Use of high throughput phenotyping and genome wide association studies (GWAS) to identify genetic determinants of meningococcal disease traits

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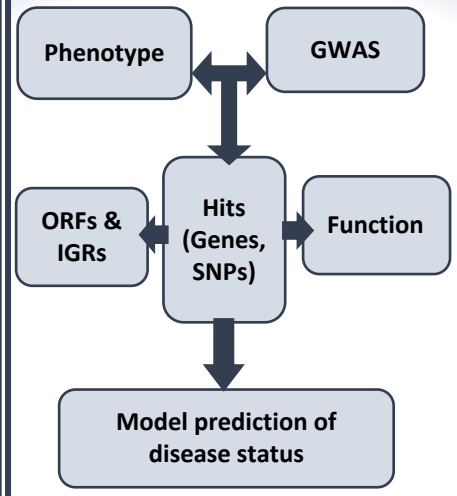
Context

Despite ongoing vaccination programmes, *Neisseria meningitidis* is still a major cause of septicemia and meningitis. Strains with cc11 MenW and cc23 MenY genotypes are both common causes of disease and frequently observed among carriers. In 2019-20, MenW and MenY isolates caused 26% of all UK invasive meningococcal disease cases (Health Protection Report 2021). High levels of genetic variation are thought to facilitate the ability to cause disease and to establish a carriage state.

Genome sequence data is generated for all meningococcal disease and carriage isolates and deposited in the MRF Meningococcal Genome Library.

Using this resource, we aim to determine how genetic variation contributes to differences in clinically important bacterial phenotypes and if specific phenotypic traits influence disease.

Plans and Goals



Case 1. MenW cc11 Phenotypic Variation

A total of 163 MenW cc11 isolates (carriage and disease) were selected for testing. Disease isolates were equally split between the original MenW cc11 variant that appeared in 2009 and a sub-variant that was first detected in 2013.

Glycerol stocks were prepared in multi-well plates from one mid-log phase growth to allow for high-throughput and reproducible testing in a range of phenotypic assays.

Findings and Progression

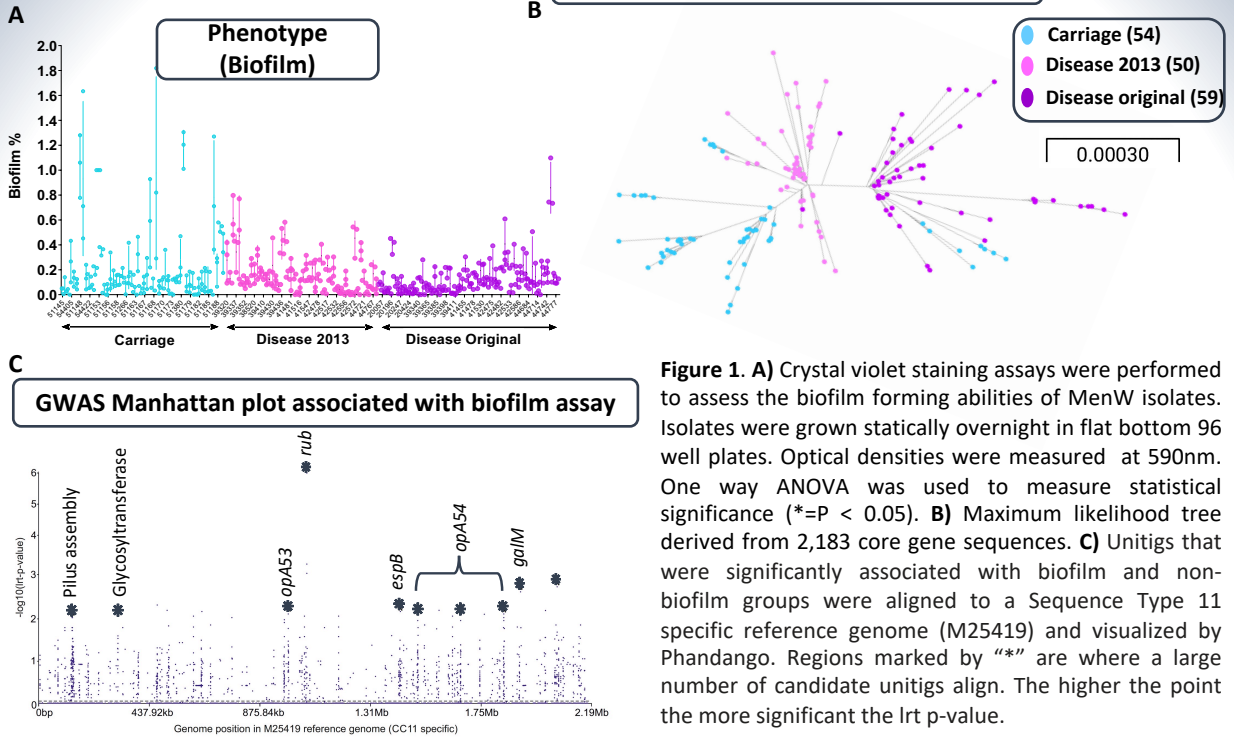
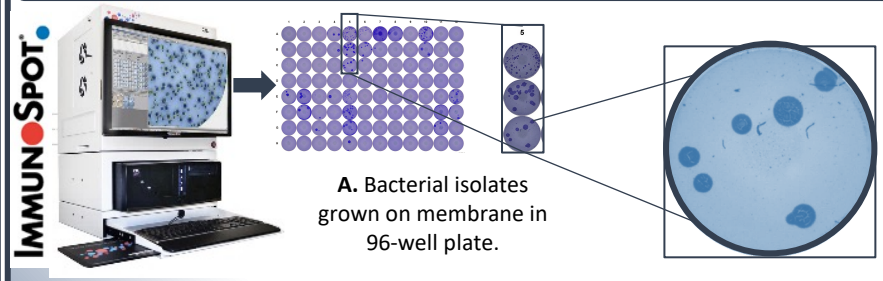


Figure 1. **A)** Crystal violet staining assays were performed to assess the biofilm forming abilities of MenW isolates. Isolates were grown statically overnight in flat bottom 96 well plates. Optical densities were measured at 590nm. One way ANOVA was used to measure statistical significance (*=P < 0.05). **B)** Maximum likelihood tree derived from 2,183 core gene sequences. **C)** Unitigs that were significantly associated with biofilm and non-biofilm groups were aligned to a Sequence Type 11 specific reference genome (M25419) and visualized by Phandango. Regions marked by "*" are where a large number of candidate unitigs align. The higher the point the more significant the lrt p-value.

Development of a new and robust method for high-throughput quantification of colony forming unit (CFU) in ImmunoSpot machine.(CTL)



A. Bacterial isolates grown on membrane in 96-well plate.

B. CFU counts as well as colony morphology could be visualise at microscopic level.

Work in Progress

Multiple phenotypic experiments have been done in conjunction with GWAS to link phenotypic variants to specific gene alleles, nucleotide variants or phase variable states. These analyses are on-going but will provide a foundation for future experimental confirmation.

Conclusions

1- Our assay systems are robust, reproducible, and easily scalable for efficient high-throughput genotypic and phenotypic testing.

2- Our preliminary results pinpoint a range of genetic variants and gene pathways that are potentially linked to each phenotype.

3- The availability of large data sets of complete genome sequences with accompanying phenotypic metadata promises to revolutionise the identification of disease-associated phenotypes and may lead to an enhanced ability to predict disease potential.

Funding
Medical Research Council
(MR/S009264/1)