SITUATIONAL ANALYSIS ON THE ANTIMICROBIAL RESISTANCE PATTERNS AMONG BACTERIAL ISOLATES FROM DISEASED SMALL ANIMALS, LIVESTOCK AND WILDLIFE

By

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

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Antimicrobial resistance (AMR) is a global threat of immense significance to the health of animals and humans. While there is an abundance of reports on AMR among clinical isolates from humans, information regarding the patterns of resistance among clinical isolates from animals is scarce. Hence a situational analysis of AMR among the clinical cases received at a veterinary diagnostic laboratory was performed to describe the pattern of resistance demonstrated by clinical isolates. Specifically, this study describes the AMR pattern of clinically important pathogens from pets and livestock using retrospective laboratory data between 2015 and 2017, and determine the resistance patterns of selected pathogens of public health significance from domestic and wildlife between 2018 and 2019. In diseased pets, *Escherichia coli* was the most commonly isolated (n=101, 13%) bacteria. Additionally, *E. coli* from the pets was highly resistant to amoxicillin (73.2%) and cephalexin (66.3%). Moreover, more than 75% of isolates from diseased pets were multi-drug resistant (MDR). Isolates from cats appeared to have higher level of resistance to multiple antibiotics compared to those from dogs. While in diseased livestock, *Escherichia coli* was the most commonly isolated (n=185, 26%) bacteria, and (>77%) were resistant to neomycin and streptomycin. Moreover, more than 76% of isolates from livestock were MDR. In general, isolates from non-ruminants have a higher level of resistance to multiple antibiotics compared to ruminants. In conclusion, this study has highlighted the trends and level of AMR among clinical isolates from pets and livestock based on routine antibiotic therapies. This study also discovered the trends of resistance among clinical isolates to those antibiotics of public health significance using the scope of testing as described by the national integrated surveillance for AMR.

Keywords: pet, livestock, wildlife, clinical isolates, AMR, MDR

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

ANALISIS SITUASI CORAK KERINTANGAN ANTIBIOTIK DI KALANGAN ISOLAT BAKTERIA DARI HAIWAN PELIHARAAN, TERNAKAN DAN HAIWAN LIAR SAKIT

Oleh

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Kerintangan antibiotik (AMR) adalah ancaman global yang sangat penting kepada kesihatan haiwan dan manusia. Terdapat banyak laporan mengenai kerintangan antibiotik (AMR) dalam kalangan isolat klinikal dari manusia, tetapi maklumat dan laporan kerintangan antibiotik dalam kalangan isolat klinikal haiwan sangat jarang diperolehi. Oleh itu, analisis situasi mengenai kerintangan antibiotik (AMR) dalam kalangan kes-kes klinikal yang diterima dari makmal diagnostik veterinar dilakukan untuk menggambarkan corak kerintangan antibiotik yang ditunjukkan oleh isolat klinikal. Secara khususnya kajian ini menerangkan corak kerintangan antibiotik (AMR) terhadap patogen bakteria penting dari haiwan peliharaan dan dari ternakan menggunakan data retrospektif makmal antara tahun 2015 dan 2017, dan menentukan corak kerintangan antibiotik dari patogen bakteria terpilih yang mempunyai kepentingan kepada kesihatan awam dari haiwan domestik dan liar antara 2018 dan 2019. Dalam haiwan peliharaan sakit, *Escherichia coli* adalah bakteria yang paling sering dijumpai (n= 101, 13%). Selain itu, isolat *E. coli* dari haiwan peliharaan mempunyai tahap rintangan yang tinggi terhadap *amoxicilin* (73.2%) dan *cephalexin (*66.3%). Tambahan pula, lebih daripada 75% isolat dari kucing dan anjing yang sakit rintang terhadap pelbagai antibiotik (MDR). Isolat dari kucing kelihatan mempunyai tahap kerintangan yang lebih tinggi terhadap pelbagai antibiotik berbanding dengan anjing. Manakala, dalam haiwan ternakan sakit, *Escherichia coli* adalah bakteria yang paling kerap dijumpai (n= 185, 26%), dan (>77%) rintang terhadap *neomycin* dan *streptomycin.* Malahan, lebih daripada 76% isolat dari ternakan adalah MDR. Secara amnya, isolat dari bukan ruminan mempunyai tahap kerintangan yang lebih tinggi terhadap pelbagai antibiotik berbanding dengan ruminan. Kajian keratan rentas dilakukan di mana *E. coli, Klebsiella pneumoniae* dan *Salmonella* spp. telah diperiksa menggunakan protokol bersepadu AMR. Analisis menunjukkan (>75%) bakteria terpilih ini rintang kepada *ampicillin.* Secara keseluruhan, 58.6% isolat ini adalah MDR. Kesimpulannya, penemuan dari kajian ini telah memberi penekanan tentang tren dan tahap AMR di kalangan isolat klinikal dari haiwan peliharaan dan ternakan berdasarkan terapi antibiotik lazim. Kajian ini juga menemui tren ketahanan antara isolat klinikal terhadap antibiotik yang mempunyai kepentingan kesihatan awam dengan menggunakan skop pengujian seperti yang dijelaskan oleh pengawasan bersepadu nasional untuk pengawasan AMR.

Kata kunci: haiwan peliharaan, ternakan, haiwan liar, isolat klinikal, AMR, MDR

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LIST OF ABBREVIATIONS

AGP	Antibiotic growth promotant
AMR	Antimicrobial resistance
ARG	Antibiotic resistance gene
AST	Antibiotic susceptibility testing
ATCC	American type culture collection
BLFVM	Bacteriology Laboratory, Faculty Veterinary Medicine
Cfu	Colony forming unit
CLSI	Clinical and laboratory standards institute
DANMAP	Danish integrated antibiotic resistance monitoring and research programme
DNA	Deoxyribonucleic acid
DOF	Department of Fisheries
DVS	Department of Veterinary Services
E. coli	*Escherichia coli*
E. faecalis	*Enterococcus faecalis*
ESBL	Extended-β-lactamese
FAO	Food and agriculture organization of the United Nations
FCR	Feed conversion ratio
I	Intermediate
K. pneumoniae	*Klebsiella pneumoniae*
Kg	Kilogram
MDR	Multidrug resistant
Mg	Milligram

MHA	Mueller hinton agar
MINDEF	Ministry of Defence
MOH	Ministry of Health
MOHE	Ministry of Higher Education
MRSA	Methicillin-resistant *Staphylococcus aureus*
MRSP	Methicillin-resistant *Staphylococcus pseudintermedius*
MVDL	Minnesota Veterinary Diagnostic Laboratory
My-AP-AMR	Malaysia action plan for antimicrobial resistance
NA	Not included
NPCB	National pharmaceutical control bureau
OIE	World Organization for Animal Health
PFGE	Pulsed field gel electrophoresis
P. mirabilis	*Proteus mirabilis*
R	Resistant
S	Susceptible
S. aureus	*Staphylococcus aureus*
S. canis	*Streptococcus canis*
S. intermedius	*Staphylococcus intermedius*
S. pseudintermedius	*Staphylococcus pseudintermedius*
SPSS	Statistical package for social sciences

UNISZA	Universiti Sultan Zainal Abidin
UPM	Universiti Putra Malaysia
VRE	Vancomycin-resistant Enterococci
WHO	World Health Organization
β-lactam	Beta-lactam

CHAPTER 1

INTRODUCTION

1.1 Background

Antimicrobial resistance (AMR) is a global health issue of immense importance for both animals and human health (Robinson *et al.,* 2016). Activities related to sustaining human health, animal production and health, and the environment are major drivers for the emergence of AMR (Berendonk *et al.,* 2015; Hernando-Amado *et al.,* 2019; Wegener, 2012). The impact of AMR on human health has been widely reported (Fey *et al.*, 2000; Friedman *et al.,* 2016; Marshall & Levy, 2011; Mund *et al.,* 2017). In the animal production and health component, vast discussions have been presented on activities related to livestock production and husbandry that drives AMR (Landers *et al.,* 2012). However, there has been less emphasis on the impact of AMR on animal health in general especially among companion animals and wildlife.

In (2003), Wegener stated that the use of antimicrobial agents in domestic animals for treating, preventing diseases and growth-promoting contributes to the selection of antimicrobial resistance. It has also been widely reported that antimicrobial resistance in food-producing animals poses risks to humans because of the transmission of resistant zoonotic bacteria via the food chain (Economou & Gousia, 2015; Rozman *et al.,* 2019; Vidovic & Vidovic, 2020). However, until very recently, little attention has been directed to the use of antibiotics in companion animal clinics and the impact of such usage on the resistance level of veterinary pathogens. In a few recent studies, companion animals are reported to be able to disseminate antimicrobial resistance due to their close contact to humans (Amadi *et al.,* 2019; Chung *et al.,* 2017; Wedley *et al.,* 2011).

Little information is available about the level of resistance among clinical isolates from diseased animals in Malaysia. In contrast, there is a growing evidence of the widespread occurrence public health significant pathogens such as vancomycin-resistant enterococci (VRE), methicillin-resistant *Staphylococcus aureus* (MRSA) and multi-drug resistant *Salmonella typhimurium* in pet animals (Guardabassi *et al.,* 2017; Iseppi *et al.,* 2015; Van Den Bunt *et al.,* 2018). There are also studies that have linked bacterial pathogen isolated in livestock and pets to those found in their owners (Subbiah *et al.,* 2020; Yamasaki *et al.,* 2012). Therefore, the gaps of information from veterinary clinical isolates will be explored through the objectives of the current study.

The AMR patterns and trends among clinical isolates from domestic and wildlife have not been well reported. This information is necessary to enhance the understanding about the AMR situation among important disease-causing agents in local animals. In Malaysia, a report from a regional government laboratory has revealed the occurrence of multi-drug resistance *E. coli* from veterinary clinical isolates (Shahaza, *et al.,* 2017). Moreover, global reports on laboratory surveillance of AMR patterns and trends from diagnostic cases have shown high levels of resistance among common disease-causing bacteria in animals (Bourély *et al.,* 2019; Rzewuska *et al.,* 2015; Saputra *et al.,* 2017; Yassin *et al.,* 2017). Therefore, laboratory surveillance data have been highly effective in providing comprehensive information on the local AMR trends and patterns (IACG, 2018; Opintan *et al.,* 2015).

1.2 Problem statement

Dramatic increase of AMR among bacterial pathogens of zoonotic potential has been reported in many countries (Garcia-Migura *et al.,* 2014; Kaesbohrer *et al.,* 2012). Some antimicrobial agents used in animals have almost completely lost their efficacy to treat common bacterial infections. The trend of AMR is dependent on the way that antibiotic is used in the population. In Malaysia, antibiotic use in animals (pets or livestock) has not been documented and AMR among isolates from diseased animals is unknown. There is a large gap in knowledge about the AMR among local pets and livestock. Previous study has reported high level of resistance among clinical isolates from domestic and wild animals (Shahaza, *et al.,* 2017). However, more analysis from available data is needed to obtain more information to describe the AMR situation on the country.

1.3 Justification of the study

Previous studies have focused on the trends of antimicrobial-resistant pathogens in clinical isolates from humans. There is limited information on the AMR trends of common bacterial species isolated from clinical veterinary isolates especially from diseased pets and livestock. Analysis of clinical data is necessary to determine the situation of AMR in veterinary clinical application. This could serve to guide future treatments of sick animals. The present study will describe the pattern and trend among clinical isolates from animals' samples submitted at the Bacteriology Laboratory of UPM Serdang. The findings from this study will emphasize the significance of antimicrobial resistance emergence among pathogens in veterinary cases and improves the understanding of the AMR among pathogens of significance in local animals.

1.4 Research questions

This study aims to address these questions:

i. What is the trend and pattern of AMR among clinically important bacterial pathogens from diseased pets (2015-2017)?

ii. What are the differences between resistance profile of bacterial isolates from diseased cats and dogs?

iii. What is the trend and pattern of clinically important bacterial pathogens from diseased livestock (2015-2017)?

iv. What are the differences between resistance profile of bacterial isolates from diseased ruminants and non-ruminants?

v. What is the pattern of antimicrobial resistance of selected sentinel pathogens (*E. coli*, *Salmonella* spp. and *K. pneumoniae*) isolated from diseased domestic and wildlife between 2018 and 2019?

1.5 Research hypothesis

i. The resistance profile of AMR is significantly different between diseased cats and dogs.

ii. The resistance profile of AMR is significantly different between diseased ruminants and non-ruminants.

iii. There is an increasing and decreasing trend of antibiotic resistance in diseased pets and livestock from 2015 to 2017.

iv. The resistance profile of AMR in selected pathogens is significantly different between diseased companion, livestock and wildlife between 2018 and 2019.

1.6 General objective

The present study was carried out with the aim of describing the patterns of resistance among bacteria isolated from clinical cases presented at a diagnostic laboratory in Universiti Putra Malaysia.

1.7 Specific objectives

i. To determine the antimicrobial resistance patterns of clinically important bacterial pathogens in clinical isolates from diseased pets (2015-2017)

ii. To identify the antimicrobial resistance patterns of clinically important bacterial pathogens in clinical isolates from diseased livestock (2015-2017)

iii. To study the antimicrobial resistance patterns of selected pathogens in veterinary clinical isolates from diseased domestic and wildlife (2018-2019).

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