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Fishing for gonorrhoae – a custom amplicon panel for detection in clinical metagenomes

Background

Culture independent diagnostic testing (CIDT) has been adapted by public health laboratories around the world resulting in faster diagnosis and cost reduction.

However, the catch of CIDTs (such as nucleic acid amplification tests) is that results are given in binary fashion (presence/absence), further information on resistance or type without additional enrichment steps or culturing is very limited (Figure 1).

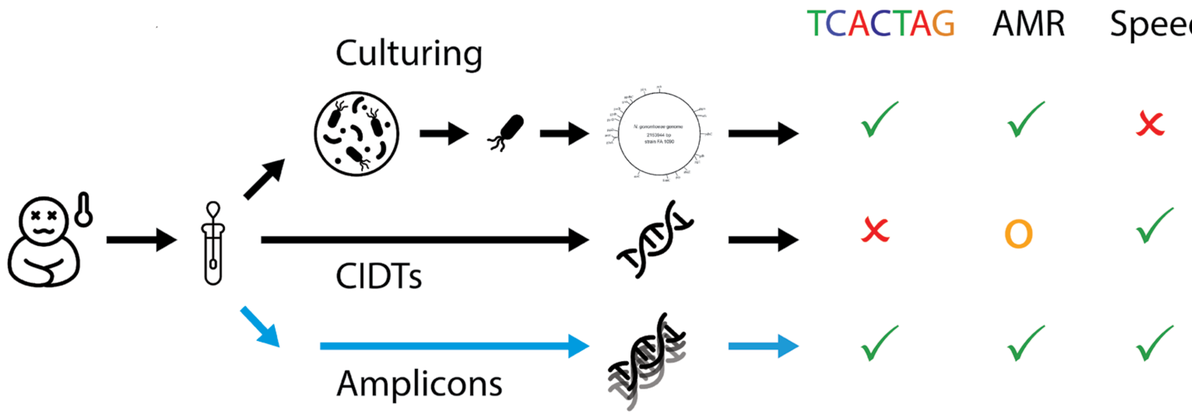

Figure 1. Overview of sample processing work flows: traditional culturing, CIDT and amplicon sequencing. AMR = Antimicrobial resistance

Approach

Panel design* → **Species specificity** → **Primer design** → **Synthetic metagenomes** → ***In silico* validation** → **Clinical validation**

* 50S ribosomal protein L6 (*rplF*) – distinction of *Neisseria* species[1] + 7 AMR genes: *gyrA*, 23S, *penA*, *mtrR*, *ponA*, *porB*, *parC* (based on *Neisseria gonorrhoeae* NG-STAR typing scheme[2])

Neisseria gonorrhoeae

Gonorrhoea is the second most common sexually transmitted disease worldwide and of growing public health concern due to its recombinant nature and ability to progressively evolve resistance to antibiotics[3].

Global gonorrhoea rates are on the rise (Figure 2). A survey of antimicrobial resistance in New Zealand *N. gonorrhoeae* (*Ngon*) is only done every few years resulting in a lack of surveillance.

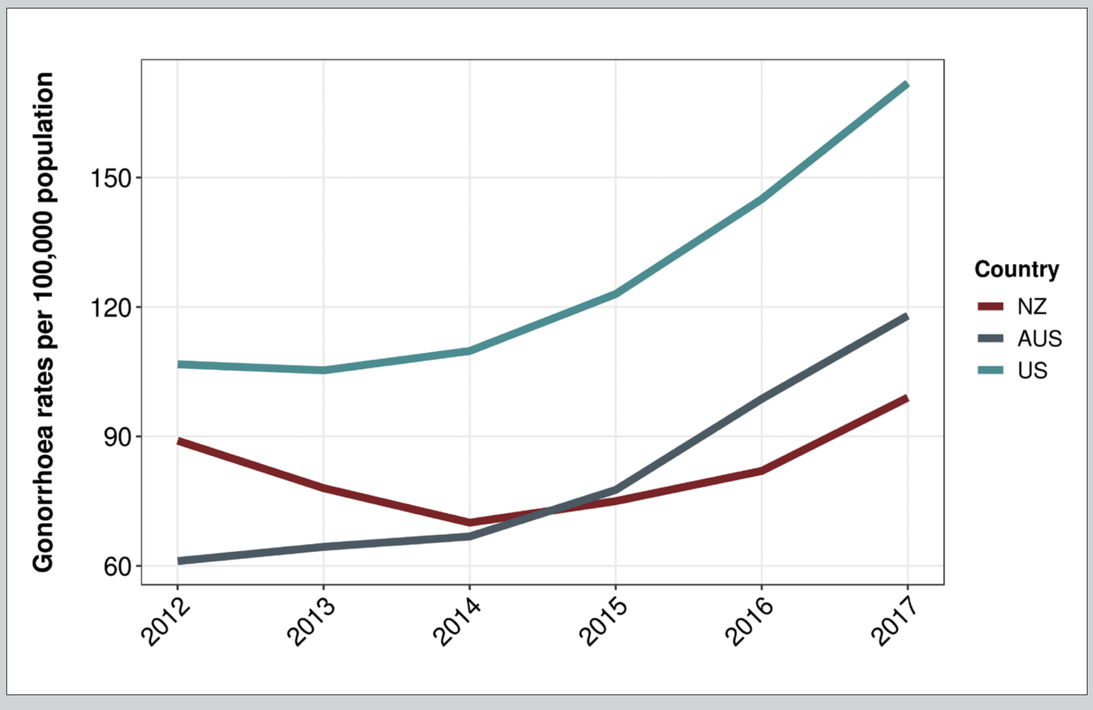

Figure 2: Comparison of gonorrhoea notification rates per 100,000 population for New Zealand[4], Australia[5] and the United States[6].

Results

A total of **158 shotgun metagenome** samples (Human Microbiome Project and Sequence Read Archive) from different body sites were blasted against custom databases containing target genes (min. ident. 70 %, e-value $10x10^{-5}$) to determine Neisseria species specificity (Figure 3). Samples from the oral cavity gave the highest number of hits, due to commensal *Neisseria* species. Classification revealed 98.1% (*gyrA*, *parC*, *rplF*) to 100% *Neisseria* specific hits (*ponA*, *penA*, *mtrR*, *porB*), whereas 23S yielded 21.5% *Neisseria* classifications.

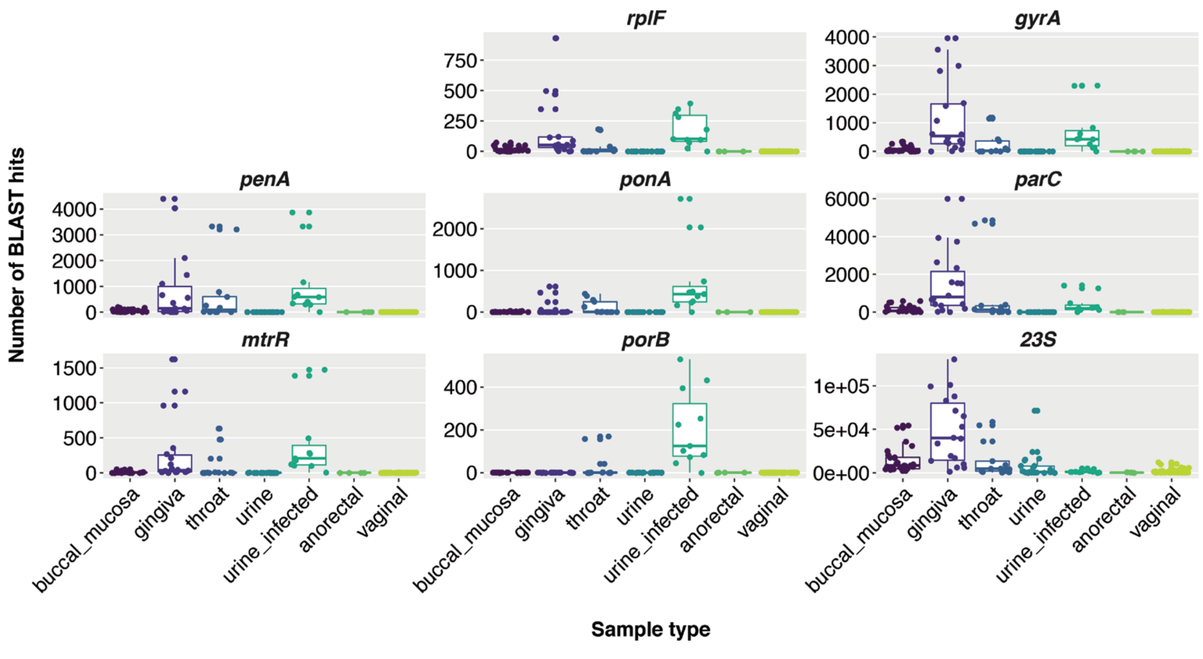

Figure 3: Raw BLAST hits for shotgun metagenome samples. Except for urine-infected data from Ngon-positive samples, all data stems from healthy patients.

Methods

- BLAST and classification of the hits using centrifuge v1.0.4[7]
- Primer pairs were designed for 2x250bp Illumina Miseq runs, covering the allele lengths in public databases to allow epidemiological inference.
- Primers were tested for *Neisseria* species specificity using an *in silico* PCR against the *Neisseria* pubMLST database[8].

Primer design caveats

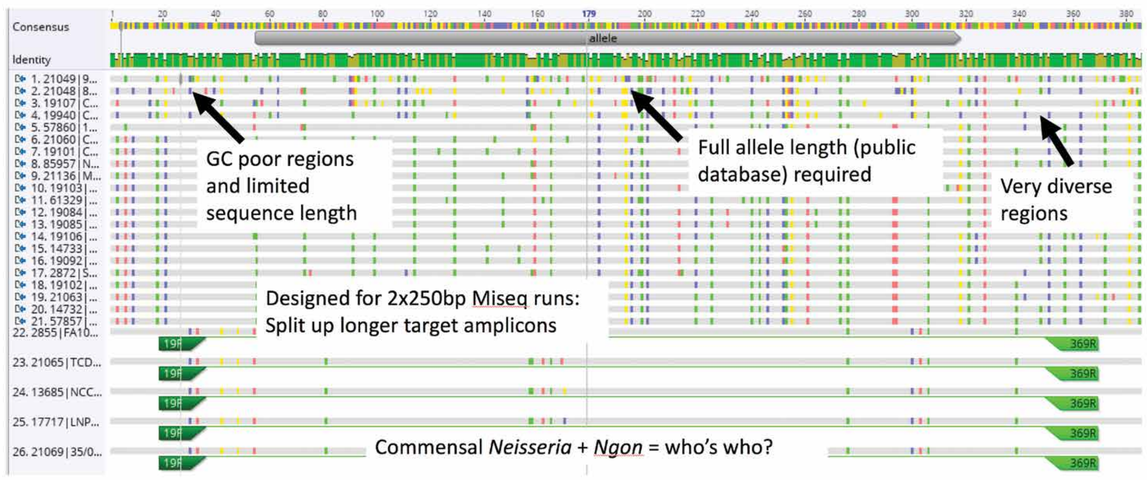

Figure 4: Illustrating caveats in primer design. Alignment shows *N. gonorrhoeae and N. meningitidis gyrA* CDS.

References:

[1] Bennett et al. 2014 J Clin Microbiol
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[3] WHO, Global Action Plan AMR in N. gonorrhoeae, 2012
[4] STI in NZ, Annual Surveillance Report 2016, ESR
[5] https://www.aihw.gov.au/reports/australias-health/australias-health-2018/contents/indicators-of-australias-health/sexually-transmissible-infections-bloodborne-virus
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[8] https://pubmlst.org/neisseria/

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