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Antimicrobial Resistant CC17 Enterococcus faecium: The Past, the Present and the Future

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Highlights

- CC17 Enterococcus faecium has emerged as a pandemic clone
- Vancomycin resistance is a critical issue
- Dissemination of CC17 E. faecium in the human-animal-environment interface has been documented
- Current epidemiology of vancomycin resistance E. faecium
- Therapeutic alternatives to vancomycin resistant CC17 E. faecium

Abstract

Enterococcus faecium are robust opportunistic pathogens that are most commonly found as commensals of the human and animal gut but can also

survive in the environment. Since the introduction and use of antimicrobials, E. faecium have been found to rapidly acquire resistance genes which when expressed can effectively circumvent the effects of most antimicrobials. The rapid acquisition of multiple antimicrobial resistances has led to the adaptation of specific E. faecium clones in the hospital environment collectively known as clonal complex (CC)17. CC17 E. faecium are responsible for a significant portion of hospital-associated infections, which can cause severe morbidity and mortality. Here, we review the history of E. faecium from commensal to a significant hospital-associated pathogen, its robust phenotypic characteristics, commonly used laboratory typing schemes and antimicrobial resistances with a focus on vancomycin and its associated mechanism of resistance. Finally, we review the global epidemiology of vancomycin resistant E. faecium and potential solutions to problems faced in public health.

Keywords

Enterococcus faecium, Enterococcus, Vancomycin resistant enterococci, Epidemiology, Antimicrobial resistance, Clonal Complex 17.

1. Introduction

Over the last three decades, Enterococcus faecium is a species of bacterium that has ranged from being considered a commensal which could be used as used as probiotic to an ESKAPE pathogen (a list of the leading causes of nosocomial bacterial infections) (1). Although ubiquitous in the environment, E. faecium is most abundant as a commensal of the human and animal gut microbiome.

However, in an immunocompromised host, E. faecium can behave as an opportunistic pathogen causing severe morbidity and mortality. Furthermore, E. faecium can resist the effect of many antimicrobials through the rapid acquisition of antimicrobial resistance genes which effectively circumvents modern day medicine. In this review, we have focused on hospital associated Clonal Complex (CC) 17 E. faecium and the impact it has on public health.

2. The Past

Thiercelin first described a bacterium termed "Entérocoque" (French) in 1899 as a diplococcus bacteria inhabiting the gut (2). The English translation, Enterococcus, was later adopted to broadly describe the genus consisting of Gram-positive bacteria that are homofermentative lactobacillales of the firmicutes phylum. The Enterococcus genus is associated with strong survival traits that can overcome broad temperature fluctuations (10 - 45°C), wide pH gradients (pH 4.5 - 10.0), high NaCl concentrations (6.5%) (3), survive heat exposure of up to 80°C for 33 minutes, and have variable tolerance to sub-clinical concentrations of chemical disinfectants such as alcohol and chlorhexidine (4, 5). The haemolytic ability of enterococci is mediated by the expression of cytolysin which is commonly encoded on plasmids but can also be found on the chromosome (6).

2.1 The rise of a genus

Before the Enterococcus genus was established, enterococci were members of the Streptococcus genus and were further classified as Group D Streptococcus using the Lancefield serological typing scheme (7). Using molecular technologies in 1984, Schleifer and Kilpper-Bälz found sufficient distinction

between S. faecalis and S. faecium with other members of the streptococci family to establish a new genus, they termed Enterococcus (8). The two species S. faecalis and S. faecium were subsequently re-named Enterococcus faecalis and E. faecium. Over 50 additional species have subsequently been re-classified or newly identified as enterococci.

Although enterococci were identified as a molecularly distinct genus, phenotypic identification using traditional laboratory tests are difficult due to the lack of common traits amongst species of the genera. Presumptive identification is made based on the isolate (i) growing in 6.5% NaCl at 45°C; (ii) hydrolysing esculin in the presence of bile salts (bile-esculin test); (iii) hydrolysing leucine-β-napthylamide by producing leucine aminopeptidase (LAPase test); (iv) and hydrolysing L-pyrrolidonyl-β-napthylamide by producing pyrrolidonyl arylamidase (PYR test). Species and genus identification for enterococci however can also be performed by a microbiology laboratory within minutes using matrix-assisted laser desorption ionization – time of flight mass spectrometry (MALDI-TOF MS) (9).

2.2 Splitting of the species

To understand why some E. faecium are clinically important while others remain commensals, two studies have examined the evolution of the species. Galloway-Peña et al. (10) and Lebreton et al. (11) described two distinct E. faecium clades, one accounting for hospital-associated (HA) isolates and the other accounting for community-associated (CA) isolates. Using synonymous single nucleotide polymorphism (sSNP) molecular clock estimate with E. coli parameters, Galloway- Peña predicted the evolutionary division occurred 1-3 million years ago.

Lebreton et al., using Bayesian evolutionary analysis on sampled phylogenetic trees (BEAST) excluding recombinations, describes a more complex evolutionary path with two divisions. The first bifurcation, which they postulated stemmed from increased urbanization and domestication of animals, was estimated to have occurred around 2,776 ± 818 years ago and divided the species into human and animal dominant clades. The animal clade further divided into an epidemic hospital clade (A1) and a clade that causes sporadic infections in animals and human in the community (A2). The division was thought to have occurred as a result of the introduction and use of antimicrobials in hospitals and animal feed approximately 74 ± 30 years ago.

2.3 Typing

Using multilocus sequence typing (MLST), which characterizes the loci within seven E. faecium housekeeping genes, (atpA, ddl, gdh, purK, gyd, pstS and adk), E. faeium can be divided into genetic lineages known as sequence types (ST) (12). ST17 was identified as the ancestral clone of the hospital associated clade (A1) which has since been re-named clonal complex (CC)17 (13). The majority of hospital associated E. faecium isolates have since been identified as members of CC17 (Figure. 1).

Although MLST is an important method for typing isolates, considerable sequence diversity has been observed between clinical isolates of E. faecium with the same ST (14, 15). Recently, Carter et. al identified several E. faecium that could not be typed by MLST due to the loss of the required housekeeping gene, pstS (16). Whole genome sequencing studies have shown genetic diversity within E. faecium may have already crossed a degree of divergence usually associated with speciation (11). As such, perhaps a more robust typing method

which takes into account genetic changes throughout the whole genome would be more appropriate for typing E. faecium isolates.

For now, the use of MLST in surveillance can still serve to signal the emergence of a new ST of E. faecium at a particular facility or geographical area. The early identification of new E. faecium STs at a hospital may lead to preemptive infection control, particularly if the STs have previously been characterized as highly pathogenic.

3. The Present

3.1 The Start of the Antimicrobial Era

As opportunistic pathogens, E. faecium infections primarily occur in immune compromised patients and therefore pose a serious threat to those in intensive care, burns, oncology and organ transplant units. In the late 1970s, enterococci infections became increasingly prominent in hospitals mirroring the introduction and use of third generation cephalosporins to which all enterococci are intrinsically resistant to. A decade later, in the United States of America (USA), the first reports of an increase in infections and outbreaks due to ampicillin resistant enterococci were published (17). As a result, vancomycin was introduced as a treatment option. However, reports of vancomycin resistance enterococci (VRE) emerged not long after .

By the early 1990s, VRE had become the second most common nosocomial pathogen in the USA (18) and was endemic in many North American hospitals (19). It has been hypothesized the increase in VRE colonization and infection in the USA has stemmed from the heavy use of vancomycin (20). Similarly, in

Europe, VRE colonization and infection dramatically increased over a short period of time. However, unlike the USA, a large community reservoir was thought to be the reason for the sudden increase in VRE colonization and infection. In the late 1980s, farmers in Europe began supplementing animal feed with avoparcin, a glycopeptide antimicrobial similar to vancomycin. Evidence of VRE colonization was soon observed in farm animals and also in the community (21). The use of avoparcin in animal husbandry was subsequently banned in Europe in 1996. However persisting VRE colonization in poultry has been reported up to eight years after the ban (22).

3.2 To survive is to adapt

The rapid adaptation to antimicrobials can be attributed to the hyper-mutable DNA of E. faecium. Studies have consistently identified multiple recombinant regions consisting up to 26% of the E. faecium genome (23). It is believed the lack of the CRISPR-CAS loci, which protects genomic DNA from extracellular DNA in other bacteria, results in the high recombination rates observed in E. faecium (24). In addition, E. faecium are able to acquire and disseminate genes rapidly through mobile genetic elements (MGEs) such as plasmids and transposons which are ubiquitous among bacteria (25). MGEs usually carry gene cassettes consisting of virulence factors and antimicrobial resistance genes.

3.3 Plasmids

Plasmids are extrachromosomal DNA encoding non-essential genes which can be transmitted through donor-recipient interactions (26). The genomic content of plasmids are plastic and dynamic and can encode for functions such as maintenance, resistance and pathogenicity (27). As a result, classification of plasmids as fixed genetic structures is difficult and unrealistic.

In 2010, a novel plasmid classification system was introduced for enterococci and other Gram-positive bacteria. The classification was based on the sequence homology of replication initiating genes (rep) which are essential for plasmid replication and maintenance (28). In the same study, plasmids identified in E. faecium were categorized into six of the 19 known rep families (2, 4, 11, 14, 18). The most prominent rep-family identified were rep2 (45%) and rep14 (31%), found in isolates from animal and human origin.

3.4 Transposons

Either composite or non-composite transposons are chromosomally encoded DNA sequences that can be excised and transferred through mechanisms similar to that of plasmids. Once transferred, the transposon is able to insert itself into the chromosome. In E. faecium, composite transposons such as Tn1547 confers vancomycin type B (vanB) resistance and are flanked by insertion sequences (IS). Tn1546, a derivative of Tn3, a replicative transposon which confers vancomycin type A (vanA) resistance, does not contain flanking IS elements (29, 30).

The ability to share MGEs allow E. faecium to accumulate and share beneficial traits that provide an advantage. As a result, E. faecium can rapidly adapt its genome to overcome stressful environmental conditions.

3.5 A pathogen is only as bad as its virulence factors

The virulence of a bacterium provides a quantitative measure of its ability to cause disease. Virulence factors are specific traits found in bacteria that results in disease to the host. These factors can be broadly classified by their function

such as bacterial toxins, cell surface adhesins that mediate bacterial attachment, protective cell surface proteins and secreted exoenzymes (31).

3.5.1 Adhesion

The adherence of the bacterial cell to host cells is the first step in establishing infection. The extracellular matrixes of host cells play an important role in cell function and are also prime targets for bacterial adhesion. Microbial surface component recognizing adhesive matrix (MSCRAMM) are a subset of adhesion factors which mediate initial attachment (14). Included in the MSCRAMM family of genes for E. faecium are ace, acm, scm and ecba, of which, ace and acm share homologous domains to cna, the collagen-binding Staphylococcus aureus MSCRAMM.

3.5.2 Aggregation substances

Aggregation substances are another class of adhesins carried by E. faecium which are encoded on inducible sex pheromone plasmids. As well as promoting adhesion to bacterial cell, in vitro aggregation substances enhance adhesion to a variety of eukaryotic cell surfaces. The enterococci surface proteins (ESP), is a high molecular weight surface protein that influences enterococci pathogenesis (32). A high correlation has been observed between the presence of ESP and the ability to form biofilms ($P<0.0001$) (33). The CC17 hospital-adapted E. faecium has been characterized by harboring an ESP containing pathogenicity island (34).

3.5.3 Exoenzymes

Exoenzymes are enzymes produced by the bacterial cell that are secreted externally and can damage host cells triggering an inflammatory process (35).

In E. faecium the gelatinase exoenzyme is a metalloendopeptidase encoded by gelE which is capable of degrading a wide range of host substrates such as insulin, casein, hemoglobin, fibrinogen, collagen and gelatin. gelE is also able to clear the bacterial surface of misfolded proteins and activating autolysin (36). A second exoenzyme present in E. faecium is hyaluronidase which can cause tissue damage by catalyzing hyaluronic acid, a component in the extracellular matrix of connective tissues. It has been suggested E. faecium produces hyaluronidase to break down host hyaluronic acid into simpler substrates which are transported and metabolized in the bacterial cell supplying it with nutrients (37). A third exoenzyme, cytolysin, which is encoded in an operon of eight genes either on a plasmid or in the chromosome, targets host erythrocytes, macrophages and polymorphonuclear leukocytes triggering an inflammatory process (38, 39). In addition to host cell destruction, cytolysin is also a bacteriocin which targets other Gram-positive bacteria (40).

3.6 Intrinsic Antimicrobial Resistance

3.6.2 Aminoglycosides

Due to its Gram-positive cell wall, all enterococci are naturally resistant to low levels of aminoglycosides (41). However, when antimicrobials with bacterial cell wall activity, such as β-lactams, are used synergistically, aminoglycoside uptake in E. faecium can be increased.

E. faecium may also expresses a chromosomally encoded 6'-N-aminoglycoside acetyltransferase which cleaves the 6’-amino group of several aminoglycosides (42). The slow rate of enzymatic activity results in a moderate level of aminoglycoside resistance. High level aminoglycoside resistance in E. faecium

may be attained by the acquisition of genes encoding for a variety of aminoglycoside modifying enzymes such as 2”-phosphotransferase-6’-acetyltransferase (ACC(6’)-APH(2”)) which allows the isolate to survive concentrations >1000 µg/mL (43). The loss of efficacy of aminoglycoside has resulted in the loss of all aminoglycoside based synergistic antimicrobials .

3.6.3 Cephalosporins

Cephalosporins are broad-spectrum β-lactam antibiotics which have low toxicity and hypoallergenic properties (44). E. faecium are intrinsically resistant to cephalosporin concentrations of >10,000 µg/ml. Cephalosporins, such as ceftriaxone, can reach biliary concentrations of 5,000 µg/ml which virtually kills all upper gastrointestinal bacteria other than E. faecium. Studies have found an increased proportion of enterococci in the gastrointestinal tract of volunteers after given oral cephalosporin (44). The removal of cephalosporin-susceptible bacteria increases the colonisable area and the risk of E. faecium infection (45).

3.7 Acquired Antimicrobial Resistance

3.7.2 Beta-lactams

Because of their ability to inhibit the synthesis of essential cell wall peptidoglycan, ampicillin and penicillin were the most effective β-lactams against E. faecium. Penicillin’s low affinity towards eukaryotic cells are an added benefit when used in vivo. Many E. faecium however have acquired high level β-lactam resistance through the modification of the penicillin binding protein (PBP) 5 gene which results in: (i) a decreased β-lactam affinity due to a modified protein product; (ii) an increased β-lactams tolerance due to an up-regulation of gene expression; (iii) or a combination of modifications (i) and (ii)

which can increase resistance exponentially (46). E. faecalis may be 10 - 100 times less susceptible to β-lactams such as penicillin compared to most streptococci and E. faecium may be resistant a further 4 - 16 times.

E. faecium can also acquire the blaZ gene coding for a β-lactamase enzyme (47). The enzyme inactivates β-lactams by cleavage of the β-lactam ring. Sequence studies have shown that the blaZ genes found in enterococci are similar to the blaZ gene found in S. aureus suggesting a cross species origin (48). However, unlike staphylococci, expression of β-lactamase in enterococci is constitutively low hence a high inoculation concentration is required to ensure sufficient β-lactamase production results in penicillin resistance .

3.7.3 Vancomycin

Preceded by an increase in infections and outbreaks caused by ampicillin-resistant enterococci, clinically significant isolates of VRE were subsequently detected in the United Kingdom (49) and Europe (50) and shortly after in the USA (18). By the early 1990s, VRE had become the second most common nosocomial pathogen in the USA (18) and was endemic in many North American hospitals (19).

In Australia, the first reported vancomycin resistant E. faecium (VREfm) was a vanA E. faecium from a liver transplant recipient in 1995 (51). Since then, the vast majority of VRE isolated in Australia have been E. faecium harboring the vanB operon (52). Although prevalence or incidence rates of VREfm in Australian hospitals are not routinely collected, several studies have shown a significant increase in the number of patients infected or colonized with vanB E. faecium (14, 53, 54). The 2016 Australian Group on Antimicrobial Resistance

(AGAR) Australian Enterococcal Sepsis Outcome Program (AESOP) reported 46.5% of E. faecium isolates vancomycin resistant, of which 58.9% were vanB resistant (see http://www.agargroup.org/surveys).

Three of the ten known van genes (vanA, vanB and vanM) carry greater clinical significance as they are able to confer intermediate to high levels of resistance towards vancomycin and are encoded on mobile genetic elements. The remaining seven known van genes (vanC, vanD, vanE, vanF, vanG, vanL and vanN) typically confer lower levels of resistance and/or are not transferable and therefore they do not pose a high risk to public health. The highest level of vancomycin tolerance for wild-type E. faecium also known as the epidemiological cut-off value (ECOFF) is 4 µg/ml (55). The vanA and vanM types characteristically encode for high levels of inducible vancomycin resistance (MIC, 64 - 1,000 µg/ml & ≥ 256 µg/ml respectively) which are clearly distinguishable from wild-type by phenotypic antimicrobial susceptibility tests (ASTs). The vanB operon, encodes for a variable level of inducible vancomycin resistance (MIC, 0.5 - ≥ 256 µg/m) which overlaps with wild-type distributions (56).

The vanA, vanB and vanM type also differ in their geographical distributions with vanA more predominant in North America, Europe, Iran, China whilst vanB is predominant in Australia, New Zealand, Singapore, England, Wales and Scotland (56-61). vanM, so far has only been reported in China and Singapore (62, 63). However the geographical distribution of vanM may be underestimated as commercial molecular test kits routinely used in microbiology diagnostic laboratories only detect vanA and vanB (62).

3.8 Mechanism of vancomycin resistance

In the normal synthesis of cell wall peptidoglycan, a racemase enzyme initially converts L-alanine to D-alanine in the bacterial cytoplasm (64). A ligase combines two D-Ala molecules together as a dipeptide which is added to uracil diphosphate–N-acetylmuramyl-tripeptide to form uracil diphosphate–N-acetylmuramyl-pentapeptid. The pentapeptide is bound to an undercaperol lipid carrier which, after the binding of N-Acetyl-D-glucosamine, is allowed to translocate to the outer surface of the cytoplasm (Figure 2). The pentapeptide is added to newly formed peptidoglycans via transglycosylation and anchored by transpeptide cross-bridges.

The key to the potent antimicrobial effect of glycopeptides on enterococci relies on the binding of the glycopeptide to the D-Ala-D-Ala at the C-Terminus end of the translocated pentapeptide. The binding prevents subsequent transglycosylation, transpeptidation and carboxypeptidase reactions. Modifications to the D-Ala-D-Ala dipeptide mediated by the van genes reduces the affinity of vancomycin binding by up to 1,000 times and thus losing its efficacy (65).

3.9 Structure of the van operon

The vanA operon consist of three major components: Regulation (vanR and vanS), glycopeptide resistance (vanH, vanA and vanX) and accessory genes (vanY and vanZ) (Figure 2). In vanA type resistance, a dehydrogenase enzyme encoded by vanH reduces pyruvate to D-Lac. The ligase encoded by the vanA gene then catalyses an ester bond between D-Ala and D-Lac (66). The resulting dipeptide can be incorporated into the peptidoglycan resulting in a severe

reduction in vancomycin affinity (65). It is important to note the simultaneous production of D-Ala-D-Ala and D-Ala-D-Lac precursors does not result in significant increase in vancomycin resistance as sufficient vancomycin binding to D-Ala ending peptidoglycans still renders the cell susceptible (66). It is therefore necessary for the removal of susceptible D-Ala-D-Ala precursors for high levels of resistance. For this to occur, a D,D-dipeptidase encoded by vanX hydrolyses the susceptible D-Ala-D-Ala dipeptide into two D-Ala peptides (67). A D,D-carboxypeptidase encoded by vanY cleaves remaining D-Ala at the C-terminus end of developing peptidoglycans left behind by vanX (68). The two enzymes coded by vanX and vanY ensures the removal of susceptible D-Ala-D-Ala binding sites for glycopeptides.

The vanB type operon's structure is similar to the vanA operon. It contains a dehydrogenase, a ligase and a dipeptidase gene component that has a 67-76% sequence homology with its vanA counterpart. Therefore, it is not surprising that the D-Ala-D-Ala peptidoglycan precursor for vanB is replaced with D-Ala-D-Lac by the same processes as described for vanA (66). Although the vanA and vanB type resistance is induced by teicoplanin and vancomycin respectively, the transcriptional activation of both operons follow the same mechanisms (Figure 4). Although the vanB type is not commonly known to carry teicoplanin resistance, evidence of a novel vanB2 teicoplanin resistant variant has been identified (69). The vanB operon has an additional vanW gene but do not have the vanZ gene compared to the vanA operon (Figure 3).

Based on the sequence difference, the vanB type operon has been subdivided into three subtypes: vanB1, vanB2 and vanB3 (70, 71). The three subtypes have no known influence on the level of vancomycin resistance. A study in 2001 by

McGregor et al. on the prevalence of the vanB2 gene examined 204 enterococci isolates from 59 hospitals in England, Wales, Scotland and the Republic of Ireland, and showed 202 (99%) isolates carrying the vanB2 gene (60). Analysis of the conjugative transposon, Tn5382, which carries the vanB2 gene, suggest horizontal gene transfer was responsible for its dominance (60). In Australia, we have identified the vanB2 subtype in 94.85% of 251 vanB positive E. faecium with the remaining isolates carrying the vanB1 subtype (unpublished data).

The vanM type resistance consists of 1,032bp encoding a 343 amino-acid protein which shares approximately 80% sequence identity with vanA. The vanM does not possess the vanZ or vanW component (72). The vanM type, like vanA, vanB and vanF confers vancomycin resistance through the inducible synthesis of precursors ending in D-Ala-D-Lac. The operon organization however mostly resembles that of vanD. Upstream of the vanM cluster lies an IS-1216-like element which may account for its dissemination akin to the IS-1216V element found widely in vanA types by transposon-mediated fusion of vanA plasmids with other plasmids (26, 73, 74).

The vanD operon which is only found in E. faecium, is exclusively located on the chromosome and cannot participate in horizontal gene transfer (75). Although vancomycin and teicoplanin resistance conferred by the vanD gene cluster are typically low, they can reach concentrations of up to 256 µg/mL and 64 µg/mL respectively. The organization of the vanD operon is similar to that of vanA, vanB and vanF and produces peptidoglycan precursors ending in D-Ala-D-Lac.

The other van operons, vanC, vanE, vanG, vanL and vanN produce peptidoglycan precursors ending in D-Ala-D-Ser to which glycopeptides have a lower binding affinity (76-78). Therefore, enterococci harbouring the vanC, vanE, vang, vanL or vanN operons are usually resistant to low vancomycin concentrations of up to 32 µg/mL. The vanC operon, intrinsically found in E. gallinarum and E. casseliflavus, provides resistance to vancomycin. The biochemically and phenotypically similar vanE operon is only found in E. faecalis.

3.10 Epidemiology

E. faecium have the ability to survive in extreme conditions, are ubiquitous in the environment and highly prevalent in the natural gut microbiome. Surveys have isolated E. faecium from wild animals including birds and insects. In the environment, soil and water bodies such as rivers, ponds and waste water have also been identified as reservoirs for E. faecium (79). In Portugal, antimicrobial resistant E. faecium were recovered from fecal samples of wild rabbits, badgers, forest wildcats, storks, quails, wolf, birds of prey and sewage (80, 81). A separate study also identified ST18 CC17 E. faecium from the fecal sample of a wild Ibreian wolf in Northeast Portugal indicating the presence of CC17 E. faecium in native wildlife (82). Elsewhere, CC17 E. faecium have been reported in wild corvid birds in USA, slovakia and the Czech republic (83, 84). Although there were many other reports of multi-drug resistant E. faecium from wildlife, most studies did not perform MLST.

In the environment, waste water is often been reported as a reservoir for CC17 E. faecium. A comprehensive study in the south coast of England focusing on treated and untreated water from municipal waste water, hospital waste water

and farm run-off water identified CC17 E. faecium belonging to an epidemic group associated with outbreaks in UK, the Netherlands, the USA and Australia (85). Two other independent studies performed on the effluent waters of two waste water treatment plants in Gdansk, Poland, and a river downstream of a plant in the northwest of France, also recovered CC17 E. faecium but noted that those isolates were in a minority (86, 87).

Besides wild animals and the environment, CC17 E. faeicum has also manage to adapt to domestic animals. The carriage of CC17 E. faecium in domestic animals results in an increased risk of zoonotic transfer to humans. The prevalence of CC17 E. faecium in companion animals is well documented internationally (88-91). In Portugal, CC17 E. faecium isolates identified in companion cats and dogs were resistant to ampicillin and/or high-level gentamicin (88). In Korea, it was reported that ampicillin and ciprofloxacin resistance were high in CC17 E. faecium isolated from companion dogs and humans while tetracycline resistance was more commonly identified in isolates from companion dogs. Additionally, vancomycin resistant isolates were only found in CC17 E. faecium isolated from humans (89). The findings suggest CC17 E. faecium may possess advantages for infecting humans and animals but their antimicrobial resistance phenotypes may have evolved independently as a result of different antimicrobials used in human and veterinary medicine in different countries.

Another potential route for the zoonotic transfer of CC17 E. faecium occurs between farm animals and humans (92). Besides direct human-animal transfer of CC17 E. faecium, as with companion animals, farm animals carrying CC17 E. faecium pose the risk of contaminating food produce. Internationally, CC17

E. faecium have been recovered in swine, chickens and cows (93-95). In Spain, CC17 E. faecium was isolated from chicken, veal and rabbit samples, with isolates from all three carrying antimicrobial resistances to vancomycin, ampicillin, erythromycin, ciprofloxacin and high-levels of streptomycin and kanamycin (96). In Canadian animal farms, low quantities of CC17 E. faecium together with MGEs carry antimicrobial resistance genes have been identified in bovine fecal samples (97).

In Portugal, CC17 E. faecium was isolated from farmed pigs and their surrounding environment; manure, waste lagoons, drinking water (91). In addition fresh vegetables sold in Portuguese supermarkets were also found to carry CC17 E. faecium including lettuce, green olives, celery and broccoli (98).

Although exposure to CC17 E. faecium in the community may appear high, E. faecium are opportunistic pathogens therefore, community associated E. faecium infections are uncommon. In Europe, despite the ban of avoparcin in the animal industry two decades ago, colonization of VREfm in people without hospital contact or history of glycopeptide use can vary between ~2-28% of adults (99, 100). Similarly, in South Korea, which also had history of avoparcin use, 4.7% of farm animals and 1% of healthy individuals are reported to carry VREfm in their gut (101). Conversely in North America, where the use of avoparcin was prohibited, VREfm was not identified in the healthy adult population sampled (102).

In hospitals, the two most commonly isolated species of enterococci are E. faecalis and E. faecium. Although E. faecalis is identified more frequently than E. faecium, a shift in trend towards a greater prominence of E. faecium has been

observed (103). Antimicrobial resistance is more often identified in E. faecium (80 - 100%) compared to E. faecalis (0 - 16%) suggesting E. faecium is able to acquire and express resistance genes more frequently (56-58, 104-106).

Critically ill patients such as those in intensive care units hold the highest risk of infection in the hospital followed by patients in hematology, neonatal and renal units (107). Amongst the patients in these wards, patients undergoing organ transplant pose the highest risk followed by patients with prolonged hospital stays. Prior therapy with antimicrobials that are ineffective against E. faecium, such as third generation cephalosporins, increases the risk of colonization and infection.

In the hospital, E. faecium remain viable on inanimate surfaces from seven days to two months which increases the risk of acquiring E. faecium through factors such as exposure to contaminated medical equipment, proximity to patients or previous bed occupant shedding E. faecium and transmission by health care workers (108-110). A previous study which reported the low recovery of VREfm from rectal swabs of healthcare workers suggest healthcare workers do not serve as major VREfm reservoirs and VREfm colonization is uncommon in healthy persons .

The spectrum of disease associated with E. faecium infection, which has remained relatively unchanged, was extensively reviewed by Murray in 1990 (107). The urinary tract is the most common point of entry for enterococci into the blood stream, which leads to bacteremia, the leading cause of E. faecium morbidity and mortality (111). Other sources of E. faecium leading to blood stream infection (BSI) include intravenous lines and abscesses. However a

significant proportion remains unknown and is assumed to originate from the intestinal microbiota (112, 113). E. faecium are able to translocate across the luminal surface of the intestines in a similar fashion to Candida albicans and Escherichia coli (114, 115). Secondary to BSI, enterococci account for 5-20% of native valve and 6-7% of prosthetic valve related bacterial endocarditis (116). Additionally, the vegetation of heart valves increases the risk of bacterial adherence enhancing the risk of infection .

In a 2016 Australia-wide surveillance of enterococcal bacteremia which included 1,058 patient-episodes, 39% of isolates were E. faecium, of which 46.5% were vancomycin resistant (117). Compared to a related survey conducted in 2005, when the prevalence of vancomycin resistance in E. faecium was reported at 7%, the 2016 data represents a seven-fold increase in prevalence (118). The distribution of vanA and vanB genes in VREfm reported in the 2016 study was 42.7% and 55.2% respectively, with four isolates carrying both sets of genes. The distribution of vanA to vanB VREfm isolates in 2010 was 1.6% and 98.4% respectively, indicating a shift in prevalence towards vanA type VREfm has occurred in Australia (http://www.agargroup.org/surveys).

In the USA, the National Healthcare Safety Network (NHSN) report on hospital-associated infections from 2011-2014 ranked E. faecium ninth overall (3.7%) for pathogens frequently reported (119). Approximately 83-86% of E. faecium collected from central-line associated blood stream infections and catheter-associated urinary tract infections were vancomycin resistant. Comparatively lower, 60-64% of E. faecium isolates collected from surgical site infections were vancomycin resistant.

The 2016 Canadian Antimicrobial Resistance Surveillance System (CARSS) report indicated a decreasing trend in the prevalence of VRE from 2012 to 2014. 60% of VRE cases reported in 2014 were characterized, of which, 99% were E. faecium and 98% carried the vanA type vancomycin resistance .

In South America, a multicenter study involving 32 hospitals from Colombia, Ecuador, Perú and Venezuela, found 31% of E. faecium isolates carry the vanA type resistance to vancomycin (120). Additionally, all representative isolates of PFGE clusters subjected to MLST were identified as members of CC17 with the most frequent ST being ST412. In Brazil, a study of 53 E. faecium isolates from patients at two university hospitals identified the vanA type vancomycin resistance in all isolates (121). Additionally, the 31 isolates selected for MLST were shown to belong to CC17 with predominantly ST412 isolates. A second Brazilian study also identified a ST412 CC17 vanA E. faecium resistant to vancomycin (>256 μg/ml) and linezolid (64 μg/ml) (122). In Cuba, two CC17 E. faecium clones, ST656 and ST262, were resistant to ampicillin, quinolones, imipenem, high-level gentamicin, erythromycin, clindamycin, vancomycin, teicoplanin, and sulfamethoxazole/trimethoprim.

In Europe, the European Union/European Economic Area (EU/EEA) population-weighted mean percentage for VREfm reported in the 2016 Annual report of the European Antimicrobial Resistance Surveillance Network (EARS-Net) was 11.8% which was not significantly different than that reported in 2013 (123). National percentages for 2016 ranged widely from 0% to 46.3% with five countries reporting zero VREfm cases while several European countries with comparatively high percentages reporting significantly increasing trends over the last four years.

In Asia, studies conducted in China predominantly reports ST78 CC17 E. faecium carrying the vanA type vancomycin resistance. However, vanM type resistance has been reported in selected areas (61, 62, 124, 125). In Malaysia, ST17, ST203 ST78 and ST601 E. faecium isolates belonging to CC17 were identified at a local hospital. In South Korea, only vanA type resistant (43/531) E. faecium isolates were identified in a three-year study of 212 nontertiary-hospitals. Of the vanA E. faecium isolates, ST78 (30.2%) was the dominant ST. Two studies in Taiwan identified vanA type E. faecium as the dominant van type with ST414, ST78, ST 17 and ST18 as the dominant STs (126, 127).

4 The Future

VREfm outbreaks not only incur a significant cost for the healthcare system but also places vulnerable patients at greater higher risk of acquiring fatal infections. Reports of successful infection control measures that control the development of outbreaks have been documented on multiple occasions (54, 128, 129). Other reports make a synonymous point on the importance of ongoing surveillance (130, 131). Mathematical modelling developed by Erika et al. predict the only preventative measure that could potentially eradicate VREfm from an institution is to prevent colonized patients from entering the hospital. This, however, is an unrealistic goal. The constant monitoring of VREfm carriage in high risk groups, such as patients admitted from long-term care facilities into vulnerable units, has been projected to reduce transmission significantly (132) and may be the only option.

Reports documenting the successful control of VREfm outbreaks often mention the importance of common general infection control procedures such as

education for healthcare workers, hand and environmental sanitization, antimicrobial stewardship, the use of sterile equipment and personal protective gear (14, 133). However, counter to these reports, it has also been reported that these protocols which are successful in containing other outbreaks such as that of methicillin resistant Staphylococcus aureus (MRSA) are inadequate for enterococci (14).

Apart from preventing the spread of VREfm, the use of alternative antimicrobial therapy is another potential strategy to consider. Currently the two leading alternatives for the treatment of VREfm are linezolid and daptomycin, with clinical success rates of 50-80% as a first-line drug and 50-59% as salvage therapy for VRE bacteraemia respectively (134-137). However, resistance to both antimicrobials have been reported in E. faecium. Antimicrobials such as tigecycline and quinupristin/dalforpristin are infrequently used due to poor oral bioavailability, greater adverse effects or reduced activity against E. faecium. New therapeutic approaches such as daptomycin-β-lactam, daptomycin-fosfomycin and daptomycin-tigecycline combination therapy may be used to increase treatment efficacy. Daptomycin-β-lactam regimens have shown most promise in in-vitro studies (138). Although new alternatives such as tedizolid, telavancin, oritavancin and dalbavancin have only been recently approved by the FDA for the treatment of VREfm, the development of new antimicrobials has been steadily declining over the years. Moreover, new antimicrobials such as tedizolid are often a derivative of older antimicrobials (linezolid) utilizing the same mechanism of action with some enhanced activity (139). As such, resistance to the original drug often provides some cross-resistance to the new antimicrobial.

The fight against chronic VREfm outbreaks in hospitals is urgent and has to be fought on many fronts. The use of technology in typing and surveillance can help identify outbreaks early, allowing infection control to limit the spread of the outbreak. Antimicrobial stewardship practices can limit the dissemination of antimicrobial resistance genes in E. faecium population extending the efficacy of current antimicrobials. However the development of new antimicrobials is required to overcome the rapid adaptation observed in E. faecium. This will prevent a scenario where E. faecium becomes resistant to all available antimicrobials which will set us back decades of medical advancements due to the risk of untreatable infections.

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Figures

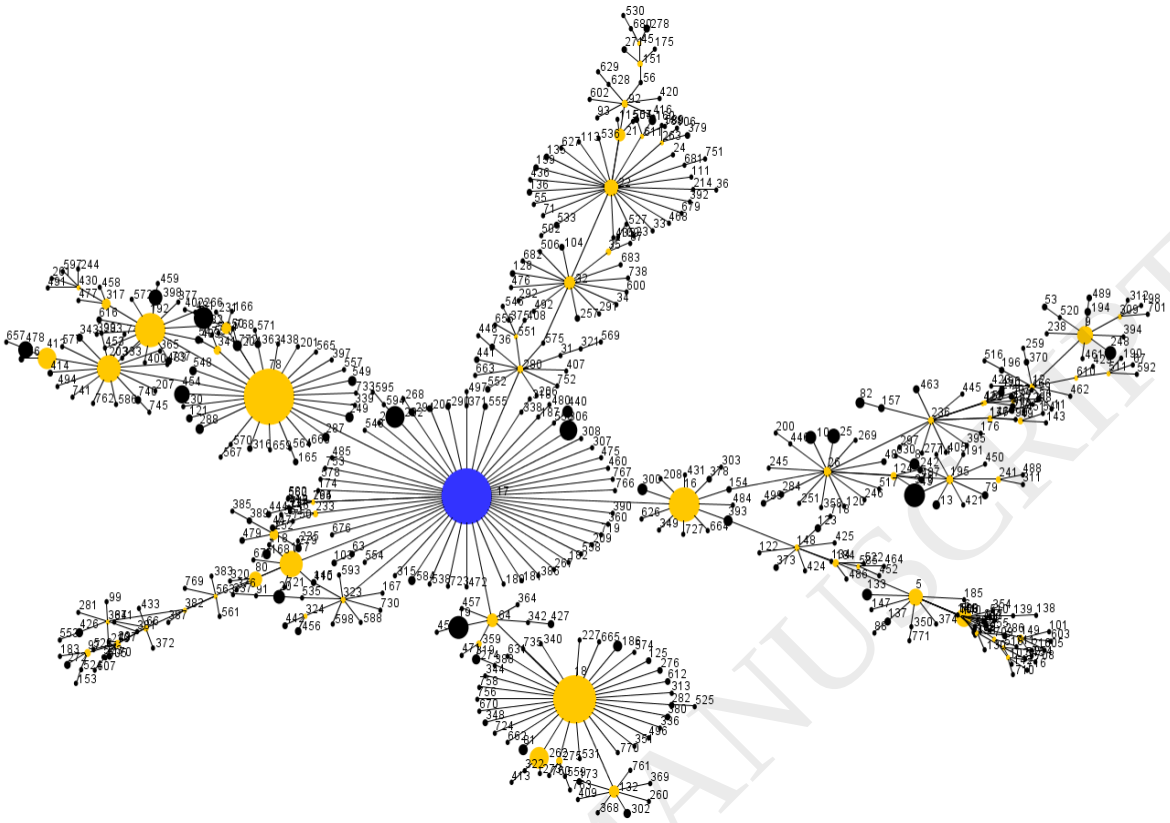

Figure 1. eBurst –generated population snapshot of E. faecium sequence types (STs) associated with Clonal Complex (CC) 17 worldwide taken as of 15 December 2017 adapted from http://efaecium.mlst.net/. Each ST is represented by a black dot. The numbers refer to a particular ST. The size of each dot reflects the number of isolates within a ST. The ancestral ST of a clonal complex is represented by a blue dot. The yellow-coloured dots represent a subgroup cofounder.

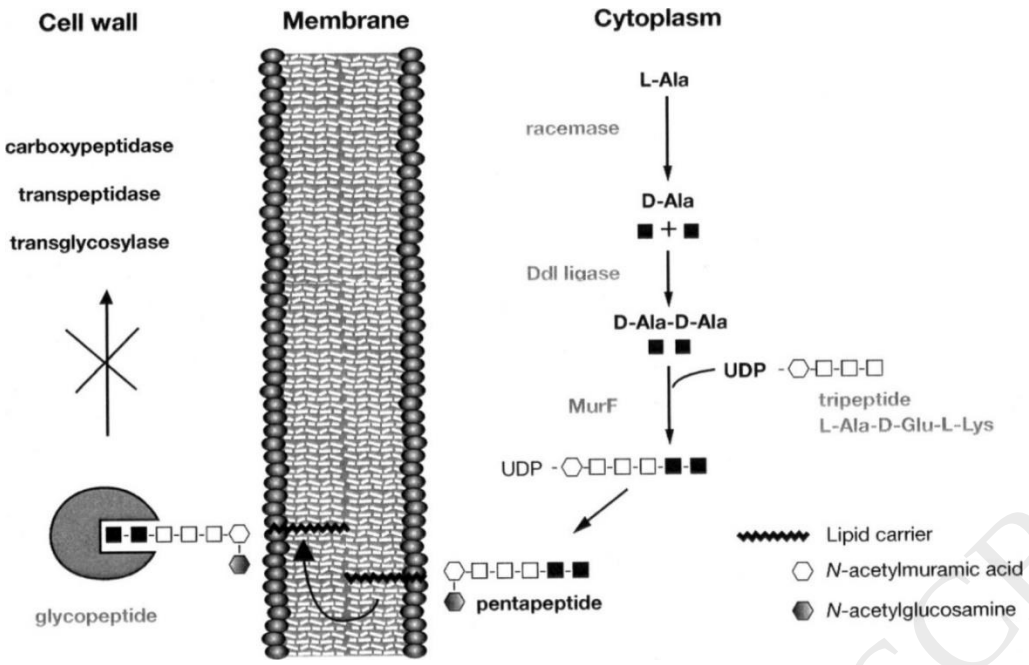

Figure 2 Peptidoglycan biosynthesis and mechanism of action of glycopeptide such as vancomycin (64).

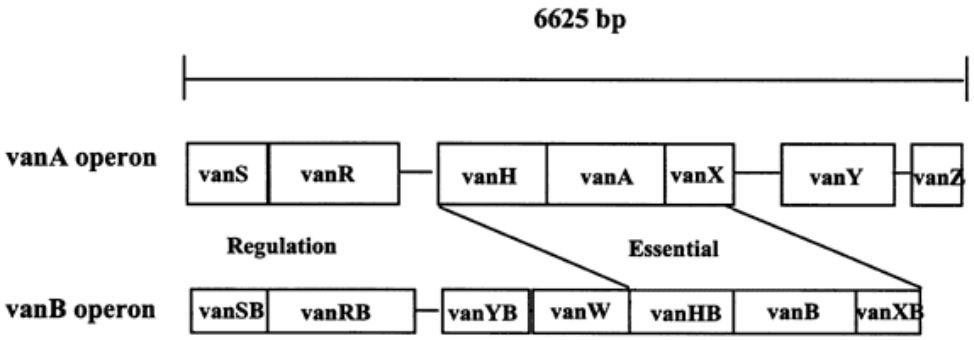

Figure 3 Comparison of vanA and vanB gene structures (140).

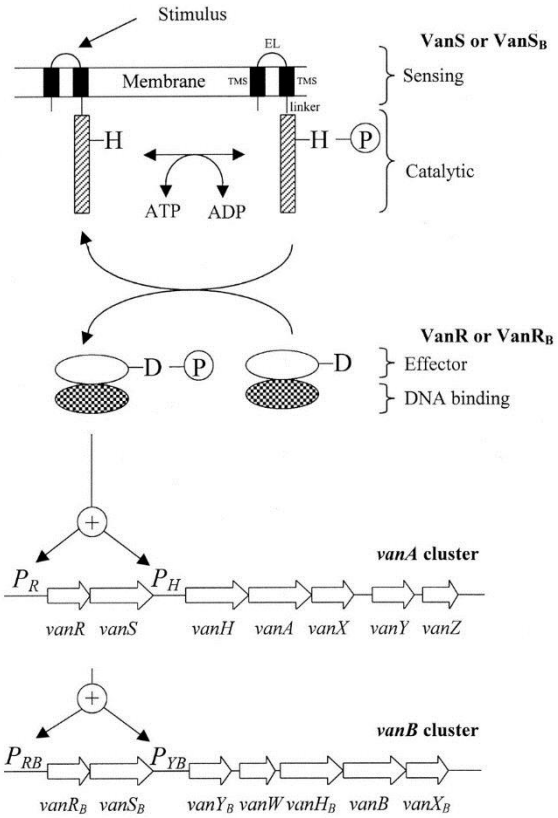

Figure 4 Transcriptional activation of the vanA and vanB gene clusters (141).