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Short Communication

Alterations in phage-typing patterns in vancomycin-intermediate *Staphylococcus aureus*

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The ability of phage-typing and *Sma*I chromosomal RFLPs to conclude appropriate strain relatedness between a collection of 12 well-characterized *in vitro*-selected vancomycin-intermediate *Staphylococcus aureus* (VISA) strains and their seven vancomycin-susceptible parent strains is reported. Generally, no *Sma*I RFLP alterations were observed in VISA strains when they were compared with their respective parent strains, and clonal relationships between isogenic strains were clearly evident. Unlike the *Sma*I RFLP results, parent strains and VISA derivatives generally did not share similar phage-typing profiles. Depending on the phage set investigated, some VISA strains even became untypable by this method. Loss of phage infectivity is probably due to cell wall (phage receptor) alterations that are expressed by the VISA strains investigated. Collectively, these findings indicate that inappropriate relationships between VISA and vancomycin-susceptible parents might be drawn if only phage-typing and antibiotic susceptibility are utilized to determine epidemiological relationships.

Introduction

Analysis of *Sma*I chromosomal RFLPs produced using PFGE is very effective in tracking *Staphylococcus aureus* clones (Jorgensen *et al.*, 1996; O'Brien *et al.*, 1999; Simor *et al.*, 2002). Phage typing is also used to track outbreak, epidemic and community strains of *S. aureus* effectively (Blair & Williams, 1961; O'Brien *et al.*, 1999; O'Neill *et al.*, 2001). While phage typing is often valuable in discerning strain relationships quickly and inexpensively, the technique is not always helpful, since non-typable *S. aureus* strains exist (Blair & Williams, 1961; Andrasevic *et al.*, 1999; Tambic et al., 1997).

The glycopeptide antibiotic vancomycin is still the major drug of choice for treatment of severe disease caused by infection with methicillin-resistant *S. aureus* (MRSA). Since 1997, vancomycin-intermediate *S. aureus* (VISA) have become an emerging clinical reality (Hiramatsu *et al.*, 1997a). Vancomycin-intermediate expression in *S. aureus* is mediated by chromosomal mutation(s) (Avison *et al.*, 2002; Pfeltz *et al.*, 2000; Sakoulas *et al.*, 2002; Walsh & Howe, 2002) and is not due to horizontal gene transfer of vancomycin-resistance determinants (*van*) from organisms such as the vancomycin-resistant enterococci. VISA strains harbour a chromosomal mutation(s) that leads to alterations in cell-wall synthesis and structure, which are thought to impart resistance by sequestering glycopeptide molecules away from their target (for review, see Walsh & Howe, 2002). PFGE and *Sma*I chromosomal RFLP analysis have been used to demonstrate that VISA can disseminate in a clonal fashion (Chesneau *et al.*, 2000; Hiramatsu *et al.* 1997b; Kim *et al.*, 2002). Two reports have now appeared on high-level vancomycin-resistant *S. aureus* (VRSA) that have acquired the enterococcal *vanA* determinant (Miller *et al.*, 2002; Sievert *et al.*, 2002). Outbreaks with VISA or VRSA have not yet become a common occurrence.

We now report on the ability of phage typing and PFGE to conclude appropriate strain relatedness between a collection of 12 *in vitro*-selected VISA and their seven vancomycin-susceptible parents.

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Abbreviations: MRSA, methicillin-resistant *S. aureus*; VISA, vancomycin-intermediate *S. aureus*; VRSA, vancomycin-resistant *S. aureus*.

Methods

Strains. The strains analysed represent various genetic backgrounds and methicillin resistance levels and their isogenic *in vitro* step-selected VISA derivatives (Pfeltz *et al.*, 2000). The subscript V in a strain designation refers to a vancomycin-intermediate derivative (e.g. $BB270_{V5}$) of a susceptible parent strain (e.g. BB270), and the number associated with the subscript V (such as V5) refers to the vancomycin concentration at which the strain was maintained directly following VISA phenotype selection (e.g. 5 μg ml^{-1} for $BB270_{V5}$) and not the actual vancomycin MIC (Pfeltz *et al.*, 2000). The MICs of the strains for vancomycin, teicoplanin and oxacillin and other characteristics of these strains were described by Pfeltz *et al.* (2000).

Phage typing and PFGE. Phage typing, performed on strains passaged in antibiotic-free medium, and PFGE of *Sma*I chromosomal digests were performed as described previously (Blair & Williams, 1961; O'Brien *et al.*, 1999). Chromosomal banding patterns were scanned with a Flour-S MultImager and analysed by Multi-Analyst/PC (Bio-Rad).

Results and Discussion

*Sma*I chromosomal RFLPs of the isogenic parents (BB255, BB270, SH108, BB399, 13136p−m+, BB568 and COL) and *in vitro*-selected VISA strains ($BB255_{V3}$, $BB270_{V5}$, $BB270_{V15}$, $SH108_{V5}$, $BB399_{V5}$, $BB399_{V12}$, 13136p−m+$_{V5}$, 13136p−m+$_{V20}$, $BB568_{V5}$, $BB568_{V15}$, COL_{V5} and COL_{V10}) and a dendrogram generated from these RFLPs are shown in Fig. 1. It should be noted that strain BB270 is a methicillin-resistant transductant of BB255, and strains BB568 and COL are isogenic (Pfeltz *et al.*, 2000); therefore, as expected, these strain pairs cluster together (Fig. 1). *Sma*I restriction profiles also revealed that, with the exception of VISA strain $BB399_{V12}$, no significant alterations occurred in the *Sma*I chromosomal RFLPs of most strains (BB255, BB270, SH108, 13136p−m+, BB568 and COL) as they were step-selected to become vancomycin-intermediate. These data confirm that major genetic alterations are not required for *S. aureus* to develop the VISA phenotype. In addition, clonal relationships between the collection of *in vitro*-selected VISA mutants and isogenic parent strains are clearly evident. Other investigators have also demonstrated that *in-vitro*-selected VISA retain parental *Sma*I RFLP patterns (Schaaff *et al.*, 2002). *Sma*I chromosomal RFLPs are also similar between VISA that arise from vancomycin-susceptible strains during vancomycin therapy in a single patient (Rotun *Rotun et al.*, 1999; Sieradski *et al.*, 1999a; Smith *et al.*, 1999).

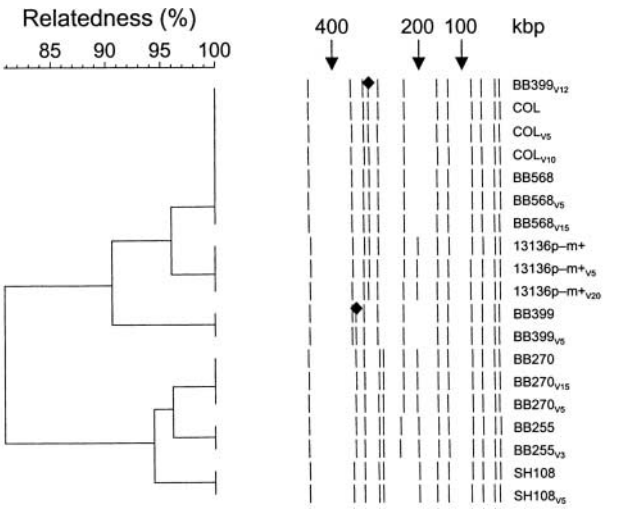

Fig. 1. Cartoon of *Sma*I-macrorestricted chromosomal RFLPs of *S. aureus* strains investigated and dendrogram to demonstrate percentage relatedness. ◆, Band alterations between BB399 and $BB399_{V12}$.

A 300 kb *Sma*I fragment present in parent strain BB399 has been replaced by a 275 kb fragment in $BB399_{V12}$ (Fig. 1), indicating the loss of ~25 kb, which actually allowed $BB399_{V12}$ to cluster with COL and BB568. Altered *Sma*I RFLP patterns have been reported to occur within *in vitro*-selected VISA strains (Riepert *et al.*, 2003). These alterations have been attributed to loss of the methicillin-resistance determinant (*mec*) as well as other genes (Riepert *et al.*, 2003; Sieradzki *et al.*, 1999b). $BB399_{V12}$, however, maintains high-level methicillin resistance, similar to parent BB399 (Pfeltz *et al.*, 2000), indicating that the former has not lost *mec*. *Sma*I RFLP alterations can result from the loss of a prophage (Smeltzer *et al.*, 1994) and, perhaps, during the selection for the VISA phenotype in $BB399_{V12}$, a prophage excised and was lost.

Unlike the *Sma*I RFLP results, parent strains and VISA derivatives generally did not share similar phage-typing profiles using the international basic phage set (IBPS), the international MRSA phage set (IMRSA) or the Australian MRSA phage set (AMRSA) (Table 1). When the IBPS was used, all VISA derivatives demonstrated altered phage types when compared with their respective parent strains. VISA mutant 13136p−m+$_{V5}$ even became non-typable by this phage set. Typing with IMRSA revealed that isogenic strain sets BB255 and $BB255_{V3}$ and BB568 and $BB568_{V5}$ had the same phage types, while strain 13136p−m+ and its VISA mutants were non-typable by this phage set. All other VISA strains exhibited altered IMRSA types compared with their respective parent strains, and $SH108_{V5}$ became untypable. Typing with AMRSA revealed that the BB255 and BB568 strain sets all had identical phage types, as did COL and its VISA derivative COL_{V5}. All other VISA mutants demonstrated altered AMRSA types compared with their respective parent strains, and $BB399_{V5}$ became untypable. Other studies have also shown alterations in phage-typing patterns following *in vitro* acquisition of a VISA phenotype in *S. aureus* (Daum *et al.*, 1992; Schaaff *et al.*, 2002).

Phage infection can be prevented in Gram-positive bacteria as a result of adsorption inhibition (Chatterjee, 1969; Tran *et al.*, 1999; Wilkinson & Holmes, 1979), restriction modification systems (Kong & Josephsen, 2002), DNA injection-blocking systems (McGrath *et al.*, 2002), lysogenization (Beard-Pegler & Vickery, 1985) and abortive infection systems (Dai *et al.*, 2001). We showed that the *in vitro* VISA investigated demonstrated minimal to no alteration in *Sma*I

Table 1. Phage-typing results of *S. aureus* strains investigated

Strain	IBPS	IMRSA	AMRSA
BB255	88/47/53/(54)/75/90	MR8/MR12/33/38	47T/90A/1648/13M
$BB255_{V3}$	88/75/90	MR8/MR12/33/38	47T/90A/1648/13M
BB270	88/47/53/54/75/77/84/90	MR8/MR12/38/56B	47T/56B/56C/90A/1648/87M/13M
$BB270_{V5}$	88/75/90	MR8/MR12/33/38	47T/90A/1648/13M
$BB270_{V15}$	88/75/90	MR8/MR12/33/38	47T/90A/1648/13M
SH108	88/6/53/54/75/83A/90	33/38/56B	47T/56B/56C/90A/1648/67R/87M/13M
$SH108_{V5}$	6	Non-typable	67R/13M
BB399	29/(52A)/(80)/90	33/38	90A/1648/13M
$BB399_{V5}$	80	33	Non-typable
$BB399_{V12}$	52A/80/90	MR8/MR12/33/38	47T/90A/1648/13M
13136p−m+	(88)/53/(54)/75/77/84/90	Non-typable	47T/(56B)/56C/90A/1648/67R/87M/13M
$13136p-m+_{V5}$	Non-typable	Non-typable	47T/(56C)/90A
$13136p-m+_{V20}$	54/85/90	Non-typable	47T/56C/(90A)/67R/13M
BB568	(52A)/(75)/77/84/90	(MR8)/MR12/33/38	47T/90A/1648/13M
$BB568_{V5}$	52A/(80)/77/84/90	MR8/MR12/33/38	47T/90A/1648/13M
$BB568_{V15}$	88/52A/(80)/(47)/90	MR12/33/38	47T/90A/1648/13M
COL	88/52A/80/77/84/90	MR8/MR12/33/38	47T/90A/1648/13M
COL_{V5}	52A/80/90	33	47T/90A/1648/13M
COL_{V10}	52A/80/90	33	47T/90A/13M

RFLPs compared with parent strains; however, alterations in phage types were common. This latter finding is probably due to modifications of phage cell-wall receptors that result from alterations in cell-wall physiology that are expressed by the VISA strains investigated (Pfeltz *et al.*, 2000). Collectively, these findings indicate that inappropriate relationships between VISA and vancomycin-susceptible parents might be drawn if only phage-typing is utilized to determine epidemiological relationships. This might be especially true in situations where VISA arise within a single infected patient being treated with vancomycin, as has previously been reported to occur (Rotun *et al.*, 1999; Sieradski *et al.*, 1999a; Smith *et al.*, 1999). Recognizing vancomycin-susceptible parent strains may be of clinical importance, since some *S. aureus* strains can mutate to become VISA with greater ease compared with other strains (Pfeltz *et al.*, 2000; Schaaff *et al.*, 2002), and the presence of these strains may therefore indicate a unique threat.

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