Journal of Global Antimicrobial Resistance 16 (2019) 36–47

Contents lists available at ScienceDirect

Journal of Global Antimicrobial Resistance

journal homepage: www.elsevier.com/locate/jgar

Review

Antimicrobial-resistant CC17 *Enterococcus faecium*: The past, the present and the future

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ARTICLE INFO

Article history:
Received 11 January 2018
Received in revised form 17 August 2018
Accepted 18 August 2018
Available online 24 August 2018

Keywords:
Enterococcus faecium
Enterococcus
Vancomycin-resistant enterococci
Epidemiology
Antimicrobial resistance
Clonal complex 17

ABSTRACT

Enterococcus faecium is a robust opportunistic pathogen that is most commonly found as a commensal of the human and animal gut but can also survive in the environment. Since the introduction and use of antimicrobials, *E. faecium* has been found to rapidly acquire resistance genes that, 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 17 (CC17). CC17 *E. faecium* are responsible for a significant proportion 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.

Contents

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https://doi.org/10.1016/j.jgar.2018.08.016

1. Introduction

Enterococcus faecium is a species of bacterium that, over the last three decades, has ranged from being considered a commensal that could be used as a probiotic to an 'ESKAPE' pathogen (a list of the leading causes of nosocomial bacterial infections comprising *E. faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* spp.) [1]. Although ubiquitous in the environment, *E. faecium* is most abundant as a commensal of the human and animal gut microbiome. However, in immunocompromised hosts, *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 that effectively circumvents modern-day medicine. In this review, we have focused on hospital-associated clonal complex 17 (CC17) *E. faecium* and its impact on public health.

2. The past

Thiercelin first described a bacterium termed 'Entérocoque' (French) in 1899 as a diplococcus bacterium 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 phylum Firmicutes. 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 *Streptococcus faecalis* and *Streptococcus faecium* with other members of the streptococci family to establish a new genus, which they termed *Enterococcus* [8]. The two species *S. faecalis* and *S. faecium* were subsequently re-named *Enterococcus faecalis* and *Enterococcus faecium*. Over 50 additional species have subsequently been reclassified or newly identified as enterococci.

Although enterococci were identified as a molecularly distinct genus, phenotypic identification using traditional laboratory tests is difficult due to the lack of common traits among species of the genus. 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-naphthylamide by producing leucine aminopeptidase (LAPase test); and (iv) hydrolysing L-pyrrolidonyl-β-naphthylamide 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 isolates and the other accounting for community-associated isolates. Using a synonymous single nucleotide polymorphism molecular clock estimate with *Escherichia coli* parameters, Galloway-Peña et al. predicted that the evolutionary division occurred 1–3 million years ago [10].

Using Bayesian evolutionary analysis on sampled phylogenetic trees (BEAST) excluding recombinations, Lebreton et al. described a more complex evolutionary path with two divisions. The first bifurcation, which they postulated stemmed from increased urbanisation and domestication of animals, was estimated to have occurred around 2776 ± 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 humans 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 ca. 74 ± 30 years ago [11].

2.3. Typing

Using multilocus sequence typing (MLST), which characterises the loci within seven *E. faecium* housekeeping genes (*atpA*, *ddl*, *gdh*, *purK*, *gyd*, *pstS* and *adk*), *E. faecium* can be divided into genetic lineages known as sequence types (STs) [12]. ST17 was identified as the ancestral clone of the hospital-associated clade A1, which has since been re-named CC17 [13]. The majority of hospital-associated *E. faecium* isolates have since been identified as members of CC17 (Fig. 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 owing to loss of the required housekeeping gene *pstS* [16]. Whole-genome sequencing studies have shown that 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 that takes into account genetic changes throughout the whole genome would be more appropriate for typing *E. faecium* isolates.

For now, 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. Early identification of new *E. faecium* STs at a hospital may lead to pre-emptive infection control, particularly if the STs have previously been characterised as highly pathogenic.

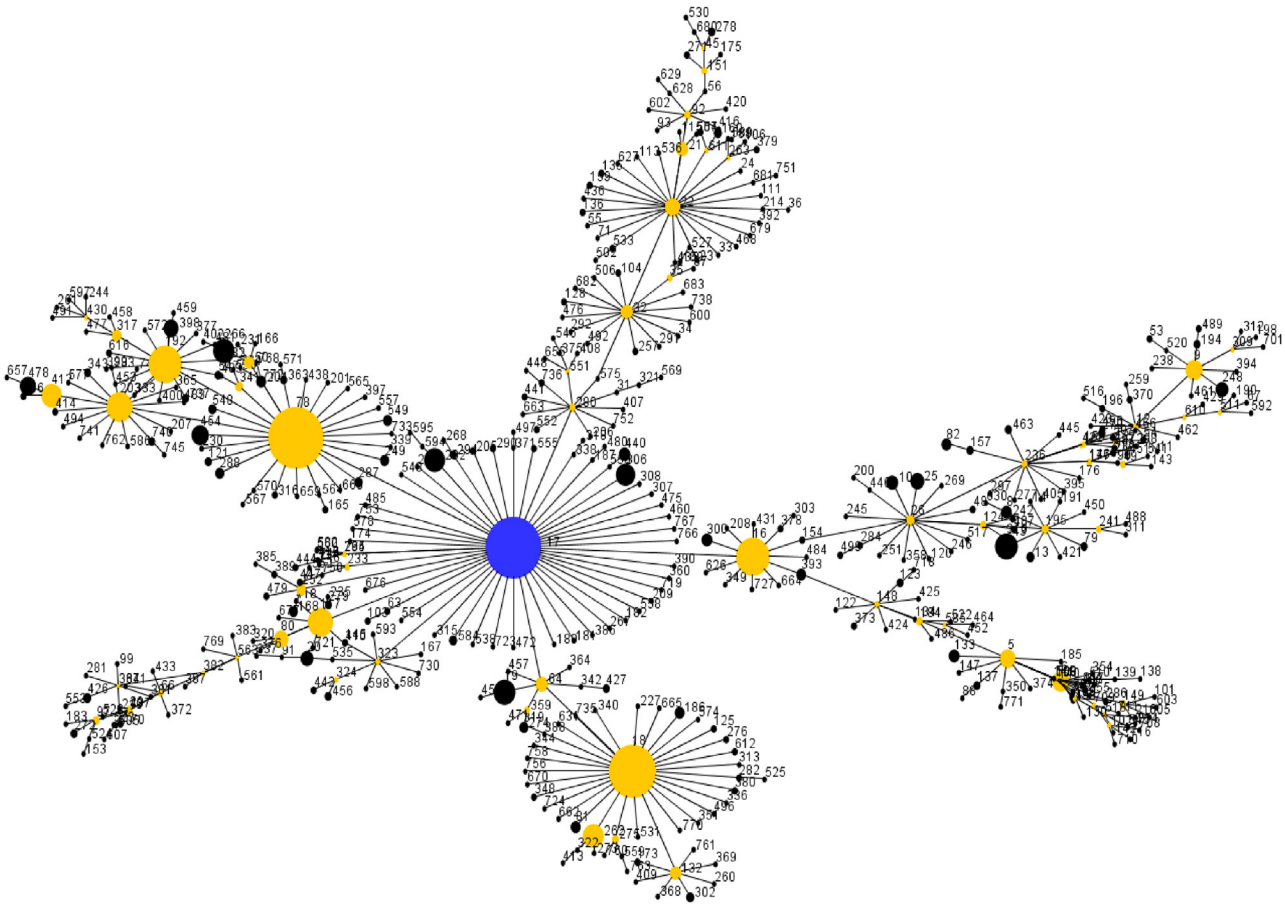

Fig. 1. eBURST-generated population snapshot of *Enterococcus faecium* sequence types (STs) associated with clonal complex (CC) 17 worldwide taken as of 15 December 2017 (adapted from http://efaecium.mlst.net/). Each ST is represented by a black dot. Numbers refer to a particular ST. The size of each dot reflects the number of isolates within a ST. The ancestral ST of a CC is represented by a blue dot. The yellow-coloured dots represent a subgroup co-founder.

3. The present

3.1. The start of the antimicrobial era

As opportunistic pathogens, *E. faecium* infections primarily occur in immunocompromised 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. A decade later in the 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-resistant enterococci (VRE) emerged not long after.

By the early 1990s, VRE had become the second most common nosocomial pathogen in the USA [18] and were endemic in many North American hospitals [19]. It has been hypothesised that the increase in VRE colonisation and infection in the USA has stemmed from the heavy use of vancomycin [20]. Similarly in Europe, VRE colonisation 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 colonisation and infection. In the late 1980s, farmers in Europe began supplementing animal feed with avoparcin, a glycopeptide antimicrobial similar to vancomycin. Evidence of VRE colonisation was soon observed in farm animals as well as in the community [21]. Use of avoparcin in animal husbandry was subsequently banned in Europe in 1996. However, persisting VRE colonisation in poultry has been reported up to eight years after the ban [22].

3.2. To survive is to adapt

Rapid adaptation to antimicrobials can be attributed to the hypermutable DNA of *E. faecium*. Studies have consistently identified multiple recombinant regions comprising up to 26% of the *E. faecium* genome [23]. It is believed that lack of the CRISPR-*cas* loci, which protect 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 that 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 that can be transmitted through donor–recipient interactions [26]. The genomic content of plasmids is plastic and dynamic and can encode 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*) that are essential for plasmid replication and maintenance [28]. In

the same study, plasmids identified in *E. faecium* were categorised into 6 of the 19 known *rep* families [2,4,11,14,18]. The most prominent *rep* families identified were *rep2* (45%) and *rep14* (31%), found in isolates of animal and human origin.

3.4. Transposons

Transposons, either composite or non-composite, 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 Tn*1547* confer vancomycin type B (vanB) resistance and are flanked by insertion sequence (IS) elements. Tn*1546*, a derivative of Tn*3*, a replicative transposon that confers vancomycin type A (vanA) resistance, does not contain flanking IS elements [29,30].

The ability to share MGEs allows *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 result 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

Adherence of 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 components recognising adhesive matrix molecules (MSCRAMM) are a subset of adhesion factors that 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 MSCRAMM of *S. aureus*.

3.5.2. Aggregation substances

Aggregation substances are another class of adhesins carried by *E. faecium* that are encoded on inducible sex pheromone plasmids. As well as promoting adhesion to bacterial cells, in vitro aggregation substances enhance adhesion to a variety of eukaryotic cell surfaces. Enterococcal surface protein (Esp) is a high-molecular-weight surface protein that influences enterococcal 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 characterised by harbouring an Esp containing pathogenicity island [34].

3.5.3. Exoenzymes

Exoenzymes are enzymes produced by bacterial cells 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* that is capable of degrading a wide range of host substrates such as insulin, casein, haemoglobin, fibrinogen, collagen and gelatin. GelE is also able to clear the bacterial surface of misfolded proteins and to activate autolysin [36]. A second exoenzyme present in *E. faecium* is hyaluronidase, which can cause tissue damage by catalysing the hydrolysis of hyaluronic acid, a component in the extracellular matrix of connective tissues. It has been suggested that *E. faecium* produces hyaluronidase to break down host hyaluronic acid into simpler substrates that are transported and metabolised in the bacterial cell thus 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 that targets other Gram-positive bacteria [40].

3.6. Intrinsic antimicrobial resistance

3.6.1. Aminoglycosides

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

Enterococcus faecium may also expresses a chromosomally-encoded 6′-*N*-aminoglycoside acetyltransferase that 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 a variety of aminoglycoside-modifying enzymes such as 2″-phosphotransferase-6′-acetyltransferase [ACC(6′)-APH(2″)], which allows the isolate to survive aminoglycoside concentrations of >1000 μg/mL [43]. The loss of efficacy of aminoglycoside has resulted in the loss of all aminoglycoside-based synergistic antimicrobials.

3.6.2. Cephalosporins

Cephalosporins are broad-spectrum β-lactam antibiotics with low toxicity and hypoallergenic properties [44]. *Enterococcus faecium* is intrinsically resistant to cephalosporin concentrations of >10 000 μg/mL. Cephalosporins such as ceftriaxone can reach biliary concentrations of 5000 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 receiving oral cephalosporins [44]. The removal of cephalosporin-susceptible bacteria increases the colonisable area and the risk of *E. faecium* infection [45].

3.7. Acquired antimicrobial resistance

3.7.1. β-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*. The low affinity of penicillin towards eukaryotic cells is an added benefit when used in vivo. Many *E. faecium*, however, have acquired high-level β-lactam resistance through modification of the penicillin-binding protein 5 (PBP5) gene that results in: (i) decreased β-lactam affinity owing to a modified protein product; (ii) increased β-lactam tolerance due to upregulation of gene expression; or (iii) a combination of modifications (i) and (ii), which can increase resistance exponentially [46]. *Enterococcus faecalis* may be 10–100 times less susceptible to β-lactams such as penicillin compared with most streptococci, and *E. faecium* may be resistant a further 4–16 times.

Enterococcus faecium can also acquire the *blaZ* gene encoding 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 the β-lactamase in enterococci is constitutively low, hence a high bacterial inoculum is required to ensure sufficient β-lactamase production to result in penicillin resistance.

3.7.2. Vancomycin

Preceded by an increase in infections and outbreaks caused by ampicillin-resistant enterococci, clinically significant isolates of VRE were subsequently detected in the UK [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*-positive *E. faecium* from a liver transplant recipient in 1995 [51]. Since then, the vast majority of VRE isolated in Australia have been *E. faecium* harbouring 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 colonised 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 as vancomycin-resistant, of which 58.9% were *vanB* resistant [55].

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 to vancomycin and are encoded on MGEs. 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 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 [56]. The *vanA* and *vanM* types characteristically encode for high levels of inducible vancomycin resistance [minimum inhibitory concentrations (MICs) of 64–1000 μg/mL and ≥256 μg/mL, respectively], which are clearly distinguishable from wild-type by phenotypic antimicrobial susceptibility testing. The *vanB* operon encodes for a variable level of inducible vancomycin resistance (MICs of 0.5 μg/mL to ≥256 μg/mL) that overlaps with wild-type distributions [57].

The *vanA*, *vanB* and *vanM* types also differ in their geographical distributions, with *vanA* more predominant in North America, Europe, Iran and China, whilst *vanB* is predominant in Australia, New Zealand, Singapore, England, Wales and Scotland [57–62]. So far, *vanM* has only been reported in China and Singapore [63,64]. 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* [63].

3.8. Mechanism of vancomycin resistance

In the normal synthesis of cell wall peptidoglycan, a racemase enzyme initially converts L-alanine to D-alanine (D-Ala) in the bacterial cytoplasm [65]. 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-pentapeptide. The pentapeptide is bound to an undecaprenol lipid carrier which, after binding the *N*-acetyl-D-glucosamine, is allowed to translocate to the outer surface of the cytoplasm (Fig. 2). The pentapeptide is added to newly formed peptidoglycans via transglycosylation and is anchored by transpeptide cross-bridges.

The key to the potent antimicrobial effect of glycopeptides against 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 1000 times, thus losing its efficacy [66].

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*) (Fig. 3). In *vanA*-type resistance, a dehydrogenase enzyme encoded by *vanH* reduces

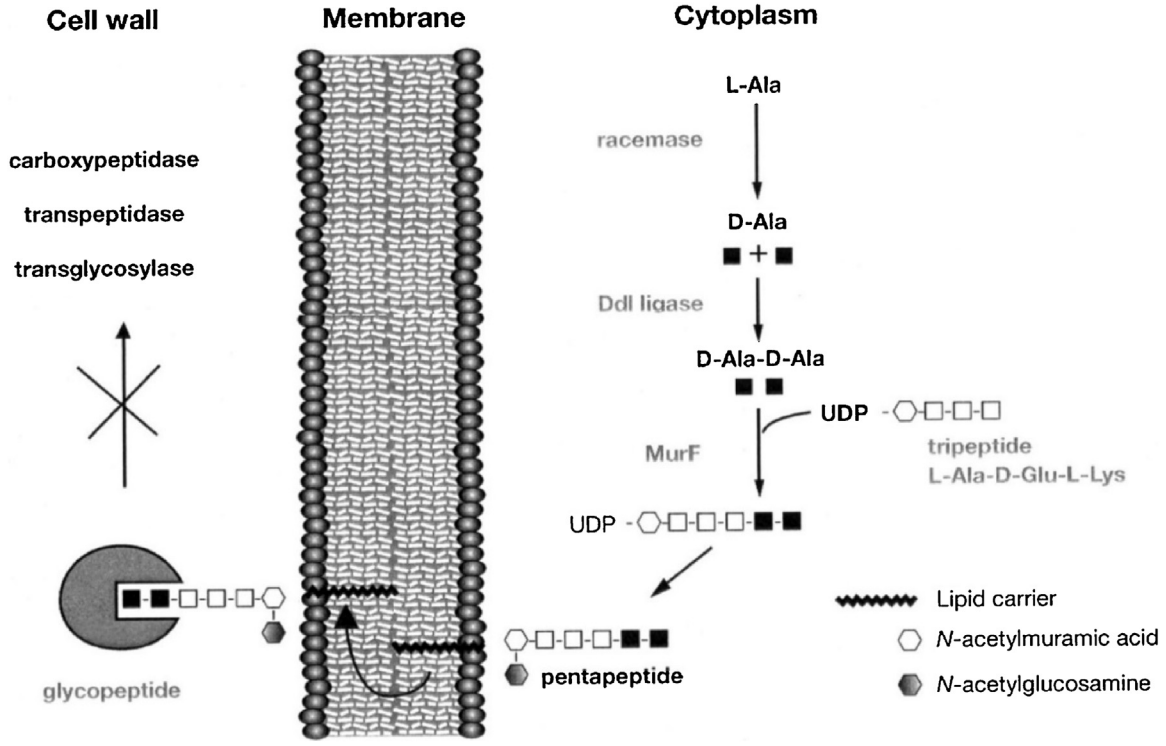

Fig. 2. Peptidoglycan biosynthesis and mechanism of action of glycopeptides such as vancomycin [64].

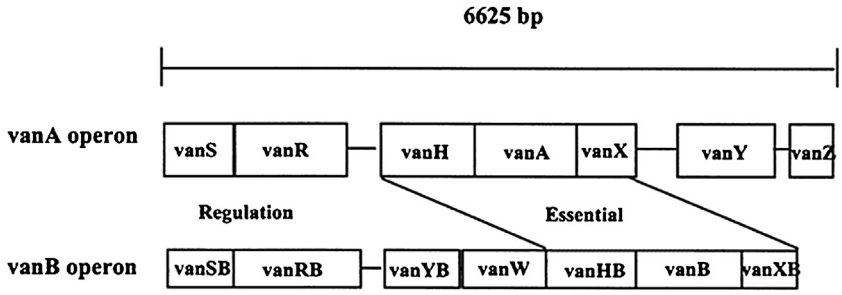

Fig. 3. Comparison of *vanA* and *vanB* gene structures [146].

pyruvate to D-Lac. The ligase encoded by the *vanA* gene then catalyses an ester bond between D-Ala and D-Lac [67]. The resulting dipeptide can be incorporated into the peptidoglycan resulting in a severe reduction in vancomycin affinity [66]. It is important to note that simultaneous production of D-Ala-D-Ala and D-Ala-D-Lac precursors does not result in a significant increase in vancomycin resistance as sufficient vancomycin binding to D-Ala-ending peptidoglycans still renders the cell susceptible [67]. 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 [68]. A D,D-carboxypeptidase encoded by *vanY* cleaves remaining D-Ala at the C-terminus end of developing peptidoglycans left behind by VanX [69]. The two enzymes coded by *vanX* and *vanY* ensure the removal of susceptible D-Ala-D-Ala binding sites for glycopeptides.

The structure of the *vanB*-type operon is similar to the *vanA* operon. It contains a dehydrogenase, a ligase and a dipeptidase gene component that has 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* [67]. Although *vanA*- and *vanB*-type resistance is induced by teicoplanin and vancomycin respectively, transcriptional activation of both operons follows the same mechanisms (Fig. 4). Even though the *vanB*-type is not commonly known to carry teicoplanin resistance, evidence of a novel *vanB2* teicoplanin-resistant variant has been identified [70]. The *vanB* operon has an additional *vanW* gene but does not have the *vanZ* gene compared with the *vanA* operon (Fig. 3).

Based on the sequence difference, the *vanB*-type operon has been subdivided into three subtypes: *vanB1*; *vanB2*; and *vanB3* [71,72]. 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 showed 202 isolates (99%) carrying the *vanB2* gene [61]. Analysis of the conjugative transposon Tn*5382*, which carries the *vanB2* gene, suggested that horizontal gene transfer was responsible for its dominance [61]. 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).

vanM-type resistance consists of 1032 bp encoding a 343-amino acid protein that shares approximately 80% sequence identity with vanA. The *vanM* operon does not possess the *vanZ* or *vanW* component [73]. The *vanM*-type, like *vanA*, *vanB* and *vanF*, confers vancomycin resistance through inducible synthesis of precursors ending in D-Ala-D-Lac. The operon organisation, however, mostly resembles that of *vanD*. Upstream of the *vanM* cluster lies an IS*1216*-like element that 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 [26,74,75].

The *vanD* operon, which is only found in *E. faecium*, is exclusively located on the chromosome and cannot participate in horizontal gene transfer [76]. Although vancomycin and teicoplanin resistance conferred by the *vanD* gene cluster is typically low, they can reach concentrations of up to 256 μg/mL and 64 μg/mL, respectively. The organisation 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 [77–79]. 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 only *Enterococcus gallinarum* and *Enterococcus casseliflavus*, provides resistance to vancomycin. The biochemically and phenotypically similar *vanE* operon is only found in *E. faecalis*.

3.10. Epidemiology

Enterococcus faecium has the ability to survive in extreme conditions, is ubiquitous in the environment and is 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* [80]. In Portugal, antimicrobial-resistant *E. faecium* was recovered from faecal samples of wild rabbits, badgers, forest wildcats, storks, quails, wolf, birds of prey and sewage [81,82]. A separate study also identified ST18 CC17 *E. faecium* from a faecal sample of a wild Iberian wolf in Northeast Portugal, indicating the presence of CC17 *E. faecium* in native wildlife [83]. Elsewhere, CC17 *E. faecium* has been reported in wild corvid birds in the USA, Slovakia and the Czech Republic [84,85]. Although there are many other reports of multidrug-resistant *E. faecium* from wildlife, most studies did not perform MLST.

In the environment, waste water is often reported as a reservoir for CC17 *E. faecium*. A comprehensive study on 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 the UK, Netherlands, USA and Australia [86]. Two other independent studies performed on the effluent waters of two wastewater 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 these isolates were in a minority [87,88].

Besides wild animals and the environment, CC17 *E. faecium* has also managed to adapt to domestic animals. 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 [89–92].

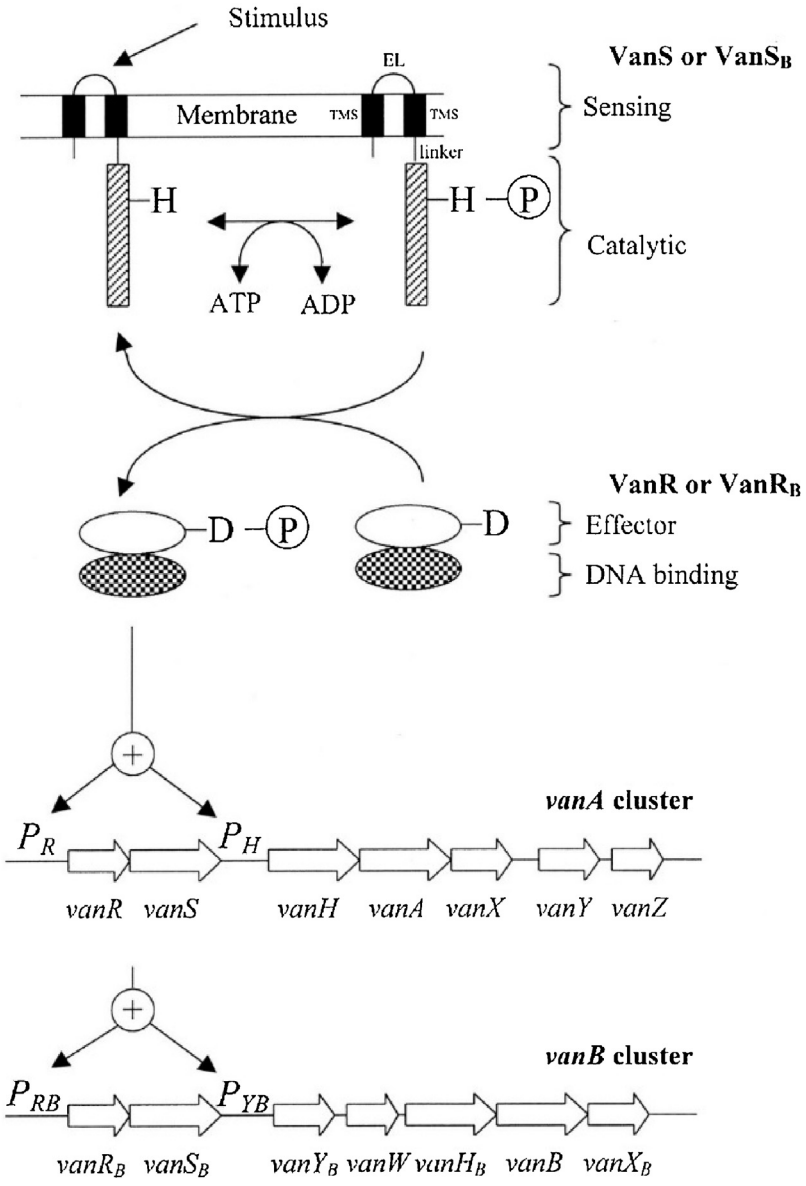

Fig. 4. Transcriptional activation of the *vanA* and *vanB* gene clusters [147].

In Portugal, CC17 *E. faecium* isolates identified in companion cats and dogs were resistant to ampicillin and/or high-level gentamicin [89]. In South Korea, it was reported that ampicillin and ciprofloxacin resistance was high in CC17 *E. faecium* isolated from companion dogs and humans, whilst tetracycline resistance was more commonly identified in isolates from companion dogs. In addition, vancomycin-resistant isolates were only found in CC17 *E. faecium* isolated from humans [90]. These findings suggest that 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 [93]. 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 [94–96]. In Spain, CC17 *E. faecium* were isolated from chicken, veal and rabbit samples, with isolates from all three carrying antimicrobial resistance to vancomycin, ampicillin, erythromycin, ciprofloxacin and high-levels of streptomycin and kanamycin [97]. In Canadian animal farms, low quantities of CC17 *E. faecium* together with MGEs carrying antimicrobial resistance genes have been identified in bovine faecal samples [98].

In Portugal, CC17 *E. faecium* was isolated from farmed pigs and their surrounding environment (manure, waste lagoons and drinking water) [92]. In addition, fresh vegetables sold in Portuguese supermarkets were also found to carry CC17 *E. faecium*, including lettuce, green olives, celery and broccoli [99].

Although exposure to CC17 *E. faecium* in the community may appear high, *E. faecium* is an opportunistic pathogen therefore community-associated *E. faecium* infections are uncommon. In Europe, despite the ban on avoparcin in the animal industry two decades ago, colonisation of VREfm in people without hospital contact or a history of glycopeptide use can vary between 2% to 28% of adults [100,101]. Similarly, in South Korea, which also has a history of avoparcin use, 4.7% of farm animals and 1% of healthy individuals are reported to carry VREfm in their gut [102]. Conversely, in North America, where the use of avoparcin was

prohibited, VREfm was not identified in the healthy adult population sampled [103].

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 [104]. Antimicrobial resistance is more often identified in *E. faecium* (80–100%) compared with *E. faecalis* (0–16%), suggesting that *E. faecium* is able to acquire and express antimicrobial resistance genes more frequently [57–59,105–107].

Critically ill patients such as those in intensive care units hold the highest risk of infection in the hospital, followed by patients in haematology, neonatal and renal units [108]. Amongst the patients in these wards, patients undergoing organ transplant pose the highest risk, followed by patients with prolonged hospital stay. Prior therapy with antimicrobials that are ineffective against *E. faecium*, such as third-generation cephalosporins, increases the risk of colonisation and infection.

In the hospital, *E. faecium* remains viable on inanimate surfaces from 7 days to 2 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 healthcare workers [109–111]. A previous study that reported low recovery of VREfm from rectal swabs of healthcare workers suggests that healthcare workers do not serve as major VREfm reservoirs and that VREfm colonisation 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 [108]. The urinary tract is the most common point of entry for enterococci into the bloodstream, which leads to bacteraemia, the leading cause of *E. faecium* morbidity and mortality [112]. Other sources of *E. faecium* leading to bloodstream infection (BSI) include intravenous lines and abscesses. However, a significant proportion remain unknown and are assumed to originate from the intestinal microbiota [113,114]. *Enterococcus faecium* is able to translocate across the luminal surface of the intestines in a similar fashion to *Candida albicans* and *E. coli* [115,116]. Secondary to BSI, enterococci account for 5–20% of native valve-related and 6–7% of prosthetic valve-related bacterial endocarditis [117]. In addition, heart valve vegetation increases the risk of bacterial adherence, enhancing the risk of infection.

In a 2016 Australia-wide surveillance of enterococcal bacteraemia that included 1058 patient episodes, 39% of isolates were *E. faecium*, of which 46.5% were vancomycin-resistant [55]. Compared with a related survey conducted in 2005, when the prevalence of vancomycin resistance in *E. faecium* was reported at 7% [118], the 2016 data represent a seven-fold increase in prevalence. 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 that 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 BSIs 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 characterised, of which, 99% were *E. faecium* and 98% carried *vanA*-type vancomycin resistance [120].

In South America, a multicentre study involving 32 hospitals from Colombia, Ecuador, Perú and Venezuela found 31% of *E. faecium* isolates carry *vanA*-type resistance to vancomycin [121]. In addition, all representative isolates of pulsed-field gel electrophoresis (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 *vanA*-type vancomycin resistance in all isolates [122]. In addition, 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) [123]. In Cuba, two CC17 *E. faecium* clones (ST656 and ST262) were resistant to ampicillin, quinolones, imipenem, high-level gentamicin, erythromycin, clindamycin, vancomycin, teicoplanin and trimethoprim/sulfamethoxazole [124].

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 [125,126]. 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 reported significantly increasing trends over the last 4 years.

In Asia, studies conducted in China predominantly report ST78 CC17 *E. faecium* carrying *vanA*-type vancomycin resistance. However, *vanM*-type resistance has been reported in selected areas [62,63,127,128]. In Malaysia, ST17, ST203, ST78 and ST601 *E. faecium* isolates belonging to CC17 were identified at a local hospital [129]. In South Korea, only *vanA*-type resistant (43/531) *E. faecium* isolates were identified in a 3-year study of 212 non-tertiary hospitals [130]. 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, ST17 and ST18 as the dominant STs [131,132].

4. The future

VREfm outbreaks not only incur significant costs for healthcare systems but also place 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 [54,133,134]. Other reports make a synonymous point on the importance of ongoing surveillance [135,136]. Mathematical modelling developed by D'Agata et al. predicted that the only preventative measure which could potentially eradicate VREfm from an institution is to prevent colonised patients from entering the hospital [137]. This, however, is an unrealistic goal. 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 [138] 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 sanitisation, antimicrobial stewardship, and use of sterile equipment and personal protective gear [14,139]. 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 *S. 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 [140–143]. However, resistance to both antimicrobials has been reported in *E. faecium*. Antimicrobials such as tigecycline and quinupristin/dalfopristin 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 [144]. Although new alternatives such as tedizolid, telavancin, oritavancin and dalbavancin have only recently been approved by the US Food and Drug Administration (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) utilising the same mechanism of action with some enhanced activity [145]. 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. 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 the *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 owing to the risk of untreatable infections.

Funding

None.

Competing interests

None declared.

Ethical approval

Not required.

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