Neisseria gonorrhoea testing in New Zealand, culture or PCR?

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Abstract

There is change afoot for the diagnosis of Neisseria gonorrhoeae in New Zealand. As was the case for Chlamydia trachomatis in years past, molecular technologies are gradually taking over and it is important that laboratories do not expect the same smooth transition. Ongoing monitoring of the specificity of methods in the face of high rates of genetic transfer in Neisseria as well as the increased cost of nucleic acid amplification tests (NAAT) testing, specialist facilities and technical skills required should be taken into account. Without a national consensus on gonorrhoea testing, concerns over false positive results from NAAT and the emergence of antibiotic resistance are being handled in different ways. Following an open discussion at the 2012 NZIMLS Annual Scientific Meeting, it was our aim to present a high level overview of the state of Neisseria gonorrhoeae testing in New Zealand, and the challenges that are being faced.

Key words: Neisseria gonorrhoeae, polymerase chain reaction, New Zealand, gonorrhoea, testing trends, Sputasol, porA/pa supplementary testing

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Introduction

Neisseria gonorrhoeae was first described by Albert Neisser in 1879. Of the eleven species of Neisseria that colonise humans, only two are pathogens. N. gonorrhoeae is the causative agent of gonorrhoea, known colloquially as "The Clap," derived from the French word "clapier" (which has variously meant "brot hel" or "pustulant sore"), and is transmitted via sexual and perinatal contact (1). This gram negative diplococcus infects the human urogenital tract, eye, throat, and rectum, and can cause systemic infections. Most gonorrhoea infections in women go undetected as the patients are asymptomatic, although the long term sequelae can be significant (2,3). In males, the majority of patients experience symptoms; however there is an understandable reluctance to undergo the invasive testing required for culture (urethral swabs) which may lead to an underestimate of prevalence.

N. gonorrhoeae bacteria are motile and possess type IV pili to adhere to surfaces similarly to a grappling hook. Through cooperation, groups of N. gonorrhoeae are able to pull up to 100,000 times their own weight, the equivalent of a human pulling 2,000 African elephants (4). In 2011, researchers at Northwestern University found evidence of a human DNA fragment in a N. gonorrhoeae genome, the first example of horizontal gene transfer from humans to a bacterial pathogen (5).

For years, gonorrhoea has been easily treated with a single oral dose of antibiotics. However, N. gonorrhoeae has progressively acquired resistance to each new agent: sulphonamides in the 1940s, penicillins and tetracyclines in the 1970s and 1980s, and fluoroquinolones by 2007. Since then, 3rd generation cephalosporins have been the only antibiotics recommended for empiric treatment of gonorrhoea (6) but clinicians now face the emergence of 3rd generation cephalosporin- resistant N. gonorrhoeae without any well-studied, effective backup treatment options (7).

Traditionally, N. gonorrhoeae detection has been performed by growing and identifying viable organisms by microbial culture before measuring antibiotic susceptibilities. Culture relies on organism viability, and detection of this is both time consuming and subjective. There has been a shift to NAAT for the diagnosis for infectious diseases, particularly for sexually transmitted infections (STIs), with detection of Chlamydia trachomatis by PCR recommended as best practice since 2008 in New Zealand (8).

However, when considering shifting to NAAT methods for detection of N. gonorrhoeae it is important that laboratories do not expect the relatively smooth transition seen previously when C. trachomatis moved from enzyme immunoassay to NAAT. N. gonorrhoeae is naturally competent for DNA transformation as well as being capable of conjugation. Both mechanisms allow the DNA of N. gonorrhoeae to undergo confornational changes, which can allow the bacteria to avoid detection if genetic changes occur in the gene targets of NAATs. The need for ongoing monitoring of the specificity of such methods, as well as the increased cost, specialist facilities and technical skills required, should be taken into account by laboratories.

New generation NAATs address some of these concerns by offering multiple genetic targets, reducing the chance of false negatives. Many molecular platforms now offer combined testing for C. trachomatis and N. gonorrhoeae. These next generation NAAT assays are more sensitive and specific than culture and predecessor PCR assays, and provide the option of screening using non-invasive specimens such as urine and self-taken vaginal swabs, although none are yet approved for use on non-genital sites. And none are yet able to offer reliable information on antibiotic susceptibilities.

National testing

During the 2012 NZIMLS ASM held in Wellington, a discussion about N. gonorrhoeae testing methods was opened to all attendees during our talk on “How do you like your gonorrhoea? Dead or alive?” There is little consensus between laboratories on how, when and whether to proceed to molecular testing for N. gonorrhoeae. The regions raised differing funding issues, instrumentation issues, laboratory space and the availability of technical skill as barriers to change. The NAAT instrumentation systems that are in use include the Roche cobas 4800 and the Roche cobas Amplicor platforms, BD Viper, and the Abbott M2000 platform. No attendees were using the Aptima Combo 2 system in October 2012. The majority of laboratories in New Zealand continue to perform culture based testing methods for routine N. gonorrhoeae detection, although many supplement this with some form of molecular testing.

Why didn’t we do it sooner?

Although dual target NAAT assays for Chlamydia and Gonorrhoea have been available in New Zealand since 1999, early versions of these assays were shown to lack specificity causing some hesitation in moving away from culture for N. gonorrhoeae. Two studies performed at Medical Laboratory Wellington did nothing to improve local confidence in the technology. In 2000, culture and Amplicor NG PCR (Roche) were compared for on 582 specimens from the Wellington Sexual Health Clinic. Positive PCR results were confirmed using the Amplicor NG 16s rRNA PCR (Roche). The positive predictive value of the confirmed cobas Amplicor NG test, however, was too low (83.3%) to consider replacing culture with PCR.
In 2006, following a case of PID in a patient post-termination of pregnancy in which *N. gonorrhoeae* was later identified as the causative agent; the Amplicor NG test was revisited to answer the question: was culture missing in cases in which *N. gonorrhoeae* would have been diagnosed. Additionally, 37% more patients were treated for *C. trachomatis* by PCR than we tested for *N. gonorrhoeae* by culture, indicating the higher acceptability of non-invasive urine and self taken vaginal swabs over cervical and urethral cultures. The validation data was reassuring enough for us to cease routine culture and move to cobas 4800 NG PCR in May 2012. Confirmatory testing for the porA pseudogene and the opa gene has been retained for non-genital positive results to acknowledge that the high rate of genetic exchange between Neisseria in the urogenital sites may lead to false positive results. Recently this testing was used to identify *N. gonorrhoeae* in a throat swab from Auckland (10).

**Fishhooks**

Two issues were identified during the study that other laboratories should be aware of. Firstly, female urine specimens have been shown to have low sensitivity for Chlamydia PCR (11,12), and the data confirmed that this is also true for *N. gonorrhoeae*, with only 86.7% sensitivity compared to a cervical or urethral culture test. Therefore urine cannot be recommended as a sole screening specimen for females.

Unexpectedly, we found a high incidence of ‘Failed’ cobas 4800 results from patients whose cultures had grown *N. gonorrhoeae*. These errors are reported by the cobas instrument when the pipettor is blocked by material in the sample. It appears that the mucopurulent discharge, commonly associated with *N. gonorrhoeae* infection, causes such interference. We eventually showed that 1:1 dilution of cobas collection buffer with Sputasol (1.4% DTT; Oxoid) could resolve a majority of these specimens without additional patient sample collection. This may lead to false positive results. Recently this testing was used to identify one such organism in a throat swab from Auckland (10).

Many local laboratories who are performing *N. gonorrhoeae* NAAT routinely, confirm positive results by either in-house assay or another commercial testing platform. Supplementary confirmatory testing needs to be carefully considered as the results of the RCPA Microbiology *C. trachomatis* / *N. gonorrhoeae* panels from 2012 showed that 30-50% of participants missed the low load *N. gonorrhoeae* samples in March 2012. The results also show that some laboratories are correctly obtaining a positive result for their screening method but a negative result using their supplementary method. This suggests some differences in assay sensitivity between laboratories which may be due to incompatibility between commercial specimen preparation systems, poor target choice or suboptimal performance of the supplementary assay (14).

**Emerging antibiotic resistance/how are we using/should we be using culture?**

The emergence of extensively drug resistant (XDR) strains of *N. gonorrhoeae* that display high level resistance to third generation cephalosporins (ceftixime and cefixime) is a major public health concern, as well as a concern for laboratories providing diagnostic testing. These organisms pose a problem for empirical treatment of patients not tested by microbial culture for *N. gonorrhoeae*. Recommendations from the CDC include outlines for enhanced antibiotic susceptibility surveillance to monitor the extent of the problem globally (15,16). These concerns have generated much debate over the wisdom of transferring *N. gonorrhoeae* diagnosis to NAAT methods. The problem for NAAT based testing methods is that to provide susceptibilities, a viable organism is required. The lysis buffers utilised for many commercial *N. gonorrhoeae* assays destroy the organism in order to gain access to the nucleic material of the cell.

Our current approach is to culture symptomatic patients selected by the Wellington Sexual Health Clinic and to monitor Ceftriaxone and Cefpodoxim susceptibility. ESR has indicated enhanced STI surveillance to include *N. gonorrhoeae* antibiotic susceptibility results will commence in 2013. Whilst this type of survey will decrease the risk of a ‘surprise’ 3rd-generation cephalosporin resistant organism emerging, there are no moves to develop a plan for patient management after it arrives.

A solution for the future may be in the development of molecular methods for the detection of sequences responsible for resistance. Until such tests are available for routine laboratory use, a duplication of testing facilities for both culture and PCR detection of *N. gonorrhoeae* is required in order to continue surveillance of antibiotic susceptibility (17). The conundrum of a Neisseria diagnostic laboratory arises when it is not economically viable to provide both tests. In this situation we pose the question, is sensitivity subordinate to surveillance?

**Best practice guidelines for testing and treatment**

At the 2012 New Zealand Sexual Health Society (NZSHS) gonorrhoea workshop, it was agreed that standardisation of testing and treatment was needed in New Zealand, and a panel of experts was convened to tackle this task on behalf of the NZSHS. The group includes sexual health physicians, technical laboratory specialists in both molecular and microbiology, and representatives from ESR.

The aim of this ‘Gonorrhoea Working Group’ is to develop a best-practice guideline document to advise diagnostic testing and clinical management of *N. gonorrhoeae* infection in New Zealand. In particular:

- To standardise testing methods for detection and reporting of infection and antimicrobial susceptibility.
- To develop an action plan for management of cases with suspected treatment failure, enhance ESR surveillance of resistance within the community and provide recommendations for outbreak management at a regional level.

Consultation with stakeholders is planned as part of this process. We look forward to the development of this guideline in 2013.

**Summary**

*N. gonorrhoeae* is an intriguing bacteria, with the strength to move elephants (relatively), change genes faster than Clarke Kent turning into Superman, and it can even use human DNA to its own advantage. Finally, we are nearing the end of the line for empiric treatment with reports of rising MIC’s for 3rd generation Cephalosporins, the last group of antibiotics known to be effective at clearing *N. gonorrhoeae* infection.
The use of NAATs to screen for infection increases the accessibility of testing for those who would otherwise refuse an invasive swab sample. Other advantages include optimal sensitivity and detection of non-viable organisms allowing for less stringent requirements for the collection and storage of specimens. However NAATs are currently unable to provide antibiotic susceptibility information data (to referring clinicians or for monitoring of resistance patterns). Gonorrhoea control relies on early detection and treatment. Consensus needs to be developed about best practice testing for infection and how to monitor the incursion of ceftriaxone resistance strains into New Zealand. Even more important is to develop an action plan for when this unwelcome visitor does arrive.

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References