Isolation of *Klebsiella pneumoniae* from Chicken Cloacal Samples and Analysis of their Antibiotic Susceptibility Pattern from Dhaka City

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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 and Bachelor of Science in Biotechnology

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Declaration

It is hereby declared that

1. The thesis submitted is our own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. We have acknowledged all main sources of help.

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Approval

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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. Our research complies with all applicable national animal welfare regulations and guidelines to ensure the ethical treatment of animals. We also took the permission from the sellers of wet markets under the supervision of microbiology department lab protocols. No animals were harmed in the research we conducted.

Abstract

Background:

Zoonotic pathogens can be transmitted from animals to humans, which represent a significant threat to human health due to the possibility of triggering infectious disease outbreaks. This study aimed to detect the prevalence of zoonotic bacteria *Klebsiella pneumoniae* (*K. pneumoniae*) in different types of chicken from Dhaka city.

Materials and Methods:

In this study, 82 chicken cloacal swabs were collected from nine well-known wet marketplaces around Dhaka city from February 2024 to June 2024. These chickens were randomly selected from four different types of chicken, which were processed with saline water under aseptic conditions and inoculated by spreading on HiCrome KPC agar medium for isolation and identification of *K. pneumoniae*. Metallic blue-colored colonies were considered presumptive *K. pneumoniae*. Then, PCR was used to confirm *K. pneumoniae* by targeting the "16S–23S internal transcribed spacer" gene. Following that, the Kirby-Bauer disk diffusion method was then used to test for antibiotic susceptibility, and the Clinical and Laboratory Standards Institute (CLSI, 2023) guidelines were followed to interpret the antibiotic susceptibility pattern.

Results:

Among the 82 samples analyzed, *K. pneumoniae* was detected in 41 (50%) cases. Randomly selected 50 isolates underwent Antimicrobial Susceptibility Testing, where 80% of the isolates were Multiple Drug-Resistant and 50% were Extensively Drug-Resistant. Isolates showed higher antibiotic resistance to Amoxicillin, Tetracycline, Piperacillin/Tazobactam, and Ciprofloxacin, with resistance rates ranging from 55% to 95% and higher sensitivity to Meropenem and Azithromycin, ranging from 45% to 75%.

Conclusion:

Findings in this study showed a high occurrence of *K. pneumoniae* in chickens, indicating that these chickens might be an important reservoir for human and animal infections and suggesting their potential threat to food safety. So, preventive measures, including enhanced biosecurity and public education, must be strengthened to mitigate the spread of zoonotic illnesses.

Keywords: Zoonotic Pathogen, Multidrug resistance, *Klebsiella pneumoniae*, Chicken cloacal swab

Dedication

“To our beloved family”

Acknowledgement

The people and organizations listed below deserve our sincere gratitude and appreciation for their important support and advice in helping me finish this thesis. We must first express our gratitude to Associate Professor Fahim Kabir Monjurul Haque, Ph.D., our supervisor, for his unflinching support, priceless insights, and constant encouragement. Additionally, his knowledge and direction were crucial in determining the focus and calibre of this research. Md. Feroze H. Haque, Ph.D., Associate Professor and Chairperson of the Department of Mathematics and Natural Sciences at BRAC University, and Program Director and Associate Professor Nadia Sultana Deen, PhD, are also praiseworthy of our sincere gratitude for their kind cooperation throughout this study as well as for their insightful comments, constructive criticism, and helpful suggestions that significantly improved the quality of this work overall. We are appreciative of Brac University's staff and faculty for creating a supportive learning environment and giving us access to the tools we needed to finish this project successfully. The Department of Mathematics and Natural Sciences' assistance has been helpful. We owe a great deal of gratitude to the study participants who voluntarily donated their time and wisdom. Their openness to sharing their insights and thoughts has greatly improved our research. We want to sincerely thank Golam Niaz Murshidi, our mentor and Research Assistant, and Ifthikhar Zaman for helping us with every aspect of our thesis. We remained focused and resolute because of their confidence in us and ongoing encouragement. Finally, we would like to thank all the researchers, academics, and writers whose work we have cited in this thesis. Their contributions have melded our perspective and given this study a strong foundation. Lastly, we are grateful for all the support, inspiration, and direction we have received and feel privileged to have been given the chance to work on this research project.

Table of Contents

List of Tables

List of Figures

List of Acronyms

K. pneumoniae	*Klebsiella pneumoniae*
MDR	Multi drug Resistance
PCR	Polymerase Chain Reaction
XDR	Extensively Drug-Resistant
MIU	Motility Urease Test
MHA	Mueller–Hinton Agar
Bp	Base Pair
DNA	Deoxyribonucleic Acid
Min	Minute
Sec	Second
TAE	Tris-acetate-EDTA

Glossary

MDR	Multidrug resistant (MDR) is acquired resistance to at least one antimicrobial agent from three or more antimicrobial groups.
XDR	Extensive drug-resistant, or XDR, is characterized as resistance to at least one antimicrobial agent in all but two or fewer antimicrobial categories.
PCR	Polymerase chain reaction is a laboratory technique for amplifying millions to billions of copies of a given section of DNA in a short period.
Isolation	Bacterial isolation is the process of isolating one species of bacteria from a mixed culture of bacteria using various plating methods such as pouring, spreading, streaking, and serial dilution.
Pathogenicity	Pathogenicity is the characteristic or state of being pathogenic, or the propensity to cause illness, whereas virulence is the capacity of an organism to cause disease, or its degree of pathogenicity within a group or species.

Chapter 1

Introduction

Zoonotic bacteria are those microorganisms which can easily spread from animals to humans while causing a wide range of diseases. These bacteria mainly originate from different animal sources, including domestic pets, wildlife, as well as livestock. Moreover, there are different types of zoonotic bacteria and they are Salmonella, Campylobacter, Escherichia coli (particularly the O157 strain), *Klebsiella pneumoniae* (*K. pneumoniae*), and *Brucella*. Here *K. pneumoniae* is a common gram-negative, zoonotic bacterium and member of the family Enterobacteriaceae, which can be found in the environment, human and animal digestive tract easily. Nevertheless, it is mainly associated with nosocomial infections. Moreover, there are many types of chickens in poultry, but two main types are layer chickens and broiler chickens. Furthermore, poultry is one of the most popular forms of consumed meat in the world food industry because of its low cost of production and the absence of any religious restrictions on how it is consumed. In addition, in the case of providing meat and eggs, chickens are an important provider of protein; although they can also carry zoonotic bacteria, which can be harmful to humans (Abbas et al., 2024) However, chickens can shelter zoonotic bacteria like *K. pneumoniae*. Moreover, the most common transmission routes are from direct contact with infected animals, consumption of contaminated food or water, and exposure to environments which are polluted with these pathogens. Furthermore, infections that are caused by zoonotic bacteria can easily lead to significant health issues, such as gastrointestinal diseases, respiratory illnesses, and, in severe cases, systemic infections. Moreover, several symptoms like diarrhea, vomiting, and abdominal pain are common, and they can also cause long-term problems or even death.

In addition, in poultry farms, insufficient hygiene procedures can easily lead to contamination during

processing, handling, and poultry management. Moreover, these infections are especially dangerous for those people who are at risk, such as those who have weakened immune systems. Additionally, cross-contamination is possible to occur if workers neglect to follow good hygiene or if raw meat comes into contact with infected surfaces. Furthermore, improper cooking techniques or temperatures may allow these viruses to persist, endangering customers. While reducing the incidence of foodborne illnesses requires strengthening food safety procedures at each of these phases (Abebe, 2020). On the other hand, the main concern here is with these infections, which are frequently made worse by antibiotic resistance, particularly as a result of ESBLs (Extended Spectrum Beta Lactamases), which is another significant challenge here. Also, *K. pneumoniae* is becoming more resistant to antibiotics, making the treatment choices more difficult as well as raising the rates of morbidity and mortality. Adopting suitable agricultural methods, ensuring secure handling, including cooking chicken, and putting in place strong biosecurity procedures on fields are all necessary to lower the frequency of *K. pneumoniae* infections. Moreover, the growing frequency of these microorganisms emphasizes how urgently better food safety procedures and public health campaigns are needed (Morands et al., 2022).

So, therefore, the prevalence of antibiotic resistance in zoonotic bacteria is increasing day by day, which presents a major risk to public health. Humans may fall sick by consuming contaminated food or getting into direct contact with animals which have been infected due to the overuse and misuse of antibiotics in agricultural sectors. Moreover, the health of humans and animals is seriously threatened by this increasing resistance, which also makes the availability of infection treatments more difficult to use. Consequently, the main focus of the study is to detect and evaluate antibiotic resistance in *K. pneumoniae*, which is a critical food-borne zoonotic pathogen from chicken samples. Also, considering the pathogen's association with severe infections and its growing resistance to antimicrobial treatments. Furthermore, this research seeks to confront significant public health concerns as well. Not only that, the results here will help offer

valuable insights, which are for strengthening food safety protocols and also reducing the risks that are associated with *K. pneumoniae* contamination in poultry goods. Also, it is crucial to set up efficient zoonotic bacteria monitoring and control mechanisms in order to guarantee food safety, safeguard the general public's health, and stop zoonotic diseases from spreading. Furthermore, such risks can be greatly reduced by raising public knowledge and encouraging hygienic food handling and animal care practices (Ghai et al., 2022).

Chapter 2

Materials & Methods

2.1 Workflow

Sample Collection

Cloacal swabs from chickens using cotton swabs

↓

Culture on MacConkey Agar

Serially diluted sample spread on MacConkey Agar

↓

Subcultured on KPC Agar

Mucoid pink colonies were subcultured on KPC agar

↓

Subcultured on Nutrient Agar (NA)

Metallic blue colored colonies Subcultured on NA

↓

DNA Extraction by Boiling Method

Extraction of bacterial DNA

↓

Polymerase Chain Reaction (PCR)

Amplification using KP_Pf primers targeting 16S–23S internal transcribed spacer" gene

↓

Agarose Gel Electrophoresis

Detection of PCR products

↓

Antibiotic Susceptibility Testing

Kirby-Bauer disk diffusion method following CLSI 2023 guideline

2.2. Collection of Samples

2.2.1 Area of Sample Collection

Between February 2024 and June 2024, samples were taken from 9 local marketplaces inside the city of Dhaka. These areas were Dcc Mohakhali, wireless (Mohakhali), North Badda, South Badda, Badda DIT, Merul Badda, Bou Bazar, Rampura, and Banasree.

2.2.2 Sample Collection Procedure

In this study, four different types of chickens were collected from different marketplaces in the aforementioned places in Dhaka. These chickens were chosen because sometimes they are frequently consumed baking, grilling, barbecuing, frying, and boiling. In our country, people mostly use “Broiler”. The 4 chicken samples collected in this study are Deshi chicken, Cross-breed Sonali (golden), Broiler/Fryer, and Layer. To prevent cross-contamination, cloacal swabs were gathered in such a manner that the cotton swab did not come into contact with any chicken fur in the anus region.

A total of eighty-two chicken cloacal samples were collected. All of the samples were collected early in the morning. To prevent cross-contamination, a sterile icebox was used to carry the cloacal samples, and sterile cotton swabs were used to collect samples from the rectal area wearing sterile hand gloves (rubbing 70% ethanol).

Chicken cloacal swab samples were collected following the method, which was described by Safika (2022) (Figure 2.1). As soon as we discovered that 90% of the swabs were yellow, we dipped these yellowish cotton swab sticks in sterile falcon tubes (filled with 5 ml of physiological saline solution) and sealed them tightly. Once the samples were labeled, they were promptly transferred from the market to the

microbiological lab in an ice box that kept the temperature at 4°C.

Figure 2.1: Samples were Collected by Taking Chicken Cloacal Swabs

2.3 Preparation of Samples

To prevent additional contamination, the rectal samples were taken out inside a laminar airflow. The samples were then prepared for the 10-fold serial dilution procedure. We processed our sample by serial dilution. Serial dilution is used to reduce a dense culture of cells to a usable concentration level that allows for the quantification of cell populations that are easier for our practical work. We diluted up to factors 10^{-1} to 10^{-3} in this dilution procedure.

The well-mixed, highly concentrated solution from the direct sample (which was submerged in five milliliters of saline in the falcon) was moved into the first dilution tube. A known volume of a saline solution was stored in the direct one. Here, 1 milliliter (1000 microliters) of the cloacal sample was drawn into the first dilution tube with diluent using a pipette. To guarantee uniform dilution, the contents of the first dilution tube were completely mixed with the 9 ml of saline in the test tube. After that, 1 ml of diluted

sample from the first dilution tube was drawn to the second dilution tube containing diluent using a clean pipette tip. Every succeeding dilution tube was treated using the same dilution technique. Utilizing a vortex machine, the contents of the second dilution tube were mixed thoroughly. Same as before, dilution was performed for factor 10^{-3}. One milliliter of diluted sample was discarded from the factor 10^{-3} test tube after vortexing. The samples were at last prepared for spreading.

2.4 *K. pneumoniae* Isolation by Culture

For the isolation of *K. pneumoniae*, processed samples were spread on MacConkey agar plates and KPC agar plates.

2.4.1 *K. Pneumoniae* Culture on MacConkey Agar

To isolate *K. pneumoniae* from the chicken cloacal sample, the spread plate method was used. The samples were diluted 10-fold using saline, and 75 µl of each ten-fold dilution was transferred and disseminated on MacConkey agar using the spread plate technique. The colonies exhibit lactose fermentation. Because of lactose fermentation, *K. pneumoniae* colonies on MacConkey agar exhibit a pink colony (Figure 2.2).

Figure 2.2: Pink Colony Formation on MacConkey Agar Plate

2.4.2 *K. pneumoniae* Culture on KPC Agar

The putative *K. pneumoniae* isolates were incubated overnight and then subcultured using the streaking method on Klebsiella pneumoniae carbapenemase (KPC) agar plates for additional confirmation. The KPC plates were then re-incubated for 24 hours at 37°C. A pure single colony was then transferred to each Nutrient Agar (NA) plate. The individual metallic blue colonies were collected the following day (Figure 2.3). Following that, each colony was streaked on NA plates to create isolated colonies, which were then incubated for 24 hours at 37°C. As we know, NA, commonly known, is regarded as a popular choice for streaking since it encourages the growth of different bacterial strains. And it provides the necessary nutrients for the effective subculture of a broad range of bacteria. We proceeded with DNA extraction and PCR after obtaining white colonies on NA.

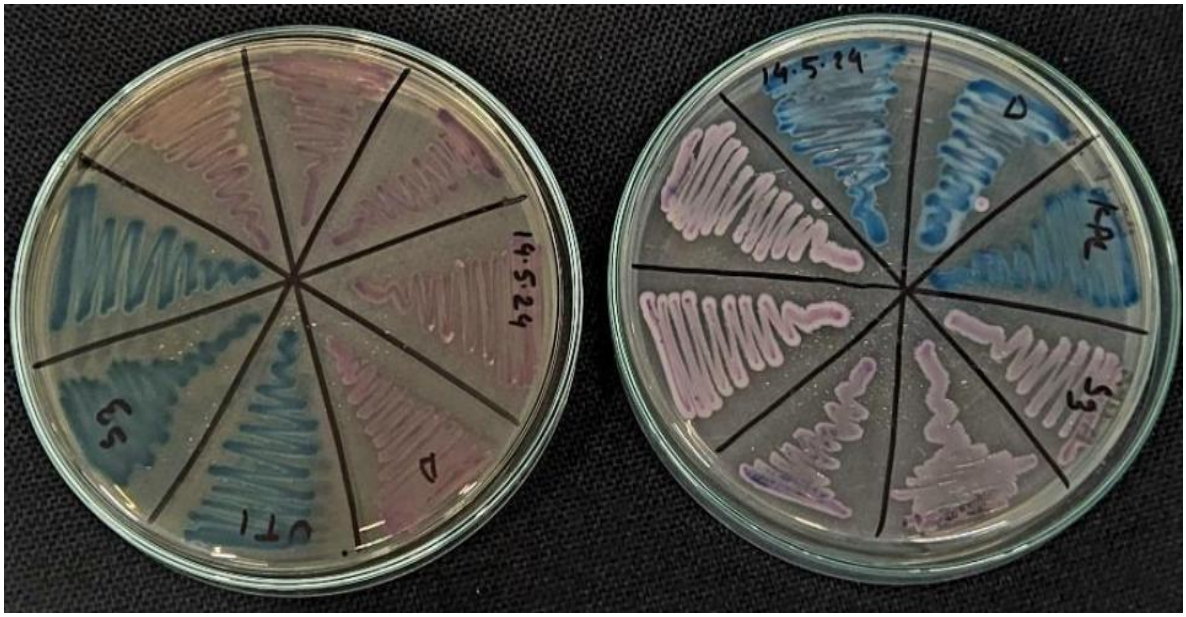

Figure 2.3: Metallic Blue Colonies Formation on KPC Agar Plate

2.5 Molecular Identification of *K. pneumoniae*

2.5.1 DNA Extraction from Isolates

To begin the DNA extraction process, all samples were subcultured on NA medium and incubated for 24 hours at 37°C (Figure 2.4).

After the 24-hour culture, a loopful of bacteria was mixed and vortexed in a 2 ml microcentrifuge tube with 150 µl Tris-EDTA (TE) buffer. The water bath machine was set at 95°C. The microcentrifuge tubes were put in the dry heat machine for 20 minutes. Twenty minutes later, the tubes were removed from the dry heat machine and centrifuged for ten minutes at 10,000 rpm. Following centrifugation, the 130 µl supernatants from every microcentrifuge tube were gathered and kept at -20°C in a second 2 ml microcentrifuge tube. These tubes contained the template DNA.

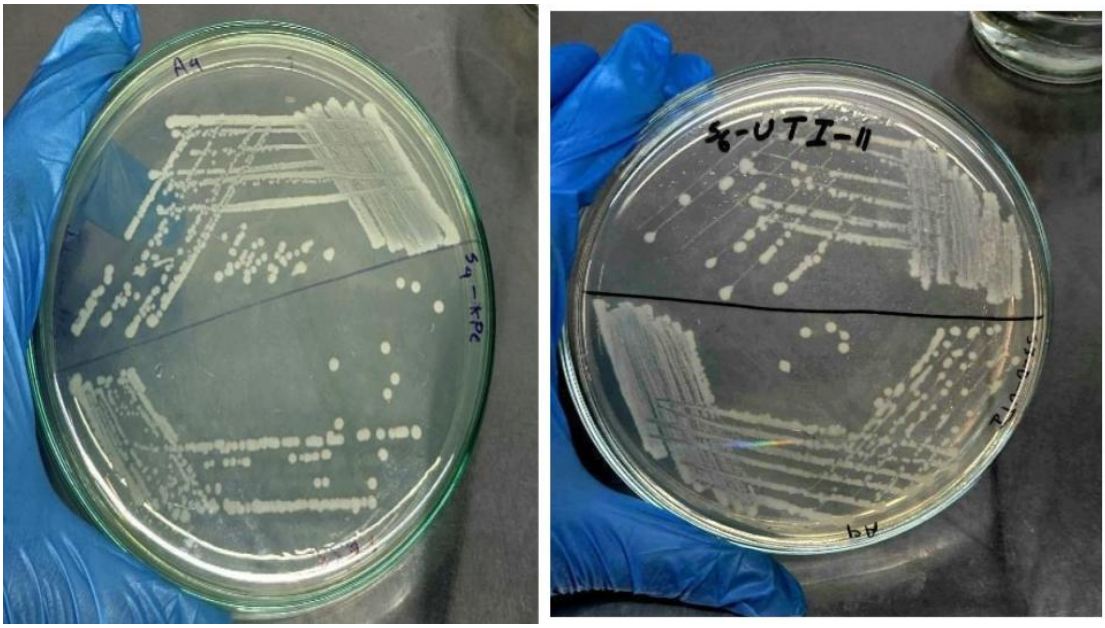

Figure 2.4: Subculture on Nutrient Agar

2.5.2 PCR (Polymerase Chain Reaction)

All the DNA extraction products are performed with polymerase chain reaction (PCR).

Master Mix Preparation

In a PCR tube, 6 µl master mix, 1 µl forward primer, 1 µl reverse primer, and 3 µl nuclease-free water were added. Then 2 µl of DNA extraction as a template was added to each PCR tube. Next, all the tubes were spun for 10 to 15 seconds. Finally, the total volume of a PCR product was 13 µl. So, the PCR products were ready to be amplified in thermal cycler PCR machines. The calculation given below is for 1 sample, and for multiple samples, the amount will be multiplied with 'n'.

Table 2.1: PCR Product Preparation for One Sample

Reagent	Total Volume
Master Mix	6 µı
Reverse Primer (RP)	1 µı
Forward Primer (FP)	1 µı
Nuclease Free Water	3 µı
DNA Sample	2 µı
Total	13 µı

PCR Product Amplification

Using "KP_Pf" primers, suspected *K. pneumoniae* isolates were examined for confirmation. Specific genes were chosen to identify *K. pneumoniae* in chicken cloacal swab samples. To identify presumptive *K. pneumoniae* isolates, the 16S–23S rDNA internal transcribed spacer gene was targeted using KP_Pf primer. For the identification of *K. pneumoniae*, the "16S–23S rDNA internal transcribed spacer" gene was used. The primer set from Table 2.2 was used for PCR amplification.

Table 2.2: Primers Used For Amplification of Resistance Genes By Polymerase Chain Reaction (PCR)

Target Gene	Primer	Primer Sequence (5'-3')	Target Organism	Amplicon Size	PCR Condition	Reference
16S–23S rDNA Internal Transcribed Spacer	KP_Pf	K_Pf-f (5′-ATT TGA AGA GGT TGC AAA CGA T3′) (25 mer) K_Pr1-R (5′-TTC ACT CTG AAG TTT TCT TGT GTT C-3′) (22 mer)	*Klebsiella pneumoniae*	130 bp	10 min at 94 °C followed by 35 cycles of 30s at 94 °C, 20s at 57 °C, and 20s at 72 °C, then 10-min hold at 72 °C	(Yin Liu.,2008)

Cycles for PCR Conditions

The cycling conditions were 10 min at 94 °C followed by 35 cycles of 30s at 94 °C, 20s at 57 °C, and 20s at 72 °C, then 10-min hold at 72 °C. After PCR, all the products were further analyzed using Gel Electrophoresis.

2.5.3 Agarose Gel Electrophoresis

The findings of a PCR reaction are typically visualized (made visible) using gel electrophoresis. The agarose gel was prepared by 1.2 g agarose powder, 2000 µL Tris-acetate-EDTA (TAE) buffer, along with 98% distilled water. TAE was used as a running buffer to migrate the DNA in the positive electrode. Next, 4µL Ethidium Bromide (EDTA) was added, which is a carcinogenic fluorescence dye that helps the bands to show under UV light.

Next, all the PCR products of 4µL were loaded into the gel by autoclaved tips. 4µL of 100 bp ladder was used to identify the size of unknown DNA molecules. Finally, the products were visualized under a UV transilluminator (Figure 2.5).

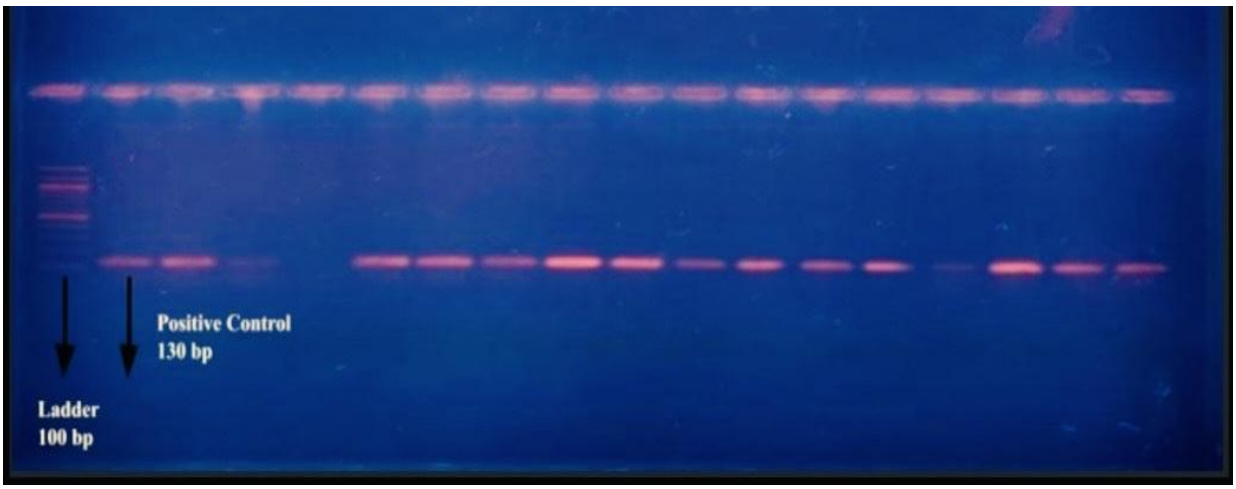

Figure 2.5: Bands were visualized under a UV transilluminator. The gel electrophoresis image of PCR product of KP_Pf (130 bp) DNA amplification in some isolates of *K. pneumoniae* under UV

2.6 Antibiotic Susceptibility Test (AST)

To determine the drug resistance pattern of *K. pneumoniae*, the Kirby-Bauer disc diffusion method was used to conduct antibiotic susceptibility tests. For antimicrobial susceptibility testing, all the isolates were subcultured and streaked in the non-selective media, NA, and incubated at 37°C for 24 hours (Figure 2.6).

Figure 2.6: Streak on Nutrient Agar Media (Non-selective)

We used 11 different antibiotics from 7 antimicrobial categories for antimicrobial susceptibility testing. Turbidity was set to 0.5 McFarland by suspending colonies in 5 ml of 0.9% NaCl solution and then swabbed on Mueller Hinton Agar (MHA) (HiMedia). The zone diameters of the selected antimicrobial agents were interpreted following the Clinical Laboratory Standards Institute (CLSI) 2023 guidelines (Standards & Testing, 2023). Single-drug resistance is defined as resistance to a single antibiotic class, while multidrug resistance (MDR) is characterized by resistance to at least one agent in three or more antimicrobial categories. Extensively drug-resistant (XDR) bacteria can be characterized using two main sets of criteria. The first set is based on the number of antimicrobial classes, or subclasses, to which a bacterium is resistant. The second set considers whether the bacterium is resistant to one or more key antimicrobial agents. These definitions follow the criteria established by the Clinical Laboratory Standards

Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) (Magiorakos et al., 2012). Antibiotics and groups for this test are as below:

Table 2.3: Concentrations and Diffusion Zones of the Antibiotics

Antibiotic group	Antibiotics	Disk Code	Disk Potency	Interpretive Criteria		
				Sensitive (mm)	Intermediate (mm)	Resistant (mm)
Aminoglycoside	Amikacin	AK 30	30	≥ 20	17-19	≤ 16
Carbapenem	Imipenem	IPM 10	10	≥ 23	20-22	≤ 19
	Meropenem	MEM 10	10	≥ 23	20-22	≤ 19
	Amoxycillin	AML 10	10	≥ 17	14-16	≤ 13
Beta-lactam	Cefixime	CFM5	5	≥ 19	16-18	≤ 15
	Ceftriaxone	CTR 30	30	≥ 23	20-22	≤ 19
	Amoxyclav	AMC 30	30	≥ 18	14-17	≤ 13
Tetracyclin	Tetracycline	TE 30	30	≥ 15	12-14	≤ 11
Beta-lactamase Inhibitor Combination	Piperacillin/ Tazobactam	PIT 100/1]	100/10	≥ 25	21-24	≤ 20
Fluoroquinolon	Ciprofloxacin	CIP 5	5	≥ 26	22-25	≤ 21
Macrolide	Azithromycin	AZM 30	30	≥ 13		≤ 12

Below we share a picture of an antibiotic disk, which was placed on MHA media:

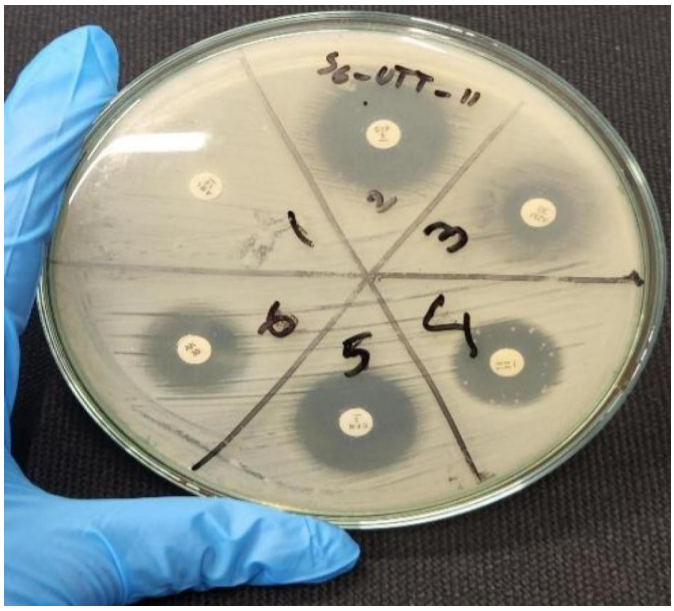

Figure 2.7: Antibiotic Susceptibility Test

This plate shows the zone of inhibition for 6 antibiotics. Then from this we observe the zone of inhibition and based on the CLSI guideline and we interpret the result.

Chapter 3

Result

In total, 82 samples were analyzed for the quantitative presence of *K. pneumoniae* in different types of chicken around Dhaka city throughout the sampling period out of which 42 samples (51%) were found positive. Out of 82 samples, 6 were from DCC Mohakhali market, 12 from Wireless market, 10 from North Badda market, 4 from South Badda market, 13 from Badda DIT market, 11 from Merul Badda market, 11 from Bou Bazar market, 10 from Rampura market, and 5 from Banasree market. These isolates were identified through morphological, biochemical, and molecular examination.

3.1 *K. pneumoniae* Samples Based on Area and Chicken Type

High levels of *K. pneumoniae* were observed, especially in Broiler chickens, where 49 samples were positive compared to 14 positive samples in Layer chickens. Further lower detection rates of *K. pneumoniae* were reported in Cross-Breed Sonali chickens (9 positive samples) and Deshi chickens (10 positive samples). Information regarding this is described in detail in Table 3.1.

Table 3.1: Distribution of *Klebsiella pneumoniae* Samples Collected from Different types of Chickens around Different Areas of Dhaka City

Market Place	Number of Isolates				
	Types of Chicken				Total
	Cross-Breed Sonali	Deshi	Broiler	Layer	
DCC Mohakhali	0	0	6	0	6
Wireless Mohakhali	0	8	0	4	12
North Badda	5	0	5	0	10
South Badda	4	0	0	0	4
Badda DIT	0	0	10	3	13
Merul Badda	0	0	9	2	11
Bou Bazar	0	0	6	5	11
Rampura	0	2	8	0	10
Banasree	0	0	5	0	5
Total	9	10	49	14	82

3.2 *K. pneumoniae* Isolates Based on Area and Chicken Type

Out of 50 isolates, 6 were from DCC Mohakhali market, 4 from Wireless market, 6 from North Badda market, 2 from South Badda market, 6 from Badda DIT market, 13 from Merul Badda market, 3 from Bou Bazar market, 8 from Rampura market, and 2 from Banasree market. Information regarding this is described in detail in Table 3.2.

Table 3.2: Distribution of *K. pneumoniae* Isolates Collected from Different types of Chickens around Different Areas of Dhaka City

Market Place	Number of Isolates				
	Types of Chicken				Total
	Cross-Breed Sonali	Deshi	Broiler	Layer	
DCC Mohakhali	0	0	6	0	6
Wireless Mohakhali	0	3	0	1	4
North Badda	3	0	3	0	6
South Badda	2	0	0	0	2
Badda DIT	0	0	5	1	6
Merul Badda	0	0	10	3	13
Bou Bazar	0	0	2	1	3
Rampura	0	3	5	0	8
Banasree	0	0	2	0	2
Total	5	6	33	6	50

3.3 Antibiotic Susceptibility Pattern of *K. pneumoniae* Isolates

The sensitivity test was carried out by calculating the diameter of the antibiotic inhibition zone formed on the Mueller–Hinton agar. In this study, a total of 50 isolates have been selected for antibiotic susceptibility testing (AST) to observe the antibiotic susceptibility pattern of *K. pneumoniae* where 5 were Cross-Breed Sonali chickens, 6 were Deshi chickens, 33 were Broiler chickens, and 6 were Layer chickens.

The antimicrobial resistance profile indicates that *K. pneumoniae* isolates detected chickens were highly resistant to Amoxycillin (92%). Contrarily, all of the isolates showed the highest sensitivity to Azithromycin (68%). *K. pneumoniae* isolates from all samples exhibited the lowest resistance to Meropenem (20%). Contrarily, all of the isolates showed the lowest sensitivity to Amoxycillin (4%). The results of the antibiotic susceptibility pattern of isolates detected in summer are shown in Table 3.3.

Table 3.3: Antibiotic Susceptibility Pattern of *K. pneumoniae* Isolates

Antibiotic Group	Antibiotic Name	Sensitive Bacteria Quantity	Sensitive (%)	Intermediate Bacteria Quantity	Intermediate (%)	Resistant Bacteria Quantity	Resistant (%)
Aminoglycosides	Amikacin	14	28%	23	46%	13	26%
Carbapenem	Imipenem	23	46%	12	24%	15	30%
	Meropenem	27	54%	13	26%	10	20%
	Amoxycillin	2	4%	2	4%	46	92%
Beta-lactam	Cefixime	25	50%	2	4%	23	46%
	Ceftriaxone	10	20%	15	30%	25	50%
	Amoxyclav	5	10%	20	40%	25	50%
Tetracycline	Tetracycline	13	26%	1	2%	36	72%
Beta-lactamase Inhibitor Combinations	Piperacillin/Tazobactam	6	12%	16	32%	28	56%
Fluoroquinolone	Ciprofloxacin	8	16%	7	14%	35	70%
Macrolides	Azithromycin	34	68%	0	0%	16	32%

Overall, *K. pneumoniae* isolates showed the highest resistance to Amoxycillin antibiotics across all samples, followed by Tetracycline and Ciprofloxacin. Contrarily, all of the isolates from all samples exhibited the lowest resistance to Meropenem, followed by Amikacin, Imipenem, and Azithromycin antibiotics. In this study, all isolates exhibited the highest sensitivity to Azithromycin, followed by Meropenem, Cefixime, and Imipenem. Contrarily, all of the isolates exhibited the lowest sensitivity to Amoxycillin, followed by Amoxyclav, Piperacillin/Tazobactam, and Ciprofloxacin. This is detailed in Figure 3.1.

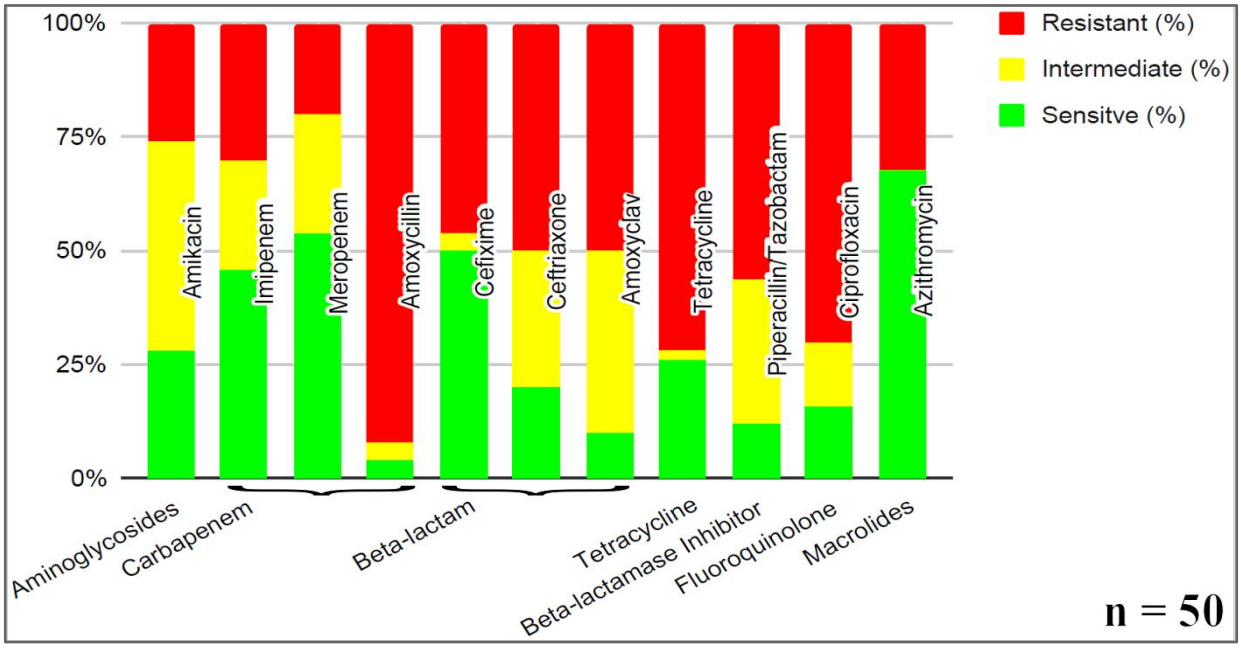

Figure 3.1: Antibiotic Susceptibility Pattern of *Klebsiella pneumoniae* Isolates

3.4 Types of Resistance Bacteria Based On Chicken Type

The highest percentage of multidrug resistance was observed in Cross-Breed Sonali chicken samples (100%). Extensively drug-resistant isolates were observed in Layer chicken samples (66%). Multiple-drug-resistant (MDR) isolates display resistance to at least three antibiotic categories. In this study, at least

three antibiotic-resistant groups were detected in 40 *K. pneumoniae* isolates. Table 3.4 describes the counts and percentages of MDR and XDR isolates of 4 different types of chickens.

Table 3.4: Chicken Type-wise Distribution of *Klebsiella pneumoniae* Samples Based on Counts of MDR and XDR Isolates in Different Areas of Dhaka City

Chicken Type	No. of Samples	No. of Isolates	No. of MDR Isolates	MDR n (%)	No. of XDR Isolates	XDR n (%)
Cross-Breed Sonali	9	5	5	100%	3	60%
Deshi	10	6	4	66%	1	16%
Broiler	49	33	26	78%	16	48%
Layer	14	6	5	83%	4	66%
TOTAL	82	50	40	80%	24	48%

n = number of isolates (%)

3.5 Types of Resistance Bacteria Based On Area

In this study, the highest percentage of multidrug-resistant isolates was observed at North Badda, South Badda, and Banasree (100%) markets. The highest percentage of extensively drug resistance was observed

at Banasree (100%) market. Table 3.5 describes the counts and percentages of MDR and XDR isolates of different types of chickens collected from 9 areas around Dhaka city.

Table 3.5: Area-wise Distribution of *Klebsiella pneumoniae* Samples Based on Counts of MDR and XDR Isolates in Dhaka City

Area	No. of Samples	No. of Isolates	No. of MDR Isolates	MDR n (%)	No. of XDR Isolates	XDR n (%)
DCC Mohakhali	6	6	5	83%	4	66%
Wireless	12	4	2	50%	1	25%
North Badda	10	6	6	100%	4	66%
South Badda	4	2	2	100%	1	50%
Badda DIT	13	6	5	83%	2	33%
Merul Badda	11	13	9	69%	5	38%
Bou Bazar	11	3	2	66%	2	66%
Rampura	10	8	6	75%	3	37.5%
Banasree	5	2	2	100%	2	100%
TOTAL	82	50	39	78%	24	48%

n = number of isolates (%)

Chapter 4

Discussion

Here, the findings of this study conducted in Dhaka, Bangladesh, indicated that *K. pneumoniae* was detected in 51% of the chicken cloacal samples collected, and emphasized its widespread presence among poultry in the region. Moreover, among the samples, the highest rate of *K. pneumoniae* was observed in Broiler chickens at 49%, 14% in Layer chickens and 9% in Cross-Breed sonali chickens, and also 10% in Deshi chickens. It indicates a significant prevalence of *K. pneumoniae* in Broiler chickens within the Dhaka area. Furthermore, when compared to similar studies from other regions, these findings provide insight into the comparative prevalence and antibiotic resistance patterns of the pathogen. In Ethiopia, for example, *K. pneumoniae* has been linked with omphalitis and other respiratory complications in poultry. Similarly, studies from Egypt reported *K. pneumoniae* in poultry suffering from different kinds of infections, with 12.5% of these isolates showing multidrug resistance (MDR). However, these results from Dhaka city suggest significantly higher rates of multidrug resistance, with 100% of the isolates from Cross-Breed Sonali chickens showing MDR and extensively drug-resistant (XDR) isolates being the most common among Layer chickens at 66%. Moreover, these comparisons suggest that the factors which are driving antibiotic resistance in Dhaka are markedly different, similarly influenced by local antibiotic use, different farming conditions and also inadequate poultry monitoring. Additionally, comparing these results with other regions globally reveals significant differences in the rates of prevalence and resistance patterns. For instance, in Norway, *K. pneumoniae* was found at a lower prevalence rate of 26% in Broiler chickens, highlighting how species differences can act as a reservoir for this pathogen. In Portugal, 42% of the fecal samples from chickens contained *K. pneumoniae*, with 90% of these results showing multidrug resistance. These findings from Portugal are somewhat comparable to Dhaka's pattern, yet the study of Dhaka shows significantly higher levels of MDR and XDR. (Asri et al.,2021).

While analyzing the antibiotic susceptibility data from Dhaka, Amoxycillin showed the highest rate at 92% which is similar to the findings that are reported to Poland, their resistance to Amoxycillin in poultry isolates also exceeded to 90%. In spite of this, in Dhaka, the isolates showed the highest sensitivity to Azithromycin which is 68%, a pattern that is similar with the observations from Kenya, where Azithromycin showed relatively higher effectiveness compared to other antibiotics. Additionally, this all represents a potential treatment option for *K. pneumoniae* infections. Comparatively, Denmark and France reported the presence of *K. pneumoniae* at high rates of 90% and 73%, respectively, however, the overall resistance in these areas was much lower than that observed in Dhaka. Furthermore, resistance patterns in Denmark and France were vastly limited to Trimethoprim (12%) and Tetracycline (9%), highlighting the effectiveness of biosecurity strategies, better antibiotic control and improved poultry management approaches. These approaches also emphasize the different strategies and successes which were achieved worldwide in addressing *K. pneumoniae* and its resistance patterns globally (Kot et al., 2024).

In addition, Dhaka's wet markets, mainly in North Badda, Banasree and South Badda really showed alarming resistance rates. Particularly here, in Banasree markets and North Badda markets had 100% MDR rates, and the highest percentage of XDR isolates were found in these markets as well. Moreover, these results highlight how localized conditions are like the cleanliness of markets, largely misuse of antibiotics as well as the lack of the strict laws of poultry guidelines are the main contributors to the spread of resistant strains. Here, the differences with the global findings especially from European countries like Denmark and France further highlight these disparities. On the other hand, while these markets of European countries maintain significantly lower levels of resistance due to other regions benefiting from strong regulatory measures and biosecurity protocols, Dhaka continues to struggle with addressing these issues effectively (Kot et al., 2024). On the other hand, the resistance data suggests that Amoxycillin and Tetracycline are the most commonly resisted drugs when the isolates demonstrate the least resistance to Meropenem, Amikacin, Imipenem, and Azithromycin. Furthermore, these data indicate that when the

resistance levels are at alarming points, there are some antibiotics that will still show the actual levels of sensitivity and will act as a treatment option even with the improvement in antibiotic stewardship efforts (Kuve, 2023).

The study here further shows that the prevalence and the patterns of multidrug resistance (MDR) and extensively drug-resistant (XDR) are concerning with 100% of the Cross-Breed sonali chickens showing the MDR. On the other hand, a significant number of the Layer chicken samples which is 66% revealed XDR, with at least these three antibiotic groups showing the resistance. As well as the comparisons between Dhaka and these different regions suggest that the local strategies that are used in markets of Dhaka should be improved urgently. Moreover, differences in biosecurity measurements, regulatory enforcement and misuse of antibiotics are main factors driving the resistance levels observed in Dhaka. On the other hand, the success of different markets of European countries sustains lower resistance rates which highlights the critical role of regulatory strategies, effective hygiene practices as well as regular monitoring. Although, markets of Dhaka mainly struggled with some issues such as the overuse of antibiotics, biosecurity protocols. Therefore, the results from this study mainly suggest that *K. pneumoniae* is a global threat to poultry sectors as well as the patterns of multidrug resistance present in Dhaka are higher than other places observed. Furthermore, these differences can be from the socio-economic challenges, poultry practices and also the insufficient enforcement of antibiotic regulations. Here, globally comparative analysis with different countries such as Kenya, Poland, Norway etc studies enhancing biosecurity measurement, poultry practices, improving wet markets hygiene and also raising consciousness about the usage of antibiotics, these can really play a significant role.

Chapter 5

Conclusion

The study on *Klebsiella pneumoniae* as a zoonotic pathogen and its antibiotic resistance patterns (AST) reveals its considerable threat to public health. *Klebsiella pneumoniae* is found in both poultry and human populations, with the potential for cross-species transmission, particularly in environments with frequent human-animal interaction, like commercial poultry farms. The research shows that *Klebsiella pneumoniae* is resistant to various antibiotics, which complicates treatment for both animals and humans. These resistance patterns highlight the importance of developing and implementing effective AST methods to manage treatment and curb the spread of resistant strains.

Basically, our findings indicate that commercially raised poultry chickens, particularly those in high-density environments, have higher levels of *Klebsiella pneumoniae* contamination and exhibit increased resistance to commonly used antibiotics, including tetracycline, ciprofloxacin, and amoxicillin. The frequent use of antibiotics in these commercial systems contributes to the emergence of resistant strains, posing a public health risk due to the potential transmission of these bacteria. Additionally, the zoonotic nature of *Klebsiella pneumoniae* stresses the need for monitoring its presence in animals to reduce the risk of human transmission. To tackle this issue, it is essential to strengthen biosecurity practices, ensure responsible antibiotic use in agriculture, and maintain comprehensive surveillance across both animal and human populations. Ultimately, the findings underscore the need for a One Health strategy, considering the close links between human, animal, and environmental health, to effectively prevent and control *Klebsiella pneumoniae* infections and their associated resistance.

References

1. Abbas, R., Chakkour, M., Zein El Dine, H., Obaseki, E. F., Obeid, S. T., Jezzini, A., Ghssein, G., & Ezzeddine, Z. (2024). General Overview of Klebsiella pneumonia: Epidemiology and the Role of Siderophores in Its Pathogenicity. Biology, 13(2), 78. https://doi.org/10.3390/biology13020078
2. Abebe, E., Gugsa, G., & Ahmed, M. (2020). Review on Major Food-Borne Zoonotic Bacterial Pathogens. Journal of tropical medicine, 2020, 4674235. https://doi.org/10.1155/2020/4674235
3. Abreu, R., Semedo-Lemsaddek, T., Cunha, E., Tavares, L., & Oliveira, M. (2023). Antimicrobial Drug Resistance in Poultry Production: Current Status and Innovative Strategies for Bacterial Control. *Microorganisms*, *11*(4), 953. https://doi.org/10.3390/microorganisms11040953
4. Enany, M., El-Mahallawy, H. S., Mohammed, W. E. I., Hassan, W. M. M., & Aboueisha, A. M. (2023). Detection of *Klebsiella pneumoniae* in broiler chickens at Ismailia City, Egypt. *Faculty of Veterinary Medicine, Suez Canal University.*
5. Ghai, R. R., Wallace, R. M., Kile, J. C., et al. (2022). A generalizable one health framework for the control of zoonotic diseases. Scientific Reports, 12, 8588. https://doi.org/10.1038/s41598-022-12619-1
6. Islam, A., Islam, S., Amin, E., Shano, S., Samad, M. A., Shirin, T., Hassan, M. M., & Flora, M. S. (2022). Assessment of poultry rearing practices and risk factors of H5N1 and H9N2 virus circulating among backyard chickens and ducks in rural communities. *PloS one*, *17*(10), e0275852. https://doi.org/10.1371/journal.pone.0275852
7. Kahin, M. A., Mohamed, A. H., Mohomed, A. A., et al. (2024). Occurrence, antibiotic resistance profiles, and associated risk factors of *Klebsiella pneumoniae* in poultry farms in selected districts of Somalia Regional State, Ethiopia. *BMC Microbiology, 24*(1), 137. https://doi.org/10.1186/s12866-024-03298-1
8. Koju, P., Shrestha, R., Shrestha, A., Tamrakar, S., Rai, A., Shrestha, P., Madhup, S. K., Katuwal,

N., Shrestha, A., Shrestha, A., Shrestha, S., K.C, S., Karki, P., Tamang, P., Thekkur, P., & Shakya Shrestha, S. (2022). Antimicrobial Resistance in E. coli Isolated from Chicken Cecum Samples and Factors Contributing to Antimicrobial Resistance in Nepal. Tropical Medicine and Infectious Disease, 7(9), 249. https://doi.org/10.3390/tropicalmed7090249

9. Kot, B., & Witeska, M. (2024). Review: Antimicrobial resistance of Klebsiella pneumoniae isolated from poultry, cattle and pigs. *Animal*, *18*(11), 101345. https://doi.org/10.1016/j.animal.2024.101345

10. Larsson, D. G. J., & Flach, C. F. (2022). Antibiotic resistance in the environment. Nature Reviews Microbiology, 20(5), 257–269. https://doi.org/10.1038/s41579-021-00649-x

11. Lemlem, M., Aklilu, E., Mohamed, M., Kamaruzzaman, N. F., Devan, S. S., Lawal, H., & Kanamma, A. A. (2024). Prevalence and molecular characterization of ESBL-producing Escherichia coli isolated from broiler chicken and their respective farms environment in Malaysia. BMC microbiology, 24(1), 499. https://doi.org/10.1186/s12866-024-03653-2

12. Magiorakos, A. P., Srinivasan, A., Carey, R. B., Carmeli, Y., Falagas, M. E., Giske, C. G., … Monnet, D. L. (2012). Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance. Clinical Microbiology and Infection, 18(3), 268–281. https://doi.org/10.1111/j.1469-0691.2011.03570.x

13. Manyi-Loh, C., Mamphweli, S., Meyer, E., & Okoh, A. (2018). Antibiotic Use in Agriculture and Its Consequential Resistance in Environmental Sources: Potential Public Health Implications. *Molecules (Basel, Switzerland)*, *23*(4), 795. https://doi.org/10.3390/molecules23040795

14. Mbah, M. I., & Anyamene, C. O. (2021). Extended spectrum beta-lactamase producing *Escherichia coli* and *Klebsiella* species isolated from layer chicken farms in Jalingo, Nigeria. *The International Journal of Engineering and Science (IJES, 10*(2), Series I, 37–44.

15. Nordmann, P., Poirel, L., & Villegas, M. V. (2011). Global spread of carbapenemase-producing Klebsiella pneumoniae. *Emerging Infectious Diseases*, 17(10), 1791–1798.

16. PERMATASARI, D. A., WITANINGRUM, A. M., WIBISONO, F. J., & Effendi, M. H. (2020). Detection and prevalence of multidrug-resistant Klebsiella pneumoniae strains isolated from poultry farms in Blitar, Indonesia. *Biodiversitas Journal of Biological Diversity*, *21*(10). https://doi.org/10.13057/biodiv/d211024

17. Podschun, R., & Ullmann, U. (1998). Klebsiella spp. as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenic features. *Clinical Microbiology Reviews*, 11(4), 589–603.

18. Rahbe, E., Watier, L., Guillemot, D., Glaser, P., & Opatowski, L. (2023). Determinants of worldwide antibiotic resistance dynamics across drug-bacterium pairs: A multivariable spatial-temporal analysis using ATLAS. *Articles, 7*(7), e547–e557

19. safika safika,Zumala Nilasari.2022. Detection of antibiotic resistance coding gene in Klebsiella pneumoniae bacteria isolated from broiler chickens in West Java, Indonesia. Retrieve from https://japsonline.com/admin/php/uploads/3634_pdf.pdf

20. Sophia Atingo Kuve, A.S.(2023)Exploring Antimicrobial Resistance in Extended- Spectrum B-Lactamase Producing Klebsiella pneumoniae Isolates from Chicken in Kunshan, China and Nairobi, Kenya.*DKU Global Health Program Duke University*

21. Vourc'h, G., Moutou, F., Morand, S., et al. (2022). Zoonoses: The ties that bind humans to animals. Versailles, France: Éditions Quae. Retrieved from https://www.ncbi.nlm.nih.gov/books/NBK596951/

22. Wareth, G., & Neubauer, H. (2021). The animal-foods-environment interface of Klebsiella pneumoniae in Germany: An observational study on pathogenicity, resistance development and the current situation. Veterinary Research, 52(1), 16. https://doi.org/10.1186/s13567-020-00875-w

23. World Health Organization. (2014). *Antimicrobial resistance: global report on surveillance*. WHO Press. Available at: https://www.who.int