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Difficulties in Differentiating *Neisseria cinerea* from *Neisseria gonorrhoeae* in Rapid Systems Used for Identifying Pathogenic *Neisseria* Species

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***Neisseria cinerea* and *Neisseria gonorrhoeae* may occur at the same body sites and may have similar colony morphologies. Ideally, systems used for rapid identification of *N. gonorrhoeae* should be able to differentiate *N. cinerea* from gonococci. We tested seven *N. cinerea* strains using the Gonochek II (Du Pont Diagnostics), Minitek (BBL Microbiology Systems), RapID-NH (Innovative Diagnostics, Inc.), RIM-N (American Microscan), and Phadebact (Pharmacia Diagnostics) systems. We found that the reactions produced by *N. cinerea* in Gonochek II, Minitek, and RapID-NH kits could be confused with the results produced by some strains of *N. gonorrhoeae*. The susceptibility of *N. cinerea* to colistin, its ability to grow on tryptic soy or Mueller-Hinton agar, and its inability to grow on modified Thayer-Martin medium help differentiate it from gonococci.**

Neisseria cinerea was first characterized in 1906 (31) and is described in the most recent edition of *Bergey's Manual of Determinative Bacteriology* (30). This organism may colonize the nasopharynx and occasionally the genital tract (J. S. Knapp, P. A. Totten, B. H. Totten, and E. W. Hook III, Abstr. Annu. Meet. Am. Soc. Microbiol. 1983, C26, p. 316). On at least one occasion, it has been recovered from conjunctival secretions. Rarely, it may act as an opportunistic pathogen (6). Since the organism may occur at the same body sites as *Neisseria gonorrhoeae*, techniques used to identify pathogenic *Neisseria* spp. should have the ability to differentiate *N. cinerea* from gonococci. Misidentification of *N. cinerea* as *N. gonorrhoeae* could lead to social or possibly legal problems (12).

N. cinerea strains do not produce detectable acid when tested in cystine-Trypticase agar (CTA) Medium (BBL Microbiology Systems, Cockeysville, Md.) (18, 19). As a result, it seems unlikely that *N. cinerea* would be confused with gonococci in laboratories that utilize CTA Medium for identifying *Neisseria* spp. However, problems with the sensitivity and specificity of CTA Medium have led some laboratories to use other methods for identification of pathogenic *Neisseria* spp. A number of newer systems designed to identify pathogenic *Neisseria* spp. in 6 h or less have been described previously (1–4, 7, 8, 10, 11, 13, 15–17, 20–29, 32–35). BACTEC Neisseria Differentiation kits (Johnston Laboratories, Inc., Towson, Md.) can identify *N. gonorrhoeae*, *Neisseria meningitidis*, and *Neisseria lactamica* in 3 h by radiometric detection of carbohydrate utilization. Interestingly, a number of *N. cinerea* isolates that we have studied yielded positive glucose reactions when tested in BACTEC Neisseria Differentiation kits (5a, 6). The positive glucose reactions observed in BACTEC Neisseria Differentiation kits could result in misidentification of *N. cinerea* as gonococci if laboratory personnel fail to observe the precautions listed by the manufacturer (12).

When we reviewed published evaluations of many other rapid systems used for identifying *Neisseria* spp., we were unable to find any information regarding the reactions produced by *N. cinerea* in such systems. This paper describes the results obtained when seven *N. cinerea* strains were tested in five systems available for rapid identification of pathogenic *Neisseria* spp. In three of the systems tested, *N. cinerea* strains yielded reactions that were similar to those produced by *N. gonorrhoeae*.

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MATERIALS AND METHODS

Strains. The strains used in the study included isolates from the University of Mississippi, Jackson, Miss. (UMC 2768), Neisseria Reference Laboratory, Seattle, Wash. (NRL 32165 and NRL 32824), Centers for Disease Control, Atlanta, Ga. (CDC F4340), EY Laboratories, San Mateo, Calif. (1901 and 601), and the type strain *N. cinerea* ATCC 14685. The strains from the University of Mississippi and from Seattle were identified at the Neisseria Reference Laboratory (courtesy of J. S. Knapp) by methods previously described (19). Six *N. gonorrhoeae* strains and five *N. meningitidis* isolates including *N. meningitidis* ATCC 13090 were also included in the study so that the reactions produced by these species could be compared with the reactions obtained with *N. cinerea*. All gonococcal and meningococcal isolates were oxidase-positive gram-negative diplococci that grew on modified Thayer-Martin medium and yielded characteristic reactions in BACTEC Neisseria Differentiation kits. Isolates were stored in tryptic soy broth with 6% glycerol at −70°C until tested.

Test procedures. All *N. cinerea* and gonococcal isolates were tested in the following systems: Gonochek II (E. I. du Pont Diagnostic and Bioresearch Systems Division, Wilmington, Del.), Minitek (BBL), RapID-NH (Innovative Diagnostics, Inc., Atlanta, Ga.), RIM-N (American Microscan, Campbell, Calif.), and Phadebact Gonococcus Test (Pharmacia Diagnostics, Piscataway, N.J.). All *N. cinerea* isolates were tested on at least two occasions in Gonochek

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II, Minitek, RapID-NH, and RIM-N kits. Organisms were tested in the above kits by methods recommended by the manufacturers. Isolates were incubated on chocolate agar for no more than 18 h before being tested in Minitek kits, and for 18 to 21 h before testing in the other four systems. With the Phadebact Gonococcus Test, the boiled colonies suspension method recommended by the manufacturer was used for preparing isolates for testing.

RESULTS

In the Minitek system, positive tests appear yellow or yellow-orange, weak positive reactions are orange, and negative reactions appear red or red-orange. In Minitek kits, three of the seven *N. cinerea* strains yielded an orange color with the glucose disk each time they were tested. The remaining four strains produced an orange color with the glucose disk on at least one occasion, but yielded a negative reaction with the glucose disk on other occasions. All seven isolates gave negative maltose, sucrose, and *o*-nitrophenyl-β-D-galactopyranoside (ONPG) reactions each time they were tested in Minitek kits. The six *N. gonorrhoeae* strains tested all produced yellow or yellow-orange color reactions with glucose disks. Except for a borderline maltose reaction produced by one isolate, all *N. gonorrhoeae* strains yielded negative maltose, sucrose, and ONPG reactions. The five *N. meningitidis* isolates tested produced positive reactions with the glucose and maltose disks and negative reactions with sucrose and ONPG.

In RapID-NH kits, each of the seven *N. cinerea* strains gave a positive reaction for proline iminopeptidase activity (PRO) and an orange color (positive) in the glucose well. All other reactions included in the kits were negative. Each of the *N. gonorrhoeae* isolates produced positive PRO and glucose reactions. With five of the six gonococci tested, glucose reactions were stronger (yellow or yellow-orange) than the glucose reactions produced by *N. cinerea* isolates. The remaining gonococcal isolate produced an orange color in the glucose well. All other reactions were negative. The five meningococcal isolates tested produced reactions characteristic for this species, including positive gamma-glutamylaminopeptidase reactions.

In RIM-N kits, all *N. cinerea* strains yielded negative glucose, lactose, maltose, and sucrose reactions. All six gonococcal isolates yielded positive glucose reactions, and *N. meningitidis* ATCC 13090 yielded positive glucose and maltose reactions. The meningococcal and gonococcal isolates yielded positive reactions within 30 min.

In Gonochek II kits, each of the *N. cinerea* isolates produced a pink or red-pink reaction, which is indicative of PRO activity. In the Gonochek II system, this reaction is interpreted as presumptive evidence of *N. gonorrhoeae*. All gonococcal isolates produced a pink or red-pink reaction.

With the Phadebact Gonococcus Test, six *N. cinerea* strains produced negative reactions, and the remaining isolate produced an uninterpretable result (coagglutination with both the gonococcal reagent and the control reagent). Five of the six *N. gonorrhoeae* isolates yielded positive reactions; one isolate produced an uninterpretable result.

DISCUSSION

N. cinerea strains resemble *N. gonorrhoeae* more closely than other commensal *Neisseria* spp. or *N. meningitidis* (19). The colony morphology of *N. cinerea* is not unlike that seen with type T3 *N. gonorrhoeae* colonies (19). Both *N. cinerea* and *N. gonorrhoeae* can occur in pharyngeal and genital tract secretions. In most instances, *N. cinerea* colonizes mucosal surfaces without producing symptomatic infection. However, to avoid problems that might arise from misidentification of *N. cinerea* as *N. gonorrhoeae*, identification schema should include tests that will differentiate the two organisms.

Several investigators have found that *N. cinerea* strains do not produce detectable acid in conventional carbohydrate utilization tests (18, 19, 31). Knapp et al. (19) have shown that the organism also appears asaccharolytic when tested in modified oxidation-fermentation medium. However, a number of *N. cinerea* strains that we have studied gave positive glucose reactions in BACTEC Neisseria Differentiation kits (5a, 6). Recently, Dossett et al. (12) also reported that an *N. cinerea* strain tested in BACTEC Neisseria Differentiation kits gave positive glucose growth indices and negative maltose and fructose growth indices.

N. cinerea strains were not included in early evaluations of the BACTEC system or other *Neisseria* identification methods that are based on radiometric or spectrophotometric detection of CO_2 produced from sugars (9, 28). Similarly, published reports dealing with tests based on rapid carbohydrate utilization (2, 3, 7, 13, 15, 17, 21–25, 27, 28, 32–35), detection of enzymes such as PRO and gamma-glutamylaminopeptidase (10, 13, 26, 32, 33), or coagglutination (1, 3, 4, 8, 11, 15, 20, 24, 26) have not described any results for *N. cinerea*.

We found that *N. cinerea* strains produced either weak positive or negative glucose reactions in the Minitek system. Gonococci usually produce strong glucose reactions in this system. However, one of the gonococci included in this study gave weak positive (orange) color reactions with the glucose disks. We feel that it would be difficult to differentiate *N. cinerea* from such gonococci on the basis of the Minitek results alone. Dossett et al. (12) also reported that a strain of *N. cinerea* produced reactions identical to those of *N. gonorrhoeae* in Minitek kits. In contrast to the reactions produced in Minitek kits, glucose reactions in RIM-N kits were negative for all of the *N. cinerea* isolates that we tested.

In RapID-NH kits, all seven *N. cinerea* isolates produced glucose reactions that were weaker than those observed with most gonococci. However, one gonococcal isolate produced an orange color in the glucose well that was identical to the glucose reactions produced by *N. cinerea*. Overall, both *N. cinerea* and *N. gonorrhoeae* gave the same profiles (positive PRO and glucose reactions) in RapID-NH kits.

The dichotomy between the negative glucose reactions observed with *N. cinerea* in RIM-N kits and in CTA medium or modified oxidation-fermentation medium and the positive reactions obtained in the BACTEC, Minitek, and RapID-NH systems has not been explained to date. Many factors may affect the results when *Neisseria* spp. are tested for carbohydrate utilization. These factors include: the peptone, cystine, and sodium sulfite concentrations of the basal medium; the amount, pH, and buffering capacity of the basal medium; the concentration, pK, color change interval, and buffering capacity of the pH indicator; the concentration and purity of the carbohydrates; the methods used for sterilizing the basal medium and sugars; the source and size of the inoculum; the methods used to inoculate the test medium; and the amount of time elapsed between inoculation of the medium and subsequent readings (5, 7, 14, 17, 18, 21, 22, 25, 27, 28, 34, 35). A number of these factors differ for CTA Medium, modified oxidation-fermentation medium, Minitek, RIM-N, RapID-NH, and BACTEC Neisseria Differentiation

kits. Also, the BACTEC system is designed to detect CO_2 production from sugars rather than acid production.

Gonochek II is a single-tube system that uses detection of PRO and gamma-glutamylaminopeptidase activities and an ONPG reaction to differentiate *N. gonorrhoeae* from *N. meningitidis* and *N. lactamica* (32, 33). In this system, gonococci give a positive reaction for PRO and negative reactions for gamma-glutamylaminopeptidase and ONPG. The *N. cinerea* strains that we tested gave the same reaction as the gonococci we tested. The two species cannot be differentiated from one another solely on the basis of the Gonochek II results.

We did not have the opportunity to test *N. cinerea* strains in the API NeIdent system (Analytab Products, Plainview, N.Y.). The API NeIdent system was designed to differentiate pathogenic *Neisseria* spp. on the basis of a number of biochemical reactions including assays for various aminopeptidases (16). Dossett et al. (12) reported that one *N. cinerea* isolate produced reactions similar to those of *N. gonorrhoeae* in API NeIdent kits.

The Phadebact Gonococcus Test is based on antigenic recognition of *N. gonorrhoeae* rather than detection of carbohydrate metabolism or preformed enzymes (11). None of the *N. cinerea* strains that we tested produced false-positive results in Phadebact coagglutination tests. However, other *Neisseria* spp. such as *N. lactamica* may give false-positive reactions in the Phadebact system. A larger number of *N. cinerea* strains should be tested in this system to make sure that cross-reactions do not occur with *N. cinerea*.

This study included a relatively small number of *N. cinerea* strains. Clearly, additional studies are needed to determine if our findings are representative of isolates from other geographic areas. Nevertheless, in view of the similarities in the reactions obtained with *N. cinerea* and *N. gonorrhoeae* in BACTEC, Gonochek II, Minitek, and RapID-NH kits, some precautions appear warranted. The manufacturers of BACTEC and Gonochek II kits emphasize that suspect *Neisseria* isolates should be checked for their ability to grow on a selective medium such as Thayer-Martin before the isolates are tested in these two kits. Only suspected *Neisseria* spp. that will grow on such selective media should be tested in BACTEC or Gonochek II kits. If this recommendation is adhered to, potential confusion between *N. cinerea* and *N. gonorrhoeae* will be avoided since most *N. cinerea* strains grow poorly or not at all on modified Thayer-Martin or Martin-Lewis medium. In view of our findings with *N. cinerea*, a similar policy may be appropriate for laboratories using Minitek or RapID-NH kits.

In situations in which identifying an organism as *N. gonorrhoeae* may raise the issue of child abuse, e.g., recovery of suspected gonococci from a prepubertal child, supplemental tests should also be considered (12). Several procedures that have been useful in differentiating *N. cinerea* from *N. gonorrhoeae* include testing isolates for their ability to grow on various plated media and colistin disk diffusion susceptibility tests (12, 19). *N. cinerea* strains are susceptible to colistin, whereas *N. gonorrhoeae* isolates are resistant. Most *N. cinerea* strains will grow on tryptic soy agar and Mueller-Hinton agar. In contrast, gonococci will not grow on tryptic soy or Mueller-Hinton agars.

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