

NOTES

Organism Resembling *Neisseria gonorrhoeae* and *Neisseria meningitidis*

DONNA S. HODGE,^{1*} FRASER E. ASHTON,² ROBERT TERRO,¹ AND AMINA S. ALI¹

Clinical Bacteriology Section, Laboratory Services Branch, Ministry of Health, Toronto, Ontario M5W 1R5,¹ and National Reference Centre for *Neisseria*, Laboratory Centre for Disease Control, Ottawa, Ontario, K1A 0L2,² Canada

Received 19 February 1987/Accepted 22 April 1987

A problem isolate resembling *Neisseria gonorrhoeae* and *Neisseria meningitidis* is reported. Growth and biochemical characteristics indicated the organism to be *N. meningitidis*, whereas serological characteristics indicated it to be *N. gonorrhoeae*. This vaginal isolate may be a genetically transformed gonococcus with the ability to utilize maltose. Conversely, it may be a meningococcus which has acquired antigenic determinants of *N. gonorrhoeae*.

In November 1986, a vaginal isolate was referred to the Toronto Ministry of Health Laboratory from a laboratory in southern Ontario, Canada. The isolate was morphologically and biochemically typical of *Neisseria meningitidis*, but it reacted with all serological confirmatory tests for *Neisseria gonorrhoeae*, including Difco GCFA (Difco Laboratories, Detroit, Mich.), GonoGen (New Horizons Diagnostics Corp., Columbia, Md.), the Phadebact Monoclonal GC OMNI test (Pharmacia Diagnostics AB, Uppsala, Sweden), and the Syva MicroTrak (Syva Co., Palo Alto, Calif.). The purpose of this note is to inform laboratories of the possibility of isolating similar strains.

The colonial morphology most strongly resembled that of *N. meningitidis*. The colonies on New York City medium (4) were of butyrous consistency, round with an entire edge, yellowish, and slightly opaque after 36 to 48 h of incubation at 36°C in 5% CO₂. Two colony variants were apparent, one small, about 0.5 mm in diameter, and one larger, 2 to 3 mm in size, after 36 to 48 h of incubation. Distinct small and large colonial variants were also seen on GC medium (7) and were more typical of *N. meningitidis* (3) than *N. gonorrhoeae*. Both colonial variants gave identical biochemical and serological results which eliminated the possibility of a mixed culture of gonococci and meningococci. On blood agar incubated at 36°C in 5% CO₂, colonies attained a diameter of 1 mm within 18 to 24 h. Early subcultures of the organism grew on blood agar at 22°C in air but poorly. Later subcultures did not grow at 22°C. There was no growth on nutrient agar at 36°C in 5% CO₂. These features are typical of *N. meningitidis*.

The organism was a gram-negative diplococcus. It was oxidase and catalase positive, whereas β-D-galactosidase (10), DNase (10), and tributyrinase (11) activities were undetected. Biochemically, the strain resembled *N. meningitidis* in that it produced acid from glucose and maltose but not from sucrose, fructose, lactose, and mannitol in three test systems: cystine-tryptic digest agar (10), modified oxidation-fermentation medium (9), and starch gelatin medium (Institut Armand Frappier, Laval-des-Rapides, Quebec,

Canada). The organism behaved as *N. meningitidis* in enzyme-based kits, the Gonocheck II test (Du Pont Co., Wilmington, Del.) and Identicult (Scott Laboratories, Inc., Carson, Calif.), showing that gamma-glutamyl aminopeptidase was produced. The strain was unable to synthesize polysaccharide from 5% sucrose (10). The culture grew on an auxotyping medium without cysteine (12). *N. gonorrhoeae* has an absolute requirement for cysteine-cystine (2) and does not grow on medium lacking these amino acids. The isolate failed to reduce 0.001% nitrite even after 5 days of incubation in heart infusion or GC broth, a phenomenon which would be highly unusual for a gonococcus (8).

The organism was resistant to 18 mg of spectinomycin per liter, with an MIC of 21 mg/liter. The clinical isolates of *N. gonorrhoeae* we encounter are most often fully susceptible to 15 mg of spectinomycin per liter. The organism was also susceptible to penicillin (0.12 mg/liter), ampicillin (0.5 mg/liter), tetracycline (1.0 mg/liter), erythromycin (1.0 mg/liter), trimethoprim-sulfamethoxazole (1.5/28.5 mg/liter), and sulfadiazine (10 mg/liter).

Serologically, the organism resembled *N. gonorrhoeae*. The fluorescent-antibody technique using the Difco GCFA conjugate was strongly positive. *N. meningitidis* is recognized to occasionally give a weak reaction with this reagent but never to fluoresce so strongly as to be misidentified as *N. gonorrhoeae*. The newer immunologic kits available for gonococcal confirmation were also strongly positive with this culture. The GonoGen and Phadebact Monoclonal GC OMNI tests, both coagglutination test systems using monoclonal antibodies to *N. gonorrhoeae*, were positive. The isolate also fluoresced brightly with the fluorescein-labeled, purified mouse monoclonal antibodies specific to *N. gonorrhoeae* of the Syva MicroTrak system. The strain coagglutinated strongly in the Gonotype 5 reagent (New Horizons Diagnostics Corp.).

The strain was nonagglutinable in meningococcal grouping sera and nontypeable with standard meningococcal typing sera 1 to 15 (5).

Ison et al. (6) remarked that it is conceivable that *N. gonorrhoeae* might be transformed to oxidize maltose and be misidentified as *N. meningitidis*. We were interested in

* Corresponding author.

knowing how this isolate would be reported by clinical laboratories. It was sent as an unknown quality control culture to 11 regional public health laboratories in Ontario. One laboratory astutely reported "*Neisseria* sp. biochemically resembling *N. meningitidis* and serologically resembling *N. gonorrhoeae*." Seven laboratories reported it as *N. meningitidis*. Three laboratories which had identified it as *N. meningitidis* made their decision based on colonial morphology and carbohydrate utilization alone. No serology had been performed. Three others identified it as *N. meningitidis* by disregarding their serological findings, which were positive for *N. gonorrhoeae*. Three laboratories identified the organism as *N. gonorrhoeae* on the strength of a positive GonoGen reaction, although the organism had been shown to utilize glucose and maltose.

The organism was stable biochemically and serologically on repeated subculture. All laboratories which handled it, including ours, recorded identical results for tests done, although some differences existed in the number of tests done in each laboratory.

In summary, the organism most closely resembled *N. meningitidis* in its colonial morphology, production of acid from glucose and maltose, growth without cysteine on a defined medium, production of gamma-glutamyl aminopeptidase, failure to reduce 0.001% nitrite, and resistance to spectinomycin. Serologically, however, it reacted strongly with the Difco GCFA conjugate and monoclonal antibodies specific to *N. gonorrhoeae*. Sparling et al. (13) discussed interspecies transformation of members of the family *Neisseriaceae* and suggested the possibility of constructing biologically interesting hybrids of the gonococcus. Based on our experience, it appears that nature may have accomplished this phenomenon. When Ison et al. (6) used *Neisseria lactamica* as a donor in transformation studies with *N. gonorrhoeae*, 0.2% of the transformants acquired the ability to oxidize maltose. They all retained the ability to oxidize glucose and, interestingly, had not acquired the ability to oxidize lactose. These researchers suggested that normal mouth flora might transform some strains of *N. gonorrhoeae* to oxidize maltose. The isolate we describe may well be such a strain. A more precise identification of this unusual isolate may be elucidated by isoenzyme analysis (1), DNA hybridization, examination of the outer membrane protein, and detection and characterization of plasmids.

We thank William Craig, MDS Laboratory, St. Catharines, Ontario, Canada, for bringing the isolate to our attention.

LITERATURE CITED

1. Braude, A. I., J. A. McCutchan, C. Ison, and P. R. Sargeant. 1983. Differentiation of *Neisseriaceae* by isoenzyme electrophoresis. *J. Infect. Dis.* **147**:247-251.
2. Catlin, B. W. 1973. Nutritional profiles of *Neisseria gonorrhoeae*, *Neisseria meningitidis*, and *Neisseria lactamica* in chemically defined media and the use of growth requirements for gonococcal typing. *J. Infect. Dis.* **128**:178-194.
3. DeVoe, I. W., and J. E. Gilchrist. 1978. Piliation and colonial morphology among laboratory strains of meningococci. *J. Clin. Microbiol.* **7**:379-384.
4. Faur, Y. C., M. H. Welsburd, M. E. Wilson, and P. S. May. 1973. A new medium for the isolation of pathogenic *Neisseria* (NYC medium). I. Formulation and comparison with standard media. *Health Lab. Sci.* **10**:44-54.
5. Frasch, C. E., and S. S. Chapman. 1972. Classification of *Neisseria meningitidis* group B into distinct serotypes. II. Extraction of type-specific antigens for serotyping by precipitin techniques. *Infect. Immun.* **6**:127-133.
6. Ison, C., A. A. Glynn, and S. Bascomb. 1982. Acquisition of new genes by oral *Neisseria*. *J. Clin. Pathol.* **35**:1153-1157.
7. Kellogg, D. S., Jr., W. L. Peacock, Jr., W. E. Deacon, L. Brown, and C. I. Pirkle. 1963. *Neisseria gonorrhoeae*. I. Virulence genetically linked to clonal variation. *J. Bacteriol.* **85**:1274-1279.
8. Knapp, J. S. 1984. Reduction of nitrite by *Neisseria gonorrhoeae*. *Int. J. Syst. Bacteriol.* **34**:376-377.
9. Knapp, J. S., and K. K. Holmes. 1983. Modified oxidation-fermentation medium for detection of acid production from carbohydrates by *Neisseria* spp. and *Branhamella catarrhalis*. *J. Clin. Microbiol.* **18**:56-62.
10. Morello, J. A., W. M. Janda, and M. Bohnhoff. 1985. *Neisseria* and *Branhamella*, p. 176-192. In E. H. Lennette, A. Balows, W. J. Hausler, Jr., and H. J. Shadomy (ed.), *Manual of clinical microbiology*, 4th ed. American Society for Microbiology, Washington, D.C.
11. Riou, J. Y., J. Buissière, M. Guibourdenche, G. Brault, and J. P. Carlier. 1981. Hydrolyse de la tributyrine par les *Neisseria* et les *Branhamella*. *Ann. Microbiol. (Paris)* **132A**:159-169.
12. Shtibel, R. 1985. Auxotypes of *Neisseria gonorrhoeae* in Toronto: relationship to penicillin resistance and to sex of patient. *J. Med. Microbiol.* **20**:187-196.
13. Sparling, P. F., G. D. Biswas, and T. E. Sox. 1977. Transformation of the gonococcus, p. 156-176. In R. B. Roberts (ed.), *The gonococcus*. John Wiley & Sons, Inc., New York.