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Vancomycin-Resistant *Enterococcus:* Issues in Human Health, Animal Health, Resistant Mechanisms and the Malaysian Paradox

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Abstract | *Enterococcus* is a major constituent of the intestinal flora and environment. They are a hardy organism and can survive harsh prevailing environmental factors and hosts. Over time, the constant and heavy usage of antibiotics like vancomycin in treating infection caused by them has resulted in their development of resistance and virulent characteristics. VRE infections in animals are uncommon, and even so in pets. The reverse is, however, the case with human VRE infection. VRE are of great importance in public health, animal and medical health. The adaptability and complexity of the VRE genes have resulted in the appearance of resistant species in a wide range of environment and hosts which will invariably allow the host and environment to act as a reservoir. This article, therefore, discusses and reviews VRE, its phenotypic and genotypic characteristics, antimicrobial resistance, virulence factors and zoonotic potential. Finally, it sheds light on the situation of VRE in Malaysia. This would be the first review to look at VRE in Malaysia. Continuous surveillance is required as these VRE could evolve into a multidrug-resistant strain.

Keywords | Vancomycin-resistant enterococcus, Vancomycin-resistant genes, public health, Antimicrobial resistance, Malaysian VRE, Animal health

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INTRODUCTION

Enterococcus occur naturally in the surroundings and are an important constituents of the gut microbes in humans and animals (van Schaik and Willems, 2010; Boehm and Sassoubre, 2014). *Enterococcus* species detected till date are over 50 (Bonacina et al., 2016; Guzman et al., 2016). In human guts, *Enterococcus faecalis* occur mostly followed by *E. faecium* while in livestock, *E. faecium* is the major species followed by *E. faecalis*, *E. cecorum* and sometimes *E. hirae* (Klein, 2003; Guzman et al., 2016). Urinary tract infections, inflammation of the endocardium, and blood infection are linked to Enterococci and these conditions are particularly worse in immunocompromised individuals (Lebreton et al., 2014; Neelakanta et al., 2015). Nosocomial and drug-resistant infections are largely caused by *E. faecium* and *E. faecalis* and these pathogens are largely accountable for human vancomycin-resistant enterococci (VRE) infections (van Schaik and Willems, 2010; Lebreton et al., 2013). The spotlight was beamed on Enterococci as a significant hospital-acquired microbe in regard to their innate resistance to various antibiotics, the rate at which they become highly infectious and the factors responsible for their multidrug resistance (Balli et al., 2014; Bourgeois-Nicolaos et al., 2014; Kristich et al., 2014). Epidemiological reports have continued to implicate VRE in regards to its effect on health, the economy and continuous infection in humans (Byappanahalli et al., 2012; Shaghaghian et al., 2012) following to their earliest documentation in the 80s (Leclercq et al., 1988; Uttley et al., 1988). Though VRE is hardly seen in pets, they rarely cause infection in animals (Willems et al., 2011).

Studies have suggested that *Enterococcus* resistant to vancomycin act as a reservoir and source of resistant genes. This article, therefore, discusses and reviews VRE, its phenotypic and genotypic characteristics, antimicrobial resistance, virulence factors and zoonotic potential. Finally, it sheds

light on the situation of VRE in Malaysia. This would be the first review to look at VRE in Malaysia.

Mechanism of Vancomycin Resistance in Enterococci

Vancomycin-resistant occur due to changes in the manner of the synthesis of peptidoglycan in enterococci leading to the replacement of D-Alanine-D-Alanine with D-Alanine-D-Lactate or D- Alanine-D-Serine (Arthur and Quintiliani, 2001; Courvalin, 2006) invariably resulting in an array of manifestation of glycopeptide resistance. The chances of drugs with glycopeptide origin to bind are far reduced when a change occurs in the D-Ala-D-Lac and D-Ala-D-Ser as compared to D-Ala-D-Ala (Sujatha and Praharaj, 2012). These changes will result in different manifestation in the forms of resistance of glycopeptide. These changes are brought about by the presence of various genetic elements carried on motile genetic components and encoded area of the chromosome of various species of *Enterococcus* (Kristich et al., 2014). Differences in the forms of Vancomycin-resistant genes and phenotypic characteristics are as a result of this phenomenon which allows us to understand the various levels at which glycopeptides resistance occur which could either be low level or high level (Kramer et al., 2018).

Vancomycin-Resistant Genes

For Enterococci, the vancomycin-resistant genes are referred to as *vanA*, *vanB*, van*C, vanD, vanE, vanG, vanL*, *vanM*, and *vanN*. The *vanF* resistant gene was identified in *Paenibacillus popilliae* (Patel et al., 2000). All these genes are peculiar in the way they are resistant to antimicrobials especially the glycopeptide groups, how they move from one gene to the other and how they can stimulate the desired effect (Xu et al., 2010; Lebreton et al., 2011).

The main culprit for a lot of VRE infections and colonization in humans is *vanA* and *vanB* (Werner et al., 2008) isolated from the environment and infected animals. The infection and colonization occurs as a result of an alteration in the synthesis of peptidoglycan caused by mobile genetic elements leading to the proliferation of *Enterococcus*. These genes are spread all over the world and are very complex (Ben Braïek and Smaoui, 2019).

Transposons (Tn) are known as jumping genes and Tn*1546* is peculiar to *vanA* (Hegstad et al., 2010). Also peculiar to *vanA* are *orf1* and *orf2* responsible for transposition and a gene (*vanZ*) for resistance to teicoplanin (Werner et al., 2011). There are a duo of promoters that exist and are solely accountable for the transcription of the nucleotide sequence of *vanA*. For *vanR* and *vanS* which are the genes for response regulator and sensor kinase respectively, the same promoter is responsible for their transcription while the other promoter takes care of other genes (Kristich et al., 2014).

A variant of *vanB* tagged as *vanB1-3* has been described as having a high vancomycin resistance to Enterococci while the most prevalent of this variant occurring globally is the *vanB-2* (Santona et al., 2018). The genotypic makeup of a normal *vanB* and *vanA* are alike.

The acquiring and/or substitution of Jumping genes like Tn*1547*, Tn*1549*, and Tn*5382* are responsible for the transposition of *vanB* resistant genes (López et al., 2009) and one of these genes Tn*1549*, which is a chromosomal one and rarely seen on plasmids (Werner et al., 2012), is known to occur among other gram-positive bacteria and considered the *vanB* type enterococci that occur most (Tsvetkova et al., 2010). Like the promoters of *vanA*, *vanB* promoters are also two and transcribe seven nucleotide sequences. Their differences lie in the fact that *vanB* possesses *vanRB* and *vanSB* a dual component signaling system and unlike *vanA* encodes *vanH*, *vanW, vanX, vanY* and D-Ala-D-Ala lig (Kristich et al., 2014).

Unlike the *vanA* and *vanB*, *vanC* is considered as having a lower virulence with different genetic makeup (Reynolds and Courvalin, 2005; Naser et al., 2006). In *E. flavescens, E. gallinarum* and *E. casseliflavus*, the *vanC-1-3* variant forms are occasionally seen as their distinguishing features and these variant forms are very much present within these species (Cetinkaya et al., 2000). The similar *vanC-2* and *vanC-1* are constituents in *E. casseliflavus* and *E. gallinarum* respectively (Courvalin, 2006) while the *vanC-4* has 93–95% nucleotide similarity with *vanC-2* and *vanC-3* (Naser et al., 2006).

The *vanD* is rarely reported but found in the *vanC E. gallinarum* (Boyd et al., 2006). It is purely chromosomal and likened to *vanA* and *vanB* (Boyd et al., 2004). The *vanD* typify an array of mutations resulting in extensive different variant forms and phenotypes that are resistant (Depardieu et al., 2009).

Though detected among small isolates of *E. faecalis* in North America and Australia (Abadía-Patiño et al., 2004), the *vanE* is similar to *vanC1* and found in *E. gallinarum*. The transferability of *vanE* is still sketchy and acquiring the *vanE* would mean that, a mutation would have occurred within the integrase genes of *vanE E. faecalis* (Ben Braïek and Smaoui, 2019)

A pair of variant forms of *vanG* from *E. faecalis* were discovered and these clusters were subsequently reported (Boyd et al., 2006) as this variant forms can be transferred through a plasmid unlike *vanE, vanC vanL* and *vanN*.

It is proposed that *vanL* gene is chromosomal (Boyd et al., 2008) as they do not possess the power to transfer or conjugate. They, however, possess about 50% nucleotide similarity to the ligases of *vanC* and *vanE*. On the other hand, *vanM* is very identical to *vanA, -B* and *–D* and had been shown to possess the transfer and conjugation ability in an *in-vitro* study in *E. faecium* (Xu et al., 2010).

The *vanN* are unequalled as solely in *E. faecium* can they be transferred through mobile genetic element. They are also identical to *vanG* and had only been lately discovered and reported from *E. faecium* making them the newest *E. faecium* gene cluster (Lebreton et al., 2011).

Of all the vancomycin-resistant genes of enterococci, *vanA, vanB*, and *vanC* are the most resistant to vancomycin and teicoplanin with *vanA* having more virulence (Ahmed and Baptiste, 2018). The ability of these VRE to cause infection is dependent upon their ability to elicit resistance and virulence characteristics and this is irrespective of the genes they tend to express (Szakacs et al., 2014). Often regarded as the species of enterococci with motility, *E. gallinarum/casseliflavus* mainly harbours the *vanC* which is known to cause the least clinical infections in humans with the least resistance (Cetinkaya et al., 2000; Zirakzadeh and Patel, 2006). A lot of human VRE infection especially those caused by *vanC*-type *E. gallinarum/casseliflavus* have shown to be nosocomial with disastrous tendencies as they do not react to treatment (Koganemaru & Hitomi, 2008). This is because VRE caused by *vanC* is on the rise and unlike the *vanA* and *vanB,* the chances of death caused by *vanC* and its worldwide occurrence is low (Tan et al., 2010).

Over time, *E. gallinarum vanC* has been isolated from hospital, livestock as well as humans possessing *vanC-1* and *vanA* gene clusters as *E. gallinarum/casseliflavus* has been reported to harbor *vanA*, *-B* and *-D* (Haenni et al., 2009; Neves et al., 2009) with a high degree of resistance to linezolid and vancomycin (Yasliani et al., 2009; Praharaj et al., 2013). This is so as *E. casseliflavus/gallinarum* is known to possess resistant inducible genes of *vanC* with *vanC* being elicited constitutively (Panesso et al., 2005).

Virulence Genes/Factors

The role virulence factors play in the pathogenicity of Enterococcus cannot be overemphasized. A lot of study has been carried on these factors over the years. The commonest of the virulence factors are adhesion to collagen (*ace*, *acm*), extracellular surface protein (*esp*), aggregation substances (*agg*, *asa1*), adhesion like endocarditis antigens (*efaAfs* and *efaAfm*), cytolysin (*cyl*), gelatinase (*gelE*) and hyaluronidase *(hyl)* (Ben Braïek and Smaoui, 2019).

The aggregation substances (*agg* and *asa1*) produces Enterococcus surface protein which is of great help during reproduction in bacteria by forming aggregate (Chajęcka-Wierzchowska et al., 2017). It not only links epithelial cells to virulent characteristics and resistant determinants via plasmid exchange and colonization but also attaches to the network of extracellular proteins (Wagner et al., 2018). Different studies have provided evidence that *E. faecalis* harbors the *agg* gene most (Guzman Prieto et al., 2016; Lins et al., 2019; Farman et al., 2019).

Cytolysin or β-haemolysin is composed mostly of a toxin made up of peptide which it uses to digest target cells in the cytoplasm by the formation of pores (Price et al., 2019). Cytolysin produced during enterococcal infection causes fivefold mortality than an enterococcal infection not producing cytolysin (Delaplain et al., 2019).

Gelatinase hydrolyses collagen, bioactive peptides and gelatin. It as an extracellular Zn-metallo-endopeptidase which causes substantial damage to host tissue by splitting fibrin resulting in the dissemination of *Enterococcus* specifically *E. faecalis* (Farman et al., 2019) and involved in biofilm formation (Aladarose et al., 2019).

Not only does the extracellular surface protein encourages colonization, found in a very protected region of the chromosome and occur more in *E. faecium*, it is also engaged in eukaryotic cells attachment and evasion of the immune response of the host (Lee et al., 2019; Song et al., 2019).

The adhesion to collagen genes *ace* is found in *E. faecalis* while the *acm* in found in *E. faecium*. They both have the tendency to attach to types I and IV collagen with *acm* possessing an extra ability to attach to laminin and belonging to the subfamily of bacterial adhesions surface called Microbial Surface Components Recognizing Adhesive Matrix Molecules (MSCRAMM) (Fiore et al., 2019).

The *efaA* are associated with endocarditis with *efaAfs* commonly found in *E. faecalis* and *efaAfm* found in *E. faecium* (Fiore et al., 2019).

The *sag* and *scm* genes are rarely found in enterococci but when they are found, they are commonly described in *E. faecium* (Kim et al., 2019). In addition, the *ebp* and *bee* gene known to promote biofilm formation are mostly described in *E. faecalis* (Estela Gaitán, 2019).

The *hyl* virulent factor encodes hyaluronidase which hydrolyze hyaluronic acid with a possible role in translocation and associated with antibiotic resistance genes and pilin genes on the plasmid (Tatsing et al., 2019).

Epidemiology of VRE

E. faecium/faecalis are the main sources of multidrug and glycopeptide resistance as they are the commonest of the enterococci species in which their resistant genes are commonly described (Gilmore et al., 2013; Kramer et al., 2018). Emaneini et al. (2016), in their review, observed a prevalence of 8–13% of human VRE resulting from *E. faecalis* in Iran and some countries in Europe. Similarly, in Southeast Asia, Europe, North America and Latin America, *vanA* and *vanB* are isolated mostly from *E. faecium* (Mendes et al., 2016; Jahansepas et al., 2018).

(Monteserin and Larson, 2016) and Kristich et al. (2014) in their study observed that *vanA* is occasionally described in *E. durans/hira*, and *E. gallinarum*, but most often described from *E. faecium/faecalis* in humans as well as livestock globally. This was further seen in research conducted by Jahansepas et al. (2018) where the prevalence of VRE was 19% and the *vanA* resistant genes were carried mostly by *E. faecium* in Iran. The occurrence of *vanB* is however sparse globally but has been reported more in countries like Germany, Poland and Sweden (Bender et al., 2016; Sadowy, 2018). These resistant genes differential occurrence is linked to the differences in the use of antibiotics by these different countries. This is seen in Australia where the resistant gene has never been isolated from animal or often from the feces of human and it is the most common human *E. faecium* (R. S. Lee et al., 2018).

It is of importance to note that VRE genes only from the *Enterococcus* motile species do not necessarily predict resistance to glycopeptides (Beukers et al., 2017). To buttress this point, in a study by Özsoy et al. (2017), they found *vanB* present in an anaerobic bacteria from the feces of humans suggesting *vanB* in this bacteria as a possible origin of enterococcus resistance. In another study by Kateete et al. (2019), they observe the presence of *vanB* in a *vanC* species of *enterococcus*. Similarly, Sun et al. (2014) and Flipse et al. (2019) reported that in humans, *vanC* was identified in *E. faecium/faecalis*.

Restriction endonuclease analysis, whole genome sequencing, multi-locus sequence typing, amplified fragment length polymorphism, pulsed-field gel electrophoresis and CRISPR are important in the typing of enterococci as well as determining clonality and phylogenetic relationships. These tools have successfully categorized *E. faecium* into clades A and B (Lebreton et al., 2013; Hullahalli et al., 2015). Clade A usually involve hospital infection related to humans and subdivided into two; that which is a species in a hospital during an epidemic referred to as A1 and that which occurs in livestock and humans in an unpredictable manner and clade B which are mostly found within the community and are not hospital-based (Mikalsen et al., 2015; Buultjens et al., 2017).

The lineage A1 is also known as CC17 (Clonal Complex 17) of *E. faecium* which is a universal clone suited for hospital and responsible for gut colonization. This A1 clade is known to possess more polypeptide resistance and virulence factors like the hyaluronidase gene *(hyl)*, the enterococcal surface protein (*esp*) and the IS16 insertion-element which than the non-A1 clade (Guzman Prieto et al., 2016). The A1 lineage also possesses sequence types (ST) that are characterized worldwide such as the ST17 and its lineage like the ST174, ST78, ST16, ST64 and ST63 (Mikalsen et al., 2015).

In contrast, the clones of *E. faecalis* like CC87, CC2 as well as CC40 are not distributed worldwide, more constricted and are community and hospital-based. The appearance of CC78 and particularly ST117 within this complex which is isolated more from European hospitals (Tedim et al., 2017) is due to the worldwide increase in *E. faecium* multidrug-resistant (MDR) isolates.

A study by Buultjens et al. (2017) observed upward surge in VanB VRE and colonization in the blood as a result of the substitution of the abundant ST17 by ST203 which had led to a modification in the occurrence and distribution of VRE in Australia. This modification is also as a result of the discovery of Tn1549 and forty other genes by genomic from ST203.

In the African context, a study by Dziri et al. (2016) discovered a new clone ST90 not related to the CC17 and reported a prevalence of 5% VanA - *E. faecium* from nosocomial sources in Tunisia lacking the *hyl* gene but possessing all other virulent genes. In another study by Elhani et al. (2014) in Tunisia, *vanA E. faecium* were found to harbor ST80 and ST18 of CC17 origin and also only IS16 element were present.

Enterococcus faecalis sequence types ST87, ST40, ST28, ST21, ST16, ST9 and ST6 are hospital-related and outbreak sequence types that are known to be dominant (Quiñones et al., 2018) and are present in the community, food and livestock (Ahmed and Baptiste, 2018) unlike *E. faecium* where the hospital-related sequence types are not found in the community (Leong et al., 2018).

VRE in Animals and Public Health Concerns

In Europe and other countries in the nineties, a glycopeptide compound avoparcin usage as supplement to improve growth was responsible for the spread of VRE in livestock production (Li et al., 2019). The spread of VRE is not however only tied to their occurrence in the nosocomial settings but in the use as avoparcin as the resistant genes of VRE particularly *vanA* was isolated in meat, community sewage as well as animal feces (Young et al., 2016; Li et al., 2019). However, avoparcin usage was never reported in

North America yet, in 2008, VRE was detected in animal (Kristich et al., 2014).

VRE has been incriminated as the bulk of nosocomial infection as a result of the vancomycin therapy in North America (Adams et al., 2016) while in Australia, the broad use of avoparcin and vancomycin in the livestock industry and humans respectively, is responsible for the occurrence of VRE (Guzman Prieto et al., 2016).

There was an upward surge in VRE cases in Europe ever since 1999 (Bender et al., 2016; Kampmeier et al., 2018) against the backdrop in the low occurrence of VRE in humans that are asymptomatic (Ahmed and Baptiste, 2018). In Europe, the prohibition of avoparcin took effect in 1997. Despite the prohibition, the damages that have already been caused by previous uses of avoparcin cannot be overlooked (Wurster et al., 2016; García-Solache and Rice, 2019). Several years following the prohibition, isolates of VRE were still detected in some farms in Europe. For instance, in the study of Kruse et al. (1999) in Norway, isolates of VRE were detected easily in poultry that used avoparcin before the prohibition while none were detected from swine and poultry after the prohibition. In another study by Borgen et al. (2000) and Borgen et al. (2001), VRE were detected in poultry farms (99% prior and 11% after) and farmers (18% prior and 1% after) that used avoparcin prior and before the prohibition respectively. Just recently, a study by Bortolaia et al. (2015) reported a 47% prevalence of VRE *vanA* resistant genes in poultry in Denmark. Even though the occurrence of VRE decreased significantly in Italy, VRE was still reported in livestock, meat either poultry or pork and human feces (Del Grosso et al., 2015). Similar observations were made in Germany (Remschmidt et al., 2018), Taiwan (Kuo et al., 2018), Malaysia (Daniel et al., 2017), China (Sun et al., 2019) and Switzerland (Buetti et al., 2019). VRE has been isolated from cattle (Tatsing and Ateba, 2019) and bovine feces (Beukers et al., 2017).

Aside from farm animals, VRE prevalence has been reported in dogs (Ossiprandi and Zerbini, 2015; Pasotto et al., 2016) and cats in a study by Bağcigil et al. (2016), this was however after the prohibition of avoparcin. In Eastern European countries like Hungary, Poland and Italy, VRE *vanA* resistant gene has been detected in horses (Pomba et al., 2016).

Occurrences of VRE following the prohibition of avoparcin, has triggered uncertainty about its transmission route. This is evident as avoparcin had never been reported to be used in animal in the United States but the hospital-acquired VRE in the United States of America is higher than in Europe (Adams et al., 2016). Over the years, the quest to establish the transmission route of VRE is almost impossible. However several studies had tried to establish correlation. For instance, Hermanovská et al. (2016) reported a similarity in the VRE isolated from animals and humans but stated that this similarity is as a result of contact between human and animal and not by eating the product. Another study by Abdelhady and Mishra, (2019) and Yamanaka et al. (2019) reported a short-lived infection when humans were infected with VRE isolated from animals in an experimental murine model. Molecular studies have also have reported a strong relationship between animals and humans VRE (Silva et al., 2018; Aslantaş, 2019; ISMB and Talebi, 2019). The presence of plants and wild animals acting as a source of infection indicates that the relationship that exists between the VRE of humans and animals does not show the causal relationship (García-Solache and Rice, 2019).

The Malaysian Paradox

The first case of VRE in Malaysia go as far back as the mid-nineties and since then, VRE has continued to be isolated from different sources. Riley et al. (1996) in their study, reported the pioneer case of VRE from a bone marrow transplant patient. Ten years later, Zubaidah et al. (2006) reported the pioneer case of VRE acquired within the hospital. This was the first case of confirmed VRE isolation from a patient with renal complications. The pioneer case of community-acquired VRE specifically *E. faecium* was reported by Raja et al. (2005) from a patient with a buccal abscess. In a similar twist, Son et al. (1999), reported the pioneer case of VRE isolated and characterized from animals in Malaysia. Two years later, Toosa et al. (2001) reported the isolation of VRE *E. faecalis* from poultry. Isolation of VRE has been reported in Malaysia in farm animals and their products (Toosa et al., 2001; Ong et al., 2002; Fifadara et al., 2003; Hassan et al., 2006; Shah-Majid et al., 2007; Chan et al., 2008; Getachew et al., 2010; Getachew et al. 2012; Getachew et al. 2013; Daniel et al., 2017). VRE has also been isolated from ducks and geese (Ong et al., 2002; Shah-Majid et al., 2004), pigs (Getachew et al., 2010; Getachew et al. 2013; Tan et al., 2018) and environment (Chan et al., 2008; Dada et al., 2013; Daniel et al., 2017). VRE has also been detected in poultry farmers (Getachew et al., 2012) and pig farmers (Tan et al., 2018). As if that is not enough, VRE has also been isolated within the hospital environment (Raja et al., 2005; Zubaidah et al., 2006; Ibrahim et al., 2011; Ibrahim et al., 2012; Weng et al., 2013; Mohamed et al., 2015; Lim et al., 2017; Daniel et al., 2017).

The use of avoparcin and vancomycin in Malaysia has been prohibited to mitigate the spread or prevalence of VRE by the National Pharmaceuticals Regulatory Agency (NPRA) and the Department of Veterinary Services (DVS). Monitoring of veterinary drug residues including antibiotics in animal feed has been implemented by DVS since the year

2013 regarding EEC Directive 1990. This will invariably involve the monitoring of two antibiotic groups; group A which consist of the banned substance like Avoparcin, Chloramphenicol, vancomycin and group B which consist of drugs with MRLs like tetracycline (NPRA, 2014).

What has brought VRE to the limelight in Malaysia is not only because of its crucial public health concerns but of its impact on the livestock sector of the economy (Fifadara et al., 2003; Hassan et al., 2006; Getachew et al., 2010; Getachew et al., 2012).

Concerning VRE within farm animals and their products in Malaysia, Fifadara et al. (2003) reported the presence of *vanA* genes isolated from all *E. faecalis* 22 vancomycin-resistant isolates from frozen beef. Another study by Ong et al. (2002) reported the isolation of *vanC-3* gene *E. flavescens*. This study reported a low prevalence of VRE (2.0%) from feces of duck, chicken and geese in a Kuala Lumpur wet market. Similarly, Chan et al. (2008) also reported a low prevalence of VRE 1.3% from poultry farms in Malaysia and three VanA each belonging to *E. faecium/faecalis* and *E. gallinarum* were identified. Interestingly, this study reported a 19.5% prevalence of enterococci resistant to the bifunctional aminoglycoside. VanB was for the very first time isolated from poultry in Malaysia by Yew et al. (2006). This study, however, reported a very high prevalence of VRE 44% (1658/3710).

Among farmers working in either piggery or poultry, the prevalence of VRE, the distribution of the resistant genes and virulence genes has been fluctuating. For instance, Getachew et al. (2012) in their study on poultry farm workers reported a VRE prevalence of 9.4% most of which were isolated from *E. faecalis/faecium* and *E. gallinarum*. In most of this isolates, VanA was isolated while VanB was only isolated from a single isolate and the *esp* and *gelE* genes were isolated from *E. faecalis* and *E. faecium* respectively. In another study, this time in pig farmers, Tan et al. (2018) reported that the farmers mostly carries *E. faecium/faecalis*. Unlike the study of Getachew et al. (2012), where resistant genes were isolated, the isolates in this study were all susceptible to vancomycin. They, however, harbour virulent genes like *efa, asaI, gelE*, *esp, cyl* and *ace* with *efa* and *asaI* occurring most in *E. faecalis/faecium*.

A lot of studies in Malaysia has reported the occurrence of VRE in nosocomial setting. As earlier stated, Zubaidah et al. (2006) reported the pioneer case of VRE acquired within the hospital. This would become the pioneer case of confirmed VRE isolation from a patient with renal complications. This study isolated *E. faecium* seemingly possessing *vanA*. Ten years earlier, Riley et al. (1996) in their study, reported the pioneer case of VRE from a bone marrow transplant patient. Another study by Ibrahim et al. (2011) reported 1% VRE cases isolated from *E. faecium, E. avium* and *E. faecalis*. These VRE all possess the *vanA* gene and four and two of the VRE were isolated from the blood and urine of the patients respectively. Contrary to all the studies cited above within the hospital environment, Weng et al. (2013) in their study did not report any VRE, they however isolated *E. faecalis/faecium* from body fluids, blood, urine and pus. In another study in a hospital, though retrospective in nature, Mohamed et al. (2015) reported a VRE prevalence of 2.88% isolated from *E. faecium*. Besides, these VRE isolates possess the *vanA* gene. In another hospital in Malaysia, four VRE from *E. faecium* was isolated from calamitous cases within the hospital (Lim et al., 2017). Daniel et al. (2017) in their study reported no VRE isolates from *E. faecalis*. They, however, reported the presence of MDR *E. faecalis*. In their study, *E. faecalis* isolates were also shown to possess the virulent gene *esp* the most followed by *asaI*.

VRE has been isolated from the environment, thus implicating it in the stabilization of VRE in the ecosystem. It is therefore thought to be a reservoir for VRE. Chan et al. (2008) in their study in Kelantan, Malaysia reported a VRE prevalence of 4% (1/25) from poultry drinking water. These VRE was isolated from *E. faecalis* and possess the *vanA* gene. Beeches are also not left out as Dada et al. (2013) reported the isolation of a large number of *E. faecalis/faecium* from two beaches in Malaysia. The resistance of these isolates to vancomycin was further reported in another study by Dada et al. (2013). In this study, while they reported a VRE prevalence of 5.8% in *Enterococcus* species other than *E. faecium* and *E. faecalis*, 4.78% prevalence was reported for *E. faecium* with the least prevalence seen in *E. faecalis*. More recently, Daniel et al. (2017) in their study to determine the molecular evolution of *E. faecalis* from different sources reported a high prevalence of (83%) of multi-resistant *E. faecalis* including vancomycin from river water closely followed by wastewater (60%). Further, they reported the possession of *vanA* gene by an isolate from river water that did not elicit and show resistance to vancomycin.

The most commonly reported virulence genes for enterococcus are serine protease (*sprE*), gelatinase (*gelE*), accessory colonization factor (*ace*), hyaluronidase gene (*hyl*), pathogenicity islands (*PAI*), enterococcal surface protein (*esp*), aggregation substance (*asaI*) and cytolysin (*cylA*) (Jett et al., 1994; Vidana et al., 2016). Several studies in Malaysia had reported the presence of these genes. For instance, Getachew et al. (2012) in their study reported 58.3% *esp* from *E. faecium* and 78% *gelE* from *E. faecalis* among poultry farmers. Dada et al. (2013) in their study on two beaches in Malaysia reported the presence of virulent characteristics like caseinase production, hemolysis of rabbit blood, slime production, hemolytic action on horse blood and gelati-

nase. Of these virulent characteristics, haemolysis of rabbit blood (3.65%) had the lowest prevalence while the highest prevalence (15.01%) was recorded in caseinase production. Further, Al-Talib et al. (2015) reported the presence of *esp, sprE, gelE, ace and PAI* in their study in a Malaysian hospital. From their study, all the five virulent genes were harboured by 22.4% *E. faecalis* isolates in comparison to the 12.8% *E. faecium* isolates. The *ace* virulent gene from this study is the most prevalent (88%), this is closely followed by *gelE* (74.8%), *sprE* (74.3%), *esp* (45.5%), and *PAI* (30.5%). More recently, in their study on the prevalence of Multidrug-resistant *Enterococcus* species in pigs, pig farmers and environment, Tan et al. (2018) reported the presence of *efa, asaI, gelE, esp, cyl* and *ace* genes where biofilm was formed by 52% of the isolates. *E. faecalis* possess most of the *efa* virulent gene (90%) followed by *asaI* (43%) possessed by *E. faecium.*

Little is known about the genetic relatedness and distribution of clones of enterococcus in Malaysia. Few studies have however tried to bridge this gap. Getachew et al. (2013) in their study using MLST reported ST203, ST17, ST55, ST79, ST29 from *E. faecium* and ST4, ST6, ST87, ST108, ST274, ST244 from *E. faecalis*. The most common clonal complex from this study was CC17 though CC2 and CC87 were also reported from humans. This study, however, suggested the possible transmission route to be from humans to poultry as ST203 was identified in single poultry isolate similar to the human isolates. In addition to MLST, Lim et al. (2017) compared the genome of two calamitous cases of VRE *E. faecium* in a hospital where ST80 and ST203 were described and reported to belong to CC17 with ST80 reported first in this study in Malaysia. Further, PFGE was used to determine the genetic relatedness of *E. faecalis* isolates in a study by Daniel et al. (2017), where they reported sixty-three pulsotypes from three sources (environmental, clinical and animal) with each source having different pulsotypes. More recently, Tan et al. (2018) in their study used the REP-PCR and PFGE. *E. faecalis* was reported to possess 126 pulsotypes in three clusters (C15, 16 and 22) while *E. faecium* possess 35 pulsotypes in a single cluster (C2) using the PFGE. Similarly, the REP-PCR profile produced 13 special patterns of *E. faecalis* merged into 24 clusters while 11 special patterns merged into 11 clusters were produced for *E. faecium*. All the isolates in this study were reported from pigs and humans and were all unique to a specific region.

CONCLUSIONS AND FUTURE THOUGHT

Enterococcus is a major constituents of the intestinal flora and environment. They are a hardy organism and can survive harsh prevailing environmental factors and hosts. Over time, the constant and heavy usage of antibiotics like vancomycin in treating infection caused by them has resulted in their development of resistance and virulent characteristics.

In animals, VRE infections are uncommon and even so in pets as illustrated only by few studies. The reverse is, however, the case with human VRE infection. The role of animals as reservoirs cannot be overemphasized, as such the surveillance of VRE should be part of every country antimicrobial surveillance program. Since it is obvious that the use of glycopeptide in animals is without any serious peril, humans should be the major user of glycopeptide.

In Malaysia, the *vanA* gene seems to be the most prevalent, however, the role of other resistant genes needs to be well defined. Three most reported virulent factors in Malaysia are the *esp, ace* and the *gelE* even though there are only a few studies that had reported the presence of virulent genes among enterococcus isolates. Reports on other virulent characteristics are sparse and further studies is needed to elucidate their role in the epidemiology of VRE in Malaysia. *E. faecium/faecalis* and *E. gallinarum* are the three most widely reported *Enterococcus* in Malaysia. Other species of *Enterococcus* in Malaysia have received little or no attention. Again, to understand the epidemiological status of VRE in Malaysia, extensive studies need to be done and must cut across the lesser-known species of enterococcus. As reported in other studies, the nosocomial CC17 is the predominant clonal complex in Malaysian hospital studies. To the best of our knowledge, no study in Malaysia had addressed the role of VRE in companion animals. The chance that this group of animals could act as reservoirs cannot be overemphasized and studies are therefore required to define their roles in the epidemiology of VRE. Summarily, a Meta analytical study of VRE in Malaysia would paint a true picture of the epidemiology of VRE in Malaysia.

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CONFLICT OF INTEREST

None declared.

AUTHORS CONTRIBUTION

Wada Y conceived and wrote the review, Harun AB, Yean CY, and Rahman ZA provided critical feedback and helped shaped the review and manuscript.

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