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Genetic basis of glycopeptide resistance in Staphylococcus aureus

Objectives The mechanisms of newly emerging antibiotic resistance, such as low-level glycopeptide resistance in staphylococci, need to be studied and understood at all levels. Only with comprehensive knowledge will we be able to efficiently combat the danger posed by multiresistant pathogens, which threatens to throw us back to the pre-antibiotic era.

Conclusions Due to strain-dependent multiplicity and the variability of gene expression patterns associated with glycopeptide intermediate resistance in *S. aureus* (GISA), it was not possible to identify a common denominator or key gene acting as a general indicator for gylcopeptide resistance. However, the activity of the membrane protein TcaA was shown to have a direct impact on glycopeptide resistance. The loss or inactivation of TcaA increases glycopeptide resistance, and it was genotypically linked to some clinical GISA isolates. The formidable plasticity and variability of the staphylococcal genome allows multiple ways for them to adapt to glycopeptide stress, yielding resistance. However since glycopeptide resistance, in the absence of antibiotic selection, can be a burden for *S. aureus*, as soon as the selection pressure is relieved "phenotypically sensitive revertants" (not true genotypic revertants but very likely forward mutants with altered properties) appeared.

Main results and findings

Glycopeptide intermediate resistance in *S. aureus* (GISA) arises intrinsically as the result of multiple accumulated mutations and/or alterations in gene expression and can occur *in vivo,* in patients exposed to prolonged glycopeptide therapy, or *in vitro,* through the successive sub-culturing of strains on increasing concentrations of glycopeptide. Phenotypes common to GISA include decreased growth rate, decreased colony size, and decreased haemolysis (on sheep blood agar). One GISA mutant analysed contained 12 genetic loci that were more than 3-fold downregulated, all of which were likely to play a role in virulence, suggesting an overall lowered virulence of the mutant.
Analysis of the transcriptional changes imposed by vancomycin stress in *S. aureus* Newman revealed that:

- sixty-nine ORFs were more than 3-fold upregulated upon vancomycin exposure (29 of which were also more than 3-fold upregulated by oxacillin, indicating that they might be part of a general cell wall stress regulon) including genes involved in cell wall synthesis and metabolism as well as many genes of poorly characterised or unknown function.
- not necessarily all genes upregulated by glycopeptides contribute to resistance (and/or that a combination of these genes has to be involved).
- some of the clinical GISA isolates showed varied levels of induction (i.e. differences in the abundance of transcript induction, lack of induction, or relatively high levels of gene expression in the absence of induction) for several of the ORFs tested, indicating that they may have a disrupted gene induction pathway, which could be contributing to resistance.

tcaA is the key gene (within *tcaRAB*) responsible for changes in glycopeptide resistance levels, and TcaA activity is the only genetic marker that has been directly linked to glycopeptide resistance in clinical GISA. TcaA analyses revealed that:

- *tcaA* gene expression is strongly inducible by cell wall targeting-antibiotics and that *tcaA* inactivation (deletion or disruption) is a relevant contributing factor to the glycopeptide resistance levels of some *S. aureus* clinical GISA isolates.
- TcaA is a predicted membrane protein of unknown function, and homologues of TcaA are found only in staphylococci and bacilli. Functional analysis of TcaA (through a series of deletions) showed which parts of TcaA are involved in glycopeptide resistance.

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