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Correlation Between Heavy Metal Resistance and Antimicrobial Resistance in Bacterial Isolates From Different Industrial Effluent Samples

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ABSTRACT

Due to rapid increase in industrialization pollution of environment with toxic heavy metals turns into the world's biggest issue. To beat these issues substantial metal safe organisms are currently used to treat such water in water treatment plant before dumping it into water bodies. These organisms indicated impervious to a few anti-infection agents alongside metal resistance. Material and Methods: Six industrial ifulent tests were gathered from various mechanical zones. The segregates were recognized on the premise of morphological, cultural and biochemical tests. The MIC of substantial metals was resolved. The anti-microbial susceptibility was checked by Kirby Bauer Disk Diffusion strategy. In the present study out of 9 segregates from various examples (66.7%) were gram positive and (33.3%) were gram negative confines. All the isolates demonstrated resistance to substantial metals. The Cadmium and Mercury were profoundly dangerous to all segregates aside from Enterobacter and Chromium was less harmful to all isolates and they endure high centralizations of Chromium. All the gram positive separates were exceedingly impervious to all anti-infection with the exception of Vancomycin and Ampicillin and all the gram negative confines were impervious to all anti-infection agents aside from Ciprofloxacin and Nalidixic acid. These substantial metal enduring microorganisms can be utilized to for the bioremediation procedure to clean our surroundings and biological system. The utilization of organisms for cleaning procedure of environment is simple and modest strategy since microorganism's exhibit all over the place and they likewise built up specific instruments to make due in such sort of contaminated environment which is extremely useful for us to utilize them for our advantages.

Keywords: *Industrial effluent, Heavy Metals, Cadmium, Chromium, Mercury, Lead, Bioremediation.*

INTRODUCTION

As the industries are advancing step by step the world is getting contaminated with harmful substantial metals (Raja, *et al.*, 2008). Heavy metals experience such synthetic changes and shape such mixes which can be harmful, cancer-causing, and mutagenic, even in a low fixation (Ruiz-Manriquez, *et al.*, 1998). For the evacuation of metals customary technique gets costly and has numerous impediments. To expel substantial metals from water bodies organic techniques are utilized as an option strategy (Raraz, 1995). Microorganisms adjusted a percentage of the exceptional instruments to endure these harmful metal focuses. These methods incorporate metal sorption, uptake and gathering, mineralization, extracellular precipitation and enzymatic oxidation or lessening to a less poisonous structure, and efflux of substantial metals from the cell (Hughes, *et al.*, 1991; Nies, 1992; Urrutia,. and Beveridge, 1993).

In the rundown of substantial metals Cadmium needs the unique consideration since it is a

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vital lethal toxin in light of its high solubility in water and genuine harmful impacts in human and other living life forms. (Tang, *et al.*, 2006).

Cadmium can tie to the respiratory chemicals and causes oxidative anxiety and malignancy.

The metal is cancer-causing, mutagenic, teratogenic, embryotoxic in nature and can bring about extreme weakness, hyperglycemia, renal harm, lung growth, osteomalacia, DNA harm, cracks and vertebral osteoporosis, fringe blood vessel sickness, maturing, neuron danger, liver harm, cardiovascular maladies, harm to conceptive framework and diminished immunopotency because of its obstruction with iron digestion system. Investigators archived that microscopic organisms, parasites and algae can bioaccumulates Cadmium, so they can be use as new bioremediation innovation for the expulsion of cadmium from waste water. (Rafatullah, *et al.*, 2012.

Chromium is a Hexavalent compound and is extremely perilous poisonous toxin of the earth. Chromium is utilized as a part of numerous businesses for the creation of numerous day by day routinely utilized items like paper, generation of paints and shades, tanning, conservation of wood, in cowhide tannery, in electroplating and in metallurgical commercial ventures. Numerous microorganisms can degrade the chromium contamination from the earth by the utilization of a few catalysts. These bacterial compounds can be use in chromate bioremediation. The component of Chromium diminishment by these microorganisms is variable and fluctuates from specie to specie. A few organisms use Chromium as the last electron acceptor in the respiratory chain, while some different strains use proteins for diminishment of chromium to less poisonous structure.

Mercury positioned sixth position in the rundown of harmful metal. It is found in the indigenous habitat. In oceanic environment the mercury present in its oxidized structure, mercuric particle (Hg2+). The inorganic type of mercury is found in water and residue and by the activity of microorganisms it changed over into methyl mercury. This methyl mercury rapidly turn into the piece food and demonstrates its sensational impacts from lower to higher living beings.

Lead is not a transient component. It is found in ocean water in low fixation then mercury (Weast, 1984). Because of its low solubility less concentration is accessible organically and is minimal lethal to microorganisms. Lead enduring microbes were secluded (Trajanovska, *et al.*, 1997) and it is watched that these microscopic organisms collect lead phosphate in their cell. Furthermore, scientists are concentrating on the utilization of these microscopic organisms for sterilizing lead tainted locales.

The two primary normal sorts of toxins including consistently in the earth from the emanating and modern waste water are the metals and anti-toxins. Conjunction of these two components in any sort of environment can be from any source like from living organisms forms release, from animal fertilizer and from poultry ranch destinations, conveying distinctive anti-microbials and crucial metals. For instance domesticated animals are fed with arsenic and anti-toxins containing nourishment supplements keeping in mind the end goal to keep them away from diseases and to advance their development. This make the gut residential creatures pre-presented to anti-microbials and metals. Then again the utilization of poultry excrement as soil manure to recapture soil fruitfulness and supplement is additionally a noteworthy reason for pre-presenting occupant microorganisms to substantial metals and anti-microbials. At the point when regular habitat having diverse microbial groups, get presented to joined tainting of substantial metals and anti-toxins it causes the event and spread of anti-infection safe organisms and at some point multidrug resistant microorganisms are advanced. There are two potential outcomes of antibiotic resistance. One is the occurrence of metal that aides in the development of occupant organisms and expansion in supplements in that environment which as of now have antibiotic resistant microorganisms. The second probability is the securing of safe qualities by anti-microbial sensitive organisms because of the co-steadiness of substantial metal and anti-microbial in the same environment.

The microscopic organisms get resistant to substantial metals and antibiotic agents by constant and delayed low level of release of these two noteworthy environmental toxins.

MATERIAL AND METHOD

Samples collection: 6 waste water samples were collected from different industrial areas. The samples were collected in sterile plastic bottles.

Sample Processing

Serial dilutions (106) of the samples were made. 0.1 ml of the sample from last dilution were spread on Nutrient Agar plates and incubated at 37°C for 24 hours.

Identification of isolates: Isolates were identified on the basis of morphological, cultural and biochemical tests.

Minimum Inhibitory Concentration: 5 different concentrations of metal salts from metal stock solution supplemented in Nutrient Agar plates. Inoculate culture obtained from the samples. Incubate plates at 37°C for 24 hours. Next day determined the MIC of heavy metal resistance.

Antibiotic susceptibility testing: Antibiotic susceptibility was performed by Kirby Bauer method and incubated the plats at 37°C for 24 hours. Next day observe the size of zone of inhibition.

RESULTS

From six samples, nine organisms were isolated. Out of these six isolates were

Table I: Morphological, Biochemical and Cultural Characteristics of Gram Positive Isolates

Isolates	Gram reaction	Arrangement	Biochemical test			OF Test (Hugs leifson media)	Selective and differential agar		Novabiocin	Organism identified
			Catalase	Coagulase	Oxidase		MSA	Blood agar		
S1a	gram positive	Cocci in clusters	+	+	Nil	O^+F^+	Mannitol fermenting colonies	ß hemolysis	Nil	S.aureus
S1b	gram positive	Cocci in clusters	+	-	Nil	O+F+	Not Mannitol fermenting	ß hemolysis	Sensitive	S. epidermidis
S2a	gram positive	Rods in chain	+	Nil	-	Nil	Nil	ß hemolysis	Nil	Bacillus. spp
S2b	gram positive	Rods in chain	+	Nil	-	Nil	Nil	ß hemolysis	Nil	Bacillus. spp
S3a	gram positive	Micro-cocci in tetrade	+	+	+	O+F-	Only yellow colonies	ß hemolysis	Nil	M.luteus
S3b	gram positive	Cocci in clusters	+	+	Nil	O+F+	Mannitol fermenting colonies	ß hemolysis	Nil	S. aureus

Table II: Morphological, Biochemical and Cultural Characteristics of Gram Negative Isolates

Isolates	Gram reaction	Arrangement	Biochemical test			OF Test (Hugs leifson media)	Selective and differential agar		Novabiocin	Organism identified
			Catalase	Coagulase	Oxidase		MSA	Blood agar		
S4	Gram negative	Short rods, scattered	+	Nil	+	Alkaline slant/Acidic butt + No gas	+	Non lactose fermenting colonies	ϒ hemolysis	Salmonella spp
S5	Gram negative	Short rods, scattered	+	Nil	+	Alkaline slant/Alkaline butt + no gas	+	Non lactose fermenting colonies	ϒ hemolysis	Pseudomonas spp
S6	Gram negative	Short rods, scattered	+	Nil	-	Alkaline slant/Acidic butt +gas	+	Lactose fermenting colonies	ϒ hemolysis	Enterobacter spp

Table III: Antibiotic sensitivity of Gram Positive Isolates

Isolates	Antibiotics Zone Of Inhibition				
	Penicillin	Vancomycin	Ampicillin	Rifampin	Lincomycin
S1a	18mm	21mm	25mm	15mm	26mm
S1b	13mm	25mm	22mm	17mm	24mm
S2a	-	18mm	15mm	14mm	21mm
S2b	-	16mm	18mm	13mm	19mm
S3a	18mm	19mm	0.0mm	0.0mm	20mm
S3b	20mm	22mm	24mm	18mm	25mm

Table IV: Antibiotic sensitivity of Gram Negative Isolates

solates	Antibiotics Zone Of Inhibition				
	Cephalexin	Streptomycin	Nalidixic acid	Trimethoprim	Ciprofloxacin
S4	0.0mm	0.0mm	16mm	10mm	24mm
S5	0.0mm	0.0mm	16mm	9mm	22mm
S6	0.0mm	11mm	20mm	15mm	14mm

gram positive organisms included *S.aureus, S.epidermidis, Bacillus spp, Micrococcus.* The other three isolated organisms were *Salmonella, Pseudomonas* and *Enterobacter*. The morphological, biochemical and cultural characteristics of these organisms were shown in Table I and Table II.

Heavy metal tolerance: In gram positive isolates, the Micrococcus has the lowest MIC of Cadmium which is 50μg/ml. Chromium has the highest MIC of 400μg/ml in all gram positive isolates, while the lowest MIC of Mercury was observed in S.aureus which is 200μg/ml.

In gram negative isolates, the Enterobacter has

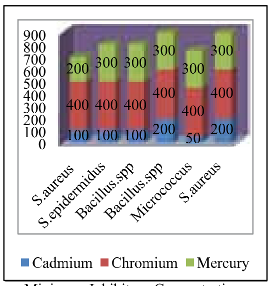
Table V: MIC of different heavy metals

Iso-lates	Different concentration of Metals														
	Cadmium					Chromium					Mercury				
	100 µg/ml	200 µg/ml	300 µg/ml	400 µg/ml	500 µg/ml	100 µg/ml	200 µg/ml	300 µg/ml	400 µg/ml	500 µg/ml	100 µg/ml	200 µg/ml	300 µg/ml	400 µg/ml	500 µg/ml
S1a	-	-	-	-	-	+	+	+	-	-	+	-	-	-	-
S1b	-	-	-	-	-	+	+	+	-	-	+	+	-	-	-
S2a	-	-	-	-	-	+	+	+	-	-	+	+	-	-	-
S2b	+	-	-	-	-	+	+	+	-	-	+	+	-	-	-
S3a	-	-	-	-	-	+	+	+	-	-	+	+	-	-	-
S3b	+	-	-	-	-	+	+	+	-	-	+	+	-	-	-
S4	+	-	-	-	-	+	+	-	-	-	+	-	-	-	-
S5	+	-	-	-	-	+	+	-	-	-	+	-	-	-	-
S6	-	-	-	-	-	+	+	-	-	-	+	+	+	+	-

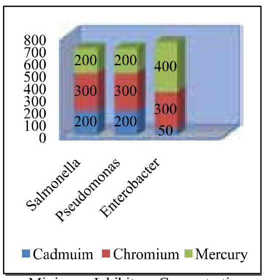
Table: 2 ; Note: (+) = Growth: (-) = No Growth

the lowest MIC of Cadmium which is 400µg/ml and the highest MIC of Mercury which is 400µg/ml. In all gram negative isolates, the MIC of Chromium is 300µg/ml. The metal tolerance and MIC of heavy metals shown by graph and table.

Different concentrations of Heavy Metals



Minimum Inhibitory Concentration
Of Heavy Metals of Gram Positive Isolates



Minimum Inhibitory Concentration
Of Heavy Metals of Gram Negative Isolates

Antibiotic sensitivity: All gram positive isolates were highly sensitive to Vancomycin and Ampicillin. Gram negative isolates were sensitive to Ciprofloxacin and Nalidixic acid. The results of Antibiotic resistance were shown in table III and table IV

DISCUSSION

In present study the species of Staphylococcus showed minimum tolerance against Cadmium i.e. 50µg/ml and against Mercury i.e. 400µg/ml and showed maximum tolerance against Chromium i.e. 300µg/ml. they were also sensitive to many antibiotics like Vancomycin, ampicillin and Lincomycin and showed resistant to common antibiotic like penicillin and Rifampin. Shakoori, *et al.*, (2000) isolated gram positive organism that isolate could tolerate a concentration of dichromate 80 mg ml-1. In this study the isolate micrococcus showed no tolerance against Cadmium even at 50µg/ml and showed maximum tolerance against chromium at concentration 300µg/ml and against mercury at concentration 200µg/ml, it was also resistant to many antibiotics like Penicillin, Ampicillin, Rifampin and Lincomycin except Vancomycin. In present study the *Bacillus species* showed the same results of metal tolerance as other gram positive isolates. One *Bacillus spp* was tolerated 100µg/ml concentration of Cadmium and the other species showed no growth at this concentration. On the other hand *Bacillus spp* tolerated high concentration of Chromium i.e. 300µg/ml and Mercury 200µg/ml. These Bacillus species were also resistant to Penicillin, Rifampin and Lincomycin, but sensitive to Vancomycin and Ampicillin. According to Chaturvedi (2011) study they isolated Bacillus strain, was found to tolerate Cr (VI) concentration as high as 4500 mg/L. According to Abbas, *et al.*, (2014) study, their all the isolates were resistant to Penicillin and Rifampin. Only one strain showed sensitivity against Tetracycline, Gentamycin and Ciprofloxacin and it was resistant to Amoxicillin, Penicillin, Cephalexin, Erythromycin and Streptomycin. In present study among gram negative isolates the isolates Salmonella and Pseudomonas tolerated least concentration of Cadmium and Mercury i.e. 100 µmg/ml and high concentration of Chromium i.e. 200µg/ml. They were also resistant to antibiotics Cephalexin, Streptomycin, Nalidixic acid, Trimethoprim except Ciprofloxacin. In this study the isolate Enterobacter was the only organism that could tolerate the highest concentration of Mercury i.e. 400µmg/ml. It could not tolerate the least concentration of Cadmium i.e. 50µg/ml and its growth was noted at 200µmg/ml concentration of Chromium. In the same way it was highly resistant to many antibiotics Cephalexin, Streptomycin, Trimethoprim and Ciprofloxacin and only sensitive to Nalidixic acid. Raja, *et al.*, (2009) studied sewage bacteria and observed that among the heavy metals Arsenic and Nickel were less toxic to isolates, on the other hand Chromium and Mercury were highly toxic to all isolates and no growth was seen. Present study showed that the Cadmium and Mercury were highly toxic to all isolates except Enterobacter and Chromium was less toxic to all isolates and they tolerate high concentrations of Chromium. Similarly most of the isolates were resistant to most of the antibiotics except Vancomycin and Ampicillin. In contrast Filail *et al.* (1999) studies waste water isolates that were *Pseudomonas. aeroginosa. Klebsiella. pneuoniae, Proteus. mirabilis* and *Staphylococcus* these all strain are resistant to heavy metals and antibiotics.(Filali, *et al.*, 2000).

CONCLUSION:

The industrial waste water along with other impurities also contains different toxic metals. These toxic metals are very lethal for all types of living organisms from microscopic organisms to higher animals. So it is very necessary to properly treat this contaminated water before dumping into sea or river to protect aquatic and land organisms form the exposure of these dangerous metals. Researchers are busy to find out easy and cheap method to get rid from this heavy metal contamination. Bioremediation is the solution of the problem. Microorganisms living in such contaminated environment adapted several mechanisms to absorb these toxic metals from the waste water and convert them into less toxic form. It is very helpful to use these microbes for bioremediation process because no harmful substances are produce in turn. Bioremediation is increasing and becomes a popular technique for treating our environment to make its free from hazardous pollutants.

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