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CMPT Clinical Bacteriology Program

Innovation, Education, Quality Assessment, Continual Improvement

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Challenge M242-5

August 2024

Joint fluid – *Neisseria gonorrhoeae*

HISTORY

A simulated joint fluid sample collected from a 40 year old male traveler was sent to category A laboratories. Participants were expected to isolate and report *Neisseria gonorrhoeae.*

CMPT QA/QC/STATISTICS

All simulated joint fluid samples are produced at CMPT according to CMPT internal protocols. The sample contained a pure culture of *Neisseria gonorrhoeae.*

The samples are assessed for homogeneity and stability using in-house quality control methods and random selection of samples before and during production, and post sample delivery. The number of random samples selected is 15% of the total production batch.

The challenge sample lot was confirmed to be homogeneous and stable for **2 days** after shipment.

Organism identification was confirmed by a reference laboratory.

All challenge components have in-house assigned values based on the most clinically appropriate result; the most clinically appropriate result is determined by expert committee evaluation. No further statistical analysis is performed on the results beyond that described under "Suitability for grading."

SURVEY RESULTS

Reference laboratories: 12/12 (100%) labs reported *Neisseria gonorrhoeae*, 1 lab indicated it does not process this type of sample

Participants: 40/47 (85%) processing labs reported *Neisseria gonorrhoeae/* species, or gram negative diplococci, refer (Table 1)

Suitability for Grading

A challenge is considered suitable for grading if agreement is reached by 80 percent of the selected reference group and at least 50 percent of the participants.

Organism identification was correctly performed by at least 80 percent of reference laboratories and greater than 50 percent of all laboratories and was thus determined to be suitable for grading.

MAIN EDUCATIONAL POINTS from M242-5

1. *N. gonorrhoeae* is isolated in only about 50% (range of 25% to 75%) of synovial fluid specimens. Nucleic acid amplification testing (NAAT) of synovial fluid is more sensitive (greater than 75%) than culture and should be performed if available.
2. Acute purulent arthritis due to other bacteria may be distinguished from gonococcal arthritis through microbiological identification from synovial fluid culture.
3. When possible antimicrobial susceptibility testing (AST) should be performed to guide antibiotic therapy. Given widespread resistance, oral cephalosporins such as cefixime are no longer recommended for empiric therapy.

Grading

Maximum grade: 4

Reporting *N. gonorrhoeae* was graded 4.

COMMENTS ON RESULTS

This challenge is graded since >80% of reference labs and >50% of participant labs reached agreement; 40/50 of labs were able to grow an isolate consistent with *Neisseria gonorrhoeae* (*Neisseria* species or gram negative cocci) for further workup to perform identification and antibiotic susceptibility testing. The 10 labs that did not achieve agreement with the expected result did not observe growth on primary plates even after extended incubation of up to 7 days. These 10 labs received no grade given the potential issue of compromised organism viability because the viability of the sample showed to be lower than expected. 3 labs did not perform any culture stating that this sample is "not normally processed".

Table 1. Identification results

Reported	Total	Grade
Neisseria gonorrhoeae ± refer	36	4
Neisseria species, refer, ± presumptive	2	4
Gram negative coccus/cocci, refer	2	4
No growth	7	ungraded
sample not normally processed	3	ungraded
Total	**50**	

ISOLATION AND IDENTIFICATION

In cases of monoarticular arthritis, aspirated joint fluid should be sent for cell count, Gram stain, and culture on media to recover the fastidious *N. gonorrhoeae*. *N. gonorrhoeae* are gram negative diplococci that are typically coffee-bean or kidney-bean shaped with a small central clearing. Gram stain of synovial fluid lacks sensitivity for the diagnosis of septic arthritis. Gram stains are positive in less than 25% of cases of gonococcal septic arthritis, while positive in 70-80% of septic arthritis due to gram positive organisms and 40-50% positive in cases of septic arthritis due to other gram negative organisms.[9, 10] Non-gonococcal septic arthritis in adults is caused mainly by gram positive cocci (75% to 80%) while gram negative bacilli are involved in 15% to 20% of the cases. *Staphylococcus aureus* is the most common organism in native and prosthetic joint infections, followed by streptococci.[10]

Cultures may also be negative due to the fastidious nature of the organism. The use of selective media is not necessary from normally sterile body sites such as blood or joint aspirates as it was the case in this survey, but these specimens should be plated to an enriched medium such as chocolate agar which will enhance the growth of *N. gonorrhoeae*.[1] Inoculated media should be incubated at 35ºC in 5% CO2 and increased humidity and examined daily for up to 72 hours.

Gonococci are more slow-growing, but round moist colonies are usually visible by 48 hours. Occasional strains will also grow on blood agar, and growth on blood agar should not be automatically used to exclude *N. gonorrhoeae* as a possibility. *Neisseria* species are oxidase positive, non-motile and are oxidative metabolizers of carbohydrates. *Neisseria gonorrhoeae* typically oxidizes glucose only; other *Neisseria* species oxidize a range of other sugars in addition to glucose.[1]

NAATs (nucleic acid amplification tests) are the cornerstone of *N. gonorrhoeae* (and *Chlamydia trachomatis*) diagnostics for sexually transmitted infections. Hologic Aptima Combo NAAT demonstrated higher sensitivity than culture for the detection of *N. gonorrhoeae* in joint fluid specimens[2]. NAAT-based identification of *N. gonorrhoeae* with the Aptima Combo 2 assay demonstrated higher detection sensitivity than culture with joint fluid specimens[2]. However, labs must validate the use of this assay for detection of *N. gonorrhoeae in* joint fluid as this is not a Health Canada approved specimen type for the Aptima Combo 2 assay. Identification with MALDI-TOF technology is possible, although the use of an extended database or confirmatory testing is recommended.[3]

While NAATs have been the cornerstone of identification for STI-related *N. gonorrhoeae* infections, emerging resistance to Ceftriaxone (the primary agent used to treat gonococcal infections) is rising in Canada, making empiric treatment challenging [6,7] and underscores the continued importance of culture and antibiotic susceptibility testing.

ANTIMICROBIAL SUSCEPTIBILITY

Culture for *N. gonorrhoeae* is essential to facilitate recovery of isolates for antimicrobial susceptibility testing. It is important to submit isolates to reference laboratories (provincial public health lab or National Microbiology Lab) to support ongoing surveillance of antimicrobial resistance. A disadvantage of solely performing molecular detection is the lack of an isolate to contribute towards complete data on regional susceptibility patterns.

In order to prepare culture for transportation to reference labs: First, obtain fresh culture by growing a pure *N. gonorrhoeae* isolate at 35°C in 5% CO2 on suitable media for gonorrhea (such as chocolate agar or modified Thayer-Martin) for 24-48 hours, until obtain visible growth. Then, prepare and submit the pure culture fresh (24-72 hr) at time of shipping as either an agar plate, agar slant or frozen cryotube of (culture in glycerol at -70° C).

AST is typically performed for Azithromycin, Ceftriaxone, Cefixime, and Ciprofloxacin. Agar dilution is the gold standard method employed by reference laboratories to detect minimum inhibitory concentrations (MICs). The Kirby-Bauer disk diffusion test and E-test labeled gradient strip method are also frequently used. However, the observed diameter of inhibition-zone by disk diffusion is relatively imprecise and cannot be converted into a concrete MIC value.

Several challenges remain for direct NAAT testing of clinical specimens to detect resistance markers to cephalosporins and azithromycin.Challenges include cross-reactivity with other bacterial species, low *N. gonorrhoeae* DNA burden, and the plethora of mutations in several different genes that can be involved in resistance to cephalosporins and azithromycin. Next-generation sequencing (NGS) overcomes many of the challenges and may be utilized in the near future for more complete AST. However, cost-reductions are essential to increase NGS adoption for AST. In the development pipeline are rapid, accurate and cost-effective point-of-care (POC) NAATs that detect *N. gonorrhoeae* and antimicrobial resistance. Such tests will be able to guide individualized gonorrhea therapy, which will improve treatment and reduce antimicrobial resistance selection pressure[11].

Emerging antimicrobial resistance has led to high prevalence of *N. gonorrhoeae* strains with resistance to previously used first line antimicrobial therapy (e.g., sulfonamides, penicillins, older cephalosporins, tetracyclines, and fluoroquinolones). In many settings worldwide, ceftriaxone is the last remaining option for empiric first-line antimicrobial monotherapy[4] however, strains resistant to the extended-spectrum cephalosporins cefixime and ceftriaxone and resistant to nearly all other available therapeutic antimicrobials have emerged.[5, 6]

While ceftriaxone injection (intramuscular or intravenous) is the preferred initial antibiotic of choice, there can be wide geographical variations in susceptibility patterns. Treatment of individuals is based on regional and national guidelines based on suscepti-

bility patterns of isolates collected from local cases. Ceftriaxone either given intravenously or intramuscularly, is now the preferred initial antibiotic of choice. Intravenous administration of 1 gm every 24 hours is usually preferred in patients presenting with purulent arthritis. Doxycycline 100 mg twice a day for seven days is usually added to cover potential coinfection with *Chlamydia trachomatis.* After 1 to 2 days of clinical improvement with ceftriaxone, a seven-day (or longer course for immunocompromised individuals) of antibiotics may be completed with daily intramuscular ceftriaxone.

Please refer to the 2023 revised Canadian Guidelines on Sexually Transmitted Infections published by the Public Health Agency of Canada for the management and treatment of gonococcal infections.[7]

CLINICAL RELEVANCE

Septic arthritis and a characteristic syndrome of poly-arthritis and dermatitis are the predominant manifestations of disseminated gonococcal infection (DGI).[8] The arthritis–dermatitis syndrome is the most common presentation of DGI. Polyarthralgias are common, usually involving the knees, elbows, and distal joints. Joint involvement is asymmetric with only a few joints involved, which helps to differentiate from immune-complex mediated arthritis which involves many joints and is more symmetrical.

The skin rash consists of papules and pustules, and this may all be associated with a mild systemic illness. Blood cultures and skin lesions can culture positive during this phase, but joint cultures will most likely be negative. This syndrome resolves but is followed by classic septic arthritis in one or two joints, ankles, knees, elbows or wrists. This final stage of septic arthritis can emerge without the prior syndrome, and in this case it is similar to septic arthritis of any etiology.

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