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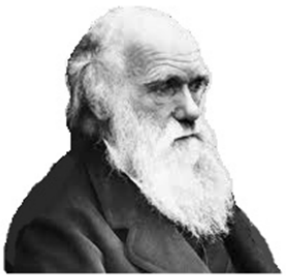

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Antibacterial activity of (PVP-ZrO_2) nanocomposite against pathogenic bacteria

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ABSTRACT

The antibacterial activity of a PVP-ZrO_2 nanocomposite was investigated against pathogenic bacteria *S. aureus* and *K. pneumoniae* 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 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% *S. aureus,* and at 80% *K. pnumoniae*. The polymer-nanocomposite was prepared by weight percentage wt. % of (PVP) being dissolved in (10) ml of distilled water, with weight percentages 5%, 10%, 15%, 20% and 25% of ZrO_2 nanoparticles added.

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

1. INTRODUCTION

Polyvinylpyrrolidone (PVP) is awater-soluble polymer made from the monomer *N*-vinylpyrrolidone, is regarded as bulky, non-toxic, colorless, non-ionic, temperature-resistant, pH-stable, biocompatibility polymer, regard as interesting polymer for its capacity to interact with enormous variety of organic and inorganic compounds(1) because of its

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solubility in water and its extremely low cytotoxicity. It is industrially used as expanded 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 acomplex called povidone -iodine that possesses disinfectant properties[2].

Zirconium is a chemical element with symbol Zr, forms avariety 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 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 in to two groups depended 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, is especially troublesome in hospitals where patients with open wounds and weakened immune systems[5]. *Klebsiella pneumoniae* it is gram negative bacteria rod-shaped, non-motile, important members 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. lipopolysaccharide layer that protects the bacteria against phagocytosis[6]. Aim of this study 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 agent studied on Muller Hinton agar by using the standard disc diffusion method according to (NCCLs, 2015) include (Amoxicillin + Clavulanic acid) Cefotaxime, Ceftriaxone, moxifloxacin, Norfloxacin, Ciprofloxacin Azithromycin, gentamicin) using overnight culture at a 0.5 McFarland standard followed by incubation at 35 °C for 16 to 18 h.

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%) of (ZrO_2) as shown in Table (1).

Table 1. Composite weight rates

Wight ratio of ZrO_2 nanoparticles %	**PVP (gm)**	**ZrO_2 nanoparticles (gm)**
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 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 cultures were serially diluted. The 0.1 ml of dilution sample was taken and spreaded on nutrient agar plates. The plates were incubated at 37 °C for 24 h. The colonies were observed in control and treatments.The colonies were counted and inhibition effect was assessed and calculated percentage of reduction of bacterial growth .using the following equation described as [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 on this study conducted by using disc diffusion test to eight type of antibiotics included azithromycine, gentamicin, amoxicillin\clavulanic acid, ceftriaxone, ciprofloxacine, cefotaxime, norfloxacine, moxifloxacin. The result showed that all isolates both *S. aureus* and *K. pneumoniae* were Sensitive (100%) to moxifloxacine. *S .aureus* resistance 80% CR, 50% CTX, (40% CN, AMC, AZM) and (30% CIP, NOR). While *K. pneumoniae* resistance 90% CRO, 80% CTX, 50% AMC, 40% AZM, 30% CIP, 20% CN and 10% NOR (Figure 1).

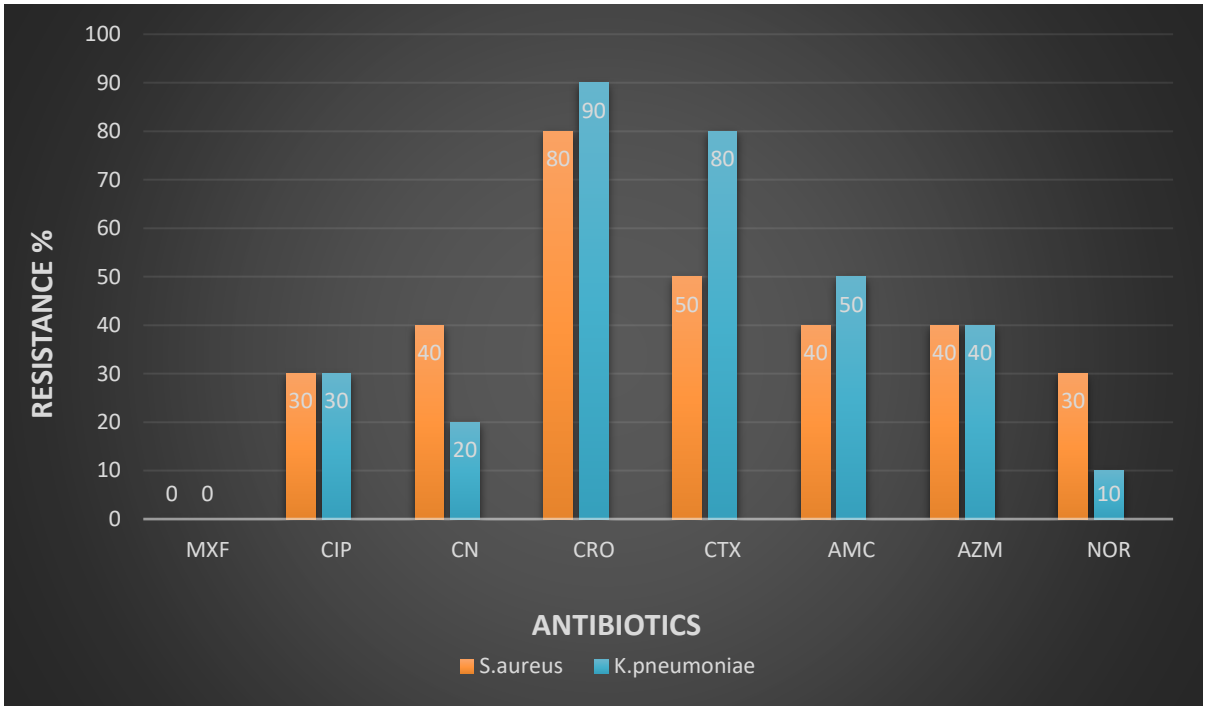

Figure 1. Susceptibility of *S. aureus and K. pneumoniae* isolates to antimicrobials agent.

3. 2. Antibacterial Activity of (PVP-ZrO_2) nanocompositeas Against Pathogenic Bacteria

Co-culture this method show high efficiency of (PVP-ZrO_2) nanoparticles against both *Klebsiella pneumoniae* and *S. aureus*. Pure PVP are effect 100% against *S .aureus* and weak growth to *K. pneumoniae* while pure ZrO_2 effect 100% against *K. pneumoniae* and weak growth to *S. aureus.* No growth observed at concentration (PVP- ZrO_2) (5%, 10%, 15%, 20%, 25%) to both type of bacteria compared with control (Figure 2a, 2b) .The reduction of *K. pneumoniae* and *S. aureus* growth reached to 100% at (5%, 10%, 15%, 20% and 25%) ZrO_2 compared with control (Figure 3a, 3b). Study by [8] revealed that the zirconia exhibits activity only against the *E. coli*, whereas, the Zr(IV) complexes exhibits activity against both the bacteria: gram -ve *E. coli* and gram +ve *S. aureus*. is quite different from that of gram −ve bacteria (*E. coli*), (*S. aureus*) in terms of charge and chemical moieties.

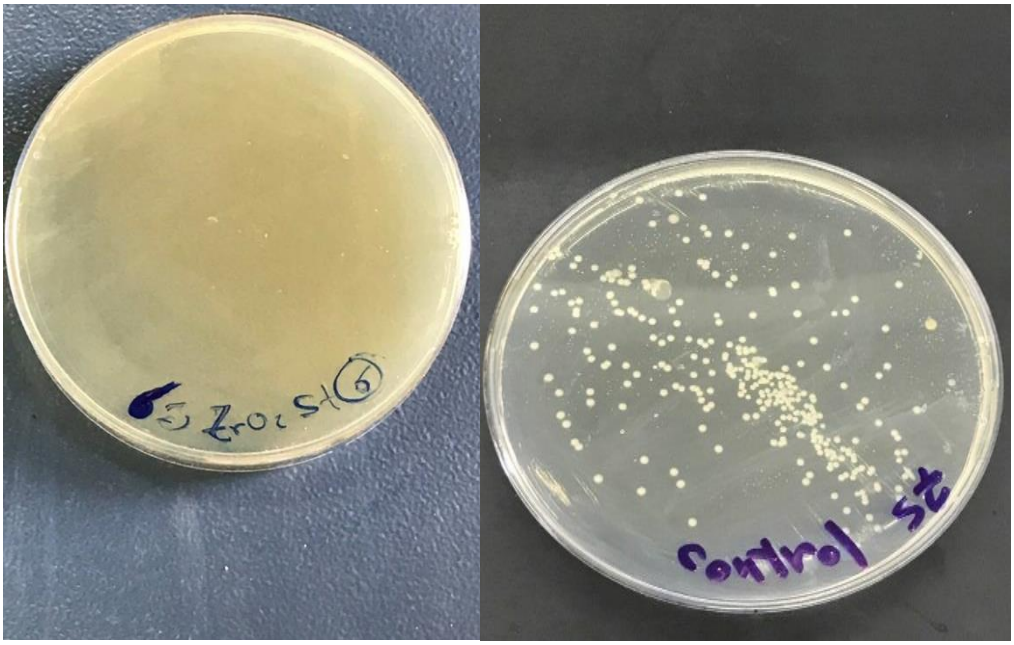

Figure 2a. Antibacterial activity of PVP-ZrO_2 nanocomposite against *Staphylococcus aureus*

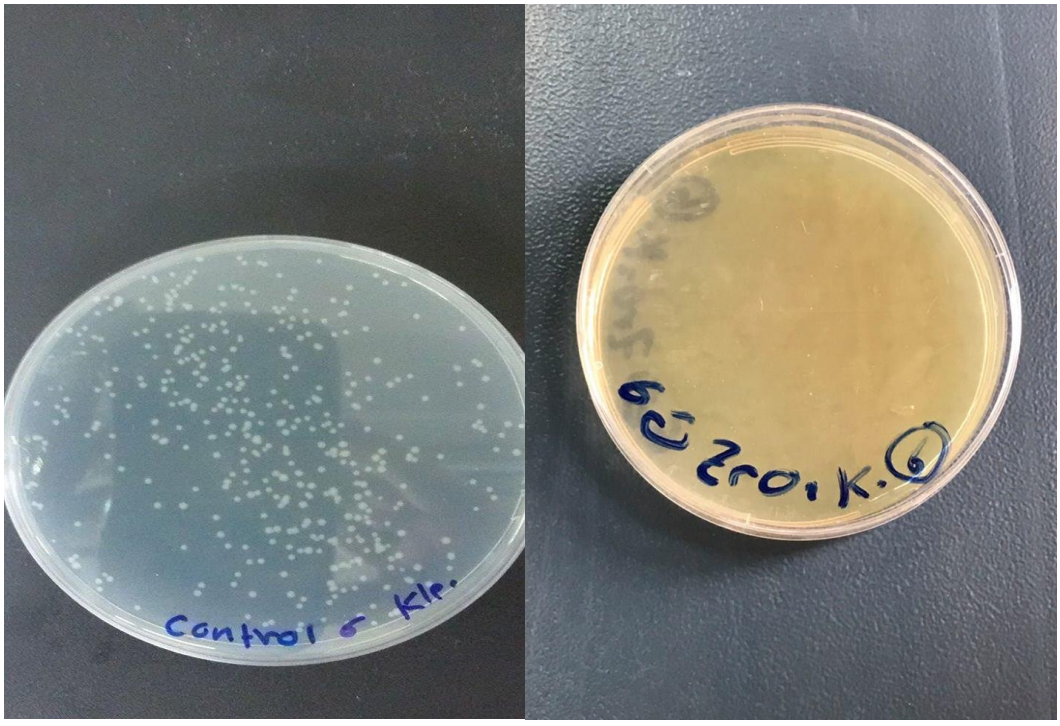

Figure 2b. Antibacterial activity of PVP-ZrO_2 nanocomposite against *Klebsiella pneumoniae*

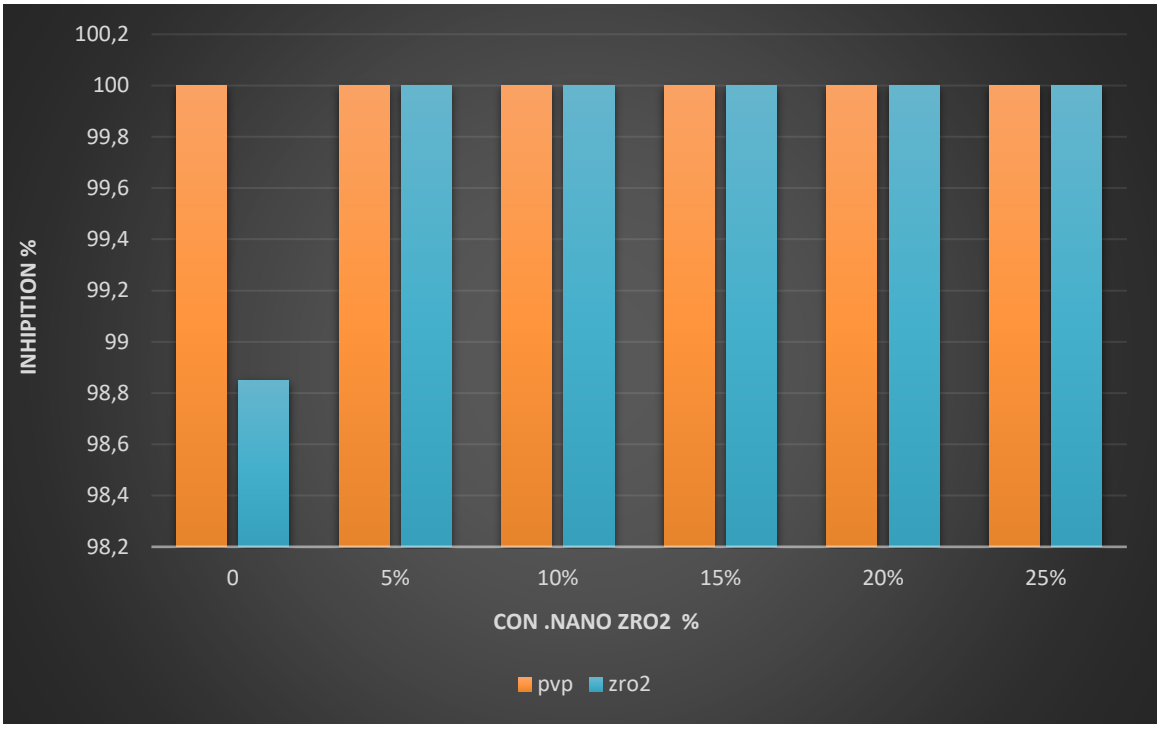

Figure 3a. Reduction of *S. aureus* growth by PVP-ZrO_2 nanocomposite.

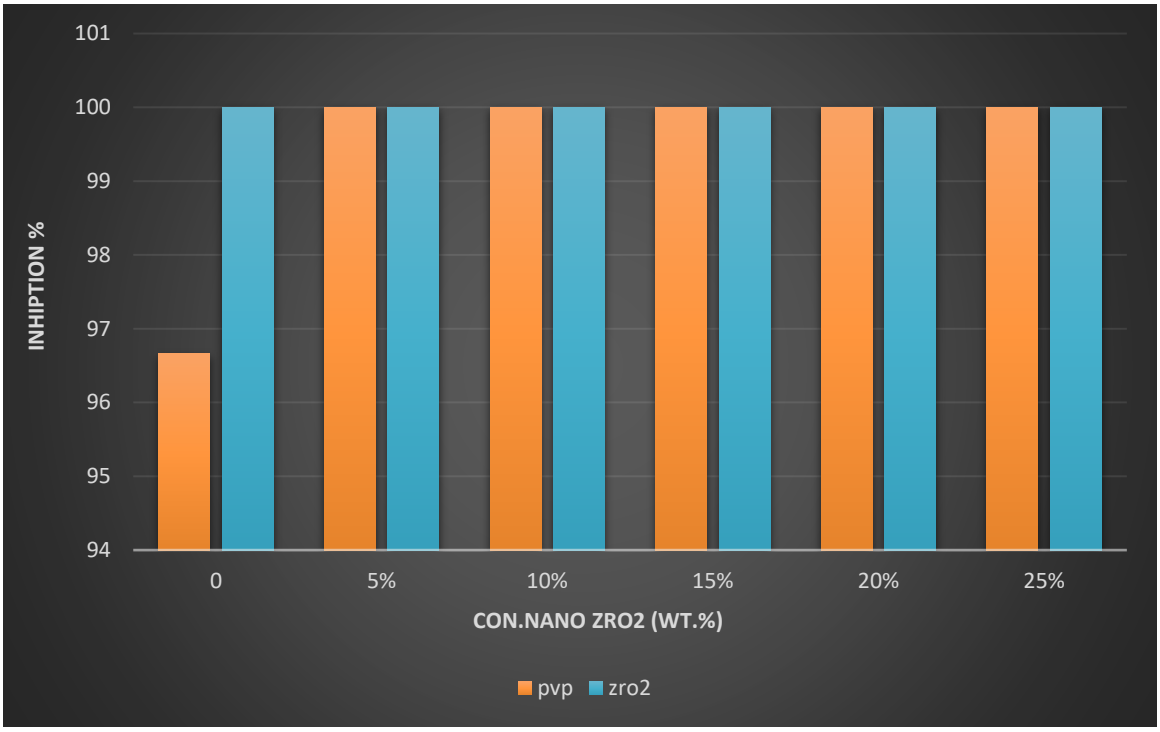

Figure 3b. Reduction *of K. pneumoniae* growth by PVP-ZrO_2 nanocomposite.

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] Zirconium oxide nanoparticles have 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 disrupts 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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