Molecular diagnostics of antimicrobial resistance in Neisseria gonorrhoeae: a state-of-play.

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Gonorrhoea is a worsening global health threat

Neisseria gonorrhoeae is a causative agent of chronic abdominal pain, pelvic inflammatory disease and disseminated gonococcal disease. Cases of *N. gonorrhoeae* infections are increasing globally; there was an overall 80% increase in notifications in 2015–2017 in Australia [1]. It disproportionately affects men who have sex with men, Indigenous Australians, people aged 15-24, and more recently, there that been a resurgence of Gonorrhoeae in urban heterosexuals.

each year in the U.S.A

~550,000

Gonorrhea infections are **resistant** to at least one antibiotic [2]

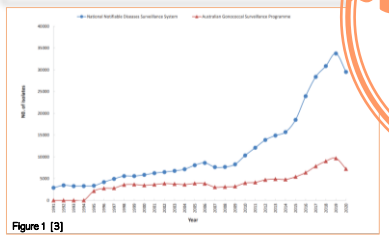

Figure 1 [3]

Molecular diagnostics of resistance are required

Traditional bacterial culture techniques remain the gold standard for *N. gonorrhoeae* antimicrobial resistance (AMR) testing. However, in the last two decades culture has been gradually replaced by more sensitive Nucleic Acid Amplification Testing (NAAT) for diagnosis of Gonorrhoea. Unfortunately, antibiotic resistance isn't easily detected via NAAT, and to our knowledge there are currently only two commercially available for this purpose. This diminishes disease surveillance efforts. There is a need for molecular AMR tests that can detect genetic mutations that confer resistance to multiple antibiotics.

Benefits of molecular testing

- Molecular AMR testing will enhance surveillance of the spread of AMR alleles in gonococcal populations, giving a greater sample size.
- Using rapid molecular testing to diagnose resistance, as opposed to culture-based testing, offers the potential for development into rapid point-of-care diagnostics.
- Enhanced capacity to detect resistance, particularly at the point of care, can facilitate resistance-guided therapy (where the results of the molecular test are used to directly inform individual patient treatment).

Barriers to development

- Commensal *Neisseria* species often have high genetic homology in genes of interest to resistance, due to horizontal gene transfer. High stringency is required to reduce cross-reactivity and false positives. This is primarily an issue affecting pharyngeal swabs,
- Mechanisms of resistance are often complex and multifactorial, and so not readily incorporated into simple rapid molecular tests. For example, compensatory mutations that are present in resistant genotypes are not necessarily the cause of resistance. Further, not all genes in a genome are expressed.
- The requirement to update tools with novel resistance mutations.

What can be done?

There are new ides that are being employed to enhance feasibility:

- Simplifying the approach by identifying wildtype sequences to predict susceptibility rather than trying to detect all known resistance mutations.
- Using whole genome sequencing-based phylogenomic associations for resistance correlations between clones.

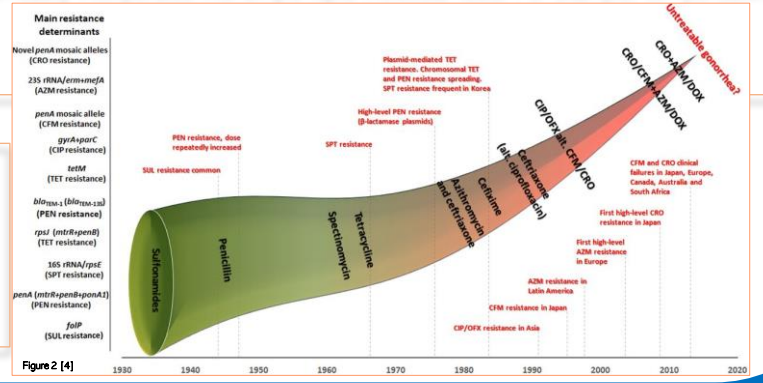

Figure 2 [4]

Future research:

More research is required to develop new molecular resistance testing methods, including:

- enhancing understanding of the molecular mechanisms and evolutionary pathways to resistance,
- improved understanding of AMR genes in other *Neisseria* species to inform assay design, thereby reducing cross-reactivity with commensal *Neisseria* species, and
- longitudinal genomic studies (particularly after molecular diagnostic implementation) to characterise the potential for novel resistance emergence.

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References: [1: Australian gonococcal surveillance programme annual report 2019], [2: CDC USA national centre for HIV/AIDS, viral hepatitis, STD and TB prevention], [3: adapted from Australian Gonococcal Surveillance Programme Annual Report, 2020], [4: M. Unemo and W. M. Shafer 2014].