Revista Argentina de Microbiología (2004) 36: 78-80

ISSN 0325-7541

Revista Argentina de Microbiología (2004) 36: 78-80

Unusual phenotypic characteristic of *Neisseria gonorrhoeae* from male patients who have sex with men

S.D. GARCÍA[1], R. MASSA[2], A. FAMIGLIETTI[1], C. VAY[1], AND G. GUTKIND*[2]

[1]*Servicio de Bacteriología Clínica, Hospital de Clínicas «Jose de San Martín»,* [2]*Cátedra de Microbiología, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Junín 956, 8º piso, (1113) Ciudad Autónoma de Buenos Aires, Argentina*

**Correspondence: e-mail: ggutkind@ffyb.uba.ar*

SUMMARY

We describe four isolates of Ng from men who have sex with men (MSM) patients that were able to grow in the abscense of CO_2, as previously was described for *N. gonorrhoeae* ssp. *kochii*. These isolates were able to grow aerobically (without any added CO_2) at 37 °C giving small colonies after 48 hs; two of them isolated from pharynx and urethra of one patient, were also able to grow without the blood supplement in the same conditions. In these unusual isolates the major outer-membrane proteins are of the same molecular weight than Ng. These isolates could be taken for other members of the genus if not confirmed by means of these (or other) methods.

Keywords: *Neisseria gonorrhoeae*, CO_2 requirements, classification

RESUMEN

***Neisseria gonorrhoeae* con características fenotípicas atípicas de pacientes homosexuales masculinos.** Cuatro aislamientos de *Neisseria gonorrhoeae* recuperados de muestras extragenitales de pacientes homosexuales masculinos fueron capaces de crecer en en medio sólido en ausencia de CO_2 en aerobiosis, tal como ya fue descrito para *N. gonorrhoeae* sub-especie *kochii*, dando origen a pequeñas colonias luego de 48 hs de incubación a 37 °C. Mas aún, dos de los aislamientos (aislados de uretra y faringe del mismo paciente) fueron capaces de crecer sin el agregado del suplemento de sangre. En estos aislamientos, los perfiles de proteínas de envoltura externa fueron idénticos a los de aislamientos genitales de la misma especie. Este hallazgo refuerza la necesidad de lograr una identificación cierta de estos microorganismos, ya que los esquemas simplificados hubieran llevado a su identificación errónea como *N. meningitidis* o su confusión con otras especies del género.

Palabras clave: *Neisseria gonorrhoeae*, requerimiento de CO_2, identificación

Even if most *Neisseria gonorrhoeae* (Ng) are isolated from typical gonococcal urethritis, it can also be recovered cases of proctitis, faryngitis, artritis, disseminated infection, meningitis, dermatitis and stomatitis (1), and unusual gonococcal infections are increasingly reported (6, 9).

Cultivation of Ng requires the use of different rich and complex media together with an humid, CO_2-containing atmosphere at the appropriate temperature (9); however, it was already reported that some strains of Ng grew in the abscense of CO_2, provided a small amount of yeast extract was included in the medium (4).

For most diagnostic laboratories, production of acid from maltose or glucose is the sole biochemical reaction used for distinguishing meningococci from gonococci. However, not all isolates are typical: a few serologically identifiable meningococci fail to produce acid from maltose and/or glucose; and some gonococci also fail to produ- ce significant amounts of acid in glucose-containing media (1).

In 1986, Mazloum, et al. reported unusual *Neisseria* spp. isolated from eye cultures of children in Egypt, which were able to grow without any extra CO_2 atmosphere, utilized only glucose (but not maltose), exhibited a positive reaction when tested both with antisera to crude antigens from *Neisseria meningitidis* and Ng, and did not react with the fluorescent antibody test for Ng or with monoclonal antibodies used to serotype gonococci. On SDS-PAGE, the major outer-membrane proteins had different patterns than those noted for comparable proteins of meningococci and gonococci. It was suggested to group similar isolates as *Neisseria gonorrhoeae* ssp. *kochii* (11).

We describe four isolates of Ng from men who have sex with men (MSM) patients that were able to grow in the abscense of CO_2, as previously was described for *N. gonorrhoeae* ssp. *kochii*.

Between January 1985 to July 1999, 121 extragenital isolates of Ng were obtained from 3948 patients with uncomplicated gonorrhea attending the Sexually Transmitted Diseases Program, Hospital de Clínicas «José de San Martín», Facultad de Medicina, Universidad de Buenos Aires.

Swab specimens from rectum, pharynx, urethra and endocervix were plated both on modified Thayer-Martin medium (Difco, Detroit, Michigan) and Columbia agar supplemented with proteose peptone # 3 and 5% sheep blood, incubated at 37 °C in 5% CO_2 atmosphere for 48 hours. Microorganisms were identified by a combination of classical morphologic and biochemical (Gram stain, oxidase, superoxol, and carbohydrate reactions on Cysteine Triptone agar) (9), structural (total and outer membrane protein profiles) and immunologic (reaction to monoclonal antibodies specific for Ng) characteristics. All extragenital strains were also tested for CO_2 requirements by sub-culturing on Nutrient Agar supplemented (or not) with 5% sheep blood, and incubated aerobically at 37 °C for 48 hours.

Four isolates were able to grow (rendering small colonies) on Columbia agar with 5% sheep blood incubated aerobically (without any added CO_2) at 37 °C for 48 hs; two of them isolated from pharynx and urethra of one patient, were also able to grow without the blood supplement in the same conditions. Clinical data from the patients are given in Table 1.

The unusual strains were serogrouped as WII-III [serotyping was done by using the monoclonal antibody reagents and latex agglutination to *N. meningitidis* serogroups A,B, C (Biomerieux, S.A., Buenos Aires, Argentina) and techniques as described previously] (7, 13); none of them reacted with specific antisera to *Neisseria meningitidis.* Physiological features of the strains are given in Table 2. The outer membrane protein profiles displayed the same pattern that Ng reference- control strain (see photography).

All isolates were penicillinase-negative (2, 12). The MIC ranges of selected antibiotics (µg/ml) were: penicillin, 0.064-0.5; ceftriaxone, 0.004; ciprofloxacin 0.004 - 0.008; clarithromycin 2 [determined using E-test strips*(11), following manufacturer's recommendations* (PDM Epsilometer, AB Biodisk, Solma, Sweden), on GC agar base (Difco, Detroit, Michigan)]. Resistance was defined accordingly to the National Committee for Clinical Laboratory Standards Guidelines (Vol 19 N° 1, 1999).

Growth in the abscense of CO_2 is very unusual in gonococci from our patients. These isolates would have been described as «*N. kochii* « or maltose negative *N. meningitidis* if additional test were not performed. Both major outer membrane proteins profile and reaction with monoclonal antibodies contributed to the differentiation (11). Differentiation of maltose negative *N. meningiti- dis* should be enforced, taking into consideration the high prevalence of meningococci among these popula-tions (5, 14).

Confusion in the literature in the identification of *N.gonorrhoeae* with *N. cinerea* has also been noted previously (3, 8). *N. cinerea* strains are slightly more pigmented than those of *N. gonorrhoeae* and are positive for nitrite reduction but negative for screening with serological reagent for *N. gonorrhoeae* and *N. meningitidis.*

Additional tests (some as protein profiles, not usually available in the clinical microbiology laboratory), should be considered for an accurate characterization of similar isolates.

Since recognition of the lack of CO_2 requirements in these samples, gonococci recovered from urethral samples were also tested, showing an increasing prevalence.

These unusual gonococci could not be differentiated when compared with typical strains in resistance to drying or cold stress (data not shown).

Acknowledgments. This work was supported in part by grants from the University of Buenos Aires, Argentina (TB 039), and SEPCYT (PICT 0693) to G.G.

G.G. is a member of «Carrera del Investigador Científico-CONICET».

REFERENCES

1. Cattin RW (1973) Nutricional profiles of *Neisseria gonorrhoeae, Neisseria meningitidis* and *Neisseria lactamica* in chemically defined me dia and the use of growth requirements for gonococcal typing. J. Infect. Dis. 128: 178-194.
2. Dillon JR (1983) Laboratory methods for *Neisseria gonorrhoeae.* Published by authority of the Minister of National Health and Elfare. Ottawa, Ontario, Canada.
3. Dossett JH, Appelbaum PC, Knapp JS, Totten PA (1985) Proctitis associated with *Neisseria cinerea* misidentified as *Neisseria gonorrhoeae* in a child. J.Clin. Microbiol. 21: 575-577.
4. Griffin PJ, Racken E (1956) The carbon dioxide requirement of *Neisseria gonorrhoeae*. J. Bacteriol.71: 717-721.
5. Janda WM, Bohnoff M, Morello JA, Lerner JA (1980) Prevalence and site-pathogen studies of *Neisseria meningitidis* and *N. gonorrhoeae* in homosexual men. JAMA 244: 2060-4.
6. Knapp JS, Houmans EH (1999) *Neisseria* and *Branhamella.* In: Murray P, Baron EJ, Pfaller MA, Tenover FC, Yolken

RH. (Eds), Manual of Clinical Microbiology, ASM Press,Washington D.C., pp. 586-603.
7. Knapp JS, Tam MR, Nowinski RC, Holmes KK, Sandstrom EG (1984) Serological classification of *Neisseria gonorrhoeae* with use of monoclonal antibodies to gonococcal outer membrane protein I. J. Infect. Dis. 150: 44-48.
8. Knapp JS, Totten PA, Mulks MH, Minshew BH (1984) Characterization of *Neisseria cinerea*, a non pathogenic species isolated on Martin-Lewis medium selective for pathogenic *Neisseria* spp. J.Clin.Microbiol.19: 63-67.
9. Koneman EW, Allen SD, Janda WM, Schereckenberger PC, Winn Jr WC (1997) *Neisseria* species and *Moraxella catarrhalis.* In: Koneman EW, Allen SD, Janda M, et al. Diagnostic Microbiology, Lippincott Williams & Wilkins Publishers, pp. 491-537.
10. Laemmli, UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. 227: 680- 685.
11. Mazloum H, Totten PA, Brooks GF, Dawson CR, Falkow S, James JF, et al. (1986) An unusual *Neisseria* isolated from conjunctival cultures in rural Egypt. J. Infect. Dis.154: 212-224.
12. O´Callagham CH, Morris A, Kirby SM, Shingler AH (1972) Novel method for detection of beta-lactamase using a chromogenic cephalosporin substrate. Antimicrob. Agents. Chemother. 1: 283-288.
13. Sandstrom EG, Knapp JS, Buchanan TB (1982) Serology of *Neisseria gonorrhoeae.* W-antigen serogrouping by coagglutination and protein I serotyping by enzyme-linked immunoabsorbent assay both detect protein I antigens. Infect.Immun. 35: 229-239.
14. Tsang RSW, Kiefer L, Law DKS, Stolz J, Shahin R, Brown S, Jamieson F. (2003) Outbreak of Serogroup C meningococcal disease caused by a variant of *Neisseria meningitidis* serotype 2a ET-15 in a community of men who have sex with men. J. Clin. Microbiol. 41: 4411-4.
15. Yasin RM, Suan KA, Meng CY (1997) Comparison of E-Test with agar dilution methods in testing susceptibility of *Neisseria gonorrhoeae* to azithromycin. Sex. Transm. Dis. 24: 257-260.

Recibido: 25/11/03 – Revisado: 24/05/04