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Characterization of *Neisseria polysacchareae* sp. nov. (Riou, 1983) in Previously Identified Noncapsular Strains of *Neisseria meningitidis*

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A study of 216 noncapsular strains of *Neisseria meningitidis* isolated from patients and carriers received in the Meningococcus Reference Laboratory between 1978 and 1984 is reported. The characterization of the strains consisted of biochemical tests for the following characteristics used for the differentiation of *Neisseria* species: oxidase, catalase, and β-galactosidase activities; sugar degradation; nitrate and nitrite reduction; DNase activity; polysaccharide production with 5% sucrose; aminopeptidase activity; and growth in Thayer-Martin and Catlin media. Of the strains studied, 50 showed characteristics of a new taxon recently described (*Neisseria polysacchareae*). Characteristics that differentiated these strains from meningococcal isolates were polysaccharide production with 5% sucrose, gamma-glutamylaminopeptidase activity, and a requirement for cysteine or cystine for growth in Catlin medium. All of the *N. polysacchareae* strains identified were isolated from the nasopharynx of healthy carriers.

Pathogenic *Neisseria* spp. (meningococcus and gonococcus) are normally identified on the bases of growth on Thayer-Martin medium and sugar utilization (7, 10). For the identification of other members of the genus *Neisseria*, there are additional characteristics, such as nitrate and nitrite reduction, DNAse, lipase, production of polysaccharide with 5% sucrose, and β-galactosidase activity, that permit the classification of the majority of *Neisseria* and *Branhamella* isolates (7, 8).

Growth on Thayer-Martin medium is considered to be a basic method for the separation of pathogenic strains from saprophytic ones. Mitchell et al. (6) described atypical meningococcal strains, later designated *Neisseria lactamica* (5), which grew on this medium. In 1983, Riou et al. (8) described 13 isolates of a new taxon of *Neisseria* sp. provisionally named *Neisseria polysacchareae*. These bacteria grew on the selective medium but differed from *N. lactamica* and *Neisseria meningitidis* in some characteristics not normally used in the identification of these species (polysaccharide production with 5% sucrose, aminopeptidase activity, and nutritional requirements in Catlin medium [2]). This last method is an epidemiological marker for *Neisseria gonorrhoeae* (auxotyping). *N. polysacchareae* requires cysteine or cystine for growth on this medium, as does *N. gonorrhoeae*, while *N. meningitidis* can grow without this amino acid.

None of the isolates of *N. polysacchareae* studied by Riou produced capsules (8).

The purpose of this study was to evaluate the possible existence of strains of this new taxon in a group of isolates identified as *N. meningitidis*. Identification was performed by conventional methods, as well as by determination of Thayer-Martin medium growth and acid production from glucose and maltose for isolates that were nongroupable by slide agglutination.

A total of 216 strains of *N. meningitidis* were studied. These strains were received in the reference laboratory for confirmation and serological studies; 102 strains were from a previously published study of the incidence of meningococcus in a school population (9), 34 strains were isolated from cerebrospinal fluid or blood of patients with meningococcal infections (meningitis or sepsis or both), and 80 strains were isolated from sporadic carriers. All the strains showed the common characteristic of being nongroupable (autoagglutinable, polyagglutinable, or nonagglutinable with antimeningococcal sera).

The following strains were used as biochemical test controls: *N. lactamica* NCTC 10617, *N. subflava* Ne44 (Centre for Recherche of Meningocoques [CIRM] Marseille *N. flavescens* Ne64 (CIRM), *N. sicca* ATCC 9913, *Branhamella catarrhalis* Ne55 (CIRM), and *N. meningitidis* MA2848 (culture collection of our reference laboratory, Majadahonda, Madrid, Spain). A reference strain of *N. polysacchareae* 462 was kindly supplied by J. Y. Riou, Pasteur Institute, Paris, France.

All strains in the study were grown on Thayer-Martin medium and were confirmed by using the reference laboratory criteria: colonial morphology on blood agar, oxidase activity, sugar utilization (glucose, maltose, sucrose, lactose, fructose, and mannitol) in CTA medium (Difco Laboratories, Detroit, Mich.) and MHBT medium (10), and β-galactosidase activity (7). The strains were preserved at −70°C in skim milk (Difco) until studied.

All of the strains previously identified as noncapsular meningococci by slide agglutination were studied, by methods described previously (7, 8), for the following characteristics: growth on nutrient agar and at 22°C, reduction of nitrates and nitrites; DNAse activity, polysaccharide production with 5% sucrose, and gamma-glutamylaminopeptidase activity. The study of nutritional requirements was made by using the Catlin auxotyping method, with arginine and cystein or cystine as metabolite markers (2). The lack of capsules in the strains was demonstrated by suspending a loop from a 24-h culture on blood agar at 37°C under a 5% CO_2 atmosphere, in 0.9% NaCl and testing with antimenin-

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gococcal sera produced in our laboratory as previously described (11).

The antimicrobial susceptibilities of *N. polysaccharea* and true noncapsular meningococci were studied by determining the MICs of various agents by using an automatic multi-inoculator (Microtiter AM80) with a concentration of microorganisms of 3×10^5 CFU/ml. The medium used was Mueller-Hinton Agar (Difco) containing 5% sheep blood. The antimicrobial agents studied were sulfadiazine, penicillin, ampicillin, rifampin, spyramicin, and chloramphenicol.

The results of phenotyping tests and the characteristics of the isolates identified as *N. polysaccharea* are shown in Tables 1 and 2. Of the 13 strains that were polyagglutinable with meningococcal sera, 6 (46%) were identified as *N. polysaccharea*. Of 73 nonagglutinable strains, 15 (20.5%) belonged to the new taxon, and of the group of 130 autoagglutinable strains, 29 (22.3%) belonged to the new taxon.

All of the strains of this species were from the nasopharyngeal tracts of carriers and showed the characteristics expressed in Table 2. The differential characteristics of the 50 *N. polysaccharea* strains identified with respect to true noncapsular meningococci were colonies perceptibly smaller on Thayer-Martin medium after 24 h of incubation, absence of gamma-glutamylaminopeptidase activity, production of polysaccharide with 5% sucrose, and requirement of cysteine or cystine for growth in Catlin medium. None of the strains required arginine for growth in the same medium; these data are similar to those obtained by Riou et al. (8) with isolates from France and Belgium.

In the study of antimicrobial susceptibility, we found *N. polysaccharea* to be more susceptible to sulfadiazine than was *N. meningitidis* (Table 3). The MICs of the antimicrobial agents studied were higher than those obtained for the meningococcus with the exception of the MIC of chloramphenicol, which was slightly higher for meningococci. The MICs obtained in our laboratory for *N. polysaccharea* were similar to those determined by Dabernat et al. (3).

The new *Neisseria* taxon resembles *N. meningitidis* phenotypically and genotypically (8) and is reminiscent of the early description of *N. lactamica* (5, 6). We find ourselves with new data to add to the controversy existing on the correct use and interpretation of carrier studies in the control of meningococcal infection, as has been discussed by several authors (1, 9, 13), who noted that the percentage of carriers in a population does not appear to be related to the incidence of the illness, whereas the existence of virulent strains in carriers does. Thus, selective control measures should be taken against virulent strains only.

As has been demonstrated for *N. lactamica* (4, 9), this new taxon could theoretically play a role in the prevention of nasopharyngeal colonization by meningococci. More extensive studies are needed to determine the incidence of this neisseria in people at risk for infection and to analyze its possible protective role.

In this regard, we examined the 50 isolates of *N. polysaccharea* by using the antiserum-agar method (12), studying the possible cross-reactivity with antimeningococcal sera. A high percentage of *N. polysaccharea* strains showed cross-reactivity, especially with serum against group B men-

TABLE 1. Strains of *N. polysaccharea* characterized from noncapsular *N. meningitidis* strains

Agglutinability[a]	No. of strains			
	N. polysaccharea		Noncapsular *N. meningitidis*	
	Carriers	Patients	Carriers	Patients
Polyagglutinable	6	0	5	2
Nonagglutinable	15	0	49	9
Autoagglutinable	29	0	74	27

[a] A total of 13 polyagglutinable, 73 nonagglutinable, and 130 autoagglutinable strains were characterized.

TABLE 2. Differential characteristics encountered between *N. polysaccharea* and noncapsular *N. meningitidis* strains

Characteristic tested	Result[a]	
	N. polysaccharea	*N. meningitidis*
Size (mm) of colonies in Thayer-Martin medium	2	3–4
Oxidase activity	+	+
Catalase activity	+	+
β-Galactosidase activity	−	−
Gamma-glutamylaminopeptidase activity	−	+
Acid produced from		
Glucose	+	+
Maltose	+	+
Sucrose	−	−
Fructose	−	−
Lactose	−	−
Reduction of		
NO_3^-	−	−
NO_2^-	−	v
DNAse activity	−	−
Polysaccharide production with 5% sucrose	+	−
Growth in Catlin medium without:		
Cysteine	−	+
Arginine	+	+

[a] At total of 50 *N. polysaccharea* strains and 166 *N. meningitidis* strains were tested. +, Positive result; −, negative result; v, variable.

TABLE 3. MICs of antimicrobial agents against *N. polysaccharea* and noncapsular *N. meningitidis* strains

Strain (*n*), antimicrobial agent	MIC range (μg/ml)	MIC_{50}[a]	MIC_{90}[b]
N. polysaccharea (50)			
Sulfadiazine	1–50	4.03	14.28
Penicillin	0.025–0.2	0.08	0.16
Ampicillin	0.1–0.4	0.16	0.33
Rifampin	0.1–0.4	0.15	0.31
Spiramycin	0.8–6.4	2.09	4.11
Chloramphenicol	0.4–0.8	0.40	0.40
Noncapsular *N. meningitidis* (74)			
Sulfadiazine	1–100	14.43	62.96
Penicillin	0.006–0.2	0.02	0.05
Ampicillin	0.012–0.2	0.03	0.09
Rifampin	0.012–0.4	0.02	0.11
Spiramycin	0.4–6.4	0.75	2.12
Chloramphenicol	0.2–1.6	0.51	0.77

[a] MIC_{50}, MIC for 50% of the strains tested.
[b] MIC_{90}, MIC for 90% of the strains tested.

ingococcus, as had been demonstrated for *N. lactamica* by several authors (4, 5, 9, 12).

The differential diagnosis of *N. polysacchareae* and *N. meningitidis*, requires the systematic determination of polysaccharide production with 5% sucrose, aminopeptidase activity, and β-galactosidase activity. By this approach, strains of neisseria that are distinct from *N. meningitidis* capable of growth on Thayer-Martin medium were detected. As has been shown, these strains occur very frequently in children (4, 9) and, if not detected, produce errors in calculations of the real percentages of meningococcus in a population. In an extensive survey carried out with children aged from 5 to 7 years (9), 102 strains were identified as noncapsular meningococci; 38 of these displayed the characteristics of *N. polysacchareae*.

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