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The study of simultaneous resistance to heavy metals and antibiotics in resistant bacteria to silver and cadmium isolated from the wastewater

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ABSTRACT

This laboratory study simultaneously was conducted to evaluate the resistance to heavy metals, silver, cadmium and a number of commonly used antibiotics. Sampling was done in 2014 of the two silver workshops in Isfahan, and the inlet of phase 2 of Shahin Shahr wastewater treatment plant, Isfahan. To isolate resistant bacteria to metal silver and cadmium and review multiple resistance to other metals in the medium PHG II agar with a concentration of 0.5 mM of metals and to determine the minimum inhibitory concentration (MIC) of growth, agar dilution method were used. To determine the resistance to several common antibiotics, disk diffusion techniques in agar with Kirby-Bauer method was used. In this study, molecular identification of bacteria was performed through gene sequencing 16S rDNA. New nucleotide sequences of the Cd1 and Cd2 isolates in GenBank's database were deposited under accession numbers of KP753912 and KP753913. Most heavy metal-resistant isolated bacteria in this study had multiple resistance (MMR) compared to the Cadmium, Silver, Copper and Zinc metals and showed simultaneous resistance pattern to several heavy metals and antibiotics. Resistant isolates to cadmium compared to resistant isolates to silver had lower resistance and minimum inhibitory concentration (MIC), they also showed less resistance to a variety of antibiotics used in this study in a way that Cd2 isolate that was recognized as Dechloromonas hortensis strain MS2 was sensitive to all tested antibiotics. Since some of the isolates were highly sensitive to some of the antibiotics, the metal-resistant isolates and sensitive to antibiotic may be a proper candidates for biological removal of these metals from contaminated wastewater in the future.

1. Introduction

The pollution of the environment with toxic heavy metals is spreading throughout the world along with industrial progress (Edward Raja et al., 2009). Since the bacteria are typically exposed to these heavy metals, create different mechanisms for resistance (Spain and Alm, 2003). Bacterial resistance to metal ions is often determined by plasmids. These plasmids often code resistance to antibiotics as well (Verma et al., 2001). This resistance also can be chromosomal (Silver and Phung, 1996). Researches have shown that the antibiotic-resistant and resistant to metal microorganisms appear in contact with environments which are contaminated to metal; these polluted environment will cause simultaneous selection of resistance factors to antibiotics and heavy metals. Microbial resistance to antibiotics and metal ions is a potential risk for health because these traits are usually associated with transmissible plasmids (Sabry et al., 1997). The prevalence of such resistant microorganisms is

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ecologically important, because under the environmental conditions having metal, metal and antibiotic-resistant microbial populations with a prevalence of R factor are adapted quickly than such conditions as mutation and natural selection, thus; this leads to a rapid increase in their number (Karbasizadeh et al., 2003).

During a survey in 2000, Fillali et al. isolated multiple resistant strains to heavy metals, antibiotics, hydrocarbons and aromatic hydrocarbons from water contaminated with metal. These bacteria included strains of *Pseudomonas fluorescens*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Proteus mirabilis* and *S. aureus*, which showed a network of multiple resistance to heavy metals, antibiotics and aromatic compounds (Fillali et al., 2000). Rajbanshi in 2008 conducted a research, and isolated 10 resistant bacteria to metal from the wastewater treatment plant that included resistant materials to chrome such as *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella sp.*, resistant materials to cadmium such as *Acinetobacter sp.*, *Flavobacterium sp.* and *Citrobacter sp.*, resistant *Bacillus sp.* to Nickel, resistant *Pseudomonas* species to Copper and resistant *Methylobacterium* species to Cobalt. All isolates showed high resistance to heavy metals and some antibiotics. These laboratory studies were conducted to determine the resistance to heavy metals and antibiotics simultaneously.

2. Materials and Methods

2.1. Isolation of resistant bacteria to metal from studied wastewater

First sampling was done from the effluent of the silver workshops and the phase 2 inlet of the Shahin Shahr wastewater treatment plant, Isfahan. Sampling was done in sterilized wide plastic bottles. Samples were transferred on ice to research laboratory of Islamic Azad University of Falavarjan to implement the rest of the various stages of work. Diffusion in agar method was used to isolate resistant bacteria to silver and cadmium; first, 1000 ml of PHG II agar medium was prepared including 4 g peptone, 1 g yeast extract, 2 g glucose, 15 g of agar (all materials needed for PHG II agar medium was prepared from Merck Company of Germany). Then wastewater samples were released in PHG II agar medium with a concentration of 0.5 mM of the respective metal. The growth of bacterial colonies were studied after 5 days of incubation at temperature of 30°C (Alboghobeish et al., 2014). The silver nitrate metal salt and cadmium nitrate were used in this study (Scharlau, Spain); it is noteworthy that metal salts used in this study have been implemented in many similar studies. Lima de Silva et al. in 2012 and Richards et al. in 2002 used silver nitrate metal salt as well to study resistant bacteria to silver, Also Zolgharnein et al. in 2007 used cadmium nitrate for isolation and study of resistant bacteria to metal. Bacteria purification was conducted with Streak Plate Method, on PHG II agar medium with the same metal concentration. Microbial spreading was prepared from pure produced colonies, and Gram stain.

2.2. Determination of minimum inhibitory concentration (MIC)

In this study, agar dilution method was used to determine the minimum inhibitory concentration of growth, in which plates containing PHG II agar medium with different concentrations (0/5, 1, 2, 4, 8, 16 and 32 mM) of heavy metal (Ag, Cd) were prepared so that each plate contained a concentration of metal (Alboghobeish et al., 2014). The metal solution with a suitable concentration was added to the culture medium, then resistant colonies were cultured on plates (Hassen et al., 1998). Plates were put at the temperature of 30°C for 48 hours, after the growth of microorganisms based on the growth or lack of growth in a concentration, in between concentrations were selected and growth in those concentrations was also examined. Finally, the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined (Coral et al., 2005; Alboghobeish et al., 2014).

2.3. Molecular identification of isolates

In this study, the molecular identification of isolates was done using the Colony PCR technique with the help of public primers designed from gene 16S rDNA, in the Knowledge Based Pars Tali Gene Company situated in the Scientific and Research Park of

Isfahan. Nucleotide sequences derived from the above technique was identified homologically after reviewing and correcting chromatogram, by BLAST server in site NCBI (http://www.ncbi.nlm.nih.gov/BLAST).

The 16S rDNA gene was amplified by using the universal bacterial Primers of OF BUN (5′-CGCATTTCACCGCTACAC-3′) and OR BUN (5′-TATGTACACACCGCCCGT-3′).

11 resistant to isolateed metal bacterial strains, were incubated again on PHG II agar medium at 30 °C; these pure colonies were used to perform PCR. In order to perform the colony-PCR technique, first, an amount of purified colonies were dissolved in 10 ml of sterile distilled water using a sterile loop, and 2 µL of it was used as a template for PCR reaction. Also 0.75 µL $MgCI_2$ 50 mM, 0.5 µl dNTP 10 mM, 0.5 µL of each directing and leading primers (20 µM), 0.2 µL Taq polymer of 5U, 2.5 µL of PCR 10X buffer, and 18.05 µL injection distilled water were used to form 25 µL of reaction mixture.

In the following the initial denaturation was done for 5 min at 94°C, there was 35 Cycle consisting of denaturation at 94°C for 45 second, annealing at 58°C for 45 second, extension at 72°C for 45 second and final extension at 72°C for 5 min. A cooling temperature of 4°C was applied.

PCR products were analyzed by 1% (W/V) agarose gel electrophoresis containing green viewer (2 µL) in 1x TBE buffer, and then, they were sequenced and compared with the National Center for Biotechnology Information (NCBI) database using the BLAST software available through the center 's website (http://www.ncbi.nlm.nih.gov/BLAST). Subsequently, new sequences of 16S rDNA were presented to the NCBI using the BankIt service.

2.4. Determination of bacteria sensitivity to antibiotics

One of the ways used to determine resistance to antibiotics, is the disk diffusion technique in agar with Kirby-Bauer method, which was used in the present study. In this method antibiotics disks included gentamicin (GM 10), erythromycin (E 15), tetracycline (TE 30), cephalothin (CF 30), sulfamethoxazole trimethoprim (SXT 25), cefoxitin (FOX 30), amoxicillin (AMX 25), vancomycin (V 30), novobiocin (NB 5), penicillin (P 10), Chloramphenicol (C 30), streptomycin (S 10), rifampicin (RA 5) (Padtan Teb, Iran).

2.5. Determination of multiple resistance to metals

To determine multi metal resistance (MMR), PHG II medium containing the desired metal was used to study resistance. To detect microbial resistance to other metals (silver nitrate, cadmium nitrate, copper sulfate, zinc nitrate), resistant colonies to relevant metal on plates with a specific concentration (0.5 mM) of the other metal were cultured radially. Then for a period of 48-96 hours was incubated at 30°C. The lack of growth of bacterial colonies in other plates was considered as their sensitivity to that certain metal, while the growth in each of the plates were considered as resistant colony to that metal (Singh et al., 2010; Egbebi and Famurewn, 2011).

3. Results

The results of the MIC and MBC of isolate resistant bacterial strains to metals of silver and cadmium, as well as the lists of bacteria resulted from the the molecular identification of isolates are presented in Tables 1 and 2.

Table 1. The results of MIC and MBC resistant bacterial isolates to silver (in mM)

Isolate number	Bacteria name	Concentration of silver (mM)	
		MIC*	MBC**
Ag1	*Pseudomonas sp.SQ7-53*	5	6
Ag2	*Pseudomonas chengduensis* strain MBR	5	6
Ag3	*Sphingobium sp.THG-S23.15*	5	6
Ag4	*Stenotrophomonas maltophilia* strain YS86	1.25	1.5
Ag5	*Stenotrophomonas maltophilia* strain MS8	6	7
Ag6	*Pseudomonas nitroreducens* strain tonekaboni	4	5
Ag7	*Pseudomonas aeruginosa* strain FRD1	3	3.5

Table 2. The results of MIC and MBC resistant bacterial isolates to cadmium (in mM)

Isolate number	Bacteria name	Concentration of cadmium (mM)	
		MIC*	MBC**
Cd1	*Azospira oryzae* strain MS1	1.5	1.75
Cd2	*Dechloromonas hortensis* strain MS2	2	2.5
Cd3	*Acidovorax avenae* strain AF132	1	1.25
Cd4	*Entrobacter cloacae* strain 34983	1.5	1.75

*: Minimum inhibitory concentration (MIC).
**: Minimum bactericidal concentration (MBC).

Table 3. The results of antibiogram resistant strains to metals (Cd, Ag) isolated from the wastewater against antibiotics disks by Kirby-Bauer method.

Isolate name	Antibiotics												
	P 10µg	E 15µg	V 30µg	TE 30µg	C 30µg	GM 10µg	CF 30µg	SXT 25µg	FOX 30µg	AMX 25µg	NB 5µg	S 10µg	RA 5µg
Ag1	R	I 18.3	R	S 41.6	S 32	S 22.3	R	S 31	R	S 24.3	R	S 19	R
Ag2	R	I 19.3	R	S 38	S 27.3	S 22.3	I 15.6	S 22.3	R	S 26	R	S 19.6	R
Ag3	S 36	S 44.6	S 39.6	S 49.3	S 42.6	S 47.3	R	S 30	R	I 16	R	R	S 31
Ag4	R	R	S 12.3	S 19.6	S 27	S 16.3	R	S 23.6	R	R	R	S 16.3	R
Ag5	R	S 28.6	S 19.3	S 25.6	S 26	S 18.3	R	S 16.6	R	R	I 19	S 20	I 17.6
Ag6	R	R	R	S 36.3	S 18.6	S 23	R	S 28.3	R	I 14.3	R	S 19.3	R
Ag7	R	R	R	S 28	I 15.6	S 24.3	R	I 13.6	R	R	R	R	R
Cd1	S 39.3	S 26.3	S 17.6	S 26	R	S 22.6	S 48.6	S 28.6	S 44	S 28	I 19.6	R	S 22.6
Cd2	S 29.3	S 35.6	S 21.6	S 31.3	S 23.6	S 27.6	S 24.6	S 33	S 39.3	S 31.3	S 25.6	S 47.3	S 33.3
Cd3	S 42.3	S 35.3	S 14.3	S 39.6	S 45	S 30.3	S 45.3	S 31.6	R	S 42	R	S 21.3	R
Cd4	R	S 25.3	S 33.3	S 40.3	S 23.3	S 42.3	S 40.3	S 33.3	S 35.3	S 31.3	S 32.6	S 35.3	S 33.3

Legend and abbreviations: R: Resistant, S: Suseptible, I: Intermediate, P: Penicilin, E: Erythromycin, V: Vancomycin, TE: Tetracycline, C: Chloramphenichol, GM: Gentamicin, CF: Cephalothin, SXT: Sulfamethoxazole Trimethoprim, FOX: Cefoxitin, AMX: Amoxicillin, NB: Novobiocin, S: Streptomycin, RA: Rifampicin. (It should be noted that the S, R and I was determined according to NCCLS (National Committee for Clinical Laboratory Standard**)** information. numbers in table are in mm).

In this study, the inhibitory effect of some antibiotics were investigated on the resistant strains to isolated metals. The average of the areola diameter of lack of growth resulted from three repetitions of strains towards used antibiotics in millimeters (mm), is presented in table 3.

The molecular analysis of isolates revealed that, the two isolates of Cd1 and Cd2 had new sequences, which were registered as *Azospira oryzae* strain MS1, and *Dechloromonas hortensis* strain MS2 with the access numbers KP753912 and KP753913 in the GenBank of the National Center for Biotechnology Information (NCBI), respectively. The phylogenetic trees of results are shown in Figures 1 and 2.

In this study, the resistant bacteria to isolated metal were also investigated in terms of strength to concentration of 0.5 mM of other metals (cadmium, silver, copper and zinc);the results are provided in tables 4 and 5.

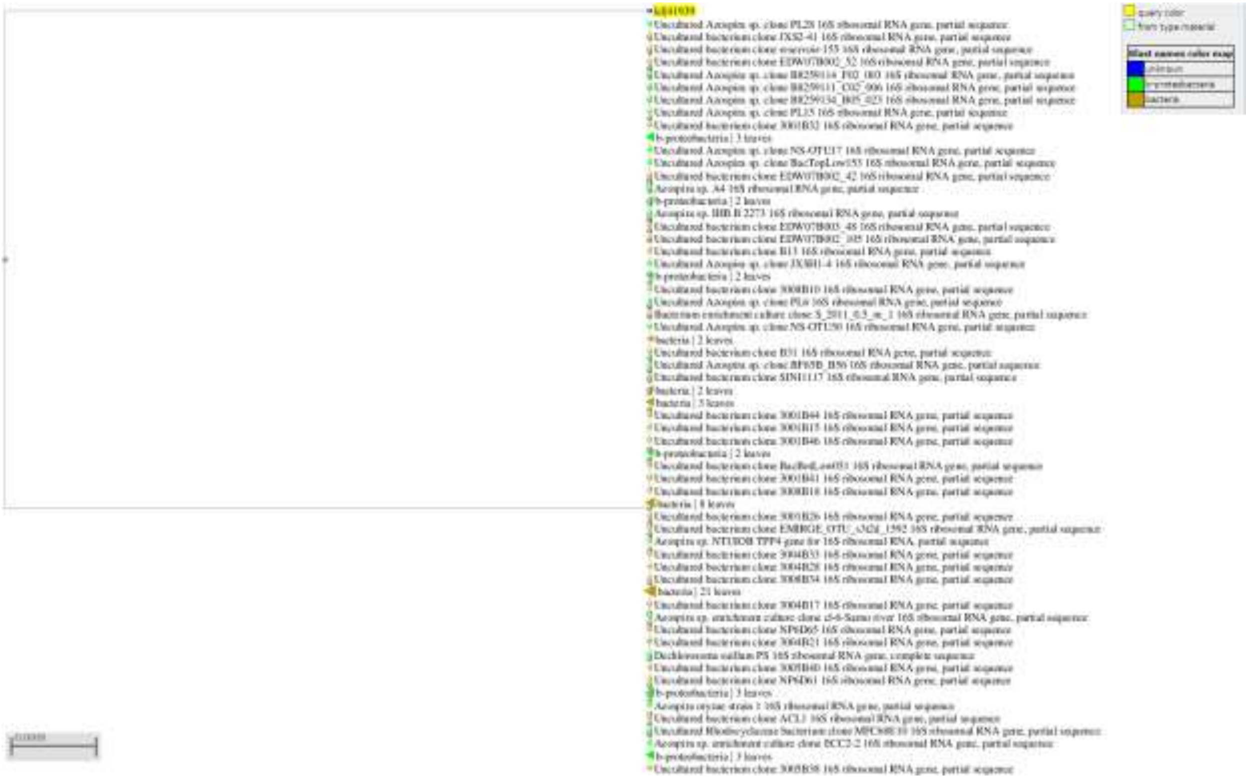

Figure 1. The phylogenetic evidence of identified isolates of Cd1; Extracted from BLAST database.

Table 4. The multi-resistant results of the resistant bacteria to silver to the 0.5 mM concentration of other metals.

Isolate number	Bacteria name \ Concentration 0.5 (mM) of metals	Cd	Cu	Zn
Ag1	*Pseudomonas sp.SQ7-53*	+	+	+
Ag2	*Pseudomonas chengduensis* strain MBR	-	+	-
Ag3	*Sphingobium sp.THG-S23.15*	-	+	+
Ag4	*Stenotrophomonas maltophilia* strain YS86	+	+	+
Ag5	*Stenotrophomonas maltophilia* strain MS8	+	+	+
Ag6	*Pseudomonas nitroreducens* strain tonekaboni	+	+	+
Ag7	*Pseudomonas aeruginosa* strain FRD1	+	+	+

Descriptions Table: -: lack of growth, +: growth

Figure 2. The phylogenetic evidence of identified isolates of Cd2; Extracted from BLAST database.

Table 5. The multi-resistant results of resistant bacteria to cadmium to 0.5 mM concentration of other metals.

Isolate number	Concentration 0.5 (mM) of metals / Bacteria name	Ag	Cu	Zn
Cd1	*Azospira oryzae* strain MS1	-	-	+
Cd2	*Dechloromonas hortensis* strain MS2	-	-	+
Cd3	*Acidovorax avenae* strain AF132	-	+	+
Cd4	*Entrobacter cloacae* strain 34983	+	+	+

Descriptions Table: -: lack of growth, +: growth

4. Discussion

The presence of heavy metals in the environment is directly related to the resistance; in a way that their participation in the environment cause simultaneous selection of resistance factors to heavy metals and antibiotics. It means that the microorganisms, develope resistance mechanisms in the presence of metals that lead to the selection of resistant species with capability of metal toxicity tolerance (Edward Raja et al., 2009; Anyanwu and Nwachukwu, 2011).

In tables 4 and 5 multiple resistance of isolated resistant bacteria to the concentration of 0.5 mM of other metals is provided; out of the resistant bacteria to silver, isolates of Ag1, Ag4, Ag5, Ag6 and Ag7 in addition to silver, were able to tolorate a concentration of 0.5 mM of metals Cd, Cu and Zn. Isolate of Ag2 was able to grow on the concentration of 0.5 mM of Cu and isolate Ag3 was able to tolerate a concentration of 0.5 mM of Cu and Zn metals. Among the resistant isolates to cadmium, in addition to resistance to cadmium isolates of Cd2 and Cd1 were able to grow on the medium containing concentration of 0.5 mM of Zn and isolate Cd3 had resistance to Cu and Zn. Also isolate Cd4 was able to tolorate all three metals of Cu, Ag and Zn. As a result, it can be concluded that in this study, the majority of resistant isolates to isolated metal had multiple resistance to other metals.

Multiple resistance to other metals among isolated isolates from sea water by Sabry et al. (1997) showed that almost all of the isolates except for a few were resistant to several metal ions.

Zhang et al. in 2014 in a study, isolated six resistant bacteria to metal from the Yangtze River in China which had multiple resistance to metals of cadmium, copper, lead, chromium and nickel. Vela-Cano et al. in 2014 achieved to isolate a kind of *Rhodococcus* that showed high resistance to metals of cadmium, copper, zinc and lead. Elsilk et al. (2014) succeeded in isolating *B. anthracis* PS2010 from industrial wastewater in Egypt that had resistance to metals such as cadmium, copper, cobalt, zinc and lead. In the study of Varadarajan and Shikha (2014), isolated isolates from gold plating wastewater, showed multiple resistance to heavy metals.

The results of this study and other researchers' studies, have shown that resistant isolates to heavy metals have shown multiple resistance to other metals. In fact, the results show that in the majority of resistant bacteria to metal, different categories of the expression genes can show the similar expression when they are exposed to different metal compounds (Hu et al., 2007). The reason of multiple tolerance to toxic compounds can be their similar toxic mechanisms and since toxic metals have similarities in their toxic mechanisms, multiple tolerance phenotype is common among resistant bacteria to heavy metal. Strains including resistance gene to isolated metal in this study, had multiple resistance to heavy metals, and probably were able to survive in the contaminated environment with several heavy metals. They could be used in the removal of heavy metals from wastewaters that are contaminated by several metals, however, this issue requires further study.

In addition, there are evidences regarding the correlation between the contamination of the bacterial medium metal and spread of resistance to antibiotics. The relationship between the amount and type of contaminant metals and specific patterns of antibiotic resistance indicates multiple mechanisms of resistance to antibiotics and heavy metals. It can be concluded that the genes responsible for resistance to both external factors are the same. Therefore, long metal contaminations can increase the antibiotic resistance factors (Baker-Austin et al., 2006).

In this study, the resistant bacteria to isolated metals from mentioned wastewater were also studied in terms of the patterns of antibiotic resistance. The results of this study (Table 3) showed that resistant isolates to cadmium compared to resistant isolates to silver had lower resistance and MIC, they also showed lower amount of resistance to the types of antibiotics used in this study. But resistant isolates to silver that showed a higher average of MIC than silver, showed more antibiotic resistance as well. In fact, most of the resistant isolates to isolated metal in this study, were also resistant to some studied antibiotics, but there were some exceptions, in a way that isolate of Cd2 known as *Dechloromonas hortensis*, were sensitive to all tested antibiotics.

A study by Whelan et al. (1997) investigated 15 strains of *Enterobacter* isolated from coastal waters in Turkey; their results showed that plasmid resistant foils have had a resistance to copper and nickel and by transferring plasmid from *Enterobacter* to *E. coli* it was found that resistant strain *E. coli* also has received the same plasmid.

Study carried out in Bucharest, on the relationship between resistance to antibiotics (gentamicin, tetracycline, ampicillin and cefotaxime) and heavy metals (cadmium, copper, chromium and nickel) in *E. coli* shows that multiple resistance to antibiotics has been developed in all strains. Further, investigation of plasmids showed that resistant strains had plasmids with a length of 3.8-50 Kilobase pair (Kbp) (Lazar et al., 2002). Singh et al. (2010) by using the Kirby-Bauer method, investigated sensitivity and antibiotic resistance of *Pseudomonas* resistant isolates to heavy metals from industrial wastewater; their results showed that resistant isolates to metal were resistant to some antibiotics.

Many researches have shown that there is a link between metal tolerance and resistance to antibiotics in the environment. Because of the possibility of the similarity of antibiotic resistance and resistance to heavy metals genes and their placement on a plasmid therefore it is most likely for them to be moved toward together in the medium. Because of the increased presence of resistant pathogenic bacteria to antibiotics, the treatment of infectious diseases has been so difficult and expensive than before. So we must not only put stress on efficient use of antibiotics in the community but also be aware of the use of other antimicrobial substances such as heavy metals that are in the environment (Spain and Alm, 2003).

So far, the relationship between metal tolerance and resistance to antibiotics has been reported several times and their results were consistent with the results of this study. according to all the reports, the encoding resistance genes to metals and antibiotics are on transferable plasmids (Rajbanshi, 2008; Edward raja et al., 2009). In addition Fillali et al. (2000) have also shown simultaneous resistance to heavy metals, antibiotics and aromatic compounds. The results of this study were consistent with the results of other researchers and the most resistant species to isolated metal had resistant to other metals as well as some antibiotics.

In a study, Jain et al. (2009) investigated the amount of resistance to metal and antibiotic in isolated bacteria from copper industries and mines. They demonstrated that metal pollution in the environment leads to the development of multi antibiotic resistance, probably through horizontal gene’s transfer to the bacterial population. To overcome this problem, they have noted the treatment of wastewater being full of metal and their immunization.

In this study, resistant bacteria to isolated metal showed the highest resistance to cefoxitin antibiotics and most sensitivity to tetracycline and gentamicin antibiotics. The majority of resistant isolates to isolated metal in this study were resistant to some of the studied antibiotics but some of the isolates had high sensitivity to antibiotics. The isolate Cd2 was sensitive to all tested antibiotics, therefore; it seems that these resistant isolates to metal and sensitive to antibiotic can be proper candidates for biological removal of these metals from contaminated wastewater in the future. Biological removal of heavy metals from industrial waste by resistant bacteria to metals can be a useful solution to the problem of environmental pollution caused by industrial plants.

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Refereces

Alboghobeish, H., Tahmourespour, A., Doudi, M., 2014. The study of Nickel Resistant Bacteria (NiRB) isolated from wastewaters polluted with different industrial sources. J Environ Health Sci Eng. 12: 1-7.

Anyanwu, C.U., Nwachukwu, O.N., 2011. Heavy metal resistance in bacteria isolated from contaminated and uncontaminated. Int. J. Res. Chem. Environ. 1(1): 173-178.

Baker-Austin, C., Wright, M.S., Stepanauskas, R., McArthur, J.V., 2006. Co-selection of Antibiotic and Metal Resistance. Trends Microbiol. 14(4): 176-182.

Coral, M.N., Korkmaz, H., Arikan, B., Coral, G., 2005. Plasmid heavy metal mediated resistances in Enterobacter spp. Isolated from sofulu landfill. Ann. Microbiol. 55(3): 175–179.

Edward Raja, C., Selvam, G.S., Omine, K., 2009. Isolation, identification and characterization of heavy metal resistant bacteria from sewage. Int. Jt. Symp. Geodisaster Prev. Geoenviron. Asia. 205-211.

Elsilk, S.E., El-Shanshoury, A.R., Ateya, P.S., 2014. Accumulation of some heavy metals by metal resistant avirulent *Bacillus antracis* PS2010 isolated from Egypt. Afr. J. Microbiol. Res. 8(12): 1266-1276.

Egbebi, A.O., Famurewa, O., 2011. Heavy metal resistance among *Klebsiella* isolates in some parts of southwest Nigeria. Asian J. Med. Sci. 3(5): 183-185.

Fillali, B.k., Taoufik, J., Dzairi, F.Z., Talbi, M., Blaghen, M., 2000. Waste water bacterial isolates resistant to heavy metals and antibiotics. Curr Microbiol. 41(3): 151-156.

Hassen, A., Saidi, N., Cherif, M., Boudabous, A., 1998. Resistance of environmental bacteria to heavy metals. Bioresour. Technol. 64(1): 7-15.

Hu, Q., Dou, M.N., Qi, H.Y., Xie, X.M., Zhuang, G.Q., Yang, M., 2007. Detection, isolation, and identification of cadmium-resistant bacteria based on PCR-DGGE. J Environ Sci. 19(9): 1114–1119.

Jain, P.K., Ramachandran, S., Shukla, V., Bhakuni, D., Verma, S.K., 2009. Characterization of metal and antibiotic resistance in the bacterial population isolated from copper mining industry. Intl J Integrative Biol. 6(2): 57-61.

Karbasizadeh, V., Badami, N., Emtiazi, G., 2003. Antimicrob heavy metal resistance and plasmid profile of coliforms isolated from nosocomial infections in hospital in Isfahan, iran. Afr. J. Biotechnol. 2(10): 379-383.

Lazar, V., Cernat, R., Balotessu, C., Contar, A., Coipan, E., Cojocaru, C., 2002. Correlation between multiple antibiotic resistance and heavy-metal tolerance among some *E.coli* strains isolated from polluted waters. Bacteriol virusol parazitol epidermiol. 47: 155-160.

Lima de Silva, A.A., Riberiode Carvalho, M.A., de Souza, S.A., Teixerira Dias, P.M., da Silva Filho, R.G., de Meirelles Saramago, C.S., et al. 2012. Heavy metal tolerance (Cr, Ag and Hg) in bacteria isolated from sewage. Braz. J. Microbiol. 43(4): 1620-1631.

Rajbanshi, A., 2008. Study on heavy metal resistant bacteria in Guheswori sewage treatment plant. Our Nature, 6: 52-57.

Richards, J.W., Krumholz, G.D., Chval, M.S., Tisa, L.S., 2002. Heavy metal resistance patterns of *Frankia* strains. Appl. Environ. Microbiol. vol. 68: 923-927.

Sabry, S.A., Ghozian, H.A., Abou-Zeid, D.M., 1997. Metal tolerance and antibiotic resistance patterns of a bacterial population isolated from sea water. J. Appl. Microbiol. 82(2): 245-252.

Silver, S., Phung, L.T., 1996. Bacterial heavy metal resistance: New Surprises. Annu Rev Microbiol. 50: 753-789.

Singh, V., Chauhan, PK., Kanta, R., Dhewa, T., Kumar, V., 2010. Isolation and characterization of *pseudomonas* resistant to heavy metal contaminants. Int. J. Pharm. Sci. Rev. Res. 3(2): 164-167.

Spain, A., Alm, E., 2003. Implications of microbial heavy metal tolerance in the environment. Rev Undergaduate Res. 2: 1-6.

Varadarajan, H., Shikha, S., 2014. Biodiversity characterization of bacterial and fungal isolates from gold electroplating industry effluents. Appl. Environ. Microbiol. 2(5): 212-219.

Vela-Cano, M., Castellano-Hinojosa, A., Vivas, A.F., Toledo, M.V.M, 2014. Effect of heavy metals on the growth of bacteria isolated from sewage sludge compost tea. Advances in Microbiology. 4: 644-655.

Verma, T., Srinatha, T., Gadpayle, R.u., Ramteke, P.W., Hans, R.K., Garg, S.K., 2001. Chromat tolerant bacteria isolated from tannery effluent .Bioresour Technol. 78(1): 31-35.

Whelan, K.F., Sherburne, R.K., Taylor, D.E., 1997. Characterization of a region of the IncHI2 plasmid R478 which protects *Escherichia coli* from toxic effects specified by components of the Tellurite, Phage and Colicin resistance cluster. J Bacteriol. 179(1): 63-71.

Zhang, Q., Achal, V., Xiang, W.N., Wang, D., 2014. Identification of heavy metal resistant bacteria isolated from Yangtze River, China. Int. J. Agric. Biol. 16(3): 619-623.

Zolgharnein, H., Azmi, M.L.M., Zamri Saad, M., Mutalib, A.R., Mohamed, A.R., 2007. Detection of plasmid in heavy metal resistance bacteria isolated from the Persian Gulf and enclosed industrial areas. Iran J Biotech. 5(4): 232-239.