Commentary

Challenges with antimicrobial susceptibility testing for *Neisseria gonorrhoeae* in the era of extensively drug-resistant gonorrhoea — molecular antimicrobial resistance testing crucial

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Invited commentary on 'Characterization of Neisseria gonorrhoeae *isolates detected in Switzerland (1998–2012): emergence of multidrug-resistant clones less susceptible to cephalosporins', by Endimiani et al.*

Gonorrhoea is a major public health problem globally and *Neisseria gonorrhoeae* is evolving into a superbug with antimicrobial resistance (AMR) to previously and currently recommended drugs for treatment. The recent occurrence of failures to treat gonorrhoea with the extended-spectrum cephalosporins (ESCs) cefixime and ceftriaxone and emergence of gonococcal strains exhibiting high-level clinical resistance to all ESCs, the last remaining options for empiric first-line antimicrobial monotherapy, have caused grave concern.[1,2] In response to this developing situation, the WHO has published a global action plan.[3] In the lack of new antimicrobials for treatment, one key component stressed in this action plan is to enhance the surveillance of gonococcal AMR (and verified treatment failures with particularly the ESCs): locally, nationally, and internationally.

A recent paper by Endimiani and colleagues[4] characterized *N. gonorrhoeae* isolates from Switzerland (1998–2012), where detailed data regarding the gonococcal population have been mainly totally lacking. This paper addressed many issues of importance, e.g. the increase in AMR during the latest decades, crucial need to substantially enhance the phenotypic AMR surveillance and obtain longitudinal AMR data, requirement to increase our understanding of genetic AMR determinants and use molecular epidemiological typing for identification of multidrug-resistant gonococcal clones spreading in Switzerland and internationally (e.g. NG-MAST ST1407). However, the paper also illustrates the difficulties to obtain an adequate number of isolates for phenotypic AMR surveillance (only 60 isolates from one institution examined), the use of different types of definitions that are essential to further standardize such as minimum inhibitory concentration breakpoints, 'less susceptible' and 'multidrug resistance', which in the study was characterized as isolates resistant to ≥ 3 antimicrobials. When antimicrobials that have not been recommended for gonorrhoea treatment in decades (e.g. penicillin and tetracycline) are included, this definition is not very relevant. More appropriate definitions of multidrug resistance and extensively drug resistance, based also on antimicrobial's use in gonorrhoea treatment, have been published.[5]

Unfortunately, there are large obstacles to enhance the phenotypic gonococcal AMR surveillance due to the lack of culture in many settings. This is particularly because in many less-resourced settings, gonorrhoea diagnosis depends upon syndromic management, which has resulted in a lack of skills to sample specimens and culture, and in many more-resourced settings, nucleic acid amplification tests (NAATs) have replaced culture for diagnosis. It is essential to strengthen the culture capacity globally and, for this, national and international financial and political commitments are essential. However, it is also urgent, which also was stressed by Endimiani *et al.*,[4] to develop rapid molecular point-of-care AMR testing, ideally combined with simultaneous detection of gonococci, which could directly provide a diagnosis and guide individually-tailored treatments.[2,6,7] Laboratory-developed molecular assays exist for detection of one or more genetic AMR determinants involved in resistance to many antimicrobials.[2,7] Unfortunately, for most AMR determinants, the sensitivity and specificity of these molecular AMR assays in their prediction of AMR are often low (particularly for the recommended ESCs with their ongoing evolution of resistance involving many different mutations, and their epistasis, in several divergent genes). Additionally, many of the AMR determinants are also present in, e.g. commensal Neisseria species, making it difficult to predict AMR in particularly pharyngeal samples. Intensified research is crucial to continue to identify and appropriately verify (e.g., with site-directed mutagenesis and/or transformation) novel genetic AMR determinants (focus on ESCs), agree on the nomenclature of AMR determinants (e.g. *penA*

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alleles), and develop an internationally accessible database containing the sequence information and, ideally, appropriate microbiological, genetic, clinical, and epidemiological data.[1,2] Despite that the molecular AMR assays might still be in their infancy, research is crucial to evaluate how such methods could be used to supplement traditional AMR testing (which never will be replaced completely) both in gonococcal AMR surveillance programmes and ideally for guiding individually-tailored treatment (by confirming resistance or susceptibility), which can ensure the rational use of antimicrobials and affect the control of both gonorrhoea and AMR. Molecular AMR methods, particularly highly multiplexed ones that can quickly be adjusted in response to novel AMR determinants, can supplement the culture-based AMR surveillance and, e.g. be used for rapid and comprehensive testing of a population for particular antimicrobials.[2,7] High-throughput genome sequencing (combined with appropriate epidemiological data) and other novel technologies might, initially in reference laboratories, revolutionize the molecular AMR testing for both gonococcal isolates and NAAT-positive samples. Genome-based characterization might also elucidate many additional issues regarding gonococcal evolution, diversity, and strain populations; national and international transmission of successful gonococcal clones, which also can predict the AMR; identify new targets for diagnostics (e.g. for rapid point-of-care tests), treatment and vaccine; and even find associations with pathogenesis (tissue tropism, type and severity of infection, etc.).[8,9]

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