

Influence of tannery wastes and the surrounding environment of tannery industries on microbial growth and proliferation

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The present study was carried out to assess the degree of microbiological proliferation in tannery wastes and the surrounding environment of the tannery industries. In this regard, a total of 8 tannery waste (n=4) and environmental (n=4) samples were tested. All the samples contained a huge load of bacteria and fungi in an average of 10^8 cfu/g or ml. An extended numbers of pathogenic bacteria were recovered. Among the pathogenic bacteria, *Staphylococcus* spp. was predominant. Most of the samples exhibited the presence of *Pseudomonas* spp. *Salmonella* spp. and fecal coliform were found each in one sample. *Bacillus* spp., *Escherichia coli*, *Klebsiella* spp. and *Vibrio* spp. were found in few samples. The average load of the pathogens was 10^4 cfu/g or ml. All the pathogenic isolates were found to be multidrug resistant. Higher resistance was found against penicillin and streptomycin. Tannery waste after lather treatment sample showed antibacterial activity against all the pathogens tested. Overall, presence of pathogenic microorganisms with multidrug resistance traits may pose serious public health threats.

Key words: Tannery wastes; Microbiological proliferation; Drug resistance; Antibacterial activity

Industrial wastes are usually originated from various industrial processes, and the amount and toxicity of waste released from industrial activities varies with the industrial processes. Noticeably, among all the industrial wastes tannery effluents are ranked as the highest pollutants (1-3). Industrial pollution remains one of the major problems of Bangladesh, especially in the urban areas where effluent discharge practices are yet too primitive (4, 5). Industrial units including tannery are mostly located along the banks of the rivers vastly due to the ease of transportation for incoming raw materials and outgoing finished products (5). These industrial units can act in generating a considerable amount of pollution by discharging untreated effluents directly into the environment (4, 5).

Tanning industry generates large quantities of solid wastes during the leather manufacturing process and subsequently during effluent treatment. During this process, different wastes such as meat separated from skin, fat tissue, hair, sludge, and chromium are generated (6). Tannery effluent is among one of the most hazardous industrial pollutants. Major problems are due to wastewater containing heavy metals, toxic chemicals, chloride and lime with high dissolved and suspended salts and other pollutants which may severely affect the growth of living organisms including microorganisms (7-9). The contamination of metals is a

major environmental problem, especially for aquatic environment (10-14). Metals that remain in contaminated sediments in the surrounding environment of tannery industry may accumulate in microorganisms that can enter into the food chain and eventually affect human well-being (15).

In addition, the organic pollutants present in tannery wastewater are not completely degraded even after the secondary treatment process and support the growth of pathogenic microbes including coliform, Streptococci, Staphylococci, anaerobic spore forming bacilli, and many other types of health hazards organisms. These pathogens can easily spread into the aquatic environment as well as the surrounding environment along with tannery wastewater and cause serious health hazards to living organisms (16). Despite large advances in water and wastewater treatment processes, waterborne diseases still pose a major worldwide threat to public health. There are several reports available regarding the presence of a variety of organic pollutants and pathogenic microbes including the drug resistant ones in various types of industrial wastewaters (16-19).

Considering all these facts, present study endeavored to estimate the possible proliferation of pathogenic microorganisms in the wastes disposed by the tannery industries and the surrounding environment of the industrial units. The present study was also carried out to determine the antibiotic resistance of the bacterial isolates. Moreover, antibacterial traits of the wastes and environmental samples were determined.

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MATERIALS AND METHODS

Study area, sampling and sample processing. Four different types of tannery waste samples (tannery wastes after lather treatment, CaCO₃ containing wastes, liquid tannery wastes and solid tannery wastes) and four different environmental samples (surface water from lake, dry soil sample from waste dumping area, solid wastes from dumping area and wet soil sample from waste dumping area) were randomly collected from a tannery industry located at Hazaribagh, Dhaka, Bangladesh and its surrounding environment from November 2015 to January 2016 following standard protocol. For the identification and enumeration of pathogenic bacteria and fungi, 10g or ml of each sample was blended with 90 ml of buffer peptone water (pH 7.2 ± 0.2) and diluted up to 10⁻⁶ for all the samples tested according to the standard guideline (20-24).

Isolation and identification of bacteria

Estimation of total viable bacteria, *Escherichia coli*, *Klebsiella* spp., *Staphylococcus* spp., *Bacillus* spp. and *Pseudomonas* spp. For each of the cases, 0.1 ml of samples from the dilution (10⁻⁴ and 10⁻⁶) for the solid and (10⁻² and 10⁻⁴) for the liquid was introduced on to the nutrient agar and sabouraud dextrose agar for the isolation of total viable bacteria and fungi, respectively. Likewise, each sample was introduced onto MacConkey agar, mannitol salt agar (MSA), starch agar and *Pseudomonas* agar for the isolation of coliforms (*Escherichia coli* and *Klebsiella* spp.), *Staphylococcus* spp., *Bacillus* spp. and *Pseudomonas* spp., consecutively. All the plates were then incubated at 37 °C for 24 hours (20-24).

Isolation of *Salmonella* spp., *Shigella* spp. and *Vibrio* spp. By considering the possible occurrence of viable but non-culturable (VBNC) cells (24-28), 10 ml of sample was transferred into 90 ml of selenite cysteine broth (SCB) and alkaline peptone water (APW) for the enrichment of *Salmonella* spp., *Shigella* spp., and *Vibrio* spp., respectively and incubated at 37 °C for 6 hours. After incubation, the samples were diluted up to 10⁻⁴ and then 0.1 ml of samples from each of the 10⁻² and 10⁻⁴ dilutions were spread onto *Salmonella-Shigella* (SS) agar and thiosulfate citrate bile salt sucrose (TCBS) agar for the isolation of *Salmonella* spp. & *Shigella* spp., and *Vibrio* spp., consecutively. Plates were incubated at 37 °C for 48 hours for the detection of typical colonies.

Antibiotic susceptibility test of the isolates. The standard agar-disc-diffusion method (Kirby Bauer technique) was used to examine the antibiotic

susceptibility of the isolates (either sensitive or resistance) on Mueller-Hinton agar (Difco, Detroit, MI) (24, 29-31). The antibiotic discs used in this experiment were Ampicillin (AMP, 10 µg), Imipenem (IPM, 10 µg), Azithromycin (AZI, 15µg), Penicillin (PEN, 10µg), Gentamycin (GEN, 10 µg), Streptomycin (STM, 10µg), Erythromycin (ERY, 15µg), Ciprofloxacin (CIP, 5µg), Cefixime (CFX, 5µg) and Chloramphenicol (CHL, 10 µg). The plates were then inverted and incubated at 37 °C for 24 hours. After incubation, the plates were examined and the zone of inhibition was measured in mm.

Antimicrobial assay. For the determination of antimicrobial activity, modified agar well diffusion method was followed using Mueller-Hinton agar plate (24, 32, 33). Suspensions of different bacteria such as *E. coli*, *Pseudomonas* spp., *Micrococcus* spp., *Vibrio* spp., *Klebsiella* spp., *Staphylococcus aureus*, and *Salmonella* spp. was introduced on to the MHA agar were prepared using normal saline, consisting of 10⁸ cfu/ml with a turbidity equivalent to that of the 0.5 mL McFarland standard, and each suspension was then subject to lawn on the Muller-Hinton agar (MHA) (Oxoid Ltd., Basingstoke, Hampshire, England). The wells were dug (8 mm²) on the inoculated Muller Hinton agar medium and 100µl or 11mg/ml of each sample were introduced. Normal saline was used as negative controls whereas antibiotic disk of Gentamycin (GEN, 10 µg) was used as positive control. The plates were incubated at 37 °C overnight and examined for the zone of inhibition. The diameter of the inhibition zone was measured in mm using slide calipers.

RESULTS AND DISCUSSION

Determination of the presence of pathogenic bacteria from the tannery wastes and environmental samples tested. Proliferation of drug resistant bacteria in the aquatic environment has been drastically accelerated due to the unregulated disposal of industrial, hospital and domestic wastes containing organic substances along with toxic chemicals (18, 34-39). In this study, all the samples were found to contain huge load of viable bacteria in

TABLE 1. Isolation and quantification of pathogenic bacteria from tannery waste and environmental samples

Sample	Microbial load (cfu/ml or g)									
	TVB	Fungi	<i>E. coli</i>	<i>Klebsiella</i> spp.	Fecal coliform	* <i>Salmonella</i> spp.	<i>Pseudomonas</i> spp.	<i>Bacillus</i> spp	* <i>Vibrio</i> spp.	<i>Staphylococcus</i> spp.
tannery wastes after lather treatment	5.0×10 ⁸	3.2×10 ⁸	0	0	0	0	1.8×10 ⁶	0	0	2.0×10 ⁶
CaCO ₃ containing wastes	3.2×10 ⁸	1.4×10 ⁸	2.5×10 ⁵	0	4.2×10 ⁴	0	2.5×10 ⁷	2.9×10 ⁵	7.2×10 ⁵	1.6×10 ⁷
liquid tannery wastes	1.5×10 ⁸	4.8×10 ⁸	2.8×10 ³	2.9×10 ⁴	0	0	0	1.8×10 ⁵	4.9×10 ⁴	2.6×10 ⁵
solid tannery wastes	3.4×10 ⁸	1.7×10 ⁸	0	1.2×10 ⁴	0	3.8×10 ⁴	3.9×10 ⁶	2.2×10 ⁴	1.9×10 ⁵	3.1×10 ⁶
surface water from lake	2.8×10 ⁸	5.7×10 ⁵	0	0	0	0	1.0×10 ⁶	0	0	3.7×10 ⁵
dry soil sample from waste dumping area	5.8×10 ⁸	3.8×10 ⁸	2.2×10 ⁴	0	0	0	2.2×10 ⁶	2.0×10 ⁵	0	1.8×10 ⁵
solid wastes from dumping area	4.3×10 ⁸	0	0	0	0	0	3.2×10 ⁵	0	0	5.3×10 ⁵
Wet wastes from dumping area	3.2×10 ⁸	0	0	0	0	0	2.6×10 ⁶	0	0	4.3×10 ⁵

TVB = Total viable bacteria

The experiments were in triplicates. Average count (cfu/ml or g) from all samples have been shown here.

*Bacterial load after enrichment (Prior to enrichment, the recovery was nil).

Shigella spp. was absent in all samples.

TABLE 2. Antimicrobial susceptibility pattern of different pathogenic isolates

Antibiotics	<i>E. coli</i> n=3		<i>Klebsiella</i> spp. n=2		<i>Bacillus</i> spp. n=4		<i>Vibrio</i> spp. n=3		<i>Staphylococcus</i> spp. n=8		<i>Pseudomonas</i> spp. n=7	
	R	S	R	S	R	S	R	S	R	S	R	S
AMP (10µg)	100%	0%	100%	0%	50%	50%	100%	0%	0%	100%	87.5%	0%
CIP (5µg)	33%	67%	50%	33%	25%	100%	0%	100%	50%	50%	0%	100%
STM (10µg)	100%	0%	100%	0%	50%	67%	33%	50%	100%	0%	100%	0%
IPM (30µg)	0%	100%	50%	50%	0%	100%	0%	100%	0%	100%	0%	100%
PEN (10µg)	100%	0%	100%	0%	ND	ND	100%	0%	0%	100%	57%	43%
GEN(10µg)	0%	100%	0%	100%	0%	100%	0%	100%	0%	100%	29%	71%
AZI (15µg)	0%	100%	50%	50%	50%	50%	0%	100%	0%	100%	29%	71%
CFX (5µg)	100%	0%	100%	0%	0%	100%	0%	100%	0%	100%	43%	57%
ERY (15µg)	100%	0%	100%	0%	50%	50%	100%	0%	0%	100%	29%	71%
CHL (10µg)	33%	67%	50%	50%	0%	100%	0%	100%	0%	100%	43%	57%

AMP= Ampicillin, CIP= Ciprofloxacin, STM= Streptomycin; IPM= Imipenem, ERY= Erythromycin, AZI= Azithromycin, GEN= Gentamycin, PEN= Penicillin, CFX= Cerfixime, STE= Streptomycin. CHL=Chloramphenicol
ND = Not done; N = Number of isolates; R = Resistant; S = Sensitive

average of 10^8 cfu/ml or g (Table 1) as found previously from tannery waste and different environmental samples (5, 16-20, 22, 24, 29, 40). A huge array of pathogenic microorganisms was found in tannery wastes of Bangladesh by Siddiquee et al. (41). Fungi were present in all samples in a range of 5.7×10^5 - 3.8×10^8 cfu/ml or g except solid and wet wastes of dumping area. Pathogenic bacteria were also recovered in significant quantities from all the samples, mostly in the CaCO_3 containing wastes and solid tannery wastes. Pathogenic proliferation was confirmed through the biochemical tests. *Staphylococcus* spp. were found to be present in all samples in a range 1.8×10^5 - 1.6×10^7 cfu/g or ml (Table 1). *Pseudomonas* spp. was the second prevalent bacteria as found in all samples except liquid tannery wastes. *E. coli* was absent in pharmaceutical and hospital waste water samples but resided in the other four samples within the range of 2.5×10^3 - 3.3×10^4 cfu/ml or g. *E. coli* and *Vibrio* spp. were found in three samples, whereas *Klebsiella* spp. and *Bacillus* spp. were recovered in 2 and 4 samples, respectively in an average of 10^4 cfu/g or ml. *Salmonella* spp. was detected only in solid tannery waste sample, while fecal coliform was present in CaCO_3 containing waste sample (Table 1). All the tested samples were free from *Shigella* spp. Contamination.

Elevated antibiotic resistance of the isolates. In cohort to the previous studies on surface water and wastes, present study recovered a significant number of multidrug resistant isolates (18, 22, 29, 40). Ali et al., (5) also found an extended frequency of drug resistant isolates in the tannery waste. The pathogen showed greater resistance against Penicillin (10µg) and Streptomycin (10µg), whereas sensitivity against Gentamycin (10µg) and Imepenem (30µg, Table 2).

While all the isolates showed resistance against Ampicillin (10µg), *Streptococcus* spp. showed sensitivity against the antibiotic. Although all *Vibrio* spp. and *Pseudomonas* spp. exhibited sensitivity against Ciprofloxacin (5µg), all the other bacteria showed resistance to some extent. *Staphylococcus* spp. was recovered as sensitive against most of the antibiotic tested.

Antibacterial activity of tannery waste and environmental samples. The tannery wastes are supposed to have antibacterial activity as powders of different heavy metals are used in leather processing at retanning stage to prepare bacterial growth resisting leather (42). Previous study by Nawaz et al. (42) evident the presence of antibacterial activity in tannery waste samples. In the current study, tannery waste after lather treatment sample showed antimicrobial activity against almost all organisms (Table 3). Among all the isolates, larger zone of inhibition was found against *Vibrio* spp. All the other samples exhibited almost no antibacterial activity. CaCO_3 containing waste sample imparted antibacterial activity only against *Salmonella* spp., whereas, dry soil sample from waste dumping area exhibited antibacterial traits against *Klebsiella* spp.

CONCLUSION

The findings of the present study revealed that all the samples contain huge array of microorganisms including pathogenic bacteria. All the isolates were found to be multidrug resistant. Only the solid waste after lather treatment sample exhibited antimicrobial activity. Presence of pathogenic bacteria with antibiotic resistance traits portrayed serious public health threats. As tannery waste and the surrounding ecosystem are known to contain metal pollution which are toxic to human beings,

TABLE 3. Antimicrobial activity of the samples tested

Herbal medicine samples	Zone of inhibition (mm) against test bacteria						
	<i>E. coli</i>	<i>Klebsiella</i> spp.	<i>Salmonella</i> spp.	<i>Vibrio</i> spp.	<i>Pseudomonas</i> spp.	<i>Micrococcus</i> spp.	<i>Staphylococcus</i> spp.
tannery wastes after lather treatment	11mm	12 mm	12mm	17 mm	11 mm	15 mm	11 mm
CaCO ₃ containing wastes	0	0	11 mm	0	0	0	0
liquid tannery wastes	0	0	0	0	0	0	0
solid tannery wastes	0	0	0	0	0	0	0
surface water from lake	0	0	0	0	0	0	0
dry soil sample from waste dumping area	0	16 mm	0	0	0	0	0
Dry wastes from dumping area	0	0	0	0	0	0	0
Wet wastes from dumping area	0	0	0	0	0	0	0

adherence of metals on microbial cell surface would enhance the health risk by many folds. Finally, it could be said that adequate preventive and regulatory measures should be taken in tannery industrial activities with a view to ensuring safe, sound and healthy environment for the greater benefit of Bangladesh.

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