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OCCURRENCE OF PLASMID LINKED MULTIPLE DRUG RESISTANCE IN BACTERIAL ISOLATES OF TANNERY EFFLUENT

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Abstract

Effluents of three different tanneries (T-1, T-2, & T-3) were investigated to isolate and scrutinize antibiotic, chromate and salinity resistant bacteria. Total 18 isolates of 9 different bacterial genera were screened out and identified; some strains established in all effluents. Amongst the three effluents tested; T-1 exhibited largest population of all isolates compared to T-2 and T-3 effluents. The T-1 effluent contained largest 4.4 $\times 10^6$ cfu/ml population of *Pseudomonas aeruginosa* followed by 3.9 $\times 10^6$ cfu/ml in T-2 effluent. The lowest 0.7 $\times 10^6$ cfu/ml count of *Aeromonas* spp. was recorded in T-3 effluent. Furthermore, antibiotic susceptibility tests were performed with 7 antibiotics which include ampicillin, sulfafurazole, ciprofloxacin, norfloxacin, tetracycline and amikacin. Three strains of *P. aeruginosa* and one strain of *Escherichia coli* deserved as multiple drug resistant (MDR). The *P. aeruginosa*T-3 and *E. coli*T-1 showed strongest MDR feature for 5 antibiotics. The response of chromate (50, 100, 200, 250 and 300 µg/ml) and NaCl concentrations (20, 40, 60 and 80 g/l) was incredible for 4 MDR isolates. Nearly each strain showed tolerance up to 300 µg/ml of chromate and 80 g/l of NaCl. The *P. aeruginosa*T-1, *P. aeruginosa*T-2, *P. aeruginosa*T-3 and *E. coli*T-1 were most tolerant isolates. Plasmid profiling of resistant strains was conducted with agarose gel electrophoresis. As consequence, plasmids from two strains of *P. aeruginosa* and *E. coli*T-1 represented different bands. At least for confirmation of plasmids nature; these were transformed and transformants were screened on medium having antibiotics. The study of plasmid transformation has confirmed the plasmid mediated resistance in isolates.

Key words: Tannery effluent, Antibiotics, Chromium, Drug resistance, Halotolerance.

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INTRODUCTION

Environmental pollution has been a major irritant to the racing industrial development. Kanpur is one of the prominent industrial cities in India, well known especially for tanneries. Out of the 2500 tanneries in the country, a cluster of about 350 are located near holy river Ganga in Kanpur district and about 75,000 tonnes of cow and buffalo hides are processed annually (13). The effluents are released to river Ganga and during many religious occasions a huge crowd of human's bath with contaminated water (26) that creates major risk for infections.

Chromium is a considerable environmental concern as it is widely used in leather tanning, electroplating, metal finishing and chromate preparation (1, 36). Extensive use of chromium (Cr) in industries has resulted in chromium contaminated soil and ground water at production sites (28, 38). The generation of pollution is significantly high in the pre-tanning operations compared to post-tanning operations (25). The tanning industry discharges effluents containing chrome salts in excess of the maximum permissible limits into environment (16). The maximum permissible level of Cr in potable and industrial wastewater is 0.1 mg/l (11).

The tannery effluents are rich in large concentrations of nutrients including inorganic nitrogen (N), N-rich organic residues (11) and sulfides. Apart from nutrients and the organic material, that releases valuable nutrients on decomposition, tannery effluent contain large amounts of Cr, pathogens and toxic organic components; all of which pose serious threats to the environment (28). Sludge deposition from such effluents, therefore, provides a natural environment for enrichment of chromium-resistant bacteria.

A number of chromium-resistant microorganisms have been reported, including *Pseudomonas* spp. (23, 24), *Desulfovibrio* sp. (22), *Enterobacter* spp. (3), *Escherichia coli* (31), *Bacillus* spp. (7) and several other bacterial isolates (14, 18). Certain colonizers of the aquatic environment, such as *Pseudomonas* spp., possess a pronounced capacity for acquisition and dissemination of resistance genes. Strains that belong to this genus are infact frequently resistant to several antimicrobial agents, with susceptibility patterns similar to those of clinical strains (27).

Tannery effluent contains large number of antibiotic resistant pathogens and toxic organic components; all of which pose serious threats to the environment (8, 34). Also at risk are aquatic ecosystems, which are largely controlled by and dependent upon, microbial organisms. Resistance of virulent bacteria in the environment due to widely used medical and veterinary prescribed antibiotics poses a potential hazard to humans and livestock (21). Antibiotic resistant strains reach in the environment through manure, liquid manure of animals, human excretions (2) as well as through animal hide in tanneries and their effluents. Although numerous chromium-resistant microorganisms from such chromium contaminated sediments have been isolated by several investigators (4, 27) but cooperative study was lacking.

The objective of the present study was firstly to define the population diversity of bacterial community that flou-

rish in the tannery effluents and secondly screening of antibiotic resistant, chromate resistant and halotolerant bacteria.

MATERIALS AND METHODS

Sample collection

Effluent samples from three different tanneries (T-1, T-2 and T-3) located in the Kanpur city (geographic coordinates: 26.467° North and 80.350° East) were collected (Figure 1). Each sample was replicated three times during sampling. Autoclavable plastic bottles with tense screw caps were employed for sample collection and samples were taken from the foremost outlet channels. After collecting samples, bottles were kept in frost box for transportation to laboratory. Samples were stored at 4 ^{0}C and all microbial examinations were performed within 8 hours from the collection.

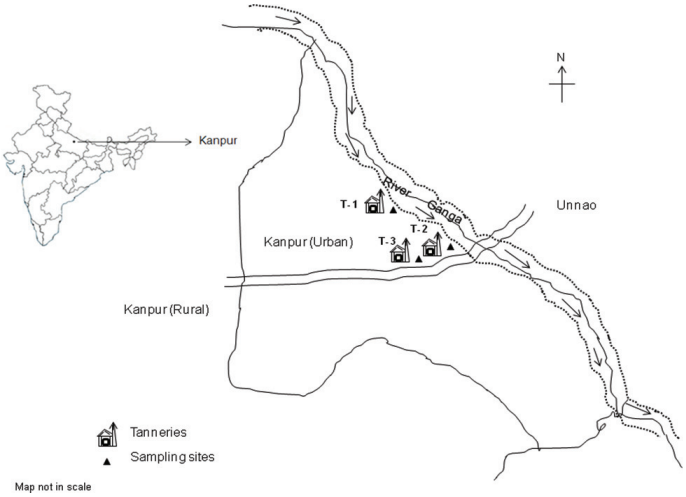

Figure 1. Showing different locations of tanneries and sampling sites for effluent collection at Kanpur city.

Isolation and purification of bacteria

For isolation and enumeration of bacteria, standard spread plate technique (3) was employed using nutrient agar medium (HiMedia Ltd.). The effluent samples were serially diluted using sterile water and a definite amount of a particular dilution was poured on LB agar (tryptone, 10 g/l; yeast extract, 5 g/l; NaCl, 10 g/l glucose, 0.1 g/l). After overnight incubation (37^{o}C), total numbers of colonies were counted to conclude colony forming units (cfu) in accurate volume of effluent. For purification of isolates, multi-streaking was performed repeatedly.

Identification of bacteria

The genera of different isolates were confirmed on the basis of diverse biochemical tests (3) which include: cell and colony morphology, Gram staining, motility, oxidase and catalase activities, indole test, Voges-Proskauer test, citrate reactions, gelatinase activity, nitrate reduction, urease test, glucose oxidation and various carbohydrate fermentations. The purified strains were recognized according to the criteria prescribed in Bergey's Manual of Systematic Bacteriology (17).

Antibiotic susceptibility test by disc diffusion method

Antimicrobial susceptibility pattern of different isolates was tested by standard disc diffusion method of Bauer *et al.* (5) on Mueller Hinton agar (MHA; HiMedia Ltd.) plates using commercial antibiotic discs (HiMedia Ltd.). Different antibiotics of variable strength employed were: ampicillin (10 mg) sulfafurazole (30 mg), ciprofloxacin (5 mg), norfloxacin (10 mg), tetracycline (30 mg), gentamicin (10 mg) and amikacin (30 mg). According to the inhibitory zone diameter around discs, isolates were categorised as sensitive and resistant followed by the criteria of Clinical Laboratory Standards Institute (9). The *Escherichia coli* ATCC 25922 were used as reference strain in all antimicrobial testing**.**

Determination of chromium tolerance

To determine the potential of chromate tolerance isolates were grown in nutrient broth (NB) medium containing different concentrations (50, 100, 200, 250 and 300 µg/ml) of $K_2Cr_2O_7$ at 37 ^{0}C for three days. Growth of the isolates was deliberated (4) by measuring optical density with Spectronic genesys-6 spectrophotometer (Thermoelectron Corporation, USA) at 540 nm using uninoculated broth as control in thermo stated quartz quvettes. Growth of each isolate was intended and articulated as percent with reference to the unsupplemented set as 100 %.

Determination of salinity tolerance

For determination of salinity forbearance, isolates were grown-up in Luria Bertani broth prepared by adding different levels of NaCl including 20, 40, 60 and 80 g/l in four replicates. Later on incubation growth of every isolate was measured spectrophotometrically as like to aforementioned chromate tolerance analysis.

Plasmid isolation

Isolation of plasmids (from three *P. aeruginosa* and one *E. coli* strains) was performed using standard alkaline lysis protocol (29). Accordingly three complex solutions were prepared; solution-I (2µM Tris-HCl, pH 8.0; 50 mM glucose; 10 mM EDTA, pH 8.0), solution-II (1% SDS; 0.2N NaOH) and solution-III (5M glacial acetic acid; 5M potassium acetate). Two ml of overnight grown cultures were separately centrifuged (10,000×g) for 10 min in a cooling centrifuge (Remi Pvt. Ltd. India). Thus bacterial pellet obtained were suspended in the solution-I and cells were lysed by the treatment of solution-II. Furthermore, the precipitation of genomic DNA was completed by the solution-III followed by re-centrifugation as aforementioned. The supernatant with plasmid DNA was extracted twice with phenol and chloroform then after treated with ethanol (for DNA precipitation) and RNAse (20µl/ml). It was finally mixed in 50 µl TE buffer (pH 8.0) and the plasmid DNA obtained was run on 0.8% agarose gel electrophoresis using ethidium bromide. The gel slab was pictured by gel documentation system (Bangalore Gennei, India).

Transformation and screening

To confirm the nature of isolated plasmids, these were transformed into non-resistant *E. coli* strains. For this purified plasmid DNA isolated from resistant strains were used for transformation into calcium chloride treated cells (29) of *E. coli* strains. The screening of transformed cells was carried out by streaking on LB agar containing pre used concentrations of different antibiotics separately. Thus, growth of cells on the medium was confirmatory to decide transformants.

RESULTS

Bacterial profile in effluents

Succession of various bacterial species was observed in the effluent samples collected from three distinct tanneries. It was observed that *Escherichia coli, Enterobacter* spp. and *Pseudomonas aeruginosa* were present commonly in each tannery (T-1, T-2 & T-3) effluent, while, *Enterobacter* spp. and *Proteus* spp. were found in T-1 and T-2 effluents. Similarly, *Aeromonas* spp. was present only in T-2 and T-3 effluents. The *Salmonella* spp. and *Acinetobacter* spp. were found in T-1 and T-3 effluents respectively (Table 1).

Total colony forming units (cfu) and pattern of antibiotic resistance

Total bacterial population having different genus and species in each effluent sample were determined and expressed as colony forming units. The population of bacterial isolates in the effluents of tanneries were extremely differed (Table 1). Amongst the three tannery effluents tested; T-1 contained largest population (4.4×10^6 cfu/ml) of all bacterial isolates in comparison to other two effluents (T-2 and T-3). The second largest population (3.9×10^6 cfu/ml) of *P. aeruginosa* was recorded in T-2 effluent. However, the lowest population (0.7×10^6) of *Aeromonas* spp. was found in T-3 effluent. Total 18 isolates of 9 bacterial genuses were identified; some strains were common in all tanneries (Table 1). The antibiotic susceptibility pattern using 7 antibiotics (ampicillin; A, tetracycline; T, sulfafurazole; Sf, ciprofloxacin; Cf, amikacin; Ak, gentamycin; G, norfloxacin; Nx) to *E. coli, P. aeruginosa, Serratia* spp., *Enterobacter* spp., *Salmonella* spp., *Enterococcus* spp., *Aeromonas* spp., *Acinetobacter* spp. were tested. All three strains of *P. aeuroginosa* showed remarkable antibiotic resistance. The *P. aeruginosa*T-3 was resistant to five different antibiotics (A, T, Sf, G and Nx) tested, while, *P. aeruginosa*T-1 was also resistant to three antibiotics (Sf, Cf and G) of distinct classes. The *E. coli*T-1 also showed novel resistance to 5 different antibiotics which include A, T, Sf, Ak and G. Thus three strains of *P. aeruginosa* and *E. coli*T-1 are resistant to more than three distinct groups of antibiotics and referred as multiple drug resistant. However, other isolates were either strictly sensitive against all antibiotics tested or resistant to less than three class of antibiotics and could not pass the criteria of multiple drug resistance. Comparing the effectiveness of the antibiotics in regard to the different isolates of the present study, ciprofloxacin and norfloxacin were stronger against various

Table 1. Community profile of bacterial species in the effluent of three different tanneries located at Kanpur city.

Tanneries/effluent	Bacterial isolates	cfu/ml
T-1	*Enterobacter* spp.	$1.5 \pm 0.34 \times 10^6$
	Enterococcus spp.	$1.6 \pm 0.42 \times 10^6$
	Escherichia coli	$3.1 \pm 0.94 \times 10^6$
	Proteus spp.	$0.9 \pm 0.13 \times 10^6$
	Pseudomonas aeruginosa	$4.4 \pm 1.34 \times 10^6$
	Salmonella spp.	$0.6 \pm 0.11 \times 10^6$
	Serratia spp.	$1.1 \pm 0.57 \times 10^6$
T-2	*Aeromonas* spp.	$1.8 \pm 0.27 \times 10^6$
	Enterobacter spp.	$1.4 \pm 0.36 \times 10^6$
	Enterococcus spp.	$1.8 \pm 0.42 \times 10^6$
	Escherichia coli	$3.4 \pm 0.82 \times 10^6$
	Proteus spp.	$1.2 \pm 0.11 \times 10^6$
	Pseudomonas aeruginosa	$3.9 \pm 0.71 \times 10^6$
T-3	*Acinetobacter* spp.	$1.3 \pm 0.08 \times 10^6$
	Aeromonas spp.	$0.7 \pm 0.03 \times 10^6$
	Enterococcus spp.	$1.8 \pm 0.47 \times 10^6$
	Escherichia coli	$2.8 \pm 0.88 \times 10^6$
	Pseudomonas aeruginosa	$3.6 \pm 0.93 \times 10^6$

T-1, T-2 &T-3; denotes isolates of three different tanneries ±; Standard deviation (n=3)

Table 2. Antibiotic susceptibility pattern of different bacterial isolates from three different tanneries (T-1, T-2 and T-3) located at Kanpur city.

Bacterial isolates	Antibiotic susceptibility pattern	
	Sensitive	Resistant
*E.coli*T-1[#]	Cf, Nx	A, T, Sf, Ak, G
*E.coli*T-2	T, Sf, Cf, Ak, G, Nx	A
*E.coli*T-3	A, Sf, Cf, Ak, G, Nx	T
*P. aeruginosa*T-1[#]	A, T, Ak, Nx	Sf, Cf, G
*P. aeruginosa*T-2[#]	T, Cf, G	A, Sf, Ak, Nx
*P. aeruginosa*T-3[#]	Cf, Ak	A, T, Sf, G, Nx
Serratia spp.T-1	A, T, Sf, Cf, Nx	Ak, G
Enterobacter spp.T-1	Ak, T, Sf, Cf, Ak, Nx	G
Enterobacter spp.T-2	Sf, Cf, Ak, G, Nx	A, T
Salmonella spp.T-1	A, T, Cf, Ak,G	Sf, Nx
Enterococcus spp.T-1	T,Cf, Ak, G, Nx	A, Sf
Enterococcus spp.T-2	A, T, Sf, Cf, Nx	Ak, G
Enterococcus spp.T-3	A, Sf, Cf, Ak, G	T, Nx
Aeromonas spp.T-2	A, T, Sf, Cf, G, Nx	Ak
Aeromonas spp.T-3	A, T, Sf, Cf, Ak, G, Nx	….
Acinetobacter spp.T-3	T, Sf, Cf, Ak, G, Nx	A
Proteus spp.T-1	A, T, Sf, Cf, Nx	Ak, G
Proteus spp.T-2	A, T, Sf, Cf, Nx	Ak, G

A; ampicillin, T; tetracycline, Sf; sulfafurazole, Cf; ciprofloxacin, Ak; amikacin, G; gentamycin, Nx; norfloxacin.
T-1, T-2 &T-3; denotes isolates of three different tanneries
[#]Multiple drug resistant isolates

isolates. According to these observations it is obvious that, *P. aeruginosa*T-1, *P. aeruginosa*T-2, *P. aeruginosa*T-3 and *E. coli* are multiple drugs resistant to multiple class drugs. Amongst all the isolates compared, *P. aeruginosa*T-3 and *E. coli*T-1 exhibited best property of multiple drug resistance (Table 2).

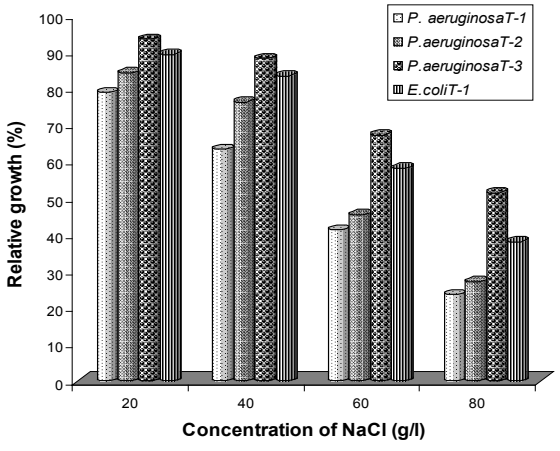

Figure 2. Salinity tolerance of different isolates based on relative growth at variable levels.

Halotolerance of isolates

Four MDR bacterial strains (*P. aeruginosa*T-1$^{Sf+Cf+G+}$, *P. aeruginosa*T-2$^{A+Sf+Ak+Nx+}$ and *P.aeruginosa*T-3$^{A+T+Sf+G+Nx+}$ and *E.coli*T-1$^{A+T+Sf+Ak+G+}$) were grown-up in four inconsistent concentrations (20, 40, 60 and 80 g/l) of NaCl to check the salinity tolerance. All strains tested were found to be salt tolerant despite showing reduced growth with the respective increase in the salt concentration. *P. aeruginosa* T-3$^{A+T+Sf+G+Nx+}$ strain showed premier growth (51.45%) and tolerance at highest 80 g/l of salt treatment. The highest tolerance with respect to the relative growth at highest 80 g/l salt treatment recorded was 51.45%, 38.07%, 27.12% and 23.60% correspondingly in *P. aeruginosa*T-3$^{A+T+Sf+G+Nx+}$, *E.coli*T-1$^{A+T+Sf+Ak+G+}$, *P.aeruginosa*T-2$^{A+Sf+Ak+Nx+}$ and *P.aeruginosa*T-1$^{Sf+Cf+G+}$ (Figure 2). The highest growth of each strain was recorded at the lowest 20g/l of NaCl concentration. The maximum 93.56% and 89.32% relative growth were found in *P.aeruginosa*T-3$^{A+T+Sf+G+Nx+}$) and *E.coli*T-1$^{A+T+Sf+Ak+G+}$ culture respectively.

Chromium tolerance of MDR isolates

The influence of varying concentrations (50, 100, 200, 250 and 300 µg/ml) of chromate ($K_2Cr_2O_7$) was observed on the growth of three different *P. aeruginosa* isolates (*P. aeruginosa*T-1$^{Sf+Cf+G+}$, *P. aeruginosa*T-2$^{A+Sf+Ak+Nx+}$ and *P. aeruginosa*T-3$^{A+T+Sf+G+Nx+}$) and *E. coli*T-1$^{A+T+Sf+Ak+G+}$ (Figure 3). The growth of each isolate was negatively affected with increase in chromate concentration. At the level of 250 µg/ml the growth was exceedingly reduced even as, only two isolates (*P. aeruginosa*T-3 $^{A+T+Sf+G+Nx+}$ and *E.coli*T-1 $^{A+T+Sf+Ak+G+}$) were able to endure 300 µg/l concentration. The highest growth during this treatment were noted by *P. aeruginosa*T-3$^{A+T+Sf+G+Nx+}$ which were 66.47%, 53.33%, 33.67%, 28.22% and 18.56% respectively in 50, 100, 200, 250 and 300 µg/ml of chromate. The *E. coli*T-1 was the next best isolate that have presented higher growth including 61.22%, 48.50%, 28.11%, 17.30% and 8.24% in 50,

100, 200, 250 and 300 µg/ml of chromate correspondingly. The lowest growth was recorded with 300 µg/ml concentration were 0.32 and 1.45% of *P. aeruginosa*T-1$^{Sf+Cf+G+}$ and *P. aeruginosa*T-2$^{A+Sf+Ak+Nx+}$ strains respectively. However the other isolates from the effluents could not tolerate this level of chromate concentration (data not shown).

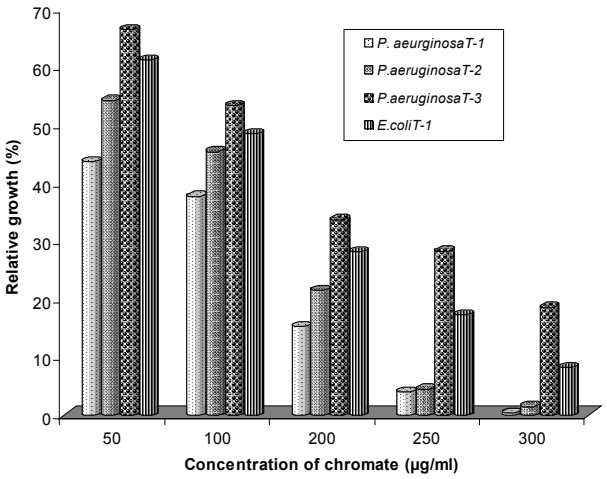

Figure 3. Chromium tolerance of different isolates on the basis of their relative growth in variable levels of chromate.

Plasmid profile of MDR bacteria

Agarose gel electrophoresis was performed to get plasmid profile of four MDR bacterial isolates. It was observed that *P.aeruginosa*T-1$^{Sf+Cf+G+}$ and *P.aeruginosa*T-2$^{A+Sf+Ak+Nx+}$ exhibited single band of plasmid DNA each of different molecular weight. However rest of the two strains *P.aeruginosa*T-3$^{A+T+Sf+G+Nx+}$ and *E.coli*T-1$^{A+T+Sf+Ak+G+}$ represented three different bands of distinct molecular weights (Figure 4).

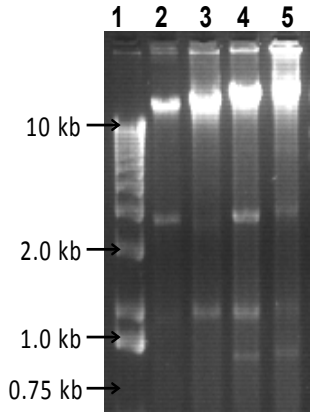

Figure 4. Plasmid profile of four multiple antimicrobial drug resistant bacteria including *P.aeruginosa*T-1$^{Sf+Cf+G+}$ (lane 2), *P. aeruginosa* T- 2$^{A+Sf+Ak+Nx+}$ (lane 3) *P. aeruginosa*T-3$^{A+T+Sf+G+Nx+}$ (lane 4) and *E. coli* T- 1$^{A+T+Sf+Ak+G+}$ (lane 5). The lane 1 shows 1kb standard DNA ladder.

Confirmation of transformants

For the confirmation of tansformants, the plasmid treated cells were grown on the medium containing antibiotics for screening. It was evident that only transformed cells were able to grow with different concentrations of antibiotic groups. This has confirmed that the property of drug resistance in the present isolates is plasmid mediated. Because of this, cells which have received plasmids via transformation were able to endure dosage of antibiotics.

DISCUSSION

A combined cooperative study was performed including studies on multiple drug resistance, chromate resistance and halotolerance along with plasmid profiling of scrutinized isolates from tannery effluent. It is well acknowledged that wide application of antibiotics in human and veterinary medicine has led to large-scale dissemination of bacteria resistant to antibiotics in the environment. Antibiotic resistant strains reach the environment through different channels and sources (27). The addition of resistant isolates through the animal hide to the tanneries and their effluents may be important channel. The increase of resistant bacteria can also be attributed to the antibiotic substances found with increasing frequency in sewage (20). In general some bacteria with the most characteristic resistance behaviour are isolated from areas frequently contaminated with antimicrobial substances (27, 12). In this report, numerous Gram negative and Gram positive bacteria isolated from tannery effluents are reported that show antibiotic resistance. Contrary to our expectations we have achieved the isolates tolerable to higher concentrations of the chromate and NaCl, which were not tested and reported elsewhere in the literature. The tolerance of higher concentrations by the isolates might be due to the facilitated use of chromate and NaCl at increased levels by the concerned tanneries. Particularly, significant is the finding that three *P. aeruginosa* isolates and another *E. coli* isolates were found to be not only halotolerant, chromium tolerant but also multiple drug resistant. In our understanding, this is the first joint report of multiple drug resistant isolates from tannery industries.

An amazing achievement of the study was indeed, multiple drug resistance of isolates. This feature of isolates is possible due to the imprudent use of broad spectrum antibiotics during the cure of animals. Accordingly, based on the observations of antibiotic sensitivity it was found that *P. aeruginosa*T-1$^{Sf+Cf+G+}$, *P. aeruginosa*T-2$^{A+Sf+Ak+Nx+}$, *P. aeruginosa*T-3$^{A+T+Sf+G+Nx+}$ and *E. coli*T-1 are multiple drug resistant to multiple drug classes. However, *P. aeruginosa*T-3$^{A+T+Sf+G+Nx+}$ was one of the most resistant bacteria which has shown highest multiple drug resistance. Similar observations were also attributed by Shrivastava *et al.* (32). They have suggested that *P. aeruginosa* is notorious for its resistance to antibiotics and is particularly a dangerous and dreaded pathogen. The natural resistance of bacterium to many antibiotics is due to the permeability barrier afforded by its outer membrane lipopolysaccharides (32). In addition to its nutritional versatility, *P. aeruginosa* resists high concentrations of salt, dyes, weak antiseptics, and many commonly used antibiotics. Moreover, *Pseudomonas* spp. maintains antibiotic resistance plasmids, both R Factors and RTFs (36). Already it has been mentioned that only a few antibiotics are effective against *Pseudo-*

monas spp. (15) and even not against all strains. Similar observations were also evident in three isolates of *P. aeruginosa*T-1$^{Sf+Cf+G+}$, *P. aeruginosa*T-2$^{A+Sf+Ak+Nx+}$ and *P. aeruginosa*T-3$^{A+T+Sf+G+Nx+}$ reported different patterns of antibiotic resistance with different antibiotics.

One of the major factors has been mentioned in literature as contributing to the resistance: mutation in common genes that thereby extend resistant genes among diverse microorganisms through plasmids (10). Plasmid borne resistance was determined in the different isolates by plasmid DNA profiling of the MDR strains. *P. aeruginosa*T-3 strain that was resistant to most of the antibiotics and chromate showed three distinct bands of plasmid. The presence of distinct plasmids is the cause of multiple drug resistance. Another fact also exerted in the study is that the same MDR isolates also presented the chromate and salinity tolerance. These properties might be also plasmid encoded phenomenon.

On other hand, extensive use of chromium in various industrial applications has caused significant environmental contamination. Industrial effluents are generally characterized by the coexistence of a large number of toxic and nontoxic cations and therefore, it is necessary to study multiple metal effects on the physiology, biochemistry and resistance pattern of microorganisms (39). The advantage of selecting for indigenous bacteria from contaminated environments may be the minimization of inhibitory effects from other compounds that may be present along with Cr (VI). Since, viable indigenous organisms will have developed some degree of resistance to these compounds (35).

In the present study, total nine bacterial isolates were isolated from three different tannery effluents having chromium resistance property. Similar trends in bacterial populations (cfu) of the Cr-contaminated sediments were also reported by Luli *et al.* (19) and Losi and Frankenberger (18). Furthermore, when screening of these isolates was performed, five strains were found chromate resistant to more than 250 µg/ml chromate. Of these five isolates, *P. aeruginosa* and *E. coli* spp. were found extremely resistant up to 300 µg/ml of chromate. Our findings are in the agreement to the Camargo *et al.* (6). Shakoori *et al.* (30) has also isolated chromate resistant *Bacillus* spp. from tannery effluent. Although various other attempts (18, 21) were also made to isolate chromate resistant isolates from tannery effluents, microorganisms only resistant at lower concentrations of Cr are reported when compared to our findings. The chromate resistance reported may be possibly due to exclusion of metal species, production of low molecular weight binding proteins, transformation, bioaccumulation, etc. (33, 37). Basu *et al.* (4) have reported that heavy metal resistance is linked with antibiotic-resistance; such type of incidence can be attributed in our finding also. The resistance pattern of chromate and antibiotics studied for different isolates appears to be correlated with each other.

The effluents studied are extremely contaminated with chromium and salinity tolerant multiple drug resistant bacteria. The major concern with the present findings is that *P. aeruginosa* and *E. coli* found in all effluents were most resistant isolates to chromium and salinity with a feature of multiple drug resistance. The multiple drugs, chromium and salinity resistance was found plasmid mediated feature in isolates.

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Other articles in this theme issue include references (40-67).

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