Reduction of Nitrite by Neisseria gonorrhoeae
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A total of 112 Neisseria gonorrhoeae strains representing protein I serogroups WI, WII, and WIII and six auxotypes were tested for their ability to reduce nitrite. All strains reduced 0.001% (wt/vol) potassium nitrite (KNO₂) within 24 h and 0.01% (wt/vol) KNO₂ within 48 h. None of the strains reduced 0.1% (wt/vol) KNO₂ even after incubation at 37°C for 5 days, and the strains could not be cultured from these tests after incubation for 24 h. Thus, all of the Neisseria gonorrhoeae strains tested were nitrite sensitive but were able to reduce subtoxic concentrations of KNO₂. Nitrite reduction is a species characteristic for N. gonorrhoeae.

Nitrite reduction is included as a differential test for the classification of Neisseria spp. and Branhamella catarrhalis (7, 9). Nitrite is reduced by all human Neisseria spp., with the possible exception of Neisseria meningitidis (6, 7). In 1961, Berger showed that neither Neisseria gonorrhoeae strains nor some Neisseria meningitidis strains could reduce 0.1% (wt/vol) nitrite (1). While characterizing Neisseria meningitidis (3), Berger subsequently reevaluated nitrite reduction by Neisseria gonorrhoeae strains (7, 9). In 1961, Berger showed that neither Neisseria gonorrhoeae strains nor some Neisseria meningitidis strains could reduce 0.1% (wt/vol) nitrite (1). While characterizing Neisseria meningitidis (3), Berger subsequently reevaluated nitrite reduction by Neisseria gonorrhoeae strains (7, 9). In 1961, Berger showed that neither N. gonorrhoeae strains nor some N. meningitidis strains could reduce 0.1% (wt/vol) nitrite (1). While characterizing N. meningitidis (3), Berger subsequently reevaluated nitrite reduction by N. gonorrhoeae and N. meningitidis (2). All five strains of N. gonorrhoeae tested reduced 0.01% (wt/vol) nitrite. However, among 58 N. meningitidis strains tested, only strains of serogroups A, D, and Y could reduce 0.01% (wt/vol) nitrite but reduced 0.01% (wt/vol) nitrite (3). Berger subsequently reevaluated nitrite reduction by N. gonorrhoeae and N. meningitidis (2).

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(The results were reported in part at the Annual Meeting of the American Society for Microbiology, New Orleans, La., March 1983 [J. S. Knapp, Abstr. Annu. Meet. Am. Soc. Microbiol. 1983, D27, p. 63].)

MATERIALS AND METHODS

Strains. A total of 112 N. gonorrhoeae strains were tested. The laboratory strains included the taxonomic type strain, strain NRL 30010 (= ATCC 19424), 15 serogroup WI, WII, and WIII serological reference strains (11), strains NRL 905 (AHU+/WI) and NRL 8038 (F62; Pro/WII), and strains isolated in The Philippines (4 strains), Europe (5 strains), and Africa (18 strains, 4 of which were β-lactamase positive). Also included were 67 cervical and urethral isolates from women and men attending an Adult Medicine Clinic in Seattle, Wash. The clinical isolates represented six auxotypes and serogroups WI, WII, and WIII (Table 1). Five Neisseria mucosa strains were compared with the N. gonorrhoeae strains in these tests. The strains were grown on supplemented GC base medium (Difco Laboratories, Detroit, Mich.), as described previously (3). Strains were stored at -70°C in a solution of 50% heat-inactivated (56°C, 30 min) gamma-globulin-free horse serum (GIBCO Laboratories, Grand Island, N.Y.) in tryptic soy broth (Difco). The tests were inoculated in triplicate with loopfuls of an overnight culture grown on supplemented GC base agar at 37°C for 24 h in 5% CO₂. A loopful of broth was removed from each test in 0.001% (wt/vol) KNO₂, plated onto supplemented GC base medium, and incubated at 37°C for 24 h to confirm that positive tests resulted from N. gonorrhoeae activity. Similarly, each test in 0.1% (wt/vol) KNO₂ was nitrite sensitive but reduced subtoxic concentrations of nitrite. Pure cultures of N. gonorrhoeae were isolated from all tests in medium which had contained 0.001% (wt/vol) KNO₂. In contrast, N. gonorrhoeae was not isolated from tests in medium which contained 0.1% (wt/vol) KNO₂ and was tested after 24 h of incubation. Thus, the N. gonorrhoeae strains were not only unable to reduce, but were killed by, 0.1% (wt/vol) KNO₂.

Detection of nitrite reduction by bacterial species is routinely made in a nutrient medium containing 0.1% (wt/vol) KNO₂ (12). Strains are tested in 4-ml volumes of medium in 15- by 125-mm tubes. Deep broth tubes are recommended because nitrite reduction occurs only in the absence of oxygen (8), and the ratio of surface area to volume is kept small to prevent oxygen from diffusing into the medium and inhibiting nitrite reduction. In this study, nitrite reduction...
TABLE 1. Characteristics of 67 clinical isolates of N. gonorrhoeae tested for their ability to reduce nitrite

<table>
<thead>
<tr>
<th>W serogroup</th>
<th>AHU⁺</th>
<th>Proto</th>
<th>Pro⁻</th>
<th>Arg⁺</th>
<th>Arg⁻</th>
<th>Pro⁻</th>
<th>PAU⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>WI</td>
<td>5</td>
<td>2</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WII</td>
<td>39</td>
<td>10</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WIII</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Serogroups WI and WIII are subgroups which belong to the Protein IB molecule group, which is distinct from the protein IA molecule group to which serogroup WI strains belong. (10, 11).

b AHU⁺, Arginine, hypoxanthine, and uracil requiring; Proto, no requirements; Pro⁻, proline requiring; Arg⁺, arginine requiring; PAU⁺, proline, arginine, and uracil requiring. All strains required cystine-cysteine for growth.

was detected easily in 1-ml volumes of medium in 12- by 72-mm tubes. I postulated that N. gonorrhoeae cells rapidly reduced any dissolved oxygen in the medium and that cells near the medium surface continued to reduce any dissolving oxygen, thus permitting nitrite reduction to occur in the anaerobic medium.

Although N. gonorrhoeae strains reduced subinhibitory nitrite concentrations, they were inhibited by, and could not reduce, the 0.1% (wt/vol) KNO₂ routinely recommended for nitrite reduction tests (12). The results obtained in this study emphasize the need to modify routinely recommended tests where necessary in order to determine whether nitrite-sensitive bacterial species can reduce nitrite at subinhibitory concentrations. Obviously, this applies not only to descriptions of new taxa, but also to reevaluation of well-established species, such as N. gonorrhoeae. Consequently, I recommend that nitrite reduction tests be made in a broth medium containing either 0.01 or 0.001% (wt/vol) KNO₂ in order to detect nitrite reduction by those bacterial species that are unable to reduce 0.1% (wt/vol) KNO₂.

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LITERATURE CITED