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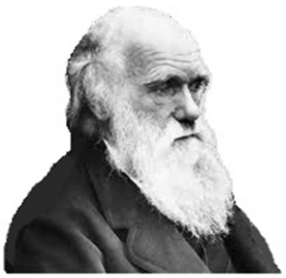

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Antibacterial activity of (PVP-ZrO_2) nanocomposite against pathogenic bacteria

Raghad Abdullatif Abdulrazaq[1], Zainab A. Al-Ramadhan[2], Hadeel H. Khalaf [2,*]

[1]Department of Biology, College of Science, Al-Mustansiriyah University, Baghdad, Iraq

[2]Department of Physics, College of Education, Al-Mustansiriyah University, Baghdad, Iraq

*E-mail address: hassan.hadeel86@yahoo.com

ABSTRACT

The antibacterial activity of a PVP-ZrO_2 nanocomposite was investigated against pathogenic bacteria *S. aureus* and *K. pneumonia*. After antibacterial sensitivity was determined and one isolate was chosen that showed more antibiotic resistance. Herein, the Co-culture technique was used to calculate percent reduction of bacteria. The results that were obtained in this method show that ZrO_2 nanoparticles have inhibitory effect against pathogenic bacteria gram negative bacteria and gram positive bacteria - with the reduction of growth reaching 100% to both, *S. aureus* and *K. pnumoniae* at 5, 10, 15, 20, and 25% ZrO_2, compared with control. The resistance patterns of *S. aureus* and *K.pnuemonia* isolates show the Moxifloxacin (MXF) is the best antibiotic for both bacteria - with sensitivity at 100%, while resistance to Ceftriaxone (CRO) is at 90% to *S. aureus*, and at 80% to *K. pnumoniae*. The polymer-nanocomposite was prepared by weight percentage (wt%) of (PVP) being dissolved in 10 mL of distilled water, with the weight percentages 5, 10, 15, 20, and 25 wt% of ZrO_2 nanoparticles added.

***Keywords*:** PVP, ZrO_2, Anti-Bacterial activity, Antibiotics, Nanocomposities, *Staphylococcus aureus, Klebsiella pneumoniae*

1. INTRODUCTION

Polyvinylpyrrolidone (PVP) is a water-soluble polymer made from the monomer *N*-vinylpyrrolidone, and is regarded as bulky, non-toxic, colorless, non-ionic, temperature-resistant, pH-stable, biocompatibility polymer, regarded as an interesting polymer for its capacity to interact with enormous variety of organic and inorganic compounds(1) because of its solubility in water and its extremely low cytotoxicity. It is industrially used as expanded

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polystyrene additive, as the gelling agents for suspension polymerization, stabilizer, and fiber treating agents, paper processing aids, adhesives, and thickening agents). PVP added to iodine forms a complex called povidone -iodine that possesses disinfectant properties[(2)].

Zirconium is a chemical element with symbol Zr, forms a variety of inorganic and organo-metallic compounds. Zirconium is a lustrous greyish-white, soft, ductile, and malleable metal that is solid at room temperature, though it is hard and brittle at lesser purities[(3)]. In a powder form, zirconium is highly flammable, but the solid form is much less prone to ignition. Zirconium is highly resistant to corrosion by alkalis, acids, salt water and other agents. However, it will dissolve in hydrochloric and sulfuric acid, especially when fluorine is present. The most common oxide is zirconium dioxide ZrO_2, also known as *zirconia*. Zirconium-bearing compounds are used in many biomedical applications, including dental implants and crowns, knee and hip replacements, middle-ear ossicular chain reconstruction, and other restorative and prosthetic devices[(4)]. Bacteria are very small organisms with size between 0.3 μm and 5μm, divided into two groups is dependent on cell wall, gram positive and gram negative bacteria. *Staphylococcus aureus* is a Gram-positive cocci, gold-colored singly, paris and cluster, it is a common type of bacteria that live on the skin and mucous membranes of humans, and is especially troublesome in hospitals where patients stay with open wounds and weakened immune systems[(5)]. *Klebsiella pneumoniae* is a gram negative bacteria rod-shaped, non-motile, and is important member of the family *Enterobacteriaceae*. One of the characteristics that distinguish *Klebsiella* spp. is the outermost layer that consists of a large polysaccharide capsule which gives the colonies their glistening and mucoid appearance on agar plates with lipopolysaccharide layer that protects the bacteria against phagocytosis[(6)]. The aim of this study is to determine antibacterial activity of (PVP-ZrO_2) nanocomposite against pathogenic bacteria in order to use them in biological applications.

2. MATERIAL AND METHOD

2. 1. Pathogenic Bacteria

Tow types of bacterial isolates used in this study included gram positive bacteria (10 isolates) *of Staphylococcus aureas* and gram negative bacteria (10 isolate) of *K pneumoniae,* were obtained from Department of Biology / College of Sciences / Al-Mustansiriyah University.

2. 2. Antimicrobial susceptibility test

All isolates were tested for eight types of different antimicrobial agents studied on Muller Hinton agar by using the standard disc diffusion method, according to (NCCLs, 2015) including (Amoxicillin + Clavulanic acid) Cefotaxime, Ceftriaxone, moxifloxacin, Norfloxacin, Ciprofloxacin Azithromycin, gentamicin), by using overnight culture at a 0.5 McFarland standard, followed by incubation at 35 °C for 16 to 18 hours.

2. 3. Preparation of (PVP-ZrO_2) Nanocomposite

Weight percentage (wt%) of (PVP) and (ZrO_2) were dissolved in 10 mL of distilled water with stirring the solution by using magnetic stirrer for about 1 hour at room temperature, adding the weight percentages (5, 10, 15, 20, and 25 wt%) of (ZrO_2), as shown in **Table 1**.

Table 1. Composite weight rates

Wight ratio of ZrO_2 nanoparticles %	PVP (g)	ZrO_2 nanoparticles (g)
0	0.1	0
0	0	0.1
5	0.095	0.05
10	0.09	0.01
15	0.085	0.015
20	0.08	0.02
25	0.075	0.025

2. 4. Antibacterial activity of (PVP- ZrO_2) nanocomposite against pathogenic bacteria: Co-Culture Method

Co-Culture technique was used for determination of antibacterial effect of (PVP -ZrO_2) nanocomposite. *Klebseilla pneumoniae* and *Staphylococcus aureus* were grown on nutrient broth with **(**PVP-ZrO_2**)** nanocomposite at the ratio 1:1 (vol:vol) (bacterial broth: PVP-ZrO_2 nanocomposite solution), the control medium contained nutrient broth only. Co-cultures and control were incubated at 37 °C for 24 h. After the incubation, 1 mL of each culture was serially diluted. The 0.1 mL of dilution sample was taken and spread on nutrient agar plates. The plates were incubated at 37 °C for 24 h. The colonies were observed in control and at treatments. The colonies were counted and inhibition effect was assessed and calculated percentage of reduction of bacterial growth, using the following equation, as described in [7]

$$R = \frac{A - B}{A} \times 100\%$$

where: *R*: is the reduction of bacterial growth
A: is the number of bacterial colonies from control
B: is the number of bacterial colonies from treatments with PVP-ZrO_2 nanoparticles composite.

3. RESULTS AND DISCUSSION

3. 1. Antimicrobial Susceptibility

All isolates in this study, conducted by using disc diffusion test to eight types of antibiotics, included azithromycine, gentamicin, amoxicillin\clavulanic acid, ceftriaxone,

ciprofloxacine, cefotaxime, norfloxacine, and moxifloxacin. The result showed that all isolates, both *S. aureus* and *K. pneumoniae* were sensitive (100%) to moxifloxacine. *S. aureus* resistance was 80% CR, 50% CTX, (40% CN, AMC, AZM), and (30% CIP, NOR), while *K. pneumoniae* resistance was 90% CRO, 80% CTX, 50% AMC, 40% AZM, 30% CIP, 20% CN and 10% NOR (**Figure 1**).

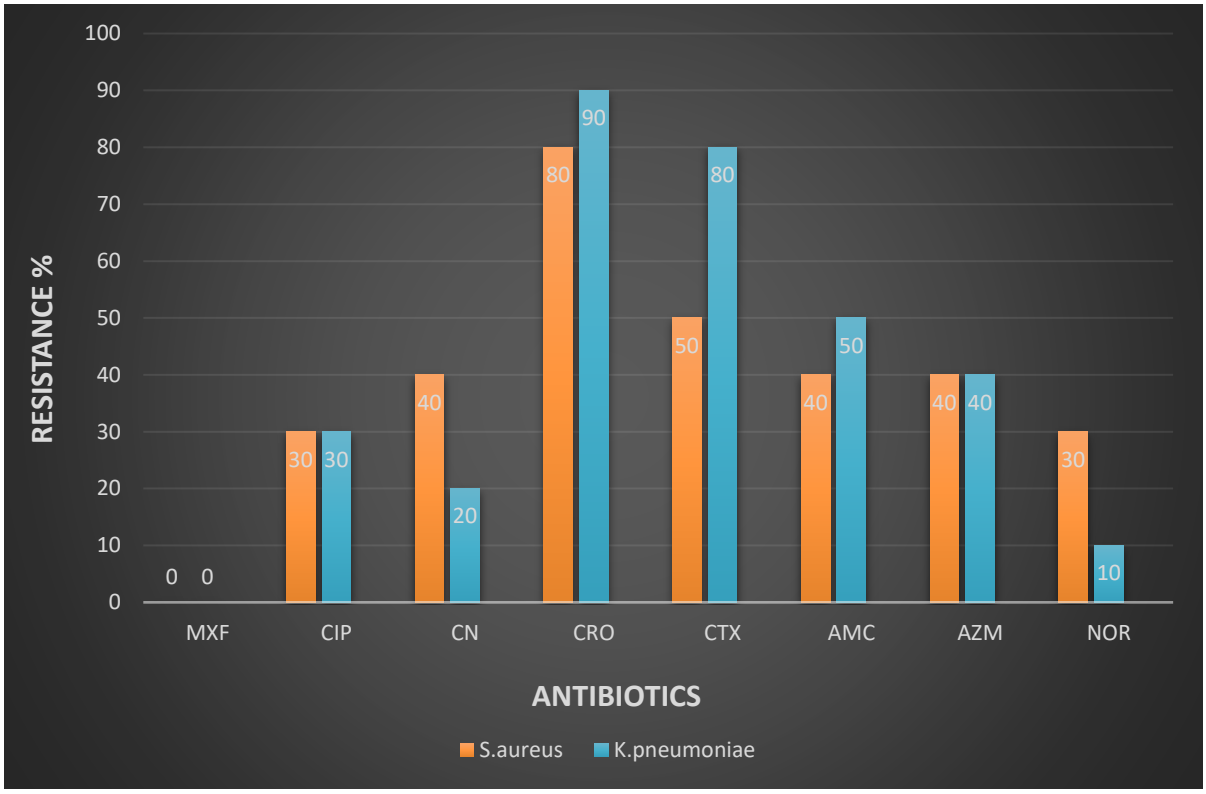

Figure 1. Susceptibility of *S. aureus and K. pneumoniae* isolates to antimicrobial agents.

3. 2. Antibacterial Activity of (PVP-ZrO_2) nanocomposites against Pathogenic Bacteria

Co-culture this method shows a high efficiency of (PVP-ZrO_2) nanoparticles against both, *Klebsiella pneumoniae* and *S. aureus*. Pure PVP was with the effect 100% against *S .aureus* and weak growth to *K. pneumonia*, while pure ZrO_2 effected with 100% against *K. pneumoniae* and weak growth to *S. aureus.*

No growth was observed at concentration (PVP- ZrO_2) (5, 10, 15, 20, and 25 wt%) to both types of bacteria compared with control (**Figure 2**a, 2b). The reduction of *K. pneumoniae* and *S. aureus* growth reached up to 100% at (5, 10, 15, 20, and 25 wt%) ZrO_2 compared with control (**Figure 3**a, 3b).

The study by[8] revealed that the zirconia exhibits activity only against the *E. coli*, whereas, the Zr(IV) complexes exhibit activity against both, the bacteria: gram -ve *E. coli* and gram +ve *S. aureus* and is quite different from that of gram −ve bacteria (*E. coli*), (*S. aureus*) in terms of charge and chemical moieties.

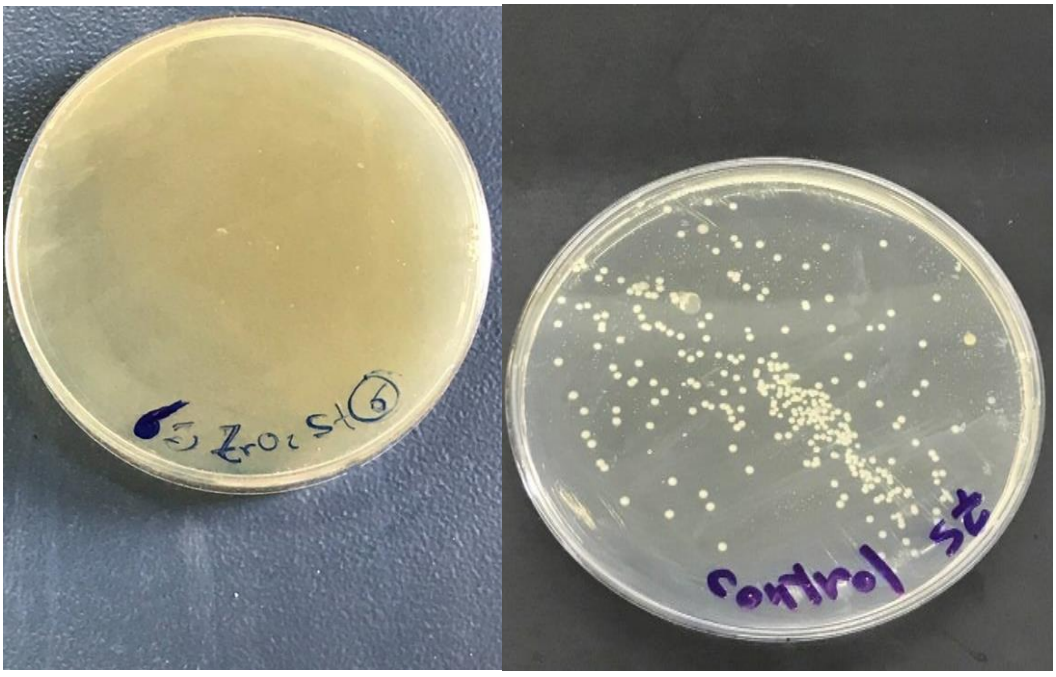

Figure 2a. Antibacterial activity of PVP-ZrO_2 nanocomposite against *Staphylococcus aureus*

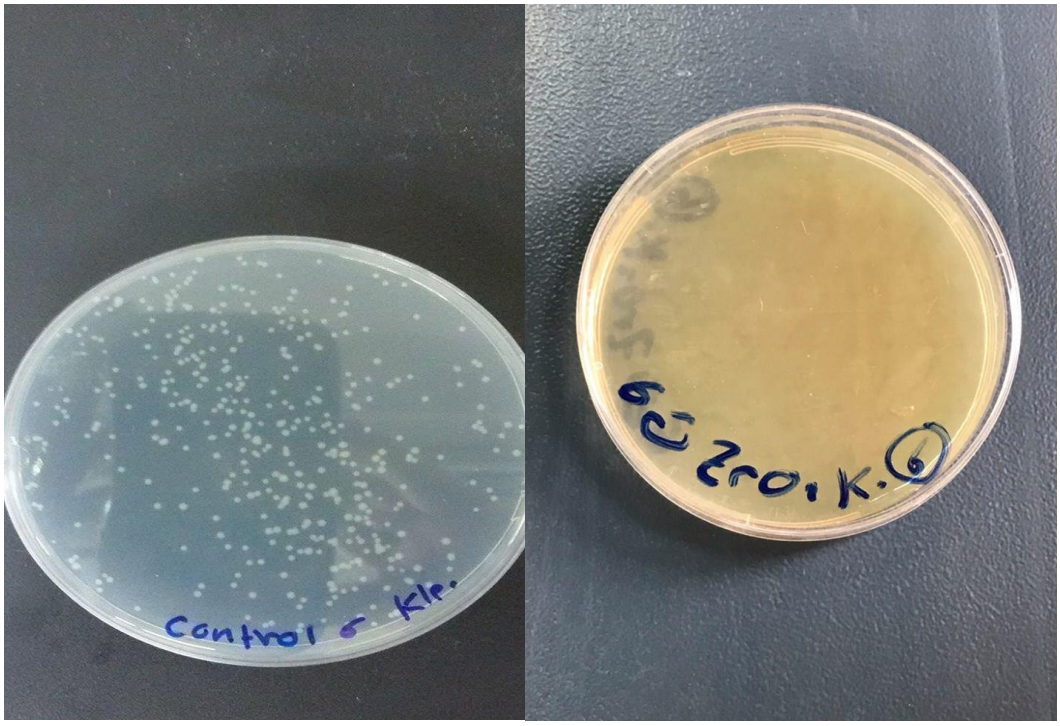

Figure 2b. Antibacterial activity of PVP-ZrO_2 nanocomposite against *Klebsiella pneumoniae*

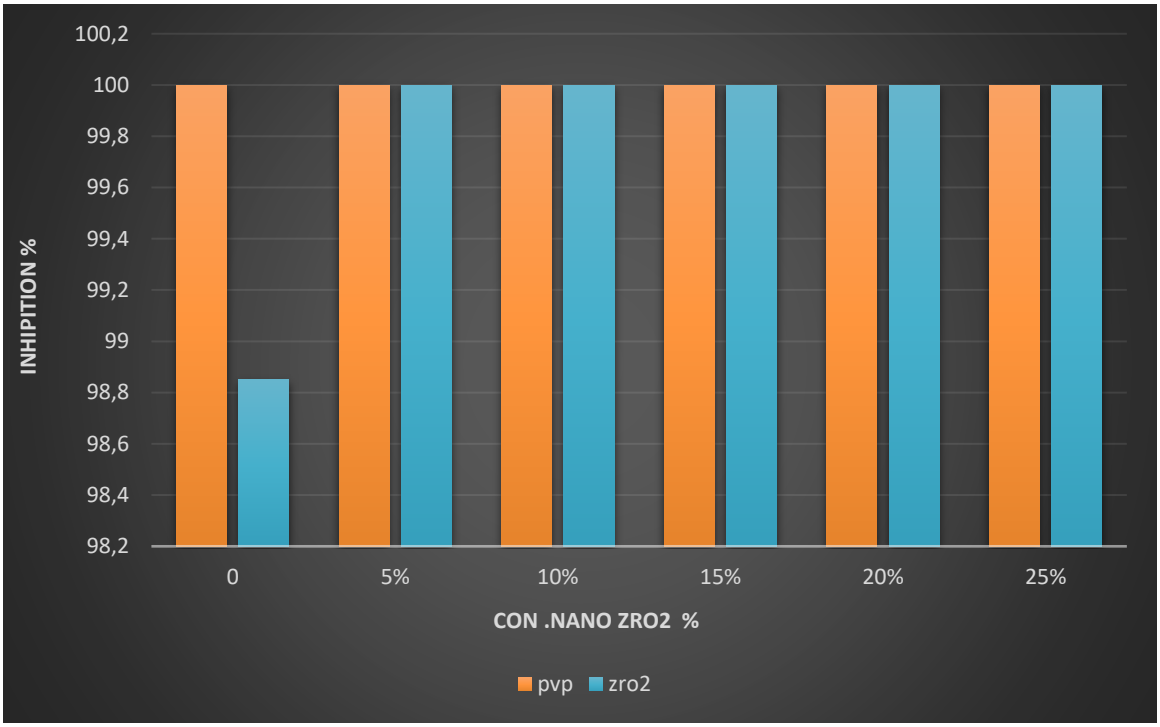

Figure 3a. Reduction of *S. aureus* growth by PVP-ZrO_2 nanocomposite.

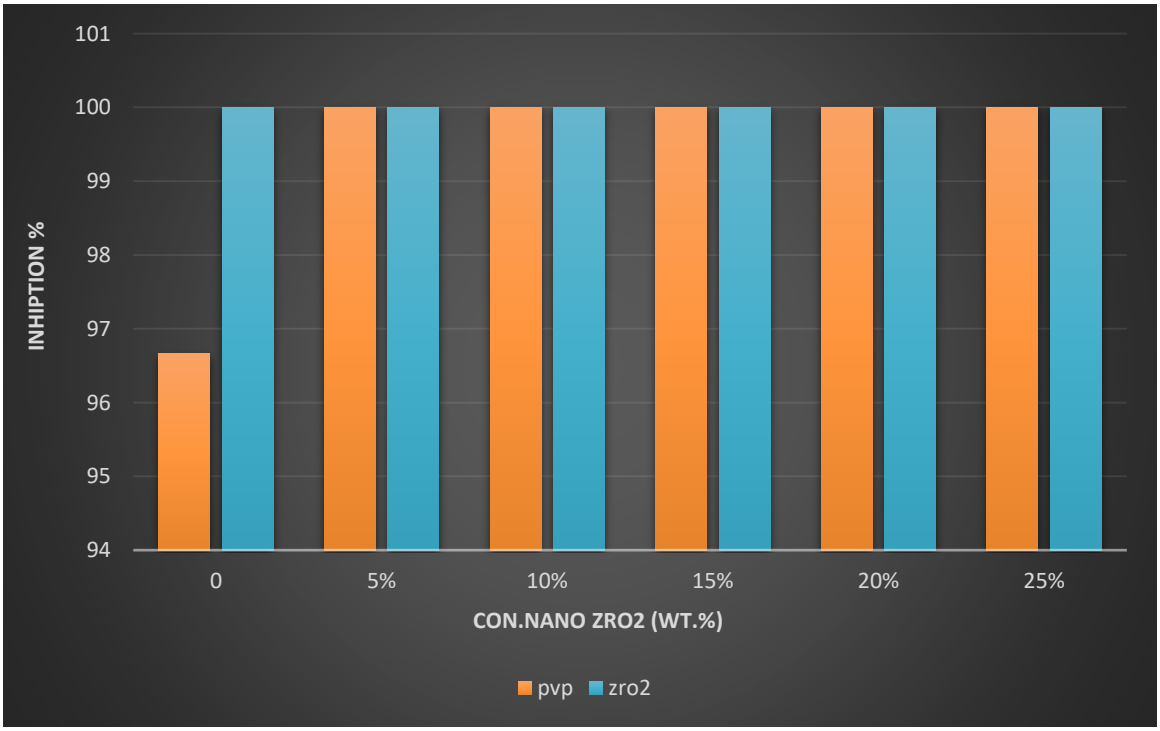

Figure 3b. Reduction *of K. pneumoniae* growth by PVP-ZrO_2 nanocomposite.

Therefore, the differences in antibacterial activity of ZrO_2 nanoparticles against *S. aureus* and *E. coli* could be attributed to the surface charge. Study by[9] on Zirconium oxide nanoparticles have revealed antibacterial activities on the isolates, the inhibition zone was 37 mm for *Staphylococcus epidermidis*, 10 mm for *Staphylococcus aureus*, 8 mm for *Klebsiella* spp. The action of nano-Zr may target the bacterial membrane, leading to change of the permeability, and disrupting the outer membrane barrier components, such as lipopolysaccharide, culminating in the perturbation of the cytoplasmic membrane[10]. Their antimicrobial effect is due to blockage of respiratory enzyme pathways, alteration of microbial DNA and the cell wall, or may be ascribed to the atomic arrangements of different exposed surfaces[11].

4. CONCLUSION

PVP-ZrO_2 nanocomposites had antibacterial activity against some of gram positived and gram negative pathogenic bacteria. Co-culture is the best method for detection of antibacterial effect of nanocomposites.

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