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Synergistic effect of silver nanoparticles extracted from *Talaromyces islandicus* and antibiotics on resistant bacteria *Ps. aeruginosa* and *E. facelius* isolated from various infections

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Abstract

Pseudomonas aeruginosa [P. aeruginosa] and *Enterococcus faecalis)* are pathogenic bacteria that are resistant to many antiobiotic. The main aim of this study was to test the synergistic effect of silver nanoparticles [AgNPs] in combination with different types of antibiotics against *P. aeruginosa* and *E. faecalis*. This study showed that the synergistic effect of silver nanoparticles is less effective than the effect of the antibiotic only. It showed that the antibiotic more effective in declining the growth of *P. aeruginosa* and *E. faecalis* than the combination mixture of silver nanoparticle and antibiotic. The synergistic effect needs more studies to enhance the Nano activity of sliver with antibiotics.

Introduction

Pseudomonas aeruginosa (*P. aeruginosa*) is a rod-shaped, gram-negative, aerobic bacterium. It hastens the loss of lung capacity and ultimately raises the mortality and morbidity rates. The ability of P. aeruginosa to produce biofilms is what allows it to survive and be virulent. In wound and urinary tract infections, Enterococcus faecalis and Pseudomonas aeruginosa are frequently coisolated. The host naturally limits the availability of iron at infection sites as a defensive measure. Opportunistic pathogens called enterococci are linked to a variety of illnesses. Silver and its compounds have different effects against microorganisms and viruses, as proven by studies, due to the small size that does not exceed 5 nm, as it increases the surface area and generates a tendency to migrate to the outer surface

of the nanoparticles with an increase in the chemical activity of the particles and an increase in the formation of free radicals and an increase in the production of reactive oxygen [1,2].

Nanoparticles can be obtained by chemical and biological methods from microorganisms such as bacteria and fungi that have the ability to produce Nanoparticles with ant microbiological properties [3].

The size of the particles is an important and essential element in the effectiveness of antimicrobial silver nanoparticles, as large molecules have been shown to be less effective against microbes than small molecules that affect more against microbes due to their ability to penetrate and enter the cell [4].

The modern nanotechnologies have opened the door to multiple and varied medical and therapeutic applications, including what is known as nanomedicine, and includes a number of modern medical technologies, including the use of nanoparticles produced by fungi as antibiotics against pathogenic bacteria and fungi, as silver nanoparticles have shown high effectiveness in this field, as shown by silver nanoparticles (AgNPs) Silver Nanoparticles] used in the manufacture of products and consumer goods such as dyes, cosmetics and food products [5].

In the twentieth century, studies proved the ability of pathological bacteria to acquire the characteristic of resistance to antibiotics used against them, due to the development of bacteria, which contributed to resistance and that the increase in bacterial resistance against antibiotics has become a real threat to human health, so scientists have resorted to the development of antibiotics through the production of new antibiotics to eliminate these forms of resistance and due to the ability of bacteria to develop new methods of resistance, these methods did not work, so the world has tended to Finding new therapeutic alternatives, including the use of compounds and nanoparticles ranging in size from 1-100 natometers, with the addition of antibiotics to the nanoparticles, which showed a synergistic effect against bacteria [6]. It showed a high percentage of the ability to inhibit bacterial growth and here appeared the importance of manufacturing silver nanoparticles mediated by fungi or bacteria as a biological nanoplant for its high ability to produce silver nanoparticles, which were used in different fields [7], and therefore scientists are currently tending to use nanoparticles and study their inhibitory effect on different bacteria instead of antibiotics and on this basis the study aimed to:

1- Isolation and diagnosis of fungi and bacteria taken from secret samples in the city of Samarra.

2- Then test the ability of three nanoparticles fungi to produce silver nanoparticles

3- Study the effect of nanoparticles on antibiotic-resistant bacteria.

5- Study the effect of interference of silver nanoparticles with antibiotics.

Materials and Methods

Collecting clinical samples

Samples were collected from different infections including skin injuries, urinary tract infections, and injuries for both sex from patients of Samarra General Hospital in Salah Al-Din Governorate for the period between January 2021 to April, 2022, diagnostic tests were

carried out, it was cultivated in the MacConkey agar and blood agar for diagnosis formal and biochemical industries.

Construction of silver nanotechnology from Mushrooms

Synthesis of the nanoparticle by *Talaromyces islandicus* was isolated from a previous disease infection and diagnosed as the first isolation in Iraq and was registered in the genebank. After planting the fungi on potato Dexfrose Agar and incubating at a temperature of 28co for 7 days, a tablet was taken from the fungal colony using a sterile cork piercer with a diameter of 7 mm and then placed in a conical flask containing 300 ml of potato Dextrose broth medium [8, 9].

Then placed in an incubator with a temperature of 26 ° C for 7-5 days to obtain the Qatari barn, then filter the fungal mass using sterile filter paper Watman filter Nol, then wash well with sterile water to remove the remnants of the medium, then put the fungal mass in a conical flask containing 100 ml distilled water and leave for 72-24 hours in an incubator, then filter the fungal mass using filter paper, then dried and then weigh 10 g of fungal mass.

Then put 100 ml of 1mm AgNo3 silver nitrate solution and place in completely dark conditions for 24 hours

Examination of AgNps nanoparticle samples 72 hours after placing the AgNps solution in the incubator under dark conditions and the temperature is 26-25 °C, 2 ml of AgNps solution was taken and an examination with a UV-visible spectrophotometer.

Then the sample was prepared for examination through by converting X-ray Powder Diffraction (XRD) it into powder by drying method using hot air at 60°C after 72 hours of filtering the fungus, the filtered solution was placed in plastic tubes with a volume of 50 ml and placed in a refrigerated centrifuge speed (7000 rpm) for 15 minutes, then poured part of the filtrate and mixed with the precipitate and put in his ceramic eyelid and placed in the hot air oven for 15-20 minutes. Then collect the dried sediment and then turn it into fine powder by grinding and drilling about 3-2 g for the X-ray examination sample and FESEM was examined [10].

The effect of antibiotics on *Pseudomonas aeruginosa and E. facelius* bacteria:

Antibiotics have been used in tablet form and antibiotics are Ceftriaxone, Ceftazidime, AZiMac, Amikacin, Gentamicin, levofloxacin, Cefolaxin, Ampicillin, Ciprofloxacin, Erythromycin.

The effectiveness of the minimum inhibitory concentration of the antibiotics

The rate of concentration of antimicrobial agents depends on the type of organism The antimicrobial agent For the selection of the full-range Mic, about 12 mitigations are selected, where the Mic test represents the breakpoint Mic, i.e. distinguish the different categories of sensitivity to antibiotics, i.e. Susceptible or moderate sensitivity Intermediate or Resistant Then the preparation of the stored solution Stock Solutions An accurate standard analytical balance is used to weigh antimicrobial agents and the powder percentage can be obtained within the concentration and effectiveness using the following equation:

Weight of powder (mg) = volume of solution (ml) x concentration (mg/L) potency of powder (mg/g).

About 12 dilutions are selected, where the breakpoint selection represents the Mic that changed the different categories of sensitivity to the antimicrobial Susceptible or Intermediate or Resistant that stimulates fear by liquid Broth dilution, where you need at least 1 ml per dilution per tube and the anti-Levofloxacin is dissolved by the solvent agent NaoH and by a diluent agent is water.

Microdilution method with a capacity of 500≥ µL per hole and then a stimulation of antibiotics was used for use in the MIC test Table [4].

The method of liquid medium cultures was used, this is done by taking a sample from the colony and transferring it to develop a nutritious broth brain heart in fusion, then incubating the broth at 37-35 ° C until the growth reaches a turbidity equal to 0.5 McFarland, then a volume of bacterial suspension, which is equal to the volume of diluted antimicrobial solution, is added to each tube or hole containing the antimicrobial agent. Synergistic effect of silver nanoparticles produced from *Talaromyces islandicus* fungus and antibiotic on bacteria. Synergistic effect of silver nanoparticles produced from *Talaromyces islandicus* and the antibiotic Levofloxacin on *Ps. Auerignosa* bacteria.

Preparation of nanoparticle solution diluant:

The used solution form after filtering it and the following concentrations was prepared:

- The first concentration I mm was prepared from the AgNPs solution 100% concentration.
- The second concentration was prepared by adding 5 ml of the initial solution and 5 ml of water only to make the concentration 50%.
- The third concentrate was prepared by adding 2.5 ml of the initial solution 7.5 ml of distilled water to make the concentration 25%.
- The fourth concentrate is prepared by adding 1 m1 ml of distilled water.

Results and Discussion

Study the effect of MIC of Nanoparticles extracted from Talaromyces against bacteria

The results detailed in Figure 1 showed that the silver nanoparticles synthesized from Talaromyces vary in their effect against bacteria, where the minimum inhibitory concentration of *E. faecalis* is (32, 64, 128, 256, and 512) mg/L respectively.

The results shown in Table 1 showed that the minimum inhibitory concentration of *P. aeruginosa* bacteria is (0.125, 0.25, 0.5, 1) mg/L respectively.

Table 1: Effect of Silver Nanoparticles Synthesized by Talaromyces against Bacteria.

Type of bacteria Concentration mg/l	*Enterococcus faecalis*	*Pseudomonas aeruginosa*
512	-	+
256	-	+
128	-	+
64	-	+
32	-	+
16	+	+
8	+	+
4	+	+
2	+	-
1	+	-
0.5	+	-
0.25	+	-
0.125	+	-

The effect of Minimum inhibition concentration of the antibiotic

The effect of four antibiotics (Erythromycin and Ciprofloxacin, Levofloxacin] in graduated concentrations by dilution method ranging between (512, 256, 128, 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25, and 0.125) mg/L respectively to measure the minimum concentration of antidotes to *Enterococcus faecalis* , *Pseudomonas aeruginosa*, and as shown in Tables 2 and 3, the results showed that the minimum inhibitory of Levofloxacin on *E. faecalis* bacteria was (512, 256, 128, 64, 32, 16) mg/L on respectively, while the results show that is resistant *Pseudomonas aeruginosa* to the anti-Levofloxacin, It is clear from the results that the bacteria *Pseudomonas aeruginosa* are resistant to the antibiotic, and the results are consistent with the findings of the researchers [12].

Table 2: Anti-Levofloxacin Effect on Isolated Bacteria

Type of bacteria *Concentration mg/l*	*Enterococcus faecalis*	*Pseudomonas aeruginosa*
512	-	+
256	-	+
128	-	+
64	-	+
32	-	+
16	-	+
8	+	+
4	+	+
2	+	+
1	+	+

0.5	+	+
0.25	+	+
0.125	+	+

The results of the study showed that the minimum inhibitory of Erythromycin (EM) was 512 mg/L against *Enterococcus faecalis* while the bacteria, were *Pseudomonas aeruginosa* resistant to EM as shown in Table 4.

Table 2: Anti-EM effect on isolated bacteria

Type of bacteria / *Concentration mg/l*	*Enterococcus faecalis*	*Pseudomonas aeruginosa*
512	-	+
256	+	+
128	+	+
64	+	+
32	+	+
16	+	+
8	+	+
4	+	+
2	+	+
1	+	+
0.5	+	+
0.25	+	+
0.125	+	+

The results detailed in Table 4 show the minimum inhibitory of, *Enterococcus faecalis* and Pseudomonas *aeruginosa* after treatment with graduated concentrations of anti-E, and the results showed that *Pseudomonas aeruginosa* bacteria are resistant to antibodies of all concentrations while the minimum inhibitory of *Enterococcus faecalis* is (8, 16, 32, 64, 128, 256, 512) respectively, and the minimum inhibitory of P. mirabilis is 512 mg/L of antibiotic E. The results indicate that the two types of bacteria are resistant to the antibiotic The results match what the researchers found, *E. faecalis* strains carrying many antibiotic-resistance genes and high antibiotic-resistance rates [14].

Table 4: Anti- E antibiotic effect on isolated bacteria

Type of bacteria / *Concentration mg/l*	*Enterococcus faecalis*	*Pseudomonas aeruginosa*
512	-	+
256	-	+
128	-	+
64	-	+
32	-	+
16	-	+
8	-	+
4	+	+
2	+	+

1	+	+
0.5	+	+
0.25	+	+
0.125	+	+

The results detailed in Table 5 show that the minimum inhibitory of *E. faecalis* from the antibiotic Ciprofloxacin is 0.25, 0.5, 1, 2, 4, 8, 16, 32, 64, 128, 256, 512 mg/L respectively, while *P. aeruginosa* was resistant to Ciprofloxacin at all concentrations. The results are consistent with [13] results where the cause of bacterial resistance is due to genetic factors.

Table 5: Anti-Ciprofloxacin Effect on Isolated Bacteria

Type of bacteria *Concentration mg/l*	*Enterococcus faecalis*	*Pseudomonas aeruginosa*
512	-	+
256	-	+
128	-	+
64	-	+
32	-	+
16	-	+
8	-	+
4	-	+
2	-	+
1	-	+
0.5	-	+
0.25	-	+
0.125	+	+

The effect of Minimum inhibition concentration (MIC] of nanoparticles extracted from fungus, the antibiotic, and the interaction between

The synergistic activity of AgNPs extracted from. with antibiotic in inhibition test

After measuring the minimum inhibitory concentration of the antibiotics Levofloxacin, Ciprofloxacin, Erythromycin, EM in graduated concentrations (512, 256, 128, 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.125) mg/L, respectively after interference with silver nanoparticles synthesized from Talaromyces in graduated concentrations (1, 0.5, 0.25) against Ps. Aeruginosa The results detailed in Table 6 showed that the bacteria are resist *Pseudomonas aeruginosa* ant to the antibiotics Levofloxacin and EM interfering with the silver nanoparticles synthesized from Talaromyces No lethal effect was shown on bacteria in all concentrations. The minimum inhibitory concentration of Ciprofloxacin interfered with silver nanoparticles of synthesis of *Talaromyces* (64, 128, 256, 512) mg/L, respectively, while the minimum inhibitory of the antibiotic Ciprofloxacin interfering with nanoparticles at a concentration of [0.5] against *Ps. aeruginosa* bacteria was (512, 256) mg/L respectively, while the minimum inhibitory of Ciprofloxacin was 512 mg/L overlapping with a concentration of 0.25 silver nanoparticles synthesized from *Talaromyces*. Similarly, for anti-E interfering with *Talaromyces* at a concentration of [1], the minimum inhibition concentration was 512 mg/L, while the minimum inhibitory concentration of anti-E was (512, 256) mg/L, respectively, after interference with Talaromyces synthesized silver nanoparticles at 0.5, 025.

Table 6: Effect of interference of silver nanoparticles synthesized from Talaromyces fungus and antibiotics on *Pseudomonas aeruginosa* bacteria

Antibiotic	LEV			CIP			E			EM		
	B.MIC	MIC	A.MIC	B.MIC	MIC	A.MIC	B.MIC	MIC	A.MIC	B.MIC	MIC	A.MIC
Nanoparticle conc. / Antibiotic conc.	1	0.5	0.25	1	0.5	0.25	1	0.5	0.25	1	0.5	0.25
512	+	+	+	-	-	-	-	-	-	+	+	+
256	+	+	+	-	-	+	+	-	-	+	+	+
128	+	+	+	-	+	+	+	+	+	+	+	+
64	+	+	+	-	+	+	+	+	+	+	+	+
32	+	+	+	+	+	+	+	+	+	+	+	+
16	+	+	+	+	+	+	+	+	+	+	+	+
8	+	+	+	+	+	+	+	+	+	+	+	+
4	+	+	+	+	+	+	+	+	+	+	+	+
2	+	+	+	+	+	+	+	+	+	+	+	+
1	+	+	+	+	+	+	+	+	+	+	+	+
0.5	+	+	+	+	+	+	+	+	+	+	+	+
0.25	+	+	+	+	+	+	+	+	+	+	+	+
0.125	+	+	+	+	+	+	+	+	+	+	+	+

The minimum inhibitory concentration of the four antibiotics Levofloxacin, Ciprofloxacin, Erythromycin, and EM was measured in graduated concentrations (512, 256, 128, 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.125) mg/L, respectively] after interference with silver nanoparticles synthesized from Talaromyces in graduated concentrations (1, 0.5, 0.25) against *E. faecalis*. the results detailed in Table 7 showed that *E. faecalis* is resistant to EM antibiotics interfering with silver nanoparticles synthesized from Talaromyces and showed no lethal effect on bacteria of all concentrations.

Table 7: Effect of Interference of Silver Nanoparticles Synthesized from Talaromyces and Antibiotics on *E. faecalis*

Antibiotic	LEV			CIP			E			EM		
	B.MIC	MIC	A.MIC	B.MIC	MIC	A.MIC	B.MIC	MIC	A.MIC	B.MIC	MIC	A.MIC
Nanoparticle conc. / Antibiotic conc.	1	0.5	0.25	1	0.5	0.25	1	0.5	0.25	1	0.5	0.25
512	-	-	+	-	-	-	-	-	-	+	+	+
256	+	+	+	-	-	-	-	-	-	+	+	+
128	+	+	+	-	+	+	-	+	+	+	+	+
64	+	+	+	+	+	+	+	+	+	+	+	+
32	+	+	+	+	+	+	+	+	+	+	+	+
16	+	+	+	+	+	+	+	+	+	+	+	+
8	+	+	+	+	+	+	+	+	+	+	+	+
4	+	+	+	+	+	+	+	+	+	+	+	+
2	+	+	+	+	+	+	+	+	+	+	+	+
1	+	+	+	+	+	+	+	+	+	+	+	+
0.5	+	+	+	+	+	+	+	+	+	+	+	+
0.25	+	+	+	+	+	+	+	+	+	+	+	+
0.125	+	+	+	+	+	+	+	+	+	+	+	+

The results detailed in table [7] that the minimum inhibitory of Levofloxacin on *E. faecalis* bacteria was (512, 256, 128, 64, 32, 16) mg/L a minimum concentration of 512 mg respectively. Overlapping with silver nanoparticles synthesized from Talaromyces at a concentration of (1, 0.5) while the rest of the concentrations were lower-bacterial inhibitors. Overlapping with silver nanoparticles synthesized from Talaromyces at a concentration of (1, 0.5) while the rest of the concentrations were bacterial inhibitors. Nanoparticles can penetrate the cell wall and membrane of bacteria and act by disrupting important molecular mechanisms. In combination with appropriate antibiotics, NPs may show synergy and help prevent the developing global bacterial resistance, it also reduces bacterial adhesion during biofilm formation which usually occurs in the early stages Several researchers have shown

that AgNPs enhance the antibacterial effect of antibiotics against both resistant and susceptible bacteria [15, 16].

The researchers [17] used the same concentrations of nanoparticles used in the study and reached the results that combination therapy can significantly reduce the concentrations of both antibiotics and eliminate the pathogen's antibiotic resistance. These findings imply that biosynthesised Ag NPs can be effective broad-spectrum antibacterial agents, possibly at lower doses than those currently used in clinical trials to treat infections

Conclusion

Silver nanoparticles produced from fungi *Talaromyces islandicus* were very effective against the bacteria used in the study Pseudomonas aeruginosa and *Enterococcus faecalis*. Where high concentrations of silver nanoparticles inhibited bacteria Enterococcus *faecalis*, and low concentrations inhibited bacteria Pseudomonas aeruginosa Bacteria Pseudomonas aeruginosa showed high resistance to the antibiotics used in the study, Erythromycin and Ciprofloxacin, Levofloxacin. As for the bacteria Enterococcus *faecalis*, it was resistant to most antibiotics, but to a lesser extent than *Pseudomonas aeruginosa*, as it resisted most of the antibiotics except for the antibiotic Ciprofloxacin. In the process of interaction between silver nanoparticles and antibiotics. The interference process showed a change in the ability of bacteria to resist antibiotics.

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التأثير التآزري لدقائق الفضة النانوية المنتجة من *Talaromyces Islandicus* والمضاد الحيوية Levofloxacin على البكتيريا *Staphylococcus aureus*

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البرازية المعوية، اختبار المواد النانوية، ضد جرثومية، الزنجارية

معلومات المؤلف

الموبايل:

الخلاصة:

بكتريا الزائفة الزنجارية وبكتريا المعوية البرازية هما بكتريا ممرضة وايضا مقاومة للعديد من المضادات الحيوية. الهدف الرئيسي للدراسة الحالية لغرض اختبار فعالية تداخل الفضة النانوية في تركيب مختلف المضادات الحيوية ضد بكتريا الزائفة الزنجارية وبكتريا المعوية البرازية. الدراسة الحالية لوحظ تأثير تداخل الفضة النانوية اقل فعالية من تأثير المضادات فقط. واتضح خلال النتائج ان المضادات الحيوية أكثر فعالية في تراجع نمو بكتريا الزائفة الزنجارية وبكتريا المعوية البرازية بعد عملية دمج الفضة النانوية والمضادات الحيوية. ان تأثير عملية التداخل ضرورية في تحسين تأثير المواد النانوية للفضة مع المضادات