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## Biography

**Dr Amy Jennison** is the Supervising Scientist of Molecular Epidemiology, Public Health Microbiology, which is the Queensland reference laboratory responsible for the molecular surveillance of notifiable bacterial pathogens and characterisation of public health related outbreaks. She is leading a team in the application of WGS to routine molecular surveillance and heads numerous research projects aimed at utilising WGS for improving molecular epidemiological investigation and addressing culture independent diagnostic testing through deep sequencing approaches.

# The role of microbiology in gonococcal control in the West: helping to understand the enemy



*David J Speers*

Department of Microbiology  
PathWest Laboratory Medicine WA  
Queen Elizabeth II Medical Centre  
Hospital Avenue  
Nedlands, WA 6009, Australia  
Tel: +61 8 9346 2197  
Fax: 08 9346 3960  
Email:  
david.speers@health.wa.gov.au

**Western Australia (WA), Australia's largest state by area, has one of the highest notification rates of gonorrhoea in the world. This is likely a reflection of the challenges of providing health services over a vast remote area combined with a unique set of sociocultural aspects. Despite this, microbiology can play a pivotal role in the public health management of gonorrhoea even if the primary health services are thousands of kilometres away from the laboratory. However, it requires new approaches to how diagnostic testing and laboratory surveillance are conducted and the repurposing of existing technologies to cater for novel demands. In this article I describe some of the microbiological approaches that have been undertaken in WA to help address the public health challenge of gonorrhoea.**

**That is, facilitating the appropriate antimicrobial management of gonorrhoea in an era of increasing resistance to prevent treatment failure, timely provision of an accurate diagnosis to inform appropriate treatment, and providing molecular insights to better understand gonococcal transmission (Table 1).**

## Mitigating antimicrobial resistance

*Neisseria gonorrhoeae* has shown a remarkable capacity to become resistant to the antimicrobial agents employed to control it. With an estimated 78 million new cases each year and the emergence of ceftriaxone and azithromycin resistance in many countries around the world<sup>1</sup>, including Australia<sup>2,3</sup>, *N. gonorrhoeae* has earned its World Health Organization and Centers for Disease Control and Prevention designation as an urgent antimicrobial resistance threat. It is critical to delay the introduction of multi-resistant *N. gonorrhoeae* strains into remote WA, with its high rate of gonorrhoea and more limited access to healthcare. Australia, like most of the world, has transitioned to a combination of ceftriaxone and azithromycin<sup>4,5</sup> for empiric gonorrhoea therapy but the emerging resistance to extended spectrum cephalosporins<sup>6</sup> and the increasing prevalence of low level azithromycin resistance in Australia<sup>3</sup> is a reminder that resistance is an inevitable consequence of widespread use of these agents. The incursion of ceftriaxone-

Table 1. Potential microbiological solutions to address challenges for gonococcal control in remote regions of Western Australia.

Challenge	Impacts	Microbiological solutions	Examples
Significant distance from person to testing laboratory	<ul style="list-style-type: none"> <li>– Delay in diagnosis for asymptomatic infections impacting treatment</li> <li>– Delayed contact tracing</li> </ul>	<ul style="list-style-type: none"> <li>– Introduction of molecular point-of-care testing (POCT)</li> <li>– Molecular testing of pooled specimens for cost-effectiveness</li> </ul>	POCT-suitable molecular testing platforms, e.g. GeneXpert CT/NG platform
Predominant culture-independent diagnostic testing	<ul style="list-style-type: none"> <li>– Unknown case antimicrobial susceptibility profile</li> <li>– Potential introduction of antimicrobial resistant strains leading to failure of empiric treatment</li> </ul>	Direct specimen molecular antimicrobial resistance detection for: <ul style="list-style-type: none"> <li>– case management</li> <li>– surveillance</li> </ul>	Direct molecular detection methods for: <ul style="list-style-type: none"> <li>– PPNG</li> <li>– Chromosome-encoded penicillin resistance (PBP2, PorB, mtrR genes)</li> <li>– Azithromycin resistance (23S rRNA gene)</li> <li>– Ciprofloxacin resistance (GyrA gene)</li> </ul>
Very high transmission rates	<ul style="list-style-type: none"> <li>– Ongoing morbidity in remote region populations</li> <li>– Potential rapid spread of introduced antimicrobial resistant strains</li> </ul>	Molecular epidemiology studies of remote region isolates to inform education and prevention strategies	MLST NG-MAST NG-STAR Whole genome sequencing

PPNG, plasmid mediated penicillin resistant *Neisseria gonorrhoeae*; NG-MAST, *Neisseria gonorrhoeae* multi-antigen sequence typing; NG-STAR, *Neisseria gonorrhoeae* sequence typing for antimicrobial resistance.

and/or azithromycin-resistant gonococci into remote WA communities would create an enormous public health challenge.

There are few places in the world where penicillins remain the first-line treatment for gonorrhoea, but remote WA is one such place. Despite gonorrhoea being highly endemic in the remote Kimberley, Pilbara, Midwest and Goldfields regions of WA, oral combinations of amoxicillin, probenecid and azithromycin have been used for many years for the syndromic management of gonorrhoea, based on the relatively high co-occurrence of *N. gonorrhoeae* and *Chlamydia trachomatis* infection and the relative susceptibility of *N. gonorrhoeae* to penicillin and azithromycin<sup>7</sup>. This has avoided the logistic and cultural issues of administering intramuscular injections in less accessible regions. As long as the combination azithromycin, amoxicillin and probenecid remains effective for locally acquired uncomplicated gonorrhoea in these remote regions its use will limit the exposure of remote WA region *N. gonorrhoeae* strains to third-generation cephalosporins, potentially delaying the establishment of resistance. To ensure this relative susceptibility to penicillins remains the case ongoing surveillance for antimicrobial resistance is essential, but these efforts are hampered by the low culture confirmation rates of gonorrhoea notifications from remote region WA, recorded at 14% in 2016<sup>8</sup>. Therefore, molecular surveillance for antimicrobial resistance in these regions would be extremely helpful.

Penicillin resistance (MIC ≥ 1 mg/L) in the remote WA regions is almost all due to penicillinase producing *N. gonorrhoeae* (PPNG), which harbours a 7426 bp multicopy TEM plasmid<sup>9</sup>. In 2016 no isolate from the remote WA regions exhibited chromosomally mediated penicillin resistance<sup>8</sup>. This provides a unique opportunity to monitor penicillin resistance in gonococci from remote WA regions by using real-time PCR to detect this plasmid on *N. gonorrhoeae* positive nucleic acid extracts, thereby not requiring viable cultures. This surveillance has been performed on all *N. gonorrhoeae* positive nucleic acid extracts at the PathWest Laboratory Medicine WA Queen Elizabeth II Laboratory, which receives most of the *N. gonorrhoeae* specimens from remote WA regions, since 2012<sup>10</sup>.

PPNG rates remained very low and below the WHO empiric therapy standard of 5% resistance in all remote WA regions<sup>6</sup> until 2016 when the PPNG molecular detection rate increased in the Mid West region from 4.7% of 43 specimens in 2015 to 23% of 121 specimens tested in 2016. This was not apparent from phenotypic susceptibility testing as only two of the 18 Mid West isolates in 2016 were PPNG<sup>8</sup>. On further investigation an incursion of PPNG around Geraldton, the major regional centre of the Mid West, had occurred. In response to this the regional public health unit was notified and empiric prescribing for locally acquired uncomplicated gonorrhoea was changed from ZAP

(azithromycin, amoxicillin, probenecid) packs to intramuscular ceftriaxone, in combination with azithromycin. In terms of laboratory response to this incursion reporting of the PPNG result in combination with the *N. gonorrhoeae* PCR detection will soon commence, together with investigation into a non-culture ciprofloxacin detection method to be run in parallel with PPNG detection, to allow the safe use of these agents when appropriate. Ciprofloxacin resistance occurred in 4.5% of remote WA region isolates in 2016<sup>8</sup>.

### Timely provision of accurate diagnosis

Making the correct diagnosis is the cornerstone of appropriate treatment and microbiology has a central role in ensuring practical yet accurate rapid diagnostic tests for *N. gonorrhoeae* detection. For diseases like gonorrhoea in WA, where the nearest tertiary molecular laboratory may be over 3000 km away, this is best achieved by molecular point of care testing (POCT). The Test, Treat ANd GO (TTANGO) trial<sup>11</sup> was a randomised controlled trial by the Kirby Institute, UNSW, with a cross-over design conducted from 2013 to 2015 to explore the benefits of molecular POCT for *N. gonorrhoeae* and *C. trachomatis* as part of routine clinical care in 12 remote Australian Aboriginal primary health services, the first study of its kind. Overall *N. gonorrhoeae* diagnostic concordance was 99.9% for the 2486 samples between the GeneXpert CT/NG<sup>®</sup> (Cepheid, Sunnyvale, CA) molecular POCT compared to reference laboratory testing<sup>12</sup>. TTANGO demonstrated that the introduction of molecular POCT for *N. gonorrhoeae* and *C. trachomatis* in the remote clinic setting requires considerable planning, attention to quality control and changes to clinical guidelines. However, the results are highly accurate with a range of benefits including more timely and targeted prescribing, more efficient contact tracing and reduced staff time spent in contact tracing. Following this pilot study TTANGO 2 commenced in 2016 as an expanded program to a wider network of remote health services (including 15 sites in WA) over a 5-year period. The overall aim of TTANGO2 is to determine whether molecular POCT combined with increased testing is a sustainable and useful strategy for *N. gonorrhoeae* and *C. trachomatis* disease control in remote communities.

One of the major drawbacks to molecular POCT in the clinic situation is affordability due to the current lack of a Medicare rebate. One option to decrease the reagent costs of molecular POCT is specimen pooling. A high prevalence cohort over 100 symptomatic men who have sex with men and contacts of those with known gonorrhoea attending an inner city sexual health clinic was chosen for a prospective comparison of the GeneXpert CT/NG<sup>®</sup> testing of pharyngeal and rectal swabs pooled with urine samples to testing of individual specimens by routine laboratory

methods<sup>13</sup>. The pooled testing was found to be 100% concordant for *N. gonorrhoeae* detection when compared to individual specimen testing, showing this method to be potentially useful as a POCT for screening such high risk groups.

### Understanding the enemy

With the recent incursion of an antimicrobial resistant *N. gonorrhoeae* strain into Geraldton in the Mid West region of WA it is important to understand the transmission networks locally, between remote regions, and between remote and more populated regions of WA. An understanding of the transmission pathways could also provide insights into the driving factors for the high *N. gonorrhoeae* transmission rates in WA. Microbiology can provide a better understanding of *N. gonorrhoeae* transmission through molecular epidemiological studies, thereby highlighting where interventions may have the greatest impact on transmission. To investigate the *N. gonorrhoeae* epidemiology in WA pulse-field gel electrophoresis (PFGE) and *N. gonorrhoeae* multi-antigen sequence typing (NG-MAST) was performed on 128 consecutive WA *N. gonorrhoeae* isolates cultured between January 2011 and December 2013<sup>14</sup>. A total of 67 distinct PFGE pulsotypes were identified with 59 NG-MAST sequence types but no predominant NG-MAST or PFGE types were found, highlighting the diversity of *N. gonorrhoeae* strains within WA. However, when the isolates were classified based on the more conserved *tbpB* gene to more clearly demonstrate linkages over time the presence of four large clusters, which accounted for 67% percent of all isolates, were identified. This suggests that the majority of the isolates originated from independent, small sexual networks with an infrequent interchange between other communities and regions. In terms of the *N. gonorrhoeae* genomogroups, some genomogroups were present throughout the study period, some disappeared and others were introduced demonstrating how the composition of the gonococcal population in remote regions is ever changing due to, as yet, unknown selection forces. It also suggests that gonococcal epidemiology in remote region WA is quite different to the major population centres and, in some cases, geographically restricted.

To further explore the gonococcal epidemiology of remote region WA core genome phylogeny with MLST was performed on over 50 WA isolates and compared to an international *N. gonorrhoeae* collection<sup>15</sup>. Two distinct population groups were found among the remote region isolates that carried no chromosomal antimicrobial resistance genotypes. In contrast, most isolates from the metropolitan region of Perth belonged to population groups that were globally distributed and were frequently multi-drug resistant, suggesting these isolates had been introduced through

international travel. This data is consistent with independent gonococcal transmission networks in remote WA regions curtailing the incursion of antimicrobial resistant strains and resulting in a distinct population structure of *N. gonorrhoeae*, which may explain the failure to establish antimicrobial resistant strains in remote WA. However, with the circulation of remote region population group isolates we may now see more widespread dissemination of the PPNG strain that appeared around Geraldton in 2016. Moreover, this information emphasises the fact that the public health measures employed to combat remote region gonococcal transmission must be culturally appropriate and relevant. Further interrogation of the sequence data is underway to identify novel markers linked to antimicrobial resistance suitable for PCR detection at the time of *N. gonorrhoeae* detection that may be able to rapidly identify gonorrhoea cases with and without antimicrobial resistance beyond PPNG.

In summary, microbiology has much to contribute to the public health management of gonorrhoea by identifying features of the pathogen and the host-pathogen interaction that can be exploited for the public health management of *N. gonorrhoeae*. An appreciation of these differences across geographic locales will help inform the measures that can be taken toward gonococcal control, tailored to a particular setting.

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## Biography

**Dr David Speers** is an Infectious Diseases Physician at Sir Charles Gairdner Hospital, Head of Microbiology, QEII Network at Path-West Laboratory Medicine WA and a Clinical Associate Professor for the Department of Medicine and Pharmacology, University of WA. He is a fellow of the Royal Australasian College of Physicians, the Royal College of Pathologists of Australasia, and the Australasian College of Tropical Medicine.

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