UNIVERSITI PUTRA MALAYSIA

MOLECULAR EPIDEMIOLOGY OF VANCOMYCIN-RESISTANT ENTEROCOCCI ISOLATED FROM SELECTED HUMAN, POULTRY AND PIG POPULATIONS IN PENINSULAR MALAYSIA

YITBAREK GETACHEW MOLLA

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By

YITBAREK GETACHEW MOLLA

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

October 2010

DEDICATION

Dedicated to my mother Shewaye Kebede and my sister Meseret Getachew

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

MOLECULAR EPIDEMIOLOGY OF VANCOMYCIN-RESISTANT ENTEROCOCCI ISOLATED FROM SELECTED HUMAN, POULTRY AND PIG POPULATIONS IN PENINSULAR MALAYSIA

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October 2010

Chairman : Latiffah Hassan, PhD

Faculty : Faculty of Veterinary Medicine

Farm animals have been implicated as source and reservoirs of vancomycin-resistant enterococci (VRE) for human colonization. However, the relative importance of the major food animal reservoirs compared to human and environmental reservoirs is difficult to quantify. In Malaysia, VRE have been a great concern to the poultry farmers in general, especially farms that export their animals to the neighbouring country because of the restriction imposed by the importing country due to the potential risk of VRE transmission to their human population. The present study was carried out to investigate the epidemiology of VRE in selected human (poultry workers, veterinary students and pig butchers), poultry and pig populations in Peninsular Malaysia. The specific objectives of the study were to detect and describe VRE, identify the epidemiological risk factors for colonization with VRE, determine the vancomycin-resistance genes and vancomycin-susceptibility pattern, detect virulence and enterocin genes, determine genetic diversity of VRE species, and

elucidate the genetic relatedness of *E. faecalis* and *E. faecium* isolates from human and animals.

VRE were detected in 28 (9.4%) of apparently healthy humans, specifically in four (4.3%) of the veterinary students, 15 (13.5%) of poultry workers and 9 (9.5%) of pig butchers. Risk factor analyses showed that previously hospitalized individuals were 4 times more likely to be VRE positive than non-hospitalized. Older (age ≥ 40 years) individuals were 5 times more likely to be colonized than younger subjects (age < 40 years). VRE were detected in 32 (12%) and 1 (0.4%) of pigs and poultry samples respectively. However, factors contributing for VRE colonization in pigs and poultry were not evident. There was no evidence to suggest that contact with animals significantly contribute to VRE among the examined human population.

Enterococcus faecalis, E. faecium, E. gallinarum, E. casseliflavus, E. durans and *E. hirae* were detected. *E. faecium* was the dominant species in human, *E. faecalis* was common in poultry while *E. casseliflavus* was abundant in pigs. Interestingly, other than VRE, highly vancomycin-resistant Gram-positive cocci (VRC) bacteria were detected. Selected VRC were identified as *Pediococcus* species. This incidental finding suggested that potentially high numbers of VRE false positive farms have been detected using the conventional VRE identification system.

Vancomycin-resistance gene *van*B was only detected in one of *E. faecalis* isolate obtained from human making it the first report in Malaysia. The gene *van*A was detected in 20 (71%), 7 (13%) and 51 (36%) humans, pigs and poultry VRE isolates respectively. The prevalence of intrinsic vancomycin resistance genes *van*C1 and *van*C2/3 was the same as *E. gallinarum* and *E. casseliflavus* respectively. However,

E. gallinarum isolates that acquired *van*A gene were observed. In general *van*A carrying VRE isolates were noted to be highly resistant (MIC ≥ 256µg/mL) while majority of *van*C isolates were intermediately resistant.

Virulence gene g*elE* was detected in 75 (95%) of *E. faecalis* and 41(71%) of *E. faecium* isolates. *CylA* gene was present in 5 (6%) *E. faecalis* and 2 (3%) *E. faecium* isolates. The *esp* gene was detected in 4 (5%) and 8 (14%) of *E. faecalis* and *E. faecium* isolates respectively. Among 79 *E. faecalis,* 3 (4%) *entA,* 4 (5%) *entB,* 6 (7.6%) *entP* and 19 (24%) *ent31* gene harbouring isolates were identified. Likewise, from 58 *E. faecium* isolates, 31 (53%), 8 (14%), 21 (36%) and 1 (0.6%) of them were positive for *entA, entB, ent31* and *entL50AB* genes, respectively. Meanwhile, enterocin genes were rare in other species. The finding highlighted the presence of trait aiding spread, survival and dominance of VRE in microbial ecology.

In this study RAPD was used to determine the genetic diversity of VRE isolates obtained from humans, pigs and poultry. High genetic diversity was generally seen in *E. faecalis, E. faecium, E. gallinarum* and *E. casseliflavus.* RAPD was a rapid technique that allowed characterizing a number of isolates at a time. However, RAPD method showed low reproducibility and failed to differentiate strains with *van*A genes from those without *van* genes.

Multilocus sequence typing of selected 14 *E. faecium* and 11 *E. faecalis* revealed six sequence types (ST) for *E. faecium* (ST203, ST17, ST55, ST29 and ST79) and *E. faecalis* (ST4, ST6, ST87, ST108, ST274 and ST244) respectively. Clustering analysis showed that more than 70% of *E. faecium* and all *E. faecalis* were hosts specific (human, pig and poultry). *E. faecium* isolates from healthy individuals had

close similarity to clinical isolate (D, 0-14), but there was high dissimilarity (D, 20) between animal and human isolates in general. Most of human *E. faecium* isolates were derivatives of the epidemic strain clonal complex (CC) -17. Two of human *E. faecalis* were derived from epidemic CC2 and CC87. Only one poultry *E. faecium* was part of the epidemic CC17. *E. faecium* strains reported from Singapore have close similarity to human isolates (D, 7) but high degree of variation (Distance 19 – 29) from animal isolates characterized in this study. Similar *E. faecium* ST17 stains are reported from 21 countries. Mainly from the Western countries, Australia and Korea. *E. faecium* ST203 is reported from four Asian and three European countries. Australia, Belgium, France, Brazil and Netherlands were countries that reported ST57 and ST29. Netherland was the only country in MLST database where ST55 was reported. Similar strain of *E. faecalis* ST4 identified in present study was reported from Thailand. The overall finding from this part of the study is highly significant in the context of refuting the claims that poultry products in Malaysia potentially lead to human VRE colonization in Singapore.

The findings from this study have contributed significantly to the understanding on the distribution and types of VRE among healthy humans, pigs and poultry. The study has elucidated the potential risk factors among humans for VRE colonization, types of resistances, virulence and enterocin genes present, genetic characteristics, and has given first insight into MLST profiles and genetic relatedness of human and animal VRE stains circulating in Malaysia. The study has provided evidence that can be used in the future should the issue on human-animal VRE transmission arise in Malaysia.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan utuk ijazah Doktor Falsafah

EPIDEMIOLOGI MOLEKULAR ENTEROKOKUS RINTANG VANKOMISIN YANG DIPENCILKAN DARI POPULASI MANUSIA, AYAM DAN BABI YANG TERPILIH DI SEMENANJUNG MALAYSIA

Oleh

YITBAREK GETACHEW MOLLA

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Haiwan ternakan telah dikaitkan sebagai punca dan takungan enterokokus rintang vankomisin (VRE) bagi manusia. Akan tetapi, kepentingan relatif haiwan ternakan berbanding dengan manusia dan persekitaran sebagai penakung organisma VRE adalah sukar untuk dinyatakan dalam bentuk kuantiti. Di Malaysia, VRE merupakan kebimbangan oleh pengusaha ladang ternakan ayam, terutamanya ladang yang mengekspot ayam ke negara jiran oleh kerana kekangan oleh negara yang mengimpot disebabkan potensi dan risiko perpindahan VRE kepada populasi manusia di negara mereka. Kajian ini dijalankan untuk menyiasat epidemiologi VRE dalam beberapa populasi manusia (pekerja ladang ayam, pelajar veterinar dan pekerja rumah sembelih babi), ayam dan babi di Semenanjung Malaysia. Objektif spesifik kajian ini adalah untuk mengesan dan menghuraikan VRE, mengenalpasti faktor risiko epidemiologi bagi pengkolonisasi VRE, mengenalpasti gen rintang vankomisin dan pola kerentanan vancomisin , mengesan gen virulen dan gen enterocin, mengenalpasti diversiti genetik VRE dan menjelaskan hubung-kait genetik isolat *E. faecalis* and *E. faecium* pada manusia dan haiwan.

VRE dikesan dalam 28 (9.4%) sampel manusia, secara spesifiknya dalam empat (4.3%) pelajar veterinar, 15 (13.5%) pekerja ladang unggas dan sembilan (9.5%) pekerja rumah sembelih babi. Analisis faktor risiko menunjukkan individu yang pernah dimasukkan ke hospital adalah empat kali lebih berkemungkinan untuk positif VRE berbanding yang tidak pernah dimasukkan hospital. Individu yang lebih tua (umur ≥ 40 tahun) adalah lima kali ganda berkemungkinan untuk dikoloni berbanding yang yang lebih muda (umur < 40 tahun). VRE dikesan dalam 32 (12%) dan 1 (0.4%) babi dan ayam. Akan tetapi, faktor yang menyumbang kepada kolonisasi VRE dalam babi dan ayam adalah tidak jelas. Tiada bukti yang mencadangkan sentuhan dengan haiwan menyumbang keertian kepada VRE dalam populasi manusia yang diperiksa.

Enterococcus faecalis, E. faecium, E. gallinarum, E. casseliflavus, E. durans dan *E. hirae* telah dikesan. *E. faecium* adalah spesis yang dominan dalam manusia, *E. faecalis* adalah umum dalam unggas manakala *E. casseliflavus* adalah dominan dalam babi. Selain dari VRE, bakteria kokus Gram-positif (VRC) yang memiliki ketahanan yang tinggi terhadap vancomisin juga dikesan. VRC yang terpilih dikenalpasti sebagai spesis *Pediococcus*. Penemuan secara kebetulan ini menunjukkan kemungkinan VRE positif palsu dikesan di ladang dengan menggunakan system pemencilan dan pengenalpastian konvensional VRE.

Gen kerintangan vankomisin *van*B hanya dikesan dalam satu isolat *E. faecalis* yang diperolehi dari manusia. Ini adalah laporan pertama gen *van*B dalam Malaysia. Gen *van*A dikesan 20 (71%), 7 (13%) dan 51 (36%) isolat VRE manusia, babi dan

unggas. Prevalen gen rintang vankomisin intrinsik *van*C1 dan *van*C2/3 adalah sama pada *E. gallinarum* dan *E. casseliflavus*. Akan tetapi, isolat *E. gallinarum* yang mempunyai gen *van*A juga dikesan. Secara amnya isolat VRE yang memiliki *van*A dikesan mempunyai ketahanan yang sangat tinggi (MIC ≥ 256 µg/mL), manakala kebanyakan isolasi *van*C mempunyai ketahanan yang sederhana.

Gen virulen g*elE* dikesan pada 75 (95%) isolat *E. faecalis* dan 41(71%) isolat *E. faecium*. Gen *CylA* wujud dalam 5 (6%) isolat *E. faecalis* dan 2 (3%) *E. faecium*. Gen *esp* dikesan pada 4 (5%) dan 8 (14%) isolat *E. faecalis* and *E. faecium*. Dalam 79 isolat *E. faecalis*, 3 (4%) *entA*, 4 (5%) *entB*, 6 (7.6%) *entP* dan 19 (24%) *ent31* dikenalpasti. Dari 58 isolat *E. faecium*, 31 (53%), 8 (14%), 21 (36%) dan 1 (0.6%) daripada mereka adalah positif gen *entA, entB, ent31* dan *entL50AB*. Pada masa yang sama, gen *enterocin* adalah jarang atau tiada dalam spesis yang lain. Keputusan ini mengenengahkan trait yang membantu penyebaran, kelangsungan hidup dan kedominan VRE dalam ekologi mikrobiologi.

Kajian ini menggunakan random amplified polymorphic DNA (RAPD) untuk menentukan diversiti genetik dalam isolat VRE yang diperolehi daripada manusia, babi dan ayam. Diversiti genetik yang tinggi dapat dilihat dalam *E. faecalis, E. faecium, E. gallinarum* dan *E. casseliflavus*. Tetapi, metod RAPD memberikan keterulangan yang rendah dan gagal untuk membezakan strain yang mempunyai gen *van*A daripada yang tidak mempunyai gen *van*A.

Jujukan penaipan pelbagai lokus (MLST) 14 *E. faecium* dan 11 *E. faecalis* merekodkan enam jenis jujukan (ST) untuk *E. faecium* (ST203, ST17, ST55, ST29 and ST79) dan *E. faecalis* (ST4, ST6, ST87, ST108, ST274 dan ST244). Analisis

pengklusteran menunjukkan lebih daripada 70% *E. faecium* dan semua *E. faecalis* adalah spesifik kepada hos (manusia, babi dan ayam). Isolat daripada individu yang sihat mempunyai kesamaan yang rapat kepada isolasi klinikal (D, 0-14), tetapi ketidakserupaan yang tinggi diantara isolat haiwan dan manusia secara amnya (D, 20). Kebanyakan isolat manusia *E. faecium* adalah berpunca daripada strain kompleks klonal epidemik (CC) -17. Dua daripada *E. faecalis* manusia adalah berpunca dari CC2 dan CC87 epidemik. Hanya satu unggas *E. faecium* adalah bahagian daripada CC17 epidemik. Strain *E. faecium* yang dilaporkan dari Singapora mempunyai kesamaan yang rapat dengan isolat manusia (D, 7) akan tetapi variasi yang tinggi daripada isolat haiwan yang dicirikan dalam kajian ini (D, 19-29). Strain *E. faecium* ST17 yang sama telah dilaporkan di 21 negara, terutamanya dari negara Barat, Australia dan Korea. *E. faecium* ST203 telah dilaporkan dari empat negara Asian and tiga negara Eropah. Australia, Belgium, France, Brazil dan Netherlands merupakan negara yang melaporkan ST57 dan ST29. Netherland adalah satu-satunya negara di dalam database MLST yang melaporkan ST55. Strain *E. faecalis* ST4 yang sama dikenalpasti dalam kajian ini telah dilaporkan di Thailand. Keseluruhan penemuan daripada bahagian kajian ini mempunyai keertian yang tinggi di dalam konteks menolak anggapan bahawa produk ayam dari Malaysia menyebabkan kolonisasi VRE pada manusia di Singapora.

Penemuan dalam kajian adalah bererti dan menyumbangkan kepada pemahaman dalam taburan dan jenis VRE dikalangan manusia, babi dan ayam yang sihat. Ia telah menjelaskan potensi faktor risiko dikalangan manusia untuk dikolonisasi VRE, jenis kerintangen, virulens dan kehadiran gen enterotocin, ciri genetik dan

menemukan profil MLST dan hubungkait genetik dalam strain VRE manusia dan haiwan. Kajian ini telah menyediakan bukti yang boleh digunakan untuk masa hadapan dalam jika isu transmisi VRE manusia-haiwan wujud di Malaysia.

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My parents deserve special thanks, especially my mother Shewaye Kebede for her care, love and advice. My eldest sister Meseret Getachew, thank you for taking great care of me and the family, you are a blessing to me. My siblings, Selamawit, Wosen and Tewodros Getachew thank you for your unstinting love, concern and support. God bless you.

APPROVAL

I certify that a Thesis Examination committee has met on ..... to conduct the final examination of Yitbarek Getachew Molla on his thesis entitled "**Molecular Epidemiology of Vancomycin-Resistant Enterococci isolated from selected Human, Poultry and Pig populations in Peninsular Malaysia''** in accordance with Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

Members of the Examination Committee were as follows:

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Title
Name of Faculty
(Chairman)

Name of Examiner 1, PhD
Title
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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of **Doctor of Philosophy**. The members of the Supervisory Committee were as follows:

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Date: December 2010

DECLARATION

I declare that the thesis is my original work except for quotation and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

YITBAREK GETACHEW MOLLA

Date: 15th October 2010

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LIST OF ABBREVIATIONS

AFLP	Amplified Fragment Length Polymorphism
AGP	Antimicrobial Growth Promoter
ATCC	American Type Culture Collection
CC	Clonal Complex
CDC	Center for Disease Control and Prevention
CI	Confidence Interval
CLSI	Clinical and Laboratory Standards Institute
DANMAP	Danish Integrated Antimicrobial Resistance Monitoring and Research Program
DF	Degree of freedom
DLV	Double Locus Variants
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide triphosphate
DVS	Department of Veterinary Service
ECDC	European Center for Disease Prevention and Control
GRE	Glycopeptide-Resistant Enterococci
MIC	Minimum Inhibition Concentration
MLEE	Multilocus Enzyme Electrophoresis
MLST	Multilocus Sequence Typing
M-PCR	Multiplex-Polymerase chain reaction
NaCl	Sodium Chloride
OR	Odds Ratio
PCR	Polymerase Chain Reaction
PFGE	Pulsed Field Gel Electrophoresis
PYR	Pyrrolidonyl -aryl-amidase
RAPD	Random Amplified Polymorphic DNA
Rep-PCR	Repetitive PCR
RFLP	Restriction Fragment Length Polymorphism
rRNA	Ribosomal ribonucleic acid
SD	Standard Deviation
SE	Standard Error

SLV	Single Locus Variant
SNPs	Single Nucleotide Polymorphisms
ST	Sequence Type
TLV	Triple Locus Variant
VRC	Vancomycin-Resistant Cocci
VRE	Vancomycin-Resistant Enterococci
VSE	Vancomycin-susceptible Enterococci
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

The species belonging to the genus *Enterococcus* are ubiquitous in nature. They are intestinal commensal bacteria of man and animals and also found in soil, surface waters, and on plant and vegetables (Giraffa 2002; Devriese *et al.* 2006). However, *Enterococcus* species rank among the leading causes of nosocomial infections, in particular *E. faecalis* and *E. faecium* are commonly found as opportunistic pathogens causing urinary tract infection, bacteraemia and endocarditis (Richards *et al.* 2000; Tendolkar *et al.* 2003). Recently, mortality both in humans and animals (Lu *et al.* 2002; Chadfield *et al.* 2004; Chadfield *et al.* 2005; Ghanem *et al.* 2007) associated with enterococci was recorded.

The *Enterococcus* emerged as common cause of hospital-acquired infections in the mid to late 1970s. The emergence coincides with, and likely related to the increasing use of broad-spectrum cephalosporins which enterococci are naturally resistant to (Ogier and Serror 2008). With its propensity to acquire new traits, such as high-level aminoglycoside, penicillin, and glycopeptide resistance, the *Enterococcus* continues to create new therapeutic challenge and dilemmas. Moreover, enterococci are able to transfer plasmids to streptococci and staphylococci which may result in the spread of penicillin and vancomycin resistance to these organisms, and other Gram-positive species (Simjee *et al.* 2006).

Vancomycin, a high-molecular-weight glycopeptide, has been used as the drug of last resort in the treatment of Gram-positive bacterial infections, especially those caused by enterococci (Dowling *et al.* 2006). However, in the late 1980th vancomycin resistant enterococci (VRE) were reported (Uttley *et al.* 1988). Since then VRE have become the second most common nososcomial pathogens with great economic and health impacts after methicillin resistant *S. aureus* (Division of healthcare Quality Promotion 2008; ECDC 2008). The increasing occurrence of VRE, however, poses a serious problem, not only in the treatment of enterococcal infections, but also because it increased the risk of resistant determinant horizontal transfer to other vancomycin-susceptible species (Tendolkar *et al.* 2003).

Hospitals are reservoirs for antimicrobial-resistant enterococci due to the use of antibiotics (Prescott and Aarestrup 2006). However the use of antibiotics in animal husbandry such as in poultry and pigs had contributed to the emergence of VRE (Bates 1997; Jensen *et al.* 2008). Most classes of antimicrobials used in animals have human analogues, and are therefore capable of selecting for human antibiotics resistance (Boerlin *et al.* 2006). Therefore, animals or animal production environments serve as the reservoirs for those antimicrobial-resistant bacteria or resistance genes (Aarestrup *et al.* 1996; Dowling *et al.* 2006). The use of avoparcin at subtherapeutic doses for growth promotion was suggested as the main factor for emergence of non-nosocomial VRE (Bates 1997).

Molecular techniques have been employed in epidemiologic investigation of VRE. Molecular typing techniques ascertain the presence or absence of different strains of

VRE by determining clonality. The identification of strain clonality during epidemiological investigations can demonstrate the association between the source of infection and clinical isolates (Conway and Roper 2000). Recent developments of various molecular typing methods have enabled better understanding of the epidemiology of VRE. Applications of the epidemiologic principles to bacterial population give new insight into the natural history of colonization and transmission of the bacteria into human host (Foxman 2007). The molecular typing rapidly determines whether an increase in VRE isolates at a given institution is due to one or multiple strains and whether the clonal dissemination of one strain is occurring. Molecular technique could provide answers to important and frequently arising questions of infection control; the modes of transmission and the sources of infection in outbreak investigations (Koblet 1987). Such information can be invaluable to interrupt the spread of VRE infection or colonization. Rapid typing can significantly reduce costs associated with treatment, containment, and decontamination (Healy *et al.* 2005)

The incidence of VRE infection and colonization among hospitalized patients has rapidly increased worldwide in the 1990s. The new bacteria have been reported in an increasing number of countries outside Europe and the USA, such as Singapore (Chlebicki and Kurup 2008), Japan (Fujita *et al.* 1998; Watanabe *et al.* 2009), Australia (Bell *et al.* 1998), China (Zheng *et al.* 2007) and Korea (Woo-Joo *et al.* 1998; Jung *et al.* 2006).

VRE is also a concern in Malaysia hospitals. Sporadic human VRE cases have been reported (Ministry of Health Malaysia 2008; Raj *et al*. 2005; Zubaidah *et al*. 2006). Occurrence of VRE in animals were reported by several authors in Malaysia (Son *et al.* 1999; Dahlia *et al.* 2000; Radu *et al.* 2001; Ong *et al.* 2002; Ooi 2003; Shah-Majid *et al.* 2004; Hassan *et al.* 2006; Chan *et al.* 2008). Ong *et al*. (2002) showed that different species of VRE in poultry wet markets in Malaysia are present at low rate (2%). Radu *et al*. (2001) recorded the occurrence of the *vanA* and *van*C2/C3 genes in *Enterococcus* species isolated from poultry sources in wet markets. In addition, Son *et al*. (1999) molecularly characterised vancomycin-resistant *E. faecium* from imported tenderloin beef samples in Selangor. The study by Hassan *et al.* (2006) documented a prevalence of 43.8% in broiler poultry. Recently, Chan *et al*. (2008) reported that highly resistant VRE occur at low (< 2%) percentage in poultry farms. In addition, Dahila *et al*. (2000) reported five VRE isolates from ducks.

VRE in poultry have been a concern to many export poultry and duck farmers in Malaysia since the link between poultry and VRE was suggested. Currently Malaysia is under pressure to produce and export VRE-free chickens and thus farmers incur additional costs from VRE screening procedures and disposal of positives products (Kamaruddin Mat Isa, Department of Veterinary Services (DVS), Putrajaya. pers. comm. July 06, 2007). In 1999, more than 90% of export farms were suspended from exporting poultry and poultry products to Singapore because of VRE and *Salmonella* (Zaini *et al.* 2000; Zaini *et al.* 2000a). VRE were first isolated in Singapore in 1994 and until 2004 were only sporadically encountered in public hospitals. A small VRE outbreak resulting from an imported case occurred once

(Ong 2005). However, in 2006 there was an outbreak of VR*E. faecium* in tertiary hospitals (Chlebicki *et al.* 2006). Currently, VRE has become established in Singapore healthcare institutions (Chlebicki and Kurup 2008). Nevertheless, Singapore maintained the strict VRE screening regulation on importing chickens, which served to create pressure on the Malaysian authorities and poultry farmers.

The scope of animal and human VRE studies in Malaysia is limited and studies have not attempted to address the epidemiological aspect of VRE in general. Roles of pigs in the epidemiology of VRE have never been studied. Moreover, molecular similarity and genetic diversity of VRE of pig, poultry and human isolates have not been described. In addition, no study has attempted to elucidate the genetic relatedness of VRE in animals to that of humans. The present study addressed some of these issues and narrowed the knowledge gap. The main objective of this study was to generate epidemiological information on VRE in human and animal population and establish the relationship between VRE found in animals to that in man. The information generated will enhance the understanding of the VRE in Malaysia.

The hypotheses of this study were;

1. Vancomycin-resistant enterococci are highly prevalent in human, poultry and pig population of Malaysia.
2. Vancomycin susceptibility pattern and resistance factors differ among VRE species.
3. Enterococci species isolated from community, poultry and pig are virulent.
4. The VRE species are genetically diverse.

5. Human, poultry and pig enterococci species originated from different geographical locations are genetically unrelated.

The specific objectives of the study were to:

1. detect and describe VRE species in selected human, poultry and pig population.
2. determine the vancomycin resistance genes and the phenotypic characteristics among the isolates,
3. determine presence of virulence and enterocin genes in VRE isolates of human, chicken and pigs.
4. elucidate genetic diversity of VRE isolates using random amplified polymorphic DNA analysis.
5. elucidate the genetic relatedness of *E. faecalis* and *E. faecium* among human and animal isolates.
6. determine the epidemiological risk factors for acquiring VRE in human and farms.

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