Office Laboratory Identification of Neisseria Gonorrhoeae

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Current literature is contradictory regarding what tests are necessary to establish an adequate presumptive diagnosis for office identification of Neisseria gonorrhoeae. This is especially true in light of recent reports of N meningitidis causing acute genital infection. This study was designed to look at the various criteria recommended and to establish guidelines for the office identification of N gonorrhoeae. Four hundred thirty-six isolates grown on Thayer-Martin selective agar were studied. Of these, 156 were found to be oxidase-positive. Gram stain of the isolates showed that 31.4 percent of the oxidase-positive isolates were gram-negative diplococci, 59 percent were yeastlike mold, and 9.7 percent were gram-negative bacilli. All of the gram-negative diplococci were confirmed to be N gonorrhoeae by sugar fermentation studies. No isolates of N meningitidis or saprophytic Neisseria were found. On the basis of this finding, it is recommended that office identification of N gonorrhoeae from genital or anal cultures should include (1) growth on Thayer-Martin (or comparable) medium, (2) positive reaction to oxidase reagent, and (3) identification of gram-negative diplococci on Gram stain of the Thayer-Martin isolate.

The recent trend to cut medical care costs has led to an increase in the use of office cultures. Throat cultures, urine cultures, and gonorrhea cultures are all being done in many offices. While much has been written about the identification of pathogenic organisms on urine and throat cultures, the literature concerning identification of N gonorrhoeae is contradictory regarding what tests are actually necessary to establish the diagnosis.

Thayer and Martin first developed a selective medium for the isolation of N gonorrhoeae in 1964 using ristocetin and polymyxin to inhibit overgrowth of other organisms.[1] In 1966 the medium was changed to use vancomycin, colistin, and nystatin instead of the ristocetin and polymyxin, with equally good results.[2] The formula was again altered in 1967 to use IsoVitaleX, a chemically defined supplement, instead of yeast extract, again with equivalent outcome.[3] The results of these studies showed from 75 to 80 percent reduction of contaminants on urethral/vaginal specimens and nearly complete inhibition of saprophytic Neis-

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seria, thus significantly simplifying the isolation of N gonorrhoeae.

The most widely accepted standards for the presumptive diagnosis of N gonorrhoeae were established by the Center for Disease Control (Atlanta) in 1970: (1) growth on Thayer-Martin selective medium, (2) positive reaction with oxidase reagent, and (3) gram-negative diplococci on routine Gram stain of the isolate.[4] These criteria will eliminate all but N meningitidis and N lactamica (a rare saprophytic Neisseria). Also, Morello and Bohnhoff state that even when the oxidase test is positive, gram-negative bacilli, such as strains of Moraxella, Eikenella, and Kingella, produce colonies resembling those of N gonorrhoeae. Furthermore, these organisms may grow on Thayer-Martin selective medium, especially if the medium is not fresh or has been incubated for longer than 48 hours.[5] Schaeffer states, however, that "growth of oxidase-positive colonies (on Thayer-Martin medium or similar medium) provides reliable presumptive evidence of N gonorrhoeae."[6] Gram stain is not included in the diagnosis. This would simplify office culture of N gonorrhoeae if acceptably accurate.

Two recent articles raise some questions concerning the sufficiency of even the criteria set forth by the Center for Disease Control. Both reported cases of acute genital tract infections with N meningitidis, an uncommon but previously reported occurrence. The source of the infection was hypothesized to be orogenital contact, with the partner carrying N meningitidis in the nasopharynx.[7,8] The significance of differentiating N meningitidis from N gonorrhoeae is that gonorrhea implies sexual contact with an infected person, which may raise the question of infidelity in a married couple, whereas meningococcal infection may be acquired from an otherwise healthy partner who carries the organism orally. The presence of meningococci can be distinguished from gonococci only by carbohydrate fermentation tests, coagglutination, or fluorescent antibody studies, which go beyond the criteria noted above.

This study was designed to investigate the different criteria recommended and to establish some basis for deciding whether Gram stain of culture isolates is necessary for identification and whether the three criteria recommended by the Center for Disease Control would lead to significant misdiagnosis of gonococcal infection due to the presence of N meningitidis or saprophytic Neisseria.

Methods

The data for this study were obtained from the results of all isolates on Thayer-Martin medium received in The Toledo Hospital Microbiology Laboratory during a three-month period. Urethral, cervical, and anal swabs were obtained from various sources, including private outpatients, inpatients, and hospital clinic patients, and were brought to the laboratory in Stuart's transport medium. All specimens were streaked on appropriate culture media within two hours.

The media used were Thayer-Martin for selective growth of Neisseria, chocolate agar as a control for vancomycin-sensitive Neisseria, and blood agar and MacConkey's medium for isolation of other organisms. The Thayer-Martin agar was prepared by laboratory personnel using 36 gm of GC agar base (Difco Laboratories, Detroit), 10 gm of hemoglobin powder (Difco), 10 ml of IsoVitaleX (BBL Microbiology Systems, Cockeysville, Md) and 10 ml of V-C-N inhibitor (BBL) to a final volume of 1 liter according to the standard instructions supplied by BBL. The cultures were incubated for 24 to 48 hours in a 5 percent carbon dioxide enriched moisture-adjusted environment and then observed for growth. Each colony type observed on Thayer-Martin or chocolate agar was tested with oxidase reagent (tetramethyl-p-phenylenediaminedihydrochloride, 1 percent, Marion Scientific Company, Kansas City, Mo), and microscopic examination of the isolate was performed using a standard Gram stain technique. All gram-negative diplococci that tested oxidase-positive were then subcultured, and further identification was done using a sugar fermentation system (Minitek, BBL) or serologically confirmed by coagglutination (Phadebact Gonococcus Test, Pharmacia Diagnostics, Piscataway, NJ). All identifications of N gonorrhoeae were confirmed by their Minitek fermentation pattern of glucose-positive fermentation and sucrose, maltose, and o-nitrophenyl-beta-D-galactopyranoside (ONPG) nonfermentation. Problem strains or "stat" cultures were confirmed serologically by coagglutination.

Results

During the period under study there were 436 colony types isolated on Thayer-Martin medium. One hundred fifty-six (35.8 percent) of the isolates

were positive when tested with oxidase reagent. These were further analyzed by Gram stain: 49 (31.4 percent of the oxidase-positive isolates) were found to be gram-negative diplococci, 92 (59.0 percent) were yeast-like mold, and 15 (9.7 percent) were gram-negative bacilli. Further analysis by carbohydrate fermentation confirmed that all oxidase-positive gram-negative diplococci were N gonorrhoeae. No isolates of N meningitidis or saprophytic Neisseria were identified on any of the cultures.

No attempt was made to identify false negative cultures by duplication or repeat culture. However, there were no isolates of N gonorrhoeae grown on chocolate agar that did not grow on Thayer-Martin agar.

Discussion

The diagnosis of gonorrhea on a cervical, anal, or urethral culture carries with it many implications, both medical and social. Since it is still generally accepted that gonorrhea can only be acquired by sexual contact with an infected person, in a monogamous relationship in which one person is infected and the other is not, it is implied that the infected person has been sexually active outside the relationship. Therefore, one must have a high degree of certainty before making the diagnosis of gonorrhea.

The results of this study clearly indicate that growth on Thayer-Martin medium and a positive oxidase reaction without Gram stain as recommended by Schaeffer[6] are not adequate criteria for diagnosis of gonorrhea. The addition of the Gram stain did result in 100 percent specificity in this study. This leads to the question of the significance of the presence of N meningitidis and N lactamica on genital and anal cultures. In this study no isolates of either were found.

This study was performed in a hospital microbiology laboratory. Nevertheless, the medium and reagents are all similar to those available commercially for office use. Therefore, the implications also hold true for the identification of N gonorrhoeae in the office laboratory. The continued use of the office culture to diagnose gonorrhea is dependent on the necessary tests being specific while remaining within the capabilities of the basic office laboratory. The addition of the Gram stain to the culture and oxidase test, while being more time consuming, should not require any materials not already present in most offices. The sugar fermentation test, however, would require additional equipment and training, and would probably make it unfeasible for most offices. While the possibility of N meningitidis and N lactamica being misidentified remains, the reports of these organisms being present on cultures from the genitals or anus remain rare. This would not necessarily be true on throat cultures for oropharyngeal gonorrhea, and these should include sugar fermentation tests or serologic confirmation for positive identification. Also, in cases where the social repercussions of this diagnosis may be particularly severe, it may be advisable to subculture the presumptively identified N gonorrhoeae isolates and send them to a reference laboratory for confirmation.

It is concluded that the identification of N gonorrhoeae from genital and anal cultures is an appropriate office procedure when the following criteria are met: (1) growth on Thayer-Martin (or comparable) medium, (2) positive reaction with oxidase reagent, and (3) identification of gram-negative diplococci on Gram stain. This is in accordance with the recommendations of the Center for Disease Control. Throat cultures for N gonorrhoeae should be sent to a microbiology laboratory for sugar fermentation or serologic studies to rule out other Neisseria.

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