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Evaluation of API NH, a New 2-Hour System for Identification of *Neisseria* and *Haemophilus* Species and *Moraxella catarrhalis* in a Routine Clinical Laboratory

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API NH is a new 2-h system (bioMérieux, La Balme-les-Grottes, France) for the identification of most *Neisseria* and *Haemophilus* spp. of clinical significance and of *Moraxella catarrhalis* and for the detection of penicillinase production. Furthermore, this system allows the biotyping of *Haemophilus influenzae* and *Haemophilus parainfluenzae*. Three hundred eighteen strains belonging to these species, previously identified by conventional methods, were tested. Among the 305 strains belonging to species included in the data base, 225 (73.8%) were identified without additional tests, 79 (25.9%) were correctly identified after extra tests, and 1 strain (0.3%) was misidentified. For 131 (90.3%) of the 145 *H. influenzae* and *H. parainfluenzae* strains, results of biotyping were in agreement with results of standard methods. API NH is an accurate and reliable method for the routine identification of these bacteria in a clinical laboratory, for biotyping of *Haemophilus* spp., and for the detection of penicillinase-producing strains. The system is ready to use and time saving; inoculation of the system and reading of results are easy.

Members of the genus *Haemophilus* are obligate parasites that constitute part of the normal flora of the human respiratory tract. In some cases, *Haemophilus* spp., mainly *Haemophilus influenzae*, may be responsible for various suppurative diseases (9). Of the members of the genus *Neisseria*, *Neisseria meningitidis* and *Neisseria gonorrhoeae* are the only species recognized as primary pathogens, while the other *Neisseria* spp. as well as *Moraxella catarrhalis* are either commensal or opportunistic pathogens (3, 10). The conventional means of identifying these bacteria are based on the use of selective and/or enriched media and nutritional requirements, in addition to biochemical tests. These methods are actually time-consuming and tedious. The present study was carried out to evaluate the new API NH system (bioMérieux, La Balme-les-Grottes, France) for the identification of *Haemophilus* and *Neisseria* spp. as well as *M. catarrhalis*, for the biotyping of *Haemophilus* spp., and for the detection of penicillinase production.

MATERIALS AND METHODS

Bacteria. The study was carried out on 318 strains belonging to the genera *Haemophilus*, *Neisseria*, and *Moraxella*, including 120 recent clinical isolates, 178 stock strains of clinical origin, and 20 reference strains. The strains were grown in a CO_2 incubator for 24 h at 37°C on chocolate agar supplemented with PolyVitex (bioMérieux).

Conventional identification. The following characteristics were determined for each strain: Gram staining, oxidase, catalase, and hemolysis. The *Haemophilus* strains were identified according to their X and V factor requirements (9). Biotyping was performed with an API 20E strip (bioMérieux) as described by Holmes et al. (6) by using erythrocyte extract (Diagnostics Pasteur, Marnes la Coquette, France) instead of Taxo XV-factor strips (BBL). The *Neisseria* and *M. catarrhalis* strains were identified by the ready-to-use *Neisseria* 4H system (Diagnostics Pasteur).

Penicillinase detection. The penicillinase detection test was performed by using the Cefinase reagent (bioMérieux).

API NH. The API NH system provides 13 tests: 4 fermentation tests (glucose, fructose, maltose, and sucrose), 8 enzymatic reactions (ornithine decarboxylase [ODC], urease, lipase, alkaline prosphatase, β-galactosidase, proline arylamidase, γ-glutamyl transferase, indol production), and penicillinase detection. The reactions either produce a spontaneous color change or are revealed by the addition of reagents. A heavy suspension in saline, adjusted to a no. 4 McFarland standard, was prepared by rolling a swab on the culture obtained on chocolate agar. The suspension was immediately inoculated into the microtubes of the strip according to the manufacturer's instructions. After a 2-h incubation period at 35 to 37°C under aerobic conditions, the reactions were read visually and identification was obtained by consulting the profile list included in the instruction manual.

RESULTS

Among the 305 strains belonging to species included in the data base, 225 (73.8%) were identified without additional tests, 79 (25.9%) were correctly identified after extra tests, and 1 strain (0.3%) was misidentified (Table 1). Ten of the 13 strains belonging to species not in the data base were misidentified and 3 were not identified (Table 2). When considering all of the 318 strains tested, the percentages presented above become, respectively, 70.8, 24.8, and 3.5%.

For 131 (90.3%) of the 145 *H. influenzae* and *Haemophilus parainfluenzae* strains, biotyping was in agreement with our reference method. One (0.3%) *H. parainfluenzae* strain was misidentified as *H. influenzae*.

The most frequently encountered pathogens (*Neisseria*

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TABLE 1. API NH identification of the 305 strains belonging to species listed in the data base

Species	No. of strains						
	Total	Stock strains	Recent isolates	Collection strains (reference)[a]	Correctly identified	Correctly identified after extra tests	Misidentified
M. catarrhalis	22	9	11	2 (NCTC 3622, ATCC 25240)	22		
N. meningitidis	33	16	14	3 (NCTC 8555, ATCC 13090, ATCC 8305)	33		
N. gonorrhoeae	27	17	8	2 (NCTC 10223, ATCC 19424	27		
N. subflava biovar *subflava*[b]	11	8	2	1 (CCUG 800)		11	
N. subflava biovar *perflava*[b]	10	6	4			10	
N. subflava biovar *flava*[b]	4		4			4	
N. mucosa	8	7		1 (DSM 4631)		8	
N. sicca	11	10		1 (CCUG 4790)		11	
N. cinerea	7	4	2	1 (CCUG 346)		7	
N. lactamica	5	4		1 (ATCC 23970)	5		
N. polysaccharea	3	2		1 (CCUG 18031)		3	
H. parainfluenzae	28	13	14	1 (NCTC 7857)	24	3	1[c]
H. influenzae	117	54	61	2 (ATCC 10211, CCUG 1892)	114	3	
H. aphrophilus	13	9	3	1 (NCTC 5906)		13	
H. paraphrophilus	6	6				6	
Total	305	165	123	17	225	79	1

[a] ATCC, American Type Culture Collection, Rockville, Md.; CCUG, Culture Collection of the University of Göteborg, Göteborg, Sweden; DSM, Deutsche Sammlung von Mikroorganismen, Braunschweig, Germany; NCTC, National Collection of Type Cultures, London, England.
[b] The three biovars were pooled as *N. subflava* in the data base. Extra tests were required to obtain a precise identification at the biovar level.
[c] Misidentified as *H. influenzae* by the API NH system.

meningitidis, *N. gonorrhoeae*, *M. catarrhalis*, and *H. influenzae*) were correctly identified. The results of the biotyping of *H. influenzae* and *H. parainfluenzae* strains on the basis of ODC, urea, and indole tests are listed in Table 3. One hundred thirty-one (90.3%) strains were assigned to the same biotype as with the reference method. Penicillinase production was correctly demonstrated in all strains except one *N. gonorrhoeae* strain, which was positive with Cefinase and for which results were doubtful within 2 h with the API NH system.

Regarding the origins of the strains tested, no difference was observed between recent clinical isolates and stock and reference strains.

DISCUSSION

API NH is a convenient system for identifying most of the pathogenic strains of *Haemophilus*, *Neisseria*, and *Moraxella* that are isolated routinely in the laboratory. In our experience, standardization of the inoculum size and careful homogenization of the bacterial suspension have proved to be particularly important for obtaining accurate results.

A rapid and correct identification was obtained for most of the strains tested (Table 1). Even one atypical maltose-negative *N. meningitidis* strain was well identified. Additional tests were generally required for the identification of the so-called nonpathogenic species. Some species were poorly represented, such as *Neisseria polysaccharea*, *Neisseria cinerea*, *Haemophilus aphrophilus*, and *Haemophilus paraphrophilus*.

None of the seven *N. cinerea* strains could be distinguished from glucose-negative *N. gonorrhoeae* by the API NH system. However, extra simple tests or information proposed by the system, such as growth on nutrient agar and the origin of the specimen, can help to avoid any possible confusion about species identity. The positive lipase test allowed differentiation of *M. catarrhalis* from all *Neisseria* and *Haemophilus* spp. *N. polysaccharea* (11) could not be distinguished from the mixed taxon *N. mucosal/N. sicca/N. subflava*. Cann and Rogers (2) noticed phenotypic similarities between these different species, the API NH system. Additional tests were also required to distinguish *H. aphrophilus* from *H. paraphrophilus*.

The results for 13 strains belonging to species not listed in the API NH data base (*Neisseria flavescens*, *Neisseria denitrificans*, *Haemophilus haemolyticus*, and *Haemophilus segnis*) are presented in Table 2. *N. flavescens*, which was particularly unreactive from a biochemical point of view, could be identified only on the basis of conventional tests. The result for *H. segnis* was a nontypeable *H. influenzae*. Additional tests including growth factor requirements were needed for its correct identification.

In relation to the other systems already available for the identification of *Neisseria*, *Haemophilus*, and *Moraxella*

TABLE 2. Results obtained for species not included in the API NH data base

Species	No. of strains	Reference strain	API NH identification (no. of strains)
N. flavescens	7	NCTC 8263	*N. cinerea/N. gonorrhoeae* (6)
		ATCC 13120	Not identified (1)
N. denitrificans	2	ATCC 14686	Not identified (2)
H. haemolyticus	3		*H. influenzae* (2), *H. influenzae/H. parainfluenzae* (1)
H. segnis	1		*H. influenzae* (1)

TABLE 3. Biotyping of 145 *Haemophilus* strains

Strain and reference biotyping result	API NH biotyping result
H. influenzae (117)[a]	
I (47)	I (47)
II (35)	II (35)
III (20)[b]	III (20)
IV (4)	IV (4)
V (3)	V (3)
VI (4)	VI (4)
VII (1)	VII (1)
Nontypeable (3)	I (1), III (1), IV (1)
H. parainfluenzae (28)	
I (8)	I (8)
II (4)	II (4)
III (6)	III (5), II (1)
IV (4)	I (3), II (1)
Nontypeable (6)	I (2), II (2), III (1), *H. influenzae* I (1)

[a] Values in parentheses are numbers of strains.
[b] Including one *H. aegyptius* strain.

spp., the API NH system compares well. Janda et al. (8) found that the HN ID kit (MicroScan Division, Baxter Diagnostics Inc., West Sacramento, Calif.) gave reliable results for *N. gonorrhoeae* and *M. catarrhalis* but was less efficient for *N. meningitidis*. Among the automated systems mentioned by Stager and Davis (12), very few were suitable for the identification of *Haemophilus* and *Neisseria* spp. and *M. catarrhalis*. None performed well in identifying members of all three genera. Durussel and Siegrist (5), studying carbohydrate utilization, noticed misidentifications and emphasized the need to know the morphology and pigmentation for correct discrimination between *Neisseria subflava* and *N. gonorrhoeae* or *M. catarrhalis*.

Biotyping of *Haemophilus* strains involved identification of all currently recognized biotypes (4). Our reference method and the API NH system were in full agreement for all typeable *H. influenzae* strains tested (Table 3). Some of the results obtained for biotypes III and IV of *H. parainfluenzae* were not in agreement with those of the reference method. Similar results were obtained by Warren et al. (13). Using two different kits to biotype *Haemophilus* spp., those investigators found discrepancies with the ODC and urea tests for 77 of the 98 strains tested. One *Haemophilus aegyptius* strain was tested and was considered to be *H. influenzae* biotype III by the API NH system. More precise identification would be of great interest for epidemiological studies, as demonstrated by Irino et al. (7).

Regarding the penicillinase production by strains of *N. gonorrhoeae*, *Haemophilus* spp., and *M. catarrhalis*, the results obtained by the use of the API NH system and Cefinase correlated well except for those for one strain of *N. gonorrhoeae*, which gave a doubtful result in the API NH system even after 4 h of incubation.

However, it must be emphasized that noninvasive strains of *H. influenzae* resistant to β-lactam drugs in a nonenzymatic manner have been described recently (1); therefore, the detection of penicillinase may be insufficient for the detection of β-lactam resistance in *Haemophilus* strains.

It must also be emphasized that, as for every reagent requiring a heavy inoculum for the detection of enzymatic reactions, it is essential to check the reliability of basic characters such as Gram staining, growth on selective media, and the purity of the inoculated strain; in fact, any contaminating agent may lead to a misidentification.

In conclusion, the API NH system is an efficient system for the identification of *Neisseria* and *Haemophilus* species as well as *M. catarrhalis*. This system appears to be well-adapted for routine use and gives rapid, accurate results, provided that the manufacturer's instructions are strictly followed.

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