A STUDY OF PREVALENCE AND ANTIMICROBIAL RESISTANCE AND PATHOGENIC ACTIVITY OF BACTERIA IN THE AIR OF CERTAIN AREAS OF DHAKA CITY

By

Anika Ashraf
ID: 18126012
Tasnuva Faria
ID: 18126051

A thesis submitted to the Department of Mathematics and Natural Sciences in partial fulfillment of the requirements for the degree of
Bachelor of Science in
Microbiology

Department of Mathematics and Natural Sciences
BRAC University
April 2022

Declaration

It is hereby declared that

1. The thesis submitted is our own original work while completing a degree at BRAC University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material that has been accepted or submitted, for any other degree or diploma at a university or other institution.
4. We have acknowledged all the main sources of help.

Student's Full Name & Signature:

Anika Ashraf	**Tasnuva Faria**
ID: 18126012	ID: 18126051

Approval

The thesis/project titled "A study of prevalence and antimicrobial resistance and pathogenic activity of bacteria in the air of Dhaka city." submitted by-

1. Anika Ashraf (18126012)
2. Tasnuva Faria (18126051)

Of Summer, 2015 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of B.Sc. in Microbiology on 04.09.2021

Examining Committee:

Supervisor:
(Member)

_____

Dr. M. Mahboob Hossain

Professor, Department of Mathematics and Natural Sciences

BRAC University

Co-supervisor:
(Member)

_____

Akash Ahmed

Lecturer, Department of Mathematics and Natural Sciences

BRAC University

Program Coordinator:
(Member)

_____

Dr. Mahbubul H. Siddiqee

Department of Mathematics and Natural Sciences

BRAC University

Departmental Head:
(Chair)

_____

A F M Yusuf Haider

Professor and Chairperson, Department of Mathematics and Natural Sciences

BRAC University

Abstract

The economic hub of Bangladesh, it's capital city Dhaka is subject to a high level of pollution all year round, which makes the air harmful to breathe. On average, around 14 m^3 of air is inhaled by an individual every day. The ubiquity of pathogenic bacteria in the air that people inhale, presents the utmost health hazard for them. The purpose of the study is the enumeration of the prevalent bacteria in Dhaka city's air, identifying the pathogens among them by analyzing their hemolysis pattern. This study was carried out in 10 bustling areas around Dhaka (Dhanmondi, Gulistan, Mohakhali, Khilgaon, Motijheel, Mohammadpur, Badda, Keraniganj, Shantinagar, Mirpur). For sample collection, different selective media (Nutrient agar, Mannitol Salt agar, MacConkey agar, EMB agar) were exposed in the air where the highest and lowest CFU were 189 and 1 respectively. Distinct colonies were chosen and sub-cultured in nutrient agar for running further tests. Pathogens were screened a through Hemolysis test in blood agar. A total of 157 isolates were screened for pathogenicity in blood agar and their hemolysis pattern showed 43 of them being capable of hemolysis. Among 157 isolates, 22 showed β hemolysis where they completely lysed the red blood cells; 12 isolates showed α hemolysis and 9 isolates showed γ hemolysis. In the DNAse test, out of 43 samples, 17 turned out to be DNAse negative, and 26 were DNAse positive. The results of the study indicate the potential presence of pathogens in the air which calls for an extension of it that would help find out the potential pathogens present in the air and act on time before the matter gets out of hand and give rise to new incidences, epidemics, or even pandemics.

Keywords: air microbiota, air pollution, pathogenicity, multidrug-resistant.

Dedication

To science, for science.

Acknowledgment

This project would not have been possible without the support of many people. Our heartfelt gratitude goes to Professor A F M Yusuf Haider, Chairperson, Mathematics and Natural Sciences Department, Dr. Mahbubul H. Siddiquee, Coordinator of the Microbiology program, for maintaining the department regulations and delivering proper education to every student including us.

We would like to convey our deepest gratitude to my supervisor, Dr. M. Mahbboob Hossain, Professor, Mathematics and Natural Sciences, BRAC University, for his patience, guidance, and support. We have benefited greatly from his wealth of knowledge and meticulous editing. We are extremely grateful that he took us on as students and continued to have faith in us over the years.

We are indebted to Akash Ahmed, Lecturer, Mathematics and Natural Sciences, BRAC University, who has been an ideal teacher, mentor, and thesis co-supervisor, offering advice and encouragement with a perfect blend of insight and motivation. We are proud of, and grateful for, our time working with him.

Most importantly, we would like to thank our parents, siblings, and numerous friends who endured this long process with us, always offering unconditional, unequivocal support; and love.

Anika Ashraf

&

Tasnuva Faria

April 22

Table of Content

List of Tables

List of Figures

List of Acronyms

MIU	Motility Indole Urease
MR	Methyl Red
VP	Voges Proskauer
Fig	Figure
MSA	Mannitol Salt Agar
NA	Nutrient Agar
MAC	Mac Conkey Agar
et al	and others
mg	Milligram
µg	Microgram
AMC	Amoxiclav
AZM	Azithromycin
CIP	Ciprofloxacin
LE	Levofloxacin
P	Penicillin
CFM	Cefixime
MXF	Moxifloxacin

TE	Tetracyclin
DO	Doxycyclin
CIP	Chloramphenicol
CXM	Cefuroxime
S	Sensitive
R	Resistance
I	Intermediate
spp	Species

Chapter 1

Introduction

1.0: Introduction

Air pollution is a matter of utmost concern in this day and age. According to a fairly recent study, Bangladesh composes the most polluted air with Dhaka securing the second position in terms of pollution (daily star). According to air visual 2018 reports [1]. Dhaka city is ranked number 17th among the world's most polluted cities. However, this study aimed to enumerate the prevalence of the microorganisms present in the air of Dhaka city to get an idea of the kind of bacteria present in such an extremely polluted arena. Every day, 14m^3 of air is inhaled by a person relatively (Kabir *el at.,* 2016) (Brochu *el at.,* 2006). If there are numerous amounts of microbes in the air people inhale, it could be health hazardous for them. Some pathogenic strains in the air and their chemical secretions have the potential to bring about acute health disorders and cause infectious diseases (Kabir *el at.,* 2016) (Sekulska *el at.,* 2007). So, it is of great significance to enumerate the type of microbes present in the air we breathe in and their hazardous effect and disease-causing capability, which is the pathogenicity, is determined. The fact that a pathogenic microbe would survive or not depends on its resistance capacity and the environmental conditions (Cernei *el at.,* 2013). The aim of the study is to find out pathogens in the air and confirm the pathogenic strain species through genetic analysis of 16srRNA gene sequences. The study also depicts the multidrug resistance of the prevalent microbes in the air. In addition to that, a comparison of colony counts with temperature and humidity respectively at the time of sample collection was done to estimate the effect of environmental factors on microbial presence.

1.1: Background of the Study

Microbiologists all over the world started finding bacteria in every nook and cranny, far and wide for a very long time ever since Leeuwenhoek first saw 'animalcules' through his hand-held microscope in the late 1600s. Starting from dental plaque to hydrothermal vents to the dead sea, no place is free from bacteria because they can survive in any kind of extreme conditions . Even the air, which can be considered one of the least hospitable environments for microbes as it holds fewer nutrients help to support relatively fewer organisms than other mediums .For this reason the aero microbiology, which is the study of living microorganisms suspended in air, traditionally gets less attention than aquatic or soil microbiology. However, the sudden emergence of airborne COVID-19 transmission has generated new interest in this aero-microbiology field. The troposphere, or the innermost closest layer of Earth's atmosphere, is the most favourable for the growth and survival of microorganisms. One reason is that it contains more water vapor than the other layers, thus preventing desiccation

of the cells and also allowing a certain level of biological activity to be retained. Unlike soil microbes, which can be regarded as native to their environment, microbes that are found in the air only get there by being introduced from another source. Such as the human activities like waste disposal, waste treatment, agriculture and industry have more chance of releasing microbes into the air. As soil is a major source of bioaerosols , one gram of soil can hold millions of bacterial and fungal cells, where some of them exist in their spore form, making it easier for them to survive the unfavourable conditions of the atmosphere when they become suspended there .

1.2: Objectives of the Study:

Pathogenic bioaerosols are dependent upon prevailing physicochemical properties of the atmosphere, such as temperature, humidity, solar radiation, wind, precipitation, and air pressure, for transport and survival. High temperature, for example, is generally unfavourable to the survival of bacteria and viruses in the air, but with a more marked effect on the latter. This study is an attempt at shedding some much-needed attention on the conditions of microbial load assessment and monitoring. This study aims to-

- Quantify the prevalence of different types of microbes in Dhaka city's air.
- Identify the microbes through a "biochemical test".
- Identify the pathogenic microbes through "pathogenicity test".
- Detect the virulence activity of the pathogens.
- Detection of the "Multi-Drug Resistant" strain among the pathogenic bacteria.

Chapter 2

Materials and Method

2.0: METHODS AND MATERIALS

This thesis work was done by covering seven different zones around Dhaka. All the samples were collected from outdoor of different zones in Dhaka. The lab work was conducted at the

Microbiology Research Laboratory of the Department of Mathematics and Natural Sciences of BRAC University.

2.1: Sample Collection

A total of 3785 samples were collected from ten different zones of Dhaka (Mohammadpur, Mohakhali, Gulistan, Shantinagar, Badda, Keraniganj, Khilgaon, Motijheel, Mirpur, Dhanmondi). The samples were collected in three different media in two different durations, one was for 3 minutes, and another was for 5 minutes. All the samples were labeled properly.

2.1.1: Sample collection on Mannitol Salt Agar:

Mannitol salt agar is a selective medium used for the isolation of pathogenic staphylococci. The medium contains mannitol, a phenol red indicator, and 7.5% sodium chloride. The high salt concentration inhibits the growth of most bacteria other than staphylococci. On MSA, pathogenic *Staphylococcus aureus* produces small colonies surrounded by yellow zones. The reason for this change in color is that *S. aureus* ferments the mannitol, producing an acid, which, in turn, changes the indicator from red to yellow. The growth of other types of bacteria is generally inhibited.

2.1.2: Sample collection on Nutrient Agar:

Nutrient agar or NA is a universal media that is generally used to support the growth of non-fastidious organisms like a variety of different bacteria and fungi. NA contains many nutrients that are generally needed for the growth and development of bacteria. The medium contains 0.5% peptone -which provides organic nitrogen and 0.3% beef extract or yeast extract which provides the water-soluble content of vitamins, carbohydrates, nitrogen, and salts.

2.1.3: Sample collection on MacConkey Agar:

MacConkey is a selective and differential culture medium which is also an indicator. MacConkey medium is designed to isolate Gram-negative and enteric bacilli and differentiate them based on their fermentation of lactose. It contains crystal violet and bile salts which inhibit the growth of other gram-positive but allows the selection and growth of gram-negative bacteria which can ferment lactose.

2.1.4: Sample collection on Eosin Methyl Blue Agar:

Eosin Methylene Blue (EMB) agar is a differential microbiological medium, which slightly inhibits the growth of Gram-positive bacteria and provides a color indicator distinguishing between organisms that ferment lactose (e.g., *E. coli*) and those that do not (e.g., Salmonella, Shigella). EMB agar was originally devised by Holt-Harris and Teague and further modified by Levine. It is thus a combination of the Levine and Holt-Harris and Teague formulae which contains a peptic digest of animal tissue and phosphate as recommended by Levine and two carbohydrates as suggested by Holt-Harris and Teague. The medium is important in medical laboratories to distinguish gram-negative pathogenic microbes in a short period of time.

2.2: Microbial culture of the samples:

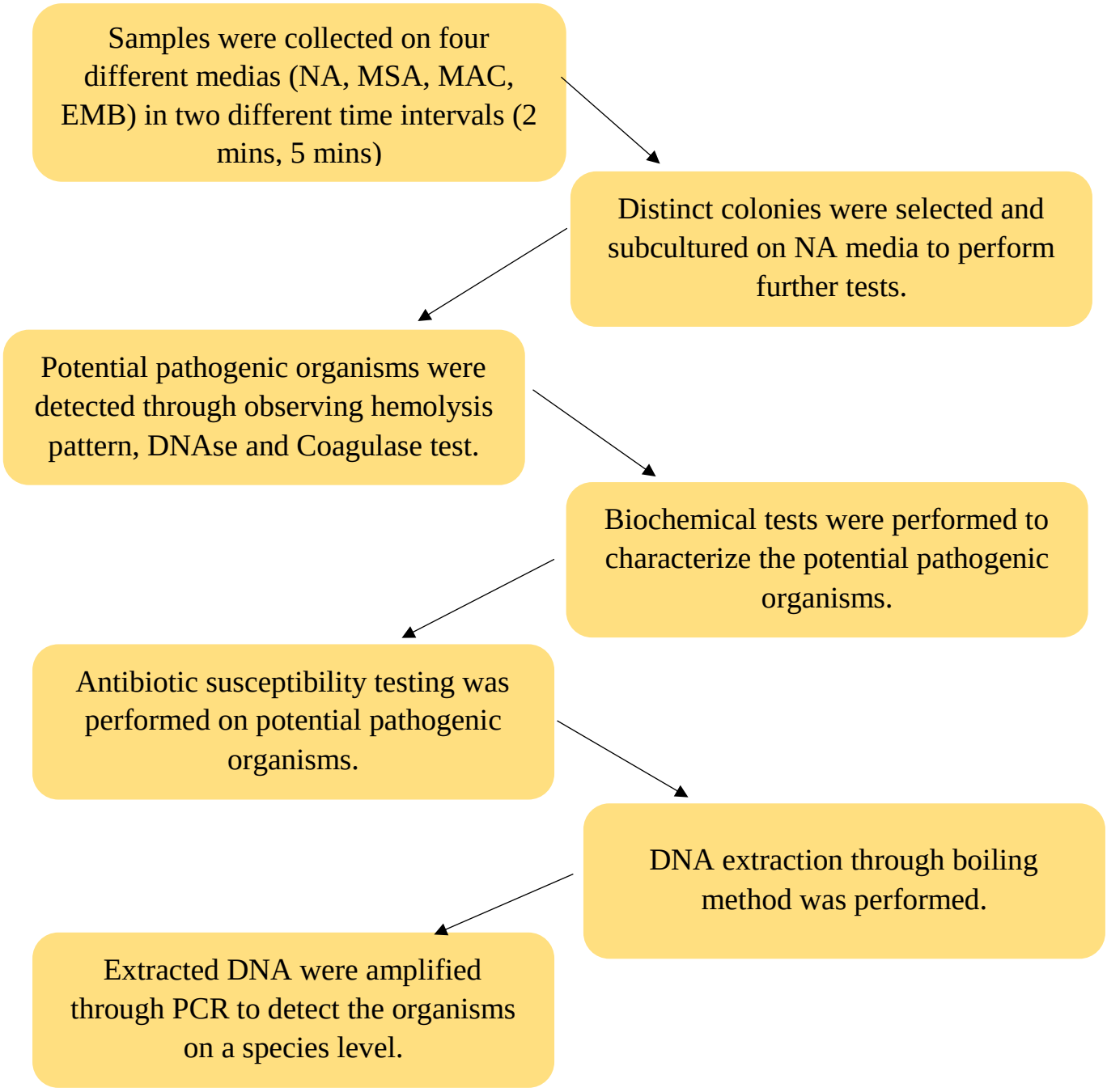
Initially, all the samples were incubated for 24 hours at 37^0C for growth. The next day after incubation selective colonies were taken from the media and inoculated on nutrient agar.

2.3: Screening of the bacteria from the samples:

These bacterial samples were isolated and then identified using the biochemical tests and put into the ABIS, a bacterial identification software for isolation of the organisms.

2.4: Experimental Workflow:

This study was carried out at the Math and Natural Sciences lab at BRAC University. The entire study methodology is briefly given below: -



2.5: The biochemical tests that were performed are-

- Gram staining
- Methyl Red (MR) test
- Voges– Proskauer (VP) test
- Citrate Utilization test
- Catalase test
- Triple Sugar Iron (TSI) test
- Motility Indole Urease (MIU) test
- Indole test
- Starch Hydrolysis
- Phenol red lactose broth
- Phenol red sucrose broth
- Phenol red glucose broth
- Coagulase test
- Telluride Glycine Agar

Biochemical Tests

2.6: Gram staining

The Gram stain procedure distinguishes between Gram-positive and Gram-negative groups. The morphology of the bacteria can also be checked using this method. In the gram staining method, different types of reagents are used such as crystal violet, saffranine, gram's iodine, etc. In this method, we smeared organisms in slides with saline solution and then added the reagents step by step. In the end, we differentiated all our organisms by seeing their cells color pink or violet. Gram-negative organisms have shown pink-colored cells under a microscope and gram-positive organisms have shown violet-colored cells.

Results of Gram staining-

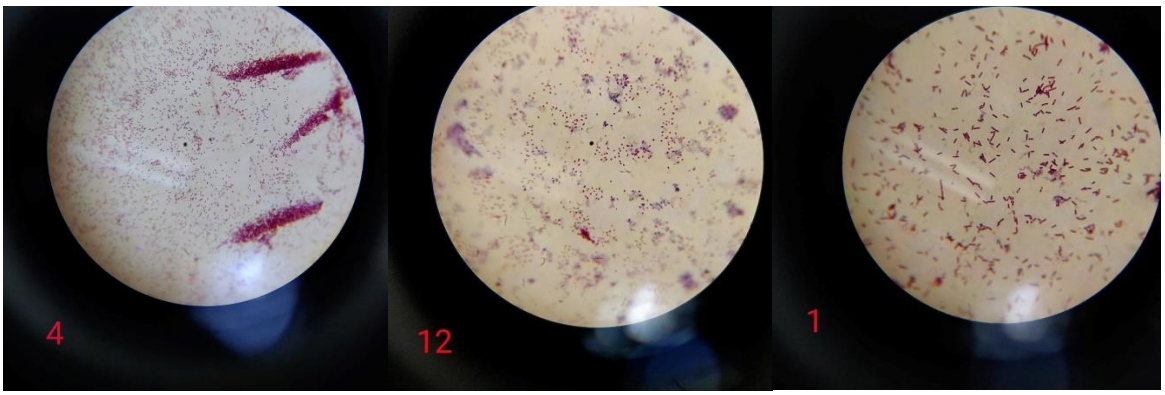


Figure 1: Gram Staining results.

2.7: Triple sugar iron test

Triple sugar iron agar is a differential medium used to determine H2S production and the type of carbohydrate fermentation. Gas from carbohydrate metabolism can also be detected. To conduct the test, an isolated colony was inoculated in the TSI medium. The results were observed after 24 hours of incubation at 37°C. (Cappuccino and Sherman

2008). In a triple iron sugar test, we inoculated the TSI media with our organisms and incubated them for 24 hours. Following the next day, we have seen different results such as

red slant/red butt which meant glucose, lactose, and sucrose non-fermenter, red slant / yellow butt meant glucose fermentation only, yellow slant/yellow butt meant all sugar fermentation. Production of Gas and H2S has shown cracked in the media and black spots in the media.

Figure 2: TSI test results.

2.8: Motility Indole Urease test

In the laboratory Motility testing using a semi-solid medium is commonly used for the identification of gram-positive bacteria. MIU test was done for determining the motility of bacteria, indole production, and urea degradation using the enzyme urease. Using an inoculating needle, a colony from a 24-hour fresh bacterial culture was picked up and inoculated in the medium. The test tubes were incubated at 37°C for 24 hours. The appearance and color of the media were observed after incubation (Cappuccino and Sherman, 2008). Motility-positive organisms have shown diffuse zone of growth flaring from the line of inoculation of the organism and on the other side urease, positive organisms have shown a pink color appearance in the media.

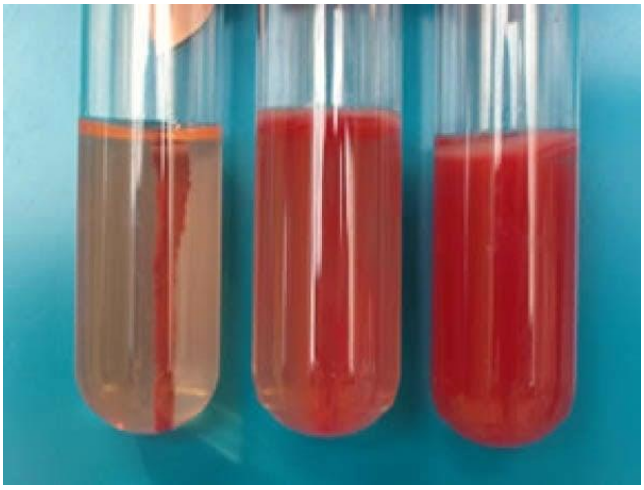


Figure 3: MIU Test results.

2.9: Catalase test

A catalase test was done to determine the ability of the bacteria to degrade hydrogen peroxide. A sterile microscopic slide was placed on a petri dish and a small amount of organism was picked using a sterile inoculating loop. Then 1 drop of 3% H_2O_2 was placed on the organism on the microscopic slide by using a dropper. Finally, the positive result indicates the presence of bubbles of oxygen gas. If no bubble formation means the result was negative. (Cappuccino et al., 2008).

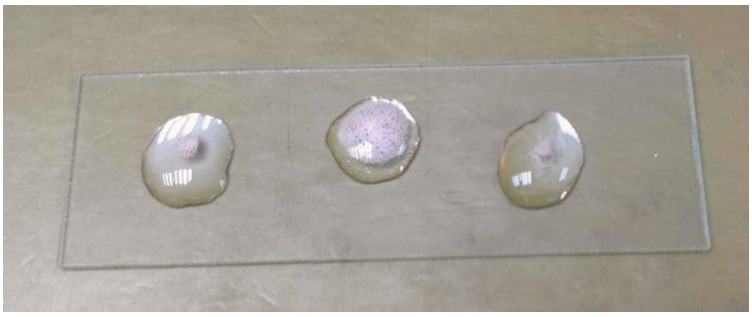


Figure 4: Catalase Test Result.

2.10: Indole test

The indole production test was done to determine the production of indole by pathogens. Only some pathogens can produce indole. For the indole test, tryptophan broth was inoculated with a bacterial culture to observe the production of indole and incubated at 37°C for 24 hours. Then

Kovac's reagent was added to the broth culture to observe the production of indole by observing the color changes to determine whether the result is positive (cheery red ring) or negative (yellow) (Cappuccino and Sherman, 2008). We have inoculated our organisms in indole broth and incubated them for 24 hours in the incubator at 37°C. Indole positive organisms have shown a pink-colored ring on the top of the media whereas negative organisms have showed yellow ring.

Figure 5: Indole test result.

2.11: MR-VP test

Methyl red test was applied to analyze the bacterial ability to produce stable acid end products.

Bacterial cultures were inoculated MR broth in clean test tubes and incubated overnight at 37°C. Then methyl red reagent was added, and the medium was observed for the immediate development of color. The appearance of a red color indicated a positive result, and the appearance of yellow color indicated a negative result. (Cappuccino and Sherman, 2008).

The Voges-Proskauer test determines the capability of producing non-acidic or neutral end products. Bacterial cultures were inoculated VP broth in clean test tubes and incubated overnight at 37°C. Then Barrett's reagent A and Barrett's reagent B were added. The tube was then allowed to remain still for 10-15mins, and the solution was observed for color changes to determine whether the result is positive (pink-red) or negative (yellow) (Cappuccino and Sherman, 2008).

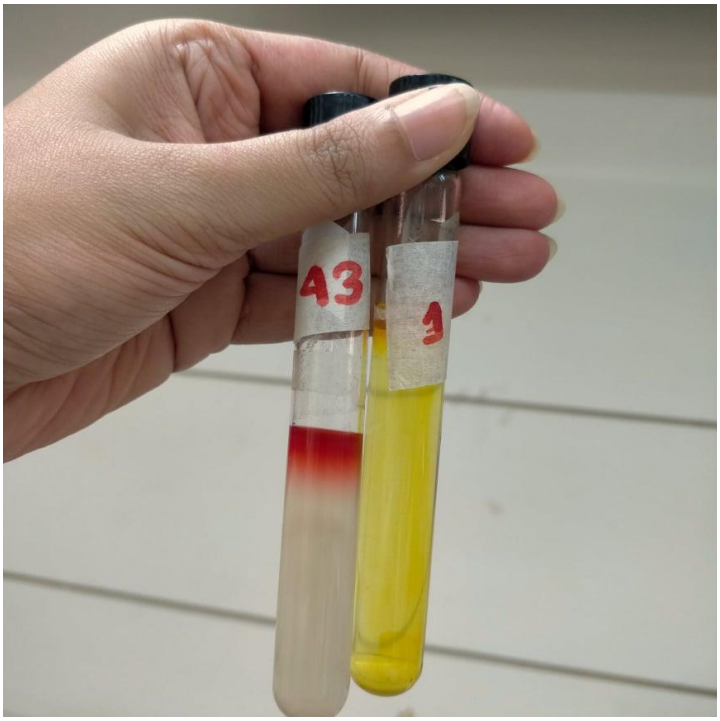


Figure 6: MR-VP test.

2.12: Coagulase Test

The coagulase test is a biochemical test that is performed to differentiate between coagulase-positive Staphylococcus aureus and coagulase-negative Staphylococcus CONS. Coagulase is an that is generally produced by *S. aureus.* Staphylococcus aureus produces this coagulase that converts the soluble fibrinogen in plasma to insoluble fibrin. Staphylococcus produces two forms of coagulase bound bound-free.

2.13: Starch Hydrolysis

Starch hydrolysis is one of the biochemical tests which is used to identify those bacteria who can hydrolyze amylose and amylopectin (present in starch) using the enzyme a-amylase and oligo-1,6-glucosidase. Starch hydrolysis is generally used to differentiate species from genre Clostridium and Bacillus because two organisms have large molecules of amylose and amylopectin. They cannot pass through the bacterial cell wall. There is no color change when organisms hydrolyze starch, to interpret the result gram's iodine is added. Based on the concentration of iodine, it can turn into blue, purple, or black in the presence of starch. A clearing area or zone around the bacterial growth indicates that the organism has hydrolyzed starch.

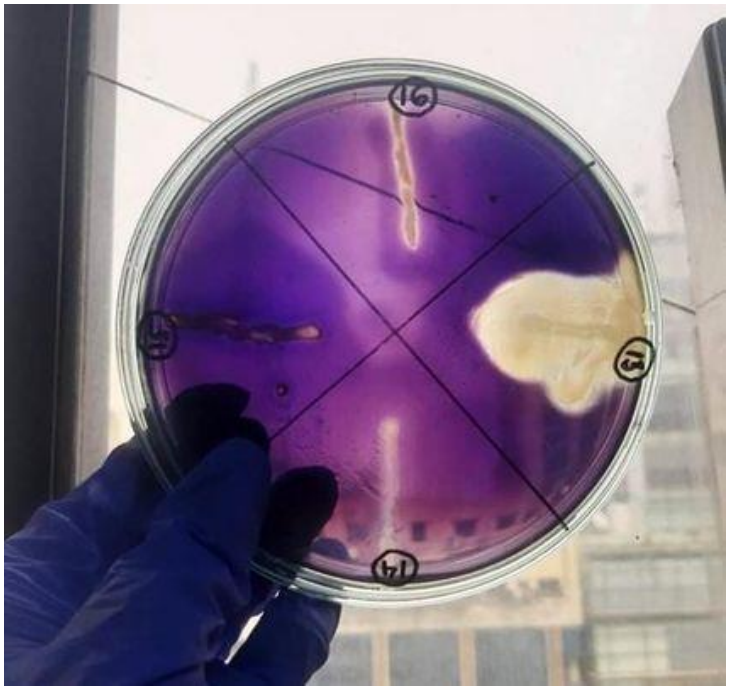


Figure 7: Starch Hydrolysis Result.

2.14: Phenol red carbohydrate (lactose, sucrose, glucose) fermentation test

Phenol red lactose, sucrose, and glucose broth are biochemical tests that are used to identify fermented organisms. Phenol red is an indicator. The broth mediums are a nutrient broth to which 0.5-1.0% lactose, sucrose, and glucose are added. The pH indicator phenol red is red at neutral pH but turns yellow at p H <6.8. After 24 hours incubation, the liquid in the tube turns yellow It indicates that there is a drop in the pH because of the production of the acid by the fermentation of the carbohydrate (sugar) present in the media.

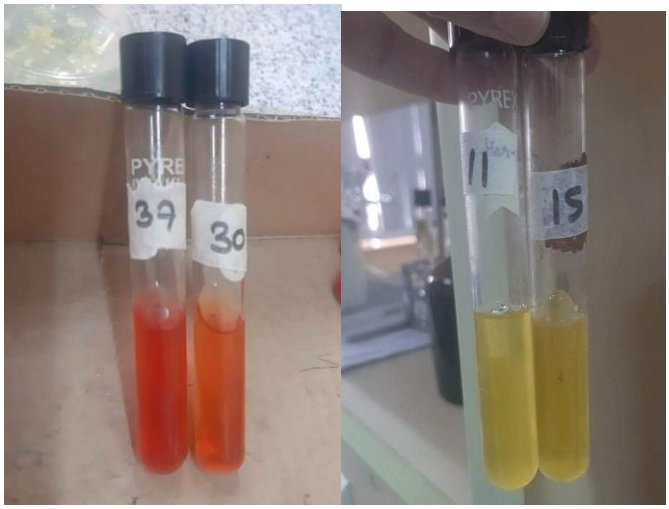


Figure 8: Phenol red carbohydrate (lactose, sucrose, glucose) fermentation test result.

2.15: Blood Agar Hemolysis Test

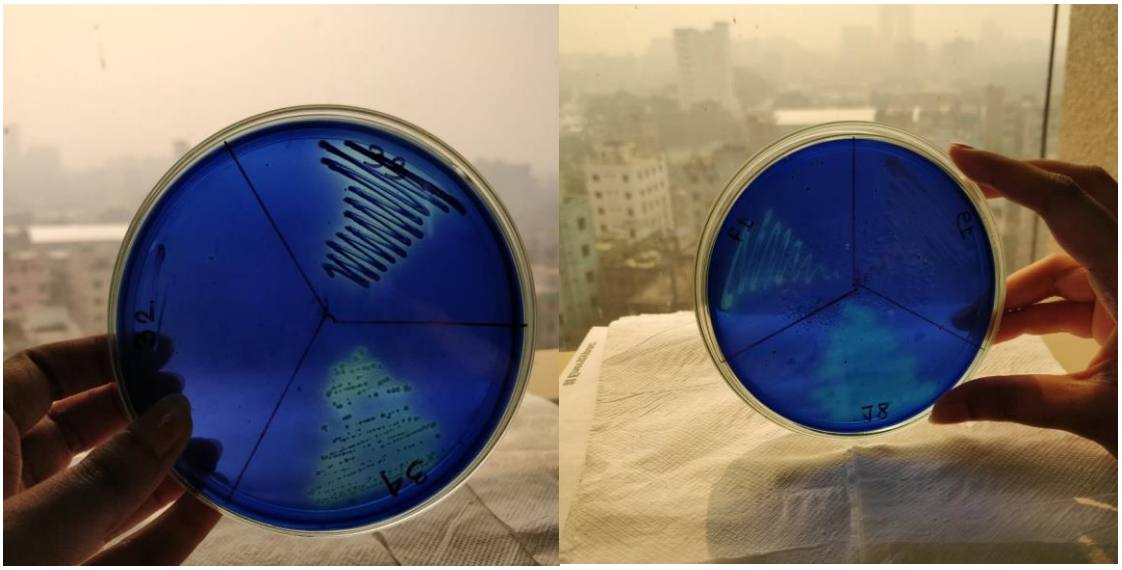
Blood agar base medium is augmented with the addition of 5% of sheep blood is generally used. This medium is called blood agar. Hemolysis in the blood agar medium is determined by the streaking of the organisms on a blood agar plate. After 24 hours of incubation, the blood agar medium is checked for signs of alpha- or beta-hemolysis. If the medium is discolored or darkened or showing green color after bacterial growth, the organism has shown alpha hemolysis. And if the agar plate has cleared surrounding undergrowth, the organism is beta-hemolytic. No discernible change in the color of the medium constitutes gamma-hemolysis (Gerhardt et al., 1994).

Figure 9: Blood agar hemolysis test result.

2.16: DNase Test

DNA hydrolysis test or Deoxyribonuclease (DNase) test is used to check the pathogenicity of an organism and whether an organism can hydrolyze DNA and utilize it as a source of carbon and energy for growth. DNase agar base, a differential medium is being used in the microbiology laboratory to test the ability of an organism to produce deoxyribonuclease or DNase. This Dnase agar medium base is generally pale blue in color because of Tolluidine

Dye (indicator) complex. It also contains nutrients for the bacteria. After the inoculation of the organism in the agar base if the organism that grows in the medium produces Deoxyribonuclease, if it does the organism will break down DNA into smaller fragment molecules. When the DNA is broken down, it no longer binds to the Toluidine blue, and the blue color fades and the colony is surrounded by a colorless clear zone.



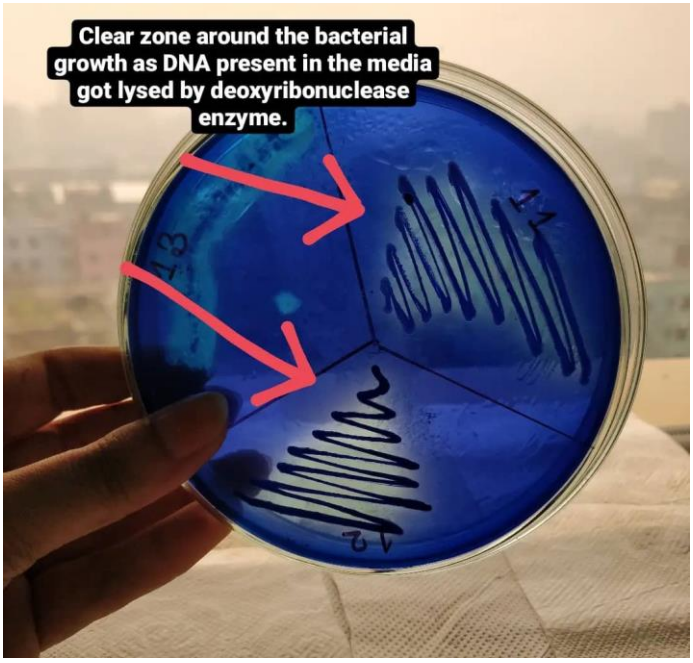


Figure 10: DNAse test results.

Chapter 3

Results

3.1: Sample Collection Area and Zones:

Among 3785 bacterial colonies, 157 distinct colonies of bacteria were isolated through different types of biochemical tests. All the air samples were collected from 10 different areas at different temperatures.

Zone	Time	TEMP	UV	Precipitation Probability	HUM	DATE
Mohammadpur	12:33 pm	30^0C	Low	40%	76%	01.09.21 Sunday
Badda	12:25 pm	24^0C	Low	43%	79%	03.10.21 Tuesday
Keraniganj	12:13 pm	35^0C	Low	10%	70%	09.10.21 Monday
Motijheel	11:55 am	35^0C	Low	20%	70%	10.10.21 Tuesday
Shantinagar	10:36 am	37^0C	Low	40%	86%	12.10.21 Thursday
Mirpur	01:48 pm	35^0C	Moderate	61%	72%	16.10.21 Monday
Khilgaon	11:30 am	32^0C	Moderate	55%	76%	12.10.21 Thursday
Gulistan	2:05 pm	32^0C	Low	50%	70%	17.10.21 Wednesday
Mohakhali	12:00 pm	35^0C	Low	63%	80%	25.10.21 Thursday
Dhanmondi	02:20 pm	33^0C	Low	60%	70%	15.10.21 Sunday

Table 1: Zone Areas, Temperature, Humidity, and Date of sample collection

Samples were collected from 10 bustling areas of Dhaka City. A few things were kept in mind during this process. The timing was roughly kept from 11 am – 3 pm, as this is the period when most people commute around the city. The highest temperature and lowest temperature recorded were 37^0 Celsius and 24^0 Celsius

respectively. Recorded UV exposure was mostly low. A 40% chance of rain means that there is a 40% chance that rain will fall somewhere within the forecasted area. Rain refers to 0.01 inch or more. Lastly, Water in the air in its gaseous state, i.e. humidity, can store very large amounts of energy and can release it again during conversion to the liquid state, rain. Due to its properties, water, as humidity in the air, stabilizes our climate and prevents large extremes of temperature. The highest and lowest humidity recorded was 86% and 70% respectively.

3.2: Colony Count:

Zone	Duration (min)	Nutrient Agar (CFU)	Mannitol Salt Agar (CFU)	MacConkey Agar (CFU)	Eosin Methyl Blue Agar (CFU)
Mohammadpur	3	107 + 4	79 + 9	2 + 0	14 + 5
	5	127 + 2	86 + 7	8 + 0	7 + 4
Badda	3	34 + 0	21 + 3	5 + 0	10 + 3
	5	79 + 0	29 + 0	7 + 0	6 + 3
Keraniganj	3	115 + 0	97 + 8	0 + 2	2 + 2
	5	132 + 2	166 + 6	2 + 1	0 + 3
Motijheel	3	127 + 3	38 + 1	1 + 1	0 + 1
	5	133 +2	94 + 0	4 + 0	10 + 1
Shantinagar	3	170 + 1	61 + 0	6 + 0	5 + 3
	5	183 + 0	133 + 0	13 + 0	5 + 8
Mirpur	3	188 + 0	47 + 0	1 + 1	5 + 0
	5	176 + 1	53 + 0	1 + 1	7 + 0
Khilgaon	3	63 + 1	34 + 2	2 + 3	0 + 2
	5	57 + 1	43 + 2	3 + 0	6 + 8
Gulistan	3	112 + 4	21 + 1	5 + 0	18 + 1
	5	80 + 6	53 + 6	3 + 0	3 + 2
Mohakhali	3	80 + 1	77 + 3	11 + 3	25 + 7
	5	124 + 2	87 + 1	11 + 2	27 + 9
Dhanmondi	3	65 + 1	16 + 8	1 + 0	3 + 3
	5	81 + 0	72 + 3	1 + 0	5 + 4

Total number of bacterial colonies isolated- 3785, fungal colonies isolated- 163

Table 2: Numbers of samples

Samples were collected for 3 and 5 minutes. The total number of bacterial colonies collected was 3785 and a total number of fungal colonies collected was 163.

3.3: Comparison between bacterial CFU and fungal CFU from air sample isolates

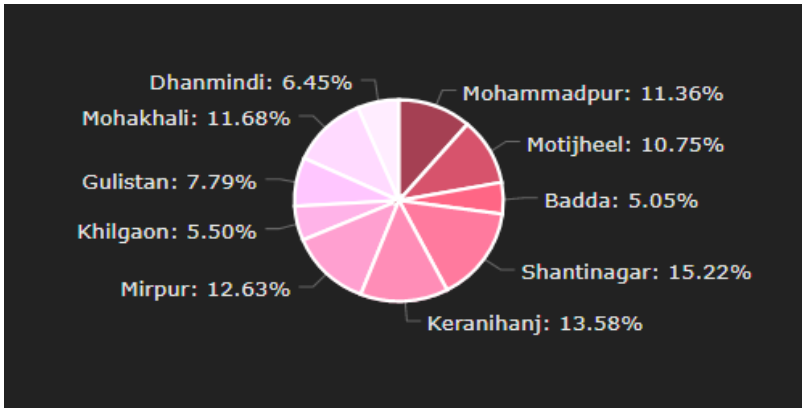


Figure 11: Isolated Bacterial colonies

Among the total 3785 bacterial colonies, Shantinagar and Keraniganj gave the greatest number of colonies which is 15.22% and 13.58% of the total isolated colonies respectively. This is indicative of the amount of biological pollutants present in the air. Among other areas- 6.54% from Dhanmondi, 11.68% from Mohakhali, 7.79% from Gulistan, 5.50% from Khilgaon, 12.63% from Mirpur, 11.36% from Mohammadpur, 10.75% from Mothijheel and 5.05% from Badda were collected.

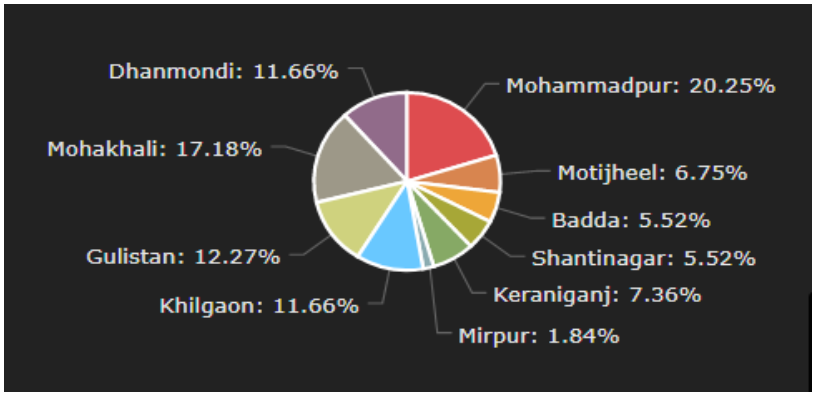


Figure 12: Isolated Fungal colonies

Along with bacterial colonies, a hefty amount of fungal colonies also made their way into this project. The greatest amount of colonies was collected from Mohammadpur. Among other areas, 11.66% in Dhanmondi, 17.18% in Mohakhali, 12.27% in Gulistan, 11.66% in Khilgaon, 6.75% in Motijheel, 5.52% in Badda, 5.52% in Shantinagar, 7.36% in Keraniganj and 1.84% in Mirpur.

3.4: Biochemical Test Results: After the subculture of the samples on nutrient agar media, the isolates were taken for biochemical test confirmation.

Sample	Catalase	MIU			MR-VP		Simone's Citrate	Starch Hydrolysis	TSI						Probable Organism
		Motility	Indole	Urease	MR	VP			Slant/butt	Glucose	Lactose	Sucrose	H_2S Production	Gas Production	
1. DMD3	+	-	+	-	+	-	-	-	S= R B= R	+	-	-	-	-	*Serratia spp*
2. MRP10	+	+	-	-	-	-	+	+	S= Y B= Y	+	+	+	-	-	*Bacillus spp*
3. STN10	+	+	-	-	-	+	+	-	S= R B= Y	-	-	-	+	+	*Salmonella spp*
4. KLG10	+	-	+	-	+	+	-	-	S= R B= Y	+	-	-	-	-	*Shigella spp*
5. MTJ1	+	-	+	-	+	-	-	+	S= R B= Y	+	-	-	-	-	*Staphylococcus spp*
6. GLS6	+	-	-	+	-	-	-	+	S= R B= R	+	-	+	-	-	*Vibro spp*
7. BDD11	+	+	-	+	+	+	+	+	S= R B= Y	+	-	-	-	-	*Bacillus spp*
8. MHK3	+	+	-	-	-	+	-	-	S= R B= Y	+	-	-	-	-	*Brevibacillus spp*

9. BDD8	+	+	-	-	+	+	-	-	S= R B= Y	+	-	-	-	-	*Bacillus spp*
10. MDP26	+	+	-	+	-	-	+	+	S= Y B= Y	+	+	+	-	-	*bacillus spp*
11. GLS9	+	-	-	-	-	-	-	-	S= R B= R	-	-	-	-	-	*Edwardsiella 80%*
12. STN1	+	+	-	-	-	-	+	-	S= R B= R	-	-	-	-	-	*Acinetobacter spp*
13. BDD18	+	+	-	-	+	-	-	-	S= R B= Y	+	-	-	-	-	*Staphylococcus spp*
14. STN2	+	-	-	-	+	-	+	-	S= R B= Y	+	-	-	-	+	*Cornybacterium spp*
15. MDP6	+	+	-	-	-	-	-	-	S= Y B= Y	+	+	+	-	+	*StreptoBacilli spp*
16. GLS5	-	+	-	-	-	-	+	-	S= R B= Y	+	-	-	-	+	*Bacillus spp*
17. KLG3	+	+	-	-	+	-	-	-	S= R B= Y	+	-	-	+	-	*Salmonella spp*
18. BDD10	-	+	-	-	+	+	-	+	S= R B= Y	+	-	-	-	-	*Bacillus spp*
19. MDP12	+	-	-	+	+	+	+	-	S= Y B= Y	+	+	+	-	-	*Staphylococcus spp*
20. STN3	+	-	+	+	+	-	+	-	S= R B= R	-	-	-	-	-	*Bacillus spp*
21. STN12	+	-	+	+	+	-	+	-	S= R B= Y	+	-	-	-	-	*Staphylococcus spp*
22. BDD7	+	-	-	+	+	-	-	-	S= R	+	-	-	-	-	*Staphylococc*

									B= Y						*us spp*
23. MDP6	+	-	-	+	+	-	-	-	S= R B= Y	+	-	-	-	-	*Streptobacillus spp*
24. STN13	+	-	-	+	+	+	-	-	S= R B= Y	+	-	-	-	-	*Bacillus spp*
25. MRP11	-	-	-	+	-	-	+	-	S= R B= R	-	-	-	-	-	*Bordetella spp*
26. GLS13	+	+	-	+	+	+	-	-	S= R B= Y	+	-	-	-	+	*Bacillus spp*
27. MTJ10	+	-	-	-	+	+	-	-	S= R B= Y	+	-	-	-	+	*Bacillus spp*
28. BDD24	+	-	-	+	+	-	-	-	S= R B= Y	+	-	-	-	-	*Staphylococcus spp*
29. STN4	+	-	+	+	+	-	+	-	S= R B= Y	+	-	-	-	-	*Staphylococcus spp*
30. MHK4	+	-	-	+	+	-	-	-	S= R B= R	-	-	-	-	-	*Staphylococcus spp*
31. MHK2	-	-	-	-	+	-	+	-	S= R B= Y	-	-	-	-	-	*Xenorhabdus spp*
32. MTJ3	+	-	-	-	+	-	-	-	S= R B= R	-	-	-	-	-	*Bacillus indcus*
33. KNG8	+	-	+	-	-	-	-	-	S= R B= R	-	-	-	-	-	*Staphylococcus spp*
34. MDP1	+	+	+	-	+	-	-	-	S= Y B= Y	+	+		-	+	*Escherichia coli*
35. BDD30	-	+	-	-	+	-	+	-	S= R B= R	-	-	-	-	-	*Bacillus sp*

36. BDD21	+	+	-	+	+	-	+	-	S= R B= Y	+	-	-	-	-	*Spirillium spp*
37. STN14	+	-	+	+	-	-	+	-	S= R B= Y	+	-	-	-	-	*Staphylococcus spp*
38. BDD4	+	+	-	-	+	-	+	-	S= R B= Y	+	+	+	-	+	*Enterobacter spp*
39. BDD6	+	-	+	+	+	-	-	-	S= R B= R	-	-	-	-	-	*Staphylococcus*
40. MRP1	+	+	-	-	-	-	-	+	S= R B= R	-	-	-	-	-	*Pseudomona spp*
41. MDP10	+	+	-	-	+	-	+	-	S= Y B= Y	+	+	+	-	+	*Vibrio /enterobacter spp*
42. GLS7	+	+	-	-	-	-	+	+	S= R B= R	-	-	-	-	-	*Pseudomonas Aeruginosa*
43. STN11	+	-	-	+	-	-	+	-	S= Y B= Y	+	+	+	-	+	*Klebsiella spp*

R=Red, Y=Yellow (acid formation)

Table 3: Biochemical tests for bacterial identification

Each species of bacteria has specific metabolic needs and relies on different enzymes to fuel those unique needs. The presence of catalase, gelatinase, oxidase, and urease, for example, can be used to identify the species of bacteria. Biochemical reactions used in biochemical tests depend on the presence of such bacteria. Various biochemical tests were performed in the isolated bacteria such as Motility test, Indole test, Urease test, Tripple sugar iron test, Simmonne's Citrate test, Starch Hydrolysis test, and Catalase test.

3.5: Antibiotic susceptibility test:

Isolate Number	AZM	TE	P	CXM	MXM	DO	CIP	LE	C30	AMC

1. DMD3	S	S	R	S	S	S	S	R	S	S
2. MRP10	S	R	R	R	S	S	I	R	S	S
3. STN10	S	S	R	S	S	S	S	S	R	S
4. KLG10	S	I	R	I	R	S	I	I	S	I
5. MTJ1	S	I	R	S	S	S	S	I	S	R
6. GLS6	S	I	R	R	S	S	R	R	S	S
7. BDD11	S	I	R	S	S	S	S	S	S	R
8. MHK3	S	S	R	R	S	S	S	S	S	S
9. BDD8	S	S	R	R	S	S	S	S	S	S
10. MDP26	I	I	R	R	S	S	I	S	S	S
11. GLS9	S	S	R	R	S	S	S	S	S	S
12. STN1	S	S	R	R	S	S	S	S	S	S
13. BDD18	I	I	R	I	R	S	S	I	I	S
14. STN2	S	S	R	R	S	S	S	S	R	S
15. MDP6	I	S	R	R	S	S	S	I	S	R
16. GLS5	R	R	R	S	I	S	S	S	S	I
17. KLG3	R	S	I	I	S	I	I	I	I	I
18. BDD10	R	R	I	R	S	S	S	I	I	R
19. MDP12	I	S	R	S	S	R	R	S	S	S
20. STN3	S	S	R	R	S	S	S	S	R	S

21. STN12	S	S	R	R	S	S	S	S	R	S
22. BDD7	S	S	R	R	S	S	S	S	S	S
23. MDP6	I	R	I	R	S	S	S	I	I	I
24. STN13	I	R	S	R	S	R	S	R	I	I
25. MRP11	S	S	R	S	S	I	S	S	S	S
26. GLS13	S	S	R	S	S	I	S	S	S	S
27. MTJ10	S	I	R	I	S	S	S	S	S	S
28. BDD24	I	S	S	S	S	R	S	S	S	S
29. STN4	S	S	R	R	S	R	S	S	R	S
30. MHK4	S	S	R	R	S	R	S	S	S	S
31. MHK2	R	S	R	R	S	S	S	S	S	S
32. MTJ3	R	S	S	R	S	S	I	I	R	S
33. KNG8	R	R	R	R	R	R	R	R	S	R
34. MDP1	S	S	S	R	S	S	S	S	S	S
35. BDD30	I	S	R	I	S	S	S	S	S	S
36. BDD21	I	R	R	I	S	S	S	S	R	S
37. STN14	R	I	R	R	S	R	S	I	I	I
38. BDD4	R	I	I	R	S	I	R	R	I	R
39. BDD6	S	S	S	R	S	I	S	S	S	S
40. MRP1	S	S	I	R	S	I	S	S	S	S

41. MDP10	I	I	R	R	S	R	S	S	S	S
42. GLS7	I	I	I	R	R	R	R	R	S	I
43. STN11	R	I	R	R	R	R	I	R	S	I

Table 4: Antibiotic Susceptibility test results of various isolates

Antibiograms are often used by clinicians to assess local susceptibility rates, as an aid in selecting empiric antibiotic therapy, and in monitoring resistance trends over time within an institution. Antibiograms can also be used to compare susceptibility rates across institutions and track resistance trends. In this project, the Kirby Bauer method was used. In Kirby-Bauer testing, bacteria are placed on a plate of the solid growth medium, and wafers of antibiotics (white disks, shown) are added to the plate. After allowing the bacteria to grow overnight, areas of clear media surrounding the disks indicate that the antibiotic inhibits bacterial growth.

3.6: Blood Agar Test Results:

Hemolysis Pattern	Isolates
α	MDP11, DMD3, GLS6, MTJ3, MTJ1, STN12, STN13, GLS13, GLS9, KLG10, BDD18, MTJ10
β	MDP1, MDP12, MDP10, GLS7, GLS5, MDP26, MRP1, STN14, STN1, STN2, STN3, STN10, STN11, BDD10, BD11, BDD6, BDD7, BDD8, MHK2, MHK3, MHK4
γ	MDP6, MRP10, BDD4, BDD30, KNG8, STN4, KLG3, BDD21, BDD24

Table 5: Blood agar test results.

Among 157 isolates, 22 showed β hemolysis where they completely lysed the red blood cells; 12 isolates showed α hemolysis and 9 isolates showed γ hemolysis. eta-hemolysin breaks down the red blood cells and hemoglobin completely. This leaves a clear zone around bacterial growth. Such results are referred to as β-hemolysis (beta hemolysis). Alpha-hemolysin partially breaks down the red blood cells and leaves a greenish color behind. This is referred to as α-hemolysis (alpha hemolysis). The greenish color is caused by the presence of biliverdin, which is a by-product of the breakdown of hemoglobin. If the organism does not

produce hemolysins and does not break down the blood cells, no clearing will occur. This is called γ-hemolysis (gamma hemolysis).

3.7: DNAse Test Results:

Test Result	Sample Name
DNAse positive	GLS6, BDD11, MHK3, BDD8, GLS9, STN1, BDD18, STN2, KLG3, BDD10, MDP12, STN3, STN12, BDD7, MDP6, STN13, MRP11, GLS13, STN4, KNG8, MDP1, BDD21, STN14, BDD4, MRP1, GLS7
DNAse negative	DMD3, MRP10, STN10, KLG10, MTJ1, MDP26, MDP6, GLS5, MTJ10, BDD24, MHK4, MHK2, MTJ3, BDD30, BDD6, MDP10, STN11

Table 6: DNAse test results.

The test is used to determine the ability of an organism to hydrolyze DNA. DNase agar is a differential medium that tests the ability of an organism to produce an exo-enzyme, called deoxyribonuclease. DNases are extracellular endonucleases that cleave DNA and release free nucleotides and phosphate. In this test, out of 43 samples, 17 turned out to be DNAse negative, and 26 were DNAse positive.

Chapter 4

Discussion

4.1: Discussion

The rising air pollution in Dhaka city is becoming one of the main reasons for many health hazards in Bangladesh. The pathogenic organisms in the air are one of the main causes behind such anomalies. The matter seems to become more serious when commercially produced antibiotics start being challenged by the pathogens. According to a report by WHO in 2014, in the year 2012,2.6 million deaths were likely to be caused due to outdoor air pollution. This scenario was most prevalent in the Southeast Asian countries and the Western Pacific countries because these are the developing countries where industrialization following air pollution is high with low to middle-income rates.

The study clearly aims at finding out what exactly are the different types of microbes that can largely prevail and survive in the air of Dhaka city despite the city being top-ranked in air pollution. The aim was to find out the pathogens in the air that the people are being exposed to daily. It was also necessary to depict the graveness of the threat of the pathogens by seeing the antibiotic susceptibility and resistance of the pathogens.

The basic findings of the study were at first all the prevalent bacteria with *Staphylococcus spp*. being the most prevalent in almost all the zones of Dhaka city. The zones were Mohakhali, Badda, Gulistan, Shantinagar, Mirpur, and Dhanmondi. These are one of the busiest areas in the city. The zones were chosen to cover north Dhaka city. However, the date of the collection time was taken, and the humidity and the temperature were jotted down. The records were important to enumerate a comparison between these factors and the total colony count. The samples were then collected in three different kinds of media. The media were nutrient agar, mannitol salt agar, MacConkey agar. NA was selected for universal growth of all organisms whereas MSA was chosen for *staphylococcus* and for some differential growth, and MacConkey was chosen for only growth of gram-negative bacteria. The media were taken on the site and exposed in the air by opening the cover of the petri dish. Precautions were taken so that, nothing touched the media. Only the growth of organisms present in the air was allowed. Here, the time of exposure played a crucial role. The time of exposure was 3 mins for each of the media (NA, MSA, Mac, EMB) and 5 mins for each of them as well. However, at first, we considered exposure for 1 min

as well but over time it was seen that there wasn't sufficient growth for 1 min exposure. So, the exposure time was kept for 2 and 5 minutes accordingly.

After the exposure, the samples were brought back to the lab and incubated at 370 for 24 hours. Growth occurred and the colonies were selected seeing the morphology. However, both bacterial and fungal growth occurred. Bacteria with similar morphology were selected and the samples were brought down to 74. These were then sub cultured in the NA plate. From there biochemical tests were performed.

4.2: Conclusion

We got about 3765 colonies of bacteria and 163 colonies of fungi in our research . The gradually we

have isolated about 157 bacteria among total of 3765 . From here, the possible future implications of the research could get included, and then can be compared to a public health issue. The gradual process or course of transmission of the pathogen in affected humans could be found out in order to understand the level of damage it can do to the health of humans and other living beings . Also, the virulence activity of pathogens could be detected as well . The findings or research could be termed as a potential environmental hazard issue and further studies related to it could be conducted in the near future. There is a more diversified form of bacteria that is born from the air outdoors which is an environment that is open greatly compared to the indoors. It is not easy but rather a tough work to find out the source of some specific bacteria in the open-air system as bacteria and other microscopic organisms are mainly there due to various carriers or means or systems. here is an influence of the environmental changes on the type of microbes present. And thus, this was a reason which made our research somewhat difficult to conduct in the outdoor environments.

References

[1] World Most Polluted Cities in 2018 - PM2.5 Ranking | AirVisual. (2019). Retrieved 23 December 2019, from https://www.airvisual.com/world-most-polluted-cities?continent=&country=&state=&page=1&perPage=50&cities=vnxvinME52vNycse5

[2] Kabir, M. S., Mridha, F., Islam, S., & Shorifujjaman, M. (2016). Microbiological pollutants in the air and antibiotic resistance profile of some bacterial isolates. Jahangirnagar University Journal of Biological Sciences, 5(1), 47-56. (Kabir *el at.,* 2016)

[3] Brochu, P., Ducré-Robitaille, J. F., & Brodeur, J. (2006). Physiological daily inhalation rates for free-living individuals aged 1 month to 96 years, using data from doubly labeled water measurements: a proposal for air quality criteria, standard calculations, and health risk assessment. Human and Ecological Risk Assessment, 12(4), 675-701. (Brochu *el at.,* 2006)

[4] Stryjakowska-Sekulska, M., Piotraszewska-Pajak, A., Szyszka, A., Nowicki, M., & Filipiak, M. (2007). Microbiological quality of indoor air in university rooms. Polish Journal of Environmental Studies, 16(4), 623. (Sekulska *el at.,* 2007)

[5] Cernei, E. R., Maxim, D. C., Mavru, R., & Indrei, L. L. (2013). Bacteriological analysis of air (aeromicroflora) from the level of dental offices in Iaşi county. Romanian Journal of Oral Rehabilitation, 5(4), 53. (Cernei *el at.,* 2013)

[6] James, C., & Natalie, S. (2014). Microbiology. A laboratory manual. Pearson Education. (James *el at.,* 2014)

[7] Kelly, F. J., & Fussell, J. C. (2015). Air pollution and public health: emerging hazards and improved understanding of risk. Environmental geochemistry and health, 37(4), 631-649.

(Kelly et al., 2015)
[8] Baron S. (1996). Staphylococcus. Galveston. University of Texas Medical Branch, 12. (Baron *el at.*, 1996)

[9]Baker S, The H C. (2018). Recent insights into *Shigella:* a major contributor to the global diarrhoeal disease burden. CURR OPIN INFECT DIS, 31(5),449–454. (Baker,2018). (Baker *et at.*, 2018)

[10]Makut M D, Nyam M A, Shehu L, Anzaku S J. (2014). A survey of the microflora of the outdoor air environment of Keffi Metropolis, Nasarawa State, Nigeria. Afr. J. Microbiol. Res, 8(27), 2650-55. (Makut *et al.*, 2014)

[11] Cappuccino J, Sherman N,(2008). Microbiology: A Laboratory Manual, SUNY, Rockland Community College.

[12] Smets W, Deny's S, Moretti S, Lebeer S, (May 2016). Airborne bacteria in the atmosphere: Presence, Past, Future. Atmospheric Environment, DOI: 10.1016/j.atmosenv.2016.05.038. (Smets et al., 2016)

[13] Gerhardt, Philipp, R. G. E. Murray, Willis A, Wood, Noel R. Krieg. 1994. Methods for General and Molecular Bacteriology. American Society for Microbiology, Washington, D. C. (p. 619, 642, 647) (Gerhardt et al., 1994)

[14] *Why Studying Microorganisms in the Air Is Vital*. (2020). ASM.Org. https://asm.org/Articles/2020/December/Why-Studying-Microorganisms-in-the-Air-Is-Vital

Appendix A.

Media Composition: Nutrient Agar

Component	Amount per Litre solution
Beef extract	3.0 g
Peptone	5.0 g
Agar	20.0 g
pH	7.0-7.2

Mac Conkey Agar :

Components	Amount per Litre solution
Peptones (meat and casein)	3.000
Pancreatic digest of gelatin	17.000
Lactose monohydrates	10.000
Bile salts	1.500

Sodium Chloride	5.000
Crystal violet	0.001
Neutral red	0.030
Agar	13.5g

Mannitol Salt Agar :

Component	Amount per Litre solution
Proteose peptone	10g
HM peptone B	1g
Sodium chloride	75g
D-Mannitol	10g
Phenol red	0.025g
Agar	15g
Final pH	7.4
Phenol Red	0.024g
Agar	12g

Triple Iron Sugar Media :

Component	Amount per Litre solution
Beef extract	3g
Peptone	20g
Yeast extract	3g
Lactose	10g
Sucrose	10g
Dextrose monohydrate	1g
Ferrous sulphate	0.200g
Sodium chloride	5g
Sodium thiosulphate	0.300g

Indole /Tryptone Water :

Component	Amount per Litre solution
Tryptone Water	10g
Sodium chloride	5g

Methyl Red /Vogus Proskauer Media :

Component	Amount per Litre solution
Buffed Peptone	7g
Dextrose	5g
Dipotassium phosphate	5g
pH	6.9

MIU Agar :

Component	Amount per Litre solution
Tryptone	10g
Dextrose	5g
Sodium chloride	1g
Phenol red	0.010g
Agar	2g

Starch Hydrolysis Agar

Component	Amount per Litre solution
Peptone	5g

Sodium chloride	5g
Yeast extract	1.5g
HM peptone	1.5g
Starch soluble	2g
Agar	15g

Tellurine Glycine Agar :

Component	Amount per Litre solution
Casein enzymatic hydroly ate	10g
Yeast extract	5g
Mannitol	5g
Di potassium phosphate	5g
Lithium chloride	5g
Glycine	10g
Agar	16g

Phenol red lactose/dextrose/sucrose broth :

Component	Amount per Litre solution
Pept ne	10g
Beef e tract	1g
Sodium chloride	5g
Lactose/Sucrose/Dextrose	5g
Pheno red	0.018g
pH	7.4

Appendix B.

The important equipment used through the study are listed below:

Autoclave, Model No: WAC-47	Korea
Balance (Core series): Adam	UK
Centrifuge, Model No: Code: 5433000.011	Eppendorf, Germany
Freezer (-20°C)	Siemens Germany
Incubator	UK
Laminar air flow	UK
Micropipettes	Eppendorf, Germany
Oven (Universal drying oven) Model: LDO-060E	Labtech, Singapore
Refrigerator, Model: 0636	Samsung

Vortex Mixture	VWR International