



**UNIVERSITI PUTRA MALAYSIA**

**A SYSTEMATIC REVIEW OF ANTIMICROBIAL RESISTANCE OF  
Escherichia coli ISOLATED FROM PIGEONS**

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*Escherichia coli* ISOLATED FROM PIGEONS**

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A project paper submitted to the  
Faculty of Veterinary Medicine, Universiti Putra Malaysia  
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## DEDICATION

This thesis is especially dedicated to:

**My parents, who has done everything to support me**

Alex Teng, Lily Sng

**My supportive supervisors, who guided me in my research**

Assoc. Prof. Dr. Siti Khairani Bejo

Prof. Dr. Jalila binti Abu

and

**All my fellow DVM friends.**

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My friends who have kept me accompany throughout my learning process has undoubtedly given me the strength and heart to complete this research. All of my friends have provided help directly or indirectly to push me through the finishing line.

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## CONTENTS

TITLE .....	i
CERTIFICATION .....	ii
DEDICATION .....	iii
ACKNOWLEDGEMENTS .....	iv
CONTENTS .....	v
LIST OF TABLES .....	vi
ABSTRAK .....	vii
ABSTRACT .....	ix
INTRODUCTION .....	1
METHODOLOGY .....	7
RESULTS .....	11
DISCUSSION .....	15
RECOMMENDATION .....	18
CONCLUSION .....	19
REFERENCES .....	21

## LIST OF TABLES

	PAGE
Table 1: Keywords, Synonym and Variation of search string.....	4
Table 2 : Search string utilised .....	5
Table 3: Inclusion and Exclusion criteria .....	6
Table 4: Percentage of total <i>E. coli</i> isolated tested against specific antibiotics.....	12
Table 5: Methods used to test for <i>E. coli</i> antibiotics susceptibility .....	12
Table 6 : AMR rate of <i>E. coli</i> samples collected from Captive or Free- living pigeons	14

**ABSTRAK**

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4901- Projek.

**SEBUAH ULASAN SISTEMATIK TENTANG RINTANGAN ANTIMIKROB  
*Escherichia coli* DIASINGKAN DARIPADA MERPATI****Oleh****Teng Huah Sheng  
2021****Penyelia: Prof. Madya Dr. Siti Khairani Bejo****Penyelia Bersama: Prof. Dr. Jalila binti Abu**

Rintangan antimikrobial (AMR) sering dianggap sebagai salah satu ancaman terbesar kepada perubatan moden. Ciri-ciri AMR telah ditemui lebih kerap dalam bakteria berbanding sebelum ini. Keberkesanan antibiotik telah diancam, mengakibatkan peningkatan kerumitan dalam rawatan menggunakan antibiotik dan kekurangan kesan antibiotik. Ini telah menyebabkan pemantauan rintangan terhadap antibiotik dalam haiwan liar menjadi lebih penting berbanding sebelum ini, terutamanya dalam haiwan synanthropic. Kertas kerja ini membentangkan tinjauan literatur sistematik dengan tujuan untuk menyiasat perbezaan antara status AMR merpati liar dan kurungan, serta trend AMR selama ini. Ini akan memberi kita cerapan tentang cara bakteria AMR yang lazim ditemui dalam haiwan synanthropic seperti merpati yang hidup berdekatan dengan manusia, dan perbezaan dalam AMR merpati liar dan merpati kurungan. Melalui analisis 19 artikel merentas tiga pangkalan data, Science Direct, SCOPUS dan PubMed, pandangan holistik *E.coli* AMR yang diasingkan daripada merpati dicapai. Antibiotik yang paling kerap diuji untuk kerentanan merentas kertas yang dipilih untuk semakan ialah Ampicillin (AMP), Ciprofloxacin (CIP), Gentamicin (GEM), Tetracycline (TET), Chloramphenicol (COT) dan Trimethoprim/ Sulfamethoxazole (SXT). Akibatnya,

merpati kurungan mempunyai kadar AMR yang lebih tinggi dalam *E.coli* diasingkan merentas antibiotik yang disebutkan secara konsisten. *E.coli* yang diasingkan daripada merpati kurungan didapati ada tahanan antimikrob pada 52.51%, 21.17%, 15.78%, 65.66%, 22.97 dan 22.91% masing-masing manakala *E.coli* yang diasingkan daripada merpati liar ialah 24.07%, 5.87%, 5.87% %, 9.85% dan 6.86% kepada AMP, CIP, GEM, TET, COT dan SXT masing-masing. Walaupun keputusan ini konsisten dengan kebanyakan penemuan yang menunjukkan bakteria dengan ciri AMR lebih kerap ditemui dalam haiwan kurungan berbanding haiwan liar, ia masih amat membimbangkan kerana *E.coli* dengan sifat AMR daripada merpati liar didapati pada kadar yang begitu tinggi. Ini mencadangkan bahawa semua orang, tidak kira pada persekitarannya ada berisiko untuk dijangkiti oleh bakteria dengan sifat AMR

Kata kunci: rintangan antimikrob; merpati; *E coli*; antibiotik

**ABSTRACT**

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfilment of the course VPD 4901 – Project.

**A SYSTEMATIC REVIEW OF ANTIMICROBIAL RESISTANCE OF  
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by

**Teng Huah Sheng**

**2021**

**Supervisor: Assoc. Prof. Dr. Siti Khairani Bejo**

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Antimicrobial Resistance (AMR) is oftentimes considered as one of the biggest threats to modern medicine. AMR traits have been found more frequently in bacteria than ever, threatening the efficacy of antibiotics, resulting in less effective and increased complexity in treatment using antibiotics. This has resulted in monitoring resistance to antibiotics in wild animals to be more important than ever, especially in synanthropic animals. This paper presents a systematic literature review with the purpose of investigating the difference between AMR status of free-living and captive pigeons, as well as the trend of AMR over the years. This will provide us with insight as to how prevalent AMR bacteria are found in synanthropic animals such as pigeons that live close to humans, and the differences in AMR of free-living pigeons and captive pigeons. Through the analysis of 19 articles across three databases, Science Direct, SCOPUS and PubMed, a holistic view of the *E.coli* AMR isolated from Pigeons is achieved. The most frequently tested antibiotics for susceptibility across the papers selected for review are Ampicillin (AMP), Ciprofloxacin (CIP), Gentamicin (GEM), Tetracycline (TET),

Chloramphenicol (COT) and Trimethoprim/ Sulfamethoxazole (SXT). Consequently, domestic or captive pigeons consistently have a higher AMR rate in the isolated *E. coli* across the antibiotics mentioned. *E. coli* isolated from captive pigeons are found to be resistant at 52.51%, 21.17%, 15.78%, 65.66%, 22.97 and 22.91% respectively while *E. coli* isolated from free-living pigeons are 24.07%, 5.87%, 7.87%, 19.80%, 9.85% and 6.86% to AMP, CIP, GEM, TET, COT and SXT respectively. While these results are consistent with most findings indicating bacteria with AMR traits are more often found in captive animals compared to free-living animals, it is still worrying to be able to isolate *E. coli* with AMR trait from wild pigeons at such a high rate, which leads to suggest that everyone, no matter the setting is at risk of being infected by bacteria with AMR trait.

**Keywords:** antimicrobial resistance; pigeon; *E. coli*; antibiotic

## INTRODUCTION

Rock Dove / Pigeons (*Columba livia*) or more commonly just called pigeons has become a common sight of the urban and suburban human population, various cultures have also domesticated it for the purpose of food production, or sports such as that of pigeon racing. Pigeons are also well adapted to the urban landscape as it naturally roosts and breed on cliff or rock ledges while in the wild, which is a characteristic that has allowed it to flourish in an urban setting where tall buildings with rock ledge like structure are aplenty, and trees are far and few between, potentially creating a space where other birds that naturally roost and breed in trees won't be as successful. The pigeon's success in the modern environment can also be seen where some attempts to control its population show almost no impact and any changes in the population in an area is almost instantly replaced with other pigeons from a nearby area (Sol and Senar, 1995). Pigeons could also potentially host several human pathogens not limited to bacteria (Haag-Wackernagel and Moch, 2004).. The combination of the pigeon being so widespread in an urban environment and its success in such an environment could potentially make pigeons a source of zoonotic disease that impacts many human lives.

Unlike most other synanthropic animals found in an urban environment such as rats, or mice, pigeons aren't met with as much disgust generally, and in fact, pigeon feeding is a pastime of many in various places of the world. This hobby of many has led to overpopulation in some places resulting in rules that had to be enforced to discourage people from feeding the pigeons (Haag-Wackernagel, 1995). There also exists some culture where people would keep and breed pigeons on the roofs of houses as a hobby such as in Iran (Ghanbarpour *et al.*, 2020). This is another reason pigeons could potentially be a source of zoonotic disease since the general population aren't typically avoidant of pigeons and may even seek out opportunities to get close to it through activities like pigeon feeding. This puts pigeons in a very unique position

where they are almost present in all major cities in the world, possess the risk to harbour zoonotic diseases, and yet aren't avoided by the general population. Zoonotic diseases caused by pigeons aren't an issue limited to regions that are developed, it can also be seen in developed region such as in Japan, where a study on contamination level of fecal samples of pigeons in public areas revealed pigeon feces as a source of several zoonotic agents for birds, animals, and humans (Tanaka *et al.*, 2005). This zoonotic risk posed by pigeons is compounded when faced with risks of antimicrobial resistance that may be present in these bacteria from pigeons, complicating treatment if one were to be infected by such a bacteria.

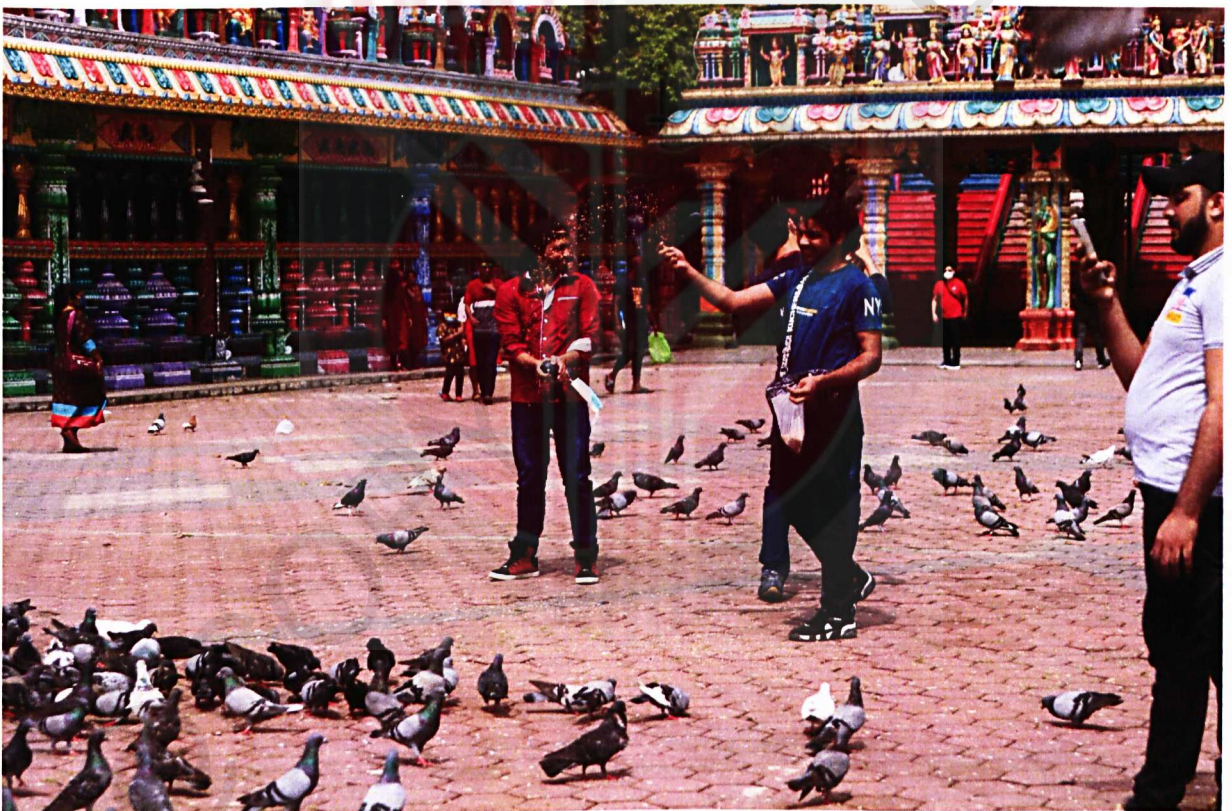


Figure 1: Tourist feeding pigeons at Batu Caves, Selangor, Malaysia.

WHO (World Health Organisation) has urged humanity to take action to prevent a return to a “pre-antibiotic era” back in 2015. This stance by WHO still holds true even now as this issue seems nowhere near solved, and will only continue to pose a risk to modern medicine if no change in practice is done on a global scale. It is also noted that indiscriminate use of antibiotics has indirectly or directly selected for bacteria with antimicrobial properties. This is

an ongoing issue that will cause treatment for diseases caused by bacteria much more complex as more bacteria become resistant to the antibiotic used. Considering diarrheagenic *E. coli* has been isolated in pigeons (Horn *et al.*, 2018), it becomes a source of potential environmental contamination which could lead to human infection. In fact, *E. coli* was also responsible for 0.9% (48 out of 5,079) of the foodborne disease outbreak in the European Union in 2017 (EFSA and ECDC). This is only considering reported cases, we could expect there to be more unreported cases throughout the world, affecting thousands of lives annually. As pigeons are widely found in areas or regions with a huge population of humans, it can constitute as a reservoir for antimicrobial resistant (AMR) bacteria, and serves as a risk for human health due to their proximity to humans and its ability to 'distribute' the AMR. *Escherichia coli* are often selected as an indicator bacteria for faecal contamination of the environment, particularly for food; it can also be used to monitor antimicrobial resistance in different animal species (Kojima *et al.*, 2009).

There are not many studies made for *E. coli* antimicrobial resistance isolated from pigeons, however most studies that were conducted have shown that AMR bacteria are often isolated from both captive and free-living pigeon populations. This suggests that AMR bacteria are not only confined to the domesticated population that interact with humans on a daily basis, but also the free-living pigeon population which may not have much interaction with humans depending on the area in which the pigeons reside. This further suggests that pigeons could serve as a sentinel of AMR bacteria, giving it the potential to disseminate AMR bacteria into the environment and serve as a point of contact where bacteria could obtain AMR quality through horizontal gene transfer such as plasmids and even facilitate new epidemics (Baker *et al.* 2018).

Monitoring resistance to antibiotics is essential to obtain more information in its trend, and also potentially inform future use of antibiotics, as well as regulations in regards to the use of antibiotics. Obtaining more information about the current resistance to antibiotics in bacteria could also inform us in potential ways these bacteria are obtaining these resistance properties, and how we can prevent the bacteria from getting more AMR and spreading it even further. Some studies have also suggested that bacteria may be co-selected for different antibiotics, indicating that no particular antibiotic has to be widely used before selection pressure for bacteria with resistant properties is developed in the bacteria (Kimpe *et al.*,2002). This indicates that regular monitoring studies should be done to obtain the most up to date information about the antibiotic most commonly resistant found in the environment and synanthropic animals in order to be informed in what antibiotics to be administered for diseases due to bacterial origin.

This systematic review aims to identify some of the most effective methods in order to test for antimicrobial resistance in the bacteria isolated. Besides that, the wider objective is to be able to compare the AMR of *E. coli* isolated from captive and free- living pigeons.

<b>Keyword</b>	<b>Synonym (similar word)</b>	<b>Variation</b>
Antimicrobial resistance (AMR)	Antibiotic resistance, superbug	Multidrug resistance (MDR)
<i>Escherichia coli</i>	<i>E. coli</i>	Pathogenic <i>Escherichia coli</i>
Pigeon	<i>Columba livia domestica</i> , Rock pigeon, Columbidae	Doves

Table 1: Keywords, Synonym and Variation of search string

Database	Search string
<b>PubMed</b>	(antimicrobial resistance[Title/Abstract] OR antibiotic resistance[Title/Abstract] OR Multidrug resistance[Title/Abstract] OR AMR[Title/Abstract] OR MDR)[Title/Abstract] AND ( <i>Escherichia coli</i> [Title/Abstract] OR <i>E. coli</i> [Title/Abstract] OR Pathogenic <i>Escherichia coli</i> )[Title/Abstract] AND (Pigeon[Title/Abstract] OR <i>Columba livia domestica</i> [Title/Abstract] OR Columbidae[Title/Abstract] OR doves[Title/Abstract] OR Rock pigeon)[Title/Abstract]
<b>ScienceDirect</b> (Title, Abstract or author - specified keywords)	( antimicrobial resistance OR superbug OR antibiotic AND resistance ) AND ( pigeon OR columba livia domestica ) AND ( <i>E. coli</i> OR <i>escherichia coli</i> )
<b>SCOPUS</b>	TITLE-ABS-KEY ( ( anti AND microbial AND resistance OR superbug OR antibiotic AND resistance OR antimicrobial AND resistance OR anti-microbial AND resistance ) AND ( pigeon OR columba AND livia AND domestica ) AND ( <i>E. coli</i> OR escherichia AND coli OR e AND coli OR e. AND coli ) )

Table 2: Search string utilised

<b>Criterion</b>	<b>Inclusion</b>	<b>Exclusion</b>
<b>Literature type</b>	Empirical article	Opinion Focused, Duplicates, Systematic Review, Meta-Analyses
<b>Language</b>	English or translated to English approved by researcher / publisher	Other languages besides English
<b>Research Type</b>	Experimental, Case study, Quantitative study	Qualitative study
<b>Publication date</b>	1990-2021	Before 1990
<b>Number of antibiotics tested for susceptibility</b>	At least 3 antibiotic, and 3 antibiotic group represented	Less than 3 antibiotics
<b>Isolates tested for antimicrobial susceptibility</b>	All <i>E. coli</i> isolated were tested	Only a specific group of <i>E. coli</i> isolate are tested

Table 3: Inclusion and Exclusion criteria

## METHODOLOGY

The review is done in consideration of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines. This standard is often used for systematic reviews, especially in fields relating with medicine. While this paper is not strictly in relation with medicine, it is close enough in its nature to greatly consider adopting PRISMA as the guideline while conducting the systematic review. PRISMA's guidelines are also relatively easy to follow, and it also provides clear instruction which would help make the process of the systematic review much smoother.

The search terms are detailed in Table 1. The terms are selected based on its similarity to the primary words selected that will give the highest possibility of obtaining papers with relevant information while eliminating papers that will not provide the information we require for the systematic review. The primary keywords are selected to fulfill the objective of this research, and synonyms as well as variations are considered on the basis that some researchers may focus on other aspects that could potentially be linked to this research objective. For example, while our objective is focused on antimicrobial resistance, research that focuses on multidrug resistance may also bring in additional data and relevant information. Another variation to be considered is the term 'doves', while many differentiate doves and pigeons based on its setting or feather colouration, they could be referring to the same animal, *Colum livia*, but chose to use the term 'doves'. The keywords are used to search and narrow down the papers chosen to be reviewed and the keywords are specifically applied to narrow down papers based on its presence in the title, abstract and keywords (usually abbreviated as TITLE-ABS-KEY in most search engines). Additionally, every paper that fit the keyword criteria was reviewed fully before being excluded based on the exclusion criteria even if the title and abstract may suggest that it is irrelevant to the current studies.

The databases are selected based on several characteristics such as its ease of use, library size and accessibility based on the university's subscribed database. The selected databases are Science Direct, SCOPUS and PubMed. Science Direct has a long history as it is a database operated by Elsevier, being one of the oldest publishing companies for scientific contents. While SCOPUS is operated by Elsevier as well, it provides a different database as ScienceDirect as the database includes abstract from other publishers other than Elsevier. It has also withstood scrutiny and came out on top with outstanding customer satisfaction and service quality (Yas *et al.*, 2020). PubMed provides a free search engine for life sciences and medical papers which are fitting for this research topic.

Utilising the search string as detailed in Table 2 for the respective databases, 24, eight, and eight papers from PubMed, ScienceDirect and SCOPUS respectively fit the search criteria, totaling to 38 papers.

One reviewer, screened through all the papers to eliminate papers based on the inclusion and exclusion criteria detailed in Table 3. The criteria are selected to best suit the objective of the research. The time frame for the paper to be included is based on the fact that antimicrobial resistance as an issue is only being brought to light in the past 10 years or so, and therefore a 20 years' time frame should allow the researcher to capture most of the most recent researches related with *E. coli* AMR isolated from pigeons. Similarly, the number of antibiotics screened for susceptibility is set to a minimum of four with at least one antibiotic in four different antibiotic groups for the purpose of being able to detect for multi drug resistant bacteria as it is one of the many points of interest when conducting this systematic review. The criteria of language are also set on the basis of readability and ability for the reviewer to understand the

paper. The research type and literature type should also be focused on empirical data and quantitative study, as the research should be based on solid data in order to arrive at a strong conclusion. Lastly, the antibiotic susceptibility portion of the research should be done on all *E. coli* isolated so as to not indirectly introduce bias to the data, skewing the result one way or the other.

Based on these criteria, a total of 19 papers are rejected due to repeated papers, not fulfilling the inclusion criteria, or excluded due to the exclusion criteria. This brings the total paper to be used for data collection down to 19, from the previous total of 38. There are papers that were rejected from all three databases used, specifically, 15, two and two papers from PubMed, ScienceDirect and SCOPUS respectively were rejected.

Once the papers are screened due to incompatibility with the inclusion and exclusion criteria, relevant information for the research is extracted from the papers. Information such as date of sample collection, the location of the pigeon in which the sample are collected from, method of obtaining the pigeon, the origin of the sample, sample transport medium/ storage medium, culture medium, method for testing antimicrobial susceptibility, number of isolated *E. coli* sample and whether the pigeon is captive/ domesticated or free-living/ feral, number of antimicrobial agents tested for susceptibility and its result, as well as number of multi drug resistance (MDR) bacteria. These information are selected to be extracted in order to be able to see a trendline in terms of AMR and MDR rate among the isolates across the years, and maybe across different geographic region, and also potentially provide insight as to the difference of AMR and MDR prevalence between the pigeons that are free living and those that are captive. This would provide us a holistic view of the current condition of the AMR

isolated from the pigeons as it gives insight to it on the basis of timeline, geographical and type of living condition.

However, as these papers might be done for a different purpose other than testing for antimicrobial susceptibility, there may be some gap in information that this research intends to extract, and therefore some level of interpretation is required when analysing the data obtained. For example, for the purpose of this research, papers where the date of sample collection is not specified, it will be assumed that the samples are collected in the same year the paper is first sent to the publisher. If the sample collection occurs across multiple years, it will be treated as if all of the samples are collected in the year the final sample is collected. If there are gaps in information during analysis of the antibiotic susceptibility rate, the particular group of samples with information gap will not be taken into consideration during analysis as to not unintentionally skew the results due to missing data.

Finally once the data is extracted, it is analysed to understand the implication of the data. Furthermore, the data is also tested using IBM SPSS Statistic 26 to test for statistical significance to lend more credibility to the data and the results obtained from said data.

## RESULTS

Firstly, the data to be analysed are grouped in different categories based on the source of the sample. It is grouped based on whether it is from captive or free-living pigeons. Captive in this case is defined as any pigeon reared for commercial purpose, or as a companion animal such as pets. On the other hand, free living pigeons are defined as pigeons that are not owned by anyone and are considered “wild” pigeons. The samples were also grouped based on the testing method used to test for the antimicrobial susceptibility.

Within the 19 papers reviewed, a total of 1418 *E. coli* (689 captive, 729 free-living) across 5 continents (South America, Asia, Oceania, Europe and Africa) are isolated and tested for antimicrobial susceptibility. This covers the majority of the continents in the world, and covers a total of 12 countries. (Brazil, China, Iran, Bangladesh, Turkey, Belgium, Qatar, Spain, Australia, South Korea, South Africa, Poland). Among the collected samples, a total of 52 different antibiotics were tested across the isolates. Most of the antibiotics tested for susceptibility are chosen on the basis of its use in veterinary, animal production and human health in the region the sample was collected from. However, as not all of the isolates were tested against the 52 different antibiotics, it is decided that only the data from the top six antibiotics tested for susceptibility across all of the isolates will be used to be analysed as these antibiotics were tested on at least 50% of the isolates. The top six antibiotic tested for susceptibility among the *E. coli* isolates were Ampicillin, Ciprofloxacin, Gentamicin, Tetracycline, Chloramphenicol, Trimethoprim/ Sulfamethoxazole at 81.52%, 62.41%, 88.15%, 73.20%, 68.55% and 89.28% of the isolated *E. coli* respectively. The mentioned 6 antibiotics also cover more than 4 antibiotic classes. These antibiotics are also widely used throughout the world, some of these antibiotics are also regarded as critically important human antibiotics such as ampicillin, chloramphenicol and ciprofloxacin (WHO, 2017). This would provide a holistic

view of the antibiotic resistance among the isolates as it covers multiple antibiotic classes as well as widely used antibiotics in the world.

Antibiotic	Number of <i>E.coli</i> tested
Trimethoprim/ Sulfamethoxazole (SXT)	1266 (89.28%)
Gentamicin (GEN)	1250 (88.15%)
Ampicillin (AMP)	1156 (81.52%)
Tetracycline (TET)	1038 (73.20%)
Chloramphenicol (COT)	972 (68.55%)
Ciprofloxacin (CIP)	886 (62.41%)

Table 4: Percentage of total *E. coli* isolated tested against specific antibiotics

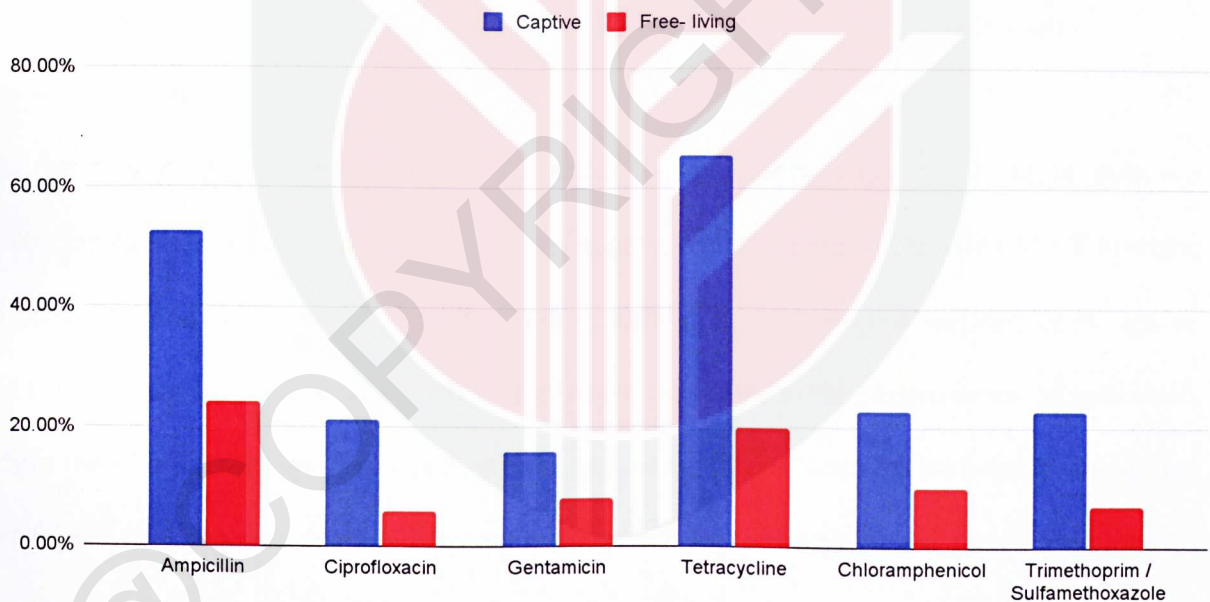
There are only two methods of testing used for the antimicrobial susceptibility test which are disc diffusion and agar dilution method. 77.93% of the *E. coli* samples were tested using disc diffusion method and 22.07% of the *E. coli* samples were tested using the agar dilution method.

Method	Number of <i>E.coli</i> isolate tested
Disc diffusion	1105 (77.93%)
Agar dilution	313 (22.07%)

Table 5: Methods used to test for *E. coli* antibiotics susceptibility

Among the *E. coli* isolated from captive pigeons, and tested against the 6 mentioned antibiotics, it is found that 52.51%, 21.17%, 15.78%, 65.66%, 22.97%, and 22.91% of the tested *E. coli* are resistant against AMP, CIP, GEN, TET, COT and SXT respectively. On the other hand, *E. coli* isolated from free living pigeons that are tested against the mentioned antibiotics are 24.07%, 5.87%, 7.87%, 19.80%, 7.32%, and 6.86% resistant against AMP, CIP, GEN, TET, COT and SXT respectively. This suggests that *E. coli* samples isolated from captive pigeons display a higher amount of antimicrobial resistance to the six most commonly tested antibiotics amongst all of the samples collected.

**Percentage of AMR *E. coli* against type of antibiotic resisted**



Bar chart 1: Percentage of AMR *E. coli* against type of antibiotic resisted.

	Ampicillin	Ciprofloxacin	Gentamicin	Tetracycline	Chloramphenicol	Trimethoprim/ Sulfamethoxazole
Captive	282/537 (52.51%)	101/477 (21.17%)	107/678 (15.78%)	413/629 (65.66%)	102/444 (22.97%)	123/537 (22.91%)
Free- living	149/619 (24.07%)	24/409 (5.87%)	45/572 (7.87%)	81/409 (19.80%)	52/528 (9.85%)	50/729 (6.86%)
Percentage increase from Free- living to Captive <i>E. coli</i> samples	118.16%	260.65%	100.51%	231.62%	133.20%	233.97%
Total	431/1156 (37.28%)	125/886 (14.11%)	152/1250 (12.16%)	494/1038 (47.59%)	154/972 (15.84%)	173/1266 (13.67%)

Table 6: AMR rate of *E. coli* samples collected from Captive or Free- living pigeons

### Statistical Analysis

The result of the difference in *E. coli* AMR status between captive and free-living pigeons are tested for its statistical significance using chi square with the help of the IBM SPSS Statistic 26 program. The null hypothesis is that both groups of *E. coli* samples isolated from captive and free-living pigeons display no difference in resistance rate to the particular tested antibiotic. While the alternative hypothesis is that both groups of *E. coli* samples isolated from captive and free- living pigeons display different resistance rates to the particular tested antibiotic. If p- value is  $<0.05$ , it suggests that there is a statistically significant difference between the two groups of data set. After running the chi square test for the six groups of data for the six antibiotic analysed, all of the results display statistically significant results conforming the alternative hypothesis, indicating that the *E. coli* samples collected from captive and free- living pigeons are different and display a different rate of AMR status.

## DISCUSSION

Pigeons are very common in all cities in the world, and there is even an overpopulation problem in some regions where population control programs had to be employed to reduce the numbers of pigeons on the streets (Haag-Wackernagel, 1995). Not only can pigeons be a nuisance due to hygiene concerns; it may even be potential carriers of many microorganisms such as *Salmonella spp.*, *Campylobacter spp.*, enteropathogenic *E. coli*, *Cryptosporidium spp.*, and even the avian influenza H5N1 virus (Carvalho *et al.*, 2020). These microorganisms that can be potentially spread by pigeons indicate that pigeons are a cause of concern in the context of public health, even more so due to their ability to also carry AMR bacteria as shown through this research. This research also shows that AMR bacteria can also be isolated from free-living pigeons that may have minimal contact with humans.

One of the first things that was looked at in this research is the methods used to test for the antimicrobial susceptibility. All of the papers reviewed used one of two methods to test for AMR qualities, disc diffusion and agar dilution method. Both of these methods can test for antimicrobial susceptibility effectively as well as it uses a similar concept to determine the bacteria's antimicrobial susceptibility. However, between these methods, the most preferred method seems to be the disc diffusion method. There's no implied reasoning as to why one method is preferable to the other amongst the researchers of the papers reviewed. There's a possibility that it is a combination of various factors that caused the researchers to favour one method over the other. For starters, there's the ease of conducting the test itself, where the disc diffusion method remains to be the easier method of the two as it is direct, and requires less expertise to conduct effectively. Furthermore, in most AMR research, the objective is to observe the rate of AMR amongst the sample and not necessarily find out the minimum inhibitory concentration (MIC) which can only be observed when using the agar dilution

method and remains as one of the core advantages of the agar dilution method when compared with the disc diffusion method. There is also the efficiency of the test in the context of the research objective where the disc diffusion method can test multiple types of antibiotics in varying levels of concentration in a single disc, saving time and money when conducting research about AMR bacteria. In terms of accuracy of the test, both tests are regarded as accurate and reliable (Liu *et al.*, 2016), which means the accuracy of the test shouldn't be the primary factor when choosing one method over the other.

The highlight of the results are definitely the data in regards to the AMR of the samples collected. There is a significant difference between the samples collected from captive birds, and those that are free living. The samples collected from captive birds displayed a higher amount of resistance to all six antibiotics being analysed. While any amount of resistance is cause for concern, it is surprising to see a difference between the samples isolated from free living and captive birds. This finding is also consistent with a few papers reviewed and the general idea that captive animals may be exposed to more bacteria with AMR properties due to selective pressure from the use of antibiotics in treatments or therapeutic use for growth promotion. In some instances, this increase in AMR is also attributed to the abundant use of antimicrobial agents in racing pigeons (Kimpe *et al.*, 2002). However, it is unclear from this research alone just how big of a factor the management of the pigeon plays in the difference in AMR rate between the captive and free-living pigeons, there may be various other factors at play that causes this difference in AMR rate. For example, free-living pigeons tend to congregate at certain areas where people feed pigeons, which may increase or decrease the likelihood of the pigeon from obtaining *E. coli* with AMR qualities, which causes the difference between the AMR qualities of *E. coli* collected to be greater or lesser.

There's a few key take away from this particular set of data analysed. The first thing to note is that there is a relatively high amount of *E. coli* with AMR properties isolated in both captive and free-living pigeons. For example, at least 20% of the *E. coli* samples that are tested against ampicillin displayed resistance, with 52.51% samples collected from captive pigeons displaying resistance towards the mentioned antibiotic. This proves worrying as it shows that ampicillin will be having limited effectiveness against *E. coli* that are isolated from pigeons especially those that are captive bred. The difference in percentage of *E. coli* resistant to tetracycline among samples collected from the captive and free-living pigeons is to be noted. This is because the difference in terms of percentage of *E. coli* resistant against the mentioned antibiotic is the greatest between samples isolated from captive and free-living pigeons, 65.66% and 19.80% respectively and it could be explained by the widespread use of tetracycline for the treatment of poultry infection due to the low cost, efficacy and lack of side effect (Filazi *et al.*, 2017) which may have spread into pigeons. The most widespread antibiotic resistance property amongst the *E. coli* tested is towards tetracycline, with 47.59% of the *E. coli* isolated and tested against tetracycline displaying resistance, which is the highest amongst all six antibiotic AMR rate analysed.

However, if we operate under the assumption the rate of resistance found in *E. coli* isolated from the captive pigeons is the baseline resistance, it would actually suggest that the resistance towards ciprofloxacin increased by 260.65% due to the change in management which is the highest increase in any of the AMR rate among all of the antibiotics analysed. This is highly concerning as ciprofloxacin is considered to be a critically important antibiotic for the treatment of *E. coli* infections (WHO, 2019). While the ciprofloxacin resistance rate of *E. coli* from free-living pigeons is at 5.87% which is higher than some of the studies conducted which found the resistance rate at only 2% (Radimersky *et al.*, 2010). The more troubling data is that of the *E.*

*coli* sample collected from captive pigeons where 21.17% of those tested against ciprofloxacin showed resistance to the antibiotic. However, without long term data to support the claim that the increase in resistance towards ciprofloxacin is due to the change in management, more research has to be done to determine if other factors are at play. This is the reason why establishing a baseline resistance rate is so important as it allows a clearer view as to how certain practices may affect the AMR rate in bacteria. Without proper data, it is difficult to select the best course of action to reduce the rate of AMR bacteria from spreading to the environment, let alone reversing it if at all possible.

While the risk of *E. coli* from pigeons infecting humans are minimal when compared to other zoonotic diseases that are much more widespread, as evident by the low number of infection cases involving pigeons, it is still important to maintain a healthy amount of surveillance on the status of AMR in synanthropic animals such as pigeons even if they rarely if ever pose a direct risk to human health. This is due to the possibilities of AMR properties from one bacteria passing on to another bacteria through horizontal or vertical transmission (Li *et al.*, 2019). The AMR properties may one day be transferred into another bacteria that poses a larger risk to public health.

## **RECOMMENDATION**

There is a lack of research done specifically on pigeons hence there is a lack of standardisation when it comes to conducting research in relation to bacteria isolated from pigeons for AMR research. This ultimately causes some data to be missing, lacking or hard to read in some reporting of results. For example, a bacteria being multidrug resistant (MDR) is defined as being resistant to at least one antimicrobial drug in three or more antimicrobial categories. However, it is either not reported in many of the papers reviewed, or in some cases, it is

reported with a different definition of MDR, which makes any attempt to do systematic review for MDR complicated and difficult as not all AMR research report on MDR numbers and it may even use a different definition of MDR making data interpretation much more confusing.

There are also various studies that present their data in bar chart or graph forms without any tables, making the data harder to interpret in some cases when specific numbers are of concern. Tables should be included to provide better readability in terms of specific numbers and graphs or bar charts should be reserved to display trends or to bring light to certain patterns observed and not be the only method of presenting the data in a structured manner.

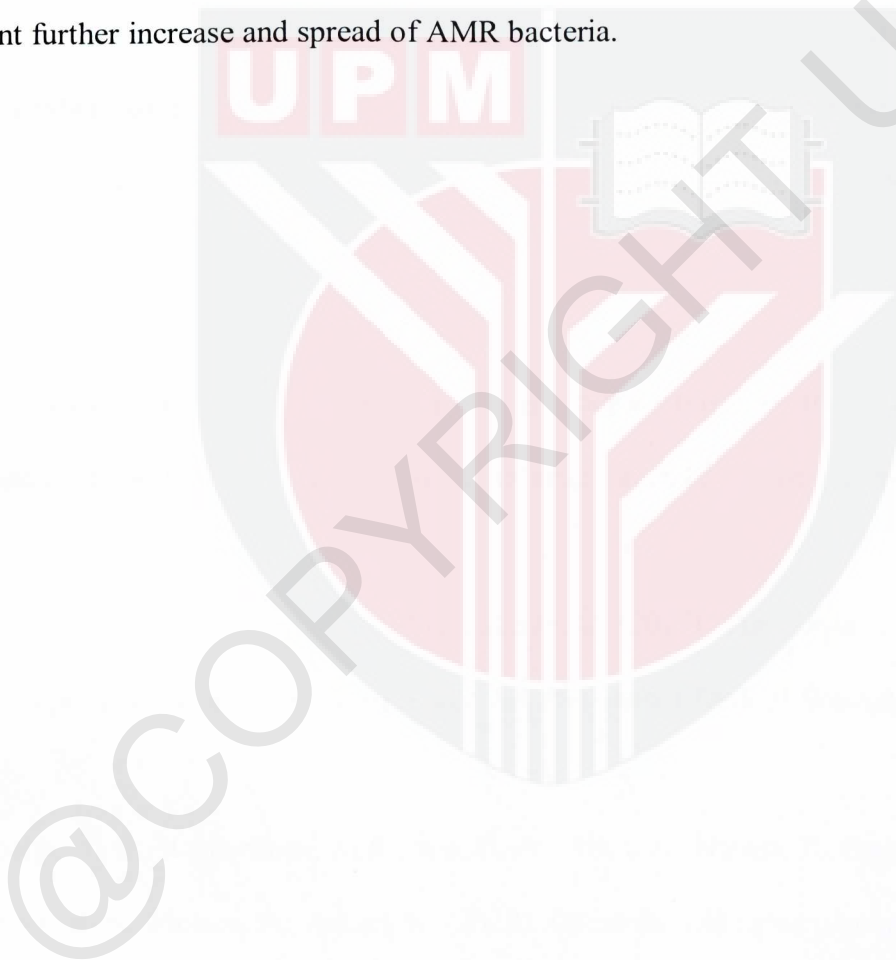
Aside from issues about definition of MDR, there is also a lack of transparency or information about how the samples were obtained from free- living pigeons. Information about trapping the pigeons and collecting samples are especially useful so that other researchers could emulate the best practices and it also increases transparency of the research to eliminate any concerns in regards to animal safety during the research process.

There is definitely a need to conduct more research on synanthropic animals such as pigeons for surveillance purposes. This will help further the understanding of the potential risk of AMR bacteria infecting humans due to interaction of humans and synanthropic animals as well as the current state of AMR bacteria in both animals from a captive and free- living setting.

## **CONCLUSION**

In conclusion, this research shows that AMR bacteria can be isolated from pigeons at a relatively high rate from both pigeons of captive and free- living origin. Not only that, it also shows that *E. coli* isolated from captive pigeons consistently showed a higher rate of AMR

when compared to *E. coli* isolated from free- living pigeons. These findings show with great confidence that AMR bacteria are not only found in wild animals that may not be found in close proximity with humans, they can also be found at a higher rate in animals that are captive and are in close contact with humans, showing how human interactions such as use of antibiotics could cause a selection pressure of AMR bacteria, further increasing the rate of AMR bacteria. The research also further suggests that there is a need for additional research on AMR of bacteria found in pigeons and to further promote the proper use of antibiotics to prevent further increase and spread of AMR bacteria.



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