A study of the prevalence of bacterial pathogens in the air of the different areas of Dhaka city

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A thesis submitted to the Department of Mathematic and Natural Sciences in partial fulfillment of the requirements for the degree of
B. Sc. In Biotechnology

Department of Mathematic and Natural Sciences
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Declaration

It is hereby declared that

1. The thesis submitted is our own original work while completing 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 which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I/We have acknowledged all main sources of help.

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Abstract:

The economic hub of Bangladesh, it is capital city Dhaka is subject to a high level of pollution all year round, which makes the air harmful to breathe. According to the Air Quality Index (AQI), Dhaka city once again topped among the cities around the world with the worst air quality recorded 316 AQI scores **(*Dhaka tribune*).** An AQI above 300 is considered “hazardous” indicating that 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 prevalence of different types of bacteria existing in Dhaka city’s air through the biochemical tests, identifying the pathogens among them by blood agar hemolysis test and DNase test. A total of 117 isolates were screened for pathogenicity in blood agar and their hemolysis pattern showed 63 of them being capable of hemolysis. Of the 117 isolates, 44 showed β hemolysis where they completely lysed the red blood cells; 13 isolates showed α hemolysis and

6 isolates showed γ hemolysis. Most isolates were from Meradia, Khilgaon, and Uttara respectively. Moreover, a comparison of CFU count including temperature and humidity records was done during the sample collection to estimate the effect of environmental factors on microbial presence. These findings call for an extension of the study that would involve genetic analysis of 16sRNA gene sequences and the virulence activity of the pathogens. It 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 microbes, Blood Agar Hemolysis, DNase

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Chapter 1:

INTRODUCTION

1.1 Overview

A few days ago, Dhaka, the capital city of Bangladesh has topped Mumbai on becoming the most polluted city in the world. This statement alone is enough to signify the extremely poor condition of the air of Dhaka city. Dhaka is the 9th largest city worldwide and in case of population, it is the 5th largest in the world and 4th largest in the continent of Asia with more than 10 million people living within the City's limit (*Dhaka Air Quality Index (AQI) and Bangladesh Air Pollution | AirVisual*, 2022). The population of greater Dhaka accounts for more than 22 million. As it is the economic hub of the country, the population density in Dhaka city is the highest in the country and due to these reasons, the city is subjected to excessive pollution annually. The megacity holds some relatively high numbers of pollution readings, making its air harmful to breathe year-round.

In 2019, Dhaka city was put in the 'unhealthy' bracket of air quality as its PM2.5 came in with a reading of 83.3 µg/m³ as the yearly average (*Dhaka Air Quality Index (AQI) and Bangladesh Air Pollution | AirVisual*, 2022). This reading alone is enough to depict the extremely poor condition of air and the health hazards of breathing this polluted air. What is alarming is that these PM2.5 readings are not going down, instead, they are on the rise on annual basis. For example, in January, a record high PM2.5 of 181.8 µg/m³ was recorded in Dhaka city, putting it into the 'very unhealthy' bracket (150.5 to 250.4 µg/m³) (*Dhaka Air Quality Index (AQI) and Bangladesh Air Pollution | AirVisual*, 2022). Today the PM2.5 concentration is 213.3 µg/m³ and the air quality index (AQI) is 263 which is very unhealthy (The World Air Quality Index project, 2022).

The sole reason for the very unhealthy condition of the air of Dhaka city is pollution and the megacity faces a lot of it. The city is subjected to pollution from all sides. Contamination from fuel-burning vehicles releases chemical compounds such as nitrogen dioxide (NO2), carbon monoxide (CO), carbon dioxide (CO_2), etc. (Siddiqui, 2020). Moreover, the vehicles are not subjected to maintenance and quality checks resulting in poor condition of the vehicles and emitting far more pollution than a

regular vehicle would (*Dhaka Air Quality Index (AQI) and Bangladesh Air Pollution | AirVisual*, 2022 *&* Williams, 2021). The ever-growing industrial side of Dhaka city is also contributing equally to the pollution level by the use of unregulated fuel sources for power (such as the burning of coal, wood, and any other combustible material), releasing excessive amounts of noxious fumes, smokes, and other contaminants such as carbon monoxide (CO), ozone (O_3), sulfur dioxide (SO_2), nitrogen oxides (NO_X), etc. into the atmosphere (Siddiqui, 2020 & Williams, 2021). Another reason for contamination includes the high dust concentration in the city (Williams, 2021). The large amounts of dust given off by poorly maintained construction sites would contain a variety of PM2.5 and PM10, such as silica dust or finely ground soil or gravel particles, all of which can cause several long-term health effects when respired (Williams, 2021).

Another type of pollution that is present but has gone unnoticed by most is the presence of airborne micro-organisms in the contaminated air. As the air is contaminated with various kinds of pollutants and dust particles, the density of the air also increases drastically. This supports the free-floating and transmission of micro-organisms from one place to another through the air. It is estimated that a person when resting, inhales 7 or 8 liters of air per minute which means 11,000 liters of air is inhaled per day (*How Much Oxygen Does a Person Consume in a Day? | Air Quality*, 2021). The presence of micro-organisms in the air not only possess a serious health hazard but also can lead to life-threatening conditions. Some pathogens hovering in the air can cause acute health disorders and cause deadly infectious diseases that can cause fatality (Kabir *el at.*, 2016 & Sekulska *el at.*, 2007). According to Cernei *el at.*, (2013), the survivability of a pathogenic micro-organism in the outside environment depends on its ability to resist environmental factors such as temperature, humidity, pressure, pH change, nutrient scarcity, UV exposure, etc. Yet, the prevalence of airborne diseases, such as influenza, pneumonia, tuberculosis, mumps, and other Lower Respiratory Infections and Chronic Obstructive Pulmonary Diseases is at an all-time high. According to a study by Karim *el at.*, (2020), a total of 900 female inhabitants of lower socioeconomic in Dhaka city, were examined from September 2013 to August 2015 and in this population, 58.44% were found to be infected, among them four types were recorded, such as influenza (25.11%), mumps (10.22%), pneumonia (17.44%) and tuberculosis (5.67%). Therefore, it is not only

important but crucial to identify what kind of micro-organism is present in the air of Dhaka city as well as their capacity to cause disease, pathogenicity, virulence, and multidrug resistance.

The study emphasizes the identification of these vicious pathogens found in the air through a series of biochemical tests and pathogenicity tests. Further genetic analysis of 16srRNA gene sequences of the potential pathogens will also be performed for accurate identification and confirmation of the pathogenic strain. The study also includes the detection of the virulence activity of the pathogens as well as identification of the "Multi-Drug Resistant" strain among the pathogenic micro-organisms. The study will also be able to show the different concentrations of micro-organisms present in different areas in Dhaka city as samples were taken from 18 different places. A statistical analysis of the colony counts of the micro-organisms collected from different areas has also been performed. Pathogenicity tests have been performed on the collected samples and the samples from Meradia, Uttara, Abdullahpur, Khilgaon, Shantinagar are found to be more pathogenic than other areas.

1.2 Aims and Objectives:

1. Quantifying the prevalence of different types of microbes in Dhaka city's air
2. Identifying the microbes through "biochemical test" and "pathogenicity test".
3. Accurate identification of pathogenic micro-organisms through DNA sequencing.
4. Detecting the virulence activity of the pathogens.
5. Detection of the "Multi-Drug Resistant" strain among the pathogenic bacteria.

Chapter 2

MATERIALS AND METHODS:

In this thesis work, a total of 18 areas of Dhaka city was covered. The areas that were covered are, Abdullahpur, Mirpur 10, Uttara, Tejgaon, Rampura, Meradia, Gulshan, Shantinagar, Khilgaon, Mohakhali, Banani, Mohammadpur, Baridhara DOHS,

Bashundhara DOHS, Nandipara, Elephant Road, Badda, Gulisthan. The samples were collected from outdoor environments that were moderately crowded and had 24/7 human traffic. All the lab works were performed at the Microbiology Research Laboratory of the Department of Mathematics and Natural Sciences of BRAC University maintaining proper precautions and safety guidelines.

2.1 Sample Collection

From the selected areas, a total of 117 samples were collected. The process of sample collection was done in 48 hours by the members of the research group. After sample collection, the samples were incubated for 48 hours. After incubation, isolation and subtraction of common bacterial colonies from media plates were performed. Four different media was used to collect the samples in three different time intervals, one minute, three minutes, and five minutes. The samples were collected from an average of five feet 2 inches above the ground. This was performed to make sure the collection of samples was done only from an average humans' nasal and oral height from the ground level. After sample collection, the samples were labeled properly.

2.2 Sample Collection on Nutrient Agar:

Nutrient Agar or NA is generally used for the exemplary growth of a wide range of non-fastidious organisms. It is a universal media that is very popular for its ability to support the growth of various types of bacteria and fungi. The components of NA support exemplary growth.

2.3 Sample Collection on Mannitol Salt Agar:

Mannitol Salt Agar or MSA is a commonly used selective and differential growth medium in microbiology that encourages the growth of a group of certain bacteria while inhibiting the growth of other bacteria. This medium serves as one of the methods of distinguishing pathogenic microbes in a short period of time. The high salt (NaCl) concentration (about 7.5–10%) inhibits most bacteria, making MSA selective against most Gram-negative and selective for some Gram-positive bacteria

like ***Staphylococcus***, ***Enterococcus,*** and ***Micrococcaceae*** that can tolerate high salt concentrations. It also functions as a differential medium for mannitol-fermenting staphylococci, containing carbohydrate mannitol and a pH indicator, phenol red for detecting acid produced by mannitol-fermenting staphylococci.

2.4 Sample Collection on MacConkey Agar:

MacConkey agar is a selective and differential media used for the isolation and differentiation of non-fastidious gram-negative rods, particularly members of the family Enterobacteriaceae and the genus Pseudomonas. The medium is used for the isolation of gram-negative enteric bacteria and the differentiation of lactose fermenting from lactose non-fermenting gram-negative bacteria.

In MacConkey agar, a pancreatic digest of gelatin and peptones (meat and casein) provide the essential nutrients, vitamins, and nitrogenous factors required for the growth of microorganisms. The fermentable source of carbohydrate here is lactose monohydrates. It is the crystal violet and bile salts that perform the selective actions, inhibitory to most species of gram-positive bacteria. The osmotic balance of the medium is maintained by sodium chloride. Neutral red functions as the pH indicator that turns red at a pH below 6.8 and is colorless at any pH greater than 6.8. Agar is the solidifying agent.

2.5 Sample Collection on Eosin Methylene Blue (EMB) Agar:

Eosin Methylene Blue (EMB) agar functions as both selective and differential culture medium. It selectively promotes the growth of Gram-negative bacteria (inhibits Gram -positive bacteria) and aids in the differentiation of lactose fermenter and non-lactose fermenting colonies. This agar medium is characterized by the existence of a combination of two dyes; eosin and methylene blue in the ratio of 6:1. These dyes are toxic to Gram-positive bacteria.

Gram-negative bacteria that ferment the lactose produce dark purple colonies. In addition, certain lactose-fermenting bacteria produce flat, dark colonies with a green metallic sheen. Other lactose fermenters produce larger, mucoid colonies, often

purple only in their center. Additionally, most of the strains of ***E. coli*** colonies show a characteristic green sheen in EMB. Lactose non-fermenters are produced either colorless or light lavender colonies.

2.6 Microbial Culture of the Samples:

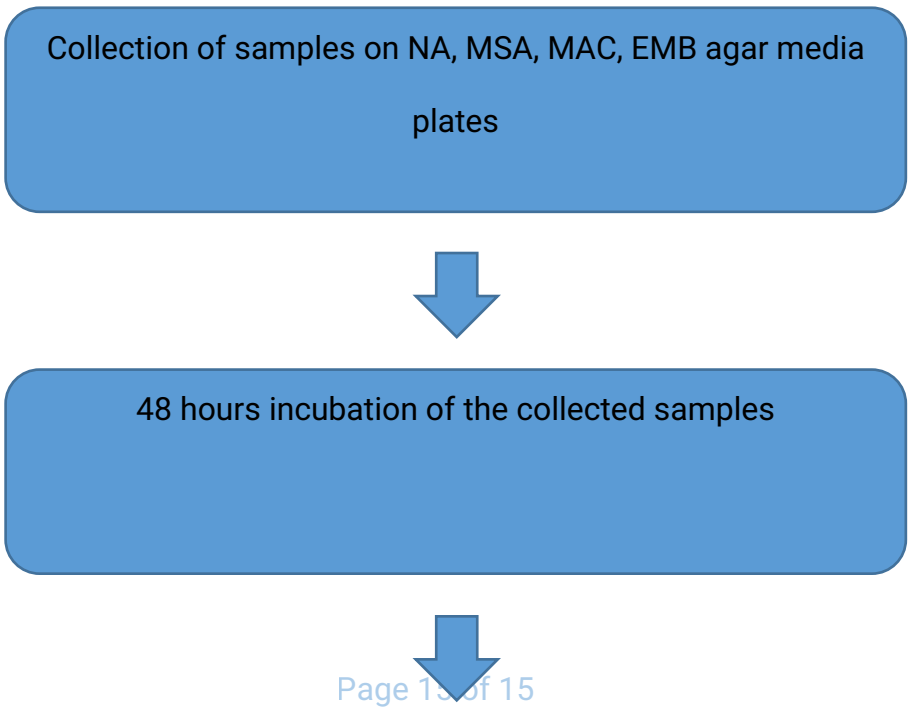
At the initial phase, after the collection of all samples on media plates, all the samples were incubated for 24 hours at 37 ºC for growth. After 24 hours of incubation, the next day, colonies were selected and were inoculated in NA media plates.

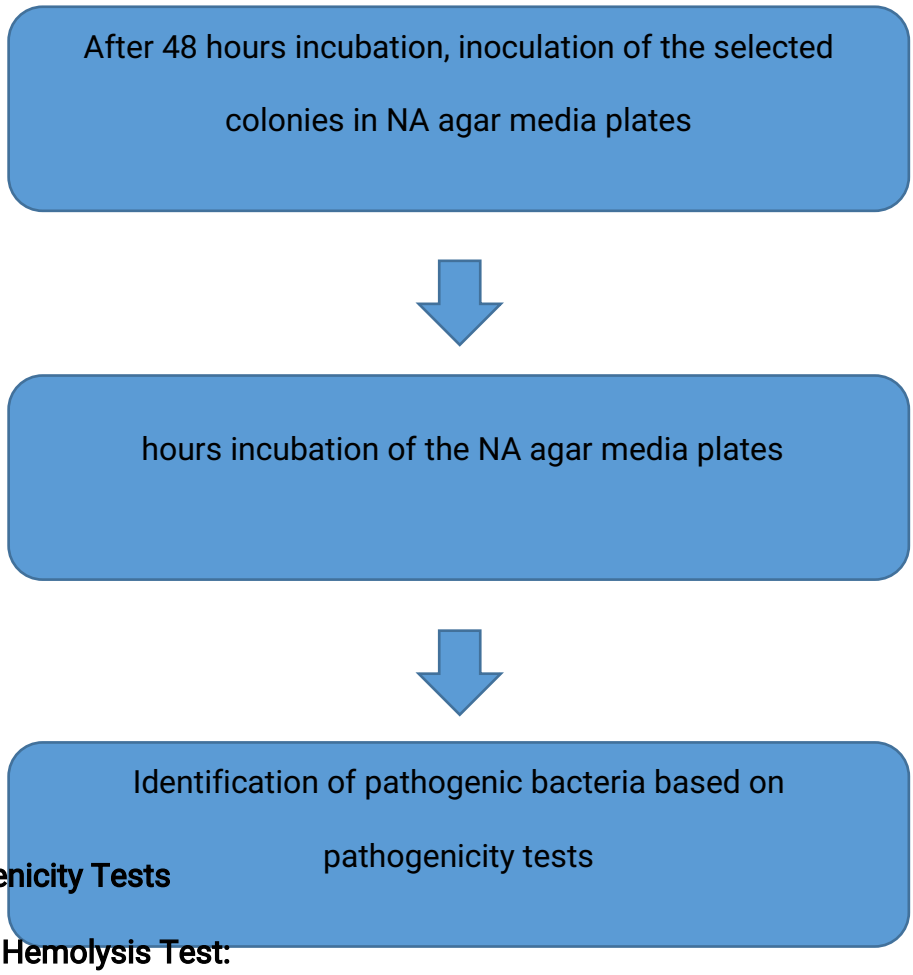
2.7 Screening of Pathogenic Bacteria from the Samples:

Pathogenicity tests were performed on the bacterial samples that were isolated. Till now two pathogenicity tests were performed.

- Blood Agar Hemolysis Test
- DNase Test

2.8 Experimental Work Flow:





2.9 Pathogenicity Tests

Blood Agar Hemolysis Test:

Blood Agar is an enriched medium provided with multiple nutrients that generally comes as a basal media for the preparation of blood agar by supplementation with blood. It is an excellent medium for the cultivation of fastidious bacteria that require particular nutrients and don't profusely grow on general media like Nutrient Agar. Blood agar, like most other nutritional media, has one or more protein sources, salt, and beef extract for vitamins and minerals. Besides these components, blood agar also contains 5% defibrinated mammalian blood. The blood is added to the autoclaved basal media.

In the LAB, a blood agar base was used along with 5% healthy human blood to prepare the blood agar. The streaking of the organisms on a blood agar plate determines the hemolysis pattern. After inoculation of micro-organism in the blood agar plate, the plates are incubated at 37 °C for 24 hours. Traces of alpha or beta-hemolysis is checked after 24 hours incubation on the blood agar plates. If the medium is discolored or darkened or shows green color after bacterial growth, the

organism has shown alpha-hemolysis. 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).

DNase Test:

DNase agar medium is used for the identification of exhibiting deoxyribonuclease activity of bacterial and fungal species, particularly for the identification of pathogenic Staphylococci. It is used to check the pathogenicity of an organism if it can break down DNA and utilize it as a source of carbon and energy for growth. The indicator Toluidine Blue is responsible for the light blue color of the DNase agar medium base. The composition of DNase medium also supports the growth of bacteria,

After micro-organism inoculation in the DNase agar, the agar plates are incubated for 24 hours. If the micro-organism can synthesize Deoxyribonuclease, it will hydrolyze the DNA around it in smaller fragments. As the DNA is fragmented, it will no longer bind to Toluidine blue, and blue color fades and the colony is surrounded by a colorless clear zone.

Chapter 3

Results:

3.1 Isolates Numbers:

Among 3620 colonies, 117 numbers of bacterial colonies were selected for the pathogenicity test. This study was carried out in 18 bustling areas around Dhaka (Gulistan, Mohakhali, Khilgaon, Motijheel, Mohammadpur, Badda, Shantinagar, Mirpur, Baridhara, Bashundhara, Uttara, Abdullahpur, Meradia, Nandipara, Rampura, Elephant Road, Tejgaon, Gulshan) at different temperatures. For sample collection, different selective media (Nutrient agar, Mannitol Salt agar, MacConkey agar, EMB

agar) were exposed in air for 1 minute, 3 minutes, and 5 minutes.

Table 1: Areas of Sample Collection, Temperatures, Humidity, and Dates

Area	Temperature	Humidity	Date
Gulistan	32°C	70%	24.10.2021 (Sunday)
Gulshan	33°C	70%	28.10.2021 (Thursday)
Mohakhali	28°C	65%	02.11.2021 (Tuesday)
Khilgaon	15°C	82%	23.12.2021 (Thursday)
Motijheel	22°C	68%	26.12.2021 (Wednesday)
Mohammadpur	25°C	79%	29.12.2021 (Saturday)
Agargaon	17°C	64%	02.01.2022 (Sunday)
Shantinagar	22°C	65%	04.01.2022 (Tuesday)
Mirpur	17°C	68%	10.01.2022 (Monday)

Baridhara	20°C	75%	11.01.2022 (Tuesday)
Bashundhara	22°C	77%	15.01.2022 (Saturday)
Uttara	21°C	69%	17.01.2022 (Monday)
Abdullahpur	23°C	70%	17.01.2022 (Monday)
Meradia	20°C	72%	18.01.2022 (Tuesday)
Nandipara	13°C	79%	18.01.2022 (Tuesday)
Rampura	17°C	77%	19.01.2022 (Wednesday)
Elephant Road	18°C	70%	20.01.2022 (Thursday)
Tejgaon	24°C	64%	20.01.2022 (Thursday)

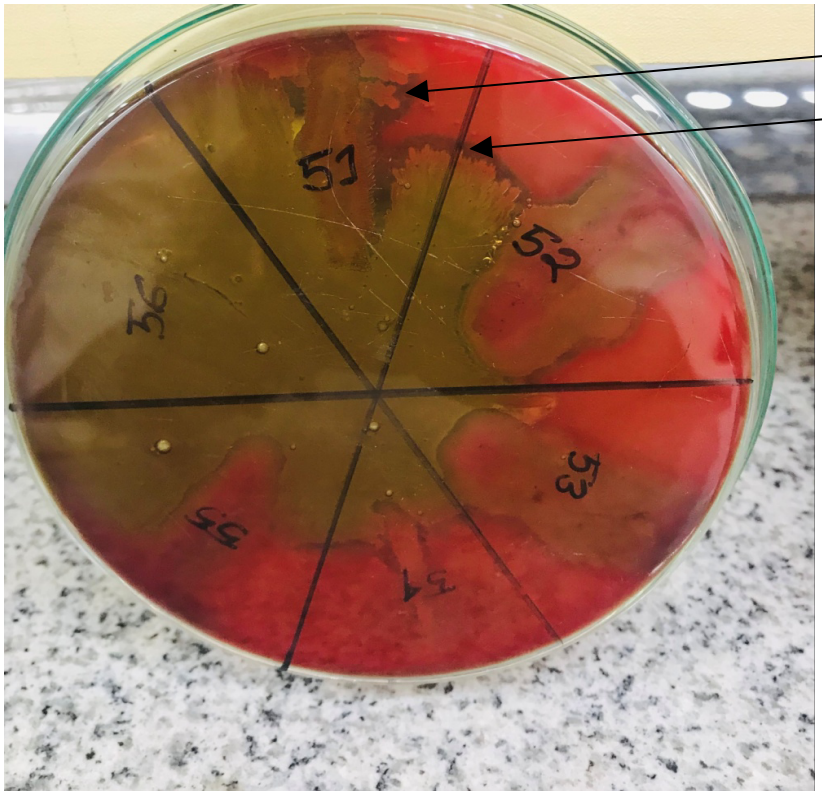
Table 2:Colony Forming Unit (CFU) at different areas of Dhaka city

Zone	Time	NA	MSA	MAC	EMB
Gulistan	1 minute	107	53	1	3
	3 minutes	150	60	5	7
	5 minutes	190	68	5	5
Mohakhali	1 minute	120	89	8	95
	3 minutes	150	105	12	102
	5 minutes	189	130	13	105
Khilgaon	1 minute	160	102	0	45
	3 minutes	189	125	0	35
	5 minutes	195	166	0	55
Motijheel	1 minute	87	13	5	0
	3 minutes	95	22	0	0
	5 minutes	105	25	3	1
Mohammadpur	1 minute	130	45	12	3
	3 minutes	125	89	8	8
	5 minutes	148	104	16	13
Agargaon	1 minute	155	35	45	68
	3 minutes	167	48	53	78
	5 minutes	190	55	65	105
Shantinagar	1 minute	145	12	5	75
	3 minutes	168	7	4	78
	5 minutes	188	18	3	83
Mirpur	1 minute	178	55	0	96
	3 minutes	190	88	2	99
	5 minutes	200	112	1	105
Baridhara	1 minute	140	18	8	25
	3 minutes	165	23	13	28
	5 minutes	167	28	13	32
Bashundhara	1 minute	88	8	0	8
	3 minutes	95	12	3	18
	5 minutes	103	15	1	23
Uttara	1 minute	96	16	0	8
	3 minutes	104	19	3	11
	5 minutes	112	21	8	14
Abdullahpur	1 minute	123	12	1	7
	3 minutes	135	8	1	9
	5 minutes	165	13	3	9
Meradia	1 minute	190	29	10	45
	3 minutes	195	38	18	69
	5 minutes	198	48	23	78

Nandipara	1 minute	98	12	0	11
	3 minutes	113	18	3	15
	5 minutes	118	13	3	21
Gulshan	1 minute	185	33	4	43
	3 minutes	188	37	9	58
	5 minutes	189	49	14	63
Rampura	1 minute	165	13	5	35
	3 minutes	183	15	12	45
	5 minutes	189	28	16	53
Elephant Road	1 minute	86	5	0	9
	3 minutes	92	12	3	11
	5 minutes	107	18	0	13
Tejgaon	1 minute	112	3	3	21
	3 minutes	165	17	8	28
	5 minutes	185	29	9	33

After 24 hours of the incubation period, 3620 colonies were found on Nutrient Agar. During this study, the highest CFU was 198 and the lowest CFU was 1. Most of the bacterial colonies had the same morphologies as a result 117 distinct colonies were screened for pathogenicity test.

3.2 Blood Agar Hemolysis Test Results:



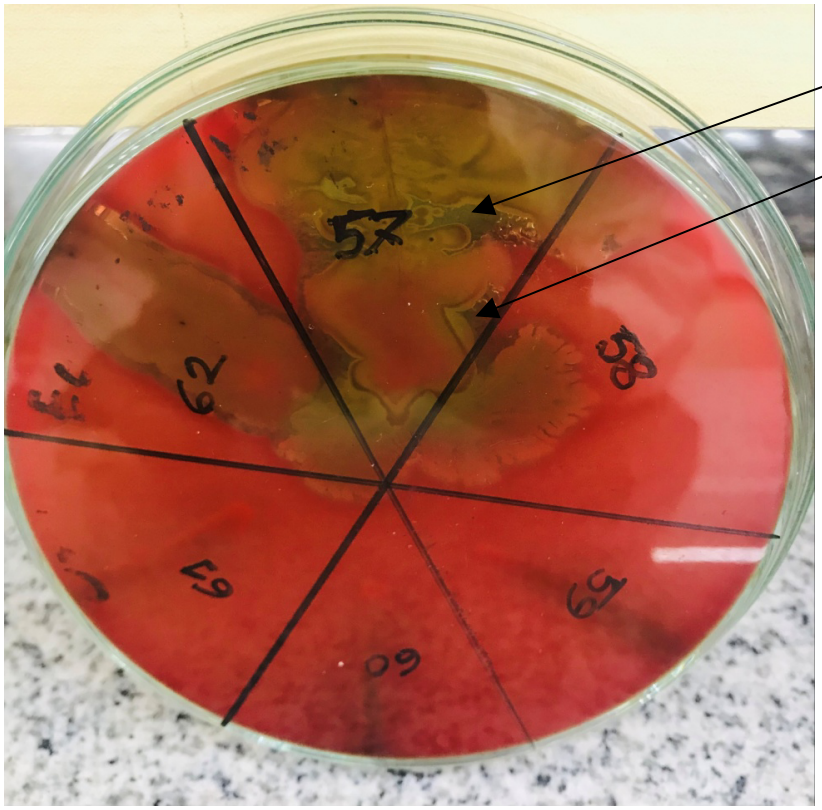


Table 3: Total Number of Different Hemolysis Patterns based on the Areas

Zone	β-hemolysis	α-hemolysis	Y-hemolysis
Gulistan	1	0	0
Mohakhali	3	1	0
Khilgaon	5	2	1
Motijheel	1	0	0
Mohammadpur	2	1	0
Agargaon	1	1	1
Shantinagar	3	0	0
Mirpur	2	1	0
Baridhara	1	1	0
Bashundhara	1	1	0
Uttara	3	2	1
Abdullahpur	2	0	0
Meradia	6	1	2
Nandipara	3	0	0
Gulshan	4	0	0

Rampura	3	1	1
Elephant Road	1	0	0
Tejgaon	2	1	0

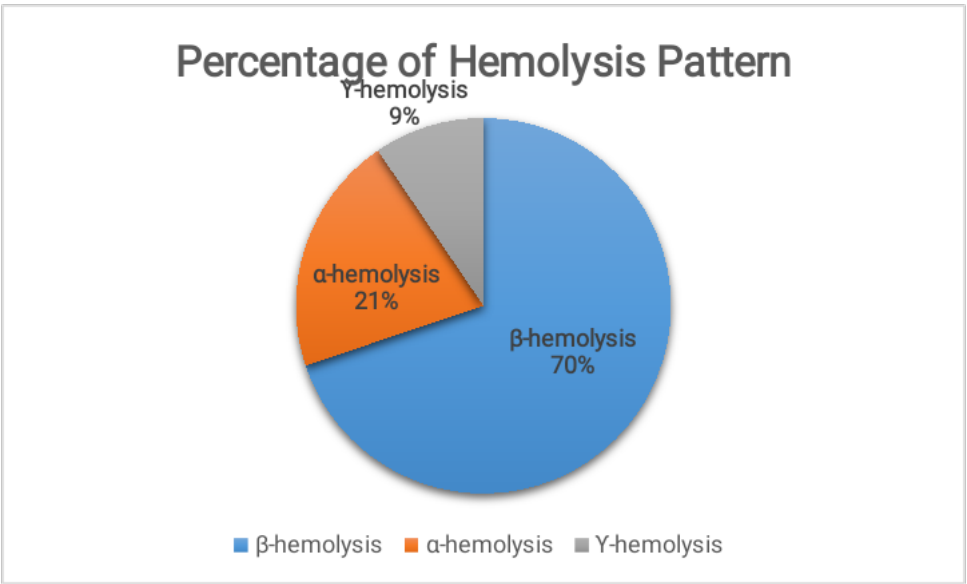


Figure 1: The Percentage of Different Hemolysis Pattern

Most of the isolates showed β-hemolysis pattern (70%).

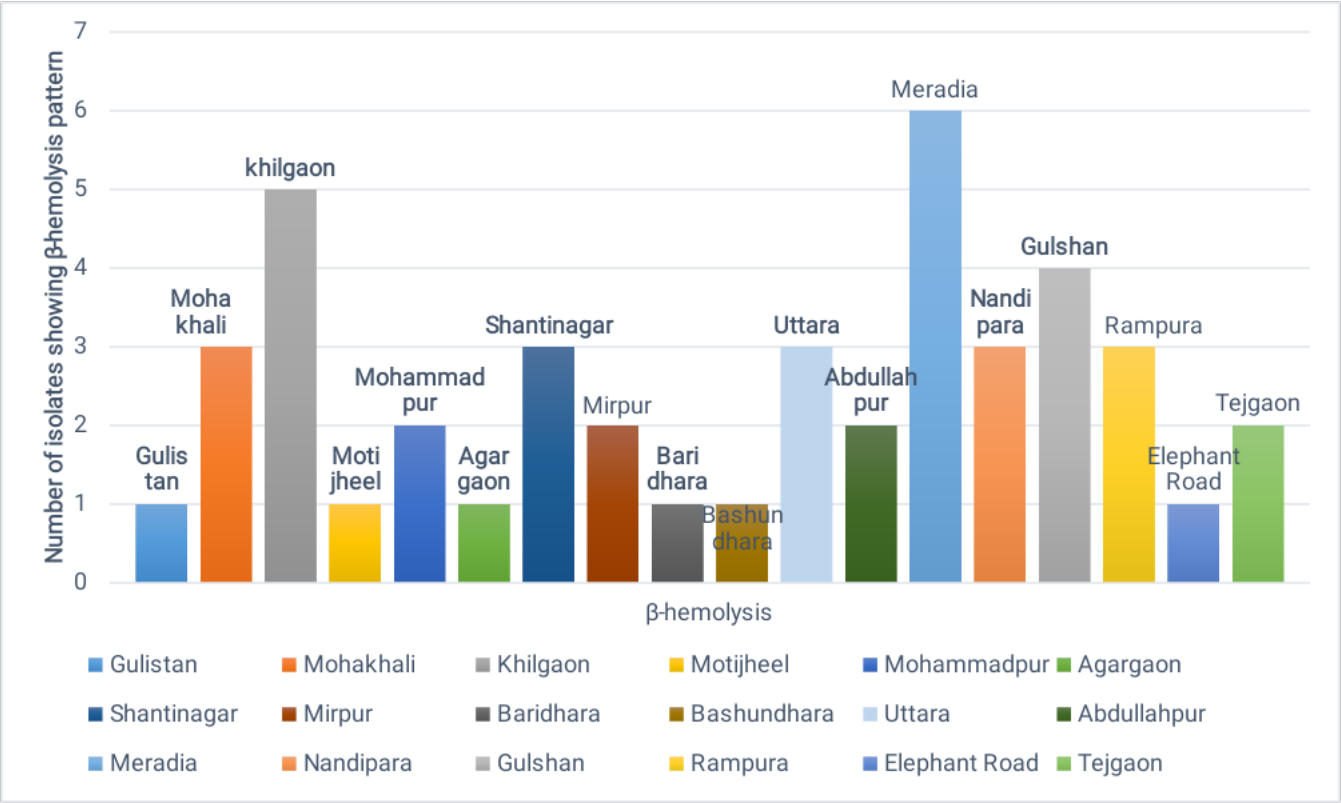


Figure 2: Comparison between the Areas based on their β-hemolysis Pattern (showing the bar chart)

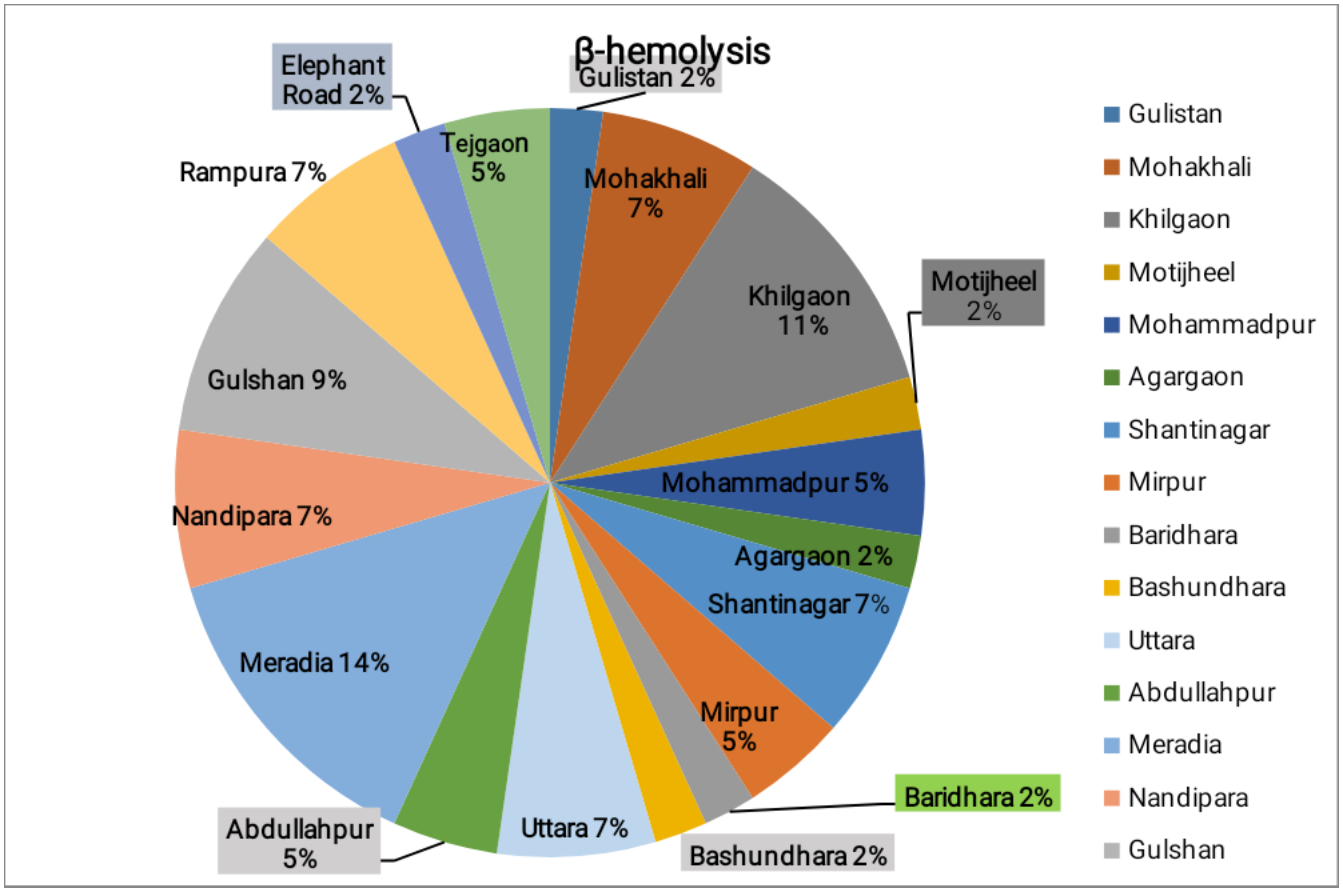


Figure 3: Percentage of total β-hemolysis pattern at 18 different zones

Among the 117 isolates, 44 of them showed β-hemolysis where they significantly lysed the Red Blood Cells. According to the data, the highest isolates were from Meradia (14%), Khilgaon (11%) and Gulshan (9%) respectively. However, the same number of isolates was found from Mohakhali, Shantinagar, Uttara, Nandipara, and Rampura (7%).

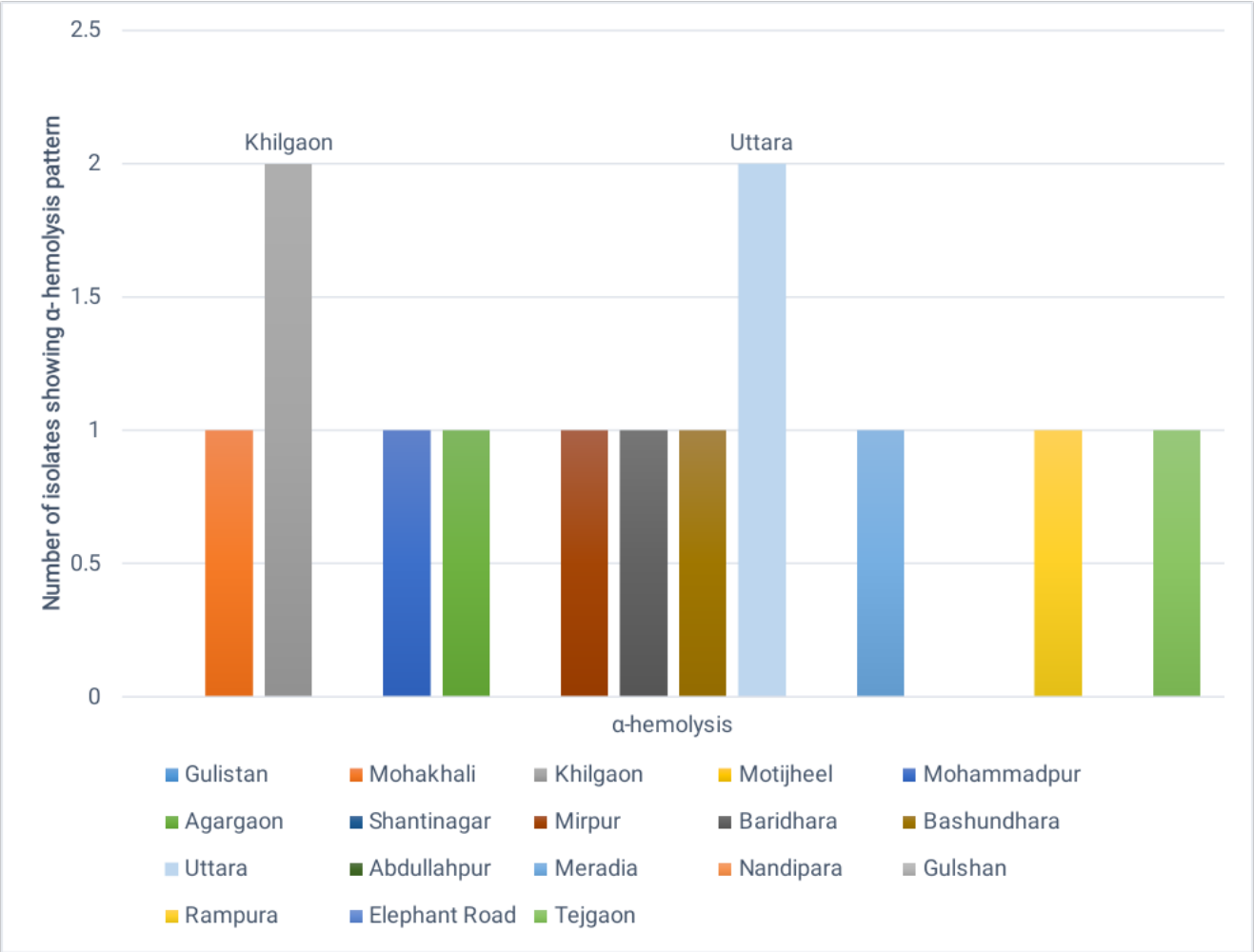


Figure 4: Comparison between the areas based on their α-hemolysis pattern

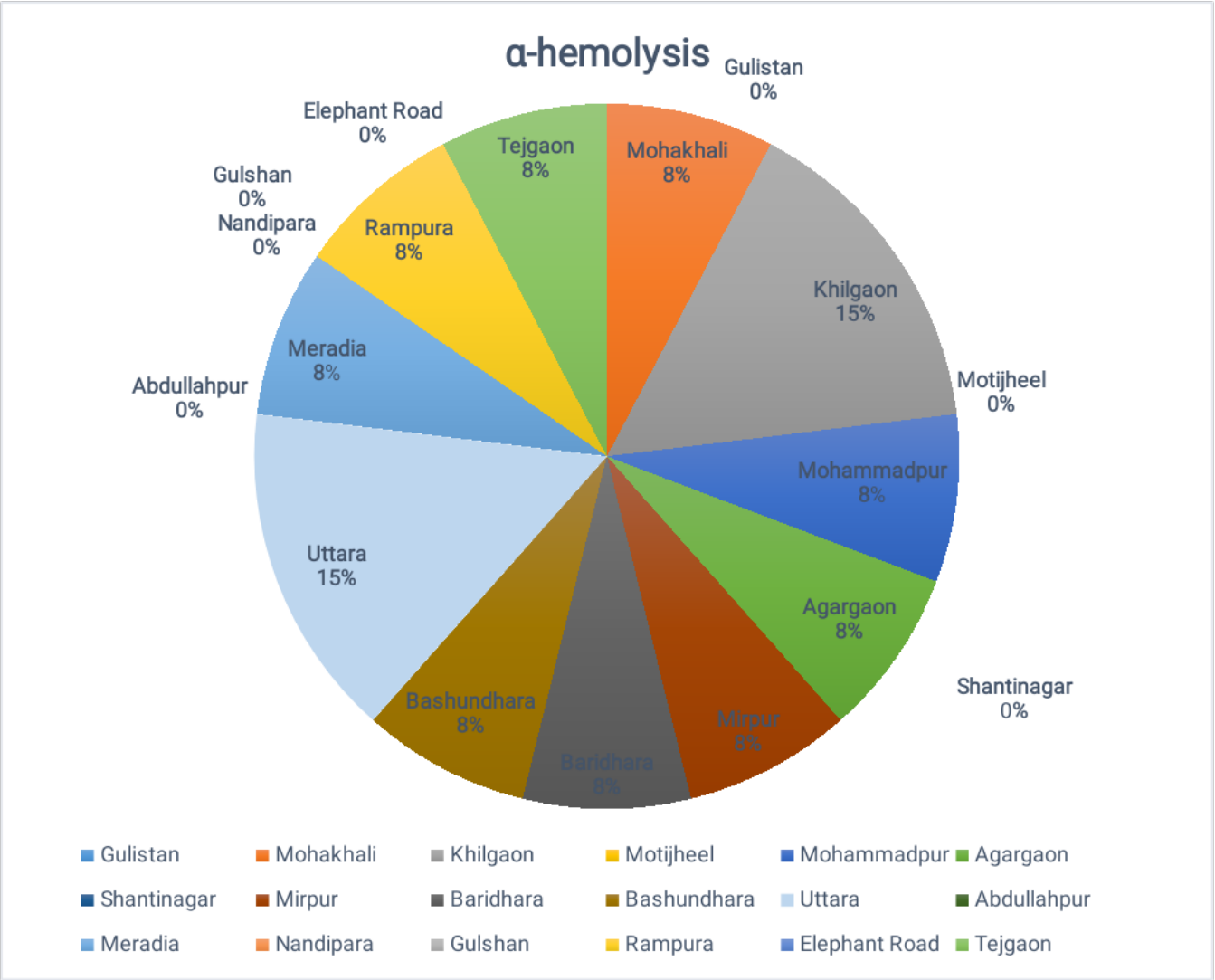


Figure 5: Percentage of α-hemolysis pattern at 18 different areas

Among 63 hemolysis patterns, 13 of them showed α-hemolysis and most of the isolates were from Khilgaon and Uttara **(15%).**

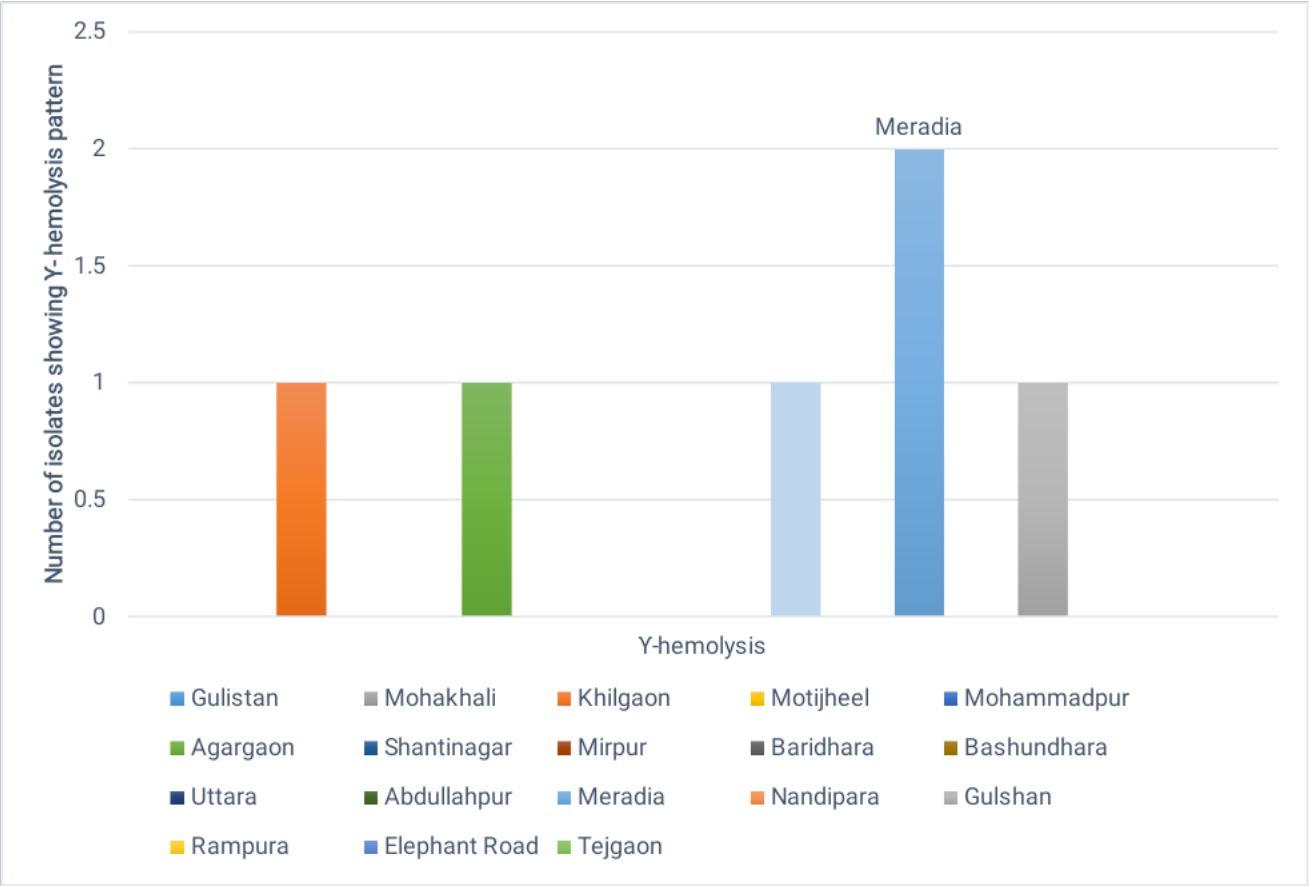


Figure 6: Comparison between the areas based on their Y-hemolysis pattern

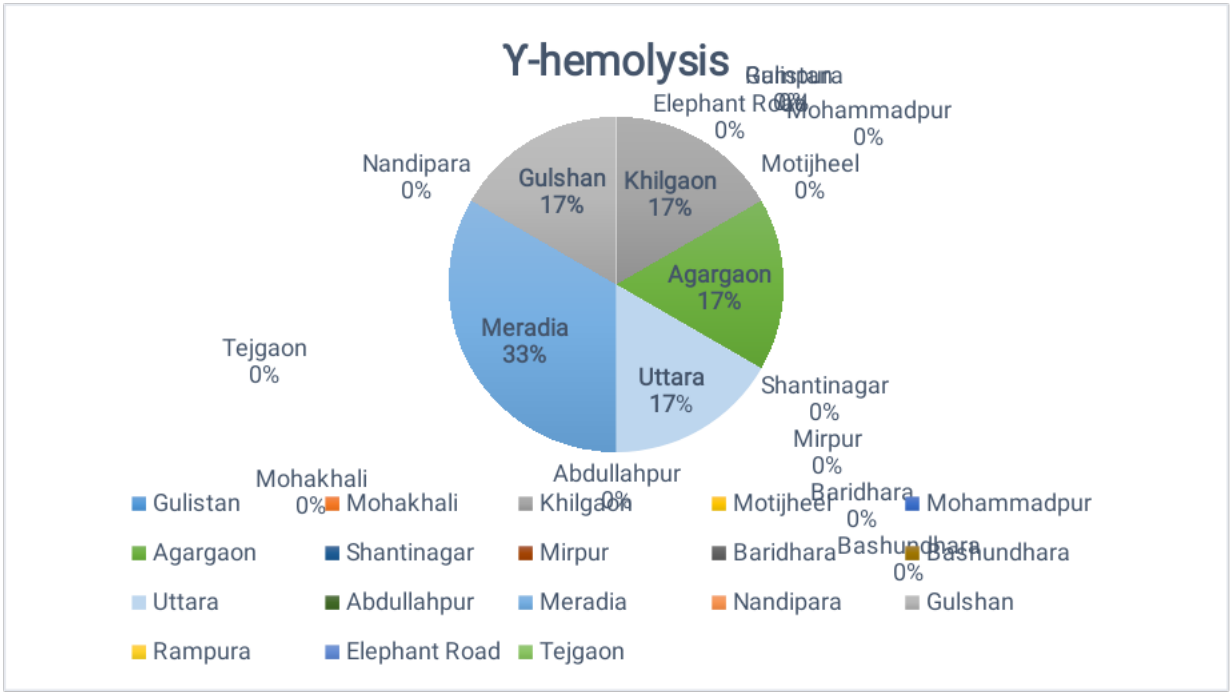
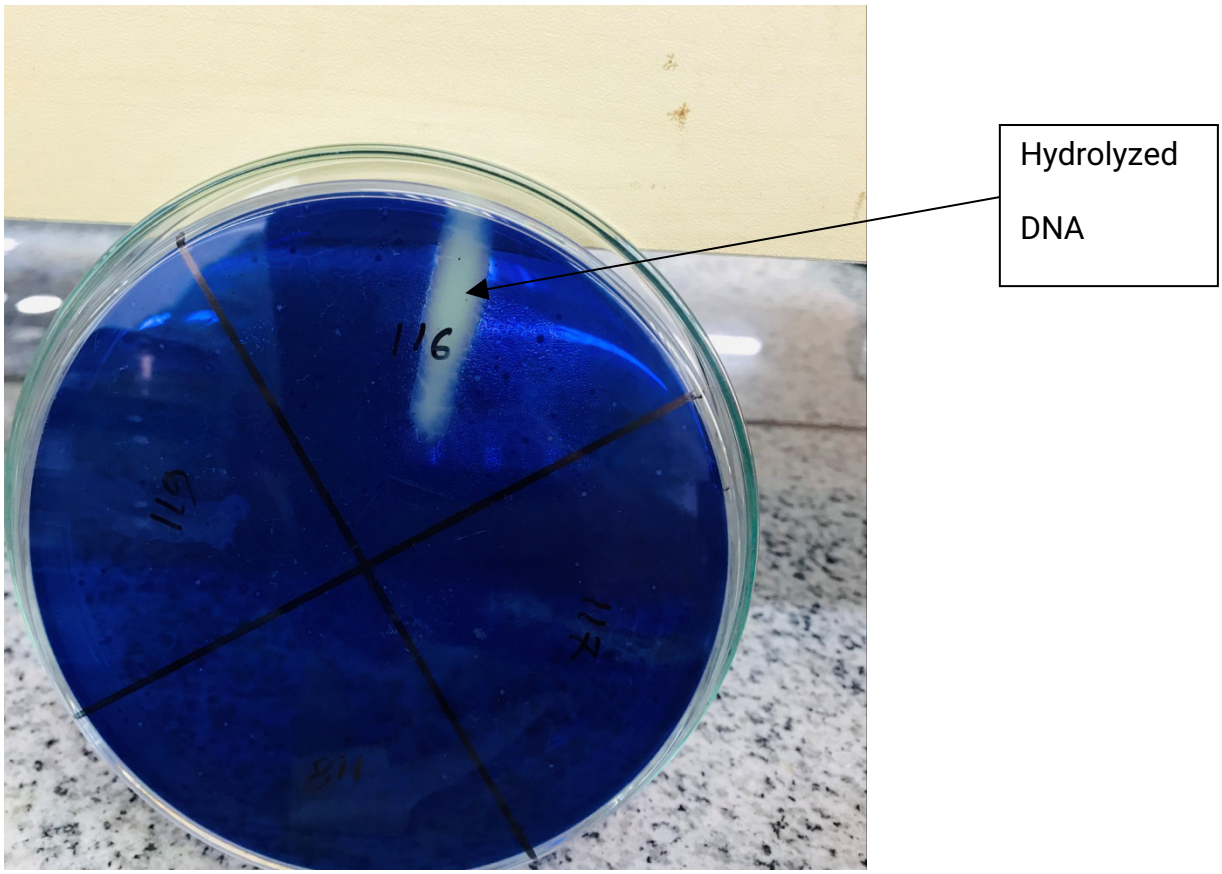


Figure 7: Percentage of Y-hemolysis pattern at 18 different area

The pie chart showed that Meradia showed 33% Y-hemolysis pattern whereas 17% Y-hemolysis pattern was retrieved from Khilgaon, Gulshan, Uttara, and Agargaon.

3.3 DNase Test Result on DNA Agar Base Media:



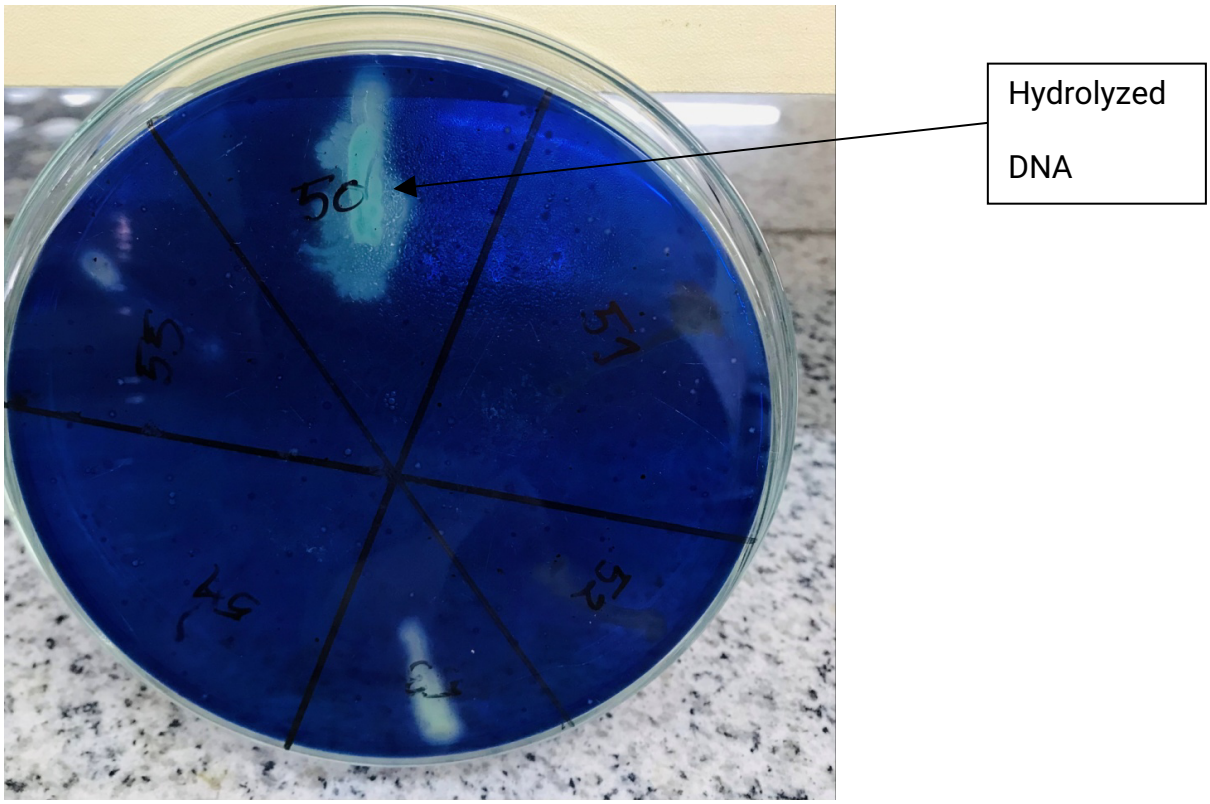


Table 4: Total Number of DNase Positive Test Results based on the Areas

Zone	DNase Positive
Gulistan	1
Mohakhali	2
Khilgaon	5
Motijheel	0
Mohammadpur	0
Agargaon	2
Shantinagar	2
Mirpur	3
Baridhara	1
Bashundhara	1
Uttara	3
Abdullahpur	2
Meradia	6
Nandipara	3
Gulshan	2
Rampura	2
Elephant Road	0
Tejgaon	1

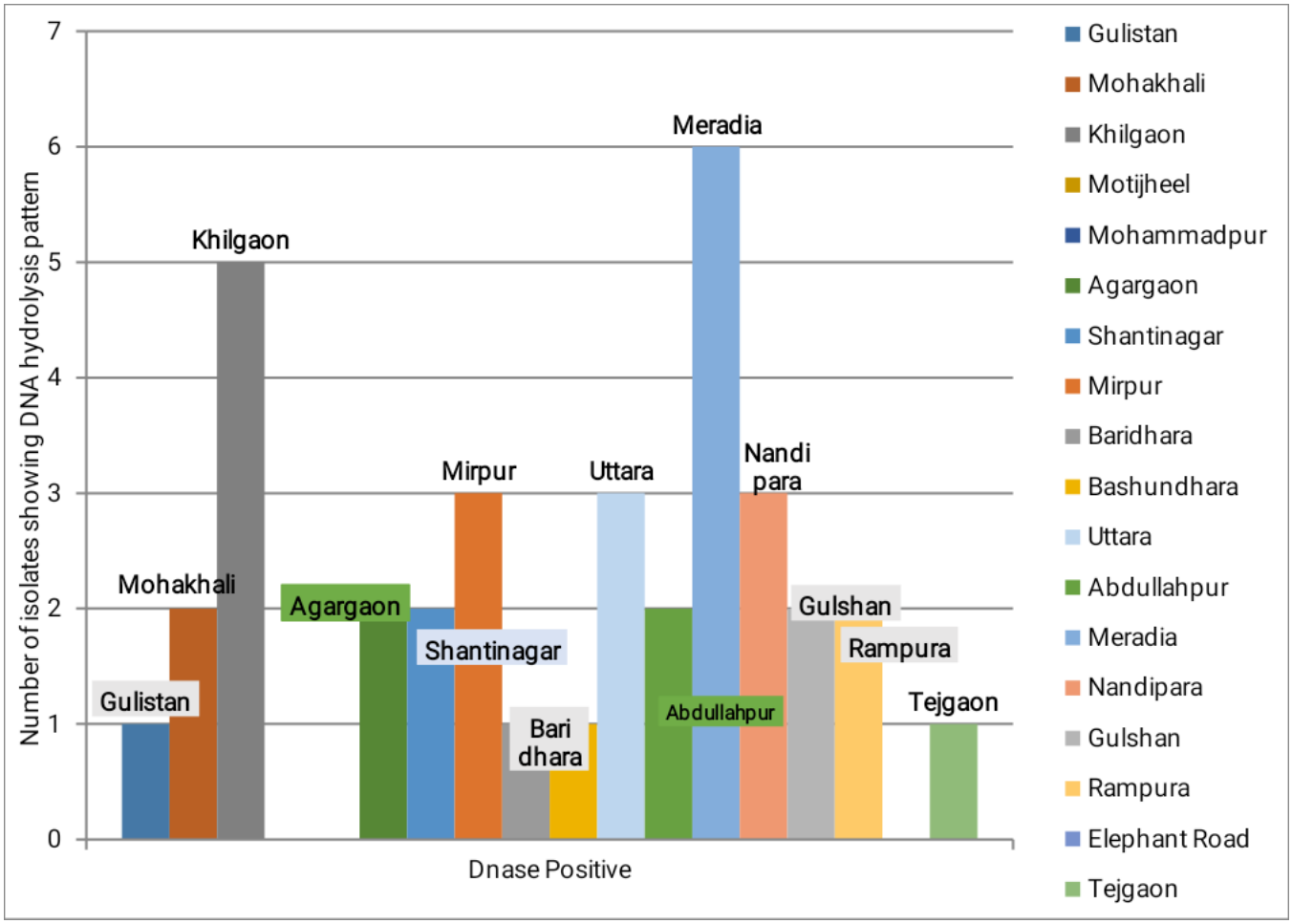


Figure 8: Comparison between the areas based on their DNase Positive Results

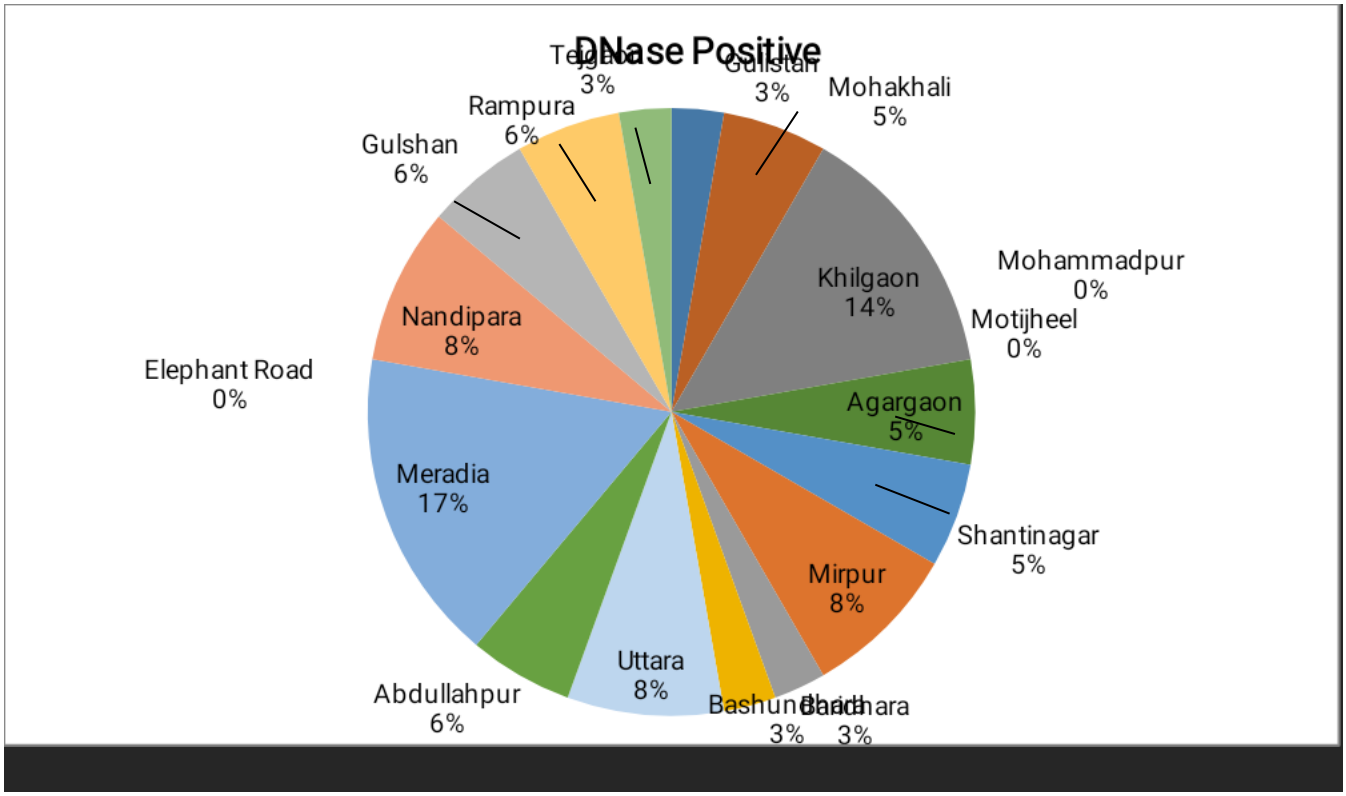


Figure 9: Percentage of DNase positive result at 18 different areas

Among 117 bacterial colonies, Meradia and Khilgaon showed the most positive result on DNA Agar base which was 17% and 14% respectively.

Table 5: Common Isolates Positive in both Blood Agar Base and DNA Agar Base

DNase Positive	Hemolysis Pattern
Mohakhali 3	α-hemolysis
Mohakhali 4	β –hemolysis
Shantinagar 9	α-hemolysis
Mohammadpur 10	β -hemolysis

Gulshan 20	β -hemolysis
Rampura 45	β -hemolysis
Rampura 49	β -hemolysis
Uttara 50	β -hemolysis
Uttara 52	β -hemolysis
Uttara 55	β -hemolysis
Nandipara 56	β -hemolysis
Nandipara 62	β -hemolysis
Nandipara 64	β -hemolysis
Baridhara 78	α-hemolysis
Mirpur 100	β -hemolysis
Mirpur 101	β -hemolysis
Mirpur 102	β -hemolysis
Mirpur 103	β -hemolysis
Mirpur 104	β -hemolysis
Abdullahpur 105	Y-hemolysis
Khilgaon 106	β -hemolysis
Khilgaon 107	β -hemolysis
Khilgaon 108	β -hemolysis

Khilgaon 109	β -hemolysis
Elephant Road 112	β -hemolysis
Elephant Road 113	β -hemolysis
Meradia 114	β -hemolysis
Meradia 115	β -hemolysis
Meradia 116	β -hemolysis
Meradia 117	β -hemolysis

The types hemolysis pattern showing DNAase positive result. In this table, it could be seen that most of the isolates showed beta-hemolysis pattern.

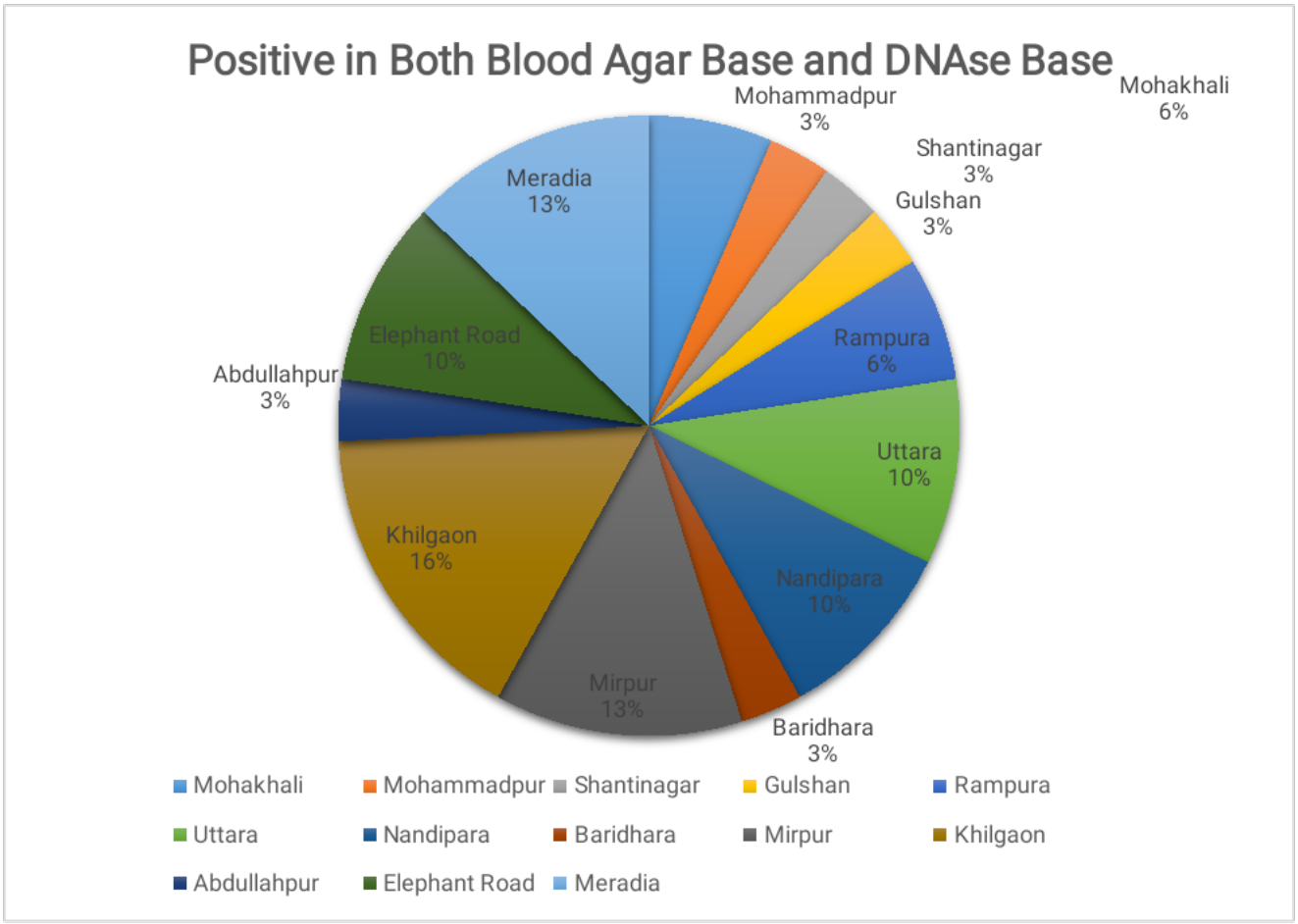


Figure 10: Percentage of Common Isolates Positive in Both Blood Agar Base and DNase Agar Base

Chapter 4

DISCUSSION:

The ever-degrading quality of air in Dhaka city is raging havoc in the city dwellers and has already started to take a toll. In 2017, the country saw 123,000 deaths due to air pollution. In 2019, the number increased by 50000, becoming 173,500. According to (Death Toll from Air Pollution Sees Alarming Rise in Bangladesh, 2020) air pollution was the 3rd leading risk of death after dietary risk factors and high blood pressure-related issues. These numbers are just the ones that are being recorded. The actual number is quite higher. It is a point to be noted that, there has been no data till now on the infection or death rate of people by airborne pathogens. This issue is both humongous and difficult at the same time. First of all, no one can exactly pinpoint the actual place, a person might have caught an air-borne pathogen in real-time. Secondly, it is very easy to term this airborne infection as waterborne or vector-borne, etc. So, it is seen that the procedure is not easy at all and here the significance of the study comes.

This study not only focuses on the collection of pathogenic micro-organisms from the air but also their identification, characterization. This characterization and identification will be performed by a series of biochemical and pathogenicity tests. The virulence and the multi-drug resistance capability of the micro-organism are also going to be checked. Through the findings of this study, results can be obtained on what type of deadly pathogens may be lurking in the polluted air, just waiting to cause infection in the suspectable host.

Till now, two pathogenicity tests have been performed on the 117 collected samples by the research group. The pathogenicity tests include,

- Blood Agar Hemolysis Test
- DNase Test

First of all, from 18 different places in Dhaka city, samples were collected on four different media plates (NA, MSA, MAC, EMB) in three different time intervals (1min, 3 min, 5min). Four different media plates were used for selective collection of pathogenic micro-organisms from the air. NA is a universal media that supports exemplary growth of all kinds of micro-organisms including bacteria, fungi, etc. MSA

media plates were used for the selective collection of some Gram-positive bacteria like ***Staphylococcus, Enterococcus,*** and ***Micrococcaceae*** that have the ability to tolerate high salt concentrations. Its high salt concentration helps to inhibit the growth of unwanted micro-organisms in the media plates making it easier for the easy isolation of gram-positive bacteria. Both the MAC agar media plates and EMB agar media plates were used for selective isolation of gram-negative enteric bacteria and for the differentiation of lactose fermenting from lactose non-fermenting gram-negative bacteria while avoiding the growth of gram-positive bacteria in the media plates. The media plates were exposed in the outside environments of the selected areas and they were kept exposed for 1 min, 3 min, and 5 min sequentially while making sure no other organism or object touches or falls on the media plates. The sample collection was done from an average height of 5 feet from the ground to avoid any kind of ground contaminations. The concentration of micro-organisms in the ground or very close to the ground is very high. To avoid that error and to resemble the average breathing heigh of a human, samples were taken from an average of 5 feet above the ground. Here three different time intervals were kept so that the micro-organisms in the air get sufficient time to randomly fall into the four distinct media plates.

After sample collection of all the 18 places, the media plates containing potential samples were taken to the lab and the plates were incubated for 48 hours in 37º C. Based on the morphologies, common bacterial colonies from the media plates were selected for screening through the pathogenicity test. After incubation, the number of obtained samples was enormous, 3620 colonies, but through screening, the number was reduced to 117. Selected colonies from these 117 were inoculated in pre-made NA media plates for the isolation of single bacterial colonies. After obtaining single colonies, the two pathogenicity tests were performed and the results of the pathogenicity test were shocking.

In the Blood Agar Hemolysis test, Among the 117 isolates, 63 showed hemolysis patterns in human blood. Among these 63 hemolysis patterns, 44 of them showed β-hemolysis where they completely lysed the Red Blood Cells, 13 of them showed α-hemolysis and 6 showed Y-hemolysis patterns. What is more shocking is that healthy human blood was used to deliberately lower the number of micro-organisms in the blood agar plates as human blood contains anti-bodies to inhibit the growth of

micro-organisms. Yet the percentage of hemolysis pattern was over 50%. The exact percentage is 58.87%. The highest number of β-hemolysis was recorded from Meradia (14%), Khilgaon (11%), and Gulshan (9%) respectively. However, the same number of isolates and same hemolysis pattern was found from Mohakhali, Shantinagar, Uttara, Nandipara, and Rampura (7%). α-hemolysis were mostly found from Khilgaon and Uttara (15%) and most Y-hemolysis patterns were recorded from Meradia. From this hemolysis pattern result, it is seen that the concentration of deadly potential pathogen is the highest in Meradia, Khilgaon, Gulshan. In this race, Shantinagar, Uttara, Nandipara, and Rampura are also not far behind. The hemolysis pattern obtained from these areas was also very deadly.

The second pathogenicity test includes DNase test. This test is performed to see if an organism if can hydrolyze DNA and utilize it as a source of carbon and energy for growth. Among 117 bacterial colonies, 36 showed positive results in the DNase test. Among the selected areas, Meradia (6) and Khilgaon (5) showed the most positive result on DNA Agar base which was 17% and 14% respectively.

From the blood agar hemolysis test, it was seen that isolates from Meradia, Khilgaon, and Gulshan showed the most severe β-hemolysis pattern. In the DNase test, isolates from Meradia (6) and Khilgaon (5) showed the highest number of hemolysis patterns and the most severe ones. From this obtained result, a hypothesis can be made that Meradia and Khilgaon contain potentially the most deadly pathogenic micro-organisms. Potentially deadly is said because in both Blood Agar Hemolysis and DNase test human blood and DNA was used and the micro-organisms from these two areas showed the most severe pattern of hemolysis. After Meradia and Khilgaon, Mirpur, Shantinagar, Uttara, Nandipara, and Rampura are also not so far behind. The hemolysis pattern obtained from both the pathogenicity tests from these areas is also bad and from these areas, potential disease-causing pathogens can be obtained.

On the contrary, identification of air microbes has been conducted from time to time covering smaller areas in Bangladesh. For example; air samples were collected from 3 different indoor sites and 3 different outdoor sites of Jahangir Nagar University premises (Kabir *el at.*, 2016). According to this study, the highest average bacterial count was 6167 CFU/m3 and the lowest average bacterial count was 577 CFU/m3

from the outdoor site. In indoor sites, the highest and the lowest average bacterial count were 5786 CFU/m3 and 764 CFU/m3 respectively. However, no pathogenicity test was performed in this study. This study revealed that the indoor and outdoor air of the different sample sites of JU Campus was highly contaminated with bacteria and fungus.

There is another similar study where air samples were collected from the different indoor and outdoor sites of Curzon Hall Campus of Dhaka University to investigate the concentration of airborne micro-flora (Uddin et al., 2019). In this study, Potato Dextrose Agar Media was exposed to the air for 10 minutes in the morning, noon and evening at four different locations in order to isolate fungal colonies. Monthly samples were recorded from September 2018- to November 2018. According to this study, 2681 fungal colonies were found in which 924, 928, and 829 colonies were found in the morning, noon and evening respectively. Moreover, the study revealed that the highest colonies were found in the month of October whereas the spore contents were comparatively less in the month of September.

Another research was conducted in general surgical and labor theatres in Federal Teaching Hospital Abakaliki (FETHA), Ebonyi State, Nigeria to determine the prevalence of airborne bacteria (IR et al., 2020). In this study, blood agar plates were exposed in the air before sub-culturing into Nutrient Agar media to obtain discrete colonies. After that, bacterial identification and antibiotic susceptibility test were done. According to this study, 138 airborne bacterial colonies were isolated among which *Staphylococcus aureu*s was the most dominant one (29%)

However, another study was carried out in 7 different crowded areas of Dhaka city to find out the pathogens present in Dhaka city's air. Thirteen potential pathogens were detected among which ***Shigella dysenteriae*** and ***Staphylococcus spp*** were found to be multi-drug resistant which is an alarming condition for human health (Nawar et al., 2021). In this study, the highest CFU was 137 and the lowest CFU was 1. Total 77 bacterial colonies were screened for pathogenicity test and bacterial identification among which ***Staphylococcus spp*** was the most abundant species. This study focused on bacterial identification and performed antibiotic-resistant tests whereas our study focuses on bacterial identification on a seasonal basis including measurement of virulence factor.

Our study is still in its infancy as a result no solid statement cannot still be made through the results obtained from pathogenicity tests. However, the result indicates the presence of **potential pathogens** in the air that are capable of causing hemolysis in blood agar. Bacterial identification tests will be performed to determine the probable pathogens. The identification of these pathogenic microbes can be applied for the primary screening method of disease identification. Furthermore, the research will give a statistical analysis of which area has which kind of microbes through "biochemical test" and find out the "**multi-drug resistant**" bacteria by performing a multi-drug resistance test. To **conclude,** the virulence factors of the microbes are also going to be measured that will contribute to accessing the severity of the disease caused by the microbes.

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Appendix:

Appendix A

Media Composition:

Composition of Nutrient Agar:

- 0.5% Peptone
- 0.3% beef extract/yeast extract
- 1.5% agar
- 0.5% NaCl
- Distilled water
- pH is adjusted to neutral (7.4) at 25 °C.

Composition of Mannitol Salt Agar:

- 5.0 g/L enzymatic digest of casein

- 5.0 g/L enzymatic digest of animal tissue
- 1.0 g/L beef extract
- 10.0 g/L D-mannitol
- 75.0 g/L sodium chloride
- 0.025 g/L phenol red
- 15.0 g/L agar
- pH 7.4 ± 0.2 at 25 °C

Composition of MacConkey Agar:

- 17g/L Peptone (Pancreatic digest of gelatin)
- 3g/L Proteose peptone (meat and casein)
- 10g/L Lactose monohydrate
- 1.5g/L Bile salts
- 5g/L Sodium chloride
- 0.03g/L Neutral red
- 0.001g/L Crystal Violet
- 13.5g/L Agar
- Final pH 7.1 ± 0.2 at 25 °C.

Composition of Eosin Methylene Blue (EMB) Agar:

- 10g/L Peptic digest of animal tissue
- 2g/L Dipotassium phosphate
- 5g/L Lactose
- 5g/L Sucrose
- 0.4g/L Eosin – Y
- 0.065g/L Methylene blue
- 13.5g/L Agar
- Final pH 7.2 ± 0.2 at 25 °C.

Composition of Blood Agar:

- 10g/L Peptone
- 10g/L Tryptose
- 5g/L Sodium chloride
- 15g/L Agar
- Final pH at 25°C: 7.3 ±0.2

To the base medium, 5% sterile mammalian blood is added after autoclaving and before pouring onto the plates.

Composition of DNase Agar:

- 15g/L Tryptone
- 5g/L Soya peptone
- 2g/L Deoxyribonucleic acid (DNA)
- 5g/L Sodium chloride
- 15g/L Agar
- Final pH 7.3 ± 0.2 at 25 °C.

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.