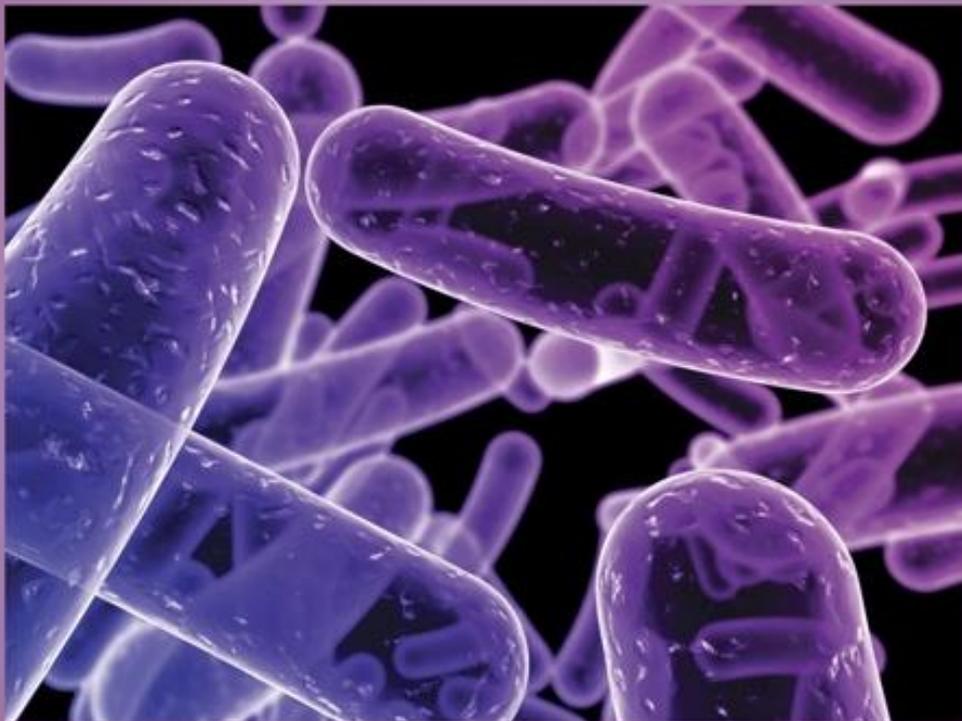




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Effect of Zinc Ferrite Nanoparticles with Antibiotics on *klebsiella pneumoniae* isolated from Infection Wound

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ABSTRACT

It is known that inflammation after surgical operations is a big problem for many people. The person is often exposed to a bacterial infection during the operation and during his visit to the hospital to change the surgical dressing or during the need to stay in the hospital to perform a surgery as an infection inside the hospital. This study included the collection of 200 samples from the site of inflammation using a cotton swab for both sexes and different ages. The isolates were diagnosed based on the phenotypic characteristics of *K. pneumoniae* bacteria, conducting biochemical tests for the isolates, and comparing the results with approved references to know the genus and type of bacteria. The diagnosis was confirmed by using the API-20 system. The results showed that 50 isolates belonging to the genus *K. pneumoniae* bacteria, with a rate of 25% of the total samples.

Examinations of the anti-inhibitory activity of zinc ferrite nanoparticles ZnFe₂O₄-NPs were carried out on *K. pneumoniae* bacteria by double dilutions (tubes) with six double concentrations (2,4,8,16,32,64) μg / ml. The highest inhibition percentage was at a concentration of 64 μg / ml, with an inhibition rate of 96.5%, and the lowest inhibitory concentration of MIC bacteria was at a concentration of 2 μg / ml.

INTRODUCTION

Bacterial infection is considered one of the most important infections that occur after surgical operations and wounds, whether the wounds are superficial or deep. It was noted that a number of surgical operations are delayed in recovery, which occurs as a result of negligence and failure to diagnose the factor causing the inflammation, or the patient's long stay in the hospital in addition to the patient's hesitation to outpatient clinics, which increases the chances of infection with bacteria (Gerard *et al.*, 2016)

Bacterial resistance to antibiotic drugs poses an increasingly serious threat to global public health along with stubborn bacteria (Mirzai *et al.*, 2020). Natural alternatives were turned to, and after the developments in genetic engineering of synthetic chemistry and biotechnology, new ways to search for alternative treatments. (Ghosh *et al.*, 2019).

Today, the world is witnessing a huge development in the field of nanotechnology, which has led to its application in a wide range of vital fields in medicine, energy, communications, air and water purification, in addition to medicines and cosmetics. (Ramanathan and Aqra, 2019).

Nanotechnology is used to produce materials of different sizes starting from 1-100 nanometers, which is equal to 10⁹, and it is obtained in multiple ways. To a large extent, the solvents and chemicals used, hence the importance of biotechnology, which is one of the most environmentally friendly technologies in the manufacture and production of nanomaterials and its use. It has recently been used to control types of pathogenic bacteria at different concentrations (Nasrollahzadeh *et al.*, 2019).

The aim of study to determining the effectiveness of ZnFe₂O₄ nanoparticles on *Klebsiella Pneumonia* at different concentrations.

MATERIALS AND METHODS

Samples were taken from the infected wound site such as caesarean section, nephrectomy, appendectomy and cholecystectomy using a sterile cotton swab containing a vector medium, and patients who were taking antibiotics were excluded. The primary determination tests included the phenotypic characteristics of bacterial growth on different media that had been previously inoculated, such as Macconkey agar media, blood agar media, and chrome agar media. Observable by colony shape, colony structure, coloration, and edges (Brooks *et al.*, 2013). biochemical tests were also conducted to check the properties of the isolate bacteria, and these tests included the indole test to verify the production of indole, the methyl red test to check the fermentation sugar and acid production, the Voges -Proscauer test to detect the acetone compound, the citrate test to verify the consumption of citrate as a single carbon source and formation of

sodium carbonate. Urease test indicating hydrolysis to form urea and ammonium, oxidase test to verify production of cytochrome c, catalase and fermentation tests for sugars (Brown and Smith, 2017).

Diagnostic of Zinc Ferrite

Nanoparticles:

Zinc ferrite nanoparticles have been extensively investigated using visible and ultraviolet light spectroscopy (UV). Fourier transform infrared (FT-IR) spectroscopy, and energy-dispersive X-ray silver nanoparticles (EDX), while the properties were evaluated using scanning electron microscopy (SEM) and transmission electron microscopy (TEM).

Testing The Inhibitory Activity of Zinc Ferrite Nanoparticles Against Bacteria (Tubes Method):

This method was based on the method of (Saginur *et al.*, 2006) as follows: 1-Add 1 ml of the nutrient broth into 8 test tubes.

2- Add 1 ml of each concentration of nanoparticles (2,4,8,16,32,64) micrograms ml to the tubes containing the nutrient broth.

3-100 microliters of bacterial suspension were added at an age of 18-24 hours at a concentration of 10⁸ * 1.5 cells ml.

4-A bacterial suspension was added to tube 7 containing the nutritious broth as a positive control.

5- 8 nutrient broth and nanoparticles were added to the tube as a negative control. Examination of bacterial growth by reading the absorbance with a spectrophotometer at a wavelength of 595 and finding the inhibition percentage for each concentration through the following equation:

$$\text{Inhibition \%} = \frac{OD_{595\ 1} - OD_{595\ 2}}{OD_{595\ 1}} \times 100$$

OD₅₉₅₁=OD for control

OD₅₉₅₂=OD for test

Testing the Inhibitory Effectiveness of Antibiotics Against Bacteria (tubes method):

This method was based on the method of (Saginar *et al.*, 2006), as follows:

- 1- Add 1 ml of the nutrient broth into 8 test tubes.
- 2- Add 1 ml of each antibiotic concentration (2, 4, 8, 16, 32,64) micrograms/ml to the tubes containing the nutrient broth.
- 3- 100 microliters of bacterial suspension were added, with an age of (18-24) hours and a concentration of $10^8 \times 1.5$ cells/cm.
- 4- The nutrient broth and bacterial suspension were added to tube No. .7 as a positive control.
- 5- The nutrient broth and antibiotics were added to tube No. 8 as a negative control. Examination of bacterial growth by reading the absorbance with a spectrophotometer at a wavelength of 595 and finding the inhibition rate for each concentration through the following equation (Hussein, 2017).

$$\text{Inhibition \%} = \frac{OD_{595\ 1} - OD_{595\ 2}}{OD_{595\ 1}} \times 100$$

OD₅₉₅1=OD for control

OD₅₉₅2=OD for test

RESULTS AND DISCUSSION

200 samples were collection from the site of infections resulting from infected surgical procedures from different sites of the body. Samples were cultured on MaCkconkey agar, blood agar, and Hi-

crome agar. Fifty isolates were isolated and diagnosing *Klebsiella pneumoniae*, which accounted for 25% of the population. Total postoperative wound samples. conducting morphological, and biochemical tests of *Klebsiella Pneumonia* according to (Kumar *et al.*, 2011; Sharmeen *et al.*,2012) and it was diagnosed by studying the phenotypic characteristics of the bacteria by cultivating it on solid MacConkey medium distinguished by its ability to ferment the sugar lactose in the medium of MaConkey. Its colonies appear pink in color, while on the Hi-Chrom agar, it appears in the form of metallic blue colonies.

Identification and Characterization of ZnFe₂O₄ NPs:

1-Visible and Ultraviolet Spectroscopy:

Ultraviolet-visible spectroscopy of nanoparticles is one of the important and widely used methods for the characterization and formation of metal nanoparticles in aqueous media. The absorption spectra of ZnFe₂O₄ nanocomposite were recorded with a wavelength range between 200-800 nm. The results showed that the ZnFe₂O₄ nanocomposite has strongest absorption at the range of 250-800 nm. Our results agree with the results of (Yashavanth Kumar *et al.*, 2012). The sharp absorption edge formed at 334 nm. As shown in Figure (1).

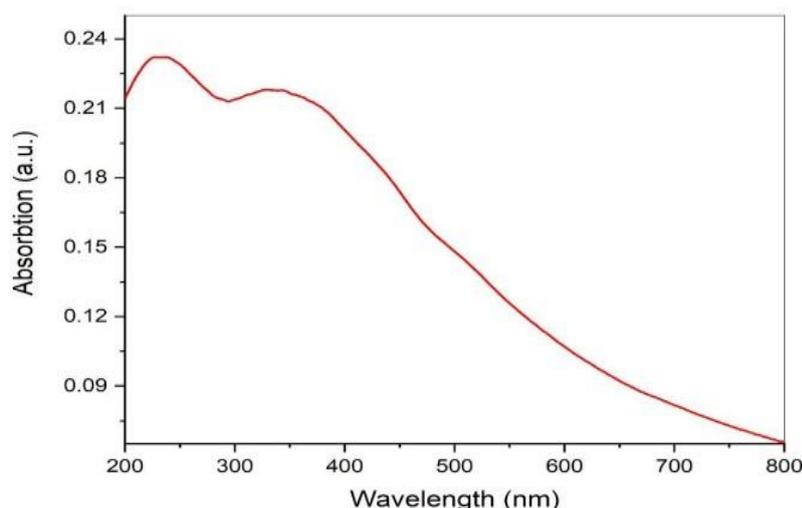


Fig. 1: Shows UV spectroscopy of ZnFe₂O₄.

2-X-Ray Diffraction (XRD) Analysis:

X-ray diffraction analysis of $ZnFe_2O_4$ nanomaterials was performed, which is an important technique to confirm the formation of crystals and determine their size. The results showed six values, which are as follows (220 (440), (511), (422), (400), (311), as in Figures (2-6). and the average crystal size was calculated by a formula Debye-Scherrer), as we can see from the following figure, the measurements of the diffraction of rays.

The X-ray of zinc ferrite nanoparticles, in which many diffraction peaks appeared, the diffraction angles of the three highest values, and it turns out that they are within the nanoscale size, and that the size of these nanoparticles is less than 100 nm, as we note from the Figure 2. The results are in agreement with the results of (Haghniaz *et al.*,2021). It was found that the average size is 1.87-1.8 nm, confirming that the nanomaterial It contains nanoparticles.

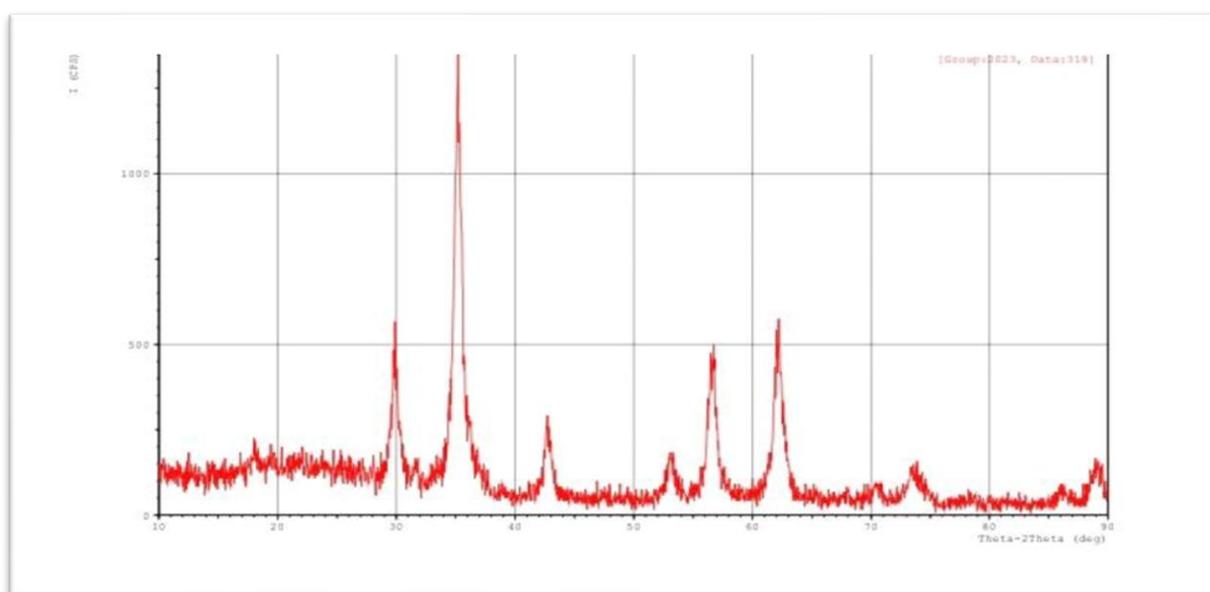


Fig. 2: X-ray diffraction (XRD) of $ZnFe_2O_4$ -NPs.

3-Fourier Transmission: FTIR Diffraction Analysis Infrared Spectroscopy:

FTIR analysis was applied to identify the biomolecules and functional groups present in the $ZnFe_2O_4$ compound

(Fig.3), (3917.85), (3779.40), (3695.08), (3430.43), (2927.04), (1628.10), (1488.79), (1391.18), (1063.35), (856.55), (566.56) cm^{-1} . The broad and strong peak was at about (566.56 -3917.85) cm^{-1} .

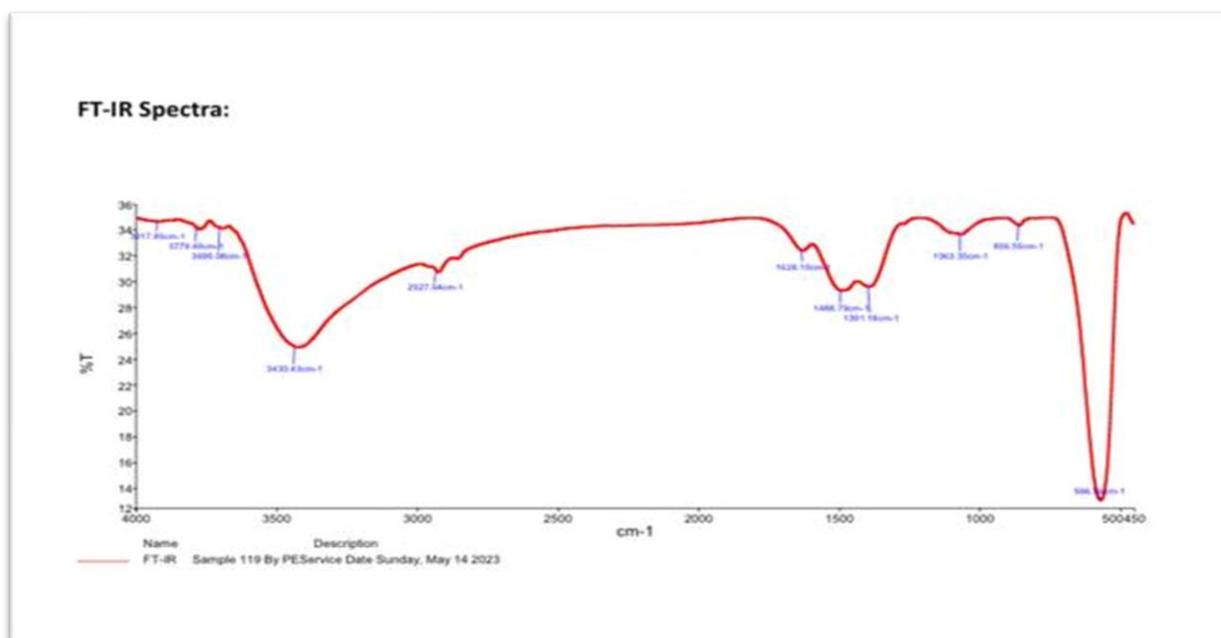


Fig. 3: FTIR of ZnFe₂O₄-NPs

4-Scanning Electronic Microscope (SEM):

The analysis shows that the average of the nanoparticles is between (48.66), (47.90), (43.59 (50.50) (48.96 nm),

where it appears that most of the particles are spherical and smooth on the surface with little agglomeration with an average particle size of 47.9 nm, as shown in t Figure (4).

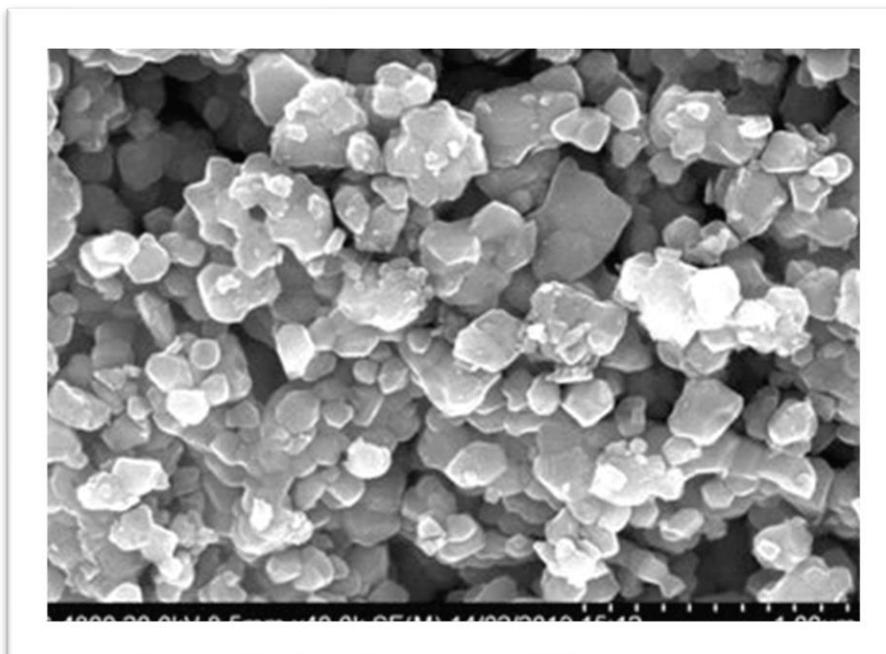


Fig. 4: FE-SEM of ZnFe₂O₄

Figure 5, shows the peaks obtained in the EDX spectrum analysis to determine the elements oxygen (O), iron (Fe) and zinc (Zn). According to the composition, where

the percentage of the measured weight was as follows (24.63) for oxygen, iron (48.13%), zinc ((27.24). No other FTIR peaks were observed indicating that the

composite NPS was free of impurities. These results were consistent with the results of (Borade *et al.*,2020)

5-Transmission Electronic Microscope (TEM):

This examination is done to understand the crystalline properties of the

nanomaterial, where the particles were observed as semi-spherical particles with smooth surfaces, and the average NPs were calculated from the estimation of more than 120 particles in the random fields of the transmission electron microscope TEM, where the average was about 44.3 nm.

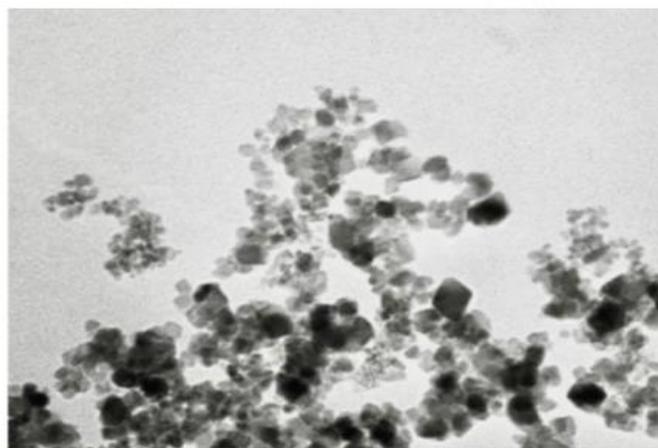


Fig. 5: TEM examination of ZnFe₂O₄

Inhibitory Effectiveness of Nanoparticles Using the Tubes Method:

The inhibitory effect of ZnFe₂O₄ nanomaterials on *K. pneumoniae* bacteria was examined using tubes method. The inhibition action against the bacteria was measured using six different concentrations of 2,4,8,16,32,64 µg/ ml. Results of the current study showed that ZnFe₂O₄ nanomaterials have a significant effect on the inhibition of *K.pneumoniae*. The

concentration of 64 micrograms/ ml showed the highest percentage of growth inhibition among the concentrations with an inhibition rate of 96.5 micrograms/ml, depending on the inhibition law mentioned in the work methods chapter, which indicates, the inhibitory activity increased with increasing the concentration of nanoparticles. The inhibition percentages for each concentration as shown in Table (1).

Table 1: Shows the effectiveness of nanoparticles against *K.Pneumoniae*.

| Substance µg/ ml | | 2 | 4 | 8 | 16 | 32 | 64 | Inhibition average |
|-----------------------|--------------------|-------|-------|-------|-------|-------|-------|-----------------------|
| Zinc iron oxide | Od | 0.384 | 0.354 | 0.124 | 0.022 | 0.019 | 0.014 | 61.79a |
| | Inhibition rate | 4 | 11,5 | 69 | 94,5 | 95,25 | 96,5 | |
| Control | Od | 0.400 | 0.400 | 0.400 | 0.400 | 0.400 | 0.400 | 0.0 D |
| | Inhibition rate | 0 | 0 | 0 | 0 | 0 | 0 | |

We note from the results of the statistical analysis that the inhibition rate of zinc ferrite nanoparticles was 61.79%, with a significant decrease in the concentration level at $0.01 > = P$ – Value. It turns out that the method of testing the inhibitory effectiveness of

zinc ferrite nanomaterials using the tubes method is the preferred method, and we have obtained good results, because the method of tubes allows nanoparticles to spread and move through the liquid medium and reach the bacterial cells to inhibit them.

Zhang and Chen (2009) indicated that the mechanism by which the nanoparticles interact in general with bacterial cells is that the microorganisms carry a negative charge, while the metal oxides carry a positive charge, differences in charge creates an electromagnetic attraction between the bacteria and the surface of the particles, and that the nanoparticles releases ions that interact with the thiol group (SH) of food-carrying proteins that protrude from the bacterial cell membrane. It reduces the permeability of the membrane and thus leads to cell death.

The results of the current study reported that zinc ferrite nanoparticles $ZnFe_2O_4$ - NPS confer excellent efficiency in the resistance of *K.pneumoniae* bacteria. The damage caused to bacteria by $ZnFe_2O_4$ is caused by reactive oxygen species (ROS), and later the occurrence of sugar and protein leakage inside the cells confirms its ability to destroy the bacterial membrane. A recent local study in

Baghdad conducted by Al-timimi, (2021) stated that other nanomaterials also showed the ability to inhibit bacterial growth.

Inhibitory Effectiveness of Antibiotics Using the Tubes Method:

The inhibitory effect of six resistance antibiotics:(Erythromycin, Gentamycin, Augmentin, Amoxicillin, Tetracycline, Ampicillin) *K. pneumoniae* bacteria was examined using tubes method, in which the inhibition action against the bacteria was measured using six different concentrations (2,4,8,16,32,64) $\mu g/ ml$ (Table 2). The results of the current study showed the inhibited activity of bacteria using powdered antibiotics in tubes. We found that powder antibiotics is better than the antibiotic tablets, and in the case of powdered antibiotics, higher concentrations were used than the tablet concentrations, and it may also be due to the spread of the powdered antibiotic and its better movement through the liquid medium and access to bacterial cells to inhibited.

Table 2: Shows the effectiveness of antibiotics against *K. pneumoniae*

| Conc. $\mu g/ ml$ | | 2 | 4 | 8 | 16 | 32 | 64 | Inhibition average |
|------------------------------|-----------------|-----------|-----------|------------|------------|------------|--------|------------------------------------|
| Augmentin | OD | 0.352 | 0.170 | 0.160 | 0.141 | 0.069 | 0.064 | 60.17 A |
| | Inhibition rate | 12 | 57.5 | 60 | 64.75 | 82.75 | 84 | |
| Tetracycline | OD | 0.364 | 0.296 | 0.162 | 0.076 | 0.069 | 0.052 | 57.54 A |
| | Inhibition rate | 9 | 26 | 59.5 | 81 | 82.75 | 87 | |
| Amoxicillin | OD | 0.392 | 0.380 | 0.354 | 0.332 | 0.300 | 0.240 | 16.75 B |
| | Inhibition rate | 2 | 5 | 11.5 | 17 | 25 | 40 | |
| Erythromycin | OD | 0.395 | 0.385 | 0.378 | 0.367 | 0.360 | 0.340 | 7.29 C |
| | Inhibition rate | 1.25 | 3.75 | 5.5 | 8.25 | 10 | 15 | |
| Gentamycin | OD | 0.390 | 0.384 | 0.372 | 0.354 | 0.334 | 0.319 | 10.25 C |
| | Inhibition rate | 2.5 | 4 | 7 | 11.25 | 16.5 | 20.25 | |
| Ampicillin | OD | 0.381 | 0.367 | 0.350 | 0.332 | 0.303 | 0.272 | 16.46 B |
| | Inhibition rate | 4.75 | 8.25 | 12.5 | 17 | 24.25 | 32 | |
| Control | OD | 0.400 | 0.400 | 0.400 | 0.400 | 0.400 | 0.400 | 0.0 D |
| | Inhibition rate | 0 | 0 | 0 | 0 | 0 | 0 | |
| Concentration average | | 4.44 F | 14.5 E | 28.13 D | 36.72 C | 42.06 B | 46.84A | P-Value= <0.01 |
| P – Value= < 0.01 | | | | | | | | |

We note from the results of the statistical analysis (Fig. 6), a significant decrease for the two antibiotics (Amoxicillin and Ampicillin, with inhibition rates of (16.75) and (16.46), respectively, for the two antibiotics (Augmentin and Tetracycline) at rates of

inhibition (60.17) and (57.54) at P – Value= < 0.01 and a significant decrease for the two antibiotics (Gentamycin (Erythromycin) by (10.25, 7.292) % compared to the two antibiotics (Amoxicillin). Ampicillin at P – Value= < 0.01.

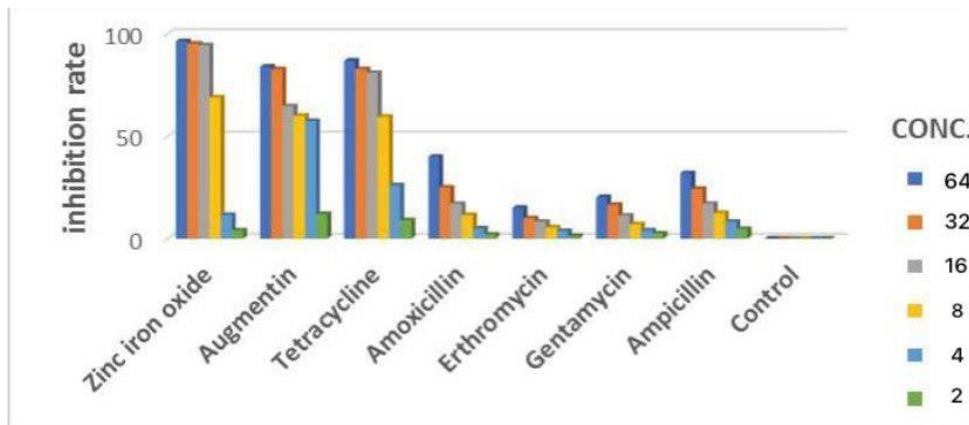


Fig. 6: Effectiveness of nanoparticles and antibiotic resistance against *klebsiella pneumoniae*

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