Research Article

Phylogenetically Diverse Escherichia coli Strains from Chicken Coharboring Multiple Carbapenemase-Encoding Genes (bla<sub>NDM</sub>-bla<sub>OXA</sub>-bla<sub>IMP</sub>)

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Carbapenem-resistant Enterobacteriaceae (CRE) has been a public health risk in several countries, and recent reports indicate the emergence of CRE in food animals. This study was conducted to investigate the occurrence, resistance patterns, and phylogenetic diversity of carbapenem-resistant <i>E. coli</i> (CREC) from chicken. Routine bacteriology, PCR detection of <i>E. coli</i> species, multiplex PCR to detect carbapenemase-encoding genes, and phylogeny of CRE <i>E. coli</i> were conducted. The results show that 24.36% (19/78) were identified as CREC based on the phenotypic identification of which 17 were positive for the tested carbapenemases genes. The majority, 57.99% (11/19), of the isolates harbored multiple carbapenemase genes. Four isolates harbored all <i>bla</i><sub>NDM</sub>, <i>bla</i><sub>OXA</sub>, and <i>bla</i><sub>IMP</sub>, and five and two different isolates harbored <i>bla</i><sub>NDM</sub> and <i>bla</i><sub>OXA</sub> and <i>bla</i><sub>OXA</sub> and <i>bla</i><sub>IMP</sub>, respectively. The meropenem, imipenem, and ertapenem MIC values for the isolates ranged from 2 μg/mL to ≥256 μg/mL. Phylogenetic grouping showed that the CREC isolates belonged to five different groups: groups A, B1, C, D, and unknown. The detection of CREC in this study shows that it has become an emerging problem in farm animals, particularly, in poultry farms. This also implies the potential public health risks posed by CRE from chicken to the consumers.

1. Introduction

Carbapenem resistance in Enterobacteriaceae is a serious emerging antimicrobial resistance (AMR) issue that has been escalating and posing challenges in treating infections caused by the resistant pathogen. Enterobacteriaceae are inhabitants of the intestinal flora and are among the most common human pathogens that cause cystitis and pyelonephritis with fever, sepsis, pneumonia, peritonitis, meningitis, and device-associated infections [1]. The bacteria are transmitted easily between human and animals, especially via fomites, food, and water. During the transmission, genetic materials are transferred through horizontal gene transfer, mediated mostly by plasmids and transposons. Enterobacteriaceae are among the common nosocomial pathogens often causing infections through medical devices that include ventilators, intravenous catheters, urinary catheters, or wounds caused by injury or surgery [2]. Such nosocomial infections commonly affect immunocompromised patients and in patients being treated using invasive devices.

Carbapenem is a broad-spectrum β-lactam antibiotic that is regarded as the last-line antibiotic, especially to be used in critically ill patients who have developed antimicrobial-resistant bacterial infections. Unfortunately, Enterobacteriaceae have developed resistance against this last resort drug and made it ever challenging to treat infections caused by these pathogens. Among the bacteria in the family Enterobacteriaceae, <i>E. coli</i>, and <i>Klebsiella pneumoniae</i> are the most commonly
According to Zaidah et al. [8], unpublished data from di-
samples collected antemortem from abattoir in Terengganu.
prevalence of 1% (2/200) from broiler chicken cloacal swab
[6]. A more recent study by Ghazali et al. [7] reported CRCE
E. coli
56.7% (85/150) of the cloacal swab samples were positive for
Resistance Patterns.
Based on the routine microbiology,
E. coli
eny, and identify the common carbapenemase genes in the
antimicrobial resistance patterns, determine the phylo-
group D, and unknown group (Figure 2 and Table 1). The results from quadruplex PCR
showed that 52% (78/150) detection rate of E. coli from the cloacal swab samples
collected. The resistance pattern of E. coli isolates showed that
87.18% were resistant to streptomycin, followed by ceftriaxone
(80%), trimethoprim-sulfamethoxazole (66.7%), ceftazidime
(33.3%), meropenem (32.05%), ertapenem (30.8%), doripe-
nem (29.5%), imipenem, and ciprofloxacin (26.9%).

2.2 Multiplex PCR Detection of Carbapenem Resistance
Encoding Genes (bla
IMP, bla
NDM, bla
KPC, and bla
OXA).
The PCR result confirmed the presence of carbapenemase genes
in the identified E. coli isolates. Out of the 78 E. coli isolates,
19 (24.36%) were positive for at least one of the carbapenem-
ase genes. Among these, about 58% (11/19) were positive
for multiple carbapenemase genes. Four isolates harbored
all bla
NDM, bla
OXA, and bla
IMP, and five and two different
isolates harbored bla
NDM and bla
OXA and bla
OXA and bla
IMP,
respectively. However, none of the isolates were positive for
bla
KPC (Figure 1 and Table 1).

2.3. Phylogenetic Analysis. The results from quadruplex PCR
showed that the CREC belong to diverse phylogroups
including group A, group B1, group C, group E, group D,
and group unknown. Among the 19 CREC isolates, nine
were identified as members of group A while five, three,
and one were, respectively, typed as group B1, group C,
group D, and unknown group (Figure 2 and Table 1).

3. Discussion
Carbapenem resistance in common bacterial pathogens has
become one of the most concerning global public health
issues since the carbapenem antibiotics are among the most
critically important antimicrobials for the treatment of
infections in humans [9]. Carbapenem have been reported
to show the broadest spectrum of antimicrobial activity
in vitro against Gram-positive and Gram-negative bacteria,
including anaerobes [10]. Because of their broad spectrum
of actions, potency, and effectiveness in treating broad range
of infections in humans, carbapenem have been recognized
as the antibiotics of last resort to treat infections caused by

2. Results
2.1 Bacterial Isolation and Identification and Antimicrobial
Resistance Patterns. Based on the routine microbiology,
56.7% (85/150) of the cloacal swab samples were positive for
E. coli. However, further confirmation using E. coli species-
detected CRE that have been posing threat to the public health
and animal health [3]. Such prevailing AMR issue has been
compromising the efficacy of antibiotics, and according to the
World Health Organization, there is a possibility for the world
to encounter an era, in which all the antibiotics become ine-
effectve thereby increasing mortality rate and increasing cost of
treatment if no intervention is done to overcome the problem.
There are also concerns that failure to counter the rising AMR
problems worldwide may lead to reemergence of previously
eradicated or controlled diseases [4].

According to the National Surveillance of Antimicrobial
Resistance (NSAR) in Malaysia, from 2006 to 2017, which
analyzed the data obtained from hospital microbiology labo-
ratories from different parts of the country, carbapenem
resistance in E. coli declined from 0.5% in 2010 to 0.2% in
2014 [5]. A recent report on the prevalence of CRE in a ter-
inary hospital in Malaysia shows that the prevalence of CRE
in 2015 and 2016 was 0.3% (5/1590) and 1.2% (17/1402),
respectively. However, none of the isolates were positive for
"Figure 1: Multiplex PCR results for carbapenem genes (bla
KPC, bla
NDM, bla
OXA, and bla
IMP) of E. coli isolates from chicken identified as
CRE phenotypically. M, 100 bp DNA marker; lanes P1-P19, test sample (E. coli) isolates.
"
lates harbored isolates, harbored multiple carbapenemase genes. Four isolates from different farms in Terengganu state. This study investigated the prevalence of CREC particularly on K. pneumoniae in broiler chickens from different farms and drinking water from the farms, and workers handling the chickens reported a prevalence rate of 15% and 6% from the broilers and water samples, respectively. Among the poultry CRE isolates (n = 15), all were blaNDM positive, while blaKPC, blaOXA48, and blaNDM genes were detected in 11 of the isolates while four isolates were positive for either blaKPC or blaNDM or blaOXA48 and blaNDM. In addition, 56% of K. pneumoniae isolates from humans harboring multiple genes suggesting a high incidence of this resistant bacteria in humans may contribute to its dissemination among food-producing animals and the livestock environment, thus increasing the risk of foodborne transmission to the consumers [7]. The presence of carbapenem resistance in bacteria from animals, including food-producing animals (pigs, bovines, and horses), has also been reported from some European countries such as Germany, France, and Belgium and Egypt [12, 13]. The identification of E. coli isolates harboring multiple (at least two) carbapenemase-encoding genes from food animal in this study differentiates it from previous similar studies which mostly reported E. coli isolates harboring one or two carbapenemase genes [14, 15].

Carbapenems are not routinely used in food animal production including poultry farming; however, carbapenem resistance has been very few. The current study reports relatively higher prevalence of CRE, 24.36% (19/78) of the total E. coli isolated from 150 cloacal swab samples collected from broiler chicken from commercial farms based on phenotypic identifications of which 17 were positive for the tested carbapenemase genes, whereas the two isolates were negative for carbapenemase genes while showing CREC-positive results on MIC test by using E-test strips. The meropenem, imipenem, and ertapenem MIC values for the isolates ranged from 2 μg/mL to ≥256 μg/mL. Most of the E. coli isolates were resistant to at least two antibiotics including meropenem, ertapenem, and imipenem showing multidrug resistance. The majority, about 58% (11/19) of the confirmed CREC isolates, harbored multiple carbapenemase genes. Four isolates harbored blaNDM, blaOXA48, and blaIMP genes, and five and two different isolates harbored blaNDM and blaOXA and blaOXA48 and blaIMP, respectively. In general, there is scarcity of reported data on the prevalence of CRE in food animals in Malaysia. A recent study investigated the prevalence of CRE in broiler chickens, ruminants, and swine from different farms in Terengganu state. This study reported a much lower CRE prevalence of 1% (2/200) from broiler chicken cloacal swab samples collected antemortem from an abattoir in Terengganu. The same study also investigated the prevalence of CREC in 151 ruminants and 100 swine faecal samples collected from different farms in the state of Terengganu; however, no CREC was reported. A study from Egypt conducted on CRE particularly on carbapenem-resistant K. pneumoniae in broiler chickens from different farms, drinking water from the farms, and workers handling the chickens reported a prevalence rate of 15% and 6% from the broilers and water samples, respectively.

### Table 1: Antimicrobial resistance profile and phylogenetic diversity of CRE isolated from cloacal swab samples from chicken.

| Isolate ID | Antimicrobial susceptibility (disc diffusion) | E-test MIC value | Carbapenem resistance (carbapenemase-encoding genes) | Phylogroup |
|------------|---------------------------------------------|-----------------|-----------------------------------------------------|------------|
|            | ETP (10 μg) MEM (10 μg) IMP (10 μg) ETP (μg/mL) MEM (μg/mL) IMP (μg/mL) blaKPC blaNDM blaOXA48 blaIMP |                  |                                                     |            |
| P1         | S R R R 8 ≥256 | 4 - + + - | B1 | |
| P2         | S S R R 6 8 16   | - - - +       | C   | |
| P3         | R R R R 32 ≥256 | 32 - + + + | A   | |
| P4         | R R R R 4 ≥256 | 4 - + + - | A   | |
| P5         | R S R R 8 32 32 | - + - - | A   | |
| P6         | R R R R 4 6 8   | - - + A       | A   | |
| P7         | R R R R 2 32 32 | - + + + | A   | |
| P8         | S S R R 4 6 32 | - - - | A   | |
| P9         | R S S S 32 8 32 | - + + | C   | |
| P10        | R R R R 16 32 ≥256 | 4 - + + + | D   | |
| P11        | R R R R 4 0.25 32 | - - + B1 | B1  | |
| P12        | R S R R 8 16 8   | - - + A       | A   | |
| P13        | R R R R 2 4 8   | - + + + B1    | B1  | |
| P14        | R R R R 2 1.5 6 | - - + | A   | |
| P15        | S R R 0.25 ≥256 | 4 - + + - | B1  | |
| P16        | S S R R 6 4 8   | - - - C       | C   | |
| P17        | R S R 0.25 32 6 | - + + - Unknown | Unknown | |
| P18        | S S R R 2 16 0.25 | - + + B1 | B1  | |
| P19        | S S R R 0.25 ≥256 | ≥256 - + | A   | |
resistance in the *E. coli* isolates might have coevolved along with resistance to other antibiotics that are commonly used against resistant strains of bacteria that may also be disseminated through direct contact, insect vectors, and other animals [11, 16, 17]. An earlier study by Poirel et al. [18] also suggested that coselection of carbapenemase genes under the selection pressure imposed by the use of aminopenicillins and aminopenicillin-β-lactamase inhibitor combinations in livestock may lead to the emergence and spread of carbapenem resistance. Reports from previous studies indicated that CRE can persist in animal production if the bacteria are adapted to animals and the farm environment and are stabilized by coexpression of further resistance genes [19, 20]. The possibility that infected or carrier humans, particularly by the farm workers, might spread resistant bacteria in farms through direct and indirect routes of transmission cannot be ruled out. This is due to the fact that humans, the farm workers in the context of the current study, are more likely to have been exposed to broad-spectrum antibiotics, and in particular to broad-spectrum β-lactams, than the chickens [17]. Since CREC can transmit through direct anthropozoonotic or zooanthroponotic routes [21], the spread of CREC in humans may pose risk for food animal production and possibly lead to the establishment of CREC in the food animal production ecosystem and may lead to subsequent further spread of these pathogens [19].

Phylogenetic grouping showed that the CREC isolates belonged to five different groups, groups A (47.37%), B1 (26.32%), C (15.79%), D (5.26%), and unknown (5.26%). In agreement with the current findings, a study by Asadi et al. [22] reported that the majority (54.21%) of *E. coli* isolates from chickens belonged to phylogroup A. However, contrary to the findings in this study, the authors reported that 32.53% and 7.22% of the *E. coli* isolates belonged to phylogroups D and B1, respectively. Coura et al. [23] reported that phylogroups A followed by B1 are the most common phylogroups of *E. coli* obtained from broiler carcasses suggesting the possibilities of contamination by commensal strains of *E. coli*. Cordoni et al. [24] reported that out the 272 *E. coli* strains analyzed, 132 were grouped in the B2 phylogroup, 61 in A1, 37 in group A, and 21 in groups B1 and D while the remaining 21 were not ascribable to any group. Ramadan et al. [25] also reported that higher frequencies of virulent phylogroups of D and B2 were found among avian pathogenic *E. coli* (APEC) isolates and phylogroup A in 25% of APEC isolates, which is predominantly associated with commensal *E. coli* which might have originated from commensal *E. coli* strains that might have acquired virulence-related genes. Interestingly, previous studies by Walk et al. [26] demonstrated that most *E. coli* strains that are able to persist in the environment belong to the B1 phylogenetic group. Earlier studies classifying the different *E. coli* phylogroups reported that the extraintestinal pathogenic strains usually belong to groups B2 and D, the commensal strains to groups A and B1, while the intestinal pathogenic strains belong to groups A, B1, and D [27]. In this study, discrepancies between the different methods for CRE detection have been observed. Some of the isolates appeared to show susceptibility towards the tested carbapenem antibiotics when tested by disc diffusion but were confirmed to be resistant as seen from the results from MIC determination by E-test and PCR detection of carbapenemase genes, whereas two isolates which showed phenotypic resistance to carbapenems did not harbor any of the carbapenemase genes tested in this study. These discrepancies can be attributed to the different levels of discriminatory abilities of the tests. In general, antimicrobial susceptibility by disc diffusion is the least reliable compared to MIC determination and PCR. Both phenotypic and molecular detection and characterization of CRE have their respective limitations and reliable monitoring of CRE from animals requires a combination of molecular and culture-based methods [21].
4. Materials and Methods

4.1. Ethics. This research was reviewed and approved by the animal research ethics committee at the Faculty of Veterinary Medicine, Universiti Malaysia Kelantan.

4.2. Sample Collection and Processing and Bacterial Isolation and Identification. A total of 150 samples of cloacal swabs from live chickens in poultry farms in Kelantan were collected and placed in transport media. All the samples were collected aseptically and were placed in an icebox during transportation and stored in a refrigerator at 4°C overnight and were processed the following day. The cloacal swabs were placed in 10 mL of Phosphate-buffered Saline (PBS) for enrichment and were aerobically incubated for 24 h at 37°C. The enriched samples were cultured on Nutrient agar (Oxoid, UK) and MacConkey (Oxoid, UK) agars were incubated at 37°C for 24 h. Following primary culture, bacterial growths showing lactose fermentation on the MacConkey agar (Oxoid, UK) and Gram negative were subcultured on MacConkey (Oxoid, UK) agar and Nutrient agar to obtain pure colonies. Following secondary culture, lactose-fermenting colonies on MacConkey agar were selected subcultured on Eosin Methylene Blue (EMB) (Oxoid, UK) agar 24 h at 37°C. Bacterial colonies with green metallic sheen on EMB agar were screened, and further biochemical tests were conducted to presumptively identify E. coli isolates. Further confirmation of E. coli was done by PCR detection of E. coli species-specific gene. All the confirmed E. coli isolates were inoculated onto chromogenic selective agar, Brilliance™ CRE (Oxoid, UK) selective agar. Inoculated plates were incubated overnight at 37°C, and presumptive CRE E. coli were identified according to the manufacturer’s guideline. Colonies with blue or pale pink colours were presumptively identified as CRE. All the isolates which did not show the expected colonial morphologies of CRE were further tested by PCR amplifications of common carbapenem-encoding genes.

4.3. Antibiotic Sensitivity Test (AST). Antibiotic sensitivity test was done using Kirby-Bauer disk diffusion method on Mueller-Hinton Agar (MHA) (Oxoid, UK) with all the identified isolates according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [28]. Escherichia coli ATCC 25922 strain was used as quality control. Disc diffusion method was used to determine antimicrobial susceptibility test, and the antibiotic discs used were streptomycin (10 μg), gentamycin (10 μg), enrofloxacin (5 μg), ciprofloxacin (5 μg), trimethoprim sulfamethoxazole (25 μg), ceftazidime (30 μg), ceftriaxone (30 μg), imipenem (10 μg), meropenem (10 μg), ertapenem (10 μg), and doripenem (10 μg). The media were incubated for 24 h at 37°C. After incubation, zone of inhibition for each of the antibiotic discs was measured and the antibiotic susceptibility was determined based on CLSI guidelines [28].

4.4. Determination of Minimum Inhibitory Concentration (MIC) Using E-Test. The MIC determination using E-test (Biomerieux, France) was done as recommended by the manufacturer. Briefly, overnight culture of E. coli was suspended in 10 mL normal saline (0.9% NaCl). The turbidity of the bacterial suspension was adjusted to that of 0.5% McFarland standard. The bacterial suspension was then uniformly streaked onto the entire surface of MHA (Oxoid, UK). Interpretations of the E-test strips (Biomerieux, France) were done according to the CLSI standards [28]. Escherichia coli ATCC 25922 strain was used as quality control.

4.5. Molecular Characterization of Carbapenem-Resistant E. coli

4.5.1. DNA Extraction. Following bacterial isolation and identification, genomic DNA extraction was performed for all the presumptive E. coli isolates using boiling method. One to two bacterial colonies from each of the isolates on Nutrient agar were resuspended in a 1.5 mL microcentrifuge tube containing 100 μL of 10 mmol/L Tris-HCl buffer (pH 8.0). The microcentrifuge tubes containing the samples were vortexed, and the suspensions were then boiled for 10 minutes to lyse the cells, followed by quickly chilling on ice for 5 minutes. Then, the tubes containing the suspensions were centrifuged at 12000 rpm for 10 minutes. Following that, 100 μL of the supernatant containing DNA from each of the microcentrifuge tubes was transferred into another 1.5 mL microcentrifuge and the DNA quality was assessed using a spectrophotometer and stored at -20°C until further use.

4.5.2. Molecular Detection of E. coli and Carbapenem Resistance Encoding Genes. PCR amplification was conducted to identify E. coli using primer Pho-F/Pho-R targeting the housekeeping genes of E. coli and carbapenem-encoding genes (blaKPC), blaOXA, and blaNDM) as described earlier [1, 29]. The PCR reaction mixture was prepared in a 0.5 mL Eppendorf tube prior to addition of templates. Each microcentrifuge tube contained 25 μL of the PCR Master Mix, 1 μL of 10 μmol/L each primer, and 18 μL of sterile nuclease-free water. Then, 5 μL of DNA template was added to each tube. Sterile nuclease-free water was used as negative control. All PCR amplifications were conducted using Thermal Cycler 1000 (Bio-Rad, USA). Amplification of an E. coli-specific gene (pho) was carried out using the following protocol: initial denaturation for 2 mins at 94°C, followed by 35 cycles consisting final denaturation for 1 min at 94°C, primer annealing for 1 min at 56°C, DNA extension for 1 min at 72°C, followed by final extension for 10 mins at 72°C, and holding at 12°C.

For the amplification of carbapenem-resistant genes, the PCR reaction constituted 25 μL of the 2x PCR Master Mix (Promega, USA), 1 μL of 10 μmol/L of the three primer pairs, and 14 μL of sterile nuclease-free water. Amplification of carbapenem-resistant genes was conducted by the following thermal cyclic conditions: activation of thermostable hot-start DNA polymerase for 10 mins at 94°C, followed by 36 cycles of amplification consisting of denaturation for 30s at 94°C, primer annealing for 40s at 52°C, and strand elongation for 50s at 72°C, with 5 mins at 72°C for the final extension, and holding for 12°C. Analysis of the PCR amplification products was done by using electrophoresis in a 1.5% agarose gel at 100 V and 400A for 40 mins in 1× TBE buffer. The DNA fragments were then visualized using
GelDoc EZ Imager (Bio-Rad, USA). The DNA size was determined using the 100 bp molecular weight ladder as a marker.

4.5.3. **Phylogenetic Analysis.** Characterization of the phylogenetic groups of the *E. coli* isolates was determined according to the protocols described by Clermont et al. [30]. Briefly, a single PCR reaction mixture contains 12.5 μL of 2x DreamTaq Master Mix (Promega, USA), 5 μL of DNA (approximately 100 ng), and 20 μM of each primer in a total volume of 30 μL. PCR amplifications were carried out in a Nexus Gradient Mastercycler (Eppendorf, USA) using the following conditions: initial denaturation at 94°C for 4 min and 30 cycles for each denaturation at 94°C for 5 s annealing at 57°C for 20 s (group E) or 59°C for 20 s (quadruplex and group C), amplification at 72°C for 1 min, and final extension at 72°C for 5 min. The PCR products were analyzed by electrophoresis in 1.5% agarose gel, and image analysis was done using GelDoc™ EZ Imager (Bio-Rad, USA).

5. Conclusions

In conclusion, the detection of CREC in this study shows that these resistant bacteria are not limited to human infections and that CREC has also become an emerging problem in farm animals, particularly in chicken farms. This may raise concerns that these carrier food animals may serve as a source of infection and/or colonization for humans. This implies the potential public health risks posed by emerging antimicrobial resistance particularly CREC in food animals and the need for appropriate control and prevention measures to minimize the spread of such resistant bacteria.

**Data Availability**

All the relevant data have been included in the manuscript.

**Disclosure**

The findings reported in this manuscript have been presented in “preprints” and can be accessed at https://www.researchgate.net/publication/346618337_Phylogenetically_Diverse_Escherichia_Coli_Strains_From_Chicken_Co-Harbor_Multiple_Carbapenemase_Encoding_Genes_blaNDM-blaOXA-blaIMP.

**Conflicts of Interest**

The authors declare no conflict of interest.

**Authors’ Contributions**

E. A. was responsible for the conceptualization. E.A., A.H., and K.K.B.S were responsible for the methodology. E. A. was responsible for the investigation. E.A. and A.H. were responsible for the analysis and resources. S.I. and N.F.K. were responsible for the sample collection. E. A. was responsible for writing—original draft preparation. E.A., A.H., K.K.B.S, and N.F.K. were responsible for writing, review, and editing.

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**References**

[1] P. Nordmann, T. Naas, and L. Poirel, “Global spread of carbapenemase-producing Enterobacteriaceae,” *Emerging Infectious Diseases*, vol. 17, no. 10, pp. 1791–1798, 2011.
[2] M. Haque, M. Sartelli, J. McKimm, and M. Abu Bakar, “Health care-associated infections—an overview,” *Infection and Drug Resistance*, vol. 11, pp. 2321–2333, 2018.
[3] F. Prestinaci, P. Pezzotti, and A. Pantosti, “Antimicrobial resistance: a global multifaceted phenomenon,” *Pathogens and Global Health*, vol. 15109, no. 7, pp. 309–318, 2015.
[4] WHO (World Health Organization), “Factsheet: antimicrobial resistance,” 2020, https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance.
[5] L. Y. Hsu, A. Apisarnthanarak, E. Khan, N. Suwantarat, A. Ghafor, and P. A. Tambahy, “Carbapenem-resistant Acinetobacter baumannii and Enterobacteriaceae in South and Southeast Asia,” *Clinical Microbiology Reviews*, vol. 30, no. 1, pp. 1–22, 2017.
[6] N. A. Mohamed, H. M. Said, H. Hussin, N. Abdul Rahman, and R. Hashim, “Carbapenem-Resistant Enterobacteriaceae: ClinicoEpidemiological perspective,” *Tropical Biomedicine*, vol. 35, no. 2, pp. 300–307, 2018.
[7] M. F. Ghazali, M. H. Chai, M. Z. Sukiman, N. M. Mohamad, and S. M. Zainal Arifin, “Prevalence of carbapenem-resistant *Escherichia coli* (CREC) within farm animals in Malaysia,” *International Journal of Infectious Diseases*, vol. 101, Suppl. 1, pp. 534-535, 2020.
[8] A. R. Zaidah, N. I. Mohammad, S. Suraiya, and A. Harun, “High burden of Carbapenem-resistant Enterobacteriaceae (CRE) fecal carriage at a teaching hospital: cost-effectiveness of screening in low-resource setting,” *Antimicrobial Resistance and Infection Control*, vol. 6, no. 1, p. 42, 2017.
[9] WHO (World Health Organization), “Critically important antimicrobials list. 5th rev,” 2017, http://who.int/foodsafety/publications/antimicrobials-fifth/en/.
[10] G. G. Zhanel, R. Wiebe, L. Dilay et al., “Comparative review of the carbapenems,” *Drugs*, vol. 67, no. 7, pp. 1027–1052, 2007.
[11] S. Bonardi and R. Pitino, “Carbapenemase-producing bacteria in food-producing animals, wildlife and environment: a challenge for human health,” *The Italian Journal of Food Safety*, vol. 8, p. 7956, 2019.
[12] EFSA Panel on Biological Hazards (BIOHAZ), “Scientific opinion on carbapenem resistance in food animal ecosystems,” *EFSA Journal*, vol. 11, no. 12, p. 3501, 2013.
[13] E. Hamza, S. M. Dorgham, and D. A. Hamza, “Carbapenemase-producing Klebsiella pneumoniae in broiler poultry farming in Egypt,” *Journal of Global Antimicrobial Resistance*, vol. 7, article S10, 2016.
[14] N. Roschanski, J. Fischer, L. Falgenhauer et al., “Retrospective analysis of bacterial cultures sampled in German chicken-fattening farms during the years 2011-2012 revealed additional
VIM-1 carbapenemase-producing *Escherichia coli* and a serologically rough *Salmonella enterica* serovar infantis,” *Frontiers in Microbiology*, vol. 9, p. 538, 2018.

[15] B. Tang, J. Chang, L. Cao et al., “Characterization of an NDM-5 carbapenemase-producing *Escherichia coli* ST156 isolate from a poultry farm in Zhejiang, China,” *BMC Microbiology*, vol. 19, no. 1, p. 82, 2019.

[16] K. Ahmad, F. Khattak, A. Ali et al., “Carbapenemases and extended-spectrum β-lactamase–producing multidrug-resistant *Escherichia coli* isolated from retail chicken in Peshawar: first report from Pakistan,” *Journal of Food Protection*, vol. 81, no. 8, pp. 1339–1345, 2018.

[17] L. Poirel, R. Stephan, V. Perret, and P. Nordmann, “The carbapenem threat in the animal world: the wrong culprit,” *The Journal of Antimicrobial Chemotherapy*, vol. 69, no. 7, pp. 2007–2008, 2014.

[18] L. Poirel, B. Berçot, Y. Millemann, R. A. Bonnin, G. Pannaux, and P. Nordmann, “Carbapenemase-producing *Acinetobacter* spp. in cattle, France,” *Emerging Infectious Diseases*, vol. 18, no. 3, pp. 523–525, 2012.

[19] A. Irrgang, N. Pauly, B. A. Tenhagen, M. Grobbel, A. Kaesbohrer, and J. A. Hammerl, “Spill-over from public health? First detection of an OXA-48-producing *Escherichia coli* in a German pig farm,” *Microorganisms.*, vol. 8, no. 6, p. 855, 2020.

[20] A. Irrgang, I. Fischer, M. Grobbel et al., “Recurrent detection of VIM-1-producing *Escherichia coli* clone in German pig production,” *The Journal of Antimicrobial Chemotherapy*, vol. 72, no. 3, pp. 944–946, 2017.

[21] R. Kock, I. Daniels-Haardt, K. Becker et al., “Carbapenem-resistant _Enterobacteriaceae_ in wildlife, food-producing, and companion animals: a systematic review,” *Clinical Microbiology and Infection*, vol. 24, no. 12, pp. 1241–1250, 2018.

[22] A. Asadi, T. Zahraei Salehi, M. Jamshidian, and R. Ghanbarpour, “ECOR phylotyping and determination of virulence genes in *Escherichia coli* isolates from pathological conditions of broiler chickens in poultry slaughter-houses of southeast of Iran,” *Veterinary Research Forum*, vol. 9, no. 3, pp. 211–216, 2018.

[23] F. M. Coura, S. A. Diniz, M. X. Silva et al., “Phylogenetic group of *Escherichia coli* isolates from broilers in Brazilian poultry slaughterhouse,” *Scientific World Journal*, vol. 2017, article 5898701, 7 pages, 2017.

[24] G. Cordoni, M. J. Woodward, H. Wu, M. Alanazi, T. Wallis, and R. M. La Ragione, “Comparative genomics of European avian pathogenic *E. coli* (APEC),” *BMC Genomics*, vol. 17, no. 1, p. 960, 2016.

[25] H. Ramadan, A. Awad, and A. Atiya, “Detection of phenotypes, virulence genes and phylotypes of avian pathogenic and human diarrheagenic *Escherichia coli* in Egypt,” *Journal of Infection in Developing Countries*, vol. 10, no. 6, pp. 584–591, 2016.

[26] S. T. Walk, E. W. Alm, L. M. Calhoun, J. M. Madonicky, and T. S. Whittam, “Genetic diversity and population structure of *Escherichia coli* isolated from freshwater beaches,” *Environmental Microbiology*, vol. 9, no. 9, pp. 2274–2288, 2007.

[27] C. Carlos, M. M. Pires, N. C. Stoppe et al., “Escherichia coli phylogenic group determination and its application in the identification of the major animal source of fecal contamination,” *BMC Microbiology*, vol. 10, no. 1, p. 161, 2010.