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Research Article

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Isolates of tannery effluent and their antibiogram from effluent plant in South India

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ABSTRACT

The tanning industry was designated as "Red category" due to the high pollution contributed to environment by its solid and liquid wastes the toxicity by heavy metals is spreading throughout the world along with industrial progress. The present study deals with isolation, identification and characterization of isolated from tannery effluent collected in and around Erode, South India. A total of 60 isolates were screened from tannery effluent. Antibiotic sensitivity pattern was studied using disk diffusion method. Most of the bacterial strains were sensitive sensitive to cotrimoxazole, gentamycin, kanamycin, nalidixic acid, ampicillin, and Penicillin showed resistance against bacteria. Staph aureus was 50% resistant to amikacin, 49% resistant to gentamycin and norfloxacin. Bacillus spp. demonstrated higher resistance for Ciprofloxacin similarly E.coli had 49.7% resistance to clindamycin. P.aeruginosa exhibited very high resistance for colistin and norfloxacin. The two major dominant isolates were Pseudomonas spp. and Bacillus spp., Staph aureus was found to be in subdominant form. It is found that domestic and industrial effluents are responsible for the development of bacterial resistance with the risk of human health and environment.

Keywords: Leather, Bacterial resistance, Antibiotics.

INTRODUCTION

In the past few decades, uncontrolled urbanization has caused a serious pollution problem due to the disposal of sewage and industrial effluents to water bodies. About 2500 tanneries located in different tanning centers of India with a total processing capacity of 600000 tones of hides/skins per year and an estimation of about 80000 m^3 /day of waste water being discharged from these tanneries have been reported. Unlike many other pollutants released into environment, heavy metals are difficult to remove from the environment [1]. Tannery effluent generated wastes frequently accumulate in the environment and is under increasing pressure from solid and liquid emanating from the leather industry. Microorganisms are screened and isolated from tannery wastes by various co-workers [2] Aquatic microbes become resistant to antibiotics and metals as a result of contamination with effluents [3]. Many studies have so far have focused on the occurrence, pathogenicity and control of the pathogens by disinfection and disposal of waste water treatment. Tanning is one of the industrial processes and the discharge of untreated water into open land causes great deterioration of ground water quality besides various sub processes in tanning like bathing, pickling, and dyeing cause water pollution. These effluents change the physical and chemical nature of the soil that ultimately affects the productivity and microbial community.

Antibiotic resistance in bacteria is more frequently associated and strongly correlated with metal resistance [4]. Pathogenic microorganisms from tannery effluents have been identified and reported [5], the propensity to all the groups of antibiotics have not been screened. From drinking water bacterial species that was tolerant to metals and antibiotics had been isolated [6]. The leather processing and manufacturing unit involves a variety of aggressive chemicals that consumes large quantity of water of which about 90% is discharged as waste water. The significant increase of Multiple Antibiotic Resistant (MAR) bacteria are observed in various aquatic systems and human infections caused by these bacteria could be difficult to treat with available drugs [7, 8]. Tannin which is present in the effluent is a soluble phenolic compound with high molecular weight that combines with organic compounds to form an insoluble complex and enriches the growth of microorganisms. Different microorganisms are isolated from tannery effluents [9]. The resistance development may be due to nonspecific mechanism with gene regulation of plasmids and chromosomes, which may be heritable or transferable due to the presence of resistance (R-factor) factor [10]. The pathogenic strains isolated varied from waste to waste of different effluents [11]. This study aimed to screen for the microorganisms from the tannery effluent and characterize its antibiogram to know the prevalence of resistance pathogens.

EXPERIMENTAL SECTION

Isolation of bacteria

The tannery effluent samples were collected from different tannery units in and around Erode, South India. The samples were collected in a sterile plastic container and transported to laboratory for bacteriological analysis. About 60 bacterial isolates were screened on Nutrient Agar (NA) plates by the standard pour plate method. Plates were incubated at 37°C /24h and colonies differing in morphological characteristics were selected and used for further studies.

Identification and Characterization of the tannery effluent bacteria

Selected tannery effluent isolates were grown on MacConkey agar (Himedia, India). The shape and color of the colonies were examined under the microscope after Gram staining. Isolates were biochemically analyzed for the activities of Oxidase, Catalase, MR-VP test, Urease test, Motility, Indole production and Citrate utilization. The tests were used to identify the isolates according to Bergey's Manual of Determinative bacteriology [12, 13].

Determination of Antibiotic resistance

The antibiotic resistance was done by standard agar disc diffusion method on MHA using commercial discs (Himedia, Mumbai) [14, 15]. 100 µl of fresh bacterial cultures were spread on MHA. The following antibiotics amikacin 10 µg, chloramphenicol 30 µg, ceftazidime 30 µg, ciprofloxacin 10 µg, ceftriaxone 30 µg, colistin 10 µg, gentamycin 10 µg, norfloxacin 10 µg, neomycin 30 µg, netillin 10 µg, ofloxacin 5 µg, sparfloxacin 5 µg, tetracyclin 30 µg, tobramycin 10 µg, ampicillin 10 µg, clindamycin 10 µg, cloxacillin 30 µg, cotrimoxazole 25 µg, methicillin 5 µg, penicillin 10 µg, vancomycin10 µg, were dispensed on the plate. The plates were incubated at 37ºC/24h. Inhibition zones in diameters were measured in mm. Strains were classified as Resistant (R), Intermediate (I) and Susceptible (S) according to the criteria recommended by the national committee for clinical Laboratory Standards, 2000[16].

RESULTS AND DISCUSSION

In the present study, the raw effluent of the tannery industry from South India was collected and their physico-chemical character was analyzed. All the parameter values like acidity, alkalinity and hardness were found relatively higher above than the tolerant limit. The mean pH value of the raw effluent was 8.2 slightly alkaline in nature. The mean and COD values are higher and found to be 18.81 mg/ml and 34.26 mg/ml respectively and lower than the tolerance limit. The bacterial colonies were isolated from the tannery effluent and dilutions ranging from 10^{-4} to 10^{-6} plated gave countable colonies, but the growth of the colonies decreased when the dilution factor increased. Most of the bacterial strains were to cotrimoxazole, gentamycin, kanamycin, nalidixic acid, ampicillin and penicillin showed resistance against bacteria. The strains were observed to be less resistant for most of the antibiotics tested. *Staph aureus* was 50% resistant to amikacin, 49% resistant to gentamycin and norfloxacin. *Bacillus* spp. demonstrated higher resistance for Ciprofloxacin similarly *E.coli* had 49.7% resistance to clindamycin. *P.aeruginosa* exhibited very high resistance for colistin and norfloxacin. The two major dominant isolates were *Pseudomonas* spp. and *Bacillus* spp. but *Staph aureus* was found to be in subdominant form. We tried to characterize for the production of

extended spectrum beta lactamase by double disk synergy test but the number is not significant accounting only 2-3% of the total isolates and no metallo beta lactamase producing strain was screened with imipenem.

Table-1: Isolates from tannery effluent and their antibiogram

Antibiotics	Concentration	Number of resistance isolates for each antibiotic			
		Bacillus spp. Total no.(60)	*E.coli* Total no.(60)	*P.aeruginosa* Total no.(60)	*Staph aureus* Total no.(60)
Amikacin	10	21	18	16	25
Chloramphenicol	30	23	20	16	21
Ceftazidime	30	19	19	24	19
Ciprofloxacin	10	25	20	11	19
Ceftriaxone	30	20	21	10	21
Colistin	10	19	8	27	22
Gentamycin	10	14	17	10	23
Norfloxacin	10	22	19	26	23
Neomycin	30	24	20	12	20
Netillin	10	17	22	22	19
Ofloxacin	5	20	16	11	21
Sparfloxacin	5	22	21	24	24
Tetracycline	30	19	18	16	21
Tobramycin	10	23	19	24	19
Ampicillin	10	8	10	21	19
Clindamycin	10	22	23	19	21
Cloxacillin	30	14	21	21	21
Cotrimoxazole	25	13	18	21	20
Methicillin	5	14	19	-	20
Penicillin	10	10	20	19	17
Vancomycin	10	-	-	-	14

The *Pseudomonas* spp. isolated predominantly might have been present in the skin and also the raw water used for the treatment [17] reported its surveillance in chromium solution of tannary treatment units. The organisms were found mostly sensitive to ceftazidime, colistin, norfloxacin, netillin, sparfloxacin and tobramycin. Fur and skin layer might have contributed for the *Bacillus* spp isolate. This organism isolated from tannery effluent had been used for the treatment of effluent. It was sensitive to chloramphenicol, ciprofloxacin, kanamycin, and neomycin unlike *Pseudomonas* spp. Bacterial species isolated from industrial zone in general have contributed for resistance to heavy metals [18, 19]. Besides tannary effluents and waste waters high in chromium are removed by biological means using barks and ashes of *Azadirachta indica, Syzygium cumini* and *Acacia Arabica* and can be counteracted [20].There are also studies of iron nanoparticles for detoxification of chromium content in water bodies[21]. Instead of treating the multidrug resistant isolates with synthetic compounds phytocomponents from plants are effective and good natural resource [22]. They have been reported to be resistant for gentamycin and penicillin [14] and if this resistance disseminates will be a threat to normal environmental isolates [23]. Raw water used for the treatment of skin and wool should be the contributing source for the *E.coli* being isolated. Chloramphenicol, ceftazidime, ciprofloxacin and many other antibiotics show sensitivity against the isolated strains. *Lao et al*., has reported the presence of antibiotic resistant plasmid harboring *E.coli* from tannery effluent water. *Staph aureus* generally have been present in epidermal and dermal layers of the animal's skin. The isolate was screened from tannary effluent and antigenic structure was detected [5]. The tannery effluents are serving as an enriched source to the growth and spread of microbial population that are resistant to different antibiotics not used in the treatment of the unit. This study helps in the identification of resistance against different antibiotics that may contribute to acquire resistance. The quality of ground water has deteriorated in many places in south India [24] and if resistant pathogens are enriched in the water resources will be a threat in future.It is a useful tool for the simultaneous monitoring of several resistant pathogens in the environment. The horizontal transfer of these genes to sensitive organism will ameliorate the existing scenario of drug resistance. It is a clear indication that domestic and industrial effluents are responsible for the development of bacterial resistance with the risk of human health and environment and has to regularly monitored.

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REFERENCES

[1] WX Ren; PJ Li; Y Geng; XJ Li, *Journal Hazardous materials*., **2009**, 167(1-3): 164-169.
[2] KV Loa; K Padmini, *Appl. Environ.Microbiol*., **1976**, 10,243-245.
[3] JF Timoney; J Port; J Giles; I Spanier, *Appl. Environ.Microbiol*., **1978**, 36: 465-472.
[4] JB Bell; GE Elliot, *Appl. Environ.Microbiol*., **1983**, 6:227-232.
[5] Abdella ; Erer. *J. of Environ Science*., **1992**, 45-53.
[6] JJ Calomiris; JL Armstrong; RJ Seidler *Appl. Environ.Microbiol*., **1984**, 47:1238-1242.
[7] G Dicuonzo; G Gherardi; G Lorino ; S Angeletti; F Battistoni; L Bertuccini, *FEMS microbial letters,*. **2001** ,201:205-211.
[8] MFS Lopes; T Riberio; M Abrantes; JJF Marques; R Tenreirom; MTB Crespo,*Int. J. Food Microbiol.*, **2005**, 103:191-198.
[9] DS Bhakani; ML Dhar; MM Dhar; BN Dawan; CN Mehrotra, *J. Environmental Biology*., **1969** (7):250-262.
[10] S Silver; M Walderhang. *Microbiol Rev*., **1992**, 5:195-228.
[11] DR Cooper, *J.S.L.T.C*, **1973,** 57 :19-25.
[12] JG Holt; NR Krig; PH. A Sneath; JT Staley; ST Williams, .Bergey's manual of determinative bacteriology (9th edn).Baltimore, Maryland:Williams andWilkins. **1994**
[13] A Manero; Blanch AR, *Appl. Environ.Microbiol*., **1999**, 65:4425-4430.
[14] AW Bauer; WM Kirby; JC Sherries; M Truck, *J.Clin. Pathol*., **1996**,45:493-495.
[15] AL Barry; C Thornsberry,*Manual of clinical microbiology*. **1981**, pp: 561-574.
[16] National Committee for Clinical Laboratory Standards: Performance Standards for antimicrobial disc susceptibility tests. 7 th ed. Approved standards M2-A6. National Committee for Clinical Laboratory Standards, Wayne Pa: **2000**.
[17] AM Osborn; K Bruce; P Strike; D. Ritchie; P Strike; DA Ritchie, *FEMS microbial Rev*., **1997,** 19:239-262.
[18] MI Ansari; A Malik, Bio Res Technol., **2007**, 98: 3149 -3153.
[19] A Tamil Selvi; E Anjugam ; R Archana Devi; B Madhan ; S Kannappan; B Chandrasekaran, *Asian J. Exp. Bio. Sci*., **2012**, 3 (1): 34 – 41
[20] V Krinshna Veni; K Ravindhranath, *J. Chem. Pharm. Res*., **2012**, 4(1):656-668.
[21] V Madhavi; A Vijaya Bhaskar Reddy; K Gangadhara Reddy; G Madhavi, *J. Chem. Pharm. Res*., **2012**, 4(3): 1539-1545.
[22] EA Makky ; M Mashitah Yusoff; MM Ibrahim, *J. Chem. Pharm. Res*.,**2012**,4(1):881-893.
[23] PW Ramteke, *Ind J. Microbiol*., **1997**, 37:177-181.
[24] C Balamurugan; MS Dheenadayalan, *J. Chem. Pharm. Res*., **2012**, 4(3):1632-1637.