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Proctitis Associated with *Neisseria cinerea* Misidentified as *Neisseria gonorrhoeae* in a Child

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An 8-year-old boy developed proctitis. Rectal swabs yielded a *Neisseria* sp. that was repeatedly identified by API (Analytab Products, Plainview, N.Y.), Minitek (BBL Microbiology Systems, Cockeysville, Md.), and Bactec (Johnston Laboratories, Towson, Md.) methods as *Neisseria gonorrhoeae*. Subsequent testing in a reference laboratory yielded an identification of *Neisseria cinerea*. It is suggested that identification of a *Neisseria* sp. isolated from genital or rectal sites in a child be confirmed by additional serological, growth, and antibiotic susceptibility tests and, if necessary, by a reference laboratory. The implications of such misidentifications are discussed.

Gonococcal proctitis in children is usually considered to be sexually transmitted, just as it is in adults. Moreover, gonorrhea in young boys is generally the result of homosexual contact with an adult male. We herein report the case of a child with prolonged proctitis and perianal inflammation from whom *Neisseria* sp. was repeatedly cultured and repeatedly misidentified as *Neisseria gonorrhoeae*. The importance of correct identification of suspicious *Neisseria* sp. colonies isolated from the genital or rectal sites of a child is stressed.

CASE REPORT

The patient is an 8-year-old boy who developed proctitis and perianal dermatitis in January 1983. He was first seen by his family physician in February with signs of anal pruritus and rectal discharge. Treatment with topical agents and systemic antimicrobial agents was without effect; culture of the discharge yielded β-hemolytic streptococci (not identified further), *Staphylococcus aureus*, and *Escherichia coli*. On 24 May, rectal discharge cultures again grew out β-hemolytic streptococci (not *Streptococcus pyogenes*) and *E. coli*. Antimicrobial therapy was continued for an additional 2 weeks. By 10 June there was still no improvement in the proctitis. Proctoscopy revealed that the inflammation was limited to the anal and perianal skin. The patient was admitted to his community hospital for parenteral penicillin therapy. Culture of rectal discharge on admission yielded β-hemolytic streptococci (not *S. pyogenes*) and *N. gonorrhoeae*. After 6 days of antibiotic therapy, rectal cultures again yielded *N. gonorrhoeae*. The organism was sent to two area reference laboratories for confirmation of identity and susceptibility testing.

The patient was referred to the Milton S. Hershey Medical Center on 5 July for evaluation of sexual abuse and therapy. On examination severe perirectal inflammation with purulent rectal discharge was noted. The remainder of his examination was normal. Culture of the discharge again grew *N. gonorrhoeae*. Based on this information, the patient was treated with systemic spectinomycin and povidone iodine rectal scrubs. The child and his parents underwent extensive questioning in an effort to identify a source of infection. No clues were found. Both parents had negative examinations and negative cultures. The patient's condition gradually improved, and by 20 July his rectum and perirectum appeared healed, with minimal perirectal scarring.

MATERIALS AND METHODS

Microbiology. In an effort to confirm the identity of the *Neisseria* sp. before assuming the perpetration of sexual abuse, the senior author (J.H.D.) contacted the four different laboratories that had identified the organism to check on methods. All four had used some combination of API (Analytab Products, Plainview, N.Y.), Minitek (BBL Microbiology Systems, Cockeysville, Md.), and Bactec (Johnston Laboratories, Towson, Md.) commercial kits, and all were confident that the organism was *N. gonorrhoeae*. Reactions obtained with the three commercial kits were as follows. (i) API gave positive results for hydrolysis of proline *p*-nitro anilide, hydrolysis of hydroxyproline-β-naphthylamide, and catalase production and negative results for hydrolysis of *p*-nitrophenyl-β-D-galactoside, hydrolysis of bis-(*p*-nitrophenyl) phosphate, fructose utilization, resazurin reduction in the presence and absence of glucose, hydrolysis of α-glutamyl-β-naphthylamide, hydrolysis of glycyl-phenylalanine-β-naphthylamide, and hydrolysis of glycyl-proline-β-naphthylamide. (ii) Minitek gave positive results for glucose oxidation and negative results for maltose, sucrose, and *o*-nitrophenyl-β-D-galactopyranoside. (iii) Bactec gave positive results for glucose oxidation and negative results for maltose, fructose, and *o*-nitrophenyl-β-D-galactopyranoside. On the basis of Gram stain, colonial morphology, oxidase reaction, and the above biochemical tests, the organism was identified as *N. gonorrhoeae* in all four laboratories. Serological testing, growth at lower temperatures, and growth on nonsupplemented media were not performed at this stage (see below).

By the time the patient was discharged, the Pennsylvania State Department of Health was involved in tracing contacts, and the organism was sent to the State Laboratory (Lionville) for identity confirmation. At this point its identity

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came into question, and it was forwarded to the Centers for Disease Control, Atlanta, Ga., and the *Neisseria* Reference Laboratory, Department of Medicine, University of Washington, Seattle, for further study.

The organism was eventually identified as *Neisseria cinerea*. Its characteristics and differentiation from *N. gonorrhoeae* are listed in Table 1. Susceptibility testing was performed in the laboratory of Clyde Thornsberry (Centers for Disease Control) and yielded the following results (MIC in micrograms per milliliter): penicillin G, 0.125; ampicillin, 0.125; kanamycin, 32; tetracycline, 1; erythromycin, 2; spectinomycin; 12; trimethoprim-sulfamethoxazole, 0.25; cefuroxime, 0.25; cefotaxime, 0.004; thiamphenicol, 2. Additionally, the organism was susceptible to a 10-μg colistin disk and resistant to vancomycin (MIC, 32 μg/ml). Tests for β-lactamase were negative. Cultures from the same single-colony isolates that were sent to the reference laboratories were retested in the Minitek and Bactec systems; identifications were still *N. gonorrhoeae*. The API method used in this study was discontinued soon after initiation of this investigation and could therefore not be retested with reference laboratory organisms.

TABLE 1. Physicochemical properties of *N. cinerea* and *N. gonorrhoeae*

Test	Reaction	
	N. cinerea	*N. gonorrhoeae*
Gram stain	Gram-negative diplococcus	Gram-negative diplococcus
Oxidase	+	+
Catalase	+	+
Acid produced from[a]:		
Glucose	−	+
Maltose	−	−
Sucrose	−	−
Fructose	−	−
Lactose	−	−
Mannitol	−	−
Polysaccharide from sucrose	−	−
Nitrate reduction	−	−
Nitrite reduction	+	+
DNase	−	−
Amylosucrase	−	−
Growth on:		
Thayer-Martin medium plus VCN[b]	−	+ (rarely −)
Trypticase soy agar	+	−
Sheep blood agar	+	− (rarely +)
Mueller-Hinton agar	+	−
Growth at:		
22°C	+ (weak)	−
25°C	+	−
37°C	+	+
Requirement for CO_2	−	Variable
Anaerobic growth (nitrite as terminal electron acceptor)	+	+
Pigment on Loeffler agar	− (poor growth)	−
Auxotype	Pro^- Arg^-; Cystine-Cysteine$^-$	Pro^- Arg^-; Cystine-Cysteine$^-$
Gonococcal coagglutination and monoclonal antibody test	−	+
DNA homology assay by single-strand endonuclease procedure (*N. cinerea* stock strain NRL32165)	*N. cinerea* (87% homology)	Not *N. cinerea* (44% homology)

[a] Cystine trypticase agar and modified oxidation-fermentation bases.
[b] VCN, Vancomycin-colistin-nystatin supplement.

RESULTS AND DISCUSSION

Confusion in the literature in the identification of *N. cinerea* with *N. gonorrhoeae* has been noted previously (3, 4). Of all *Neisseria* spp., *N. cinerea* is most likely to be confused as a glucose-negative strain *N. gonorrhoeae* (4). *N. cinerea* may be further differentiated from *N. gonorrhoeae* by lack of production of immunoglobulin A protease (not tested in the current study), colistin susceptibility, and growth on tryptic soy and Mueller-Hinton agars (4). All *N. cinerea* strains thus far tested yield negative gonococcal serology with commercial tests, grow on tryptic soy and Mueller-Hinton agars, and are susceptible to a 10-μg colistin disk. However, preliminary information indicates that colistin susceptibility may be simpler and more discriminatory than agar growth, since a few *N. cinerea* strains take several days to grow well on tryptic soy agar; additionally, one strain of *N. cinerea* has been encountered which only grew on tryptic soy and Mueller-Hinton agars after several subcultures over several months (1; Knapp, unpublished data). All *N. gonorrhoeae* strains tested thus far do not grow on simple agars and are resistant to colistin, but commercial serological diagnostic tests for *N. gonorrhoeae* do not detect all gonococcal strains (2; J. P. Libonati, R. L. Leilich, and L. Loomis, Abstr. Annu. Meet. Am. Soc. Microbiol. 1983, C19, p. 314). Therefore, for problem strains such as the one described here, we recommend primary screening for *N. gonorrhoeae* with a commercial serological reagent; strains with negative results should then be tested further for colistin susceptibility and growth on agars, and only organisms that are susceptible to colistin and grow on simple agars should be sent to a reference laboratory for identity confirmation. In the current case, owing to lack of knowledge of the existence of *N. cinerea*, the additional tests outlined above were not studied in the laboratories that first encountered the organism. In retrospect, performance of these simple tests would have shed light on the nongonococcal nature of the *Neisseria* sp. at an earlier stage, obviating the evaluation for sexual abuse.

Positive glucose reactions for *N. cinerea* in commercial kits, as noted in the current study, are an additional source of confusion between the two species. Recently, Boyce and co-workers (J. M. Boyce, E. B. Mitchell, Jr., and J. S. Knapp, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 1984, Washington, D.C., abstr. no. 123, p. 111) have found that five strains of *N. cinerea* were positive for glucose and negative for maltose and fructose when tested by the Bactec *Neisseria* spp. differentiation kit. Results showed that *N. gonorrhoeae* produced ^{14}C-labeled gas significantly faster than did *N. cinerea*, suggesting that the two organisms use different pathways for glucose metabolism. Further studies of ^{14}C-labeled gas production by *N. gonorrhoeae* and *N. cinerea* are needed to permit better differentiation of the two species by the Bactec method. Thus, the Bactec system may provide a means for differentiating *N. cinerea* from the other neisserias that fail to produce acid in conventional media such as those used in the current study (Table 1). We have no details on the exact pathway of glucose metabolism by *N. cinerea* at this time or on the reasons for positive glucose reactions in the Minitek system. The reactions listed in Table 1 represent standard conventional carbohydrate oxidation methodology performed in

reference laboratories. Definitive reports of clinical isolation of *N. cinerea* are relatively recent (1, 3, 4), and further clarification of discrepancies between reactions in conventional and commercial sugar methodology must await further studies. However, the problem of glucose degradation may be alleviated in a clinical setting in that the above-mentioned tests recommended to identify problem *Neisseria* spp. strains (serology, colistin susceptibility, growth on simple agars) do not require glucose oxidation. No API *Neisseria* spp. identification kit is available at this time; in the event of such a kit being released, it will be important to test it with both organisms to define accuracy of differentiation.

N. cinerea has been isolated as a commensal frequently from the naso- and oropharynx and less commonly from genital sites (4); a pathogenic role for this organism in human infections has been suggested in one report of lymphadenitis in an immunocompromised 4-year-old boy (1). In our patient, no firm conclusions could be drawn as to its pathogenicity. The clinical features of the lesions (confined to the skin) were atypical for *Neisseria* sp. proctitis, where the mucosal surface is primarily involved. The exact microbiological nature of the patient's disease is unclear. A synergistic infection could have occurred in the anal area between many colonic organisms (aerobes and anaerobes). It is not surprising that penicillin was ineffective in clearing the infection, since most of these colonic bacteria produce β-lactamase(s), which may protect penicillin-susceptible neisserias and other organisms. Although multiple colonies were picked and identified, the additional possibility of mixed flora of >1 *Neisseria* spp. cannot be excluded. Failure to isolate *N. cinerea* in earlier culture attempts also underscores the doubtful pathogenicity of the organism in our patient.

The isolation of *N. gonorrhoeae* from the rectum of a child should appropriately lead clinicians to a presumptive diagnosis of sexual abuse. Such a presumptive diagnosis demands a thorough investigation of potential sexual contacts so that abuse can be interpreted and remedial psychotherapy can be begun. The significance of this isolation of *N. cinerea* is that it was repeatedly misidentified as *N. gonorrhoeae*, with resultant initiation of evaluation for sexual abuse. Clearly, currently available commercial methods for identification of *Neisseria* spp. are inadequate in cases with such far-reaching psychosocial implications. Such kits have great value in cases of genital infection in which history and physical findings suggest sexually transmitted *N. gonorrhoeae* infection. However, if the only evidence for sexual abuse is isolation of a *Neisseria* sp., its identity should be confirmed by commercial serological methods, growth on simple agars, and colistin susceptibility and, if necessary, by a reference laboratory before proceeding with a diagnosis of sexual abuse.

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