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Article

EVALUATION OF ANTIBACTERIAL ACTIVITY OF cobalt (Co) NANOPARTICLES AGAINST ESCHERICHIA COLI

U. R. Hameed[1], and L. A . Yaaqoob[2]
Dept. Biot., Coll. Scie., University of Baghdad, Iraq
* *Correspondence: usama.rasheed1206a@sc.uobaghdad.edu.iq. laith.yaaqoob@sc.uobaghdad.edu.iq*
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ABSTRACT
It is the goal of this work to evaluate the utilization of Pseudomonas aeruginosa's pyocyanin pigment as a stabilizing and reducing agent for (Co) nanoparticles and their antibacterial effectiveness, multidrug-resistant Escherichia coli obtained from diverse clinical sources. Testing for antibiotic resistance revealed that E. coli isolates are resistant to Aztreonam, Nitrofuration, Ceftazidime, and Amoxicillin\Clavulanic.
Acid, Trimethopim Sulphamethoxazole, Tobramycin, Piperacillin, Meropenem, Gentamicin, Cefotaxime, and Ertapenem. However, they are susceptible to amikacin, Piperacillin\tazobactam, levofloxacin, and imipenem. Cobaltous chloride hexahydrate (5g) and pyocyanin (50 ml) are used to create cobalt nanoparticles (Co). Cobalt (Co) was characterized using various techniques, including XRD, AFM, UV-VIS, and FTIR.
Keywords: antibacterial action, E. coli, cobalt nanoparticles (Co) NPS.

INTRODUCTION

Nanotechnology studies tiny structures, typically between 1 and 100 nm in size that exhibit extraordinary properties compared to microscopic-sized particles [3]. The cobalt nanoparticles (Co) NPS have gained appeal because of their diverse properties, including electron transfer capabilities,supercapacitance, electrocatalysis, and exceptional chemical stability. Consequently, several physical and chemical techniques have been successfully employed to fabricate cobalt nanoparticles (Co) NPS [16]. High surface area (Co) is a family of nanostructured materials that have lately gained attention due to their intriguing features and a wide variety of applications in catalysis, biology, materials chemistry, and sensing.

The most straightforward method for creating nanoparticles is the green synthesis method. This environmentally friendly method uses plant extracts (leaves, flowers, seeds, and peels), bacteria, fungi, and enzymes to synthesize cobalt oxide nanoparticles rather than large quantities of chemicals [1].

The synthesis of cobalt nanoparticles (Co) NPS using bacteria and fungal extracts offers significant benefits over chemical and physical approaches due to its ease

of processing, low cost, and scalability for large-scale manufacturing. This procedure did not require high pressure, which was costly.

Machinery, high temperatures, or harmful chemicals. The pigment was found to be formed by gram-negative Pseudomonas aeruginosa. Pseudomonas aeruginosa [8]. The ability of Pseudomonas aeruginosa to produce the blue-green pigment pyocyanin is one of its defining characteristics. Pyocyanin is a member of the phenazine family. It seems to be heterocyclic compounds produced naturally but have side chains changed at various locations around their rings by various bacteria. [4] Pyocyanin is plentiful in the sputum of people with cystic fibrosis who are infected with P. aeruginosa and also has a role in the pathogen's pathogenicity. [20] Escherichia coli often colonizes the gastrointestinal tracts of newborn neonates within a few hours after birth. Typically, E. coli and its human host have existed in great health and mutual benefit for decades. [10] Additionally, some E. coli strains are the primary causes of sickness and death in infections linked to medical devices, such as urethral and other intravascular catheters, prosthetic joints, shunts, and prosthetic grafts.

[7] Biofilms, by enclosing organisms in an extracellular biochemical matrix, enable them to evade the host's immune response, increase their virulence, and contribute significantly to the development of antibacterial treatment resistance. [11] According to a study conducted by the National Health Institutes, biofilms are It is present in more than 80% of all bacterial illnesses. Biofilms have been associated with various medical conditions, including upper respiratory tract infections and urogenital diseases. For oral plaque infections, this work aimed to employ pure pyocyanin from Pseudomonas aeruginosa to biosynthesize cobalt (Co) nanoparticles as a reducing and stabilizing agent. Additionally, we will investigate the antimicrobial properties of the produced nanoparticles in vitro.

Against human pathogenicbacteria (E.coli).

MATERIALS AND METHODS

Between DECEMBER 15TH, 2021 and February 1st, 2022, bacterial isolates were obtained from patients suffering from urinary tract infections at hospitals (Al-Yarmouk Educational Hospital, Al Karama Educational Hospital, Mahmudiyah Hospital, City of Imamine Kadhimen). All isolates of bacteria were identified using standard biochemical techniques. The antibacterial activity and other properties of cobalt (Co) \ nanoparticles were examined using multidrug-resistant E. coli strains [17].

Pyocyanin pigment production

Each Sample of P. aeruginosa isolate was

collected is inoculated on LB broth media

[15] and incubated at 37˚C for 72 hrs.

Extraction of pyocyanin

Extraction of pyocyanin by autoclaving the broth solution for 15min at 120˚C, then centrifuging at 6000 rpm for 12 min, taking up the supinated that contains pyocyanin [14].

Synthesis of cobalt (Co) nanoparticles

This is the first study to use pyocyanin for the Biosynthesis of cobalt (Co) nanoparticles; the Process of Cobalt oxide was used to synthesize cobalt (Co) nanoparticles. The synthesis is done by dispersing(5g) of cobaltous chloride hexahydrate in 50 ccs of pyocyanin. In a flask, shake it overnight in a darkroom. The mixture was then centrifuged for 9 minutes at 4500 rpm. The precipitate of a solution containing all the cobalt (Co) nanoparticles was twice rinsed with deionized distilled water to remove any remaining pyocyanin pigment. The

precipitated nanoparticles were dried in an oven at 37°C for OVERNIGHT. Finally, the black powder was sealed in a dark container to prevent it from evaporating.

Characterization and uses.

Antibacterial activity of cobalt (Co) Nanoparticles

The agar well diffusion method assessed bacterial susceptibility to cobalt (Co) NPs. The Mueller Hinton medium was used in this test. E. coli cultivated overnight at 37°C. After incubation, the standard inoculum for each bacterial isolate at a concentration of 1.5 X 10^8 CFU / mL was formed according to the standard solution of 0.5 McFarland.

A small sterile swab was dipped inside the tube containing the suspension and subsequently inoculated on the Muller Hinton agar (MH) plate to cover the bacteria on the plate surface evenly. On MH agar plates, wells with a diameter of 6 mm were prepared aseptically, and 0.2mL of different concentrations(0.2,0.1,0.05,0.02,0.01,0.006,0.003) of cobalt (Co) NPs were distributed into separate wells followed by overnight incubation at 37°C. Following incubation, the widths of the bacterial susceptibility zones were measured and reported. A well containing only sterile distilled water was used as a negative control (18).

RESULTS

Bacterial isolation in culture media:

One hundred sixty samples of bacteria grown on MacConkey agar (Figure1), 110 lactose fermenting E.coli isolates (68.75%) appear in dry circular form, dark pink color. A dark pink region of precipitated bile salts always surrounds them. At the same time, 30 isolates of Klebsiella pneumonia (18.75%) appear as large mucus and dark pink colonies on MacConkey agar, and 20 isolates (12.5%) of pink or red growing colonies of Enterobacter spp. It could be said that E. coli is the major cause of UTIs in patients In Iraq. Similar results were mentioned when they isolated E.coli from the same source in a survey conducted, and Mohamed et al., 2017 reported that E.coli was the most common agent whose percentages (50.4%, 37.9%, 49%, 27.5%, 64.4%, 46.7%, 43.9%, and 47.4%).

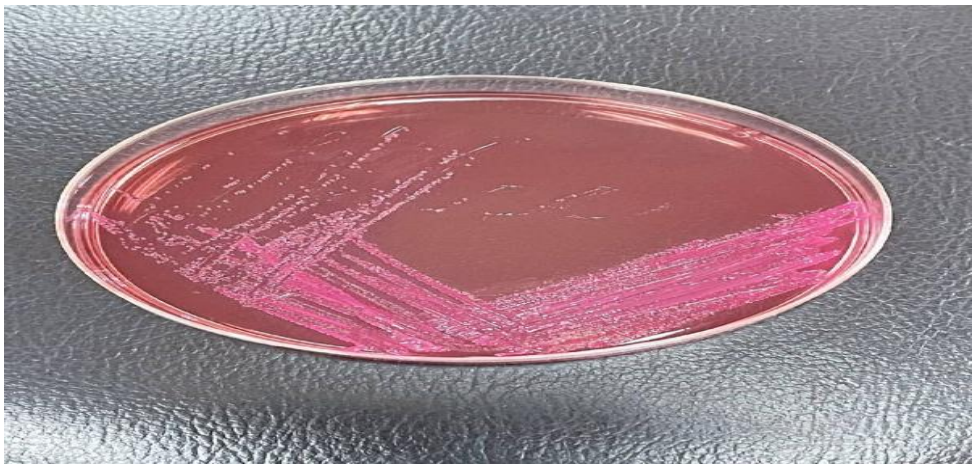

Figure 1. *E.coli* on MacConkey agar

Antibiotic Susceptibility Test of UPEC

All the isolates underwent the susceptibility test for fifteen antibiotics using the disc diffusion method recommended by the Clinical and Laboratory Standards Institute (CLSI, 2021) guidelines. The results are in (Table (. Showed varying levels of resistance

Antibiotic type	**Biofilm producer E. coli (n = 107)**		**Biofilm nonproducer E. coli (n = 33)**	
	Frequency	**%**	**Frequency**	**%**
Aztreonam	103	96.2%	30	90.9%
Nitrofurantion	18	16.8%	5	15.1%
Piperacillin\Tazobactam	13	12.1%	2	6.0%
ceftazidime	81	75.7%	17	51.5%
Amoxicillin\clavulanic acid	75	70%	17	51.5%
imipenem	73	68.2%	19	57.5%
Trimethopim\sulphamethoxazol	10	9.3%	3	9.0%
tobramycin	83	77.5%	29	87.8%
piperacillin	87	81.3%	27	81.8%
meropenem	76	71.0%	26	78.7%
Gentamicin	83	77.5%	29	87.8%
Levofloxacin	13	12.1%	2	6.0%
Cefotaxime	73	68.2%	19	57.5%
Ertapenem	87	81.3%	27	81.8%
Amikacin	10	9.3%	3	9.0%

Table 1. Antimicrobial resistance of biofilm-producing and nonproducing *E. coli*

Pyocyanin pigment extraction

The green pigment appeared on the surface

of the broth, and after shaking the flask, the green pigment was distributed in the broth

medium (Figure2).

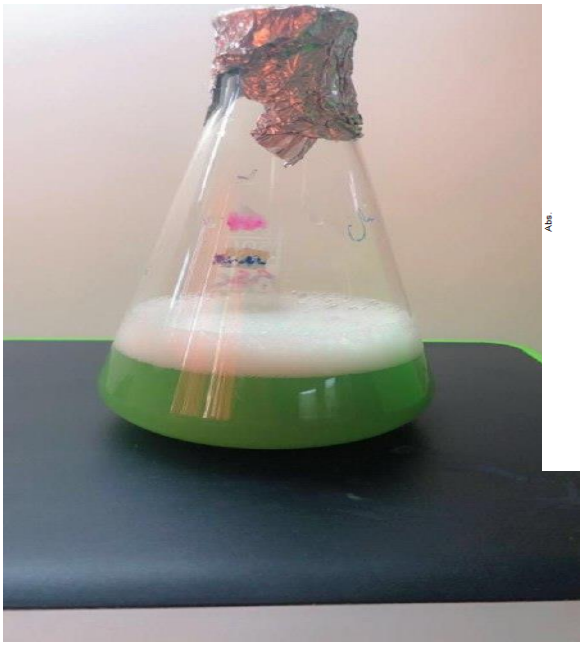

Figure 2. pyocyanin pigment

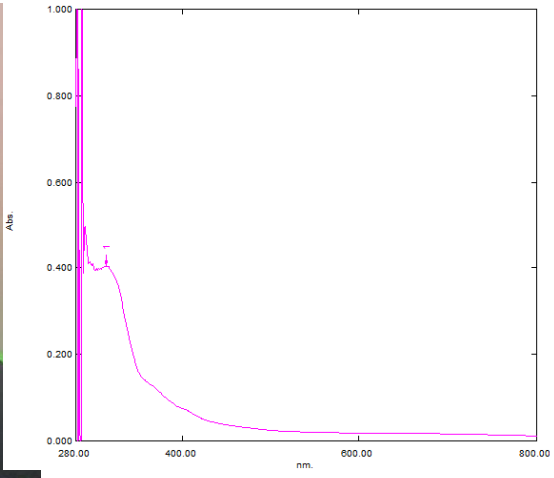

Figure 2.A. The UV-VIS of pyocyanin

UV–VIS spectral analysis of Cobalt (Co) nanoparticles

is characterized by scanning a UV-visible spectrophotometer (Shimadzu, Japan) in (Figure 3B) to detect the maximum absorption[23]. The result showed the absorbance of Cobalt (Co) nanoparticles at 200 nm

UV–VIS spectral analysis pyocyanin

The pyocyanin developed by pseudomonas aeruginosa is characterized by scanning a UV-visible spectrophotometer (Shimadzu, Japan) in (Figure) to detect the maximum absorption[23]; the result showed the absorbance of pyocyanin pigment at 315nm.

	Compound class of functional groups	**bonds**	**Frequency of Absorption(cm^{-1})**
Pyocyanin	**Alcohol and hydroxy compound** **Alcohol and hydroxy compound** **Alkenyl** **Amine** **Tertiary alcohol**	**o-H stretch** **o-H stretch** **C=C** **stretch** **N-H Bend** **O-H** **Bend**	**3429.2-3411.8** **3284.5** **1633.5** **1575.7** **1409.8**
(Co) NPs	**Heterocyclic amine** **Polymeric** **Carboxylic acid** **Alkenyl** **Alkenyl** **Vinyl**	**N-H stretch** **oH** **stretch** **C=C stretch** **C=C stretch** **C-H in-plane bend**	**3452.3-3425.3** **3280.6-3257.5** **1704.4-1685.6** **1637.4** **1552.5** **1411.8**

Table 2. Compound class of functional groups

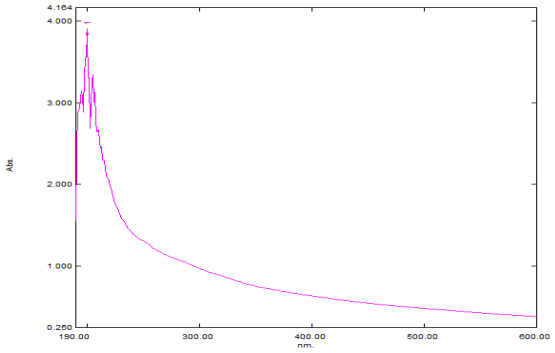

Figure 3. B. UV-VIS of Cobalt (Co) nanoparticles

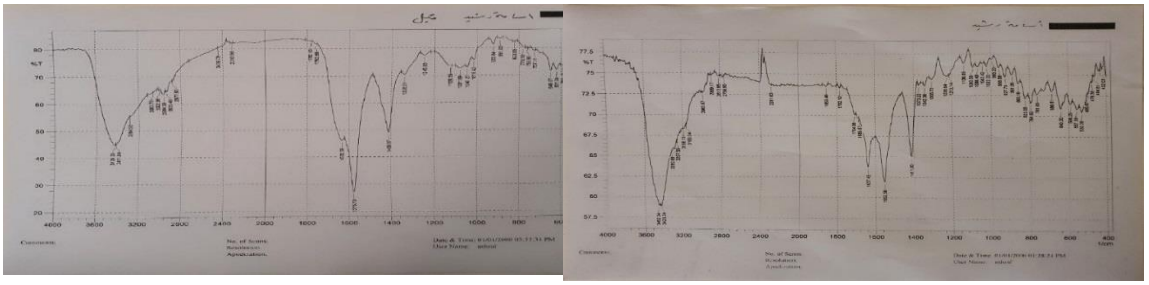

Figure 4. FTIR images of (Co) NPs synthesized using pyocyanin pigment, A: pyocyanin pigment. B: (Co) NPs

Atomic force microscopy (AFM):

The surface shape formation of the (Co) NPS was studied by atomic force microscopy to show that (Co) NPS 2D and 3D(22). (Figure 7). AFM images show that the biosynthesized (Co) NPS are spherical.

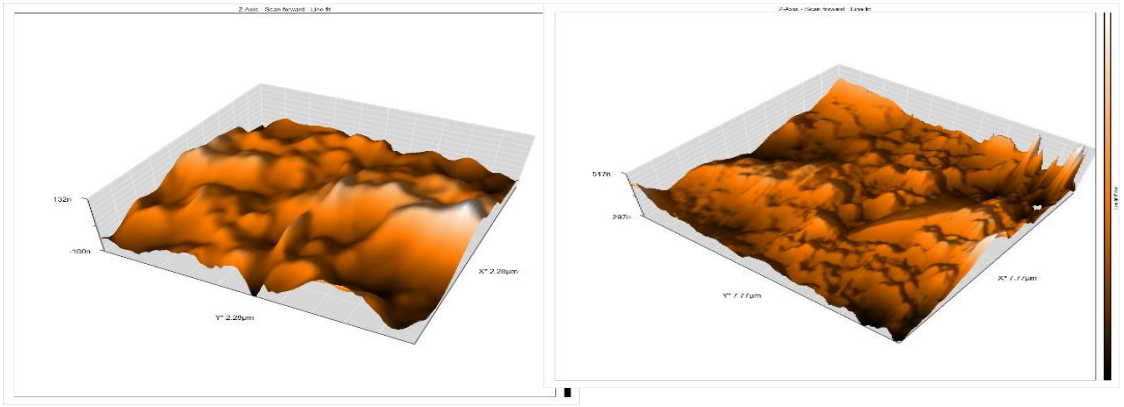

Figure 5. Atomic force microscopy (AFM) of cobalt (Co) NPs synthesized using pyocyanin illustrates

Antibacterial susceptibility test

This is the first study to use (Co) nanoparticles as an antibacterial for MDR- E. coli. The results of using (Co) nanoparticles as antibacterial agents were found to be directly dependent upon the (Co) nanoparticles concentration. (Table (shows that the maximum inhibition zones of E.coli were 37mm at a concentration of 0.2 mg/ml of (Co) nanoparticles.

Whereas the minimum inhibition zones were located at 0.006 mg/ml of (Co) nanoparticles concentrations, the inhibition zone depended on the concentration of (Co) nanoparticles. The main mechanism of (Co) nanoparticle toxicity is

potentially associated with metal oxides carrying a positive charge even though the microorganisms. The antimicrobial effect of (Co) nanoparticles against fungi and bacteria has been demonstrated and communicated in modern research, see (Figure10)

No	(Co-TiO2) con. (mg/ml)	Zone of diameter (mm)
1	0.2	37
2	0.1	34
3	0.05	29
4	0.025	27
5	0.012	20
6	0.006	17
7	0.003	No **inhibition zone**

Table 3. The inhibition zone of Antibacterial effect of (Co-TiO2) NPS on *E.coli*

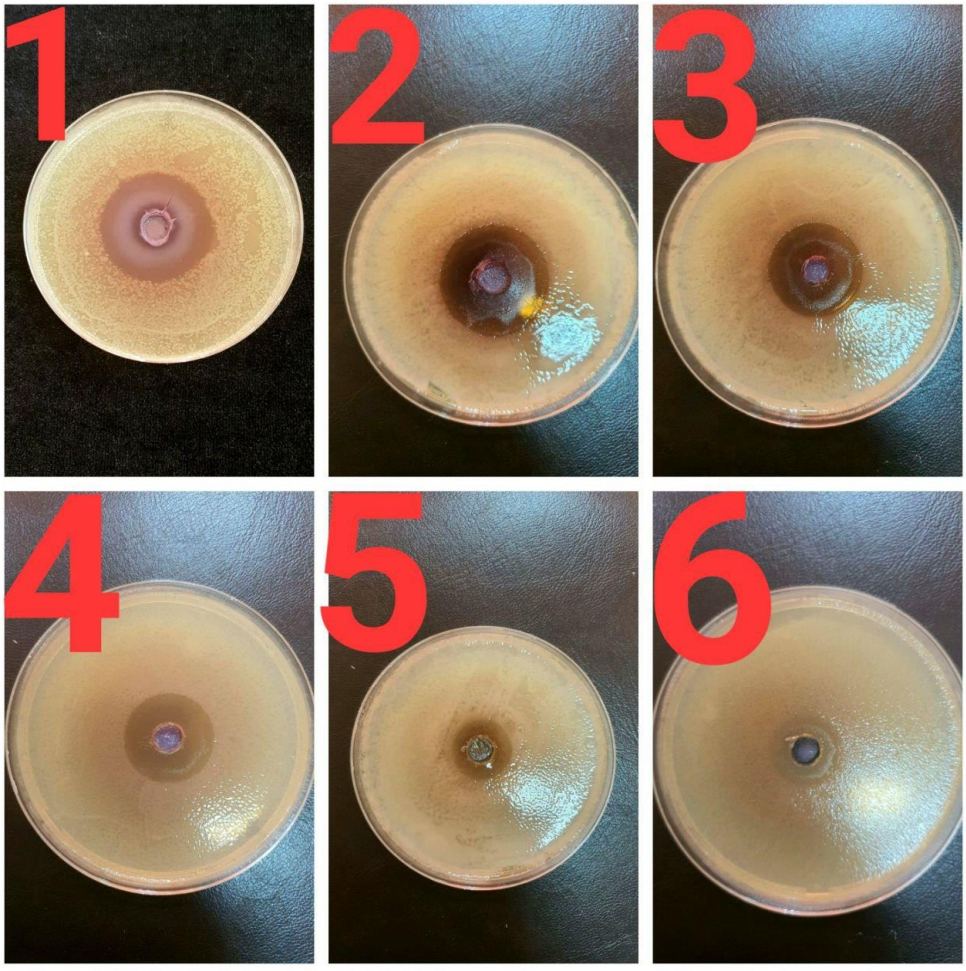

Figure 6. Antibacterial activity of (Co-TiO2) NPS on *E.coli*

DISCUSSION

The result showed the absorbance of pyocyanin pigment at 315nm and agreed with [13].

The size of an average diameter of 79.84 nm (Table 3) was also measured by AFM (Figure 8). This result agrees with [20]

Negative charges result in electromagnetic interaction between microbes and metal oxides, leading to oxidation and, finally, death of microbes [19]. The bactericidal action of (Co) nanoparticles on bacteria is of extreme importance due to the ability of pathogenic bacteria to join the body [5].

CONCLUSIONS

It was shown that the maximum inhibitory zones for E. coli were 37 millimeters in diameter based on the wavelength of the Cobalt (Co) at 200 nm, the average diameter at 79.84 nm, and the concentration of (Co) NPS in the solution at 0.2 mg/ml. Pyocyanin-coated (Co) nanoparticles have been demonstrated to be effective against E. coli.

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