Mechanisms of gram-positive vancomycin resistance (Review)

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Received October 4, 2021; Accepted November 15, 2021

DOI: 10.3892/br.2021.1490

Abstract. Vancomycin-resistant bacteria (VRB) are important consideration in medicine and public health as they can cause life-threatening infections that appear to be resistant to therapy and persist in the body after medication. A wide spectrum of antimicrobial resistance characteristics, as well as various environmental and animal settings underlie the evolution of the most prevalent the most prevalent van genes in the VRB genome, indicating significant gene flow. As illnesses caused by VRB have become increasingly complex, several previously effective therapeutic techniques have become ineffective, complicating clinical care further. The focus of this review is the mechanism of vancomycin resistance in *Enterococci*, *Staphylococci* and *Lactobacilli*.

Contents

1. Introduction

Gram-positive bacteria, such as the *Enterococcus* species, *S. aureus* and *Clostridium difficile* can be treated with vancomycin and similar glycopeptides, which are medications of last resort. Vancomycin's bactericidal effect was long hypothesized to be impervious to resistance as it binds to the bacterial cell envelope rather than to a protein target, as is the case for other antibiotics. Vancomycin's therapeutic efficacy is threatened by two types of complex resistance mechanisms, each involving a multi-enzyme route, that have arisen and are becoming extensively more common in pathogenic species. Precursor degradation and substitution with DAla-D lac or D-Ala-D-Ser alternatives, which vancomycin has a poor affinity for, constitute the mechanisms of resistance. Vancomycin resistance has been studied extensively for >30 years, and significant progress has been made in the understanding of the molecular biology of the enzyme cascades involved (1,2). The large size and high molecular weights of glycopeptides make them ineffective against gram-negative bacteria as they cannot penetrate past the outer membrane. Vancomycin is effective against all gram-negative bacteria, but nearly all gram-positive bacteria are vulnerable to glycopeptides (3). Inhibition of the bacterial cell wall by glycopeptides results in exposure of the fragile cytoplasmic membranes surrounding the bacterial cells, resulting in their bactericidal activity. There is an increase in osmotic pressure inside bacterium cells, causing them to quickly expand until they burst open (4). This review summarizes the mechanisms of gram-positive vancomycin resistance, which may be useful in the cure of illnesses caused by these pathogens.

2. Mechanisms of vancomycin resistant *Enterococci* (VRE)

vanA. Novel therapies for VRE are urgently required according to the World Health Organization (4). Vancomycin resistance is potent [Minimum Inhibitory Concentration (MIC) 64 μg/ml], and resistance to teicoplanin is modest (16-51 μg/ml) in *Enterococci*. *E. faecalis*, *E. faecium* and *E. casseliflavus* are the most common sources of vanA *Enterococci*. However, this type of resistance is also found, although more rarely, in *E. avium*, *E. durans*, *E. gallinarum* and *E. raffinosus* (5). A gene cluster on the transposon Tn1546 controls vanA resistance. Transposons are normally found on plasmids, although they can also be situated on bacterial chromosomes and vice versa (6). The resistance genes can be passed on to various *Enterococci* species (6) and even to other genera, such as *Staphylococci*, through conjugation (6,7). It has been established that when the vanA cluster of genes is expressed, peptidoglycan precursors are synthesized that end in a depsipeptide termed D-Alanyl-D-lactate (D-Lac) rather than the usual D-Ala-D-Ala end. Vancomycin has a far lower affinity for D-Ala-D-Lac than it does for a regular

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Key words: mechanisms, resistance, vancomycin, gram-positive bacteria

dipeptide product (8-10). When the vanA protein is activated, it produces the depsipeptide D-Ala-D-Lac as a byproduct (11). To use the protein coded by vanH in the above reaction, pyruvate must first be converted to D-lactate by reducing it with a D-hydroxy acid dehydrogenase enzyme (12). When it comes to the enzymes that break down D-Ala-D-Lac, the vanX protein is inactive. In order to reduce competitive pentapeptide synthesis, D-hydroxy acid dehydrogenase cleaves the D-Ala-Dla generated by conventional ligase activity (13,14).

Additionally, the vanA cluster contains the regulatory genes vanS and vanR, which control the transcription of the aforementioned vanH, VanA and vanX genes, in addition to the crucial genes stated above. As the gene's protein product is a membrane-associated sensor protein that detects the presence of vancomycin via an unknown method, the gene was named vanS (15). A sensor protein that has been triggered by vancomycin signals the cytoplasmic vanR protein (the response regulator), which functions as a transcriptional activator and activates the promoter located between the vanS and vanH genes, to send signals. This then stimulates co-transcription of the chief glycopeptide resistance genes, vanH, VanA and xenopus tubulin (16).

Vancomycin resistance is formed by the united bustle of the three enzymes represented by the three primary resistance genes: vanH, vanA, and vanX, and cannot be produced by the vanA gene alone. VanR and vanS are important regulatory genes that govern the primary resistance genes (15). An additional two genes, vanY and vanZ, are carried by the transposon Tn1546 and are positioned downstream of the gene cluster vanA. These two genes can aid glycopeptide resistance, although they are not required for it (17). With regard to the vanY protein (also known as D,D-carboxypeptidase), the vanA cluster appears to be the key regulator. Transcriptional control of the vanA cluster has been shown to be complicated, as evidenced by the fact that the vanY protein is produced when vanA is present. vanY eliminates any normal membrane-bound peptidoglycan precursors that evade the action of the vanA gene cluster by cleaving the D-Ala terminus. As a result of vanY activity, glycopeptide antibiotic resistance is increased (17). The vanZ protein raises the MICs for teicoplanin, but not for vancomycin; however, the mechanism underlying this remains to be elucidated (18).

vanB. Vancomycin-resistant Enterococcal bacteria become vancomycin resistance at low levels of resistance (MIC >8 μg/ml), but are sensitive to teicoplanin. Only vancomycin, but not teicoplanin, induces vanB resistance, whereas the previously described vanA resistance is induced after treatment with either vancomycin or teicoplanin. Most often, the vanB phenotype is found in *E. faecalis*, although it can also be found in other *Enterococcal* species on rare occasions (8). The gene cluster controlling vanB resistance is the same as that controlling vanA resistance (vanB gene and related genes). Tn1547 is the transposon on which the vanB class gene cluster resides. While it can also be found on plasmids, this transposon is more commonly found in the bacteria's chromosomes (19). Some *Enterococci* (20) and other genera, such as *S. bovis*, carry the vanB type of vancomycin resistance gene through conjugation (21). This gene's protein product (vanB) is physically related to the vanA ligase but functions abnormally (76% amino acid homology). Using the pentapeptide ending in the depsipeptide D-Ala-D-Lac, vanB ligase promotes the formation of aberrant peptidoglycan precursors (22).

Genetic resistance genes of the vanB type, which correspond to vanA type, are classified as follows: vanRB, vanSB, vanHB and vanXB. However, unlike the vanA class vanS protein, which does recognize teicoplanin and is responsible for inducing vanA resistance, the vanSB protein does not detect this glycopeptide. vanB-resistant bacteria lack the vanZ gene, which is only found in vanA-resistant bacteria (23). The vanB gene reveals DNA sequence variability, allowing the subtyping of the vanB gene into three subtypes: vanB-1, vanB-2 and vanB-3 (24,25). vanB-2 is the mst potent gene of the vanB family (26).

vanC. Unlike vanA and vanB resistance, which can be acquired and transferred, the vanC type of resistance was found in *E. gallinarum*, *E. casseliflavus*, and *E. flavescens* as an innate constitutive feature (27). This type of resistance is characterized as resistant to vancomycin (MIC 8-32 μg/ml) but sensitivity to teicoplanin (8 μg/ml). Genes on the bacterial chromosome govern this sort of resistance, which cannot be passed from one individual to another (27,28). The vanC gene's protein output is a dipeptide called D-Alanyl-D-Serine, which terminates in a pentapeptide called vanC. This change in the peptidoglycan production impairs vancomycin binding and abrogates its function (29). In vanC-resistant bacteria, different quantities of D-Ala-D-Ser are present in comparison to normal levels, which may explain the varying levels of vancomycin resistance (30,31). *E. gallinarum* has a vanC-1 gene, *E. casseliflavus* has a vanC-2 gene and E. flavescens has a vanC-3 gene (19). Vancomycin resistance (MIC >256 μg/ml) and teicoplanin resistance (vanA genes have been found in several *E. gallinarum* and *E. casseliflavus* strains), and the number of genes conferring resistance was higher than expected (32).

vanD. In 1997, researchers discovered a novel gene which conferred vancomycin resistance which they named vanD. When tested against vancomycin and teicoplanin, the resistant strain of *E. faecium* showed a MIC of 64 and 4 μg/ml, respectively (33). Further research showed that the vanD gene is a ligase gene that is distinct from, but comparable to, the vanA and vanB genes, and the amino acid sequence of vanD showed 67% homology to the vanA genes. *Enterococci* cannot share the same vanD gene as it is situated on chromosomes (34). According to more recent research, vanD-type *Enterococci* resistant to vancomycin synthesize only peptidoglycan originators lacking the D-Ala-D-Lac, which confers constitutive resistance to vancomycin on those bacteria. The vanD gene encodes a ligase similar to the vanA and vanB types. Another gene belonging to the vanD cluster has been discovered that is analogous to the vanA-type genes. This gene, which encodes a dehydrogenase (vanHD), was termed vanHD. The dipeptidase (vanXD) gene has been termed vanXD (35).

vanE. *E. faecalis* BM4405, which is resistant to low concentrations of vancomycin (MIC 16 μg/ml) but susceptible to teicoplanin (MIC 0.5 μg/ml), possesses a more recently discovered vancomycin resistance gene named vanE (36).

This novel type of resistance resembles that conferred by the intrinsic vanC type. A larger proportion of amino acid sequence similarity may be found in vanE (55%) than in other van proteins (45%) (36).

3. Vancomycin resistance in *Enterococci* is conferred by several genes

There has been evidence of both vancomycin resistance and dependency in some *Enterococci* strains of the vanA and vanB vancomycin resistance classes. The vancomycin-dependent *Enterococci* have been isolated by plating them on vancomycin-containing agar, such as that used for the separation of Campylobacter or Gonococci, from clinical samples that seemed to be culture negative (36). If these organisms stop producing D-Ala-D-Ala, one hypothesis is that they will be unable to continue grow without a new structure to replace it. It is only when vancomycin is present that most vanA and vanB types of resistance occur, as vancomycin induces the creation of vanH dehydrogenase and of D-ala-D-lac-ligase (30). As long as vancomycin is present, bacteria may be unable to produce D-Ala-D-Ala, and even vanX destroy bacteria. This may explain why bacteria are unable to synthesize cell walls whilst D-Ala-D-Ala is present. As soon as the vancomycin is removed, the cell stops growing and replicating as it needs to either produce D-Ala or D-Ala D-Lac in order to continue growing and replicating (36,30). If vancomycin independence is restored, it may be explained by a mutation leading to constitutive D-Ala-D-Lac production or via (re-)activation of the standard mechanism for synthesizing D-Ala-DAla (36,37).

4. Mechanisms of resistance to vancomycin in *Staphylococci*

S. aureus strains, particularly methicillin-resistant strains, have been found to exhibit tolerance to vancomycin, according to early findings (38). Tolerance has been proposed as the reason for the failure of vancomycin treatment of infections caused by these bacteria despite the presence of MIC-detected susceptibility. Studies have also indicated that these resistant *S. aureus* strains increase mortality in individuals with bacteremia or endocarditis, despite vancomycin therapy (38,39). Vancomycin-resistant *S. aureus* (VRSA) strains have been identified, and these bacteria have *in-vitro* MIC values >4 μg/ml. Vancomycin resistance has been observed in both *S. aureus* and coagulase-negative staphylococci (40). VRSA is more prevalent in Africa and Asia than in Europe and the United States, and vanA and SCCmec II were the most common genetic factors associated with VRSA (41).

The emergence of VRSA was particularly noticeable in institutions with a high prevalence of methicillin-resistant *Staphylococci* and a policy of indiscriminate glycopeptide administration. The extensive use of glycopeptides (vancomycin or teicoplanin) has permitted the selection of resistant strains from both *S. aureus* and coagulase negative *Staphylococci*, primarily from *S. epidermidis*, followed by *S. hemolyticus*, *S. hominis* and *S. warneri* (42-44). Additionally, glycopeptide-resistant mutants of *S. aureus* have been selected experimentally by gradually increasing the quantity of vancomycin present during *in vitro* development, supporting this theory (9). Furthermore, the discovery that vancomycin heteroresistance is a widespread occurrence among *Staphylococci* also supports this theory (45,46). When vancomycin is used to treat bacterial infections, the presence of heteroresistance may allow for the selection of resistant strains in the body.

Due to the discovery of the vancomycin heteroresistance phenomena in *Staphylococci*, the procedures employed in the clinical microbiology laboratories to distinguish vancomycin resistance may need updating/adapting. The inoculum tested must be large enough for justification of the occurrence of the phenotype. *Staphylococci* vancomycin resistance has been shown to be reversible in the lab. S. aureus isolates with vancomycin resistance were repeatedly cultured on non-selective media in an effort to demonstrate this phenotypic reversal. Moreover, the vancomycin-resistant subpopulations were eliminated from several obtained strains. Reversion of vancomycin resistance phenotype may account for the difficulty in obtaining vancomycin-resistant clinical *Staphylococci* isolates from patients who do not respond to vancomycin therapy, as well as some of the challenges in identifying these isolates within the clinical microbiology laboratory (47). Primary testing of a vancomycin-resistant *S. epidermidis* strain was performed in a case study. A second test was accomplished on the isolate cultured to validate the first result, and the outcome was that the strain remained sensitive to vancomycin even after multiple tests. As it was unclear whether the initial primary test was flawed, researchers performed a second round of testing on the same patient (48). According to the data, there was no error in the primary susceptibility test, but there was a problem with an obscure phenotype (49). These findings may lay the groundwork for a novel approach to the clinical laboratory cultivation of *Staphylococci*. Vancomycin-resistant isolates can be maintained on the normal media as well as a medium containing low levels of the antibiotic, which may be preferable. The basic results of vancomycin susceptibility testing, not the results from subcultures, should be taken into account as well. The method by which *Staphylococci* develop resistance to vancomycin remains unknown at present, to the best of our knowledge. However, *in vitro* experiments and mouse skin transfers of the vanA gene cluster from *Enterococci* to *S. aureus* have been demonstrated in the laboratory. Clinical isolates of *Staphylococci*, which frequently co-colonize wound infection sites with *Enterococci*, may exhibit this route of genetic transmission (50). However, no investigation has shown the presence of any van gene clusters in clinical *Staphylococci* isolates, and hence a mechanism of this nature has not been observed (51).

Vancomycin-resistant *Staphylococci* in a liquid media form bacterial cell aggregates when exposed to vancomycin. Following this, the drug is no longer detected in the medium, and the bacterial aggregates break down into single cells with the same appearance as cells grown in an antibiotic-free environment. Electron microscopy examination of the bacterial aggregates revealed the development of substantial amounts of extracellular material with staining qualities comparable to those of the cell wall. Bioactive vancomycin may be extracted from cell wall material. These findings suggest that bacteria may sequester antibiotic molecules in a bound form on an additional cell wall substance, making them resistant to the activity of vancomycin (52-54).

A total of 16 vancomycin-resistant *S. aureus* clinical isolates from 7 countries were tested in a drug-free medium. Vancomycin-susceptible strains (MIC 4 μg/ml) were obtained after 10-84 days of these passages, but reversion to vancomycin resistance was achieved by a single step of vancomycin selection. All vancomycin-resistant strains had thicker cell walls that became thinner when vancomycin resistance was lost and thick again when vancomycin resistance was regained in a laboratory setting. *S. aureus* vancomycin resistance can develop under the selective pressure of long-term vancomycin use, but the resistance can be overcome by discontinuing the drug's use. It is possible, according to this research, that vancomycin resistance is caused by thickening of the bacterial cell wall, or some property associated with this (55).

Even though vancomycin-resistant *Staphylococci* thickening has been linked to complex reorganization of cell wall metabolism (10), with additional wall material displaying lower peptidoglycan cross-linking of the side chains of D-Ala (56). The complex reorganization of cell metabolism and lower peptidoglycan cross-linking prevents vancomycin from reaching the intracellular target molecules as it binds to these free termini outside the cell wall (54). In these bacteria, the thickening and metabolic reorganization of the cell wall are likely acquired through a genetic mutation (38,39). In order to better understand how these strains develop vancomycin resistance, additional research is needed to improve our understanding of vancomycin resistance in these strains and highlight potential therapeutic options.

5. Mechanisms of resistance to vancomycin in lactic acid bacteria

The lactic acid bacteria *Lactobacillus*, *Leuconostoc* and *Pediococcus* are more likely to exhibit/attain vancomycin resistance. Human commensal lactic acid bacteria have been isolated from the digestive and genitourinary tracts of individuals, as well as from dairy products. Vancomycin is ineffective against lactic acid bacteria as these bacteria are genetically resistant to it, due to the weak affinity of vancomycin binding to their cell walls (32,33). This weak binding was shown to be caused by the establishment of peptidoglycan precursors terminating in the depsipeptide D-Ala-D-Lac observed in *Enterococci*'s vanA and vanB resistance classes to vancomycin, highlighting a potential direction for analysis of the peptidoglycans present in their cell walls. DNA probes for the vanA or vanB genes failed to hybridize with glycopeptide-resistant lactic acid bacteria. These findings suggested that these natively glycopeptide-resistant bacteria included a natural ligase that catalyzes the production of D-Ala-D-Lac instead of D-Ala-D-Ala, to which vancomycin binds with high affinities (24,43).

6. Conclusions

There have been significant breakthroughs in our understanding of the molecular processes and genetics of vancomycin resistance in gram-positive bacteria. Despite this, it remains unknown where the resistance genes have originated from. A potential source of resistance for glycopeptide-producing organisms is the presence of genes encoding homologues of vanA, vanH, vanR, vanS and vanX in these species' secondary metabolic products. For infections with multi-resistant strains of *Staphylococci*, Streptococci and *Enterococci*, glycopeptides, alone or in combination with another antibacterial, are typically the sole mode of treatment. Vancomycin-resistant gram-positive bacteria can become resistant to all antibiotics if they develop and spread significant levels of resistance to the drug. Therefore, knowledge of the molecular and structural vancomycin resistance pathways may highlight potential avenues for addressing this serious issue.

Acknowledgements

The author would sincerely like to thank the Jouf University, Kingdom of Saudi Arabia, for their unbounded support.

Funding

No funding was received.

Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

SS wrote and revised the manuscript. The author has read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The author has declared that no competing interest exists.

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