The recognition of two tentative novel species among haemolytic *Acinetobacter* strains

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Objectives. The genus *Acinetobacter* currently consists of 32 (genomic) species and a number of as yet unclassified strains. The aim of the present study was to classify haemolytic *Acinetobacter* strains of unknown taxonomic status isolated mostly from human clinical specimens.

Methods. Twenty-eight haemolytic strains that could not be identified as belonging to any known (genomic) species were studied by phenotypic analysis, amplified rDNA restriction analysis (ARDRA), AFLP fingerprinting and 16S rDNA comparative sequence analysis. Type or reference strains of all known *Acinetobacter* (genomic) species were included.

Results. Using a polyphasic approach, the investigated strains were classified into two well-separated phenetic groups, termed phenon 3 (*n*=8) and phenon 7 (*n*=15), five isolates remaining ungrouped. Each of the two phenons contained non-glucose-acidifying strains that showed identical or highly similar phenotypic properties and ARDRA profiles, and formed distinct AFLP clusters at a similarity level of >50 % which is generally the species delineation level. In addition, 16S rDNA sequence analysis of three and two strains of phenons 3 and 7, respectively, indicated that these groups formed two separate lineages within the genus *Acinetobacter*. The phenons could be distinguished phenotypically from each other and from all known (genomic) species. The strains of phenon 3 were isolated exclusively from human clinical specimens whereas the phenon 7 strains originated from human (*n*=8) and equine (*n*=2) clinical specimens, hospital environment and staff (*n*=2), or from soil (*n*=2). The human clinical specimens were mostly represented by tracheal aspirate of hospitalised patients.

Conclusion. This study has shown that most of the unclassified haemolytic strains belong to two phenons distinct from all described (genomic) species of the genus *Acinetobacter*. These phenons probably represent two novel species as indicated by the presented results and by partial DNA-DNA reassociation data available from the previous taxonomic studies. The available epidemiological and clinical data suggest that the strains of both phenons may play a role in infections of hospitalised patients.