Study the Synergistic Effect of AgNPs with Antibiotics on *Klebsiella Pneumoniae* Isolates Which Diagnosed by Molecular Methods

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Abstract

The current study was conducted to study the synergistic effect of AgNPs with anti-biotics on *Klebsiella* pneumonia isolates, where 50 samples were collected from different pathological conditions (wounds - burns - urine - ear - and excretion) on the Maconkey Agar . To the *Klebsiella* , it was diagnosed microscopically and biochemically and was also diagnosed by the Vitek2 device, whose results were identical with the biochemical diagnosis, and the isolates were also subjected to molecular diagnosis for confirmation by using the PCR technique based on the diagnostic gene 16SrRNA, which is characterized by being good, stable and low heterogeneity for a long time in the bacterial type Ten types of antibiotics were selected and immersed in silver nanoparticles At a concentration of 20Mm, a test was conducted for the synergy between the silver nanoparticles AgNPs and the antagonists towards 12 isolates of *Klebsiella* bacteria, as they were applied to Muller-Hunton medium and after they dried up, the anti-saturated tablets were fixed with a silver particle solution, and after the expiration of the incubation period, the diameter of the inhibition area around the tablets was measured in mm According to the standard results, the results of the statistical analysis showed that the interaction of silver nanoparticles with the antibiotics had a significant effect on increasing the sensitivity of the isolates towards the synergistic antagonists, as the general rate of inhibition diameters of the antagonists (Impenem, Gentamicin, Amikacin Ciprofloxacillin) was (32.59, 21.6, 18.98, 36.18), respectively. , This activity is due to the ability of the nanoparticles to bind to the surface of the bacterial cell The binding causes structural damage and changes such as permeability, which facilitates the entry of the antagonist into the cell and thus its death. As for the synergies with the antagonists Tetracycline, Vancomycin, Erythromycin did not show efficacy in the direction of *Klebsiella* isolates did not give good results of synergy as it has a weak effect towards negative bacteria and it is as a non-synergistic antagonist because it does not It has effective binding sites for silver nanoparticles.

Keywords: *molecular methods; health; death; toxicity; synergy*

Introduction

Nanotechnology or nanoparticle technology, which is the science that studies the treatment of matter on the atomic and molecular scale. Nanotechnology is interested in creating new techniques and means whose dimensions are measured in nanometers, which is a thousandth of a micrometer, i.e. a part of a millionth of a millimeter, nanotechnology deals with measurements between 100 1 nanometer, i.e. it deals with atomic assemblies ranging from five to one thousand atoms, which are of dimensions much less than those of bacteria and a living cell (1) Silver nanoparticles are one of the most common types of nanoparticles known, with multiple applications, lower costs, and have antibacterial efficacy for a wide range of pathogenic and antibiotic resistance. As some antibiotics have become useless in the treatment of diseases, especially acute and pathogenic diseases, as increasing the dose beyond the required limit or the frequent use of antibiotics or cutting treatment without completing it allows the bacteria to become more resistant to those antibiotics, so it has become necessary to find alternative treatment methods to produce effective active substances. Health benefit without side effects, and these alternative solutions

include nanoparticles, including silver nanoparticles [2] One of the characteristics of *Klebsiella* is that it has a capsule, so its colonies are mucous and sticky, and it is also able to release large quantities of the mucous gel on the surface of the core of the components in it, where a strand of mucus can be observed when touching the colony with the [3] .One of the common diseases that this bacteria cause to humans are burn and wound. It also causes pneumonia, which causes inflammation of the air sacs in the lungs, if these sacs or vesicles are filled with liquid waste and pus and cause bronchitis and severe pneumonia [3] The genus *Klebsiella* spreads in humans in the form of throwing organisms in the nasopharynx and intestinal tract, as the bacteria are swallowed with food and drinks contaminated with bacteria or by contact and transmitted through the spray of respiratory patients from one patient to another [4] The ability of the bacterium to cause infection is due to its possession of many virulence factors that play an important role in causing disease and enable it to reduce and multiply within the host's body, including the capsule and the adhesion factors that enable it to attach to the surface of the host cell [5] *Klebsiella* possesses virulence genes that have the ability to evade and mask the process of phagocytosis by large phages to enable them to resist antibiotics [6] Bacterial resistance to antibiotics is a real threat to patients, as some antibiotics have become preferred food for some types of bacteria and their activity increases. In addition to the agricultural environment, the reason for this resistance is due to the widespread and frequent use of antibiotics, and this problem is one of the most medical problems facing the world, which leads to lack of control of diseases, and from a genetic point of view there are genetic elements called plasmids, which are an important vector for genes responsible for factors Virulence in bacteria, such as biofilm and hemolysin production, is the main cause of bacterial cysts events [7]

Materials and Methods

Sample collection

30 samples were collected from different pathologies. The samples included 10 samples from urine, 5 samples from wounds, 6 samples from burns, 5 samples from the ears, and 4 samples from the exit. The samples were planted directly in the planning method on suitable agricultural environments such as the environment Acids for 24 hours at a temperature of 37 ° C, and after the first transplantation of samples and purification of colonies, the samples were then diagnosed by biochemical and microscopic diagnosis, and *Klebsiella* was negative for the oxidase test, the indol test, the methyl red test, and positive for the Catalase test, and the Fox-Proscor test. Proskauer-Voges, also has the ability to ferment the sugar lactose [8]

Diagnosis was made based on the culture, microscopic and biochemical characteristics.

Molecularly diagnosed by PCR method

DNA was extracted and purified using a ready-made DNA extraction kit and according to the instructions of the American manufacturer Promega The concentration and purity of the DNA genetic material was measured using the Fluor meter Quintus device, which has high accuracy and sensitivity compared to the Nano drop, and then set and programmed the PCR device according to the optimum temperature of the mutant and correlation Prefixes and number of cycles. Then, 5 µL of the product of gene duplication was transferred to electrophoresis on an acarose gel. Where DNA loading was carried out in the gel pits and electrocuted on a horizontal surface. Then, 5 µl of the product of gene duplication was transferred to electrophoresis on an acarose gel The antibiotics were used (Ciprofloxacin, Gentamicin, Tetracycline, Amikacin, Imipenem, Vancomycin, PiPeraciclin, Chloramphenicol is, Erythromycin) where these antibiotics were placed in sterile boxes of 8 boxes and the name of the antagonist was recorded on each carton containing a solution of silver nanoparticles at a concentration of 20 mM)

To test the synergy between silver nanoparticles and antibodies

The synergy test between AgNPS silver nanoparticles was tested against 12 isolates of *Klebsiella* pneumonia, as they were transferred (5-3) a pure colony into tubes containing5ml) of a physiological salt solution and the resulting suspension turbidity was compared

with a standard McFarland turbidity of 0.5, after which a sterile cotton swab was submerged with the bacterial suspension and spread over Muller-Hunton's medium. The plates were incubated at a temperature of 37 ° C for 24 hours, and then the diameter of the inhibition zone around the disks was measured in mm (mm) for the silver saturated nanoparticles, and then compared with the standard results.

Results and Discussion

After conducting biochemical tests for bacterial isolates isolated from Ramadi Teaching Hospital, 30 isolates of *Klebsiella* pneumonia were diagnosed out of 50 samples, which were isolated from different pathological conditions that included (wounds, burns, diuresis, ears, excretions) and diagnosed based on the culture, microscopic and biochemical characteristics as well as Use a Vitek2 device. As between the microscopy and staining with the Cram stain, they are negative bacilli of the Gram stain organized in the form of short chains, and the *Klebsiella* was characterized by rather large colonies and mucous in consistency because they contain the capsule fermented for lactose sugar, and the biochemical examination showed that it is negative for the indole test and negative for the Proaskaure Vogas test. Citrate utilization And it is positive for the Uerase test, and it was confirmed by the Vitek2 device, as it gave results identical to the results of the biochemical examination Sanders and Sanders, 1997 The number of *Klebsiella* pneumonia bacteria in urine samples was the highest rate of 33%, while the rates of wounds and burns were 20, 16% (16%) respectively, and this result is close to what the world reached [9] as the percentage of *Klebsiella* pneumonia in urine samples was 21.8, The reason for their prevalence in hospital environments is due to their ability to develop different methods of resistance to disinfectants and their possession of virulence factors that resist antibiotics, and the results obtained by the two researchers are also converging [10] Molecular identification, whereby all bacterial isolates were subjected to molecular diagnosis by using PCR technology based on the 16SrRNA diagnostic gene, which is good, stable and has little heterogeneity for a long time in the bacterial species. It is also the main key to identifying among several thousand genes inside the cell. Bacterial [11] The results of DNA extraction of *Klebsiella* isolates that were isolated from different pathological conditions, as 12 isolates were selected for molecular diagnosis, showed that there are clearly packages of the isolates after the DNA transposition extracted on the acarose gel, as the molecular diagnosis matched the biochemical diagnosis. The results of the current study are consistent with the findings of the researcher [12] who showed that the percentage of identical molecular diagnosis of *Klebsiella* bacteria was 98%.

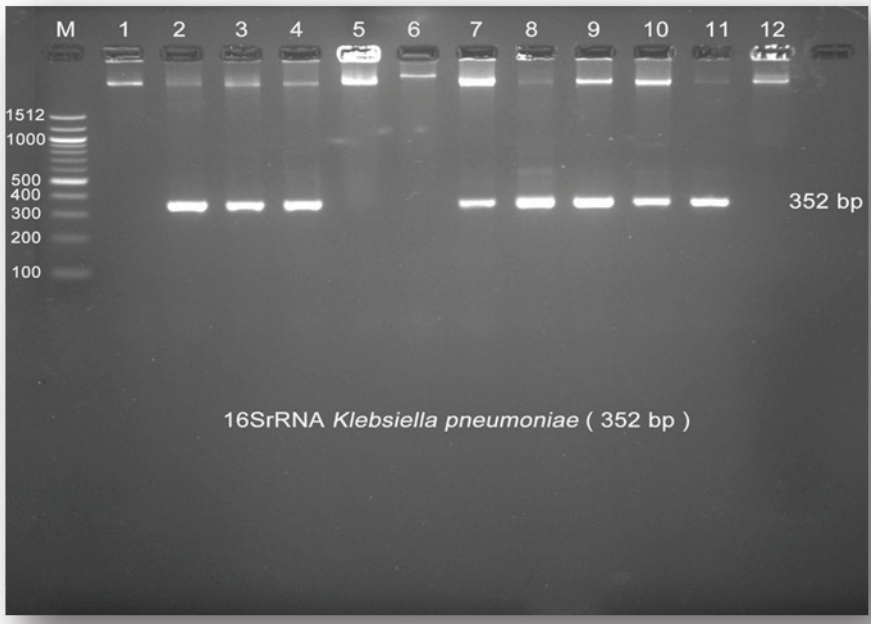

Figure 1 DNA electrophoresis on an agarose gel of 12 bacterial isolates

The effect of overlapping silver nanoparticles with antibiotics against *Klebsiella* pneumonia

The results of the statistical analysis showed that the interaction of AgNPs silver nanoparticles with antibiotics had a significant effect on increasing the sensitivity of isolates towards antibiotics, as the general rate of antagonist inhibition diameters with AgNPs was reached (Cip-Ag, Imp-Ag, AK-Ag, GN-Ag) (32.59, 36.18, 21.6, 18.98 respectively)

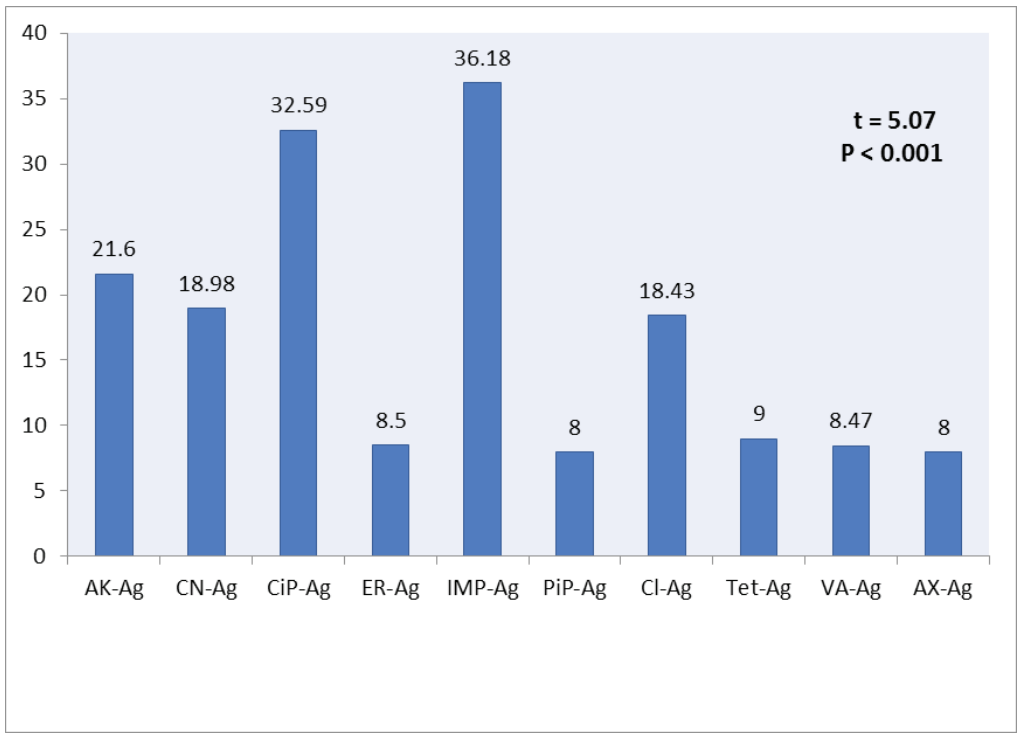

Figure 2 The effect of overlapping silver nanoparticles with antibiotics against *Klebsiella pneumonia*

Table 1 Rates of diameters of inhibition of synergy between antibiotic and silver nanoparticles

No.	AK-Ag	CN-Ag	CIP-Ag	ER-Ag	IMP-Ag	PIP-Ag	Cl-Ag	Tet-Ag	VA-Ag	Am-Ag
K1	21	18.3	32.6	10	37	10	10	11	8.6	**10.3**
K2	22	18	35.3	8	36	8	21.6	10	8	**8**
K3	24	17	25	10	37	12.5	12	12	7.3	**8**
K4	20	20	35	8	30.4	8	15	13	10	**9**
K5	20	22	23	8.5	35	14	15	10.3	10	**8.5**
K6	23	21	34	8.3	35	10	21	10	9.5	**11**
K7	21.5	17	38	7	37.2	14	22	20	6	**8**
K8	21	15.6	40	8	37	12	13	20	8	**8**
K9	20.4	20	38	10	34	10	15	10	10	**8**
K10	21	20	36	10	39	8.3	23	18	8	**6**
K11	23.6	21	24	8	38	14	15	8.46	8	**8**
K12	22	18.5	28	8.3	38.5	8	25	8	8	**8**
G-M	21.6	18.98	32.59	8.78	36.18	10.79	17.43	12.57	8.47	**8.57**
L.S.D	1.736	1.804	1.79	1.711	1.692	1.624	1.798	1.665	1.848	**1.72**

While there was no significant effect of the interaction of AgNPs with the antagonists (Tet-Ag, Er-Ag,) the general rate of inhibition diameters was (Er-Ag 8.4 VA-Ag, 9.1 Tet-Ag, 8.5) as these antagonists did not show efficacy against isolates, because It has a weak effect against negative bacteria and is non-synergistic as it does not have effective binding sites that bind with nanoparticles. Many researches indicate the synergism between nanoparticles and antibiotics, and the increased sensitivity of bacteria to the effect of antibiotics mixed with nanoparticles. As the antibiotic molecules have many active groups such as hydroxyl and amide groups that easily interact with silver nanoparticles by chelation. . The silver nanoparticles bind to the surface of the bacterial cell and this association causes damage and structural changes that affect the biological functions of the bacterial cell, such as permeability, causing gaps and pits that allow the antibiotic to penetrate the bacterial cell, leading to its death [13] As the researcher (2014) explained The role of nanoparticles in enhancing antibodies), Amikacin Imipenem, towards Gram-negative bacteria, as it is one of the modern strategies in treating multiple bacteria resistant to antibiotics, as he found that the use of the antibiotic with silver nanoparticles is more Efficient in killing bacteria, the particles also act as a carrier medium for antibiotics. The anti-synergistic antagonists with Imipenem Ciprofloxacin silver nanoparticles showed the highest significant effect ratio (32.50, 36.00), respectively, in the direction of K. pneumonia bacteria isolates. This study

agrees with the findings of [14] indicating strong efficacy. Particles that are synergistic in inhibiting the growth of a group of multiple antibiotic-resistant bacteria, including (Salmonella typhi, Pseudomonas aeruginosa Klebsiella pneumonia), and he explained that the particles mixed with the antagonists behave an anti-bacterial behavior towards many types of negative bacteria by causing a defect in the cellular membrane correlation. Bigger for nanoparticles in bacterial cells. The synergistic effect of Chloramphenicol with AgNPs increased the susceptibility to infection of bacteria and its effect was clear against Pneumoniae K, as the study agrees with the results reported before. [15] who confirmed the increasing efficacy of synergistic antibiotics and explained this to the efficiency of silver nanoparticles in transporting the antibiotic to the inside of the bacterial cell to interfere with the protein synthesis mechanism to prevent bacterial growth.

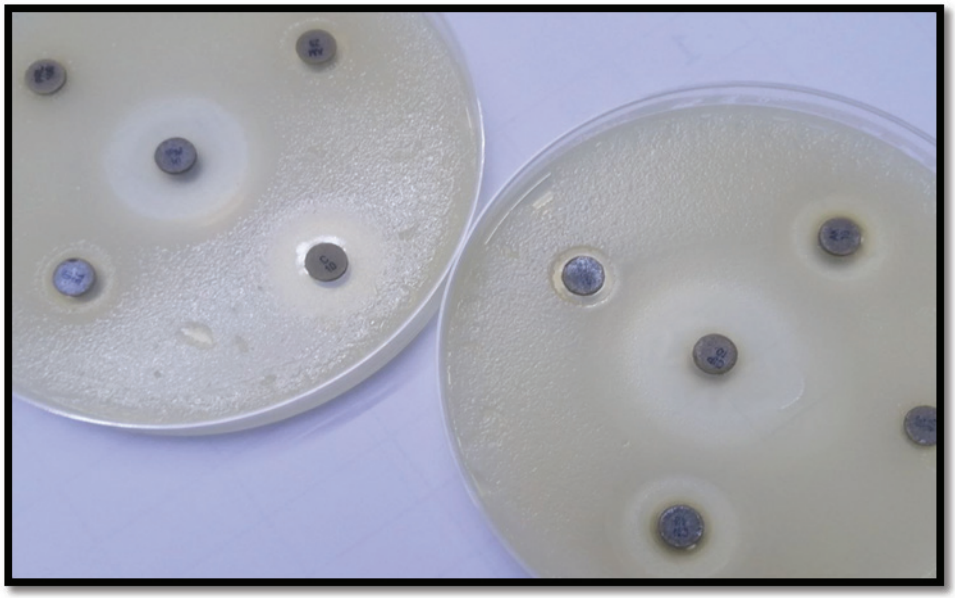

Figure 3 inhibition zone of Antibiotic inhibition with silver nanoparticles

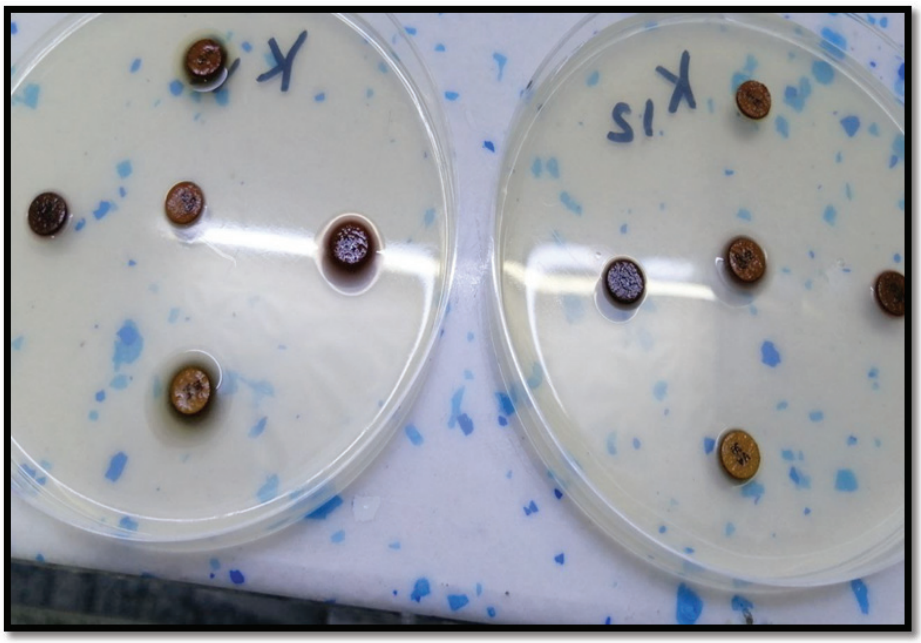

Figure 4 inhibition zone of Antibiotic inhibition with silver nanoparticles

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

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