Differences in expression of virulence genes amongst invasive and colonizing isolates of meticillin-resistant Staphylococcus aureus.

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Citation
Differences in expression of virulence genes amongst invasive and colonizing isolates of meticillin resistant *Staphylococcus aureus*

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Introduction

The prevalence of meticillin-resistant *Staphylococcus aureus* (MRSA) is an important global concern in healthcare due to potentially life-threatening infections and difficulties in treatment. The organism can colonize the mucosa, e.g. anterior nares or result in invasive infections, e.g. bloodstream infections, through the coordinated expression of extracellular and cell-bound virulence factors (Goerke *et al.*, 2000; Novick *et al.*, 1993). The transcription profiles of virulence genes vary *in-vivo* depending on the dynamic interaction between the host environment and the pathogen. Since the dynamic environment may be differentially altered in MRSA carriage compared to infection, the expression of *S. aureus* virulence genes may also vary. We previously found no significant correlation between the presence of 17 virulence genes and invasiveness of MRSA isolates (O'Donnell *et al.*, 2008) and this non-association of specific genes or combinations of genes with invasive isolates is in agreement with other larger studies (Lindsay *et al.*, 2006). The lack of a definitive correlation between invasive *S. aureus* strains and the carriage of virulence genes, suggests that the expression, rather than carriage of virulence determinants *in-vivo*, may mediate pathogenicity.

We investigated the *in vitro* expression of selected virulence genes; gamma-haemolysin (*hlg*), collagen-adhesion encoding gene (*cna*) and staphylococcal exotoxin a (*sea*), in colonizing compared with invasive MRSA clinical isolates, using the same isolate collection used in our previous study (O'Donnell *et al.*, 2008). *Hlg* and *sea* were representative of toxin genes and *cna* was representative of adhesion genes. These genes were selected because they were among those present in all isolates from our previous study. We also investigated the expression of one regulatory gene, *RNAIII*, the effector molecule of the accessory gene regulator (*agr*) system which is reported to up-regulate toxin genes and down-regulate adhesion genes.
Twenty isolates were recovered from patients with device-related bloodstream infections between January 2002 and June 2005 and twenty isolates were from nasal swabs, collected from patients that were MRSA positive on first hospital admission (between September 2006-June 2007) but with no apparent signs of infection. The spa type of each isolate was used to infer the sequence type (ST) or clonal complex (CC) from which the isolate originated and the staphylococcal chromosomal cassette mec (SCCmec) type was also determined (O'Neill et al., 2007). RNA was isolated from cultures grown to OD = 2 (3-5 h, determined from preliminary experiments to correspond to the mid-late exponential phase) in brain heart infusion (BHI) broth supplemented with commercially available pooled human serum (5 %) to mimic the in-vivo environment. Real-time RT-PCR was performed using specific primers for each of the four genes and expression levels were normalized to the expression of gyrB as an internal control. The absolute quantity of each gene in isolates could not be determined without appropriate standards, therefore calibrator-normalized relative quantitation was used. The relative expression level of each gene was the ratio of its normalized value in the test isolates to its normalized value in isolate BH16. Isolate BH16 was chosen arbitrarily from the collection as the calibrator isolate. The relative expression levels of the four virulence genes in invasive vs. colonizing isolates (including the gene regulator, RNAIII) are shown in Figure 1. There was a high level of variation in the relative expression of virulence genes among both groups of isolates. Comparing the two groups, colonizing and invasive, there was no significant alteration in the transcription of RNAIII, in invasive isolates vs colonizing isolates (P value = 0.0859 by Mann Whitney test using GraphPad Prism version 4.00 for Windows, GraphPad Software, San Diego California USA). Transcription of cna was up-regulated in colonizing isolates compared to invasive isolates (P value = 0.0110) whereas transcription of hlg was up-regulated in invasive isolates compared to
colonizing isolates (P value = 0.0193 by Mann Whitney). Although a greater number of invasive isolates expressed relatively higher levels of the sea transcript than colonizing isolates, this was not statistically significant (P value = 0.1456). Comparing the relative expression of all four genes in each isolate (data not shown), the transcription of RNAIII was accompanied by a reduction in transcription of cna and increased transcription of hlg in only 4 invasive isolates and none of the colonizing isolates. RNAIII did not increase transcription of sea but the sea transcript was apparently expressed at low levels in most isolates or was not detectable.

Genotyping of isolates used in this study revealed that both colonizing and invasive groups were highly clonal, belonging to either ST22-MRSA-IV (100% and 65%) or ST8-MRSA-II (0 % and 35%). Of the four agr types that have been described, ST22-MRSA-IV and ST8-MRSA-II isolates belong to agr type 1 (Monecke et al., 2008). Figure 2 shows that within the invasive isolates group ST8-MRSA-II isolates differed significantly in their transcription levels to ST22-MRSA-IV, for cna (P=0.005) and sea (P= 0.019) but not for hlg (P=0.064) or RNA III (P= 0.062). When the ST8-MRSA-II isolates were omitted from the overall analysis, only the transcript levels of sea were significantly increased (P=0.013) in the invasive group compared to colonizing. Although in the invasive isolates the transcripts level of cna were decreased and that of hlg were increased, these failed to reach significance (P=0.22 and 0.19) when only ST22-MRSAIV isolates were analyzed (data not shown).

Virulence factors that promote binding to the mucosal layers are important in S. aureus colonization of the host, whereas factors that promote tissue damage and provoke damaging immune responses, are important during S. aureus infection (Cheung et al., 2004; Novick et al., 1993). In the present study we found significant differences in the relative expression levels of virulence genes in colonizing vs. invasive MRSA isolates when the clinical isolate was grown to
mid-late exponential phase, in the presence of human serum in an attempt to mimic the in-vivo conditions. In the presence of human serum, a greater number of invasive isolates expressed lower levels of the cell-associated transcript, \textit{cna} and higher levels of the toxin gene \textit{hlg} but not \textit{sea}. A greater number of colonizing isolates expressed relatively high transcript levels of \textit{cna} with \textit{hlg} transcripts also being relatively high in some and \textit{sea} transcription was low or beyond the limit of detection. Our data also indicate that the origin of the isolate cannot be considered independently of other genetic factors relating to the isolate as these may make a significant contribution to pathogenicity. It has been shown that the carriage of virulence genes is highly clonal but here we have also demonstrated an association between clonal type and transcription of virulence genes (Fig. 2). Although the differences in the pattern of expression of virulence associated genes may contribute to the pathogenicity of MRSA in invasive vs. colonizing isolates, this study shows that colonizing isolates may also have the capacity to promote host damage in some situations. Alterations in the local environment, such as pH or proteolytic activity, may affect the activity of expressed proteins, or differences in the immune response to the organism may determine the overall pathogenesis of MRSA.

Expression of \textit{RNAIII}, in the late exponential phase, positively correlated with the expression of \textit{hlg} and negatively correlated with the expression of \textit{cna}, for only a small number of invasive isolates. Similarly, other authors have shown that positive correlation between transcription of the toxin genes and the regulator gene \textit{RNAIII} may be isolate-specific (Papakyriacou \textit{et al.}, 2000; Sabersheikh & Saunders, 2004) or may vary with the phase of growth studied as \textit{RNAIII} is a quorum sensing effector. Alternatively, expression of \textit{RNAIII} may not be critical for expression of \textit{S. aureus} toxins and this has been confirmed in one of the few studies that has measured \textit{in-vivo} transcription of \textit{RNAIII} in an animal model of infection (Yarwood \textit{et
It is also possible that virulence gene expression may be regulated by other regulator genes or a combination of these. Alternative regulatory systems identified in S. aureus include sar, sigB, rot, arlR/S, svrA, saeR/S (Bronner et al., 2004; Novick & Jiang, 2003).

We have found that under in vitro conditions using human serum that may partly mimic what occurs in patients (at least in infection), variations in the transcription of some virulence genes occurred, that correlated with the isolate source (colonizing or from infection) and within the invasive group, ST8-MRSA-II isolates showed decreased transcription of cna and increased transcription of sea compared to ST22-MRSA-IV isolates. Because environmental conditions in the nares of patients differ from those in the bloodstream, further work in this area should aim to mimic more closely the local host environment during colonization and infection (e.g. bacterial growth phase, pH, osmolarity and/or host-niche-specific factors, e.g. presence of polymorphonuclear neutrophils (PMNs) and/or platelets) or to investigate transcription of virulence genes when isolates are grown in the presence of the patient’s own serum. These studies should also be extended to other virulence genes and other gene regulators. Accurate direct measurement of gene expression levels in-vivo presents a challenge, but this research area may benefit from recent improvements in microgenomic detection of bacterial RNA in human tissues (Klitgaard et al., 2007). These approaches should further our understanding of the properties of S. aureus that allow it to transform from transient commensal to pathogen.

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Legend Figure 1
Fold change in transcript levels of RNAIII, cna, hlg and sea in MRSA colonizing isolates (●) and invasive isolates (▲) using real-time PCR with relative-quantitation with respect to an arbitrarily chosen isolate. Horizontal lines indicate the median value for each data set. Statistically significant changes in transcript levels are indicated.

Legend Figure 2.
Fold changes in transcript levels of RNAIII, cna, hlg and sea in MRSA invasive isolates when correlated with the clonal type of the isolate, ST-22 (▲), ST-8 (▲). Horizontal lines indicate the median value for each data set. Statistically significant changes in transcript levels are indicated.

References


Figure 1.
Figure 2.

**P=0.005

*P=0.019

Fold change in expression

RNAIII  cna         hlg      sea

gene