

Investigation of virulence genes in *Aerococcus urinae* and *Aerococcus sanguinicola* strains causing infective endocarditis, urinary tract infection and urosepticaemia using whole-genome sequencing

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Objectives

Infective endocarditis (IE) is a serious heart disease with a high mortality rate. Strains belonging to the genus *Aerococcus* are known, however rare, etiologic agents of IE. *Aerococcus* strains are known as common pathogens in urinary tract infections (UTI). It is unknown how a common pathogen for UTIs with few identified toxins and virulence factors can be a successful pathogen for a serious infection like IE. In this study strains of *Aerococcus urinae* (*A. urinae*) and *Aerococcus sanguinicola* (*A. sanguinicola*) were whole genome sequenced (WGS) to investigate pathogenicity.

Methods

Five isolates of *A. urinae* and three *A. sanguinicola* strains from urine (N=5) and blood (N=3) samples and were obtained from four patients with verified IE, UTI and urosepticemia in Denmark. Their genomes were sequenced together with the respective type strains using Illumina MiSeq. Species identifications were routinely performed with MALDI-ToF mass spectrometry. The reads were assembled into contigs using the CLC-Bio assembler and then annotated with the online genome annotation tool Rapid Annotation using Subsystem Technology, RAST (<http://rast.nmpdr.org/rast.cgi>). Virulence gene matrix for each strain was generated based on the annotated genomes.

Results

Aerococcus genome sizes were between 1.9-2.1 Mb and the number of contigs varied from 22 to 99. 1721-1861 protein encoding genes were annotated in the *A. urinae* genomes and 1812-1886 in the *A. sanguinicola* genomes. Plasmids were not present in any of the genomes.

The number of virulence, disease and defense (VDD) genes annotated varied from 21 to 26 for *A. urinae* and were exactly 27 for *A. sanguinicola* genomes including the respective type strains. Sixteen VDD genes were found in both *Aerococcus* species that were involved in the following RAST-subcategory: 1) adhesion, 2) resistance to antibiotics and toxic compounds and 3) invasion and intracellular resistance. All strains of *A. sanguinicola* shared seven VDD annotated genes, whereas two other VDD genes only were found in the *A. urinae* genomes. These nine VDD annotated genes were associated in bile hydrolysis, cobalt-zinc-cadmium resistance, copper-homeostasis and multidrug resistance efflux pumps.

Three invasion and intracellular resistance associated VDD genes were found in all clinical and type strains of *Aerococcus*, except for two clinical strains of *A. urinae* which were isolated from the same patient. These genes were linked to protein synthesis of *Mycobacterium* virulence operon. A fibronectin-binding protein encoding gene was only found in one clinical *A. urinae* strain and the corresponding type strain.

Conclusions

Species-specific genomic differences between strains of *A. urinae* and *A. sanguinicola* according to VDD genes were observed by using WGS and RAST annotation. The functional meaning of these observations needs to be elucidated further. WGS of another 40 clinical *A. urinae* and *A. sanguinicola* strains have been planned to further identify key factors associated with disease outcome.