Neisseria lactamicus sp. n., a Lactose-fermenting Species Resembling Neisseria meningitidis

DANNIE G. HOLLIS, GERALDINE L. WIGGINS, AND ROBERT E. WEAVER
Bacterial Reference and Serology Unit, National Communicable Disease Center, Atlanta, Georgia 30333

Received for Publication 21 October 1968

The biochemical and serological characteristics of lactose-utilizing strains of Neisseria were determined. These organisms were found in the nasopharynx of man and grew well on Thayer-Martín Selective Medium. They were compared with N. meningitidis to ascertain whether they were variants of this species. Differences between the lactose-using strains and the recognized species of Neisseria were considered significant enough to warrant designation of a new species, Neisseria lactamicus. This group has not been widely recognized as being separate from N. meningitidis; therefore, the normal incidence and clinical significance of these organisms has not been fully established. These organisms are oxidase-positive and positive for B-D-galactosidase activity; they demonstrate fermentation in King Oxidation-Fermentation Medium; and they produce acid from only glucose, lactose, and maltose, of the 27 substrates incorporated in Cystine Trypticase Agar. Individual strains vary in their ability to grow on Nutrient Agar at both 25 and 37 C and in their pigmentation on Loefler Medium. Results indicated that these organisms are serologically distinct from the N. meningitidis serogroups. Only 34 of 116 strains of N. lactamicus were smooth and could be tested by slide agglutination. None of the 34 could be grouped as N. meningitidis group A, B, C, D, X, Y, or Z. Thirty-one of these strains could, however, be specifically grouped with antisera prepared with N. lactamicus strains. Cross absorptions confirmed that N. lactamicus is serologically distinguishable from N. meningitidis.

As early as 1934, Jessen (6) reported a strain of Neisseria which used lactose, as well as glucose and maltose, but this organism has not received much attention in the literature until recent years. Mitchell, Rhoden, and King (10) studied several strains of the organism serologically, using fluorescent antibody (FA) and slide agglutination procedures. On the basis of reactions obtained with these two tests, they reported that some of the strains were serologically indistinguishable from group B N. meningitidis. Other authors (2, 8) have also dealt with these organisms, but none has made a definite proposal concerning their proper nomenclature.

These organisms are frequently encountered in carrier studies, and the number of carrier surveys has increased since the recognition of sulfonamide-resistant strains of N. meningitidis. As a result, the number of lactose-using strains submitted to the National Communicable Disease Center (NCDC) for identification or confirmation has increased.

We reviewed the results of the examinations of a number of these strains, including approximately 10 serologically smooth cultures, and noted that several had been submitted with a presumptive identification as nongroupable N. meningitidis. Frequently, the referring laboratories had not tested them for lactose utilization. In contrast to an earlier report (10), none of the smooth strains could be grouped as N. meningitidis by slide agglutination. These observations prompted an evaluation of the serological and biochemical characteristics of the Neisseria strains that utilize lactose.

We attempted to determine whether the lactose-utilizing Neisseria strains were variants of N. meningitidis group B, whether they constituted an additional serogroup of N. meningitidis which was different from groups A, B, C, D, X, Y, and Z, or whether these organisms were a distinct species that was different from any other species of Neisseria. Data of the serological and biochemical studies of lactose-utilizing strains of Neisseria are reported.

These strains were compared with strains of N.
meningitidis and a strain of N. subflava, since these are the species to which they are most similar.

The authors propose that this group of organisms which utilize lactose be designated a separate species, Neisseria lactamica, sp. n.

MATERIALS AND METHODS

Strains. Strains of N. lactamica were received from 17 states in the United States and from Canada. The neotype strains of N. meningitidis suggested by Branham (1) and the type strains of Slaterus (12) were also used. Other strains of N. meningitidis studied were random selections of those cultures which state health laboratories had sent to this laboratory for identification or confirmation. A culture of N. subflava (ATCC no. 19243) was obtained from American Type Culture Collection. One previously studied lactose-utilizing strain of Neisseria (10), no. SD9, was obtained from the Bacterial Chemistry Unit, NCDC.

Antisera. Antisera for N. meningitidis groups A, B, C, D, X, Y, and Z were obtained from the Biological Reagents Section, NCDC, and antisera to groups A, B, C, and D were purchased from two commercial sources.

Rabbit antisera preparation. Following the method of Hollis et al. (4), three strains of N. lactamica (A2894, A5906, and A7515) were used to prepare antisera.

Absorption of antisera. Strains were removed directly from storage at -40 C to plates of 5% rabbit blood Heart Infusion Agar (HIA). The surfaces of the plates were moistened with approximately 0.25 ml of Heart Infusion Broth (HIB). The organisms were harvested following incubation at 37 C for 18 to 24 hr in a candle jar. The antisera to be absorbed were diluted 1:2 in distilled water, and the procedure described previously (4) was employed.

Sero logical procedures. Serological methods described by Hollis et al. (4) were used.

Biochemical procedures. The production of acid from 27 substrates was determined in Cystine Trypticase Agar (CTA) Medium. Stock solutions of substrates which had been Seitz-filtered were added aseptically to give final concentrations of 1%. The substrates tested included glucose, xylose, mannitol, lactose, sucrose, maltose, glycerol, salicin, L-arabinose, adonitol, dulcitol, D-galactose, levulose, mannose, rhamnose, trehalose, raffinose, sorbitol, inositol, cellobiose, inulin, dextrin, glycogen, erythritol, melibiose, melezitose, and starch. Both 1 and 3% concentrations of ethyl alcohol, isopropyl alcohol, isononyl alcohol, methanol, and lignin in CTA medium were also tested. Optimal pH and pH tolerances were determined in HIB with pH values ranging from 5 to 10. Sodium chloride tolerance was determined in Nutrient Broth (NB) with varying percentages of NaCl (0.5 to 6.0%). Growth at 25 and 37 C was determined on Nutrient Agar (NA) with a minimal inoculum (one loopful of 24-hr HIB or a light suspension of organisms in HIB). Loeffler Medium was used in pigment studies. Victoria Blue Fat Medium, as modified by Davis and Ewing (3), was used to detect hydrolysis of corn oil. Tests for β-D-galactosidase activity on o-nitrophenyl-β-D-galactopyranoside (ONPG) were performed according to the method outlined by LeMinor and Ben Hamida (9), except that 5% rabbit blood HIA and 5% rabbit blood HIA with 1% lactose were substituted for Lactose-Glucose SH2 Medium. Oxidative versus fermentative (OF) studies were done with King OF Medium [0.5% Casitone (Difco), 0.3% agar, 0.003% phenol red aqueous; pH 7.3], Difco OF Basal Medium, and CTA Medium with 1% glucose, mannitol, lactose, and maltose. The media used for the OF studies were boiled and cooled in an ice bath just before inoculation. Duplicate sets of media were inoculated with each strain, and one set was overlayed with sterile petrolatum. Shake tubes containing 12 ml of CTA Medium were used to determine the oxygen requirements. The remainder of the biochemical methods employed were outlined by King (7). These included the use of MacConkey Agar and Simmons Citrate Agar, and catalase, oxidase, nitrate, and indole tests.

Examination for capsules was carried out with India ink.

Drug sensitivity. Sulfadiazine sensitivity tests were performed according to a modified method of P. F. Frank, U.S. Naval Medical Research Unit, Great Lakes, Ill. Briefly, this procedure consisted of incorporating various amounts of sulfadiazine in 20 ml of Mueller-Hinton (MH) Agar. The agar was poured into plates which were marked off in pie-shaped sections. The inoculum consisted of a suspension of approximately 10⁸ organisms from an 18-hr HIA slant containing 5% rabbit blood. The inoculum was streaked on plates containing 0.05, 0.1, 0.5, 1.0, 3.0, 5.0, 10, and 20 mg of sulfadiazine per 100 ml. Plates were incubated for 18 to 24 hr in a candle jar. Penicillin sensitivity tests were performed as described above, except that plates containing 0.01, 0.25, 0.05, and 0.1 µg of potassium penicillin (1,600 units/mg) per ml were used. Other antibiotic sensitivity tests were performed with commercial antibiotic discs. Plates of MH Agar were streaked evenly over the surface with a sterile cotton swab moistened in a 24-hr broth culture. Results were read after 24 hr of incubation.

Selective medium. Thayer-Martin (TM) Selective Medium employing either vancomycin, colistimethate, and nystatin (VCN) or ristocetin with polymyxin B was used in MH agar.

RESULTS

N. lactamica organisms were gram-negative cocci and diplococci which were strongly oxidase positive. The size of the colonies varied with the strain and ranged from 0.5 to 1.5 mm in 24 hr on HIA with 5% rabbit blood. Colonies were low convex to convex, entire, smooth, glossy, butyrous, and semitranslucent to semiopaque. Yellowish pigment was observed with some strains.

Biochemical tests were carried out to determine whether reactions, other than the formation of
acid from lactose, could be found that would distinguish the lactose-utilizing strains from \textit{N. meningitidis} strains and from the other species of \textit{Neisseria}.

Glucose, mannitol, lactose, sucrose, maltose, and levulose in CTA Medium are the substrates routinely used in this laboratory for identifying possible \textit{Neisseria} species. \textit{N. lactamicus} produced acid from glucose, maltose, and lactose, whereas the \textit{N. meningitidis} produced acid only from glucose and maltose. As has been reported (8, 11; H. A. Fox and M. S. Goodson, Bacteriol. Proc., p. 89, 1967), an occasional strain of \textit{N. meningitidis} failed to produce acid from either glucose or maltose. No acid was produced by either species in 26 other substrates tested. The pH range and NaCl tolerance studies did not reveal a consistent difference between the strains of \textit{N. lactamicus} and \textit{N. meningitidis}. With both of these species, no growth was observed on MacConkey Agar or Simmons Citrate Agar. Nitrate was not reduced to nitrite, indol was not produced, and corn oil was not hydrolized. Yellowish pigmentation on Loeffler Medium was observed with some of the lactose-utilizing strains, but some strains of \textit{N. meningitidis}, including some neotype strains, were also yellowish.

At 37 C, 10 lactose-utilizing strains which had been transferred only 2 to 3 times following isolation grew on NA, but at 25 C only 2 of 10 grew.

Of an additional 103 strains of \textit{N. lactamicus} (transferred an undetermined number of times), 18.4\% grew on NA at both 25 and 37 C, 59.2\% grew at 37 C only, and 22.3\% failed to grow on NA at either temperature. We have observed no strains of \textit{N. meningitidis} which grow at 25 C on NA, and only an occasional strain has grown at 37 C on this medium.

Thirty \textit{N. lactamicus} strains were tested for \textbeta-D-galactosidase activity, and all gave positive ONPG reactions within 10 min. The neotype strains of \textit{N. meningitidis} groups A, B, C, D, X, Y, and Z, \textit{N. subflava} (ATCC No. 19243) grown on lactose-containing medium, and several other \textit{N. meningitidis} cultures gave negative ONPG results at 48 hr.

OF studies were conducted with 10 \textit{N. meningitidis} strains, including the 7 neotype strains, and 9 \textit{N. lactamicus} strains. When King OF Medium was used, \textit{N. lactamicus} strains were definite fermenters of glucose, lactose, and maltose (acid was produced in both the open and overlayered tube); however, when CTA medium was used, acid was produced in glucose, lactose, and maltose in open tubes, but only slightly acid or neutral reactions were seen in the overlayered tubes. In contrast, the OF studies with \textit{N. meningitidis} strains showed less growth and, therefore, less reaction in King OF Medium. The results obtained with these strains, with the exception of groups B and Z neotype strains, indicated an oxidative, with a questionable fermentative, reaction with CTA Medium. Groups B and Z neotype strains gave a definite fermentative reaction with maltose in CTA Medium. With Difco OF Basal Medium, we observed no growth to very slightly questionable reactions with both species.

Oxidation requirements determined with shake tubes indicated that \textit{N. lactamicus}, \textit{N. meningitidis}, and \textit{N. subflava} are aerobic to microaerophilic organisms.

No definite capsules were observed with \textit{N. lactamicus}, and no Quellung reaction was obtained with the homologous or heterologous antisera.

Three lactose-utilizing strains and two strains of \textit{N. meningitidis} were tested for drug susceptibility to 27 chemotherapeutic agents by use of commercial discs. Results were similar in that both the \textit{N. lactamicus} and the \textit{N. meningitidis} strains were sensitive and resistant to the same antibiotics. \textit{N. meningitidis} and \textit{N. lactamicus} strains were further studied with sulfadiazine and penicillin with a plate dilution method. All of the lactose-utilizing strains were sensitive to 0.1 \textmu g of penicillin per ml as were the \textit{N. meningitidis} strains. Only 4 (5.4\%) of 74 strains of \textit{N. lactamicus} were resistant to 1 mg of sulfadiazine per 100 ml, whereas a much greater percentage of \textit{N. meningitidis} strains have been reported to be resistant to sulfadiazine (5).

Since many of the \textit{N. lactamicus} strains received recently were isolated from TM plates, we decided to determine whether strains which had been isolated before the introduction of TM would also grow on this medium. All grew well on TM using either VCN or polymyxin B with ristocetin. Viability counts, with MH Agar and the antibiotics suggested by Thayer and Martin, showed that \textit{N. lactamicus} strains grew equally as well as those of \textit{N. meningitidis}. Approximately one-half of the organisms of both the \textit{N. lactamicus} and \textit{N. meningitidis} strains were inhibited.

\textit{N. subflava} strain was streaked on TM, and except for a few colonies in the primary area of greatest inoculum, it failed to grow. This strain grew well on NA at both 25 and 37 C and was yellow on Loeffler Medium.

The serology of the lactose-utilizing strains was studied with antisera prepared from three strains (A2894, A5906, and A7515). Table 1 gives tube agglutination titers obtained by use of these antisera with \textit{N. meningitidis} A, B, C, D, X, Y, and Z antigens and with the homologous \textit{N. lactamicus} strains.

Serum A2894 did not agglutinate any of the
neotype strains of the various serogroups of *N. meningitidis*. Serum A5906 produced complete agglutination of the group D antigen and partial agglutination of the group Z antigen only at the 1:20 dilution. Partial agglutination also occurred at the 1:20 dilution when the group D and Z antigens were tested in serum A7515.

When the three *N. lactamticus* strains were tested with antisera to the same *N. meningitidis* strains in the tube agglutination, there was no agglutination at the 1:20 dilution of serum or at greater dilutions.

The *N. lactamticus* antisera were absorbed with the homologous strains of *N. lactamticus* and with groups A, B, C, D, X, Y, and Z strains of *N. meningitidis*. Table 2 shows the results of the tube agglutination test with these sera following absorption and testing with their homologous antigens.

Absorptions of the three antisera with *N. meningitidis* groups A, B, C, D, X, Y, and Z strains showed no reduction in titers. The detectable agglutinins of A2894, A5906, and A7515 antisera were removed or greatly reduced when these antisera were absorbed by the homologous *N. lactamticus* strains.

When the antisera to *N. meningitidis* groups A, B, C, D, X, Y, and Z were absorbed with the three *N. lactamticus* strains, the homologous titers of the A, B, C, D, X, Y, and Z antisera remained unchanged by the absorptions.

There were 116 strains of *N. lactamticus* available for testing by slide agglutination in the antisera for the 7 serogroups of *N. meningitidis* and the 3 *N. lactamticus* antisera. Only 34 of these strains were smooth and, therefore, suitable for examination. These strains were tested with groups A, B, C, D, X, Y, and Z *N. meningitidis* antisera and with the three *N. lactamticus* antisera (Table 3).

The *N. lactamticus* strains were not agglutinated by the *N. meningitidis* groups A, B, C, D, X, Y, and Z antisera, except for three strains which reacted as follows: two strains agglutinated with groups C and X and with two *N. lactamticus* antisera, and one strain reacted with group Y and with all three *N. lactamticus* antisera. The remaining 31 strains agglutinated specifically with *N. lactamticus* antisera. Approximately 100 *N. meningitidis* strains have been tested by slide agglutination with A2894, A5906, and A7515 *N. lactamticus* antisera and none agglutinated. In addition, one lactose-utilizing strain, No. SD9, studied by Mitchell et al. (10), was available for serological study. This strain agglutinated with A5906 antisera, but not with the *N. meningitidis* antisera.

Of 116 *N. lactamticus* strains, 70.7% were autoagglutinable when received for testing in this laboratory. We attempted to determine whether this rough reaction might be due to repeated transferring of the cultures before testing. Slide agglutination tests were performed on 11 cultures which had been transferred only once following isolation. Eight of these 11 strains autoagglutinated.

### Table 1. Tube agglutination titers with *N. lactamticus* antisera and *N. meningitidis* and *N. lactamticus* strains

<table>
<thead>
<tr>
<th>Antigens</th>
<th>N. lactamticus antiserum strain no.</th>
<th>A2894</th>
<th>A5906</th>
<th>A7515</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>N. meningitidis</em> serogroup</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A&lt;sup&gt;a&lt;/sup&gt;</td>
<td>—&lt;sup&gt;b&lt;/sup&gt;</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>B</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>C</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>D</td>
<td>—</td>
<td>20&lt;sup&gt;d&lt;/sup&gt;</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>X</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Y</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Z</td>
<td>—</td>
<td>—&lt;sup&gt;d&lt;/sup&gt;</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Homologous <em>N. lactamticus</em> strain</td>
<td>1:1,280</td>
<td>1:80</td>
<td>1:320</td>
<td></td>
</tr>
</tbody>
</table>

* Branham and Slaterus neotype strains used.

<sup>a</sup> No agglutination at serum dilutions of 1:20 or greater.

<sup>b</sup> The reciprocal of the dilution.

<sup>c</sup> Partial agglutination.

<sup>d</sup> Minimal reaction (<3+) at 1:20.

### Table 2. Tube agglutination titers of *N. lactamticus* antisera with the homologous antigens following absorptions

<table>
<thead>
<tr>
<th>Serogroup used for absorption</th>
<th>N. lactamticus antiserum strain no.</th>
<th>A2894</th>
<th>A5906</th>
<th>A7515</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>N. meningitidis</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2,560&lt;sup&gt;b&lt;/sup&gt;</td>
<td>80</td>
<td>640</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>2,560</td>
<td>80</td>
<td>640</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>1,280</td>
<td>80</td>
<td>640</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>2,560</td>
<td>80</td>
<td>640</td>
<td></td>
</tr>
<tr>
<td>X</td>
<td>2,560</td>
<td>80</td>
<td>640</td>
<td></td>
</tr>
<tr>
<td>Y</td>
<td>2,560</td>
<td>80</td>
<td>640</td>
<td></td>
</tr>
<tr>
<td>Z</td>
<td>2,560</td>
<td>80</td>
<td>640</td>
<td></td>
</tr>
<tr>
<td>Homologous <em>N. lactamticus</em> strain</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td></td>
</tr>
<tr>
<td>Homologous titer unabsorbed</td>
<td>1,280</td>
<td>80</td>
<td>320</td>
<td></td>
</tr>
</tbody>
</table>

* Branham and Slaterus neotype strains used for absorptions.

<sup>a</sup> The reciprocal of the dilution.
**Table 3. Slide agglutination results with 116 strains of N. lactamicus**

<table>
<thead>
<tr>
<th>N. meningitidis antisera group</th>
<th>N. lactamicus antisera</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Groupable</td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>19.8</td>
</tr>
<tr>
<td>6</td>
<td>5.2</td>
</tr>
<tr>
<td>1</td>
<td>0.9</td>
</tr>
<tr>
<td>1</td>
<td>0.9</td>
</tr>
<tr>
<td>Nongroupable</td>
<td></td>
</tr>
<tr>
<td>82</td>
<td>70.7</td>
</tr>
<tr>
<td>2</td>
<td>1.7</td>
</tr>
<tr>
<td>1</td>
<td>0.9</td>
</tr>
</tbody>
</table>

*a Symbols: ag, autoagglutinable; -, agglutination did not occur; +, agglutination occurred.

**Table 4. Number of N. lactamicus strains by geographical origin and source**

<table>
<thead>
<tr>
<th>Geographical origin</th>
<th>Clinical source</th>
<th>Biochemical tests</th>
<th>Neisseria species</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nasopharynx</td>
<td>Spinal fluid</td>
<td>Lung autogamy</td>
</tr>
<tr>
<td>Areas of U.S.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Northeast</td>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Middle Atlantic</td>
<td>6</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>East North Central</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>South Atlantic</td>
<td>50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>East South Central</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>West South Central</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mountain</td>
<td>3</td>
<td>3*</td>
<td>1</td>
</tr>
<tr>
<td>Pacific</td>
<td>9</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Canada</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Totals</td>
<td>83</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>

*a All three of these strains were from the same patient.

The geographical origin and the clinical source of the *N. lactamicus* strains are shown in Table 4. The strains were from eight different areas of the United States and Canada. Of 116 cultures, 83 were reported to be from the nasopharynx, three from the lung (same patient), one from spinal fluid, and one from amniotic fluid.

A summary of some of the distinguishing characteristics of some of the *Neisseria* species is given in Table 5. The species included are *N. meningitidis*, *N. lactamicus*, and *N. subflava*.

**DISCUSSION**

Biochemical studies showed that *N. lactamicus* strains are not identical to any of the other *Neisseria* species. The two most distinctive reactions of the *N. lactamicus* strains are fermentation of lactose and production of β-d-galactosidase (ONPG reaction). According to *Bergey's Manual*, none of the *Neisseria* species use lactose. The two species of *Neisseria* which appear to be most similar to *N. lactamicus* biochemically are *N. meningi-
tidis and N. subflava. All three of these species use glucose and maltose and fail to form acid from 29 other substrates in CTA Medium.

Studies employing King OF Medium indicate that N. lactamica is a fermentative organism. A few strains of N. meningitidis appeared to ferment maltose in CTA Medium, but none demonstrated definite fermentation in King OF Medium. Fermentative reactions were not observed with glucose in either medium inoculated with N. meningitidis. The N. lactamica strains did not appear to grow as well in CTA Medium as they did in the King OF Medium.

The production of acid by some of these strains is not always evident in CTA Medium until after 48 hr. For this reason, the CTA Medium tubes should be held for 1 week before the final readings are taken. The ONPG test can be used for a rapid indication of lactose-utilizing strains. As reported by Corbett and Catlin (2), it is not necessary to grow the organisms on a lactose-containing medium before determining the ONPG reaction.

Although to the experienced worker colonial differences between most strains of N. lactamica and N. meningitidis may be apparent on certain media, we feel they should not be relied upon for distinguishing between species.

Thayer and Martin (13, 14) reported that their medium inhibits the growth of Neisseria species other than N. gonorrhoeae and N. meningitidis. However, they are now aware (personal communication) that lactose-utilizing strains are being isolated on their medium. Our results showed that all of the N. lactamica strains grew well on both of the antibiotic formulas recommended by these authors and that a N. subflava strain grew only in the primary area of heavy inoculum.

In this laboratory, approximately 1,500 groupable N. meningitidis strains have not grown at 25 C on NA, and only an occasional strain has grown at 37 C on NA. The N. lactamica strains studied varied in their ability to grow at both 37 and 25 C on NA. Therefore, as a group, N. lactamica strains vary from this species in this characteristic; however, individual strains cannot be reliably differentiated just on the basis of their ability to grow on NA.

Since N. lactamica may or may not exhibit yellow pigmentation on Loeffler Medium, this characteristic also cannot be used to separate these Neisseria species.

In considering whether the lactose-utilizing Neisseria are variants of N. meningitidis, it is pertinent to note that H. A. Fox and M. S. Goodson (Bacteriol. Proc., p. 89, 1967) were unable to demonstrate spontaneous lactose-negative variants of the lactose-utilizing strains. Similarly, lactose-utilizing variants of N. meningitidis strains were not detected.

Another factor we considered in attempting to determine the proper taxonomical speciation of these organisms was their serology. Results of agglutination, both slide and tube, showed a distinguishable difference between three antisera prepared from N. lactamica strains and antisera to N. meningitidis serogroups A, B, C, D, X, Y, and Z. Cross absorptions showed that these N. lactamica strains were not identical with the N. meningitidis strains.

Only one strain of N. lactamica which was studied by Mitchell et al. (10) was available for examination. This strain was reported to have been agglutinated by group B antiserum upon primary isolation and, subsequently, to have become nonreactive in A, B, C, or D grouping sera. When reexamined in this study, it failed to agglutinate in the N. meningitidis grouping sera, but was agglutinated by N. lactamica antiserum A5906.

It should be noted that none of the 34 smooth strains of N. lactamica in the present study, including three strains examined at the time of primary isolation, were agglutinated by N. meningitidis group B antiserum. Of the 34 smooth strains, 31 were agglutinated specifically by one or more of the N. lactamica antisera. Three strains gave cross reactions: two strains were agglutinated by both group C and X antisera and one by group Y antisera. These three strains were also agglutinated by at least two of the three N. lactamica antisera. This is in contrast to the findings of Mitchell et al. (10), who reported that six of eight lactose-utilizing strains were serologically indistinguishable from N. meningitidis group B. At present, no explanation for this difference in results is evident. Mitchell and co-workers also found that after several laboratory subcultures, often as few as three passages, the lactose-utilizing strains were no longer agglutinated by group B antiserum, and they noted that this loss of agglutinability had not been observed with nonlactose-fermenting strains of group B N. meningitidis. We also made this latter observation.

Of 116 N. lactamica strains, 70.7% autoagglutinated. Although some strains of N. meningitidis are autoagglutinable, the percentage is appreciably below 70.7%, even when strains isolated from throat and nasopharynx only are included (4, 5).

Neisseria cultures which have been isolated from either blood or spinal fluid are submitted to NCDC by laboratories in all sections of the United States. Of approximately 1,200 strains studied over a 15-year period, a lactose-fermenting organism has been implicated in only one case.
of meningitis. We feel that further study of the
N. lactamicus strains is needed. The carrier rate
should be determined, and data should be col-
lected on any possible clinical manifestations con-
nected with the isolation of this organism. We
feel that these organisms should not just be
"lumped" with N. meningitidis and that their
clinical significance should be adequately de-
termined.

Because the clinical significance of N. lactami-
cus appears to be different from that of N. menin-
gitidis and since these organisms appear to be bio-
chemically and serologically distinct from other
Neisseria species, we feel that the designation of
these organisms as a new species is warranted.
The selection of the specific epithet lactamicus is
derived from the Lating words lactis meaning milk
and amicus meaning friend or friendly.

Representative strains of N. lactamicus were
deposited with the American Type Culture Col-
lection (ATCC) and National Collection of
Type Cultures (NCTC). They were assigned
ATCC and NCTC numbers as follows: N. lactam-
icus, NCDC A2894—ATCC 23972—NCTC
10616; N. lactamicus, NCDC A7515—ATCC
23970—NCTC 10617; and N. lactamicus, NCDC
A5906—ATCC 23971—NCTC 10618. Strain
A7515 is designated as the type strain.

ACKNOWLEDGMENTS

We thank William H. Ewing, Enteric Bacteriology Unit,
NCDC, for his guidance in the selection of a species name. We
also thank Dwane L. Rhoden, Bacterial Reference Unit,
NCDC, for providing primary subcultures of 11 strains of N.
lactamicus.

LITERATURE CITED

1. Branham, S. E. 1958. Reference strains for the serologic groups
8:1—15.
88:16—19.
logical studies of ungroupable Neisseria meningitidis. J.
5. Ivler, D., J. M. Leeson, A. W. Mathies, Jr., J. C. Fremont, L.
S. Thrupp, E. Portnow, and P. F. Wehrle. 1965. Correlates of
sulfadiazine resistance in meningococci isolated from
358—365.
Pathol. 31:241—247.
resistance and the failure to ferment maltose in Neisseria
recherche de la B-galactosidase sur celle de la fermentation
du lactose en milieu complexe dans le diagnostic bacterio-
Pasteur 102:267—277.
fermenting organisms resembling Neisseria meningitidis.
11. Pelczar, M. J., and N. D. Raymond. 1949. On the direct fer-
m entation of maltose. Science 110:256.
12. Satterius, K. W. 1963. Types of meningococci isolated from
carriers and patients in a non-epidemic period in the Nether-
29:265—271.
for the cultivation of Neisseria gonorrhoeae and Neisseria
selective cultivation of Neisseria gonorrhoeae and Neisseria