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VIEWPOINTS

Resistance-Guided Therapy for *Neisseria gonorrhoeae*

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Antimicrobial-resistant *Neisseria gonorrhoeae* infections are a threat to public health. Novel strategies for combating such resistance include the development of molecular assays to facilitate real-time prediction of antimicrobial susceptibility. Resistance to ciprofloxacin is determined by the presence of a single mutation at codon 91 of the gyrase A gene; molecular assays to guide therapy are commercially available. Resistance to cefixime is conferred via 1 of 6 critical mutations in either the mosaic *pen*A gene or specific loci in the nonmosaic region. Resistance to ceftriaxone is conferred through mutations in 1 of 4 genes: *pen*A, *pon*A, *pen*B, and *mtr*; however, the ability to predict reduced susceptibility based on those genes varies by geographic region. Here, we highlight the work done toward the development of 3 such assays for ciprofloxacin, cefixime, and ceftriaxone, discuss the status of our current understanding and ongoing challenges, and suggest future directions.

Keywords. antimicrobial resistance; cefixime; ceftriaxone; ciprofloxacin; *Neisseria gonorrhoeae*.

Antimicrobial-resistant *Neisseria gonorrhoeae* poses an urgent threat to public health [1]. *N. gonorrhoeae* has developed resistance to all antibiotics used in its treatment. Recent reports of resistance to third-generation cephalosporins have spurred concerns that we are approaching an era of untreatable infection [2]. Current Centers for Disease Control and Prevention (CDC) guidelines on the treatment of *N. gonorrhoeae* recommend a single dose of ceftriaxone for all suspected or confirmed infections, without determination of antimicrobial susceptibility [3]. The continued emergence of resistance [4] is thought to be driven by selective pressure conferred by cumulative antibiotic exposure, resulting in the accumulation of genetic mutations that gradually reduce efficacy of various antibiotics even without overt treatment failure [5].

The World Health Organization (WHO) has put forth action plans to combat the emergence of antimicrobial resistance, including calls for the development of molecular assays designed for detection of pathogens and genetic mutations that confer reduced susceptibility to specific antibiotics [6]. Use of genetic markers to guide therapy, known as resistance-guided therapy, is not a new concept; molecular assays detect genetic markers of resistance in *Staphylococcus aureus* [7], *Mycobacterium tuberculosis* [8], and many other pathogens. However, as the diagnosis of *N. gonorrhoeae* infections is primarily achieved through the use of nucleic acid amplification tests, these infections are uniquely suited for the coupling of pathogen detection with molecular resistance assays. Here, we summarize the work done thus far in identifying potential genetic markers associated with resistance for 3 antibiotics: ciprofloxacin, cefixime, and ceftriaxone, progress toward the development of molecular resistance assays, and remaining challenges.

RESISTANCE-GUIDED THERAPY FOR CIPROFLOXACIN

Ciprofloxacin inhibits topoisomerase II (or DNA-gyrase) and topoisomerase IV [9]. Analogously, various specific allelic mutations within the 2 genes that encode DNA-gyrase and topoisomerase IV, *gyrase* subunit A (*gyr*A) and *par*C, respectively, have been associated with ciprofloxacin resistance among *N. gonorrhoeae* [10]. Notably, despite the association of various mutations with phenotypic ciprofloxacin resistance, it is the absence of a single mutation at the serine 91 codon of the *gyr*A gene (Figure 1) that has been shown to be both necessary and sufficient to predict susceptibility [11].

Consequently, molecular assays have been developed for the rapid determination of mutation in codon 91 of *N. gonorrhoeae* [12], and those assays have been implemented in clinical practice [13]. Further, a multicenter clinical trial evaluating resistance-guided therapy using *gyr*A genotyping demonstrated 100% treatment efficacy (1-sided 95% confidence interval [CI], 97.5%–100%) of ciprofloxacin where *gyr*A genotyping predicted ciprofloxacin susceptibility [14]. In light of those findings, the sexually transmitted infections treatment guidelines in the United States, the United Kingdom, and Australia have included statements permissive of the use of ciprofloxacin in certain contexts and when susceptibility has

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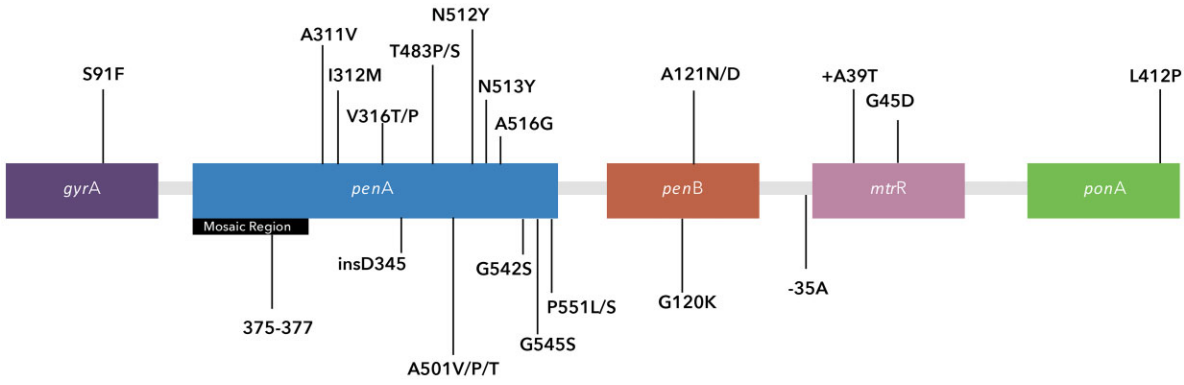

Figure 1. Mutations within *Neisseria gonorrhoeae* implicated in conferring reduced susceptibility to ciprofloxacin, cefixime, and ceftriaxone.

been confirmed through either culture or molecular detection methods [3, 15, 16].

Beyond potentially slowing the emergence of cephalosporin-resistant strains through the sole use of cephalosporins, the use of ciprofloxacin has several other benefits. While treatment with ceftriaxone requires a clinic visit for injection, a single oral dose facilitates care in nonclinical settings [17]. Additionally, expedited partner therapy would be facilitated in regions that allow practitioners to provide extra medication or a prescription for a patient's sex partner, as currently recommended by the CDC [3]. Both nonclinical care and expedited partner therapy would facilitate increased treatment of individuals who otherwise might not present to care and thereby decrease transmission of *N. gonorrhoeae* within the community.

Importantly, the prevalence of ciprofloxacin resistance in *N. gonorrhoeae* continues to rise, with many countries reporting a prevalence of greater than 30% and some greater than 70% [18]. In settings with a prevalence greater than 80%, the utility of predicting ciprofloxacin susceptibility may be reduced. Moreover, the routine use of assays to predict ciprofloxacin resistance in those settings might not be cost-effective. Studies on the cost of such assays have concluded that both the prevalence of ciprofloxacin resistance as well as the frequency of testing will impact the overall cost–benefit ratio of assay implementation [19]. However, none of the above studies have been able to comprehensively account for the systematic costs of rising antimicrobial resistance.

RESISTANCE-GUIDED THERAPY FOR CEPHALOSPORINS

The mechanisms for *N. gonorrhoeae* resistance to cephalosporins are significantly more complex and heterogeneous than for ciprofloxacin; the 4 principal genes involved in cephalosporin resistance are *penA*, *penB*, *mtrR*, and *ponA* (Figure 1) [20]. *pen*A encodes penicillin-binding protein 2, for which mutations in 83 amino acid positions have been associated with decreased ceftriaxone susceptibility [20]. Notably, there are 2 regions within the *pen*A gene: a mosaic region that is composed of inserted DNA sequences from other commensal *Neisseria* subspecies, and a nonmosaic region specific to *N. gonorrhoeae*. Mutations within each region have been shown to be associated with decreased cephalosporin susceptibility. Further, there are different mosaic strains of *N. gonorrhoeae*, with *pen*A34 being the most common form in North America [21]. The labeling nomenclature of the *pen*A gene is different from the labeling notation of mutations within the gene that reflect amino acid substitutions.

In addition to *pen*A, *pen*B encodes PorB, an outer membrane porin; amino acid alterations in G120 and A121 sites decrease permeability of antibiotics [20]. *mtrR* encodes a transcriptional repressor of the gene locus known as *mtr*, which in turn encodes an efflux pump. Deletion of a single adenine residue from the promoter region of the *mtrR* gene results in upregulation of the efflux pump and thereby reduced susceptibility to cephalosporins [20]. Finally, the *ponA* gene, which encodes penicillin-binding protein 1, may contain the amino acid alteration L421P, which has been associated to a lesser extent with resistance to cephalosporins [20]. The resulting multitude of mechanisms for conferring resistance to cephalosporins has posed numerous challenges for predicting resistance phenotypes.

Molecular Determinants of *N. gonorrhoeae* Reduced Susceptibility to Cefixime

The penA34 mosaic insertion within the *penA* gene has been repeatedly associated with cefixime resistance in *N. gonorrhoeae*; that sequence was present in 98% of 270 isolates with reduced cefixime susceptibility in 1 US study [21]. Wong et al developed an assay to detect penA34 mosaicism, which had a high sensitivity and specificity among isolates collected in North America [22]. However, non-penA34 alleles are more common in Europe and Asia [23]. Further, nonmosaic

penA mutations are also associated with cephalosporin resistance [24]. Non-*penA* mutations, such as those in *penB* (G120K and A121N/D), *mtrR* (−35A deletion in the promoter region, +A39T, and G45D), and *ponA* (L412P), likely contribute to the degree of cefixime resistance but are neither necessary nor sufficient to reduce susceptibility independent of *penA* [25].

Specific loci of mutations within the *penA* region that appear to be frequently associated with mosaic *penA* patterns include I312M, V316T, N512Y, and G545S. Importantly, studies using gene transformation techniques demonstrated that those mutations are not sufficient alone to confer reduced susceptibility to cefixime. However, reversion back to the wild type in a strain with cefixime resistance resulted in reduction of the minimum inhibitory concentration (MIC) to levels comparable with those of wild-type *penA* strains [26]. Thus, other mutations are likely important in addition to those found in the mosaic *penA* region. Wild-type *penA* can be distinguished from other mosaic forms of *penA* (with the exception of *penA*49) by determining the amino acid sequence of region 375–377 [25]. Nonmosaic *penA* mutations that appear to be critical to conferring reduced susceptibility to cefixime include point mutations within A501V/P/T, G542S, and P551L/S [25].

Peterson et al developed a multiplex polymerase chain reaction assay for determining *N. gonorrhoeae* resistance to several antibiotics including cefixime [27]. That assay used the following genetic alterations in *penA* (A311V, A501V/P/T, N513Y, G545S), *ponA* (L421P), *penB* (G120/A121), and *mtrR* (−35delA) and found a 98.2% (95% CI, 96.8%–99.1%) sensitivity and a 90.1% (95% CI, 88.6%–91.5%) specificity for predicting cefixime susceptibility in the absence of more than 3 mutations. Notably, however, that assay was assessed among strains isolated in Canada, and studies among larger sample sizes and strains from diverse geographic regions are needed.

Deng et al proposed that susceptibility to cefixime could be reliably predicted by detecting the absence of mosaic substitutions within the *penA* gene amino acid positions 375–377 and the absence of 3 critical mutations in the nonmosaic region of the *penA* gene: 501, 542, and 551. The authors conclude that an assay that detects any of the above resistance mutations would have a 99.5% (95% CI, 98.3%–99.9%) sensitivity for predicting reduced susceptibility to cefixime [25]. Subsequent work applied an analysis of the same mutations to an external dataset of *N. gonorrhoeae* strains from the United States and found a 95.9% (95% CI, 97.1%–99.4%) sensitivity for the determination of decreased cefixime susceptibility [28]. Importantly, the reported 95.9% sensitivity equated to a failure of capturing reduced susceptibility to cefixime among 8 strains. Those 8 strains did not contain the expected mosaic *penA* mutations [28]. Such a finding suggests the potential importance of an additional mutation within a non-*penA* gene or the importance of an additional locus within the mosaic *penA* region beyond 375–377.

The assay described above is promising; however, such an assay is still in development, and no clinical trials have been done to confirm test performance. Additionally, while the majority of strains with reduced susceptibility to cefixime may be captured by the assay described above, other mutations are clearly important, and ongoing surveillance and sequencing work is needed to characterize those mutations.

The benefits of an assay with the ability to predict susceptibility to cefixime would be far-reaching. Much like ciprofloxacin, as treatment with cefixime can also be given as a single-dose oral pill, care in nonclinical settings and expedited partner therapy will be greatly facilitated [17]. In fact, expedited partner use of cefixime for treating the sex contacts of a patient with *N. gonorrhoeae* infection is recommended in the most recent CDC guidelines when partner therapy with ceftriaxone is not an option [3]. Further, cefixime is recommended by the WHO in resource-limited settings when the community prevalence of resistance is low [29]. In addition, oral cefixime is considered safe in pregnancy. Finally, rare but potentially serious side effects of ciprofloxacin may limit its use in some populations, thus favoring cefixime. Importantly, single-dose cefixime may be inadequate for the treatment of pharyngeal *N. gonorrhoeae* infections [3], while a recent trial demonstrated near 100% microbiologic cure for susceptible pharyngeal infections treated with ciprofloxacin [14].

Molecular Determinants of *N. gonorrhoeae* Decreased Susceptibility to Ceftriaxone

As with cefixime, previous reports have strongly associated the presence of *penA* mosaicism with decreased susceptibility to ceftriaxone. However, unlike cefixime, a substantial number of strains have been identified in which *penA* mosaicism is neither necessary nor sufficient to predict reduced susceptibility to ceftriaxone [30]. A series of alterations in both *penA* and non-*penA* alleles have been identified as potentially important targets: *penA* (A311V, A501V/P/T, A516G, N512Y, N513Y, G542S, G545S, I312M, P551L/S, V316T/P, insD345), *ponA* (L421P), *penB* (G120/A121), and *mtrR* (−35delA) [20, 27].

An assay designed against strains isolated from Canada that screened all 4 alleles reported a sensitivity of 98.3% (95% CI, 93.9%–99.8%) and a specificity of 66.7% (95% CI, 57.6%–74.9%) for predicting decreased susceptibility to ceftriaxone when 3 or more mutations were present [31]. However, reports are conflicting about which mutations and in what combination have the most discriminatory predictive performance. Some of the discrepancy is likely due to the heterogeneity in geographic region (and therefore in dominant clonal population) from which the strains originated. de Korne-Elenbaas et al reported strains of *N. gonorrhoeae* from Amsterdam with and without decreased susceptibility to ceftriaxone [32],

noting that all strains with resistance had a *pen*A allele with an A501V mutation and *pen*B G120K/A121D mutations, which were lacking in susceptible strains. Pinto et al reported that strains with reduced susceptibility to ceftriaxone from Portugal contained a nonmosaic *pen*A allele containing mutation G542S as well as *pen*B mutation G120K and A121D [33]. Various mutations such as V316T, G545S, I312M, and N512Y may contribute to reduced susceptibility, as reverting these alterations *in vitro* reduces the observed MIC [26]. However, they do not appear to be key drivers of resistance among reported strains from epidemiological studies [20].

One molecular assay for the determination of intermediate susceptibility to ceftriaxone among *N. gonorrhoeae* isolates developed by Peterson et al reported a 99.8% (95% CI, 99.0%–100.0%) sensitivity and an 89.0% (95% CI, 87.5%–90.4%) specificity for the prediction of ceftriaxone susceptibility in the absence of 3 or more of the genetic alterations in *penA* (A311V, A501V/P/T, N513Y, G545S), *ponA* (L421P), *penB* (G120/A121), and *mtrR* (−35delA) [27]. Doná et al report the development of an assay detecting lack of insD345 and G545S in mosaic *pen*A10 and *pen*A34, finding a near 100% sensitivity (though there was only 1 isolate with cephalosporin resistance and no false negatives) and 81.7% specificity (95% CI, 72.4%–89.0%) among isolates from Switzerland [34].

Lin et al developed 4 testing algorithms from strains isolated in 23 countries (predominantly the United States [30%], Russia [14%], Canada [12%], and New Zealand [11%]) [20]. Two algorithms used *penA* genes, 1 with and 1 without mosaicism determination; the other 2 algorithms used non-*penA* genes, 1 with and 1 without mosaicism determination. Among mosaic strains (as determined by amino acid substitutions in the 375–377 region), they highlight *pen*A mutations A311V, T483P/S, A501V/P/T, and P551S, which showed low sensitivities but high specificities for reduced ceftriaxone susceptibility. Further, those mutations have been associated with high-level ceftriaxone resistance when present [26]. Among nonmosaic strains, Lin et al reported A501V/T as being strongly associated with reduced susceptibility to ceftriaxone, while G542S, P551L/S, and insD354 were not, which is in contrast to previous reports [35, 36]. Subsequently, a study using DNA microarray technology was developed for nearly 6000 isolates, concluding that the largest contribution to reduced MIC came from the *pen*A mutations A501P, A311V, G545S, and insD345, as well as *por*B (G120A) [37].

Regarding non-*pen*A mutations, a consensus appears to be that the non-*pen*A mutations [*ponA* (L421P), *penB* (G120/A121), and *mtrR* (−35delA)] are all important in contributing to reduced ceftriaxone susceptibility specifically among nonmosaic strains [20]. There may be interactions between alleles at those loci and nonmosaic alleles that contribute to the development of decreased ceftriaxone susceptibility, which should be the subject of future work.

In follow-up studies, Lin et al applied the findings by Peterson et al with regard to detecting decreased ceftriaxone susceptibility to their global dataset and reported a comparable sensitivity (98.4%; 95% CI, 97.1%–99.2%) but lower specificity (67.3%; 95% CI, 65.6%–68.9%) [38]. Likewise, Lin et al reported application of their algorithms among strains reported by de Korne-Elenbaas et al and also reported a high sensitivity but low specificity [39]. Such discrepant specificities likely reflect consequences of heterogeneity in molecular markers associated with decreased susceptibility across various geographic locations. That heterogeneity poses an important practical challenge for implementation of such assays. In areas with low prevalence of decreased susceptibility, the positive predictive value of an assay with a low specificity would be exceedingly low. Thus, tailoring of the molecular targets used in any such assay should be done with consideration of local differences in the genetic epidemiology of *N. gonorrhoeae*. Importantly, such work will be greatly facilitated by increased genomic epidemiological surveillance through increased laboratory and research capacity as well as increased reporting of the complete genomic sequences in the scientific literature.

FUTURE DIRECTIONS

The utility of resistance-guided therapy will be directly impacted by the frequency of antimicrobial resistance in the population and by how effective reduction of antibiotic use is in alleviating the selective pressure toward the emergence of resistance. The latter, importantly, is relatively unknown, with limited data from other settings suggesting a potential benefit [40], thus warranting further study. For areas in which the prevalence of resistance to ciprofloxacin is low, implementation of *gyr*A genotyping in conjunction with pathogen detection may prove to be cost-effective. Such efforts can be facilitated by the incorporation of resistant marker determination into molecular point-of-care tests [41, 42].

For assays that are used to determine *N. gonorrhoeae* reduced susceptibility to cefixime, simultaneous mutation detection of at least 6 amino acid locations will be essential. It will be important to assess the validity of such assays in geographically distinct regions with strains of *N. gonorrhoeae* carrying different mosaic alleles. Combining such an assay with pathogen detection would facilitate tailored treatment decisions at the time of diagnosis. Notably, there appear to be a minority of circulating strains that are not captured by the 6 amino acid mutations proposed. Toward the development of an assay to predict susceptibility to ceftriaxone, increased capacity and funding for and reporting of genetic epidemiology will be essential. It may be necessary for future studies to evaluate country-level characteristics of the dominant circulating mosaic strain.

For all of those assays, real-world analyses among varying specimen types will be essential in order to validate performance metrics. Further, the context in which such implementation occurs will likely vary depending on the intended use of the assay. For example, a point-of-care assay will be most useful for symptomatic patients, while reflex molecular diagnostics might be of greater use for populations that present for screening in a setting that is already reliant on nucleic acid amplification tests. Additionally, careful consideration of systematic costs and continued monitoring for the development of resistance will be important for evaluation of the potential benefit of those assays.

CONCLUSIONS

In summary, a growing body of research has laid the groundwork for the development of molecular assays capable of predicting susceptibility of *N. gonorrhoeae* to several antibiotics. The development of such assays is an important tool for combating the emergence of multidrug-resistant *N. gonorrhoeae* strains. Assays that predict *N. gonorrhoeae* susceptibility to ciprofloxacin by way of identifying the absence of mutation at codon 91 of the gyrase A gene are commercially available in some areas. Prediction of susceptibility to cefixime is thought to be possible by determining the absence of mutation at any of 6 critical loci, and a molecular assay is currently in development; however, there are likely a subset of strains that will not be identified with determination of those mutations alone. Reduced susceptibility for ceftriaxone appears to be determined by various mutations in 1 of 4 genes; the precise mutations, however, likely vary geographically. Any molecular assay aimed at determining susceptibility to ceftriaxone must account for local genetic epidemiology.

Notes

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Potential conflicts of interest. J. D. K. has patents pending regarding the use of molecular assays in the prediction of antimicrobial resistance and has received consulting fees from Roche, Abbott, Cepheid, SpeedX, Visby Medical, Curative, and Biomeme in the past 12 months. L.-T. A.-B. reports consulting fees from Curative Inc. The remaining author: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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