Review and Management of Glycopeptide Resistance in *Enterococcus* and *S. aureus* in the Era of Multi-Drug Resistant Organism (MDRO)

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Abstract

The evolution of *Enterococcus* and *Staphylococcus aureus* in the modern antibiotic era has been define by distinct strains emergence events, many of which include acquisition of antibiotic resistance. The high worry of methicillin-resistant *S. aureus* (MRSA) in healthcare and community settings is a major concern worldwide. Here, we review in brief progress made toward understanding the acquisition of vancomycin resistance in *S. aureus* and their Epidemiology.

Objective of the Study: The objective of this study is to determine the frequency of the antimicrobial resistance of *Enterococci* and *Staphylococcus* isolated in hospitalized patients, the importance role of infection control to stop their spread in the facility and in the community and to stop the abuse of antibiotics.

Keywords: Staphylococcus aureus; Enterococcus; Antibiotic Resistance; Vancomycin; VRE; hVISA; VISA; VRSA

Abbreviations

MSSA: Methicillin-susceptible *S. aureus*; MRSA: Methicillin-resistant *S. aureus*; VRSA: Vancomycin-resistant *S. aureus*; VISA: Vancomycin- intermediate *S. aureus*; hVISA: Heterogeneous Vancomycin- intermediate *S. aureus*; VRE: Vancomycin-resistant *Enterococcus faecium* and *Enterococcus faecalis*; D-Ala: D-alanine; MICs: Minimum inhibitory concentration; GlcNAc: N-acetyl-D-glucosamine; (MurNAc): N-Acetylmuramyl acid

Introduction

The first vancomycin-resistant clinical isolates of *Enterococcus faecium* and *Enterococcus faecalis* were reported in Europe in 1988 [1,2]. The same strains were later detected in hospitals on the East of the United States [3]. Since that time, vancomycin-resistant *Enterococci* have spread rapidly and are now found in most hospitals of the world [4]. Actually, the best choice to treat methicillin-resistant *Staphylococcus aureus* (MRSA) and *Enterococci* infections in case of penicillin allergic patients are the Glycopeptides especially vancomycin. The frequent use of vancomycin mainly due to increase of MRSA infections in hospital and in the community. Unfortunately, the heavy use of glycopeptide could be the main cause on the isolation of *S. aureus* with reduced susceptibility to vancomycin. Several studies suggest that vancomycin treatment failure could be associated with reduced vancomycin susceptibility *S. aureus*. Various forms of glycopeptide resistance have appeared in MRSA strains and reported, including high-level resistance, homogeneous and heterogeneous intermediate resistance. While vancomycin-resistant *S. aureus* (VRSA) strains are limited and vancomycin- intermediate *S. aureus* (VISA) strains remain rare; the more common isolates are heterogeneous (hVISA) strains. The present article highlight the actual knowledge concerning the history, epidemiology and the detection methods of 'glycopeptide resistance in *S. aureus* and *Enterococcus* and discusses therapeutic options for the treatment of the infections caused by them.

Mode of action of vancomycin

The peptidoglycan synthesis and the production of bacterial cell walls are required several steps. In the cytoplasm, L-alanine converted to D-alanine (D-Ala) by a racemase, then by a ligase, 2 molecules of D-Ala are joined and forming the dipeptide D-Ala-D-Ala, which is then added to uracil diphosphate-N-acetylmuramyl-tripeptide to form uracil diphosphate-N-acetylmuramyl-pentapeptide. This structure is bound to a lipid carrier, the undecaprenol, which, after the addition of GlcNAc from uracil diphosphate-GlcNAc, allows transferee the precursors to the exterior surface of the cytoplasmic membrane. The disaccharide-pentapeptide is then incorporated into the preexisting peptidoglycan by two enzymatic reactions transglycosylation and by transpeptidation. The high affinity of Vancomycin to the D-Ala-D-Ala C-terminus of the pentapeptide leads to block the addition of the new precursors to the preexisting peptidoglycan by inhibition of the two reactions. As vancomycin does not penetrate into the cytoplasm; the interaction with its target cannot take place only after the transfer of the precursors to the surface exterior of the cytoplasmic membrane [5,6].

Vancomycin was isolated from a Gram-positive filamentous Actinomycete called *Amycolatopsis orientalis* and was approved for use by the U.S. Food and Drug Administration in 1958 [7,8] Vancomycin inhibits cell wall synthesis. Until now, nine types of acquired resistance genes have been identified in *Enterococci*. Eight of them (VanA, VanB, VanD, VanE, VanG, VanL, VanM, and VanN) belong to acquired resistance whereas VanC in of *E. gallinarum* and *E. casseliflavus* is an intrinsic resistance. Glycopeptide resistance in *Enterococci* results from the production of modified peptidoglycan precursors ending in D-alanyl-D-lactate (D-Ala-D-Lac) (VanA, VanB, VanD, and VanM) or D-alanyl-D-serine (D-Ala-D-Ser) (VanC, VanE, VanG, VanL, and VanN) to which vancomycin binds with low affinity and the elimination of high-affinity precursors ending in D-Ala-D-Ala [9,10]. In VRSA isolates the resistance is caused by the horizontal transfer (plasmid carrying the *vanA*) from vancomycin-resistant *E. faecalis* [11,12]. Vancomycin is known to inhibited transpeptidation by binding to the terminal

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D-Ala-D-Ala of peptidoglycan in the bacterial cell wall. VRSA strains acquired *vanA* gene complex became enables to synthesize cell wall precursors terminating in D-Ala-D-Lac for which vancomycin has a very low affinity. In the presence of vancomycin, the peptidoglycan assembly continued by the novel cell wall precursors [12,13]. So the replacement of the new Target D-Ala-D-Lac depsipeptide replaces the D-Ala-D-Ala dipeptide in peptidoglycan synthesis, resulting the decreases affinity of the molecule for Glycopeptides considerably [10]. Until today $VanA$ is the only phenotype of resistance detected in $Staphylococcus\ aureus$ and the most frequently in $Enterococci.$ The $vanA\ gene\ has\ been\ found\ in\ E.\ faecium, E\ faecalis, E.\ avium\ and\ E.\ durans,$ and in highly resistant to vancomycin and teicoplanin atypical isolates of $E.\ gallinarum$ and $E.\ casseliflavus.$ VanB phenotype of resistance functioning as the same way of VAN A (synthesis of peptidoglycan precursors ending in the depsipeptide D-Ala-D-Lac) instead of D-Ala-D-Ala [14], but differs in its regulation because of vancomycin, but not teicoplanin.

Glycopeptide-dependent *Enterococcus* strains

Vancomycin dependence is an important clinically phenomenon has developed in some VanA- and VanB-type *Enterococci*. These strains are not only resistant to vancomycin and teicoplanin, but also require their presence for growth. These strains have been isolated *in vitro*, in animal models and from patients treated with vancomycin for longtime [15,16]. In these strains, *vanA* or *vanB* encoded D-Ala: D-Lac ligase is induced on the presence of vancomycin which lead to the defect in synthesis of peptidoglycan precursors ending in D-Ala-D-Ala because of the missing of a functional Ddl following various mutations in the ddl gene and, thus, permits growth of the bacteria.

History of VRSA, VISA and hVISA and mechanism of resistance to vancomycin

In 1997, a MRSA strain with vancomycin MIC of 8 μg/ml (Mu50, VISA) was isolated from the surgical wound infection from a 4 month-old male infant who had undergone cardiac surgery [17]. Later on, infections due VISA were reported in two patients from USA and one from France. After the evolution of VISA, a novel phenotype of vancomycin resistance (hVISA) was described in 1997 [18]. The first hVISA strain Mu3 was isolated from the sputum of a 64-year-old patient with MRSA pneumonia who failed vancomycin therapy. Similar and First of hVISA Strain (D958) with reduced susceptibility to Glycopeptides was isolated from blood culture in Saudi Arabia and reported 2010 [35]. hVISA strains are susceptible to vancomycin by the standard broth microdilution reference method (vancomycin MIC ≤ 2 μg/ml) but contain subpopulations of cells (one in every 10^5 -10^6) for which the vancomycin MIC is in the intermediate range, currently defined as 4 - 8 μg/ml by the Clinical and Laboratory Standards Institute (CLSI) [19]. Actually, hVISA strains are more common than VISA and different rates are reported from different countries. In 2002, the first (VRSA), vancomycin MIC ≥ 16 μg/ml] was reported from United States (U.S). Study from Brazil showed that *vanA*-containing pBRZ01 plasmid described in MRSA was acquired by MSSA strain isolate from the same patient [20]. hVISA could be is the phase before the development of VISA and vancomycin has a selective pressure to leads to growth of VISA uniform subpopulations [21]. Thickened cell wall and reduced peptidoglycan cross-linking are the most common phenotypic changes observed in hVISA/VISA [7,21,22] which leads to an increase in free D-Ala-D-Ala residues (binding sites for vancomycin). It is assumed that vancomycin binds to these free residues of D-Ala-D-Ala in the exterior layers of the thickened cell wall and is unable to reach its site of action at the cell membrane [14]. The trapped vancomycin molecules within the cell wall clog the peptidoglycan and form a barrier towards further incoming vancomycin molecules. So, collaboration of the clogging and cell wall thickening could be leading to glycopeptide resistance [23,24]. Also thickened cell wall, hVISA/VISA strains present other changes including reduced autolytic activity, which produced hemolytic activity and slow growth *in vitro* [11,21]. The *vanA* gene complex, which induced high-level resistance to Glycopeptides in *Enterococci*, was detected in VRSA isolates. The molecular mechanisms of glycopeptide resistance in hVISA/VISA are still not clear until now and need more investigations.

Detection of hVISA, VISA and VRSA in the laboratory

As the CLSI MIC criteria (MIC: 4 - 8 μg/ml for VISA and MIC ≥ 16 μg/ml for VRSA), detection of VISA and VRSA strains is easier than detection of hVISA. These criteria have been determined by using the broth microdilution (BMD) method. Results obtained by the other methods to define the MIC should be confirmed with BMD [25]. Disk diffusion (Kirby-Bauer) method is not acceptable for vancomycin susceptibility testing of *S. aureus.* In the most of clinical microbiology laboratories, detection of hVISA is big problem. Absence of a specific definition and established method makes the detection of hVISA very difficult [25]. With the traditional testing methods, hVISA strains appear susceptible to vancomycin (MIC ≤ 2 μg/ml) but contain subpopulations (1 per 105 - 106 organisms) that express reduced vancomycin susceptibility (MIC ≥ 4 μg/ml). The Antibiogram Committee of the French Society for Microbiology recommends using MHA medium with 5 μg/ml teicoplanin (MHA5T) for the detection of hVISA. Growth of one or more colonies by using an inoculum of 10 μl of a 2.0 McFarland standard suspension after 48h of incubation is considered positive. In our study Extensive genotyping and molecular characterization was don; Multilocus sequence typing (MLST) was performed as previously described. In addition, Multilocus variable number of tandem repeats analysis (MLVA) was used to evaluate the genomic content of strains as previously described. MLVA was performed

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with 10 primer pairs targeting different loci with limited variability in the number of repeats, and the results showed higher resolution than that obtained by MLST, allowing sub-clustering of clonal complexes defined by using MLST. The reference strains obtained from the NARSA collection (http://www.narsa.net/) were USA100 to USA800 and included two fully sequenced strains, USA300 and USA400. Reference strains were subjected to hybridization simultaneously with strains of ST239 and D958 in order to evaluate relatedness. The microarray was manufactured by in situ synthesis of 11,454-oligonucleotide 60-mer probes (Agilent, Palo Alto, CA), selected as previously described. Briefly, the microarray covers 95% of eight sequenced isolates, including their respective plasmids. Population analysis to detect heteroresistance to vancomycin was performed as previously described by using susceptible isolate, VISA Mu50 and hVISA Mu3 strains as control phenotypes [35].

Epidemiology

hVISA strains are more common, the VRSA strains are limited and VISA strains remain rare to be reported [14,26,27]. The true prevalence of hVISA is unknown, this due to non-standardized detection methods or missing of routine hVISA screening and variation in interpretation in the different clinical setting, geographical region, and differing patient populations [28]. The rates of hVISA globally among MRSA isolates have been ranged from 0 to 73.7% [29]. Several studies, considered that the only isolates suspected as hVISA by screening methods have been considered to PAP-AUC. hVISA predominantly reported for MRSA but it can be detected among methicillin-susceptible *S. aureus* (MSSA) strains [7,30]. High-level resistance to vancomycin in MSSA is very rare, some study from Brazil report characterization of MSSA vancomycin resistant isolated from blood culture [20]. The rate of *S. aureus* isolates demonstrating that the increases of heteroresistance correlated with increasing vancomycin MICs within the susceptible range, some studies demonstrated that heteroresistance has been reported in strains with MICs as low as 0.5 μg/ml [25,31]. MRSA colonization and exposure to vancomycin appear to be the main risk factors for hVISA and VISA infection. Most of hVISA/VISA infections developed in patients with weakened immune system and suffering from serious diseases such as malignancy, renal failure and diabetes, or in patients who have undergone major surgery [7,14]. Outbreaks caused by VISA or hVISA and nosocomial spread are rare and have been reported [27].

Importance of hVISA/VISA and VRSA clinically

Some authors consider that hVISA/VISA could be responsible for treatment failure [35] and also linked hVISA and higher mortality rate. Persistent infection and isolation of MRSA despite the glycopeptide therapy, or relapse of infection after glycopeptide therapy can suggest an infection with hVISA or VISA [35]. It is difficult to determine the significance of hVISA/VISA in clinc because of the lack of defined and controlled prospective studies [7,25]. Most common infections caused by hVISA/VISA including vancomycin treatment failure such as bacteremia, endocarditis, deep abscesses, osteomyelitis, and prosthetic device infections [7,25,28,29].

Options of antimicrobial therapy

The evolution of hVISA/VISA clinical isolates has stimulated the search for new antibiotics. At the same time, there are no guidelines concerning alternative antimicrobial therapy. Actually exist a number of antimicrobial agents which could be used in treatment of hVISA/VISA infections.

Daptomycin: A lipopeptide antibiotic with activity against Gram-positive bacteria. For hVISA and VISA, Daptomycin showed higher bactericidal activity than vancomycin [32].

Linezolid: A synthetic antibacterial agent of the oxazolidinone class. Resistance to linezolid has been reported in *S. aureus* isolates, but the rates of resistance remain very low. Linezolid was found to be useful for the treatment of hVISA/VISA infections [2,10].

Tedizolid: Recently developed OXZL for the treatment of skin and skin structure infections [33].

Tigecycline: A glycylcycline antibiotic for intravenous infusion for the treatment of skin and skin structure infections. The *in vitro* results have shown that tigecycline is active against hVISA/VISA as well as VRSA [34].

Ceftaroline and Ceftobiprole: New cephalosporins have been shown to be active against hVISA and VISA in vitro and in animal studies, but their clinical utility for infections caused by hVISA or VISA remains unknown [21].

Other antimicrobial agents potentially active against hVISA/VISA include lipoglycopeptides (Dalbavancin, Oritavancin and Telavancin), quinupristin-dalfopristin, rifampin and fusidic acid. Monotherapy use of rifampin or fusidic acid known to be develops resistance rapidly, so it's recommended to use these agents in combination with another anti-staphylococcal agent.

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Conclusion

For over four decades, Glycopeptides and especially Vancomycin and teicoplanin are the drug of choice to treat serious beta-lactam-resistant gram-positive infections. However, the emergence and spread of resistance to glycopeptide agents among clinically important gram-positive cocci like (*Enterococcus* and *Staphylococci*) species has made difficult to treat serious infections caused by such pathogens. Today, it is important to look for alternatives antibiotics to treat serious gram-positive infections. It is also more important to prevent the spread and emergence of glycopeptide resistance by taking proper infection control measures.

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