Antibiotic and Chromium Resistant *Escherichia coli* Isolated from the Tannery Effluent

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Abstract— The effluents of the five different tanneries (TE-1, TE-2, T-3, TE-4 and TE-5) were investigated for the determination of *Escherichia coli* population along with to check the antibiotic, chromate and salinity resistance in isolates. All the samples contained high population of *Escherichia coli* isolates. The highest population was observed from the sample of tannery-2 (TE-2) which was 3.8 ± 0.96 ×106 cfu/mL. The *Escherichia coli* TE-2 isolate from TE-2 represented highest tolerance of chromate and salinity. This strain too exhibited the characteristics of multiple drug resistance (MDR) against several anti-microbial agents. Since the tannery discharges have huge population of harmful, antibiotic resistant strains, their discharge in water reservoirs and frequently in open environment should be processed prior to its release.

Key words: Tannery effluent; Antibiotics; Chromium; Multiple drug resistance; *Escherichia coli*

I. INTRODUCTION

Tannery industries are well known for its microbial system resistant to broad spectrum drugs and high concentration of chromate and salinity. Kanpur is one of the prominent industrial cities in India, well known for huge number of tanneries. Various tanaries are located near holy river Ganga in Kanpur and Unnao districst and about 75,000 tonnes of cow and buffalo hides are processed annually (Gupta and Sinha, 2006). The effluent released in open environment, which created great health concerns to human, and animal both as the risk of microbial infections (Ram et al., 2007).

High concentrations of chromium are widely used in the processing of tides including tanning, electroplating, metal finishing and chromate preparation (Altaf et al., 2008). The tanning industry discharges effluents containing chrome salts above to the maximum permissible limits (Khasim and Nand Kumar, 1989) which, is 0.1 mg/l Cr for potable and industrial wastewater (Goyal et al., 2003).

In addition tannery effluent can contain large amounts of Cr, pathogens and toxic organic components; all of which pose serious threats to the environment (Alvarez et al., 1999). Sludge deposition from such effluents, therefore, provides a natural environment for enrichment of chromium-resistant bacteria (Naraian et al., 2012). Number of chromium-resistant microorganisms have been reported, including *Escherichia coli* (Shen and Wang, 1993) sustain in the tannery effluents. Tannery effluent contains large number of antibiotic resistant pathogens and toxic organic components; all of which pose serious threats to the environment (Clark, 1994). Also at risk are aquatic ecosystems, which are largely controlled by and dependent upon, microbial organisms.

Therefore, the present study was designed to evaluate the population and evaluation of antibiotic, chromate and halo tolerance in *Escherichia coli* isolates from tannery effluents.

II. MATERIAL AND METHODS

A. Sample Collection

Samples of effluents from five different tanneries (TE-1, TE-2, TE-3, TE-4 and T-5) located in the Kanpur city were collected and transported to laboratory in autoclavable plastic bottles with tight screw caps in ice box. Samples were stored at 4 ^{o}C and all microbial examinations were performed within 8 hours from the collection.

B. Isolation and Purification of Bacteria

solation and purification of bacteria was conducted employing standard spread plate technique (APHA, 1992) on nutrient agar medium (HiMedia Ltd.). A definite volume of serially diluted effluent samples 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 enumerated to determine colony forming units (cfu) in accurate volume of effluent.

C. Identification of Bacteria

Identification of the purified strains was performed according to the criteria prescribed in Bergey's Manual of Systematic Bacteriology (Krieg, 2005). The isolates were confirmed on the basis of distinct biochemical tests including: cell and colony morphology, Gram staining, motility, oxidase and catalase activities, indole, Voges-Proskauer, citrate reactions, gelatinase activity, nitrate reduction, urease test, glucose oxidation and various carbohydrate fermentations.

D. Antibiotic Susceptibility Test

Antimicrobial susceptibility of different isolates was investigated by the disc diffusion method of Bauer et al. (1966) on Mueller Hinton agar (MHA; HiMedia Ltd.) plates using commercial antibiotic discs (HiMedia Ltd.). The antibiotics employed were: ampicillin (10 mg) sulfafurazole (30 mg), ciprofloxacin (5 mg), norfloxacin (10 mg), tetracycline (30 mg), gentamicin (10 mg) and amikacin (30 mg). The isolates were categorised as sensitive and resistant followed by the criteria of Clinical Laboratory Standards Institute (2005).

E. Determination of Chromium Tolerance

To determine the influence of chromate tolerance isolates, isolates were grown in nutrient broth (NB) medium added with different concentrations (50, 100, 200, 250 and 300 µg/ml) of $K_2Cr_2O_7$ at 37 ^{0}C for three days. Cell growth of the isolates was determined (Basu et al., 1997) through Spectronic genesys-6 spectrophotometer at 540 nm and growth was expressed in percent with reference to control.

F. Determination of Salinity Tolerance

The influence of salinity was also investigated by growing isolate in Luria Bertani broth supplemented with different

levels (10, 15, 30, 40, and 80 g/L) of NaCl. The growth of all isolates was measured spectrophotometrically and expressed in percent (%).

III. RESULTS

A. Total Colony Forming Units (CFU)

Total colony forming units (cfu) were determined from the samples collected from five different tanaeries. The total population of bacterial isolates in different effluents of tanneries were tremendously differed (Table 1). Amongst the five tannery effluents investigated in the study; TE-2 represented highest 3.8 ± 0.96 $\times 10^6$ cfu. The *Escherichia coli* isolated from the TE-5 effluent showed second largest cfu (3.2 $\pm 0.62 \times 10^6$). Data obtained are presented in the table 1.

Tanneries/effluent	Bacterial isolates	cfu/ml
TE-1	*Escherichia coli*TE1	2.1 $\pm 0.81 \times 10^6$
TE-2	*Escherichia coli*TE2	3.8 ± 0.96 $\times 10^6$
TE-3	*Escherichia coli*TE3	2.9 $\pm 0.73 \times 10^6$
TE-4	*Escherichia coli*TE4	1.9 $\pm 0.57 \times 10^6$
TE-5	*Escherichia coli*TE5	3.2 $\pm 0.62 \times 10^6$

Table 1: Community profile of bacterial species in the effluent of three different tanneries located at Kanpur city.
TE-1, TE-2,TE-3, TE-4 &TE-5; denotes effluent samples from five different tanneries
±; Standard deviation (n=3)

B. Antibiotic Resistance in Different Isolates

The antibiotic susceptibility for five different isolates was conducted employing 7 variable antibiotics (ampicillin; A, tetracycline; T, sulfafurazole; Sf, ciprofloxacin; C, amikacin; Ak, gentamycin; G, norfloxacin; Nx). The results based on the nature of isolates are represented as sensitive and resistant. The *Escherichia coli* TE-2 isolate form the sample of tannery TE-2 showed remarkable resistance against maximum antibiotics used. The *Escherichia coli* TE-2 found resistant to five different antibiotics (A, T, Sf, G and Nx) of variable group., while, the *Escherichia coli* TE-3 showed lowest resistance with only amikacin. In comparisons to the effectiveness of the antibiotics about the different isolates of the present study, ciprofloxacin and norfloxacin were stronger against various isolates. Therefore, based on the observations *Escherichia coli* TE-2 represented a characteristic of multiple drug resistance (Table 2).

Bacterial isolates	Antibiotic susceptibility pattern	
	Sensitive	Resistant
E.coli TE-1	A, Sf, Cf, Ak, G, Nx	T, G
E.coli TE-2[#]	Cf, Nx, Sf	A, T, Nx, Ak G
E.coli TE-3	T, Sf, Cf, A, G, Nx	Ak
E.coli TE-4	Sf, Cf, Ak, G, Nx	A, T
E.coli TE-5	A, T, Ak, Nx,Cf	Sf, G

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

C. Chromium Tolerance

The influence of different concentrations (50, 100, 200, 250 and 300 µg/ml) of $K_2Cr_2O_7$ was analysed on the growth of five different *E. coli* isolates. Increasing the concentrations of chromate in the medium, growth of each isolate was negatively influenced and it was much reduced at 300 µg/l concentrations. The optimal growths of all five isolates were recorded with the treatment of 200 µg/l chromate concentration; while it was reduced at their higher concentrations. The lowest growth of almost every isolate was observed in the treatment on 300 µg/l chromate (Figure 1.).

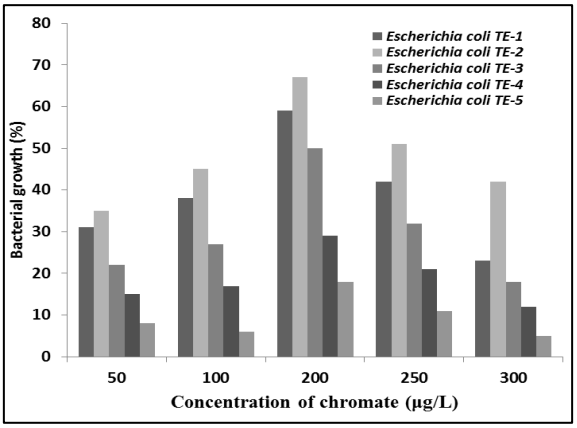

Fig. 1: Tolerance of different concentrations of chromate on the relative growth of different *E. coli* isolates.

D. Salinity Tolerance of Isolates

The salinity tolerance of five *Escherichia coli* isolates was tested. All isolates were cultured on different concentrations of the NaCl and their relative growth with reference to evaluate the the salinity tolerance was investigated. All strains tested were found to be salt tolerant despite showing reduced growth with the respective increase in the salt concentration. The highest tolerance in form of the relative growth at highest 80g/l salt treatment recorded was 54 %, in *Escherichia coli* TE-2 which was followed to the45 % of *Escherichia coli* TE-1. However, the lowest tolerance (27 %) was recorded in *Escherichia coli* TE-5 (Figure 1). As compared, all the tested isolates showed their optimal growth at 30 (%) salt treatment and further it was declined in their higher concentrations. In consequence, it was obvious that the *Escherichia coli* TE-2 was one of the most salt tolerant bacterial isolate and showed maximum growth in response to the salt treatments at variable levels (Figure 2).

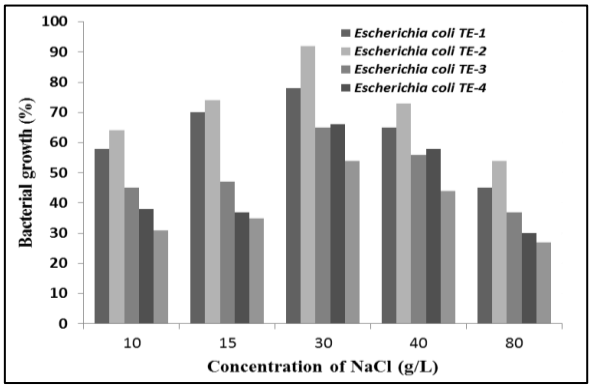

Fig. 2: Tolerance of different concentrations of salinity on the relative growth of different *E. coli* isolates.

IV. DISCUSSION

Wide application of antibiotics in human and veterinary medicine has led to large-scale dissemination of bacteria resistant to antibiotics in the environment. Most often antibiotic resistant microbial strains reach the environment through different channels (Reinthaler et al., 2003). The addition of resistant isolates through the animal hide to the tanneries and their effluents may be important channel (Naraian et al., 2012). In general some resistant bacteria are isolated from areas contaminated with antimicrobial substances (Reinthaler et al., 2003). In the present study five (05) *E. coli* isolates were isolated from five different tannery effluents. The charecteristics of antibiotics resistance, salinity tolerance and chromate resistance investigated showed remarkabke results. Over to the expectations we have achieved the *E. coli* isolates tolerable to higher concentrations of the chromate and NaCl, which significant achievement of the study. 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 (Naraian et al., 2012). In addition *E. coli* isolates was found to be not only halotolerant, chromium tolerant but also multiple drug resistance.

As the effluents of all five tanneries tested contained high population of *E. coli* isolates flourishing in the high concentrations of chromate, salt and the antibiotics. Therefore, in the present it was found that *E. coli* strain surviving in the TE-2 had characteristics of the multiple drug resistance. This is only possible due to the imprudent use of broad spectrum antibiotics during the cure of animals. Shrivastava et al. (2004) also attributed similar observations. 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 (Shrivastava et al., 2004). The presence of distinct plasmids is the cause of multiple drug resistance (Naraian et al., 2012).

V. CONCLUSIONS

Based on the findings of the present study it can be concluded that the effluents studied are extremely contaminated with chromium and salinity tolerant multiple drug resistant *Escherichia coli*. The presence of multiple drug resistant *E. coli* is a serious environmental problem. Therefore, release of such type of industrial effluents; should be strictly prohibited to protect our ecosystem and especially the human and animal health.

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