Human pathogenic bacteria are a common cause of infection and a recurrent disease complication, causing millions of deaths and bringing considerable costs to clinical facilities. Timely identification of the bacteria involved in infection is essential to clinical diagnosis and thus, disease management. Often, colony morphology observation complements conventional microbial diagnosis as means to detect phenotypic heterogeneity. Colony morphology variants, including rough, small and mucoid phenotypes, have been isolated from several human infections. Notably, *P. aeruginosa* mucoid phenotype and *S. aureus* small colony variants (SCV) are of relevance in the study of cystic fibrosis, showing a markedly more resistant phenotype to antibiotics. Several other antibiotic resistant morphotypes have been identified in bacteria related to chronic and acute infections and even to device-associated infections.

Clinical decision making relies on the ability to profile pathogenic behaviours, to interpret such behaviours adequately, and to monitor and control new occurrences. Therefore, colony morphology profiles could be useful to anticipate the response (at least, to a certain extent) to be expected of a bacterial community to antimicrobial treatment.

Colony morphology features can be viewed as part of a pathogenic signature. By collecting signatures across diseases and species, predictive and associative data mining can be used to debrief these signatures regarding occurrence-consequence relationships. Moreover, the correlation of morphological manifestations with particular regulatory responses (and pathways) can be explored by further detailing the signatures in terms of genome and proteome. Overall, these efforts originate a rich and varied set of features for decision making.

The aim of this work was to evaluate the morphotyping ability of image processing tools, both and to maximise characterisation abilities. The case study relates to previously described pathogenic morphotypes, publicly available at the colony morphology database MorphoCol (http://morphocol.org/). Evaluation addressed the inter-annotation agreement between expert visual inspection and automatic image processing using the publicly available ImageJ tool. The level of agreement obtained was sufficient to consider the creation of a semi-automatic image annotation pipeline. Since automatic processing is focused on technical aspects of the images (e.g. pixel intensity), it is unbiased towards the characterisation of multiple species or diseases and does not implicate expert knowledge. Then, MALDI-TOF MS analyses could be used to obtain molecular signatures and support the analysis of meaningful pathogenic regulatory cascades. Preliminary results on this side of the pipeline showed promising insights on the biology of pathogenic morphotypes.

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