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# Antibiotic susceptibility of *Escherichia coli* and *Bacillus* bacteria and its impact on waste workers health: A case study based on Dhaka City

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#### ABSTRACT

Solid waste, primarily consisting of residential and commercial waste, poses significant health risks in Bangladesh, particularly due to the presence of bacteria like *Escherichia coli* (*E. coli*) and *Bacillus*. These bacteria thrive in municipal solid waste and are common causes of bacterial infections, especially among waste workers who come into direct contact without proper safety measures. This lack of protection exposes them to dangerous bacteria, leading to diseases that reduce their work efficiency and, in some cases, result in death. A study conducted in Dhaka City Corporation from July 2019 to March 2020 identified and isolated *E. coli* and *Bacillus* from municipal solid waste using specific growth media – Eosin Methylene Blue (EMB) for *E. coli* and Mannitol Egg Yolk Polymyxin (MYP) for *Bacillus*. Both bacteria demonstrated susceptibility to antibiotics, but their presence highlights the severe health risks associated with poor waste management. With rapid urbanization and inadequate solid waste management systems, these risks are only increasing. The spread of such bacteria endangers not only waste workers but also the surrounding communities. Addressing this issue requires urgent improvements in municipal solid waste handling and worker safety protocols to prevent further public health crises.

Keywords: E. coli, Bacillus, solid waste, antibiotic, media.

#### INTRODUCTION

Bangladesh, with a population of approximately 162.7 million people (BBS, 2018) living in just 147,610 square kilometers, faces significant challenges in managing municipal solid waste. The rapid growth in population, urbanization, industrialization, and improved living standards have dramatically increased the generation of municipal solid waste in developing countries like Bangladesh (Khupe, 1996). Waste becomes particularly dangerous when it contains viable microorganisms or their toxins that can cause diseases in humans and animals (Vardakas et al., 2012). The improper management of these wastes creates fertile ground for bacteria to thrive, posing serious risks to human health, particularly for those involved in waste collection and disposal. A primary challenge for ecologists and municipal authorities is developing eco-friendly and effective waste management strategies. In Bangladesh, the population growth rate is 1.37% (BBC, 2024), with 23.3% of the population living in urban areas and 76.7% in rural areas. Rapid urbanization and industrialization contribute to better lifestyles, but they also generate vast amounts of

solid waste. As the population increases, so does the volume of waste and the number of harmful microbial organisms that can thrive in it, threatening human health. Waste collectors and carriers are particularly vulnerable, as the lengthy waste collection process often leaves waste exposed in secondary transfer stations. This delay gives bacteria the opportunity to proliferate and potentially spread via air or leachate, affecting both waste workers and passersby. The result can be the exposure of thousands of people to harmful bacteria, which can have severe health consequences (Dehghani et al., 2021). The objective of this research is to identify and isolate E. coli and Bacillus bacteria from municipal solid waste, to determine their antibiotic susceptibility, and to assess their impact on human health. E. coli and Bacillus bacteria are known to be dangerous to humans and are commonly found in municipal solid waste. E. coli is a type of bacteria that typically lives in the intestines of humans and animals, but certain strains can cause severe health problems. E. coli is responsible for 75% to 95% of urinary tract infections, and it can also cause abdominal cramps, vomiting, and bleeding. In severe cases, particularly in children, it can lead to acute kidney failure (Jahan et al., 2021). Bacillus, a rodshaped, gram-positive bacterium, is found widely in soil and water and is known to cause foodborne illnesses. It can generate toxins that cause two forms of gastrointestinal illness: emetic (vomiting) and diarrheal. Due to poor solid waste management, bacteria like E. coli and Bacillus have a high likelihood of growing in municipal waste. This study aims to isolate and identify these bacteria in secondary waste stations in Dhaka City and to determine their antibiotic susceptibility. Although these bacteria are common in Bangladesh, no research has specifically focused on their prevalence in municipal solid waste. E. coli and Bacillus have been studied in other countries, but Bangladesh has yet to conduct comprehensive research in this area (Hasan et al., 2021). Understanding the prevalence of these bacteria in waste stations is crucial for developing effective waste management strategies and protecting public health. Based on this research, the study will aim to scientifically prove the presence of E. coli and Bacillus in municipal waste stations, highlighting the urgent need for improved waste management practices (Akter et al., 2019). These findings will provide vital data for policymakers and health authorities to address the health risks posed by

bacteria in solid waste and implement required safety measures for waste workers and societies exposed to these hazards.

### MATERIALS AND METHODS

#### Study area

The study area was chosen according to the density of people in an area. Because the more people in an area, the more waste will generate from that place and hence the more chances of finding *E. coli* and *Bacillus* bacteria in the dumping station. The locations of the sample station are Kallayanpur Secondary waste transfer station, Mirpur Secondary waste transfer station, Jatrabari Secondary waste transfer station and Rayerbazar Secondary waste transfer station (Figure 1).

#### Agar media preparation

The samples needed to be swabbed to the agar media as soon as they were taken to avoid contamination. So, the agar media needed to be prepared before the sample was taken. The first step of the process was to prepare the agar media. The process is given below:

- 40 ml distilled water, 0.8 g agar, and 1.2 g peptone powder were taken and put in a conical flask. The flask was stirred until the powder had been dissolved into the water (Basu et al., 2005; Jilani et al., 2007).
- 2. After the mixture, three petri dishes and one beaker were filled with distilled water and wrapped securely in foil paper. Then, they were put in the autoclave for sterilization. After steadily rising to 121 °C, the temperature dropped for 22 minutes. It helps to ensure that the materials in it are properly sterilized when the temperature reaches 70 °C/60 °C (Basu et al., 2005; Jilani et al., 2007; Washington, 1991).
- 3. When the mixture, petri dishes, and the beaker were sterilized, it was immediately put in the laminar flow to cool down (Basu et al., 2005; Jilani et al., 2007; Washington, 1991).
- 4. After 5–10 minutes, the agar media mixture was poured into the petri dishes and kept for a while to solidify. After it had become solid, it was taken and put in the incubator at 37 °C for 24 hours (Basu et al., 2005; Jilani et al., 2007; Washington, 1991).



Figure 1. Sample collection points of Dhaka city for the study

# **Collection of samples**

The samples were collected from five (5) different secondary waste transport stations mentioned in Figure 1. The process of sampling that was followed is described below:

- 1. At very first, the safety kit (lab coat, gloves, double masks and headcover) was worn to maintain safety (Basu et al., 2005; Griffith, 2016).
- 2. Secondly three inside parts of each station were chosen (Edmonds, 2009).
- 3. Then, wooden cotton buds were taken and samples were taken through those buds. After that the cotton buds were put into different petri dishes so it doesn't get mix up.
- 4. Lastly, the petri dishes were wrapped up with foil paper and brought back to the laboratory for experiment (Basu et al., 2005; Jilani et al., 2007).

# Swab and storage

After collecting the sample from the station, it was necessary to bring it to the laboratory as soon as

possible. If not, there is a high chance of contamination. The swab and storage process are given below:

- 1. First a test tube was taken in the laminar flow and distilled water was poured from the beaker that has been sterilized previously along with petri dishes (Griffith, 2016).
- 2. Then the cotton buds were put in to the test tube and stirred (Basu et al., 2005).
- 3. After mixing, laboratory flame loop was taken and dipped in the test tube. Then samples were taken and swab in the agar media which was already prepared beforehand.
- 4. After swabbing, the media dishes were kept in the incubator for bacteria growth at 37 °C for 24 hours (Basu et al., 2005; Edmonds, 2009; Yamayoshi et al., 1984).

# Selective media preparation and streaking

One of the main focuses of the experiment was to identify the bacteria that were present at the secondary waste station's wastes. For this paper, it was needed to identify the presence of *E. coli* and *Bacillus* bacteria at the waste stations and to figure it out, one of the most effective methods was through selective media which has been used in this experiment (Figure 2). For *E. coli* bacteria, Eosin methylene bacteria (EMB) and for *Bacillus*, Mannitol egg Yolk Polymyxin (MYP) agar media was taken as these medias were very effective to identify *E. coli* and *Bacillus* bacteria (Edmonds, 2009; Exum et al., 2017; Griffith, 2016; Washington, 1991; Yamayoshi et al., 1984). The process of preparing these selective media is same and is given below:

- 1. First a conical flask was taken where 40 ml of distilled water, 1.0984 gm of EMB powder was taken according to the universal measurement (27.46 g/1000 ml) and stir it until the powder is dissolved with the water (Yamayoshi et al., 1984).
- 2. Following the mixing, a conical flax; three Petri dishes were covered securely in foil paper and sterilized in the autoclave. Before that, it was tested to see if there was enough water in the autoclave's bottom compartment. The lid was carefully closed once the flax and dishes were placed, and the start button was pressed. The temperature will automatically rise at 121 °C. And after reaching at 121 °C, it will cool for 22 minutes. It means when the temperature will reach at 70 °C/60 °C, then it can be assured that the materials inside has been sterilize properly (Exum et al., 2017).
- 3. After the sterilization, the dishes were taken directly to the laminar flow. Then the selective medias were transferred to the petri dishes from the conical flax and waited for them to cool down and become semi solid. The bacteria containing nutrient agar was taken out of the incubator and placed in the laminar flow. Then the flame loop was taken and the colony from the nutrient agar media of the previous day were taken off through the loop and was swabbed to the selective medias. The swap is also called streaking which we need to do very carefully (Exum et al., 2017; Park et al., 2017).
- 4. After the streaking, it was kept in the incubator at 37 °C for 48 hours to get the result.

# Muller Hinton broth and Muller Hinton agar preparation

To take the study further, antibiotic tests have been done on these bacteria. To conduct the antibiotic activity, Muller Hinton agar and Muller Hinton broth needed to prepare (Figure 2) and the process was:

- 1. First 1.14 gm of Muller Hinton agar and 1.05 gm of Muller Hinton broth powder were taken into two different conical flasks. Then mixed up with 30 ml and 50 ml distilled water respectively (Park et al., 2017).
- 2. After that, two conical flasks were taken along



1" Phase: Culture Bacteria from wastes of secondary dumping station

2<sup>nd</sup> Phase: Specific Media Prepare to culture specific Bacteria that is E coli and Bacillus

3rd Phase: Antibiotic placement on specific Bacteria to see their reaction

Figure 2. Conceptual framework of the study

with two Petri dishes and put in the autoclave for sterilize.

- 3. After the sterilization, it was taken out and put it in the laminar flow and cooled for a minute.
- 4. After cooling, it was poured in the Petri dish and wait till it became semi solid. When the solidification is done, it was taken and put in the incubator at 37 °C for 24 hours.

# **Antibiotic placement**

About 8 antibiotics were taken for the experiment. Keeping the Muller Hinton agar and Muller Hinton broth for 24 hours, it was ready for use. Now it needs to be swabbed in the bacteria from the selective media (Buttner et al., 2007; Hogan et al., 2015; Maes et al., 2017) and the process that followed was:

- 1. First the agar and broth media were taken from the incubator to be normal. When they adapt a normal temperature, colony bacteria were taken from the selective media with laboratory flame loop and mix it in the Muller Hinton broth for more growth of the bacteria.
- 2. After that it was wrapped with foil paper tightly and put in the incubator for 2 hours.
- 3. Two hours later, it was seen that the Muller Hinton broth has increased the number of bacteria. Now it was time to place antibiotic in the Muller Hinton agar. A process which was followed and the process was:
- the broth was taken out from the incubator and let cool for a moment. Then a cotton bud was taken that has been sterilizing before and put it into the broth. After taking the sample, it was swab in the Muller Hinton agar slowly (Sanderson et al., 2002).
- after swabbing, a cross mark was drawn and for dots in the Petri dish containing agar because it would help to place the antibiotic at an equal distance (Brown et al., 2007).
- after that, single dose of the antibiotics was placed according to the drawing. As there were two Petri dishes, 5 antibiotics were placed in a dish and 3 in another one.
- after placing the antibiotics in the Petri dishes, it was kept in the incubator at 37 °C for 24 hours (West et al., 2023).

#### Instrumental techniques

In the experiment, 303 series incubator was used to keep the optimum temperature, moistures

and other conditions, including inside the atmosphere's  $CO_2$  and oxygen content. A laminar flow (EQU/02-EHC) was used so that that the biological samples or any particle-related sensitive material have no contamination. A An autoclave (XFH-CA series) with pressure and steam was used to reach and maintain an excessively high temperature to live on microorganisms or spores. Digital analytical balance (EK600i model) was used to determine the weight or mass of a sample and Digital Colony Counter of BTronic group was used to count bacterial colonies.

### **RESULTS AND DISCUSSION**

#### **Isolation of bacteria**

Under the objective one, the isolation of the bacteria was done through nutrient agar. In case of isolation, samples were taken from five secondary waste transport stations. Then those samples were put in to the sterilized water prepared previous day and swab it into the nutrient agar media by flame loop. After this process the agar media was kept in the incubator at 37 °C for 24 hours.

It was found, after a day of incubation, that the nutrient agar in the media developed white colonies (Figure 3). Bacterial cells adhered to the surface on the agar plate and the majority of cells were bound by layers of slime. The colonies were in plain shape and the shapes were different because different types of bacteria can create colonies with different looks, some colonies may be colored, some colonies are circular in form, and others are irregular (Figure 3 and Figure 4).

### Identification of bacteria

Identification in the term microbiology means identification of certain specific bacteria by extending from the agar nutrient to the selective media. Every media has a different selective media only to make the particular bacteria grow.

In Objective one also stated the identification of bacteria. In case of *E. coli*, the growth of single, pairs and irregular clusters of *E. coli* bacteria on Eosin methylene blue (EMB) media was demonstrated in the test for different microbes carried on a selective medium. Some of the colonies where transparent, and some where layers of slime. In media EMB, *E. coli* gave colonies with distinctive metallic shades of green color. *E. coli* has evolved well in the EMB media. In case of *Bacillus*, MYP agar media was used and well-defined colonial lines of Bacteria growth were seen. From that selective media growth, it can be said that *E. coli* and *Bacillus* bacteria has the ability to exist in solid municipal waste which are pathogenic.

#### Antibiotic testing

The different antibiotics showed different results according to the reaction. For example, the result shows that Tetracycline antibiotic is resistance to E. coli (Table 1). It means, if this antibiotic is given to a human host which is affected by E. coli, then this Tetracycline will not work on the bacteria and will not kill it. In case of susceptibility, it is vice versa. Again, the result shows Imipenem antibiotic is sensitive to Bacillus (Table 2). It means, if this antibiotic is given to a human host which is affected by Bacillus, then this Imipenem will work on the bacteria and will be able to kill it. And if an antibiotic shows intermediate, it means the antibiotic will kill the bacteria but will need higher dose. A total number of eight antibiotics were used in this research. These antibiotics has been chosen because they are quite available on Bangladesh for use. Also, they are the most common types of antibiotics in Bangladesh. It is also very cost effective. They are:

- Imipenem (IMP),
- Ceftazidime (CAZ),
- Ceftriaxone (CTR),
- Tetracycline (TE),
- Amikacin (AK),
- Ciprofloxacin (CIP),
- Ampicillin (AP),
- Sulfanilamide Trimethoprim (COT).

### E. coli

In case of *E. coli*, these antibiotics showed varying results for *E. coli* bacteria. Some exhibited resistance and some exhibited vulnerability. The diameter of the resistance or susceptibility to these bacteria was also different. Their behavior towards bacteria has shown in the Table 1.

The above table defines that antibiotic Ceftazidime and Tetracycline showed resistance on *E. coli* bacteria which means when Ceftazidime and Tetracycline antibacterial has been inserted in the Muller Hinton agar for 24 hours, *E. coli* has shown resistance to this antibiotic, indicating that if this Ceftazidime is present in any media, *E. coli* will grow.

Similarly, When Amikacin and Sulfanilamide – Trimethoprim antibacterial was put in the Muller Hinton agar, *E. coli* showed strong sensitivity towards these antibiotics (Figure 3). It means that if this Amikacin and Sulfanilamide – Trimethoprim is present, then *E. coli* won't be able to develop.

Also, *E. coli* bacteria showed sensitivity in Imipenem, Ceftriaxone, Ciprofloxacin and Ampicillin antibacterial drug. But the sensitivity was intermediate level. It means *E. coli* will need stronger dose of Imipenem, Ceftriaxone, Ciprofloxacin and Ampicillin drug in order to be killed (Figure 3).

# Bacillus

In case of *Bacillus*, different antibiotic showed different results. Some showed resistance and some showed sensitivity and some showed intermediate result (Table 2).

In case of *Bacillus*, similar antibiotic tests were done and the result states that Imipenem and Ceftazidime showed sensitivity on *Bacillus* bacteria Which means when Imipenem and Ceftazidime antibacterial was put in the Muller Hinton

SI no.	Bacteria	Antibiotic name	Reaction
1	E. coli	Imipenem	Intermediate
2		Ceftazidime	Resistance
3		Ceftriaxone	Intermediate
4		Tetracycline	Resistance
5		Amikacin	Sensitive
6		Ciprofloxacin	Intermediate
7		Ampicillin	Intermediate
8		Sulfanilamide – Trimethoprim	Sensitive

 Table 1. Antibiotic tests used on Escherichia coli



 Table 2. Antibiotic tests used on Bacillus

SI no.	Bacteria	Antibiotic name	Reaction
1	- - - Bacillus	Imipenem	Sensitive
2		Ceftazidime	Sensitive
3		Ceftriaxone	Intermediate
4		Tetracycline	Intermediate
5		Amikacin	Resistant
6		Ciprofloxacin	Resistant
7		Ampicillin	Resistant
8		Sulfanilamide – Trimethoprim	Resistant

agar, *Bacillus* showed sensitivity towards these antibiotics. Amikacin, Ciprofloxacin, Ampicillin and Sulfanilamide– Trimethoprim, these four antibiotics showed resistance towards *Bacillus* bacteria when inserted in the Muller Hinton agar for 24 hours, *Bacillus* has shown resistance to this antibiotic, indicating that if these four bacteria are present in any media, *Bacillus* will grow.

Lastly, *Bacillus* bacteria were sensitive to the antimicrobial drugs Ceftriaxone and Tetracycline. However, the sensitivity was only on the intermediate level. *Bacillus* will require a higher dose of Ceftriaxone and Tetracycline to be destroyed (Figure 4).

#### **Health impact**

*Bacillus* and *E. coli* are widely distributed throughout the environment, particularly in soil, air, and decomposing plant residue. It has shown a capacity to grow over a wide range of temperatures including that of the human body, especially in waste workers as they can be expose directly by these bacteria (Wang et al., 2024). Given its

ubiquity in nature and the environmental conditions under which it is capable of surviving, *Bacillus* could be expected to temporarily inhabit the skin and gastrointestinal tract of humans (Nicholson et al., 2000) and *E. coli* in the stomach or intestines.

Waste workers get infected with *E. coli* predominantly by come in contact with them directly. Infection can also be spread by feces contamination of water and other foods, as well as cross-contamination during food preparation. These bacteria induce diarrhea by damaging the lining of the small intestine (Navab-Daneshmand et al., 2018).

*Bacillus* and *E. coli* bacteria found in the secondary solid waste system can cause severe damage to the waste workers work there and even the passersby. For example, most of the workers there, work with bare hands and touch the wastes without any protection. Workers can be exposed to *Bacillus* and *E. coli* bacteria through that and as a result, this can lead to stomach infection. They can also be affected by respiratory problem, skin



disease, etc. Besides, *E. coli* and *Bacillus* bacteria can cause high fever, dry cough, breathing problem, rash, heart problem, lung disease and kidney failure. These bacteria can even cause death as well (Joshi et al., 2020).

# CONCLUSIONS

The study result shows that both E. coli and Bacillus bacteria were found in the municipal solid waste. They might be tiny organisms but the negative impact they put on human health is beyond imaginable. These wastes are touched by the waste workers every day. Apart from that, these unmanage wastes are also affecting human health greatly. To accomplish a maintainable Municipal Solid Waste the specialists must anticipate what sums and synthesizes the produced waste will have. Waste management must involve a focal job in city arranging for example at a similar level as ensuring security of civilians. In the instance of waste, it is a test of obliging a large portion of a huge amount of trash each year. Right now, the areas that center around are five parts of Dhaka; Municipal Solid Waste of these five areas leads to the natural effects, jobs and duties, and obstructions and openings in Dhaka city. Worker's health should also be a concern for the municipality authorities. Necessary steps should be taken by both the govt. and the authorities of municipal solid waste management. Besides that, general people should start from their side to take responsibility of the workers by supply them the safety kits or educating them about the harmfulness of their working without safety tools. That's how a city will manage to beatify without waste and unhealthiness.

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