Post-translational regulation of alginate export by an oxygen sensor in *Pseudomonas aeruginosa*

A. Schmidt1, A.S. Lipps1, M. Bastian1, M. Merighi2, F. Aktirk1, S. Mailänder1, J. Madlung1, K. Lapouge4, C. Poschgen1, R. Simm1, K.R. Acharya1, U. Kömling1, M. Ulrich1, B. Macek3, L. Wiehlmann4, G.B. Pier5, S. Lory2, D. Haas4, D. Döring1.

1 University of Tübingen, Institute of Medical Microbiology and Hygiene, Tübingen, Germany; 2 Harvard Medical School, Department of Microbiology and Molecular Genetics, Boston, MA, United States; 3 University of Tübingen, Interfaculty Institute for Cell Biology, Proteome Centre, Tübingen, Germany; 4 Université de Lausanne, Département de Microbiologie Fondamentale, Lausanne, Switzerland; 5 MTC, Karolinska Institutet, Stockholm, Sweden; 6 University of Bath, Bath, United Kingdom; 7 Medizinische Hochschule Hannover, Hannover, Germany; 8 Harvard Medical School, Channing Laboratory, Boston, MA, United States.

To respond to environmental oxygen limitation, many facultative anaerobic bacteria use elaborate oxygen sensing mechanisms coupled to signalling cascades which allow optimizing a microaerobic/anaerobic life style. In *Pseudomonas aeruginosa* oxygen limitation causes up-regulation of the exopolysaccharide alginate stimulating a sessile life style. The molecular mechanisms leading to this phenotypic change are largely unknown. Here we describe a bacterial three component system (OraB/OraA/SadC) in *Pseudomonas aeruginosa* which up-regulates expression and export of the exopolysaccharide alginate under oxygen limitation via the second messenger cyclic bis-(3′,5″)-diguanylate monophosphate (c-di-GMP). During aerobic growth, cytosolic OraA blocked the activity of the membrane-bound diguanylate cyclase SadC. Anaerobic conditions relieved the inhibition of SadC by OraA leading to c-di-GMP production and high alginate expression. Mutations in *sadC* or *oraB* in strain PA01 resulted in a profound loss of alginate synthesis, whereas mutations in *oraA* allowed constitutive alginate synthesis under aerobic and anaerobic growth conditions. In the mucoid *P. aeruginosa* variant PDO300 carrying a mucA mutation, a sadC mutation abrogated alginate production under aerobic and anaerobic conditions, whereas overexpression of oraA prevented alginate production under aerobic growth. This novel post-translational regulatory mechanism links oxygen sensing with c-di-GMP-coupled signal-transduction and exopolysaccharide production in *P. aeruginosa*.

Prevalence of MRSA in patients with cystic fibrosis (CF) in R. Macedonia

T. Jakovska-Maretti1, S. Fustik2, L. Spirevski2, 1 University Pediatric Clinic, CF Center, Skopje, Macedonia, the Former Yugoslav Republic of; 2 University Pediatric Clinic, Skopje, Macedonia, the Former Yugoslav Republic of

Aim: Increasing prevalence of MRSA in CF population is an emerging problem. This study is aimed to determine the rate of MRSA in our CF patients in previous 3 years.

Methods: The study included 156 patients (mean age 15±8) with MRSA (17.8%) in 2009, 6 (6.6%) in 2008 and 3 (3.33%) in 2007 retrospectively. Multidrug resistant MRSA was detected in 10 patients (9%). One child died from MRSA sepsis (8 y) and one has bacterial endocarditis (16 y). Eradication therapy was performed to all patients using combined dual IV antibiotic treatment accompanied by hygienic directives over 3 weeks. This was followed by a 4 week period with oral antibiotic therapy.

Conclusion: Prevalence of MRSA in our CF patients in the last year was very high in comparison with previous years. We suspect that the reason is transmission between the patients, because we have limited space and we have no opportunity to separate our patients.

Comparison and characterization of MRSA isolates from paediatric CF patients

D.F. Gilpin1, S.J. McGrath1, J. McAughan2, M. Muhlebach3, R. Skov4, A. Reid5, J.S. Elborn1, M.M. Tunney1, 1 Queen’s University, School of Pharmacy, Belfast, United Kingdom; 2 Belfast Health and Social Care Trust, Northern Ireland; 3Pixdaus CF Centre, Belfast, United Kingdom; 4 University of North Carolina, Chapel Hill, NC, United States; 5 Statens Serum Institut, Copenhagen, Denmark; 6 Belfast Health and Social Care Trust, Adult CF Centre, Belfast, United Kingdom.

Introduction: Although MRSA is increasingly cultured from the respiratory tract of CF patients, its contribution to declining lung function remains unclear. This study aimed to characterize MRSA isolated from CF patients at two paediatric centres using molecular typing methods. Levels of delta-haemolysin (d-hly), the translation product of the major effector molecule of the agr system, were also determined.

Methods: Isolates were characterized by pulsed-field gel-electrophoresis (PFGE), SCCmec typing (multiplex PCR), agr typing (PCR-RFLP) and spa typing. Levels of d-hly produced by isolates grown under both anaerobic and aerobic conditions were compared by measuring in vitro haemolysis assays.

Results: PFGE determined that isolates from each centre formed distinct clusters with spa types similar to those in circulation in local hospital populations. Levels of d-hly were greater under anaerobic vs aerobic conditions for 19/28 isolates (67.9%), with no significant difference apparent for 8/28 (28.1%) isolates. Initial results suggest that this was most marked among isolates with agr II.

Conclusions: MRSA cultured from CF patients appear to reflect strains circulating in the local hospital background, as suggested by SCCmec and spa typing, and additional work is therefore required to investigate routes of transmission or exposure to MRSA in the CF clinic. The implications of increased production of d-hly under anaerobic conditions are under further investigation, as this may result in the down-stream modulation of other virulence factors, which may potentially play a key role in MRSA infection in CF.