CHARACTERIZATION OF POTENTIALLY PATHOGENIC Escherichia coli ISOLATED FROM CHICKEN FARMS IN MALAYSIA

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FPV 2019 9

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By

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Thesis Submitted to School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Master of Science

June 2019

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment for the degree of Masters of Science

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June 2019

Chairman: Assoc. Prof. Siti Khairani Bejo, PhD
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Escherichia coli is a part of normal floral in intestinal tract in various animal species. *E. coli* always considered as non - pathogenic, however certain *E. coli* strain can cause infection when they harbour certain virulent properties. In poultry, *E. coli* is the most predominant and important strains affecting poultry industry due to the significant economic loss as result of high mortality and morbidity. This study was conducted to determine the phenotype and genotype characteristic of 125 *E. coli* isolates available in Veterinary Research Institute (VRI) which are isolated from chicken farms with significant clinical signs and abundant growth upon isolation on growth medium. There was no concrete information regarding *E. coli* isolated from chicken available elsewhere in the country.

This study showed that *E. coli* isolates recovered from chicken farms were found to have diverse biochemical properties, with no single features was specific for *E. coli* identification. All the isolates were further categorized into 12

distinct groups based on their biochemical profiles including haemolysis morphology. Serotyping of the *E. coli* isolates revealed that 69.6% (87/125) isolates in this study cannot be assigned to any serogroups tested. Other serotypes identified were 14.4% O1:K1 (18/125), 10.4% O78:K8O (13/125) and 5.6% O2:K1(7/125) Phylogenetic analysis demonstrated that most of the *E. coli* isolated from chicken farms in this study belonged to group B1 (36.0%) and group D (28.0%), which is associated with non- virulent strain.

Multiplex PCR analysis demonstrated that the most prevalent virulence genes identified were *iss* 52.0% (65/125), followed by *iucD* 36.0% (45/125) *tsh* 32.0% (40/125), *vat* 14.4% (18/125), *astA* 12.0%(15/125), *papC* 12.0% (15/125), *irp2*

9.6% (12/125), and the least is *cva/cvi* gene (0%). None of the isolates harbored more than four virulence genes. Further analysis showed that presence of virulence genes among the isolates were highly diverse regardless their biochemical profiles, serotype and phylogenetic groups.

Antibiogram analysis revealed that 81.6% (102/125) of the *E. coli* isolates showed multidrug resistant profiles to different antibiotics. Most of the *E. coli* isolates were highly resistant to erythromycin 52.8% (66/125), followed with tetracycline 52.0% (65/125), streptomycin 40.0% (50/125), spectinomycin 39.2% (49/125), trimethoprim 38.4% (48/125) and flumequin 37.6% (47/125).These findings also demonstrated that most of the isolates were susceptible to antibiotics commonly used for *E. coli* infections treatment in poultry with lowest resistant score against polymyxin B (92.8%) and colistin (92.0%). There is no association with the multidrug resistant profiles of the isolates with serotypes, phylogenetic groups and virulence genes profiles observed in this study.

Macrorestriction analysis of selected *E. coli* isolates resulted in heterogenous Pulse Field Gel Electrophoresis (PFGE) pattern. Construction of cluster dendogram of 56 isolates with 60% coeffiecient similarity showed 41 genotypes consists of various serotypes with different biochemical profiles, serotypes, phylogroups and virulence genes profiles. This finding indicate that *E. coli* isolated from chicken farms in the country derived from different clones which display heterogenous profiles including antimicrobial resistant profiles. In conclusion, this study suggested that *E. coli* strain isolated from chicken farms was potentially pathogenic with highly diverse phenotype and genotypes. They potentially can cause disease in chicken even though initially they are harmless normal floral in gut as they able to inherit virulence genes from other bacterial strains in gut which enable them to cause disease.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Sarjana Sains

PENCIRIAN BERPOTENSI PATOGENIK *Escherichia coli* YANG DIASINGKAN DARI LADANG AYAM DI MALAYSIA

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Escherichia coli adalah bakteria Gram negatif yang merupakan sebahagian bakteria dari flora normal saluran usus dalam pelbagai spesis haiwan. *E. coli* selalunya dianggap sebagai bukan patogenik, namun begitu terdapat strain *E. coli* tertentu yang boleh menyebabkan jangkitan sekiranya mengandungi ciri virulen tertentu. Pada ayam, *E. coli* merupakan strain yang paling dominan dan penting yang menjejaskan industri unggas kerana kerugian ekonomi yang jelas lantaran dari kematian dan kerosakan yang tinggi. Kajian ini dijalankan untuk menentukan karakter fenotip dan genotip 125 pencilan *E. coli* yang dipencilkan dari ayam dengan tanda klinikal yang signifikan serta pertumbuhan yang banyak atas media pertumbuhan. Tiada maklumat yang jelas berkenaan ciri *E. coli* diasingkan dari ayam yang didapati di negara ini.

Berdasarkan hasil kajian ini, pencilan *E.coli* yang diasingkan dari ayam mempunyai ciri biokimia yang pelbagai di antara satu sama lain, dan tiada ciri yang tunggal dan spesifik yang khusus untuk pengenalpastian *E.coli*. Kesemua pencilan *E.coli* yang diuji dikategorikan kepada 12 kumpulan berbeza berdasarkan profil ujian biokimia yang dijalankan termasuklah ciri hemolisis atas agar darah. Penserotipan semua pencilan tersebut menunjukkan bahawa 69.6% (87/125) pencilan yang diuji tidak dapat dikelaskan dalam mana-mana serotip yang diuji. Serotip lain yang dikenalpasti adalah O1:K1 (14.4%), O78:K8O (10.4%), dan O2:K1 (5.6%).

Analisis filogenetik ke atas pencilan-pencilan tersebut mendedahkan bahawa sebahagian besar *E. coli* yang diasingkan dari ladang ayam dalam kajian ini terdiri dari kumpulan B1 (36.0%) dan kumpulan D (28.0%), yang dikaitkan dengan strain yang tidak virulen atau merbahaya.

Analisis PCR multipleks menunjukkan bahawa gen virulen yang paling banyak dikenalpasti adalah *iss* 52.0% (65/125), diikuti oleh *iucD* 36.0% (45/125), *tsh* 32.0% (40/125), *vat* 14.4% (18/125), *astA* 12.0% (15/125), *papC* 12.0% (15/125), *irp2* 9.6% (12/125), dan yang paling sedikit adalah gen *cva/cvi* (0%). Setiap kumpulan filogenetik diwakili dengan kombinasi gen virulen yang berbeza dan pelbagai, dan tiada satu kombinasi gen virulen yang spesifik dikenalpasti mempunyai kaitan dengan kumpulan filogenetik pencilan *E. coli*.

Analisis antibiogram menunjukkan bahawa 81.6% (102/125) pencilan *E. coli* mempunyai profil kerintangan pelbagai terhadap antibiotik yang berbeza. Kebanyakan *E. coli* menunjukkan kerintangan yang tinggi keatas eritromisin 52.8% (66/125), diikuti dengan tetrasiklin 52.0% (65/125), streptomisin 40.0% (50/125), spektinomisin 39.2% (49/125), trimethoprim 38.4% (48/125) dan flumequin 37.6% (47/125). Penemuan ini juga menunjukkan bahawa kebanyakan *E. coli* berkesan terhadap antibiotik yang seringkali digunakan dalam mengatasi jangkitan *E. coli* pada ayam dengan tahap kerintangan yang paling rendah terhadap polymyxin B (92.8%) dan colistin (92.0%). Tiada kaitan dilihat antara profil kepelbagaian kerintangan antibiotik dengan serotip, kumpulan filogenetik dan profil gen virulen dalam kajian ini.

Analisis sekatan makro keatas pencilan *E. coli* yang pilihan menunjukkan corak Pulse Field Gel Electrophoresis (PFGE) yang pelbagai. Pembinaan kumpulan dendogram keatas 56 pencilan dengan kesan 60% koefisien setara menghasilkan 41 genotip yang terdiri dari pelbagai serotip dengan pelbagai profil biokimia, kumpulan filogenetik dan profil gen virulen. Penemuan ini menyatakan bahawa *E. coli* yang diasingkan dari ladang ayam berpotensi patogenik serta mempunyai ciri fenotip dan genotip yang sangat pelbagai, *E. coli* berpotensi menyebabkan jangkitan pada ayam walaupun pada asalnya tidak merbahaya dan merupakan bakteria normal flora dalam usus memandangkan mereka berupaya untuk mewarisi gen virulen dari bakteria lain dalam usus yang menyebabkan jangkitan.

ACKNOWLEDGEMENTS

بسم الله لرحمن الرحيم

In the name of Allah the most compassionate and the most merciful.

First and foremost, along with humblest feeling, I would like to express the most gratitude to Almighty Allah, Who gives me life, health, strength and opportunity to further the masters study. Alhamdulillah, with the permission of Allah, this research study was completed.Secondly, my earnest thanks goes to my family especially my dearest husband and daughter, parents and siblings for their sacrifices and supports during the course of this study and the preparation of this thesis.

Next, my utmost appreciation and thankfulness goes to Assoc. Prof. Dr. Siti Khairani Bejo, Department of Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine, Universiti Putra Malaysia, the chairman of Supervisory Committee for her earnest in guidance, advice and support to make this hopes achieved, so that I can established my knowledge and skills in Department of Veterinary Services for a better services to the country, Insya Allah.

I am indebted to supervisory committee members, Prof. Dr. Zunita Zakaria, Department of Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine, Universiti Putra Malaysia and Dr. Ramlan Bin Mohamed A.M.N (PhD), Research and Development Division, Department of Veterinary Services, Putrajaya who always assist me in terms of ideas, recommendation, and support throughout studies and research work.

I would like to take this opportunity to acknowledge Veterinary Research Institute (VRI) which allowed me to continued my lab works in such a pleasant place, and kind staff who always there to help and assist me. My utmost thanks also goes to all staff of VLSU (Veterinary Laboratory Services Unit), Faculty of Veterinary Medicine, Universiti Putra Malaysia particularly Bacteriology Lab for their kindness and guidance throughout my studies and lab work. Furthermore, I am indebted to JPA (Jabatan Perkhidmatan Awam) which selected me for continuing study in Master Science at Universiti Putra Malaysia besides provides sponsors and allowance for my study.

Last but not least, deepest thanks to all those people who directly or indirectly involved in my life, that always make me always feeling blessed and fortunate.

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LIST OF ABBREVIATIONS

AFLP	Amplified Fragment Length Polymorphism
AMP	Ampicillin
APEC	Avian Pathogenic *E. coli*
APR	Apramycin
α	Alpha
astA	Enteroaggregative toxin gene
ATCC	American Type Culture Collection
AZM	Azithromycin
bp	Base Pair
β	Beta
BSA	Bovine Serum Albumin
CDC	Centre of Disease
ChuA	Gene require for heme transport
CLSI	Clinical Laboratory Standard Institute
CN	Gentamicin
CSB	Cell Suspension Buffer
CT	Colistin
cva/cvi	Colicin V Plasmid Operon gene
°C	Degree Celcius
DNA	Deoxyribonucleic Acid
DVS	Department of Veterinary Services
E	Erythromycin
ECOR	*E. coli* Reference Collection
EFT	Ceftiofur

EHEC	Enterohaemorrhagic *E. coli*
IB	Infectious Bronchitis
Iss	Increased serum survival gene
Irp2	Iron repressible protein gene
iucD	Aerobactin gene
ExPec	Extraintestinal Pathogenic *E. coli*
ESBL	Extended SpectrumBeta Lactamase
gyrA	DNA gyrase gene
K	Kanamycin
Kb	Kilobases
MIC	Minimum Inhibitory Concentration
MLST	Multilocus Sequencing Typing
mm	milimetre
MR	Methyl Red
MRSA	Methicilin Resistant *Staphylococcus aureus*
MHA	Mueller Hinton Agar
MVLA	Multilocus Variable Number Tandem Repeat Analysis
ND	Newcastle Disease
NMEC	Neonatal Meningitis *E. coli*
NPCB	National Pharmaceutical Bureau
PAIs	Pathogenicity islands
parC	Topoisomerase IV gene
PB	Polymyxin B
papC	P fimbrae gene
PCR	Polymerase Chain Reaction

PFGE	Pulse Field Gel Electrophoresis
RAPD	Random Ampified of Polymorphic DNA
S	Streptomycin
SH	Spectinomycin
stx	Shiga toxin gene
TBE	Tris-Borate EDTA
TE	Tris- EDTA
TE	Tetracycline
tsh	Temperature sensitive hemagglutinin gene
TSPE4.C2	Gene from published substractive library of *E. coli*
µl	Microlitre
UB	Flumequin
UPEC	Uropathogenic *E. coli*
UPGMA	Unweight Pair Group Method Using Arithmetic Averages
vat	Vacuolating autotransporter toxin gene
VP	Voges Proskeur
VRI	Veterinary Research Institute
W	Trimethoprim
WHO	World Health Organization
yjaA	Gene initially identified in genome sequence of *E. coli* K-12 strain

CHAPTER 1

INTRODUCTION

Escherichia coli is considered a member of normal microfloral of most human beings and other mammals and birds (*Ahmed et al., 2013*). This bacterium usually considered as commensal bacteria and harmless, but some strains enable to cause fatal diseases in human as well as mammals and birds (Branko *et al*., 2011). The incidence of *E. coli* infection has increased tremendously over the years (Jafari *et al.,* 2012) and has become an important issues particularly in human due to the formation of superbug multidrug resistance strains arising from those commensal strains of *E. coli*. Despite, *E. coli* has been highlighted as a major health treat due to their ability to cause severe problems in human with the most recent and fatal outbreak in Germany causing high morbidity and mortality (Buccholz *et al*., 2011).

Since ancient times, *E. coli* has been linked to the cause of diseases in poultry, but has not received any special attention since this strain is often overlooked as contaminants. However, they also have been reported as aetiology agent of various diseases in poultry such as swollen head syndrome, colisepticemia, embryonic mortality, dermatitis, cellulitis, salphingitis and septicemia (Khoo *et al*., 2010). The most important infection caused by *E. coli* in poultry is colibacillosis. Colibacillosis has become a major problem in poultry industry worldwide due to the significance economic loss through high mortality and morbidity, loss in weight gain and poor carcass quality (Dziva *et al*, 2008).

Based on Department of Veterinary Services (DVS) annual reports, *E. coli* was isolated from 70% of veterinary samples submitted for diagnosis including chicken samples (Mat Amin *et al*., 2011; Khoo *et al*., 2014). Most of these samples exhibit significant clinical signs such as high mortality, respiratory distress and low body weight. Abundant of *E. coli* are isolated from all visceral organs. It's showing that *E. coli* is one of the major problems in the poultry industry that needs to be addressed. Although *E.coli* is often regarded as contaminant and non-pathogenic, but due to a large number of *E.coli* isolates from poultry particularly chickens with significant clinical signs, it has become an alarming signal to the poultry industry in the country that need to be highlighted. However, it is unknown whether *E. coli* is indeed pathogenic and really causative agent causing the disease in the chicken, or simply non-pathogenic bacteria.

In limited information on *E. coli* itself, controlling the infection become a big challenge in many countries. In addition, antibiotic resistance issues in *E. coli* in the poultry also contributed to complicates treatment againts *E. coli* infection, resulting in ineffective disease control (Mooljuntee *et al*., 2010; Diarra and Malouin, 2014). Multidrug resistant in *E. coli* strain in food animals particularly in chicken continues to be debated in many countries including Malaysia, with the emergence and spread of multidrug resistant among *E. coli* and other commensal bacteria to newer antimicrobial compounds thus becoming a global threat (Marshall and Levy, 2011; Ong *et al*., 2014).

Though there are many study on characterization of *E. coli* has been done elsewhere, most of the studies are limited in specific serotypes and involved certain location. Based on the previous information available, *E. coli* characteristics on virulence properties, phylogenetic grouping, antimicrobial resistant and genotyping profiles in different geographical locations were heterogenous (Clermont *et al*., 2000; Ewers *et al*., 2005). The diagnosis method and recommended vaccine available were unreliable to be used in the country considering that the features of *E. coli* local strains are very different in different with other countries (Kwon *et al*., 2008; Ahmed *et al*., 2013; Zainal Abidin *et al*., 2013; Schouler *et al*., 2012). Therefore there are still no effective disease control approach can be apply to resolve the infection. Despite, most of studies on phenotyping and genotyping of *E. coli* conduct in the country involved limited to scope and certain geographical area (Apun *et al*., 2008; Khoo *et al*., 2010; Geidam *et al*., 2012b; Zainal Abidin *et al*. 2013; Khoo *et al*., 2014). As a result, there is no concrete information regarding phenotyping and genotyping profiles of *E. coli* including in chicken in the country. The information on *E. coli* local isolates will provide detail information about the disease in the country thus useful for future works in establishment of effective diagnosis and disease control programmes. It is hypothesized that *E. coli* strains isolated from chicken in Malaysia is highly diverse and still not fully establish.

Therefore, the objectives of this study were:

i. To determine the most prevalent *E. coli* serotypes isolated from chicken in Malaysia
ii. To detect the presence of virulence genes in the *E. coli* isolates by using multiplex PCR
iii. To determine phylogenetic grouping of *E. coli* isolates by using triplex PCR.
iv. To determine multidrug resistance profiles of *E. coli* isolates from chicken
v. To characterize selected *E. coli* isolates using Pulse Field Gel Electrophoresis (PFGE).

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