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Evaluations Of Antibacterial Efficiency of $Nife_2o_4$ Nanoparticles Alone and in Combination With Some Antibiotics Against Multidrug Resistant Proteus Mirabilis

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ABSTRACT

Proteus mirabilis bacteria have a unique ability to contaminated wounds and lead to severe host tissue damage. The aimed of our research to evaluate the antibacterial efficacy of nickel ferrite nanoparticles alone and in mixed with antibiotic against multidrug-resistant Proteus mirabilis utilizing Kirby-Bauer and molecular methods. included collection of 50 burned wound sample from patients in Tikrit Teaching Hospital and external clinics. (from October 2018 to March 2019). results revealed that 40(80%) from samples were react positively for *Proteus mirabilis*. Bacterial isolates (*Proteus mirabilis*) Doxycycline hydrochloride, Penicillin, CO-Trimoxazole, Ciprofloxacin, Cephalosporin, and Penicillin resistance were found. results also revealed the (NiFe2O4) inhibits strongly growth of bacteria isolated at various mini-mum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) The MBC concentricity equal 256 while the MIC concentration was 128(mg.I-1) (mg.I-1) signifi-cant alterations recorded within DNA were observed before and after processing with nano-particles, according to the findings.

1. INTRODUCTION

Because of a few variables such as the nature of the consume harm itself, the patient's re-sistant bargained station, the patient's age, the scope of damages, and bottom of the con-sume in mixing pathogenic character like the type and number of creatures, protein also poi-son creation, colonization of the consume damage area, and foundational [8]. Wounds can be classified as coincidental, compulsive, or non-workable, Virus connection of pathogens to entering cells, and they multiply, colonize, and develop better positioned to produce harm effects to infected tissues, regardless of the concept of the injury [9]. Microorganisms ranging from bacteria to organisms and parasites can contaminate wounds [5]. B-hemolytic Strept. Strept. pyogenes and Staph. aureus are the two most common gram-positive organic organisms. Proteus species and Pseudomonas aeru-ginosa are gram negative high-impact bacilli. Enterobacter, Escherichia coli, and

Klebsiella species are among the facultative anaerobes. Candida species and molds (Aspergillus spe-cies) are parasitic living forms [20].

Proteus species belong to the Enterobacteriaceae. There are many species of Proteus like Proteus mirabilis, P. vulgaris, P. penneri, P. hauseri, P. myxofaciens, P.alimentorum, P. cibarius, P. columbae, P. inconstans,P. morganii, P. terrae, and P. rettgeri [23].

P.mirabilis is reactive negatively to gram stain , it had rod-shaped, facultative anaerobic, non-capsule production, spore production , can be motile bacterium. It is most often seen under normal conditions and is responsible for tainting of the respiratory system, gastrointestinal tract, derm, ophthalmic, ears, nose, and renal system, as well as digestive. P. mirabilis is a 3ed common cause of hospitalization infections it is about 90% of infections [4].

The ever-increasing antibiotic resistance of P. mirabilis is a major concern in injury infections. P. mirabilis developed a resistant to beta lactam after acquiring heterologous -lactamase genes (In the writing, a multidrug-safe P. mirabilis clone with chromosomal Amp C-type beta-lactamase was accounted for. P. mirabilis might be non-sensitive to a wide type of -lactam antibiotics, in-cluding penicillins and cephalosporins. Proteus sppdestructiveness.'s is based on a variety of damaging components that are organized by a few characteristics encoded in operons.P. mira-bilis eats the urease complex, which were imported in pathophysiology of urolithiasis [22].

2. Material and Methods

2.1 Nickel Ferrite Nano particles

NFNPS was gifted by faculty of Science's, Chemistry branches and it was prepared using the Sol-Gel Auto Combustion Method [25].

2.2 Samples Collection

The samples were collected from fifty patients who had burn wounds that were contami-nated with hygiene swabs and transferred for testing in autoclaved BHI broth that was grow-ing at 37C0 for at least one to tow days to increase the chance of isolation [17].

2.3 Isolation and Identification of P. mirabilis

Specimens were cultured in BHI broth for 24 hours before being transferred to (Mac-Conkey agar) and Proteus media. To obtain new colonies for Gram staining and biochemical testing, all isolates were re-cultured twice (Indole, MR-VP, Simmon Citrate, Oxidase Test, and Catalase reaction).

2.4 Bacterial identification Using The Biomerieux Vitek® 2 System

After the first biochemical tests have identified bacterial isolates, this method is utilized to identify them. The VITEK® 2 system was utilized as directed by the manufacturer, and ID-Gram negative bacilli cards (GN ID Card; bio Mérieuxx) were employed for typing. The GN ID card is a 64-well with 18 hollow wells for fluorescent and restraint tests and 46 wells for fluo-rescent and inhibitory tests [16].

2.5 Antimicrobial sensitivity test

According to (CLSI) standards, the Kirby-Bauer technique is used to evaluate antibiotic sensitivity of isolated pathogenic bacteria. The standard inoculum was made by reducing overnight bacterial growth with hygienic Mueller-Hinton broth until the turbidity was com-parable to the 0.5 which is equal (1.5 x 108 CFU / ml)

depending on McFarland standards tube, then sample over Mueller-Hinton agar with a curved glass rod then dehydrated for around 30 minutes. Antibiotic discs located on surface of media with sterile forceps and in-cubated for 18–20 hours at 37°C. Following that, inhibitory zone diameters were measured and compared using CLSI-accepted standard criteria.

2.6 Nanoparticles solution

Nanoparticle suspension is a process in which nanoparticles are suspended in a liquid.

2.7 Antibiotics / nanoparticles solution mixture

A one µg from each antibiotics:1µg Nano setting up with the last focuses: 2, 4, 8, 16, 32, 64 ,128, 256, 512 and1024 µg/ml. The final fixations were:2,4,8,16,32,64,128,256,512, and 1024 g/ml, which were prepared by dissolving nanoparticles in Sterilize distal Water using vortex.

2.7.1 Determination of MIC

P. mirabilis was inoculated into cerebrum heart combination stock (last focus 1.5X108 CFU/ml) with nanoparticles fixing2,4,8,16,32,64,128,256,256,512, and 1024 g/ml, then brooded at 37oC for 24 hours. The MIC is the smallest focus tube in which development is not visible [1].

Molecular characterization of Proteus mirabilis:

· The DNA of Proteus mirabilis was secluded by Gentra puregene Bact. /unit. Adhering to the producer's directions as follows:

· Lysogeny stock (LB) stock (5ml) was immunized with single settlement of Proteus mira-bilis and hatched for the time being at 37°C.

· A volume of 500µl of the way of life (containing 0.5-1.5x108 cells contrasted McFarland tube) was moved with a 1.5 ml miniature axis tube on ice centrifuged for 5 sec.at 8000 rpm, the suspension was squander.

· An aliquot of 300µl cell lysis arrangement was added to the pellet, and blended by pipet-ting all over. The example was hatched at 60°C for 5 min., 1.5µl RNase fluid was added, and malediction by switching multiple times, the example arrangement was brooding for 30 mintes. at 37°C., at that point the example was hatched for 15 minutes. on stick to rap-idly cool the example.

· Protein precipitation arrangement (100µl) was added to the example arrangement and vortexed enthusiastically for 20 sec. at rapid. Centrifuged for 3 min. at 8000 rpm. The pel-let was discarded (DNA was encouraged by adding 300µl of isopropanol to the superna-tant from the past advance, which was blend by turning around delicately multiple times. Centrifugation for 1min. at 8000 rpm. The supernatant was dismissed and channel the cylinder by switching on a perfect part of retentive paper.

· An aliquot of 300µl of 70% ethanol was extra to the pellet and the substance was turned around various occasions to flush the DNA pellet, and centrifuged for 1min. at 8000 rpm. The supernatant was disposed of, and channel the cylinder on a spotless piece of spongy paper, a volume of 50µl DNA hydration arrangement was added and vortexed for 5 sec. at medium speed, it was put away at - 20°C for longer time-frames [28].

2.8 Random Amplified Polymorphic DNA (RAPD

Three business 10-mer preliminaries (BIONEER Company, Korea) were used in the PCR reac-tion, with the specific 5'-3' groupings: (OPH-14)3 ACCAGGTTGG 5 ACCAGGTTGG 5 AC-CAGGTTGG 5 ACCAG (OPO-11)3-GACAGGAGGT-GACAGGAGGT-GACAGGAGGT-GACAGGAGGT-GACA (OPP-4)3-GTGTCTCAGG-GTGTGTCTCAGG-GTGTGTCTCAGG-GTGTGTCT The total re-sponse volume was 25 liters, with 2 liters of layout DNA and 47.5 million groundworks. The following PCR protocol was used:

94°C for 5 m., then 30 cycles of 94°C for 45 sec., 55°C for 1 min. and 72°C for 1 min., then 72°C for 5 min. On a 1.5 percent agarose gel electrophoresis, PCR products were seen [18].

3. Results and discussion

3.1 Isolation and identification

At Tikrit Teaching Hospital, 50 burn wound specimens were collected in total. P. mirabilis was found in 40(80%) of the samples, whereas 10(20%) were negative. Swab samples were grown on MacConkey culture dish and proteus media during 18-24 hours and then incubated at 37 degrees Celsius. Then, using morphological and biochemical testing, select a colony that is can not -fermentative of lactose sugar in MacConkey media. Gram stain, oxidase, both negative and citrate negative / positive, urea hydrolysis and catalase both give positive results, indole synthesis, and voges proskaeur negative, at last methyl red reaction were positive. VITEK-2 sys. used to confirm the laboratory diagnosis of P. mirabilis. In immune-compromised individuals, P. mirabilis can be a source of both community and hospital-acquired contaminations. According to a research conducted by (Brown, 1999), Proteus is the third most common causative in hospital-acquired infections and a big contributory to wound contamination etiology. In this investigation, 480 different damage dermis samples were examined, with 13.3% of them testing positive for P. mirabilis and 86.6 percent testing negative. ill men wounds (8.3% of positive samples) were more infected with P. mirabilis than sick women injuries (5 percent). [2], P. mirabilis had a greater impact on men than on women., according to their findings. P. mirabilis was found to affect people of all ages in this study, with the most common age groups being 16 to 30 years (6.70 percent), 5 to 10 years (5 percent), and lastly 30 to 50 years (1.60 percent).

3.2 Antibiotic Susceptibility Test

Multi antibiotic were used to test the sensitivity of bacterial isolates to five antibiotics. As demonstrated in Table, all Proteus mirabilis isolates were resistant to all antibiotics tested (1).

Table (1) Antibiotic Susceptibility Test

bacterial isolate	Doxycycline hydrochloride	CO- Trimoxazole	Ciprofloxacin	Cephalosporin	Penicillin,
P. mirabilis	**Resistant**	**Resistant**	**Resistant**	**Resistant**	**Resistant**

Results of investigation showed, that all of the colony were MDR, which is somewhat higher compared to previous study's result [20]. They discovered that 82 per-cent of isolates were MDR. In addition, [26] discovered 100% MDR isolates in burns victims. Antibiotic sensitivity studies revealed that Proteus species are re-sistant to a wide variety of drugs.

This might be due to the presence of an additional protoplasm membrane that surrounds a lipid dual layer, lipo-proteins, poly, and LPS, as well as antibiotic abuse and misuse. Since this antibiotics susceptibility form of each type range has resulted in the selection of resistant mutants or the transferring of resistance factor from other enterobacteriaceae individuals, infection should be led by the sensitivity outcome [27].

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3.3 MIC and MBC of Nanoparticle Solution ($Nife_2o_4$) For P. mirabilis

The results revealed that the effective concentrations of nanoparticles (NFNPS) that in-hibited the rate of pathogens growth were MIC (128g/mill) and MBC (256g/ml) to P. mar-bilis, respectively. Table (2).

Table (2) showed the Effect of MIC and MBC for (NFNPS) on P. marbilis

Bacterial isolates	nanoparticle	con. (µg/ml)											
	NFNPS	1024	512	265	128	64	32	16 11 6	8 8	4	2	11	0
P .Mirabilis	MIC	-	-	-	-	+	+	+	+	+	+	+	
	MBC	-	-		+	+	+	+	+	+	+	+	

MIC=No growth (-) and turbidity (+), (0) Control (Muller-Hinton Broth), MBC=growth (+) and sterile(-).
Active efflux pumps, chromosomal and inducible beta-lactamases, plasmid-mediated ESBLs, and altered permeability are all part of the drug resistance pathway in P. mirabilis [15]. Nicles ions infiltrate bacterial cells, denature ribosomes, and disrupt the expression of enzymes and proteins required for ATP production, causing cell problems, ac-cording to work on the method of ability of Ag nano particles. Nickel can also inhibit DNA re-laxation by adhering to it, stopping bacteria from duplicating. The dissipation of proton the driving power is also a result of targeting the bacterial membrane [13].

3.4 DNA Isolation

As indicated in the image, DNA was extracted from resistant P. mirabilis and transplanted onto an agarose gel (1).

At 260 and 280 nanometers of wave length in UV absorption by using the (Nanodrop) was used to assess the amount and purity of DNA. the quantity of DNA reproducer was adequate to accomplish interactions (RAPD-PCR) with purity 1.69 to 1.6 and a range of 25-50 ng per microliter used.

3.5 Results of RAPD-PCR interactions

These findings were achieved after many experimental studies to arrive at 's significance circumstances in which the developmental process were regulated (dNTPs, Taq DNA poly-merase, MgCl2, DNA concentration as well as the intensity of same primers, thermocycler system appropriateness, and dropper decree used), arising in products that were two times clear and replicable. As a result, the RAPD associations for each user's genes were duplicated. The nanoparticles have a noticeable influence on the bacteria, and there are significant differences in the various primers. Some have resulted in full mutagenization of the gene, while others have resulted in the plurality current mutations in aspect and disappearance of specific bands, as well as variations in band positions. In the P. mirbilis 2nd and 3rd primers, note the full aspect of the gene and the variety in the size of the bands. Nanoparticles can potentially boost the effectiveness of antimicrobial medicines by fully obliterating the gene or inducing mutations, Deletions, substitutions, and additions that act in a change in the order of rules complementary to the initiation series are known as mutations. as shown in

the fig-ure (2, 3).

The consequences of the current examination revealed the impact of nanoparticles and its ability to repress development and slaughtering of anti-infection safe pathogens con-fines. The collection data of investigation Agree with [3], Ni nanoparticles in explicit have affirmed wide-range antibacterial products against both G+ and G - microscopic organisms. e.g Staph. aureus, E. coli, Proteus mirabilis and Pseudomonas aeruginosa. The antibacterial hardware demonstration of NPs is by and large characterized as holding fast to one of three models: oxidative pressure commencement, metal particle opportunity, or non-oxidative machines. These three sorts of instruments can happen all the while. Certain examinations have suggested that NiNPs brief equilibrium at the surface electric charge of the bacterial film and change its penetrability, at last prompting bacterial demise. Additionally, the age of (ROS) forestalls the cancer prevention agent guard framework and makes mechanical damage the cell film. As per existing examination, the headliners fundamental the antibacterial possessions of NPs are as per the following: a- production of bacterial cell film; b- duration of ROS production; c- dissemination of bacterial layer; and d- over-view of intracellular antibacterial impacts, incorporating associations with DNA and proteins. e- the ability of nickel ferrates to incite holes and pits which makes the layer of the bacterium partition. the technique results Great obstruction level of anti microbials by Gram neg. bacilli and Gram pos. cocci secluded might be because of boundless medication safe micro-organisms or self-medicine [11].

The system by which the nanoparticles infiltrates into microorganisms isn't seen totally, yet examines suggest that when P. mirbilis was treated with nickel, adjustments occurred in its layer shape and delivered a critical expansion in its penetrability influencing appropriate vehicle through the plasma film, leaving the bacterial cells unfit of appropriately controlling vehicle through the plasma layer, coming about into cell passing. It was seen that Nickel nano-particles have entered inside the microscopic organisms and accepted to have caused hurt by interfacing with phosphorous and sulfur containing mixtures, for example, DNA [24]. Nickel will in general have an incredible proclivity to response with such mixtures. The possible component of activity of Ni NPs is viewed as that DNA may have missing its du-plication capacity and cell proteins become a latent after treatment. Another explanation would be the release of Ni particles from nanoparticles, which will have an additional association to the bactericidal viability of Nickel nanoparticles. contemplated the antibacterial activity of Ni nanoparticles in mix with various antimicrobials "antibiotic medication and ciprofloxacin erythromycin and chloramphenicol. against Gram positive and Gram-negative microscopic organisms "Streptococcus pneumoniae Staphyloccus aureus, Shigella. flexneri and proteus mirabilis" with repressed of bacterial biofilm act about 65%.

A report by [25] hello discovered powerful antimicrobial activity of NINPs in combination with streptomycin and kanamycin, oxytetracycline against Staph. aureus, Esch-erichia coli proteus mirabilis range between 80–90 % restraint of bacterial biofilm product. In this examination, NiNPs explained amazing antibacterial activity in front of multidrug-safe Gram-negative and Gram positive bacilli disengaged from a medical procedure wound contaminations, outcomes are reliable plus various of research have archived antibacterial activities of NiNPs to both types of bacterial according to stain with gram with close results of MIC values.

The synergistic enemy of bacterial and hostile to biofilm activity among NiNPs and assigned anti-toxins may be attributed to various hardware of activity of antimicrobials and NiNPs impact atomic targets not the same as chosen treatment and it including 1) NiNPs enter microbial cell, denaturation ribosomes and overwhelm outflow of chemicals and amino acid essential to ATP creation, consequently prompting cell problem. 2) NiNPs has likewise the ca-pacity to stay away from DNA unwinding by restricting to them, subsequently restraining the reiteration of microorganisms. 3) Targeting the bacterial film additionally prompts scattering

of proton rationale power [21].

In light of the analyzed papers, it is reasonable to conclude that nanocomposites containing dressings are promising and a wonderful restorative choice in damage repair. Higher recovery rates, wound withdrawal reduction, hemostatic effect, antibacterial activity, and brief cytotoxicity were among the information explained or possibly certain in research [21]. Despite the fact that this exact audit established that nanoparticle-based yields have explained benefits at damage therapy, there has no examination on people, indicating the presence of fresh research for done in clinically with safe way. When treated with nanoparticles, multiple variables seen on anticipated bacterial disengaged, such the emergence and disappearance of DNA and its different quantities. The dam-age of mice with Proteus marbilis anti-toxin blockage arose after about five days, with symp-toms including warmth, rabor, and expansion of dermis, as well as appearance with yellow-ish and greenish colors purulent discharges from the damaged site. The hour of recovery for contaminated mice treated with nanoparticles and anti-infection medicines combined was exceeded hour of healings for mice received nanoparticles alone.

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