IMPROVING THE ANTIBACTERIAL ACTIVITY BY THE COMBINATION OF ZIRCONIUM OXIDE NANOPARTICLES (ZrO_2) AND CEFTAZIDIME AGAINST KLEBSIELLA PNEUMONIAE

***Sarah Naji Aziz* [1*] *,Karrar Jasim Al-Sallami* [1,2] *,Suhad Yassein Abd* [1] *,Ali Muhsin Ali Al-Musawi* [1] *Murtadha Adnan Mohammed* [1] *,Al-Maamoon H. Abed* [1] *& Sundus Qasim Mohammed* [1]**

[1]*College of Science, Mustansiryiah University, Baghdad, Iraq*
[2]*College of Science, Mansuora University, Egypt*

Corresponding author: sarahnaji2015@gmail.com

Abstract

Introduction: *Klebsilla pneumoniae* is one of must opportunistic pathogens that causes nosocomial infection, UTI, respiratory tract infections and blood infections. ZrO_2 nanoparticles have antimicrobial activity against some pathogenic bacteria and fungi. Ceftazidime is one of third generation cephalosporins groups of antibiotecs, characterized by its broad spectrum on bacteria in general and particularly on Enterobacteriaceae family like *Klebsiella* spp.
Method: Diverse clinical samples of *Klebsilla pneumoniae* were isolated from several hospitals in Baghdad – Iraq and ZrO_2 nanoparticles was investigated against it. Ceftazidime was also investigated against *K. pneumoniae*. Both of ZrO_2 nanoparticles and ceftazidime were mixed together and investigated against *K. pneumoniae*.
Results: The result showed that ZrO_2 nanoparticles were effectivity on inhibiting opportunistic pathogens. By using zirconium oxide nanoparticles on *Klebsiella pneumonia* isolates in 24h. of incubation time, inhibition zones were (38,34,10,10,8,0) mm respectively on agar plates. By using ceftazidime alone against the same bacteria inhibition zones were (40,32,10,9,8,0) mm. respectively.
Conclusion: The present study results that the antibacterial activity of ceftazidime against bacteria was increased when combination between ZrO_2 nanoparticles and the antibiotic had done, because, inhibition zones in case of mixing both of ZrO_2 nanoparticles and ceftazidime were (43,40,12,12,10,0) mm respectively. So that we can conclude that the combination of zirconium oxide nanoparticles (ZrO_2) and ceftazidime was a useful method for the treatment of *Klebsilla pneumonia* that cause nosocomial infection, UTI, respiratory tract infections and blood infections.

Keywords: ZrO_2 nanoparticles, *Klebsiella pneumonia*e, antibacterial activity, ceftazidime.

Introduction

Klebsilla pneumonia, a part of *enterobacteriaceae* family, a gram-negative, motile rod-shaped bacteria. Typical *Klebsiella pneumoniae* is an opportunistic bacteria that cause nosocomial infections, urinary tract infections, respiratory tract and blood infections (Vasaikar et al., 2017). This bacteria found in the skin, mouth and intestines, as well as in hospital settings and medical devices. Nanoparticles are particles with a diameter of 10^{-9} mm. Because of their small size and other unique mechanical, chemical, electric and magnetic features, these particles can easily enter the cell and interfere in its metabolic natural process (Arefian et al., 2015). Nanomaterials were used in biomedicine, pharmaceuticals and many biotechnologies (Gowri, Gandhi and Sundrarajan.,2014). Silver nanoparticles were used as antibacterial for both Gram negative and positive bacteria, and Silver nanoparticles can affect about 16 species of bacteria, including *E. coli*. Nanoparticles were used in the manufacture of teeth and in coating of medical devices (Sondi and Salopek-Sondi, 2004). Nanomaterials were used in personal care products, food containers, clothing washing materials, paintings tools, household appliances as well as water treatment, medical, gene delivery, bone delivery, artificial limbs and implantable materials, sensors devices (Yang et al., 2013). It was found that the use of oxide nanoparticles can eliminate several diseases that caused by Pathogenic bacteria such as *Klebsiella* spp. *Staphylococcus* spp. *Salmonella* spp.(Ravikumar and Gokulakrishnan., 2012) ZrO_2 nanoparticles were reported as broad spectrum bioactivity agents and safety, with compared with superior and heat resistance when compared with traditional antibacterial agents (Sant et al., 2012).

In previous study, (Bansal et al., 2004) researchers founded that ZrO_2 nanoparticles have antifungal activity on *Fusarium oxysporum*, which causes plant diseases as well as its effectiveness against *E. coli*, *S. aureus*, *C. albicans* and *A. niger*. ZrO_2 is a non-toxic, efficient, environmentally friendly and low-cost. (Gowri, Gandhi and Sundrarajan., 2014), thus this study aimed to detect the fact that there are antibacterial activity produced by the combination of ZrO_2 and ceftazidime against *Klebsiella pneumonia* bacteria.

Ceftazidime, one of third generation cephalosporins groups of antibiotecs that characterized by its broad spectrum on gram-positive, gram-negative and aerobic bacteria in general and is particularly antibacterial activity on Enterobacteriaceae family like *Klebsiella* spp. (Richards and Brogden.,1986).

Methods

Diagnosis of bacteria

The samples were collected from different diseases cases (infections of burns, wounds, blood). The isolates were diagnosed using traditional specific culture media and biochemical test IMVIC and API 20E test that done according to directives of the company (BioMeriux/France).

Preparation of nanoparticles solutions

ZrO_2 nanoparticles were suspended in distilled water by using ultrasound device for 15 min. that prepared (1g /100 ml) in concentration (Haghi et al., 2012).

Preparation of ceftazidime concentration

A stock buffer solution of ceftazidime were prepared by dissolving 1g of the ceftazidime powder in 100 ml of the distilled water, sterilized the antibiotic had happened by millipore filter to get (1g / 100 ml) in concentration. (Olaleye 2007)

Preparation of combination of nanoparticles solution and ceftazidime antibiotic

1ml of ZrO_2 nanoparticles were suspended with 1ml of ceftazidime by using ultrasound device for 15 min. that prepared (1g /100 ml) in concentration (Haghi et al., 2012).

Studying of inhibitory activity

Agar Wells Diffusion Method used by the Agar Wells Diffusion Method to study the effect of both nanoparticles and ceftazidime alone and when mixed together. (Olaleye 2007)

Fourier Transform Infrared Spectroscopy (FT-IR)

The FT-IR spectroscopy to the ZrO_2 nanoparticles solutions under study were carried out by using (Perkin 8300 FT-IR Shimadzu Spectrophotometer) where the spectrum wavelength ranges in 400 cm^{-1} to 4000 cm^{-1}.

X-Ray Diffraction Analysis (XRD) analysis

ZrO_2 powder used for measuring the X-ray diffraction (XRD) by using (Shimadzu XRD-6000) and the analysis had run in the University of Baghdad.

Transmission electron microscopy (TEM) technique

ZRO_2 nanoparticles composition was also studied by TEM (fig.) by using (jem-2100 electron microscope) in mansoura university to determine the shape, size and distribution of nanoparticles.

There are two different shapes for the ZrO_2 nanoparticles, the first one is rod-shaped or nanotubes long narrow closed at the both ends, while the other shape looks smaller and clustered "flower shape". Supposedly the small clusters of the ZrO_2 nanoparticles grow to show nanotubes that could be observed in TEM image.

Results

Antibacterial activities of ZrO_2 nanoparticles had studied against six isolates of *Klebsiella pneumonia* bacteria and results showed that five isolates of *K. pneumonia* had inhibition zones (38,34,10,10,8,0) mm, and the antibacterial activities of ceftazidime had tested against the same isolates of *K. pneumonia*, results showed the following inhibition zones (40,32,10,9,8,0) mm. respectively. When a combination between ZrO_2 nanoparticles and ceftazidime antibiotic had done, results showed a protective effect against these five isolates of *Klebsiella spp.* The diameters of the inhibition zone in mixed were (43,40,12,12,10,0) mm respectively as Shown in table 1 and Fig. 1.

Table 1: Diameters of inhibition zone for ZrO_2 nanoparticles alone, ceftazidime alone, and mixed of ZrO_2 nanoparticles on *Klebsiella pneumonia*

Isolates of *K. pneumonia*	Diameter of inhibition zone in (mm)			
	ZrO_2 nanoparticles	Ceftazidime	Combination of ceftazidime ZrO_2 Nanoparticles	Control (D.W)
K. 1	38	40	43	0
K2	34	32	40	0
K. 3	10	10	12	0
K. 4	10	9	12	0
K. 5	8	8	10	0
K.6	0	0	0	0

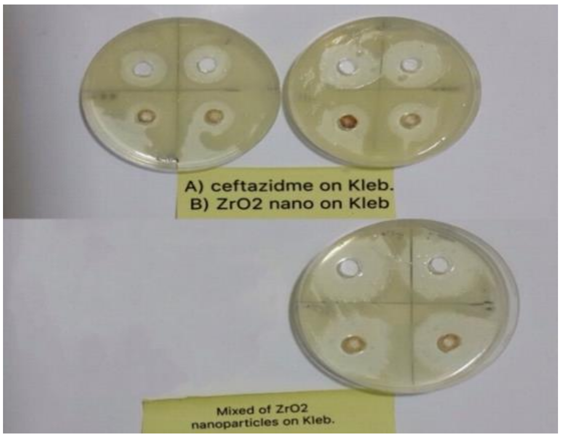

Figure 1: inhibition zone for ZrO_2 nanoparticles alone, ceftazidime alone, and mixed of ZrO_2 nanoparticles on *Klebsiella pneumonia*

The analysis of (FT-IR) to ZrO_2 nanoparticles showed that this solution contains chloride group at (592 cm^{-1}), amide CN group at (1637 cm^{-1}), O_2 group at (2065 cm^{-1}) and OH hydroxide group at (3448 cm^{-1}) as shown in Fig. 2

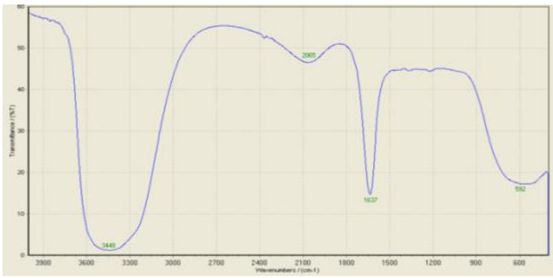

Figure 2: (FT-IR) to ZrO_2 nanoparticles solution.

The analysis of (FT-IR) to Ceftazidime showed that this solution contains chloride group at (584 cm^{-1}), amines group at (1637 cm^{-1}), O_2 group at (2065 cm^{-1}), and alcohol group (1223 cm^{-1}) and (1039 cm^{-1}) as shown in Fig. 3

Figure 3: (FT-IR) to ceftazidime solution

The analysis of (FT-IR) of ZrO_2 nanoparticles and Ceftazidime showed that this solution contains chloride group at (596 cm^{-1}), amide CN group at (1633 cm^{-1}), O_2 group at (2073 cm^{-1}), amine group at (3429 cm^{-1}), alcohol group at (1215 cm^{-1}) and the alkyl nitro groups group at (1367 cm^{-1}).
The analysis of The XRD analytical technique to ZrO_2 nanoparticles were performed and results of X-ray diffraction analysis show the crystallization or calcification of zirconium.

Figure 4: (FT-IR) to the mixed ZrO_2 nanoparticles and ceftazidime solution

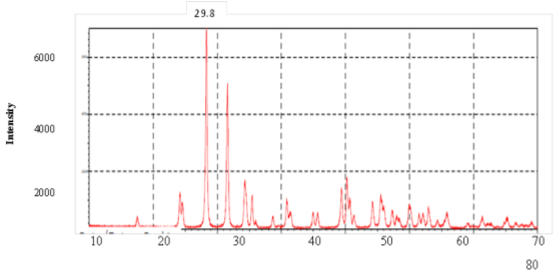

Figure 5: shows X-ray diffraction patterns of ZrO_2 nanoparticles and its intensity.

Discussion
These results of inhibition zones in our study seem concurrence with (Gangra et al 2012) which proved that ZrO_2 nanoparticles was effective against both Gram-negative and Gram-positive bacteria. In addition, ZrO_2 nanoparticles was effective against fungi, including *A. niger*. The difference in effectiveness of ZrO_2 nanoparticles against different bacterial species is due to the arrangement of atoms on the surface of the cells, which results in a difference in the shape of the molecule.

The results of (FT-IR) analysis were agreed with (Gowri, Gandhi and Sundrarajan.,2014) because they found that -OH group in the ZrO_2 nanoparticles solution when measuring the FT-IR and amide links between the amino acids and the carbonyl groups (C = O) and the CO group associated with the polyphenolic compound and the OH group found in the carboxylic acid Phenolic groups and the Carbonyl group of amino acids and proteins, which have a strong ability to bind metals that cover nanoparticles to prevent aggregation and thus play biological molecules as a hydrogenation agent for nanosecond.
While (Haghi et al., 2012) was found in infrared spectroscopy of Zirconium oxide containing Zr-O-Zr at the frequency of cm^{-1} (613) and cm-1 (819) accompanied by disappearance of protein amide. Some studies have shown that there is special interest in nanocrystalline nanoparticles due to its highly stable mechanical and electronic properties. Therefore, it is used in many applications as a reduction agent, anti-bacterial and anti-oxidant (Gowri, Gandhi and Sundrarajan., 2014)

$$D = K \lambda / \beta \cos \theta$$

ZrO_2 nanoparticles was exposure to The XRD analytical technique that performed as shown in Figure 5. The diffraction peaks indicated the small size of these crystals. Average size of these particles can be simply calculated by using Scherrer equation (Langford et al 1978)

Where:
K= Shearer constant with a value of 0.9^{-1} (form factor)
λ = wavelength of x-ray (1.5418 Å)
β = peak width of XRD in the middle of the length
θ = Bragg angle
D is the particle size

According to Sheer's equation results showed that the mean size of ZrO_2 nanoparticles molecules under study were 29.8 nanometers. The results were compared with (Vasaikar et al., 2017). They showed that the size of the particles (20 nanometers) when measured according to the Shearer equation and the highest value of the X-ray oxides measured by XRD. While (Arefian et al., 2015) the XRD value of ZrO_2 was 35 nanometers. The XRD measurement is used to identify the crystallization of molecules. In some cases, the crystallization of these molecules is not perfect, due to the insufficient thermal processor and time during the preparation process.
The results of X-ray diffraction analysis show the crystallization or calcification of zirconium, and the removal of the protein improves the biopolymerization of zirconium (Haghi et al., 2012). It is also used to detect the nature of particulate matter.(Gowri, Gandhi and Sundrarajan., 2014) The results of this study showed that the molecules of zinc oxide have a crystalline nature.

Conclusion

The present study results that the use of the ZrO_2 nanoparticles as antibacterial agent are successfully effective with wide range of microorganisms. The combination of both ZrO_2 nanoparticles and ceftazidime had caused increasing the activity of ceftazidime against *Klebsilla pneumonia* which proved a useful method for this bacteria that causes nosocomial infection, UTI, respiratory tract infections and blood infections.

The difference in effectiveness of ZrO2 nanoparticles against different bacterial species because of the differential arrangement of atoms on the surface of cells

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