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Multidrug resistance pattern of bacteria isolated from domestic and tannery waste

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Abstract: *The objective of this study was to screen for the microorganisms in the domestic wastes and tannery effluents and characterize it's antibiogram to know the prevalence of resistant pathogens. A total of 109 isolates of 16 different genera were isolated from 38 samples by aerobic culture method. Fourteen and ninety-five isolates were obtained from six domestic wastes and 32 tannery waste samples, respectively. The isolates belonged to the genus Micrococcus (18.3%), Alcaligens (15.6%), Staphylococcus (11.0%), Enterobacter (4.6%), Shigella (14.7%), Klebsiella (6.4%), Haemophilus (4.6%), Citrobacter (3.7%), Actinobacillus (3.7%), Escherichia (4.6%), Corynebacterium (4.6%) and others (8.2%). It was interesting to notice that most of the isolates were Gram-negative bacillus (63.3%) and few were Gram-positive cocci (36.7%). Pathogenic microorganisms from domestic wastes and tannery effluents have been identified and reported. Most of the isolates were resistant to chloramphenicol, nalidixic acid, nitrofurontoin and cefixime. Levofloxacin and imipenem were effective against 108 (99.5%) of the isolates. Multi drug resistance was observed in most of the isolates. Some isolates were found in both domestic and tannery waste samples, but their antibiotic resistance patterns were not similar. Serratia spp. and two Bacillus spp. with different antibiogram pattern were found only in tannery waste samples. The significant number of Multiple Antibiotic Resistant (MAR) bacteria was observed in both the samples. Human infections caused by these bacteria could be difficult to treat with available drugs.*

Keywords: *Antibiogram, biological oxygen demand, chemical oxygen demand, contamination, waste.*

I. Introduction

One of the most critical problems of developing countries is improper management of vast amount of wastes generated by various anthropogenic activities. More challenging is the unsafe disposal of these wastes into the ambient environment. Water body (especially freshwater reservoirs) is mostly affected. This has often rendered these natural resources unsuitable for both primary and/or secondary usage [1]. Industries are the major sources of pollution in all environments. Based on the type of industry, various levels of pollutants can be discharged into the environment directly or indirectly through public sewer lines. Wastewater from industries includes employees' sanitary waste, process wastes from manufacturing, wash waters and relatively uncontaminated water from heating and cooling operations [2]. Total dissolved solids (TDS), total suspended solids (TSS), toxic metals such as Cd, Cr, Ni and Pb and fecal coliform makes such water unsuitable for drinking, irrigation and aquatic life. Industrial wastewaters range from high biological oxygen demand (BOD) from biodegradable wastes such as those from human sewage, pulp and paper industries, slaughter houses, tanneries and chemical industry. Others include those from plating shops and textiles, which may be toxic and require on-site physiochemical pre-treatment before discharge into municipal sewage system [3, 4, 5].

With competing demands on limited water resources, industrial pollution remains one of the major problems in Bangladeshi cities [6]. Effluent discharge practices in Bangladesh are yet too primitive and society is in danger, especially in the industrialized part of the cities. Many hazardous and potentially dangerous industries are situated in the cities of Bangladesh. Industrial units are mostly located along the banks of the rivers. There are obvious reasons for this such as provision of transportation for incoming raw materials and outgoing finished products. The development of industry, new technologies absorbing and producing enormous amount of chemicals, organic and inorganic compounds and increasing urban agglomerations have resulted in increased sewage-polluted natural waters. The growing deficit of good quality water has spurred the need to utilize not only subterranean waters, but also fresh waters at maximal risk of microbiological and chemical pollution.

The tanning industry also has one of the highest toxic intensity per unit of output [7]. During tanning process, about 300 kg chemicals are added per ton of hides [8]. Tannery effluent is among one of the most hazardous pollutants of industry. Major problems are due to wastewater containing heavy metals, toxic chemicals, chloride and lime with high dissolved and suspended salts and other pollutants [9]. Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH and high concentrations

of suspended solids, BOD, COD, tannins including chromium [10]. The contaminant from the discharge is directly related to the nature of the industry. For example, in the textile industry, the discharge is usually high in chemical oxygen demand (COD), biological oxygen demand (BOD) and color point; the tannery industry produces discharges that have high concentration of metal such as cadmium. The industrial discharge carries various types of contaminants to the river, lake and groundwater. The quality of freshwater is very important as it is highly consumed by human activities such as drinking, bathing, irrigation. The presence of contaminants from industrial waste within the water may reduce the yield of crops and the growth of plant and it will also be harmful to the aquatic living organisms [11].

The contamination of metals is a major environmental problem. This is especially for aquatic environment. Even at very low concentrations, some metals are potentially toxic or carcinogenic. This can be hazardous to human health if entered the food chain. Metals are usually dissolved into the aquatic system through natural or anthropogenic sources. Metal ions are distributed thoroughly during their transport in different compartments of the aquatic ecosystems, in biotic or abiotic compartment such as fishes, water, sediment and plant. Metals that remain in contaminated sediments may accumulate in microorganisms that can enter into the food chain and eventually affect human well being [12].

This study was performed to analyze the physicochemical parameters of tannery wastes and domestic wastes collected from different locations of Dhaka city to analyze the correlation of total bacterial count with BOD and COD and also to study the multidrug resistance pattern of isolated bacteria.

II. Materials and Methods

2.1. Sample collection

Domestic waste samples were collected from the waste disposal bins in local areas such as Mohammadpur, Krishi market, Town Hall, Shyamoly, Agargaon of Dhaka, Bangladesh. In addition tannery waste samples were collected from different type of tannery units at Hazaribag, Dhaka, Bangladesh. The samples were collected in sterile bottles and kept in cool boxes at 4°C temperature and transported to the research laboratory within one hour.

2.2. Sample preparation

After collection, 1 mL liquid sample was transferred to a sterile test tube containing 9 mL normal saline to make 10^{-1} dilution. All dilutions were mixed well with vortex mixture (Digosystem, VM-1000, Taiwan). Serial dilutions from 10^{-1} to 10^{-5} were used for tannery waste and 10^{-1} to 10^{-12} were used for domestic waste.

2.3. Determination of physico-chemical indicators

2.3.1. pH

The pH was determined electrometrically by using the technique recommended in the Standard Methods [13].

2.3.2. Biological oxygen demand (BOD)

The biological oxygen demand determination of domestic waste and tannery waste samples in mg/L was performed at the Environmental Microbiology Laboratory, (the International Centre for Diarrheal Disease Research, Bangladesh) using standard methods [14]. BOD was calculated after the incubation period.

2.3.3. Chemical oxygen demand (COD)

Determination of chemical oxygen demand was carried out using closed reflux method [14].

2.4. Microbiological analysis

For isolation, 0.1 mL each of diluted samples was spread on nutrient agar plates and streaked on MacConkey agar plates and incubated at 37°C for 24 hours. Growth appearing on the media was isolated for the study. The isolates were confirmed by microscopic, cultural and standard biochemical tests (indole, MR-VP, citrate utilization, urease, lactose fermentation, sucrose fermentation, mannitol fermentation, catalase and oxidase test) according to Bergey's Manual of Determinative Bacteriology, [15] (9^{th} Edition, 1994) for further analysis.

2.5. Antimicrobial resistance testing

The antimicrobial resistance test was carried out by the standard disc-diffusion [16] method. Cultures grown overnight were used for the test. The antibiotic discs (Oxoid, UK) used in this study were ciprofloxacin (5 µg), ceftriaxone (30 µg), cefuroxime sodium (30 µg), nalidixic acid (30 µg), levofloxacin (5 µg), azithromycin (15 µg), chloramphenicol (30 µg), imipenem (10 µg), cefixime (5 µg), nitrofurantoin (100 µg).

III. Result and discussion

3.1. Physicochemical characteristics and total bacterial count (TCC) of domestic waste and tannery waste

All the parameter values such as acidity and alkalinity were above the WHO pH tolerance limit of between 6.0-9.0 for wastewater to be discharged into channel into stream [17]. The pH range of the domestic waste was 4.5-5.2 and the raw effluent of tannery industry showed a pH range of 3.0 to 10.0. The pH value of domestic waste samples and tannery waste samples was not similar. Domestic waste samples were highly acidic and tannery waste samples were found to be either highly acidic or alkaline, but primarily were alkaline. The range of BOD values of domestic samples were 1370-1418 mg/L and COD values were 6150-6173 mg/L. The range of BOD values of tannery samples were 1080-1308 mg/L and COD values were 3610-4197 mg/L. The BOD and COD value of domestic waste samples and tannery waste samples were higher than WHO values of 50 mg/L and 1000 mg/L respectively, for the discharged of wastewater into stream [17]. The value of BOD and COD were higher in domestic waste samples than the tannery waste samples.

3.2. Different types of bacteria isolated from waste samples

Fourteen isolates were obtained from the six domestic waste samples. A total of ninety-five isolates were obtained from tannery waste samples. Among them were Micrococcus (18.3%), Alcaligens (15.6%), Staphylococcus (11.0%), Enterobacter (4.6%), Shigella (14.7%), Klebsiella (6.4%), Haemophilus (4.6%), Citrobacter (3.7%), Actinobacillus (3.7%), Escherichia (4.6%), Corynebacterium (4.6%) and others (8.2%) (Figure 1).

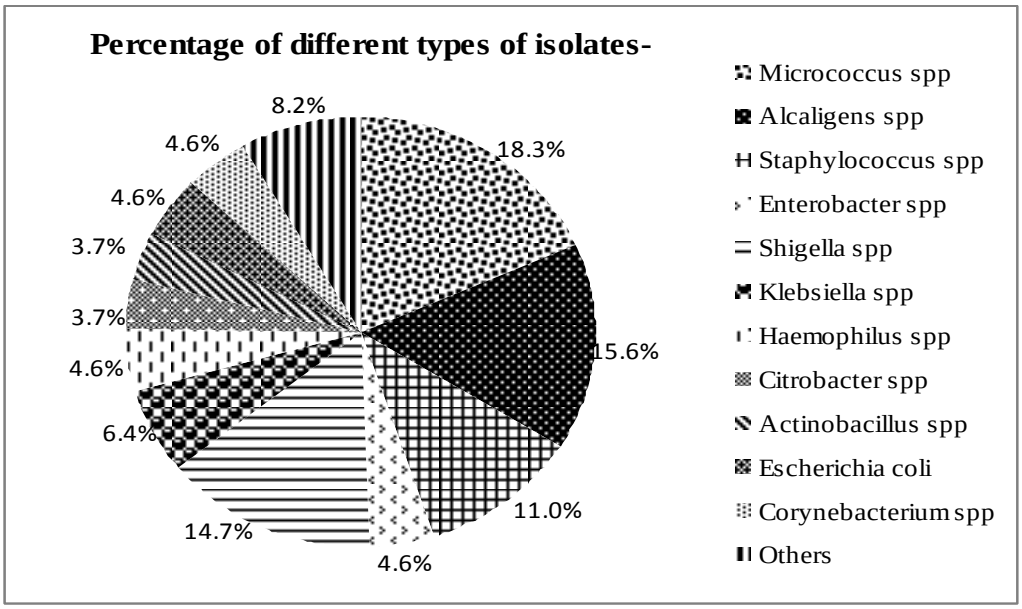

Figure 1. Percentage of different types of isolates

3.3. Gram-positive and Gram-negative bacteria isolated from waste samples

The isolates were examined under light microscope using Gram's staining. It was interesting to notice that the majority of the (63.3%) isolates were Gram-negative bacillus and the rest (36.7%) of the microorganisms were Gram-positive cocci.

3.4 Antibiotic resistance pattern of the isolates

Detected isolates were tested against ten commercially available antibiotics (Table 1, 2 and 3). Most of the isolates were resistant against chloramphenicol, nalidixic acid, nitrofurontoin and cefixime but were sensitive against levofloxacin and imipenem. Most of the bacterial isolates were moderately sensitive to Ciprofloxacin. Some isolates were found in both domestic waste samples and tannery waste samples but their antibiotic resistance patterns were not similar.

Table 1. Percentage (%) Sensitivity to antibiotics

Name of isolates	Ciprofloxacin	Ceftrixone	Cefuroxime Sodium	Nalidixic Acid	Livofloxacin	Azithromycin	Chloramphenicol	Imipenem	Cefixime	Nitrofurantoin

Micrococcus spp.	40 (8/20)	70 (14/20)	0	15 (3/20)	95 (19/20)	15 (3/20)	20 (4/20)	90 (18/20)	5 (1/20)	20 (4/20)
Alcaligens spp.	50 (8/16)	69 (11/16)	6 (1/16)	19 (3/16)	100 (16/16)	19 (3/16)	13 (2/16)	100 (16/16)	13 (2/16)	31 (5/16)
Staphylococcus spp.	58 (7/12)	25 (3/12)	8 (1/12)	33 (4/12)	92 (11/12)	25 (3/12)	25 (3/12)	92 (11/12)	8 (1/12)	25 (3/12)
Enterococcus spp.	100 (1/1)	100 (1/1)	0	100 (1/1)	100 (1/1)	0	0	100 (1/1)	0	0
Enterobacter spp.	50 (2/4)	25 (1/4)	0	0	100 (4/4)	0	50 (2/4)	100 (4/4)	0	25 (1/4)
Shigella spp.	19 (3/16)	44 (7/16)	6 (1/16)	19 (3/16)	100 (16/16)	6 (1/16)	19 (3/16)	100 (16/16)	0	12 (2/16)
Klebsiella spp.	29 (2/7)	14 (1/7)	0	0	100 (7/7)	14 (1/7)	14 (1/7)	100 (7/7)	14 (1/7)	0
Proteus spp.	0	33 (1/3)	0	0	100 (3/3)	0	0	100 (3/3)	0	0
Haemophilus spp.	60 (3/5)	40 (2/5)	0	20 (1/5)	100 (5/5)	0	0	100 (5/5)	0	0
Citrobacter spp.	33 (1/3)	67 (2/3)	0	0	100 (3/3)	0	0	100 (3/3)	0	0
Actinobacillus spp.	25 (1/4)	50 (2/4)	0	0	100 (4/4)	25 (1/4)	25 (1/4)	75 (3/4)	0	0
E. coli spp.	20 (1/5)	20 (1/5)	0	20 (1/5)	80 (4/5)	0	20 (1/5)	100 (5/5)	0	0
Edwardsiella spp.	0	0	0	0	100 (2/2)	0	0	100 (2/2)	0	0
Bacillus spp.	100 (1/1)	0	0	100 (1/1)	100 (1/1)	100 (1/1)	100 (1/1)	100 (1/1)	0	0
Corynebacterium spp.	100 (5/5)	0	0	20 (1/5)	100 (5/5)	60 (3/5)	100 (5/5)	100 (5/5)	0	40 (2/5)
Serratia spp.	0	0	0	0	100 (1/1)	0	0	100 (1/1)	0	0

Table 2. Percentage (%) Moderately sensitive to antibiotics

Name of isolates	**Ciprofloxacin**	**Ceftrixone**	**Cefuroxime Sodium**	**Nalidixic Acid**	**Livofloxacin**	**Azithromycin**	**Chloramphenicol**	**Imipenem**	**Cefixime**	**Nitrofurantoin**
Micrococcus spp.	60 (12/20)	10 (2/20)	70 (14/20)	0	5 (1/20)	15 (3/20)	0	0	15 (3/20)	40 (8/20)
Alcaligens spp.	50 (8/16)	0	38 (6/16)	12 (2/16)	0	19 (3/16)	12 (2/16)	0	6 (1/16)	25 (4/16)
Staphylococcus spp.	42 (5/12)	50 (6/12)	42 (5/12)	0	8 (1/12)	33 (4/12)	0	8 (1/12)	9 (1/12)	17 (2/12)
Enterococcus spp.	0	0	100 (1/1)	0	0	100 (1/1)	0	0	0	0
Enterobacter spp.	50 (2/4)	50 (2/4)	0	0	0	0	0	0	0	0
Shigella spp.	81 (13/16)	50 (8/16)	31 (5/16)	0	0	13 (2/16)	0	0	13 (2/16)	19 (3/16)
Klebsiella spp.	71 (5/7)	86 (6/7)	29 (2/7)	14 (1/7)	0	0	14 (1/7)	0	14 (1/7)	14 (1/7)
Proteus spp.	100 (3/3)	67 (2/3)	33 (1/3)	0	0	0	0	0	67 (2/3)	0
Haemophilus spp.	40 (2/5)	40 (2/5)	20 (1/5)	0	0	40 (2/5)	0	0	0	20 (1/5)
Citrobacter spp.	67 (2/3)	33 (1/3)	33 (1/3)	0	0	0	0	0	0	33 (1/3)
Actinobacillus spp.	75 (3/4)	50 (2/4)	25 (1/4)	0	0	25 (1/4)	0	25 (1/4)	25 (1/4)	50 (2/4)
E. coli spp.	60 (3/5)	40 (2/5)	20 (1/5)	0	0	20 (1/5)	0	0	20 (1/5)	40 (2/5)
Edwardsiella spp.	100 (2/2)	100 (2/2)	0	0	0	0	0	0	0	0
Bacillus spp.	0	0	0	0	0	0	0	0	0	0
Corynebacterium spp.	0	20 (1/5)	0	60 (3/5)	0	0	0	0	0	20 (1/5)
Serratia spp.	100 (1/1)	100 (1/1)	0	0	0	0	0	0	0	0

Table 3. Percentage (%) Resistant to antibiotics

Name of isolates	Ciprofloxacin	Ceftrixone	Cefuroxime Sodium	Nalidixic Acid	Livofloxacin	Azithromycin	Chloramphenicol	Imipenem	Cefixime	Nitrofurantoin
Micrococcus spp.	0	20 (4/20)	30 (6/20)	85 (17/20)	0	70 (14/20)	70 (14/20)	10 (2/20)	80 (16/20)	40 (8/20)
Alcaligens spp.	0	31 (5/16)	56 (9/16)	69 (11/16)	0	62 (10/16)	75 (12/16)	0	81 (13/16)	44 (7/16)
Staphylococcus spp.	0	25 (3/12)	50 (6/12)	67 (8/12)	0	42 (5/12)	75 (9/12)	0	63 (10/12)	58 (7/12)
Enterococcus spp.	0	0	0	0	0	0	100 (1/1)	0	100 (1/1)	100 (1/1)
Enterobacter spp.	0	25 (1/4)	100 (4/4)	100 (4/4)	0	100 (4/4)	50 (2/4)	0	100 (4/4)	75 (3/4)
Shigella spp.	0	6 (1/16)	63 (10/16)	81 (13/16)	0	81 (13/16)	81 (13/16)	0	87 (14/16)	69 (11/16)
Klebsiella spp.	0	0	71 (5/7)	86 (6/7)	0	86 (6/7)	72 (5/7)	0	72 (5/7)	86 (6/7)
Proteus spp.	0	0	67 (2/3)	100 (3/3)	0	100 (3/3)	100 (3/3)	0	33 (1/3)	100 (3/3)
Haemophilus spp.	0	20 (1/5)	80 (4/5)	80 (4/5)	0	60 (3/5)	100 (5/5)	0	100 (5/5)	80 (4/5)
Citrobacter spp.	0	0	67 (2/3)	100 (3/3)	0	100 (3/3)	100 (3/3)	0	100 (3/3)	67 (2/3)
Actinobacillus spp.	0	0	75 (3/4)	100 (4/4)	0	50 (2/4)	75 (3/4)	0	75 (3/4)	50 (2/4)
E. coli spp.	20 (1/5)	40 (2/5)	80 (4/5)	80 (4/5)	20 (1/5)	80 (4/5)	80 (4/5)	0	80 (4/5)	60 (3/5)
Edwardsiella spp.	0	0	100 (2/2)	100 (2/2)	0	100 (2/2)	100 (2/2)	0	100 (2/2)	100 (2/2)
Bacillus spp.	0	100 (1/1)	100 (1/1)	0	0	0	0	0	100 (1/1)	100 (1/1)
Corynebacterium spp.	0	80 (4/5)	100 (5/5)	20 (1/5)	0	40 (2/5)	0	0	100 (5/5)	40 (2/5)
Serratia spp.	0	0	100 (1/1)	100 (1/1)	0	100 (1/1)	100 (1/1)	0	100 (1/1)	100 (1/1)

IV. Conclusion

This study examines the extent of microbial pollution in domestic waste and tannery waste. The waste that enters into receiving water bodies in Bangladesh invariably results in the presence of high concentration of pollutants in the water and sediment. The pollutants have been shown to be present in concentration that may be toxic to different organisms. The effluents also have considerable negative effects on the water quality of the receiving water bodies and as such, they are rendered unsafe for human use [18]. The effects of industrial discharge to the environment and human health and lastly the corrective action that could be taken to minimize the negative impact of the discharge are a point of public health. Many ways have been proposed to protect the environment from contamination including enforcement of rules and regulations because the discharge from some industries is still exceeding the permissible limits. Before designing good corrective actions, the knowledge of effects to environment and human health, and the interaction between the contaminants and biotic and abiotic compartment must be investigated. In short, corrective actions for industries contamination clean up are important to implement.

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