BACTERIOLOGICAL STUDY OF HOUSEFLY (*Musca domestica*) ACROSS DHAKA CITY AND THEIR ANTIBIOTIC RESISTANCE 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 my own original work while completing my 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 have acknowledged all main sources of help.

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Ethics Statement

No individuals or animals were involved in this study. Therefore, only verbal permission was taken from concerned authorities for sample collection, for example, shop owners, buyers, security personnel in residential areas etc.

Abstract/ Executive Summary

Houseflies (*Musca domestica*) act as mechanical vectors for pathogens, facilitating the fomite-mediated spread of bacteria in urban environments. This study evaluated the external bacterial load on houseflies in Dhaka, Bangladesh, and characterized the antibiotic susceptibility profiles of the isolates.

Thirty-six housefly specimens were collected from street food stalls, fish markets, and residential areas across the city. Individual flies were washed in phosphate-buffered saline, serially diluted, and cultured on MacConkey, mannitol salt, and cetrimide agars for preliminary isolation.

Molecular analysis verified *Escherichia coli* in 14.7% of samples, *Staphylococcus aureus* in 10.8%, *Klebsiella pneumoniae* in 9.3%, and *Pseudomonas aeruginosa* in 0.5%. Antibiotic susceptibility testing via Kirby–Bauer disk diffusion revealed highest overall sensitivity to meropenem (81.8%), followed by ceftriaxone (70.5%), doxycycline (70.5%), and amikacin (54.5%). Vancomycin exhibited a 62.5% susceptibility rate against *S. aureus* itself (5 out of 8 isolate) but a 22.7% susceptibility over all isolates, considering both Gram-positive and negative bacteria. In contrast, amoxicillin showed only 4.5% susceptibility and 95.5% resistance. High resistance rates were also noted for cefixime (77.3%) and ciprofloxacin (30.6%) with ceftriaxone showing the least resistance (13.6%).

The detection of multidrug-resistant bacteria on housefly surfaces underscores their role in disseminating clinically significant pathogens. These findings highlight the urgent need for improved sanitary measures, vector control strategies, and antibiotic stewardship to mitigate the public health risks posed by synanthropic flies.

Keywords: Housefly; public health; molecular identification; antimicrobial susceptibility testing; antibiotic resistance.

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List of Acronyms

Abbreviation	**Acronyms**
E. coli	*Escherichia coli*
S. aureus	*Staphylococcus aureus*
P. aeruginosa	*Pseudomonas aeruginosa*
K. pneumoniae	*Klebsiella pneumoniae*
PBS	Phosphate Buffered Saline
CFU	Colony Forming Unit
TNTC	Too Numerous To Count
DNA	Deoxyribonucleic Acid
TE	Tris-EDTA
TBE	Tris Borate EDTA
MCT	Microcentrifuge Tubes
RPM	Revolutions Per Minute
PCR	Polymerase Chain Reaction
AGE	Agarose Gel Electrophoresis
RFLP	Restriction Fragment length Polymorphism
NA	Nutrient Agar
EtBr	Ethidium Bromide

Glossary

Term **Definition (Source)**

Multidrug resistance (MDR)- acquired non-susceptibility to at least one agent in three or more antimicrobial categories (Magiorakos et al., 2012).

Public Health- the science of protecting and improving the health of people and their communities by promoting healthy lifestyles, researching disease and injury prevention, and detecting, preventing and responding to infectious diseases (*What Is Public Health?*, n.d.)

Chapter 1

Introduction

1.1. Background

Science, innovation and discovery are the greatest tools of humankind. But if survival has to face even the greatest challenges, it always holds a higher possibility to win. And such is antibiotic resistance in bacteria. Alexander Fleming had discovered penicillin almost by accident, saving numerous wounded soldiers in World War II but the future might just take us a century backwards (Quinn, 2012). Bacteria are constantly evolving at a rate faster than our understanding of science can comprehend. The frequent and unnecessary exposure of antibiotics on not only human bodies but also our ecosystem has created repeated selective pressure on bacteria. Thus, evolution took over to facilitate survival, that is, the acquisition of genes, often expressed in forms of antibiotic resistance. Besides, horizontal gene transfer between both pathogenic and non-pathogenic bacteria has contributed to the yielding of multiple drug resistant organisms a lot handier. Through this process, the bacteria which had to go through the trial-and-error process of evolution, now can simply acquire the resistant gene from a neighbouring organism that achieved success through the process. In brief, the gene segment that allowed survival under selective pressure could be copied and shared with nearby bacteria within descendants under certain phylogenetic origins. The World Health Organisation (WHO) has declared antibiotic resistance as a top health and economic threat globally (World Health Organization: WHO, 2023). Therefore, treating antibiotic-resistant bacteria in the future will not come at the expense of simply penicillin only. However, prioritising public health, prevention of infection and a controlled use of antibiotics could minimize the impact and most importantly, save lives. In underdeveloped countries like Bangladesh, public health has been a major concern. In 2023, Bangladesh was ranked as the most polluted city in the world (CREA – Centre for Research on Energy and Clean Air, 2025). Nevertheless, vectors like flies and mosquitoes added with the densely populated aspect of Dhaka city multiplies the transmission rate of infectious agents by many folds.

Houseflies or *Musca domestica* is a synanthropic species that thrives in close proximity to humans and mammals. Hence, these are found in abundance in locations, such as, fish markets,

street food, slaughterhouses, farms, raw markets, hospitals and clinics etc. Microorganisms are carried by its exoskeleton, appendages and alimentary canal as it travels places and consumes, regurgitates and/ or defecates the bacteria. Although a housefly may seem tiny to the human eye, the presence of intricate detail in its delicate exoskeletal structures, like bristles, allow the carrying microorganisms to have a greater surface area for adhesion (Gioia et al., 2022).

Houseflies are infamous for spreading several enteric communicable diseases, including salmonellosis, shigellosis, cholera as well as some skin infections (Shehata et al., 2024). As we are dealing with the public health concern of houseflies as a physical vector for the transmission of bacteria, several bacteria were anticipated to be present in the samples. For example, *E. coli, K. pneumoniae, P. aeruginosa, S. aureus, Shigella, Salmonella, V. cholerae, V. parahaemolyticus* etc. (Gioia et al., 2022). Among these, the World Health Organization (WHO) has listed *Enterobacteriacea* and *Pseudomonas aeruginosa* as "critical priority" organisms, whereas *S. aureus* as "high priority" organism based on the urgent necessity of a new antibiotic drug to treat its multi-drug resistant infections in the healthcare sector (World Health Organization: WHO, 2017). According to Banna et al. (2021), approximately 30 million people every year suffer from gastrointestinal tract infections in Bangladesh while the most common causative agents are *E. coli, Salmonella* and *S. aureus*.

Escherichia coli (*E. coli*) are Gram- negative bacteria which appear as safranin red short rods upon Gram staining under light microscope. These are facultative anaerobes, the feature that allows them to live in mammalian gut as normal microbiota in symbiosis (Griffiths, 2005). As this paper explores the isolation of *E. coli* only, MacConkey agar media was used as a selective as well as differential media to serve our purpose.

Klebsiella pneumoniae is another bacillus Gram- negative bacteria (Ashurst & Dawson, 2023). In Bangladesh is a significant public health concern—spanning hospitals, communities, pediatric cases, and agricultural reservoirs. In developing regions, it can cause up to 15% of community-acquired pneumonia. Distinguished by its ability to cause severe, invasive infections (e.g. liver abscesses, meningitis) even in healthy individuals. Stool carriage of the bacteria is said to be 61% in children, 81% in adults (Kar et al., 2024). *K. pneumoniae* is detected in 64% of household water and 85% of standing water. These are areas where houseflies reside and can indirectly spread the bacteria. This is concerning because it produces beta-lactamase enzymes, including Extended-Spectrum Beta-Lactamases (ESBLs) and carbapenemases, rendering many

antibiotics ineffective. Carbapenem-resistant *K. pneumoniae* (CRKP) is now considered a critical-priority pathogen by WHO. In early 2024, WHO GLASS-EAR requested urgent data collection on hvKp ST23 isolates carrying carbapenemase genes (World Health Organization, 2024).

Staphylococcus aureus or *S. aureus* is a Gram-positive, facultative anaerobic bacteria that appears as purple cocci when viewed under light microscope after Gram-staining. It is widely found in the environment and is a normal part of human flora, especially on the skin and in the nasal mucosa of healthy people. They are capable of causing a wide spectrum of diseases from localised infections to life-threatening systemic ones (Sato et al., 2019). For *S. aureus* isolates, mannitol salt agar or MSA which is a selective and differential media was utilised.

Pseudomonas aeruginosa or P. aeruginosa are Gram-negative, aerobic and non-spore forming bacteria which also emerge as safranin red short rods upon Gram staining under light microscope (Wilson & Pandey, 2023). Prevalent among human or animal-impacted environments, they are a potent opportunistic pathogen responsible for many ailments and deaths in immunocompromised hosts. They are especially difficult to treat due to their various antibiotic resistance mechanisms and multicellular biofilms (Diggle & Whiteley, 2019). For this organism, cetrimide agar media, a selective and differential media, was used to isolate *P. aeruginosa.*

1.2. Literature Review

Several different countries across the globe have explored the bacteriological profile of housefly specimens, including Nigeria, Iraq, South Africa, the USA and many regions of Southeast Asia. Antibiogram and virulence gene identification were also included in some of the studies to provide a conclusive picture in terms of public health risks.

In Bangladesh, only two independent research publications could be traced; one in Dinajpur and the other in Mymensingh district. The Hajee Mohammad Danesh Science and Technology University, Dinajpur carried out a similar project spanning from June 2019 to July 2020. To observe the microbiological and antimicrobial susceptibility profile, both the exoskeleton as well as the digestive tract of *Musca domestica* were considered. From the exterior of a total 140 housefly samples, 63 isolates were selected (Zuhora et al., 2023). Among which, *E. coli* (19.04%), *Pseudomonas spp*. (7.93%), and *Salmonella typhimurium* (15.87%) were confirmed through molecular detection. The antibiotic susceptibility analysis revealed that 100% of the

isolates were consistently resistant to erythromycin, gentamicin, and bacitracin, whereas 80% were resistant to kanamycin and methicillin. On the other hand, 100% of the 63 isolates were sensitive to ciprofloxacin, chloramphenicol, and azithromycin, along with 85.71% being sensitive to tetracycline and amoxicillin. Another study by Bangladesh Agricultural University, Mymensingh during July to December 2018 collected a total of 140 housefly samples resulting in the detection of 275 isolates. Out of 275 isolates, 110 were *Staphylococcus aureus* (40%), 93 were *Salmonella spp*., (33.82%), and 72 were *E. coli* (26.18%). The antibiogram study revealed that majority of the isolates were highly resistant to erythromycin, tetracycline, penicillin, and amoxicillin, whereas sensitive to ciprofloxacin, ceftriaxone, chloramphenicol, gentamicin, and azithromycin (Akter et al., 2020). In conclusion, both the studies characterised its isolates to be perpetually resistant to the antimicrobial effects of erythromycin. On the bright side, the studies converge in terms of its isolates being sensitive to ciprofloxacin, chloramphenicol, and azithromycin invariably.

In the context of South Asia, a systematic review and meta-analysis done by Monyama et al. (2021) explored several different studies and their outcomes. A study in India (1992) revealed the presence of 19.5% *E. coli*, 21.2% *S. aureus* and 4.4% *P. aeruginosa*. A similar study replicated in India (1992) observed 18.8% *K. pneumoniae* in housefly samples. In a Northern Indian study in 2025, they isolated *E. coli* (ESBL-producing) from poultry farms, which was around 50% to 70% ampicillin and/tetracycline, falling under the MDR category (Odetoyin et al., 2020). Another newer study in Nepal (2017) reported 8% and 4% of PCR confirmed *E. coli* and *S. aureus* isolates respectively.

Within the continent, several South-East Asian studies were also evaluated by researcher Monyama et al. (2021). A study in Japan (2013) confirmed the isolation of 45.1% *E. coli* from housefly samples (*Musca domestica*). However, the updated study published by the same researcher in 2015 reported a reduced number of *E. coli* isolates (17.6%). Similarly, a study in Thailand (2014) confirmed the presence of 6.96% *E. coli*, 0.96% *S. aureus* and 7.2% *P. aeruginosa* through molecular identification. This study had quite a unique finding as every other study observed a higher percentage of *S. aureus* than *P. aeruginosa*, except for this one in particular. In contrast, another newer study conducted in Thailand in 2019 found that 100% of their selected isolates were, in fact, *E. coli*. In a study carried out in China by Cao et al., (2013) in China found that all their isolates were resistant to at least eight antibiotics. All of the isolates

showed resistance to amoxicillin, ticarcillin, cephalothin and cefuroxime while all were susceptible to meropenem and imipenem. A range of resistance was also observed to antibiotics among which some notable ones are ceftazidime 1 (93.8%), piperacillin (93.8%), ciprofloxacin (54.2%), gentamicin (45.8%), ceftazidime (22.9%), amikacin (16.7%), amoxicillin-clavulanate (6.3%) and piperacillin-tazobactam (2.1%) among many others. All their *E.coli* isolates showed resistance to amoxicillin, piperacillin, ticarcillin, cephalothin, cefotaxime and cefuroxime while none were resistant to amoxicillin-clavulanate, piperacillin-tazobactam, meropenem and imipenem.

Internationally, especially in Africa, similar issues have been examined in a number of works in addition to the Bangladeshi studies. Noteworthy of which was conducted in South Africa, Monyama et al. (2023) used next-generation sequencing (NGS) to characterise the bacterial population in houseflies collected from hospice contexts. They discovered dominating genera like *Streptococcus*, *Escherichia-Shigella*, *Enterobacter*, and *Providencia*. Crucially, they found important resistance genes like *ermB, tetA, blaSHV*, and *blaTEM*, which raise serious concerns about flies serving as antibiotic resistance vectors in clinical settings and may indicate horizontal gene transfer. Additionally, Odetoyin et al. (2020) studied bacteria on the surface of houseflies that were gathered from slaughterhouses, hospitals, restaurants, and garbage areas in Akure, Nigeria. From 25 houseflies, they found 67 isolates of *Staphylococcus aureus*, *Salmonella*, *Klebsiella pneumoniae*, and *E. coli*, the majority of which were multidrug resistant.

1.3. Objective

Although there were previous studies on bacteriological and antibiogram analysis of housefly samples in several countries as well as Bangladesh, ours are distinct on several terms: **(i)** we focused on Dhaka city, one of the most polluted, busy and densely populated cities in the world, where public health is a concern; **(ii)** sample collection was done from a wide range of locations encouraging the inclusivity of diverse outcomes; **(iii)** our study was not only limited to bacteriological analysis but also the antibiogram profile of them, that is, we explored the cause as well as the potential cure. Therefore, marking the novelty of our study.

This paper will aim to isolate several of the leading infection causing bacteria from housefly samples around Dhaka city and its antibiotic susceptibility profile. The main objective being identification of any antibiotic resistance patterns and raising public awareness about it.

Chapter 2

Methodology

2.1. Sample collection and processing

A total of 36 housefly samples were collected between January- February 2025. Our sample collection was limited within Dhaka city only, however, diversity of locations was ensured by collecting samples from three different locations: fish markets (n=2), street foods (n=2) and residential areas (n=2) within each area. Samples were collected from Badda (n=6), Uttara (n=6), Tejgaon (n=6), Gulshan (n=6) and Hatirjheel (n=6) areas considering all three locations within each area.

Housefly samples were collected from fish markets, street food shops and residential areas by tying clear polythene bags around a metal fly catching bat. Collected flies were transferred into sterilized zip lock bags, labelled appropriately, and brought to the BRAC University thesis laboratory using ice boxes within 2 hours of sample collection. Personal protective equipment, for example, wearing hand gloves and masks were maintained thoroughly.

Once the samples were carried to the laboratory, the housefly samples were transferred into 10ml of autoclave sterilised Phosphate Buffered Saline or PBS buffers in each falcon tube with soft hands using flame sterilized forceps. This step was done under sterile conditions under a laminar air flow to avoid any possible contaminations.

Figure 2.1. Houseflies immersed in sterilized PBS buffers.

The falcon tubes were vortexed to loosen and detach the microorganisms attached to its body parts into the PBS buffer. Thus, a suspension of the microorganisms that was carried on the fly's

exoskeleton could be obtained. The resulting suspensions were spread plated under sterile conditions on MacConkey, Mannitol Salt, Cetrimide and Nutrient agar media. Nutrient agar was used as an all-purpose media to promote the growth of all kinds of microorganisms present in that particular sample, including fastidious organisms. On the other hand, MacConkey agar was used to selectively promote the growth of Gram-negative bacteria only. Besides, MacConkey agar media is also a differential media. Therefore, it also differentiates between the bacteria growing on it based on the pH of their metabolic by-products. The differentiation could be visually identified due to the presence of methyl red, a pH indicator, as an ingredient of the media composition. Therefore, **(i)** a presumptive identification of *E. coli* and/or *K. pneumoniae* based on color change could be obtained, **(ii)** ease in picking pure isolated colonies for downstream steps. For *S. aureus* mannitol salt agar or MSA, which is a selective and differential media that gives yellow colonies due to phenol red, which shows a colour change as the isolates ferment to produce acid and decrease the pH. It also contains a high concentration of sodium chloride, NaCl salt which inhibits the growth of most bacteria but enables halophilic *S. aureus* to multiply (Shields & Tsang, 2006). The media cetrimide contains components that promote the production of *P. aeruginosa'*s characteristic pigments blue-green pyocyanin and the yellow-green pyoverdine which glows under UV so this was selected *P. aeruginosa* isolates.

In general, for medium and large sized petri dishes, 20-25 ml and 25-30 ml media were poured respectively. Similarly, a standardised amount of the sample suspension was inoculated with a microliter pipette. That is, for medium and large sized petri dishes, 80µl and 100µl sample suspensions were used respectively. If we were to keep track of the CFU (colony forming unit) count for each sample, these measurements would have been significant in terms of the calculation. However, since in the majority of our collected samples, the observed growth appeared to be TNTC (too numerous to count) even at 10^8 fold dilution, the CFU of each sample could not be measured coherently. Optimum incubation conditions, that is, 37°C temperature for 24 hours was ensured for an optimum bacterial growth.

2.2. Sample Storage and Revival

The sample suspension, along with the housefly immersed in it, was stored in a 4°C freezer for a week. The falcon tubes were discarded after a week as the bacterial load increased, proving it to be inconsistent with the original load (Pil, 2025). The bacterial colonies picked from

MacConkey, mannitol salt and cetrimide agar media were streaked on nutrient agar (NA). The petri dishes were incubated at 37°C for 24 hours.

Soft agar media were prepared, transferred into glass vials, autoclave sterilized, and allowed to solidify at room temperature. The NA-grown pure colonies were picked with a flame-sterilised needle and stabbed into the sterilized soft agar vials. Each bacterial isolate was designated to one vial only. Each time the needle was flame sterilized to pick up a pure colony to stab at least three times for each individual isolate. Following inoculation, the caps were put slightly loose so that any gas produced during incubation can escape without breaking the glass vials. All of these were done inside a laminar air flow hood to ensure sterile conditions and avoid contamination. The vials were incubated in an upright position at 37°C for 24-48 hours to ensure an optimum growth condition. After 24-48 hours, the vials were taken out of the incubator and brought to the laminar air cabinet carefully. Each vial cap was undone to place 200µL of autoclave sterilized glycerol using a microliter pipette carefully. The caps were put back on and the necks of the vials were sealed by wrapping parafilm paper around. Finally, the vials were stored at room temperature in an upright position.

Figure 2.2. Sample isolates stored in soft agar media vials.

In case of future requirements of the soft agar stored isolates, the vials were brought back inside the laminar air flow cabinet. The parafilm wrap was taken off and a flame sterilized needle was inserted along the previously inoculated stab line. The needle was then used to streak onto MacConkey, mannitol salt or cetrimide agar media, in accordance with the selected isolates and incubated at 37°C for 24 hours. The following day, pure isolated colonies from the aforementioned media grown isolates were restreaked on NA media. Similarly, the petri dishes were incubated 37°C for 24 hours. After 24 hours, the plates were taken out of the incubator and our required isolates were successfully revived. Alternatively, the stored samples were directly

streaked onto NA media for revival. However, to eliminate any possibilities of the presence of contaminants, the isolates were first streaked on its base agar media.

2.3. Molecular Identification

Although our selected isolates grew into pink colonies on MacConkey agar media, this cannot confirm the presence of *E. coli* only as many other Gram-negative bacteria also appear similar, for example, *K. pneumoniae*. Similarly, *S. aureus* growth on mannitol salt agar can only be 94% confirmed as *Staphylococcus haemolyticus* or *saprophyticus* appear similar on MSA (Kateete et al., 2010). *Pseudomonas* fluorescens can glow under UV light or *Pseudomonas putida* can show similar morphology as *P. aeruginosa* on cetrimide agar (Gholami et al., 2016). Besides, misinterpretation in crowded plates or color change later on stored refrigerated plates may occur. Biochemical tests were also ticked-off of our protocol as these tests could not provide satisfactory specificity, and demanded added labour and time. In contrast, molecular approaches, like PCR amplification and agarose gel electrophoresis (AGE) could provide sensitivity, specificity as well as time efficiency (Luca, 2025). Molecular approaches can also identify closely related species within the phylogenetic tree which cannot be done through preliminary biochemical tests. Therefore, PCR amplification and AGE was included in our protocol due to its overall alignment with our project goals.

2.3.1. DNA Extraction

Before we move forward to identify an organism, first, we need to extract the DNA from intact cells. Since our samples are bacterial cells, we have a cell wall and a cell membrane to disrupt in order to release the intracellular content out. Moreover, as bacterial cells are prokaryotes, the release of the cellular contents will simultaneously release the bacterial genome or DNA. Therefore, there is no need for additional disruption of nuclear membranes like eukaryotic cells, for example, fungi or human cells.

Boiling method was used to extract DNA from intact bacterial cells grown on nutrient agar media. This method yielded satisfactory outcomes to move forward with our project, because: **(i)** it provided us with a large amount of DNA, **(ii)** it was inexpensive and incorporated mechanical disruption of bacterial cells avoiding the use of costly chemical reagents, and **(iii)** this method allows contamination, hence, we cannot obtain highly pure DNA. However, the purity of DNA

would not affect our downstream experiments, that is, PCR (Polymerase Chain Reactions) amplification and AGE (Agarose Gel Electrophoresis). The reason is, these experiments do not require highly pure DNA content like some other molecular experiments, for example, cloning and RFLP (Restriction Fragment length Polymorphism).

The boiling method was used to extract DNA from all 204 isolates. An optimised protocol adjusted by the BRAC University Microbiology thesis lab was used. The protocol that was followed is attached below.

Step 1: A loopful of colony (1µl) was introduced in 400µl of TE buffer in an MCT.

Step 2: The suspension was vortexed for 15 seconds.

Step 3: The mixture was centrifuged at 13000 RPM for 10 mins at 25°C.

Step 4: The supernatant was discarded and the pellet was resuspended with 400µl TE buffer.

Step 5: Step 2 was repeated.

Step 6: The MCTs were put in heat blocks at 100°C for 10 minutes.

Step 7: The MCTs were kept at room temperature for 5-10 minutes.

Step 8: Step 3 was repeated.

Step 9: The supernatant was transferred to a new MCT.

Step 10: The obtained DNA extracts were stored at -20°C freezer.

Tris-EDTA or TE buffer was used as Tris maintains the osmotic balance while EDTA, as a chelating agent, sequesters divalent metal ions, like Mg^{2+}, which act as the cofactor of nuclease enzymes. Therefore, the TE buffer maintains the overall integrity of the stored content, while -20°C slows down enzymatic activity.

2.3.2. Polymerase Chain reaction (PCR)

PCR is a molecular technique that incorporates a mixture of reagents along with a template DNA in a thermal cycler machine to yield amplified numbers of DNA segments. The following reagents were mixed to produce the generalized PCR mix for every sample. The extracted DNA for each isolate was added into each PCR tube labelled accordingly. For the positive control, the extracted DNA of a previously molecular confirmed positive DNA sample was added in the same amount as our test DNA samples, whereas, in negative control, PCR mix, master mix or NFW of the same amount was added to reach the homogenous volume requirement. The positive control was incorporated to ensure that the mixture was working and was useful to produce a

meaningful amplification, that is, identify false-negative outcomes due to experimental or personnel error. On the other hand, the negative control was brought into the setting to ensure that there is no contaminating DNA, that is, to identify false-positive results due to prior contamination. The reagents as well as the extracted DNA were kept in ice block MCT holders throughout the experiment to avoid denaturation or non-specific binding possibilities within the respective tubes.

Once the PCR mix was prepared, the PCR tubes were subjected to a short spin to break down any bubble formed during the process of preparation. After ensuring that the suspensions were devoid of any bubbles and homogenous in volume and condition, the PCR tubes were loaded into the thermal cycler swiftly. The following conditions were set on the screen of the thermal cycler based on optimisation of BRAC University thesis laboratory conditions and employed resources.

Figure 2.3.2. Pre-programmed PCR conditions for ECO and NUC primer.

Table 2.3.2(c). Primer sequence & band size.

Primer	Direction	DNA Sequence	Band size (bp)
ECO	Forward	5'-GACCTCGGTTTAGTTCACAGA-3'	585 (Punom et al., 2020)
	Reverse	5'-CACACGCTGACGCTGACCA-3'	
Pf/ Pr2	Forward	5'-ATTTGAAGAGGTTGCAAACGAT-3	260 (Osman et al., 2020)
	Reverse	5'-CCGAAGATGTTTCACTTCTGATT-3'	
NUC	Forward	5'-GCGATTGATGGTGGATACGGT-3'	275-280
	Reverse	5'-AGCCAAGCCTTGACGAACTAAAGC-3'	
PASS	Forward	5'-GGGGGATCTTCGGACCTCA-3'	956
	Reverse	5'-TCCTTAGAGTGCCCACCCG-3'	

Finally, clicking the “start” option sets the reaction in motion. The duration showed 1.5-2.5 hours based on the cycle time length, number of samples loaded and the thermal cycler we used for that particular round.

Table 2.3.2(b). PCR conditions for amplification using appropriate primers.

Primer	**Temp./ Time**	***ID**	***D**	***A**	***E**	***FE**	***FH**	**Cycle number**
ECO	**Temp. (°C)**	95	94	58	72	72	4	30
	Time	3 min	45 sec	45 sec	1 min	3 min	∞	
Pf/ Pr2	**Temp. (°C)**	94	94	57	72	72	4	35
	Time	10 min	30 sec	20 sec	20 sec	10 min	∞	
NUC	**Temp. (°C)**	95	95	57	72	72	4	32
	Time	5 min	40 sec	30 sec	45 sec	10 min	∞	
PASS	**Temp. (°C)**	95	94	58	72	72	4	30
	Time	5 min	30 sec	30 sec	35 sec	2 min	∞	

***ID= initial denaturation; D= denaturation; A= annealing; E= elongation; FE= final elongation; FH= final hold.

After the reaction was completed, the PCR tubes were carefully taken out and the PCR products were subjected to agarose gel electrophoresis (AGE) in order to visualise the length of the amplicons.

Table 2.3.2(a). PCR mix preparation in correspondence to suspected organisms.

Suspected Organism (Primer name)	Reagents (µl)					Total volume (µl)
	Master mix	NFW	Forward Primer	Reverse Primer	Template DNA	
***E. coli* (ECO)**	6	3	1	1	2	13
***K. pneumoniae* (Pf/ Pr2)**	7.5	2.5	0.5	0.5	2	13
***S. aureus* (NUC)**	6.5	3.5	0.5	0.5	2	13
***P. aeruginosa* (PASS)**	7	4.5	0.75	0.75	2	15

2.3.3. Agarose Gel Electrophoresis (AGE)

The PCR itself would not hold any value in terms of interpretation of results. Thus, agarose gel electrophoresis is carried out to visually observe the amplicon length and compare it to the corresponding desired length. To carry out this experiment, at first agarose gel was prepared by mixing agarose powder (1.5- 1.7g) with 100 ml 1M TBE buffer. Similarly, agarose powder (1.5-1.7g) can also be added to 2 ml 50 M TBE buffer and 98 ml distilled water. The concentration of agarose was optimised on the basis of our primer band size. For instance, in the case of AGE of PASS PCR amplicons (band size= 956 bp), the 1.5% agarose gel concentration was used to avoid band smearing. In contrast, 1.7% agarose concentration was used to perform the AGE of NUC PCR amplicons (band size= 275=280 bp). Therefore, differentiation of smaller DNA fragments takes place distinctively under UV light visualisation. Either way, the mixture is microwaved until it heats up to turn clear from its cloudy appearance at room temperature. The conical flask was constantly swirled to avoid the characteristic three dimensional matrix conformation at molecular level before pouring. Once the solution cools down considerably, 4µl ethidium bromide (EtBr), a DNA intercalating agent, was added to the solution using micropipette. The conical flask was swirled again for a couple of minutes to solubilise the added reagent to finally

pour into the casting tray previously set by putting the comb in. The gel was allowed to cool down and set into its porous three dimensional conformation. Once set, the combs were carefully pulled out of the solid gel and gel was transferred to the gel electrophoresis apparatus. The gel was then immersed by pouring a 1 M TBE buffer as the running buffer in the Biometra apparatus.

Once the agarose gel was set and immersed in a running buffer, the PCR amplicons were loaded into the wells one by one. The leftmost well was designated to load 6µl of 100 or 50 bp ladder (BioLabs- Purple ladder) depending on the band size of the corresponding primer. The following two wells were loaded with positive and negative controls, 5µl each. And the rest were loaded with 5µl PCR products of our samples. The sequence of loading the samples was kept track of by noting them down in a notebook. The electrodes were placed in alignment and lastly, 500 mA and 100V was set for 40 minutes before starting the electrophoresis. The sample DNA ran from the negative electrode towards the positive. The porous conformation of the agarose gel differentiates the DNA fragments on the basis of its size.

After the electrophoresis was completed, the agarose gel was placed under a UV illuminator. The UV light was turned on after sealing the cover properly. The DNA bands were visualized in bright orange stains under UV illumination by EtBr as the DNA amplicons were intercalated by it. The band size was visually observed by comparing with the ladder column and the migration of the positive control.

2.4. Antibiotic Susceptibility Testing (AST)

Antibiotic susceptibility testing (AST) was done by the standard Kirby- Bauer or disc diffusion method. In this method, Mueller Hinton agar (MHA) media is used due to its porous composition in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2023). This particular feature allows the diffusion of antibiotic discs placed on the media to provide standardized outcomes. The autoclave sterilized MHA media are prepared and poured to solidify in petri dishes inside laminar airflow cabinets. Then a small amount of sample isolate grown on NA media is picked up using a loop and added into a 10 ml autoclaved saline solution in the test tube and vigorously vortexed to form a homogenous solution. The suspension was then visually compared with 1.0 McFarland standard solution to ensure an optimum bacterial concentration for the experiment. An autoclave sterilised cotton swab was dipped into the

prepared bacterial solution, squeezed out the excess by pressing against the inside of the test tube, and used to lawn onto the surface of the MHA media. The lawning was done as uniformly as possible followed by the placement of the antibiotic discs using a flame sterilized forceps. The petri dishes were incubated at 37°C temperature for 16-18 hours. After 16-18 hours, the plates were taken out of the incubator and the bacterial zone of inhibition (ZOI) around the antibiotic discs were measured using a ruler in millimeters (mm). To determine the sensitive, resistant or intermediate status of that particular sample for the employed antibiotics, the ZOI were compared with the CLSI guideline chart for AST. Any microorganism showing resistance to three (3) or more antibiotics of different classes will be classified as Multi-Drug Resistant (MDR). The list of antibiotics that were used along with the antibiotic content is presented in the table below.

Table 2.5. Antibiotics used and its content.

*Antibiotic	Content (µg)	Brand name	Class of Drug	Inhibits the Biosynthesis of (Tortora et al., 1956)		
				Cell wall	Protein	*NA
AMC	30	Oxoid	Penicillin- like	✔		
AMX	30	HiMedia	Penicillin- like	✔		
AK	30	HiMedia	Aminoglycoside		✔	
AZM	15	Oxoid	Macrolide		✔	
CFM	5	HiMedia	Cephalosporin	✔		
CIP	5	HiMedia	Fluoroquinolone			✔
CRO	30	Oxoid	Cephalosporin	✔		
DO	30	Oxoid	Tetracycline		✔	
MEM	10	Oxoid	Carbapenem	✔		
VA	30	HiMedia	Polypeptide	✔		

*AMC= amoxiclav; AMX= amoxicillin; AK= amikacin; AZM= azithromycin; CFM= cefixime; CIP= ciprofloxacin; CRO= ceftriaxone; DO= doxycycline; MEM= meropenem; VA= vancomycin.

*NA= nucleic acid

Chapter 3

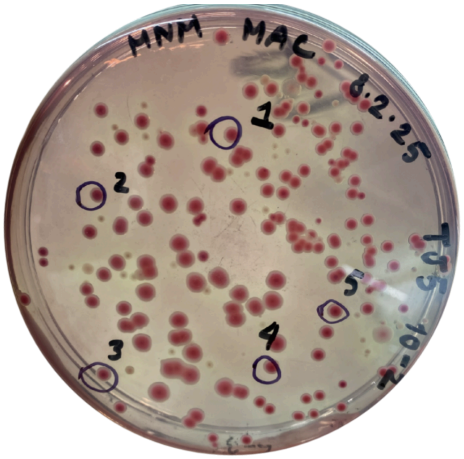
Results

3.1. Bacteriological Analysis

3.1.1. Culture and Selection of Isolates

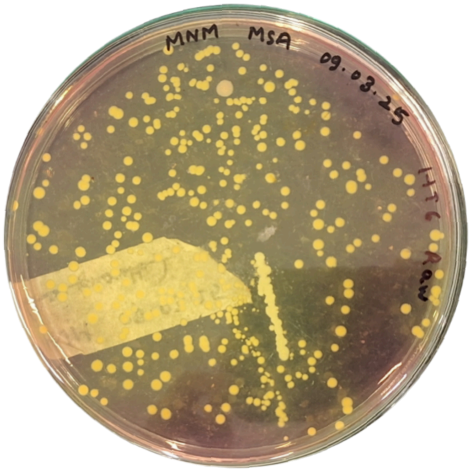
Initial selection of the suspected isolates was based upon visualisation of colony morphology and color produced on selective media. Around 2 to 5 suspected isolates were selected from each plate. In total, 311 isolates were selected, however, of these 311 we are reporting on 204 isolates. That is excluding any thiosulfate citrate bile salts sucrose agar (TCBS) or xylose lysine deoxycholate agar (XLD) grown isolates for the purpose of this thesis.

Figure 3.1.1(a). Suspected *E. coli* & *K. pneumoniae* on MacConkey Agar.



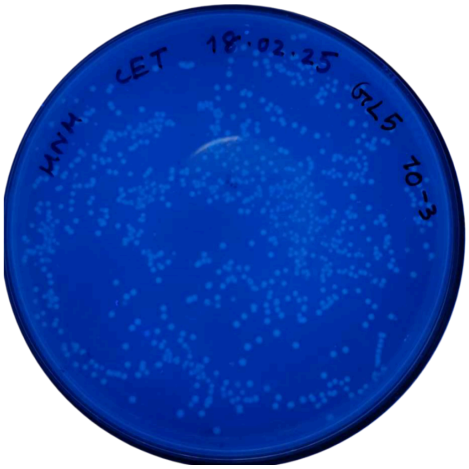
About 75 isolates were selected from MacConkey agar (MAC) plates in total. Both suspected *E. coli* and *K. pneumoniae* being lactose fermenting in nature, produced a pink color colony. However, *K. pneumoniae* colonies in general were larger in size and mucoid or glossy looking at times. While *E. coli* colonies were smaller with a brighter pink shade.

Figure 3.1.1(b). Suspected *S. aureus* on Mannitol Salt Agar.



About 74 isolates were selected from Mannitol Salt agar (MSA) plates in total. Suspected *S. aureus* colonies were salt-tolerant, producing medium-sized yellow color colonies and fermented mannitol to turn the red media surrounding the colony into yellow color as well.

Figure 3.1.1(c). Suspected *P. aeruginosa* on Cetrimide Agar under UV light.

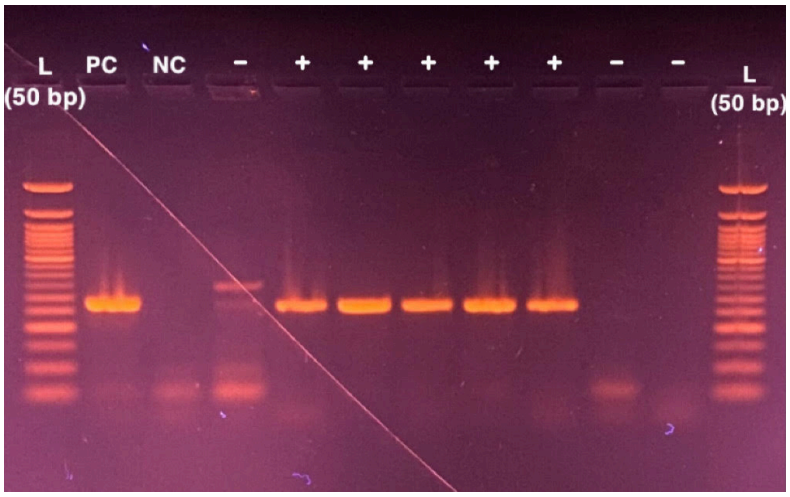


About 41 isolates were selected from cetrimide agar (CA) plates in total. These media plates only produced small white colonies. Suspected *P. aeruginosa* did not actually show any particular color change upon naked-eye visual inspection, but under UV-light, slight glow could be observed from some colonies.

3.1.2. Molecular Identification of Isolates

Molecular identification of housekeeping gene sequence in bacterial cells is considered to be the gold standard of species level bacterial identification. The reason lies in the highly conserved property of this sequence among all species. As a result, bacteria within closely positioned phylogenetic trees can also be precisely identified.

Figure 3.1.2(a). Molecular identification of *S. aureus* (Band size= 275-280 bp).

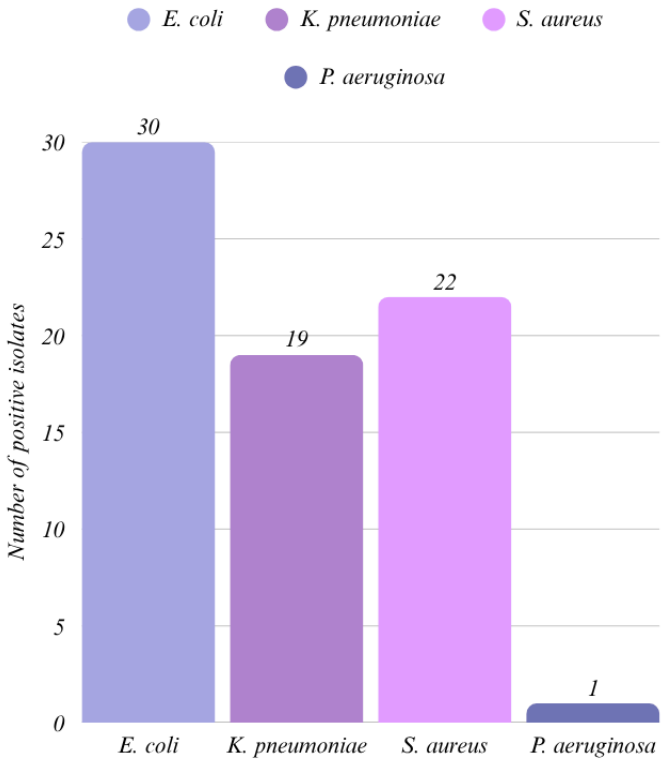


For our study, we conducted PCR and AGE under varied conditions for each primer. Our total isolate count was n=204. Among which, 30 ECO positive (14.71%)), 19 Pf/ Pr2 positive (9.31%), 22 NUC positive (10.78%) and 1 PASS positive (0.49%) isolates were confirmed through molecular identification. Therefore, our study identified housefly samples to be carrying the highest number of *E. coli* (14.71%) followed by *S. aureus* (10.78%) and *K. pneumoniae* (9.31%). The least number of bacteria isolates were identified to be *P. aeruginosa* (0.49%).

Table 3.1.2. Percentage distribution of the confirmed organisms found.

Organism Name	**Number of isolates**	**Percentage**
E. coli	30	14.71%
K. pneumoniae	19	9.31%
S. aureus	22	10.78%
P. aeruginosa	1	0.49%
Unidentified	132	64.71%
Total	204	100%

Figure 3.1.2(b). Bar chart representation of confirmed isolate numbers.



3.2. Antibiotic Susceptibility Testing (AST)

Upon the completion of PCR and gel run for the molecular identification of our isolates, our study moved on to AST or antibiogram. In the antibiogram, it was found that 42 isolates out of 44 showed complete resistance against amoxicillin. Both *K. pneumoniae* and *E. coli* showed the highest susceptibility to Meropenem with the antibiotic having the highest susceptibility among all isolates as well. Vancomycin, a narrow spectrum antibiotic for Gram-positive bacteria only also showed susceptibility to *K. pneumoniae* and *E. coli* at 1 (6.67%) and 4 (20%), respectively.

Table 3.2(a). Percentage distribution of sensitivity of all isolates.

	***S**		***I**		***R**	
***Name of Antibiotic**	***n**	**%**	***n**	**%**	***n**	**%**
AMC	30	68.2	2	4.5	12	27.3
AMX	2	4.5	0	0	42	95.5
AK	24	54.5	13	29.5	7	15.9
AZM	22	50	0	0	22	50
CFM	8	18.2	2	4.5	34	77.3
CIP	21	47.7	9	20.5	14	31.8
CRO	31	70.5	7	15.9	6	13.6
DO	31	70.5	3	6.8	10	22.7
MEM	36	81.8	1	2.3	7	15.9
VA	10	22.7	0	0	34	77.3

*S = Susceptible, I = Intermediate, R = Resistant

*AMC= amoxiclav; AMX= amoxicillin; AK= amikacin; AZM= azithromycin; CFM= cefixime; CIP= ciprofloxacin; CRO= ceftriaxone; DO= doxycycline; MEM= meropenem;

VA= vancomycin

*n = total number of isolates

Figure 3.2(a). Bar chart presentation of the sensitivity of all isolates.

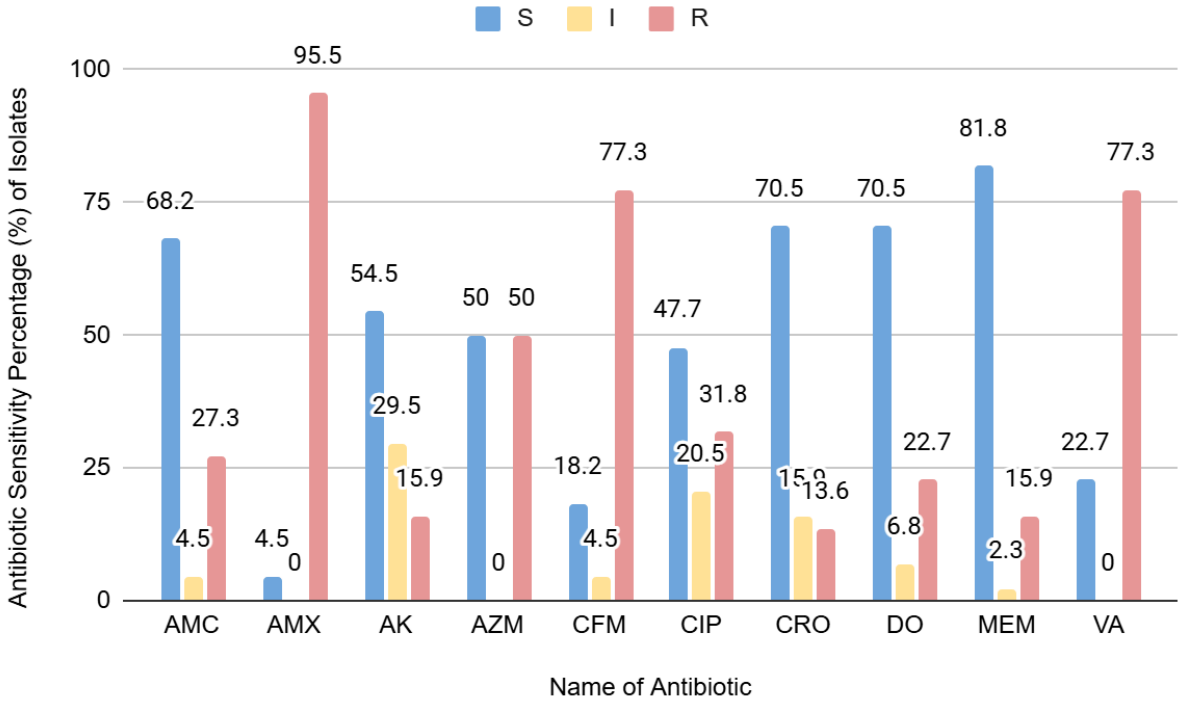


Table 3.2(b). Percentage distribution of sensitivity of *Klebsiella pneumoniae.*

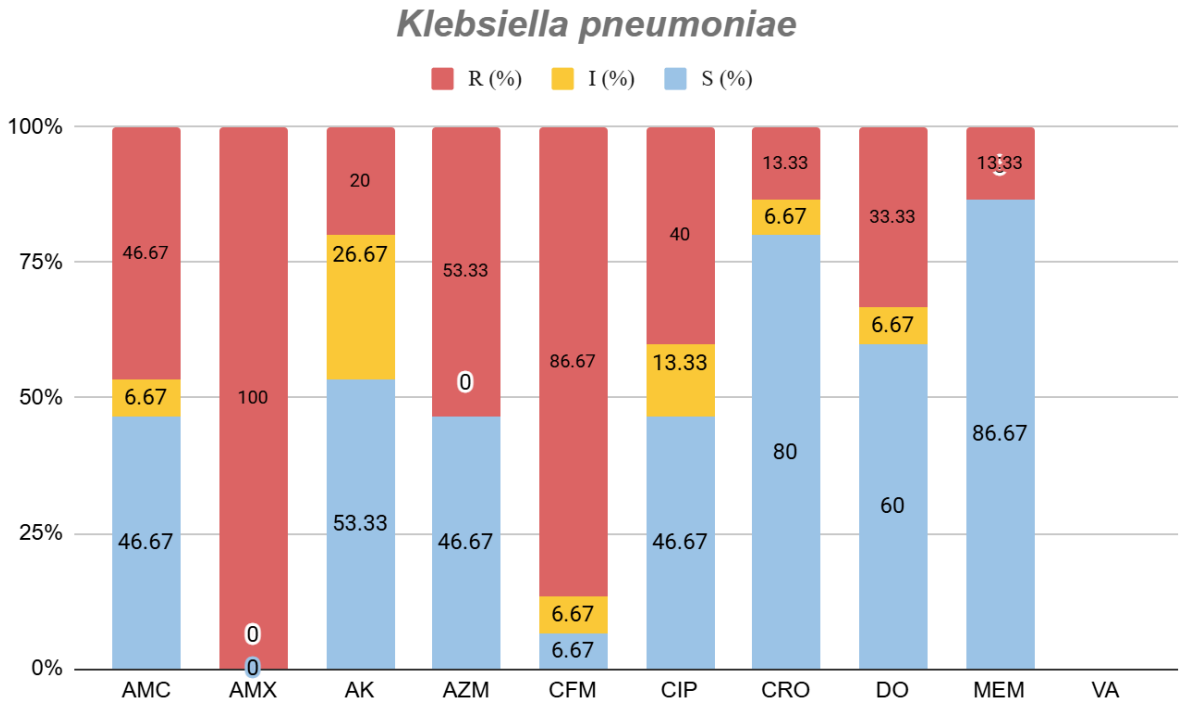
	Klebsiella pneumoniae (n=15)					
	***S**		***I**		***R**	
***Name of Antibiotic**	**Percentage (%)**	**n**	**Percentage (%)**	**n**	**Percentage (%)**	***n**
AMC	46.67	7	6.67	1	46.67	7
AMX	0	0	0	0	100	15
AK	53.33	8	26.67	4	20	3
AZM	46.67	7	0	0	53.33	8
CFM	6.67	1	6.67	1	86.67	13
CIP	46.67	7	13.33	2	40	6
CRO	80	12	6.67	1	13.33	2
DO	60	9	6.67	1	33.33	5
MEM	86.67	13	0	0	13.33	2

*S = Susceptible, I = Intermediate, R = Resistant

*AMC= amoxiclav; AMX= amoxicillin; AK= amikacin; AZM= azithromycin; CFM= cefixime; CIP= ciprofloxacin; CRO= ceftriaxone; DO= doxycycline; MEM= meropenem

*n = number of isolates

Figure 3.2(b). Bar chart presentation of sensitivity of *Klebsiella pneumoniae.*



For *K. pneumoniae,* we observed the highest resistance of 15 isolates (100%) against Amoxicillin followed by Cefixime- 13 isolates (86.67%) which was only susceptible towards 1 (6.67%). It was most susceptible towards Meropenem - 13 isolates (86.67%), followed by Ceftriaxone - 12 isolates (80%). Amikacin and Azithromycin showed approximately equal susceptible and resistance patterns against *K. pneumoniae*.

Table 3.2(c). Percentage distribution of sensitivity of *Escherichia coli.*

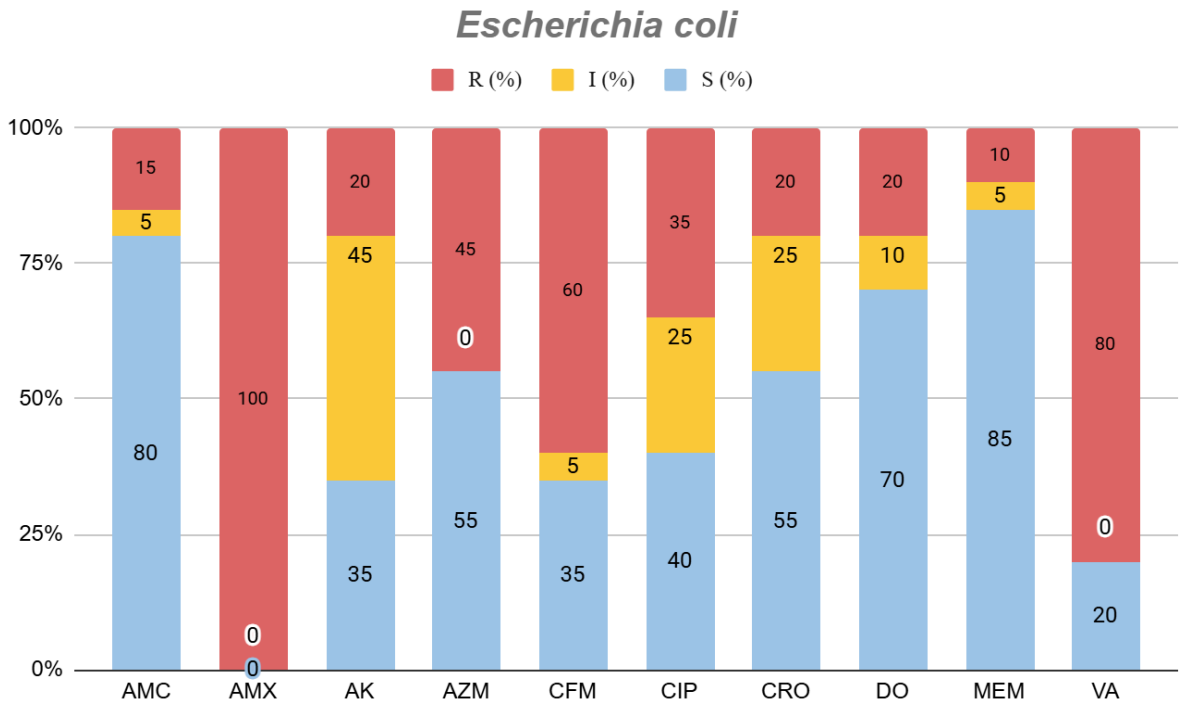
	***Escherichia coli* (n = 20)**					
	***S**		***I**		***R**	
***Name of Antibiotic**	**Percentage (%)**	**n**	**Percentage (%)**	**n**	**Percentage (%)**	***n**
AMC	80	16	5	1	15	3
AMX	0	0	0	0	100	20
AK	35	7	45	9	20	4
AZM	55	11	0	0	45	9
CFM	35	7	5	1	60	12
CIP	40	8	25	5	35	7
CRO	55	11	25	5	20	4
DO	70	14	10	2	20	4
MEM	85	17	5	1	10	2
VA	20	4	0	0	80	16

*S = Susceptible, I = Intermediate, R = Resistant

*AMC= amoxiclav; AMX= amoxicillin; AK= amikacin; AZM= azithromycin; CFM= cefixime; CIP= ciprofloxacin; CRO= ceftriaxone; DO= doxycycline; MEM= meropenem; VA = vancomycin

*n = number of isolates

Figure 3.2(c). Bar chart presentation of sensitivity of *Escherichia coli.*



E. coli showed complete resistance- 20 isolates (100%) against Amoxicillin which, just like *K. pneumoniae,* was followed by Cefixine- 12 isolates (60%). Highest susceptibility was observed by Meropenem- 17 isolates (85%) and then Amoxicillin-clavulanate or by Amoxiclav - 16 isolates (80%). *E.coli* also showed susceptibility towards vancomycin 4 (20%) although it is supposed to be effective against Gram-positive strains.

Table 3.2(d). Percentage distribution of sensitivity of *Staphylococcus aureus*

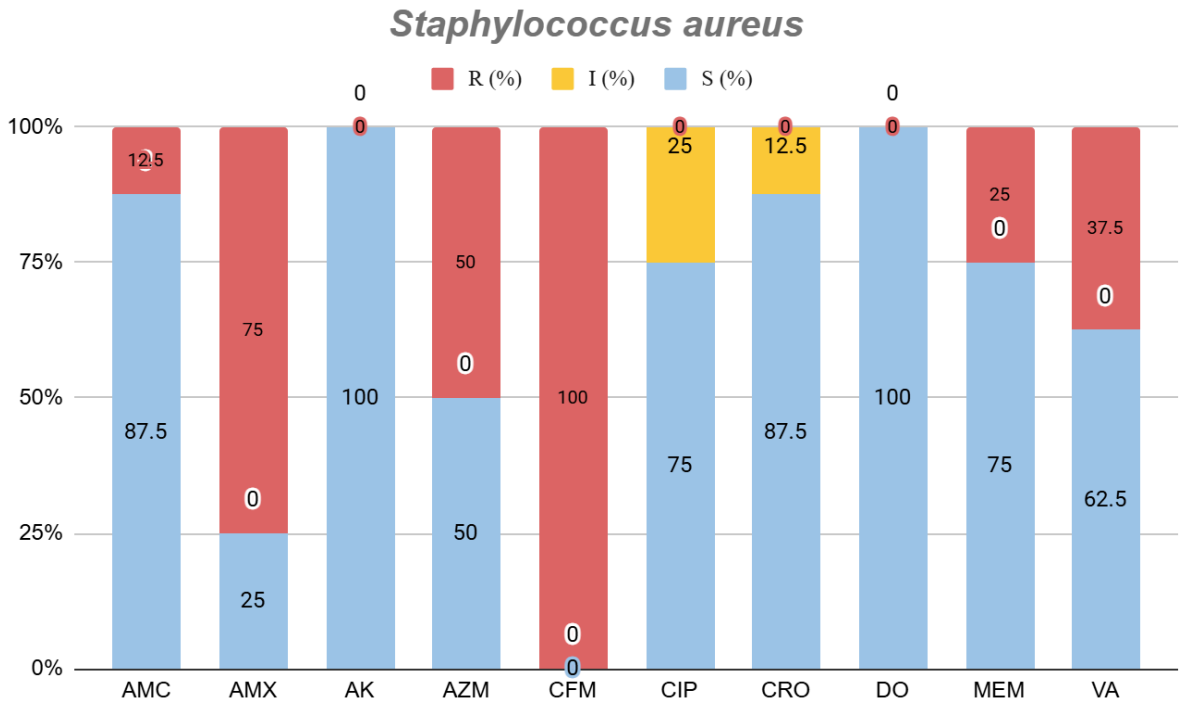
	***Staphylococcus aureus* (n = 8)**					
	***S**		***I**		***R**	
***Name of Antibiotic**	**Percentage (%)**	***n**	**Percentage (%)**	***n**	**Percentage (%)**	***n**
AMC	87.5	7	0	0	12.5	1
AMX	25	2	0	0	75	6
AK	100	8	0	0	0	0
AZM	50	4	0	0	50	4
CFM	0	0	0	0	100	8
CIP	75	6	25	2	0	0
CRO	87.5	7	12.5	1	0	0
DO	100	8	0	0	0	0
MEM	75	6	0	0	25	2
VA	62.5	5	0	0	37.5	3

*S = Susceptible, I = Intermediate, R = Resistant

*AMX= amoxicillin; AK= amikacin; AZM= azithromycin; CFM= cefixime; CIP= ciprofloxacin; CRO= ceftriaxone; DO= doxycycline; MEM= meropenem; VA= vancomycin

*n = number of isolates

Figure 3.2(d). Bar chart presentation of sensitivity of *Staphylococcus aureus.*



For *S. aureus*, our only Gram-positive isolate, all the isolates 8 (100%) were susceptible towards Amikacin and Doxycycline followed by Ceftriaxone and Amoxiclav at 7 (87.5%). It was completely resistant against Cefixime 8 (100%), followed by Amoxicillin at 6 (75%). They were also found to be equally susceptible and resistant against Azithromycin 4 (50%) and 4 (50%), respectively. For Vancomycin, *S. aureus* isolates were 5 (62.5%) susceptible and 3 (37.5%) were resistant.

Table 3.2(e). Percentage distribution of sensitivity of *Pseudomonas aeruginosa.*

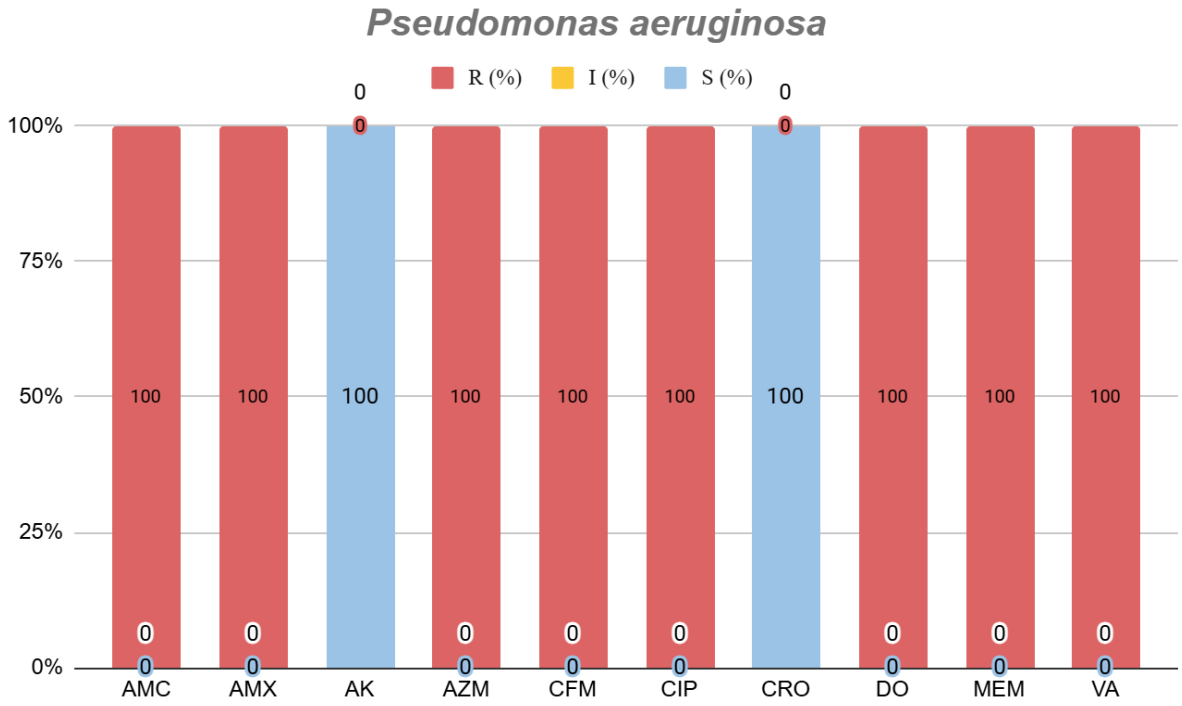
	***Pseudomonas aeruginosa* (n=1)**					
	***S**		***I**		***R**	
***Name of Antibiotic**	**Percentage (%)**	***n**	**Percentage (%)**	***n**	**Percentage (%)**	***n**
AMC	0	0	0	0	100	1
AMX	0	0	0	0	100	1
AK	100	1	0	0	0	0
AZM	0	0	0	0	100	1
CFM	0	0	0	0	100	1
CIP	0	0	0	0	100	1
CRO	100	1	0	0	0	0
DO	0	0	0	0	100	1
MEM	0	0	0	0	100	1
VA	0	0	0	0	100	1

*S = Susceptible, I = Intermediate, R = Resistant

*AMC= amoxiclav; AMX= amoxicillin; AK= amikacin; AZM= azithromycin; CFM= cefixime; CIP= ciprofloxacin; CRO= ceftriaxone; DO= doxycycline; MEM= meropenem

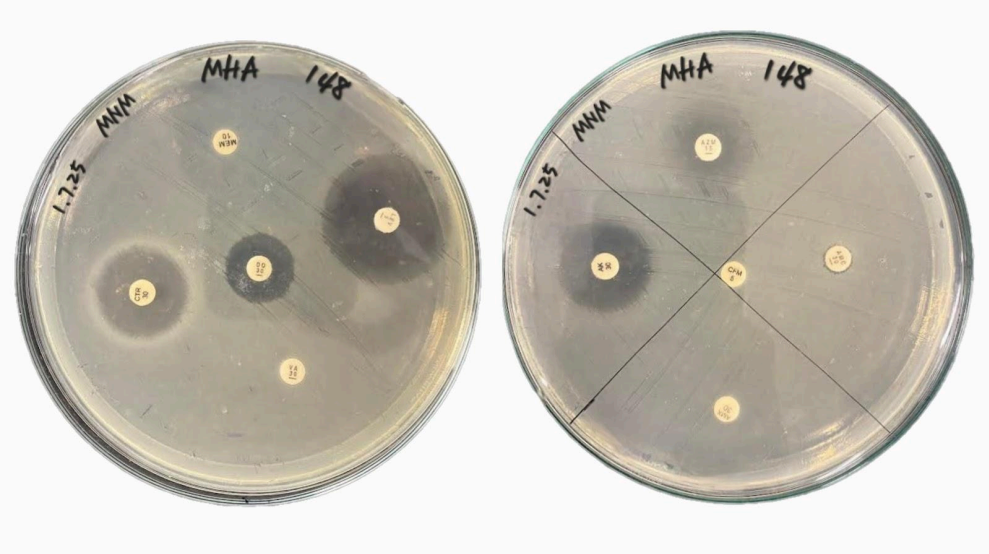
*n = number of isolates

Figure 3.2(e). Bar chart presentation of sensitivity of *Pseudomonas aeruginosa.*



For our single isolate of *P. aeruginosa*, susceptibility could only be observed towards Amikacin and Ceftrixone, with it being resistant against all the other antibiotics.

Figure 3.2(f). AST of an isolate selected from Cetrimide agar media.



Chapter 4

Discussion

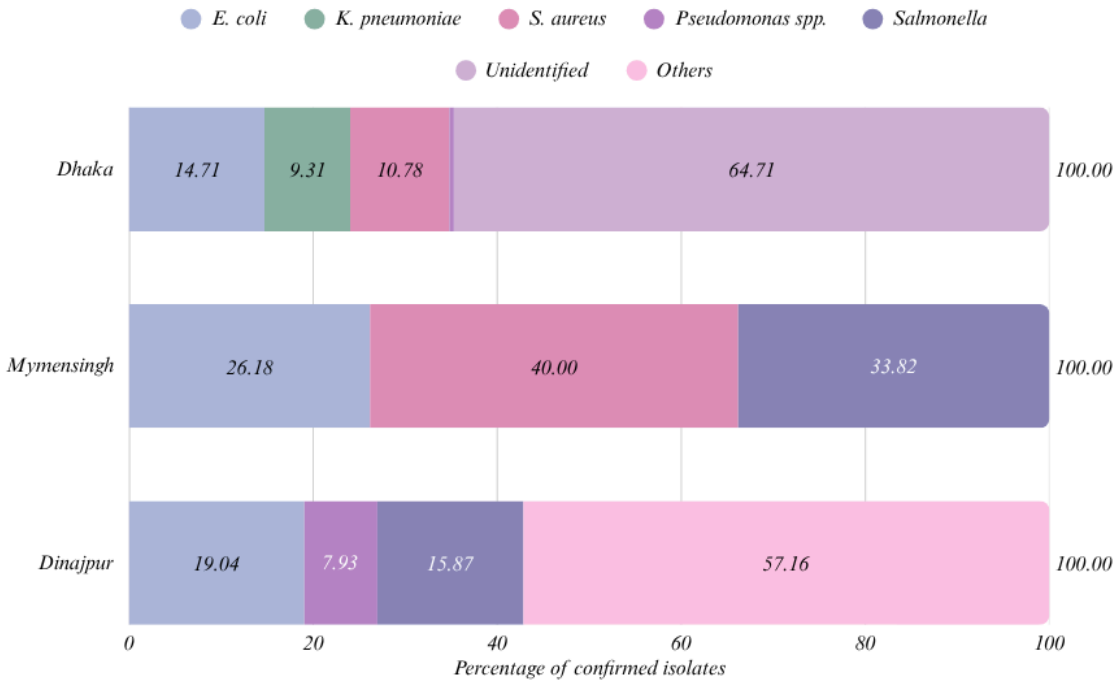
Overall, at the beginning, isolates from around 10^3 and 10^4 dilutions plates were selected and they showed the most separated (single/pure) colonies. We used media that was both selective in what it allows to grow, and differential in allowing us to distinguish between different genera or even species because it yielded faster results inexpensively (Prinzi & Rohde, 2020). Number of suspected isolates selected from each plate are as follows, 75 small pink *E. coli* and 74 large mucoid pink *K. pneumoniae* from MacConkey agar, 22 small yellow *S. aureus* that turned the media around it yellow from mannitol fermentation in mannitol salt agar and 41 small, glowing under UV light *P. aeruginosa* from cetrimide agar.

As molecular identification through housekeeping genes is considered as the gold standard for species identification, this is what we opted for to identify selected isolates in our project. To begin with, we have done PCR with ECO and Pf/ Pr2 primers for each isolate selected from MacConkey agar and nutrient agar media. Consequently, 14.71% and 9.31% of the total isolates (n=204) were positive for *E. coli* and *K. pneumoniae* respectively. Similarly, PCR was done with NUC and PASS primers resulting in our conclusion of 10.78% and 0.49% of confirmed *S. aureus* and *P. aeruginosa* respectively.

In a study conducted in Mymensingh Sadar by Akter et al. (2020), they confirmed the presence of *S. aureus* (40%) at a higher percentage than *E. coli* (26.18%). Our findings show a higher percentage of *E. coli* (14.71%) than *S. aureus* (10.78%) in housefly samples from Dhaka in 2025. However, if we compare the locations and seasonal differences in these two studies, **(i)** the study by Akter et al. (2020) collected samples from more specified locations, for example, hospitals, veterinary facilities as well as poultry and dairy farms. On the other hand, our study focused on a more generalised crowd of population in the context of Dhaka city, like street food, fish market and residential areas. Therefore, the inclusion of hospitals and veterinary facilities might have contributed to their finding a higher percentage of *S. aureus*, as several strains, like methicillin-resistant *S. aureus* (MRSA), are a global concern for secondary infections, especially in hospital settings. **(ii)** The sample collection period for Akter et al. (2020) spanned from July through December 2018 emphasising heavily on summer times in Bangladesh. Whereas, our samples were collected in January-February of 2025, during the winter months in Bangladesh.

This could have contributed to the difference in bacteriological prevalence in our environment as a whole due to the difference in temperature, humidity, and on a broader term, the ecosystem. Another study in Dinajpur conducted by Zuhora et al. (2023) confirmed the presence of *E. coli* (19.04%) as the highest number of isolates on housefly samples, while *Pseudomonas spp*. (7.93%) as the least. Their findings align with ours in this context. However, out of 204 isolates, only 1 isolate was positive for *P. aeruginosa* in our study. This can be due to our difference in primer selection. Zuhora et al. (2023) employed Ker-P primer, which amplifies the DNA segment of *P. aeruginosa* encoding for its characteristic keratinase enzyme synthesis. In contrast, we utilised PA-SS primer that amplifies a part of the housekeeping gene that is highly conserved within species. To conclude, the use of PA-SS primer is more specific in terms of species identification. Nevertheless, PA-GS primer could be used in our study to identify the presence of *Pseudomonas spp.* at genus level.

Figure 4: Stacked-Row chart representations of PCR confirmed bacteriological findings



For our study we conducted antibiotic sensitivity test (AST) or antibiogram to identify the sensitivity patterns of the isolates. We found that 42 isolates out of 44 isolates (95.5%) showed complete resistance against Amoxicillin. Both *K. pneumoniae* and *E. coli* showed the highest susceptibility to Meropenem. This aligns with a study carried out by Cao et al., (2013) in China

where they collected flies from Chengdu Shuangliu International Airport where 100% of the isolates were also detected as resistant to amoxicillin. They also found that all their isolates were susceptible to meropenem and imipenem. From their *E.coli* and *K. pneumoniae* isolates, all the isolates were susceptible to amoxicillin-clavulanate and meropenem which also lines up with our data. Moreover, in researches conducted by Akter et al. (2020) in Mymensingh city, Bangladesh and Zuhora et al. (2023) in Bangladesh, they found that most of the isolates were susceptible to ceftriaxone, ciprofloxacin, and azithromycin. In our research, although 70.5% (n=31) were susceptible to ceftriaxone, they were only 47.7% (n=21) and 50% (n=22) susceptible to the other respective antibiotics. Researcher Zuhora et al. (2023), also found that all their *E.coli* isolates were 100% susceptible to azithromycin and ciprofloxacin. However, in our study, we found them to be only 55% and 40% susceptible, respectively. Their study also found high susceptibility to amoxicillin which was completely contradicted by our findings. A study carried out by Davari et al. (2010) in Sanandaj City, West Iran isolated microorganisms, such as, *K. pneumoniae, P. aeruginosa* and *E. coli* from *M. domestica*. In this specific study, 32.5% of the isolates were resistant to the antibiotics cephalexin, chloramphenicol, ampicillin, and tetracycline. In another study carried out by Odetoyin et al. (2020) in Nigeria found that all isolates of *Pseudomonas aeruginosa*, *Salmonella* spp, and *Proteus mirabilis* were resistant to streptomycin and cotrimoxazole, augmentin and amoxicillin respectively with only 1 (9.1%) of *S. aureus* isolates being resistant to Ciprofloxcin. All their isolates were multi-drug resistant (MDR). On the other hand, we have yet to identify any microorganism as MDR. Even though *P.aeruginosa* did show resistance to multiple antibiotics, as the isolate was only one (n=1), further antibiograms with a larger number of isolates are required before any definite conclusions can be made. As for Vancomycin which showed susceptibility against *E. coli*, it was detected by Stokes et al. (2016) that incubation of *E.coli* at lower temperatures, especially at 5°C–7°C made them susceptible to vancomycin. Susceptibility could also be observed after mutagenesis as observed by Shlaes et al. (1989) and Wykes et al. (2023), but as neither of these were carried out in our study, it is difficult to determine yet the cause of susceptibility.

4.1. Limitations

Our study was not short of limitations. Firstly, uncontrollable conditions, be it rainy weather or religious holidays (Ramadan time) made timely sample collecting difficult. On top of that, due to

time constraints, a lot of the initial presumptive tests (Gram staining or biochemical) had to be scratched out of our study. Inclusion of these tests could have simplified our process of elimination while moving on to the confirmatory tests, like PCR. Additionally, our current laboratory facility is not well-equipped to conduct advanced molecular experiments, like whole genome sequencing. Moreover, many primarily selected isolates were excluded due to contamination or overgrowth on media plates, which could have affected the overall robustness of our findings. Lastly, several isolates could not be revived from stock (confirmed isolates stored in soft agar) due to fungal contamination. It is to be noted that, even after maintaining every precautionary measure, we experienced contaminations in a large number of stock samples, for example, working inside laminar air flow cabinets, wearing gloves, wiping the work station with 70% ethanol, etc. Therefore, it is very likely that due to sharing an incubator for samples from different projects, the contaminating agents were accidentally inoculated inside the incubator. As a result, a large number of sample isolates with confirmed molecular identification have been dropped off of our project.

4.2. Future Prospects

Our project has brought out a significant public health concern in light. The presence of antibiotic resistant species on the exterior of house fly samples confirms its transfer to places. Ideally, bacteria travels in fomite routes, however, houseflies provide it with a ground to fly. This project has intrigued us to explore several internal analyses. Firstly, the presence of antibiotic resistant patterns among bacteria of shared species is observed in our study. In the future, the molecular identification of virulence genes (if present) could clarify the status of these bacteria in terms of pathogenicity, infectivity and the extent of public health concern. Secondly, several antimicrobial molecules are found on the right wing of houseflies (Niode et al., 2022). The synthesis of secondary metabolites by several bacteria and the presence of bacteriophages contributes to the establishment of this attribute in houseflies. Therefore, this motivated us to examine the antimicrobial collection on its right wing at molecular level. Alongside, whether or not there is a causative factor for the antimicrobials present on the right wing could also be studied. Thirdly, the presence of other WHO characterised priority organisms, for instance, *Salmonella* and *Shigella spp.* could be included in our study (World Health Organization: WHO, 2017). Lastly, the prevalence of region specific priority organisms, for example, in the context of

Bangladesh, the presence of *Vibrio* genus and *Vibrio cholerae* could be evaluated in housefly samples, especially, during summer and from residential areas.

Chapter 5

Conclusion

Houseflies (*M. domestica*) act as a vector to many pathogenic and non-pathogenic bacteria and even resistant genes. This study focuses on the isolation of bacteria from houseflies (*M. domestica*) from different locations and areas of Dhaka city and to identify their antibiotic sensitivity profile. In a populated and polluted country like Bangladesh, where there is an abundance of houseflies almost everywhere, they pose a big threat as a carrier of many infections and diseases.

In our study, we were so far able to isolate and do the antibiogram *K.pneumoniae, E. coli, S. aureus* and *P. aeruginosa*. These were carried out through various morphological and molecular analysis and antibiotic sensitivity testing through Kirby-Bauer disc diffusion method and comparing that with the CLSI standard. As of yet, we have been able to observe a few resistant and susceptibility patterns. However, as this is an ongoing study, the detection of more isolates and the analysis of their antibiotic sensitivity and antibiotic resistant genes are yet to be done.

To combat the battle against antibiotic resistance, antimicrobial stewardship (AMS) programmes can be implemented. According to the World Health Organization, (2019), antimicrobial stewardship has been defined as a “coherent set of actions which promote the responsible use of antimicrobials.” These programs promote the usage of proper antibiotics, at appropriate dosages, for the required duration and only when required. It is aimed at reducing the misuse of antibiotics which is fueling the resistance of the microorganism and decreasing the efficacy of the antibiotics. Proper medicinal practices and education among the general public can greatly aid in this.

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