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MECHANISMS OF VANCOMYCIN RESISTANCE IN STAPHYLOCOCCUS AUREUS, ARTICLE REVIEW

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ABSTRACT

Staphylococcus aureus is a major cause of potentially life threatening infections acquired in health care and community settings. It has developed resistance to most classes of antimicrobial agents with dramatic increase in the number of health care associated infections due to vancomycin resistant *S. aureus* (VRSA). Two mechanisms of staphylococci resistance to vancomycin were studied. The first one, by having the *van* genes which was detected in resistant isolates by polymerase chain reaction (PCR). The only gene found in the selected VRSA isolates was *van*A gene. Vancomycin has been used successfully for over 50 years for the treatment of *Staphylococcus aureus* infections, particularly those involving methicillin-resistant *S. aureus*. It has proven remarkably reliable, but its efficacy is now being questioned with the emergence of strains of *S. aureus* that display hetero resistance, intermediate resistance, and, occasionally, complete vancomycin resistance. More recently, an association has been established between poor outcome and infections with strains of *S. aureus* with an elevated vancomycin MIC within the susceptible range. Vancomycin is a glycopeptide antibiotic used for the treatment of Gram-positive bacterial infections.

KEYWORDS: *Staphylococcus aureus*, Pathogenicity, Vancomycin Resistance.

INTRODUCTION

Staphylococcus aureus has been recognized as an important cause of human disease for more than 100 years. It is recognized as a cause of a wide range of infections. These infections range from minor skin infections and chronic bone infections to devastating septicemia and endocarditis.[1,2] vancomycin, a glycopeptide with activity against a wide range of Gram-positive organisms, was discovered in 1952 and by 1958 was registered by the U.S. Food and

Drug Administration for treatment of penicillin-resistant and methicillin-resistant *Staphylococcus aureus*. Unlike the rapid appearance of *S. aureus* resistant to penicillin and semisynthetic penicillins, reduced susceptibility or resistance to vancomycin took over 3 decades to emerge. Strains of *S. aureus* displaying vancomycin heteroresistance (hVISA) and vancomycin intermediate resistance (VISA) were first isolated in Japan in 1996.[3,4] hVISA strains are phenotypically susceptible using broth microdilution (BMD); however, testing in greater detail reveals subpopulations of cells with reduced susceptibility to vancomycin.

Vancomycin-resistant *S. aureus* (VRSA) strains whose resistance is due to acquisition of the *vanA* resistance determinant from enterococci were subsequently reported in the United States, in 2002.[5] VRSA has also been described in Iran and India, although it remains rare worldwide. *Reduced vancomycin susceptibility and resistance in methicillin-resistant Staphylococcus aureus*. By the end of the 1990s the relatively few multidrug-resistant and highly epidemic clones of methicillin resistant *Staphylococcus aureus* (MRSA) had become the most frequent causative agents of *S. aureus* disease in both hospitals and communities.[6] In spite of the availability of several structurally different antibacterial agents, the therapy most frequently used for treatment of MRSA infections has remained the glycopeptides antibiotics, primarily vancomycin.[7] From 1980 on, there was an abrupt and continued increase in the use of vancomycin in the United States and several countries,[8] which seems to parallel the increasing frequency of MRSA infections in hospitals. This illustrates the enormous selective pressure highly focused on MRSA strains worldwide. *Increased vancomycin MIC and problems in chemotherapy*. At first sight, the high frequency of failed vancomycin therapy,[9] is surprising given the rather modest increase in the MIC value of the isolates and the fact that VISA strains showed decreased virulence potential when tested in a variety of animal models.[10,11]

Mechanisms of Vancomycin Resistance

Experimental test of the “false target” hypothesis described above, fluorescence-labeled vancomycin was used to determine the amount of antibiotic bound by resistant and susceptible *S. aureus* isolates. Both laboratory mutants and VISA isolates bound more vancomycin; however, the amounts bound were not proportional to the MIC value of the bacteria.[12] In a more sophisticated design of the vancomycin binding experiment, it was possible to demonstrate that in VISA strains, the rate of arrival of vancomycin molecules to sites of staphylococcal cell wall synthesis at the bacterial septum was delayed in resistant

bacteria due to the presence of excess D-ala-Dala residues, which could capture and slow down the progress of the antibiotic to the site of cell wall biosynthesis. In this model, the false binding sites (D-ala-D-ala residues) that actually contribute to the increased MIC value are located close to the cell wall synthetic sites at the bacterial septum.

Modification of the model taken into account, the vancomycin binding test produced data that were consistent with the vancomycin MIC value of the bacteria.[13] Sequential mutations in VSSA lead to the emergence of hVISA and ultimately VISA. The hallmark changes are alterations in the bacterial cell wall resulting in reduced autolytic activity and wall thickening. This is thought to result in an impaired ability of vancomycin to reach its binding site and occurs specifically during the cell cycle when the division septum is being formed. These changes are particularly noted after prior exposure to vancomycin.[14,15] Multiple genetic mutations have been implicated in the pathogenesis of these cell wall modifications, usually occurring in genes important for cell wall metabolism such as *vra RS,*[16] and *graRS*; however, recent attention has focused on the essential *S. aureus* regulator *walKR*[17] Accessory gene regulator locus.

Differences in the accessorygene regulator (*agr*) quorum-sensing-system locus have also been associated with vancomycin heteroresistance. *agr* types I and II have been associated with vancomycin resistance,[18] while altered *agr* function leading to reduction in RNA III transcription and deltahemolysin production has also been linked with resistance. Other changes in h VISA. Often, multiple small sequential changes lead to stepwise generation of hVISA and VISA.[19] A number of other changes have been connected with vancomycin heteroresistance, including metabolic changes, altered surface proteins or muropeptides, reduced growth kinetics, and attenuated virulence . While the genetic determinants of hVISA and VISA are partially understood, the relative contributions of these mutations—and of altered cellular processes that contribute to hVISA and VISA—in determining the vancomycin MIC are unknown.[20] The underlying mechanism by which an elevated vancomycin MIC in VSSA causes inferior outcomes has not yet been elucidated. It has been suggested that vancomycin treatment be avoided in these situations, as it is presumed that the continuum of changes that lead to reduced vancomycin susceptibility and hVISA is implicated in the development of resistance. In addition, reduced vancomycin bactericidal activity in VSSA isolates with an elevated vancomycin MIC *in vitro* has been previously noted.[21]

There may be relevant clues when evaluating growth characteristics on conventional agar plates, as hVISA strains may have altered growth kinetics. Careful observation may reveal smaller-sized colonies or mixed small-colony variants (SCV) among normal colonies in a pure culture, reduced pigmentation and hemolysis, and slower growth. These changes may be subjective and are not diagnostic, and their observation should not replace confirmatory testing.[22,23] frequency of vancomycin-intermediate subpopulations in hVISA infections, the low inoculums required for BMD MIC testing is insufficient to detect these subpopulations. Consequently, methods for hVISA detection use a higher inoculum, prolonged incubation (to promote growth of resistant subpopulations), or more nutritious agar. The macromethod Etest (MET) is a screening test for hVISA that uses a higher inoculum (2 McFarland standard) and a longer incubation (48 h). A positive test is reported if the teicoplanin MIC is _12 _g/ml or if the teicoplanin MIC is _8 _g/ml and the vancomycin MIC is also_8 _g/ml. The actual MIC result cannot be reported, because the method differs from the standard MIC calculation.[24]

Minimum Inhibition Concentration of Vancomycin & *VanA* Operon

Clinical factors associated with an elevated vancomycin MIC are similar to those associated with the development of hVISA and include prior vancomycin exposure, prior methicillin-resistant *S. aureus* (MRSA) bacteremia, and increased patient age. Although rates of hVISA detection by PAP/AUC analysis increase as the vancomycin MIC determined by BMD increases,[25] increased mortality and treatment failure have also been reported in infections with VSSA isolates with an elevated vancomycin MIC in the fully susceptible range.

Typically, these isolates have MICs near the susceptibility breakpoint such as 1.5or 2 _g/ml, as determined using different MIC methodologies. This has created debate about whether vancomycin susceptibility breakpoints should be reduced further; however, it is difficult to ascertain an appropriate new breakpoint from these studies, as they represent significant heterogeneity in clinical features and infection types, different MIC testing methods, and different MIC values associated with inferior outcomes. In addition, hVISA was not assessed in many of these studies.[26] The underlying mechanism by which an elevated vancomycin MIC in VSSA causes inferior outcomes has not yet been elucidated. It has been suggested that vancomycin treatment be avoided in these situations, as it is presumed that the continuum of changes that lead to reduced vancomycin susceptibility and hVISA is implicated in the development of resistance. In addition, reduced vancomycin bactericidal

activity in VSSA isolates with an elevated vancomycin MIC *in vitro* has been previously noted.

However, differences in bacterial genotype may be an alternative explanation, with recent publications highlighting associations between genotype and elevated vancomycin MIC. This is particularly relevant, as elevated vancomycin MIC and inferior outcomes in treatment of VSSA infections are not merely confined to MRSA but also occur in cases of methicillin-susceptible *S. aureus* (MSSA) infection, including patients receiving beta-lactam therapy. An elevated vancomycin MIC may therefore be a surrogate marker for an unknown mechanism leading to treatment failure.[27] In May 1996, the first documented clinical infection due to *S. aureus* with the intermediate resistance to vancomycin (minimum inhibitory concentration [MIC] equal to 8 μg/ml) was reported from Japan.5 Later, vancomycin-intermediate *S. aureus* (VISA) strains were isolated in USA, Australia, Europe and other Asian countries. 2 The first clinical MRSA isolate exhibiting high-level resistance to glycopeptides (vancomycin MIC>256 μg/ml; teicoplanin MIC=128 μg/ml) due to acquisition of the *vanA* operon was detected in 2002 from Michigan.6 Although there are few reports of vancomycin-resistant *Staphylcoccus aureus* (VRSA) worldwide, it seems that Iran is a hot spot region for the emergence of these isolates.[28] During the past decade VRSA did not spread rapidly and there were only a few reports of this superbug. Until the end of 2012, 33 cases of *vanA*-type VRSA have been reported worldwide: 13 from the United States, 16 from India, 3 from Iran (2 from Tehran, 1from Mashhad) and 1 from Pakistan.7 Limited spread of VRSA is attributed to the highly-costly *vanA* operon for *S. aureus*, which can be acquired from enterococcal conjugation.[29,30]

CONCLUSION

Vancomycin resistance genes (vanA, vanB) is very high in *Staphylococcus aureus* strains (VRSA) isolated from clinical samples. Vancomycin has been the workhorse antibiotic for MRSA infections for over 50 years. Although the area of vancomycin heteroresistance and susceptibility testing has become complicated, rates of VRSA and VISA are still relatively low whereas hVISA is more common. In order to facilitate more-rapid testing for hVISA and VISA, further understanding of the molecular changes associated with heteroresistance is required. Additional knowledge about the relationship between elevated vancomycin MIC and treatment outcome in cases of VSSA infection is urgently required to clarify the ongoing role of vancomycin in the treatment of serious *S. aureus* infections.

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