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Stemming the tide of drug-resistant *Neisseria gonorrhoeae*: the need for an individualized approach to treatment

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Drug-resistant *Neisseria gonorrhoeae* poses a significant public health challenge. In recent years, gonococci resistant to first- and second-line antibiotics have spread worldwide and new strains have developed that are increasingly resistant to third-generation cephalosporins, which are currently our last line of available treatments. Given the timeline required to develop new drugs or an effective vaccine for *N. gonorrhoeae*, a top priority is to use the drugs that are available as effectively as possible. Currently, clinical management of gonorrhoea is based upon treatment guidelines informed by international gonococcal antimicrobial susceptibility surveillance programmes. This approach, although currently the most practical, is subject to a number of limitations since surveillance data inherently provide population-level information. As a result, basing treatment guidelines on these data can result in the prescription of more aggressive or broader treatment than is needed by individual patients and hence inadvertently contribute to the development and spread of resistance to important drugs. Clearly, methods are needed that provide patient-specific drug susceptibility information in a time frame that would allow clinicians to prescribe individualized treatment regimens for gonorrhoea. Fortunately, in recent years, there have been a number of advances in the development of rapid methods for characterizing both the genotype and the drug resistance phenotype of *N. gonorrhoeae* strains. Here, we review these advances and propose additional studies that would help facilitate a transition towards an individualized treatment approach for gonorrhoea.

Keywords: antibiotic resistance, gonorrhoea, public health, combination therapy, nucleic acid amplification tests, antimicrobial susceptibility testing, guidelines, molecular surveillance

Introduction

New strains of MDR *Neisseria gonorrhoeae* have emerged globally over the past 10 years. Treatment failures and *N. gonorrhoeae* strains with reduced susceptibility to antibiotics are being reported across Asia, Europe and now North America (Figure 1). These reports reveal an alarming waning efficacy of third-generation cephalosporins, the last line of antibiotics available to treat gonorrhoea.[1,2] Moreover, the prevalence of gonococci that resist both first- and second-line antibiotics has dramatically increased in recent years.[3] Under the selection pressure imposed by using broad-spectrum antibiotics, horizontal gene transfer from other *Neisseria* species, especially those that live in the oropharynx, enables *N. gonorrhoeae* to acquire new drug resistance mechanisms;[4] the *penA* mosaic gene, which has a high positive predictive value for cephalosporin resistance, is one such example.[2,5] The ability of *N. gonorrhoeae* to acquire and sustain chromosomally mediated forms of drug resistance poses a unique public health challenge to the control of sexually transmitted infections. For example, HIV targets mucosal lymphocytes that become exposed when gonococci infect and destroy columnar epithelial cells.[6] Since infection with gonococci can facilitate HIV transmission,[7–11] gonorrhoea treatment failures may increase the risk of HIV transmission in populations heavily impacted by gonococcal infection, especially MSM.

To keep physicians apprised of the latest trends in drug resistance in gonorrhoea, the ECDC reports surveillance data describing trends in changing antimicrobial susceptibility by country as part of the European Gonococcal Antimicrobial Surveillance Programme (Euro-GASP).[12] In the UK, gonococcal surveillance data appear in the annual report from the Gonococcal Resistance to Antimicrobials Surveillance Programme (GRASP).[13]

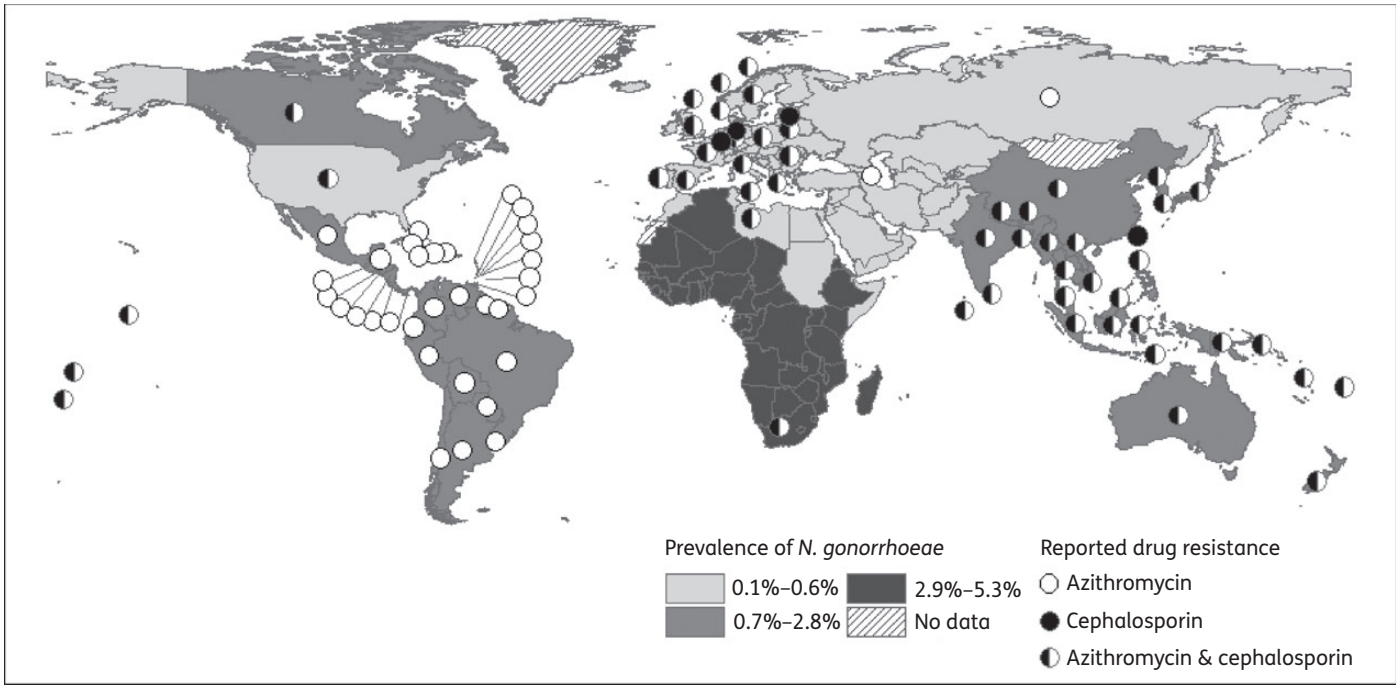

Figure 1. Gonorrhoea global prevalence and reported drug resistance. This map shows the WHO estimated prevalence of gonorrhoea globally using a shading gradient. Countries that have reported *N. gonorrhoeae* strains with reduced susceptibility to azithromycin are identified with a white circle. Countries that have reported *N. gonorrhoeae* strains with reduced susceptibility to cephalosporins are shown with a black circle. Countries that have reported *N. gonorrhoeae* strains with reduced susceptibility to both azithromycin and cephalosporins are shown with a half-black and half-white circle. Fluoroquinolone resistance has been documented and is well distributed globally, but was not included in this map to preserve clarity. Countries shaded with a diagonal striped pattern (e.g. Greenland, Mongolia etc.) were not included in the WHO prevalence data. Countries without circles have not yet reported strains of *N. gonorrhoeae* with reduced susceptibility to azithromycin or cephalosporins.[53,63–92]

Similarly in the USA, the CDC issues treatment guidelines based on data collected from the Gonococcal Isolate Surveillance Project (GISP).[14] The CDC revises treatment guidelines when treatment failures are observed and a high prevalence of resistant or multi-resistant strains are reported in the surveyed population.[15] The WHO recommends that only drugs with an expected efficacy of ≥95% should be used as first-line therapy to treat gonorrhoea.[16] The current US treatment guidelines recommend combination therapy with ceftriaxone and azithromycin or doxycycline.[15] The ECDC has published comparable treatment recommendations as informed by Euro-GASP.[12] These guidelines encourage clinicians to treat all gonorrhoea cases very broadly by prescribing these antibiotics even where little or no drug resistance has been reported. As a result, even those gonococcal infections that could still be effectively treated with other drugs such as amoxicillin would receive the broadest treatment available.[17] Historically, this has been problematic because *N. gonorrhoeae* drug resistance has quickly followed the introduction of new antibiotics (Figure 2). In general, widespread use of an antibiotic decreases its efficacy over time by promoting drug resistance through selective pressure.[18] Conversely, decreasing the use of an antibiotic may reduce the prevalence of drug resistance to that particular antibiotic over time.[19]

We would be able to slow the development of resistance to extended-spectrum cephalosporins if each patient was treated with the narrowest, but still effective, antibiotic for their own infection. To be able to implement that approach, however, clinicians would need to know the drug susceptibility or resistance phenotype for their patients' infections in real time, or near real time, so that this information could inform their prescription choices. This approach, which requires the development of rapid susceptibility testing methods, has been shown to reduce antibiotic usage[20] and could extend the utility of the drugs we currently have to treat gonorrhoea.

What are the challenges associated with individualized drug susceptibility testing?

Ideally, real-time assays or near real-time tests would best inform the clinician of the patient's gonorrhoea drug phenotype while the patient is still in the clinic. Although we have reliable molecular methods for detecting *N. gonorrhoeae*, there is no commercially available molecular method to characterize *N. gonorrhoeae* drug susceptibility. Moreover, little has been done to improve and speed up culture-based antimicrobial susceptibility testing (AST). Agar dilution is the current gold standard for performing AST on *N. gonorrhoeae* as recommended by the US CDC and CLSI.[21,22] Despite the reliability of the data it produces, agar dilution is time consuming and labour intensive, which poses a problem for smaller microbiology or hospital laboratories. Other AST culture-based methods such as the Epsilometer test (Etest) and disc diffusion still take 24 h to generate data and must be performed by experienced laboratory technologists or

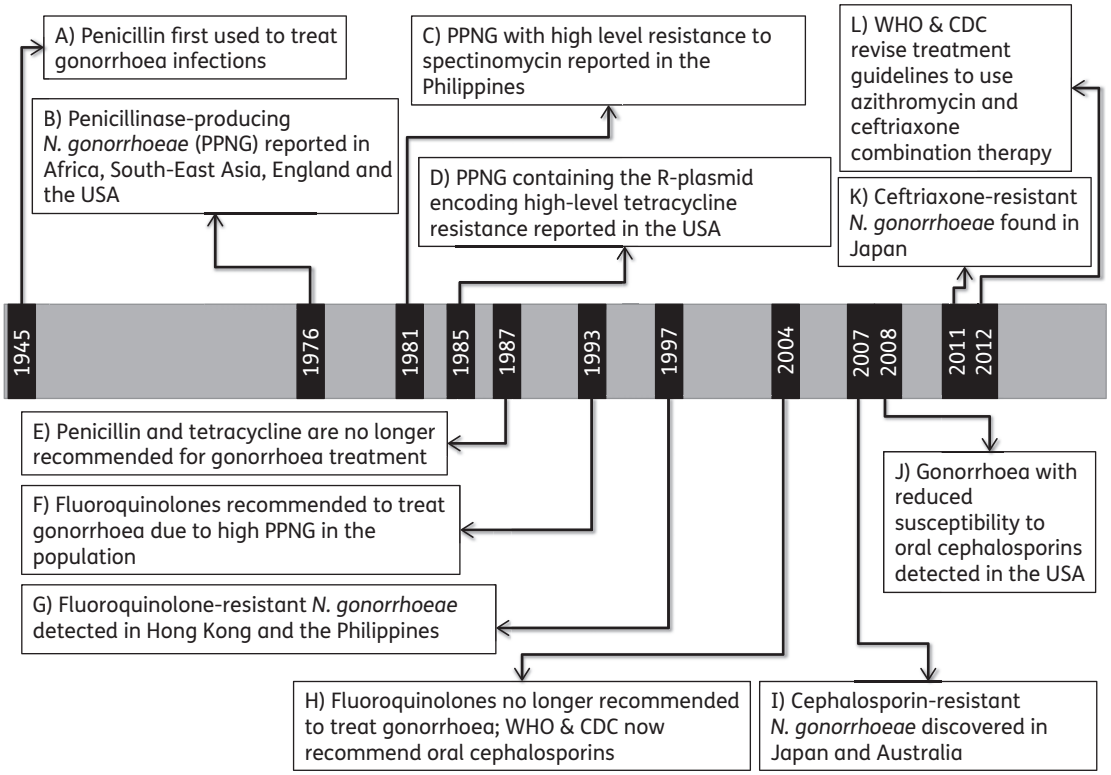

Figure 2. Timeline of gonorrhoea antibiotic treatments and treatment guidelines. This timeline shows when antibiotics such as penicillin, spectinomycin, tetracycline, fluoroquinolones and cephalosporins were used to treat gonorrhoea and when drug resistance was first reported for each of these antibiotics. We observe a pattern that drug resistance quickly follows the widespread use of antibiotics. The WHO, ECDC and US CDC changed the corresponding treatment guidelines based on documented *N. gonorrhoeae* strains with reduced susceptibility to those antibiotics. However, changing the treatment protocol imposes new selection pressure on the bacterium, resulting in natural selection favouring the persistence of *N. gonorrhoeae* strains with reduced susceptibility to the current antibiotic treatment.[15,32,56,93–99]

microbiologists to ensure results comparable to the gold standard. Broth microdilution methods for AST of *N. gonorrhoeae* have yet to be robust enough for clinical application. However, recent studies have shown progress towards developing media that support the growth of this fastidious bacterium.[23–26] Without additional enhancement to the existing AST methods for gonorrhoea, little can be done to improve drug susceptibility testing and surveillance response times.

Another consideration is that most clinical laboratories no longer receive clinical specimens allowing for the isolation and subsequent routine performance of AST on *N. gonorrhoeae*, as the introduction of molecular diagnostics for the detection of gonorrhoea has extensively replaced culture and isolation methods.[27] Instead, gonorrhoea surveillance in the USA is conducted by the CDC in concert with a network of clinicians and public health laboratories through GISP. GISP reference laboratories characterize the drug susceptibilities of each isolate collected from sentinel sites across the USA using the agar dilution method.[28] Performing this assay on aggregated isolates submitted by participating public health laboratories in most cases holds back results for submitted cultures for 3–6 months. This lag time in reporting AST values back to local public health authorities means that it is impractical to use individual GISP results to optimize treatment choices for specific patients. Instead, the goal of the GISP programme is to provide population-level information. The CDC reports the aggregate GISP data annually, showing the current trends in gonococcal drug susceptibility and demographics in populations across the USA.[29] These results are used to modify treatment guidelines and recommendations in the *Morbidity and Mortality Weekly Report*.[15] Similarly, the GRASP surveillance data[13] have led the British Association for Sexual Health and HIV to amend the guidelines for gonorrhoea treatment in the UK.[30] Further, the ECDC reports their surveillance data annually and describes gonococcal resistance rates in Europe.[12]

Because these gonorrhoea surveillance programmes provide data at the population level, sampling biases can confound observed trends.[12] Rates of drug resistance in gonorrhoea are higher in MSM than in men who have sex with women (MSW) due to different behaviour in these subpopulations.[8,31,32] Although some overlap occurs between sexual networks, *N. gonorrhoeae* strains are more or less conserved among MSM compared with MSW.[33,34] Depending on the percentage of MSM presenting for treatment at sentinel sites, there may be oversampling resulting in an apparent overall higher incidence of drug-resistant gonorrhoea.[13] A recent study traced the spread of gonococci with the *penA* mosaic gene largely through an MSM sexual network across the USA.[35] With the inclusion of samples taken from MSM patients, the GISP programme successfully identified emerging *N. gonorrhoeae* with reduced susceptibility to cephalosporins in the USA. This sampling design favours the

sensitive and early detection of MDR *N. gonorrhoeae* strains circulating in the population. However, the current sampling strategy may not reflect the actual distribution of gonococci with reduced susceptibility to cephalosporins in the overall population. The ECDC has recognized this form of sampling bias as a limitation to these surveillance strategies.[12] By encouraging clinicians to treat patients based upon the incidence of cephalosporin-resistant *N. gonorrhoeae* circulating in the general population rather than tailoring treatment to individual patients, we decrease the utility of our already limited antibiotic arsenal.

Implications of nucleic acid amplification testing

Ideally, drug susceptibility information would be gathered for individual patients when they are tested for gonorrhoea. However, gonorrhoea AST relies on culture-based methods, such as agar dilution or disc diffusion, which most laboratories no longer perform. Although primarily used by international surveillance programmes and for legal purposes, culture is not the main method used by laboratories to detect gonorrhoea infections. In the USA and the UK, public health and clinical microbiology laboratories have moved from routinely performing culture-based gonorrhoea testing to using a variety of nucleic acid-based assays, in particular nucleic acid amplification tests (NAATs), especially where there are high volumes of patient samples (Figure 3).[27,36–39] These NAATs have replaced bacterial culture due to their high degrees of sensitivity and specificity, which can detect asymptomatic infections during routine patient screening.[40–42] In addition to these benefits, NAATs have been designed as multiplex assays, many of which can detect *Chlamydia trachomatis* in addition to *N. gonorrhoeae*.[43] Moreover, NAATs have the capability to detect gonococci at body sites from which it would often be difficult to isolate pure *N. gonorrhoeae* cultures.[43–45]

NAATs, however, have their own set of limitations. NAATs are susceptible to false positive results due to the high amount of genetic exchange among commensal *Neisseria* species in pharyngeal samples.[42,46] Complicating matters, some researchers have reported false negatives by competitive inhibition, or competition between multiple targets, for a finite number of reagents in multiplex NAAT assays for detecting *N. gonorrhoeae* and *C. trachomatis*.[46–48]

The commercial application and practical utility of PCR-based NAATs that can detect both organism and resistance genes has been demonstrated. For example, Cepheid has developed a GeneXpert assay to quantify infection with *Mycobacterium tuberculosis* and to detect rifampicin resistance genes in primary sputum samples in both low- and high-prevalence populations.[49,50] A validation study of the Cepheid assay reported sensitive detection of *M. tuberculosis* and rifampicin resistance in <2 h in samples

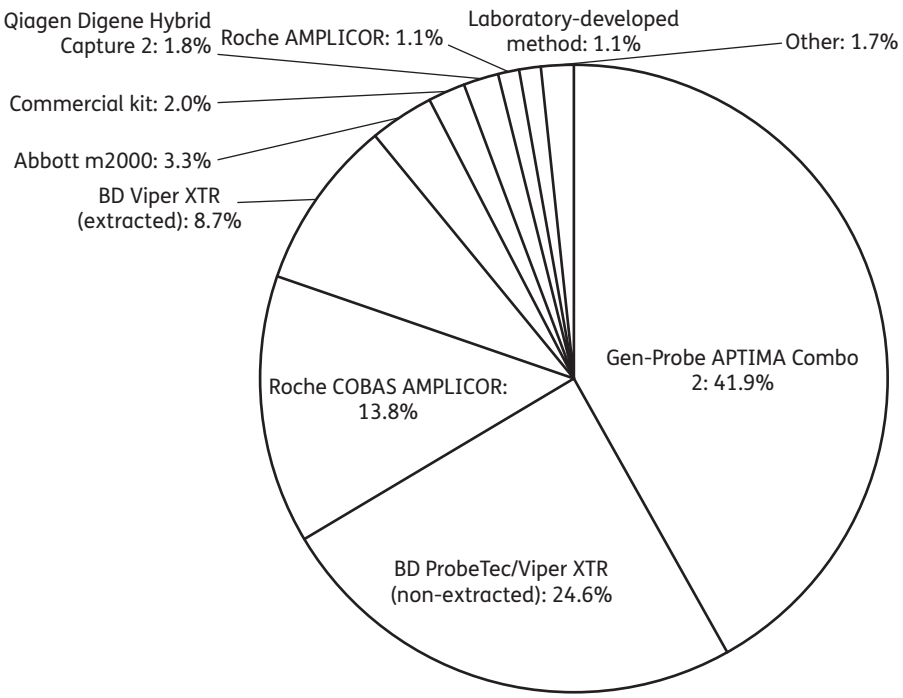

Figure 3. 2013 College of American Pathologists (CAP) survey results for gonorrhoea NAAT testing. This chart illustrates the percentage of different NAAT platforms used to detect *N. gonorrhoeae* in the 2013 CAP Proficiency Survey. Nine hundred and three CLIA-certified laboratories participated in this proficiency survey (where CLIA stands for Clinical Laboratory Improvement Amendments). Most laboratories performed transcription-mediated amplification using the APTIMA Combo 2 Assay (378) followed by strand displacement assay using the BD ProbeTec/Viper XTR (222) and PCR using the Roche COBAS AMPLICOR platform (125). The other CLIA laboratories that participated reported using the BD Viper XTR with extracted samples (79), the Abbott m2000 (30), commercial kits (18), the Qiagen Digene Hybrid Capture 2 assay (16), the Roche AMPLICOR (10), in-house laboratory-developed methods (10) and other manufacturers identified by <10 participating laboratories (15).[100]

from high-prevalence areas.[50] The decreased turnaround time and improved sensitivity of the *M. tuberculosis*–rifampicin combined assay demonstrates how a NAAT test can be used to quickly and effectively identify drug-resistant organisms compared with traditional culture-based methods. Given the limitations previously described for culture of gonococci, the sensitivity and specificity of NAAT technologies has great potential for moving towards guided treatment for gonorrhoea.

Presently, no NAAT platforms that provide drug resistance information for gonorrhoea are commercially available,[36,46,51] although several PCR-based NAATs have been developed to detect resistance markers in *N. gonorrhoeae*. PCR-based assays have been validated to detect the *gyrA* and *parC* mutations associated with reduced susceptibility to fluoroquinolones[52,53] as well as the 23S rRNA and *mtrR* mutations associated with elevated azithromycin MICs.[54] The previously described mosaic *penA* gene has been widely associated with decreased susceptibility to cephalosporins, making these alleles a major target for developing and refining NAATs to specifically detect cephalosporin resistance.[55–59] With this approach, NAATs would enable clinicians to individualize patient treatment by detecting common drug resistance markers.[37,60] Once available, the use of new NAATs for identifying drug resistance markers in clinical samples would be easy to implement in hospital and clinical laboratories that already rely on NAATs for clinical diagnosis.

While the prospect of using NAATs to obtain antibiotic resistance information in near real time shows promise, the rapid evolution of *N. gonorrhoeae* remains an ongoing challenge. Gonococci easily acquire new plasmid-mediated and chromosomal resistance genes to counteract new antibiotics. Multiplex PCR and other NAATs can be used to detect known drug resistance markers, but will not detect novel ones.[46] The recent discovery of the mosaic *penA* gene demonstrates that not all of the drug resistance mechanisms employed by *Neisseria* spp. have been clearly defined. For example, variability between *penA* mosaic alleles can lead to different MICs, requiring genomic sequencing to identify those alleles that contribute to cephalosporin resistance.[5] Moreover, most *penA* mosaics originate in commensal *Neisseria* spp., making pharyngeal detection of drug-resistant gonorrhoea problematic.[5] However, NAAT testing can be employed to perform molecular surveillance for well-known drug resistance markers in the population. Speers *et al.*[17] demonstrated that NAATs could be used to determine local drug resistance patterns in Western Australia, resulting in evidence-based treatment guidelines for patients in that area. Employing molecular surveillance for gonorrhoea drug resistance markers could elucidate these patterns across the globe, enabling local communities to generate evidence-based treatment guidelines.

While the Western Australia case study demonstrates that NAATs can be used to develop evidence-based treatment guidelines for local jurisdictions, this example also highlights why NAATs cannot be the entire solution. NAATs can be used to reliably detect the TEM-1 and Tet(M) plasmids conferring penicillin and tetracycline resistance, respectively, as well as mutations in the *gyrA*, *parC* and *mtrR* genes using PCR-based methods.[61] However, NAATs are less reliable for detecting cephalosporin resistance and azithromycin resistance because of the high sequence variability in *penA* and 23S rRNA alleles in *N. gonorrhoeae*.[5] Until we can design reliable NAATs to characterize these genetic determinants for cephalosporin and azithromycin resistance, we should expand our culture-based surveillance system and enhance with molecular surveillance. Using this approach, health jurisdictions can better identify the resistance patterns within their communities while informing local treatment guidelines with molecular and culture-based data. This requires isolation of gonococci from patients, which can be problematic for laboratories that have abandoned culture in order to exclusively run NAATs. However, the presented approach is paramount to understanding the changing landscape of *N. gonorrhoeae* drug resistance across the globe.

Recommendations

Based on the presented challenges of current drug susceptibility testing and the implications of NAAT testing for gonorrhoea, new strategies are needed to detect and treat gonococcal infections while maintaining active surveillance programmes to detect new resistance patterns. Adding drug resistance detection markers to current NAAT gonorrhoea detection platforms could fundamentally alter patient management from surveillance-based treatment guidelines to individualized treatment based on timely laboratory data. Switching the paradigm to an individualized treatment strategy would lower the evolutionary selection pressure on last-line antibiotics while simultaneously gaining greater longevity for our older antibiotics. Patients without MDR gonorrhoea could be treated with combination therapy using these older antibiotics so they do not develop resistance to our last-line drugs.[62] This is particularly important for gonorrhoea because many forms of gonococcal drug resistance are chromosomal and well maintained despite discontinuation of older antibiotics.[5] Since these chromosomal forms of resistance persist,[5] it is important not to use discontinued antibiotics such as ciprofloxacin in monotherapy to treat uncomplicated gonorrhoea; rather, we should use older antibiotics in combinations that would delay the development of resistance. Speers *et al.*[17] demonstrated that combination therapy using multiple drugs (amoxicillin, azithromycin and probenecid) paired with molecular surveillance for penicillinase-producing *N. gonorrhoeae* (PPNG) was effective at delaying the emergence of PPNG in Western Australia. Combination therapies customized to the individual patient's circumstances would slow the ability of *N. gonorrhoeae* to develop and maintain drug resistance towards a singular antibiotic or antibiotic class.[62] We argue that there is a need for localized molecular surveillance to aid in identifying endemic drug resistance patterns to better inform treatment approaches. However, until commercial NAATs can rapidly and reliably detect molecular resistance markers for individual patients, we must supplement these data with culture-based AST.

To keep up with the evolution of *N. gonorrhoeae*, we must develop and validate new drug susceptibility testing methods for characterizing gonococci in a shorter time scale. As previously discussed, research groups have worked towards broth microdilution for cultivation and AST of *N. gonorrhoeae*.[23–26] Introducing an effective broth microdilution AST system for gonorrhoea would decrease the amount of time required to detect drug-resistant isolates while simultaneously reducing required materials, storage space and labour. Furthermore, broth microdilution has the potential for establishing high-throughput screening for antibiotic resistance. A high-throughput screening system would greatly enhance current gonorrhoea surveillance across the globe by

reducing the lag time for generating results and allowing for larger datasets to be collected and tested. Until such a system has been validated and implemented, combining the current international surveillance systems with improving NAATs and molecular surveillance for use in laboratories is our best option for stemming the tide of drug-resistant gonorrhoea.

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Transparency declarations

None to declare.

References

1 Bolan GA, Sparling PF, Wasserheit JN. The emerging threat of untreatable gonococcal infection. *N Engl J Med* 2012; **366**: 485–7.

2 Whiley DM, Goire N, Lahra MM *et al*. The ticking time bomb: escalating antibiotic resistance in *Neisseria gonorrhoeae* is a public health disaster in waiting. *J Antimicrob Chemother* 2012; **67**: 2059–61.

3 Ison CA, Hussey J, Sankar KN *et al*. Gonorrhoea treatment failures to cefixime and azithromycin in England, 2010. *Euro Surveill* 2011; **16**: pii=19833.

4 Hess D, Wu A, Golparian D *et al*. Genome sequencing of a *Neisseria gonorrhoeae* isolate of a successful international clone with decreased susceptibility and resistance to extended-spectrum cephalosporins. *Antimicrob Agents Chemother* 2012; **56**: 5633–41.

5 Unemo M, Nicholas RA. Emergence of multidrug-resistant, extensively drug-resistant and untreatable gonorrhea. *Future Microbiol* 2012; **7**: 1401–22.

6 Galvin SR, Cohen MS. The role of sexually transmitted diseases in HIV transmission. *Nat Rev Microbiol* 2004; **2**: 33–42.

7 Kirkcaldy RD, Zaidi A, Hook EW *et al*. *Neisseria gonorrhoeae* antimicrobial resistance among men who have sex with men and men who have sex exclusively with women: the Gonococcal Isolate Surveillance Project, 2005–2010. *Ann Intern Med* 2013; **158**: 321–8.

8 De Vries HJ, Van Der Helm JJ, Schim Van Der Loeff MF *et al*. Multidrug-resistant *Neisseria gonorrhoeae* with reduced cefotaxime susceptibility is increasingly common in men who have sex with men, Amsterdam, the Netherlands. *Euro Surveill* 2009; **14**: pii=19330.

9 Carpenter RJ, Refugio ON, Adams N *et al*. Prevalence and factors associated with asymptomatic gonococcal and chlamydial infection among US Navy and Marine Corps men infected with the HIV: a cohort study. *BMJ Open* 2013; **3**: e002775.

10 Skerlev M, Culav-Koscak I. Gonorrhea: new challenges. *Clin Dermatol* 2014; **32**: 275–81.

11 Ndowa F, Lusti-Narasimhan M. The threat of untreatable gonorrhoea: implications and consequences for reproductive and sexual morbidity. *Reprod Health Matters* 2012; **20**: 76–82.

12 ECDC. *Gonococcal Antimicrobial Susceptibility Surveillance in Europe, 2012*. http://ecdc.europa.eu/en/publications/Publications/gonococcal-antimicrobial-susceptibility-surveillance-Europe-2012.pdf.

13 Public Health England. *GRASP 2012 Report: The Gonococcal Resistance to Antimicrobials Surveillance Programme*. http://webarchive.nationalarchives.gov.uk/20140714084352/http://www.hpa.org.uk/webc/HPAwebFile/HPAweb_C/1317140152190.

14 CDC. *Sexually Transmitted Diseases Treatment Guidelines, 2010*. http://www.cdc.gov/STD/treatment/2010/STD-Treatment-2010-RR5912.pdf.

15 CDC. Update to CDC's sexually transmitted diseases treatment guidelines, 2010: oral cephalosporins no longer a recommended treatment for gonococcal infections. *MMWR Morb Mortal Wkly Rep* 2012; **61**: 590–4.

16 WHO. *Guidelines for the Management of Sexually Transmitted Infections*. http://apps.who.int/iris/bitstream/10665/42782/1/9241546263_eng.pdf?ua=1.

17 Speers DJ, Fisk RE, Goire N *et al*. Non-culture *Neisseria gonorrhoeae* molecular penicillinase production surveillance demonstrates the long-term success of empirical dual therapy and informs gonorrhoea management guidelines in a highly endemic setting. *J Antimicrob Chemother* 2014; **69**: 1243–7.

18 Levy SB. Balancing the drug-resistance equation. *Trends Microbiol* 1994; **2**: 341–2.

19 Seppala H, Klaukka T, Vuopio-Varkila J *et al*. The effect of changes in the consumption of macrolide antibiotics on erythromycin resistance in group A streptococci in Finland. Finnish Study Group for Antimicrobial Resistance. *N Engl J Med* 1997; **337**: 441–6.

20 Kerremans JJ, Verboom P, Stijnen T *et al*. Rapid identification and antimicrobial susceptibility testing reduce antibiotic use and accelerate pathogen-directed antibiotic use. *J Antimicrob Chemother* 2008; **61**: 428–35.

21 Jorgensen JH, Hindler JF. New consensus guidelines from the Clinical and Laboratory Standards Institute for antimicrobial susceptibility testing of infrequently isolated or fastidious bacteria. *Clin Infect Dis* 2007; **44**: 280–6.

22 Recommendations for the laboratory-based detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae*—2014. *MMWR Recomm Rep* 2014; **63**: 1–19.

23 Takei M, Yamaguchi Y, Fukuda H *et al*. Cultivation of *Neisseria gonorrhoeae* in liquid media and determination of its in vitro susceptibilities to quinolones. *J Clin Microbiol* 2005; **43**: 4321–7.

24 Cartwright CP, Stock F, Gill VJ. Improved enrichment broth for cultivation of fastidious organisms. *J Clin Microbiol* 1994; **32**: 1825–6.

25 Wade JJ, Graver MA. A fully defined, clear and protein-free liquid medium permitting dense growth of *Neisseria gonorrhoeae* from very low inocula. *FEMS Microbiol Lett* 2007; **273**: 35–7.

26 Wade JJ, Graver MA. A liquid medium permitting anaerobic growth of *Neisseria gonorrhoeae*. *J Microbiol Methods* 2009; **79**: 364–6.

27 Ahrens K, Bradbury KJ, Bauer HM *et al*. Trends in the use of sexually transmitted disease diagnostic technologies in California, 1996–2003. *Sex Transm Dis* 2007; **34**: 513–8.

28 Kirkcaldy RD, Papp J, Weinstock HS. *Gonococcal Isolate Surveillance Project (GISP) Protocol*. http://www.cdc.gov/std/gisp/gisp-protocol07-15-2010.pdf.

29 CDC. *Sexually Transmitted Disease Surveillance 2011*. Atlanta: US Department of Health and Human Services, 2012.

30 Bignell C, FitzGerald M. Guideline Development Group; British Association for Sexual Health and HIV UK. UK national guideline for the management of gonorrhoea in adults, 2011. *Int J STD AIDS* 2011; **22**: 541–7.

31 Benson PA, Hergenroeder AC. Bacterial sexually transmitted infections in gay, lesbian, and bisexual adolescents: medical and public health perspectives. *Semin Pediatr Infect Dis* 2005; **16**: 181–91.

32 CDC. Cephalosporin susceptibility among *Neisseria gonorrhoeae* isolates—United States, 2000–2010. *MMWR Morb Mortal Wkly Rep* 2011; **60**: 873–7.

33 Bernstein KT, Marcus JL, Barry PM *et al*. Characteristics of males infected with common *Neisseria gonorrhoeae* sequence types in the

Gonococcal Isolate Surveillance Project, San Francisco, California, 2009. *Am J Epidemiol* 2013; **178**: 1289–95.

34 Buono S, Wu A, Hess DC *et al*. Using the *Neisseria gonorrhoeae* multiantigen sequence-typing method to assess strain diversity and antibiotic resistance in San Francisco, California. *Microb Drug Resist* 2012; **18**: 510–7.

35 Grad YH, Kirkcaldy RD, Trees D *et al*. Genomic epidemiology of *Neisseria gonorrhoeae* with reduced susceptibility to cefixime in the USA: a retrospective observational study. *Lancet Infect Dis* 2014; **14**: 220–6.

36 Alloba F, Alawattegama A, Jones C. Use of nucleic acid amplification tests for the detection of *Neisseria gonorrhoeae*: survey of genitourinary medicine clinics in England. *Int J STD AIDS* 2007; **18**: 652–3.

37 Goire N, Sloots TP, Nissen MD *et al*. Protocol for the molecular detection of antibiotic resistance mechanisms in *Neisseria gonorrhoeae*. *Methods Mol Biol* 2012; **903**: 319–28.

38 California Department of Public Health. *Sexually Transmitted Diseases Testing in California: 2009 Clinical Laboratory Survey Summary*. http://www.cdph.ca.gov/data/statistics/Documents/STD-Data-LabSurvey.pdf.

39 Dicker LW, Mosure DJ, Steece R *et al*. Laboratory tests used in US public health laboratories for sexually transmitted diseases, 2000. *Sex Transm Dis* 2004; **31**: 259–64.

40 Farrell DJ, Sheedy TJ. Urinary screening for *Neisseria gonorrhoeae* in asymptomatic individuals from Queensland, Australia: an evaluation using three nucleic acid amplification methods. *Pathology* 2001; **33**: 204–5.

41 McNally LP, Templeton DJ, Jin F *et al*. Low positive predictive value of a nucleic acid amplification test for nongenital *Neisseria gonorrhoeae* infection in homosexual men. *Clin Infect Dis* 2008; **47**: e25–7.

42 Tabrizi SN, Unemo M, Limnios AE *et al*. Evaluation of six commercial nucleic acid amplification tests for detection of *Neisseria gonorrhoeae* and other *Neisseria* species. *J Clin Microbiol* 2011; **49**: 3610–5.

43 Bachmann LH, Johnson RE, Cheng H *et al*. Nucleic acid amplification tests for diagnosis of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* rectal infections. *J Clin Microbiol* 2010; **48**: 1827–32.

44 Bachmann LH, Johnson RE, Cheng H *et al*. Nucleic acid amplification tests for diagnosis of *Neisseria gonorrhoeae* oropharyngeal infections. *J Clin Microbiol* 2009; **47**: 902–7.

45 Harryman L, Scofield S, Macleod J *et al*. Comparative performance of culture using swabs transported in Amies medium and the Aptima Combo 2 nucleic acid amplification test in detection of *Neisseria gonorrhoeae* from genital and extra-genital sites: a retrospective study. *Sex Transm Infect* 2012; **88**: 27–31.

46 Whiley DM, Tapsall JW, Sloots TP. Nucleic acid amplification testing for *Neisseria gonorrhoeae*: an ongoing challenge. *J Mol Diagn* 2006; **8**: 3–15.

47 Gaydos CA, Quinn TC, Willis D *et al*. Performance of the APTIMA Combo 2 assay for detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in female urine and endocervical swab specimens. *J Clin Microbiol* 2003; **41**: 304–9.

48 Hamilton MS, Otto M, Nickell A *et al*. High frequency of competitive inhibition in the Roche Cobas AMPLICOR multiplex PCR for *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. *J Clin Microbiol* 2002; **40**: 4393.

49 Marlowe EM, Novak-Weekley SM, Cumpio J *et al*. Evaluation of the Cepheid Xpert MTB/RIF assay for direct detection of *Mycobacterium tuberculosis* complex in respiratory specimens. *J Clin Microbiol* 2011; **49**: 1621–3.

50 Boehme CC, Nabeta P, Hillemann D *et al*. Rapid molecular detection of tuberculosis and rifampin resistance. *N Engl J Med* 2010; **363**: 1005–15.

51 Low N, Unemo M, Skov Jensen J *et al*. Molecular diagnostics for gonorrhoea: implications for antimicrobial resistance and the threat of untreatable gonorrhoea. *PLoS Med* 2014; **11**: e1001598.

52 Siedner MJ, Pandori M, Leon SR *et al*. Detection of quinolone-resistant *Neisseria gonorrhoeae* in urogenital specimens with the use of real-time polymerase chain reaction. *Int J STD AIDS* 2008; **19**: 69–71.

53 Uehara AA, Amorin EL, Ferreira Mde F *et al*. Molecular characterization of quinolone-resistant *Neisseria gonorrhoeae* isolates from Brazil. *J Clin Microbiol* 2011; **49**: 4208–12.

54 Wu A, Buono S, Katz KA *et al*. Clinical *Neisseria gonorrhoeae* isolates in the United States with resistance to azithromycin possess mutations in all 23S rRNA alleles and the *mtrR* coding region. *Microb Drug Resist* 2011; **17**: 425–7.

55 Ochiai S, Ishiko H, Yasuda M *et al*. Rapid detection of the mosaic structure of the *Neisseria gonorrhoeae* penA gene, which is associated with decreased susceptibilities to oral cephalosporins. *J Clin Microbiol* 2008; **46**: 1804–10.

56 Pandori M, Barry PM, Wu A *et al*. Mosaic penicillin-binding protein 2 in *Neisseria gonorrhoeae* isolates collected in 2008 in San Francisco, California. *Antimicrob Agents Chemother* 2009; **53**: 4032–4.

57 Whiley D, Bates J, Limnios A *et al*. Use of a novel screening PCR indicates presence of *Neisseria gonorrhoeae* isolates with a mosaic *penA* gene sequence in Australia. *Pathology* 2007; **39**: 445–6.

58 Unemo M, Golparian D, Nicholas R *et al*. High-level cefixime- and ceftriaxone-resistant *Neisseria gonorrhoeae* in France: novel penA mosaic allele in a successful international clone causes treatment failure. *Antimicrob Agents Chemother* 2012; **56**: 1273–80.

59 Whiley DM, Jacobsson S, Tapsall JW *et al*. Alterations of the *pilQ* gene in *Neisseria gonorrhoeae* are unlikely contributors to decreased susceptibility to ceftriaxone and cefixime in clinical gonococcal strains. *J Antimicrob Chemother* 2010; **65**: 2543–7.

60 Watchirs Smith LA, Hillman R, Ward J *et al*. Point-of-care tests for the diagnosis of *Neisseria gonorrhoeae* infection: a systematic review of operational and performance characteristics. *Sex Transm Infect* 2012; **89**: 320–6.

61 Goire N, Lahra MM, Chen M *et al*. Molecular approaches to enhance surveillance of gonococcal antimicrobial resistance. *Nat Rev Microbiol* 2014; **12**: 223–9.

62 Campos-Outcalt D. CDC update on gonorrhea: expand treatment to limit resistance. *J Fam Pract* 2011; **60**: 736–40.

63 Chisholm SA, Neal TJ, Alawattegama AB *et al*. Emergence of high-level azithromycin resistance in *Neisseria gonorrhoeae* in England and Wales. *J Antimicrob Chemother* 2009; **64**: 353–8.

64 Domeika M, Savicheva A, Sokolovskiy E *et al*. Guidelines for laboratory diagnosis of *Neisseria gonorrhoeae* infections in Eastern European countries—results of an international collaboration. *Euro Surveill* 2007; **12**: E071206.3.

65 WHO. Global incidence and prevalence of selected curable sexually transmitted infections: 2008. *Reprod Health Matters* 2012; **20**: 207–9.

66 Cole MJ, Unemo M, Hoffmann S *et al*. The European Gonococcal Antimicrobial Surveillance Programme, 2009. *Euro Surveill* 2011; **16**: pii=19995.

67 Van de Laar M, Spiteri G. Increasing trends of gonorrhoea and syphilis and the threat of drug-resistant gonorrhoea in Europe. *Euro Surveill* 2012; **17**: pii=20225.

68 Fjeldsoe-Nielsen H, Unemo M, Fredlund H *et al*. Phenotypic and genotypic characterization of prolyliminopeptidase-negative *Neisseria gonorrhoeae* isolates in Denmark. *Eur J Clin Microbiol Infect Dis* 2005; **24**: 280–3.

69 Whiley DM, Limnios EA, Ray S *et al*. Diversity of *penA* alterations and subtypes in *Neisseria gonorrhoeae* strains from Sydney, Australia, that are less susceptible to ceftriaxone. *Antimicrob Agents Chemother* 2007; **51**: 3111–6.

70 Garcia S, Casco R, Perazzi B *et al*. Ciprofloxacin resistance of *Neisseria gonorrhoeae* according to sexual habits. *Medicina (B Aires)* 2008; **68**: 358–62.

71 Jakopanec I, Hassfjord JJ, Nilsen O *et al*. A local outbreak of quinolone-resistant gonorrhoea in Norway, January 2008. *Euro Surveill* 2008; **13**: pii=18897.

72 Mendez Ede L, Morano ST, Mollerach AS *et al*. Resistance surveillance of *Neisseria gonorrhoeae* in a hospital in Santa Fe province, Argentina: 1997–2004. *Rev Argent Microbiol* 2008; **40**: 173–9.

73 Galarza PG, Alcala B, Salcedo C *et al*. Emergence of high level azithromycin-resistant *Neisseria gonorrhoeae* strain isolated in Argentina. *Sex Transm Dis* 2009; **36**: 787–8.

74 Alzahrani AJ, Obeid OE, Hassan MI *et al*. Screening of pregnant women attending the antenatal care clinic of a tertiary hospital in eastern Saudi Arabia for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infections. *Indian J Sex Transm Dis* 2010; **31**: 81–6.

75 Chen PL, Lee HC, Yan JJ *et al*. High prevalence of mutations in quinolone-resistance-determining regions and *mtrR* loci in polyclonal *Neisseria gonorrhoeae* isolates at a tertiary hospital in southern Taiwan. *J Formos Med Assoc* 2010; **109**: 120–7.

76 Florindo C, Pereira R, Boura M *et al*. Genotypes and antimicrobial-resistant phenotypes of *Neisseria gonorrhoeae* in Portugal (2004–2009). *Sex Transm Infect* 2010; **86**: 449–53.

77 Galarza PG, Abad R, Canigia LF *et al*. New mutation in 23S rRNA gene associated with high level of azithromycin resistance in *Neisseria gonorrhoeae*. *Antimicrob Agents Chemother* 2010; **54**: 1652–3.

78 Golparian D, Hellmark B, Fredlund H *et al*. Emergence, spread and characteristics of *Neisseria gonorrhoeae* isolates with in vitro decreased susceptibility and resistance to extended-spectrum cephalosporins in Sweden. *Sex Transm Infect* 2010; **86**: 454–60.

79 Huang CT, Yen MY, Wong WW *et al*. Characteristics and dissemination of mosaic penicillin-binding protein 2-harboring multidrug-resistant *Neisseria gonorrhoeae* isolates with reduced cephalosporin susceptibility in northern Taiwan. *Antimicrob Agents Chemother* 2010; **54**: 4893–5.

80 Kubanova A, Frigo N, Kubanov A *et al*. The Russian gonococcal antimicrobial susceptibility programme (RU-GASP)—national resistance prevalence in 2007 and 2008, and trends during 2005–2008. *Euro Surveill* 2010; **15**: pii=19533.

81 Aldous WK, Robertson JL, Robinson BJ *et al*. Rates of gonorrhea and *Chlamydia* in US military personnel deployed to Iraq and Afghanistan (2004–2009). *Mil Med* 2011; **176**: 705–10.

82 Glazkova S, Golparian D, Titov L *et al*. Antimicrobial susceptibility/resistance and molecular epidemiological characteristics of *Neisseria gonorrhoeae* in 2009 in Belarus. *APMIS* 2011; **119**: 537–42.

83 Hughes G, Nichols T, Ison CA. Estimating the prevalence of gonococcal resistance to antimicrobials in England and Wales. *Sex Transm Infect* 2011; **87**: 526–31.

84 Yuan LF, Yin YP, Dai XQ *et al*. Resistance to azithromycin of *Neisseria gonorrhoeae* isolates from 2 cities in China. *Sex Transm Dis* 2011; **38**: 764–8.

85 Heymans R, Bruisten SM, Golparian D *et al*. Clonally related *Neisseria gonorrhoeae* isolates with decreased susceptibility to the extended-spectrum cephalosporin cefotaxime in Amsterdam, the Netherlands. *Antimicrob Agents Chemother* 2012; **56**: 1516–22.

86 Hjelmevoll SO, Golparian D, Dedi L *et al*. Phenotypic and genotypic properties of *Neisseria gonorrhoeae* isolates in Norway in 2009: antimicrobial resistance warrants an immediate change in national management guidelines. *Eur J Clin Microbiol Infect Dis* 2012; **31**: 1181–6.

87 Lahra MM. Surveillance of antibiotic resistance in *Neisseria gonorrhoeae* in the WHO Western Pacific and South East Asian regions, 2010. *Commun Dis Intell Q Rep* 2012; **36**: 95–100.

88 Lawung R, Cherdtrakulkiat R, Charoenwatanachokchai A *et al*. Antimicrobial resistance markers as a monitoring index of gonorrhoea in Thailand. *Acta Microbiol Immunol Hung* 2012; **59**: 157–69.

89 Velicko I, Unemo M. Recent trends in gonorrhoea and syphilis epidemiology in Sweden: 2007 to 2011. *Euro Surveill* 2012; **17**: pii=20223.

90 Hottes TS, Lester RT, Hoang LM *et al*. Cephalosporin and azithromycin susceptibility in *Neisseria gonorrhoeae* isolates by site of infection, British Columbia, 2006 to 2011. *Sex Transm Dis* 2013; **40**: 46–51.

91 Kirkcaldy RD, Bolan GA, Wasserheit JN. Cephalosporin-resistant gonorrhea in North America. *J Amer Med Assoc* 2013; **309**: 185–7.

92 Lewis DA, Sriruttan C, Muller EE *et al*. Phenotypic and genetic characterization of the first two cases of extended-spectrum-cephalosporin-resistant *Neisseria gonorrhoeae* infection in South Africa and association with cefixime treatment failure. *J Antimicrob Chemother* 2013; **68**: 1267–70.

93 Gorwitz RJ, Nakashima AK, Moran JS *et al*. Sentinel surveillance for antimicrobial resistance in *Neisseria gonorrhoeae*—United States, 1988–1991. The Gonococcal Isolate Surveillance Project Study Group. *MMWR CDC Surveill Summ* 1993; **42**: 29–39.

94 Walsh C. *Antibiotics: Actions, Origins, Resistance*. Washington, DC: ASM Press, 2003.

95 Umland B. Penicillinase-producing *Neisseria gonorrhoeae*. *West J Med* 1988; **148**: 197–8.

96 Rice RJ, Aral SO, Blount JH *et al*. Gonorrhea in the United States 1975–1984: is the giant only sleeping? *Sex Transm Dis* 1987; **14**: 83–7.

97 Knapp JS, Fox KK, Trees DL *et al*. Fluoroquinolone resistance in *Neisseria gonorrhoeae*. *Emerg Infect Dis* 1997; **3**: 33–9.

98 CDC. *1993 Sexually Transmitted Diseases Treatment Guidelines*. http://www.cdc.gov/mmwr/preview/mmwrhtml/00023296.htm.

99 Lind I. Antimicrobial resistance in *Neisseria gonorrhoeae*. *Clin Infect Dis* 1997; **24** Suppl 1: S93–7.

100 College of American Pathologists. *HC6-A C. trachomatis and N. gonorrhoeae by Nucleic Acid Amplification: Participant Summary*. Northfield, IL: CAP, 2013.