EDUCATIONAL COMMENTARY – REVIEW OF NEISSERIA GONORRHOEAE

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Learning Outcomes

On completion of this exercise, the participant should be able to

- describe the pathogenesis of Neisseria gonorrhoeae;
- discuss the methods used to diagnose gonorrhea;
- justify the use of transport media when trying to isolate N gonorrhoeae;
- evaluate the current recommended treatment of gonorrhea;
- determine the effects that specific mutations have on drug resistance in N gonorrhoeae;
- describe testing assays used to determine the antimicrobial susceptibility pattern of N gonorrhoeae; and
- evaluate steps used to control the development of multidrug-resistant N gonorrhoeae.

Introduction

A number of infectious agents can be sexually transmitted. While some produce disease primarily in the genitourinary tract, others produce disease at other sites. In 2013, the Centers for Disease Control and Prevention (CDC) estimated that at any time, 110 million Americans have a sexually transmitted infection, or STI (Table 1). The CDC also estimated there were approximately 20 million newly diagnosed STIs annually. New infections are a large financial burden on the US health care system, with an estimated annual financial impact of $16 billion. An additional problem is the evolution of multidrug-resistant bacteria responsible for STIs, in particular Neisseria gonorrhoeae, the cause of gonorrhea.

Table 1. Estimated new and existing STIs in the United States, 2008.

<table>
<thead>
<tr>
<th>Sexually Transmitted Infection</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human papillomavirus</td>
<td>79,100,000</td>
</tr>
<tr>
<td>Herpes simplex virus 2</td>
<td>24,100,000</td>
</tr>
<tr>
<td>Trichomoniasis</td>
<td>3,710,000</td>
</tr>
<tr>
<td>Chlamydia</td>
<td>1,570,000</td>
</tr>
<tr>
<td>Human immunodeficiency virus</td>
<td>908,000</td>
</tr>
<tr>
<td>Hepatitis B virus</td>
<td>422,000</td>
</tr>
<tr>
<td>Gonorrhea</td>
<td>270,000</td>
</tr>
<tr>
<td>Syphilis</td>
<td>117,000</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>110,197,000</strong></td>
</tr>
</tbody>
</table>
Gonorrhea Pathology

Owing to the relative fragility of *N. gonorrhoeae* outside the human body, the bacteria require genital contact to spread between human hosts. Upon contact with the mucous membrane lining the urethra or endocervix, *N. gonorrhoeae* invade the epithelial cells and persist as facultative intracellular parasites. After an incubation period of approximately 2 to 7 days, they produce acute urethritis characterized in males by a purulent discharge and dysuria. In females, infection results in cervicitis and dysuria. Women are more likely to have an initially asymptomatic infection, which can lead to complications such as pelvic inflammatory disease and disseminated gonococcal infection.

Diagnosis

**Isolation of *Neisseria gonorrhoeae***

In the past, gonorrhea was diagnosed strictly by culture isolation and biochemical testing. The most common specimens submitted for isolation of *N. gonorrhoeae* from culture are male urethral and female endocervical swabs. Swabs with plastic or wire shafts and Dacron, alginate, or rayon tips should be used. The swab should be inserted 2-3 cm into the male urethra or 1-2 cm into the female endocervical canal and rotated 2-3 times. In males, urethral exudate is also acceptable. If bedside inoculation is not an option, a transport medium must be used. The bacteria remain viable for approximately 48 hours in a transport medium at room temperature. It is recommended that the inoculated transport medium be taken to the laboratory as soon as possible to maintain maximum bacterial viability.

In some cases, Gram stains along with patient symptoms can be enough information to determine treatment. In symptomatic males, Gram stains of urethral exudate are highly sensitive and specific and can be diagnostic for *N. gonorrhoeae* if gram-negative intracellular diplococci are seen. However, the absence of gram-negative intracellular diplococci is not sufficient to rule out gonorrhea. Gram stain of other material is not sufficiently sensitive or specific and is not recommended to be used alone for diagnosis. Therefore, culture isolation is usually necessary.

Several culture media are available for the isolation of *N. gonorrhoeae*. Thayer-Martin, Martin-Lewis, and New York City are selective media and should be used for culture of nonsterile sites. Chocolate agar is a nonselective medium that should be used for culture of sterile sites. Inoculated media are incubated at 35°C under increased carbon dioxide (5%) and examined at 24 and 48 hours. Isolates recovered from genital specimens that are oxidase-positive, gram-negative diplococci should be subjected to further testing. Confirmatory identification is often based on antigen detection, carbohydrate utilization pattern, or the presence of preformed enzymes. Because *N. gonorrhoeae* is slow-growing, the combination of culture and confirmation was time consuming and could result in treatment delays. Today, most laboratories in the United States use a direct nucleic acid amplification test (NAAT) for diagnosis.
Currently, several manufactured combination NAATs have US Food and Drug Administration (FDA) approval for detecting both *N gonorrhoeae* and *Chlamydia trachomatis*. These assays differ in method, approved specimens for testing, and transport and storage conditions. Laboratories must evaluate the testing system to determine which would work best with their workflow. Most assays have been approved for vaginal and endocervical swabs, urethral swabs from men, and first morning voided urine from women and men. NAATs are highly sensitive and specific and provide results sooner than culture isolation. However, because the bacteria are not recovered, antimicrobial susceptibility testing is unavailable.

### History

When the “golden years” of antimicrobial development began in the 1930s, gonorrhea could be easily treated with sulfonamides, and after resistance to these drugs emerged in the 1940s, with penicillin. Penicillin remained effective for several decades; however, penicillin resistance became problematic in the 1970s as *N gonorrhoeae* underwent genetic changes that required larger and larger penicillin doses for effective treatment. The chromosomal changes occurred mainly in three genes: *penA*, *mtr*, and *penB*. The gene *penA* codes for an altered penicillin-binding protein, PBP-2. The protein product of this gene is a transpeptidase involved in cell-wall synthesis. Penicillin binds to PBPs, inactivating them and inhibiting cell-wall synthesis. The altered PBP-2 has a lower affinity for β-lactams such as penicillin. The gene *mtr* (multiple transferable resistance) codes for an efflux pump that pumps penicillin out of the cell. The *penB* gene encodes proteins involved in porin production. Porins are channels in the cell wall of bacteria that facilitate transport of molecules into the cell. The porins made from the altered protein PenB have decreased permeability for penicillin.

In the mid-1970s, some *N gonorrhoeae* isolates had become totally penicillin resistant by a different mechanism, β-lactamase production. This class of enzymes attacks the β-lactam ring found in the penicillins and cephalosporins. More than 200 β-lactamases have been identified; some are antibiotic-specific whereas others render multiple drugs inactive. Penicillin resistance in *N gonorrhoeae* was generally due to the plasmid-borne TEM-1 β-lactamase. Isolates that contained this plasmid and produced the enzyme were referred to as penicillinase-producing *N gonorrhoeae*. The enzyme destroys penicillin, rendering the bacteria resistant to the drug. Because this resistance mechanism was located on a plasmid, it spread more quickly than the chromosomally-mediated drug resistance that had occurred before.

With the development and spread of multidrug-resistant *N gonorrhoeae*, improved surveillance was needed. In 1986, the CDC established the Gonococcal Isolate Surveillance Project (GISP). The program monitors antimicrobial susceptibility patterns of *N gonorrhoeae* isolated from the urethrae of males who
attend participating sexually transmitted disease clinics. With the widespread failure of the penicillins, the fluoroquinolones became first-line drugs for gonorrhea. The fluoroquinolones were safe, effective, inexpensive, and available in oral forms. The compounds work by interfering with the activity of DNA gyrase and topoisomerase, enzymes necessary for DNA replication. However, the GISP noted resistance to these compounds emerging in the 1990s and 2000s. Low-level quinolone resistance was due to alterations in cell permeability and efflux pumps. High-level resistance in *N gonorrhoeae* was due to mutations in the genes *parC* and *gyrA*, which produced enzymes with decreased affinity for fluoroquinolones. By 2007, more than 5% of GISP isolates were quinolone-resistant *N gonorrhoeae* (QRNG). The incidence of QRNG remains disproportionately higher among men who have sex with men. At that time, the CDC updated its guidelines to remove penicillin and fluoroquinolones as recommended treatments for gonorrhea. This left the cephalosporins as the only recommended treatment. This class of drugs includes oral cefixime and injectable ceftriaxone.

### Current Treatment Recommendations

Because it is not uncommon for an individual with gonorrhea to be infected with *Chlamydia trachomatis* as well as *N gonorrhoeae*, the CDC 2010 guidelines recommended combination therapy with a cephalosporin (cefixime or ceftriaxone) plus oral azithromycin or doxycycline (a tetracycline). While the percentage of gonococcal isolates exhibiting resistance to doxycycline was relatively high at approximately 20%, enough isolates were still susceptible that another goal of cotreatment was to slow the development of further resistance to cephalosporins.

However, between 2006 and 2011, the minimum inhibitory concentration (MIC) of cefixime, the lowest concentration able to inhibit in vitro growth of *N gonorrhoeae*, increased worldwide. In the United States, *N gonorrhoeae* isolates with elevated MICs (≥0.25 µg/mL) increased from 0.1% in 2006 to 1.5% in 2011. Among men who have sex with men, the percentage increased from 0.2% in 2006 to 3.8% in 2011. Despite these relatively low percentages, there were concerns that the effectiveness of cefixime in treating gonorrhea was waning. In order to preserve the efficacy of ceftriaxone, the use of cefixime as a first-line treatment for gonorrhea is no longer recommended. The CDC’s latest recommendation, issued in 2012, is combination therapy with injectable ceftriaxone and either oral azithromycin or oral doxycycline.

### Limiting the Development of Drug Resistance

It is probably not possible to prevent multidrug-resistant bacteria from developing. However, actions can be taken that might slow the development of drug resistance, particularly to ceftriaxone. An important step in controlling drug resistance is thorough monitoring of antimicrobial susceptibility to alert public health authorities when commonly used drugs are no longer effective. Although the GISP is the primary monitoring system for gonococci in the United States, surveillance can by enhanced through collaboration.
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with local and state laboratories. It is critical that patients are treated with the most effective drugs to eliminate bacteria before additional drug-resistant mechanisms can be acquired, and physicians should monitor patients for treatment failure. Laboratories that perform antimicrobial susceptibility testing should notify physicians and local public health officials when \textit{N} gonorrhoeae isolates show decreased susceptibility to ceftriaxone (MIC $\geq 0.125$ µg/mL) or cefixime (MIC $\geq 0.25$ µg/mL). Isolates that are less susceptible to antimicrobial agents than the Clinical and Laboratory Standards Institute criteria for susceptible organisms should be sent to the CDC for confirmation.

\textbf{Antimicrobial Susceptibility Testing}

Because fewer laboratories in the United States are relying on cultures for the diagnosis of gonorrhea, it is more difficult to collect data on emerging drug resistance. In addition, patient isolates are not available to guide treatment. Physicians often must treat patients empirically. \textit{N} gonorrhoeae isolates that exhibit resistance to penicillin, tetracycline, and fluoroquinolone are distributed widely throughout the United States and the world. These drugs are no longer recommended for treating gonorrhea, so susceptibility testing of these agents is not necessary.

Agar dilution is the preferred method for antimicrobial susceptibility testing of \textit{N} gonorrhoeae. In this assay, serial dilutions of antimicrobial agents are made in molten agar, poured into plates, and cooled to solidify. Isolates are inoculated on the media, and the MIC can be determined based on bacterial growth. The lowest drug concentration that inhibits growth of the bacteria is the MIC. Disk diffusion and E-test are simpler assays to perform.

In the disk diffusion assay, commercially prepared filter paper disks contain an antimicrobial agent. Bacteria are lawned onto the surface of an agar plate and several disks containing several different drugs are then added. After incubation, the diameters of the zones of inhibition are measured and compared to a reference chart. Results are recorded as \textit{sensitive}, \textit{intermediate}, or \textit{resistant}.

The E-test is based on commercially prepared filter paper strips with a concentration gradient of a drug. As with the disk diffusion test, bacteria are lawned onto a plate and the antibiotic strips are added. After incubation, an MIC can be determined based on the location of the zone of inhibition around the strip.

\textbf{Summary}

Gonorrhea continues to be a significant cause of morbidity. The control of gonorrhea depends on rapid diagnosis and effective treatment. The control of this disease is complicated by the ability of \textit{N} gonorrhoeae to acquire genes that induce drug resistance. Patients with persistent infections should be retested, and efforts should be made to evaluate patient’s sexual contacts from the past 60 days and treat them if necessary. \textit{N} gonorrhoeae has a history of developing drug resistance, and there is no
reason to think that the trend will halt. It is hoped that measures can be taken to slow the progression of further drug resistance.

References


