ASSESSING BACTERIOLOGICAL CONTAMINATION OF PAPER CURRENCIES OF BANGLADESH COLLECTED FROM PUBIC PLACES OF DHAKA CITY

By

Mohammad Ullah Emon
17126005

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

DEPARTMENT OF MATHEMATICS AND NATURAL SCIENCES
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Declaration

It is hereby declared that

1. The thesis submitted is my/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.

Student's Full Name & Signature:

Mohammad Ullah Emon, 17126005

Student Full Name
Student ID

Approval

The thesis/project titled “Assessing bacteriological contamination of paper currencies of Bangladesh collected from public places of Dhaka city” submitted by

1. Mohammad Ullah Emon (ID- 17126005)

of Fall, 2022 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Science in Microbiology on 29th December, 2022

Examining Committee:

Supervisor:
(Member)

_____

Dr. Fahim Kabir Monjurul Haque
Associate professor, Mathematics & Natural Science
BRAC University

Program Coordinator:
(Member)

_____

Nadia Sultana Deen
Associate professor, Mathematics & Natural Science
BRAC University

Departmental Head:
(Chair)

_____

Professor A F M Yusuf Haider
Professor and Chairperson, Mathematics & Natural Science
BRAC University

Abstract/ Executive Summary

Paper currency is known to have bacteria on its surface that can transmit to individuals when it is exchanged from one person to another. Consequently, the current study was carried out to assess the current situation of bacterial contamination of Bangladeshi paper currency. We have divided the area of Dhaka into seven sectors and each sector had five zones: hospital, super shop, wet market, bank, and transportation. Thirty-five currency notes were collected from the places mentioned above of Dhaka and each currency notes were collected in sterile falcon tubes. After processing, the samples were cultured on selective & non-selective media. Bacteria colonies were primarily selected based on colony morphology and cultural traits. The isolates were further identified through biochemical tests. Antibiotic susceptibility tests of the isolates were performed following the Kirby-Bauer disk diffusion method. A total of 247 confirmed isolates of bacteria were selected from 35 different samples where *Escherichia coli* (22.67%) and *Vibrio cholerae* (10.12%), *Vibrio metschnikovii (9.71%),* were the predominant species, followed by *Staphylococcus delphini* (8.90%), *Shigella spp* (1.61%), *Klebsiella spp* (8.90%) and *Citrobacter* (15.38%) among 247 isolates. Among 185 gram-negative isolates, about 26.98% of species were resistant against Penicillin, 47.34% to Peptide, 20.93% against Carbapenem, and 22.72% against 4^{th} generation Cephalosporin. On the other hand, among 62 gram-positive isolates, 44.75% were resistant to Penicillin, 64.10% to Glycopeptide, about 64.55% to Monobactam, and 23.87% were resistant to Carbapenem group. Among all the detected 247 isolates, 57.89% were multidrug-resistant bacteria. Moreover, 20.97% MDR species showed beta hemolytic pattern. Multiple antibiotic-resistant pathogenic bacteria are prevalent in paper currency regardless of their sources. This study shows that contaminated paper currency could be potential carrier for disease causing and antibiotic resistant bacteria.

Keywords: Paper currency, Antibiotic susceptibility test, Multidrug resistant bacteria, Gram positive bacteria, Gram negative bacteria, Pathogenic bacteria.

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Chapter 1

Introduction

Paper currency is one of the top used and exchanged items in the world. Everyone from every economic background use this item in their daily life for many purposes. Throughout its circulation, paper currency is frequently exchanged. Human hands carry different types of bacteria and some of them are pathogenic and can cause disease to human. When a paper currency is handled by uncleaned hands it gets introduced to many bacteria and paper currency easily absorbs moisture from hand, which allows bacteria to grow on paper currency, as it continues to change hands from one person to another, every time it gets introduced to new bacteria and the amount of bacterial contamination increases. In this way, a paper currency becomes a carrier for many bacteria and those bacteria can cause disease to immune compromised person. In addition, it is now more challenging to treat many diseases due to the advent of microorganisms that are resistant to antibiotics. Paper money could be involved because contact with fomites can cause the spread of infectious illnesses. As paper currency is continuously exchanged from one person to another person it is very easy for a disease-causing organism to spread widely when human hand contacts with the surface of paper currency (Capt et al., 1408).

Droplets from coughing, sneezing, touching other infected objects or surfaces, and placing paper currency on a filthy surface can contaminate the currency. People from many different groups frequently touch paper cash when transacting (O.G. Oyero & B.O. Emikpe, 2007). The big surface area and the material that is used to make paper currency is perfect breeding ground for bacteria and it is proven (Ayandele AA & Adeniyi SA, 2011). Various study around different countries on paper currency have been done and they all almost concluded the same problem, which states that paper currency carries pathogenic bacteria and those bacteria can

easily cause disease to public health. 80% of the thirty old two-taka notes tested positive for coliform contamination in Bangladesh due to bacterial infestation (Jakir Hosen et al., 2006), 94% of the One-dollar notes from western Ohio were found to have pathogenic or potentially pathogenic bacteria (Capt et al., 1408), 97% of Pakistani currency notes were contaminated with 11 different types of bacteria (Ejaz et al., 2018), Similar research on 12 different currency notes was done in India, and it turned out that every single one of the notes was infected with microbes. *Staphylococcus aureus, Klebsiella spp. pneumonia, Pseudomonas aeruginosa,* and *Escherichia coli* were among the species that were isolated (Pradeep NV et al., 2012). It was thought that after entering the antibiotic era the mortality and morbidity rate will get low but the death rate from infectious disease increased by 58% from 1980 to 1992 (G L Armstrong et al., 1996). Now the world is facing another massive problem which is multidrug resistant bacteria, because of this problem thousands of people are dying each year.

In Bangladesh similar studies on paper currency have been done but the number of research is quite low and not many of them are recent studies. On top of that, like other countries, Bangladesh is also witnessing multidrug resistant bacteria and it became a major issue for the public health, especially after the Covid 19 pandemic. There is almost slim to none data on recent condition about contamination of paper currency of Bangladesh and paper currency is something that with the increasing population it is being used more and because of that the possibility of paper currency carrying pathogenic and multidrug resistant bacteria is increasing. The aim of this study was to look into the likelihood of bacterial contamination of Bangladesh paper currency notes and their antibiotic resistance, also investigate the possibility to find out Multidrug resistant bacteria. Results from this research will help people to be aware of possible disease-causing bacteria while handling paper currency.

Chapter 2

Methods

Location selection: This study was conducted in Dhaka district and to cover the whole district, seven sectors were selected and each sector was selected based on locations which contained five zones which were Super shop, Wet market, Hospital, Bank and Transport terminal.

Sample collection: Total 35 paper currency were sampled from total 35 zones. Samples were collected by wearing sterile latex gloves and dropped into sterile falcon tubes which were autoclaved (Allan et al., 2018). The falcon tubes were sealed immediately as soon as the paper currency was dropped and a replacement denominator was given to the individuals (Shakir et al., 2010). Within 2 hours the samples were transported to the laboratory for sample processing.

Sample identity (ID): When samples arrived to the laboratory, they were labeled according to their location and denomination and prior to processing, each sample was given a sample ID number for easier tracking.

Bacterial culture: After samples were labeled, each sample was submerged into 20ml Brain Heart Infusion (BHI) broth and agitated for 2 minutes by using vortex machine and then incubated overnight at 37℃ (Ejaz et al., 2018). After the incubation time is done each sample broth is diluted from 10^{-1} to 10^{-5} magnitude with 9ml normal saline. After the broth dilution each sample were cultured using spread plate method and for spread plating MacConkey agar (MAC), Mannitol Salt Agar (MSA), Thiosulfate-Citrate-Bile Salts-Sucrose (TCBS) agar, Xylose-Lysine-Deoxycholate (XLD) agar, Eosin methylene blue agar (EMB), Salmonella Shigella agar (SS), Nutrient Agar were used. Every single dilution was cultured and for each dilution separate media plate was used and the aim was to get single colony culture. After

spread plating all the media plates, they were incubated at 37℃ for 24 hours. After the incubation period the plated were observed for colony growth and from each plate single colonies were selected and then with a sterile loop each of the single colony were picked and sub-cultured in Nutrient agar at 37℃ for 24 hours to get pure culture. The pure cultures were then identified by biochemical tests which includes Methyl Red (MR) test, Voges-Proskauer (VP) test, Triple Sugar Iron (TSI) test, Catalase test, Oxidase test, Citrate test, Motility test, Indole test, Urea test, Gram stain test.

For the identification of different organism biochemical tests were done and the results are shown in **table 1** for better understanding and interpretation.

Table 1: Biochemical test interpretation for different organisms.

Organism	MR test	VP test	TSI test	Catalase test	Oxidase test	Citrate test	Motility test	Indole test	Urea test	Gram stain test	Morphology	Hemolysis
Klebsiella spp.	(-)	(+)	Yellow slant, Yellow butt, Gas, No h2s	(+)	(+)	(+)	Motile	(-)	(-)	(-)	Rod	Alpha/Gamma
Escherichia coli	(+)	(-)	Yellow slant, Yellow butt, Gas, No h2s	(+)	(-)	(-)	Motile	(+)	(-)	(-)	Rod	Alpha/Beta/ Gamma
Citrobacter	(+)	(-)	Yellow slant, Yellow butt, Gas, No h2s	(+)	(-)	(+)	Motile	(+)	(-)	(-)	Rod	Alpha/Beta/ Gamma
Vibrio metschnikovii	(+)	(-)	Yellow slant, Yellow butt, Gas, h2s	(+)	(-)	(+)	Motile	(-)	(-)	(+)	Cocci	Alpha/Beta/ Gamma
Enterobacter	(-)	(+)	Yellow slant, Yellow butt, Gas, No h2s	(+)	(+)	(-)	Non-motile	(-)	(-)	(-)	Rod	Alpha/Beta/ Gamma
Shigella spp.	(+)	(-)	Yellow slant, Yellow butt, No gas, h2s	(+)	(+)	(+)	Non-motile	(-)	(-)	(-)	Rod	Gamma
Vibrio cholerae	(-)	(+)	Red slant, Yellow butt, No gas, No h2s	(+)	(+)	(+)	Motile	(-)	(-)	(-)	Comma	Alpha/Beta/ Gamma
Staphylococcus delphini	(+)	(-)	Yellow slant, Yellow butt, Gas, No h2s	(+)	(+)	(-)	Non-motile	(+)	(+)	(+)	Cocci	Alpha/Beta/ Gamma
Enterococcus	(-)	(+)	Yellow slant, Yellow butt, No gas, No h2s	(+)	(+)	(+)	Non-motile	(+)	(-)	(+)	Cocci	Alpha
Streptococcus spp.	(-)	(+)	Red slant, Yellow butt, Gas, No h2s	(+)	(+)	(+)	Non-motile	(+)	(+)	(-)	Rod	Alpha/Gamma
Micrococcus flavus	(+)	(-)	Yellow skant, Yellow butt, Gas, No h2s	(+)	(+)	(+)	Non-motile	(-)	(-)	(+)	Cocci	Alpha/Beta/ Gamma

example 1:Biochemical test interpretation for different organisms.

Table 1: Biochemical test interpretation for different organisms.

Table 2: Biochemical test interpretation for different organisms.

In this table all the biochemical tests that are done in this research study is mentioned with their results. This result helps to confirm the identification of the organisms. In this table (+) means positive and (-) means negative.

Antibiotic susceptibility testing: After identifying the microorganisms, antibiotic susceptibility test was performed using Kirby-Bauer disc diffusion technique. For this process, bacterial colony from the pure culture plates were picked and suspended into sterile normal saline and the turbidity were observed and adjusted according to the McFarland 0.5 turbidity standards (Ejaz et al., 2018). Mueller Hinton agar (MHA) was used for the antibiotic susceptibility test, to perform this process a sterile cotton swab was used for the streaking of the suspension. After streaking on the Mueller Hinton agar plates, antibiotic discs were placed on the media plates and incubated at 37°C for 24 hours. Antibiotic discs were divided into two panels according to the Gram-positive and Gram-negative bacteria. Antibiotic resistance was observed against the Gram positive and Gram-negative bacteria. For Gram positive Ampicillin, Cloxacillin, Penicillin, Amikacin, Gentamycin, Cefepime, Ciprofloxacin, Imipenem, Meropenem, Colistin, Linezolid, Vancomycin, Tigecycline, Aztreonam, TazobacPiperacilin, Azithromycin, Erythromycin antibiotic were used. For Gram negative Ampicillin, Cloxacillin, Penicillin, Amikacin, Gentamycin, Cefepime, Ciprofloxacin, Imipenem, Meropenem, Colistin, TazobacPiperacilin, Azithromycin, Erythromycin antibiotic were used.

The Clinical and Laboratory Standards Institute (CLSI) criteria were followed for interpreting the zones of inhibition, which were measured in millimeters. After measuring the inhibition area of organism against antibiotic Multidrug resistance organisms were identified, in order to characterize acquired resistance profiles in multidrug-resistant organisms, the term "multidrug resistance" was developed in accordance with international terminology standards. This nomenclature was developed by a team of international specialists under the combined effort

of the European Center for Disease Control and the Center for Disease Control. Total 143 multidrug resistant (MDR) organisms were found. The MDR organisms were then labeled and pathogenicity test were done on these multidrug resistant organisms and Alpha, Beta, Gamma hemolysis were found.

Chapter 3

Results

For this research, Dhaka district was divided into seven sectors and each sector has five zones which included Super shop, Wet market, Hospital, Bank and Transport terminal. From each zone one sample was collected, which sums up into total 35 samples. **Figure 1** shows the seven sectors in different colours according to their sector numbers from where samples were collected and each of the zones are colour coded, which is mentioned at the bottom of the figure.

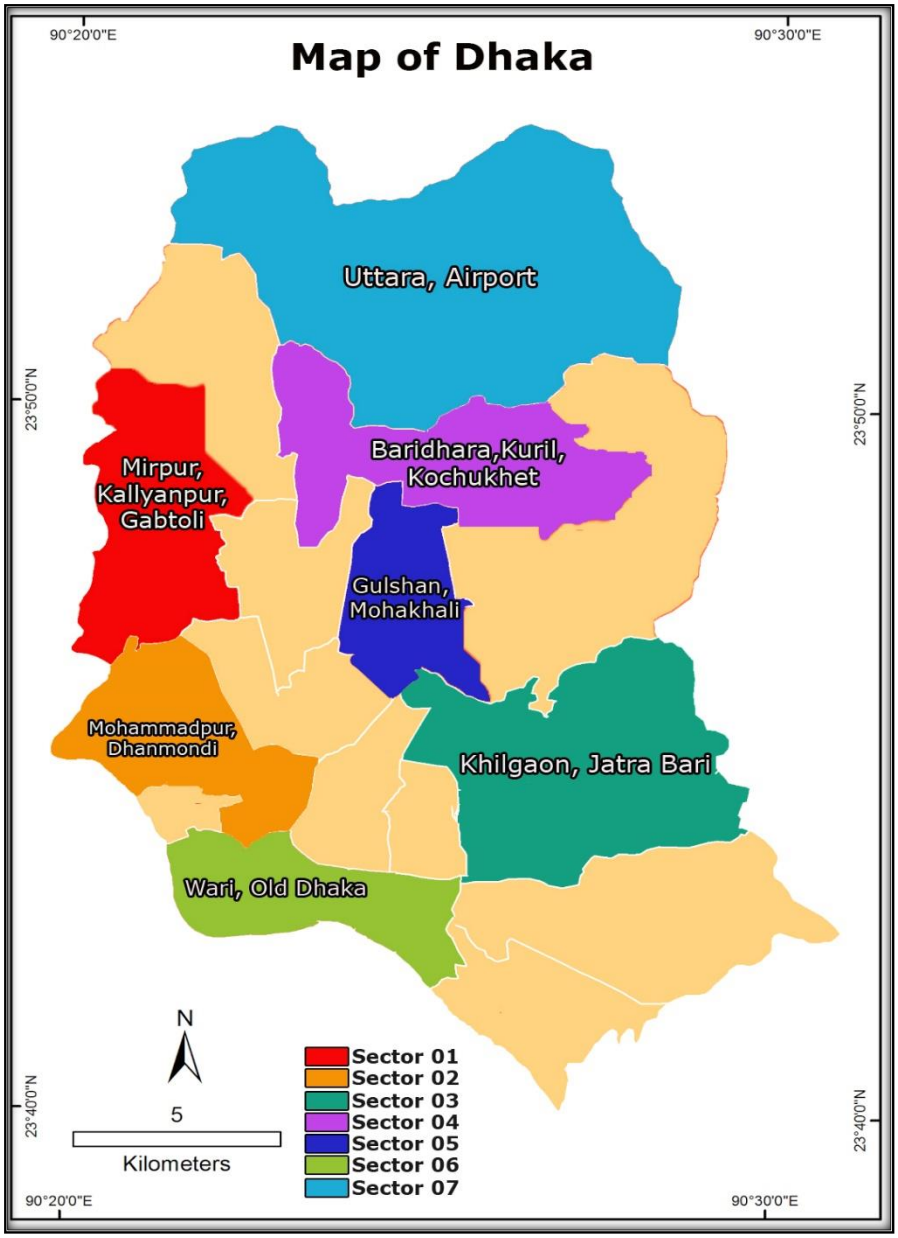

Figure 1: Sampling areas, Dhaka district. Seven sample collection sectors are colour coded.

After collecting the samples, they were cultured in selective and non-selective media at 37℃ for overnight and then after getting the growth of the organisms and selecting single colony from the culture growth, biochemical tests were done and 11 different organisms were identified, which were *Klebsiella spp., Escherichia coli, Staphylococcus delphini spp., Vibrio cholerae spp., Enterococcus, Citrobacter, Streptococcus spp., Micrococcus flavus, Vibrio metschnikovii spp., Enterobacter, Shigella spp.*.

Based on the identified organisms, a data table was established where the number of the bacteria contaminated samples are organized and this table is also based on area from where the samples were collected. In the **table 1(a),** the bacteria contaminated sample number and their collection areas are mentioned, also the identified organisms are also mentioned.

Table 1(a): Area based isolated bacteria.

Organism name	**Sector 01** (Kallyanpur, Mirpur, Gabtoli)	**Sector 02** (Dhanmondi, Mohammadpur)	**Sector 03** (Khilgaon, Jatra Bari)	**Sector 04** (Kuril, Baridhara, DOHS, Kochukhet)	**Sector 05** (Mohakhali, Gulshan)	**Sector 06** (Wari, Old Dhaka)	**Sector 07** (Uttara, Airport)	**Total**
Klebsiella spp.	04	01	04	02	00	03	02	**16**
Escherichia coli	04	04	04	03	04	01	03	**23**
Staphylococcus delphini	02	01	04	01	04	03	00	**15**
Vibrio cholerae	02	02	03	00	03	03	02	**15**
Enterococcus	01	00	00	00	00	00	00	**01**
Citrobacter	04	04	04	04	01	04	02	**23**
Streptococcus spp.	04	01	00	01	00	00	00	**06**
Micrococcus flavus	02	04	02	03	04	03	03	**21**
Vibrio metschnikovii	02	02	03	02	04	02	01	**16**
Enterobacter	00	03	01	03	00	00	01	**08**
Shigella spp.	00	00	01	00	01	00	01	**03**

In the **table 1(a),** contaminated sample numbers according to their sectors and areas are mentioned and at the very end of each row the total number of the contaminated samples are shown. Each sectors had five sample collecting areas and the total samples were 35 from seven sectors in total.

For the identification of different organism biochemical tests were done and the results are shown in **table 1** for better understanding and interpretation.

After identifying the organisms, 247 isolates were randomly selected based on their colony morphology. In **figure 2** the percentage of identified organisms are shown.

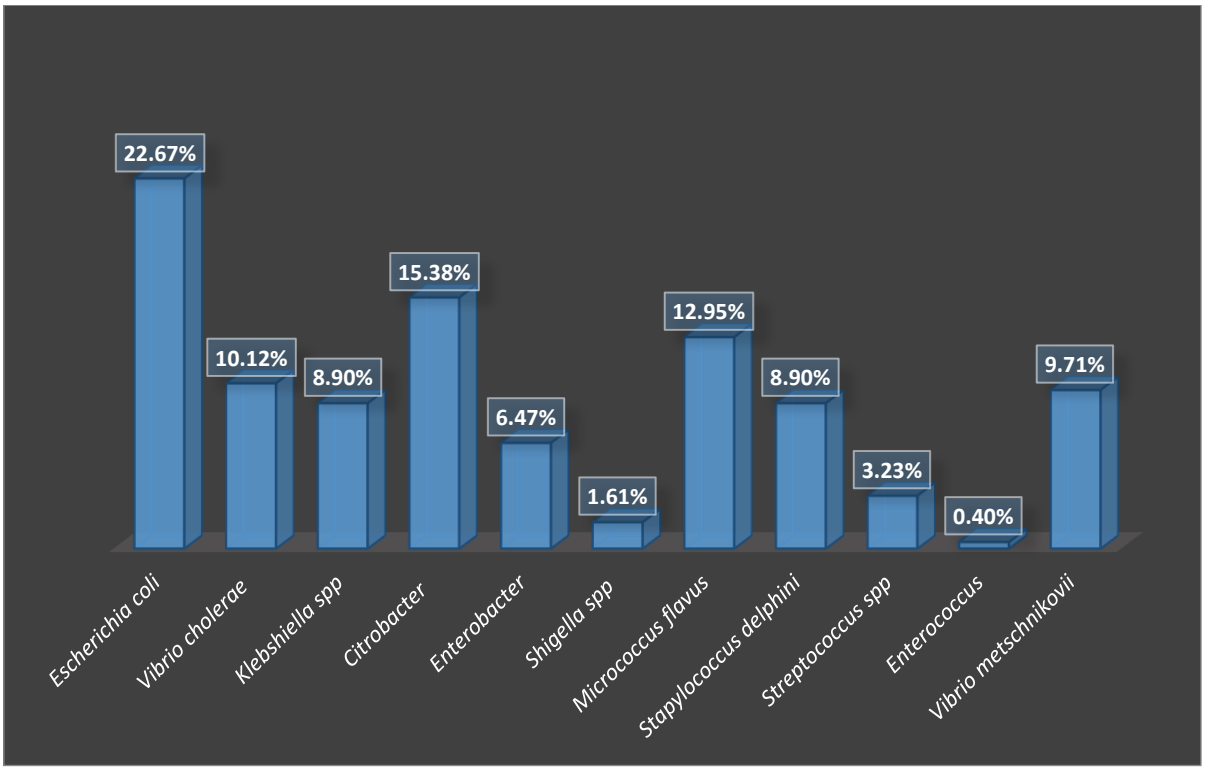

Figure 2: Percentage of selected bacteria isolates.

Based on the 35 samples and 247 isolates the identified organism percentage are *Escherichia coli* (22.67%), *Klebsiella spp.* (8.90%), *Staphylococcus delphini.* (8.90%), *Vibrio cholerae.* (10.12%), *Vibrio metschnikovii (9.71%), Enterococcus* (0.40%), *Citrobacter* (15.38%), *Streptococcus spp.* (3.23%), *Micrococcus flavus* (12.95%), *Enterobacter* (6.47%), *Shigella spp.* (1.61%). The isolates were selected based on the previous researches done on similar topic and paper currency studies.

After identifying the organisms, they were separated into two groups based on Gam-positive and Gram-negative organism. Antibiotics were selected based on Gram-positive and Gram-negative groups to perform antibiotic susceptibility test. After completing the antibiotic susceptibility test, data was collected after measuring the diameter of zone of inhibition and based on these data sensitive, intermediate and resistant was identified. All the collected data on antibiotic susceptibility testing are shown in **Table 2** and **Table 3**. In the **Table 2**, data on antibiotic susceptibility of Gram-negative organisms are shown and in the **Table 3** data on antibiotic susceptibility of Gram-positive organism are shown.

Table 2: Antibiotic susceptibility profile of Gram-negative bacteria

Antibiotic group	Antibiotic	Gram Negative microorganism. No.(%) isolates.																				
		Klebsiella spp. (N=22)			*Escherichia coli* (N=56)			*Citrobacter* (N=38)			*Vibrio metschnikovii* (N=24)			*Enterobacter* (N=16)			*Shigella spp.* (N=4)			*Vibrio cholerae* (N=25)		
		S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
Penicillin	Ampicillin	8 (36.36)	1 (4.54)	13 (59.09)	19 (33.92)	2 (3.57)	35 (62.5)	15 (39.47)	7 (18.42)	16 (42.10)	6 (25)	6 (25)	12 (50)	0	2 (12.5)	14 (87.5)	1 (25)	0	3 (75)	16 (64)	2 (8)	7 (28)
	Penicillin	12 (54.54)	4 (18.18)	6 (27.27)	39 (69.64)	7 (12.5)	10 (17.85)	21 (55.26)	6 (15.78)	11 (28.94)	15 (62.5)	5 (20.83)	4 (16.66)	-	-	-	-	-	-	19 (76)	0	6 (24)
Aminoglycoside	Amikacin	12 (54.54)	4 (18.18)	6 (27.27)	29 (51.78)	9 (16.07)	18 (32.14)	15 (39.47)	8 (21.05)	15 (39.47)	16 (66.66)	4 (16.66)	4 (16.66)	6 (37.5)	5 (31.25)	5 (31.25)	4 (100)	0	0	9 (36)	5 (20)	11 (44)
	Gentamycin	11 (50)	3 (13.63)	8 (36.36)	34 (60.71)	2 (3.57)	20 (35.71)	20 (52.63)	8 (21.05)	10 (26.31)	19 (79.16)	3 (12.5)	2 (8.33)	9 (56.25)	3 (18.75)	4 (25)	4 (100)	0	0	12 (48)	3 (12)	10 (40)
Cephalosporine	Cefepime	13 (59.09)	3 (13.63)	6 (27.27)	31 (55.35)	6 (10.71)	19 (33.92)	22 (57.89)	5 (13.15)	11 (28.94)	18 (75)	4 (16.66)	2 (8.33)	10 (62.5)	0	6 (37.5)	4 (100)	0	0	11 (44)	5 (20)	9 (36)
Fluoroquinolones	Ciprofloxacin	15 (68.18)	2 (9.09)	5 (22.72)	28 (50)	11 (19.64)	17 (30.35)	16 (42.10)	11 (28.94)	11 (28.94)	20 (83.33)	3 (12.5)	1 (4.16)	10 (62.5)	4 (25)	2 (12.5)	4 (100)	0	0	14 (56)	6 (24)	5 (20)
Carbapenem	Imipenem	17 (77.27)	1 (4.54)	4 (18.18)	39 (69.64)	11 (19.64)	6 (10.71)	27 (71.05)	4 (10.52)	7 (18.42)	20 (83.33)	2 (8.33)	2 (8.33)	12 (75)	4 (25)	0	4 (100)	0	0	24 (96)	0	1 (4)
	Meropenem	17 (77.27)	1 (4.54)	4 (18.18)	30 (53.57)	11 (19.64)	15 (26.78)	32 (84.21)	2 (5.26)	4 (10.52)	18 (75)	2 (8.33)	4 (16.66)	13 (81.25)	1 (6.25)	2 (12.5)	4 (100)	0	0	16 (64)	2 (8)	7 (28)
Peptide	Colistin	9 (40.90)	0	13 (59.09)	22 (39.28)	0	34 (60.71)	10 (26.31)	0	28 (73.68)	15 (62.5)	0	9 (37.5)	5 (31.25)	0	11 (68.75)	4 (100)	0	0	8 (32)	0	17 (68)
Tazobactam + Piperacillin	TazobacPiperacilin	10 (45.45)	6 (27.27)	6 (27.27)	26 (46.42)	9 (16.07)	21	16 (42.10)	6 (15.78)	16 (42.10)	16 (66.66)	2 (8.33)	6 (25)	8 (50)	6 (37.5)	2 (12.5)	4 (100)	0	0	14 (56)	4 (16)	7 (28)
Macrolide	Azithromycin	7 (31.81)	1 (4.54)	14 (63.63)	18 (32.14)	9 (16.07)	29 (51.78)	10 (26.31)	4 (10.52)	24 (63.15)	7 (29.16)	5 (20.83)	12 (50)	4 (25)	2 (12.5)	10 (62.5)	0	2 (50)	2 (50)	8 (32)	4 (16)	13 (52)
	Erythromycin	13 (59.09)	3 (13.63)	6 (27.27)	43 (76.78)	6 (10.71)	7 (12.5)	23 (60.52)	9 (23.68)	6 (15.78)	21 (87.5)	2 (8.33)	1 (4.16)	-	-	-	-	-	-	17 (68)	5 (20)	3 (12)

This table explains the total number of each gram-negative organism **(N)** and their susceptibility profile and their percentage **No. (%),** against specific antibiotic groups. It is important to mention that, in the table by S, I, R it means **sensitive, intermediate, resistant** and by (-) means the antibiotic was not used because in that group only one type of antibiotic was used.

Table 3: Antibiotic susceptibility profile of Gram-positive bacteria

Antibiotic group	Antibiotic	Gram Positive microorganism. No.(%) isolates.											
		Staphylococcus delphini (N=22)			*Enterococcus* (N=1)			*Streptococcus spp.* (N=8)			*Micrococcus flavus* (N=32)		
		S	I	R	S	I	R	S	I	R	S	I	R
Penicillin	Ampicillin	-	-	-	0	0	1 (100)	4 (50)	0	4 (50)	-	-	-
	Cloxacillin	6 (27.27)	0	16 (72.72)	1 (100)	0	0	3 (37.5)	2 (25)	3 (37.5)	9 (28.12)	0	23 (71.87)
	Penicillin	1 (4.54)	0	21 (95.45)	1 (100)	0	0	5 (62.5)	0	3 (37.5)	4 (12.5)	0	28 (87.5)
Aminoglycoside	Amikacin	-	-	-	0	1 (100)	0	5 (62.5)	3 (37.5)	0	24 (75)	8 (25)	0
	Gentamycin	18 (81.81)	4 (18.18)	0	1 (100)	0	0	5 (62.5)	3 (37.5)	0	21 (65.62)	11 (34.37)	0
Cephalosporine	Cefepime	14 (63.63)	3 (13.63)	5 (22.72)	1 (100)	0	0	4 (50)	2 (25)	2 (25)	12 (37.5)	1 (3.12)	19 (59.37)
Fluoroquinolones	Ciprofloxacin	19 (86.36)	3 (13.63)	0	1 (100)	0	0	5 (62.5)	3 (37.5)	0	25 (78.12)	7 (21.87)	0
Carbapenem	Imipenem	20 (90.90)	2 (9.09)	0	0	0	1 (100)	7 (87.5)	0	1 (12.5)	32 (100)	0	0
	Meropenem	13 (59.09)	0	9 (40.90)	0	0	1 (100)	3 (37.5)	2 (25)	3 (37.5)	24 (75)	0	8 (25)
Oxazolidinone	Linezolid	6 (27.27)	0	16 (72.72)	1 (100)	0	0	5 (62.5)	0	3 (37.5)	8 (25)	0	24 (75)
Glycopeptide	Vancomycin	4 (18.18)	0	18 (81.81)	0	1 (100)	0	6 (75)	0	2 (25)	7 (21.87)	0	25 (78.12)
Tigecycline	Tigecycline	12 (54.54)	8 (36.36)	2 (9.09)	1 (100)	0	0	8 (100)	0	0	21 (65.62)	8 (25)	3 (9.37)
Tazobactam + Piperacillin	TazobacPiperacilin	14 (63.63)	8 (36.36)	0	0	1 (100)	0	6 (75)	2 (25)	0	27 (84.37)	5 (15.62)	0
Macrolide	Azithromycin	-	-	-	0	0	1 (100)	4 (50)	0	4 (50)	-	-	-
	Erythromycin	0	1 (4.54)	21 (95.45)	1 (100)	0	0	6 (75)	0	2 (25)	3 (9.37)	2 (6.25)	27 (84.37)

According to this table, each gram-positive organism has a total number (N), a sensitivity profile, and a percentage (%) of them that are resistant to various antibiotic groups. It is

important to mention that, in the table by S, I, R it means **sensitive, intermediate, resistant** and by (-) means the antibiotic was not used because in that group only one type of antibiotic was used.

Total sample number was 35 and all of the 35 sample currency notes showed bacteria growth. Among the bacteria growth samples, there were sensitive, intermediate, and resistant against the antibiotic. The percentage of antibiotic susceptibility is displayed in **figures 3** and **figure 4**.

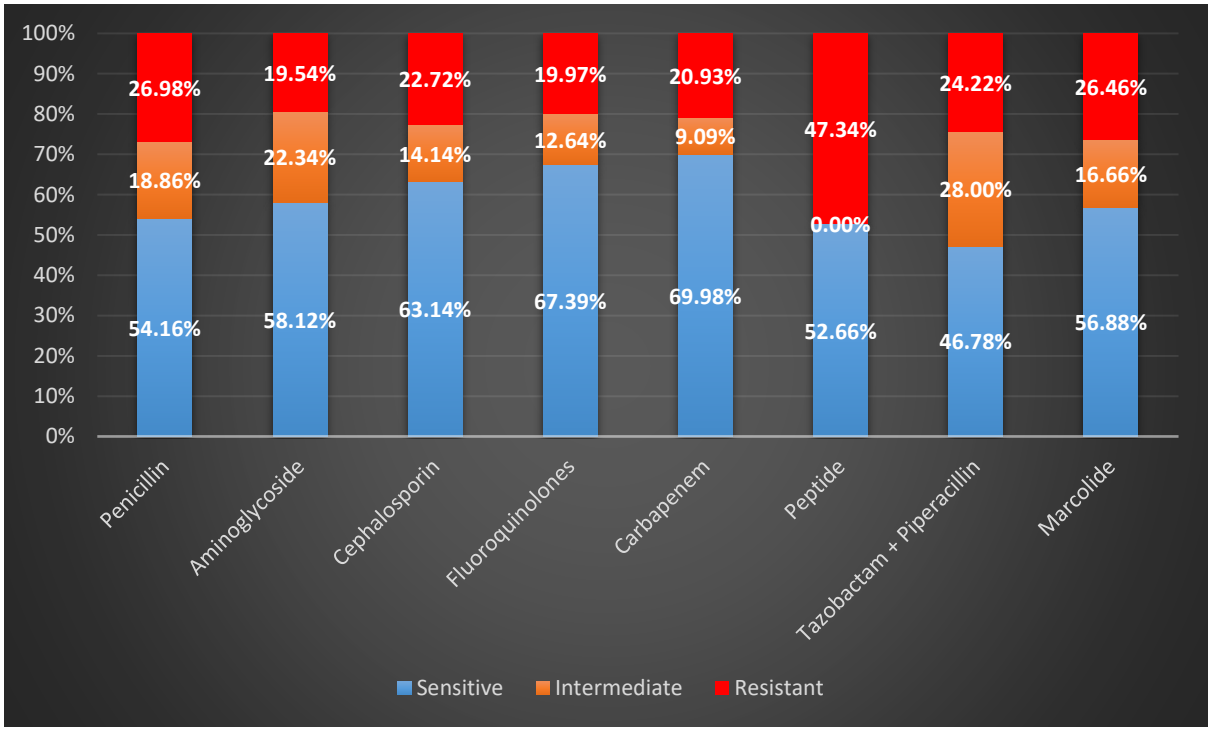

Figure 3: Antibiotic susceptibility profile of Gram-negative bacteria

In the **figure 3**, antibiogram results showed that gram-negative organisms are resistant against many antibiotics and it is indicated in red colour. This figure shows that, antibiotic resistance in the Penicillin group is (26.98%), Aminoglycoside group is (19.54%), Cephalosporin group (22.72%), Fluoroquinolones group (19.97%), Carbapenem group (20.93%), Peptide group

(47.34%), Tazobactam + Piperacillin (24.22%), Macrolide group (26.46%). With the help of the percentage data, it is fairly clear that high percentage of gram-negative bacteria are resistant against antibiotics.

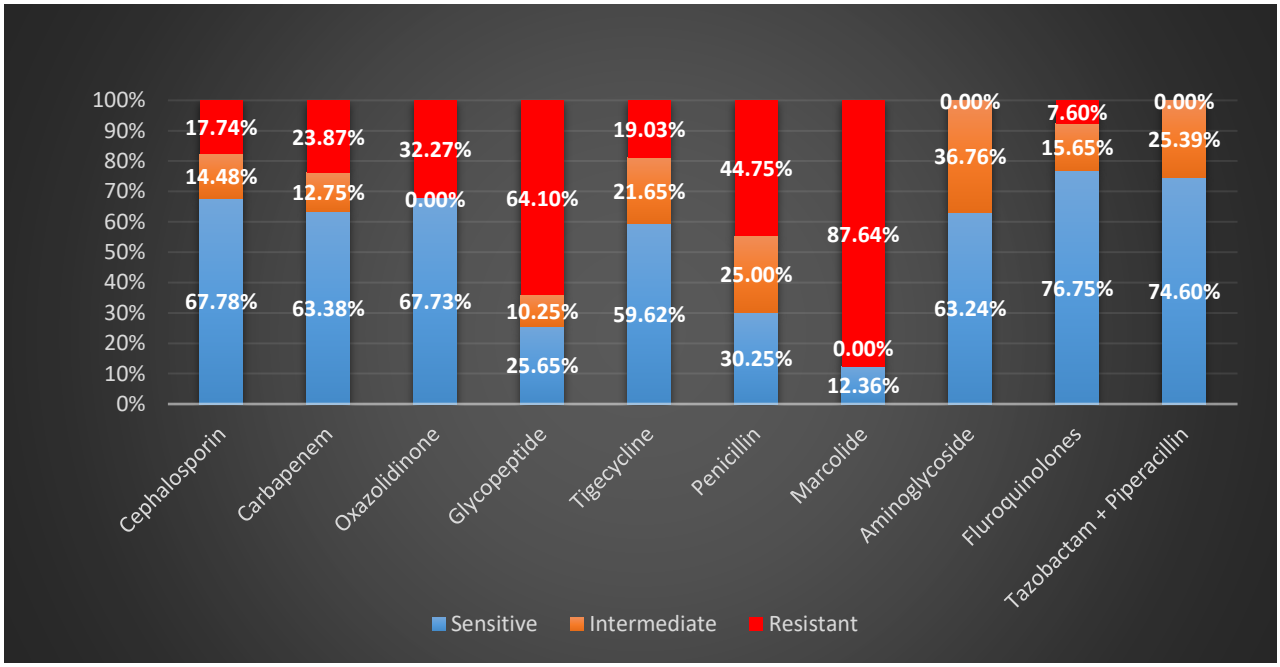

Figure 4: Antibiotic susceptibility profile of Gram-positive bacteria.

Figure 4 displays the antibiogram findings, which are highlighted in red, to illustrate that gram-positive organisms are antibiotic-resistant to all of them. This figure shows that, antibiotic resistance in the Cephalosporine group is (17.74%), Carbapenem is (23.87%), Oxazolidinone is (32.27%), Glycopeptide is (64.10%), Tigecycline is (19.03%), Penicillin is (44.75%), Macrolide is (87.64%), Aminoglycoside is (0%), Fluoroquinolones is (7.60%), Tazobactam + Piperacillin is (0%). The percentage data makes it reasonably obvious that a significant portion of gram-positive bacteria are resistant to practically all antibiotic groups.

With the antibiotic susceptibility data, Multidrug resistant organisms were identified. It is known that, antimicrobial resistance to at least one antimicrobial drug in three or more antimicrobial categories is known as multiple drug resistance (MDR) or multidrug resistance

(Magiorakos et al., 2012). Based on the data total 143 multidrug resistant (MDR) bacteria was identified. The total number of each bacterium and their percentage are indicated in **table 4**.

Table 4: Multidrug resistant bacteria profile.

Organism name	MDR (Total 143)
	n (%)
Klebsiella spp.	**11** (7.69%)
Escherichia coli	**31** (21.67%)
Staphylococcus delphini	**17** (11.88%)
Vibrio cholerae	**12** (8.39%)
Enterococcus	**01** (0.69%)
Citrobacter	**18** (12.58%)
Streptococcus spp.	**06** (4.19%)
Micrococcus flavus	**27** (18.88%)
Vibrio metschnikovii	**11** (7.69%)
Enterobacter	**08** (5.59%)
Shigella spp.	**01** (0.69%)

After gathering the multidrug resistance data, pathogenicity test was done by conducting DNase test then Hemolysis test to observe and determine whether MDR organisms were harmful or not. After getting the hemolysis results it was observed that **alpha hemolysis**, **beta hemolysis** and **gamma hemolysis** was present. Hemolysis profiling with DNase positive and negative findings are displayed in **table 5**.

Table 5: Hemolysis and DNase pattern of the isolates.

Sector No.	Area name	Alpha hemolysis				Beta hemolysis				Gamma hemolysis			
		Sample code	Organism name	DNase test	Total No.	Sample code	Organism name	DNase test	Total No.	Sample code	Organism name	DNase test	Total No.
Sector 01	Kallyanpur, Mirpur, Gabtoli.	Sample_006	*Klebshiella spp.*	Negative	**05**	Sample_020	*Escherichia coli*	Negative	**05**	Sample_007	*Micrococcus flavus*	Negative	**14**
		Sample_011	*Streptococcus spp.*	Negative		Sample_212	*Escherichia coli*	Positive		Sample_008	*Staphylococcus delphini*	Positive	
		Sample_014	*Enterococcus*	Negative		Sample_213	*Citobacter*	Positive		Sample_009	*Micrococcus flavus*	Negative	
		Sample_018	*Streptococcus spp.*	Negative		Sample_218	*Staphylococcus delphini*	Positive		Sample_010	*Streptococcus spp.*	Negative	
		Sample_223	*Vibrio cholerae*	Positive		Sample_221	*Staphylococcus delphini*	Positive		Sample_012	*Vibrio cholerae*	Negative	
										Sample_013	*Vibrio cholerae*	Negative	
										Sample_016	*Escherichia coli*	Negative	
										Sample_023	*Streptococcus spp.*	Negative	
										Sample_027	*Streptococcus spp.*	Negative	
										Sample_028	*Vibrio metschnikovii*	Positive	
										Sample_030	*Citobacter*	Negative	
										Sample_192	*Escherichia coli*	Positive	
										Sample_234	*Escherichia coli*	Positive	
										Sample_235	*Escherichia coli*	Positive	
Sector 02	Dhanmondi, Mohammadpur	Sample_061	*Citobacter*	Negative	**05**	Sample_070	*Enterobacter*	Negative	**02**	Sample_064	*Citobacter*	Negative	**09**
		Sample_072	*Escherichia coli*	Positive		Sample_184	*Micrococcus flavus*	Positive		Sample_065	*Citobacter*	Positive	
		Sample_073	*Enterobacter*	Positive						Sample_066	*Enterobacter*	Positive	
		Sample_196	*Escherichia coli*	Positive						Sample_074	*Escherichia coli*	Negative	
		Sample_198	*Citobacter*	Positive						Sample_075	*Escherichia coli*	Positive	
										Sample_076	*Enterobacter*	Negative	
										Sample_079	*Micrococcus flavus*	Positive	
										Sample_083	*Micrococcus flavus*	Negative	
										Sample_190	*Escherichia coli*	Positive	
Sector 03	Khilgaon, Jatra Bari.	Sample_118	*Klebshiella spp.*	Negative	**10**	Sample_122	*Escherichia coli*	Negative	**04**	Sample_115	*Citobacter*	Negative	**15**
		Sample_119	*Escherichia coli*	Negative		Sample_136	*Escherichia coli*	Positive		Sample_116	*Vibrio metschnikovii*	Positive	
		Sample_126	*Vibrio cholerae*	Negative		Sample_137	*Staphylococcus delphini*	Positive		Sample_120	*Escherichia coli*	Negative	
		Sample_129	*Citobacter*	Positive		Sample_231	*Citobacter*	Positive		Sample_121	*Escherichia coli*	Negative	
		Sample_131	*Vibrio cholerae*	Positive						Sample_123	*Escherichia coli*	Negative	
		Sample_138	*Micrococcus flavus*	Positive						Sample_127	*Klebshiella spp.*	Positive	
		Sample_139	*Staphylococcus delphini*	Positive						Sample_128	*Klebshiella spp.*	Positive	
		Sample_195	*Escherichia coli*	Positive						Sample_132	*Klebshiella spp.*	Positive	
		Sample_217	*Micrococcus flavus*	Positive						Sample_133	*Escherichia coli*	Positive	
		Sample_220	*Staphylococcus delphini*	Positive						Sample_135	*Enterobacter*	Positive	
										Sample_193	*Citobacter*	Positive	
										Sample_201	*Klebshiella spp.*	Positive	
										Sample_203	*Vibrio metschnikovii*	Positive	
										Sample_226	*Escherichia coli*	Positive	
										Sample_228	*Vibrio metschnikovii*	Positive	
Sector 04	Kuril, Baridhara, DOHS, Kochukhet	Sample_088	*Staphylococcus delphini*	Negative	**04**	Sample_091	*Micrococcus flavus*	Positive	**03**	Sample_086	*Micrococcus flavus*	Negative	**12**
		Sample_090	*Micrococcus flavus*	Positive		Sample_098	*Escherichia coli*	Negative		Sample_087	*Micrococcus flavus*	Negative	
		Sample_099	*Citobacter*	Positive		Sample_106	*Enterobacter*	Negative		Sample_089	*Staphylococcus delphini*	Negative	
		Sample_103	*Citobacter*	Negative						Sample_092	*Streptococcus spp.*	Negative	
										Sample_093	*Enterobacter*	Negative	
										Sample_095	*Escherichia coli*	Negative	
										Sample_096	*Enterobacter*	Negative	
										Sample_100	*Escherichia coli*	Negative	
										Sample_109	*Vibrio metschnikovii*	Negative	
										Sample_110	*Escherichia coli*	Negative	
										Sample_111	*Vibrio metschnikovii*	Negative	
										Sample_187	*Micrococcus flavus*	Positive	
Sector 05	Mohakhali, Gulshan	Sample_035	*Staphylococcus delphini*	Positive	**08**	Sample_043	*Vibrio cholerae*	Negative	**04**	Sample_033	*Staphylococcus delphini*	Negative	**13**
		Sample_036	*Staphylococcus delphini*	Negative		Sample_047	*Vibrio metschnikovii*	Positive		Sample_034	*Micrococcus flavus*	Negative	
		Sample_040	*Staphylococcus delphini*	Negative		Sample_214	*Staphylococcus delphini*	Positive		Sample_038	*Micrococcus flavus*	Negative	
		Sample_054	*Escherichia coli*	Negative		Sample_219	*Micrococcus flavus*	Positive		Sample_039	*Micrococcus flavus*	Negative	
		Sample_055	*Vibrio metschnikovii*	Negative						Sample_042	*Escherichia coli*	Negative	
		Sample_236	*Escherichia coli*	Positive						Sample_044	*Vibrio cholerae*	Positive	
		Sample_239	*Citobacter*	Positive						Sample_046	*Escherichia coli*	Positive	
		Sample_245	*Vibrio cholerae*	Positive						Sample_215	*Staphylococcus delphini*	Positive	
										Sample_230	*Vibrio metschnikovii*	Positive	
										Sample_241	*Vibrio cholerae*	Positive	
										Sample_242	*Vibrio cholerae*	Positive	
										Sample_246	*Micrococcus flavus*	Positive	
										Sample_248	*Micrococcus flavus*	Positive	
Sector 06	Wari, Old Dhaka	Sample_224	*Citobacter*	Positive	**04**	Sample_164	*Micrococcus flavus*	Positive	**06**	Sample_171	*Citobacter*	Positive	**08**
		Sample_225	*Citobacter*	Positive		Sample_165	*Staphylococcus delphini*	Positive		Sample_176	*Klebshiella spp.*	Positive	
		Sample_237	*Vibrio metschnikovii*	Positive		Sample_166	*Micrococcus flavus*	Positive		Sample_177	*Klebshiella spp.*	Positive	
		Sample_244	*Klebshiella spp.*	Positive		Sample_167	*Staphylococcus delphini*	Positive		Sample_179	*Vibrio metschnikovii*	Positive	
						Sample_170	*Vibrio cholerae*	Positive		Sample_181	*Escherichia coli*	Positive	
						Sample_222	*Micrococcus flavus*	Positive		Sample_216	*Staphylococcus delphini*	Positive	
										Sample_233	*Vibrio cholerae*	Positive	
										Sample_240	*Klebshiella spp.*	Positive	
Sector 07	Uttara, Airport	Nil	Nil		**00**	Sample_141	*Micrococcus flavus*	Positive	**06**	Sample_150	*Citobacter*	Positive	**06**
						Sample_142	*Micrococcus flavus*	Positive		Sample_151	*Escherichia coli*	Positive	
						Sample_143	*Micrococcus flavus*	Positive		Sample_152	*Escherichia coli*	Positive	
						Sample_144	*Micrococcus flavus*	Positive		Sample_156	*Citobacter*	Positive	
						Sample_145	*Micrococcus flavus*	Positive		Sample_157	*Klebshiella spp.*	Positive	
						Sample_146	*Micrococcus flavus*	Positive		Sample_161	*Shigella spp.*	Positive	

According to the **table 5**, out of total 143 multidrug resistant bacterial hemolysis total alpha hemolysis was 36 (25.17%), beta hemolysis was 30 (20.97%) and gamma hemolysis was 77 (53.84%). In **figure 5**, the percentage of the hemolysis is shown.

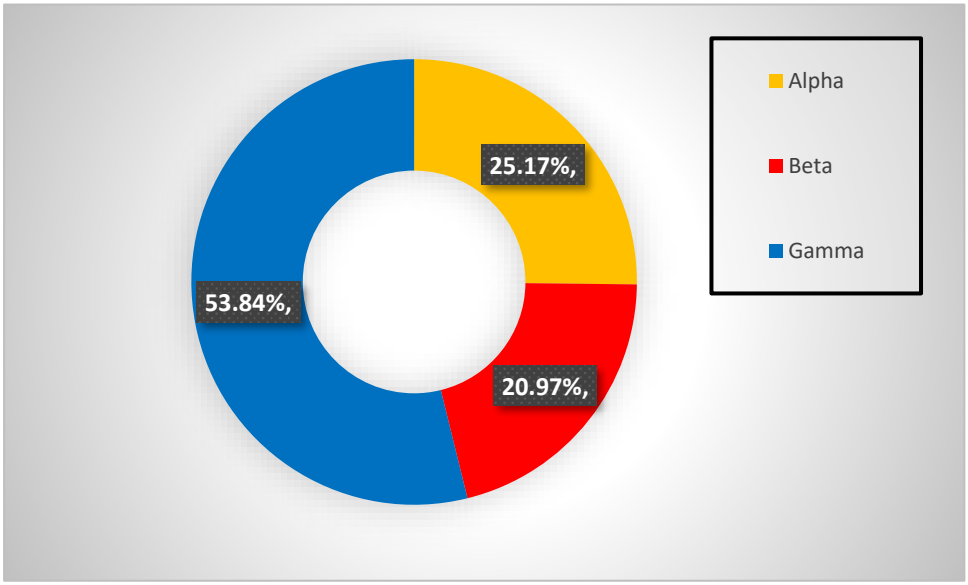

Figure 5: Hemolysis test data of Multidrug resistant bacteria.

Figure 5, Beta hemolysis, alpha hemolysis, and gamma hemolysis are each depicted in different colours: yellow for alpha hemolysis, red for beta hemolysis, and blue for gamma hemolysis.

In **table 6,** the hemolysis pattern of each bacterium is shown.

Table 6: Hemolysis pattern of multidrug resistant organism.

Organism name		Hemolysis pattern		
		Alpha hemolysis	**Beta hemolysis**	**Gamma hemolysis**
Gram Negative	*Klebsiella spp.*	03	00	08
	Escherichia coli	06	05	20
	Citrobacter	08	02	08
	Vibrio metschnikovii	02	01	08
	Enterobacter	01	02	05
	Shigella spp.	00	00	01
	Vibrio cholerae	04	02	06
Gram Positive	*Staphylococcus delphini*	06	06	05
	Enterococcus	01	00	00
	Streptococcus spp.	02	00	04
	Micrococcus flavus	03	12	12

In this table, the total number of **alpha hemolysis** is 36, **beta hemolysis** is 30 and **gamma hemolysis** is 77 out of 143 multidrug resistant organisms. In **table 5,** hemolysis and DNase result of all the **MDR** organisms are shown but for better understanding **table 6,** shows hemolysis number of each bacterium.

Chapter 4

Discussion

Paper currency is one of the most exchanged items in the world and in Bangladesh people from every economic background use this item in their day-to-day life multiple times in a day. This research on paper currency revealed that contaminated paper currency carries many disease causing and multidrug resistant bacteria and because of this reason paper currency can easily become a carrier for contagious bacteria which has high possibility to spread disease into broad spectrum of people within a matter of time. The findings of this study showed different types

of bacteria is found in paper currency and after doing biochemical tests 11 different types of bacteria is identified(Ejaz et al., 2018). Antibiotic susceptibility test confirmed the antibiotic resistance of the isolates, which further helped to determine the multidrug resistant bacteria (Magiorakos et al., 2012). Total 143 multidrug resistant bacteria is found and pathogenicity tests explained more about the condition of the MDR bacteria and helped to determine the percentage of alpha, beta and gamma hemolysis, which is a key information for the future research on the paper currency of Bangladesh.

In this study, samples were collected from seven different sectors which covered Dhaka city and sectors are shown in different colours in ***figure 1.*** This study revealed that all the collected samples were contaminated and the sample details are explained in the ***table 1(a)***, total 247 random isolates were selected and similar isolate selection can be seen in different research paper on paper currency(Boidya et al., 2015; Shakir et al., 2010). In this study *Escherichia coli* (22.67%), *Klebsiella spp.* (8.90%), *Staphylococcus delphini.* (8.90%), *Vibrio cholerae.* (10.12%), *Vibrio metschnikovii (9.71%), Enterococcus* (0.40%), *Citrobacter* (15.38%), *Streptococcus spp.* (3.23%), *Micrococcus flavus* (12.95%), *Enterobacter* (6.47%), *Shigella spp.* (1.61%) is found, similar identification process was seen in different research study on paper currency(Boidya et al., 2015; Capt et al., 1408; Pradeep NV et al., 2012; Shakir et al., 2010), bacteria percentages are related with the antibiotic susceptibility profile which is shown in ***table 2*** and ***table 3*** which shows the **gram-negative** and **gram-positive** bacteria antibiotic susceptibility test results. With this data antibiotic susceptibility group profile is shown in ***figure 3*** and ***figure 4***, here it is seen that in gram-negative bacteria, antibiotic resistance in Peptide group (47.34%) is highest and in gram positive bacteria, antibiotic resistance in Macrolide group (87.64%) is highest. Based on these data MDR profile was established (Magiorakos et al., 2012), which is shown in ***table 4***, in which *Escherichia coli 21.67%* shows the highest resistance. Total 143 MDR isolates were identified and based on this data

pathogenicity test (Hemolysis test and DNase test) is done and shown in ***table 5***, in which beta hemolysis is found 30 (20.97%), which is alarming. In this research pathogenicity test is done only in MDR bacteria. In the MDR profile it is seen that in alpha hemolysis ***Citrobacter*** is highest, in beta hemolysis ***Micrococcus flavus*** is highest and in gamma hemolysis ***Escherichia coli*** is highest.

In this study on paper currency, all these data indicates that contaminated paper currency can be a dangerous source for disease causing bacteria. Research on paper currency done in Bangladesh and other countries reflects same conclusion that paper currency carries disease causing bacteria and a very similar study was done in Bangladesh which stated similar statement and showed the source of different disease-causing bacteria transmission was paper currency (Shakir et al., 2010). In this current study all the data shows that when paper currency is transferred from one hand to another hand it carries bacteria form the pervious environment or previous carrier hand, because as a human we do all our activities with our hand and with every contact our hands gets introduced to new bacteria, which can be pathogenic or non-pathogenic and by touching paper currency we transfer those bacteria on the surface of the paper currency and being absorbable paper it easily becomes a breeding place for bacteria and multiplies and money is exchanged so rapidly that one single paper currency can carry hundreds of bacteria, which can be dangerous for human health. This research explored this hypothesis and later proved that paper currency is a carrier for disease causing and multidrug resistant bacteria.

Conclusion

From this research on paper currency, it is clear that Bangladeshi paper currency is contaminated with pathogenic bacteria and this study supports that a huge percentage of this organisms are multidrug resistant. After surviving the covid pandemic it is clear to us that how easy it is for bacteria to spread at a fast pace. This study proved that paper currency can be the carrier source of pathogenic bacteria and a simple mutation of multidrug resistant bacteria can cause another pandemic and paper currency can carry that bacteria and create a devastative threat to human life. We should do more research on this topic and find an alternative medium of paper currency that all classes of people can use.

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