Isolation of *Klebsiella pneumoniae* and *Acinetobacter baumannii* from street vended cut fruits sold in Dhaka city of Bangladesh and their antibiotic susceptibility pattern.

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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

Bachelor of Science in Microbiology

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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 the main sources of help.

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Ethics Statement

For completion of this study, samples from selected venues were collected following all the necessary precautions. All the experiments were done in BRAC University Laboratory. It should also be noted that no animal or human models were used in this study.

Abstract

The consumption of street vended ready-to-eat cut fruits is very common in Dhaka City. Since fresh produce is eaten raw, it can also pose a public health risk. So, this study was undertaken for the isolation and identification of *Acinetobacter baumannii* and *Klebsiella pneumonia*e from different street-vended fresh-cut fruits of 11 different areas of Dhaka city to investigate the occurrence of pathogen in fruits from water or hand of the vendor. In this study, 68 *Acinetobacter baumannii* and *Klebsiella pneumoniae* were found from 50 samples. Three categories of samples (fruits, hand swab and water) were collected from each vendor. Polymerase chain reaction (PCR) test was done using selective primers for both of the organisms. 61 out of 69 (88.4%) isolates of *Klebsiella pneumoniae* and 7 out of 12 (58.3%) isolates of *Acinetobacter baumannii* were confirmed. *Klebsiella pneumonia*e were found from all four type of fruits. *Acinetobacter baumannii* were found in all types of fruits except papaya. Among them, highest and lowest isolates of bacteria were found from pineapple and hog plum respectively. *Klebsiella pneumoniae* was found from fruit water and/or hand swab samples in 9 out of 11 vendors. *Acinetobacter baumannii* was found from both water and fruit samples from only one vendor. Antibiotic susceptibility test was done by using 10 antibiotics of different groups. All the isolates were resistant to Amoxicillin (78.5%), Tetracylin (82.3%), Imipenem (18%), Amikacin (17.72%), Levofloxacin (12.65%), Colistin (50.63%), Nalidixic acid (15.2%), Ceftriaxone (20.25%), Ceftazidime (29.11%) and Gentamycin (13.92%). In fact, 43% of *Acinetobacter baumannii* and 13.43% of *Klebsiella pneumoniae* were found as multidrug resistant.

Keywords: Street vended cut fruit, Dhaka city, Acinetobacter baumannii, Klebsiella pneumoniae, Polymerase chain reaction, Antibiotic susceptibility test.

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List of Acronyms:

PCR: Polumerase Chair Reaction

AST: Antibiotic Susceptibility Test

TSB: Tryptic Soy Broth

Chapter 1

Introduction

1. Introduction

Recently, fresh fruits and vegetables are generally consumed by people of different age. All the busy areas of Dhaka city where street foods are sold are very inexpensive and easily available in various streets. As fruits have a great nutritional value people are interested to eat readily cut fruits. Also, vendors make it more delicious by adding spices. As a result, past few years, the ready to eat fresh fruit consumption has increased in Bangladesh. Therefore, it has resulted in increase in food borne diseases associated with these fruits and vegetables. That's how food borne pathogen got attention in developing countries.

In the last few years, outbreaks of human infections related with the ingestion of fresh or minimally processed fruits and vegetables have increased, in spite of their nutritional value. (Beuchat, 2002) Studies said that, the bacterial contamination of fruits has increased which has caused outbreaks. (NEELIMA GARG, 1990) These organisms are transmitted through various sources. Therefore, microbial contamination can frequently be exposed to fruits and vegetables during harvesting or pre harvesting season and also by dust, dirt or water. (Carlin, 1994) Tropical fruits are usually sold after the fruits are washed, peeled, sliced and packaged in polyethylene bags. However, the ingestion of freshly cut fruits processed and vended in open markets may constitute human health risks due to microbial contamination.

Globally, more than 20 kinds of fruits are sold as minimally processed or fresh-cut fruits. In Bangladesh, watermelon, pineapple, Guava, hog plum, pomelo are among the most common street foods which are consumed regularly. However, the consumption of these fruits in open markets may constitute health risks that can lead to microbial contamination.

In developing countries, where sanitary conditions are still not so improved, GI tract infections are so common. These infections include dysentery, diarrhea, vomiting, enteric fever etc. are generally transmitted through waters and foods.

A significant opportunistic pathogen and frequent contributor to nosocomial illnesses, is *Klebsiella pneumoniae.* Although, *K. pneumoniae* infections can happen almost anywhere in the body, respiratory tract infections and urinary tract infections are the most common. Gastrointestinal colonization commonly precedes infections, and the gastrointestinal system is thought to be the main site of bacterial transmission. (Carsten Struve, 2004)

According to various investigations, *Klebsiella pneumoniae* isolates from the environment have many phenotypic characteristics with clinical isolates. Most intriguingly, a recent study

found that *K. pneumoniae* isolates from surface waters share virulence characteristics with clinical isolates, indicating that environmental populations of the bacteria may be just as aggressive as clinical strains. (R. Podschun, 2001)

Acinetobacter spp. have become a pathogen of significant public health concern due to their growing resistance to antibiotics and their associations with a variety of nosocomial infections, community-acquired diseases, and infections. Although it is acknowledged that this organism is widespread, nothing is known about how common the several pathogenic species in this genus are in food and water sources. The role of food as a source of various species, notably the *Acinetobacter baumannii* group, has been clarified with the adoption of molecular techniques. Additionally, *Acinetobacter spp.* isolated from food products showed multidrug resistance. Foods are important potential sources of *Acinetobacter spp.* transmission across the community and clinical settings, and thus emphasizes the need for more research on the possible health hazards associated with *Acinetobacter spp.* as foodborne pathogens. (P.Nasr, 2020)

Acinetobacter baumannii is a Gram-negative bacteria that has evolved into a pathogen in healthcare facilities that is extremely harmful due to its capacity to resist many classes of antimicrobial drugs. (Caitlin L. Williams, 2020) Numerous illnesses, such as bacterial infections of the skin and soft tissues, pneumonia, osteomyelitis, and meningitis, can be brought on by this pathogen. (Galleo, 2016)

The ability of *A. baumannii* to adapt to unfavorable environmental conditions, desiccation resistance, antibiotic resistance, and genome flexibility were some of the features that made it successful as a nosocomial pathogen. Additionally, *A. baumannii* can endure months of dry conditions and may endure exposure to commonly used disinfectants including phenols and chlorhexidine. (AGNESE LUPO, 2014)

Despite being mostly known as a nosocomial pathogen, *A. baumannii* has also been identified from a variety of sources, including food, water, soil, and animals .*A. baumannii* contamination in food is regarded as a severe issue since it could allow the bacteria to enter healthcare facilities and increase the number of nosocomial infections brought on by this pathogen. (Ana Carvalheira, 2020).Fresh produce consumption (fruits and vegetables) has increased recently as a result of agricultural methods being modernized and an increase in production.

An investigation was undertaken for the isolation and identification of food borne microorganisms from different street-vended fresh-cut fruits. In this study, the bacteriological safety of fresh cut fruits which are available in between September to December in streets of

crowded places are included for the research. Total 50 samples from different areas of Dhaka city were collected. Three categories of sample were collected: fruit sample, hand swab sample and water and then analyzed for bacterial contamination by standard bacterial culturing methods. Bacterial isolates were further detected through conventional PCR methods. Then, antibiotic susceptibility test was done against the bacteria, *Klebsiella pneumoniae* and *Acinatobacter baumannii* were the main concerns for isolation and study as they are emerging pathogens.

1.1 Objectives of the study

- To isolate and identify *Klebsiella pneumoniae and Acinetobacter baumannii* from guava, pineapple, hog plum, papaya or any tropical fruits.
- To determine prevalence rates of the microbe from three categorized sample: water, hand swab, fruit.
- To determine the antibiotic susceptibility patterns of the isolates.
- To propose ways of reducing any potential food safety risks associated with the studied foods.

Chapter 2

Materials and Methods

2 Materials and Method:

2.1 Work Plan:

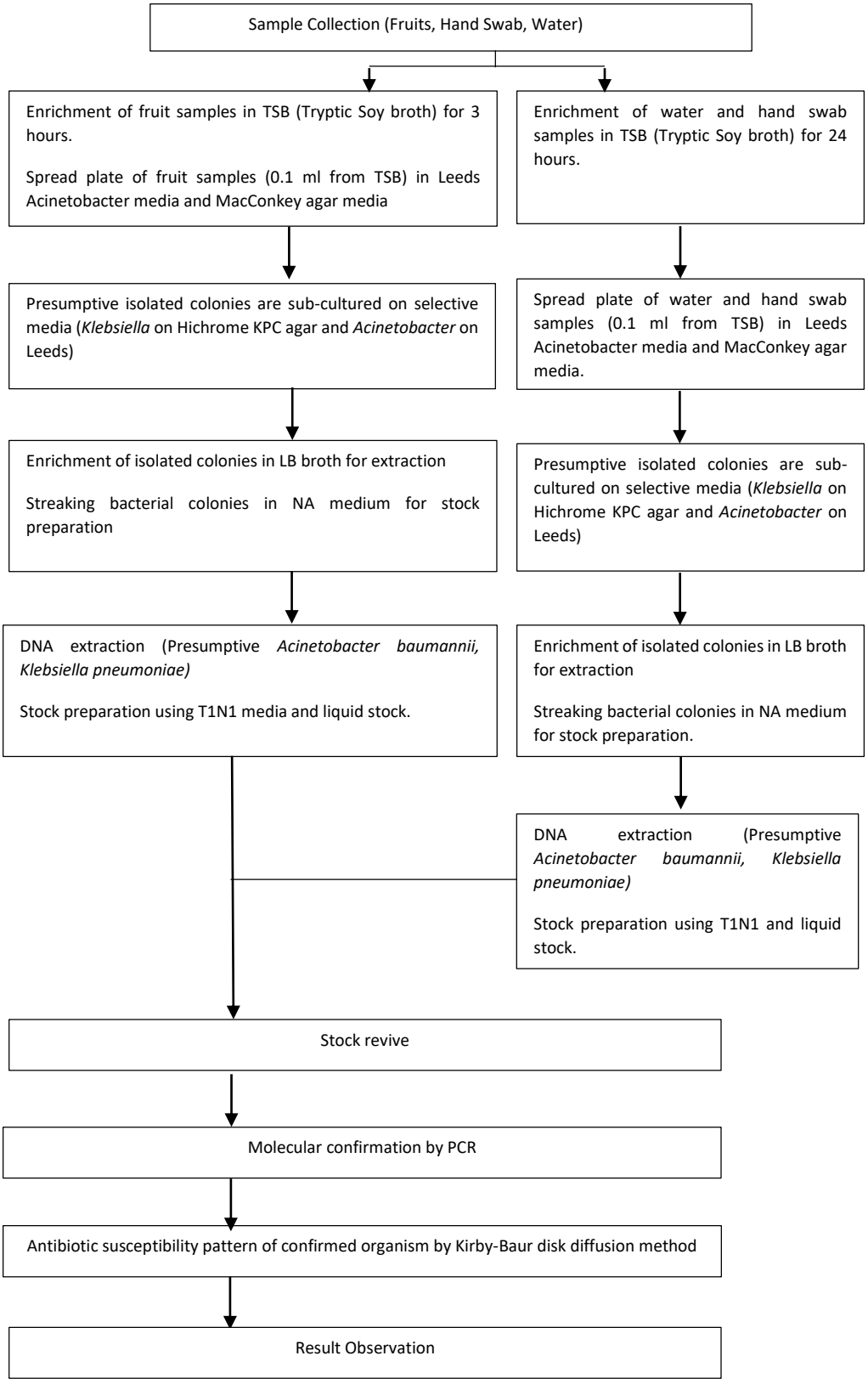

Figure 1: Work flow

2.2 Sampling sites:

Samples were collected from 11 different northern areas of Dhaka city such as Mirpur (1, 2, 10), Mohammadpur, Mohakhali, Gulshan (1, 2), Banani, Badda, Kallyanpur and Agargaon. The samples were collected from October to December 2022, mostly from crowded areas. From each area, samples were collected from one vendor. In total, 50 samples including fruits, hand swabs and water samples were collected.

Figure 2: Sampling sites

2.3 Sample collection:

50 samples were collected from different parts of Dhaka (North) city. Three categories of samples were collected from each vendor which are fruits, hand swab of the vendor and water which is used to wash the fruits. Four categories of seasonal fruits were generally found from all vendors which are papaya, guava, pineapple and hog plum. All fruit samples were collected in separate sterile zip lock bags to avoid contamination. Fruit samples were then carried to the lab in a sterile ice box.

For hand swab samples, test tubes containing 10 ml of Buffered Peptone Water and cotton swab wrapping in foil paper were autoclaved to sterilize on the day before sample collection.

While collecting hand swab, sterile cotton swab was first dipped into sterile buffered peptone water, rubbed on the hand of the vendor and dipped back into the Buffer Peptone Water.
For the water samples, approximately 100 ml of water that is used by the vendor to wash fruits, were collected in a sterile water bottle. All the samples were transported to the lab within 2 hours of collection.

2.4 Sample processing:

For processing, fruit samples were cut with sterile surgical knives. 10 gm of each sample were measured with a weighing scale and inserted into the conical flask containing 50 ml of TSB (Tryptic Soy broth). For hand swab samples, 1 ml of buffered peptone water in which the cotton swab was dipped, was added in 49 ml of TSB. Before that, the hand sample was mixed by a vortex mixer to mix the sample well. Membrane filtration method was used for water sample processing. In this method, water is passed through a membrane filter with a pore size of 0.45 µm which is later transferred with a sterile forcep into the 50 ml of TSB containing conical flask. The flasks were sealed properly and placed into the shaking incubator. Fruits were incubated for 3 hours at 37°C. On the other hand, hand swab and water samples were incubated for 24 hours at 37°C.

Figure 3: Samples in TSB broth after processing

Four Media were prepared which were used in spread plate technique by following manufacturer's instructions. MacConkey agar (MAC) and Leeds Acinetobacter Agar were used for culturing *Klebsiella Pneumoniae* and *Acinetobacter baumannii*. Briefly, all media were sterilized by autoclave at 121° C for 15 minutes.
After incubating fruit samples for 3 hours, 0.1ml of fruit samples were transferred to Leeds media to culture *Acinetobacter baumanii* and later incubated at 44°C for 24 hours. Again, 0.1ml of fruit samples were transferred to MacConkey media to culture *Klebsiella Pneumoniae*.

For water and hand swab samples, 1 ml from TSB were added to a test tube of 9 ml of sterile 0.9%. 10 fold serial dilution were done until the final dilution for the bacteria were 10^{-6}. From 10^{-6} test tubes, 0.1 ml solution was transferred to the media and cultured in the similar process.

Presumptive isolated colonies are then sub-cultured on selective media (K*lebsiella Penumoniae* on KPC media and *Acinetobacter baumannii* on Leeds Acinetobacter media). Later, presumptive isolated colonies were transferred and mixed in LB broth for DNA extraction. DNA of presumptive *Acinetobacter baumannii and klebsiella pneumoniae* were extracted by boil extraction method. Bacterial colonies were also streaked in Nutrient agar (NA) medium for stock preparation and further stocked in T1N1 and liquid stock. After that, for confirmation, molecular detection by PCR was done for each category of bacteria. After confirming the genus and species, antibiotic susceptibility test was done by using 11 antibiotics of different groups.

2.5 Stock Preparation:

Glycerol stock:

For liquid stock, 700µl of Luria Bertani (LB) broth were collected in micro-centrifuge tubes. A single colony of each bacterium from the subculture plate were inoculated in LB broth and incubated at 37°C overnight. With sterile micropipette and tips, 300 µl of 50% glycerol was added in each tube. The vials are then stored at -20°C until further use.

T1N1 stock:

For T1N1 stock, 1gm tryptone, 1gm of NaCl, and 1.5 gm agar were mixed with 100ml of water and boiled until dissolved completely. 2 ml of the solution were then added in glass vials and sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes. The media then kept in straight position until solidified. After solidification, a single colony of each bacteria were picked and stabbed in T1N1agar with a sterile inoculating needle. Lastly, 200µl of paraffin oil were added on top of the agar, sealed properly and kept outside until further use.

2.6 DNA Extraction:

This study involved molecular identification of isolated *Klebsiella pneumoniae* and *Acinetobacter baumannii*. So, DNA were extracted from the isolated colonies by boil extraction method.

A single colony of each bacteria was inoculated in 1ml of Luria Bertani (LB) broth and incubated at 37°C overnight. Then, 700 µl of broth were collected in micro-centrifuge tubes and centrifuged at 13000 rpm for 10 minutes. After centrifugation, the supernatant was discarded from top and the pellet was washed with 300 µl PBS (phosphate buffered saline). The pellet was then vortexed to mix and wash. MCT containing pellets were centrifuged again at 14000 rpm form 5 minutes. The supernatant was later discarded and 200 µl of Tris-EDTA (TE) buffer was added. These samples were then boiled at 100°C for 15 minutes and then cooled for 10 minutes. After that, the samples were centrifuged at 13000rpm for 5 minutes. In this process, cell debris precipitated at the bottom. Supernatants were collected carefully in separate MCTs (Microcentrifuge tubes). The supernatant contained extracted DNA and stored at - 20°C.

2.7 Confirmation of *Klebsiella pneumoniae* and *Acinetobacter baumannii* by PCR:

Preparation of primer:

Bla-oxa51 primer for *Acinetobactcer baumannii* and KP Pf & KP Pr1 primer for *Klebsiella pneumoniae* were used for PCR confirmation. From stock solution, working solution (10µM) were prepared for each primer. For each primer, forward and reverse primers were taken in two different micro-centrifuge tubes.

Preparation of working solution:

For each bacterial sample, 25 µL of final solution was used for PCR. For that, 12.5 µl Master Mix, 4.5µl of nuclease-free water, 2µl forward primer, 2µl reverse primer and 4µl template DNA were used.

Primers and PCR Conditions used in this Study:

Name of Bacteria	Primer	Primer Sequence	Product size (bp)	PCR conditions
Klebsiella pneumoniae	KP Pf-F KP Pr1-R	5′-ATTTGAAGAGGTTGCAAACGAT-3′ 5′-TTCACTCTGAAGTTTTCTTGTGTTC-3′	130	The cycling conditions were 10 min at 94 °C followed by 35 cycles of 30 s at 94 °C, 20 s at 57 °C and 20 s at 72 °C, followed by a 10 min hold at 72 °C.
Acinetobactcer baumannii	Bla-oxa51-F Bla-oxa51-R	5´-TAA TGC TTT GAT CGG CCT TG-3´ 5´-TGG ATT GCA CTT CAT CTT GG-3´	353	The cycling conditions were: 94°C for 3 min followed by 35 cycles at 94°C for 45 s, at 55°C for 45 s, and at 72°C for 1

				min, followed by a final extension at 72°C for 5 min.

Table 1: Primers and PCR conditions

2.8 Gel Electrophoresis:

After PCR, conventional agarose gel electrophoresis was performed to confirm the presence of the amplified product. The amplicon was separated by agarose gel electrophoresis with 2% agarose gel stained with ethidium bromide at 90 voltages for 1 hour. The gel was then visualized by UV transilluminator. To determine the size of the amplicon, 50bp DNA ladder was used.

2.9 Antibiotic Susceptibility Test:

This test was done following the Kirby-Bauer disc diffusion method. After PCR confirmation, for this test, bacteria were first culture in Nutrient agar (NA) from stock at 37°C for 24 hours. After that, a loopful of fresh pure culture was taken and mixed it with 0.9%Saline in a test tube. The turbidity of the suspension was checked and compared with the 1.0 McFarland turbidity standard. Then, a sterile swab was dipped into the test tube containing suspension and lawn on a fresh Mueller Hinton Agar (MHA). Then, 10 antibiotic discs of different groups were picked and placed onto the agar with a forcep. 10 antibiotics of different groups were used in this study: Amoxicillin, Tetracylin, Imipenem, Amikacin, Levofloxacin, Clindamycin, Colistin, Nalidixic acid, Certriaxone, Ceftazidime and Gentamycin. The MHA plates were then placed into the 37°C incubator for 24 hours.

Serial no	Antibiotic	Group	Effective against	Disc code	Disc potency (µg)	Organism	Interpretative Criteria		
							Sensitive mm or more	Intermediate mm	Resistant mm or less
1	Gentamicin	Aminoglycoside	Gram-positive and Gram-negative	GEN	10		15	13-14	12
2	Tetracycline	Tetracyline	Gram-positive and Gram-negative	TE	30		15	12-14	11
3	Ceftazidime	Cephalosporin	Gram-negative	CAZ	30	Enterobacteriaceae	21	18-20	17
						Acinetobacter spp.	18	15-17	14
4	Imipenem	Carbapenem	Gram-positive and Gram-negative	IMI	10	Enterobacteriaceae	23	20–22	19
						Acinetobacter spp.	22	19-21	18
5	Amikacin	Aminoglycoside	Gram-negative	AK	30		17	15–16	14
6	Colistin	Polymyxin E	Gram-negative	CT	10		-	11-17	-
7	Amoxicillin	Penicillin	Gram-positive and Gram-negative	AMX	30				
8	Ceftriaxone	Cephalosporin	Gram-positive and Gram-negative	CTR	30	Enterobacteriaceae	23	20-22	19
						Acinetobacter spp.	21	14-20	13
9	Levofloxacin	Quinolone	Gram-positive and Gram-negative	LE	5		17	14-16	13
10	Nalidixic acid	Quinolone	Gram-negative	NA	30		19	14-18	13

Table 2: List of antibiotic used in the experiment

CHAPTER 3

RESULTS

3 Results

3.1 Identification of Organisms:

A total of 50 samples (28 fruits, 11 hand swab and 11 water) were collected from street vended cut fruit sellers to investigate the presence *Klebsiella pneumoniae, Acinetobacter baumanni*. From these samples, 81 presumptive colonies were isolated.

3.2 Identification of *Klebsiella pneumoniae*:

Isolates picked from MacConkey agar plate, were sub-cultured on KPC agar media. On the basis of agar colony morphology, bluish green round colonies were considered as *Klebsiella pneumoniae.*

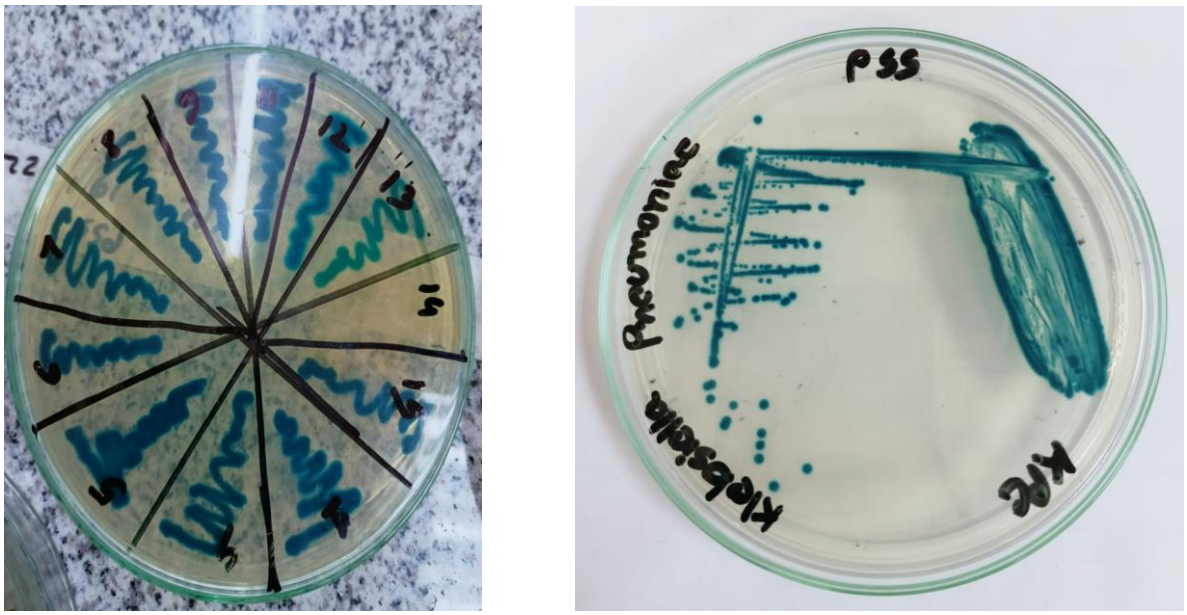

Figure 4: Klebsiella pneumoniae on KPC Agar

3.3
Identification of *Acinetobacter baumannii:*

Isolates picked from Leeds Acinetobacter media, were sub-cultured on the same agar media. On the basis of agar colony morphology, round, convex, light pink mucoid colonies with a pink to mauve color diffusion around the colony were considered as *Acinetobacter baumannii.* However, in this study round, smooth yellow colonies were also picked and confirmed as *Acinetobacter baumannii.*

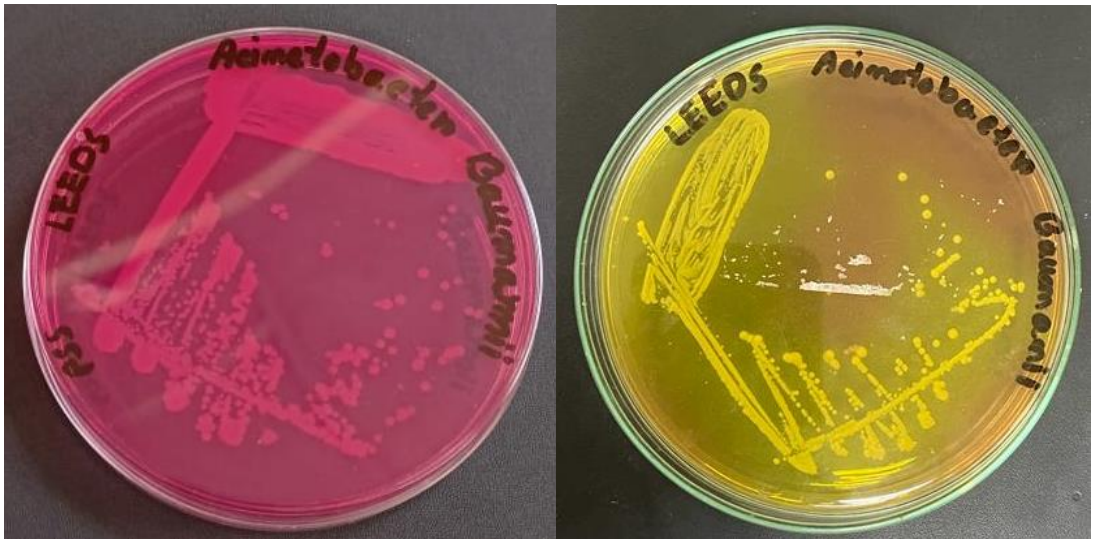

Figure 5: Acinetobacter baumannii on Leeds Acinetobacter media

3.4
PCR Results:

Polymerase chain reaction (PCR) was done for all the isolates of *Klebsiella pneumoniae* and *Acinetobacter baumannii* by using two primer pairs. **KP Pf-F and KP Pr1-R** for *Klebsiella pneumoniae* confirmation. **Bla-oxa51-F and Bla-oxa51-R primer** were used for ***Acinetobacter baumannii*** confirmation**.**

Positive result based on PCR	
Klebsiella pneumoniae	***Acinetobacter baumannii***
(*n=69)	(*n= 12)
61(88.4)	7 (58.3)
Data are presented as absolute values (percentages). *n refers to samples.	

Table 3: PCR results of Klebsiella pneumoniae & Acinetobacter baumannii

3.5
PCR result of *Klebsiella pneumoniae*:

61 isolates were confirmed as *Klebsiella pneumoniae* in PCR. 50 bp ladder was used to confirm the band. All the positive *Klebsiella pneumonia*e isolates gave band at 130 bp.

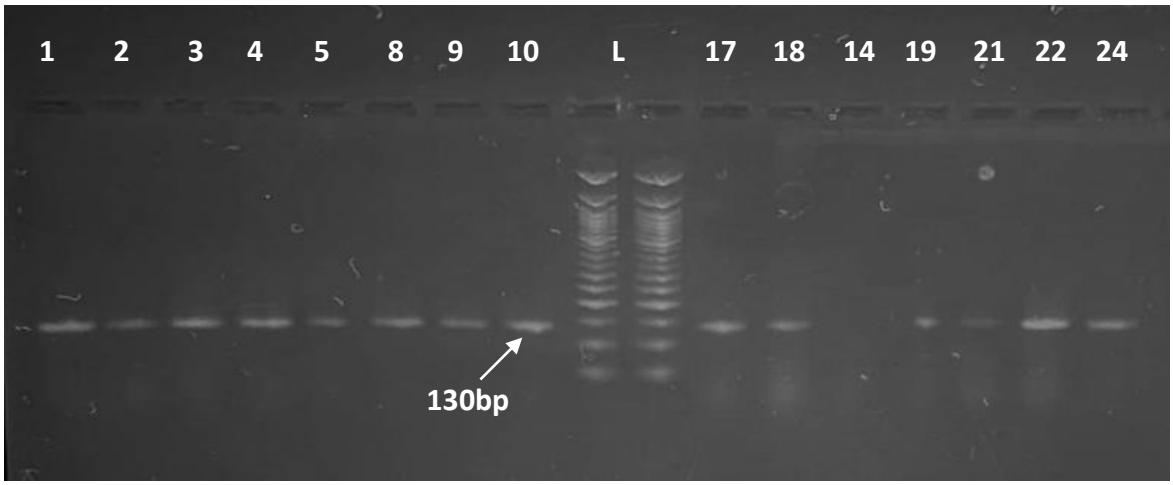

Figure 6: Gel electrophoresis result of Klebsiella pneumoniae

PCR amplified product of *Klebsiella pneumoniae* isolates. Here, 50 bp DNA ladder is marked as L. All the DNA samples showed band at 130 bp except for 14.

3.6
PCR result of *Acinetobacter baumannii*:

7 isolates were confirmed as *Acinetobacter baumannii* in PCR. 50 bp ladder was used to confirm the band. All the positive *Acinetobacter baumannii* isolates gave band at 353 bp.

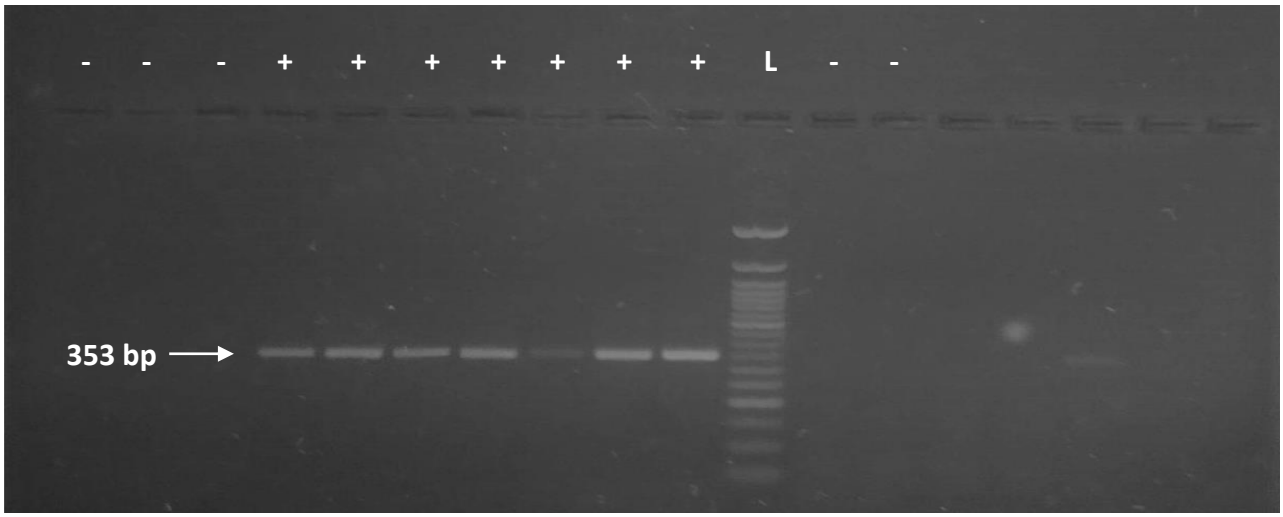

Figure 7: Gel electrophoresis result of Acinetobacter baumannii

PCR amplified product of Acinetobacter baumannii isolates**.** Here, 50 bp DNA ladder is marked as L. 7 DNA samples showed band at 353 bp which is showed as (+) and 5 samples gave negative result showed as (-)

3.7 *Klebsiella pneumoniae and Acinetobacter baumannii* found in number of samples throughout the study

From 35 out of 50 different samples of fruits, hand swab and water, *Klebsiella pneumoniae and Acinetobacter baumannii were found.*

Sample Type (n)	Name of the bacteria	Bacteria found in number of samples	Total
Papaya (6)	*Klebsiella pneumoniae*	6	6
	Acinetobacter baumannii	0	
Pineapple (10)	*Klebsiella pneumoniae*	6	7
	Acinetobacter baumannii	1	
Guava (5)	*Klebsiella pneumonia*	4	5

	Acinetobacter baumannii	1	
Hog plum (7)	*Klebsiella pneumoniae*	2	3
	Acinetobacter baumannii	1	
Hand Swab (11)	*Klebsiella pneumoniae*	6	6
Water (11)	*Klebsiella pneumoniae*	7	8
	Acinetobacter baumannii	1	
Total			35

Table 4: Klebsiella pneumoniae and Acinetobacter baumannii found in number of samples

3.8 Percentage of isolates in fruit samples:

In this study, highest percentage of isolates were found from papaya and the lowest percentage of bacteria were found from hog plum.

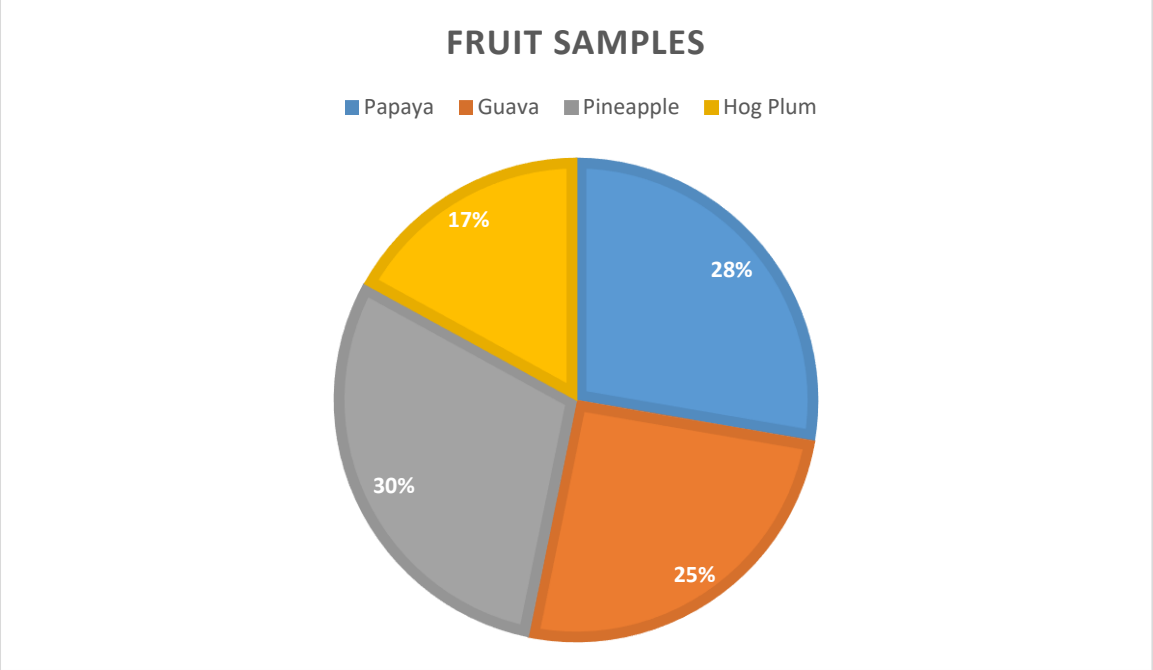

Figure 8: percentage of isolates in different fruit samples

3.9 *Klebsiella pneumoniae and Acinetobacter baumannii* found in fruits, hand swab and water from same vendor:

Out of 11 vendors, *Klebsiella pneumoniae were* identified from one or more fruits, hand swab and water or, fruit, hand swab or water from 9 vendors. From one vendor, *Klebsiella pneumonia*e were only confirmed from fruits. From another vendor, *Klebsiella pneumonia*e was not found from hand swab and water. From only one vendor, *Acinetobacter baumannii* was found and confirmed from both water and fruit samples.

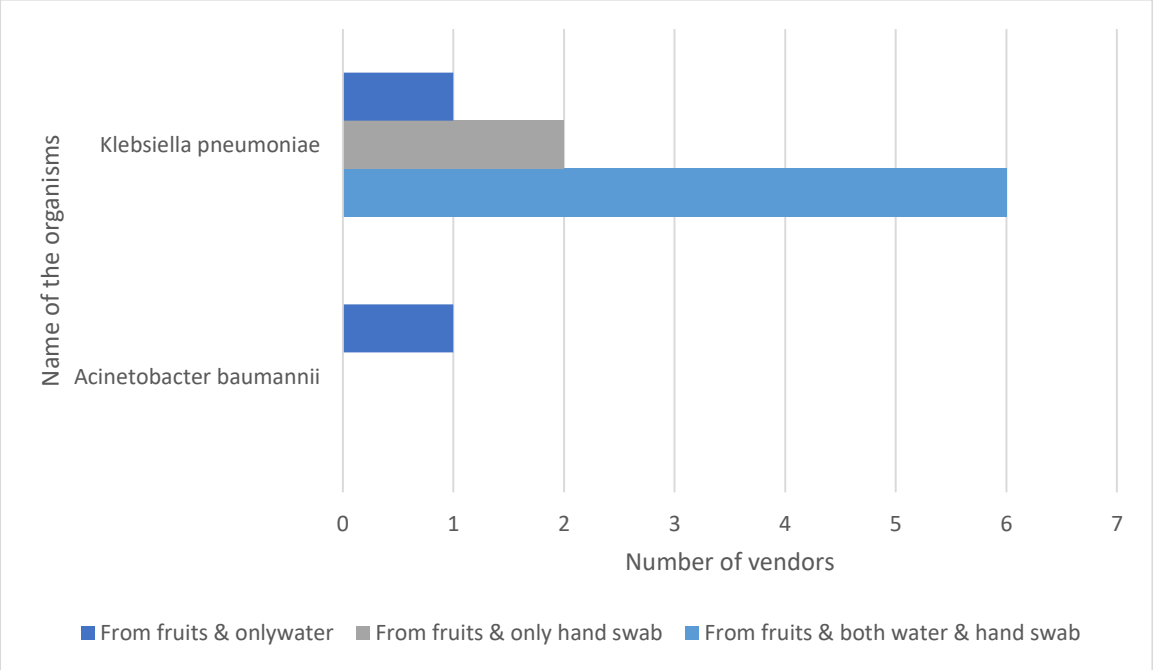

Figure 9: Klebsiella pneumoniae and Acinetobacter baumannii found in fruits, hand swab and/or water from same vendor

3.10
Antibiotic Susceptibility Test Result:

Antibiotic susceptibility test was done using Kirby - Bauer disk diffusion method. According to the CLSI guidelines, resistant, intermediate, or sensitive results were interpreted. This observation is represented in the figure. Here, *Acinetobacter baumannii* is resistant to some of the antibiotics.

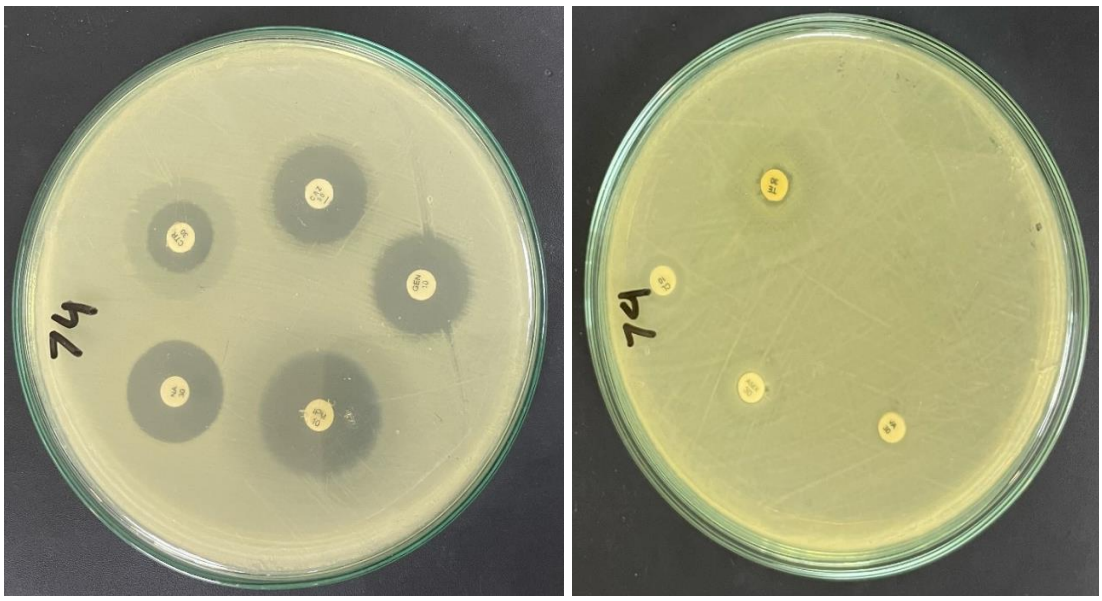

Figure 10: Antibiotic susceptibility test of Acinetobacter baumannii

Again, below figure represents the antibiotic susceptibility pattern of one of the *Klebsiella pneumoniae* isolates from this study. Here, *Klebsiella pneumonia* is resistant to some of the antibiotics.

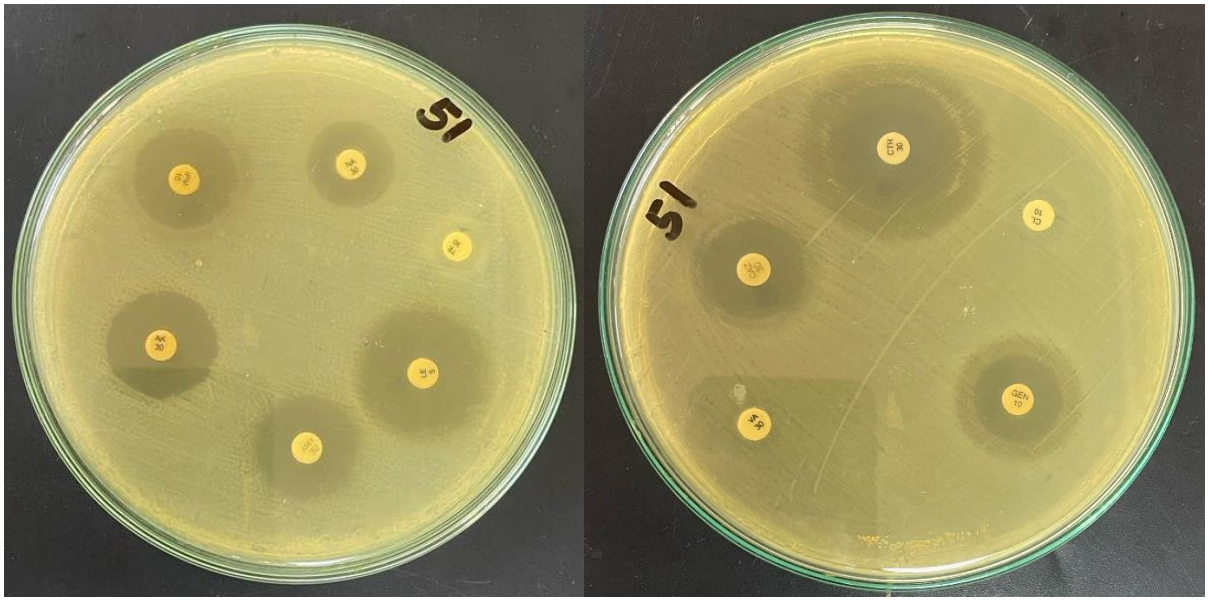

Figure 11: Antibiotic susceptibility test of Klebsiella pneumoniae

3.11 Antibiotic susceptibility pattern of all isolates:

In this study, only 12.65% of isolates were found resistant against Levofloxacin. High percentage of resistance is shown against Amoxicillin and Tetracyclin. For colistin, only isolates that showed no zone are considered as resistant.

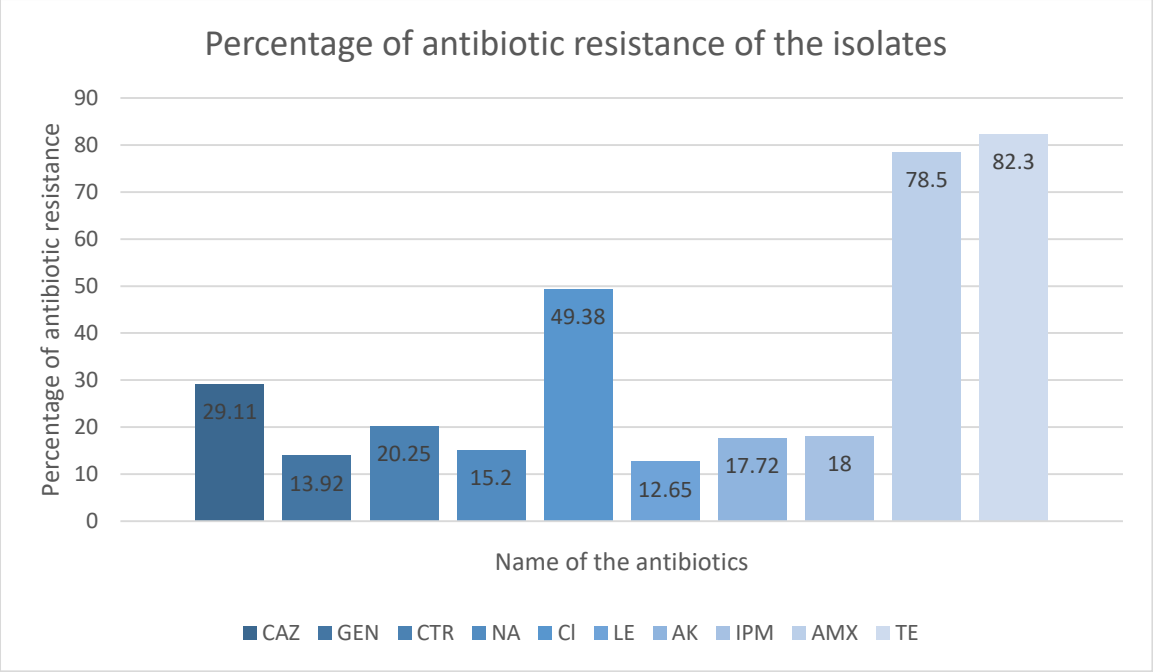

Figure 12: Percentage of antibiotic resistance of the isolates

3.12
Antibiotic susceptibility pattern for colistin

Disc diffusion method for antimicrobial susceptibility to colistin is complex because of the poor diffusion of polymyxins through agar. Both EUCAST and CLSI recommend broth microdilution (BMD) for antimicrobial susceptibility testing of colistin which is rarely used in routine microbiology laboratories. So, only isolates that showed no zone are considered as resistant in this study.

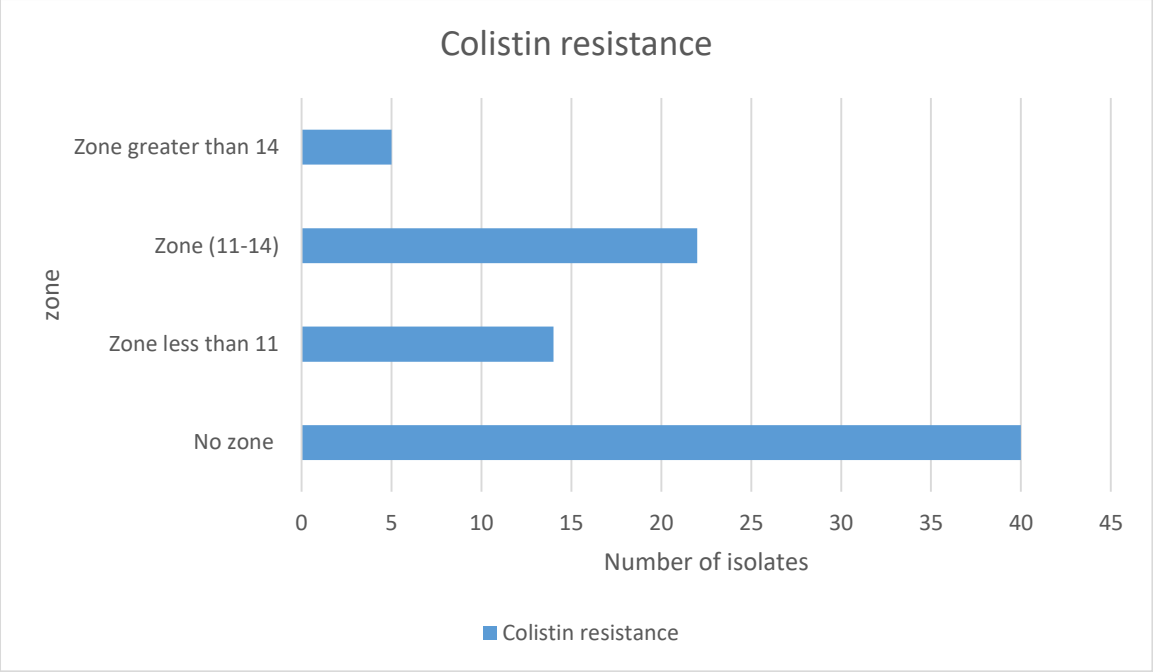

Figure 13: Antibiotic susceptibility pattern of colistin

3.13
Antibiotic susceptibility pattern of *Acinetobacter baumannii:*

All the *Acinetobacter baumannii* isolates were sensitive against Ceftazidime, Gentamycin and Ceftriaxone and Imipenem.

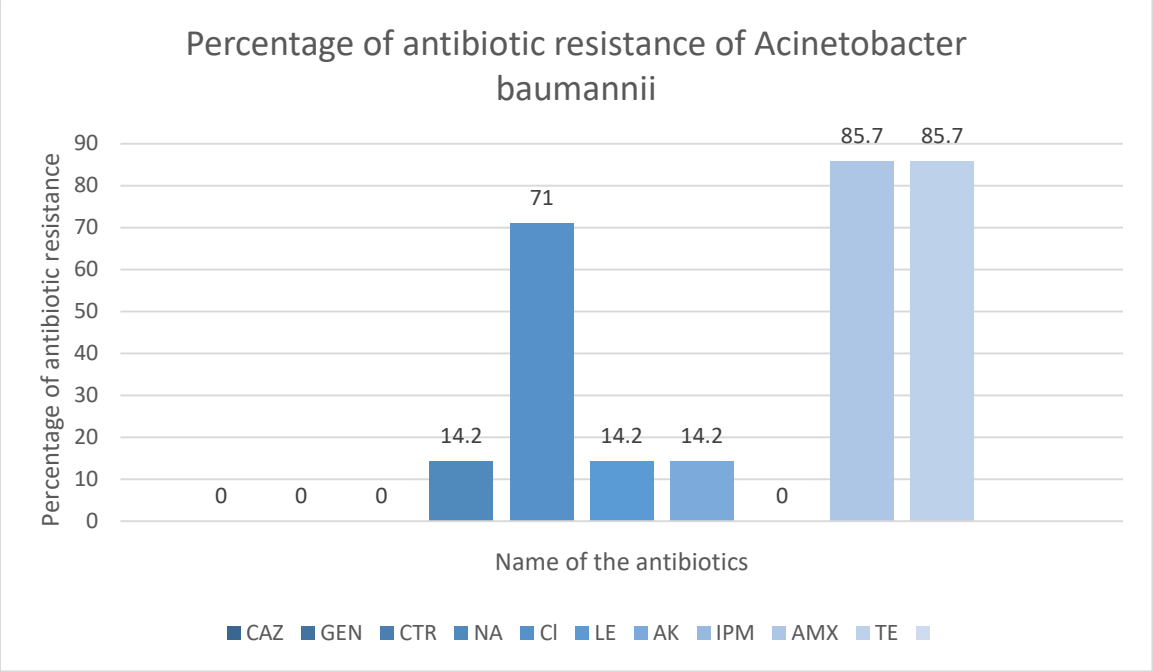

Figure 14: Antibiotic susceptibility pattern of Acinetobacter baumannii

3.14 Antibiotic susceptibility pattern of *Klebsiella pneumoniae:*

Most of the *Klebsiella pneumoniae* samples were sensitive against Levofloxacin and Gentamycin. High percentage of resistance pattern was observed against Amoxicillin and Tetracyclin.

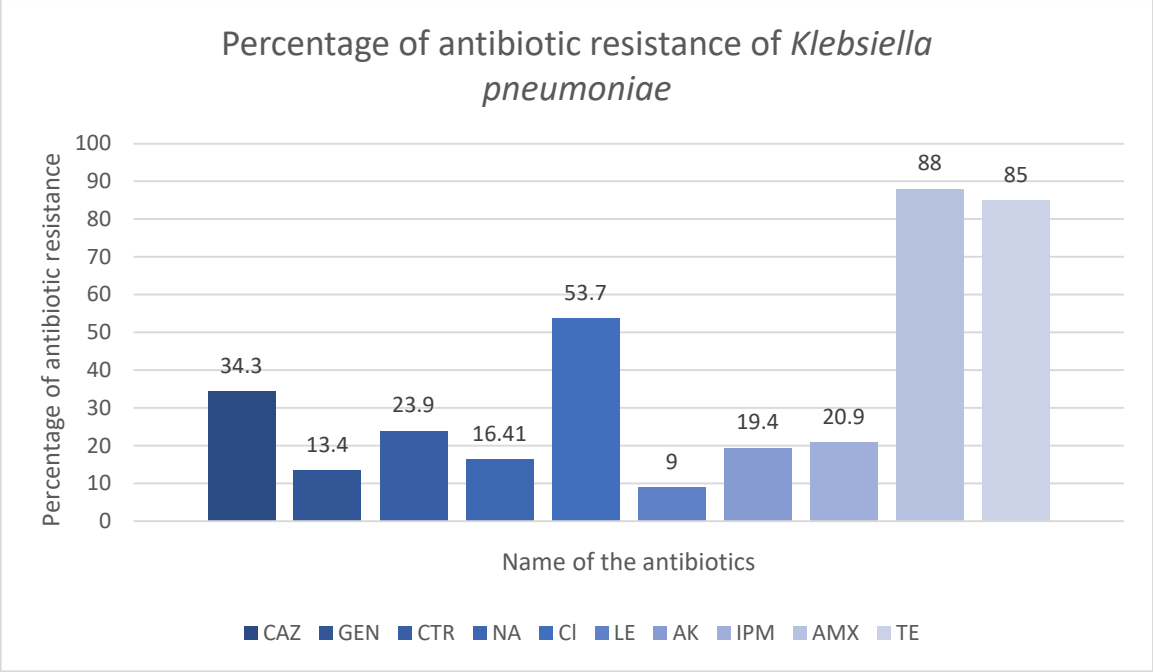

Figure 15: Antibiotic susceptibility pattern of Klebsiella pneumoniae

3.15 Multi-drug Resistance of the isolates:

In this study, it is found that, 43% *Acinetobacter baumannii were* multidrug resistant. On the other hand, only 13.43% of *Klebsiella pneumoniae* were multidrug resistant.

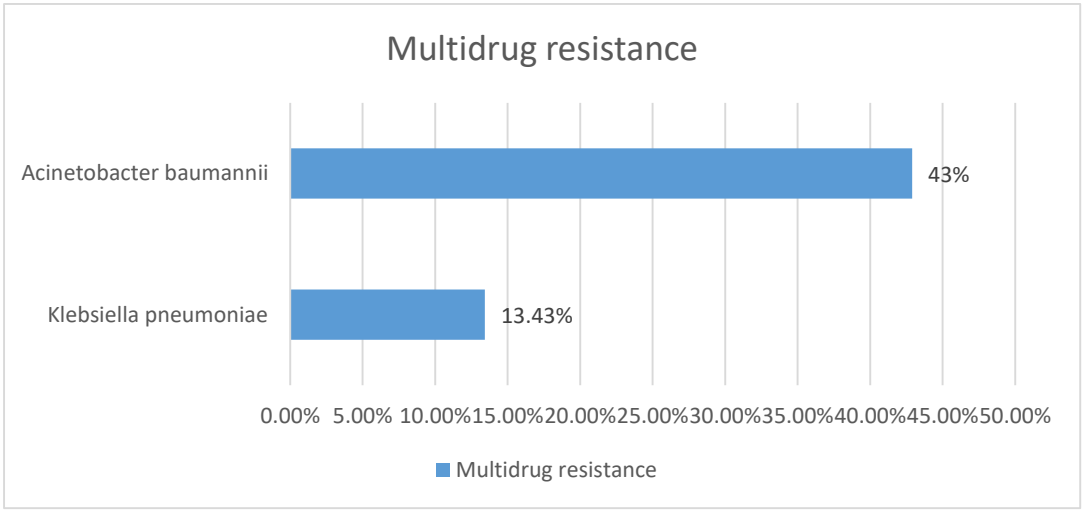

Figure 16: Multidrug resistance of the isolates

Chapter 4

Discussion

4 Discussion:

This result highlights the fact that fresh fruits could be contaminated with pathogenic bacteria and thus could possibly act as a transmission vehicle of many diseases.

The study confirmed the occurrence of *Klebsiella pneumoniae* and *Acinetobacter baumannii* on four tropical fruits which are sold in crowded places of Dhaka city. In this study, *Acinetobacter baumannii and Klebsiella pneumoniae* were found from 35 out of 50 samples from 11 different areas of Dhaka city. 68 bacterial isolates were found from all the samples. 61 *Klebsiella pneumoniae* and 7 *Acinetobacter baumannii* were found by confirmation of polymerase chain reaction (PCR). In this study, highest number of *Klebsiella pneumoniae* were found from pineapple and from hog plum, the number is low which can be an indication of low bacterial growth than other fruits. In fact, highest and lowest number of both type of bacteria were found from pineapple and hog plum respectively. Two desired bacterial isolates, *Klebsiella pneumoniae* were found in all four types of fruit samples whereas *Acinetobacter baumannii* were found in all types of fruits except papaya. No *Acinetobacter baumannii* were found from papaya which could demonstrate the lower *Acinetobacter baumannii* presence in papaya than other fruits.

Bacterial isolates were found form 8 out of 11 water samples and 6 out of 11 hand swab samples. No *Acinetobacter baumannii* were found from hand swab. However, from water samples, both bacteria were found in high range which is alarming. Pathogenic isolates can spread through water in fruits. Again, bacterial isolates were found from 6 out of 11 hand swabs of the vendor. This is an indication that, there is a greater possibility where beside water, contamination with pathogen can occur from handling of the fruits. *Acinetobacter baumannii* found from all the samples except papaya. Despite being mostly known as a nosocomial pathogen, *A. baumannii* has also been identified from a variety of sources, including food, water, soil, and animals. (AGNESE LUPO, 2014) *A. baumannii* contamination in food is regarded as a severe issue since it could allow the bacteria to enter healthcare facilities and increase the number of nosocomial infections brought on by this pathogen. Contamination due to handling from vendor or contamination during cutting fruits or washing them with unpurified water caused bacterial contamination both in water, fruit and hand swab sample. That means, poor hygiene is one cause of bacterial contamination. Another reason is using unpurified or waste water from cannels or tap causes bacterial growth in fresh fruits even if the fruit is not contaminated. In a study, resemblance in the expression of virulence factor of *Klebsiella pneumoniae is* found between surface water and clinical isolates. (R. Podschun, 2001) In this

study, it could be estimate that, both *Acinetobacter baumannii* and *Klebsiella pneumoniae* could contaminate fruit through handling the fruit or from water used. *Klebsiella pneumoniae* was found from fruit water and/or hand swab samples 9 out of 11 vendors. The fact that, *Klebsiella pneumoniae* found in surface water of which virulence factor similar to clinical isolates makes it a possibility that pathogens can disease by entering through food or water. Again, *Acinetobacter baumannii* were found from both water and fruit samples from same vendor. This is an indication that, *Acinetobacter baumannii* and *Klebsiella pneumonia*e and other bacteria could be contaminated from water which is used to wash the fruits or through handling of the fruits by vendor. After assessing the results from antibiotic susceptibility testing, high resistance pattern can be observed from this study of both the bacteria against Amoxicillin and Tetracyclin. Although, all the isolates of *Acinetobacter baumannii* were sensitive against Ceftazidime, Gentamycin and Ceftriaxone, most of the isolates were also showed resistance against Amoxicillin and Tetracyclin. 40 of total isolates were considered as resistant against Colistin since they provided no zone. Antibiotic susceptibility pattern of colistin may be subjected to error. Antimicrobial susceptibility to colistin is complex because of the poor diffusion of polymyxins through agar, which compromises the performance of both disc diffusion methods. In this study, 43% *Acinetobacter baumannii* and 13.43% of *Klebsiella pneumonia* were found as multidrug resistant. Multidrug resistance (MDR) was defined as non-susceptibility to at least one agent in three or more antimicrobial categories following the definition by Clinical Laboratory Standards Institute (CLSI), the European Committee on Antimicrobial Susceptibility Testing (EUCAST), and the United States Food and Drug Administration (FDA). (A-P Magiorakos, 2012) These emerging problems of antibiotic resistance in pathogens can cause disease in people by turning into opportunistic pathogen.

5 Conclusion

This study demonstrated the alarming presence of pathogenic bacteria among the most common and popular fresh fruits in several crowded areas. The result also indicated the current hygiene condition of selling and buying zones of fresh fruits in Dhaka. This study provided a general overview of the microbiological quality of fresh fruits and vegetable in Dhaka which will help to take necessary step to make sure the hygiene condition during fruits and vegetables selling and buying. In this study, it is found that, both *Acinetobacter baumannii* and *Klebsiella pneumoniae* could contaminate fruit through handling the fruit or from water used. This is an

indication that, *Acinetobacter baumannii* and *Klebsiella pneumonia*e and other bacteria could be contaminated from water which is used to wash the fruits or through handling of the fruits by vendor. Further characterization of the isolates by whole genome sequence could identify if the pathogens are same strain.

6 References

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