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RESEARCH PAPER

Use of PCR Technology in Diagnosis of *Pseudomonas Aeruginosa* Isolated from Filters of Factory Health Water Production and Analysis of Antibacterial Activity of Iron Oxide Nanoparticles against Resistance Bacteria

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ABSTRACT

Most species of *Pseudomonas aeruginosa* can be detected in water treatment systems, hospital environments. The result of court study found four isolates of *P. aeruginosa* from 30 sample of health waterin Baghdad City. PCR result show four isolates were producing gyr B gene with amplified size 367 bp. Results of the FTIR analysis showed that Iron Oxide Nanoparticles had six bands at (3253, 2941, 1477, 1001, 685 and 587) cm^{-1}. AFM analysis shows a three-dimensional and two-dimensional image for Fe_3O_4 NPs with molecular clusters was up 15.60 nm and peaks 39. Results of SEM and TEM shows inclusion of numerous nanoparticles with spherical Fe_3O_4 nanostructures and size of particles 30 nm. Fe_3O_4 NPs resulted in 9 mm inhibition at a concentration of 10 mg/ml, which increased to 16 mm with a concentration of 15 mg/ml, respectively. The study aimed to detection of *Pseudomonas aeruginosa* from Filters of Health Water then used iron oxide nanoparticles against *Pseudomonas aeruginosa*.

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INTRODUCTION

Drinking water is essential for human survival and health, as it maintains body temperature, helps eliminate toxins and waste, and enhances the functions of all body systems. In addition, it is important in agriculture, industry, and maintaining ecosystems [1]. Water is one of the most important natural resources on Earth. It represents the primary means of sustaining life, as it is involved in many essential vital activities such as drinking, food, agriculture, and industry. Statistics indicate that water taken from natural sources [2]. As it is the primary resource for ensuring production and growth in these sectors, water is used in agriculture to irrigate plants and provide the necessary moisture, as well as in the food and beverage industry. In industry, water is used in various industrial processes, such as the metal, wood, paper, gasoline, and oil industries, in addition to generating electricity [3]. Water filters are important for producing clean water for many purposes; filters are used to produce clean drinking water [4]. without water filters, water used for everyday use drinking, bathing, etc. would be harmful to our health and the environment. Without water purification, water cannot be

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consumed because it contains harmful bacteria, which are often found in water contaminated with sewage, as well as dangerous metals such as lead, zinc, and cadmium [5- 6]. Magnetic nanoparticles have proven effective in isolating and purifying RNA from biological samples, a critical step in many biological and biomedical applications, these magnetic nanomaterial-based methods offer significant savings compared to the complex, traditional methods used in biological laboratories, controlling the preparation of nanoparticles in different ways will be an important factor in advancing bacterial DNA isolation, as nanomaterials derive their effectiveness from their small size and large surface area, and the interactions of biological molecules with surfaces are of great importance in biotechnology [7 - 8]. Polymerase chain reaction is a biological process or test-tube system for DNA replication that allows a "target" DNA sequence to be selectively amplified by several million folds within a few hours. PCR amplifies a predetermined DNA fragment that can range in length, for example, from (100- 1,000 bp) [9]. The study aimed to detection of *Pseudomonas aeruginosa* from Filters of Health Water in Baghdad city by PCR and the antagonistic effectiveness of iron nanoparticles against this bacterium.

MATERIALS AND METHODS

Samples collection

Thirty potable water samples were randomly collected from water filters in Baghdad city (fifteen samples from Karkh and fifteen samples from Rusafa) between Aprils to May 2025. The samples were stored in a sterile box, wrapped in paper, at refrigerator temperature to prepare for testing.

Preparation of Cetrimide agar Cultural Media

A type of selective media for isolating gram-negative bacteria *Pseudomonas aeruginosa*. As the name suggests, it contains cetrimide, which is a selective element for other commensal bacteria and enhances the production of dyes from the bacteria, such as pyocyanin and fluorescein, which appear blue-green or green-yellow, respectively. as mentioned [10].

Isolation of Pseudomonas aeruginosa

Pseudomonas aeruginosa was isolated from drinking water filter samples using membrane filtration. Water samples were filtered using cellulose nitrate filter papers, using a buchner funnel and under pressure using a vacuum device. 100 ml of each sample was filtered through the filter paper under sterile conditions. The filter paper was then transferred using sterile forceps to the surface of the sterile a caracetic acid selection medium poured into Petri dishes. The dishes were then incubated at 37°C, as the nutrients in the medium diffuse through the holes in the filter paper to reach the bacteria trapped on the filter paper. After 18-24 hours of incubation, Pseudomonas aeruginosa colonies are observed if present in the sample growing on the surface of the filter paper, which can be observed by the production of the blue-green pigment pyocin [11].

Diagnosis of Pseudomonas aeruginosa
Microscopic Characteristics

Microscopic examination described by [12] stained the colony of sample to see the surface and color.

Cultural Characteristics

The cultural characteristics of the bacterial isolates of *Pseudomonas aeruginosa* were studied by their ability to grow on Macconkey agar and blood agar, as well as on the selection medium, Cetrimide agar, and their ability to produce pyocyanin pigment. The colonies growing on the aforementioned media were selected in addition to the ability of the bacteria to β-hemolysis, and they were not inhibited on the selection medium, Cetrimide agar. [13].

Polymerase Chain Reaction analysis
DNA Extraction

The preceding means that genomic DNA was produced in accordance with [14]. Upon establishing 10 ml cultures from a single colony in broth media for 12 hours, cells were pelleted by rotating cells for 5 minutes at 6000 rpm. Pellets were then thirty mg/ml lyso, then they were nursed for 2 h at 37ºC. Cell protein was then destroyed for a single hour using TE buffer with Proteinase K (1 mg/ml). the whole thing was incubated for one hour at 37°C. After 30 minutes in a shaker with a proportionate amount of phenol/chloroform/ isoamyl alcohol (24/24/1) added, solution was centrifuged for 5 minutes at 6000 rpm. Before putting the supernatant into a clean microtube, 10% of the volume of ammonium acetate was added, as well as an identical bulk of unemotional, so that hurried DNA.

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Application of Polymerase Chain Reaction

The PCR was applied utilizing sets of primers. The gyr B gene, which amplifies a 367 bp sequence, was the target of the first set of primers, comprising the forward primer 5'AAGTACGAAGGCGGTCTGAA3' and the reverse primer 5'GTTGTTGGTGAAGCAGAGCA3'. Genes were specific to *P. aeruginosa*. One cycle at 95°C for a total of two minutes, thirty cycles of 92°C for sixty seconds, annealing temperatures are fifty nine °C for detect gene and seventy two °C for one minute, in conclusion this round at 72°C for eight minutes comprised the amplification program.

Preparation of (Fe_3O_4 NPs)

The synthesis described by [15]. pH variety through combination of Iron nanoparticles ought (8-11) by keeping molar proportion of Fe^{3+}/ Fe^{2+} (2:1). Temperature at the beginning of synthesis on the particle size of Fe_3O_4 NPs was investigated from 25 to 85°C. inspiring amount enlarged from 400 to 800 rpm, the energy transferred to the suspension medium is also increased, the reaction solution dispersed into smaller droplets, and the size was reduced.

Characterization of of Iron oxide nanoparticles

The following methods were adopted for its diagnosis, methods included: Fourier-transform infrared spectroscopy, The spectral rang of iron oxide nanoparticles were recorded from a wave number range of 400 to 4000 cm-1 [16]. Atomic Force Microscopy (AFM) [17] and Scanning Electron Microscopy (SEM).

Antibacterial activity of Iron oxide nanoparticles

This method was described by [18]. The inhibitory ability of Iron oxide nanoparticles against *Pseudomonas aeruginosa* was tested in culture plates using agar well diffusion methods,0.1 ml of the study strain's bacterial suspension was seeded into 20 milliliters of Mueller Hinton agar medium.

RESULTS AND DISCUSSION

Isolation of *Pseudomonas aeruginosa* bacteria depend on macroscopically and microscopically appearance, four bacterial isolates were obtained from a total of 30 samples from sanitary water filters, as shown in (Table 1).

Results of study was agreed with [19] regarding the isolation of *Pseudomonas aeruginosa* from

Table 1. Shown samples of water were obtained from Baghdad City.

N	Sample location	Number of samples	Number of isolates	Percentage of number of isolates
1	Karkh	15	3	75
2	Rusafa	15	1	25
	Total	30	4	100%

Fig. 1. Agarose gel electrophoresis of PCR products for *P. aeruginosa* isolates. Lane (M): (100 bp DNA Ladder). Lane N: Negative control. Lanes (1-4) of *P. aeruginosa* isolates positive gyr B gene results (amplified size 367 bp).

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drinking water in Nineveh Governorate, where 7 isolates of this bacterium were obtained from 900 drinking water samples.

Polymerase Chain Reaction has proven the ability to presence of gyr B gene, respectively, in *Pseudomonas aeruginosa*. . It was concluded that four *Pseudomonas aeruginosa* isolates, four isolates were producing gyr B gene with amplified size 367 bp. (Fig. 1).

This result was agreement with [19]. Who was detection *P. aeruginosa* 100% in all sample of water with gyr B gene. *P. aeruginosa* has a single polar flagellum that plays a key role in locomotion, as well as long thread-like structures called pili that extend from the outer surface of the bacterial cell. They include several types, but type IV pili are the most important, as they are primarily responsible for adhering bacteria to cell membranes and non-living surfaces, in addition to their role in locomotion, biofilm formation, antibiotic resistance, and the host's immune system.

In current study, synthesized using the chemical method. color of the mixture before addition of NaOH solution was yellow but after treatment, it showed a gradient color changing from a yellow to black suspended mixture upon heating at 80 C for 15 min. This indicated the formation of MIONPs. Color gradient resulted from the oscillation of the conduction band of electrons in nanoparticles. MNPs showed that the dark black color in the aqueous was due to collective fluctuation of free electrons present in the reduced magnetic iron oxide nanoparticles, color of nanoparticles was found to depend on the shape and size of the NPs and dielectric constant of the surrounding medium.

This study was in agreement with [20], Counter ions got further dragged in by the electric field generated through the surface charges. An electrified doubly covering featured a layer of opposing ions and contact forces.

Fig. 2 shows the FTIR spectrum of Fe_3O_4 NPs with absorption bands located in the region of 4000 cm^{-1} to 500 cm^{-1}, characterized by strong bands at (3253, 2941, 1477, 1001, 685 and 587) cm^{-1}.

The band at (3253 cm-1). represented C-H groups , band at 2941 cm^{-1} was assigned to OH stretching, while band at 1477 cm^{-1} was carbonyl indicating its interaction with nanoparticles. band at 1001 cm-1 indicated the stretching of (COO-). Band at 685 cm^{-1} and band at 587 cm^{-1} demonstrated (C-O) stretch. This result was agreement with [15, 16], who found nearer to those reported.

The surface morphology, and hence particles size distribution of synthesized Fe_3O_4 NPs prepared by co-precipitation obtained by using atomic force microscopic images. One drop of the colloidal suspension was deposited on glass substrate at 70°C. pictures indicated revealed iron oxide nanoparticles collaborated wisely to form distinct aggregates (Fig. 3).

Fig. 3 depicts a slice of the Fe_3O_4 NPs' surface with high molecular clusters up to 15.60 nm in both three and two degrees. There were rather many peaks in the average diameters and sizes of the Fe_3O_4 NP composite; the most significant peak was (37) from (7-91) nm.

Fig. 4 demonstrates the inclusion of numerous nanoparticles with spherical Fe_3O_4 nanostructures in the sample is clearly visible. The obtained sample had an average size of about 30 nm.

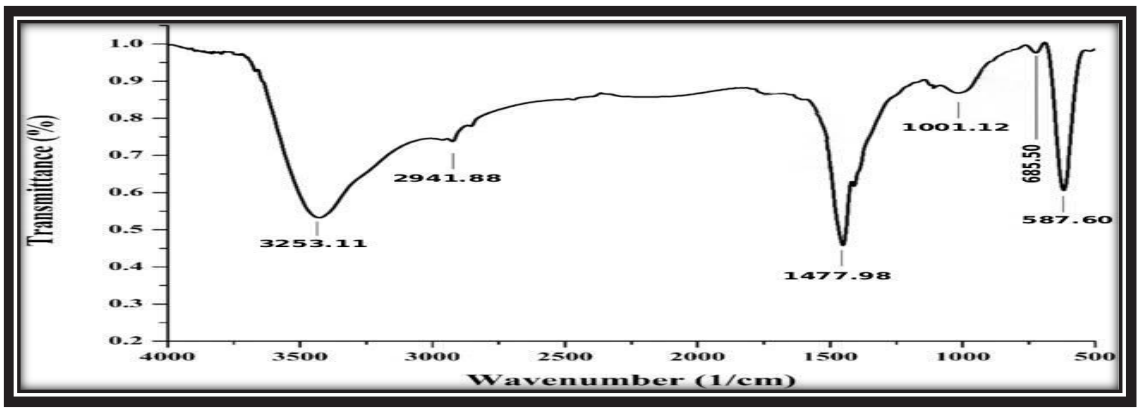

Fig. 2. FTIR analysis of Iron oxide nanoparticles.

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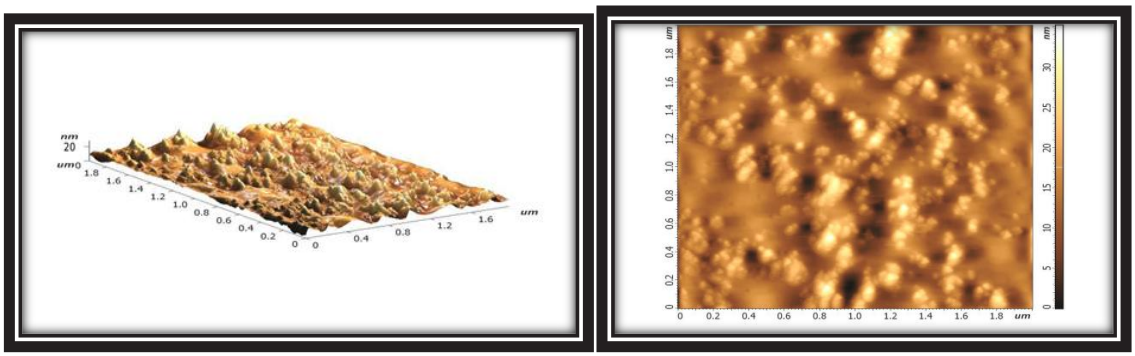

Fig. 3. AFM images: 3-Dimensional, 2-Dimensional Fe_3O_4 NPs.

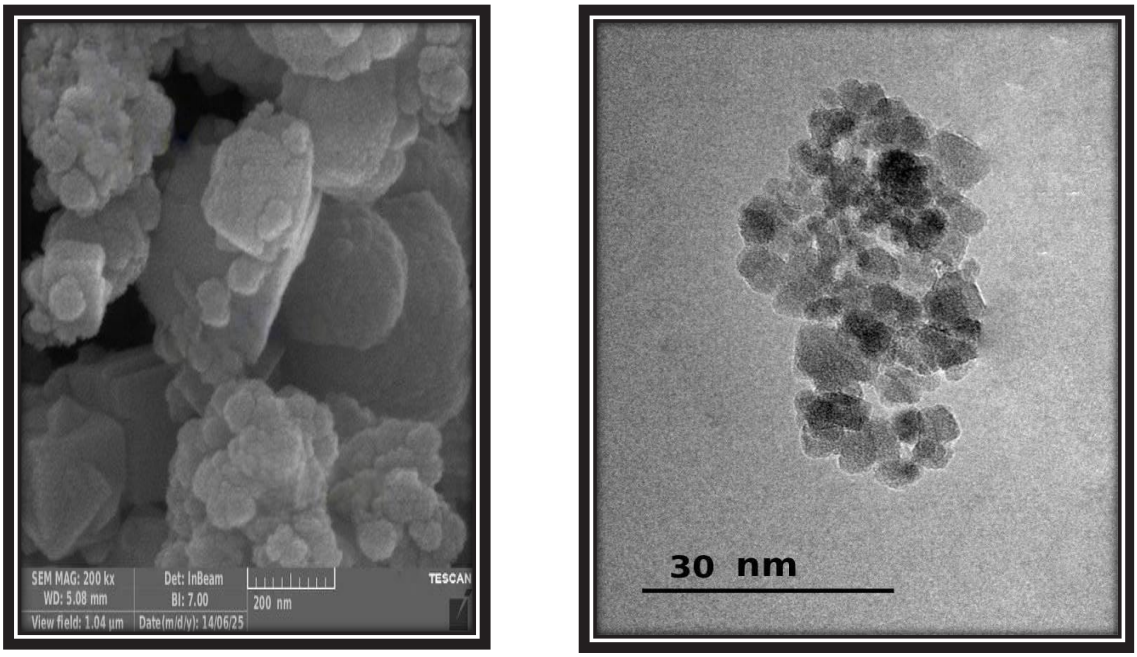

Fig. 4. A) SEM, B) TEM image of Iron Oxide Nanoparticles.

This demonstrated that it is possible to produce homogeneous magnetite nanoparticles with a rather acceptable size distribution. Here decreases coagulation of magnetite particle crystallites. It is in good agreement with previous research of [15]

Antibacterial activity of Fe_3O_4 NPs against Pseudomonas aeruginosa

The results of study showed varying inhibitory effectiveness in inhibiting *Pseudomonas aeruginosa* bacteria for Fe_3O_4 NPs at concentrations of 10 and 15 mg/ml in Mueller Hinton agar medium. resulted in 9 mm inhibition for strength of 10 mg/ml, increased to 16 mm with deliberation of 15 mg/ml, respectively, paralleled to the power treatment.

That result was agreement with [18,19] which found the main components responsible for the antimicrobial effect may interact specifically with the bacterial cell wall.

CONCLUSION

The detection of most species of is crucial for reducing pollution and protecting society and humans from the harmful effects of bacteria.

CONFLICT OF INTEREST

The authors declare that there is no conflict

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of interests regarding the publication of this manuscript.

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