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Occurrence of drug resistant bacteria in household waste samples

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Current study was carried out to investigate the presence of drug resistant bacterial isolates in the biodegradable household waste samples. In this respect, a total of six domestic waste samples including three kitchen waste samples and one sample each of home dust, dustbin waste and liquid waste were collected. Samples were analyzed for quantification of bacterial load and along with their drug susceptibility pattern. Huge array of total viable bacteria was present in all the samples (in average of 10^8 cfu/g or ml). Among the specific bacteria, *Bacillus* spp. was predominant and *Vibrio* spp. was found in almost all samples except liquid waste. Presence of *Staphylococcus* spp., *E. coli*, *Klebsiella* spp., *Pseudomonas* spp., *Salmonella* spp. and fecal coliform were evident in some samples. All isolates were found to be multidrug resistant. Notably, 100% resistance was documented against cefuroxime and amoxicillin. All the isolates showed sensitivity against meropenem, amikacin and ceftriaxone. Presence of drug resistant bacteria in household waste samples in present study critically raises the requirement for proper management and disposal of the accumulated domestic wastes by the municipal and government authorities.

Keywords: Household waste, Municipal waste, Kitchen waste, Home dust, Microbiological analysis, Drug resistance.

INTRODUCTION

For environmental sustainability, waste generation and its control have played an important role. The quantity of municipal waste generated increases at an alarming rate with population doubling and changing lifestyle patterns of the inhabitants. Any type of wastes produced from a domestic source is called domestic or household waste, which represents more than two-third of the solid waste stream of municipal area (1, 2). These are in either solid or semisolid form and usually devoid of hazardous industrial wastes. In general, household waste can be classified into five broad categories; (a) Biodegradable waste: food and cooking waste, green waste (vegetables, flowers, fruits, leaves), paper (can also be recycled), (b) Recyclable material: paper, cans, bottles, tubes, metals, other materials, etc., (c) Inert waste: waste from construction and demolition, dirt, rocks, debris, (d) Composite waste: waste from clothing, tetra packs, plastics such as toys, and (e) Domestic or household hazardous waste and toxic waste: medication, paints, e-waste, light bulbs, chemicals, fluorescent tubes, fertilizer and pesticide containers, spray cans, shoe polish etc. (3).

Household wastes, especially kitchen wastes are rich in nutrients, or eutrophic, those contain high level of carbohydrates, lipids, proteins and other organic molecules that can sustain abundant microbial populations (4). In the household waste environment, there is a syntrophic and nutritionally mutualistic relationship occurs among the microorganisms establishing an anaerobic food web. Hydrolytic enzymes split complex molecules into monomers that fermentative bacteria can use. Fermentation products further reduces to methane by methanogens (5). *Pseudomonas aeruginosa* also produces a biosurfacant named rhamnolipid that may improve the bioavailability of nutrients for other bacteria in the kitchen and other household wastes (6). Competitive and cooperative interactions among bacterial populations in the nutrient rich household waste environment help them to thrive. Household dust, on the other hand is a complex mixture of various substances of 0.001-l mm in diameter, such as fibers, dander, hair, paint chips, combustion products, sand, dead insects, pollen, algae, fungal spores and bacteria (7). Several studies were carried out for investigating microbial presence in household dust (7-9).

Improper dumping of untreated waste in rivers, drainages and highways from factories, clinics and treatment plants of domestic waste water, especially in developing countries, is the main source of antibiotic pollution of surface water which causes a major public health problem (10, 11). Presence of antibiotics in trace amount aids in selecting and establishing antibiotic resistance in microbes for their long time exposure in such environment (12). Furthermore, when such selective pressure leads to the persistence and spread of resistant genes, natural environments become repositories of resistant bacteria as well as resistance genes (13). Considering the facts, present study endeavored to estimate the presence of bacterial contaminants in different biodegradable domestic waste samples. Drug susceptibility pattern of the isolates was determined as well.

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MATERIALS AMD METHODS

Study period and sampling. The study was carried out at the Microbiology Laboratory, Stamford University Bangladesh from October 2018 to December 2018. Six different domestic waste samples including three kitchen waste samples and one sample each of home dust, dustbin waste and liquid waste were randomly collected in sterile PET bottle or jar and transported to the laboratory at the earliest convenient. For the isolation and enumeration of pathogenic bacteria, 10 g or ml of each sample was homogenized in 90 ml normal saline and diluted to 10^{-6} according to the standard guidelines (14-19).

Isolation and identification of bacteria

Estimation of total viable bacteria, *Escherichia coli*, *Klebsiella* spp., *Staphylococcus* spp. and *Bacillus* spp. From the dilutions 10^{-4} and 10^{-6}, 0.1 ml of each sample was spread onto the nutrient agar (NA) media for the enumeration of total viable bacteria. Likewise, 0.1 ml of each sample from the dilutions 10^{-2} and 10^{-4} were introduced onto MacConkey agar, Mannitol Salt agar (MSA), Starch agar and Cetrimide agar for the isolation of coliforms (*Escherichia coli* and *Klebsiella* spp.), *Staphylococcus* spp., *Bacillus* spp. and *Pseudomonas* spp., consecutively. All plates were then incubated at 37°C for 24 h (15-21).

Isolation of *Salmonella* spp., *Shigella* spp. and *Vibrio* spp. By estimating the possible presence of viable but non-culturable (VBNC) cells (19, 21-25), 10 ml of sample was inoculated into 90 ml of Selenite Cysteine broth (SCB) and Alkaline Peptone Water (APW) to enrich *Salmonella*, *Shigella*, and *Vibrio* spp., respectively and incubated at 37°C for 6 h. The enriched samples were diluted to 10^{-4} and then 0.1 ml from 10^{-2} and 10^{-4} dilutions were spread onto *Salmonella-Shigella* (SS) agar and Thiosulfate Citrate Bile salt Sucrose (TCBS) agar to isolate *Salmonella* spp. and *Shigella* spp., and *Vibrio* spp., consecutively. Plates were incubated at 37°C for 48 h for the detection of typical characteristic colonies.

Antibiotic susceptibility test of the isolates. The disc diffusion method was used to examine the antibiotic susceptibility of the isolated bacteria (either sensitive or resistance) on Mueller-Hinton agar (MHA) (Difco, Detroit, MI) (18, 20, 21, 26-28). The commercial antibiotic discs employed in this experiment were: amoxicillin (AMX, 30 μg), amikacin (AK, 10 μg), meropenem (MEM, 10 μg), azithromycin (AZM, 30 μg), ciprofloxacin (CIP, 5 μg), cefixime (CFM, 5 μg), cefuroxime (CXM, 30 μg), ceftriaxone (CTR, 30 μg), trimethoprime-sulfamethoxazole (COT, 25 μg) and Ceftazidime/Clavulanic acid (CAC, 30/10 μg). After bacterial lawn and placing antibiotic discs on MHA, the plates were incubated at 37°C for 8 h. Zone of inhibition was measured in mm for respective bacterial isolates.

RESULTS & DISCUSSION

Determination of the presence of bacterial isolates in the tested domestic waste samples. In present study, all the samples were found to contain huge load of viable bacteria on average 10^8 cfu/ml or g (Table 3.1) as found previously from different environmental samples (18, 22, 26, 29). Atalia *et al*. (2015) reported a large numbers of microorganisms in household solid wastes in their study on microbial biodiversity in such samples (30). Higher microbial load in household wastes was found by other researchers (31) using DNA microarray and other molecular techniques. Several studies also reported different pathogenic bacteria in the different domestic waste samples (31-34). As found in previous investigations, specific bacterial isolates were recovered in significant quantities from all the samples in present study (Table 1). *Bacillus* spp. was predominant and found in all types of samples in an average of 10^7 cfu/ml or g. *Vibrio* spp. was also present in almost all samples excluding the liquid waste. Whereas, *Pseudomonas* spp. was found in four samples (Table 1). *Klebsiella* spp., *E. coli* and fecal coliform each were present in three samples. Two samples contained *Staphylococcus* spp. and *Salmonella* spp. All the samples were devoid of *Shigella* spp. (Table 1). The higher microbial load found in the household waste samples was possibly due to the presence of accessible nutrients (30).

Antibiotic susceptibility pattern of the isolates. In current study, all tested isolates were found to be multidrug resistant (resistant against at least two or more drugs) (Table 2). All the isolates were found to be resistant against cefuroxime and amoxicillin. On the other hand, meropenem, ceftriaxone and amikacin sensitivity were observed for all isolates. Majority of the isolates were sensitive to azithromycin. Isolates of *Klebsiella* spp. and *Bacillus* spp. showed resistance against ceftazidime/clavulanic acid, ciprofloxacin and cefixime. Ceftazidime/clavulanic acid and cefixime resistance were exhibited by *Staphylococcus* spp. *Salmonella* spp. and *Vibrio* spp. were found to be resistant against trimethoprime/sulfamethoxazole (Table 2). Similar to the present study, Adieze et al. (2015) found multidrug resistant bacteria in the domestic and hospital waste samples (35). In India, Pandey et al. (2011) found multidrug resistant bacteria in waste water samples (36). Kummerer (2004) also reported bacterial antibiotic resistance in different types of waste samples (37). Unregulated disposal of domestic waste containing organic substances, together with toxic chemicals inclusive of antibiotics and pathogenic bacteria, can play a major role in the accumulation of bacterial drug resistance (4, 38-40).

Table 1. Isolation and quantification of pathogenic bacteria from different domestic waste samples

Domestic waste Samples	TVB (cfu/g or ml)	*Staphylococcus* spp. (cfu/g or ml)	*Klebsiella* spp. (cfu/g or ml)	*E. coli* (cfu/g or ml)	TFC (cfu/g or ml)	*Bacillus* spp. (cfu/g or ml)	*Pseudomonas* spp. (cfu/g or ml)	**Salmonella* spp.	**Vibrio* spp.
Kitchen waste 1	4.3×10^8	0	5.0×10^6	0	0	4.8×10^7	9.5×10^6	+	+
Kitchen waste 2	1.6×10^8	0	0	6.0×10^6	5.0×10^6	4.0×10^7	0	-	+
Kitchen waste 3	2.5×10^8	4.0×10^5	0	0	0	7.2×10^7	4.0×10^5	-	+
Home dust	5.4×10^8	4.5×10^6	1.2×10^6	0	3.6×10^6	4.1×10^7	0	+	+
Dustbin waste	5.5×10^8	0	2.0×10^7	2.2×10^6	4.0×10^6	5.0×10^7	1.6×10^6	-	+
Liquid waste	1.0×10^8	0	0	3.3×10^6	0	2.0×10^7	7.6×10^6	-	-

TVB = Total viable bacteria; TFC = Total fecal coliform; + = Present; - = Absent
*Presence or absence of bacteria after enrichment
Shigella spp. were absent in all samples.

Table 2. Antibiotics susceptibility pattern of the isolates from the domestic waste samples

Isolates (n)	Antibiotics (% resistant isolates)									
	CAC	**MEM**	**COT**	**CIP**	**AZM**	**CFM**	**CTR**	**CXM**	**AMX**	**AK**
Staphylococcus spp. (2)	100	0	0	50	0	100	0	100	100	0
Salmonella spp. (2)	0	0	100	50	0	100	0	100	100	0
Pseudomonas spp. (4)	25	0	25	0	0	50	0	100	100	0
Vibrio spp. (5)	20	0	80	40	0	40	0	100	100	0
Klebsiella spp. (3)	100	0	0	100	33.3	100	0	100	100	0
Bacillus spp. (6)	100	0	33.3	100	0	100	0	100	100	0
E. coli (3)	25	0	0	33.3	33.3	33.3	0	100	100	0

n = number of isolates
CAC = Ceftazidime/Clavulanic acid (30/10 µg); MEM = Meropenem (10 µg); COT = Trimethoprime/sulfamethoxazole (25 µg); CIP = Ciprofloxacin (5 µg); AZM = Azithromycin (30 µg); CFM = Cefixime (5 µg); CTR = Ceftriaxone (30 µg); CXM = Cefuroxime (30 µg), AMX = Amoxicillin (30 µg); AK = Amikacin (10 µg).

CONCLUSION

The findings of the current study revealed that all the samples contained a huge number of bacterial isolates. Multidrug resistance was found in all the isolates of different bacterial species. Presence of pathogenic bacteria with antibiotic resistance traits portrayed serious public health threats. Antibiotic resistant pathogens may extend the hazards related to the poorly managed wastes. Unplanned dumping of untreated domestic liquid and solid wastes into municipal drain and dump sites may continually accelerate this problem. Proper management of household waste should therefore be ensured. Thus, this study urges the need for a national policy on household waste management.

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