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Effect of heavy metals and some selected antibiotics on bacteria isolated from Shatt Al- Arab River

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Abstract

This study is carried out to determine the effect of heavy metals and some antibiotics on bacteria and study the ability of aquatic bacteria isolated from Shatt Al- Arab River to tolerate or resist the presence of certain selected heavy metals: Pb+, Zn2+ and Fe2+ and some antibiotics. Identification tests for the bacterial isolates revealed them to the genera *Pseudomonas*, *Aeromonas*, and *Proteus species*. These bacteria strains are checked for resistance against heavy metals by culturing them in basal medium in which varying concentrations of heavy metal compounds were incorporated. All the bacteria strains show resistance against heavy metals with Minimal Inhibitory Concentration (MIC) values ranging from (0.5-2.25 mg/L) , respectively. Generally, all the organisms had low MIC values for Pb+ and high MIC value for Zn2+ and Fe2+. This indicates average and low toxicity respectively of the heavy metals to the organisms. On the other hand, the isolates also exhibited high tolerance to most of the antibiotics like Gentamycin (87.8 %), Rifampicin (75.0 %) and Oflaxacin (62.0 %). The ability of these organisms to resist the presence of both metals and antibiotics could present some very serious health implication because of the ability of these organisms to pass these resistant genes via R-plasmids to the next cell around will affect a whole bacterial population thereby complicating treatment.

Key words: Bacteria, Heavy metals, Antibiotics, water

1. INTRODUCTION

Some of the heavy metals are essential and are required by the organisms as micro nutrients (Cobalt, Chromium, Nickel, Iron , manganese and Zinc etc.) and are known as 'trace elements' [1]. Heavy metals are often defined as a group of metals whose atomic density is greater than 5 g/cm3 [2] , and play a vital role in the metabolic processes of the biota. They are involved in redox processes, in order to stabilize molecules through electrostatic interactions, as catalysts in enzymatic reactions, and regulating the osmotic balance [3]. Water pollution is a major problem in the global context. It has been suggested that it is the leading worldwide cause of deaths and diseases [4] and that it accounts for the deaths of more than 14,000 people daily. The main sources of pollution particularly by heavy metals is usually linked with areas of intensive industry and high automobiles use. The aquatic environment is more susceptible to the harmful effects of heavy metal pollution because aquatic organisms are in close and prolonged contact with the soluble metals [5]. Heavy metals as natural components of the earth's crust are increasingly found in microbial habitat due to several natural and anthropogenic processes. However, microbes have evolved mechanisms to tolerate the presence of heavy metals either by efflux, complexation or reduction of metal ions or to use them as terminal electron acceptors in anaerobic respiration [6,7]. These heavy metals influence the microbial population by affecting their growth, morphology, biochemical activities and ultimately resulting in decreased biomass and diversity [8]. 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 [1]. 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 [9,1]. Due to the selective pressure from the metal in the growth environment, microorganisms have evolved various mechanisms to resist the heavy metal stress. Several metal resistance mechanisms have been identified: exclusion by permeability barrier, intra and extra cellular sequestration, active transport, efflux pumps, enzymatic detoxification, and reduction in the sensitivity of the cellular targets to metal ions [9,1]. Heavy metal contamination in the environment has become a serious problem due to the increase in the addition of these metals to the environment. Natural sources as well as the anthropogenic sources account for this contamination, which has become a threat to public health. Cadmium, copper and zinc are among those heavy metals that are being released to the environment [10]. Retaining suitable concentrations of essential metals such as copper, while rejecting toxic metals like lead and cadmium was probably one of the toughest challenges of living cells [11]. The first response to toxic metal contamination is a large reduction in microbial activity [12]. This is confirmed by the fact that habitats that have had high levels of metal contamination for years still have microbial populations and activities that are smaller than the microbial populations in uncontaminated habitats. [13] argued that resistance mechanisms do not offer protection at extremely high levels of free metal ions and with a lethal toxic effect. Resistance of essential metals against increased/toxic concentrations of essential metals e.g., Cu, Zn, Ni and Co, confront the cell with a special problem because of their requirement to accumulate some of these cations at trace levels and at the same time to reduce cytoplasmic concentrations from potential toxic levels. Resistant bacterial strains solve these problems by a careful regulation that results from the interaction between chromosomally determined cation transport systems and metal resistance

systems that are mostly determined by plasmids [14]. Many bacterial-resistant systems for toxic metals are encoded by plasmids [15]. Plasmids are small circular DNA molecule that can move from one bacterial cell to another [15]. However, bacterial plasmids contain genes that provide extra functions to the cells among which resistances to toxic metal is very important. It has been shown that a correlation exists between metal tolerance and antibiotic resistance in bacteria because of the likelihood that resistance genes to both (antibiotics and heavy metals) may be closely located on the same plasmid in bacteria and the presence of the organisms that possess specific mechanisms of resistance to heavy metals increases destruction or transformation of toxic substances in the natural environment [16]. Consequently, the range of genes carried on these plasmids (frequently associated with these heavy metal resistant determinants) was shown to extend far beyond those coding for antibiotic resistance [1]. Environmental deterioration often caused by oil spills and discharges resulting from various industrial activities (that may contain a high level of heavy metals) are common phenomenon. Various approaches have been used to detoxify and clean up these metals in any habitat, such as the use of certain chemicals which in turn, cause secondary pollution and physical methods that require large input of energy and expensive materials. Also there is the use of different types of microorganisms such as algae, fungi and bacteria that remove metals from solution [17]. The study aims at determination of heavy metals and some selected antibiotics on bacteria isolated from shatt Al-Arab river.

2. MATERIALS AND METHODS

2.1. Collection of water samples:

The water samples are collected in sterile screw-capped bottles. Three samples are obtained from three different locations: Al-Sendibad , Al-khoura and Al-Sarrajy on Shatt Al-Arab river (surface water) of Shatt Al-Arab River. The samples are transported to the Microbiology Laboratory, Marine Biology, Marine Science Center, University of Basra and analyzed within 24 h.

2.2. Processing of samples:

Serial dilution of the water samples are carried out using sterile distilled water as in [18]. All the different dilutions are properly labelled and used for total plate count.

2.3. Isolation of pure cultures:

The water samples are examined bacteriologically using culture techniques as in [18] . Each water sample is examined by culture technique using streak plate and spread plate technique. A sterile wire loop is used to collect a loop full of each undiluted water sample and inoculated on the surface of nutrient agar, blood agar, MacConkey agar and Eosine Methylene Blue agar. The inoculated plates are subsequently sub-cultured on fresh nutrient agar, MacConkey agar and blood agar plates to obtain pure cultures as well as study their morphological characteristics. They are incubated at 37°C for 24 h. The pure culture isolates are sub-cultured in nutrient agar slant and incubated at 37°C for 24 h and are then stored in the refrigerator until required for further use.

2.4. Identification of bacteria isolates:

The bacteria isolates are subjected to various tests as: growth morphology on different agar media and different microbiological identification tests such as gram staining and motility tests and biochemical identification tests such as catalase, coagulase, oxidase, citrate utilization, urease, indole production, hydrogen sulphide production, nitrate and nitrite reduction, methyl red, Voges Proskeur and sugar fermentation tests.

2.5. Heavy metal resistance tests:

Nutrient agar medium containing varying concentrations (0, 0.25, 0.5, 0.75 and 1.0 mg/L, respectively) of the different heavy metal compounds (Zn2+, Pb+ and Fe2+) are prepared. A sterile wire loop is used to collect a loop full of the pure isolate and directly streaked on the surface of the heavy metal incorporated medium. The process is repeated for each microorganism on the media incorporated with the selected heavy metals. The plates are incubated at 37°C for 24 h. After the incubation period, the plates are observed for any kind of growth.The isolated and distinct colonies on these selective media are subcultured repeatedly on the same media for purification. The pure culture is identified on the basis of their morphology and biochemical characters. The control experiment is carried out by inoculating the pure isolates on basal media without the heavy metals.

2.6. Determination of Minimum Inhibitory
2.6.1. Concentration (MIC):

Minimum Inhibitory Concentration (MIC) of the heavy metal resistant bacteria isolates are determined by gradually increasing the concentration of the heavy metals by 0.25 mg/L each time on the nutrient agar plate until the strains failed to give colonies on the plate. Minimal inhibitory concentration is noted when the isolates failed to grow on the plates after incubation [19].

2.6.2. Antibiotic susceptibility/testing:

Each isolate from the test samples is examined for antibiotic susceptibility using commercially prepared Antibiotic discs (ABTEC). The antibiotics resistance of the isolates is determined with the following discs containing Ceftriaxone 30 µg, Rifampicin 20 µg, Ofloxacin 10 µg, Ciprofloxacin 10 µg, Gentamicin 10 µg and Levofloxacin 5 µg, respectively. A standard inoculum of each isolate from overnight culture on nutrient agar is made by inoculating a discrete colony in 10 mL of sterile peptone water and incubating for 3 h at 37°C. Thereafter, 0.1 mL is spreaded evenly on the surface of solid nutrient agar medium. A sterile forceps is used to place multi-antibiotic discs containing the selected drugs over the surface of each inoculated plate. This procedure is repeated for all the isolates. The plates are labeled and incubated at 37°C for 24 h after which they are examined for growth inhibition. The zone (diameter) of growth inhibition for each antibiotic on the different isolates is measured in millimeters using a transparent metric rule and recorded.

3. RESULTS

Table 1 presents the results of heavy metal resistance test. There is a decline in the growth of the organisms as concentrations of the metals increase in contrast to the situation in the control i.e., 0.0 mg/L of the metals where there is a profuse/heavy growth by all the organisms. At 0.1 to 0.5 mg/L of lead concentration, there is growth in all the organisms while growth increase from 0.75 to 3.0 mg/L of lead in all the organisms. However, more organisms tolerate the presence of zinc even at high concentrations. Peudomonas species continued to grow up to 2.0 mg/L, *Aeromonas species* does not show any growth in zinc from 0.75 mg/L. Proteus species show mild resistance up to 2.25 mg/L and faile to grow from 2.5 mg/L. For iron, *Peudomonas*, *and Proteus species* showed scanty growth at concentrations of 0.1 to 1.5 mg/L . *Aeromonas species* grew moderately at 0.1 and 0.25 mg/L and growth is scanty at 0.5 to1.75 mg/L, it died off at 2.0 mg/L.

Table 1: Heavy metal tolerance testing

		Heavy metal concentration in mg/L of medium														
		0.0	0.1	0.25	0.5	0.75	1.0	1.25	1.5	1.75	2.0	2.25	2.5	2.75	3.0	3.25
Heavy metals	Bacteria isolates															
Lead	*Pseudomonas*	++	+	+	+	-	-									
	Aeromonas	++	+	+	+	-	-									
	Proteus species	++														
Zinc	*Pseudomonas*	++	+	+	+	+	+	+	+	+	+	-				
	Aeromonas	++	+	+	+	-	-									
	Proteus species	++														
Iron	*Pseudomonas*	++	+	+	+	+	+	+	+	-						
	Aeromonas	++	+	+	+	+	+	+	+	+						
	Proteus species	++														

-: No growth; +: Scanty growth; ++: Medium growth

Table 2 shows the minimum values required to inhibit the growth of the microorganisms. The lowest MIC for lead on is 0.5 mg/L for *Peudomonas*, *Aeromonas*, *Proteus* organisms and the MIC for zinc is 0.5 mg/L for *Aeromonas* and 2.25 mg/L on Proteus species. The MIC for iron on Peudomonas and Proteus is 1.5 and 1.75 mg/L for *Aeromonas* .

Table 2: Result of minimal inhibitory concentrations of bacteria isolates in mg/L.

Microorganism	Lead	Zinc	Iron
Pseudomonas	**0.5**	**2.00**	**1.50**
Aeromonas	**0.5**	**0.50**	**1.75**
Proteus specie	**0.5**	**2.25**	**1.75**

The results of antibiotics sensitivity tests as shown in Table 3 indicate that the microorganisms exhibited very low minimum inhibition to most of the antibiotics especially to CEF: 6.8%, LEV: 9.7% and CF: 36.9%, respectively. However, majority of the tested organisms were particularly very resistant to most of the antibiotics as in *Pseudomonas* spp. that was 100% resistant to Levofloxacin and 88.9% to Ceftriaxone, *Aeromonas* spp., showed 77.3% resistant to Ciprofloxacin, 100% to LEV and CEF. while Proteus spp was 100% to CF, LEV and CEF and also 66.7% resistant to OFX. These data were deduced from the number of the organisms that survived the effect of the antibiotics.

Table 3: Antibiotic susceptibility (growth inhibition) pattern of the bacterial species isolated from Shatt Al-Arab River in Basra.

		No. resistant to antibiotics (%)					
Isolates	**No. examined**	**CN**	**CF**	**LEV**	**CEF**	**OFX**	**RD**
Pseudomonas	**16**	**14(87.8)**	**8(50.0)**	**1(6.4)**	**3(18.12)**	**10(62.0)**	**12(75.0)**
Aeromonas	**20**	**12(60.0)**	**5(25)**	**0(0.0)**	**1(5)**	**8(40.0)**	**9(45.0)**
Proteus specie	**4**	**3(75.0)**	**0(0.0)**	**0(0.0)**	**0(0.0)**	**2(50.0)**	**4(100)**

CEF: Ceftriaxone; CF: Ciprofloxacin; LEV: Levofloxacin; OFX: Ofloxacin;RD: Rifampicin; CN: Gentamycin

4. DISCUSSION

This study was initiated with the aim of identifying indigenous bacteria having potential for heavy metal and antibiotic resistance. Three bacteria species (*Pseudomonas*, *Aeromonas* and *Proteus species*) were isolated from shatt Al-Arab River. The microbial level of resistance or tolerance of each concentration of heavy metal was depicted by the level of growth on the agar. The microbial load decreased with an increase in the concentration (0.25 mg/L) of heavy metal indicating the toxic effect of the heavy metals on the growth of microorganisms as earlier stated by [12]. However, no observable growth of microorganisms at high concentrations explains the theory earlier stated by [13] that resistance mechanisms do not offer protection at extremely high levels of free metal ions and a lethal toxic effect is observed. [12] stated that bacterial Resistance or Tolerance can be used to minimize the effect of heavy metals on total biological activity of the ecosystem.Generally, contamination with a specific metal increases the level of resistance of the bacterial community to that metal [12]. Minimal Inhibitory Concentration (MIC) is the highest concentration of the heavy metal required to inhibit the growth of microorganisms. Thus, lower MIC values indicate more toxic metals and higher MIC values indicate less toxicity. The resistance test indicated that among the three experimented heavy metals, average maximum resistance was shown to Zn, showing growth of microorganisms (Proteus species) up to 2.25 mgL and minimum tolerance to lead showing no growth above 0.5 mg/L of the heavy metal. Microorganisms showed an average to high resistance of 2.25 mg/L to zinc incorporated basal medium. These could be explained by the fact that generally, zinc and iron have been classified to have low toxicity on microbes while Lead has high toxicity on microorganisms [7] . The high resistance of Escherichia coli to Fe2+ is probably because the organism possess an additional high affinity ABC-transport system for ferrous iron (Fe2+) encoded by feo ABC gene [20]. Varying microbial resistance levels to heavy metals has been attributed to a variety of resistance mechanisms such as differences in uptake and/or transport of the toxic metal while in other cases, the metal may be enzymatically transformed by oxidation, reduction, methylation or demethylation into chemical species which may be less toxic or more volatile than the parent compound. These mechanisms are sometimes encoded in plasmid genes facilitating the transfer of toxic metal resistance from one cell to another [15]. In a heavy metal contaminated environment, increased uptake of these heavy metals could lead to bioaccumulation of the metals within the organisms. Therefore, removal of these organisms from the environment will help in the bioremediation of these metals. Also the metals could be biotransformed to less toxic or more volatile forms thereby decontaminating the environment [15]. In this study, correlation was found to exist between the resistance of E. *coli* to iron and Proteus to zinc to and antibiotics. The resistance of the organisms to the antibiotics confirms the correlation between resistance metal ions and antibiotics. This has also been reported by several other researchers in bacterial species from different sources [21,22,19] . Many have speculated and have even shown this to be as a result of the likelihood that resistance genes to both antibiotics and heavy metals could be closely located on the same plasmid in bacteria and are thus more likely to be transferred together in the environment [23]. The most important features of R-plasmid is that they can be transferred to heavy metal resistant bacteria, to confer resistance to several antimicrobial agents [24]. This means that if R-plasmid is present in one species in a mixed population of these organisms, other cells will receive the R-plasmid and become resistance to a variety of antimicrobial agents [25]. Many antibiotic resistant genes are located on

mobile genetic elements (e.g., plasmids, transposons and integrons), some of which are easily exchanged among phylogenetically distant bacteria. Many of these mobile genetic elements encode resistance to multiple antibiotics, heavy metals and other compounds. It is therefore possible that selective pressure by such compound indirectly selects for the whole set of resistances. This also explains the phenomenon of conjugation whereby genetic material is transfer between two bacterial cells (of the same or different species). One cell donates the DNA and the other receives it [26]. Transfer of multiple antibiotic resistances by conjugation has become a major problem in the treatment of certain bacterial diseases. Since the recipient cell becomes a donor after transfer of a plasmid it is easy to see why an antibiotic resistance gene carried on a plasmid can quickly convert a sensitive population of cells to a resistant one. And when infections caused by resistant microbes fail to respond to treatment, it may result in prolonged illness and a greater risk of death. Treatment failures also lead to longer periods if infections, which increase the number of infected people moving into the community and thus expose the general population to the risk of contracting a resistant strain of infection.

5. REFERENCES

1. Hughes, M.N.; Poole, R.K. (1989). The functions of metals in micro-organisms. In: Hughes, M.N.; Poole, R.K. (eds). *Metals and microorganisms*. Chapman and Hall, London, p. 1-38.
2. Trevors, J.T; Oddie, K.M.; Belliveau, B.H. (1985). Metal resistance in bacteria. *FEMS Microbiol. Rev.* 32 (1): 39-54.
3. Foster T.J. Plasmid-determined resistance to antimicrobial drugs and toxic metal ions in bacteria.Microbiol. Rev. 1983;47(3):361–409.
4. Larry, W., 2006. World Water Day: 'A Billion People Worldwide Lack Safe Drinking Water. Retrieved from: http: // environmentbout.com /od /environmentalevents/ awaterdaysqa.htm.
5. Shoeb, E., 2006. Genetic basis of heavy metal tolerance in bacteria. Pak. Res. Repository, 11: 389-490.
6. Gadd, G.M., 1992. Metals and Microorganisms: A problem of definition. FEMS Microbiol. Lett., 100: 197-204.
7. Nies, D.H. and S. Silver, 1995. Ion efflux systems involved in bacterial metal resistances. J. Ind.Microbiol., 14: 186-199.
8. Hassen A., Saidi N., Cherif M., Boudabous A. Effects of heavy metals on *Pseudomonas aeruginosa* and*Bacillus thuringiensis*. Bioresourse Technol. 1998;65(1–2):73–82.
9. Lima e Silva A.A., Hofer E. Resistance to antibiotics and heavy metals in *Escherichia coli* from marine fish. Environ. Toxicol. Water. Qual. 1993;8(1):1–11.
10. Lima e Silva A.A., Pereira M.P., Silva Filho R.G., Hofer E. Utilization of phenol in the presence of heavy metals by metal-tolerant nonfermentative Gram-negative bacteria isolated from wastewater. Rev. Latinoam. Microbiol. 2007;49(3–4):68–73.
11. Gatti, D., B. Mitra, B.P. Rosen, 2000. Mini review: Escherichia coli soft metal ion translocating ATPases. J. Bio. Chem.. 275(44): 34009-34012.
12. Badar, U., R. Abbas and N. Ahmed, 2000. Characterization of copper and chromate resistant bacteria isolated from Karachi tanneries effluents. J. Ind. Env. Bio., 39: 43-54.
13. Konopka, A., T. Zakharova, M. Bischoff, L. Oliver, C. Nakastu and R.F. Turco, 1999. Microbial biomass and activity in lead contaminated

soil. J. Appl. Env. Microbiol., 65(5): 2256-2259.
14. Brown, N.L., D.A. Rouch and B.T. Lee, 1999. Copper resistance determinants in bacteria. J. Mol. Microb., 27: 41-51.
15. Silver, S., 1996. Bacterial resistance to toxic metal ionsa review. J. Env. Health Perspective, 105(1): 98-102.
16. Philp, J.C., R.M. Atlas and C.J. Cunningham, 2001. Bioremediation. Nature Encyclopedia of Live Science, pp: 1-10.
17. Dubey, R.C., 2006. A Textbook of Biotechnology. S. Chad and Co. Ltd., India, pp: 569-583.
18. Chesbrough, M., 2002. Medical Laboratory Manual for Tropical Countries. Butterworths and Co. Ltd., UK, pp: 21-32.
19. Rajbanshi, A., 2008. Study on heavy metal resistance bacteria in guesswork sewage treatment plant. Our Nature, 6: 52-57.
20. Kammler, M., C. Schon, K. Hantke, 1993. Characterization of the ferrous ion uptake system of Escherichia coli. J. Bacteriol., 175: 6212-6219.
21. Cenci, G., G. Morozzi, F. Scazzocchio and A. Morosi, 1982. Antibiotics and metal resistance of Escherichia coli isolates from different environmental sources. Zentralblatt fur Bakteriologil und Hygiene 1. Abteilung Originale C, 3: 440-449.
22. Grewal, J.S. and R.P. Tiwari, 1990. Resistance to metal ions and antibiotics in Escherichia coli isolated from foodstuffs. J. Med. Microbiol., 32: 223-226.
23. Nies, D.H., 1999. Microbial heavy metal resistance. J. Appl. Microbiol. Biotechnol., 51: 730-750.
24. Peter, P., 1993. Biotechnology: A Guide to Genetic Engineering. Brow Publishers, USA, pp: 105-108.
25. Eugene, W.N., G. Denise, C.E. Aderson, N.N. Roberts Jr, S. Pear, T. Martha and H. David, 2004. Microbiology: A Human Perspective. Bacteria Pathogens, Mcgraw-Hill, pp: 616-617.
26. Campbell, N.A., J.B. Reece, J.B. Urry, M.L. Cain, S.A. Wasserman, P.V. Minorsky and R.B. Jackson, 2008. Biology. 8th Edn., Pearson Cummings, pp: 562.

تاثير بعض المعادن الثقيلة وبعض المضادات الحياتية على البكتريا المعزولة من مياه شط العرب

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الخلاصة:

أنجزت هذه الدراسة لتحديد تاثير المعادن الثقيلة وبعض المضادات الحياتية على البكتيريا المعزولة من مياه ثلاث محطات من شط العرب . حددت قابلية البكتيريا المائية المعزولة على تحمل ومقاومة المعادن الثقيلة والمضادات الحياتية . اختبارات التعرف على البكتيريا أثبتت وجود أجناس البكتيريا , سيودوموناس , أيروموناس, بروتياس . هذه البكتيريا تم فحصها لمقاومة المعادن الثقيلة وذلك بزراعتها وتنميتها على أوساط أساسية تحتوي على تراكيز مختلفة من المعادن الثقيلة . أظهرت كل البكتيريا مقاومة للمعادن الثقيلة بأقل تركيز مثبط يتراوح من (0.5-2.5 mg /L) بالتتابع . بصورة عامة كل البكتيريا أظهرت أقل تثبيط للرصاص وأعلى تثبيط للخارصين والحديد. من جانب أخر أظهرت البكتيريا مقاومة عالية ضد أغلب المضادات الحياتية مثل الجنتامايسين 87.8% و ريفامبسين 75% واوفلاكساسين 62% هذه القابلية للبكتيريا على مقاومة المعادن الثقيلة والمضادات الحياتية قد تسبب بعض المخاطر على الصحة بسبب قابلية هذة البكتيريا لتمرير هذه الجينات عبر البلازميدات نوع R الى الخلايا اللاحقة لاصابة المجاميع الاخرى من البكتيريا المحدثة للمعالجة وتصنيع الادوية.

الكلمات المفتاحية: بكتريا، معادن ثقيلة، مضادات حياتية، ماء