UNIVERSITI PUTRA MALAYSIA

COLISTIN SUSCEPTIBILITY PATTERN OF MULTI DRUG-RESISTANT *ESCHERICHIA COLI* FROM POULTRY FARMS IN MALAYSIA

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CERTIFICATION

It is hereby certified that we have read this project paper entitled " Colistin Susceptibility Pattern of Multidrug-Resistant *Escherichia coli* from Poultry Farm in Malaysia.", by Khor Shu Neng 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 – Project

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DEDICATIONS

This project paper is dedicated

To my family,

Grandmother

Father

Mother

Brother and Sister

And to all my teachers who have committed themselves towards the noble cause of education.

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It is with deepest appreciation and gratitude to all those who have made this project paper a reality.

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LIST OF ABBREVIATIONS

%	Percent
μg	Microgram
AMR	Antimicrobial resistance
MDR	Multi drugs resistant
E.coli	*Escherichia coli*

ABSTRAK

CORAK KENCENDERUNGAN *COLISTIN*KETAHANAN PELBAGAI ANTIBIOTIK *ESCHERICHIA COLI* DARI LADANG AYAM YANG BERBEZA DI MALAYSIA

oleh

Khor Shu Neng

2016

Penyelia: Prof. Datin Paduka Dr. Aini Ideris

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Colistin adalah antibiotik amat penting dalam perubatan manusia. Walau bagaimanapun, mekanisme rintangan colistin yang plasmid-pengantara dalam haiwan dan manusia telah ditemui meluas di China pada tahun 2015, dan sejak itu telah dikesan di negara-negara lain pada masa penulisan ini. Industri ternakan ayam sangat penting di Malaysia.Oleh itu, kajian ini bertujuan untuk menggambarkan corak kecenderungan colistin daripada ketahanan pelbagai antibiotik*Escherichia coli* dari ladang ayam di Malaysia. Dengan kerjasama makmal swasta, data 1 Januari 2014 hingga 1 Disember 2015 untuk 69 profil kecenderungan pelbagai antibiotik*Escherichia coli* yang tertakluk kepada ujian kepekaan antibiotik dengan menggunakan kaedah Kirby-Bauer cakera

penyebaran telah diambil. Antibiotik utama dinilai dalam kajian ini adalah colistin. Data dianalisis dengan menggunakan perisian SPSS versi 20. Berdasarkan keputusan dihasilkan, pelbagai antibiotik*Escherichia coli* menunjukkan kecenderungan tertinggi kepada *colistin* (90%). Ini diikuti oleh *fosfomycin* (57%) dantidak menunjukkan kecenderungan untuk *amoxicillin / asid Clavulunic*, *amoxicillin* dan *tilmicosin*. Di samping itu, tidak ada hubungan yang ketara antara corak kecenderungan colistin dan kedua-dua tahun atau corak rintangan kepada antibiotik yang lain. Penggunaan berhemat *colistin* dalam ternakan amat disyorkan untuk mencegah penyebaran rintangan *colistin*.

Kata kunci: *Colistin*, ayam, pelbagai ubat tahan, *E. coli*, corak kecenderungan

ABSTRACT

Abstract of the project presented to the Faculty of Veterinary Medicine in partial requirement for the course VPD 4999-Project

COLISTIN SUSCEPTIBILITY PATTERN OF MULTIDRUG- RESISTANT *ESCHERICHIA COLI*FROM DIFFERENT POULTRY FARMS IN MALAYSIA

By

Khor Shu Neng

2016

Supervisor : Prof. Datin Paduka Dr. Aini Ideris

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Colistin is a critically important antibiotic in human medicine. However, the plasmid-mediated colistin resistance mechanism in animal and human was discovered widespread in China in 2015, and has since been detected in other countries at the time of writing. As poultry livestock industry is important in Malaysia, this study aimed to describe the colistin susceptibility pattern of multidrug-resistant *Escherichia coli*(MDR *E.coli*)from different poultry farms in Malaysia. With the cooperation of a private laboratory, data from 1st January 2014 to 1 December 2015 for 69 antimicrobial susceptibility profile of MDR *E.coli*which was subjected toantibiotic sensitivity testing by using Kirby-Bauer disk diffusion method was retrieved.The major antibiotic evaluated in this study was colistin.The data was analysed by using SPSS version20. Based on the results generated, MDR*E.coli* showed highest susceptibility to colistin(90%). This was followed by fosfomycin (57%). The MDR *E.coli* showed total non-susceptibility to amoxicillin/clavulanic acid, amoxicillin and tilmicosin. In addition, there is no significant association between colistin susceptibility pattern and both years or resistance pattern to other tested antibiotics. Prudent use of colistin in livestock is highly recommended to prevent spread of colistin resistance.

Key words: Colistin, poultry, Multidrug-resistant, E. coli, Susceptibility pattern

1.0 INTRODUCTION

Colistin is the only effective drug in human medicine in the context of treatment of infections caused by MDR *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Escherichia coli*(Schorr, 2009).Due to the critical importance of colistin for use in human medicine, there is a need to focus on the possible consequences of veterinary use of colistin for human public health (WHO,2014).

Despite the abundant use of colistin in veterinary medicine for over 50 years, available information on colistin resistance transmission via horizontal gene transfer or sustained clonal expansion has not been reported(EMA, 2012). However, during a routine surveillance project which was carried out in 2015 on antimicrobial resistance in commensal *Escherichia coli* from food animals in China, a major increase of colistin resistance was observed.This report the emergence of the first plasmid-mediated polymyxin resistance mechanism, MCR-1, in Enterobacteriaceae for both animals and human (Liu *et al*.,2015).

There is evidence which revealed that antimicrobial resistant genes can transfer from food animal to human by mobile plasmid (Geidam*et al*, 2012).Thus, since the amount and pattern of antimicrobials for food animals is the major determinant for the propagation of resistant bacteria in the animal reservoir(Aarestrup *et al*., 2008), the resistant bacteria in livestock has important public health significant in order to prevent failure of critically important drugs for human medicine.

Poultry industry is important to the Malaysian livestock industry, the understanding for antibiotic susceptibility pattern especially on colistin susceptibility of isolated *E.coli*from poultry is vital for both livestock and humans. However, colistin sensitivity test is not routinely done for every sample. Hence, this study was undertaken to fulfill the following objectives:

1. To determine the colistin sensitivity pattern of MDR *Escherichia coli* (*E.coli* from different poultry farm.
2. To retrospectively evaluate the associations between colistin susceptibility, years and other tested antibiotics susceptibility pattern ofMDR *E.coli.*

2.0 LITERATURE REVIEW

2.1Usage of colistin in human medicine

Colistin is a cationic, multicomponent lipopeptide antibacterial agent and critically important drug for human medicine (WHO, 2014).Severe infections due to multidrug-resistant (MDR) Gram-negative bacteriahas accounted for high morbidity and mortality. For infections caused by MDR *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and Enterobacteriaceae (*Escherichia coli*, *Klebsiella pneumoniae*), colistin is the only effective drug in human medicine in the context of treatment for these infections(Schorr, 2009).In human medicine, the two salt forms of polymyxin E (colistin) are widely commercially available, namely colistin sulphate and colistimethate sodium (syn colistin methanesulphate, colistin sulphonyl methate, pentasodium colistimethanesulphate). Colistimethate sodium (CMS) is microbiologically inactive (Bergen et al., 2006) and less toxic than colistin sulphate (Li et al., 2006). It is administered predominantly as parenteral formulations and via nebulisation (Falagas and Kasiakou, 2005). After administration, CMS is hydrolysed to colistin, which is the base component that is responsible for its antibacterial activity (Lim *et al*., 2010).

2.2Colistin resistance *Escherichia coli* in poultry

There is limited epidemiological study of colistin resistance *E.coli*in poultry. Indeed, the lack of emergence of resistance should be addressed with caution since in depth epidemiological surveys in veterinary medicine are scarce(Lo-Ten-Foe et al., 2007). According to Geenen *et al*., (2011), resistance in *E. coli* from broilers is increasingly becoming multi-resistance, but, the colistin sensitivity is still there.

However, after the report of plasmid mediated colistin resistance reported in China, in German, early results from resistance monitoring conducted at the Federal Institute for Risk Assessment also showed that the transferrable gene *mcr-1* which was first detected in China and which caused resistance to the antibiotic colistin, is also widespread in intestinal bacteria in farm animals. Most frequently, this colistin resistance is detected in *E.coli* of fattening poultry (Bfr ,2016). More countries have revealed similar finding at the time of writing.

2.3Multi drug resistance (MDR)

Antimicrobial resistance is resistance of a microorganism to an antimicrobial drug that was originally effective for treatment of infections caused by it. The natural selection pressure can cause antimicrobial resistance and the use and misuse of antimicrobial drugs accelerates the emergence of antimicrobial resistance strains(WHO, 2014). Thus, a draft global action plan to combat antimicrobial resistance which has been submitted to the sixty-eighth World Health Assembly, took place in May 2015. This has reflected the important of monitoring antibiotic resistance pattern in livestock like poultry.

Antimicrobial resistance (AMR) in humans is inter-linked with AMR in other populations, especially farm animals, and in the wider environment.Surveillance of bacterial disease, drug usage and resistance in livestock is still relatively poor in poultry . An important step towards assessing any threat to public health from AMR in farm animals is to determine levels of resistance in those populations. Yet there has been no systematic, international review of levels of AMR in farm animals(Mark *et al*.,2014).According to Saif*et al*. (2000), in poultry, the antimicrobial dugs are used extensively to control losses. The parallel usage of antimicrobial drug has resulted in progressive development of antimicrobial resistance.

2.4 Public health significance of avian pathogenic E coli

E. coli is a gram-negative, non-acid-fast, uniform staining, non-spore-forming bacillus that grows both aerobically and anaerobically and may be variable in size and shape. Many strains are motile and have peritrichous flagella. *E. coli* is considered as a member of the normal microflora of the poultry intestine, but certain strains, such as those designated as avian pathogenic *E. coli*, spread into various internal organs and cause colibacillosis characterized by systemic fatal disease.(Lutful Kabir, 2010).

Most avian pathogenic *E. coli* isolated from poultry are specific clonal type that are pathogenic only for birds. However,they shares characteristics with mammalian *E.coli*. In addition, serotypes, virulence factors and antimicrobial resistance of avian pathogenic *E. coli* in other animals is often shared via gene transfer mechanism involving plasmid. Besides that, it alsoemphasizesthat although most of the antimicrobial resistance problems in human stem from overuse, there is evidence that antimicrobial resistant enteric bacteria can transfer from animal to human and thereby establishing a reservoir of resistant genes. (Kabir, 2010). In addition, an unexpected finding in a current study was that majority of *E.coli* isolates from retail poultry products was more consistence with avian pathogenic *E. coli* than commensal *E.coli*.(Saif *et al*. 2000) Thus, more research should be done on public health significance of avian pathogenic *E. coli.* Recently, numerous studies have highlighted similarities between human and avian pathogenic *E. coli*, particularly in their virulence genes, suggesting that poultry products could serve as a source of avian pathogenic *E. coli*that causes infections in human (Manges and Johnson, 2012).

3.0 MATERIALS AND METHODS

3.1 Data Retrieval

A retrospective study on69antimicrobial susceptibility profile of MDR *E.coli*from different poultry in Malaysia from 1st of January 2014 to 1st of December 2015 was reviewed and cross checked with the private laboratory staff on the laboratory methods. The samples types included visceral organs, feces and swabs from poultry sources submitted to the private laboratory were analysed *for E.coli* via microbiological methods using selective media such as MacConkey agar and Chromocult Coliform Agar. The isolated *E.coli* were subsequently tested for antibiotic resistance using Kirby-Bauer disk diffusion method.

The number of tested antibiotics evaluated in this study depended on request from the clients which were 10, 15 or 20. To be included for analysis, the antimicrobial susceptibility profile must be inclusive of colistin susceptibility test.

3.2 Statistical analysis

The data regarding the antimicrobial susceptibility profile of isolated MDR *E.coli* from clinical cases were presented in tables and graphs. The association between colistin susceptibility pattern, years and resistance to othertested antibioticswere determined at 95% confidence and significant at $p<0.05$ using Chi Square Test.All the statistical analyses were performed using SPSS software version 20. All the categorical values were analysed using non parametric test.

4.0 RESULTS

4.1 Antimicrobial susceptibility profile

Table 4.1:

Anmicrobial Susceptibility Pattern

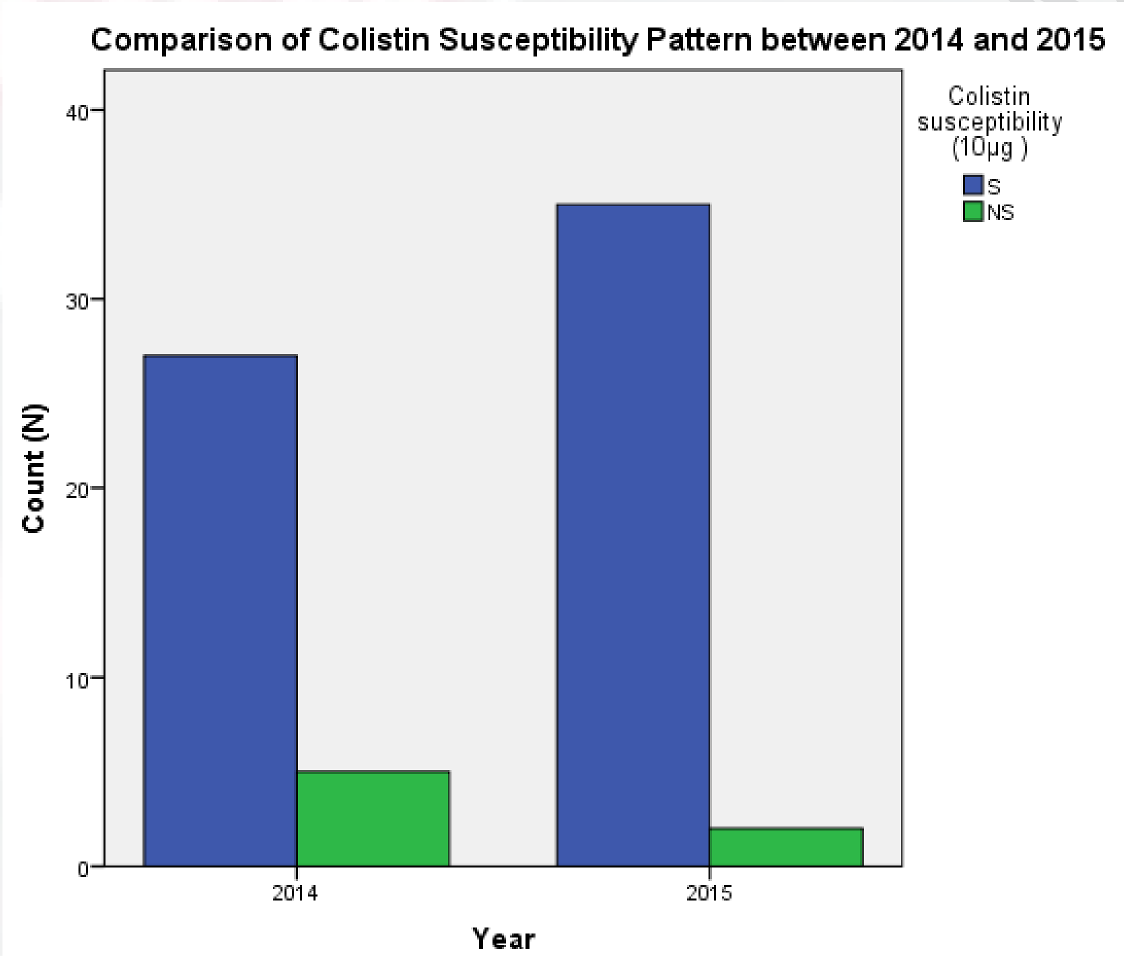
Tested antibiotics		Count	Column N %
Amoxicilin (25µg)	S	0	0%
	NS	67	100%
	Total	67	100%
Amoxicilin/clavalunic acid (20/10µg)	S	0	0%
	NS	33	100%
	Total	33	100%
Cephalexin (30µg)	S	9	16%
	NS	46	84%
	Total	55	100%
Ciprofloxacin (5µg)	S	0	0%
	NS	6	100%
	Total	6	100%
Colistin (10µg)	S	62	90%
	NS	7	10%
	Total	69	100%
Doxycycline (30µg)	S	7	11%
	NS	57	89%
	Total	64	100%
Enrofloxacin (5µg)	S	18	27%
	NS	49	73%
	Total	67	100%
Erythromycin (15µg)	S	2	11%
	NS	16	89%
	Total	18	100%
Florfenicol (30µg)	S	19	32%
	NS	40	68%

	Total	59	100%
Flumequine (30µg)	S	5	10%
	NS	43	90%
	Total	48	100%
Fosfomycin (200µg)	S	37	57%
	NS	28	43%
	Total	65	100%
Norfloxacin (10µg)	S	18	35%
	NS	34	65%
	Total	52	100%
Spectinomycin (100µg)	S	4	36%
	NS	6	55%
	Total	11	100%
Streptomycin (10µg)	S	2	11%
	NS	16	89%
	Total	18	100%
Tetracycline (30µg)	S	1	2%
	NS	46	98%
	Total	47	100%
Tilmicosin (15µg)	S	0	0%
	NS	19	100%
	Total	19	100%
Trimethoprim (5µg)	S	4	36%
	NS	7	64%
	Total	11	100%
Trimethroprim sulphamethoxazole (1.25+23.75µg)	S	18	31%
	NS	41	69%
	Total	59	100%

S= Susceptible

NS= Non-Susceptible

4.2 Bar graph for comparison of colistin susceptibility profile between 2014 and 2015



S = Susceptible
NS = Non susceptible

The bar graph showed that the susceptibility pattern of colistin for both years remain similar with high proportion of MDR *E.coli*remains susceptible toward colistin.

4.3 Crosstabulation for association between colistin susceptibility pattern and resistance pattern to other tested antibiotics

			Resistance to all tested antibiotics		Total
			No	Yes	
Susceptibility_colistin	S	Count	54	8	62
		Expected Count	53.9	8.1	62.0
	NS	Count	6	1	7
		Expected Count	6.1	.9	7.0
Total		Count	60	9	69
		Expected Count	60.0	9.0	69.0

S = Susceptible
NS = Non susceptible

This result of crosstabulation revealed no significant association between colistin susceptibility pattern and resistance pattern to other tested antibiotics.

5.0 DISCUSSION

MDR *E.coli* showed highest susceptibility to colistin(90%). This was followed by fosfomycin (57%). The MDR *E.coli* showed total non- susceptibility to amoxicillin/clavulanic acid, amoxicillin and tilmicosin. The resistance from these antibiotics may be due to continuous usage as prophylactic treatment rather than therapeutic treatment due to their cheaper cost compared to the antibiotic, whereas, colistin recorded lowest resistance may be due to its molecule which is not absorbed into the body system (Tang *et al*., 2015).

In the study of relationship among different years or antibiotics resistant pattern with colistin susceptibility pattern, the result revealed no significant association (p>0.05), between year and colistin susceptibility and between antibiotic resistant pattern and colistin susceptibility pattern. This might be concluded that colistin susceptibility pattern has stayed much the same for both years. The same goes to association between colistin susceptibility pattern and other tested antibiotic resistance pattern.

However, from the graph of antimicrobial pattern depends on susceptibility of colistin, the result revealed relatively few alternative of other antibiotics which are cephalexin, enrofloxacin, florfenicol, flumequine, fosfomycin, norfloxacin and trimethroprime+sulphamethoxazole when the colistin was tested non-susceptible. When the colistin was tested susceptible, the result revealed, the fosfomycin is one of the alternative promising antibiotic against infection. The alternatives to colistin depend on the resistance situation in a particular country(CVMP, 2009). Thus, the understanding of antimicrobial profile will aid in decision making of alternative antibiotics for colistin replacement.

From the cross table of antibiotic resistant pattern against colistin susceptibility pattern, the result revealed 1 sample resistance to all tested antibiotics included colistin and 8 samples are resistant to all tested antibiotics but colistin tested susceptible. Although these are only about 6 outof 69, these groups of samples are important. Since they revealed limited antimicrobial treatment, they should be monitored closely. Further investigation in term of history taking and others will aid in understanding of the scenario. In addition, the transition to increased colistin resistance was not accompanied by changes in susceptibility to the other antibiotics tested. Proper intervention should be taken if discovered emergence of plasmid mediated colistin resistant mechanism.

6.0 CONCLUSION

In conclusion, according to the retrospective study on colistin susceptibility pattern of multidrug-resistant *E.coli* from various poultry farms in Malaysia from 1st of January to 1st of December 2015, the colistin susceptibility test revealed highest sensitivity level compared to other tested antibiotic. There is no significant finding for association between colistin susceptibility patterns, year and resistance to all other antibiotics, but, the presence of low level of colistin resistance still warrant further monitoring and analysis. These population of colistin resistance MDR *E.coli* may carry Mcr1 gene. To aid on understanding on trend of colistin susceptibility pattern, a larger samples size from various reliable source will give a better picture on the trend.

7.0 RECOMMENDATIONS

Firstly, as plasmid mediated colistin resistance mechanism has been detected, a constant and systematic surveillance study on colistin resistance with the aid of extensive statistical analysis on retrospective data should be carried out for poultry and other livestock in order to better understand the distribution of colistin resistance bacteria in Malaysian livestock. Optimally, the molecular study on colistin resistance gene should be involving wide range of pathogens and zoonotic bacteria from every livestock farms in Malaysia.In addition, the transparency in the sales and usage of colistin willenhance the surveillance process as well.

Secondly, due to the complexity of antimicrobial web, one-health approaches is important in preventing spread of colsitin resistance and same should be apply to other type of antibiotic resistance. The use of antibiotic in livestock is not the sole contribution to the antibiotic resistance issue. Thus, more related stake holders should take responsible in improving this scenario before the antibiotic apocalypse really arrives. The sharing of all information on antibiotic resistance from different fields can aid in understanding in the complexity of antimicrobial web which in turn ease the establishment of proper antibiotic stewardship, the surveillance of antibiotic.

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