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Prevalence of Pathogenic Microorganisms and Determination of Possible Presence of Antimicrobial Residues in Industrial and Medical Wastes

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Abstract

Waste materials discharged from any industries without any treatment may cause serious environmental problems which may consequently affect public health. Present study design to screen the proliferation of microorganisms in different waste and environmental samples near industrial areas along with the assessment of the presence of antibacterial substances. The existence of the total viable bacteria and fungi was estimated up to 10^7cfu/g and 10^4 cfu/g, respectively, in solid wastes. For liquid wastes, the total viable bacteria were recovered up to 10^7 cfu/mL and fungi were observed up to 10^5 cfu/mL. Both types of samples were found to be contaminated with an array of pathogenic bacteria including *Klebsiella spp*., *Staphylococcus spp*., and *Vibrio spp*. Conversely, some medical and pharmaceutical waste samples were found to inhibit the growth of laboratory isolates tested which indicate the presence of antibiotics residues. Finally, the huge microbial load and the possible presence of antibiotic or other antimicrobial residues render the samples a major public health concern.

Keywords: Microbiological Quality; Antimicrobial Residues; Pharmaceutical Waste; Medical Waste

Abbreviations: TVB: Total Viable Bacteria; NA: Nutrient Agar; SDA: Sabouraud's Dextrose Agar; MFC: Membrane Fecal Coliform; MSA: Mannitol Salt Agar; TFC: Total Fecal Coliform; SCB: Selenite Cysteine Broth; APW: Alkaline Peptone Water; TCBS: Thiosulfate Citrate Bile Salt Sucrose.

Introduction

The increase of industrialization in Bangladesh contributes to various kinds of environmental pollution, which may directly or indirectly cause ecological imbalance within the environmental ecosystem as well as seriously damage the biosystem [1]. With the increase of population, the demand for various rubbers, plastic, cloths, papers, and many other products is also increasing day by day. Therefore, to achieve the extensive requirement of a huge population in Bangladesh, much more industries are developing gradually [2]. Different organic and inorganic pollutants and many xenobiotic compounds are introduced to environments from these industrial toxic wastes [3-5]. Discharge of this untreated or partially treated industrial waste in the aquatic environment may cause a significant change in the behavior of aquatic life. Moreover, when they release onto the soil it can change the physicochemical and biological properties of soil color which is responsible for sexual mutation in fish, amphibians, and birds [6,7].

Solid and liquid waste can also be generated from domestic, medical, commercial, or agricultural exploits, surface runoff, or storm water [8]. About 75 to 90 percent of total wastes

can be classified as domestic wastes, which are usually non-hazardous wastes generated by administrative, food, and cleaning services, among others. The remaining 10 to 25 percent are considered hazardous wastes mainly healthcare-related wastes which are referred to as hospital or medical wastes [9]. Medical wastes consist of considerable amounts of chemicals and microbial agents including microbiological cultures, infectious blood samples, human body parts, etc. [10]. Healthcare and clinical settings are considered to be the reservoirs for large numbers of pathogenic microorganisms [11]. Along with their undesirable environmental effect, these wastes can similarly cause serious health problems to humans [12]. These wastes may establish the perfect site for the exchange of resistance genes between medical and environmental pathogens [13,14]. Furthermore, the pathogens in wastewater are exposed to a wide range of biocides that could act as a selective pressure for the evolution of resistance [15]. However, antibiotic residues from hospital waste that may enter into the environment may gradually accumulate in the environmental segment and are responsible for increasing antibiotic resistance [13]. The presence of antibiotic-resistant bacteria in the waste or environmental samples is one of the biggest health concerns as they can transmit resistant genes through horizontal gene transfer to human pathogens of environmental origin [16].

Various solid and liquid wastes or effluents contain pathogenic microbes along with different chemical agents which induce the resistance traits and make them able to escape easily from water treatment plants. Similarly, municipal landfills are major contamination sources for superficial and drinking water systems, particularly when located in urban areas [17,18]. A better knowledge of landfill-associated microbial communities may provide valuable insights on the bioremediation of wastes and promote pathogen monitoring. Microbial activity inside landfills leads to decomposition of inorganic and organic substances to stabilize the waste by oxidation of ammonium [19], reduction of nitrous oxide [20], and hydrolysis of cellulose [21,22].
Considering all these facts, we designed the present study to identify the pathogenic microbial population and investigate their antimicrobial traits from different waste samples collected from different areas in Bangladesh.

Method and Materials

Study Area, Sampling and Sample Processing

A total of 16 environmental samples were collected from the different areas of Bangladesh following standard protocol [23,24]. The samples were prepared for the microbiological assay according to the standard methods as described by Cappuccino & Sherman in 2005. For the identification and enumeration of pathogenic bacteria from solid samples, 10g of each sample was blended with 90 ml of buffer peptone water (pH 7.2 ± 0.2) and for liquid samples, 1 ml sample was mixed with 9ml normal saline then diluted both types of samples up to 10^{-8} according to the standard guideline.

Microbiological Analysis of Total Viable Bacteria and Fungi

For the enumeration of total viable bacteria (TVB) and the total fungal load, 0.1 ml of each sample from the dilutions 10^{-2} and 10^{-4} was inoculated onto the nutrient agar (NA) and Sabouraud's dextrose agar (SDA) plates, respectively. Plates were incubated at 37°C for 24 hours and at 25°C for 48 hours for total viable bacteria and fungi, respectively [23-28].

Estimation of Fecal Coliform, *Escherichia coli*, *Klebsiella spp.*, *Staphylococcus spp.*, and *Pseudomonas spp.*

From the dilutions 10^{-2} and 10^{-4}, 0.1 ml of each sample was spread onto the membrane fecal coliform (MFC) agar and MacConkey agar for the enumeration of total fecal coliform (TFC), and coliforms (especially, *Escherichia coli* and *Klebsiella spp*.), respectively. Plates were incubated for 24 hours at 44.5°C and 37°C for fecal coliform and coliforms, correspondingly. Likewise, *Staphylococcus spp*. and *Pseudomonas spp*. were isolated onto Mannitol Salt Agar (MSA) and Pseudomonas agar, respectively by adding 0.1 ml of diluted sample each, and all the plates were then incubated at 37°C for 24 hours [23-28].

Isolation of *Salmonella spp.*, *Shigella spp.* and *Vibrio spp.*

Ten (10) ml of sample was transferred into 90 ml of selenite cysteine broth (SCB) and alkaline peptone water (APW) for the enrichment of *Salmonella, Shigella*, and *vibrio spp*., respectively, and incubated at 37°C for 6 hours. After incubation, the samples were diluted up to 10^{-6}, and then 0.1 ml of samples from each of the 10^{-3} and 10^{-5} dilutions were spread onto Salmonella-Shigella (SS) agar and thiosulfate citrate bile salt sucrose (TCBS) agar for the isolation of *Salmonella spp*. and *Shigella spp*., and *Vibrio spp*., consecutively. Plates were incubated at 37°C for 48 hours for the detection of typical colonies. Finally, all the isolates were biochemically examined following standard procedures as described earlier [23-28].

Biochemical Tests for the Confirmative Identification

Finally, the standard biochemical tests were performed for the identification of all the pathogenic isolates found in

the samples by the previously described methods [29].

Preparation and Testing of the Samples for Antimicrobial Assay

Anti-bacterial properties of the tested samples were observed against different pathogenic strains such as *Escherichia coli*, *Pseudomonas spp.*, *Listeria spp.*, *Vibrio spp.*, *Klebsiella spp.*, *Staphylococcus aureus*, *Bacillus spp.*, and *Salmonella spp*. [30]. At first, the lawns of bacterial suspensions (10^5 cfu or 0.5 OD measured by spectrophotometer) including each of the mentioned bacteria were prepared and 100µl of homogenized samples were introduced into the wells. Absolute ethanol and methanol were used as negative controls while the antibiotic discs of gentamicin (10 µl) were used as a positive control. Plates were incubated at 37°C for 12-18 hours and examined for formation of the zone of inhibitions (mm) [30].

Results & Discussions

Most of the developing countries of Asia are most heavily populated and quickly moving towards urbanization [31]. But due to shortcomings in waste management, financial and some other challenges, contributes to making this situation more critical. According to Das, et al. [32] large amounts of industrial waste are released into the environment without treatment.

Recovery of Microorganisms from the Industrial Waste and Environmental Samples

Most of the samples of both categories (solid and liquid wastes) were found to be highly contaminated by bacterial and fungal flora 10^2-10^7 cfu/g or cfu/ml (Table 1). In the case of industrial samples, the contamination rate of total viable bacteria and fungi was noticed within the range of 10^5-10^7 and 10^2-10^4 cfu/ml, respectively. Whereas in the medical wastes, the total viable bacterial and fungal count were estimated within the range of 10^3-10^6 and 10^2-10^4 cfu/g or cfu/ml, respectively. On the other hand, the estimated total viable bacterial and fungal count of water samples were 10^6-10^7 and 10^4-10^5 cfu/ml, respectively. In the case of the agricultural land soil sample, 10^6 cfu/g bacteria and 10^4 cfu/g fungi were counted. In garden soil samples, counted viable bacterial and fungal loads were 10^7 and 10^4 cfu/g, respectively. *Staphylococcus spp*. and *Pseudomonas spp*. were predominantly found in the majority of the samples in an average of 10^4 cfu/g or mL (Tables 1 & 2). *Vibrio spp*. was only found in a few solid and liquid industrial waste samples. Some solid and liquid waste and environmental samples were found to contain *klebsiella spp*. which can contribute to health-related problems if any contaminate our ground and surface water.

However, all the samples were devoid of the growth of *E. coli*, *Salmonella spp*., and *Shigella spp*. (Tables 2&3). Achudume and Olawale, et al. [33] reported the presence of bacterial species including *Pseudomonas, Mirococcus*, *Actinomyces*, *Neisseria*, *Bacillus*, and *Klebsiella* in waste dump areas. Park, et al. [34] identified several opportunistic pathogenic bacteria and viruses in medical waste samples. Munshi, et al. [35] tested different waste samples near including hospital, pharmaceutical, domestic and municipal wastes, and found a huge load of pathogenic bacteria. Das, et al. [32] found huge bacterial and pathogenic load in the tannery waste and environmental samples in their study in 2017. Akter, et al. [36] reported a viable bacterial count up to 10^8 cfu/g in the household waste samples.

Samples		Microbial load (cfu/g or mL)					
		TVB	Fungi	*Klebsiella* spp.	*Staphylococcus* spp.	*Pseudomonas* spp.	*Vibrio* spp.
Solid samples	Industrial waste 1	4.5×10^7	3.6×10^3	1.9×10^3	3.5×10^3	1.8×10^2	4.5×10^2
	Industrial waste 2	8.7×10^7	1.1×10^4	5.8×10^3	0	8.0×10^7	0
	Industrial waste 3	9.5×10^6	2.0×10^4	5.5×10^6	1.2×10^4	3.6×10^4	0
	Medical waste 1	1.0×10^4	4.5×10^3	0	2.8×10^3	3.0×10^3	0
	Medical waste 2	2.0×10^4	2.5×10^3	0	1.2×10^3	1.1×10^3	0
	Medical waste 3	3.1×10^4	1.2×10^3	0	1.8×10^3	1.2×10^3	0
	Garden soil 1	7.6×10^7	6.0×10^4	2.8×10^2	3.0×10^2	3.0×10^3	0
	Garden soil 2	1.6×10^7	7.0×10^4	0	1.5×10^3	6.5×10^4	0
	Agriculture soil	1.2×10^6	1.8×10^4	0	3.5×10^4	2.8×10^4	0

Liquid samples	Industrial waste 1	3.5×10^{6}	5.0×10^{4}	0	5.6×10^{2}	1.4×10^{3}	0
	Industrial waste 2	2.8×10^{5}	8.0×10^{2}	0	3.0×10^{2}	1.8×10^{3}	0
	Industrial waste 3	3.7×10^{5}	6.0×10^{2}	0	0	8.8×10^{3}	2.2×10^{2}
	Industrial waste 4	2.9×10^{5}	3.6×10^{2}	0	0	1.2×10^{3}	3.2×10^{2}
	Medical waste 1	7.0×10^{6}	7.8×10^{4}	1.3×10^{3}	1.3×10^{3}	4.8×10^{3}	0
	Medical waste 2	3.7×10^{4}	1.0×10^{2}	1.5×10^{2}	0	0	0
	Medical waste 3	5.3×10^{5}	1.4×10^{2}	0	2.5×10^{2}	3.1×10^{4}	0
	Medical waste 4	3.2×10^{3}	5.3×10^{2}	0	0	1.0×10^{1}	0
	Pond water 1	4.6×10^{6}	3.0×10^{4}	3.7×10^{2}	6.0×10^{3}	1.5×10^{3}	0
	Pond water 2	4.8×10^{7}	1.5×10^{5}	0	6.5×10^{3}	4.0×10^{2}	0
	River water 1	5.5×10^{7}	2.4×10^{5}	0	2.1×10^{3}	3.8×10^{2}	0

TVB – Total Viable Bacteria

E. coli, *Salmonella spp*., and *Shigella spp*. were absent in all the samples.

Table 1: Microbiological analysis of waste and environmental samples.

In vitro Anti-bacterial Activity of the Waste Samples

Among all industrial wastes, only a few solid waste exhibited antimicrobial activity against *E. coli* and *Listeria spp*. (Table 3). On the other hand, solid medical waste, control soil, and water samples could not able to retard the growth of laboratory isolates. However, almost all liquid medical samples were found to inhibit the growth of most laboratory organisms. Among them, sample 3 showed the highest zone of inhibition against all the test organisms (Table 3). The presence of antibacterial activity indicates waste samples may contain some degree of antibiotics or other chemicals that might come from untreated hospital wastes. This is very alarming for us because it may increase the antibiotic resistance of pathogenic microbes as well as environmental microbes [37]. Le Page, et al. [38] detected antibiotics in waste and surface water samples. Martin, et al. [39] found antimicrobial potential in the industrial waste samples.

Assumed	TSI			H_2S	Indole	MR	VP test	Citrate	Catalase	Oxidase
Organism	slant	Butt	gas	reaction	test	test		test		test
Klebsiella spp.	Y	Y	+	-	-	-	+	+	+	-
Pseudomonas spp.	R	R	-	-	-	-	-	+	-	-
Staphylococcus spp.	Y	R	+	+	-	+	-	+	+	-
Vibrio spp.	Y	Y	-	-	+	+	-	+	+	+

TSI Triple Sugar Iron Test
Y Yellow (Acid)
R Red (Alkaline)
MR Methyl red
VP Voges-Proskauer

Table 2: Confirmative biochemical tests for the isolates.

Samples		*E. coli*	*Klebsiella* spp.	*Bacillus* spp.	*Pseudomonas* spp.	*Vibrio* spp.	*Staphylococcus* spp.	*Listeria* spp.	*Salmonella* spp.
Solid samples	Industrial waste 1	0	0	0	0	0	0	0	0
	Industrial waste 2	10 mm	0	0	0	0	0	16 mm	0
	Industrial waste 3	0	0	0	0	0	0	11 mm	0
	Medical waste 1	0	0	0	0	0	0	0	0
	Medical waste 2	0	0	0	0	0	0	0	0
	Medical waste 3	0	0	0	0	0	0	0	0
	Garden soil 1	0	0	0	0	0	0	0	0
	Garden soil 2	0	0	0	0	0	0	0	0
	Agriculture soil	0	0	0	0	0	0	0	0
Liquid samples	Industrial waste 1	0	0	0	0	0	0	08 mm	0
	Industrial waste 2	0	0	0	0	0	0	08 mm	0
	Industrial waste 3	0	0	0	0	0	0	13 mm	0
	Industrial waste 4	0	0	0	0	0	0	0	0
	Medical waste 1	0	0	0	0	0	0	0	0
	Medical waste 2	17 mm	0	17 mm	17 mm	26 mm	21 mm	11 mm	29 mm
	Medical waste 3	15 mm	16 mm	14 mm	14 mm	14 mm	16 mm	13 mm	16 mm
	Medical waste 4	17 mm	0	17 mm	17 mm	26 mm	21 mm	11 mm	20 mm
	Pond water 1	0	0	0	0	0	0	0	0
	Pond water 2	0	0	0	0	0	0	0	0
	River water 1	0	0	0	0	0	0	0	0

Table 3: Antibacterial activity of waste and environmental samples.

Conclusion

Although industrial wastes might be disposed directly into the environment for decades, the potential environmental impacts are less understood and have only recently become a topic of research interest due to the rapid emergence of diseases and multidrug-resistant bacterial strains. The present study revealed the waste directly exposed to the environment may contain chemical compounds or antibiotics residues. Such activity could be responsible for the multidrug resistance of microbial isolates, which could pose a serious threat to human and animal health. So governments should take responsibility to implement proper rules against open dumping of infectious medical waste as well as municipal waste in unsecured landfills for public health and environmental safety.

Conflicts of Interest

Authors have no potential conflict of interest

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