UNIVERSITI PUTRA MALAYSIA

ANTIMICROBIAL RESISTANCE OF BACTERIA ISOLATED FROM THE CLOACAL SWAB OF PIGEONS IN THE RESIDENTIAL AREA OF PUTRAJAYA.

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ANTIMICROBIAL RESISTANCE OF BACTERIA ISOLATED FROM THE CLOACAL SWAB OF PIGEONS IN THE RESIDENTIAL AREA OF PUTRAJAYA.

NUR AININA BINTI AB MANAP

A project paper submitted to the
Faculty of Veterinary Medicine, Universiti Putra Malaysia in partial fulfilment of the requirement for the DEGREE OF DOCTOR OF VETERINARY MEDICINE
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It is hereby certified that we have read this project paper entitled “Antimicrobial Resistance of bacteria isolated from the cloacal swab of pigeons in the residential area of Putrajaya”, by Nur Ainina Binti Ab Manap and in our opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfilment of the requirement for the course VPD 4999 – Final Year Project.

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DEDICATION

This thesis is especially dedicated to:

My parents, who has done everything to support me

Ab Manap Bin Ab Rahim, my late mother, Hamidah Binti Abdul Wahab

My supportive supervisors, who guided and helped me in my project study

Assoc. Prof. Dr. Siti Khairani Binti Bejo

Prof. Dr. Jalila Binti Abu

and

All my fellow DVM24 friends.

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CONTENTS

TITLE…… i

CERTIFICATION…… ii

DEDICATION…….. iii

ACKNOWLEDGEMENTS…… iv

CONTENTS…… v

LIST OF TABLES…… vii

LIST OF FIGURES…….. viii

ABSTRAK…… ix

ABSTRACT……... xii

1.0 INTRODUCTION…… 1

2.0 LITERATURE REVIEW…… 3

2.1 Antimicrobial resistance and it's status in Malaysia…….. 3

2.2 Pigeons act as carrier of AMR bacteria……... 4

2.3 Pigeon feeding…… 6

2.4 Impacts of pigeon feeding…….. 6

2.5 Awareness of AMR carried by pigeons among communities…… 7

3.0 MATERIALS AND METHODS…… 8

3.1 Cloacal swabs collection…….. 8

3.2 Isolation of bacteria procedure…….. 8

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3.3 Identification of bacteria procedure…… 9

3.4 Antibiotic Susceptibility Test…… 11

3.5 Descriptive analysis…….. 14

4.0 RESULTS…….. 15

5.0 DISCUSSION…… 20

6.0 CONCLUSION…….. 24

7.0 REFERENCES…….. 25

APPENDICES…… 28

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LIST OF TABLES **PAGE**

Table 1: List of antibiotics used for gram-negative and gram-positive …….. 12

Table 2: List of antibiotic groups …….. 13

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LIST OF FIGURES **PAGE**

Figure 1: Summary of total isolates from 30 cloacal swab samples …… 15

Figure 2: Percentage of AMR in four pathogenic bacteria …… 17

Figure 3: Percentage of AMR in gram-negative bacteria …….. 18

Figure 4: Percentage of AMR in gram-positive bacteria ……... 19

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ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999 -Projek Tahun Akhir.

PENGASINGAN BAKTERIA KERINTANGAN ANTIMIKROB (AMR) DARI SAMPEL KLOAKA BURUNG MERPATI DI KAWASAN KEDIAMAN PUTRAJAYA

Oleh

Nur Ainina binti Ab Manap

2023

Penyelia : Assoc. Siti Khairani binti Bejo

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Kerintangan antimikrob (AMR) telah menjadi ancaman utama di seluruh dunia, samada dalam perubatan manusia dan haiwan. Ciri AMR menjadikan bakteria kurang terdedah kepada antibiotik yang biasa digunakan seperti ampisilin. Akibatnya, kos penjagaan kesihatan meningkat, terutamanya dalam mencipta antibiotik baharu yang biasanya mengambil masa yang lama. Haiwan liar seperti burung yang berhijrah boleh menjadi penyebar bakteria AMR ini kepada manusia kerana ia biasanya tinggal di kawasan rumah. Kajian ini bertujuan untuk menilai bakteria AMR yang diasingkan daripada calitan kloaka burung merpati di kawasan perumahan di Putrajaya. Tiga puluh ekor burung

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merpati telah ditangkap, dan sampel kloaka diambil dari setiap burung merpati. Sampel telah diinokulasi ke atas agar darah untuk mengisolasi bakteria. dari sampel tersebut. Sampel juga telah diinokulasi ke dalam media Rappaport-Vassiliadis (RV) dan terus diinokulasi diatas agar Xylose Lysine Deoxycholate (XLD) untuk pengasingan *Salmonella* sp. Semua bakteria yang diasingkan telah diuji dengan ujian kerintangan antimikrob. Semua bakteria gram-negatif telah diuji dengan 12 antibiotik berbeza iaitu amoxicillin-clavulanic acid, doxycycline, kanamycin, norfloxacin, trimethoprim /sulfamethoxazole, polymyxin b, ampicillin, chloramphenicol, tetracycline, cephalothin, streptomycin and gentamicin. Manakala, bagi bakteria gram-positif, mereka diuji dengan enam antibiotik iaitu vankomisin, gentamisin, kloramfenikol, trimethoprim/sulfamethoxazole, eritromisin, dan ampisilin. Sembilan puluh satu pengasingan bakteria diperoleh daripada sampel. Kesemua sampel adalah negatif kepada *Salmonella* sp. *Escherichia coli* telah diasingkan daripada 96.7% (29/30) sampel, *Enterobacter* sp. 23.3% (7/30), *Klebsiella* sp. 13.3% (4/30), *Proteus mirabilis* dan *Aeromonas* sp. 10% (3/30) dan masing-masing 3.3% (1/30) bagi *Morganella morganii, Acinetobacter lwoffi*, dan *Pasteurella* sp. *Bacillus* sp telah diasingkan daripada 46.7% (14/30) sampel, *Actinomyces* sp. 10% (3/30), *Corynebacterium* sp. 26.7% (8/30), *Staphylococcus* sp. 20% (6/30), *Streptococcus* sp. 10% (3/30) dan *Enterococcus* sp. 26.7% (8/30) masing-masing. Kebanyakan bakteria gram-negatif sensitif terhadap gentamicin 98% (48/49), kloramfenikol 85.7% (42/49) dan asid amoxicillin-clavulanic 79.6% (39/49) tetapi menjadi tahan terhadap ampicillin 94% (46/49) . Manakala kebanyakan bakteria gram-positif, sensitif terhadap gentamicin 92.9% (39/42),

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kloramfenikol 88.1% (37/42), dan trimethoprim sulfamethoxazole 69% (29/42) tetapi menjadi tahan terhadap ampicillin 83.3% (35/42). . Kesimpulannya, kebanyakan bakteria sudah pun tahan terhadap ampicillin 89% (81/91) tetapi masih terdedah kepada antibiotik lain yang biasa digunakan, seperti kloramfenikol dan gentamicin.

Kata kunci: burung merpati; kloaka, bakteri, kerintangan antimikrob

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ABSTRACT

This abstract project paper was presented to the Faculty of Veterinary Medicine as part of fulfilment of the course VPD 4999 – Final Year Project

ANTIMICROBIAL RESISTANCE OF BACTERIA ISOLATED FROM CLOACAL SWAB OF PIGEONS IN THE RESIDENTIAL AREA OF PUTRAJAYA

by

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2023

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Antimicrobial resistance (AMR) has become a major threat worldwide, both in human and animal medicine. Antimicrobial resistance (AMR) traits make the bacteria less susceptible to antibiotics commonly used such as ampicillin. As a result, increased healthcare costs, particularly in inventing new antibiotics that usually take a long time. Wild animals such as migratory birds could be potential disseminators of these AMR

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bacteria to humans as they usually lived in the house compound. This study aimed to evaluate the AMR bacteria isolated from cloacal swab of pigeons in the residential area in Putrajaya. Thirty pigeons were trapped, and cloacal swabs were collected from each pigeon. The swabs were inoculated onto blood agar for bacteria isolation. The samples were also inoculated into Rappaport-Vassiliadis (RV) enrichment broth and continued to be plated on Xylose Lysine Deoxycholate (XLD) agar for isolation of *Salmonella sp.* All isolates were subjected to antimicrobial susceptibility test. All gram-negative bacteria were tested with 12 different antibiotics namely amoxicillin-clavulanic acid, doxycycline, kanamycin, norfloxacin, trimethoprim /sulfamethoxazole, polymyxin b, ampicillin, chloramphenicol, tetracycline, cephalothin, streptomycin and gentamicin. Whereas, for gram- positive bacteria, they were tested with six antibiotics which are vancomycin, gentamicin, chloramphenicol, trimethoprim /sulfamethoxazole, erythromycin, and ampicillin. Ninety-one bacteria isolates were obtained from the samples. All samples were negative to *Salmonella sp*. *Escherichia coli was isolated from 96.7% (29/30) of the samples, Enterobacter sp.* 23.3% (7/30), *Klebsiella sp.* 13.3% (4/30), *Proteus mirabilis and Aeromonas* 10% (3/30) and *Morganella morganii*, *Acinetobacter lwoffi*, and *Pasteurella sp*. 3.3% (1/30), respectively. *Bacillus sp. was isolated from 46.7% (*14/30) of the samples, *Actinomyces sp. 10% (*3/30), *Corynebacterium sp*. 26.7% (8/30), *Staphylococcus sp.* 20% (6/30), *Streptococcus sp.* 10% (3/30) and *Enterococcus sp. 26.7% (*8/30) respectively. Most of gram-negative bacteria were sensitive towards gentamicin 98% (48/49), chloramphenicol 85.7% (42/49) and amoxicillin- clavulanic acid 79.6% (39/49) but became resistant towards ampicillin

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94% (46/49). Whereas most gram-positive bacteria, were sensitive towards gentamicin 92.9% (39/42), chloramphenicol 88.1%
(37/42), and trimethoprim sulfamethoxazole 69% (29/42) but became resistant towards ampicillin 83.3% (35/42). In conclusion, most isolated bacteria were already being resistant towards ampicillin 89% (81/91) but still susceptible to other commonly used antibiotics, such as chloramphenicol and gentamicin.

Keywords: pigeons, cloacal, bacteria, antimicrobial resistance.

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1.0 INTRODUCTION

Antimicrobial resistance (AMR) is defined as the ability of one microorganism to grow despite the presence of drugs that are designed to inhibit their growth. According to the World Health Organisation (WHO), AMR has become one of the top 10 global public threats in the human and animal medicine field. Antimicrobial resistance (AMR) is easily being spread globally through direct contact, human to human or between human and animal including food consumption of animal products. Hence, AMR has caused major economic losses as it increased healthcare costs and prolonged the treatment period.

Since AMR is easily spread globally through direct contact- human to human or between human and animal, migratory birds and resident birds have become significant carriers of zoonotic and AMR bacteria. Around 5 billion migratory wild birds migrate across continents twice a year, causing global spread of numerous diseases through migration (Elsohaby et al, 2021). In Malaysia, pigeons are commonly found at recreational parks and roofs of houses which have close proximity with human contact. Interaction of the migratory wild bird with our local bird or contamination of faecal material to the environment causing spreading of AMR bacteria, results to a death threat to our community. Hence, pigeons and wild birds could be a potential reservoirs and disseminator of resistant bacteria to human (Wang et al., 2017)

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In a recent study, *E.coli* isolated from wild birds, chickens, environment and humans in Malay village , Kedah , Malaysia showed 100% resistance towards at least one antibiotic tested. It's reported that *E.coli* isolates from the wild bird showed high resistance towards erythromycin (100%) and tetracycline (56%). Meanwhile, *E.coli* isolates from the environment showed resistance against erythromycin (97%) and (57%) to tetracycline (Mohamed et al.,2022). However, no studies have been done yet to know the status of AMR in pigeons. Thus, this project aims to detect AMR bacteria, isolated from the cloacal swabs of pigeons in the residential area of Putrajaya.

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2.0 LITERATURE REVIEW

2.1 Antimicrobial resistance and it's status in Malaysia

The emergence of AMR may be driven due to misuse of the antimicrobials such as overuse of the antibiotics in treating a disease. Some people, not following the exact prescription that should be taken by the patient, worsen the emergence and spread of resistance bacteria. According to WHO, misuse of antibiotics in humans and animals has accelerated the development of antibiotic resistance (WHO,2020).

Antimicrobial resistance (AMR) bacteria has caused a lot of impacts such as treatment failures in pneumonia and tuberculosis infection. It's reported that these infections had already built some resistance towards current antibiotics which increase morbidity and mortality. Apart from that, it also increased healthcare costs, particularly in inventing new antimicrobials which usually take a long time. Based on several studies, AMR could cost more than $1 trillion annually by 2050 worldwide due to intensive treatments in treating patients with severe infections (Dadgostar P, 2019).

According to CDC, AMR has become top 10 global public health threats as it has killed at least 1.27 million people worldwide and has been associated with 5 million deaths in 2019. It also reported that AMR has killed more people than HIV/ AIDS. Based on statistics that have been done by the University of Washington in 2019,

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AMR was found to be responsible for 17,500 deaths in Malaysia. From these studies, it showed that there are more AMR deaths in Malaysia than deaths from neurological disorders, diabetes, kidney disease, transport injuries, chronic respiratory diseases, and digestive diseases. There are 6 pathogens that associated with AMR which led to death namely *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, *Acinetobacter baumannii, Pseudomonas aeruginosa* (Aguilar et al.,2023).

2.2 Pigeons act as carrier of AMR bacteria

Rock Dove also known as Pigeon (*Columba livia*) has become a common creature that we can see in urban and suburban areas. According to Shapiro and Domyam (2013), pigeons have become a symbol of religion, arts, and act as messengers during war. Unlike other synanthropic animals such as rats and cockroaches that most people dislike, people viewed pigeons as a lovely animal. In Iran, people would keep and breed pigeons on the roofs of their houses (Ghanbarpour et al, 2021).

Based on Weber et. al. (1995), pigeons that lived in the city transmit more than 30 diseases to humans through air and their faecal material. Whereas, a study from Geigenfeind et al. (2012) reported that pigeons carry at least 110 zoonotic pathogens within their droppings, and it can be transmitted to humans through direct contact. According to several studies, *Salmonella typhimurium*, *E. coli, Streptococcus* spp.,

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Chlamydophila psittaci, *Enterococcus* spp., and *Corynebacterium* spp. have been successfully isolated from the pigeons (Oh et. el, 2023). Based on Ghanbarpour et al. (2020b), pigeons should be considered as vector of resistant *E.coli* strains. *Escherichia coli,* and *Salmonella typhimurium* have been classified as foodborne pathogens bacteria as they can produce toxins that cause disease and in severe cases, lead to death (Heredia & García, 2018).

Apart from transmitting diseases, it's proven that pigeons also carry AMR bacteria in their droppings and contaminate the environment. Based on Dorota et al. (2021), pigeons have become carriers for multi-drug resistance in urban regions. From the case study, it was found that *Staphylococcus aureus* isolated from the oral cavity swab of pigeon showed resistance towards many groups of antibiotics such as phenicol, tetracycline and beta lactam antibiotics. Apart from that, *E.coli i*solates showed resistance towards all antimicrobials tested. These findings correspond with a study from Karim et. al (2021) reported that *E.coli* isolates showed resistant towards many group of antibiotics namely amoxicillin, ampicillin, azithromycin, erythromycin, nalidixic acid, gentamicin, and tetracycline. In a different study, *Salmonella* sp. isolates from pigeons showed highest resistance against tetracycline (100%), followed by nalidixic acid (81.82%), erythromycin (45.45%), amoxicillin (36.36%), ampicillin and azithromycin (27.27%) and levofloxacin (18.18%) respectively (Karim et al., 2020b).

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2.3 Pigeon feeding

The activity of pigeon feeding has grown in favour in recent years (Karim et al., 2020). Most of them think that pigeon feeding is an enjoyable activity as the pigeons look lovely and will come near to them to eat. They are raised near the community's house compound (Karim et al., 2020) and roofs (Ghanbarpour et al, 2021). This hobby has led to overpopulation in some places resulting in rules that had to be enforced to discourage people from feeding the pigeons (Gandhiok, 2019). Although people think pigeon feeding is enjoyable, this activity could harm them because it has been proven that pigeon has become a disease carrier that carries pathogenic bacteria that develop AMR (Dorota et al.,2021).

There are many reasons why people do pigeon feeding. Most of them said that they felt empathy towards the birds as they lived in the wild, worried that they did not get enough food. Usually, people encounter wild pigeons and their droppings in public areas, roosting sites and during outdoor activities (Oh et al.,2023).

2.4 Impacts of pigeon feeding

As they feed these pigeons, the birds will fly away and leave behind their droppings. These droppings not only carry a lot of pathogenic bacteria, but also harbouring fungus that can cause diseases such histoplasmosis, cryptococcosis and candidiasis (Gandhiok, 2019). According to the CDC, Histoplasmosis is an infection

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caused by *Histoplasma capsulatum* fungus, commonly found in pigeon droppings, that are rich with dampness and organic matter, a suitable place for fungus to thrive.

Apart from becoming a vector for pathogenic bacteria, it is reported that faecal material from pigeons can damage structures and buildings. Pigeon droppings cause buildings to deteriorate faster and increase the maintenance costs (Pigeons Facts and Figures— OvoControl®, n.d.). Since their excrement has a high acidity level, accumulation of it can cause erosion and softens the building materials (Blog, 2022). According to Gandhiok (2019), it is estimated that each pigeon excretes 11.5 kg annually, causing damage to the buildings and spreading the *Salmonella* germs.

2.5 Awareness of AMR carried by pigeons among communities

Antimicrobial resistance (AMR) is an increasing problem in human and veterinary medicine. According to Elsohaby et al (2021), migratory birds carry AMR bacteria from contaminated habitats and have become a reservoir and potential disseminators of resistant bacteria through migration. Although it is proven pigeons carry a lot of pathogenic bacteria in their droppings and feathers, people still feed them. This might be due to people still not aware about the potential threats that could be given by the pigeons to them. Based on Gandhiok (2019), people who feed these pigeons might get diseases without them realising it. Thus, it can be said that awareness of AMR carried by pigeons among the community is still poor and this issue must be raised to a higher level as it involves public health matters.

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3.0 MATERIALS AND METHOD

3.1 Cloacal swabs collection

Thirty (30) cloacal swabs of pigeons were collected around the residential area of Putrajaya. Sterile transport media was used to collect the samples and kept in a cooling box containing ice packs, maintained at low temperature at 4℃ before being processed. All feathers around the cloacal area were plucked out and sanitised with 70% alcohol to minimise contamination from the feathers before inserting the sterile swab. Each sample was labelled accordingly and processed within 24 hours.

3.2 Isolation of bacteria procedure.

The samples were brought to the Bacteriology Laboratory in the Faculty of Veterinary Medicine, University Putra Malaysia (UPM) for isolation and identification of bacteria.

All 30 cloacal swab samples were subjected to be inoculated onto blood agar for isolation of bacteria by using 4 quadrant streaking method. The swab samples were also inoculated into buffered peptone water (BPW) and Rappaport-Vassiliadis (RV) enrichment broth and continued plated on Xylose Lysine Deoxycholate (XLD) agar for isolation of *Salmonella sp.* Then, from the blood agar

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and XLD agar, each isolated colony was further subculture onto nutrient agar to obtain a pure culture. All inoculated agars were incubated at 37℃ for 24 hours.

3.3 Identification of bacteria procedure

Gram staining

Gram staining was performed on all isolated bacteria for identification. One drop of normal saline was placed on a clean glass slide and mixed with a loop of isolated colony by using a loop. The smear was then air-dried and stained with crystal violet for about 1 minute. The slide was washed with tap water and the slide was stained with Lugol's Iodine for another 1 minute. The slide was washed with acetone for 2-3 seconds and then washed with tap water immediately. The slide was stained with carbol fuchsin for 1 minute and washed with tap water. The slide was then ready to be observed under microscope.

Oxidase test

The isolated colony was added and mixed with oxidase reagent (Tetramethyl-p-phenylenediamine dihydrochloride) on filter paper. A positive result showed formation of dark purple colour within 10 seconds.

Catalase test

A drop of hydrogen peroxide was placed on a clean glass slide, mixed with a loop of isolated colony. A positive result showed production of gas bubbles.

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Coagulase test

A drop of normal saline was placed on a clean glass slide, mixed with a loop of isolated colony. Then, a drop of rabbit plasma was added, and the glass slide was tilted slowly to observe any formation of clumps/coagulation within the suspension which indicate positive result.

Triple Sugar Iron (TSI) test

The isolated colony was stabbed into the TSI agar medium, until reaching the tube 's butt, and then streaked across the surface of agar slant. The positive result was obtained when there were changes in colouration of both agar's slant and butt, presence of air bubbles indicates gas production and black pigmentation which indicate sulphide production.

Sulfide Indole Motility (SIM) test

The isolated colony was stabbed into the SIM agar medium. After 24 hours of incubation, Kovac's reagent was added. The positive result was obtained when there were blackening of the agar (indicate production of hydrogen sulphide), development of christmas tree or increased turbidity (indicate motility) within the medium and formation of red layer on top of medium after Kovac's reagent been added (indicate indole positive).

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Urease Broth test

The isolated colony was streaked onto the slant of agar. A positive result showed colour changes from yellow to pink magenta.

Citrate test

The isolated colony was streaked onto the slant of agar. A positive result showed colour changes from green to blue.

3.4 Antibiotic Susceptibility Test

The susceptibility of the isolates to antimicrobial agents was tested by standard disc diffusion method. All gram-negative bacteria were tested with 12 different antibiotics whereas, gram- positive bacteria were tested with six antibiotics. The list of antibiotics used was shown in Table 1.

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Table 1: List of antibiotics used for gram-negative and gram-positive.

Gram Negative	Gram Positive
• Amoxicillin-clavulanic acid (30 µg) • Doxycycline (30 µg) • Kanamycin (30 µg) • Norfloxacin (10 µg) • Trimethoprim /sulfamethoxazole (25 µg) • Polymyxin b (300 units) • Ampicillin (10 µg) • Chloramphenicol (30 µg) • Tetracycline (30 µg) • Cephalothin (30 µg) • Streptomycin (25 µg) • Gentamicin (10 µg)	• Vancomycin (5µg) • Gentamicin (10 µg) • Chloramphenicol (30 µg) • Trimethoprim /sulfamethoxazole (25 µg) • Erythromycin (5 µg) • Ampicillin (10 µg)

A loopful of pure culture from nutrient agar was mixed with 2 ml of sterile water in a test tube until a turbidity of 0.5 MacFarland standard suspensions was achieved. A swab from the mixture was streaked evenly onto Muller- Hinton agar plate and left to semi dry. Then, antibiotic discs were dispensed onto the agar plate by using an antibiotic bell dispenser manually. Each plate consists of 6 antibiotics, thus for gram- negative bacteria, 2 sets of agar plates were required for 1 isolate.

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The plates were incubated at 37°C for 24 hours. The zones of inhibition were measured by using digital vernier callipers and the measurements were rounded off to the nearest whole number. WHONET software was used to determine the breakpoints for classifying the isolates as susceptible, intermediate, or resistant to the antibiotics tested. The list of antibiotic groups was shown in Table 2.

Table 2: List of antibiotic groups.

Group of antibiotics	Antibiotic	Concentration of disk
Aminoglycosides	•Streptomycin (S) •Gentamicin (CN) •Kanamycin (K	25 µg 10 µg 30 µg
B- lactams	•Ampicillin (AMP) •Amoxicillin/ Clavulanic acid (AMC)	10 µg 30 µg
Cephalosporins	•Cephalothin (KF)	30 µg
Fluoroquinolones	•Norfloxacin (NOR)	10 µg
Glycopeptides	•Vancomycin (VA)	5 µg
Lipopeptides	•Polymyxin B (PB)	300 units
Macrolides	•Erythromycin (E)	5 µg
Sulfonamides	•Sulfamethoxazole/ Trimethoprim (SXT)	25 µg
Tetracycline	•Tetracycline (TE) •Doxycycline (DO)	30 µg 30 µg
Phenicol	•Chloramphenicol	30 µg

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3.5 Descriptive analysis

Descriptive statistical analysis was conducted to determine the frequency of resistant bacteria isolated from the cloacal swab of the pigeons.

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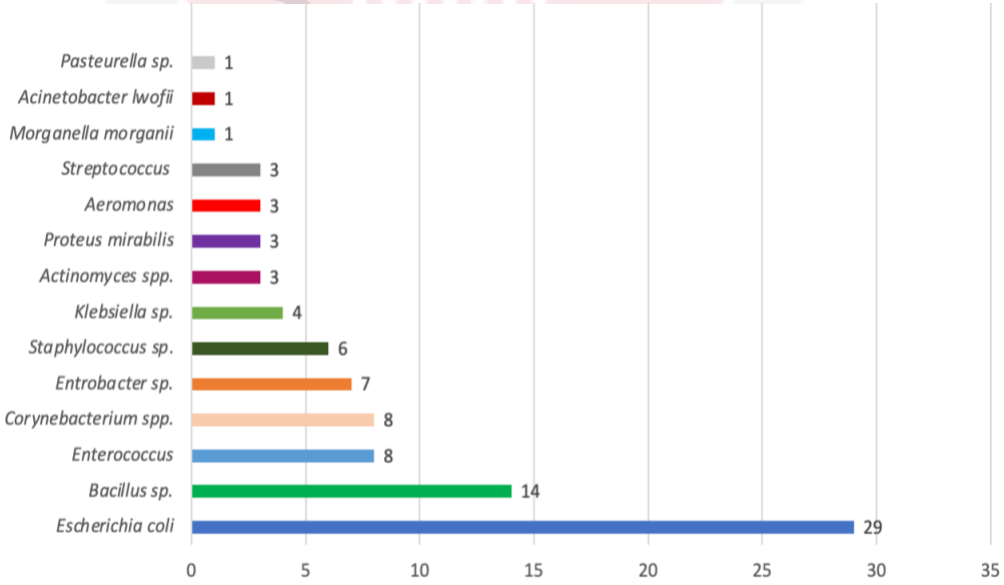
4.0 RESULTS

4.1 Isolation of bacteria from cloacal swabs sample of pigeon

Ninety-one isolates (91) consists of 14 genus of bacteria that were isolated from 30 cloacal swabs of pigeons around the residential area of Putrajaya. The highest bacteria isolated from the samples was *E. coli* with 96.7% (29/30). All samples were negative to *Salmonella sp*. Bacteria isolated were *Enterobacter sp.* 23.3% (7/30), *Klebsiella sp.* 13.3% (4/30), *Proteus mirabilis* 10% (3/30), *Aeromonas* 10% (3/30), *Morganella morganii* 3.3% (1/30), *Acinetobacter lwoffi* 3.3% (1/30), and *Pasteurella sp*. 3.3% (1/30). *Bacillus sp was isolated from 46.7% (*14/30), *Corynebacterium sp*. 26.7% (8/30), *Enterococcus 26.7% (*8/30), *Staphylococcus* 20% (6/30), *Streptococcus* 10% (3/30) and *Actinomyces sp. 10% (*3/30) of the samples (Figure 1).

Figure 1: Summary of total isolates from 30 cloacal swab samples.



4.2 Antimicrobial Resistant (AMR) profile of bacteria isolated from cloacal swab of pigeons.

4.2.1 Antimicrobial Resistant (AMR) profile of four pathogenic bacteria.

Escherichia coli, Enterococcus sp., Enterobacter sp. and *Klebsiella sp,* showed 100% (29/29), 87.5% (7/8), 85.71% (6/7) and 50% (2/4) resistance against ampicillin respectively (Figure 2). *Escherichia coli* showed resistant towards six other antibiotics namely cephalothin 27.59% (8/29), doxycycline 14% (4/29), sulfamethoxazole / trimethoprim 10.3% (3/29), tetracycline 10.3% (3/29), chloramphenicol 3.45% (1/29) and gentamicin 3.45% (1/29). Next, *Enterococcus sp* showed resistant towards four other group of antibiotics, namely, vancomycin 62.5% (5/8), sulfamethoxazole / trimethoprim 62.5% (5/8), gentamicin 25% (2/8) and erythromycin 12.5% (1/8). *Enterobacter sp.* showed resistant towards five other antibiotics namely cephalothin 57.14% (4/7), amoxicillin-clavulanic acid 28.75% (2/7), kanamycin 14.29% (1/7), polymyxin b 14.29% (1/7) and streptomycin 14.29% (1/7). Lastly, *Klebsiella sp.* showed resistance towards one other antibiotic namely tetracycline 25% (1/4).

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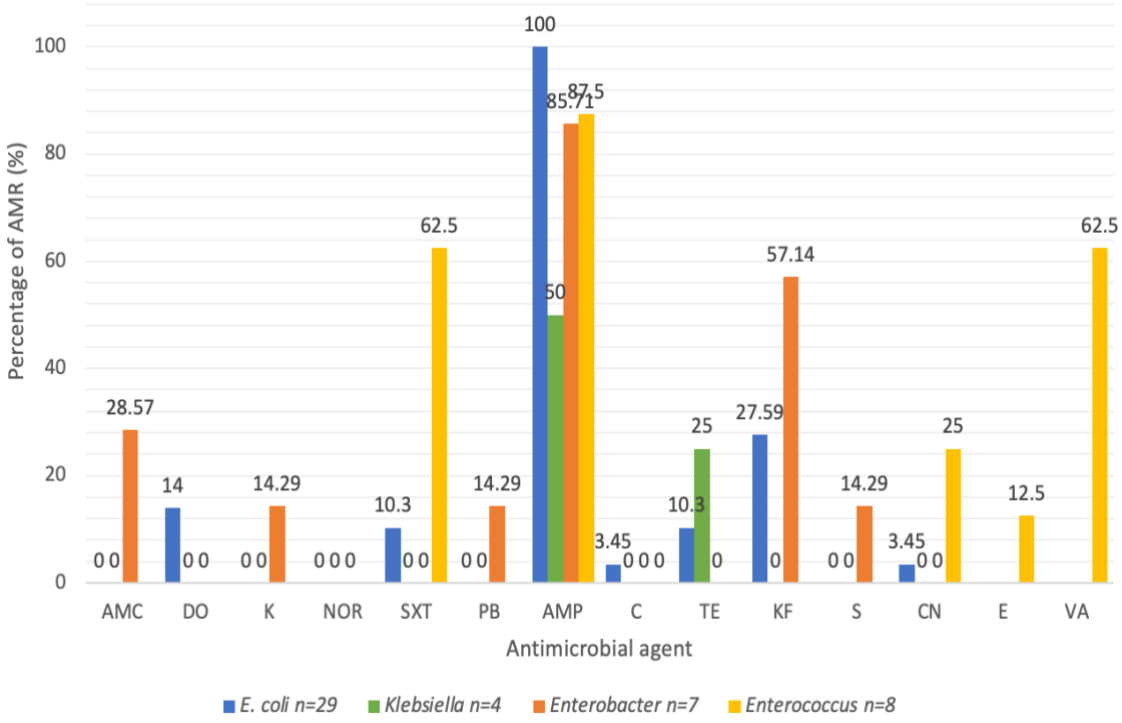
Figure 2: Percentage of AMR in four pathogenic bacteria.



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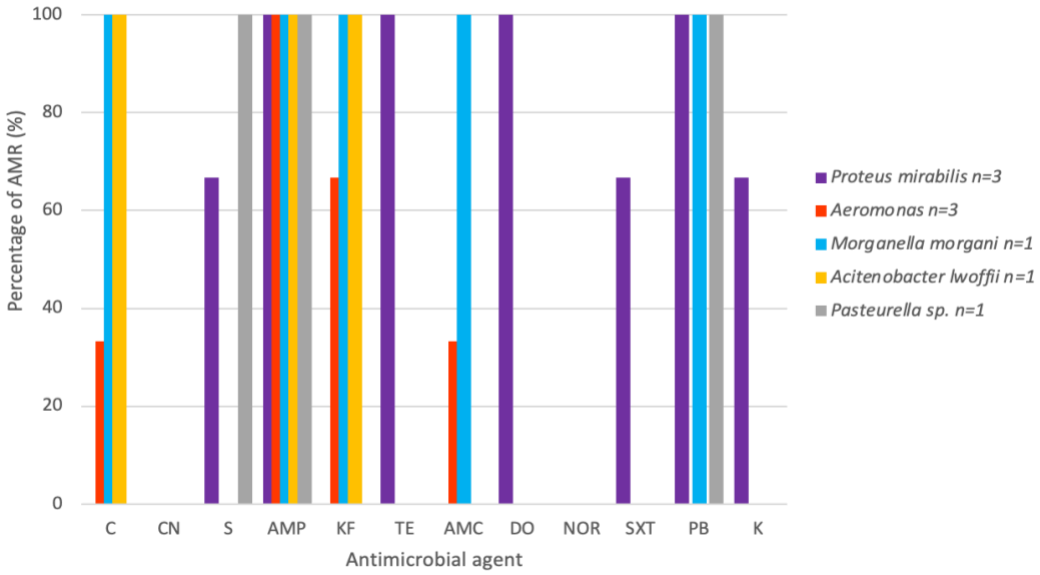
4.2.2 Antimicrobial Resistant (AMR) profile of gram-negative bacteria.

All isolates of *Proteus mirabilis* (3/3), *Aeromonas sp* (3/3), *Morganella morganii* (1/1), *Acinetobacter lwoffii (1/1) and Pasteurella sp.* (1/1) showed resistance towards ampicillin 100% (Figure 3). Furthermore, *Proteus mirabilis* was also showed resistant towards six other antibiotics namely tetracycline 100% (3/3), doxycycline 100% (3/3), streptomycin 66.67% (2/3), sulfamethoxazole / trimethoprim 66.7% (2/3), polymyxin b 100% (2/3) and kanamycin 66.7% (2/3). Meanwhile, *Aeromonas* showed resistant towards chloramphenicol, cephalothin and amoxicillin- clavulanic acid which were 33.33% (1/3), 66.7% (2/3) and 33.33% (1/3) respectively.

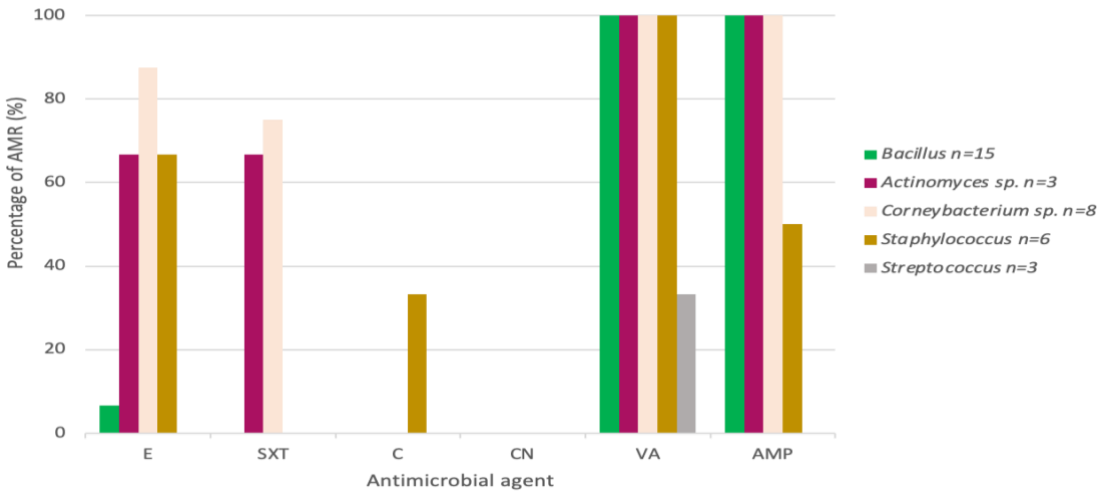
Figure 3: Percentage of AMR in gram-negative bacteria.



4.2.3 Antimicrobial Resistant (AMR) profile of gram-positive bacteria.

All gram-positive isolates showed resistance towards vancomycin where *Bacillus 100%* (15/15), *Actinomyces sp.* 100% (3/3), *Corynebacterium sp*. 100% (8/8), *Staphylococcus* 100% (6/6) and *Streptococcus* 33.33% (1/3) resistance *respectively*. *Bacillus sp,* (15/15)*, Actinomyces sp.* (3/3)*, and Corynebacterium sp.* (8/8) *showed* 100% resistance towards ampicillin but 50% (3/6) resistant in *Staphylococcus* sp (Figure 4).

Figure 4: Percentage of AMR in gram-positive bacteria.



5.0 DISCUSSIONS

The spread of infections from animals to people has been partially linked to an increase in AMR cases in humans. According to WHO in 2017, *E.coli*, *Enterococcus faecium, Klebsiella pneumoniae* and *Enterobacter species* had been classified as highest "priority status" in developing AMR , that could give potential threats to humans (Mancuso et al., 2021). In this study, all these 4 pathogenic bacteria were successfully isolated from the cloacal swab of pigeons in the residential area of Putrajaya and showed resistance to at least one group of antibiotics that were tested and these antibiotics were commonly used in animals and humans.

In this study, 29 *E. coli* isolates from pigeons were obtained and the resistance of these isolates to 12 commonly used antibiotics was monitored. In one study, *E.col*i has become clinically significant in veterinary medicines as they showed resistance to many groups of antibiotics such as penicillin, aminopenicillin, and cephalosporin which are recognised as veterinary medications (Poirel et al., 2018). These findings consistent with the results where *E. coli* has shown resistance towards seven group of antibiotics where ampicillin showed the highest resistance (100%), followed by cephalothin (27.59%), doxycycline (14%), sulfamethoxazole / trimethoprim (10.3%), tetracycline

(10.3%), chloramphenicol (3.45%), and gentamicin (3.45%). People who are not responsible for disposing of antibiotics might dump all these expired antibiotics to the nearby lakes or water bodies. The pigeons might have picked up the resistant bacteria from the environment where the bacteria had undergone pressure selection due to long exposure of the antibiotics. Thus, proper handling of antibiotics should be practised to stop unnecessary exposure of antibiotics to the bacteria in the environment. Based on Mohamed (2021a), *E. coli* acquires resistance to antibiotics faster than any other bacteria.

Klebsiella sp, Enterobacter sp, and *Enterococcus sp* isolates showed resistance towards ampicillin with values of (50%), (85.71%) and (87.5%) respectively. Based on Mancuso et al. (2021), *Enterobacter* species has caused opportunistic infections in immunocompromised patients. In this study, it showed that *Enterobacter* has built resistance towards six groups of antibiotics and among these antibiotics, polymyxin b was tested and it showed resistance of (14.29%). Based on a previous study, the prevalence of polymyxin resistance in gram negative bacteria is 10% worldwide (Silva et al., 2022) and from this study, it exceeds this value. Note that, polymyxin b antibiotic is being classified as a reserved group of antibiotics that only could be used after culture and susceptibility tests had been done. Since polymyxin b has become the last line antimicrobial agent for treatment of pan- drug resistance caused by gram negative bacteria, emergence of resistance to this antimicrobial has raised a lot of concerns worldwide (Modupe et al.,2021).

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Vancomycin- resistant bacteria has been observed in this study where *Bacillus, Actinomyces sp., Corynebacterium sp.* and *Staphylococcus* showed *(100%)* resistance whereas *Enterococcus* showed (62.5%) resistance. According to Levitus (2023), vancomycin-resistant Enterococcus (VRE) is hard to manage since the bacteria has built up resistance against many groups of antibiotics. Based on Mancuso et al. (2021), penicillins and cephalosporins were used way before vancomycin, to combat the *Enterococcus* infection in hospital settings. However, due to overuse of these group antibiotics in the healthcare field, the bacteria built up resistance by expressing a higher β-lactamase enzyme which led to development of resistance to β-lactam antibiotics (Mancuso et al. ,2021). In the 1990s, vancomycin-resistant enterococci (VRE) was notified as the second most common nosocomial pathogen due to large usage of vancomycin. According to Levitus (2023), alteration in the formation of peptidoglycan that makes up the bacterial cell wall is a mechanism on how vancomycin-resistant *Enterococcus* developed.

Based on Levitus (2023), vancomycin-resistant *Enterococcus* can be found on the skin and faecal material and these resistant bacteria can be spread via contaminated surfaces. According to the National Healthcare Safety Network, enterococci species was reported as the second most common healthcare-associated infection between 2011 until 2014. In this study, *E. faecalis* and *E.*

faecium were managed to isolate from the pigeon's cloacal swab. Mancuso et al. (2021) reported that *E. faecium has* shown resistance against many antimicrobial agents than *E. faecalis*, despite the latter being regarded as the most pathogenic species.

Overall, as the pigeons lived close to human houses, it increased the possibility of the AMR bacteria transmission through direct contact or airborne. According to Gandhiok (2019), inhalation of fungus from the pigeon's droppings can cause Histoplasmosis in immunocompromised patients. People who love to do pigeon feeding are prone to get these resistant bacteria as they attract the birds to live near to their houses. Additionally, they will breed on the roofs and leave out their droppings near to the house compound. Based on Geigenfeind et al. (2012), pigeon's faecal material carries at least 110 zoonotic pathogens and can be transmitted to humans through direct contact.

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CONCLUSION

The present study found that *E. coli*, *Enterobacte*r sp, *Klebsiella* sp and *Enterococcus sp* showed high prevalence of AMR towards ampicillin with (92.5%). This result should not be taken lightly even though ampicillin is being classified as first line antibiotics. Apart from that, *Enterococcus* sp showed high resistance against vancomycin with (62.5%). Note that vancomycin is being classified as Critically Important Antimicrobials (CIA) in human medicine, and should be used prudently to avoid emergence of vancomycin-resistant *Enterococcus* sp. Thus, authorities and people who are responsible in using antimicrobials should be more aware about this AMR issue by practising good antimicrobial stewardship. People and community from Putrajaya are advisable not to do pigeon feeding anymore as it is proven that these pigeons carry a lot of pathogenic bacteria that develop AMR within their faecal material.

RECOMMENDATION

The limitation of time for sampling the cloacal swab of pigeons. Therefore, a longer period is required to conduct the next study to obtain more sample size that can represent the target population. It was recommended, a survey can be conducted among people who did pigeon feeding to know their health status or history of getting any diseases. Apart from that, faecal samples from humans that have close contact with pigeons and environments that are contaminated by pigeon's droppings can be taken to determine their AMR status.

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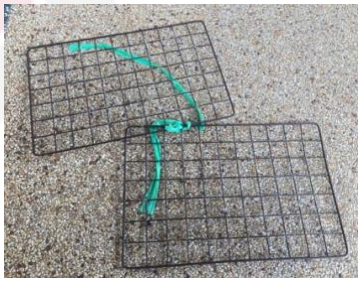
APPENDICES

Appendix A: Pigeons were waiting for their feeding time around house compound.

Appendix B: Procedure of catching and sampling cloacal swab of pigeons. Example of baits and trap that were used to catch the pigeons.



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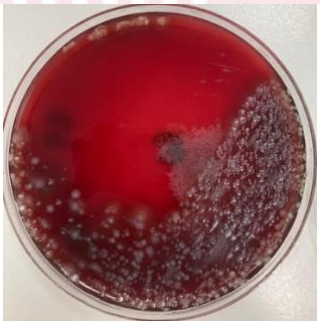
Process of sampling cloacal swab of pigeons

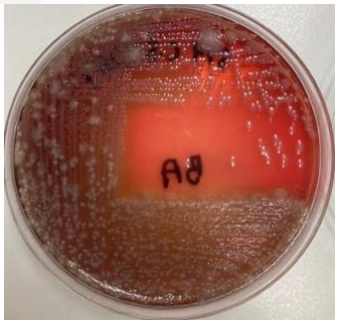
The feathers were plucked out to minimize the contamination from the feathers before inserting the sterile swab. The cloacal was swab with 70% alcohol to sanitize the area.

Appendix C: Isolation and identification of bacteria.

Blood agar was used as the primary culture media to isolate all species. Then, XLD agar was used to isolate specific bacteria such as *Salmonella* sp. Nutrient agar was used to obtain a pure culture.

Blood agar





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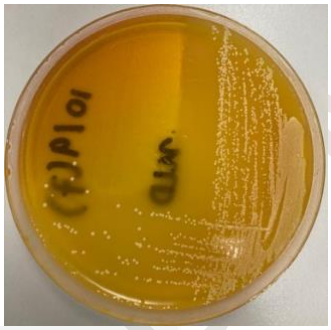
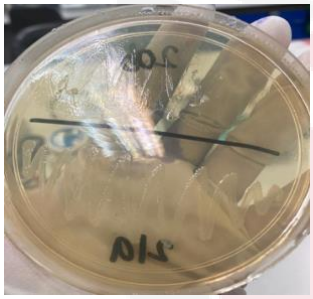
XLD agar



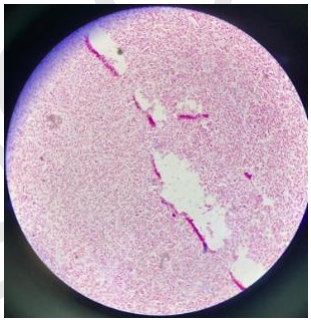
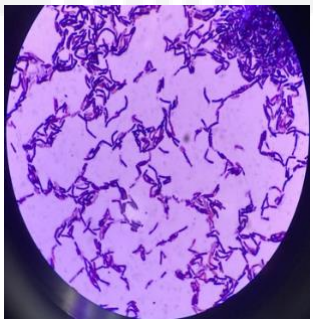
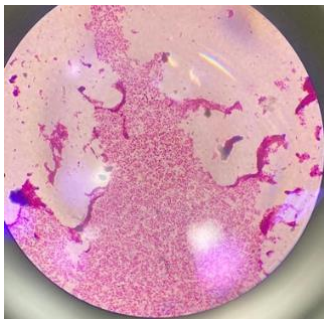
Nutrient agar



Cell Morphology

The isolated colony was stained with Gram staining and observed under microscope.

Microscopic morphology

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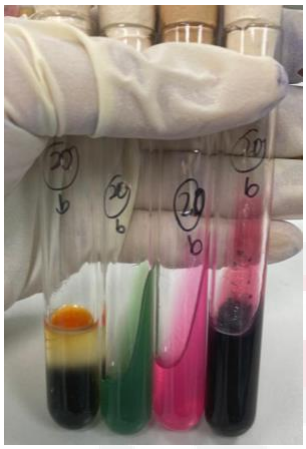
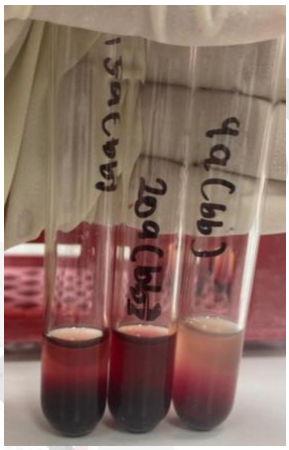
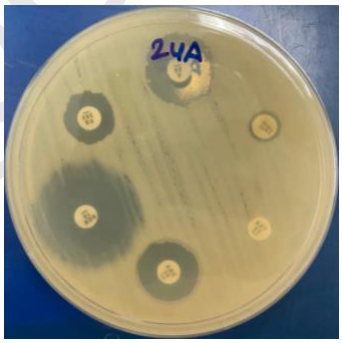
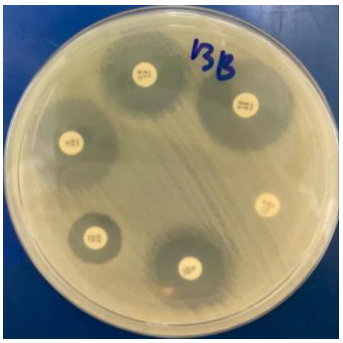
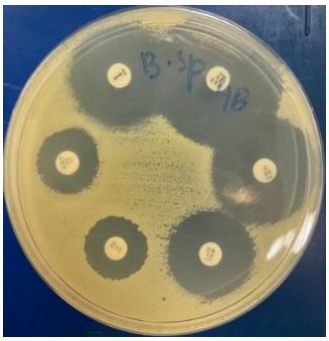
Biochemical test





Appendix D: Antibiotic susceptibility test

Example of antibiotics used

Antibiotic susceptibility test (AST) results on MHA agar