**4. Microbiology**

**136** *Pseudomonas aeruginosa* genotyping to control the efficacy of hygienic measures to segregate CF patients by *P. aeruginosa* status

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The CF clinic Hannover introduced segregation measures between *Pseudomonas aeruginosa* – positive and negative patients at the CF ward in 1986 and at the outpatients clinic in 1991. Early antipseudomonal chemotherapy has become routine since the early 1990s. Since 1998 we collect sequential *P. aeruginosa* isolates from all newly colonized patients since the onset of colonization.

We genotyped 125 first and 59 2- to 3-year isolates from 87 CF patients who had their first *P. aeruginosa*-positive throat swab or sputum in the 1998–2004 time period. *P. aeruginosa* isolates were typed by an informative microarray that identifies strains by SNP genotype of the core genome and the presence of variable elements of the accessory genome.

Sixty clones were only detected in single patients. There were seven cases where two patients and four cases that trios shared the same clone. Five clones in total clustered in groups of four, five, six, seven, and nine patients, respectively.

The two most common clones belong to dominant clones of the *P. aeruginosa* population and patients' isolates from the third and fourth most common clones were heterogeneous in their composition of the accessory genome suggesting that the nosocomial acquisition from a common source is unlikely. However, in numerous cases patients became concomitantly newly colonized with the same uncommon *P. aeruginosa* clone. Despite strict segregation measures the acquisition of *P. aeruginosa* from a common source was apparently not always prevented in our patient cohort. In summary, our new microarray is an appropriate endpoint measure for the surveillance of the *P. aeruginosa* epidemiology and of the efficacy of hygienic measures at CF clinics.

**137** The mobile gene island pKLC102 generates genome diversity of *Pseudomonas aeruginosa* in CF lungs

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Clinical isolates of *Pseudomonas aeruginosa* clone C and clone PA14 harbour different gene islands, e.g. PAGI-2, PAPI-1 and pKLC102. The genome islands are part of the accessory genome of *P. aeruginosa*. One part consists of strain-specific ORFs and the second part is made up of a syntenic set of conserved ORFs of the majority of which is classified as conserved hypotheticals of unknown function.

Different types of genome islands sharing this syntenic core have meanwhile been identified in more than a dozen proteobacteria. Dot blot experiments of more than 70 *P. aeruginosa* clones of diverse habitats and origin were analyzed for the presence of 84 ORFs of the mobile genome island pKLC102. Positive hybridization signals for 60 – 84 ORFs were found in the majority of tested strains suggesting that this island is abundant in *P. aeruginosa*. In CF clone C isolates pKLC102 was found either as an episomal plasmid or integrated into the rRNA locus adjacent to pIIA. Analysis of single isolates recovered from the same CF sputum or in vitro subcultures of single strains by combinatory PCR revealed a heterogeneous bacterial population carrying either no, only plasmid copies, only chromosomal copies or both episomal and chromosomal copies of pKLC102 indicating that pKLC102 self-mobilizes and transfers at high rates.

In summary, pKLC102 substantially contributes to the genome diversity of clone C in CF lungs and represents a new type of genome island that is rapidly integrated and excised from the chromosome by site-specific recombination.

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**138** Genomic islands from *Pseudomonas aeruginosa* CF-isolates spread the barrier of bacterial species

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*Pseudomonas aeruginosa* strains show remarkable genomic diversity. More than 20% of the genome can be made up by (pro)phages, plasmids and other mobile elements, which are specific for single strains or subgroups. When integrated into the chromosome, such variable blocks of DNA appear as "genomic islands" within the "core genome".

We have sequenced several genomic islands with sizes of more than 100 kb from different *P. aeruginosa* strains isolated from the lungs of CF-patients or from environmental habitats. The islands show significant homology among many of their putative genes. These homologues were also positioned in the same synteny.

In addition, the genomic islands contain blocks of non-conserved DNA ("cargo") coding for proteins with various functions like metabolic enzymes or pathogenicity factors. Therefore these gene islands appear as related genetic elements sharing a bipartite structure.

Similar genomic islands was also found in other *P. aeruginosa* strains and even in other bacterial species. We detected similar DNA in more than 40% of the strains from a *P. aeruginosa* collection (CF-isolates, other clinical and environmental isolates). In addition, database comparisons revealed more than 30 similar islands among bacterial genomes. They appear in closely related species (*P. fluorescens*, *P. syringae*) and also in different genera, e.g. *Burkholderia* or *Haemophilus*.

Therefore, we consider these gene islands to be members of a widespread family of related integrative elements, which confer a broad variety of "cargo" to the host genome. This "cargo" could contribute significantly to the special characteristics of a single bacterial isolate.

**139** DNA-chip based high throughput analysis of the population structure of *Pseudomonas aeruginosa*

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A cost-effective and fast microarray device for high throughput geno- and pathotyping of *P. aeruginosa* has been developed allowing insight into its population structure, spread, local epidemics and evolution. The DNA based analysis covers pathogenicity associated genes and SNPs of highly conserved *P. aeruginosa* genes, yielding an identification fidelity >99.8%. In addition the prevalence of the most common genome islands is surveyed.

The chip is commercially available and attractive to generate, store and exchange typing data in a standardised, universally applicable format. Thus, population structures and the spread of clones at domestic, continental and global levels can be monitored.

We examined more than 200 European *P. aeruginosa* isolates from CF patients, ICUs and environmental sources. Interestingly, only 15 strains covered more than 50% of all isolates, with the sequenced PAO1 and PA14 strains representing two of the major clones. We identified the most common strains in samples of various origins, with no prevalence to any specific habitat.

Co-colonizing strains of *P. aeruginosa* were unrelated in most cases. Nevertheless, we found for each of these strains closely related clonal variants in other samples, sometimes from different countries or even continents. The appearance of gene islands, however, can vary in every clone even within one habitat. This shows that the molecular clock is running at significantly different speeds for the core and the accessory genome.