparticles by *Pleurotus sajorcaju* of size range 5-50nm and Narasimha *et al*,(2011) who reported that their size range 5-50nm Ag-NPs by *Agaricus bisporus*, while Karwa *et al*,(2011) who obtained Ag-NPs prepared by *Ganoderma lucidium* showed the polydisperse nature of their nanoparticles with size range (10 to 70)nm. On the other hand, the morphology of Ag-NPs is apparently spherical particles, sized range (4.5-35)nm-poly-dispersed by TEM Figure(3-3B). The that a peak of 430 nm indicates that the NPSs have spherical form (Sudhakar *et al* ., 2014)



A



B

Figure (3.3 A,B): Image of TEM and SEM of Ag-NPs synthesized by *Agaricus bisporus* showing shape and size at: (Isfahan university-engineering college /Iran and Al kuffa university- college of science research center/ Iraq) respectively

3.3.4.Fourier transforms infrared spectroscopy (FTIR)

These results confirm the presence of primary and secondary amines bonds; C=O, N=O, C=N and COOH bonds of proteins (Ramezani *et al*., 2012).The amide linkages between amino acids residues in polypeptides and proteins give rise to well-known signatures in the infrared region ,the representative spectra in the region of 3500 to 450cm $^{-1}$ revealed the presence of different functional groups like 3290 secondary amide (N-H stretch ,H-bonded), 2928 (CH-stretching), 2161 (C=C stretching),1771 an hydride (C=O stretching),1613 (C=C stretching), 1538 aromatic (C-C stretching), 1386 ,1313 and 1080 primary alcohol (C-O stretching), and 1333cm (CH_3)

correspond to carbon hydrogen bending vibration respectively Figure (3-4). Proteins present in the extract can bind to Ag-NPs through either free amino or carboxyl groups in the proteins. The potential bimolecular in cell free extract acts as stabilizing and capping agent . Singh *et al* (2014) reported different functional groups absorb. Characteristic frequencies of FTIR radiation . similar results were also represented by Haq *et al* (2015). the carbonyl groups from the amino acid residues and peptides of proteins have a stronger ability to bind metal, so that the proteins could form a coat over the metal nanoparticle to prevent gathering of the particles(Ramezani *et al*., 2012).It can be concluded that proteins or peptides have formed a coating on silver nanoparticles, which in turn support their stability(Dhanasekaran *et al.*,2013)

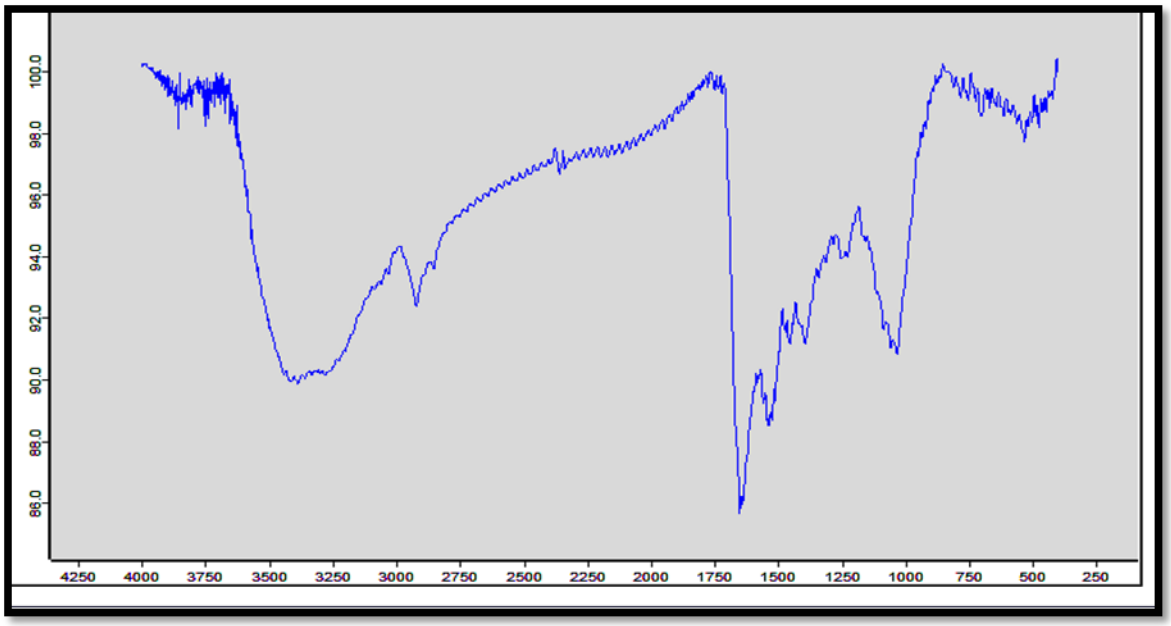


Figure (3-4):Image of spectrum of biosynthesized silver nanoparticles by *Agaricus bisporus* .

3.4. Effect of optimum volumes and concentrations of Ag-NPs

It has been used several volumes and concentration prepared Ag-NPs to get the optimal results for synthesis silver nanoparticles at 20, 30,40 and 50 *µl* was evaluated against strains bacteria by testing 17*mg/ml* Ag-NPs , 10 *mg/ml* Ag-NPs and 85 *mg/ml* Ag-NPs Table (3-7). Antibacterial activity of

Ag-NPs was measured as the diameter of inhibition zone in (mm) by agar-well diffusion methods against standard bacteria and MDR isolates were examined according to Sudhakar *et al*(2014).

The results found that the Ag-NPs of volume of 50 ml of mushroom extract with 50ml $AgNo_3$ of 17 *mg/ml* was the most effective concentration against the growth of standard strains Table(3-7) and MDR isolates Figure (3-5). On the other hand the results concluded that the inhibition zone in diameters were increased by using 50 *µl* Ag-NPs by edible mushroom *Agaricus bisporus*. The exact mechanism behind the conversion of $AgNo_3$ to Ag-NPs by mushroom extract was not known (Raja and Singh,2012). The results showed inhibition zone obtained in bacteria strains; *E. coli* ATCC28739 (≥20) mm *Ps. aeruginosa* ATCC27853 (≥18) mm *P. mirabilis* ATCC 16404 (≥15) mm and less inhibition zone it was *MRSA* ATCC 43300 (≥14)mm. respectively Table (3-7).

Table (3-7) Zone of growth inhibition (mm) of standard strains tested with Ag-NPs .

standard strains / Nanoparticles	Zone of growth inhibition (mm)			
	***MRSA* ATCC 43300**	***E. coli* ATCC 28739**	***P. mirabilis* ATCC 16404**	***P. aeruginosa* ATCC 27853**
	Mean ± SD	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**
$AgNO_3$ (control)	**10** ± 1	**16** ± 4.36	**12** ± 3.46	**12** ± 3
Nanoparticles (Ag-NPs) 17.0 mg/**ml**	**14*** ± 4	**20*** ± 2.65	**15** ± 4.58	**18*** ± 6
Nanoparticles (Ag-NPs) 10mg/ml	**12 ± 2**	**15 ± 2.01**	**10 ± 2.1**	**14 ± 4.16**
Nanoparticles (Ag-NPs) 85mg /ml	**12 ± 1.73**	**13 ± 3.3**	**10 ± 1.1**	**15 ± 3.11**

***= Significant association (p <0.05), SD = Standard Deviation**

Table 3-7 showed that 17mg/ml concentration of Ag-NPs more effected on bacterial isolates in compared with other concentration of Ag-NPs,

especially on *E. coli* (Mean of Zone of inhibition is 20mm and SD±2.65), *P. aeruginosa* (Mean of Zone of inhibition is 18mm and SD±6), *P. mirabilis* (Mean of Zone of inhibition is 15mm and SD±4.58). and *MRSA* (Mean of Zone of inhibition is 14mm and SD±4).

The results revealed that the Ag-NPs were quite effective to inhibiting the growth of all tested organisms and it found that by giving inhibition zone against MDR isolates, the inhibition zone obtained in *E.coli* (≥16mm) , *P. aeruginosa* (≥15mm) , while the lowest inhibition zone obtained with *P. mirabilis*(≥13mm) and MRSA of (≥13 mm) respectively as in Table(3-8) , Figures (3-6). The effect of silver nanoparticles on gram negative bacteria was more as compared to gram positive bacteria. The reason behind this might be the cell wall composition of gram positive bacteria as described by Birla *et al*. (2009). The current study disagree with Sudhakar *et al*.,(2014) who showed that inhibition zone was 19 mm by using Ag-NPs by *Agaricus bisporus* mushroom. The present study agreed with Haq *et al*.,(2015) who synthesized Ag-NPs using *Pleurotus sajor caju (*Mushroom) was tested against *Ps. aeruginosa* and *P.mirabilis* produced zone of inhibition 12mm.

The present study approaching to Nithya *et al*.(2009) who synthesized Ag-NPs using *pleurotus* (mushroom) was tested against *E. coli*, *P. aeruginosa S. aureus* produced zone of inhibition of (12mm), (14mm) and (11mm) respectively. Whereas Rahi & Barwal (2015) revealed that Ag-NPs synthesized by the *Ganoberma applanatum* (mushroom) measured as the inhibition zone of *MRSA* 30mm,*S. aureus* 20mm , *P. aeruginosa* and *E. coli* 24mm. Devika *et al* (2012) found that the inhibition zone of in *E. coli* (13 mm) and for each *Ps. aeruginosa and S. aureus* (8 mm) antibacterial Ag-NPs by using *Pleurotus ostreatus* .Haq *et a l.* (2015) suggested that the Ag-NPs from *Agaricus bisporus* explored medicinally and nutritionally important species of dried mushroom. pH has play an important role in NPs formation and protein denaturized so that protein will lose its activity in low pH

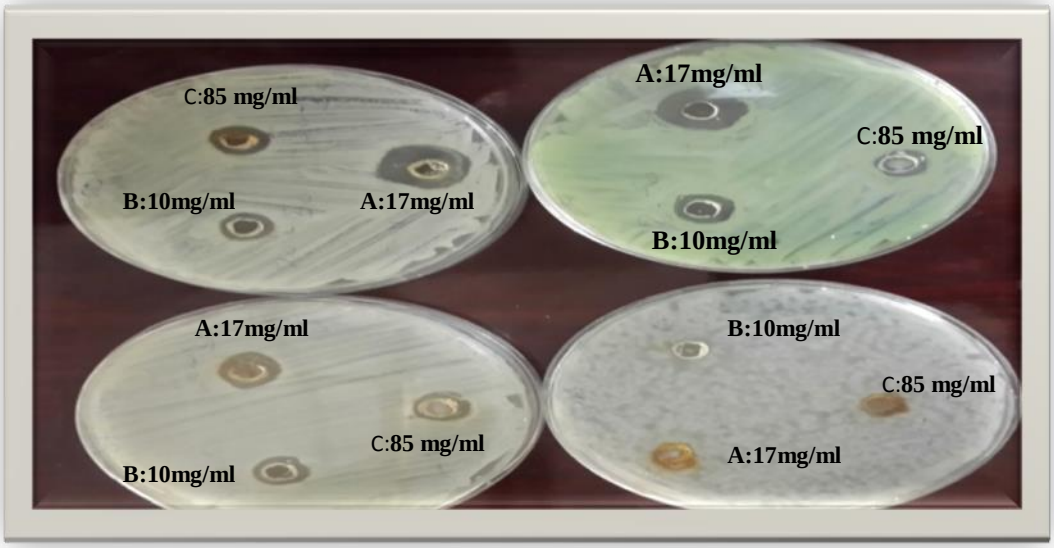
whereas at high pH, SNPs become smallest and more regular in compared of that of low pH (Banu *et al.*,2011).The bactericidal effect of metal nanoparticles has been attributed to their small size and high surface to volume ratio, which allows them to interact closely with microbial membranes and is not merely due to the release of metal ions in solution (Morones *et at*.,2005).

There is a fact that fungi produce many proteins and enzymes involved in synthesis of Ag-NPs and are simpler to grow both in laboratory and industrial level and also the yield is high(Janardhanan and Nayana,2000).Although beneficial as antimicrobial agents, silver nanoparticles have adverse effects on cells such as the production of reactive oxygen species which are toxic to both bacteria and eukaryotic cells (Park *et al.* 2009; Carlson *et al.* 2008).

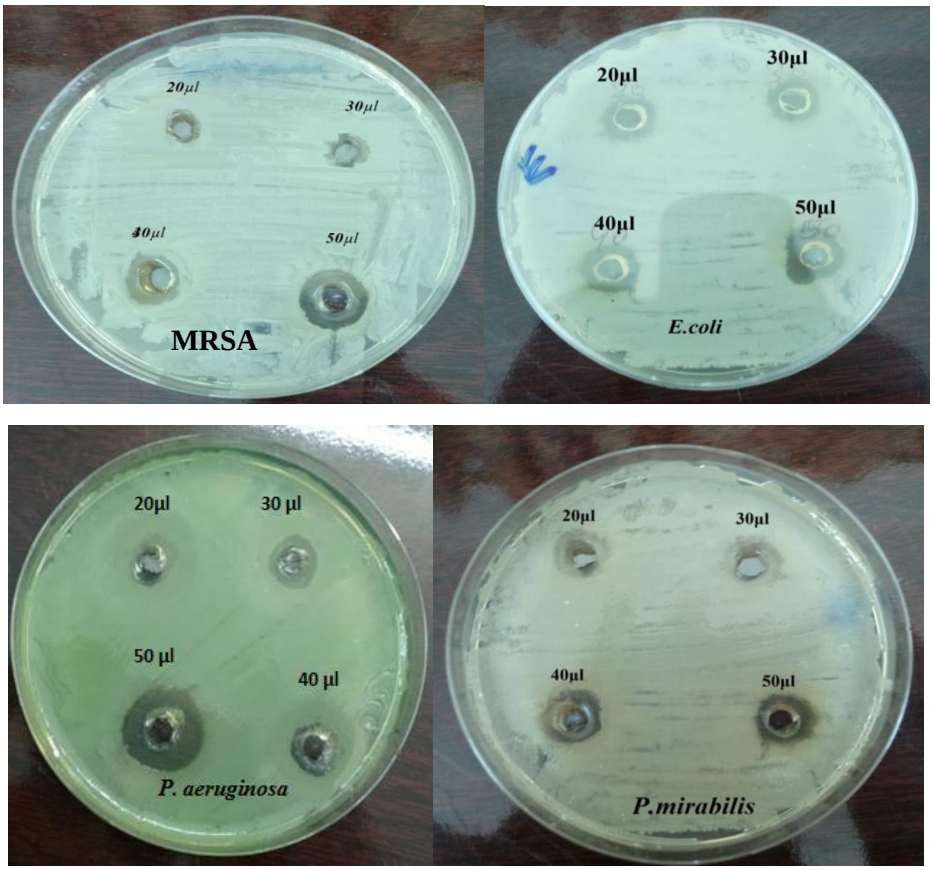
Table(3-8): Zone of growth inhibition (mm) of different concentration(Ag-NPs) against MDR bacterial isolates

MDR Bacterial isolates	Ag-NPs zone of inhibition(mm) 50 *µl*				X^2	P – value
	No. of isolate	17 mg/ml	10 mg /ml	85 mg /ml		
		Mean ± SD	Mean ± SD	Mean ± SD		
MRSA	2,3,7,10,12	13±3.3	11±1.22	10±0.90	0.478	>0.05 [NS]
E. coli	4,8,6,12,14,18,22,23,25	16±5.1	14±2.79	11±1.23		
	1,4,6	15±4.4	13±2.03	11±1.99		
P. mirabilis	1,2,5,6,7,13,14,15,17,19	13±2.7	11±0.9	10±0.87		
P .aeruginosa	6,9,10,11,16,18,20, 22,23	15±3.9	13±2	14±3.011		
	19,20,10,23	14±3.03	12±1.09	12±1.88		

NS= NonSignificant (p > 0.05), SD= Standard Deviation



Figure(3-5):Zone of growth inhibition of different concentration (Ag-NPs) against isolated MDR , A= 17mg/ml , B= 10mg/ml and C= 85 mg/ml



Figure(3-6):Zone of growth inhibition (mm) bacterial isolates MRSA, *E. coli* , *P .aeruginosa* and *P. mirabilis* with 17mg /ml Ag-NPs with 20,30,40,50*µl.*

3.5. Comparison between $AgNO_3$ solution and (Ag-NPs) nanoparticles:

The viability of $AgNo_3$ solution and Ag- nanoparticles using *Agaricus bisporus* extract were examined in the inhibition growth of bacterial isolates by well diffusion in agar method. Inhibition growth of bacterial isolates by Ag-NPs was compared with $AgNO_3$ solution and found that Ag-NPs was the most efficient of $AgNO_3$ solution in the inhibition of bacterial isolates (Table 3-9 and Figure 3-7) showed the inhibition zones for bacterial isolates treated with $AgNO_3$ solution and Ag-NPS, Sudhakar *et al*.(2014) found that mushroom extract plays role in the reduction of silver nitrate to silver nanoparticles their by producing bactericidal properties. Rasheed (2014) from Iraq revealed that increases the inhibition zone around the implanted bacteria reached to 15 mm for *Proteus*, Remarkable increment of antibiotics when silver nanoparticles with laser ablation added to improve the antimicrobial effect against gram-negative and gram-positive bacteria

The most popular of *Agaricus bisporus* is considered safe for most people to eat because it is grown in controlled, sterilized environments (Singh *et al*., 2011), so the use of fungi especially mushrooms in synthesis of nanoparticles is a less time and in fact, fungi produce many proteins and enzymes involved in synthesis of Ag-NPs and are simpler to grow both in laboratory and industrial level and also the yield is high (Janardhanan and Nayana 2000). Rasheed (2014) found that the most sensitive bacteria for silver nanoparticles was *Proteus* and the minimum inhibition concentration of l5 mg/ml was obtained for *S. aureu*.

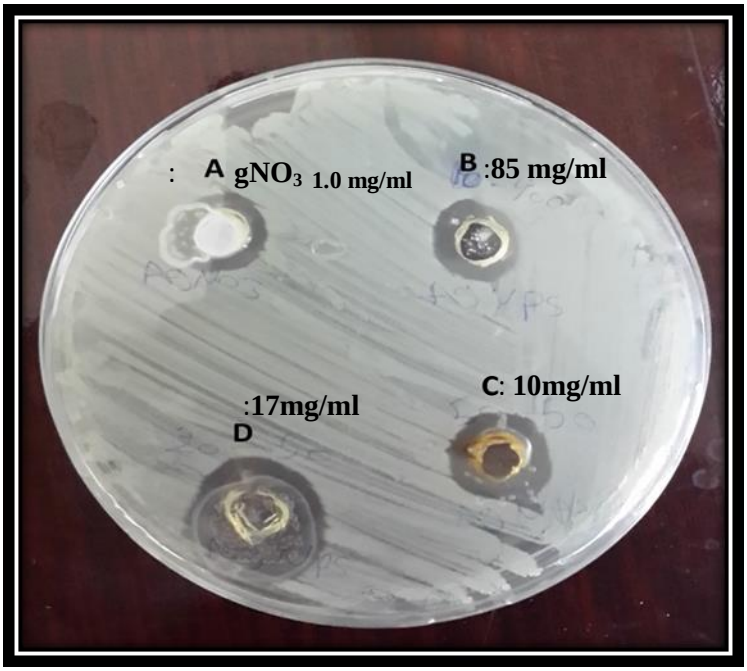


Figure (3.7) Zone of growth inhibition (mm) of *E. coli* with $AgNO_3$ 1.0 mg/ml and Ag-NPs (A:. $AgNO_3$ ~~alone~~, B: 85mg/ml Ag-NPs, C: 10 mg/ml Ag-NPs, D: 17mg/ml Ag-NPs.

Table (3-9) Demonstrated that bacterial isolates more sensitive to Ag-NPs 17mg/ml compared with $AgNO_3$ (X^2= 7.603, p<0.05).

MDR Bacterial isolates		Zone of growth inhibition(mm)		X^2 test	P – value
		$AgNO_3$ 50µl	Ag-NPs 17mg/ml 50µl		
		Mean ± SD	Mean ± SD		
1	MRSA	0.0± 0	13±4	7.603	<0.05
2	*E. coli*	10±2	16±4.12		
3	*P.mirabilis*	9±1.7	13±3.61		
4	*Ps .aeruginosa*	9±2.65	15±4.04		

SD = Standard Deviation

3.6. Estimation the combination effect of antibiotic with Ag-NPs

Thirty five MDR isolates out of 150 isolates were subjected for antibiotic susceptibility test using disc diffusion methods . The combination effect between antibiotic and silver nanoparticles by disc diffusion method gave an increasing fold diameters of inhibition zone of bacterial isolates in comparison with antibiotic alone. The Combinations of antibiotics and Ag-NPs resulted in average fold-area increases in antibacterial activity. The zone of inhibition in *E.coli* to antibiotic amoxicillin clavulanic acid ,ampicillin ,piperacillin and Azethronam were(11-18 mm),(11-15 mm), (12-18 mm) and (14-18 mm) respectively with deferent average fold-area(0.3-3.5) ,(0.2-5.2) ,(0.4-5.2)and (0.2-5.2) respectively [Table 3-10and Figure 3-8] .

This result is in agreement with Kaviya *et al.*, (2011) who reported that silver nanoparticles exhibited good antibacterial activity against both gram negative and gram positive bacteria, they showed higher antibacterial activity against *E.coli* and *P.aeroginosa* (Gram negative) than *S.aureus* (Gram positive) .

The study concluded that Ag-NPs showed variable effectiveness against bacterial isolates, the inhibition zones observed for *P. aeruginosa* with antibiotics piperacillin and Azethronam were (14-18mm) and (15-19mm) respectively and fold-area (0.5-4.4)and (0.5-5.2) respectively Table(3-11) Figure (3-9). Most *Pseudomonas* spp. are naturally resistant to Penicillin and the majority of related beta-lactam antibiotics (Cobos-Trigueros *et al* .,2015).

This result do not agree with Rahi and Barwal (2015) found that inhibition zone to amoxicillin and ampicillin increase of activity against bacterial pathogens of *E. coli* and *Ps. aeruginosa* isolates when using Ag-NPs by mushroom *Ganoderma* , which was 17.7 for *E. coli* and 10.11 *Ps. aeruginosa* against amoxicillin and 6.11 for *E. coli* toward Ampicillin either *Ps. aeruginosa* was 12.4 mm.

The high bactericidal activity is certainly due to the silver cations released from Ag nanoparticles that act as reservoirs for the Ag+ bactericidal agent.

Silver nanoparticles have ability to anchor to bacterial cell wall and subsequently penetrate it, thereby causing structural changes in cell membrane like the permeability of the cell membrane and death of cell. There is a formation of 'pits' on cell surface, and there is accumulation of the nanoparticles on the cell surface.(Mahmood ,2012) .

In contrast, *P.mirabilis* isolates showed same inhibitory to Ag-NPs with edible mushroom by inhibition zone average (12-18mm),(12-15mm),(14-19mm) and (10-15mm) respectively ,for amoxicillin clavulanic acid , ampicillin, piperacillin and Azethronam. The average fold area were (0.4-5.2),(0.4-3.5) (0.5-4.4) and (0.2-3.5) of each one respectively Table(3-12) Figure (3-10). This result is high than Buszewski *et al*.,(2016) who found that combination of Ag-NPs with ampicillin (9.3mm) zone inhibition against *P.mirabilis*. Tawfeeq e*t al.*2017 proved that results showed the synergistic action of Ag-NPs and antibiotics against (MDR) bacteria were also leading to enhance antibacterial activity.

Table (3-10)Zone of inhibition (mm) of different antibiotics against 10 isolates MDR , (in absence and in presence of Ag-NPs at content of 50 µl per disc)A: antibiotic ,B: antibiotic with Ag-NPs, C: increase fold area=(B2-A2)/A2 .

Antibiotic	No. *E.coli* isolates																											
	1			4			8			6			12			14			18			22			23			25		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Amoxicillin Clavulanic acid	13	18	0.9	13	18	0.9	7	15	3.5	9	11	0.4	7	12	1.9	7	12	1.9	7	12	1.9	12	15	0.5	7	12	1.9	11	13	0.39
Ampicillin	6	15	5.2	11	13	0.39	9	15	1.7	6	10	0.22	6	11	2.3	7	15	3.5	6	10	0.22	7	15	3.5	7	15	3.5	6	15	5.2
Cefipime	7	19	6.3	13	18	1.3	10	12	0.4	10	18	2.2	6	15	5.2	10	18	2.2	10	18	2.2	7	19	6.3	15	18	0.4	7	15	3.5
Cefotaxime	7	12	1.3	11	13	0.39	11	13	0.39	6	10	0.22	11	14	0.5	11	14	0.5	9	14	1.4	8	16	3	9	14	1.4	8	16	3
Gentamicin	8	15	2.5	8	18	4	8	15	2.5	6	10	1.7	8	18	4	10	18	2.2	10	18	2.2	12	15	0.5	12	15	0.5	7	15	3.5
Nalidaxic acid	13	19	0.3	12	16	0.77	9	14	1.4	13	16	0.3	10	18	2.2	9	14	1.4	7	15	3.5	7	15	3.5	7	15	3.5	10	13	0.5
chloramphenicol	8	16	3	9	15	1.7	9	15	1.7	9	15	1.7	9	15	1.7	8	16	3	9	15	1.7	9	15	1.7	8	16	3	8	16	3
Nitrofurantoin	9	14	1.4	9	18	3	12	15	0.5	12	15	3.5	12	18	1.2	12	15	0.5	12	18	1.2	12	18	1.2	6	15	5.2	12	15	0.5
Piperacillin	11	16	0.4	10	18	2.2	-	15	5.2	7	12	1.9	12	15	0.5	7	12	1.9	10	18	2.2	7	12	1.9	14	18	0.5	10	15	1.2
Azethronam	6	15	5.2	12	15	1.2	12	14	0.2	12	16	0.7	12	14	0.4	10	18	2.2	6	15	5.2	10	18	2.2	12	15	0.5	11	14	0.5

Table (3-11)Zone of inhibition (mm) of different antibiotics against 10 isolates MDR, (in absence and in presence of Ag-NPs at content of 50 µl per disc)A: antibiotic ,B: antibiotic with Ag-NPs, C: increase fold area=(B2-A2)/A2 .

Antibiotic	No. *P. aeruginosa* isolates																											
	1			4			8			6			12			14			18			22			23			25		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Cefipime	8	16	3	6	12	3	11	12	0.3	11	12	0.3	6	12	3	6	12	3	6	12	3	8	16	3	6	12	3	6	12	3
Gentamycin	6	10	0.22	7	14	3	6	10	0.22	11	16	1.11	7	14	3	7	14	3	7	14	3	7	14	3	7	14	3	6	10	0.22
Ciprofloxacin	10	18	2.2	7	15	3.5	7	15	3.5	10	16	1.5	9	16	2.1	7	15	3.5	9	16	2.1	9	16	2.1	7	15	3.5	7	15	3.5
Piperacillin	6	14	4.4	6	14	4.4	10	18	2.2	12	15	0.5	10	18	2.2	12	15	0.5	10	18	2.2	6	14	4.4	12	15	0.5	10	18	2.2
Azethronam	6	15	5.2	7	15	3.5	6	15	5.2	7	15	3.5	12	15	0.5	6	15	5.2	12	19	1.5	12	19	1.5	12	15	0.5	12	15	0.5

In contrast, Sondi and Salopek -Sondi (2004) reported that the antimicrobial activity of silver nanoparticles on Gram-negative bacteria was dependent on the concentration of silver nanoparticles, and was closely associated with the formation of pits in the cell wall of bacteria. Ag-Nps accumulated in the bacterial membrane caused the permeability, resulting in cell death. Singh *et al* .,(2012) suggested that electron spin resonance spectroscopy studies that silver nanoparticles when contact with bacteria, and these free radicals have an ability to damage cell membrane and make it porous which can ultimately lead to cell death.

A high occurrence of resistance of *S .aureus* against penicillin G, were (100%).The results showed that bacteria have become sensitive and there is an increase in the inhibition of antibiotic tablets regions with Ag-NPs as by edible mushroom , average inhibition zone (10-18mm) while average fold-area (0.5-2.2) presented in Table (3-11).Figure (3-113). Rahi and Barwal (2015) noted that inhibition zone of penicillin G ,amoxicillin and ampicillin increase inhibition zone against the isolates of MRSA were (11,7,7)with fold area(2.3,6 and13) respectively Birla *et al*.,(2009) were indicated the synergistic activity observed was better in *E. coli* and *Ps. aeruginosa* than *S. aureus*. Fayaz *et al*. (2010) showed that Ag-NPs can act synergistically with several antibiotics, preferentially against Gram-negative bacteria .Mahmood (2012) revealed that a synergistic effect was observed between Ag-NPs and both amoxicillin and penicillin for Gram-positive isolates

Table (3-12)Zone of inhibition (mm) of different antibiotics against 10 isolates MDR, (in absence and presence of Ag-NPs at concentration of 50 µl per disc)A: antibiotic ,B: antibiotic with Ag-NPs, C: increase fold area=(B2-A2)/A2 .

Antibiotic	No. *P.mirabilis* isolates																											
	1			2			5			6			7			13			14			15			17			19		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Amoxicillin Clavulanic acid	10	18	2.2	6	15	5.2	10	12	0.44	10	12	0.44	10	18	2.2	6	12	3	6	14	4.4	6	12	3	10	12	0.44	6	15	5.2
Ampicillin	7	15	3.5	7	15	3.5	6	12	3	10	12	0.44	10	12	0.44	6	12	3	10	15	1.2	10	15	1.2	7	15	3.5	10	15	1.2
Cefipime	12	15	0.5	6	10	1.7	6	12	3	7	15	3.5	6	14	4.4	6	12	3	6	10	1.7	6	10	1.7	6	10	0.22	6	10	0.22
Cefotaxime	6	10	1.7	6	10	1.7	6	10	1.7	6	10	0.22	6	10	1.7	6	10	1.7	6	10	0.22	6	10	1.7	6	10	0.22	6	10	1.7
Gentamicin	6	10	1.7	8	16	3	6	10	1.7	8	16	3	7	13	2.5	7	13	2.5	8	16	3	8	16	3	8	16	3	6	10	1.7
Nalidaxic acid	6	10	1.7	9	17	2.5	9	17	2.5	6	10	1.7	10	13	0.5	10	13	0.5	9	17	2.5	9	17	2.5	9	17	2.5	10	13	0.5
Nitrofurantoin	6	10	1.7	6	12	3	10	15	1.2	12	18	1.2	7	16	4.2	10	15	1.2	7	16	4.2	10	15	1.2	7	16	4.2	10	15	1.2
Piperacillin	12	15	0.5	7	15	3.5	6	14	4.4	7	15	3.5	6	14	4.4	9	19	3.4	6	14	4.4	12	15	0.5	6	14	4.4	12	15	0.5
Azethronam	6	10	0.22	6	10	0.22	7	15	3.5	6	10	1.7	7	15	3.5	6	10	1.7	6	10	1.7	7	15	3.5	6	10	1.7	7	15	3.5
Chloramphenicol	6	10	1.7	10	15	1.2	7	15	3.5	6	10	1.7	10	15	1.2	7	15	3.5	6	10	1.7	6	10	1.7	6	10	1.7	7	15	3.5

Table (3-13)Zone of inhibition (mm) of different antibiotics against 10 isolates MDR, (in absence and in presence of Ag-NPs at concentration of 50 µl per disc) A: antibiotic ,B: antibiotic with Ag-NPs, C: increase fold area=(B2-A2)/A2 **.**

Antibiotics	No. MRSA isolates														
	12			2			3			7			10		
	Ab	Ab-Ag	I.F	Ab	Ab-Ag	I.F	Ab	Ab-Ag	I.F	Ab	Ab-Ag	I.F	Ab	Ab-Ag	I.F
Penicillin G	8	10	0.59	8	10	0.59	12	16	0.93	8	10	0.59	10	18	2.24
Cefipime	9	11	0.49	11	13	0.39	9	15	1.77	10	12	0.44	8	16	3
Cefotaxime	6	10	0.22	10	14	0.96	7	10	1.5	6	10	0.22	9	15	1.77
Ciprofloxacin	6	13	3.6	12	15	0.5	6	13	3.6	9	17	2.55	12	15	0.5
Gentamicin	6	10	0.22	7	13	2.5	6	10	0.22	6	10	0.22	7	13	2.5
Nitrofurantoin	6	13	3.6	12	15	0.5	6	13	3.6	9	17	2.55	12	15	0.5
Rifampicin	6	12	1.5	8	10	0.59	8	10	0.59	9	15	1.77	9	15	1.77
chloramphenicol	8	10	0.59	7	13	2.5	7	13	2.5	8	10	0.59	7	13	2.5

The bacterial cells which in contact with silver ions, inhibit several functions in the cell and damage the cells, there is a generation of reactive oxygen species, which are produced possibly through the inhibition of a respiratory enzyme by silver ions and attack the cell itself. Silver is a soft acid, and there is a natural tendency of acid to react with a base in this case, a soft acid to react with a soft base (Yuan *et al* .,2007) . Hwang *et al.* (2012) proposed that silver nanoparticles modulate the phosphor tyrosine profile of putative bacterial peptides that can affect cellular signaling, which leads to growth inhibition in bacteria. Samantha (2015) showed that silver nanoparticles have reactive antibacterial activity against test pathogens including *S. aureus, P. mirabilis* and *E. coli* .

According to Yuan *et al* . (2007) silver nanoparticles can be used as effective broad spectrum antibacterial agents to Gram-negative and Gram-positive bacteria, including antibiotic-resistant bacteria, for *P. aeruginosa* and *S.aureus* , Ag-NPs act as efficient barriers against antimicrobial agents and the host immune system to protect the bacterial colony. The present study clearly indicates that Ag-NPs showed a good antibacterial action against MDR isolates ,all isolates gave a clear inhibition zone so the average of zone inhibition obtained in *E.coli* (12-16mm) and (12-19mm) to cefotaxime and Cefipime, the fold area were (0.2-3),(0.4-6.2), while *P. aeruginosa* zone inhibition against Cefipime were (12-16mm) and the fold area were (0.3-3). *P.mirabilis* zone inhibition (10mm),(10-15mm) with fold area (0.2-1.7) ,(0.2-4.4) cefotaxime and Cefipime .while average inhibition zone to cefotaxime and

Cefipime against MRSA were(10-15mm), (11-16mm) with fold area (0.2-1.7), (0.3-3) respectively. Haq *et al*.,(2015) reported the fold area were 8.6% by Ag-NPs using *Agaricus bisporus* with Ceftriaxone, the combination of antibiotics with Ag-NPs have better antimicrobial activities, the antibacterial activity of

Cephalexin was increased by AgNPs with Cephalexin antibiotic against *E.coli and S. aureus*. Singh and Raja.(2012). Mahmood (2012) proposed that a synergistic effect was observed between Ag-NPs and ceftriaxone for Gram-negative isolates .

The combination between Gentamycin and Ag-NPs showed a good effect on both *E. coli*, *Ps. aeruginosa* and *P.mirabilis* and the average inhibition zone was observed when Ag-NPs were combined with antibiotic Gentamycin against *E.coli* the effectiveness increased with inhibition zone (10-18mm) with fold area (0.5-4). ***P. aeruginosa*** were found to response to combination with inhibition zone(10-16mm) increased fold area (0.2-3). This result is less than of Birla *et al* (2009) found that (0.5, 0.1) increased fold area against Gentamycin of *E. coli* and *Ps. aeruginosa* isolates. *P.mirabilis* represented show combination between Gentamycin and Ag-NPs, isolates showed diameter average (10- 16 mm) and fold area (1.7-3).the results of Buszewski *et al* .,(2016) noted that inhibition zone of Ag-NPs and Gentamycin (8.3) against *P.mirabilis* . The results of combination assay for MRSA were 10-13mm and rate fold area (0.2- 2.5). The result was approaching of results reported by Birla *et al* (2009) were indicated the fold area (0.36) against Gentamycin synthesized Ag-NPs using *Phoma glomerata* and Haq *et al*.,(2015) reported that fold area were 23.8% by Ag-NPs using *Agaricus bisporus. aeruginosa* is a nosocomial pathogen of particular clinical concern not only because of its extraordinary resistance mechanisms armamentarium but also for its formidable ability to 44 Infection control adapt very well to the hospital environment (Moradali *et al*.,2017).

The main reason why nanoparticles is considered an alternative to antibiotics is that nanoparticles can effectively prevent microbial drug resistance in some cases because the bacteria have powerful ability of developing resistance against antibiotics,. The widespread use of antibiotics has

led to emergence of numerous hazards to public health, such as superbugs that do not respond to any existing drug and non-medicated epidemics. (Khameneh *et al* ., 2016)

Gurunathan *et al*. (2015) proposed that silver nanoparticles inhibit bacterial growth by inactivating the proteins. Silver atoms bind to thiol groups (SH) in enzymes which deactivates the enzymes. Silver alters the function of compounds in cell membrane which is important in transmembrane energy generation and ion transport, by forming a stable S-Ag bond with the thiol group of the compounds. Silver acts as a catalyst in the formation of disulfide bonds in the reaction of oxygen molecules in cell and hydrogen atoms of thiol groups (R-S-S-R). including *P.aeruginosa, Shigella flexneri, S. aureus, Streptococcus pneumoniae, Helicobacter pylori, Helicobacter felis, E. coli, K. pneumoniae,* and *Bacillus subtilis* .Quinolone group include Ciprofloxacin and Nalidixic acid .

The results indicated that the percentage of inhibition zone diameters were increased when used Ag-NPs by mushroom with Nalidixic acid against each of *E. coli* and *P.mirabilis* , the average inhibition zone were (15-18mm) and (10-17mm) respectively .

On the other hand increasing the antibacterial activities in fold area were (0.3-3.5) and (0.5-2.5). while inhibition zone diameters with Ciprofloxacin when used Ag-NPs by mushroom against *Ps. aeruginosa* and MRSA the average of inhibition zone were(13-19mm) and (13-17mm) , increasing the antibacterial activities in fold area were (2.1-3.5) and (0.5-3.6) respectively . Sotiriou and Pratsinis ,(2010).hypothesized that bactericidal activity of silver nanoparticles is attributable to Ag+ ions which enter the cell and intercalate between the purine and pyrimidine base of DNA. It is also possible for Ag-NPs not only interact with the surface of the membrane, but also penetrate inside the

bacteria and inactivate DNA replicating ability causing the devastation of the cell.

The results of isolates showed that Nitrofuration gave the highest average inhibitory zone of *E.coli* which was (12-18 mm) with fold area (0.5-3) by using AgNps, compared with using disc diffusion alone. Silver nanoparticles target protein synthesis, nucleic acid synthesis, and Gram positive cell wall synthesis, which explains why these bacteria were more susceptible to silver nanoparticles. Indeed, silver nanoparticles attach to the surface of the cell membrane and disturb its function, penetrate bacteria and release silver ions (Sondi *et al.,* 2004).Combination effect of Ag-NPs with edible mushroom were observed against *P.mirabilis* isolates by increasing the antibacterial activities of Nitrofuration with inhibition zone (10-18mm) with increases fold arae(1.2- 4.2) .

This study appeared that the high occurrence of resistance of *S .aureus* against Nitrofuration so the results showed that the bacteria have become more sensitive and there is an increase in the inhibition of the antibiotic tablets regions with Ag-NPs as by edible mushroom, became (13-17mm), the results of the combination assay were (0.5-3.6) .

The most important problem caused by the chemical antimicrobial agents is multidrug resistance. Therefore, an alternative way to overcome the drug resistance of various microorganisms is needed desperately, especially in medical devices(Rahi *et al.*,2013)

The high resistance here with antibiotics Rifampicin were (100%), against MRSA, on the other hand all isolates became susceptible to this antibiotic with rate (10-15mm) by using Ag-Nps with folded area(0.5-1.7). This resistance was presumed to be due to mutation(s) in the gene encoding the beta-subunit of DNA-dependent RNA polymerase, the target of the rifampin class of

antibiotics. Ag-NPs can cause further damage to bacterial cells by permeating the cell, where they interact with DNA, proteins and other phosphorus- and sulfur-containing cell constituents (Marambio-Jones and Hoek 2010).Results have demonstrated that MDR isolates *E.coli* and *P.mirabilis* have notably rates of resistance to chloramphenicol .The results showed that chloramphenicol gave inhibitory zone of *E.coli* which was (15-16mm) with folded area (1.7-3) by using Ag-Nps, compared with using disc diffusion alone. However, the results indicated that the percentage of inhibition zone (diameter) in *P. mirabilis* were increased when used Ag-Nps to reach a rate of 15mm and the folded area were (1.2-3.5) respectively.

Results approach to Mahmood (2012) revealed that a combination effect was detected between Ag-NPs and chloramphenicol for Gram-negative isolates was 14mm. *Proteus* is widespread in the environment and makes up part of the normal flora of the human gastrointestinal tract. *Proteus species* are the major cause of diseases acquired outside the hospital, where many of these diseases eventually require hospitalization are often the cause of urinary tract infections (UTIs) (Schaffer and Pearson, 2015).

The isolates of MRSA showed resistant to chloramphenicol which was determined (Table 3-10). all isolates were increased with AgNps in zone inhibition (10-13mm) so the folded area were (0.5-2.5). However, the resistance rates in the present study were higher than those reported by others (Hwang *et al*.,2012), who found that the synergistic interactions of Ag-Nps and chloramphenicol were found only against *Enterococc faecium* and *P. aeruginosa* while no Synergistic to *E.coli* and *S.aureus.* AgNPs are able to physically interact with the cell surface of various bacteria. This is particularly important in the case of Gram-negative bacteria where numerous studies have observed the adhesion and accumulation of AgNPs to the bacterial surface. Many studies have reported that AgNPs can damage cell

membranes leading to structural changes, which render bacteria more permeable. This effect is highly influenced by the nanoparticles' size, shape and concentration and a study using *Escherichia coli* (Periasamy *et al*, 2012). they accumulation on the membrane cell creates gaps in the integrity of the bilayer which predisposes it to a permeability increase and finally bacterial cell death. (Rai *et al*.,2014)

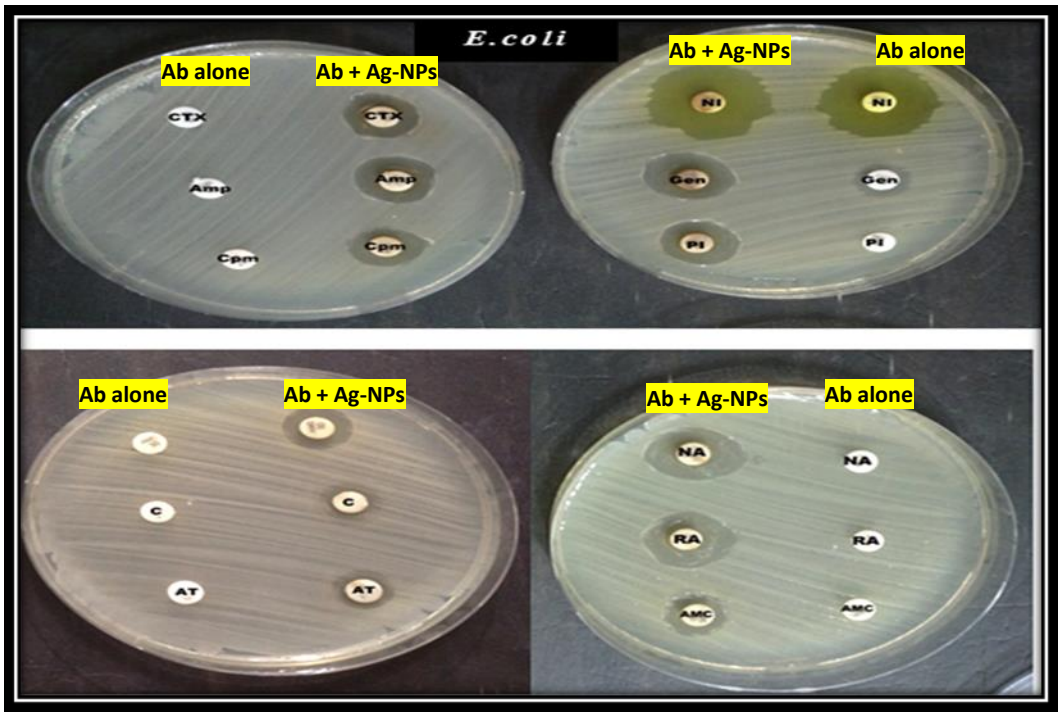


Figure (3-8) Zone of growth inhibition (mm) of *E.coli* with antibiotic alone and in combination with Ag-NPs .(Ab= Antibiotic)

Ampicillin =Amp

Amoxicillin-clv =Amc

Cefipime =Cpm

Cefotaxime =Ctx

Gentamycin =Gen

Azethronam =At

Nalidixic acid =Na ; Chloramphenicol = C ;Nitrofurantoin =Nit and Piperacillin =PI

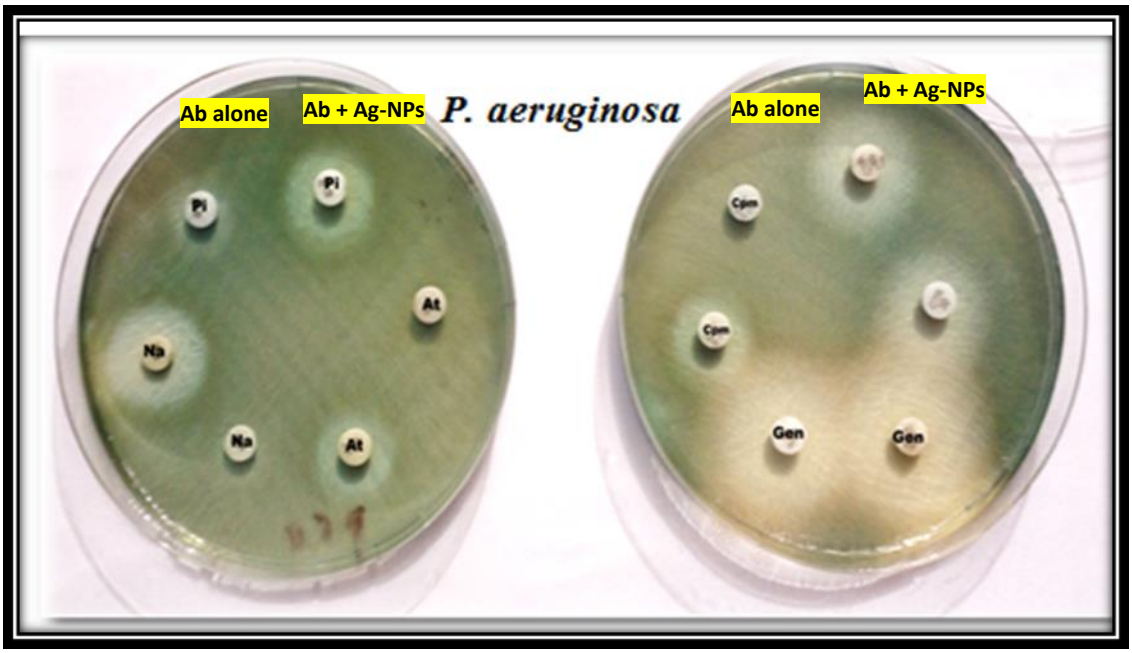


Figure (3-9) Zone of growth inhibition (mm) of *P. aeruginosa* with antibiotic alone and in combination with Ag-NPs .(Ab= Antibiotic)

Cefipime = CPM

Gentamycin = Gen

Azethronam = At

Ciprofloxacin= Cip

Piperacillin = Pi

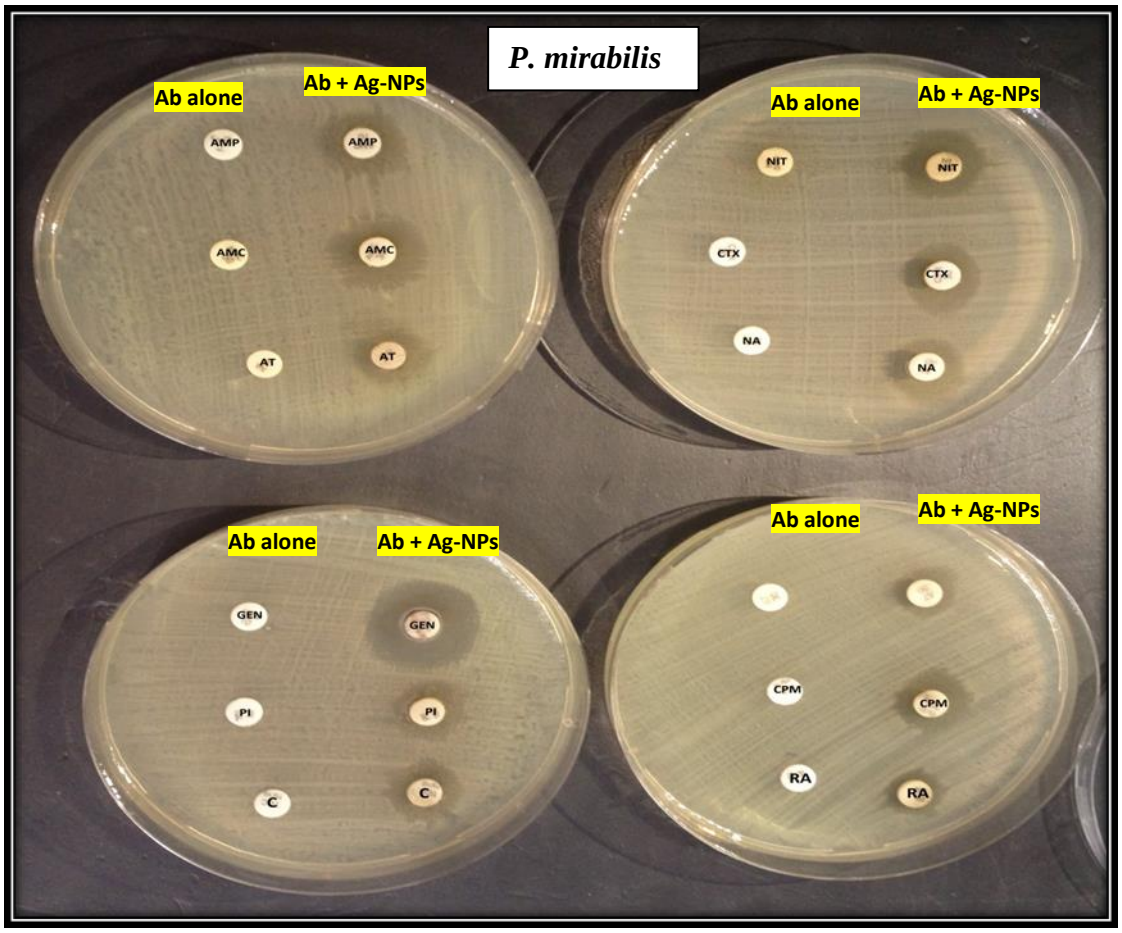


Figure (3-10) Zone of] growth inhibition (mm) of *P.mirabilis* with antibiotic alone and in combination with Ag-NPs .(Ab= Antibiotic)

Ampicillin = Amp

Amoxicillin-clv =Amc

Cefepime = Cpm

Cefotaxime = Ctx

Gentamycin = Gen

Azethronam = At

Nalidixic acid = Na

Chloramphenicol = C

Nitrofurantoin = Nit and Piperacillin = PI

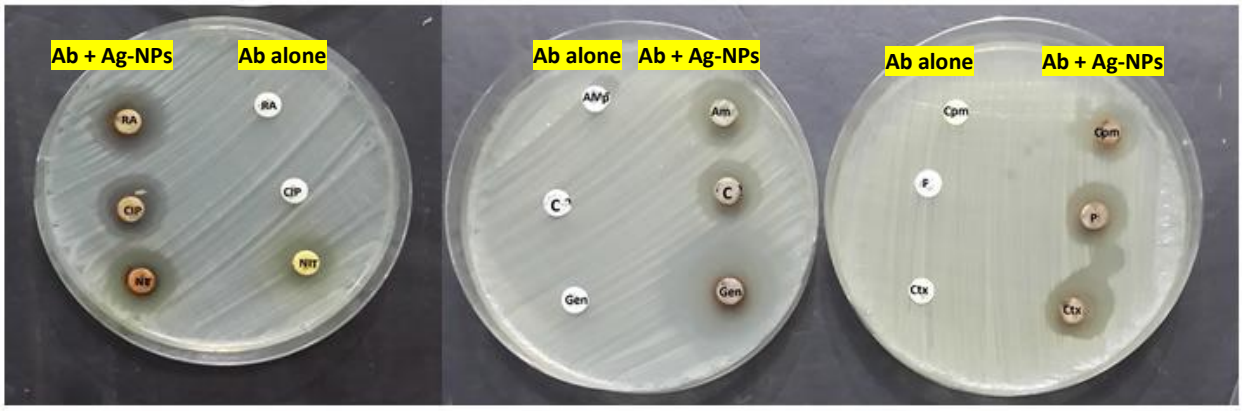


Figure (3-11) Zone of growth inhibition (mm) of MRSA with antibiotic alone and in combination with Ag-NPs . .(Ab= Antibiotic)

Cefipime =Cpm

Cefotaxime =Ctx

Gentamycin =Gen

Chloramphenicol = C

Nitrofurantoin =Nit

Piperacillin =PI

Ciprofloxacin= Cip

Rifampicin =RA

3.7. Detection of virulence factors

Investigation of some virulence factors that are important in the process of settlement and the continuation of infection, biofilm formation and ATPase important virulence factors in bacteria. Because there are no local studies on how nanoparticles affect the inhibition of both properties, this study chose these two factors and studied them for several reasons :

Most chronic and persistent bacterial infections are associated with biofilm growth, a strategy that has accelerated the emergence and rapid spread of MDR bacteria. It has been known for decades that biofilm associated bacteria is much more difficult to be eradicated by bactericidal antimicrobials than planktonic cells (Lewis,2001). Among the four general mechanisms that cause antibiotic resistance including target alteration, drug inactivation, decreased permeability and increased efflux, drug extrusion by the multidrug efflux pumps serves as an important mechanism of MDR. Efflux pumps not only can expel a broad range of antibiotics owing to their poly-substrate specificity, but also drive the acquisition of additional resistance mechanisms by lowering intracellular antibiotic concentration and promoting mutation accumulation (Sun *et al*.,2014). Biofilm formation as a mechanism to remain in a favorable nook .

Humans have developed intricate immune systems for one critical reason: microorganisms are continually trying to inhabit their bodies. The body, or at least parts of it, is nutrient rich and relatively stable with respect to water content, oxygen availability, and temperature. Consequently, there is a never-ending race between the development of the host immune system and the progression of bacterial strategies to evade it. In some cases, a compromise has been made, and as such, the body is inhabited by a large number of commensals, many of which exist as biofilms. As the body is obviously an appealing place for bacteria to live, it may be that the primary motivation for switching to the biofilm mode of growth is to remain fixed. (Lewis,2001) . A second possibility, which supports the biofilm as a mode of defense is that bacteria may have evolved to interpret elevated glucose levels as a cue that it is in the bloodstream, and that it needs to form a biofilm to remove itself from circulation and protect itself from the immune system.

Alternatively polysaccharide production may function as a mechanism of glucose storage during times of plenty, and/or as a mechanism to augment the accumulation phase so that when the organism finds itself in an environment rich in nutrients it can occupy that niche. With all of the complex mechanisms that pathogenic and commensal bacteria have evolved to survive in the human body, it is clear that the benefits that we afford them outweigh the hurdles synthesis (Sun *et al*.,2014) .

3.7.1. Detection of bacterial ability to produce biofilm by tissue culture plate and tube methods .

Biofilm(s) are groups of microorganisms coated in an exopolymeric coat. They have been associated with variety of persistent infections that respond poorly to conventional antibiotics (Bose *et al*.,2009). This method was achieved by Christensen *et al*. (1985) to detect the slime layer production by bacteria.

In the present study bacterial ability to produce biofilm were applied on 65 isolates shown in table (3-14) TM method and TCP, Optical density (OD) of stained adherent biofilm was obtained using ELISA auto reader at wavelength 590 nm, and the interpretation of the results were conducted . A standard method discovered 35(53.84%) as strong, 10(15.38%) as moderate and 20(30.67%) as weak/non biofilm producers by TCP table (3-15) while the results detected By TM the number of strong biofilm producers were 30(46.15%) , moderate were 18(27.69%) and weak or non-biofilm producers were 17(26.15%) table (3-16).

Table(3-14)Comparison between tube and tissue culture plate method for detection biofilm

Biofilm formation	Tissue Culture Plate	Tube Methods	X^2	P - value
Strong	35(53.84 %)	30(46.15%)	2.914	0.2330 [NS]
Moderate	10 (15.38%)	18(27.69%)		
Weak	20 (30.67%)	17(26.15%)		
Total number	65	65		

NS= Not Significant (p > 0.05), SD= Standard Deviation

Table(3-15) Positive and negative biofilm production with tissue culture plate method

Bacterial isolates		Biofilm formation(TCP)		P – value
		Positive biofilm %	Negative biofilm %	
1	*MRSA (n= 5)*	5 (100)	0 (0)	<0.00001*
2	*E.coli (n=65)*	45 (69.23)	20 (30.67)	0.0013*
3	*P.mirabilis (n=30)*	22 (73.33)	8 (26.66)	0.0003*
4	*P. aeruginosa* (n=35)	20 (57.14)	15 (42.85)	0.011*

***= Significant association (p <0.05), n=number**

Table(3-16) Positive and negative biofilm production with Tube method

bacterial isolates		Biofilm formation (TM)		P – value
		Positive biofilm (%)	Negative biofilm (%)	
1	*MRSA (n=5)*	5(100)	0 (0)	<0.00001*
2	*E.coli (n=65)*	35 (53.84)	30(46.15)	>0.05 [NS]
3	*P.mirabilis (n=30)*	16(53.33)	14(46.66)	>0.05[NS]
4	*P. aeruginosa* (n=35)	17(48.57)	18(51.42)	>0.05[NS]

***= Significant association (p <0.05), NS= Non Significant (p > 0.05), n=number**

The results approaching to Ahmed (2013) demonstrated that out of 118 Gram-positive bacteria the percentage of biofilm producers by TCP method was 95 (80.5%) higher than in TM were 78 (66.10%) producer as (moderate and strong) , in another study Hassan *et al*., (2011) they described the incidence of biofilm producers among 81 isolates of Gram-positive and Gram-negative bacteria were 51 (63%) strong bacteria biofilm and 30 (37%) moderate bacteria biofilm , in which Gram-positive bacteria generally higher than Gram-negative bacteria in producing biofilm. Depending on comparison between the results of biofilm production ,its appear that TCP is the more sensitive than TM methods as in Figure 3.18 so it depends, the results showed strong and moderate biofilm are 45 (69.23%) isolates *E. coli, 20* (57.14%) *P. aeruginosa* ,*P.mirabilis* and MRSA were 22 (73.33%) and 5 (100 %) respectively while by TM Figure (3.19) the majority 9of the organisms associated with biofilm production were *E. coli* 30 (46.15%) ; 17 (48, 53%) for *P. aeruginosa* and 16(53.33) *P.mirabilis* and MRSA were 5(7.69%).

These isolates were tested on antibiotics and found to be MDR. Similar results reported by Pramodhini *et al.*, (2012) who showed that there was a

relationship between biofilm production and MDR 80% of strains producing biofilm were multi drug resistant phenotypic . Strong biofilm production was caused by urinary catheter tips. Bakir & Ali (2016) in consistent finding that for biofilm production by TCP method as (moderate and strong) by bacteria *P. aeruginosa* were (81.8%) and TM (45.5%). There is an association between biofilm production with persistent infection and antibiotic therapy failure(Bose *et al* .,2009).They exhibit resistance to antibiotics by various methods like restricted penetration of antibiotic in to biofilm, decreased growth rate and expression of resistance genes (Sasirekha *et al*., 2012).

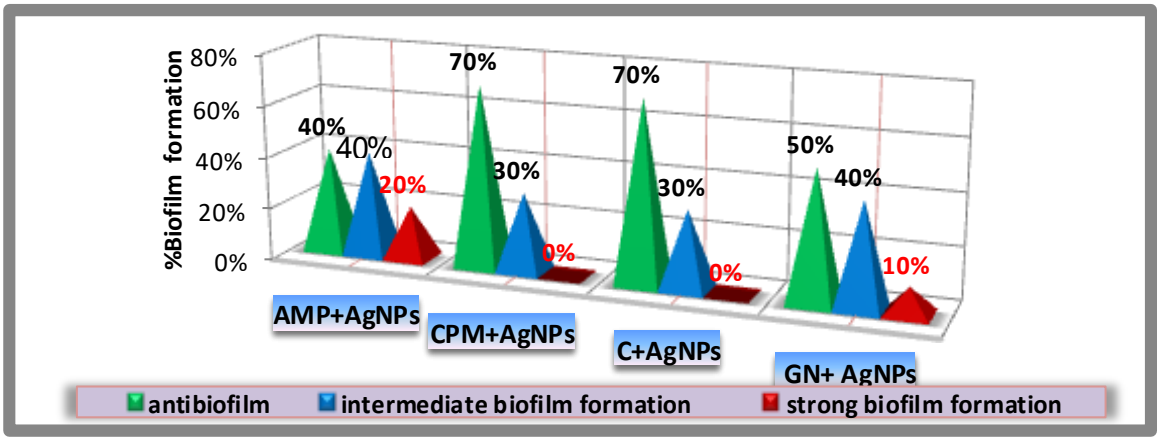
A suitable and reproducible method is necessary for screening of biofilm producers in any healthcare setup and this TCP method can be recommended (Bose *et al* .,2009).Tube method was good for strongly biofilm producing isolates but it was difficult to discriminate between weak and biofilm negative isolates due to the variability in observed results by different observers. Consequently, high variability was observed and classification in biofilm positive and negative was difficult by tube method (Mathur *et al.,* 2006).

3.7.2. Evaluation of biofilm inhibition assay

This study examined the effects of Ag-NPs by *Agaricus bisporus* at 30µl in combination with several antibiotic was evaluated against bacterial isolates adopting biofilm inhibition spectrophotometric assay. The antibiotics used in our study belong to several classes and have various cellular targets, modes of action and bacterial resistance mechanisms penicillin G, Ampiciline , cefotaxim , chloramphenicol and rifampicin.

These 35 isolates selected according to multi-drug resistance pattern TM and TCP as the most isolates obtained the highest OD value, Combination of Ag-NPs with antibiotics to inhibit the biofilm formation was performed according to Hwang *et al.* (2012) with few modifications.

The readings of OD have decreased within the range (0.120) ,whereas the OD of strong biofilm were ≥ 0.240 and the non-biofilm were ≤ 0.120.Gram positive cultures of bacterial isolated was *S.aureus* (*MRSA*) while of Gram negative *E.coli*, *Ps. aeruginosa* and *P.mirabilis* .The isolates showed different response to the antibiotics after they were treated, the highest response to Cefipime and chloramphenicol were 70% by *E. coli*, while Ampicillin and Gentamycin were 40%,50% respectively Figure3.12 and Table 3.17.This result agreed with the results obtained by Hwang *et al.* (2012) they revealed that combinations of Ag-NPs with conventional antibiotics (Ampicillin, Kanamycin and chloramphenicol) also appeared to actively inhibit biofilm formation to varying degrees. There is an association between biofilm production and antibiotic resistance. Therefore UTI caused by biofilm producing *E. coli*, (Gopinathl *et al* 2015).



Figure(3-12): Biofilm inhibition of *E. coli* determined using TCP method, the effect of addition of Ag-NPs in combination with antibiotics(antibiofilm =sensitive).

Table (3-17) The optical density value of Biofilm activity in TCP obtained by using antibiotics alone and in combination with Ag-NPs for *E.coli*

Isolated Bacteria Number / Antibiotics-Ag-NPs	*E.*1	*E.*4	*E.*8	*E.*6	*E.*12	*E.*14	*E.*18	*E.*22	*E.*23	*E.*25
Control	0.380	0.405	0.475	0.380	0.395	0.399	0.338	0.200	0.346	0.255
AMP	0.200*	0.209**	0.380*	0.300*	0.187**	0.243	0.260*	0.322*	0.240*	0.290*
AMP- Ag-NPs	0.104**	0.150**	0.199**	0.095**	0.104**	0.177**	0.102**	0.166*	0. 378*	0.380*
CPM	0.185*	0.176**	0.200**	0.199**	0.300*	0. 299*	0. 279*	0.250*	0.249*	0.288*
CPM- Ag-NPs	0.100**	0.099**	0.089**	0.085**	0.110**	0.070**	0.173**	0.130*	0.234*	0.087**
C	0.230*	0.440*	0.500*	0.190**	0.120**	0.194**	0.277*	0.204	0.232*	0.255
C-Ag-NPs	0.110**	0.109**	0.060**	0.100**	0.079**	0.104**	0.145**	0.130*	0.170**	0.103**
GEN	0.210*	0.230*	0.300*	0.244*	0.245**	0.294*	0.289*	0.254*	0.269*	0.255
GEN-Ag-NPs	0.070**	0.079**	0.115**	0.111**	0.150**	0.100**	0.145**	0.155*	0.270*	0.130**

****Significant ($p < 0.001$) in compared with control, *=Significant ($p < 0.05$) compared with control.**

The highest response in *P. aeruginosa* was 60% for both Piperacillin and Cefipime ,while Gentamycin were 50% Figure (3.13) and Table (3.18).Hwang *et al.* (2012)reported differentiate results between inhibition of biofilm formation by TCP method were effective than the various conventional antibiotics Ampicillin, chloramphenicol , Kanamycin. This opportunistic pathogen is able to form biofilm and is characterized by its multifactorial and increasing antibiotic resistance (Rewatkar and Wadher, 2013). Ag-NPs are also active against bacterial biofilms (Kalishwaralal *et al*., 2010), so they may prove effective in combatting biofilm-mediated. It was shown that biofilm formation is inhibited by the ability of Ag-NPs to prevent the initial steps in their development, microbial adhesion to various surfaces (Monteiro *et al*., 2012). Biofilm producing bacteria that have been isolated from urinary catheter tips were also multi drug resistant. Microbial biofilm have been associated with a variety of persistent infections which respond poorly to conventional antibiotic treatment (Rewatkar and Wadher, 2013).

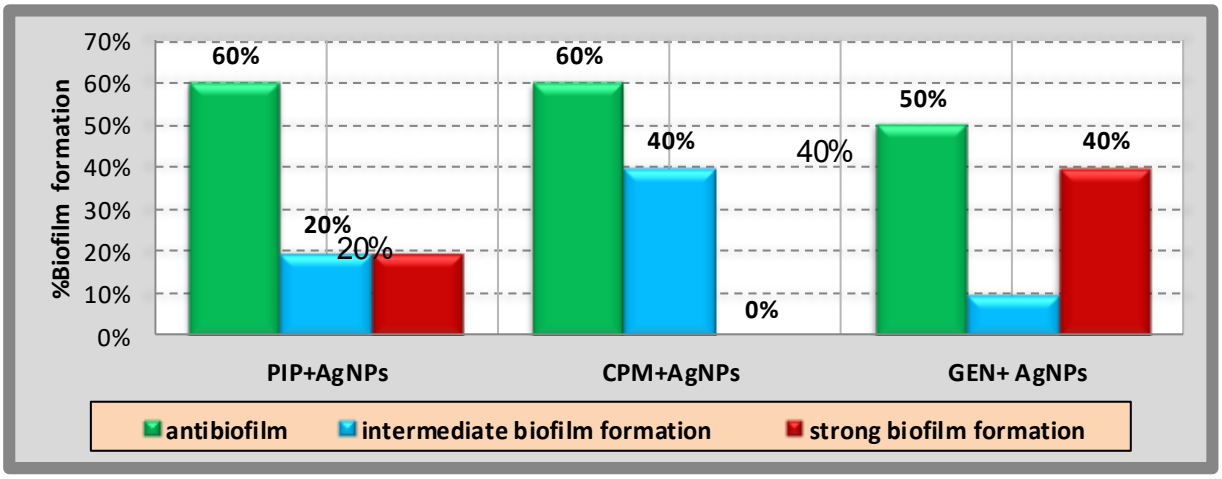


Figure (3-13) Biofilm inhibition of *P. aeruginosa* determined using TCP method, the effect of addition of Ag-NPs **in combination antibiotics (antibiofilm =sensitive).**

Table (3-18) The optical density value of biofilm activity in TCP obtained by using antibiotics alone and in combination with Ag-NPs for *P. aeruginosa*

Isolated Bacteria Number / Antibiotics-Ag-NPs	*PS*.6	*PS* .9	*PS* 10	*PS* .11	*PS*. 16	*PS*. 18	*PS* .19	*PS* .20	*PS* .22	*PS* .23
control	0.344	0.345	0.269	0.350	0.333	0.309	0.399	0.300	0.322	0.282
PIP	0.230*	0.222*	0.320	0.340	0.286*	0.344*	0.360*	0.245*	0.289*	0.270
PIP- Ag-NPs	0.094**	0.059**	0.099	0.075**	0.112**	0.177**	0.100**	0.146*	0.270*	0.280
CPM	0.209*	0.206*	0.210	0.299*	0.278*	0. 288	0. 229*	0.299	0.256*	0.232*
CPM- Ag-NPs	0.060**	0.087**	0.088	0.092**	0.094**	0.073**	0.153**	0.144*	0.134**	0.145**
GEN	0.224*	0.255*	0.278	0.243*	0. 240*	0.298	0.281*	0.255*	0.261*	0.205*
GEN-Ag-NPs	0.088**	0.072**	0.047	0.087**	0.100**	0.140**	0.265*	0.251*	0.260*	0.244*

****Significant ($p < 0.001$) compared with control, *=Significant ($p < 0.05$) compared with control**

The highest level of inhibition for *p. mirabilis* showed in Figure (3.14) 60% for Ampicillin and Cefipime, 50% for chloramphenicol and Gentamycin Additionally the result showed that the OD values acquired by Ag-NPs with antibiotics were less than the OD values obtained by antibiotics alone Table(3.19) Which confirms that the nanoparticles united with the antibody molecules to be compounded to reduce the process of forming biofilm. Bacterial biofilm has long been considered as a virulence factor contributing to infection associated with various medical devices and causing nosocomial infection(Bose *et al* .,2009).

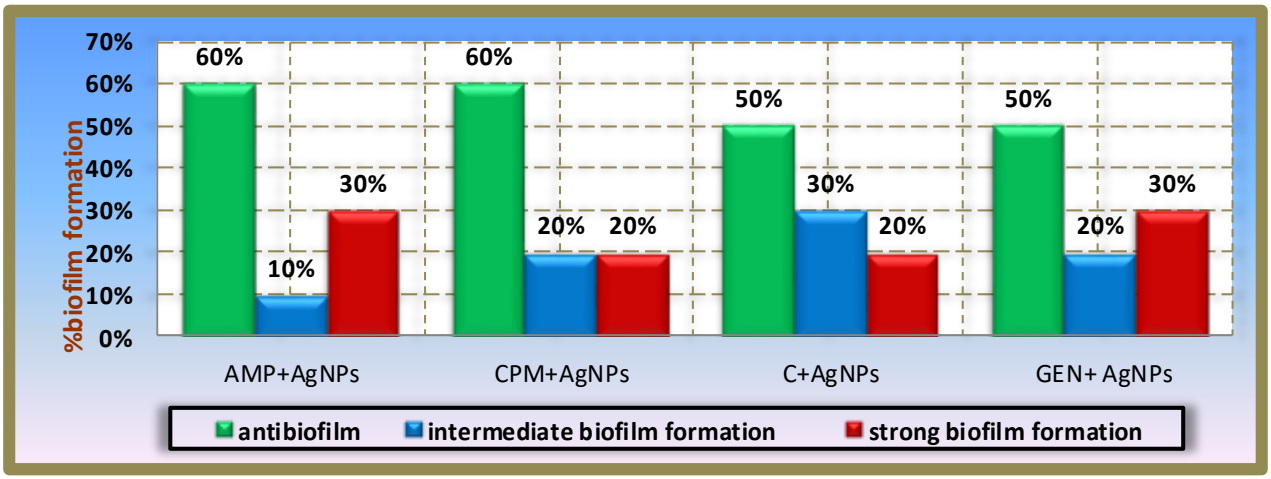


Figure (3-14): Biofilm inhibition of *P.mirabilis* determined using TCP method. addition of Ag-NPs **in combination with antibiotics (antibiofilm =sensitive).**

Table (3-19) The optical density value of biofilm activity in TCP obtained by using antibiotics alone and in combination with Ag-NPs for *P.mirabilis*

Isolated bacteria Number / Antibiotic Ag-NPs	*P.1*	*P.2*	*P.5*	*P.6*	*P.7*	*P.13*	*P.14*	*P.15*	*P.17*	*P.19*
Control	0.323	0.358	0.345	0.390	0.383	0.376	0.389	0.350	0.326	0.250
AMP	0.300	0.222*	0.348	0.240*	0.386	0.344*	0.260*	0.245*	0.189**	0.280*
AMP- Ag-NPs	0.045**	0.077**	0.088**	0.075**	0.100**	0.077**	0.287*	0.156**	0.245*	0.260
CPM	0.299*	0.206*	0.293*	0.288*	0.378	0. 289	0.229*	0.295*	0.254*	0.244
CPM- Ag-NPs	0.067**	0.080**	0.093**	0.067**	0.294*	0.073**	0.093**	0.274*	0.144**	0.135*
C	0.293*	0.340	0.286*	0.289*	0. 304*	0.299*	0.309*	0.340	0.298*	0.265
C-Ag-NPs	0.299*	0.055**	0.299*	0.093**	0.150*	0.110**	0.103*	0.089**	0.177**	0.187*
GEN	0.248*	0.282*	0.245*	0.223*	0. 199*	0.198**	0.128**	0.388*	0.242*	0.245
GEN-Ag-NPs	0.266*	0.289*	0.066**	0.076**	0.099**	0.112**	0.165**	0.141**	0.160**	0.274*

****Significant (p < 0.001) in compared with control, *=Significant (p < 0.05) compared with control**

S.aureus can readily form biofilm on artificial surfaces, such as stents, prostheses and catheters in recent years, implanted medical devices have been crucial in the advancement of patient care and the management of serious medical conditions. however, They are embedded in a matrix of extracellular polymeric substances (EPS) they have produced, and exhibit an altered phenotype with respect to growth rate and gene transcription. (Donlan and Costerton,2012).

The response of MRSA for Penicillin G 40% and 60% for each Cefipime and chloramphenicol, while 20%for Rifampicin Figure (3.15) and Table (3.20).Khalid *et al.* (2015) revealed that Ag-NPs had an inhibitory activity on biofilm formation greater than 55%. Combinations of Ag-NPs and antibiotics, showed a greater inhibitory activity than Ag-NPs alone, yet ampicillin did not show any increase in activity and anti-biofilm activity was due to Ag-NPs.

(Bose *et al* .,2009) reported anti biofilm effect of biologically synthesized silver nanoparticles against *S.aureus* and its synergistic effect with chemotherapeutics products ,Their studies reveals biogenic synthesized silver nanoparticles inhibited biofilm of both the tested organism and the inhibition was increased .Gurunathan *et al.*(2014) they were revealed Ag-NPs and Vancomycin Combination inhibited biofilm activity in Gram-negative and Gram-positive bacteria by 55% and 75%, respectively. Bacterial cells were grown to form biofilm and then treated with Ag-NPs in combination with antibiotics There are many mechanisms used bacteria to resist antibiotics. It is possible to have more than one mechanism in one organism. It may be a recipe to be a biofilm. Ag-NPs are also active against bacterial biofilm(s) (Kalishwaralal *et al*.,2010).

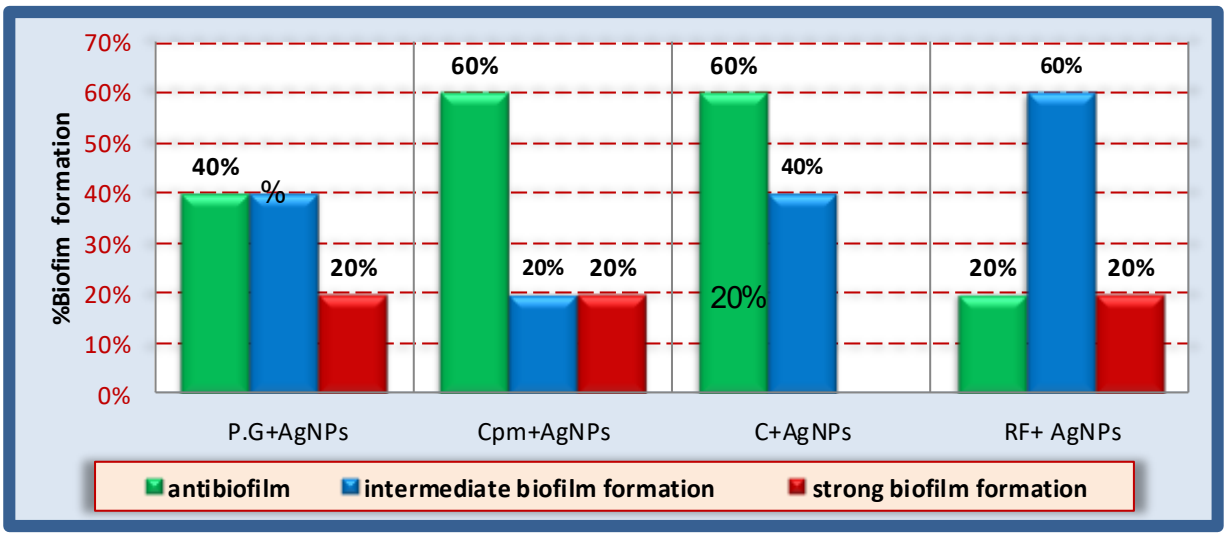


Figure (3-15): Biofilm inhibition of *MRSA* determined using TCP method. The effect of addition of Ag-NPs **in combination with antibiotics (antibiofilm =sensitive).**

Table (3-20) The optical density value of biofilm activity in TCP obtained by using antibiotics alone and in combination with Ag-NPs for MRSA

Isolated Bacterial Number / Antibiotics-Ag-NPs	MRSA-12	MRSA 2	MRSA 3	MRSA 7	MRSA-10
Control	0.251	0.299	0.240	0.280	0.233
P.G	0.266	0.223*	0.245	0.255*	0.245
P.G-Ag-NPs	0.234*	0.166**	0.156	0.081**	0.047**
CPM	0.287*	0.269*	0.253	0.276	0.223
CPM-Ag-NPs	0.100**	0.144**	0.051**	0.242*	0.089**
C	0.265	0.243*	0.283*	0.234*	0.238
C-Ag-NPs	0.083**	0.075**	0.067**	0.130**	0.143*
RF	0.244	0.254*	0.253	0.287	0.200*
RF-Ag-NPs	0.282*	0.052**	0.076**	0.080**	0.099**

****Significant ($p < 0.001$)** in compared with control, *=**Significant ($p < 0.05$) compared with control**

The main problem is that biofilms are extremely resistant to host defense mechanisms and antibiotic treatment, comparing to their planktonic analogues, making biofilm-related infections a major cause of morbidity and mortality. (Bookstaver *et al*. 2009). So they may prove effective in combatting biofilm-mediated, drug-resistant and device-centred infections. It was shown that biofilm formation is inhibited by the ability of Ag-NPs to prevent the initial steps in their development, i.e. microbial adhesion to various surfaces (Monteiro *et al.*, 2012) .

The results of optical density readings of bacterial culture showed that the presence of nanoparticles with antibiotics inhibited the growth of these bacterial isolates and thus inhibited their ability to form biofilm with the presence of antibiotics The selected antibiotics showed best compatibility with the Ag-NPs on the biofilm inhibition .

The results of TCP method by using Ag-NPs with antibiotics were effective than antibiotics alone. Therefore, combining Ag-NPs with different antibiotics at lower concentrations has the potential to become an effective anti-biofilm and antibacterial treatment Gurunathan *et al* (2014).

3.7.3. Antibacterial activity with detergents and ATPase inhibitors.

ATPase is an assay designed to indicate the nature of the interaction between the compound and the transporter. ATPase assays are used in two different modes: ATPase activation and ATPase inhibition. Transported substrates increase baseline atpase activity, while inhibitors or slowly transported compounds inhibit baseline atpase activity (Glavinas *et al.*,2008).

ATPase is a membrane assay that indirectly measure the activity of efflux transporters. ATP Binding Cassette or efflux transporters mediate the transport of substrates cross cell membranes against a concentration gradient. ATP cleavage is tightly linked to substrate translocation, as the energy for the substrate translocation is derived from ATP hydrolysis. ATP hydrolysis yields inorganic phosphate (Pi), which can be measured by a simple colorimetric reaction. The amount of (Pi)liberated is directly proportional to the activity of the transporter. (Glavinas *et al.*,2008).Combination of Ag-NPs with inhibitors in MDR bacteria was performed according to the Hwang *et al.* (2012) with few modifications. To elucidate whether the antibacterial activity of Ag-NPs was associated with the altered membrane permeability or the action of adenosine triphosphatase (ATPase), we examined the antibacterial activity of Ag-NPs in the presence of detergents and ATPase-inhibiting agents.

To determine the detergent-induced permeabilization, a particular concentration of Ag-NPs was determined using detergents, TX-100 , Tris and NaN3 .

This study examined the NaN_3, tris, triton X-100 ,alone and the effects of Ag-NPs by edible mushroom in combination compared to control (Fig 3-16). The results clarified that there was only little changes in the optical density following treatment with NaN_3,Tris,Triton X-100 alone in comparison whit combination Ag-NPs ,showed high changes in the optical density when using Ag-NPs in comparison between using of each inhibitors alone.

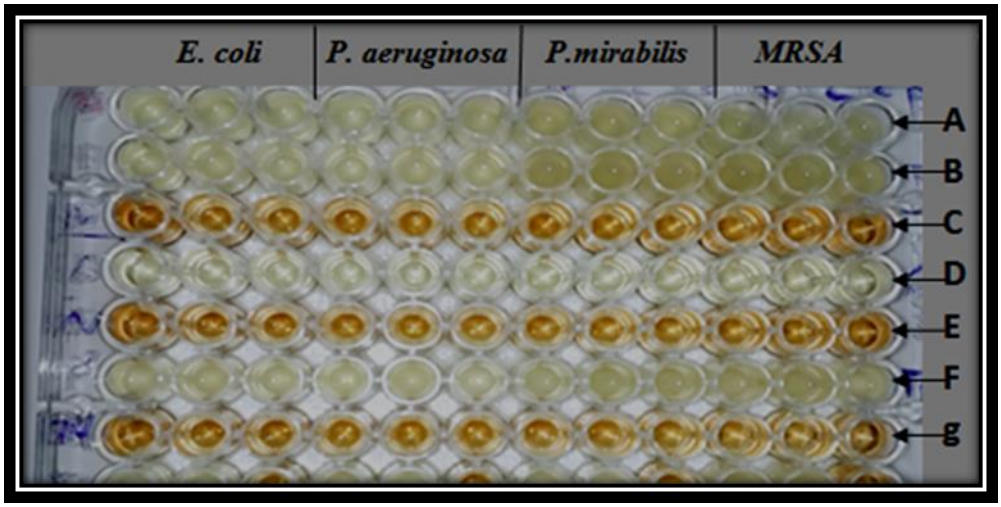


Figure (3-16): Effect of addition Ag-NPs in combination with ATPase inhibitors determined using the TCP method, various conventional antibiotics. (a)Control , (b)Tris, (c) Tris+ Ag-NPs,(d) NaN3 ,(e) NaN3+ Ag-NPs ,(f) Triton ,(g)Triton + Ag-NPs.

The isolates showed different response after they were treated Tables [(3-21),(3-22), (3-23),(3-24)]. The result showed that OD values acquired by NaN_3 ,alone and the OD values in combination with Ag-NPs .The level of inhibition of NaN_3showed in Figure (3-17) 60% ,50%, 40% and40% respectively against each of *E. coli*, *Ps. aeruginosa* , *P .mirabilis* and *MRSA*, *Ps. aeruginosa* gave the highest response . NaN3 metabolic inhibitors that can decrease ATP levels by disrupting electrochemical proton gradients in a bacterial environment were used as an inhibitor of ATPase (Swallow *et al*.,1990).

Renau *et al.*(1999).suggested that the difference in susceptibility of antimicrobial agents against Gram-positive and Gram-negative determinants present in all microorganisms. With few exceptions,they are chromosomally encoded and present a conserved. bacteria has been caused by a permeability barrier, such as an outer membrane or MDR pumps. Bacterial multidrug efflux pumps are antibiotic resistance.Bacterial multidrug efflux pumps are antibiotic resistance.

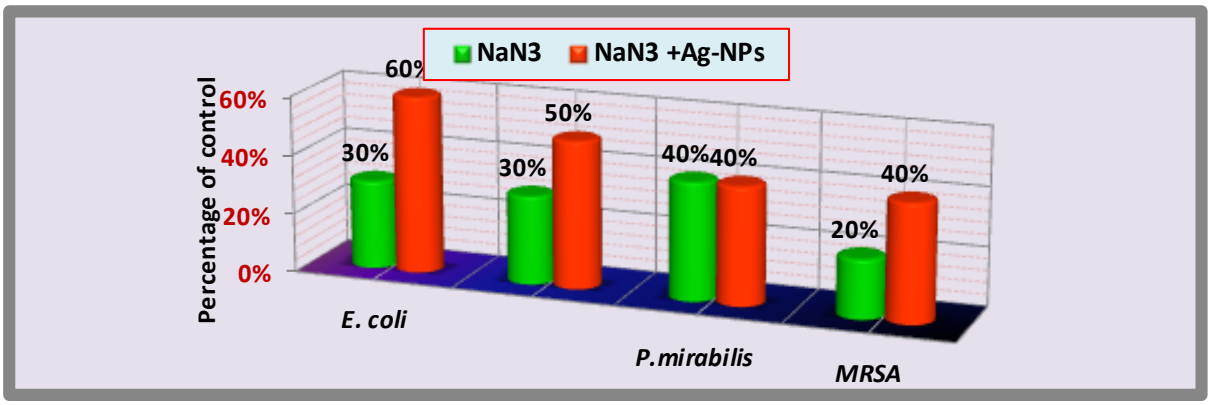


Figure (3.17):Effects of membrane-permeabilizing agents on the susceptibility of bacterial isolates. The presence of NaN_3 alone and in combination with Ag-NPs

Organization both at genetic and at protein levels that multidrug efflux pumps are ancient elements encoded in bacterial genomes long before the recent use of antibiotics for human and animal therapy Blanco- Andujar *et al.* (2016). The results showed changes in the optical density when using Ag-NPs with Triss Figure (3.18) the highest response to Tris were 80% by *E. coli*, while *Ps. aeruginosa*, *P.mirabilis* and MRSA were 60%,50% and 40%respectively, while the result acquired by Triton X-100 with Ag-NPs showed percentage 20%,30% and 10% respectively against *E. coli*, *Ps. aeruginosa* and *P.mirabilis*, While MRSA growth was not inhibited by combination Figure(3.19) .

The increased effect of detergent-induced membrane permeability on the activity of Ag-NPs. strains used in experiments.

The effect of the membrane-permeabilizing agent Triton X-100 (TX-100) on the susceptibility of the four microorganisms to Ag-NPs and treatments. compared to the OD 600 value of alone . The Binding of inhibitors and Peptidoglycan . Hwang *et al.* (2012) revealed that Ag-NPs had an inhibitory activity influenced by ATP associated with metabolism rather than by the permeability of the outer membrane.

The permeability of outer membrane was not remarkably affected by the activity of triton x-100 in combination Ag-NPs, and additionally the result exhibited low response of Gram-negative bacteria against Tris in combination with Ag-NPs, as compared to Gram-positive bacteria multidrug efflux pumps have been increasingly found to be associated with clinically relevant drug resistance. On the other hand, accumulating evidence has suggested that efflux pumps also have physiological functions in bacteria and their expression is subject tight regulation in response to various of environmental and physiological signals (Sun *et al*.,2014).

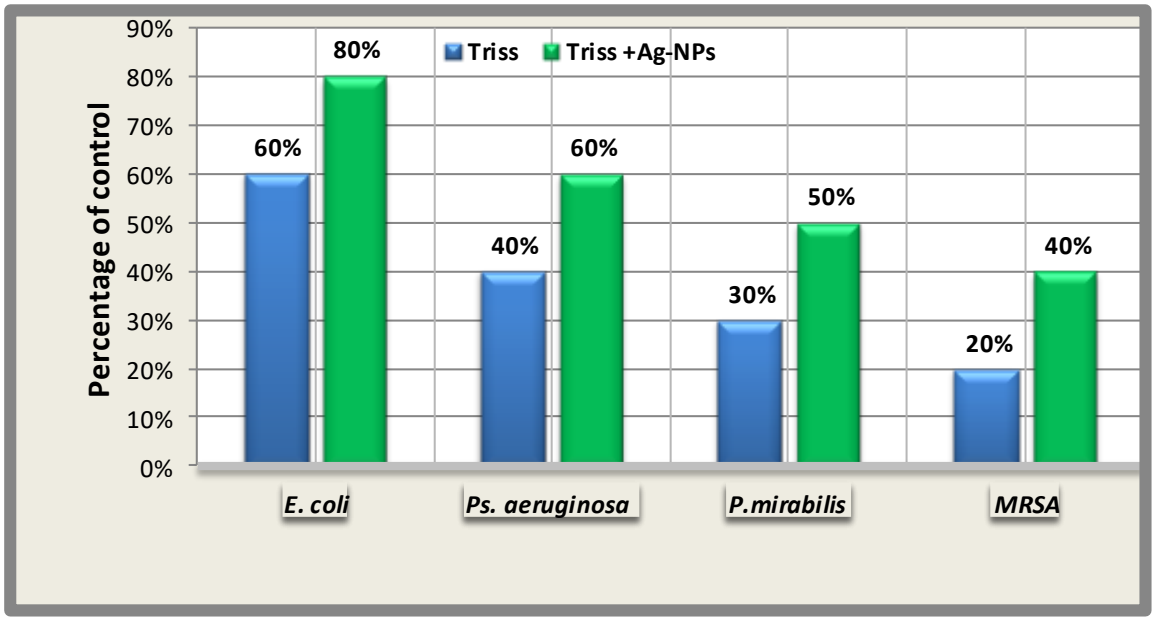


Figure (3-18):Effect of Tris alone and in combination with Ag-NPs on the susceptibility of bacterial isolates

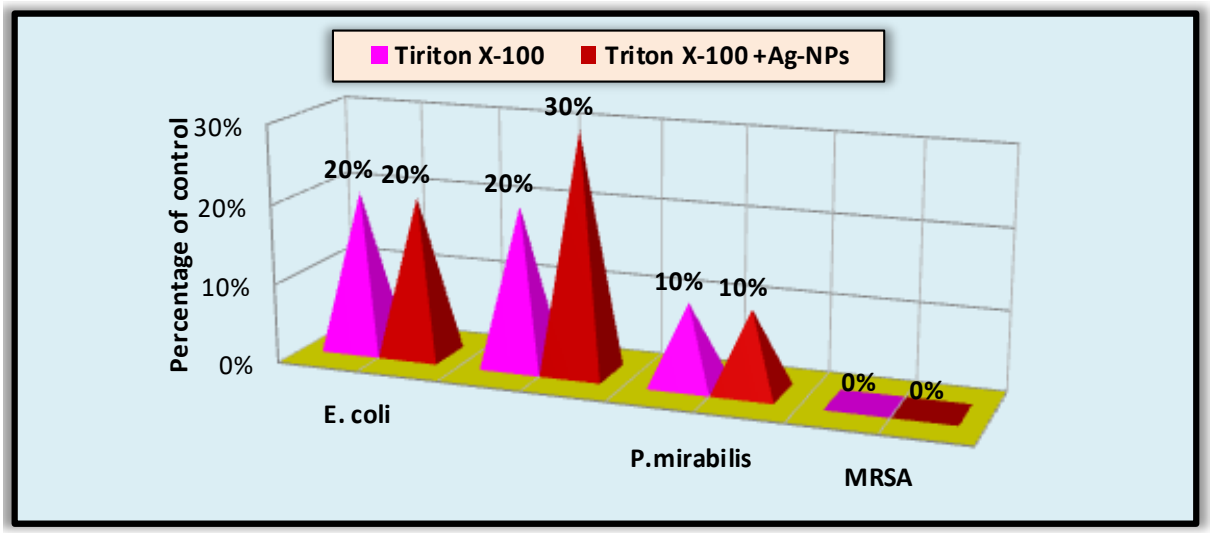


Figure (3-19):Effect of Triton X-100 alone and in combination with Ag-NPs on the susceptibility of bacterial isolates

However, to facilitate their clinical applications, future bids to lower their improve solubility, as well as search for the candidates that can inhibit different classes of efflux pumps is necessary Efflux pumps are prominent in terms of both their high efficiency of drug extrusion and broad substrate specificities, underlying their roles in MDR (Piddock *et al* .,2010). FoF1-ATPase catalyzes ATP synthesis coupled with the proton flow across the energy-transducing membranes such as the bacterial plasma membranes, mitochondrial inner membranes and chloroplast thylakoid membranes (Boyer ,1997) AgNPs can interact with bacterial membranes and increase membrane permeability, thereby causing changes in membrane structure and inducing DNA damage and enzyme inactivation, which eventually leads to cell death (Vardanyan *et al*.,2015).

Table (3-21) The optical density value of ATPase activity in TCP obtained by using inhibitors alone and inhibitors -Ag-NPs for *E. coli*

Isolated Bacteria Number / Inhibitors-Ag-NPs	*E.*1	*E.*4	*E.*8	*E.*6	*E.*12	*E.*14	*E.*18	*E.*22	*E.*23	*E.*25
Control	0.224	0.405	0.475	0.180	0.195	0.099	0.138	0.200	0.146	0.155
Triss	0.167/ 0.033*	0.209/ 0.00086*	0.180/0.0007**	0.100/ 0.0104*	0.187	0.163/ 0.027*	0.278/ 0.0201*	0.301/ 0.0311*	0.099/ 0.0151*	0.194/ 0.020*
Triss Ag-NPs-	0.114/ 0.00061**	0.150/0.0003**	0. 075/ 0.00001**	0.095/ 0.005*	0.150/ 0.031*	0.187/ 0.044*	0.106/ 0.049*	0.170/ 0.032*	0. 078/ 0.0280*	0.180/ 0.0341*
NaN3-	0.195/ 0.0341*	0.196/0.00344**	0.500/ 0.047*	0.099/ 0.0055*	0.200	0. 129/ 0.011*	0. 172/ 0.036*	0.225/ 0.0277*	0.179/ 0.0411*	0.173/ 0.0203*
NaN3 Ag-NPs-	0.107/ 0.0001**	0.150/0.0003**	0.630/ 0.0008**	0.085/ 0.0033*	0.210	0. 079/ 0.046*	0. 073/ 0.022*	0.100/ 0.024*	0.244/ 0.0002**	0.187/ 0.0310*
Triton X-100-	0.230	0.440/ 0.042*	0.500/ 0.047*	0.190	0.120/ 0.026*	0.194/ 0.0007**	0.187/ 0.0288*	0.208	0.152	0.155
Triton X-100Ag-NPs	0.220	0.400	0.460	0.200/ 0.033*	0.099/ 0.005*	0.184/ 0.0004**	0.141	0.220/ 0.0461*	0.150	0.163

****Significant ($p < 0.001$) in compared with control, *=Significant ($p < 0.05$) compared with control.**

Table (3-22) The optical density value of ATPase activity in TCP obtained by using inhibitors alone and inhibitors -Ag-NPs for *P. aeruginosa*

Isolated Bacteria Number / InhibitorsAg-NPs	PS.6	PS .9	PS 10	PS .11	PS. 16	PS. 18	PS .19	PS .20	PS .22	PS .23
Control	0.473	0.306	0.350	0.402	0.306	0.374	0.250	0.374	0.367	0.280
Tris	0.204/ 0.0006**	0.383/ 0.0101*	0.361	0.212/ 0.011*	0.383/ 0.009*	0.190/ 0.0008**	0.124/ 0.00012**	0.373	0.391/ 0.0430*	0.250/ 0.0355*
Tris- Ag-NPs-	0.154/ 0.0003**	0.390/ 0.0100*	0.159	0.107/ 0.0003**	0.140/ 0.00055**	0.198/ 0.002*	0.100/ 0.00026**	0.360	0.351	0.273
NaN3-	0.275/ 0.00022**	0.377/ 0.029*	0.355	0.414	0.377/ 0.038*	0.350/ 0.0009**	0.245	0.176	0.170	0.270
NaN3-Ag-NPs-	0.240/ 0.00082**	0.316	0.362	0.125/ 0.00041**	0.299/ 0.0402*	0.131/ 0.00028**	0.241	0.168	0.120/ 0.008*	0.222/ 0.0244*
Triton X-100-	0.250/ 0.0013*	0.358/ 0.030*	0.120/ 0.0003**	0.474/ 0.0131*	0.358/ 0.0433*	0.361/ 0.0001**	0.261	0.341/ 0.0422*	0.365	0.215/ 0.0133*
Triton X-100Ag-NPs	0.100	0.361	0.102	0.389	0.161	0.361	0.257	0.368	0.355	0.200/ 0.011*

Significant (p < 0.001) in compared with control, *=**Significant (p < 0.05)** compared with control

Table (3-23) The optical density value of ATPase activity in TCP obtained by using inhibitors alone and in combination with Ag-NPs for *P. mirabilis*

Isolated bacteria Number / Inhibitors-Ag-NPs	P.1	P.2	P.5	P.6	P.7	P.13	P.14	P.15	P.17	P.19
Control	0.274	0.254	0.286	0.293	0.206	0.259	0.261	0.299	0.220	0.209
Triss	0.112/ 0.0003**	0.190/ 0.0077*	0.274	0.158/ 0.0075*	0.200	0.270	0.285/ 0.047*	0.322/ 0.0084*	0.216	0.232/ 0.0380*
Triss- Ag-NPs	0.107/ 0.00013**	0.211/ 0.0061*	0.285	0.187/ 0.0062*	0.107/ 0.0066**	0.290/ 0.0075*	0.266	0.291	0.240/ 0.037*	0.101/ 0.0001**
NaN3	0.167	0.262	0.286	0.297	0.130/ 0.0133*	0.176/ 0.005*	0.130/ 0.0007**	0.335/ 0.0066*	0.230	0.266/ 0.0310*
NaN3-Ag-NPs	0.059/ 0.006*	0.249	0.296	0.298	0.108/ 0.0076*	0.120/ 0.004*	0.101/ 0.0004**	0.384/ 0.0051*	0.263/ 0.029*	0.150/ 0.0091*
Triton X-100	0.157	0.275/ 0.038*	0.279	0.290	0.189	0.069/ 0.0003**	0.280/ 0.025*	0.289	0.234	0.199
Triton X-100Ag-NPs	0.107/ 0.00013**	0.267	0.289	0.222/ 0.010*	0.194	0.127/ 0.00026**	0.256	0.302/ 0.0033*	0.230	0.187

****Significant (p < 0.001) in compared with control, *=Significant (p < 0.05) compared with control**

Table (3-24) The optical density value of ATPase activity in TCP obtained by using inhibitors alone and in combination with Ag-NPs for MRSA

Isolated Bacterial Number / Inhibitors-Ag-NPs	MRSA-12	MRSA 2	MRSA 3	MRSA 7	MRSA-10
Control	0.251	0.250	0.238	0.285	0.212
Triss	0.242	0.252	0.256	0.281	0.109/ 0.00051**
Triss- Ag-NPs	0.286/ 0.0355*	0.243	0.105/ 0.00020**	0.288	0.101/ 0.0006**
NaN3-	0.255	0.119/ 0.00036**	0.257	0.299	0.211
NaN3- Ag-NPs	0.100/ 0.00044**	0.109/ 0.000022**	0.271/ 0.008*	0.352/ 0.006*	0.289/ 0.0072*
Triton X-100-A	0.065/ 0.00011**	0.245	0.283/ 0.0033*	0.354/ 0.0071*	0.238/ 0.008*
Triton X-100Ag-NPs-	0.123/ 0.00025**	0.255	0.237	0.278	0.243/0.0077*

****Significant ($p < 0.001$)** in **compared with control, *=Significant ($p < 0.05$) compared with control**

3.7.4. Antibacterial Activity of ATPase in TCP obtained by using inhibitors in combination with Ag-NPs and drugs by using standard strains

To elucidate whether the antibacterial activity of Ag-NPs was associated with either the altered membrane permeability or the mechanism of multidrug resistance (MDR), we examined the antibacterial activity of Ag-NPs in the presence of detergents and ATPase-inhibiting agents, respectively. To determine the detergent-induced permeabilization, a particular concentration of Ag-NPs was determined using the detergent Tris and Triton X-100 (Cordwell *et al* .,2002) .

NaN3, a metabolic inhibitor that can decrease ATP levels by disrupting electrochemical proton gradients in a bacterial environment, were used as inhibitors of ATPase(Shibata *et al* .,2012). The antibacterial activity of Ag-NPs was measured in the presence of 30mM Triss, 0.01% TX-100, and 0.001% ,NaN3

Generally, antibacterial drugs inhibit bacterial growth in different targets, including inhibition of cell wall synthesis, disruption of cell membrane function, inhibition of protein, and nucleic acid synthesis (Cordwell *et al* .,2002). According to our results, Ag-NPs showed the increasing cytoplasmic membrane permeability and inhibiting ATPase Bacterial strains viability was highly decreased when Ag-NPs (30 μg/mL), Ampicillin and inhibitors were used together.The effect of the membrane-permeabilizing agent Tris and Triton X-100 (TX-100) on the susceptibility of Bacterial strains to AMP treatment. The viability of bacteria was determined spectrophotometrically optical density at 600 nm, OD600 (sun *et al*.,2014).The data is represented as an average of three independent experiments. $P < 0.001$ as compared to the control alone.Comparison with the antibacterial activity of Ag-NPs in the presence of Tris was markedly increased (Steinfels *et al*.,2004). Actually, it has been reported that 0.001% NaN3 significantly increased susceptibility toward AMP in clinical *P. aeruginosa* isolates (Jung and Lee,2008).

Mechanism of antibacterial activity of AMP involved the membrane disruption and cell lyses. The results of AMP treatment in combination with TX-100 and NaN3 showed that AMP has role in increasing cytoplasmic membrane permeability and decreasing activity of ABC transporter. These results show the promising effect for the use of AMP based products in the treatment Further, *in vivo* experiments are needed for the clinical use of AMP on different infectedpatients. Ag-NPs attach to the cell surface, alter the physical and chemical properties of the cell membranes and the cell wall and disturb important functions such as permeability, osmoregulation, electron transport and respiration (Marambio-Jones and Hoek 2010). Another study NaN3 is a metabolic inhibitor, which reduces ATP level by disrupting electrochemical proton gradients in a bacterial cell . Actually, it has been

reported that 0.001% NaN3 significantly increased susceptibility (Jung and G. Lee,2008).

Table (3-25) The optical density value of ATPase activity in TCP obtained by using inhibitors in combination with Ag-NPs and AMP for stander strains.

Isolated Bacterial Number / Inhibitors-Ag-NPs	*E. coli* ATCC- 28739	*P. mirabilis* ATCC- 16404
Control	0.270	0.268
Tris-AgNps	0.122/ 0.00012	0.196/ 0.00033
Tris- Ag-NPs- AMP	0.130/ 0.0015	0.105/ 0.00020**
NaN3-AgNps-	0.119/ 0.00036**	0.107/ 0.00035
NaN3- Ag-NPs-Amp	0.109/ 0.00002**	0.200/ 0.00018*
Triton X-100-AgNps	0.205/0.012	0.283/ 0.0033*
Triton X-100Ag-NPs-AMP	0.155/0.0011	0.137/ 0.00018

****Significant ($p < 0.001$) in compared with control, *=Significant ($p < 0.05$) compared with control**

Conclusions and *Recommendations*

Conclusions

1. Most of bacterial isolates were Multi-drug resistant thought anti biotic resistant biofilm and ATPase inhibition .
2. Edible mushroom (*Agaricus bisporus*) has positive efficiency in biosynthesis of slivernanoparticles due to its containing compound which have play major role in acting as a reductant and capping material in order to synthesis of a novel Ag-NPs.
3. The biogenic of Ag-NPs showed antibacterial activity against MDR pathogenic bacteria under study and a reduction in biofilm production and ATPase activity.
4. Ag-NPs have potential applications in the biomedical field and the straightforward procedure used has several advantages for a large- scale commercial production.

Recommendations

1. As a result of increasing the resistance of microbes to the antibiotics specially of those nosocomial infection ,it is necessary to invade a new methods to stopped or militated of this problem ,nanotechnology involved to resolution sooner.
2. Synthesized of Ag-NPs showed comprehensive bactericidal activity against *Eschershia coli, Pseudomonas aeruginosa, protues mirabilis and Methicillin resistant Staphylococcus aureus*. Ag-NPs enhanced the antimicrobial activity of antibiotics. synthesis of nanoparticles using *Agaricus bisporus* can potentially eliminate the problem of chemical agents, which may have adverse effects in its application, thus making nanoparticles more biocompatible
3. Further studies must be done to make in vivo by mammals against Gram-negative and Gram-positive bacteria in combination with AgNps in Iraq, will be helpful in monitoring and evolution antimicrobial resistance and to guide interference to minimize its occurrence and to amusement of the toxicity .
4. Screening of other microorganisms to select the efficient in the production of silver nanoparticles.
5. Determination and the compound that responsible of production of nanoparticles.

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Appendix (1) Ddiagnostic tests for *E.coli* and *P. mirabilis* using API 20 E (Bio Merieux, API 20 E system)

TEST	REACTION	NEGATIVE	POSITIVE
ONPG	β-galactosidase	Colourless	Yellow (maybe pale)
ADH	Arginine dihydrolase	Yellow	Orange or red
LDC	Lysine decarboxylase	Yellow	Orange or red
ODC	Ornithine decarboxylase	Yellow	Orange or red
CIT	Citrate utilisation	Light green	Blue-green or blue
H_2S	H_2S production	Colourless	Black
URE	Urea hydrolysis	Yellow	Pink
TDA	Tryptophan deamination	Yellow	Dark brown
IND	Indole production	Colourless reagent	Pink
VP	Acetoin production	Colourless	Pink or red
GEL	Gelatin hydrolysis	Colourless	Black diffuse pigment
GLU	Glucose fermentation	Blue	Yellow
MAN	Mannitol	Blue	Yellow
INO	Inositol	Blue	Yellow
SOR	Sorbitol	Blue	Yellow
RHA	Rhamnose	Blue	Yellow
SAC	Sucrose	Blue	Yellow
MEL	Melibiose	Blue	Yellow
AMY	Amygdalin	Blue	Yellow
ARA	Arabinose	Blue	Yellow
Oxidase	Cytochrome oxidase	Colourless	Purple

Appendix (2) Diagnostic tests for *P. aeruginosa* using API 20 NE(Bio Merieux, API 20 NE system)

Test	Substrate	Reactions/Enzymes	Resultats	
			Negative	Positive
No3	Potassium nitrate	Reduction of nitrate to nitrite	NIT1 + NIT2/5min	
			Colour less	red
TRP	Tryptophane	Indole production	James / immediate	
			Colour less	red
GLU	Glucose	Fermentation/oxidation	blue to green	Yellow
ADH	Arginine	Arginine - dehydrolase	Yellow	red/ orange
URE	Urea	Urease	Yellow	red/ orange
ESC	esculin ferric citrate	Hydrolysis (B-glucosidase) (Esculin)	Yellow	grey/brown /black
GEL	gelatin (bovine origin)	Hydrolysis (Protease) (Gelatin)	no pigment diffusion	diffusion of black pigment
PNPG	4-nitrophenyl-βD-galactopyranoside	β – galactosidase (Para-Nitrophenyl- βD – Galactopyr-anosidase	colourless	yellow
GLU	D - glucose	assimilation (Glucose)	Transparent	Opaque
ARA	L – arabinose	assimilation (Arabinose)	=	=
MNE	D – mannose	assimilation (Mannose)	=	=
MAN	D – mannitol	assimilation (Mannitol)	=	=
NAG	N-acetyl-glucosamine	assimilation (N-Acetyl-Glucos amine)	=	=
MAL	D – maltose	assimilation (Maltose)	=	=
GNT	Potassium gluconate	assimilation (Potassium gluconate)	=	=
CAP	Capric acid	assimilation (Capric acid)	=	=
ADI	adipic acid	assimilation (Adlic acid)	=	=
MLT	Malic acid	assimilation (Malate)	=	=
CIT	Trisodium citrate	assimilation (Trisodium citrate)	=	=
PAC	Phenylacetic acid	assimilation (phenyl Acetic acid)	=	=

Appendix (3)Diagnostic test for *S. aureus* API staph (Bio Merieux, API 20system)

Tests	Substrate	Reactions/ Enzymes	Result	
			Negative	Positive
0	No substrate	Negative Control	Red	-
GLU FRU MNE MAL LAC TRE MAN XLT MEL	D-Glucose D-Fructose D-Mannose Maltose Lactose D-Trehalose D.Mannitol Xylitol D.Melibiose	(Positive Control) Acidification due to Carbohydrate Utilization	red	Yellow
NIT	Potassium nitrate	Reduction of nitrate to nitrite	NIT1+NIT_2 - 10 min	
			Colourless	red
PAL	B-naphthylacid phosphate	Alkaline phosphatase	ZYMA+ZYMB - 10 min	
			Yellow	Violet
VP	Sodium Pyruvate	Production of acetylmethyl carbinol	Vp1 + Vp_2 - 10 min	
			Colourless	Violet – pink
RAF XYL SAC MDG NAG	Raffinose Xylose Saccharose *a* - methyl - D -glucoside N-acetyl-glucosamine	Acidification dueto carbohydrate utilization	red	Yellow
<u>ADH</u> <u>URE</u>	Arginine Urea	Arginine dihydrolase Urease	Yellow Yellow	Red Red - Violet

الخلاصة

اجريت الدراسة الحالية لتقييم التصنيع الحيوي لجسيمات الفضة النانوية (AgNps) silver nanoparticles باستخدام فطر المشروم الصالح للاكل *Agaricus bisporus* ،وتأثيرها كعامل مثبط لوحده او بخلطها مع المضادات الحيوية تجاه بعض البكتريا ذات المقاومة المتعددة للأدوية .جمعت 250 عينة سريريه من المرضى (الذكور والاناث) من الراقدين في مستشفى الديوانية العام ومستشفى الاطفال التعليمين في مدينة الديوانية للفترة من تشرين الاول(2016) ولغاية شباط (2017) لغرض عزل البكتريا ذات المقاومة المتعددة, فضلا عن استحصال العزلات القياسية للبكتريا (*Escherichia coli*، *Staphylococcus aureus, Proteus mirabilis Pseudomonas aeruginosa*).
اظهرت نتائج العزل ان 150 (60%) من العزلات هي بكتيريا ايجابيا و 100 (40%) من العينات لم تعطي نموا و واعتمادا على مصدر العينات اظهرت النتائج أن العزلات البكتيرية توزعت الى 60 (40%) الادرار، 48 (32%) القسطرة، 26 (17.3%) الجروح 16 (10.6%) دمامل جلدية و مسحات الأذن.
اوضحت نتائج الزرع والاختبارات الكيموحيوية ان البكتيريا المعزولة كانت 65 (34%) منها تعود الى *Escherichia coli* ، و35 (23%) تعود الى *Pseudomonas aeruginosa* ، و 30 (20%) تعود الى *Proteus mirabilis* و 20 (14%) تعود الى *Staphylococcus aureus*.
تم استخدام الفطر *Agaricus bisporus* كمختزل حيوي في تصنيع دقائق الفضة النانوية ، و تم الكشف عن Ag-NPs من خلال تغير اللون من الاصفر الى اللون البني الغامق .تم توصيف دقائق Ag-NPs باستخدام جهاز الاشعة فوق البنفسجية والاشعة تحت الحمراء والمجهر الالكتروني النافذ TEM والماسح SEM اظهرت ان جزيئات Ag-NPs ذات شكل كروي بحجم 5-35 نانوميتر من خلال المجهر الإلكتروني النافذ و 5-50 نانوميتر من خلال المجهر الإلكتروني الماسح ،فيما أظهر تحليل FTIR ان

أطياف Ag-NPs بين 250 إلى 4000 سم -1 .بينما اظهر التحليل الطيفي ان الدقائق النانوية المحضرة ذات امتصاصية 430 نانوميتر .اظهرت

دقائق Ag-NPs احتوائها نشاطا مضاد ضد العزلات البكتيرية المرضية بالمقارنة مع فعالية المضادات الحيوية لوحدها. اختبرت 35 عزلة بكتيرية بالاعتماد على مقاومتها العالية للمضادات الحياتية لغرض قياس الفعالية المضادة للبكتريا لجسيمات الفضه النانوية المخلوطة مع المضادات الحيوية لكل من Methicillin- resistance, *S. aureus* (*MRSA*), *P. aeruginosa, E. coli* and *P. mirabilis*

اظهرت النتائج وجود زيادة في منطقة التثبيط (Increase fold area) للنمو مابين (0.2- 4) لبكتريا *E. coli* و(0.2-4.4) لبكتريا *P. aeruginosa* و(0.2- 3.6) لبكتريا (MRSA) and *P. mirabilis* .

من ناحية أخرى، تم تثبيط قدرة العزلات البكتيرية لإنتاج الغشاء الحيوي biofilm كعامل ضراوة باستخدام صفيحة الزرع النسيجي وطريقة ألانبوب . وأظهرت النتائج أن جميع العزلات البكتيرية التي تم اختبارها كانت منتجة biofilm وكانت طريقة صفيحة الزرع النسيجي هي الطريقة الأكثر حساسية في الكشف عن الغشاء الحيوي للبكتيريا ذات المقاومة المتعددة. بالإضافة إلى ذلك فإن دقائق Ag-NPs المخلوطة مع المضادات الحيوية أظهر انخفاضا ملحوظا في إنتاج البكتريا للغشاء الحيوي بالمقارنة مع تأثير المضادات الحيوية لوحدها, وقد اظهر التحليل الاحصائي وجود فروقات معنوية **(p< 0.05).** بالمقارنة مع معامل السيطرة (control) .

تم التحري عن قابلية Ag-NPs على تثبيط ATPase باستخدام مثبطات الجدار الخلوي Tris ، Triton X100 و NaN3. و اوضحت النتائج ان اعلى مستوى تثبيط كان هو مع المثبط Tris مع Ag-NPs . استنتجت الدراسة ان دقائق الفضة النانوية بواسطة فطر*Agaricus bisporus* قد برهنت على امكانية استخدامها كمضادات بكتيرية للتغلب على مشكلة التأثيرات الجانبية للمضادات الحياتية المستخدمة .

وزارة التعليم العالي
والبحث العلمي
جامعة القادسية
كلية الطب
فرع الاحياء المجهرية

التصنيع الحيوي لجسيمات الفضة النانوية باستخدام مستخلص الفطر *Agaricus bisporus* وفعاليته كمضاد بكتيري ضد البكتيريا المتعددة المقاومة للمضادات

أطروحة

مقدمة إلى مجلس كلية الطب /جامعة القادسية
وهي جزء من متطلبات نيل درجة دكتوراه فلسفة في
الأحياء المجهرية الطبية

من قبل
عقيل عباس كريم الخفاجي

بكالوريوس علوم في علوم الحياة - 2005
ماجستير أحياء مجهرية طبية - 2010

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