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

By

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

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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 of the main sources of help.

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Abstract

Air pollution in Dhaka city is a burning question now. A new study suggests that Bangladesh composes the most polluted air with Dhaka securing the second position in terms of pollution (daily star). According to air visual 2018 reports, Dhaka city is ranked number 17th amongst the world's most polluted cities. The purpose of this study is the enumeration of the prevalence of different types of microbes existing in the air of Dhaka city. This identification was done through biochemical tests and their antibiotic resistance pattern was also analyzed. The organisms were then screened for pathogenicity by blood agar hemolysis and DNase test. After the detection of some potential pathogens, in order to make the biochemical identification more rigid, 16srRNA sequencing of the organisms was done to conclusively identify the pathogenic species in air. Thirteen potential pathogens were detected among which two organisms, namely, *Shigella dysenteriae*, *Staphylococcus spp*. were also found to be multidrug-resistant by performing disc diffusion test against most commonly used antibiotics. Alongside, a comparison of total CFU count with the temperature and humidity record respectively at the time of sample collection was done. This helped to build a comparative study of the effect of important factors like temperature and humidity on the growth of air microbes.

Keywords: 16srRNA, air microbiota, pathogenicity, multidrug-resistant

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List of acronyms :

MIU	Motility Indole Urease
MR	Methyl Red
VP	Vogus 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

1.1Overview

Air pollution in Dhaka city is a burning question now. A new study suggests that Bangladesh composes the most polluted air with Dhaka securing the second position in terms of pollution (daily star). According to air visual 2018 reports [Air Visual December 2019]. Dhaka city is ranked number 17^{th} amongst the world's most polluted cities. However, the aim of this study was to enumerate the prevalence of the microorganisms present in the air of north 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 (Kabir *el at.,* 2016) (Brochu *el at.,* 2006). If there are numerous amount 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 their 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. Side by side the study also depicts the multidrug resistance of the prevalent microbes in the air.

In addition to that, a comparison of colony count with temperature and humidity respectively on the time of sample collection was done to estimate the effect of environmental factors on microbial presence. thirteen pathogenic strains were detected in the process from different locations. Among them, two of the organisms were found pathogenic as well as resistant to

most of the antibiotics used which are namely, *Shigella dysenteriae, Staphylococcus spp.* Both of the strains were found in the Mirpur area of Dhaka city. These two have been particularly because they were the pathogenic strains that showed resistance to the maximum number of antibiotics.

12 : Staphylococcus spp: *Staphylococci* are mostly gram-positive. They are found in nasal passages and axillae. Many types of infections might be caused by *Staphylococcus spp*. Their diameter is 1 μm. The pathogenic form of *S.aureus* shows some virulence factor-like tissue-damaging toxin, proteins that cause colonization of host tissue, etc. *S.aureus* and *S.epidermis* show multiple antibiotic resistance. (Baron *et al.*1996)

13 :Shigella dysenteries: *Shigella* genus is rod-shaped, facultative anaerobic and gram-negative. They are the causative agent of *Shigellosis* which is an intestinal disease. *Shigella* is a major contributor to diarrhoeal disease. Shigella species are turning very challenging for most of the antibiotics and they manipulate the host defenses. New technology must be introduced to reduce *Shigellosis.* (Baker et al., 2018)

Aims and objectives:

i . Enumeration of the prevalence of different types of microbes existing in the air of Dhaka city.

ii. An antibiotic resistance pattern of microbes were also analyzed followed by screening them for pathogenesis.

iii. In order to make the biochemical identification more rigid, DNA sequencing of the organism was done to conclusively identify the pathogenic species in air.

iv. To shed a light on the presence and threats by different microbes in air of Dhaka city

Chapter 2

MATERIALS AND METHODS

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

Seventy four samples were collected from seven different zones of Dhaka (Mirpur 10, Uttara, Dhanmondi, Farmgate, Mohakhali, Banani, Gulshan). The samples were collected in three different media in two different time intervals, one was for two minutes and another was for five minutes. All the samples were labeled properly.

2.2 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.

2.3 Sample collection on nutrient agar:

Nutrient agar or NA is a universal media which 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 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 od vitamins, carbohydrates, nitrogen, and salts.

2.4 Sample collection on Mac Conkey agar:

Mac Conkey is a selective and differential culture medium which is also an indicator. Mac Conkey 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 has the ability to fermentate lactose.

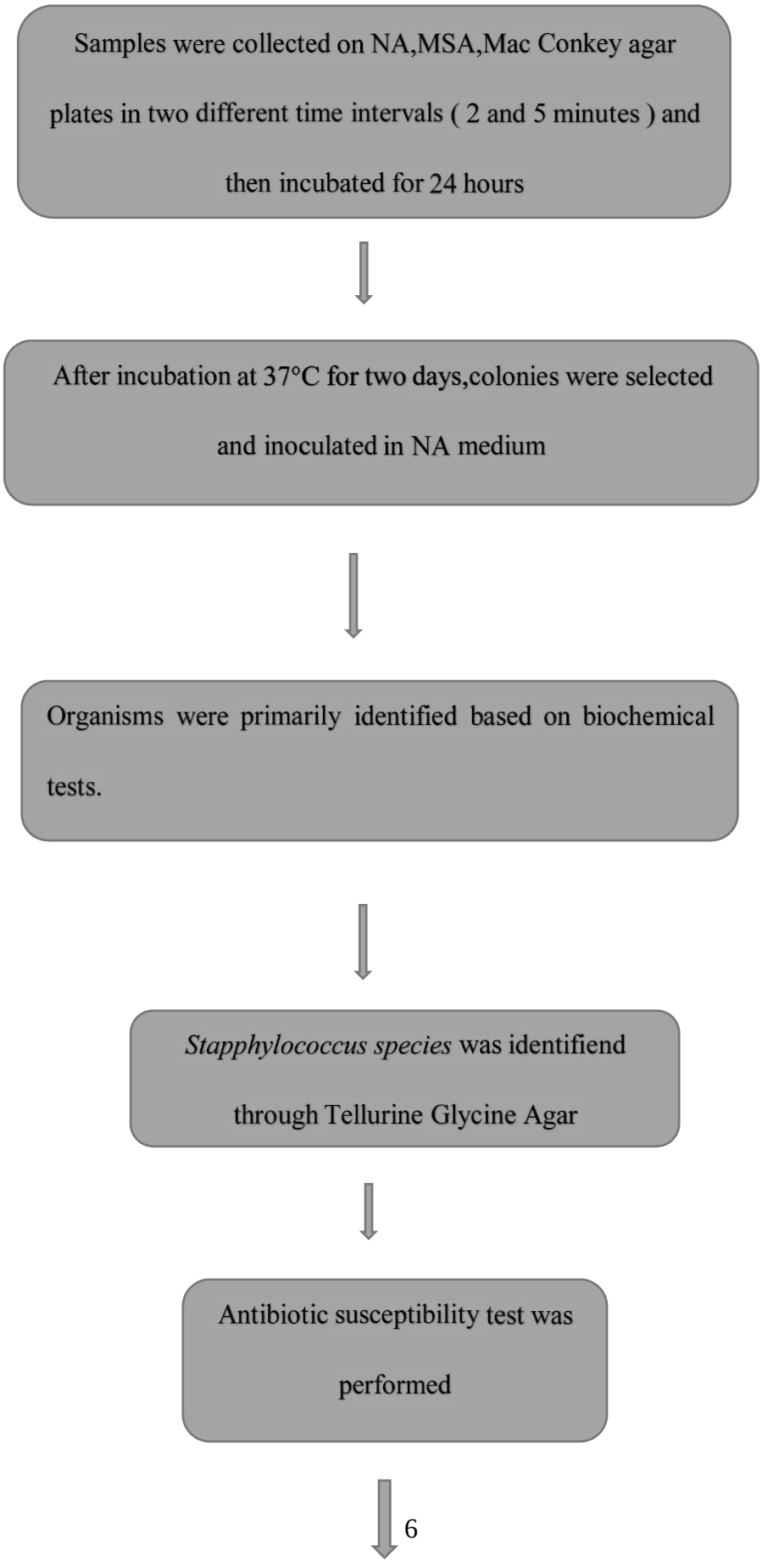
2.5 Microbial culture of the samples:

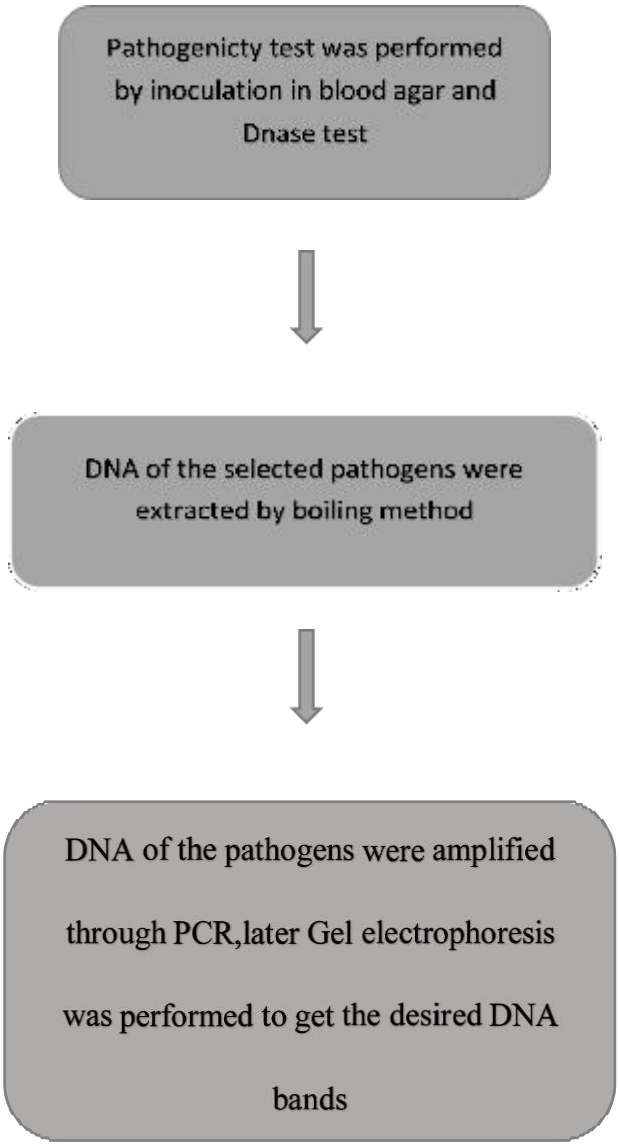
Initially, all the samples were incubated for 24 hours in 37℃ for growth. The next day after incubation selective colonies were taken from the media and inoculated on nutrient agar.

2.6 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.

2.7 :Experimental Work Flow




Pathogenicty test was performed by inoculation in blood agar and Dnase test
DNA of the selected pathogens were extracted by boiling method
DNA of the pathogens were amplified through PCR,later Gel electrophoresis was performed to get the desired DNA bands

2.8 :The biochemical tests 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

2.9 :Gram staining

Gram staining was done using standard method (Cappuccino *et al.,* 2008) .

2.10 :Triple sugar iron test

Triple sugar iron agar is a differential medium used to determine H_2S 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 have inoculated the TSI media with our organisms and incubated them for 24 hours. Following the next day, we have seen different results like 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 H_2S has shown cracked in the media and black spots in the media.

2.11 :Motility Indole Urease test

In 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 was observed after incubation (Cappuccino *et al.*, 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 pink color appearance in the media.

2.12 :Catalase test

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 bacterial colony 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 there is no bubble formation it means the result was negative. (Cappuccino et al., 2008)

2.13 :Indole test

The indole production test was done to determine the production of indole by the organisms. 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 pink-colored ring on the top of the media whereas negative organisms have shown the yellow ring

2.14 :Methyl Red (MR) test

Methyl red test was applied to analyze the bacterial ability to produce stable acid end products. Bacterial cultures were inoculated to 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).

2.15 :Voges–Proskauer (VP) test

The Voges-Proskauer test determines the capability of producing non-acidic or neutral end products. Bacterial cultures were inoculated to VP broth in clean test tubes and incubated overnight at 37°C. Then Barrit's reagent A and Barrit's reagent B was added. The tube was

then allowed to remain still for ten to fifteen minutes and the solution was observed for color changes to determine whether the result is positive (pink-red) or negative (yellow) (Cappuccino and Sherman, 2008).

2.16 :Coagulase Test

Coagulase test is a biochemical test that is performed to differentiate between coagulase-positive *Staphylococcus aureus* and coagulase-negative *Staphylococcus CONS.* Coagulase is an enzyme which 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 and bound free.

2.17 :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 can not 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.

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

Phenol red lactose, sucrose and glucose broth are biochemical tests that are used to identify 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 medium.

2.19 :Tellurine Glycine Agar

Tellurine glycine agar-based is used for the quantitative detection of Staphylococci from foods and other sources like soil, air, skin, etc. After the inoculation of organisms, the Petri dishes are placed into the incubator at 37°C for 24 hours. If there is any black colored colony is seen in the media, it is considered as a positive result.

2.20: Antibiotics used in this experiment

Amoxiclav, Ciprofloxacin, Levofloxacin, Penicillin G, Moxifloxacin ,Cefixime

Cefuroxime, Azithromycin, Tetracyclin, Doxycilin and Chloramphenicol . These antibiotics were used to detect antibiotic sensitivity pattern of the bacteria .

2.21 :Pathogenicity tests results

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 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).

2.22 : DNase Test

DNA hydrolysis test or Deoxyribonuclease (DNase) test is used to check the pathogenicity of an organism 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, it is broken down DNA into smaller fragment molecules. When the DNA is broken down, it no longer binds to the Toluidine blue, and blue color fades and the colony is surrounded by a colorless clear zone.

2.23: PCR and Gel Electrophoresis:

A polymerase chain reaction is an in vitro method to produce a million copies of specific DNA sequences by amplifying it in less than two hours while gel-electrophoresis is the method to analyze DNA and RNA strand by separating the genetic material by size.

Chapter 3

RESULTS :

3.1 Isolates Numbers :

Among 1124 bacteria colonies and 51 fungus colonies, 74 number of colonies of bacteria were isolated through different types of biochemical tests. All the air samples were collected from 7 different areas at different temperatures.

Table 1: Sample Collection Areas, Temperature, Humidity and Dates

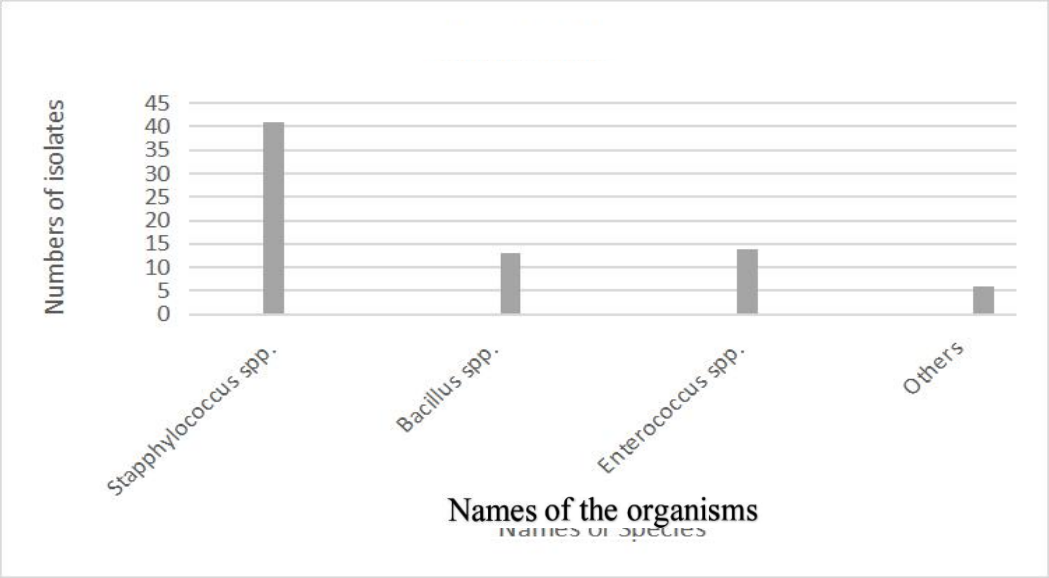
ZONE	TEMP	HUM	DATE
Mohakhali	21^0C	76%	20.01.19 Sunday
Banani	24^0C	79%	31.01.19 Thursday
Gulshan	34^0C	63%	16.04.19 Tuesday
Farmgate	35^0C	66%	8.07.2019 Monday
Uttara	33^0C	86%	18.09.2019 Wednesday
Mirpur	35^0C	94%	18.09.2019 Wednesday
Dhanmondi	29^0C	76%	1.10.19 Tuesday

Table 2: Numbers of samples

Zone name:	Time	NA	MSA	MAC
Mohakhali	1min	26	7	0
	2min	33+3	9	0
	5min	89+4	21	0
Banani	2min	71+6	5	0
	5min	103+4	3	1
Gulshan	2min	40+5	9+2	2
	5min	56+7	7+2	2
farmgate	2min	28+3	18+2	0
	5min	25+2	16+1	4
mirpur	2min	70+1	10	1
	5min	124	34+2	7+1
uttara	2min	77+1	25+1	1
	5min	137+2	32+1	2
dhamondi	2min	12	0	0
	5min	17	6	0

Total 1124 number of bacterial isolate and 51 fungus isolates were found during the study in the nutrient agar ..

3.2 : Comparison between air sample isolates



Figures 1: **Numbers of isolates and their identity**

Among seventy four isolated bacterial strain , 42 samples were isolated as *Stapphylococcus spp,* 13 samples were isolated as *Bacillus spp.* , 14 samples were isolated as *Enterococcus spp*. , and other 5 different kind of bacterial strain were found .

Table 3.3 :Biochemical Test Results : After subculture of the samples on nutrient agar media, the isolates were taken for biochemical test for identification of the organisms.

Isolates no.	Isolates	Catalase test	Indole	MIU			MRVP		Simmon's citrate	Starch hydrolysis	TSI						Probable organism Interpretation by using ABIS online software
				Motility		Urease	Methyl Red	VogesProskauer			Slant/ Butt	Glucose	Lactose	Sucrose	H_2S production	Gas production	
1.	MKI 1(5min Na1)	+	-	+		+	-	-	-	+	B=y,S=R						*Stapphylococcus spp.*
2.	MK2 (5min Na2)	+	-	+		+	-	-	-	+	B-Y, S=R	-	-	-	-	-	*Paenibacillus panacisoli*, similarity : 82.6%
3.	MK3(5min MSA)	+	-	-		-	-	-	-	-	B=Y,S=Y	+	-	-	-	-	*Clostridium putrifaciens* Similarity : 91.3%
4.	MK4 (2min NA)	+	-	+		-	-	-	-	-	B=YS=R	-	-	-	-	-	*Bacillus farraginis* Similarity : 92
5.	MK 5(1min MSA)	+	-	+		-	-	-	-	-	B=Y,S=Y	+	-	+	-	-	*Stapphylococcus spp.*
6.	BN1(2min MSA1)	+	-	+		+	-	-	-	-	B=R,S=R	-	-	-	-	-	*Bacillus farranginis* Similarity : 90.8%
7.	BN2(2 MSA 2)	+	-	+		+	-	-	-	-	B=R,S=R	-	+	-	-	-	*Paenibacillus residue* Similarity 90.3%
8.	BN3(5min MSA)	+	-	+		+	-	-	-	-	B=Y,S=Y	-	-	-	-	-	*Bacillus farranginis* Similarity : 91.5%
9.	BN4 (2MIN NA)	+	_	-		-	-	-	-	-	B=R,S=R	-	-	-	-	-	*Stapphylococcus spp.*
10.	BN 5 (5MIN NA1)	+	-	+		-	-	-	-	-	B=Y,S=Y	-	-	-	-	-	*Stapphylococcus spp.*
11.	BN6 (5MIN NA2)	+	-	+		-	-	-	-	+	B=R,S=R	+	+	+	-	-	*Stapphylococcus spp.*
12.	BN7(5MIN NA3)	+	-	-		-	+	+	-	-	B=Y,S=R	+	-	-	-	-	*Stapphylococcus spp.*
13.	BN8(5MIN MAC)	+	-	+		+	-	-	-	-	B=R,S=R	-	-	-	-	-	*Stapphylococcus spp.*
14.	GK1(2min NA1)	+	-	+		-	-	-		-	B=Y, S=Y	+	+	+	-	-	*Bacillus nealsoni* Similarity : 89.5%

15.	GK2(2min NA 2)	+	-	+		-	-	-		-	B=Y,S=R	+	-	-	-	-	*Brevibacillus Brevis* Similarity: 99%
16.	GK3(2min NA4)	+	-	-		-	-	-		-	B=R,S=R	-	-	-	-	-	*Viridibacillus neidei* Similarity: 89.2%
17.	GK4(2min NA5)	+	-	-		+	+	-		+	B=R,S=R	+	-	-	-	-	*Yersina aleksiciae* Similarity: 90.9%
18.	GK5(2min NA6)	+	-	+		+	-	-		-	B=Y,S=Y	+	-	-	-	-	*Paenibacillus residui* Similarity: 96.3%
19.	GK6(2min MSA1)	+	-	-		-	-	-		-	B=R,S=R	+	-	-	-	-	*Bacillus oleoronius* Similarity: 96.3%
20.	GK7(2min MSA2)	+	-	-		-	-	-		-	B=yR,S=R	+	-	-	-	-	*Stapphylococcus spp.*
21.	GK8(2min MSA3)	+	-	+		-	-	-		-	B=Y,S=Y	-	-	-	-	-	*Bacillus farranginis* Similarity : 99% (*Viridibacillus neidei 99%)*
22.	GK9(5MIN MSA1)	+	-	-		+	+	-		-	B=Y,S=R	-	-	-	-	-	*Stapphylococcus spp.*
23.	GK10(5MIN MSA3)	+	-	-		+	+	-		-	B=Y,S=R	-	-	-	-	-	*Stapphylococcus spp.*
24	Gk11(5MIN MAC1)	+	-	+		+	+	-		-	B=R,S=R	+	-	-	-	-	*Stapphylococcus spp.*
25	GK12(5MIN MAC2)	+	-	-		+	-	-		-	B=R,S=R	-	-	-	-	-	*Stapphylococcus spp.*
26	FM1 (2MIN NA1)	+	-	-		-	-	-	-		B=Y,S=R	-	-	-	-	+	*Leminorella richidi* _similarity (90%) *Salmonella spp* Similarity-82.7%
27	FM2(2MIN NA2)	+	-	+		+	-	-	-		B=Y,S=Y	-	-	-	-	-	*Brevundimonas dimunuta* Similarity:88.1% *PSudomonas tuomurensis* Similarity: 88.1%
28	FM3(2MIN NA3)	+	-	-		+	-	-	-		B=Y,S=R	-	-	-	-	+	*Leminorella richidi* _similarity (90.1%) *Salmonella spp (*Possibility of *serovar gallinarum* Similarity: 81.2 %
29	FM4(5MIN NA 1)	+	-	-		+	-	-	-		B=Y,S=R	-	-	-	-	-	*Bacullus indcus* Similarity : 88.1%

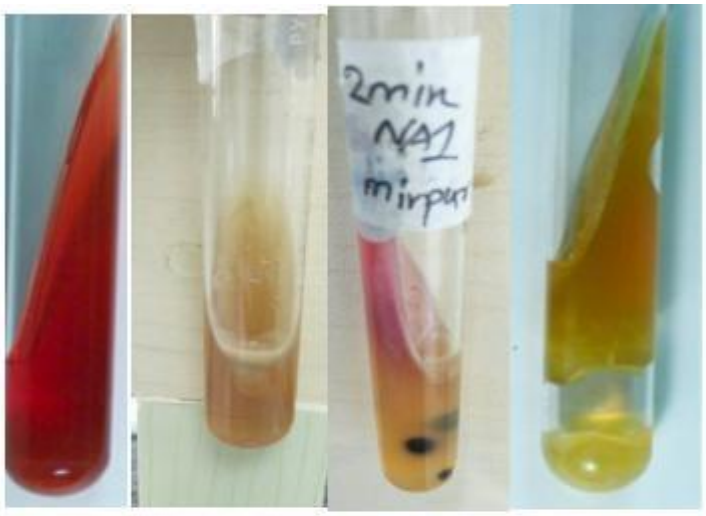
30	FM5(5MIN NA 2)	-	-	-		+	-	-	-		B=R,S=R	-	-	-	-	-	1.*Xenorhabdus beddingii* *2, xenorhabdus bovieni* *3. Xenorhabdus japonica* *4. xenorhabdus nematophila* Similaritry : 84.3 %
32	FM7(5MIN NA4)	-	-	-		+	-	-	-		B=Y,S=Y	-	-	-	-	-	1.*Xenorhabdus beddingii* *2, xenorhabdus bovieni* *3. Xenorhabdus japonica* *4. xenorhabdus nematophila* Similaritry : 84.3 %
33	FM8(5MIN MAC1)	+	-	+		+	-	-	+		B=R,S=R	-	-	-	-	-	1 .*Photorhabdus asymbiotica subsp. Asymbiotica* Similarity: 90.1% 2. *Photorhabdus asymbiotica subsp. Australis* Similarity: 90.1%
34	FM9(5MIN MAC2)	+	-	-		-	-	-	-		B=Y,S=R	+	-	-	-	-	*Edwardsiella ictaluri* Similarity : 94.5%
35	FM10(5MIN MSA1)	-	-	+		+	-	-	-		B=Y,S=Y	+	-	-	+	-	*3. Xenorhabdus japonica 85.6%*
36	FM11(5MIN MSA2)	+	-	+		-	-	-	-		B=Y,S=Y	-	-	-	-	-	*Photorhabdus temperata* Similarity: 84.8%
37	FM12(5MIN MSA3)	+	-	-		+	-	-	-		B=Y,S=Y	-	-	-	-	-	*1 .Obesumbacterium proteus* *2.Obesumbacteium pseudoproteus* Similarity: 89.3 %
38.	UT1(2min Na1)	+	-	-		+	-	-	-	-	B=Y,S=R	+	-	-	-	-	*Edwardsiella ictaluri* Similarity: 87.7%
39	UT2(2min NA2)	+	-	+		-	-	-	-	-	B=Y,S=Y	-	-	+	-	-	*Stapphylococcus spp.*
40	UT3(2min Na3)	+	-	-		-	+	-	-	-	B=Y,S=Y	-	-	-	-	-	*Stapphylococcus spp.*

41	UT4(2min MSA1)	+	-	-		-	-	-	-	-	B=Y,S=Y	-	-	-	-	-	*Stapphylococcus spp.*
42	UT5(2min MSA2)	+	-	+		+	-	-	-	-	B=Y,S=R	-	-	-	-	-	*Stapphylococcus spp.*
43	UT6(2min MAC)	+	-	+		-	-	-	-	-	B=R,S=R	+	-	-	-	+	*Stapphylococcus spp.*
44	UT7(5 min Na1)	+	-	-		-	-	-	-	+	B=Y,S=Y	-	-	-	-	+	*Rathyibacter iranicus* Similarity; 88.3w%
45	UT8(5min Na2)	+	-	-		-	+	-	-	-	B=Y,S=Y	-	-	-	-	-	*Brevibacterium pityocampae* similarity: 88%
46	UT9(5min Na3)	+	-	+		-	-	-	-	-	B=Y,S=Y	-	+	-	-	-	*Stapphylococcus spp.*
47	UT10(5min Msa1)	+	-	-		-	+	-	-	-	B=Y,S=R	-	-	-	-	-	*Stapphylococcus spp.*
48	UT11(5min Msa2)	+	-	+		-	-	-	-	-	B=Y,S=Y	-	-	+	-	-	*Stapphylococcus spp .*
49	UT12(5min Msa3)	+	-	+		+	-	-	-	-	B=Y,S=Y	+	-	+	-	-	*Stapphylococcus spp.*
50	UT13(5min Mac)	+	-	-		+	-	-	-	+	B=Y,S=Y	+	-	-	-	+	*Leminorella richidi* _similarity : 83.3%
51	MR1(2min Na1)	+	-	+		-	-	-	-	+	B=Y,S=R	-	-	-	-	+	*Cornybacterium afermentans* Similarity: 88.2%
52	MR2 (2min Na2)	+	-	+		-	+	-	-	+	B=Y,S=R	+	-	+	-	-	*Stapphylococcus spp.*
53	MR3(2min Na3)	+	-	+		+	-	-	-	-	B=Y,S=Y	-	+	+	-	-	*Stapphylococcus spp.*
54	MR4(2min MSA1)	+	-	-		+	-	-	-	-	B=Y,S=Y	-	-	+	-	-	*Stapphylococcus spp.*
55	MR5(2min MSA2)	+	-	+		-	-	-	-	-	B=Y,S=Y	-	-	-	-	-	*Stapphylococcus spp.*
56	MR6(5min Na1)	+	-	-		-	+	-	-	+	B=Y,S=Y	-	+	-	-	-	*Shigella dysenteriae* Similarity: 86%
57	MR7 (5min Na2)	+	-	+		+	+	-	-	-	B=Y,S=Y	-	-	-	-	-	*Stapphylococcus spp.*
58	MR8(5min Na3)	+	-	+		-	-	-	-	-	B=Y,S=Y	+	-	-	+	-	*Stapphylococcus spp.*
59	MR9(5min NA4)	+	-	-		-	-	-	-	+	B=Y,S=Y	-	-	-	-	+	*Bacillus carboniphylus* similarity: 90%
60	MR10(5min MSA1)	+	-	+		+	+	-	-	-	B=Y,S=Y	-	+	-	-	-	*Stapphylococcus spp.*
61	MR11(5min MSA2)	+	-	-		-	+	-	-	-	B=Y,S=Y	-	+	-	-	-	*Stapphylococcus spp.*
62	MR12(5min MSA3)	+	-	-		+	+	-	-	-	B=R,S=R	-	-	-	-	-	*Stapphylococcus spp.*
63	MR13(5min MAC1)	+	-	+		+	-	-	-	+	B=Y,S=Y	+	-	+	+	-	*Morganella morganii subsp.sibonii* similarity: 86%
64	MR14(5min MAC2)	+	-	-		+	+	-	-	-	B=Y,S=Y	-	-	-	-	-	*Stapphylococcus spp.*

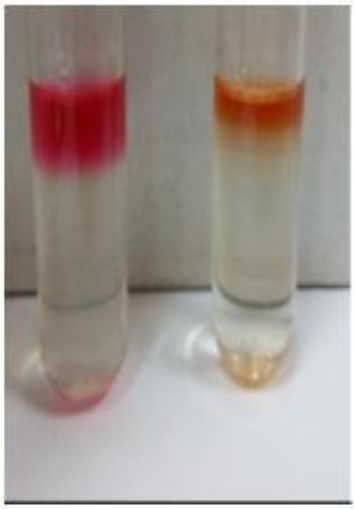
Continuation of table 3.3

65	MR15 (5min MAC3)	+	-	-		-	-	-	+	-	B=R,S=R	-	-	-	-	-	*Stapphylococcus spp.*
66	MR16(2min MAC)	+	-	-		-	-	-	-	-	B=R,S=R		-	-	-	-	*Stapphylococcus spp.*
67	DM1(2min NA1)	+	-	-		+	+	-	-	-	B=Y,S=Y	-	-	-	-	-	*Stapphylococcus spp.*
68	DM2(2min NA2)	+	-	-		-	+	-	-	-	B=Y,S=Y	-	-	-	-	-	*Stapphylococcus spp.*
69	DM3(2min NA3)	+	-	+		+	+	-	-	-	B=Y,S=Y	-	-	-	-	-	*Stapphylococcus spp.*
70	DM4(5min Na1)	+	-	+		+	-	-	-	-	B=Y,S=Y	+	+	-	-	-	*Stapphylococcus spp*
71	DM5(5min Na2)	+	-	+		-	-	-	-	-	B=R,S=R	+	-	-	-	-	*Stapphylococcus spp.*
72	DM6(5min Na3)	+	-	+		-	-	-	-	-	B=Y,S=Y	-	+	-	-	-	*Stapphylococcus spp.*
73	DM7(5min MSA1)	+	-	+		+	-	-	-	-	B=Y,S=Y	-	-	-	-	-	*Stapphylococcus spp.*
74	DM8(5min MSA2)	+	-	-		+	-	-	-	-	B=Y,S=Y	+	-	-	-	-	*Stapphylococcus spp.*

3.4 :Biochemical Test Results :

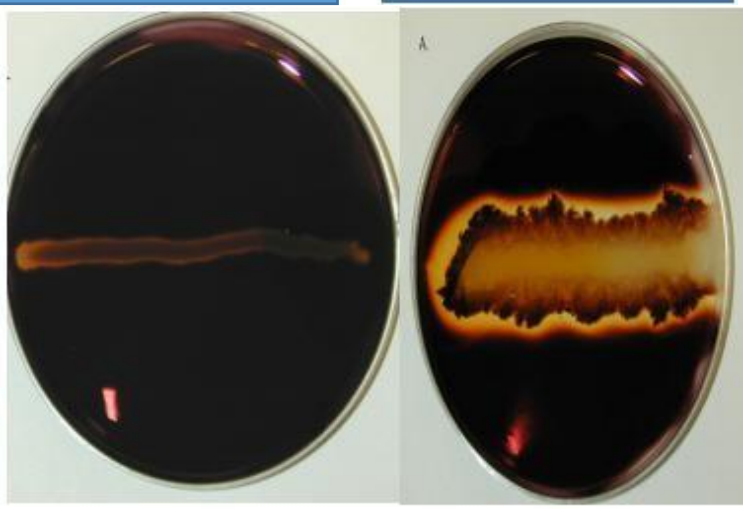


TSI Control , all sugar fermentation,H2SO4 and gas production

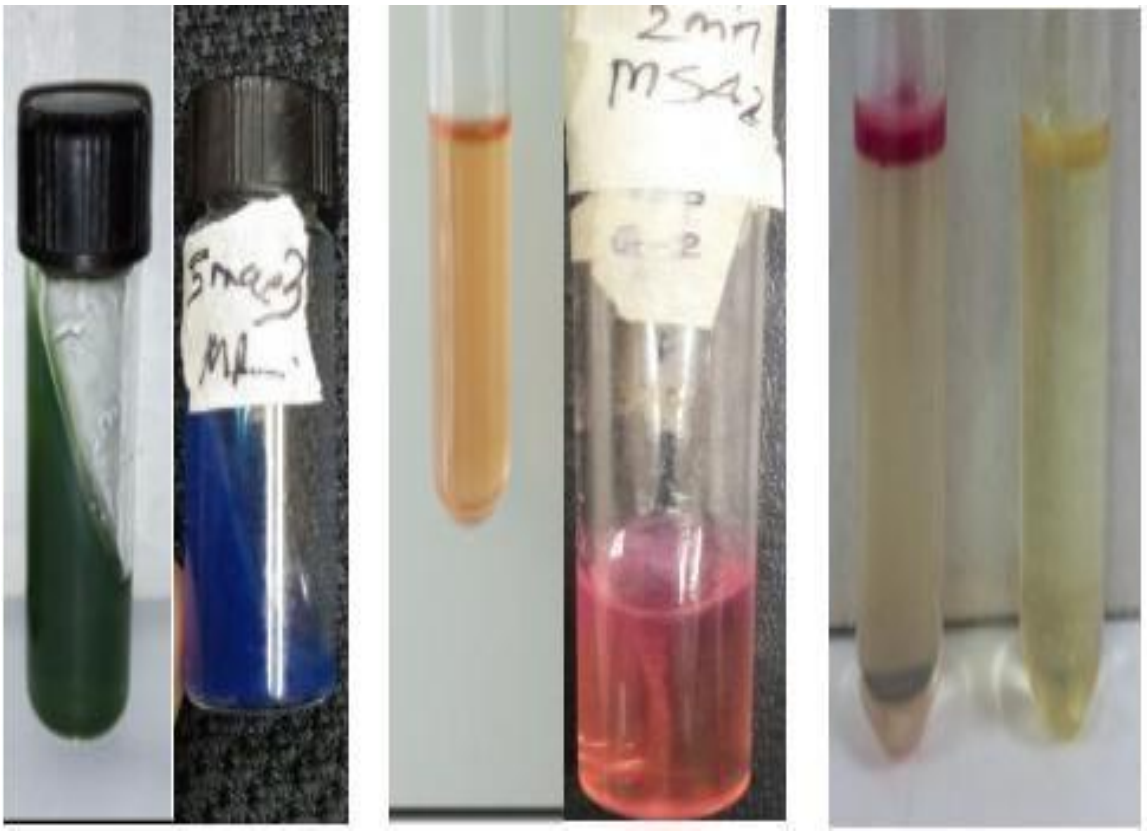


MR Positive and Negative

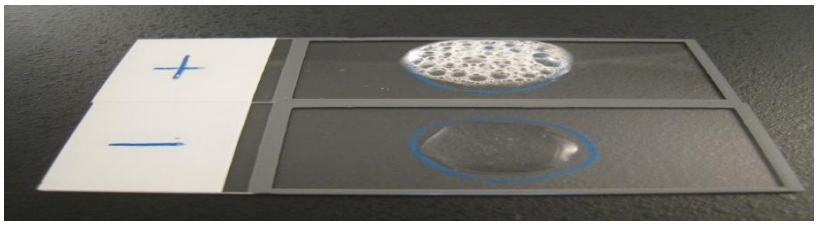
Lactose Fermentation



Starch Hydrolysis Negative and Positive



Citrate test negative and postive, MIU negative and positive , Indole positive and negative



Catalase test positive and negative

Figure 2 : Biochemical Test Results

3.5 :Antibiotic susceptibility test:

Table 3.5 : Antibiotic Susceptibility

Isolates No	AZM 10µg	TE 30µg	P 10µg	CFM 5µg	MXF 5µg	DO 30µg	CIP 5µg	LE 5µg	C 30µg	AMC 30µg	CXM 30µg
1.	R	S	R	R	S	S	S	S	S	S	R
2	S	I	R	R	S	S	S	S	S	R	R
3	S	S	S	S	S	S	S	S	S	S	S
4	R	S	S	R	S	S	S	S	R	R	R
5	S	S	R	R	I	S	S	S	S	R	S
6	S	S	S	S	S	S	S	S	S	S	S
7	S	S	S	R	S	S	S	S	S	S	S
8	R	S	R	R	I	S	S	S	R	S	R
9	I	S	S	R	S	S	S	S	S	S	S
10	S	S	S	S	R	S	S	S	S	R	S
11	R	S	R	R	S	S	S	S	R	S	R
12	R	S	S	R	S	S	S	S	S	S	S
13	S	S	R	R	S	S	R	S	S	R	R
14	S	S	S	R	S	S	S	S	S	S	S
15	S	S	S	R	R	S	S	S	S	S	S

S=Sensitive,R=Resistant,I=Intermediate

16	**S**	**R**	**R**	**R**	**R**	**R**	**S**	**S**	**R**	**S**	**R**
17	**I**	**S**	**R**	**R**	**S**	**S**	**S**	**S**	**S**	**S**	**R**
18	**S**	**S**	**R**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**
19	**I**	**S**	**R**	**R**	**S**	**S**	**S**	**S**	**R**	**R**	**R**
20	**S**	**S**	**S**	**R**	**S**	**S**	**S**	**S**	**S**	**S**	**R**
21	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**I**	**R**	**I**	**R**
22	**R**	**S**	**R**	**R**	**S**	**S**	**S**	**S**	**S**	**S**	**I**
23	**S**	**S**	**R**	**R**	**S**	**R**	**S**	**S**	**S**	**S**	**S**
24	**S**	**S**	**R**	**R**	**S**	**S**	**S**	**S**	**S**	**S**	**R**
25	**S**	**S**	**R**	**R**	**S**	**S**	**S**	**S**	**S**	**S**	**R**
26	**S**	**S**	**S**	**R**	**S**	**S**	**S**	**S**	**S**	**I**	**S**
27	**S**	**S**	**R**	**R**	**S**	**S**	**S**	**S**	**S**	**S**	**S**
28	**S**	**S**	**S**	**R**	**S**	**S**	**S**	**S**	**S**	**S**	**S**
29	**I**	**S**	**S**	**R**	**S**	**S**	**S**	**S**	**S**	**S**	**S**
30	**S**	**S**	**S**	**R**	**S**	**S**	**S**	**S**	**S**	**S**	**S**
31	**R**	**S**	**S**	**R**	**S**	**S**	**S**	**S**	**R**	**S**	**S**
32	**R**	**S**	**R**	**R**	**S**	**S**	**S**	**S**	**S**	**S**	**R**
33	**S**	**S**	**R**	**R**	**S**	**S**	**S**	**S**	**I**	**S**	**R**
34	**S**	**S**	**R**	**R**	**S**	**I**	**S**	**S**	**S**	**S**	**S**

S=Sensitive,R=Resistant,I=Intermediate

35	**S**	**S**	**S**	**I**	**S**	**S**	**S**	**S**	**S**	**S**	**S**
36	**R**	**S**	**R**	**R**	**S**	**S**	**S**	**S**	**I**	**S**	**I**
37	**R**	**S**	**R**	**R**	**S**	**S**	**S**	**S**	**S**	**S**	**S**
38	**S**	**S**	**S**	**R**	**S**	**S**	**S**	**S**	**S**	**S**	**R**
39	**S**	**S**	**S**	**R**	**S**	**S**	**S**	**S**	**S**	**S**	**R**
40	**R**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**I**
41	**I**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**R**
42	**R**	**S**	**S**	**R**	**S**	**S**	**S**	**S**	**S**	**S**	**R**
43	**S**	**S**	**R**	**R**	**S**	**S**	**S**	**S**	**S**	**S**	**R**
44	**S**	**S**	**S**	**R**	**S**	**S**	**S**	**S**	**S**	**S**	**R**
45	**S**	**S**	**R**	**R**	**S**	**S**	**S**	**S**	**S**	**S**	**R**
46	**R**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**R**
47	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**R**	**R**
48	**R**	**S**	**R**	**R**	**S**	**I**	**S**	**S**	**I**	**S**	**R**
49	**R**	**S**	**S**	**R**	**S**	**S**	**S**	**S**	**S**	**S**	**R**
50	**S**	**S**	**R**	**S**	**S**	**S**	**S**	**S**	**R**	**S**	**R**
51	**I**	**S**	**R**	**R**	**S**	**S**	**S**	**S**	**R**	**S**	**R**
52	**R**	**S**	**R**	**R**	**S**	**S**	**S**	**S**	**S**	**S**	**R**
53	**I**	**S**	**R**	**R**	**S**	**S**	**S**	**S**	**S**	**S**	**R**

S=Sensitive,R=Resistant,I=Intermediate

54	**R**	**S**	**R**	**R**	**S**	**S**	**S**	**S**	**S**	**S**	**R**
55	**R**	**S**	**R**	**R**	**S**	**S**	**S**	**S**	**S**	**S**	**R**
56	**I**	**R**	**R**	**R**	**R**	**R**	**R**	**S**	**R**	**R**	**R**
57	**R**	**S**	**R**	**R**	**R**	**S**	**S**	**S**	**I**	**I**	**R**
58	**R**	**R**	**R**	**R**	**I**	**R**	**S**	**S**	**S**	**I**	**R**
59	**I**	**S**	**R**	**R**	**S**	**S**	**S**	**S**	**R**	**S**	**I**
60	**R**	**R**	**R**	**R**	**R**	**R**	**I**	**I**	**I**	**I**	**I**
61	**R**	**S**	**R**	**R**	**S**	**S**	**S**	**S**	**S**	**S**	**S**
62	**R**	**S**	**R**	**R**	**S**	**S**	**S**	**S**	**S**	**S**	**S**
63	**R**	**I**	**R**	**R**	**S**	**S**	**S**	**S**	**S**	**I**	**S**
64	**R**	**S**	**R**	**R**	**R**	**S**	**S**	**S**	**S**	**S**	**R**
65	**R**	**S**	**R**	**S**	**S**	**S**	**S**	**S**	**R**	**S**	**R**
66	**I**	**S**	**R**	**S**	**S**	**S**	**S**	**S**	**R**	**S**	**I**
67	**R**	**S**	**R**	**R**	**S**	**S**	**S**	**S**	**S**	**S**	**R**
68	**R**	**S**	**S**	**R**	**S**	**S**	**S**	**S**	**S**	**S**	**S**
69	**S**	**S**	**R**	**R**	**S**	**S**	**S**	**S**	**S**	**S**	**R**
70	**R**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**R**	**S**	**S**
71	**R**	**S**	**R**	**R**	**S**	**S**	**S**	**I**	**R**	**S**	**R**
72	**I**	**S**	**S**	**R**	**S**	**S**	**S**	**S**	**R**	**S**	**R**

S=Sensitive,R=Resistant,I=Intermediate

73	**R**	**S**	**S**	**R**	**S**	**S**	**S**	**S**	**S**	**S**	**R**
74	**R**	**S**	**R**	**R**	**R**	**R**	**S**	**R**	**R**	**I**	**R**

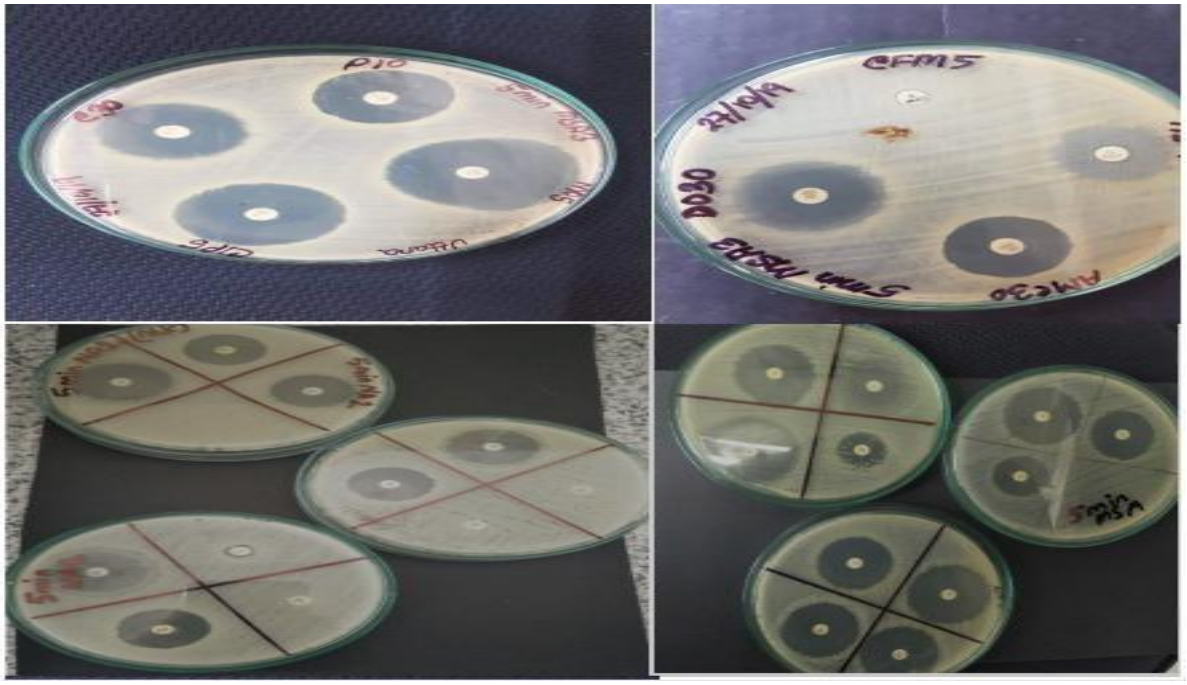


Figure 3 : Antibiotic Susceptibility test by disc diffusion method

3.6 : Comparison of antibiotic susceptibility

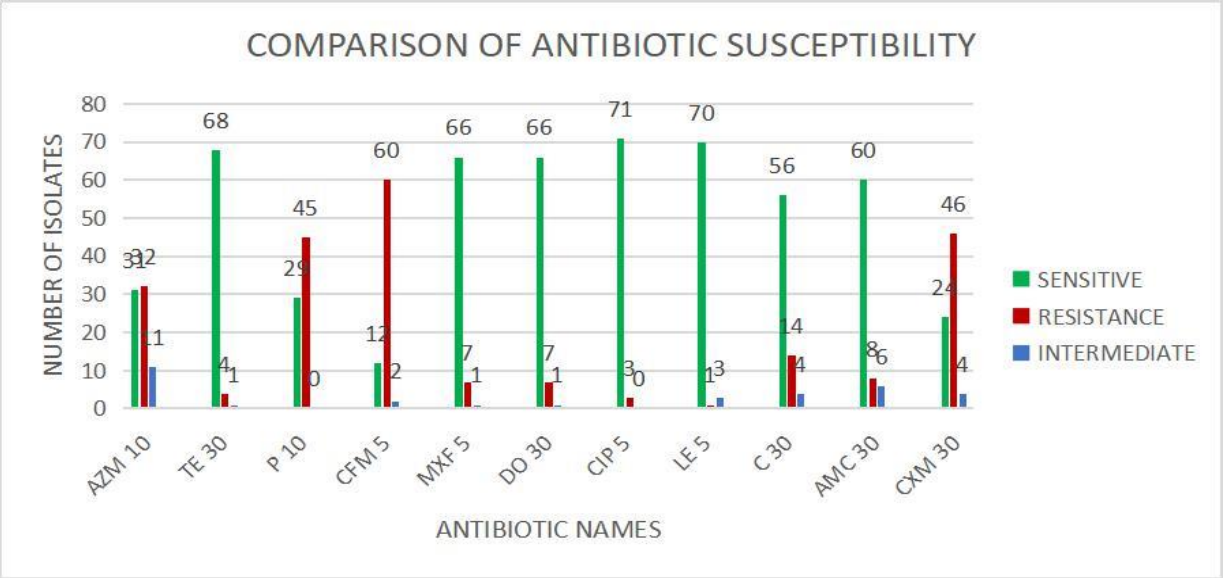


Figure 4: **antibiotic susceptibility pattern**

Among our isolated bacterial strain , most of the bacteria have shown sensitivity towards Tetracyclin 30 µg, Moxifloxacin 5 µg , Doxycyclin 30 µg , Ciprofloxacin 5 µg, Levofloxacin 5 µg, Amoxiclav 30 µg, Chloramphenicol 30 µg and have shown resistance to Penicillin 10µg , Cefixime 5µg , Azithromycin 10 µg and Cefurixime 30 µg .

3.7 : Pathogenicty Test Results:

Pathogenicity test was done through blood agar test and DNase agar base.

Names of Pathogens :

5min Na1 (UTT) - *Rathyibacter iranicus* Similarity; 88.3%

2min Na1 (UTT)- *Edwardsiella ictaluri* Similarity : 87.7%

5min Na4 (MRP) - *Bacillus carboniphylus S*imilarity : 90%

2min Na1 (MRP) - *Cornybacterium afermentans* Similarity : 88.2%

5min Na1 (MRP) -*Shigella dysenteriae* Similarity : 86%

5min Na3 (MRP)- *Staphylococcus spp.*

5min Na2 (UTT)- *Brevibacterium pityocampae* Similarity : 88%

2min Na1 (FM)- *Leminorella richidi* similarity (90%)

Salmonella spp Similarity-82.7%

5min Mac (BN)-*Staphylococcus spp.*

5min Na1 (BN)- *Staphylococcus spp.*

2min Na5(GL)- *Yersina aleksiciae* Similarity 90.9%

5min Na2 (MK)- *Paenibacillus panacisoli,* similarity : 82.6%

2min MSA2(BN)-*Paenibacillus residue* Similarity 90.3%

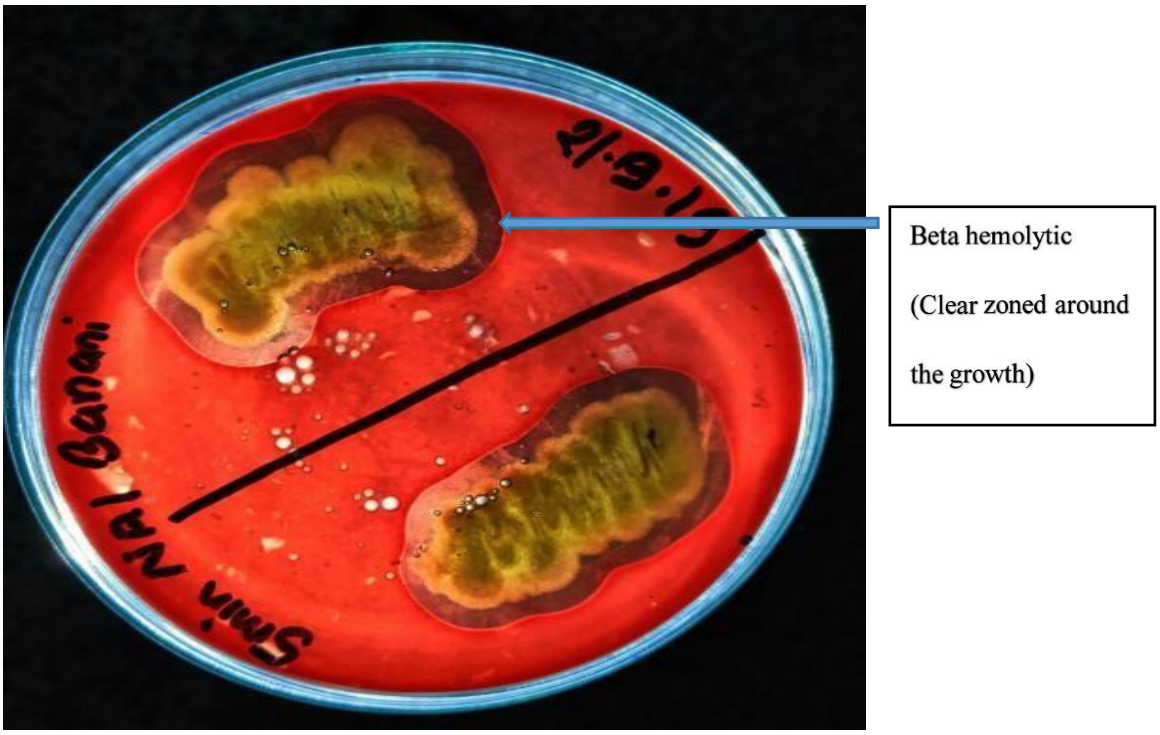


Figure 5: Haemolytic activity test on blood agar base

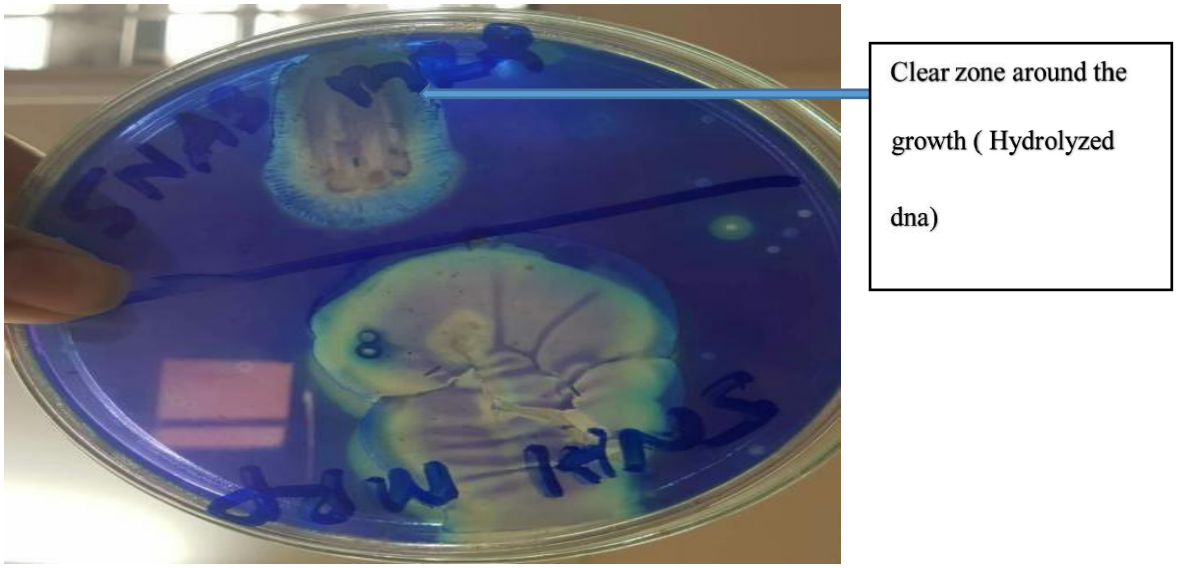


Figure 6: Dnase Test on DNase Agar Base

3.8: Table 4 : Hemolysis ,Dnase and Coagulase test results :

Beta Hemolytic	Dnase Positive	Coagulase Positive
2min MSA2 (BN)-*Paenibacillus residue*	-	+
5min Na1 (UTT) - *Rathyibacter iranicus*	-	+
2min Na1 (UTT)- *Edwardsiella ictaluri*	-	-
5min Na4 (MRP) - *Bacillus carboniphylus*	-	-
2min Na1 (MRP)-*Cornybacterium afermentans*	-	+
5min Na1 (MRP)-*Shigella dysenteriae*	+	-
5min Na3 (MRP)- *Staphylococcus spp.*	+	+
5min Na2 (UTT)- *Brevibacterium pityocampae*	+	-
2min Na1 (FM)- *Leminorella richidi*	-	-
5min Mac (BN)-*Staphylococcus spp.*	+	-
5min Na1 (BN)- *Staphylococcus spp.*	+	+
2min Na5(GL)- *Yersina aleksiciae*	-	-
5min Na2 (MK)- *Paenibacillus panacisoli*	+	+

Thirteen pathogens showed beta hemolysis , six were Dnase positive and six were coagulase positive and one of them was all tests positive .

3.9 :Table 5 : Antibiotic Susceptibility of Pathogens:

Isolates Names	AZM 10	TE 30	P 10	CFM 5	MXF 5	DO 30	CIP 5	LE 5	C30	AMC 30	CXM 30
5min Na1 (UTT)	S	S	S	R	S	S	S	S	S	S	R
2min Na1 (UTT)	S	S	S	R	S	S	S	S	S	S	R
5min Na4 (MRP)	I	S	R	R	S	S	S	S	R	S	I
2min Na1 (MRP)	R	S	R	R	S	S	S	S	S	S	R
5min Na1 (MRP)	I	R	R	R	R	R	R	S	R	R	R
5min Na3 (MRP)	R	R	R	R	I	R	S	S	S	I	R
5min Na2 (UTT)	S	S	R	R	S	S	S	S	S	S	R
2min Na1 (FM)	S	S	S	R	S	S	S	S	S	I	S
5min Mac (BN)	S	S	R	R	S	S	R	S	S	R	R
5min Na1 (BN)	S	S	S	S	R	S	S	S	S	R	S
2min Na5(GL)	I	S	R	R	S	S	S	S	S	S	R
5min Na2 (MK)	S	I	R	R	S	S	S	S	S	R	R
2min MSA2 (BN)	S	S	S	R	S	S	S	S	S	S	S

Among all the 13 potential pathogens it was found that the isolates , 5NA1 MRP and 5NA3 MRP have shown resistance against most of the antibiotics .

3.10 : Comparison of antibiotic susceptibility between pathogens:

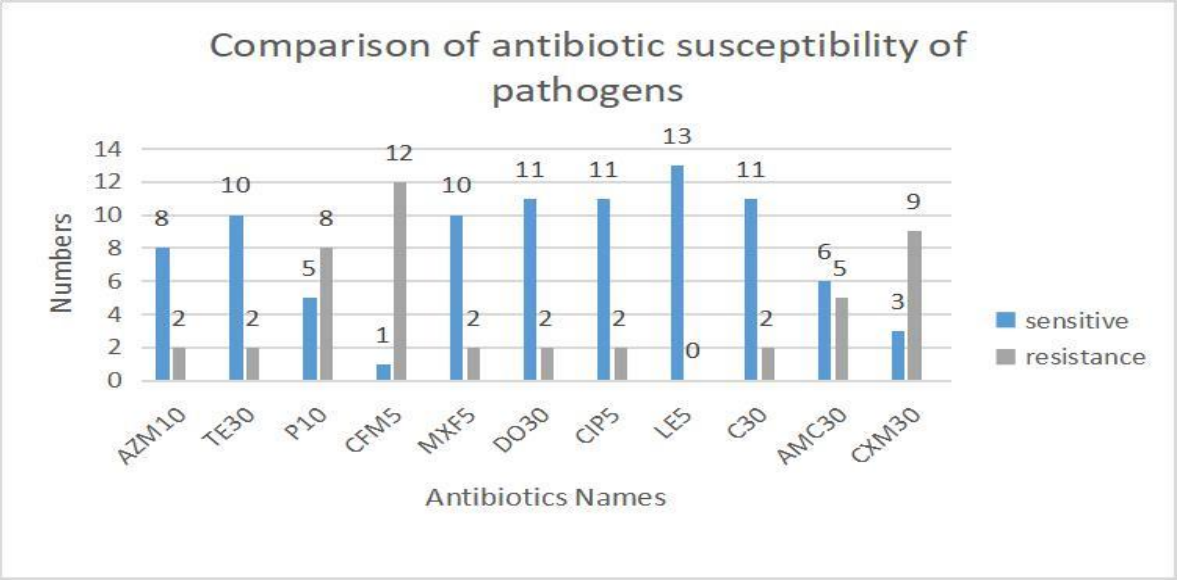


Figure 7: Pathogens antibiotic susceptibility

Among eleven antibiotics ,most of the organisms showed sensitivity to TE 30 , MXF 5, DO 30 , CIP 5, LE 5 and C 30 and others resistant to CFM 5, P 10 , CXM 30 , AZM 10 .

3.11 :PCR and gel electrophoresis: After pathogenicity and antibiotic susceptibility test, PCR and gel electrophoresis was performed using 16srRNA primer.

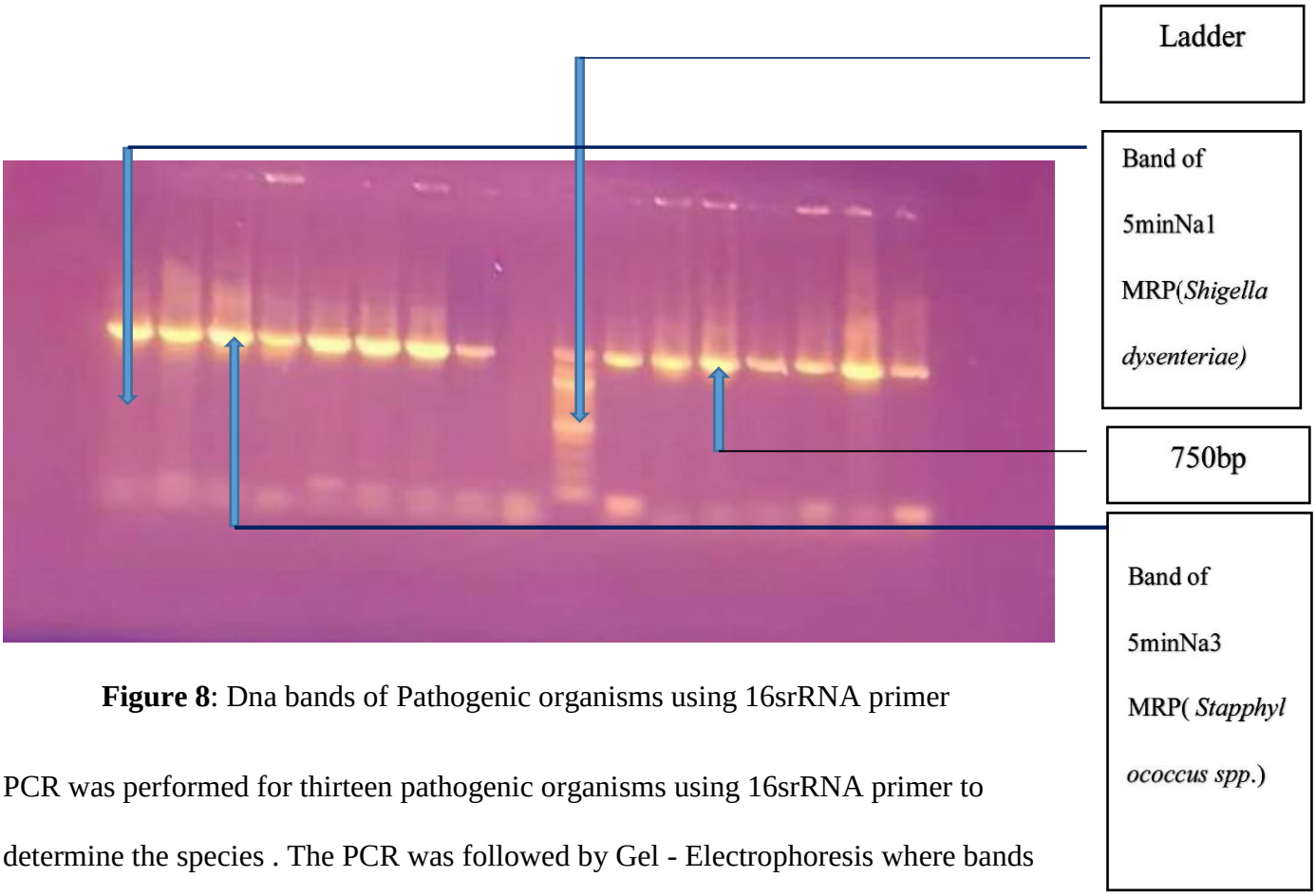


Figure 8: Dna bands of Pathogenic organisms using 16srRNA primer

PCR was performed for thirteen pathogenic organisms using 16srRNA primer to determine the species . The PCR was followed by Gel - Electrophoresis where bands sized 750 base pair were found .

CHAPTER 4:

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 anomaly. The matter seems to become more serious when the commercially produced antibiotics start being challenged by the pathogens. According to a report of 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 South-east 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 (Kelly *et al.*, 2015) .

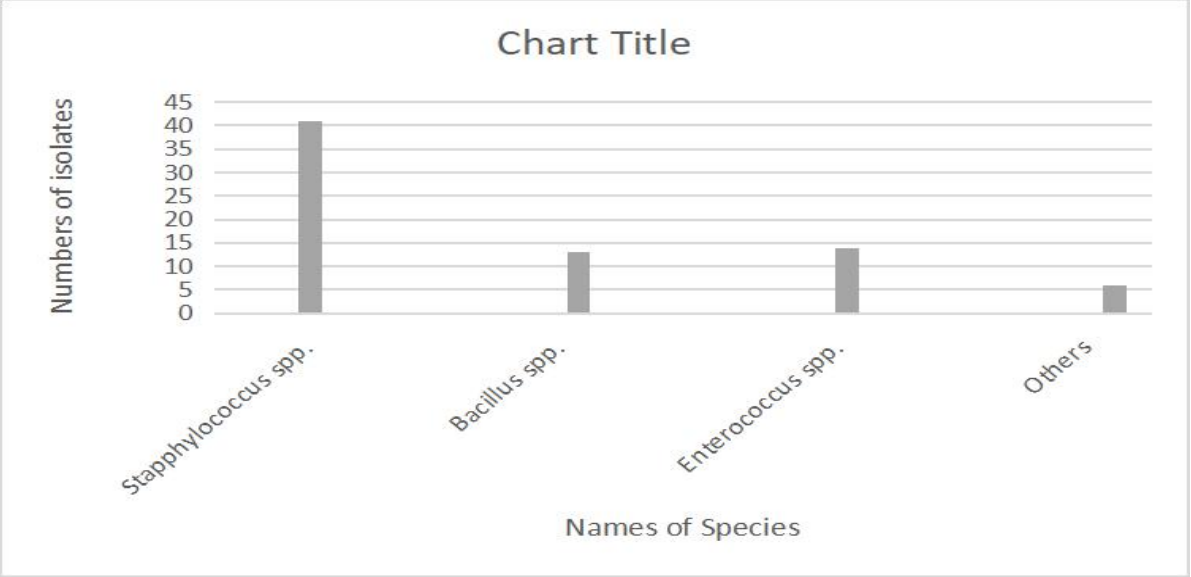
The study clearly aims at finding out what exactly are the different types of microbes that can largely prevail and survive in the air of Dhaka city despite the city being top-ranked on air pollution. The aim was to find out the pathogens in the air that the people are being exposed to on a daily basis. 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, Banani, Gulshan, Farmgate, Uttara, Mirpur, and Dhanmondi. These are one of the busiest areas in the city. The zones were chosen to cover the north Dhaka city. However, the date of the collection time was taken and the humidity and the temperature were noted 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, Mackonky agar. NA was selected for universal growth of all organisms whereas MSA was chosen for *staphylococcus* and for some differential growth, and Mackonky 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 2 mins for each of the media (NA, MSA, Mac) 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 37-degree Celcius for 24 hours. Growth occurred and the colonies were selected seeing the morphology. However, both bacteria and fungal growth occurred. Bacteria with similar morphology were selected and the samples were brought down to 74. These were then subcultured in the NA plate. From there biochemical tests were performed. After the biochemical test, the samples were screened for pathogenicity and 13 pathogens were found. Out of the 13, 2 pathogens were multidrug-resistant which were namely, *Shigella dysenteriae, Staphylococcus spp.* The first one is the causative agent of Shigellosis, and the second one is the causative agent of many other infections. So they were thought to possess some serious health risks to be on the air. However, 16srRNA sequencing was done for all the pathogens after isolation of their DNA using a boiled method and then the following amplification by PCR and gel electrophoresis. This was done to specifically confirm the pathogenic species present in the air.

According to (Makut et al., 2014), "Six bacterial species were isolated at varying frequencies of occurrence. The bacterial species with their respective frequencies of occurrence were *S.aureus (100%), S.pyogenes (100%), E.coli (90%),Bacillus spp.(100%), Enterobacter aerogenes (40%) and Shigella spp. (50%).*"



During our research, we have got 1124 colonies of bacteria and 51 colonies of fungi. Among 1124 bacteria, we have selected 74 isolates for identification. From all of our isolated strain, we have got 42 *Staphylococcus spp.* , 13 *Bacillus spp.* , 14 *Enterococcus spp.* , and other 6 species.

On the other hand, another research that has been conducted to find out the prevalence of bacteria in indoors showed a contrasting result. According to (Sidra *el at.*, 2015) , *Stapphylococcus spp* was the most abundant species of bacteria out of the thirty sites where the sampling has been done . *Micrococcus spp , Bacillus spp* and *Serratia spp* and some gram negative and gram positive rods and cocci were found in some sites which were very less in number .

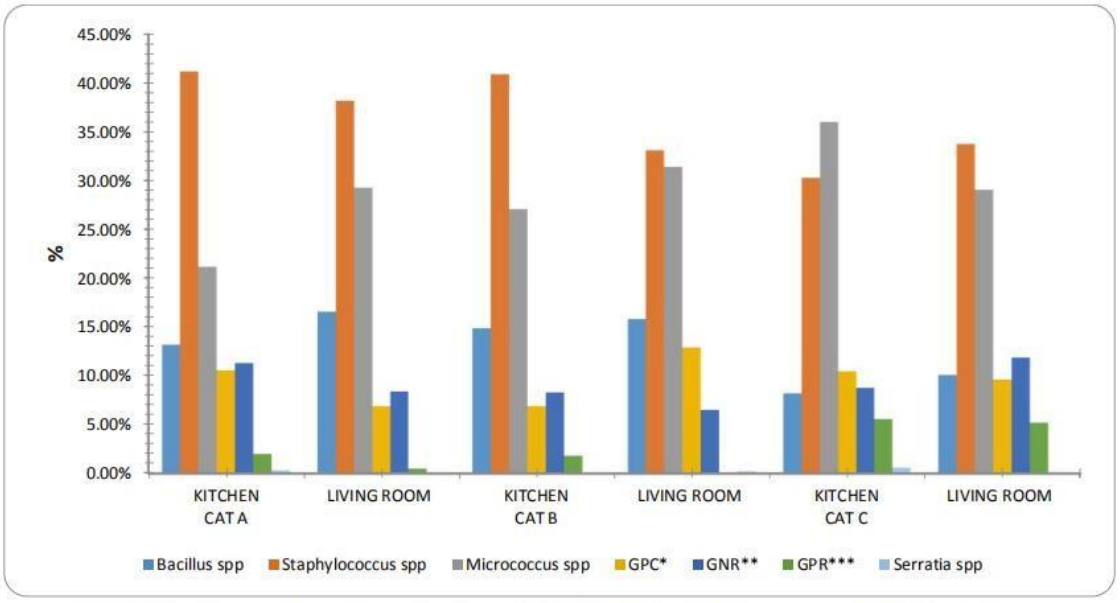


Fig. 2. Percentage of bacterial species observed in the kitchens and living rooms of different categories.

Figure 9 : (Sidra *et al.*, 2015)

According to our experimental study in outdoor areas , we have also found *Stapphylococcus spp* as the most abundant species among all the bacterial species we found .

According to (Kumer K *et al.*, 2018)“ Organisms that are often associated with the hospital acquired infection are *Staphylococcus aureus*, *Micrococcus sp.*, *Pseudomonas sp.*, *Proteus sp.*, *Aspergillus spp.*, and viruses. *Pseudomonas aeruginosa* has been particularly incriminated in nosocomial infection because of its intrinsic resistance to most antibiotics and its ability to survive and multiply at low temperatures and in disinfectant solutions .

Another research in Ebonyi State University's male and female hostel in Nigeria, bacterial isolates, the dominant species were members of the genera *Escherichia coli*(25.00% am and 25.00%pm), *Micrococcus spp*.(33.80% am and 16.20% pm) and *Klebsiella spp.* (23.65% am and 26.35%pm) for both afternoon and morning period while *Corynebacterium spp.* (6% am and 0% pm) *Pseudomonas spp.* (50.00% am and 0.00% pm) *Staphylococcus spp.* (0.00% am and 50.00% pm) (Ibiam U *et al* ., 2016)

Possible future implications of the research could include, after understanding the prevalence of different pathogens in the air, it could be compared to a public health issue. The course of transmission of the pathogen to humans could be found out in order to understand how harmful it is for the health of humans and animals. The virulence activity of pathogens could be detected too. The 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 and exposed. It is more problematic to find out the source of certain bacteria in the open-air system because bacteria and other microscopic organisms are carried by various carriers or means or systems so there is an influence of the environmental changes on the type of microbes present. This was the reason that made our research a bit difficult to conduct in outdoor environments.

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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
Dextose	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 hydrolysate	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
Peptone	10g
Beef extract	1g
Sodium chloride	5g

Lactose/Sucrose/Dextrose	5g
Phenol 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