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The evolution of vancomycin intermediate *Staphylococcus aureus* (VISA) and heterogenous-VISA

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ABSTRACT

Resistance to new antimicrobials is generally recognized in *Staphylococcus aureus* soon after they are released for clinical use. In the case of vancomycin, which was first released in the 1950s, resistance was not reported until the mid 1990s, with the description of vancomycin-intermediate *S. aureus* (VISA), and heterogenous-VISA (hVISA). Unraveling the complex genetic and cell wall structural changes conferring low-level vancomycin resistance in *S. aureus* has proved challenging. However the recent advances in high throughput whole-genome sequencing has played a key role in determining the breadth of bacterial chromosomal changes linked with resistance. Diverse mutations in a small number of staphylococcal regulatory genes, in particular *walKR*, *graRS*, *vraSR* and *rpoB*, have been associated with hVISA and VISA. Only a small number of these mutations have been experimentally proven to confer the resistance phenotype and some of these only partially contribute to resistance. It also appears that the evolution of VISA from VSSA is a step-wise process. Transcriptomics studies, and analysis of host pathogen interactions, indicate that the evolution of vancomycin-susceptible *S. aureus* to VISA is associated not only with antibiotic resistance, but with other changes likely to promote persistent infection. These include predicted alterations in central metabolism, altered expression of virulence associated factors, attenuated virulence *in vivo*, and alterations in susceptibility to host innate immune responses, together with reduced susceptibility to other antibiotics. In fact, current data suggests that hVISA and VISA represent a bacterial evolutionary state favoring persistence in the face of not only antibiotics, but also the host environment. The additional knowledge of staphylococcal biology that has been uncovered during the study of hVISA and VISA is significant. The present review will detail the current understanding of the evolutionary process in the generation of hVISA and VISA, and explore the diverse additional changes that occur in these strains.

1. Introduction

The global threat of antimicrobial resistance in clinically important bacteria continues to escalate. While a number of bacterial species contribute to this emerging issue, *Staphylococcus aureus* remains one of the key challenges for clinicians and scientists in this regard. *S. aureus* is a major opportunistic human pathogen, causing a wide spectrum of clinical infections, from relatively mild skin infection to devastating septicaemia (Lowy, 1998). Attempts to develop a successful vaccine against this pathogen have so far been unrewarding (Bagnoli et al., 2012), so effective antimicrobial therapy is our principal means to combat serious *S. aureus* infections. Early *S. aureus* isolates were susceptible to penicillin, and effectively treated by this agent (Lowy, 2003), however resistance to this drug evolved rapidly after the introduction of penicillin into clinical practice in the 1940's (Rammelkamp and Maxon, 1942). Similar patterns of rapidly evolving resistance, initially in hospitals and then in the community, have occurred for subsequent classes of antimicrobials active against this organism, including quinolones and macrolides (Chambers, 2001; Lowy, 2003). The most significant antimicrobial resistance issue in *S. aureus* has been the evolution, and widespread dissemination, of methicillin-resistant *S. aureus* (MRSA) due to the acquisition of genes leading to production of an altered penicillin binding protein, encoded by genes on the mobile element SCC*mec* (Katayama et al., 2000).

For many years after the development of multidrug resistance in *S. aureus*, in particular MRSA, the glycopeptide antibiotics

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vancomycin and teicoplanin remained the last line agents for intravenous therapy. It was therefore a significant concern when the first strains of *S. aureus* with reduced susceptibility to vancomycin were reported from Japan in 1997 (Hiramatsu et al., 1997a, b). These strains demonstrated moderate reductions in susceptibility to glycopeptides, and were distinct from fully glycopeptide-resistant strains of *S. aureus* (vancomycin-resistant *S. aureus*; VRSA), first reported from the USA in 2002, due to conjugative transfer of the *vanA* operon from *Enterococcus faecalis* (2002, Perichon and Courvalin, 2009). The uncertainty regarding the optimal laboratory detection method for strains of *S. aureus* with subtle reductions in susceptibility to glycopeptides (heterogeneously-resistant strains) has made it difficult to understand the epidemiology, clinical impact, and evolution of reduced glycopeptide susceptibility in this bacterial species. For the purposes of this review we will use the terminology vancomycin-intermediate *S. aureus* (VISA), and heterogenous-VISA (hVISA) because these names are most widely used in the literature. It should be noted, however, that not all isolates of *S. aureus* demonstrating reduced susceptibility to teicoplanin also demonstrate the same level of reduced vancomycin susceptibility (Kaatz et al., 1990; Manquat et al., 1992), so the terms glycopeptides-intermediate *S. aureus* (GISA) and VISA cannot necessarily be used interchangeably.

There have been changes in international guidelines for laboratory detection of VISA, because of growing data demonstrating poor vancomycin efficacy when the MIC was $\geq$4 mg/L (Tenover and Moellering, 2007), and the current recommendations are summarized in Table 1. Heterogenous-VISA refers to a strain of *S. aureus* that has a vancomycin minimum inhibitory concentration (MIC) within the susceptible range, however more detailed testing using a higher inoculum or prolonged incubation is able to detect a resistant subpopulation with a higher MIC (Hiramatsu, 2001). There are no current approved methods for the confirmation of heterogenous-VISA, however population analysis profile testing (PAP), which is used to detect these resistant subpopulations, is generally considered the gold standard (Howden et al., 2010). Population analysis profile involves testing a high inoculum of an overnight culture of *S. aureus* on media containing a range of vancomycin concentrations, using a spiral plater. The colonies are counted at each of the vancomycin concentrations after 48 h incubation and the area under the resulting population analysis curve (AUC) calculated. The reference hVISA strain, Mu3 (ATCC700698), is used as a control in these experiments, and the AUC ratio of the test organism calculated compared to Mu3 (PAP-AUC ratio). If the ratio is >0.9 and the vancomycin MIC is in the susceptible range then the isolate is hVISA (Wootton et al., 2001). Because PAP is time consuming and labour intensive a range of screening media and assays based on a high inoculum and using vancomycin and teicoplanin Etest strips have been developed that allow the identification of presumptive hVISA (Howden et al., 2010).

Significant effort has gone into understanding the genetic determinants of hVISA and VISA, aided in part by the recent availability of high throughput genome sequencing technologies, and the genetic determinants of resistance are becoming more defined. Additionally, it is becoming clear that a number of selective pressures, potentially including the *in vivo* milieu promote the evolution of VISA, and while antimicrobial resistance is the key phenotypic outcome, other effects on host pathogen interactions frequently occur, such as reduced expression of virulence genes, and attenuated virulence in animal models. It's been suggested that VISA strains employ a stealth strategy, not only to combat the effect of vancomycin therapy, but also to promote a phenotype better equipped to avoid the human immune response (Gardete et al., 2012). Here, we review the current understanding of selective pressures and genomic changes driving the evolution of hVISA and VISA, and review the other features of VISA strains, with a focus on more recent discoveries.

2. The emergence of hVISA and VISA

2.1. Initial reports and epidemiology

Strains of *S. aureus* that evolved teicoplanin resistance *in vivo* during failed therapy were first reported in the early 1990's from the USA and Europe (Kaatz et al., 1990; Manquat et al., 1992). In both cases, teicoplanin resistance emerged but the strains remained vancomycin susceptible by MIC testing. Subsequently, in 1997, Hiramatsu et al. reported the isolation of clinical isolates of *S. aureus* that demonstrated a vancomycin-intermediate phenotype (Mu50 strain) (Hiramatsu et al., 1997b), and a strain that demonstrated a hVISA phenotype (Mu3 strain) (Hiramatsu et al., 1997a). These reports resulted in significant interest in the issue of vancomycin resistance in *S. aureus*, and led to the isolation and characterization of hVISA and VISA from many countries around the world (see (Howden et al., 2010) for summary). Subsequent retrospective analyses detected previously unrecognized hVISA and VISA isolates from the USA and Europe, at least back to the mid 1980s (Robert et al., 2006; Rybak et al., 2005), and from Japan in 1990, before vancomycin was available in that country (Yamakawa et al., 2012).

Clonality testing, initially using pulsed field gel electrophoresis and then multilocus sequence typing, demonstrated that VISA strains were not clonal (Fridkin et al., 2003; Howe et al., 2004; Smith et al., 1999). However, many reported hVISA and VISA strains are from clonal complex 5 or 8, in particular ST5 (CC5) and ST239 (CC8), likely reflecting their success as hospital adapted MRSA clones. The majority of hVISA and VISA are reported in hospital MRSA strains, rather than community MRSA or MSSA strains, presumably because the selective pressures promoting hVISA and VISA are greater in the hospital environment. However, VISA has also been reported infrequently in methicillin-susceptible *S. aureus* (Pillai et al., 2009), and in community clones of MRSA (Gardete et al., 2012). It has been clearly demonstrated, not surprisingly, that as the vancomycin MIC of *S. aureus* isolates increases, the proportion that will test hVISA positive increases (Musta et al., 2009). We have also noted that hospital adapted clones of *S. aureus* (such as ST239 [CC8], and ST5[CC5]) have a higher median vancomycin MIC compared to other clones (unpublished observation). This is therefore likely to be reflected in a higher rate of hVISA, and subsequently VISA, among these clones compared to other MRSA genetic

Table 1
Summary of definitions for reduced vancomycin susceptibility in *S. aureus*.

Classification	*S. aureus* broth MIC (mg/L)		Comment
	EUCAST	CLSI	
Susceptible (VSSA)	$\leq$2	$\leq$2	hVISA within this category. Requires additional testing (e.g. PAP)
Intermediate (VISA)		4–8	EUCAST, no intermediate category
Resistant (VRSA)	$\geq$4	$\geq$16	

Note. PAP, population analysis profile testing; EUCAST, european committee on antimicrobial susceptibility testing; CLSI, clinical and laboratory standards institute.

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