Dr. Pierre Vaudaux
Division des Maladies Infectieuses
Hôpital Cantonal Universitaire de Genève (HUG)
24, Rue Micheli-du-Crest
1211 Genève 14
pierre.vaudaux@hcuge.ch

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Prof. Dr. Jacques Schrenzel
Laboratoire de Recherche Génomique, Service des Maladies Infectieuses,
Hôpitaux Universitaires de Genève (HUG)
Jacques.schrenzel@hcuge.ch

Mechanisms of glycopeptide resistance in staphylococci

Objectives The recent emergence of *S. aureus* strains resistant to the glycopeptide vancomycin is a serious public health issue. Vancomycin was until recently the drug of "last resort", the only uniformly effective agent against all staphylococcal infections. This project aims to identify the genes responsible for resistance to teicoplanin, another glycopeptide closely related to vancomycin, and to develop a simple and reliable screening assay for glycopeptide-intermediate *S. aureus* (GISA) strains.

Conclusions Substantial evidence was provided that acquisition of teicoplanin resistance by *S. aureus* may be linked with multiple changes in the expression and regulation of virulence genes. In line with a recent report showing that a majority of GISA isolates were recovered from device-associated infections, the link between emergence of teicoplanin resistance in *S. aureus* and increased attachment to fibronectin may be of significant clinical relevance if it could be extended to clinical GISA strains. In fact, fibronectin-binding proteins play a prominent role in *S. aureus* attachment and colonisation of host tissue and implanted biomaterials.
The approach for the design of whole-genomic oligoarrays that was provided is applicable to gene expression profiling, comparative genome hybridization, molecular epidemiology and gene deletion mapping. Relying on parameters shown to affect the hybridisation of nucleic acids, software was designed (freely available at www.genomic.ch) and its performance experimentally validated on the bacterial pathogen *S. aureus*.
In the treatment of orthopaedic infections, the impact of the long-term vancomycin therapy for the eradication of colonisation by methicillin-resistant *S. aureus* (MRSA) on the emergence of vancomycin-intermediate *S. aureus* was studied.
In another *in vitro* study, it was shown that screening on glycopeptide-containing agar (2mg/L vancomycin or 5mg/L teicoplanin on Brain Heart Infusion Agar) provides a simple, reliable and low-cost screening assay for MRSA exhibiting intermediate susceptibility to glycopeptides (GISA). This method, which can be easily implemented in clinical microbiology laboratories, is adequate for minimising the risk of missing potential homogeneous or heterogeneous GISA isolates and can be used for prospective screening of decreased susceptibility to glycopeptides in any hospital setting.
In an experimental model for therapy of chronic foreign body infections due to *S. aureus* (rat model of subcutaneous implanted tissue cages), daptomycin was at least equivalent to vancomycin, though drug dosage should be optimized to obtain inflammatory fluid levels of daptomycin, minimising emergence of resistant populations.
Generally, treatment of an infected prosthesis should consider the best surgical strategy associated with optimal antibiotic therapy, tailored to the individual patient. Novel therapeutic approaches for hip prosthetic infections should include the type of microorganism, antibacterial susceptibility, clinical presentation (age, comorbidities, etc.), thus combining less aggressive surgical techniques with antibiotic therapies based on drug combinations against biofilm-associated bacteria, including rifampicin (particularly with quinolones) with excellent bioavailability, which allows prolonged and efficient therapy.

Main results and findings

Development and validation of a real-time quantitative PCR (TaqMan®) This method allowed the evaluation of gene expression in a teicoplanin-resistant strain of *S. aureus* (compared to its isogenic teicoplanin-susceptible parental strain or teicoplanin-susceptible revertant strain). It was shown that:

- there is an increase in levels of expression and surface display of fibronectin-binding proteins.
- there is a significant change in other virulence factors, like reduction of α-toxin, reduction of protein, downregulation of *agr,* upregulation of *sarA* and *sigB.*
- most of the altered parameters returned to "parental" levels in the teicoplanin-susceptible revertant strain.
- this real-time quantitative PCR is also suitable for analysing significant changes in global regulator transcript levels and activities.

Microarray A reliable and fully validated oligoarray (microarray technology) was developed for the identification of *S. aureus* genes, whose induction/repression may play a key role for expression of glycopeptide resistance *in vivo.* The flexible microarray design allows easy update with new

published genomes, different probe design strategies, mobile elements, intergenic regions, etc. It is useful for gene expression (transcription) profiling, comparative genome hybridisation, gene deletion mapping, molecular epidemiology, for strains derived from other backgrounds; and the software is freely available (www.genomic.ch).
Analysis of trancriptomic changes in key autolysis-regulating genes of a teicoplanin-resistant (TeiR) strain of *S. aureus* showed that:

- the TeiR strain was far more resistant to autolysis, although putative effector genes (*atl, cidA, lytM*) of bacterial autolysis showed no significant decline in transcript levels but even increased transcript levels for some of them (*cidBC*).
- changes in *cidABC* levels are compatible with decreased transcript levels of their own negative regulators (*lytSR* and *lrgAB*).
- the autolysis-deficient phenotype TeiR strain is compatible with increased transcript levels of global regulators (*mgrA, arlRS,* and *sarA*) combined with a strong decline in *agr* RNAII/RNAIII.

GISA Screening A simple and reliable screening assay for MRSA exhibiting intermediate susceptibility to glycopeptides (GISA) was shown. Concentration of 2 mg/L vancomycin in Brain Heart Infusion Agar (V2-BHI) or 5 mg/L teicoplanin in Brain Heart Infusion Agar (T5-BHI) proved to be suitable (validation by testing two panels of 94 and 95 consecutive MRSA bloodstream isolates). The results for V2-BHI, compared to Etest MICs (Minimum Inhibitory Concentration) with 0.5 McFarland on Mueller-Hinton Agar (MHA) (according to CDC, Centres for Disease Control and Prevention), were of lower sensitivity (<30%, <65%) but high specificity (100% and 99%) on period A and B isolates, respectively. Comparison with Etest MICs using a higher inoculum, 2 McFarland, on Brain Heart Infusion Agar (BHIA) revealed a higher sensitivity (57% and 81%) but lower specificity (91% and 65%) on period A and B isolates, respectively. Surprisingly, neither the teicoplanin susceptibility testing method on T5-BHI nor any teicoplanin Etest MIC was a good predictor of reduced vancomycin susceptibility (in contrast to other studies).

Experimental therapy An experimental model of therapy with alternative antimicrobial agents that can overcome glycopeptide resistance (rat model of subcutaneous implanted tissue cages) revealed that:

- *in vitro,* in the presence of 50% tissue cage fluid, elimination of a spontaneous methicillin-susceptible revertant strain (showing equivalent virulence to its methicillin-resistant parent) was more rapid with 4 mg/L daptomycin compared to vancomycin.
- intraperitoneal administration of 30 mg/kg daptomycin once-daily or 50 mg/kg vancomycin twice-daily produced antibiotic levels above MBC (Miminal Bactericidal Concentration).
- after 7 days of therapy with daptomycin or vancomycin, counts of the revertant strain decreased, compared to untreated animals, but were not significantly different from each other.
- in daptomycin-treated rats, 3 out to 28 cages yielded subpopulations with reduced susceptibility to daptomycin.

Clinical aspects of resistance therapy Novel therapeutic approaches for hip prosthetic infections should take into account the type of microorganism, antibacterial susceptibility and clinical presentation (age, comorbidities, etc.). These approaches allow less aggressive surgical techniques and the use of antibiotic combinations (particularly with quinolones) active against biofilm-associated bacteria, including rifampicin, and with excellent bioavailability allowing prolonged and efficient therapy.

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