Original Article

Antibiotic Susceptibility in metal tolerant *Pseudomonas Spp.* from Tannery waste water

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ABSTRACT: In the present study total ten heavy metal resistant *Pseudomonas* sp were isolated from Tannery waste water from Unnao (U.P) India, against Nickel, Chromium and Zinc. All the isolates exhibited high resistance to heavy metals with minimum inhibitory concentration (MIC) for heavy metals ranging from 100µg/ml to 1200µg/ml. All isolates showed multiple tolerances to heavy metal and were multi antibiotic resistant. Heavy Metal Tolerance Test indicated maximum microbial tolerance of *Pseudomonas* sp (Ps-6&9) to Nickel (400 µg/ml) and no growth was found at 200 µg/ml to Zinc. All the Pseudomonas isolates were found to have a wide range of multi-drug and multi-metal resistant property indicating the tannery waste effluents are highly enriched which supports and spread of Antibiotics and heavy Metal tolerant microbes in the environment.

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INTRODUCTION

Heavy metal is described as those elements that occur at very low levels of few parts per million in a given system and having atomic density greater than 5g-cm3 [1]. Heavy metal pollution is a global environment problem because heavy metals cannot be degraded or destroyed and hence, tends to accumulate in soil and water. The non-biodegradability of heavy metal is responsible for their long term persistent in environment and subsequent bio-accumulation in food chain [2-3].

Many industries can generate heavy metal pollution such as electroplating industry, mining industry, and leather industry, tanning industry, pigment manufacturing and dye industry. Large number of heavy metals is released from tanneries, chromium with other heavy metals is found in high level in waste water in tanneries.

Presence of such metal (Cr, Ni, Cd etc) and other metal ions is substantially toxic to plants, animals as well as micro-organisms [4].

Microbes develop various types of resistance mechanism in response to heavy metals and keep their cell from homeostasis against the damage induced by heavy metals [5]. Antibiotics are widely used in human and veterinary medicines for disease treatment and prophylaxis.

Presence of antibiotic in waste water treatment plants are well source of antibiotic [6]. Due to their long exposure to antibiotics, microbes develop resistance to which they were originally susceptible. Micro-organism can produce enzyme that destroy the antibiotic activity or modify the plasma membrane which reduces its permeability [7].

Micro-organism resistant to metal and antibiotic appears because of exposure to metal and antibiotic contaminated environment, which cause coincidental and co selection of resistance factor for both heavy Metal and antibiotics. Heavy metal and antibiotic resistance is generally confined to plasmid DNA of bacteria, but sometimes it can also be coupled to chromosomal DNA [8, 9]. To survive under metal stressed condition bacteria, develop several types of mechanism to tolerate the uptake of heavy metals ions. This study was performed to determine the antibiotic and heavy metal resistance pattern of bacterial spp. of *Pseudomonas*, which were isolated from tannery waste water.

MATERIALS AND METHODS

Collection of Tannery effluent from CTEP

The tannery effluent samples were collected in a sterilized conical flask (capacity 2L) from the two-step aeration lagoon i.e., initial and final CTEP of tannery industries located at Unnao (UP), India.

Isolation and identification of heavy metal resistant bacteria

For the selective isolation of heavy metals resistant bacteria, heavy metals incorporated media were used. The *Pseudomonas* isolation agar incorporated with heavy metals like Cr^{6+}, Ni^{2+}, and Zn^{2+} were prepared. The concentration of each heavy metal was maintained at 50 µg/ml of the medium. The wastewater sample directly streaked on (P.I.A.) media and incubated at 37°C for 24 h. After the incubation period the plates were observed for any kind of growth on the media. The isolated and distinct colonies of *Pseudomonas* spp. on the selective media were sub cultured and obtain in the form of pure culture and identified on the basis of their morphology and biochemical characters.

Determination of Minimum Inhibitory Concentration (MIC)

A varying mode of growth pattern was recorded among all Nickel, Zinc and Chromium tolerant isolates of *Pseudomonas* from Tannery waste water at their varying concentrations. *Pseudomonas* isolates showed full, moderate and less growth at varying concentration of Nickel, Chromium and Zinc.

Determination of antibiotic sensitivity and resistance pattern

Determination of antibiotic sensitivity and resistance pattern Antibiotic sensitivity and resistance of the isolated heavy metal resistant isolates were assayed according to the Kirby-Bauer disc diffusion method given by Bauer *et al.* (1966) [10]. 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.

RESULTS AND CONCLUSION

Isolation and identification of heavy metals resistant bacteria

Zinc, Nickel and Chromium Resistant *Pseudomonas* populations were recorded from the Tannery Wastewater (Site-I, II and III) at their varying concentrations (50, 100, 200, 400, 800, 1200 µg/ml).

A decreasing trend was observed in viable count of metal tolerant *Pseudomonas spp* with increase in metal concentration as compared to control. Maximum population was observed 1.95×10^5 CFU/ml and minimum by 1.2×10^4 CFU/ ml at50 and 400 µg/ml against nickel respectively. Zinc was observed more toxic than Nickel. Chromium showed highest toxicity to *Pseudomonas* population by 1×10^4 CFU/ml at 50 µg/ml concentration. Control Plate showed higher number of viable count as -compared to the viable number on metal amended plates. (Table 1, Fig. 1)

Table 1: Viable Count of Metal Tolerant *Pseudomonas spp.* Isolated from the Tannery Waste Water

Metal	Concentration (µg/ml)	Cfu/ml
Control	No Metal	2.2×10^5
Ni^{3+}	50	1.95×10^5
	100	8×10^4
	200	1.9×10^4
	400	1.2×10^4
	800	-
	1200	-
Zn^{+2}	50	4.2×10^4
	100	3.5×10^4
	200	2×10^4
	400	-
	800	-
	1200	-
Cr^{+6}	50	1×10^4
	100	6×10^4
	200	
	400	
	800	
	1200	
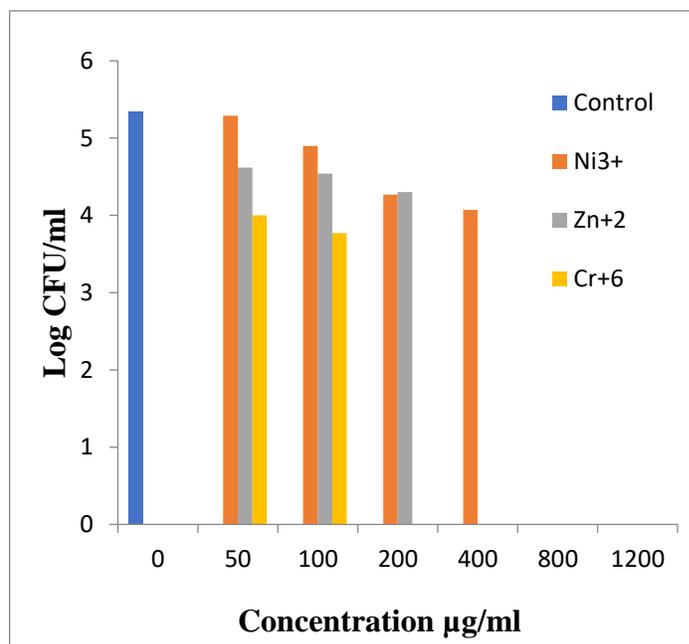
Fig. 1: Viable count of metal tolerant population of *Pseudomonas spp.* from Tannery waste water.

Determination of Minimum Inhibitory Concentration (MIC)

A varying mode of growth pattern was recorded among all Nickel, Zinc and Chromium tolerant isolates of *Pseudomonas* from Tannery waste water at their varying concentrations. *Pseudomonas* isolates showed full, moderate and less growth at varying concentration of Nickel, Chromium and Zinc. (Table 2) Isolate number PS-02, 03, 06 and 09 showed full growth at 100µg/ml concentration against Nickel. Isolates PS- 01, 03, 04, 06 and 10 exhibited less growth at 200 µg/ml concentration of Chromium. No growth was observed at higher concentrations 800-1600 µg/ml against Nickel, Zinc and Chromium. In case of Zinc, isolate PS-02, 04 and 09 showed less growth at 100 µg/ml concentrations, Isolates PS-01 and 05 were completely inhibited at 100µg/ml of Zinc. (Table 2).

Table 2: Growth patterns of *Pseudomonas* isolates against heavy metals isolated from the Tannery waste water

Metals	Conc. (µg/ml)	Isolates									
		Ps-1	Ps-2	Ps-3	Ps-4	Ps-5	Ps-6	Ps-7	Ps-8	Ps-9	Ps-10
Ni^{+3}	100	++	+++	+++	++	+	+++	++	++	+++	+
	200	+	+	++	+	+	+++	++	-	+++	+
	400	++	+	+	-	+	+++	++	-	+++	-
	800	-	-	-	-	-	-	-	-	-	-
	1200	-	-	-	-	-	-	-	-	-	-
Cr^{+6}	100	++	++	+	+++	++	+	++	+	+++	+++
	200	++	++	++	++	+++	++	-	-	+++	+
	400	-	-	-	-	-	-	-	-	-	-
	800	-	-	-	-	-	-	-	-	-	-
	1200	-	-	-	-	-	-	-	-	-	-
Zn^{+2}	100	-	+	++	+	-	++	++	++	++	++
	200	-	-	-	-	-	-	-	-	-	-
	400	-	-	-	-	-	-	-	-	-	-
	800	-	-	-	-	-	-	-	-	-	-
	1200	-	-	-	-	-	-	-	-	-	-

Antibiotic sensitivity of heavy metals resistant isolates

Pseudomonas isolates showed a variable resistance against different antibiotics tested. All the isolates showed 100% resistance against Oxacillin, 30% resistance to both Amoxycillin and Ampicillin.

All the isolates were recorded sensitive against Gentamycin, ofloxacin, Kanamycin, Streptomycin, Ciprofloxacin, Erythromycin and Tetracycline. (Table 3, Fig. 2)

Table 3: Percentage of Resistance against Antibiotics in 10 heavy metals tolerant isolates of *Pseudomonas* isolates isolated from tannery waste water

| Antibiotics	Concentration (µg/disc)	No. of resistant strains	Percentage
Ciprofloxacin	5	0	0
Oxacillin	1	10	100
Amoxycillin	10	3	30
Ofloxacin	2	0	0
Kanamycin	30	0	0
Streptomycin	25	0	0
Ampicillin	10	3	30
Erythromycin	15	0	0
Tetracycline	10	0	0
Gentamycin	50	0	0
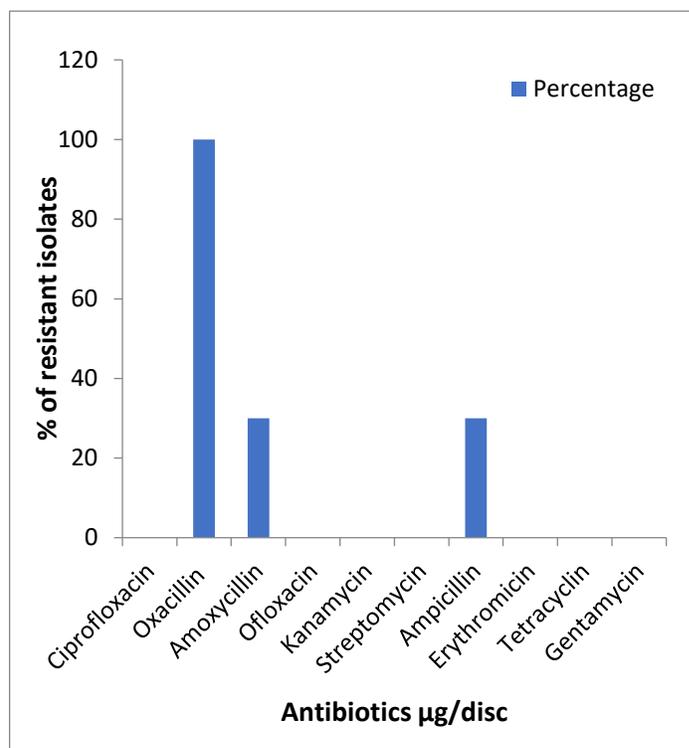
Fig. 2: Frequency of antibiotic resistance among metal tolerant *Pseudomonas* isolates isolated from Tannery waste water

DISCUSSION

Heavy metal pollution is ubiquitous and contamination of the aquatic system by toxic heavy metal is a serious pollution problem [11]. This study investigated the concentrations of heavy metals in water samples from Tannery waste water at Unnao city. In this study, the metals detected exceeded the permissible limit which supports microbial life in aquatic environment. In line to this investigation, Oyetibo *et al.,* (2010) reported that the concentrations of the heavy metals obtained for the water sample from Alaro River generally exceeded maximum allowed limits [12].

The results indicated the influence of human activity on abundances of heavy metals in water samples. Sampling site, which contain high concentrations of heavy metals was a potential source for metal-resistant bacteria. It is expected that such environment foster adaptation and selection for heavy metal resistance.

In this study, a slight decline in *Pseudomonas* population was seen in all the metal supplemented media as compared to the control without metal amendment. Hence, the lower population count suggested that the presence of metal in the medium has the inhibition effect on the bacterial growth. The bacterial growth decreased with the increase in concentration of heavy metals showing toxic effect of the heavy metals on growth of bacteria [13]. The effect of increasing concentrations (0, 50, 100, 200, 400 and 800 μg mL^{-1}) of heavy metals on the growth of the *Pseudomonas* population observed that as the concentration of heavy metal increased the number of metal resisting bacteria decreased.

The efficiency of the different bacterial isolates to grow in a specific metal containing medium varied with the type of the bacterial isolates. This varying response of the isolates to a specific tested metal could be owing to variations in cell wall structure of different bacterial isolates or as a result of difference in resistance mechanisms [14]. Yet, the decrease in bacterial growth in the presence of increased concentration of the metals used in this study was evident from the toxicity of metals in this study.

MIC determined for the metals were between 100 μg/ml and 400 μg/ml, varied depending on the metal and *Pseudomonas* isolates. Based on MIC, among three heavy metals, maximum tolerance was shown to Nickel, showing growth of *Pseudomonas* isolates up to 400 µg/ml and minimum tolerance to chromium (VI) showing no growth of *Pseudomonas* isolates *above* 200 µg/ml concentration.

Pseudomonas isolates showed high resistance of 400 µg/ml MIC to both nickel and zinc and minimum resistant of 200 µg/ml to chromium incorporated medium this result supports the finding of Virender Singh *et al.,* (2010) who showed that maximum microbial tolerance of *Pseudomonas spp.* to Copper (300 µg/ml) and lowest to Chromium (60 µg/ml) [15]. This study showed a high incidence of metal resistance for the *Pseudomonas* isolates. Many bacterial species isolated from industrial zones had been shown to develop resistance to heavy metals [16].

The antibiotic susceptibility testing of *Pseudomonas* isolates isolated from Tannery waste water in our study showed that a considerable proportion was resistant to antibiotics. All the isolates of *Pseudomonas* were sensitive to Ciprofloxacin and Ofloxacin supports the finding of Singh *et al.,* (2010) which showed tolerance against all antibiotics except Ciprofloxacin. High resistance of *Pseudomonas* Isolates in this study to Kanamycin and Tetracycline supports the finding of Virender Singh *et al.,* (2010) who showed that 75 % and 50% of *Pseudomonas* isolates from waste water treatment plant demonstrated antibiotic resistance to Kanamycin and Tetracycline respectively [16].

CONCLUSION

Bacteria resistant to both heavy metals and antibiotics can readily be isolated from the natural environment, such as Blue Nile River, Bahir Dar, Ethiopia. In this study it is evident that these bacteria resistant to high levels of heavy metals proved the contamination of the study location by metal species. Further, the concentrations of heavy metals detected (Cu^{2+}, Zn^{2+} and Cr^{6+}) slightly exceeded the permissible limits as per the United State Environmental Protection Agency (USEPA) guideline for maximum contamination levels for heavy metal concentration in aquatic life. From this study, it can be concluded that all isolates were resistant to eight of metals tested. Out of the eight metal resistant isolates, seven of them were resistant to more than one antibiotic. The statistical analysis carried out showed a high correlation between the resistance of heavy metals and antibiotics tested. However, further study is needed to recognize the specific resistance determinants involved in the association between metal exposure and antibiotic resistance.

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CONFLICT OF INTEREST

No conflict of interests

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