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Considering the Potential Application of Whole Genome Sequencing to Gonorrhea Prevention and Control

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Increasingly applied to identify mutations conferring antimicrobial resistance (AMR), disease outbreaks, and pathways of disease spread, whole genome sequencing (WGS)—the process of determining the complete DNA sequence of an organism's genome at a single time—has emerged as a powerful tool for public health. Genomic analyses played central roles in recent outbreak investigations, such as of a high-profile outbreak of carbapenem-resistant *Klebsiella pneumoniae* at the US National Institutes of Health Clinical Center, the 2010 outbreak of cholera in Haiti, the 2014–2015 HIV outbreak in Indiana, the epidemic of Zika virus in the Americas, and large outbreaks of foodborne and waterborne illness.[1–7] Whole genome sequencing findings have informed development of novel molecular diagnostics and explorations of human microbiomes.[8,9] Whereas DNA sequencing methods were painstakingly performed manually decades ago, the development of automated methods in the 1990s, followed by rapidly accelerating speed of sequencing, plummeting cost, increasing computational capacity, growing number of sequences in publically available repositories (e.g., GenBank), and increasing availability of bioinformatics tools in the past decade, have supported a dramatic expansion of WGS.

Recently, WGS has been used to investigate *Neisseria gonorrhoeae,* the bacterium that causes gonorrhea. Gonococcal AMR to nearly all therapies used for gonorrhea treatment has been detected across the world; *N. gonorrhoeae* has been designated as an urgent AMR threat by the US Centers for Disease Control and Prevention and a high-priority pathogen by the World Health Organization.[10,11] Thus, most WGS-based studies of *N. gonorrhoeae* focused on exploring genetic determinants of AMR or combined WGS data with patient demographic and behavioral information to describe clusters and provide insights into *N gonorrhoeae* and AMR geographic and temporal spread through different sexual networks. Over just the past 4 years, these studies have been conducted in multiple countries and the sample sizes of each study increased to include more than 1000 isolates.[12–19] In this commentary, we probe the questions of how such genomic data might inform gonorrhea

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public health prevention and control efforts. In particular, we consider the potential use of WGS for (1) molecular detection of AMR for informing clinical decision making and enhancing surveillance, and (2) informing programmatic action for gonorrhea control. We also consider what knowledge gaps need to be addressed and what barriers need to be overcome for WGS to be useful for gonorrhea control.

DETECTING AMR

The most direct application of *N. gonorrhoeae* WGS to date has been molecular AMR detection. With widespread use of nucleic acid amplification testing (NAAT) for gonorrhea diagnosis and corresponding declines in use of culture (and resulting limited availability of local phenotypic antimicrobial susceptibility testing outside sentinel surveillance) in many developed countries, molecular susceptibility testing (i.e., identification of the presence or absence of resistance mutations to predict susceptibility) has become increasingly attractive. Although well-validated molecular susceptibility tests are not yet commercially available, substantial research has been conducted to (1) develop WGS approaches to predict susceptibility and (2) use WGS to identify resistance determinants (which can be incorporated as targets into polymerase chain reaction [PCR] assays and used with NAATs). In a study of 680 sequences from England, the United States, and Canada, collected in 1989 through 2014, and reference strains, Eyre and colleagues[18] demonstrated that WGS and multivariate linear regression analyses based on multiple resistance determinants could predict minimum inhibitory concentrations of five antimicrobials within one doubling dilution for 93% of *N. gonorrhoeae* isolates. The models demonstrated high-level accuracy for penicillin, ciprofloxacin, and azithromycin, but lower accuracy for cefixime (16% of phenotypically resistant isolates had WGS-predicted susceptibility) and tetracycline (13% of phenotypically susceptible isolates had WGS-predicted resistance). Although WGS of *N. gonorrhoeae* is currently limited by the need for DNA isolation from culture-based isolates, WGS-based susceptibility testing might become a useful tool once sequencing can be performed routinely from nonculture clinical NAAT specimens such as urine or extragenital swabs. Recent work by Graham and colleagues[20] demonstrated technical feasibility by successfully sequencing *N. gonorrhoeae* from 11 urine specimens. A next step could include comparisons of results from nonculture specimens to results from simultaneously collected-culture specimens. Investigators conducting such comparisons should bear in mind that seemingly discordant results might occur due to the nature of the specimens themselves: unlike culture specimens—which probably will contain single strains because of the process of subculturing, nonculture specimens that may be more likely to harbor multiple strains. Analyses of genomic data from nonculture specimens, which are likely to harbor multiple organisms and multiple strains of organisms, are expected to be more complex than analyses of culture specimens (which are grown on selective media to limit growth of other organisms), yet valuable in monitoring resistance genotypes.

Identification of resistance determinants through WGS also supports the development of PCR assays for resistance. Investigators in Australia developed a molecular assay for penicillin susceptibility and implemented it in the Northern Territories (where penicillin resistance remains rare) to inform therapy.[21] Several groups have developed real-time gyrA PCR assays for ciprofloxacin susceptibility; investigators at the University of California, Los

Angeles, investigated use of a same-day reflex PCR assay in a large health care system to allow for the use of ciprofloxacin for gonorrhea treatment and are conducting further clinical validation in other health care settings (NCT02961751).[22–26] Assays to predict susceptibility to extended-spectrum cephalosporins and azithromycin have also been developed.[27–30] However, although there are correlations between mosaic penA XXXIV and reduced extended-spectrum cephalosporin susceptibility, and mutations in mtr operon and 23 s rRNA and reduced azithromycin susceptibility, the correlations are imperfect and assays for these mutations might not be highly sensitive or specific, at least in the United States.[17]

Based on current test technologies and treatment approaches, use of molecular susceptibility testing (either WGS-based or NAAT and reflex PCR based) is unlikely to be broadly applicable to gonorrhea treatment. Gonorrhea treatment, particularly in men with symptomatic urethral infections or exposed partners of persons with gonorrhea, is often administered during the clinical encounter—before the availability of laboratory results—and based on established national guidelines. However, for persons for whom laboratory results would be available at the time of treatment, such as asymptomatic persons found to be infected based on screening, molecular susceptibility testing could inform clinical decision making. Targeted patient-specific treatment may allow for the use of antimicrobials that are no longer recommended for routine treatment, including oral and low-cost regimens such as ciprofloxacin. If molecular susceptibility testing becomes available in clinical settings, it seems likely that the testing will be performed with PCR assays, rather than WGS. An important consideration is that molecular susceptibility testing approaches only detect already-defined resistance markers; novel mutations conferring resistance will be missed. Thus, changes in the mutations conferring resistance or the emergence of new mutations could compromise the predictive accuracy of the assays. There are many unanswered questions related to the temporal and geographical stability of these markers; a more complete understanding of stability is critical to the robust expansion of a reliable NAAT-based diagnostic tool for gonococcal AMR. Expanded sequencing of previously collected phenotypically resistant isolates can advance the understanding of previous stability over time and across populations. Moving forward, stability of markers included in molecular susceptibility testing and identification of the need for inclusion of new markers should be continuously evaluated by pairing existing culture-based phenotypic surveillance —needed to identify novel resistant strains and mutations—with WGS of a representative sample of resistant strains.

Incorporation of molecular susceptibility testing into gonorrhea treatment decision making raises several important issues and questions. Gonorrhea prevention and control efforts in the United States have focused on, in part, improving provider adherence to current Centers for Disease Control and Prevention Sexually Transmitted Diseases (STD) Treatment Guidelines to ensure that patients are treated with the most effective regimen. In the United States, many health care providers, particularly those practicing in private settings, face challenges in adopting updates in gonorrhea treatment guidelines[31]; how would new patient-specific diagnostics be incorporated into clinical care and current treatment guidance? Will these tests provide greater clarity for providers or greater confusion? How should results be provided to providers to maximize clarity and without sacrificing timeliness of treatment? Also, will changes in treatment approaches resulting from implementation of these tests

influence bacterial susceptibility? As noted earlier, it is anticipated that molecular assays will not detect strains with novel resistance genotypes, so what are the treatment and control implications of the emergence of novel strains that may be inadequately treated?

A second potential use of WGS is to enhance and expand surveillance of AMR mutations (and perhaps strain types). As technology improves (such as allowing for WGS from nonculture specimens), molecular surveillance may allow for monitoring of susceptibility, for example, through monitoring the prevalence of individual mutations, to expand beyond the constraints of culture-based surveillance, which is often limited to sentinel surveillance in STD clinics and largely symptomatic males with urethritis. Implementation of molecular surveillance would, however, require thoughtful consideration of the objectives of such surveillance, the optimal approach (sentinel or population based? periodic cross-sectional evaluation or continuous surveillance?), feasibility of data collection, management, and transmission, anticipated representativeness, and cost. Whole genome sequencing-based surveillance would require considerable infrastructure upgrades (such as powerful computing capacities and large amounts of data storage) to handle the massive amounts of data generated, and capacity building (such as training the STD workforce to manage, analyze, and interpret WGS data). These challenges would be substantial—many local and state STD programs in the United States have limited resources and are struggling to implement or maintain existing surveillance systems to monitor disease morbidity based on case and laboratory reports. In addition, many state and local STD programs currently lack the epidemiologic expertise to analyze these relatively basic case-based morbidity data and translate findings into programmatic action. It is worth noting that other approaches to molecular characterization and strain typing, such as multilocus sequence typing (MLST), *N. gonorrhoeae* multiantigen sequence typing (NG-MAST), and *N. gonorrhoeae* sequence typing for AMR (NG-STAR) generate results that might be easier to interpret and do not generate the massive amounts of data that WGS does. However, these typing schemes do not necessarily correlate with phylogenetic relationships or AMR and do not provide the high resolution of WGS,[12] and the usefulness of these approaches to inform public health action still needs to be demonstrated.

DETECTION OF GONOCOCCAL OUTBREAKS AND UNDERSTANDING TRANSMISSION

Might WGS have a role in informing local, state, or federal programmatic action for gonorrhea control and prevention? As noted earlier, WGS has been used to guide responses to outbreaks of other pathogens; the applicability of WGS to guide field investigations of gonorrhea outbreaks, particularly outbreaks of resistant strains, needs to be explored. If conducted in a timely manner and with sufficient sequencing of the underlying gonococcal population for context, phylogenetic analyses of WGS data may support identification of clusters of resistant strains. The analyses might also inform the outbreak response. For example, the response to a small highly related or clonal cluster (suggesting recent transmission among a small number of persons) might focus on intensive and focused partner services, whereas the recognition that resistant infections appear in multiple phylogenetic lineages (suggesting previously undetected and now widespread transmission

in multiple sexual networks) might call for a broader response. Whole genome sequencing was used in 2 recent investigations of isolates with high-level azithromycin resistance, but understanding how best to apply WGS to *N. gonorrhoeae* outbreaks is still very much a work in progress.[13,19] Pilot work applying WGS to investigations should address whether phylogenetic analyses can be used to prioritize patients or contacts for investigation (such as those who appear important for transmission), how quickly phylogenetic analyses need to be performed to be useful for guiding investigations, and whether the necessary speed required is at all realistic.

Because fewer STD programs in the United States interview persons with gonorrhea for partner services, combining WGS data with available demographic data might allow public health officials to better understand characteristics of local sexual networks. Elucidation of networks might, for example, identify individuals who are central to a network or who connect subnetworks. Incorporation of geospatial and additional epidemiologic data could further strengthen these analyses. Exploration of interventions based on such findings may be of value.

More broadly, WGS, particularly when combined with epidemiologic, network, and other laboratory data, may be a powerful tool for elucidating transmission patterns of disease and the speed of spread, within and across populations and across geographic boundaries.[12,16] Potential programmatic uses of transmission models include estimating the source of an identified strain (such as a highly antimicrobial-resistant strain), where and within what time frame it may spread (roughly akin to hurricane tracking), and based on these estimates, deployment of public health resources (e.g., enhanced surveillance, screening, or control efforts) to address a rapidly developing cluster of genetically related gonococcal infections. When genomic data point to regional or cross-jurisdictional transmission patterns not otherwise known because of epidemiologic data limitations, transmission models may also foster regional or cross-jurisdictional collaborative prevention and control efforts. Greater understanding of the degree of genetic similarity between isolates from recent transmission events would move the STD field toward these goals. For example, studies comparing genetic relatedness of isolates collected from patients, their named partners, others in their sexual network, and persons unlikely to be in the patients' sexual networks can assist with development of models that determine the likelihood of transmission between persons or groups of people.

A critical consideration for the previously mentioned applications is where sequencing and genomic analyses would be performed—at the local, state, regional, or federal level? If conducted at the local or state level (which might facilitate more rapid analyses and closer integration into a local response), substantial investments in data systems, storage, and computing power, software packages, and analytic expertise would be needed. It also may be worth considering the ethical, legal, and scientific issues that will arise if WGS analyses are used in legal and forensic investigations of sexual assault and abuse that involved gonorrhea transmission.

In this commentary, we frame the necessary dialogue and highlight the important questions that need to be addressed to fully take advantage of the opportunities that WGS may offer to

public health detection and response for gonorrhea. As technologies have improved and scaled-up, WGS has become faster and cheaper. In an era of diminishing public health and STD program resources, investments in new approaches must be made thoughtfully. Whole genome sequencing is a potentially powerful tool for controlling gonorrhea and slowing the spread of AMR, but like any new technology, thoughtful consideration should be given to if, when, and how WGS should best inform gonorrhea treatment, surveillance, and programmatic action.

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