How to Cite:
Al-Khateeb, M. B. S., Al-Wakeel, M. I., & Al-Ghanimi, A. A. A. (2022). Effect of nanoprepared levofloxacin in inhibiting of Klebsilla pneumoniae Isolated from respiratory tract infections. *International Journal of Health Sciences*, *6*(S4), 9720–9730. https://doi.org/10.53730/ijhs.v6nS4.12049

Effect of nanoprepared levofloxacin in inhibiting of Klebsilla pneumoniae Isolated from respiratory tract infections

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***Abstract*---**Objective: Global statistics revealed that respiratory tract infections (ARTIs) are one of the main causes of mortality. Klebsiella pneumonia is considered one of the main causes of ARTIs. Levofloxacin has a wide spectrum effect against gram negative and gram positive bacteria, and its inhibitory activity can by improved through nanobiotechnology. Method: A nanohybrid antibiotic was synthesized by intercalating levofloxacin between magnesium oxide layers. The prepared antibiotic was characterized by fourier transform infrared spectroscopy (FT-IR), X-ray diffraction (XRD), scanning electron microscope (SEM) and atomic force microscope (AFM). Results: FT-IR spectrum showed the appearance of new groups in the Lev-MgO spectrum, which indicate the creation of a new compound. XRD spectrum revealed the presence of new diffraction planes which support the results obtained from FT-IR technique. SEM images demonstrated that MgO particles were converted to structure similar to coins arranged in irregular mode. AFM images showed the formation of spherical nanoparticle that indicates the formation of a new compound. Conclusion: The nanohybrid antibiotic was efficient in inhibiting twelve Klebsiella pneumonia isolated from respiratory tract infections.

***Keywords*---**characterization, Klebsiella pneumonia, levofloxacin, nanohybrid.

Introduction

Currently acute respiratory tract infections (ARTIs) are considered as an important cause of mortality in low-income countries and they represent the fifth most leading causes of mortality (1). *Klebsiella pneumonia* possess the ability to cause ARTIs due to its different virulence factors. These factors enable *K. pneumonia* to invade the host such as capsular polysaccharide, serum resistance, fimbriae, siderophore production, production of urea and enterotoxin as well as its resistance to wide spectrum of antibiotics (2,3).

During the last decades, several types of antibiotics used for the treatment of ARTIs. Quinolones are considered as a suitable drugs to treat this type of infections as they are characterized by ease of administration, excellent oral absorption, high penetration of tissues, prolonged half-lives besides their efficiency and safety (4). Levofloxacin which belongs to the third generation of fluoroquinolones, is characterized by wide-spectrum effect against gram positive and negative bacteria especially the causes of ARTIs (5,6).

Nanobiotechnology received recently more attention due to its role in combination biological principles with physical and chemical processes to manufacture nano-sized particles with improved characteristics. Nanoantibiotics stand at the forefront due to reduction toxicity, low cost and efficiency in inhibiting drug resistant microorganisms (7,8). The current study aimed to prepare, characterize a new nanohybrid antibiotic and evaluate its efficiency in inhibiting *K. pneumonia* isolated from ARTIs.

Material and Methods

Preparation of nanohybrid levofloxacin

The nanohybrid levofloxacin was prepared according to the method that described by Bashi *et al.* (2013) (9) as follows:

a- Preparation of levofloxacin solution
   This solution was prepared by dissolving 1.2 gm of levofloxacin in 40 ml of deionized distilled water and the volume completed to 50 ml with deionized distilled water, too.
b- Preparation of carrier:
   Carrier solution was prepared by dissolving 1 gm of magnesium oxide (MgO) in 50 ml of 50% ethanol.

Briefly, fifty ml of levofloxacin solution were added drop by drop to the MgO solution with stirring. The mixture was stirred by magnetic stirrer at room temperature for 24 hrs, and the mixture was placed in an incubator at 40 °C for 18 hrs. The precipitate was separated by centrifuge at 5000 rpm for 20 min, washed with deionized water for several times and was dried at 50 °C. Finally the dried precipitate was grinded well and gave the symbol Lev-MgO.

Characterization of synthesized nanohybrid levofloxacin

The nanohybrid levofloxacin of present study was characterized by using several methods according to (10,11) as follows:

i. Fourier Transform Infrared Spectroscopy (FT-IR):
The infrared spectrum study for each of nanohybrid levofloxacin and levofloxacin free from as well as MgO was carried out by making disks through mixing with potassium bromide (KBr) after grinding well. The infrared spectrum was measured in a wave number range (4000-400) cm-1.
ii. X-ray diffraction spectrum (XRD):
The nanohybrid levofloxacin of the present study was characterized using XRD which explains the difference in the layer before and after the intercalating process by using Bracg's law.
iii. Scanning Electron Microscope(SEM):
The outer surface of Lev-MgO nanohybrid antibiotic as well as the surface of the MgO layers were studied using SEM.
iv. Atomic force microscope (AFM)
The current study included measuring the diameter, size and aggregation of the nanohybrid Levofloxacin nanoparticles by using AFM.
v. Energy Dispersive Spectroscopy (EDS):
Analysis by EDS was used to investigate the presence of Mg, Oxygen and Carbon elements in the composition of the nanohybrid Lev-MgO.

Detection of inhibitory activity of free and nanohybrid levofloxacin

The inhibitory activity of free and nanohybrid form of levofloxacin was detected against *Klebsiella pneumonia* isolated from respiratory tract infections according to the well diffusion method (12) by using antibiotic concentration of (16-512) µg/ml.

Results and Discussion

Characterization of nanohybrid antibiotics
Characterization by FT-IR

FT-IR results of free Levofloxacin are shown in Fig. 1. The two bands that appeared at (3441 and 3267) cm^{-1} were attributed to a stretch vibration of the hydroxyl group. The aliphatic (C-H) stretch was noticed through the appearance of the bands at frequencies of (2937 and 2848) cm-1. The band which appeared at 1724 cm^{-1} is attributed to stretch of the strong carbonic group (C=O). In addition, the appearance of the band at 1620 cm^{-1} indicates the stretch of ketone. The structural stretch of benzene was noticed at frequencies of (1519 and 1450) cm-1, whereas a band at 802 cm^{-1} is due to aromatic bending of (C-H) (10).

As shown in Fig. 2 few absorption bands were appeared in the FT-IR spectrum of magnesium oxide. The band at 3437 cm^{-1} is due to the stretching vibration of O-H group which belongs to the physically adsorbed water, whereas the band at 1514 cm^{-1} indicates the bending vibration of surface of O-H group of the mentioned absorbed water. The band at 1429 cm^{-1} is due to the asymmetric stretching of

carbonate types (CO3-2). The vibration of metal bond was noticed at (680-580) cm^{-1} (13).

Fig. 3 shows a combination FT-IR spectrum of both the MgO carrier and Levofloxacin. The sharp band at 3699 cm^{-1} is due to the surface OH group that belongs to the magnesium. The typical broad absorption band that appeared at 3444 cm^{-1} refers to the stretch of the hydroxyl group which is slightly shifted. The C-H stretching band was noticed at 2904 cm-1. The stretch of the ketone group which was noticed at 1649 cm^{-1} and the band was shifted to higher frequency. The appearance of broad band at 1427 cm^{-1} indicates the structural stretch of benzene (10).

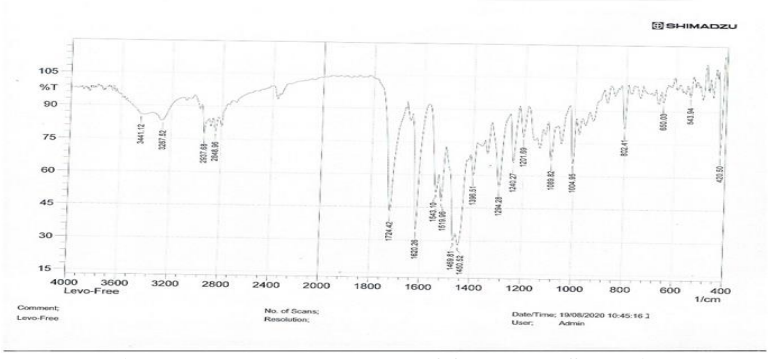

Fig. 1 FT-IR spectrum of free Levofloxacin

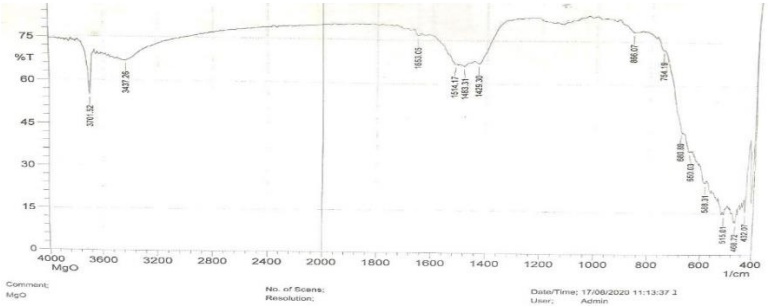

Fig. 2 FT-IR spectrum of Magnesium oxide

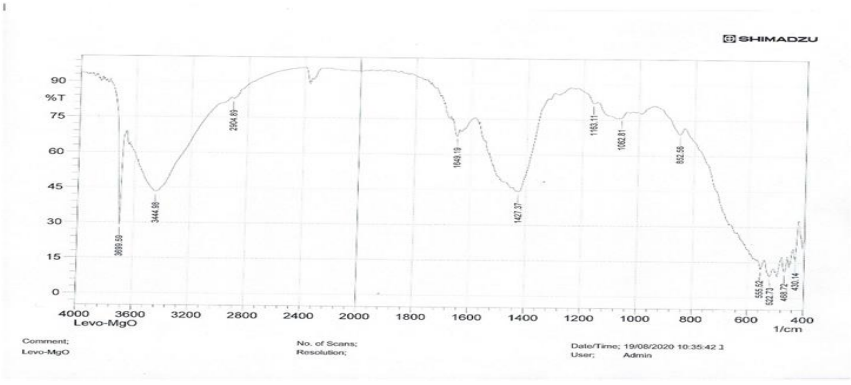

Fig. 3 FT-IR spectrum of nanohybrid Levofloxacin (Lev-MgO).

Characterization by XRD

The current study included the X-ray diffraction spectrum of Lev- MgO as well as MgO to obtain the differences in the thickness of the layer before and after intercalation between MgO layers by using Brag's law. Fig. 4 shows XRD analysis of MgO. The spectrum showed many diffraction planes included (lll) at 37.95° with crystalline distance of 0.23 nm, (200) at 42.95° with crystalline distance of 0.21 nm, (220) at 62.37° with crystalline distance of 0.14 nm, (311) at 74.75° with crystalline distance of 0.126 nm, and the diffraction plane (222) at 78.66° with crystalline distance of 0.121 nm (13). After the ion exchange between MgO layers and Levofloxacin, the diffraction plane (003) was appeared at 18.54° with crystalline distance of 0.47 nm while other plane (006) was appeared at 37.92° with crystalline distance of 0.23 nm (Fig. 5). Results of XRD gave good indication for the intercalations of Levofloxacin within magnesium oxide to prepare Lev-MgO.

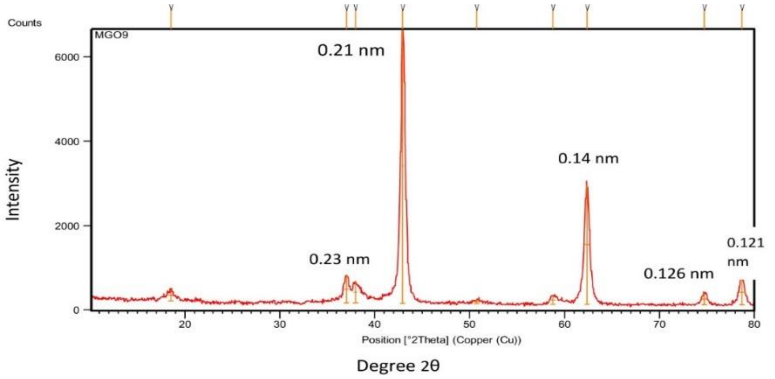

Fig. 4 XRD spectrum of Magnesium oxide

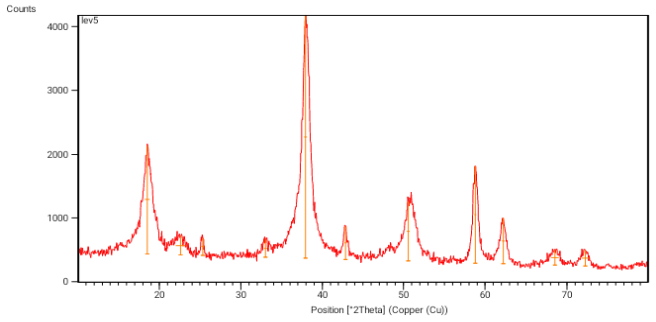

Fig. 5 XRD spectrum of nanohybrid Levofloxacin (Lev-MgO).

Characterization by SEM and EDS

Scanning electron microscope images of MgO (Fig. 6) showed that MgO particles appeared similar to cork granules. A large part of these granules were converted to structures, similar to coins arranged in irregular mode (Fig.7). The morphology conversion of these particles is a good indication for Levofloxacin intercalation between MgO layers and synthesizing a nanohybrid compound. Previous study

demonstrates the conversion of MgO particles to structures similar to thin sponge pieces when Ciprofloxacin was intercalated between MgO layers (14) while the intercalation of chlorohexidine between MgO resulted in the formation of structures similar to the beehive (15).

Energy Dispersive Spectroscopy confirmed the preparation of nanohybrid antibiotic (Lev-MgO) through the appearance of magnesium in the spectrum of Lev-MgO (Fig. 8). EDS technique is useful tool in different biomedical fields due to its high sensitivity in detecting various types of elements in tissues (16).

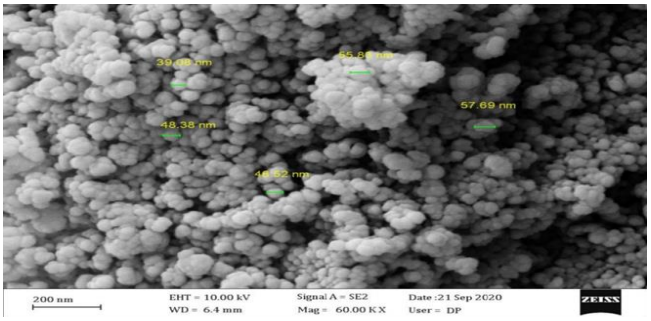

Fig. 6 Scanning Electron Microscope image of Magnesium oxide.

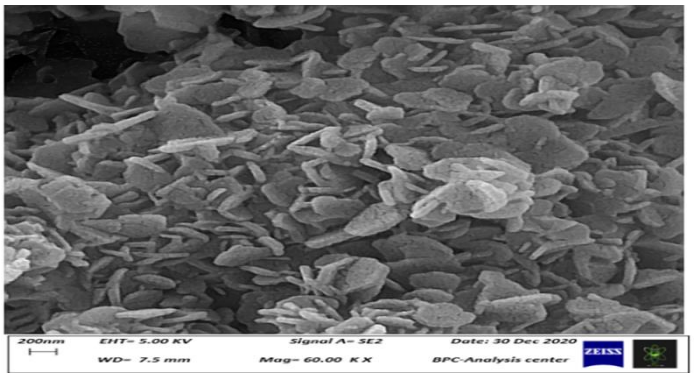

Fig. 7 Scanning Electron Microscope image of nanohybrid Levofloxacin (Lev-MgO).

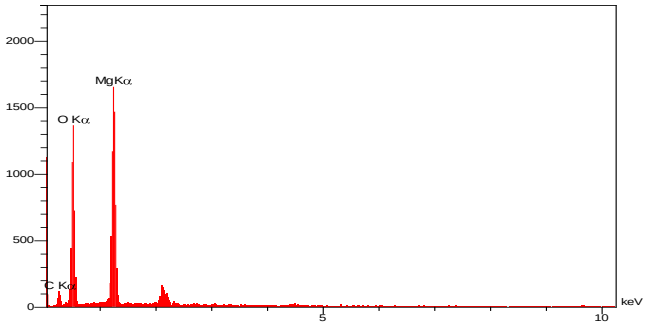

Fig. 8 Energy Dispersive Spectrum analysis of nanohybrid Levofloxacin (Lev-MgO)

Characterization by AFM

Atomic force microscope images scanned the outer surface of Lev-MgO. The three dimensional image of a section of the prepared nanoantibiotic are shown in Fig. 9. The nanoparticles were spherical in shape and its height reached to 296 nm. Fig. 10 showed the granularity cumulation distribution chart of Lev-Mgo. The highest percentage of the nanoparticles was 8.7% that belongs to the nanoparticles with diameter of 90 nm. The means of particle size of Lev-Mgo was 120 nm. The result of current study agreed with those obtained by Al-Khafagi (2015) (15) as the average diameter of the prepared chlorhexidine nanoparticles was 121 nm.

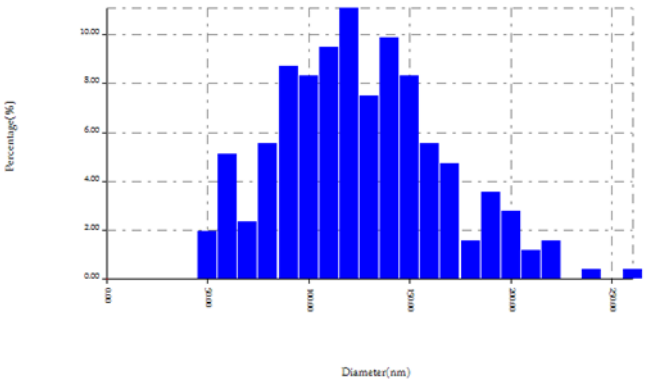

Fig. 9 Three Dimensional image of nanohybrid Levofloxacin (Lev-MgO) by Atomic Force Microscope

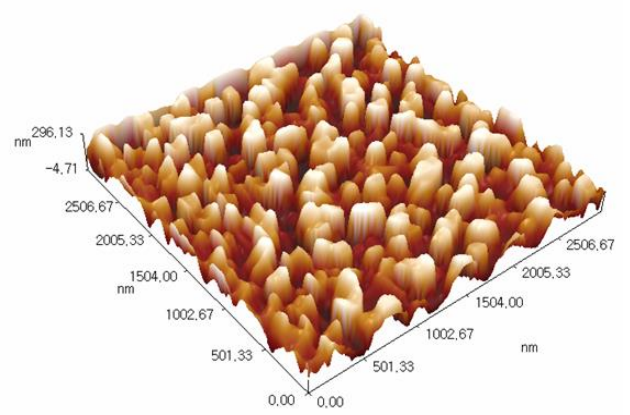

Fig. 10 Granulity Cumulation Distribution Chart of nanohybrid Levofloxacin (Lev-MgO) by Atomic Force Microscope

The inhibitory activity of the nanohybrid antibiotic against *K. pneumonia*

The inhibitory action of the free and the nanohybrid antibiotic (Lev-MgO) was studied against 12 isolates of *K. pneumonia* isolated from respiratory tract infections. Results in tab.1 showed that inhibitory activity of free levofloxacin increased with the increase in the antibiotic concentration. *Klebsiella pneumonia*

19 was the most sensitive isolate where the inhibition zone diameter reached to 27.5 mm by using 512 µg/ml of levofloxacin, while *K. pneumonia* 22 was the most resistant isolate to the action of the antibiotic under study where the inhibition zone diameter reached to 22 mm by using the same concentration of the antibiotic.

On the other hand it is evident from tab. 2 that *K. pneumonia* 15 and 16 were the most sensitive isolates to the action of the nanohybrid antibiotic Lev-MgO with an inhibition diameter of 24 mm by using 512 µg/ml antibiotic concentration. Results from the same tab. showed that *K. pneumonia* 22 was the less sensitive isolate to the inhibitory action of the nanohybrid antibiotic used in this study with an inhibition diameter of 19 mm. Levofloxacin is a broad spectrum antibiotic. It inhibits DNA-gyrase in susceptible microorganisms, which then cause breakage of DNA strands and in turn inhibits the relaxation of supercoiled DNA (18). Several studies demonstrated the inhibitory activity of nanohybrid antibiotics against pathogenic bacteria. Al-Fatlawi (19) found that the nanohybrid azithromycin was very efficient in inhibiting *K. pneumonia* isolates. In addition to, doxycycline loaded on silver nanoparticles was more efficient in inhibiting *K. pneumonia* isolates than free doxycycline (20).

Tab.1: Inhibitory activity of free levofloxacin

A.B. Conc.		16	32	64	128	256	512
Bacterial isolate							
K.pneumonia	1	13	13.5	17	19.5	24	27
K.pneumonia	2	11	13.5	16	19	22	24.5
K.pneumonia	3	10.5	13	16.5	18	21.5	24
K.pneumonia	4	11	13	16	18.5	21	24
K.pneumonia	5	12	15	17.5	20	22.5	25
K.pneumonia	6	13	15.5	18	22	25	26.5
K.pneumonia	7	11.5	14.5	16.5	19	22	25.5
K.pneumonia	8	12	13.5	16.5	20	22.5	25
K.pneumonia	9	15	18	21	23	25	27.5
K.pneumonia	10	14	16.5	18.5	21	23.5	26
K.pneumonia	11	13	15.5	19.5	21.5	24	25
K.pneumonia	12	8.5		13.5	17	19.5	22

Tab.2: Inhibitory activity of nanohybrid Levofloxacin (Lev-MgO)

A.B. Conc.		16	32	64	128	256	512
Bacterial isolate							
K.pneumonia	1	9	12	13.5	16	18	21
K.pneumonia	2	12.5	14	15.5	18.5	20.5	23
K.pneumonia	3	10	12	14.5	16	17.5	20
K.pneumonia	4	11	12.5	15	17.5	20	22.5

K.pneumonia	5	13	16	17.5	20.5	21.5	24
K.pneumonia	6	10.5	14.5	15.5	18.5	21	24
K.pneumonia	7	10.5	12.5	15	17	19.5	23
K.pneumonia	8	8	11	13.5	15.5	17.5	20.5
K.pneumonia	9	9.5	11.5	13	16	18	19.5
K.pneumonia	10	11.5	13.5	15.5	17.5	19.5	22
K.pneumonia	11	8.5	12	14.5	17	19	21.5
K.pneumonia	12	7.5	11	13.5	14	16.5	19

Tab.1: Inhibitory activity of free levofloxacin

A.B. Conc.		16	32	64	128	256	512
Bacterial isolate							
K.pneumonia	1	13	13.5	17	19.5	24	27
K.pneumonia	2	11	13.5	16	19	22	24.5
K.pneumonia	3	10.5	13	16.5	18	21.5	24
K.pneumonia	4	11	13	16	18.5	21	24
K.pneumonia	5	12	15	17.5	20	22.5	25
K.pneumonia	6	13	15.5	18	22	25	26.5
K.pneumonia	7	11.5	14.5	16.5	19	22	25.5
K.pneumonia	8	12	13.5	16.5	20	22.5	25
K.pneumonia	9	15	18	21	23	25	27.5
K.pneumonia	10	14	16.5	18.5	21	23.5	26
K.pneumonia	11	13	15.5	19.5	21.5	24	25
K.pneumonia	12	8.5		13.5	17	19.5	22

Tab.2: Inhibitory activity of nanohybrid Levofloxacin (Lev-MgO)

A.B. Conc.		16	32	64	128	256	512
Bacterial isolate							
K.pneumonia	1	9	12	13.5	16	18	21
K.pneumonia	2	12.5	14	15.5	18.5	20.5	23
K.pneumonia	3	10	12	14.5	16	17.5	20
K.pneumonia	4	11	12.5	15	17.5	20	22.5
K.pneumonia	5	13	16	17.5	20.5	21.5	24
K.pneumonia	6	10.5	14.5	15.5	18.5	21	24
K.pneumonia	7	10.5	12.5	15	17	19.5	23
K.pneumonia	8	8	11	13.5	15.5	17.5	20.5
K.pneumonia	9	9.5	11.5	13	16	18	19.5
K.pneumonia	10	11.5	13.5	15.5	17.5	19.5	22
K.pneumonia	11	8.5	12	14.5	17	19	21.5
K.pneumonia	12	7.5	11	13.5	14	16.5	19

Conclusion

It can be concluded that magnesium oxide could be suitable carrier in the synthesis of nanohybrid antibiotics due of the presence of great number of highly reactive ages which then shows high reactivity of MgO. The prepared nanohybrid antibiotic Lev-MgO was efficient in inhibiting *K.pneumonia* isolated from respiratory tract infections.

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