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Point of care antibiotic susceptibility testing for gonorrhoea – improving therapeutic options and sparing the use of cephalosporins.

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Key Words: *Neisseria gonorrhoeae*, Antibiotic Resistance; Point of Care Technology, Penicillin, Fluoroquinolone.

Key Messages:

1. Point of care susceptibility testing for gonococcus allows individualised antibiotic treatment despite high local resistance rates, sparing cephalosporin use.
2. Most known genetic markers of resistance currently have a limited role in predicting phenotypic resistance but evidence suggests their absence can accurately predict susceptibility.
3. Modern microengineered nanotechnologies may provide the basis for point of care susceptibility testing in the near future.
4. Rapid tests remain dependent upon surveillance programmes to monitor for new emerging resistant strains of gonorrhoea.

Competing Interest: None declared.

Antimicrobial therapy for *Neisseria gonorrhoeae* is a major public health concern with high rates of resistance to penicillin, tetracycline and fluoroquinolones detected in England and Wales in the Gonococcal Resistance to Antimicrobials Surveillance Programme (GRASP) (GRASP 2008 Report: Trends in Antimicrobial Resistant Gonorrhoea. Health Protection agency. <http://www.hpa.org.uk/Publications/InfectiousDiseases/HIVAndSTIs/0906GRASP2008/> Last accessed 26/07/2010). Currently, third generation oral cephalosporins, such as cefixime or injectable ceftriaxone, are recommended as first-line therapy. However, low-level increases in minimum inhibitory concentrations (MIC) for cephalosporins are now widely reported¹ and reports of azithromycin resistance² further highlight reduced therapeutic options. Recommendations to limit spread of cephalosporin resistance may well include combination and even prolonged therapy, but these undermine clinical desirability for a single observable dose.

However, there is still significant local variation in resistance rates and most circulating gonococcal strains continue to be susceptible to fluoroquinolones and penicillin in most settings. With the emergence of rapid diagnostic technologies for STIs and other infections, we ask whether point of care (PoC) antimicrobial susceptibility testing might soon be ready to exploit this aspect of gonococcal epidemiology? The answer will depend on; i) current knowledge of the molecular basis of gonococcal resistance; ii) availability of molecular diagnostic platforms supporting detection of multiple molecular resistance markers; and iii) validation of these assays.

Recent progress in understanding mechanisms of gonococcal resistance has been significant. Known chromosomal mutations associated with penicillin resistance include those altering structure and function of penicillin binding proteins (PBPs) and efflux pump activity genes, including *penA*, *ponA*, *penB*, *mtrR* regulator and possibly *penC*^{3 4 5}. The other classic resistance mechanism involves TEM-1 type penicillinase production, encoded by the plasmid mediated *bla*_{TEM-1} gene. Tetracycline resistance may involve acquisition of the *tet(M)* gene⁶, or a combination of *mtrR*, *penB* and *rpsJ* gene mutations⁷ whereas fluoroquinolone resistance is mediated by mutations in *gyrA*^{8 9} and *parC*. Molecular mechanisms that have given rise to cephalosporin resistance include multiple mutations in PBP2, acquired most often through mosaic gene exchange with other Neisserial species, as well as single point mutations.

A comprehensive validation analysis of multiple resistance markers for penicillin, tetracycline and fluoroquinolones including *penA*, *penB*, *mtrR* and *bla*_{TEM1}, *rpsJ*, *tet(M)*, *gyrA*, *parC*, provides insight into their value as a diagnostic test for resistance and susceptibility¹⁰. Resistance rates to antimicrobials (with defined MICs) in 464 gonococcal isolates were: penicillin, 30% (1mg/ml); fluoroquinolone, 45% (1mg/ml) and tetracycline, 38% (2mg/ml). The presence of specific resistant-gene combinations predicted phenotypic resistance in 40%, 90% and 54% of isolates, respectively. However, in isolates where resistance associated genes were completely absent ("susceptible genotypes") susceptibility to penicillin (MICs <0.5mg/l), fluoroquinolones (MIC < 0.25mg/l) and tetracycline (MIC < 2mg/l) was 100% (95% confidence interval 94%-100%), 100% (C.I. 98%-100%) and 98% (C.I. 94%-100%), respectively. When more stringent MIC values for low level resistance were used (= 0.12mg/l, = 0.12mg/l and = 0.5mg/l) these predictive values for susceptibility fell to 90%

(C.I. 80%-96%), 99% (C.I. 96%-100%) and 83% (C.I.74%-89%). The rates of penicillin, ciprofloxacin and tetracycline susceptible genotypes in this study were 11.6%, 32.2% and 19.6%, respectively.

Clearly more work is required to establish the diverse molecular mechanisms of resistance, particularly for cephalosporins and for second line drugs such as azithromycin, where mechanisms may be diverse and complex¹¹. However these data allow us to speculate on possible benefits of PoC antibiotic susceptibility tests. For example, of 1,276 men and women with gonorrhoea in GRASP, June to August 2008, 28% had ciprofloxacin resistant strains. Using a susceptibility genotype prevalence of 32.2% as in the study above (which had a higher phenotypic resistance rate to that in London), PoC susceptibility testing based on absence of markers might have identified 410 patients treatable by ciprofloxacin. Significant numbers of individuals with penicillin susceptible gonorrhoea may also have been identified, depending on the degree of overlapping resistance. Immediate, directly observable fluoroquinolone and penicillin therapy could then become available as a therapeutic option for significant numbers of patients, alongside other strategies for managing cephalosporin resistance such as using combination therapy.

It is unclear whether PoC susceptibility testing would contain spread of cephalosporin resistance, as the main drivers such as community prescribing of antimicrobials and importation of resistant strains from resource poor settings, are likely to be unaffected. In addition, PoC susceptibility tests would need to become available before resistance becomes more widespread, particularly as increasing cephalosporin use is likely to result in increased chromosomally mediated resistance to tetracyclines and penicillins¹² and because fluoroquinolone resistance may persist, possibly because of fitness advantages of resistance strains. This requires industry, academia and regulators to collaborate closely to bring innovative diagnostics to market and it is encouraging that this may soon be the case for a number of novel, affordable microengineered rapid STI tests following recent investment to support such collaborations (<http://www.innovateuk.org/ourstrategy/innovationplatforms/detectionandidentificationofinfectiousagents.ashx>; last accessed July 2010). These models of support could be used to accelerate the development of PoC nucleic acid amplification test (NAAT) based susceptibility tests, which have been made possible by advances in microengineering¹³.

Laboratory based techniques for detecting molecular resistance markers such as spectrometric methods, real time PCR¹⁴, sequencing and microarrays may be useful as gonococcal culture is replaced by NAATs for diagnosis. However the turnaround times for these methods (hours rather than minutes) is still too long for providing immediate treatment to patients with gonorrhoea. Furthermore, centralised tests will be less cost-effective in settings of low gonococcal prevalence. PoC susceptibility tests will have to be accurate and flexible, so that markers for new resistance mechanisms can be rapidly added as they are discovered and validated. This will require collaboration with ongoing national surveillance and research programmes. Performance of tests will be challenged by low levels of DNA from genital and non-genital samples, although, one real time PCR assay for *gyrA* resistant genotype in first void female urine samples was able to give a result in 70% of cases¹⁴. Nanotechnology platforms have the potential for single molecule detection¹⁵ and may thus overcome poor sensitivity of the assay. Ultimately however, molecular based testing, whether

centralised or PoC, will not be able to detect emergence of new resistance strains and should not be seen as an alternative to strong phenotypic antimicrobial resistance surveillance programs. These will need to remain in place to monitor for the ongoing threat of resistance.

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