Isolation of Heavy Metal Resistant *Pseudomonas spp.* from Hospital Sewage Water in Mosul City

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Abstract

This study was designed to isolate heavy metal resistant *Pseudomonas* from different hospital sewage waste water in Mosul city; sixty isolates were collected as of their ability to grow in the presence of mercury, lead and nickel. Isolates were identified .Results revealed that they were belong to *Pseudomonas* , Six isolates were selected for further investigation. Growth rate at different temperature and PH, antibiotic sensitivity and minimum Inhibition concentration for each heavy metal was studied Results Showed that optimal growth rate was at 30° C and PH 7 . Isolate were resist to Penicillin, Cefuroxim, Tetracycline and Chloromphenicol,. Also result exhibited that MIC for mercury, lead and nickel. Were (50,100 for and 250-300 µg/ml) respectively.

Introduction

Soil and Waste water pollution by heavy metal is a significant environmental problem [1]. Wastewaters from the industries and sewage sludge applications have permanent toxic effects to human and the environment [2]. Metals play a vital role in the metabolic processes of the biotechnology. Some of the heavy metals are essential and are required by the organisms as micro nutrients (cobalt, chromium, nickel, iron manganese and zinc etc.) are known as 'trace elements' [3]. They are involved in redox processes, in order to stabilize molecules through electrostatic interactions, as catalysts in enzymatic reactions, and regulating the osmotic balance [4],[5]. On the other hand some other heavy metals have no biological role and are detrimental to the organisms even at very low concentration (cadmium, mercury, lead etc.). However, at high levels both of the essential and non essential metals become toxic to the organisms. These heavy metals influence the microbial population by affecting their growth, morphology, biochemical activities and ultimately resulting in decreased biomass and diversity [6].

Heavy metals can damage the cell membranes, alter enzymes specificity, disrupt cellular functions and damage the structure of the DNA. Toxicity of these heavy metals occurs through the displacement of essential metals from their native binding sites or through ligand interactions [3]. Also, toxicity can occur as a result of alterations in the conformational structure of the nucleic acids and proteins and interference with oxidative phosphorylation and osmotic balance [7],[3].

Lead (Pb) a major pollutant that is found in soil, water and air is a hazardous waste and is highly toxic to human, animals, plants and microbes [8].

Nickel (Ni) is the 24th most abundant element in the earth crust and has been detected indifferent media in all parts of the biosphere. Ni is classified as the borderline metal ion because it has both soft and hard metal properties and can bind to sulfur, nitrogen and oxygen groups [9]. Ni has been implicated as an embryo toxin and teratogen [10]. Mercury is one of the most toxic metals in the environment. It has been released into environment in substantial quantities through natural events and anthropogenic activities [11]. Genetic diversity can identify individual organisms from some unique part of their DNA or RNA providing definitive information on its biodiversity [12]. Resent development in nucleic acid technology such as the polymerase chain reaction (PCR), and analysis of 16SrRNA [13] has resulted in new methods that can be used for genotype analysis of bacteria.

This study aimed to isolate and characterization of *Pseudomonas spp* resistant bacteria to heavy metals from hospital waste water

Material and Methods

Isolation of bacteria

The sewage water samples were collected from hospital waste water. The samples were collected in sterile glass container and transport to laboratory for bacteriological analysis. The bacterial isolates were screened on nutrient agar with 0.3% cetriamid supplemented with 5 mg/l concentration of each metal (Hg^{+2}, Pb, Ni) one time by the standard pour plate method. Plates were incubated at 30ºC for 2 days and distant colonies of pseudomonas spp. were sub culture and obtain them in form of pure culture.

Identification and characterization of the bacteria

The distant colonies were sub cultured and obtain in form of pure culture and identified on the basis of their morphology and biochemical characters. Selected sewage isolates were grown on nutrient agar with 0.3% cetriamid and MacConkey agar media (Hi Media). The shape and color of the colonies were examined under the microscope after Gram staining. Isolates were bio chemically analyzed for the activities of Oxidase, Catalase test Urease test , gelatin hydrolysis, motility, indole production and citrate utilization .The isolates were identify according to Bergey 's Manual of Systematic Bacteriology [14].

Determination of optimal growth conditions

The optimal growth conditions with reference to pH and temperature were determined. The isolates were grown in Nutrient Broth medium with different pH

values (5, 6, 7, 8, and 9) and incubation was carried out at temperature (25 °C, 30 ° C, 37 ° C and 42 ° C). The optical density of the log phase growing cultures (8-10h) condition was noted at 600 nm to determine the growth rate.

Determination of MIC

MIC of the heavy metal resistant Pseudomonas *spp* was determined by gradually increasing the concentration of the heavy metal 50 µg/ml each time on nutrient agar plate until the strains failed to give colonies on the plate. The starting concentration used was 50µg/ml. The culture growing on the last concentration was transferred to the higher concentration by streaking on the plate. MIC was noted when the isolates failed to grow on plates [15].

Determination of antibiotic resistance

Antibiotic sensitivity and resistance of the heavy metal resistant isolates were assayed according to the Kirby-Bauer disc diffusion method. After incubation, the organisms were classified as sensitive or resistant to an antibiotic according to the diameter of inhibition zone given in standard antibiotic disc chart.[16].

Results & discussion:

Isolation & characterization of heavy metal resistant *pseudomonas spp*.

In this study we identify and characterized heavy metal resistant bacteria isolated from hospital waste water. Sixty colonies were sub cultured on heavy metal nutrient Agar medium supplemented with 0.3% cetriamid to obtain in pure culture. The percentage of the *Pseudomonas spp.* heavy metal resistant bacteria compared with control culture (heavy metal free cetriamid medium) were counted as shown in Table(1) the result reviled that highest resistance isolate to Ni ione was(71.28). Finally six isolates were selected based on high degree of heavy metals were used for further studies. Result in table (2) Showed that was multiple resistant to more one heavy metal.

Table (1) Percentage of bacterial heavy metal resistance in waste water.

No. of sample	% Resist to Hg	% Resist to Ni	% Resist to Pb	% Resist to Mixed (Hg Ni, bp)
9	25.64	71.28	12.8	28.2
16	24	34	16	20
23	0	2.71	1.08	3.37
35	0	3.5	0	3.57
47	15	16.6	10	0
53	11.5	0	3.8	15.3

*Percentage Calculated as= [No. of cfu's grow on heavy metal /No. of cfu's grow on control media] x100%. [17].

Table(2) Resistant of *Pseudomonas* isolates to heavy metal

Name of bacteria	Resistant to
Ps1	Hg, Pb, Ni
Ps2	Pb
Ps3	Hg
Ps4	Hg. Pb. Ni
Ps5	Ni
Ps6	Hg, Pb, Ni

All isolates were Gram-negative, rod shaped motile bacteria. The sewage isolates showed optimum growth at 30°C and pH 7.0. The biochemical characteristics of sewage bacteria and growth rate were shown in table (3) .

Table (3) Some Biochemical tests for heavy metal resistant *Pseudomonas* Isolates

Biochemical tests & Growth Rate	Result
Oxidize test	Positive(+ve)
Catalase test	Positive(+ve)
Urease Test	Positive(+ve)
Gelatin Hydrolysis	Positive(+ve)
Motility	Positive(+ve)
Indol Production	Negative(-ve)
Citrate Utilization	Positive(+ve)
Growth Rate °C	
25	50%
30	100%
37	75%
42	0

MIC of heavy metal and antibiotic sensitivity

All isolate s of *Pseudomonas spp*. showed very high degree of resistance to all heavy metalsand MIC values varying from one isolate to another as shown in table(4). Among the heavy metals nickel was less toxic, where as mercury and lead were highly toxic to

all isolate studied .Results revealed that isolates were resistance to some antibiotics and sensitive to another

Table (4) : MIC and antibiotic sensitivity.

Heavy metal ions	MIC
Hg	50 μg/ml
Pb	100 μg/ml
Ni	250-300 μg/ml
Antibiotic(Oxoid)	resistance
Ampicillin 100mg	Sensitive
Penicillin 10mg	Resistance
Pipracillin 50mg	Sensitive
Imipenen 50mg	Sensitive
Ticracillin10mg	Sensitive
Amikicin 30mg	Sensitive
Ciprofloxacin10mg	Sensitive
Streptomycin10mg	Sensitive
Cefuroxime 30mg	Resistance
Tetracycline 100mg	Resistance
Chloramphenicol 30mg	Resistance

The wastewater coming from hospital and industrial sources is the appropriate environment where the microorganisms can develop resistance to heavy metals and antibiotics. The presence of small amount of antibiotics and heavy metals in the wastewater induce the emergence of antibiotic and heavy metal resistant microorganisms. Some the isolates in the present study showed multiple tolerances to both heavy metals and antibiotics like (ps1,ps4 and ps6). The microbial resistance to heavy metal is attributed to a variety of detoxifying mechanism developed by resistant microorganisms such as complexation by exo polysaccharides, binding with bacterial cell envelopes, metal reduction, metal efflux etc. These mechanisms are sometime encoded in plasmid genes facilitating the transfer of toxic metal resistance from one cell to another. Filali [l8] studied wastewater heavy metal and antibiotic resistant isolates *Pseudomonas aeroginosa*, *Klebsiella pneumoniae*, *Proteus mirabilis* and *Staphylococcus* resistant to heavy metals and antibiotics.also, Sharma [19] isolated highly cadmium resistant *Klebsiella* that was found to acte a potential agent for bioremediation of heavy metals pollution.

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عزل بكتريا السيدوموناس المقاومة لبعض العناصر الثقيلة من مياه مجاري المستشفيات لمدينة الموصل

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الملخص

صممت الدراسة لعزل بكتريا السيدوموناس المقاومة للمعادن الثقيلة. جمعت 60 عزلة من مصادر مختلفة من مياه فضلات مجاري المستشفيات لمدينة الموصل اعتماداعلى قابليتها على النمو بوجود الزئبق، الرصاص، والنيكل وتم تشخيصها.اظهرت النتائج ان هذه العزلات تعود الى جس السيدوموناس بعدها اختيرت ستة عزلات مقاومة للمعادن الثقيلةالمستخدمة في التجربة. درس معدل النمو بدرجات حرارية وقيم PH مختلفةوحساسيتها للمضادات الحيوية واقل تركيز مثبط للنمو من المعادن الثقيلة. .أظهرت النتائج ان افضل معدل للنمو كان عند درجة حرارة 30°C و PH 7 وكانت العزلات مقاومة للبنسيلين والسيفروكسام والتتراسيكلين والكلورومفينيكول . اما اقل تركيز مثبط للزئبق كان 50 مايكروغرام / مل و 100 مايكوغرام /مل للرصاص و 250 –300 مايكروغرام/مل للنيكل .