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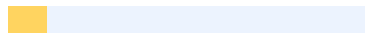
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Isolation of Multidrug-resistant bacteria from raw hides, salted hides,and tannery wastewater and their heavy metal (Cr) tolerance

ABSTRACT

Numerous leather products are created from cattle hides and skins. However, the processing of these products can release toxic heavy metals, including chromium, into the environment, leading to health hazards and environmental pollution. This study aims to isolate and identify multidrug-resistant bacteria from rawhide, salted hides, and tanneries in different areas of the Savar region in Dhaka City, Bangladesh. The secondary objective is to determine the heavy metal tolerance and Minimum Inhibitory Concentration (MIC) of Cr against all isolated bacteria. The study found 206 bacterial isolates from three areas: Polashbari Kathaltola, Polashbari Bazar, and SavarHemaythpur. Rawhide yielded 100 (48.54%) bacterial isolates, salted hide yielded 80(38.83%) isolates, and tannery wastewater yielded only 26(12.62%) isolates. Further, Bacillus spp. (60) was the most commonly isolated bacteria. The other identified bacteria were Pseudomonas spp. (46), Staphylococcus spp. (40), Proteus spp. (40), and Escherichia coli (20). The isolated strains showed multidrug resistance, with Staphylococcus spp. Being highly resistant to Erythromycin (100%), followed by Trimethoprim (95%) and Tetracycline (88%). Pseudomonas spp. were highly resistant to Azithromycin (100%) and Gentamycin (100%). The MIC and the MIC breakpoint determined the screening and evaluation of Cr tolerance against bacteria, and among the isolated bacterial isolates, Bacillus spp. showed a MIC breakpoint of 600 ppm (11.52mM) for Cr tolerance. The isolated heavy metal-resistant bacteria are useful in the bioremediation process and can aid in recovering and removing heavy metals from environmental effluents, thus preventing adverse effects on humans and animals. The government and leather companies should take legal responsibility for proper leather processing to ensure a preventative approach for safe public health. Otherwise,carcinogenic heavy metals can cause cancer in humans.

INTRODUCTION:

It is worth noting that China, Brazil, Argentina, India, Russia, and the European Union are significant producers of cattle scales, while Australia, China, New Zealand, and the EU produce sheep and lamb skin. Developing countries control more than 78% of the global cattle herd, and they generate 64% of hide and 65% of sheepskin worldwide (Dixit et al. 2015). In total, around 522,600 9 tons of heavy leather and 1,185 million square meters of light leather, including split leather, were produced from 6.0 million wet-salted raw hides worldwide. The Bangladeshi government has granted five top companies permission to export 10 million square feet of rawhide from July 2021 to June 2022, earning a total of $151.37 million in foreign exports in 2021-2022 (Sajjad 2022). In the 1940s, RP Sahain established 17 the first tannery in Narayanganj, Bangladesh. Later, it was moved to

Hazaribag in Dhaka. Currently, there are over 220 small and large tanneries operating in Bangladesh. Various preservation methods like cooling, salting, and drying are used in the country to process hides. Soaking methods are found to be the most suitable for removing microbes from the hides. Different techniques are applied to remove unwanted raw skin components from hides during the tanning process (Islam et al. 2015). In the leather industry, chromium is used for various purposes, and this heavy metal is mixed with environmental wastewater, which can have negative effects on humans, animals, and the ecosystem. Heavy metals can accumulate in agricultural soil, aquatic bodies, and plants and ultimately enter the human and animal bodies through the food chain. Therefore, disposing of hides that contain heavy metals in the environment can be considered an environmental pollutant. Proper wastewater treatment is necessary before releasing these heavy metals into the environment (Kookhaee et al. 2022). Removing heavy metals from tannery wastewater is an expensive and time-consuming process. Among the available methods, using bacteria is a cost-effective and eco-friendly approach for removing heavy metals from tannery wastewater effluents. Previous research has identified several bacterial isolates, including E. coli, Pseudomonas spp., and Bacillus spp., from raw hides, salted hides, and tannery wastewater effluents (Kookhaee et al. 2022). According to Mohamed et al. (2016), Staphylococcus spp., Micrococcus spp., Bacillus spp., Pseudomonas spp., and E. coli are the most predominant drug-resistant and heavy metal-resistant bacteria associated with hide and skin damage during processing in tanning industries. Chromium-resistant bacteria were found in tannery wastewater effluents, which help reduce Cr from environmental effluents. These bacteria have chromate reductase genes that synthesize reductase enzymes or induce proteins, which catalyze the reduction of hexavalent Cr (VI) to trivalent chromium Cr (III) (Wani et al. 2018) and help in the bioremediation process of Cr (Sanjay et al., 2020). Tons of rawhides are processed into final goods every year via the tanning process without proper waste management, resulting in the transfer of heavy metals Cr (III, VI) to tannery wastewater from processed hide. In many parts of the world, treated water from

tanneries is used for agricultural or irrigation purposes. Sometimes, this water is mixed with other water bodies. Improper treatment of this wastewater can cause chromium to transfer to agricultural land or water bodies, which can then enter the food chain of humans or animals. Chromium is a dangerous pollutant that can cause a range of health issues, including renal impairment, neural cell injury, hemolysis, liver dysfunction, reduction of antioxidant enzymes, and motor activity. It can also modulate the immune response through immunostimulatory or immunosuppressive processes and cause various types of cancer in humans (Chauhan et al. 2022). In addition, when exposed to the skin, it can cause irritant and allergic contact dermatitis (Liljedahl 2021). Keratinocytes are the first target cells affected by chromium in the development of contact dermatitis. Freshwater fishes are also at risk of heavy metal and infection. Moreover, chromium is toxic to plants and can cause chlorosis. In the 21st century, leather finishing has undergone significant changes, particularly with regard to the use of water-based treatments due to environmental concerns (Wanyonyi and Mulaa 2020). However, earlier studies have shown that the increased presence of multidrug-resistant bacteria in tannery wastewater may be linked to the removal of heavy metals from the same wastewater (Kookhaee et al. 2022). Therefore, the objective of this research is to isolate, identify, and characterize bacteria from rawhide, salted hide, and tannery wastewater and to assess the heavy metal resistance potential and minimum inhibitory concentrations (MIC) of these isolated microbes.

MATERIALS AND METHODS:

Selection of study area and research design

The research was conducted between January and June 2018 by the Department of Microbiology at Gono Bishwabidyalay in Savar, Dhaka. The study focused on Polashbari Kathaltola, Polashbari Bazar, and Savar Hemaythpur tanneries, where samples were collected from raw cow hides, salted hides, and tannery wastewater. Sterilized

sample collection tubes were used, and a total of sixty samples were collected from each category aseptically. These samples were then transferred to the laboratory for further microbiological analysis (Table 1).

Table 1: Types of samples and their collection location

Type of sample	Location	Sample number
Raw hide	Polashbari bazaar and Kathaltola	20
Salted hide	Savar Hemayathpur Tannery	20
Tannery waste water	Savar Hemayathpur Tannery	20
Total samples		60

Isolation and identification of bacterial isolates

The process of primary isolation was carried out using the serial dilution method. The samples were diluted in Phosphate-Buffered Saline (PBS) with 10-3, 10-4, and 10-5 dilution factors. Then, they were spread on Plate Count Agar (PCA) and incubated at 37ºC for 24 hours. Following this, the isolated bacterial cultures were subcultured on Eosin Methylene Blue agar (EMB agar), Cetrimide agar, Mannitol Salt Agar (MSA agar), and Mackconkey agar until pure cultures were obtained in terms of morphology (shape, size, surface texture, edge, and elevation, color, opacity, etc.) (Kundu et al. 2021). Once

pure cultures were obtained on selective media, the bacteria's cultural morphology was identified using Gram staining. Biochemical characteristics were then determined by performing a group of tests such as the catalase test, coagulase test, Methyl Red–Voges Proskauer (MR-VP) test, Indole, 6 Triple Sugar Iron (TSI), Kingler Iron Agar (KIA), Motility Indole Urease (MIU), and sugar fermentation. These tests were used to identify the pure isolates (Kookhaee et al. 2022).

Screening of Chromium (Cr) tolerance in isolated bacterial isolates

After isolating all the samples, a pure culture of 100 µL from the Nutrient broth was 1 spread on Nutrient agar plates mixed with heavy metal Cr concentrations ranging from 100 ppm to 700 ppm (1.92 mM to 13.44 mM). 11 The plates were then incubated at 37°C overnight. Purified colonies were preserved for further processing after overnight incubation. Nutrient agar plates without Cr were used as control plates (Kookhaee et al. 2022).

Detection of Cr tolerance in isolated bacteria

The Cr tolerance test was conducted on isolated bacterial strains using 1 the agar dilution method described by Kookhaee et al.(2022). 100 µL of bacterial suspensions was transferred in 0.5 McFarland standards (1.5 X 108) onto Nutrient agar plates with concentrations of Cr ranging from 100 ppm to 700 ppm (equivalent to 1.92 mM to 13.44 mM). A Nutrient agar plate without Cr was used as a control.

Antibiotic Sensitivity Tests Evaluation and MDR Indices

After biochemical test confirmation, all the bacterial isolates isolated from different areas were tested for antibiotic sensitivity tests. According to CLSI-2017, a 14 Kirby-Bauer disc diffusion method was applied for the antibiotic sensitivity test with Muller- Hinton agar plates. A total of 15 commercially available antibiotics including Azithromycin (30µg), Penicillin (10µg), Carbapenems (10µg), Gentamicin (10µg), Tetracycline (30µg),

Erythromycin (15μg), Chloramphenicol (30μg), Trimethoprim (5μg), Salfamethoxazole (10μg), Streptomycin (10μg), Amoxicillin (30μg), Nitrofurantoin (30μg),Vancomycin (30μg), and Nalidixic acid (30μg) were utilized. For this, 100 μl of pure bacterial culture was spread on Moller-Hinton agar (Oxoid, TM, UK) with a glass spreader for the antibiotic sensitivity test. Then, the antibiotic disc was placed on a cultured plate and incubated at 37ºC for 24 hours.After overnight incubation, the zone of inhibition was measured with a millimeter scale according to manufacturer guidelines. The following formulation calculated antibiotic resistance (R): %R= AB ×100. Where A= Number of positive resistance isolates on a specific antibiotic; B = number of total test isolates. Multidrug-resistant (MDR) bacterium was defined as being resistant to three or more antibiotics. The following formulation estimated the MDR indices for Cr-tolerant isolates: MDR= A/B. Where A= Number of resistant antibiotics; B=Number of tested antibiotics.

Determination of Minimum Inhibitory Concentration (MIC) of Cr

The MIC of Cr was evaluated by Kookhaee et al. (2022). To prepare the stock solution, 0.283 g of potassium dichromate (dried for 60 minutes at 100°C) was added to 100 ml of distilled water in a volumetric flask, resulting in 1000 ppm Cr. From this, daily applicable solutions were created through dilution. The pH of the medium was adjusted to between 6.7-7.6. For the determination of the MIC breakpoint of isolated bacteria, seven different concentrations of Cr (ranging from 100 ppm to 700 ppm) were mixed with Brain Heart Infusion agar, inoculated with 100 μl of bacterial culture, and incubated at 25°C for 24 hours. After overnight incubation, metal tolerance was evaluated for different isolates, such as MIC of Cr with growth resistance or growth sensitivity. A control plate with no Cr concentration was used for comparison. The experiment was conducted in triplicate for all the isolates.

Statistical Analysis

The statistical analysis was performed using the SPSS software, version 25.0 (New York,

USA). To determine the standard error, the Skewness and Kurtosis tests were applied. If the data did not follow a normal distribution, it was represented as the mean ± standard deviation (SD), and $P< 0.05$ was considered statistically significant.

RESULTS:

Isolation, identification, and prevalence of bacterial isolates from cattle hides

A total of 206 bacterial isolates were obtained from raw hides, salted hides, and tannery wastewater collected from three different locations in Savar, Dhaka, Bangladesh. All the isolates were identified using a combination of cultures and confirmed with biochemical tests. Specific culture media were used to detect particular isolates, along with biochemical tests (refer to Figure 1).

Figure 1: Cultural 20 characteristics of E. coli on EMB (A); Proteus spp. on MAC (B); Pseudomonas spp. on CA (C); Staphylococcus spp. On MSA (D) and Bacillus spp. on NA (E). Biochemical test result of E. coli positive in Indole (F); Proteus spp. positive in Citrate test (G);
Pseudomonas spp. positive in MR (H); Staphylococcus spp. positive in TSI (I) and Bacillus spp. positive in Sugar fermentation (J); EMB: Esoin Methylene Blue agar; MAC: MacConkey agar; CA: Cetrimide agar; MSA: Mannitol Salt Agar; NA: Nutrient Agar; MR: Methyle Red;TSI: Triple Sugar Iron; Yellow color indicate corrections and added update information.

Among the isolated 206 bacterial isolates, 60 were identified as Bacillus spp. (29.12%), followed by 46 Pseudomonas spp. (22.33%), 40 Staphylococcus spp. (19.41%), 40 Proteus spp. (19.41%), and 20 Escherichia coli (9.71%) (Table 2). The analysis showed a strong significant correlation (0.00) between E. coli and Proteus spp., whereas there was a significant correlation (0.002) between E. coli and Pseudomonas spp. (Table 3). The gram staining revealed that 100 (48.54%) of the isolated bacterial isolates were Gram-positive bacteria, and 106 (51.45%) were Gram-negative. The distribution of bacteria varied among different types of samples. A total of 100 (48.54%) bacteria were isolated from 20 rawhide samples, including 40 (40%) Gram-positive and 60 (56.60%) Gram-negative bacteria. 80 (38.83%) bacterial isolates were obtained from 20 salted hide samples, and among these, 40 (40%) were Gram-positive, and the remaining 40 (37.73%) were Gram-negative. 26 (12.26%) bacterial isolates were found in 20 tannery wastewater samples.Among these, 20 (20%) were Gram-positive, and 6 (5.66%) were Gram-negative bacteria.

Out of the 206 bacterial isolates, 100 (48.54%) were isolated from PolashbariKathaltola and Polashbari Bazar from cow raw hides, whereas 80 (38.83%) bacterial isolates were isolated from salted hides of Savar Hemaythpur tannery areas, and 26 (12.62%) bacterial isolates were isolated from tannery wastewater in Savar Hemaythpur tannery areas (Table 4). The primary bacterial isolates in the rawhide samples were E. coli, Proteus spp., Pseudomonas spp., Staphylococcus spp., and Bacillus spp., and these bacterial strains were found in all collected samples. In the case of salted hide samples, the presence of Proteus spp., Pseudomonas spp., Staphylococcus spp., and Bacillus spp. were found in 20 (25%) samples, but no E. coli was detected. In samples collected from tannery wastewater in Savar Hemaythpur, Bacillus spp. was found in 20 (76.92%) samples, whereas Pseudomonas spp. was found in only 6 (23.08%) samples. The presence of E. coli, Proteus spp., and Staphylococcus spp. were not detected in the collected samples (Table

2).

Table 2: Prevalence [1] of isolates based on type of samples collected from different areas

Bacterial species	Number of isolates (%)			
	Polashbari bazaar and Kathaltola (Raw hides)	Savar Hemaythpur tannery (Salted hides)	Savar Hemaythpur tannery (Tannery waste water)	Percentage (%)
Escherichia coli	20(20%)	0(00%)	0(00%)	20 (9.71%)
Proteus spp.	20(20%)	20(25%)	0(00%)	40 (19.41%)
Pseudomonas spp.	20(20%)	20(25%)	6(23.08%)	46 (22.33%)
Staphylococcus spp.				

20(20%)

20(25%)

0(00%)

40 (19.41%)

Bacillus spp.

20(20%)

20(25%)

20(76.92%)

60 (29.12%)

Total

100

80

26

206

Table 3: The correlation between different bacterial species

Correlations

E. coli

Proteus spp.

2 Pseudomonas spp.

Staphylococcus spp.

Bacillus spp.

E. coli

Pearson Correlation

1

.500**

.390**

.500**

.b

Sig. (2-tailed)

0

0.002

0

.

N

60

60

60

60

60

Proteus spp.

Pearson Correlation

.500**

1

.780**

1.000**

.b

Sig. (2-tailed)

0

0

0

.

N

60

60

60

60

60

Pseudomonas spp.

Pearson Correlation

.390**

.780**

1

.780**

.b

Sig. (2-tailed)

0.002

0

0

.

N

60

60

60

60

60

Staphylococcus spp.

Pearson Correlation

.500**

1.000**

.780**

1

.b

Sig. (2-tailed)

0

0

0

.

N

60

60

60

60

60

Bacillus spp.

Pearson Correlation

.b

.b

.b

.b

.b

Sig. (2-tailed)

.

.

.

.

**.Correlation is significant at the 0.01 level (2-tailed).

b. Cannot be computed because at least one of the variables is constant.

Table 4: Prevalence of Gram positive and Gram negative isolates isolated from raw hides, salted hide and tannery waste water

Type of sample

No. of sample

collection

No. of Gram positive isolates

(%)

No. of Gram negative isolates

(%)

No. of total isolates

(%)

Raw hides

20

40 (40%)

60 (56.60%)

100 (48.54%)

Salted hides

20

40 (40%)

40 (37.73%)

80 (38.83%)

Tannery waste water

20

20 (20%)

6 (5.66%)

26 (12.62%)

Total

60

100 (48.45%)

106 (51.45%

206

Antibiotic resistance patterns

After confirming the identity of bacterial isolates through biochemical tests, antibiotic sensitivity tests were performed to determine their resistance patterns. The results (Table 5) showed that among the major bacterial strains, E. coli displayed the highest resistance to Sulfamethoxazole (100%), followed by Gentamycin (90%) and Penicillin (75%). Proteus spp. [1] was found to be highly resistant to Penicillin (100%) but was least resistant to Erythromycin (85%). Pseudomonas spp. [2] showed high resistance to Azithromycin and Gentamycin (100%) but was least resistant to Tetracycline (25%) and Amoxicillin (15%). Staphylococcus spp. was highly resistant to Erythromycin (100%), Trimethoprim (95%), and Tetracycline (85%). Finally, Bacillus spp. showed high resistance to Penicillin, Trimethoprim, and Sulfamethoxazole (100%).

Table 5: Antibiotic resistance pattern of isolates

Name of antibiotic with

E. coli (n=20)

Proteus spp.

Pseudomonas

Staphylococcus spp. (n=20)

Bacillus spp. (n=20)

disc concentration (µg)

(n=20)

spp.(n=20)

%R

%R

%R

%R

%R

Azithromycin (30µg)

0

-

20/20 (100%)

-

0

Penicillin (10µg)

15/20 (75%)

20/20(100%)

-

15/20 (75%)

20/20 (100%)

Carbapenems (10µg)

8/20 (40%)

0

-

-

Gentamicin (10µg)

18/20 (90%)

18/20 (90%)

20/20 (100%)

-

0

Tetracycline (30μg)

14/20 (70%)

5/20 (25%)

17/20 (85%)

0

Erythromycin (15μg)

-

17/20 (85%)

-

20/20 (100%)

-

Chlramphenicol (30μg)

-

-

-

14/20 (70%)

12/20 (60%)

Trimethoprim (5μg)

0

-

-

19/20 (95%)

20/20 (100%)

Salfamethoxazol (10µg)

20/20(100%)

-

-

-

20/20 (100%)

Streptomycin (10µg)

11 -

-

-

-

0

Amoxicillin (30µg)

0

-

3/20 (15%)

-

-

Nitrofurantoin (30µg)

0

-

0

-

-

Vancomycin (30µg)

-

-

10/20 (50%)

15/20 (75%)

0

Nalidixic acid (30µg)

 -

 -

10/20 (50%)

-

-

%R= Resistance percentage; - = Not tested antibiotics

Multidrug-resistant isolates

The term "multidrug-resistant" (MDR) refers to strains of bacteria that show 5 resistance to three or more antibiotics from different classes on the Muller-Hinton agar plate. In a recent study, 70% of Pseudomonas spp. (7 out of 10) and 90% of Staphylococcus spp. (9 out of 10) 1 were found to be MDR when tested with commercially available antibiotics on the Muller-Hinton agar plate. Out of the 10 species of Pseudomonas spp. tested, 7 showed multidrug resistance to different antibiotics. Some of them were resistant to three antibiotics on the Muller-Hinton plate, while some showed resistance to four antibiotics. Similarly, out of the 10 Staphylococcus spp. tested, 9 showed MDR to different antibiotics on the Muller-Hinton agar (Table 6).

Table 6: MDR isolates isolated from different sources

Antimicrobial agents

No. of MDR isolates in Percentage

Staphylococcus spp. (n=10)

E, TE, P

2 (20%)

TE, T, SLX

2 (20%)

T, SLX, TE, C

2 (20%)

V, C, P, E

1 (10%)

TE, V, P, E, C,

2 (20%)

Total

9 (90%)

Pseudomonas spp. (n=10)

AZ, GEN, TE

2 (20%)

GEN, NA, V

1 (10%)

TE, AMX, V, GEN

2 (20%)

NA, GEN, V, AMX, AZ

2 (20%)

Total

7 (70%)

Determination of MIC of Cr/ Screening of Cr tolerance

In this research, heavy metal (Cr) was used to determine the tolerance level of MDR (multidrug-resistant) isolates on Brain Heart Infusion Agar. **1** The bacterial isolates were purified and tested for heavy metal (Cr) tolerance at different concentrations ranging from 100 ppm to 700 ppm to determine the minimum inhibitory concentration breakpoints for

each isolate from raw hides, processed hides, and tannery wastewater. The heavy metal (Cr) tolerance isolates with minimum inhibitory concentrations are presented in Table 7. The results showed that most of the MDR 6 isolates were able to grow well in low concentrations of Cr and gradually decreased in number as the concentration of Cr increased. In this research, E. coli was able to grow up to 500 ppm (9.6 mM), Proteus spp. up to 400 ppm (7.68 mM), Pseudomonas spp. up to 400 ppm (7.6 mM), Staphylococcus spp. up to 400 ppm (7.6 mM), whereas Bacillus spp. tolerated up to 600 ppm (11.52mM) of Cr concentrations. However, above 600 ppm of Cr concentration, all isolates were unable to tolerate the heavy metal(Table 7).

Table 7: 1 Minimum Inhibitory Concentration (MIC) detection with chromium

Minimum Inhibitory Concentration (MIC)/ breakpoints

Isolates

100 ppm

(1.92mM)

200 ppm

(3.84mM)

300 ppm

(5.76mM)

400 ppm

(7.68mM)

500 ppm

(9.6mM)

600 ppm

(11.52mM)

700 ppm

(13.44mM)

Escherichia coli

G

G

G

G

G

NG

NG

Proteus spp.

G

G

G

G

NG

NG

NG

Pseudomonas spp.

G

G

G

G

NG

NG

NG

Staphylococcus spp.

G

G

G

G

NG

NG

NG

Bacillus spp.

G

G

G

G

G

G

NG

DISCUSSION:

In recent years, hides have received significant attention as they are processed to produce finished goods like shoes and handbags. 17 The leather industry has been established worldwide, with many tanneries now in operation. Bangladesh exports hides to foreign countries and earns foreign currency. However, contamination of cow hides by putrefaction bacteria is a significant obstacle to international trade. Additionally, tannery wastewater is harmful to the environment and public health, making it an alarming issue worldwide. To address these concerns, our research aims to measure bacterial loads, identify bacteria from raw hides, salted hides, and tannery wastewater, and 10 determine the minimum inhibitory concentration (MIC) from different Cr concentrations.

The study identified a total of 106 (51.45%) Gram-negative bacteria and 100 (48.55%) Gram-positive bacteria. These results are 5 consistent with the findings of Ahmed et al. (2016). Raw hides, salted hides, and tannery wastewater had five major genera of bacteria isolated. The prevalence of bacteria in this study is similar to that of Ahmed et al. (2016), who also identified Staphylococcus spp. (53.12%) as the most dominant and Bacillus spp. (4.66%) as the least. Similarly, Olukitibi et al. (2017) reported S. aureus in 44% of samples,

followed by Bacillus spp. (14%) and E. coli (8%). Obeng et al. (2013) found similar microorganisms in their research, which confirms 3 the results of this study. Clarence et al., (2009) found that Gram-negative isolates were predominant in their study, while Gram-positive bacteria were the least. 1 The findings of this study are consistent with those of Clarence et al. (2009).

Various infectious diseases can spread to humans and animals due to the isolated pathogens that can easily transmit from tannery wastewater to fresh water, agricultural land, and the environment. The first priority for proper treatment and prevention of these pathogens should be effective antibiotics. However, the indiscriminate use of commercial antibiotics can promote the multidrug-resistant power of bacteria, leading to adverse consequences for populations. This issue may be caused by naturally resistant microbes to commercial antibiotics, and the sub-therapeutic antibiotics in animal feeds can be chosen for resistant strains. The resistance of 4 Gram-positive and Gram-negative bacteria to antibiotics may be due to virulence factors and the intrinsic nature of their cell walls. Our research reported that E.coli showed higher resistance to Salphamethanoxol (100%),followed by Gentamycin (90%) and Tetracycline (70%). These findings were similar 2 to a previous study by Iroha et al. (2011). Additionally, Pseudomonas spp. showed resistance to Azithromycin (100%) and Tetracycline (25%), which agreed with Ali et al. (2010). The finding 12 of this study is that Pseudomonas spp. and Staphylococcus spp. were multidrug-resistant. Similarly, Ali et al. (2010) and Iroha et al. (2011) found that Pseudomonas spp. were resistant to Gentamycin, Tetracycline, Amoxicillin, Azithromycin, and Vancomycin.

3 The results of this study are significant as they have identified four bacterial species, i.e., E. coli, 2 Pseudomonas spp., Staphylococcus spp., and Bacillus spp. are resistant to Chromium (Cr). These findings are similar to those of Camargo et al. (2003). Additionally, the study identified different minimum inhibitory concentrations (MIC) for all four bacterial

isolates. Metal-resistant bacteria can quickly grow and spread on industrial effluents, which provide them with a nutritional source for multiplication. Identifying resistance to metallic substances can help in monitoring multiple harmful contaminants at the same time. Bacterial resistance and its impact on human health and the environment are directly linked to household and industrial waste. The isolated bacterial isolates have demonstrated adaptive tolerance to all of the tested heavy metal concentrations. Chromium, which is used in the hide industry, poses a significant threat to the environment, particularly to freshwater and soil. Furthermore, Multidrug- Resistant (MDR) isolates from raw hides, salted hides, and tannery wastes can affect the environment, fresh, agricultural land, and ultimately, humans and animals by contaminating vegetables and other foods from agricultural land.

CONCLUSION:

In this study, we aimed to isolate and identify multidrug-resistant (MDR) bacteria from raw hides, salted hides, and tannery wastewater in the Savar area of Bangladesh. We used various methods and media to isolate the bacteria and determine their minimum inhibitory concentration (MIC) against chromium (Cr). During this study, five bacterial isolates were identified through morphological and biochemical tests, all of which exhibited MDR. Among them, Bacillus spp. showed the highest tolerance to Cr. These preliminary results suggest that Bacillus spp. may be helpful in the bioremediation of Cr in tannery wastewater. Bioremediation is a promising approach for removing environmental contaminants, but it is not commonly used in developing countries like Bangladesh. Tannery wastewater containing Cr can contaminate surface and ground waters and pose a public health risk. Therefore, tannery companies must adequately treat and manage their wastewater to prevent environmental damage. The best available techniques must be used, and an environmental management system (EMS) should be implemented to improve the overall environmental performance of tanneries.

ACKNOWLEDGEMENT

Conflict of Interest

The authors have no conflict of interests.

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