Section A -Research paper

Effect of Phytosynthesis silver oxide nanoparticles on multidrug-resistant *Klebsiella pneumonia* isolated from children

Mohammed Oudah Hamad
Department of Ecology, Faculity of Science, University of Kufa
E-Mail: mohammeda.alkhafaji@uokufa.edu.iq

Muthik A.Guda
Department of Ecology, Faculity of Science, University of Kufa
E-Mail: meethakha.almithhachi@uokufa.edu.iq

ABSTRACT

The purpose of this research is to find an alternative to chemically manufactured drugs such as antibiotics used in the treatment of bactria infections, through nanotechnology manufactured from plant sources (green manufacturing), which is characterized by abundance and low economic cost, as well as having an effective inhibition of multidrug-resistant *Klebsiella pneumonia* isolated from children growth. These experiments were carried out in the laboratories of the College of Science, Department of Ecology from September to January 2022 -2023. Silver nanoparticles were synthesized using the crude aqueous extract of *Schanginia aegyptiaca*, and their properties were diagnosed. Field emission scanning electron microscopy (FE-SEM) was used to determine the surface, shape and compositional size of biosynthetic silver nanoparticles. The results showed that the sizes of nanoparticles ranged between 56.89 - 87.78 nm. The atomic force microscope was used to find out the shapes, topography, roughness and protrusions of the surfaces of the silver nanoparticles, as the average roughness of the nanoparticles was 71.44 nm. The X-ray diffraction (XRD) test was used to measure the size and crystalline nature of the above materials, as the average size of the silver nanoparticles was 75.03 nm, and these sizes were calculated according to the Debye-Sparker equation. Atotal of ninty five children patients suffering from asthma were enrolled in current study, sputum samples were collected and cultured according to standard methods.11(11.6 %) isolates of *K.Pneumoniae* were isolated and identified based on its cellular, cultural and biochemical characteristics. The activity of 20 antibiotics were detected against the current isolates by using Kirby Bauers disk diffusion method, the results showed high degree of resistance for most antibiotics by present isolates.The impinem,meropenem, siftrixone,azethromycin ,ciprofloxacin and amikacin were found more effective. In the present study, seven antibiotics were tested for minimum inhibitory concentrations (MICs)of isolates were higher level.

Keyword: *K.pneumonia* , Children , Multidrug resistant (MDR), silver nanoparticles, *Schanginia aegyptiaca*.

INTRODUCTION

K. pneumonia was belonging to Enterobacteriaceae family, gram- negative straight rod organismhave 0.6-6.0μm as length and 0.3-1.0 μm in diameter, facultatively anaerobic, arranged singly , pairs or short chains and non motile, living in 37C°,it catabolized D-glucose

and other carbohydrates with the production of acids and gas. negative and positive for oxidase and catalese respectively. indole, methyl red, voges-proskauer, and simmons citrate reactions vary among species ,also reduces nitrates .Several species hydrolyze urea . H2S is not produced through its grow on KCN.One of causes upper and lower respiratory tract infections for inpatients hospitalized and outpatients especially in children, its colonies large and heavy mucoid.it is resistant to antibiotics, sterilizers and disinfectants especially in hospital and it is from causes of nosocomial infections, so it is among the most prominent bacteria and common in hospital infections and hospital acquired pneumonia, UTI ,burns and wounds infections. It has ability to release fimbriae, capsule, polysaccharide, endotoxins and enzymes,siderophores and lipopolysaccharide, these pathogenic factors enables it to attack and invade host cells,also these bacteria are well-known for cause of health-care associated infections and its ability to produce biofilm and enzymes such as Extended Spectrum β-Lactamase (ESBLs) and carbapenemase which contribute to its pathogenicity and which considered one causes of high levels antibiotic resistance (Kim *et al*.,2016; Nirwati *et al*.,2019). It is possesses an important capsule in its virulence, as well as protecting it against cellular devouring and ingestion by immune cells (neutrophile important in killing pathogenic bacteria by phagocytosis). Also *K.pneumonia* is produce of aerobactin as an iron-binding protein (Munita and Arias ,2015; Bengoechea and Pessoa, 2019). Some strains of *K.pneumonia* produce hemagglutinins, which may be associated with its pathogenicity(Bailey, 2018). .

K.pneumonia is one of the most common pathogens and harmful to asthmatic children when infected with it. genera, species and drug-resistant bacterial strains of these bacteria pose serious problems in hospitals for patients especially allergic asthmatic children , their companions and workers in hospitals(Ayatollahia *et al*., 2020). One of the causes resistance to drugs by common pathological bacterial strains in Iraq the practices of some doctors, health staff, health care professionals, errors in laboratory identification of the bacterial type and the appropriate treatment to eliminate it, as well as patients' failure to adhere to the prescribed period to complete the course of treatment, and some mistakes practices lead to multidrug resistant by bacteria, the microorganism, including pathogenic bacteria, develops resistance by constantly adapting its presence to a changes microroganism environment in trying to survival (Hamad,2018). *K.pneumonia* has evolved capacity for enzymatic destruction of the drugs and changes in metabolic pathways and aimes sites for antibiotics, reduce and slow the bacterial taking of the antibiotics. Also has modify sites less attractiveness to antibiotics(Reyes, 2019). Genetic material is important for spreading microbial resistance to antibiotics. Effects of prolonged hospitalization and frequent hospitalization expose some patients to particular risks for drug-resistant infections,most vulnerable are children and elderly who suffering from chronic diseases such as asthma, weak immune defenses, as well as those who need surgical intervention, which leads to their weakened immune system, which makes it more susceptible to bacterial infections, including *K.pneumonia* which multidrugresistant. Persons who take antibiotics may have an increased risk of infection with resistant microorganisms because antibiotics kills sensitive and beneficial microorganisms for the human body, allowing resistance to it by strains that take root little by little and become dominant, when resistant bacteria spread, especially the treatment in a safe from the excessive and multi-resistance. whether they are not actually needed or the wrong return to them for the purpose of treatment when contracting some diseases that are not treated with antibiotics, such as viral infections and some bacterial infections that is treated with one or more antibiotics is not suitable for such infections, as well as not referring to the bacteriological specialists for diagnosing the pathogen such as bacteria, or incorrect identification of the bacterial,viral or fungal cause, as well as the

continuous treatment given to patients who have been hospitalized for a long period, which leads to the dangers of multiple drug resistant by bacteria as a result of mutations in the bacterial genetic material, especially gram-negative strains such as *K.pneumonia*, which may cause an exacerbation of the health problem with regard to the randomly use of antibiotics by patients, which are described according to a prescription by a specialist doctor,as a result of continuous taken antibiotics , randomly , and given it by people who are not specialized in this aspect of medical science, also giving treatment before identification bacteria and appropriate treatment to kill it through bacteria culture and identification, and then conducting antibiotcs sensitivity test and avoiding direct administration of the antibiotic by the doctor, pharmacy, or health staff, for these bacteria capable of producing capsule, which is severe the mucous and viscosity, which qualifies it to repel the host's defenses, in addition to the difficulty of spreading the antibody towards the bacteria in the places where bacteria are present within the affected tissue or organ (Hamad ,2018;Hamad,2020 ;Miftode *et al*.,2021).

through what was mentioned above, the present study aimed to investigate an important clinical aspect for patients, which is the isolation and identification of clinically important *K. pneumonia* from children and detection of its resistance pattern to antibiotics.Therefore, this research aims to manufacture silver nanoparticles from the aqueous extract of Schanginia aegyptiaca and to characterize them by chemical and physical methods in comparison with the crude aqueous extract. Growing identified pathogenic fungi, Aspergillus flavus and Fusarium oxysporium, and testing their sensitivity to different antibiotics. Testing the inhibition activity of biosynthetic silver nanoparticles on growth using minimal concentrations.

MATERIALS AND METHODS

1.Collection of specimens

The current study was conducted on children, male and female, who they are having signs and symptoms of chest infection, including 95 sputum samples were collected from them, these samples were placed in sterile container,individually and then transferred to the laboratory for the next experiments. Standard methods were used for samples handling, transportation, culture, incubation and laboratory examination to isolate *K. pneumoniae* causing infection and identification it,also examination sensitivity for antibioitcs.

2. Microscopic Diagnosting

Film were prepared by intak a litle amount of isolates colonies from a *K.pneumonia* culture on a clean glass slide and mixing present bacterial colonies with a small drop of deionized distilled water, pigmented with classical Gram stain and tested under light Microscope.

3. Bacterial Characteristics in Cultur

Cultural&microscopical criteria of current isolates were studied with10&40X lens.

4.Bacterial Isolates

K. pneumoniae causing upper and lower infections was isolated after culture of sputum samples on media including nutrient agar, blood agar and MacConkey agar(Himedia) by streaking,identification depending on cultured and microscopic traits and biochemical examination(Macfaddin ,2000).

5.Detection of Capsule

A swab of the bacterial suspension was made on a clean glass slide without thermal fixation and then dried. A solution of crystal violet dye was poured over the slide quietly at a concentration of 1% and left for 4 minutes, then the swab was washed with 20% of copper sulfate prepared from dissolving 20 g of copper sulfate. In a small volume of distilled water,

the volume was completed to 100 ml, dried in air and then examined under the compound light microscope
as bacteria cells appeared in the form of a very small purple spot surrounded by a transparent halo that represented the capsule (Forbes *et al*, 2007).

6. Antibiotic resistance test

The isolates were tested against 20 antibiotics prepared in the form of ready-made disks of Bioanalyse-Turky company by Kirby Bauers disk diffusion method and the MICs of antibiotics was estimated by Macro-dilution method based on what was mentioned in Guidelines (CLSI,2019).

7. Plant sample collection and preparation of the plants extract

The tartaea plant was collected from the wilderness of Najaf Governorate during September 2022, and its classification was confirmed by the National Herbal Authority / General Authority for Agricultural Research. The crude extract was prepared using the method (Guda *et al*., 2016) by thoroughly washing the vegetative part of the plant with water to remove contaminants from the surface and drying it well with dry air. 50 g of the plant powder was ground, then mixed in 250 ml of deionized water and heated. The mixture was immersed in a water bath at 45°C for 30 minutes, after which the extract was filtered using Whatman filter paper, and stored at 4°C for later use.

8.**Preparation of AgNO3 Solution and Biosynthesis of silver nanoparticles green synthesis**

0.016987 gm of silver nitrate was dissolved in 100 ml of deionized water to obtain a 1mM silver nitrate solution ready for use. Silver nanoparticles of taraei extract were synthesized according to the method (Hamad *et al*., 2023).

9. **Identification of biosynthesized silver nanoparticles**

The prepared samples were characterized by a French scanning electron microscope MIRA3 FE-SEM to determine the shape and size of the particles in the prepared samples (Vanmathi selvi and Sivakumar, 2012) by placing approximately 5 microliters of the prepared solutions for examination on a gold and carbon mesh electron microscope stand, and leaving it in the room temperature to dry and tested using different magnifying powers. The atomic force microscope (Angstrom Advanced AA2000) was used to determine the surface morphology and roughness of the prepared silver nanoparticles, and to determine their size and diameter.

Results and Discussion

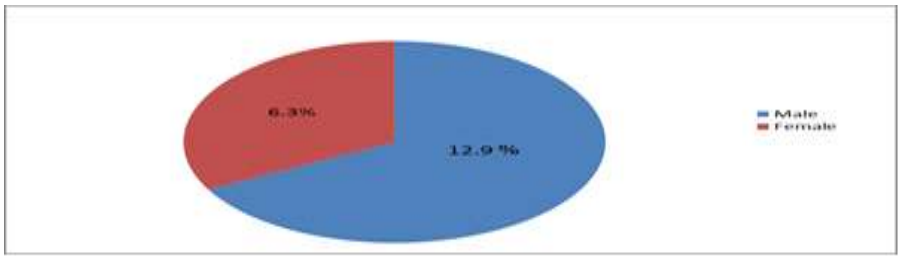
Eleven bacterial isolates of *K.pneumonia* were isolated from asthma children, its numbers and percentages were showed in Table 1. The current isolates were identified by its cultural ,microscopical and biochemical criteria in Table 3.

Table1. Numbers and percentages of *K.pneumonia* isolates isolated from asthmatic children

Age & Gender		**Number Samples**		**Number total samples**	**Number total isolates**	**%for isolates**
		-ve	**+ ve**			
Children (1- 12 years)	Male	19	43	62	8	12.9
	Female	8	25	33	3	6.3

Total		27	68	95	11	11.6

The present results declared that the total bacterial isolation rate was (11.6%) from children including male and female , aged(1-12) years and percentage of it showed in figure 1. While Ahmed *et al*.,(2020) showed That the most predominant bacteria were *K.pneumoniae*(31.8%)and Streptococcal pneumoniae (18.2%) while *Staphylococcus aureus, Acinetobacter baumannii* complex, methicillin-resistant *S. aureus Pseudomonas aeruginosa* and Enterobacter aerogenes are equally distributed (9.1%).also stated by Nirwati *et al.*, (2019) in *K. Pneumonia* isolated from patients with body infections was 167 out of 962 clinical bacterial isolates, with a percentage of (17.36%) while Miftode *et al.*, (2021) was able to isolate different types of bacteria that cause infections in human, including the bacteria under study, and a percentage of (45.3%) out of 75 patients. An increasing antimicrobial resistance among *K. pneumonia* has been rapidly growing. The cross-sectional, analytical and descriptive study was conducted to investigate the multidrug resistance(MDR) of *K. pneumoniae* isolated from respiratory tract infection. One hundred and fifty-one *K. pneumoniae* isolates were recovered from 330 sputum specimens (45.75%).The results of the current study confirm what many studies have stated that upper and lower respiratory tract infections are common at different ages especially in children, but they are more frequent in males, especially during asthma.



Figure(1):% of male and females asthmatic children with *K.pneumonia* infection.

K.pneumonia infections plays important role in acute asthma in children, some asthmatic children are treated with some antibiotics because found bacterial inflmation, many studies numerous atopic inflammatory cells such as refered that more bacterial pathogens are able of stimulating bronchial epithelial, mast and eosinophils cells .Additionally, some cells such as mast cells express TLR4that important a receptor to *K.pneumonia* lipopolysaccharide, After trigger of TLR4 ligands, mast cells trigger subclasses of genes. Some cytokines and chemokines that attract Th2 and eosinophils cells, Moreover, some viral infections such as rhinovirus and cynsitial virus is often noticed in association with serological indicator of bacterial inflmation assume that pathogenic bacteria may increase immunoglobulin levels secondary to infections by virus through access to the underlying immune system by the ifection mucosa (Ahmed *et al*.,2017 ;El Rifai and Rizk, 2018).

The isolates under study were distinguished by its distance from the effects of most antibiotics, which led to the characterization of multidrug resistance, especially the widely used antibiotics, especially those available in government and local pharmacies such as penicillins, cephalosporins,carpenemes and other antibiotics. From a variety of mechanisms individually or collectively, of antibiotics and increasing the expression of multiple efflux pumps of antibiotics, its production of enzymes that neutralize the effect of antibiotics by breaking them down such as pencillinase, third generation of cephalosporinase and aminoglycoside, the lack of porosity of its outer membrane, which prevents the entry of the

drug, as well as the production of β- Lactamase destroys beta-lactam antibiotics. Many bacterial species have the ability to produce these enzymes (Aladag *et al*.,2013). The isolates showed high levels of resistance to the most of antibiotics and intermediate resistance prevalence against imipenem and meropenem random and misuse of antibiotics Lead to the emergence of superbugs prospective studies must focus on new strategies for facing of thetsunami of drug resistance.

Table2. Biochemical tests for *K. pneumonia*

Bacterial isolate / ***Test***	***K. pneumoniae***
Produce Catalase	+
EMB Growth in	Central dark
Oxidase	–
haemolysis	γ
Motility test	–
Indol test	–
Methyl red test	-
Voxproskauer test	+
Citrate utilization	+
Urease	+
Growth on kligler iron	Acidic/Acidic
H2S	–
Gas production/ Glucose fermentation	+/+
Lactose fermentation	+
Sucrose fermentation	+
Maltose fermentation	+
Mannose fermentation	+
Xylose fermentation	+
Trehalose fermentation	+
fermentation Manitol	+
Phenyl alanine deaminase	-
Decarboxyl from arginine	+

.

Symbole:+Positive,-:Negative,complete haemolysis:β,not found haemolysis:γ

Also, the misuse of antibiotics in quality and quantity and in an unscientific manner and without consulting specialists by patients or their families, or taking antibiotics that are not

appropriate for the disease situation and without relying on laboratory identification, or making serious mistakes in identification bacteria in the laboratory and antibiotics sensitivity tests, which leads to the survival of bacteria under study live and emergence of multiple antibiotic-resistant mutated strains(Hamad, 2017;Hamad, 2020;Ayatollahia *et al*.,2020).Table4showed that cefotaxime norfloxacin, and lomfloxacin had a resistance ratio 72.0%, 79.0% and 62.0%, respectively. The high resistance of the isolates under study is due to continuity of their exposure to antibiotics,in addition to environment characterized by the continuous presence of antibiotics that containing these bacteria.

Table 3. Antibiotics susceptibility among current bacterial isolates

Antibiotic	**Sensitive (N)%**	**) %Intermediate (N**	**Resistant (N)%**
Lomfloxacin	6(54.5)	1(9.0)	4(36.4)
Azithromycin	8(72.2)	0(0.0)	3(27.3)
Amoxiclave	5(45.5)	0(0.0)	6(54.5)
Meropenem	10(90.9)	0(0.0)	1(9.0)
Impenem	9(81.8)	0(0.0)	2(18.2)
Ampiclox	2(18.2)	2(18.2)	9(81.8)
Cifipiem	6(54.5)	1(9.0)	4(36.4)
Ticarcillin	2(18.2)	1(9.0)	8(72.2)
Amoxcillin	1(9.0)	1(9.0)	9(81.8)
Aztronam	7(63.6)	0(0.0)	4(36.4)
Colstin	6(54.5)	1(9.0)	4(36.4)
Gentamycin	5(45.5)	2(18.2)	4(36.4)
Cloramphenicol	2(18.2)	2(18.2)	7(63.6)
Sifitrixon	9(81.8)	0(0.0)	2(18.2)
Ciprofloxacin	5(45.5)	3(27.3)	3(27.3)
Amikacin	8(72.2)	0(0.0)	3(27.3)
Cefotaxime	7(63.6)	0(0.0)	4(36.4)
Norfloxacin	3(27.3)	2(18.2)	6(54.5)
Ampicillin	0(0.0)	0(0.0)	11(100)
Nitrofurantion	6(54.5)	1(9.0)	4(36.4)

And their use is not affiliated with scientific rules and basis and without consulting specialists, encouraging the occurrence of mutations in the chromosome and bacterial plasmid, and this is in agreement with what was confirmed ,the current results refered to the highest resistance rate to ampicillin and ticarcillin by *K.pneumonia* was 96% and this is agreement with many studies , use of meropenem and impinem antibiotics was effective with a percentage of 20.6% and 31.0%, respectively, (Aladag *et al*.,2013) indicated the high activity of meropenem among 16 antibiotics used in examining drug sensitivity towards *K.pneumonia* isolates isolated from asthmatic children, but we note the beginning the increased resistance of these antibiotics by most of the current isolates compared to what was mentioned , While Hirsch and Tam pointed out the role of *K.pneumonia* by producing carbapenemases (KPCs), their effects appear quickly by causing multiple infections that are resistant to antibiotics all over the world, as bacterial strains containing these enzymes are degraded or degraded for a wide range of antibiotics containing the *B*-lactam ring, cephalosporins, carbapenems and monobactam pencillin.The present study was showed

effectiveness of carpenemes antibiotics including impenem and meropenem against bacteria, as it has a significant therapeutic effect for many bacterial infections, including respiratory infections, especially those resistant to antibiotics. While most of the isolates were resistant to colistin, cefipime and aztronam at a rate of 75.9%, 62% and 82.8%, respectively, but some of these antibiotics had high activity against gram-negative and gram-positive bacteria Ayatollahia *et al* ., (2020) refers to in recent years, becuse the randomly use of drugs, antibiotics resistance have increased in pathogenic bacteria, especially Klebsiella spp. antibiotics resistance is related with an increase in morbidity , mortality and treatments costs. Therefore, detection of drug resistance profile for taking choosing the suitable theraputic for infections coming via these bacteria looked important, *K. pneumoniae* causes health problems, especially in hospitals in most countries of the world, &thus results in complications for patients, especially with regard to treatment options& limitations of treatment that are important to eliminate it. It is one of the important bacterial pathogens that are resistant to many antibiotics in hospitals and this is in agreement with what was indicated by Manjula *et al*., (2014) that determining the prevalence of *K. pneumoniae* isolates that produce antibiotic-destroying enzymes, including ESβL, with determining the pattern of their resistance to antibiotics in clinical samples from females, especially pregnant women with urinary tract infection, is very important, the frequency of resistance to beta-lactams is due to the production of beta-lactamase enzymes that encode it in a plasmid or chromosome, which may be transmitted from one type or bacterial strain to another or within the other type and destroy these antibiotics, as well as having other mechanisms related to the anatomical aspect of the bacteria cell ,the secreted mucous substances by the bacteria represented by the important capsule in protecting bacteria from external influences such as antibiotics, disinfectants and sterilizers, and this is what was referred to(Ayatollahia et al.,2020).

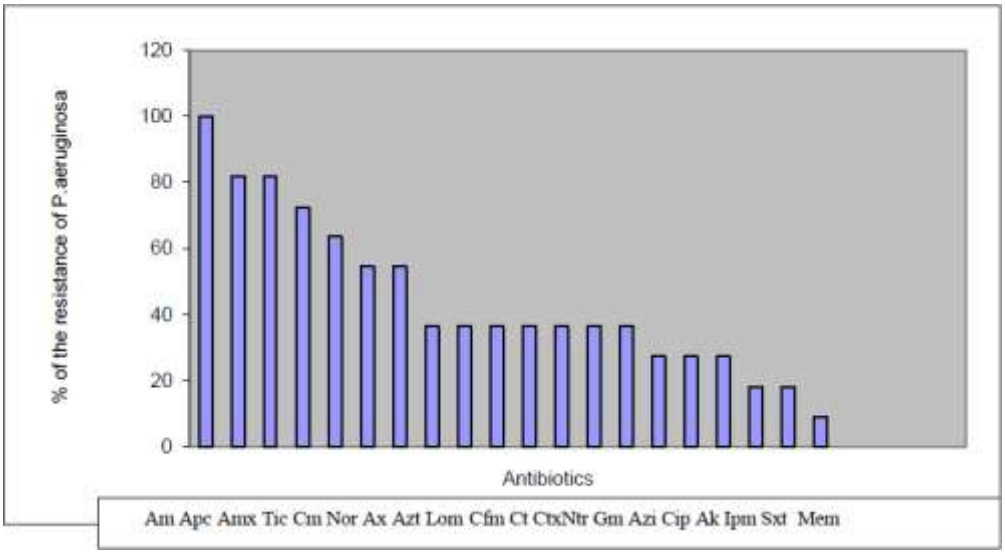


Figure 2. The Rates of Resistance of *K.pneumonia* for Antibiotics

The present results refers to the resistance of current isolates to ciprofloxacin was 62.0%, and that antibiotic resistance by bacteria produces because of the lack of knowledge of antibiotics as a fact and a scientific achievement by some of its users and those who are ignorant of their health importance in the medical field and its consequences with regard to the economic aspect of the individual and the state, as their use without prior knowledge of them leads to their misuse as well as the use that is not subject to scientific grounds or speed in its use and

description from before the doctor or pharmacist and without referring to the drug sensitivity examination, it is very important to anticipate the emergence of bacterial resistance to infection antibiotics, not to mention continuing to prescribe it repeatedly and for a long time for chronic infections and.The current results were that some of the current isolates were very resistant to most of the treatments commonly used by patients admitted and discharged from hospitals and some studies refers to most of bacterial isolates infections show complications in its infection with upper and lower tract infection through theraputic and prolonging the treatment time. Also, one of the reasons for resistance of current bacterial isolates may be the transfer of genetic material carrying genes responsible for antibiotic resistance, such as a plasmid (R-plasmid or a transposon, or a piece of DNA from resistant bacterial cells such as, *Staphylococcus aureus, Pseudomonas aeruginosa* and some strains belonging to other bacterial genera of Enterobacteriaceae that are sensitive to antibiotics , Also Hamad (2017) mention that increase in the lowest inhibitory concentrations of the seven antibiotics used against isolates were 64->128µg ml for some isolates and 32->128 for others
Reyes *et al*. 2019 indicated that carpenems-producing by *K.pneumoniae* (Cp-Kpn) strains pose a challenge to clinical workers due to its increasing prevalence in hospital settings &resistance to multiple antibiotics. In recent years, as a result of the unscientific random use of antibiotics, resistance of gram-negative bacilli, such as *K. pneumoniae,* has increased.

Table 4. MICs of antibiotics towards *K.pneumonia* isolates isolated from asthmatic children.

Antibiotcs	**% for resistant**	**MIC for *K.pneumonia***
Meropenem	9.0	32 -0.5
Ciprofloxacin	27.3	1-128
Azthromycin	27.3	1- >128
Nitrofurantion	36.4	128 -16
Sifitrixone	18.2	32 -1
Amikacin	27.3	64 -1
Ampcillin	100	128- > 32

Identification of biosynthesized silver nanoparticles

Possible some bacterial types such as *Pseudomonas* aeruginosa killed or inhibition by Onion extract and H2o2 (Hamad, 2016)Atomic force microscopy allows knowledge of the shapes, topography, roughness, and protrusions of the surfaces of different particles and molecules represented by surface heights and surface structure. This technique refers to digital images that allow quantitative measurements of surface features and two- and three-dimensional images and their analysis from different perspectives (Guda *et al*., 2021). Figure 3 shows that the size of the biologically manufactured silver nanoparticles ranged between (0 - 12.7 nm), with an average of 4.96 nm, and the surface roughness was 75.54 nm, and the root mean square value was 14.08 nm. The biosynthetic nanoparticles have a high rate of roughness, which works to increase its antibiotic activity (Salman and Abd Atae, 2021). The maximum cumulative rate of 94 manufactured nanoparticles reached 51.87, and the presence rate was 55.03%.

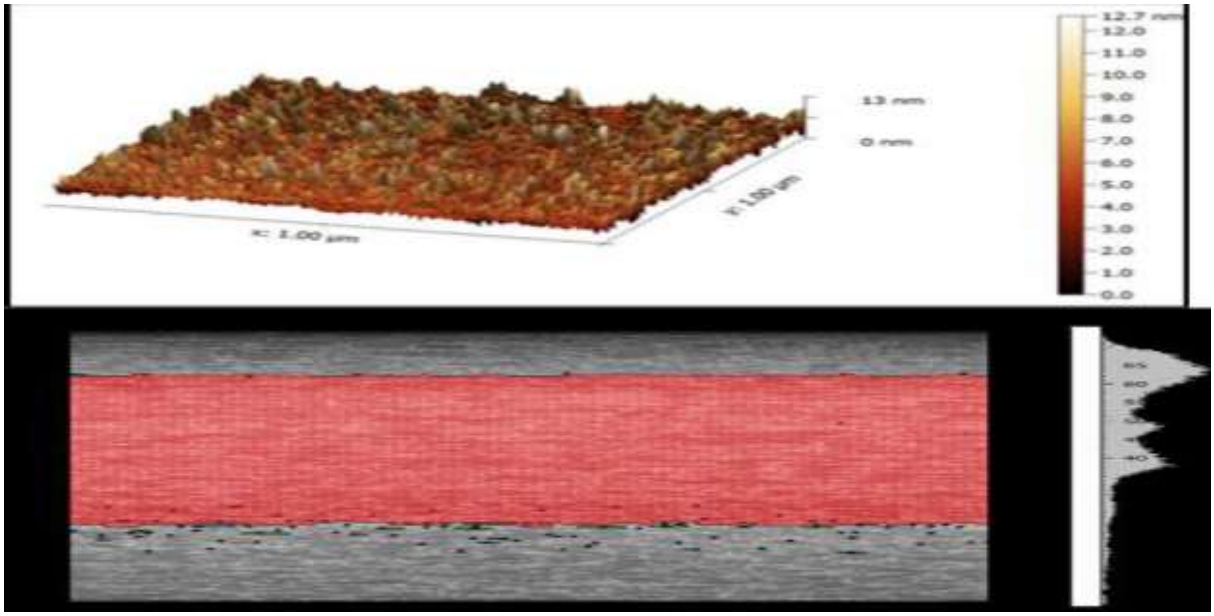


Figure 3 shows the size and surface roughness of silver nanoparticles synthesized from taraea plant extractAnti- bactrial activity of silver nanoparticles bioscopic and Aqueous extract on the studied bactria compared to the standard antibiotic

The results shown in Figure 4 show that there are significant differences between the studied bacteria. The first isolate recorded the highest inhibition rate, reaching 31 mm in Figure 4. The second isolate recorded the lowest inhibition rate, reaching 25.2 mm. with a significant increase of 0.41 mm, except for silver nanoparticles, compared to plant extract and antibiotic, which recorded 27 mm and 22 mm, respectively.

Figure (4) shows the effectiveness of the aqueous extract and bio-synthesized silver nanoparticles against Klebsiella bacteria, in comparison with the antibiotic tablet (Chloromphenicol (30 μg) and distilled water (DW).

REFERENCES

Ahmeda,A.H.; Galala,M.M.; Eltrawya,H.H.and Abdelazizb,S.Y. (2022).Sputum bacterial

identification in patients with bronchial asthma Exacerbation.J. Al-Azh.Ass. Med.18(4):440-448.

Ahmed, S.M.K.; Embarek, M.S.E.and Hassan, A.T.(2017). Burden of bacterial exacerbation in bronchial asthma in Assiut University Hospitals, Egypt. J. Egy. Intern. Med. 29:71–76.

Aladag,M.O.;Uysal,A.;Dundar,N.and Gunes,E.(2013).Characterization of *K.pneumonia* Strians Isolated from Urinary Tract Infections:Detection of ESBL Characteristics,Antibiotic Susceptibility and RAPD Genotyping.J.Microb.62(3):401- 409.

Alduhaidahawi, F.J., Guda, M.A., Obaid, J.K. (.2019) . The spatial variation study of spread the lupus erythematous disease in Najaf and Babylon governorates , Indian Journal of Forensic Medicine and Toxicology.

Al-Edhari, A.H., ALRegawi, S.M., Sardar, A.S., Guda, M.A., Almayahi, B.A. (2018) . Morphological study for valerianella kotschyi boiss. And v. Muricata (Stev.) Baxt. (Valerianaceae) in Kurdistan, Iraq , Plant Archives.

Al-Khafaji, B.A.H., A.guda, M., Al-Edhari, A.H., Altamimi, A.J.T. (2019) .Taxonomic study for the genus bupleurum L. (apiaceae) in Iraq using chloroplast gene RPL16 , Indian Journal of Public Health Research and Development .

Ayatollahia ,J;. Sharifyazdib,M. Fadakarfard,R. Shahcheraghi,S. H.(2020). Antibiotic resistance pattern of *Klebsiella pneumoniae* in obtained samples from Ziaee Hospital of Ardakan, Yazd, Iran during 2016 to 2017. J. IBERO. MED.2: 32-36.

Bailey , D.C.(2018). Structural and functional delineation of aerobactin biosynthesis in hypervirulent *Klebsiella pneumonia*.J. Bio.Chm.18.(20):7841-7852.

Bengoe chea,J.A.and Sa Pessoa, J.(2019).Klebsiella pneumoniae infection biology: living to counteract host.defen defences. J.FEMS. Microbiol. Rev.43(2):123–44.

Clinical and L aboratory S tandars Institute (CLSI) .(2010). Performance standars for antimicrobial susceptibility testing; 20 ed. approved standars, M 100-S20 and M100-S19, U.S.A.

Forbes, B. A., Daniel F. S. and Alice S. W. (2007). Bailey and Scott's Diagnostic microbiology. 12th ed. ,Mosby Elsevier company , USA.

Guda, M. A., Hakeem, J. I., Alabassi, M. M., and Almayahi, B. A. (2017). Effects of Environmental Stress on Nutrients of Typha domingensis Pers. Plant in Najaf, Iraq. Annual Research and Review in Biology, 1-6.

Guda, M. A., Merza, T., and Almayahi, B. (2016). Response of non-enzymatic antioxidants to phragmites Australis (Cav.) Trin. Ex. Steudel Plants of the Environmental Stresses in Baher Alnajaf, Iraq. Plant Cell Biotech. Molec. Biol.; 17: 140, 148.

Guda MA , Hadi SJ , Madhloom AAF and Almayahi BA. (2020) .Phytohormones Responses of Ricinus communis to Saline Soils and Regulation of Biosynthetic Hormone Pathways, La Prensa Medica Argentina journal Vol. 106 (2). Pp.106-179.

Hamad,M.O. . (2016) . Effect of onion extract and hydrogen peroxide *on Pseudomonas aeruginosa* isolated from urinary tract infection . J.Ban.She.Mu. 9(4): 205-207.

Hamad ,M.O.(2018). Clinical Study of Burkholderia Cepacia isolated From Body Infections. J.Plan.Arch.18 (1):65-72.

Hamad, M.O. (2018).Susceptibility of Porphyromonas Gingivalis Isolated from Patients Suffering from Periodontitis to Antibiotics.J. Glob. Pharma. Techno.10(8)..531-536.
2020).

Effect of AntibioticsCombinationon Escherichia coli Contaminated.(Hamad,M.O Pacifier used by children suffering from diarrhea. J. AL-Qadis. Med. 16(1): 1-9.

Hamad.M.O.(2017). Detction of Susceptibility of Bacteria Causing Gastroenteritis in Children. Used Pacifier for Antibiotics.J.Biochem. Cell. Arch.17.(2). 689-696.

Hakeem G.L. alkareemaw , Muthik A. Guda , Maytham M. Alabassi , Mikdad abdol elah taher, Attyaf J. T. Altamimi , Hazim A.N.Alhadrawi,(2020).Use of wild plant species as indicator of some heavy metals in the soil of General Company for tire industry in Najaf Governorate, IOP Conf. Ser.: Mater. Sci. Eng. 870 012099.

Harlow, B.L.and Bavendam, T.G.(2018) .The Prevention of Lower Urinary Tract Symptoms (PLUS) Research Consortium: A transdiciplinary approach toward promoting bladder health and preventing lower urinary tract symptoms in women across the life course J. Wom. Heal. 27(3):283-289.

Hasan A.Qazmooz, Muthik A. Guda, Jaafar B. Algburi, Sadiq K.L. Al-Zurfi and Thulfiqar Ali Al-Graiti ,(2020). Determination of heavy metal in samples of tirmania nivea fungi in different soils, plant archives , 20(2):313-317.

Hirsch ,E.B. Tam ,V.H. Detection and treatment options for *Klebsiella pneumonia* carbapenemases(KPCs): an emerging cause of multidrug-resistant infection.J.Antimicrob.Chemother.10(108). Khattab,R. Shaker,G. Askoura,M. and Abbas ,H. (2020). Multidrug resistant *Klebsiella pneumoniae* isolated from respiratory tract infection.J.Rec. Pharm. Biomed. Sci. 4 (1), 1- 5.

Kim J, J.; Chukeatirote, E.and Ahn, J.(2016).Assessment of antibiotic resistance in *Klebsiella pneumoniae* exposed to sequential in vitro antibiotic treatments.J.Ann. Clin Microbiol Antimicrob. 15(1):60.

Kudo, M., Ishigatsubo, Y. and Aoki, I. (2013). Pathology of asthma. J. Microbio 4. 263.
Macfaddin ,J. F. (2000). Biochemical tests for identification of medical bacteria. 3rd ed. The Williams and Wilkins co. Baltimore. USA.

Manjula N.G.; Math,G.C.;Nagshetty,A.(2014).Antibiotic Susceptibility Pattern of ESβL Producing *Klebsiella pneumoniae* Isolated from Urine Samples of Pregnant Women in Karnataka. J.Clin Diag. Res. 8(10): 8-11.

Miftode ,I.; MIFTODE,R.and IANCU,L.S.(2021). Insights into multidrug‑resistant *K. pneumonia* urinary tract infections: From susceptibility to mortality.J. Exper. Ther. Med. 22: (1086).1-9.

Muthanna F Ali1, Alyaa H Salman2, Muthik A Guda3, Ali Abid Abojassim3 and B Almayahi. (2020) . The Hydro Climatic Effects of Thermal Pollution on Surface waters in Iraq and its Biological Effects, La Prensa Medica Argentina journal Vol. 106 (2). Pp.106-189.

Nihad H Mutlag , Muthik A Juda , Marwa L Hussein , Haneen N Hassan (2019) . Assessment of Bombax ceiba Leaves Extract and Pleurotus ostreatus Fungus Filtrate on treatment of some Isolated Dermatophytic Fungi. 2nd International Science Conference IOP Conf. Series: Journal of Physics: Conf. Series 1294 (2019) 072022.

Muthik A. Guda, Hakeem G.L. alkareemaw, Maytham M. Alabassi, Mohammed O. Hamad Hazim A. Naji.(2019), Investigation use irradiation to treat west water and their effects on plants. Journal of Al-Hussein Bin Talal University for Research, a scientific journal of the Dorit Court issued by the Deanship of Scientific Research and Higher Studies, 5 (5).

Muthik A. Guda, Hakeem J. I., Maytham M. Alabassi , B. A. Almayahi., (2020) .Phytoremediation Of Some Heavy Metals In The Soil Of General Company For Tire Industry In Najaf Governorate By Wild Plant Species, Indian Journal of Environmental. Protection ,40(7):758-763.

Muthik A. Guda , Jawad K. Obaid , Faris j. Alduhaidahawi , Helen J. A. Alkurdi , Hakeem G.L. alkareemaw , Hayder M. Salih , Hussein F.M. AL.Isawi, (2020), The role of street and park trees to CO2 removal and improving air quality and climate in urban areas in Najaf (Iraq), IOP Conf. Ser.: Mater. Sci. Eng. 870 012087.

Munita, J.M.; Arias, C.A.(2016). Mechanisms of Antibiotic Resistance J.Microbiol4(2).1-37.

Nirwati,H ;Sinanjung,K Fahrunissa, F.; Wijaya,F; Napitupulu,S. and Hati,V.P. (2019). Biofilm formation and antibiotic resistance of *Klebsiella pneumoniae* isolated from clinical samples in a tertiary care hospital Klaten, Indonesia.J. BMC Proc.13(11):1-8.

Muthik, A Guda, Merza T, Almayahi B. (2016) Response of non-enzymatic antioxidants to phragmites Australis (Cav.) Trin. Ex. Steudel Plants of the Environmental Stresses in Baher Alnajaf, Iraq. Plant Cell Biotech. Molec. Biol. ;17:140-148.

Muthik A. Guda . Afyaa S. Nasir , Azha, Sh. Younus and ,Attyaf J. T. Altamimia. (2018) . Antioxidant Enzyme Responses of Juncus aschers. (EtBuch.) Adoms to Some of Environmental Stresses and use it as Indicators, Indian Journal of Public Health Research & Development: 9.12

Muthik A. Guda,Nihad .H.Mutlag and Kasim Kadhim Alasedi. (2018). The Potential Use of Atriplex nummularia plant as Contamination Indicators of Heavy Metal in different soils, Plant Archives , 18(2) , pp. 2372-2378

Muthik A.Guda , Mikdad A. Taher, Basim Almayahi .(2019) . Anatomical characteristics of vascular bundles associated with heat tolerance in Phragmites australis . Analele Universităţii din Oradea, Fascicula Biologie2(2) pp. 136-139.

Nasir SA , Jaber SH , Guda MA and Almayahi BA .(2020) .Oxidative Stress in the Male Rats Treated with Metronidazole, La Prensa Medica Argentina journal Vol. 106 (2) Pp.105-180.

Rizk,H.I and El Rifai,N.M. (2018).Use of antibiotics for asthma attacks Egyptian.(2018). J.Egy. Pedi. Alle. Immu. 16(2):41-44.

Zainab H. Thajeel , Muthik A. Guda , Nihad H. Mutlag , Bashaer S. Jabbar . (2020). Investigation the effect of irrigation with magnetized saline water to improve the vegetative growth and the anatomical characteristics of the tomato plant (*Lycopersicon esculentum* L.), International Journal of agriculture and statistical science , 16: (1) 815-823.

Muthik A. Guda, Nihad. H. Mutlag, and Anam A. Tsear. (2020).The use of Atriplex nummularia plant as the hyperaccumulators of silver. AIP Conference Proceedings 2290, 020041 (2020); https://doi.org/10.1063/5.0027565.

Muthik A Guda, Sheimmaa J Hadi, Kasim Kadhim Alasedi, Faris J Alduhaidahawi, Laith Saheb, & Muthanna F Ali. (2021). Antioxidant enzyme response of medical plant Persian Fenugreek (Trigonella foenum-graecum L.) to irrigation with microwaves treated water. International Journal of Research in Pharmaceutical Sciences, 12(1), 442-445. https://doi.org/10.26452/ijrps.v12i1.4087.

Mohammed Oudah Hamad, Alaa Yaqoop Rahi, Muthik A. Guda Study of Staphylococcus spp. isolated from Clinical Cases and determination of its multidrugresistant to antibiotics and phytosynthesized ZnO (NPs) . Cardiometry; No.26 February 2023; p.227-235; DOI: 10.18137/cardiometry.2023.26.227235; Available from: https://www.cardiometry.net/issues/no26-february-2023/study-of-staphylococcus-spp-isolated-from-clinical-cases.

Muthik A. Guda , Enas Abd al-Raouf Ammar Semysim.2023.Response Of A Medicinal Plant Peganum Harmala To Iron Oxide Nanoparticles F3O4(Nps).Journal of Pharmaceutical Negative Results ¦ Volume 13 ¦ Special Issue 8 ¦ 2022

Recurrent,J.; Lee ,U.and Ackerman ,L.(2019).Uncomplicated Urinary Tract Infections in Anger Women : AUA/CUA/SUFU Guideline.

Reyes , J. Aguilar, A. C. and Caicedo , A . (2019). Carbapenem - Resistant *Klebsiella pneumonia* Microbiology Key Points for Clinical Practice. Intern. J. Gene. Medi.12 :437-446. 119-125.

Robinson , J. L.; Finlay,J.C. Lang, M. E.and Bortolussi , R.(2014). Urinary tract infection in infants and children: Diagnosis and management. J.Paedia.Chi. Hea.19(6) :315–319.

Romolo, G., L. Facep, M.A. Warcester and B. Gideon.(2004). Complicated urinary tract infection: Risk stratification, clinical evaluation and evidence-based antibiotic therapy for out management. Update. J. American Health Consultants. 68(30): 74-230.

Valet ,R.S.; Gebretsadik, T.and Carroll, K.N.(2011). High asthma prevalence and increased morbidity among rural children in a Medicaid cohort.J. Ann. Alle.
Ast. Im. 106:467–73.