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Diagnosis and antimicrobial treatment of bacterial *Neisseria gonorrhea* infections: Update review article

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Abstract

Sexually transmitted infections (STIs) are caused by a wide spectrum of bacteria, viruses and parasites. These agents can be easily transmitted by direct genital or oral sexual contact. Recently, the World Health Organization (WHO), reported that more than 1 million STIs are acquired every day worldwide. Each year, there are an estimated 357 million new infections with one of the following four STIs: chlamydia, gonorrhea, syphilis and trichomoniasis. There are few studies and official reports published on the prevalence of STIs in most Arab countries. However, a few recent studies showed indicate an increased prevalence of certain STIs in some Arab countries, and few data is available on their antimicrobial susceptibility.

Introduction

Gonorrhea is the second most prevalent bacterial sexually transmitted infection (STI) with a global estimated incidence of 78 million cases annually [1,2], with probably a large number of cases that remain unreported [2]. In the United States of America, *N. gonorrhea* is the second most commonly reported communicable disease, with more than 350,000 cases reported annually [3]. A recent study from Saudi Arabia reported that nongonococcal urethritis, trichomoniasis, and HIV were the most commonly reported STIs in the Kingdom of Saudi Arabia over the period 2005-2012 , and the study showed that gonococcal urethritis accounted for 4.4% of the total STI cases [4].

Obligate pathogenic organisms of STIs such as gonorrhea and syphilis can be recognized clinically and confirmed by rapid laboratory test in men and women. Whereas, other potential bacterial pathogens of STIs represented by *Chlamydia trachomatis*, *Mycoplasma genitalium* and *Ureaplasma urealyticum* are among the most prevalent agents found in the genital tract of humans [5-6]. These organisms are more difficult to detect and to prove their presence the actual cause of genital infections, since they can contribute to asymptomatic and symptomatic clinical diseases alone or in association with *N.*

gonorrhea [5-7]. Prompt and accurate treatment of STIs is essential measurement to cure patients and prevents their complications . In addition, rapid detection of positive cases of STIs will help to control and prevent the spread of their harmful agents in the communities. It is important to mention that there is no available vaccine against any type of STIs.

Clinical manifestations of *N. gonorrhea* infections

The majority of urethral infections caused by *N. gonorrhea* among men produce symptoms that require medical examination [8]. Among women, gonorrhea does not produce recognizable symptoms until complications occur [9]. Because gonococcal infections among women are frequently asymptomatic, the Centers for Disease Control and Prevention (CDC/USA) recommended that clinicians screen all sexually active women, including those who are pregnant, for gonorrhea infection if they are at any increased risk [10]. In general, risk factors of gonorrhea infections include age younger than 25 years, a previous gonorrhea infection, other STIs, and new or multiple sexual partners [8-10].

In Western countries, 10 to 30 % of patients with gonorrhea will have a concomitant Chlamydia infection [6,11-12]. The disease is associated with high morbidity and socioeconomic consequences and remains a public health problem worldwide. *N. gonorrhea* can also infect other mucosal surfaces, including the pharynx, rectum and conjunctiva, but may rarely invade the bloodstream, causing disseminated infection [8,11,13]. In women, the primary gonococcal infection is in the endocervix, with concomitant urethral infection, and it is frequently asymptomatic, but if the infection causes symptoms, the most common is purulent vaginal discharge , which is a result of endocervicitis [8,10-11]. Gonorrhea infections do not cause vaginitis, but other concomitant infections may produce vaginal findings [8]. Ascending infection may occur in 10–20% of infected women and can result in acute pelvic inflammatory disease (PID) or salpingitis that may manifest as endometritis, and tubo-ovarian abscess, all of which can lead to scarring, ectopic pregnancy, infertility, and chronic pelvic pain [8,13-14]. In men, gonococcus is known to cause urethritis, which is rarely complicated by infections of other parts of the genital tract [11,13]. Unlike women, men with urogenital infections are usually symptomatic. The normal incubation period is two to six days after exposure, and symptoms include purulent penile discharge and dysuria. The possibility always remains of untreated gonorrhea in men leading to prostatitis, epididymitis, epididymo-orchitis, urethral stricture, and infertility [14,15-16]. Rectal infection with *N. gonorrhoeae* may occur through direct inoculation by receptive anal intercourse and is typically seen in homosexual males but can also appear in women. In addition, patients often do not experience symptoms (two thirds of patients), except for possible occurrences of anal pruritus, painless rectal discharge, and tenesmus. Pharyngeal infections caused by *N. gonorrhoeae* usually occur after orogenital exposure; symptoms are mild or absent [11,13]. Adult ocular gonococcal infection is very rare, with transmission usually being by autoinoculation or direct inoculation from the genital secretions of an infected partner [17]. Disseminated infection is rare but it can occur in 1 to 3 % of adults who have gonorrhea. It can occur in both sexes with higher frequency in women [8,11-12]. Symptoms of disseminated infection can range from slight joint pain, a few skin lesions, and no fever to overt polyarthritis and a high fever [11-12]. Disseminated gonorrhea may also present as bacterial endocarditis and meningitis; yet, the incidences of these presentations have declined with the introducing of antibiotic therapy [11]. During the neonatal period and the first year of life, gonorrhea infections can cause neonatal conjunctivitis (ophthalmia neonatorum), pharyngitis, rectal infections, and, in rare cases, pneumonia [8,11]. Preadolescent children most commonly contract gonococcal infec-

tions through sexual abuse [8]. Rapid diagnosis and administration of antimicrobial therapy are the main measurement to reduce and control of gonorrhea infection in the community and prevent its complications in infected persons [3,18] .

Laboratory Diagnosis

Specimen collection

The specimen collection method depends on the testing technique used in a laboratory and the age, sex and sexual orientation of the patient. Specimens should be collected with dacron or rayon swabs because calcium alginate may be toxic to gonococci. Fatty acids inhibit the growth of gonococci; therefore, cotton swabs that do not list acceptable manufacturer specifications should not be used [19]. To minimize the inhibitory effects of unknown substances in the specimen, the swabs should be inoculated directly onto growth medium or placed in swab transport medium immediately after sampling [20].

Gram-stain and culture

Until the late 1980s, laboratory diagnosis of gonorrhea was limited to gram stain and bacterial isolation. Gram staining may be performed directly on exudates from several sites, such as the male urethra, cervix, rectum, synovial fluid, eye discharge, vagina in prepubertal girls, skin lesions, and cerebrospinal fluid. The presence of intracellular gram- negative kidney-shaped diplococci in polymorphonuclear leukocytes on microscopy suggests the diagnosis of a gonococcal infection [8,12]. The gram stain is a rapid tool and has comparable sensitivity to bacterial culture for symptomatic urethral gonorrhea in men. However, it is relatively insensitive for specimens collected from women and from extragenital sites where the specificity of gram stain may also be affected by the presence of commensal *Neisseria* species [20]. The primary specimens should be inoculated onto nonselective chocolate agar and selective agar (Thayer-Martin) containing antimicrobial agents that inhibit the growth of commensal bacteria and fungi. The inoculated plates should be incubated at 35°C to 37°C in a moist atmosphere enriched with CO2 (3% to 7%)[19]. After 18 h to 24 hours culture should be used as the inoculum for additional tests. Plates should not be incubated for longer than 48 h because most old cultures would not survive storage conditions. Autolysis may occur during prolonged incubation, and growth from agar plates becomes difficult to suspend in solutions.

Confirmation of isolates

Several *Neisseria* and related species may be misidentified as *N. gonorrhea* unless appropriate differential tests are performed, especially in specimens obtained for direct smear or isolates from endocervical secretions of women. Therefore, it is essential to confirm *N. gonorrhea* isolates by biochemical tests: *N gonorrhea* can be differentiated from other *Neisseria* species, *Moraxella* species, *Kingella* species and other commensals based on its ability to grow on appropriate selective and nonselective media, produce acid from glucose, maltose,

lactose, sucrose and fructose, reduce nitrate, produce polysaccharide from sucrose and exhibit DNase production. The Rapid Identification Method-*Neisseria* (Remel Inc, USA) is a commercially available rapid acid production test that compares well with the conventional method, but may also not differentiate between *N gonorrhea* and *N cinerea* [21].

Chromogenic enzyme substrates

These tests are based on the presence of preformed chromogenic enzyme in the culture and, thus, require a heavy inoculum of the organism grown on selective medium to permit rapid speciation of isolates.

Evaluation of eight methods for identification of pathogenic *Neisseria* species: Neisseria-Kwik, RIM-N, Gonobio-Test, Minitek, Gonochek II, GonoGen, Phadebact Monoclonal GC OMNI Test, and Syva MicroTrak Test [21].

Coagglutination tests

Coagglutination tests can be performe on primary culture and, therefore, confirmed results can be obtained one day earlier than tests that require subculturing from primary culture plates, by using molecular techniques [22], or the Maldi Tof technique [23]. There is no specific and sensitive serological test available to detect recent gonococcal infections through the demonstration of *N. gonorrhea*-specific antibodies or antigens in patients' sera [19].

Molecular detection Methods

Over the last 20 years, nucleic acid amplification methods (NAATs) have gradually replaced traditional methods for the detection of sexually transmitted infections. NAAT technology comes with some important gains for diagnosis, increased sensitivity, and rapid result for screening of symptomatic and asymptomatic individuals using various clinical specimens [19]. However, NAAT tests have some technical problems. False-negative and false-positive results have been reported using various tests. Additionally, NAAT methods can facilitate detection of *C. trachomatis*, *N. gonorrhoeae*, *M. genitalium* and *T. vaginalis* [24]. Single detection systems or dual detection tests for *C. trachomatis* and *N. gonorrhea* are now commercially available The hybridization assays include the Gen- Probe PACE II (Gen-Probe, USA) and the Digene Hybrid Capture II assays (Digene Corp., USA) [25]. These assays use a specific oligonucleotide probe to hybridize directly to *N. gonorrhea* nucleic acid present within a specimen . Reported sensitivity and specificity values of the hybridization assays showed that these may be below that of bacterial culture [25]. Several commercial *N. gonorrhea* NAATs are available since 10 years, and make use of different technologies: Polymerase Chain Reaction (PCR) (Roche Diagnostics, USA), Transcription-Mediated Amplification (TMA) (Gen-Probe, USA), Strand Displacement Amplification (SDA) (Becton Dickinson, USA), and the Ligase Chain Reaction (LCR) (Abbott Laboratories, USA) [12]. Each of these assays has used a unique *N. gonorrhea* gene target [25]. Multiplex qualitative real-time PCR assay that detects *C. trachomatis* and *N. gonorrhea* and contains an internal control has been developed. A real-time PCR assay has the advantage of reducing the detection time of regular PCR procedures [24]. In addition to the commercial assays, numerous in house *N. gonorrhoeae* NAAT assays have also been described. Gene targets for in-house PCR assays have included the ORF1, CppB, OMPIII, Opa genes as well as the porA pseudogene [24].

There are several advantages of *N. gonorrhea* NAATs; first, they offer improved sensitivity compared with bacterial culture. The increased sensitivity of NAATs makes them particularly suitable for screening, enabling accurate diagnosis of both symptomatic and asymptomatic gonococcal infections, which is critical to the control of the disease [24]. Second, specimens collected for NAATs do not require the organism to be viable for detection and so require less stringent transport conditions compared with those collected for bacterial culture. Finally, NAATs can be used effectively on noninvasive specimens such as urine and self-collected specimens [6, 24]. *N. gonorrhea* NAATs do have some limitations; these include the typical problems associated with the use of NAAT protocols, such as high cost, carryover contamination, inhibition of the reaction, high quality control requirements, and the absence of antibiotic resistance data [25]. In addition, DNA amplification tests may not be suitable for test-of-cure because gonococcal DNA may be present for weeks after successful treatment of an infection [25].

Recommendation: The Centers for Disease Control and Prevention (CDC,USA) now promotes nucleic acid amplification testing, also known as NAAT, as the preferred method to detect these STDs in men and women in its latest recommendations to

clinical laboratories [26]. The NAAT method detects the DNA genes of the bacteria causing the infection. It does this in part by amplifying or making numerous copies of the genetic material so that the detection system can identify the presence of the bacteria. It is theoretically able to detect as little as a single copy of bacterial nucleic acid in a sample taken from the individual tested [26].

Antimicrobial treatment

The widespread distribution of *N. gonorrhea* strains which are resistant to commonly and previously used antibiotics is a significant global public health problem, and it is a major threat to reducing the impact of STIs worldwide [27-31]. Recent studies from different continents reported increased antibiotic resistance to most previously used drugs such as penicillin, tetracycline and fluoroquinolones [31-34]. Resistance to ciprofloxacin has reached 52.9% in Europe, 74.5% in Russia, 86.5% in the USA and 93.8% in China [30-31]. Moreover, a meta-analysis conducted in China and a report from the French National Reference Centre (CNR) have described an important resistance rate to tetracycline (82.4% and 86%, respectively) [32].

Few studies have recently reported on antibiotic resistance among *N. gonorrhea* isolates in Arab countries [35,36]. A study carried by Hamze *et al.*, 2016 [35], indicated that a significant increase in the prevalence of gonorrhea in Tripoli/ North of Lebanon , a city suffering from a decline in the current socioeconomic situation with a high unemployment rate, extreme poverty and the influx of thousands of Syrian refugees. All their s *N. gonorrhea* trains were isolated from male patients . The study demonstrated *N. gonorrhea* strains showed significant rates of resistance to quinolone, macrolide and tetracycline drugs, with a resistance prevalence rates of 72.3% to nalidixic acid, 40.4% to ofloxacin, 38.3% to ciprofloxacin, 40.4% to azithromycin, 23.4% to tetracycline and 21.3% to minocycline [35]. While a recent study from Morocco reported that a total of 72 isolates were examined, and a significant resistance to tetracycline (92.8%) and ciprofloxacin (86.8%), which were used as first-line treatment in gonococcal infections in Morocco. However, no resistance was found to spectinomycin, ceftriaxone or cefixime in all the isolates [36].

Currently, the treatment of gonococcal infections in many countries is limited to the use of third-generation cephalosporins such as ceftriaxone and cefixime [22-26]. The results of a recent study showed that the gonococcal genotypes persisting in the population and fluctuated significantly within a 3 year period, with numerous other genotypes appearing or disappearing. Significant fluctuations in the most common genotypes accounted for the majority of observed increases in both ciprofloxacin and penicillin resistance. Single-year genotypes contributed to ~20% of ciprofloxacin and penicillin resistance in each year. The study assumed that these changes determines *N. gonorrhea* antimicrobial resistance levels within the population [27].

It is important to note that some patients with gonococcal infection should be treated for possible *C. trachomatis* coinfection if chlamydia has not been ruled out [11]. For infections acquired via consensual sexual activity, patients should be advised to be abstinent for at least one week after treatment to prevent reinfection. Patients should also be advised to contact their sexual partners and to encourage their treatment. This strategy greatly reduces the public health burden of gonococcal disease [11].

The epidemiology of antimicrobial resistance in *N.gonorrhea* has changed decisions about gonococcal treatment recommendations because of shifts in antimicrobial resistance patterns in many countries over the world. The emergence of fluoroquinolone-resistant *N. gonorrhea* in the United States prompted the CDC in 2007, to stop recommending fluoroquinolones for treatment of gonorrhea, leaving cephalosporins as the only remaining class of antimicrobials available for treatment of gonorrhea in the United States [36]. Criteria for resistance to cefixime and cef-

triaxone have not been defined by the Clinical and Laboratory Standards Institute (CLSI). However, isolates with cefixime or ceftriaxone MICs ≥0.5 μg/mL are considered to have decreased susceptibility [37]. Because of the prevalence of tetracycline resistance among *N. gonorrhea* isolates worldwide, particularly those with elevated cefixime MICs, the use of azithromycin as the second antimicrobial is preferred. However, decreased susceptibility of *N. gonorrhea* to cephalosporins and other antimicrobials is expected to continue; local and country surveillance for antimicrobial resistance is essential for guiding therapy recommendations [38].

Recommendation

The new CDC guidelines recommend either the association of a cephalosporin with azithromycin or a higher ceftriaxone dose. Gonococcal strains with elevated MICs to cefixime also are likely to be resistant to tetracyclines but susceptible to azithromycin in the United States. Consequently, only one regimen, dual treatment with ceftriaxone and azithromycin, is recommended for treatment of gonorrhea [39]. However, in the case of azithromycin allergy, doxycycline (100 mg orally twice a day for 7 days) can be used in place of azithromycin as an alternative second antimicrobial drug when used in combination with ceftriaxone or cefixime [39].

In conclusion, gonorrhea infection is still highly important public health issue and should be controlled by rapid diagnostic methods and treated without any delay with effective antimicrobial drugs.

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