Studies on a Collection of Gram-Negative Bacterial Strains Showing Resemblance to Moraxellae: Examination by Conventional Bacteriological Methods

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A collection of 36 gram-negative bacterial strains, showing some resemblance to moraxellae and possibly belonging to *Moraxella* or related genera, was examined by a number of conventional tests in three laboratories by members of the ICSB Subcommittee on the Taxonomy of *Moraxella* and Allied Bacteria. Eight of the strains were oxidase negative and could be classified as belonging to *Acinetobacter*. Of the 28 oxidase-positive strains, two were yellow-pigmented cocci, but differed markedly from neisseriae. A group of 11 rod-shaped strains, also pigmented to a greater or lesser extent, appeared related to *Flavobacterium*. Four unpigmented, mesophilic strains differed sufficiently to be unlikely members of *Moraxella*. Nine unpigmented psychrophilic strains, including two strains of Thornley's Phenon 3, showed similarities to moraxellae, although most of them oxidized aldose sugars like acinetobacters. Further taxonomic studies of the psychrophilic group will be of particular interest. The strains described by Bijsterveld (1970) and by Sutton et al. (1972) appeared to be nearly identical, but differed from moraxellae sufficiently to be unlikely members of *Moraxella*.

During the meetings of the ICSB Subcommittee on the Taxonomy of *Moraxella* and Allied Bacteria at the Xth International Congress for Microbiology in Mexico City in 1970, it was thought that certain organisms resembling moraxellae deserved study to see if they actually belonged to *Moraxella* or to other genera tentatively placed in the family *Neisseriaceae*. It was decided to gather a collection of such strains for joint studies by those members of the Subcommittee who desired to take part. It was agreed that the participating laboratories were free to select methods according to their own choice.

Three laboratories were interested in carrying out examination of the strain collection by conventional bacteriological methods, and this paper reports this part of the investigations. The results obtained by more highly specialized methods are expected to follow in later reports.

MATERIALS AND METHODS

The designations, sources, and origins of the strains are presented in Table 1. Each participating laboratory used tests and methods of its own choice. There are, consequently, considerable differences between the procedures used by the three laboratories. The designations of the laboratories, A (Oslo), B (Copenha-

gen), and C (London) are used to indicate which methods were used by each laboratory. If the medium used in the test is not specified, it is to be understood that blood agar was used, with 5% human blood (A), 10% horse blood (B), and 5% horse blood (C).

Cell morphology. Cell morphology was determined by microscopy of Gram-stained films from 20- and 48-h-old cultures at room temperatures and at 35 C (A), after 48 h at 30 C (B), and after overnight incubation at 33 C or 22 C (C). Films stained by the method of Butt et al. (5) (A), or wet India ink preparations from mucoid colonies only (B), were examined for capsules.

Colony morphology. Colony morphology was observed daily for several days (A), after 48 and 72 h (B), or after overnight incubation (C) at the temperatures indicated above.

Pigmentation. Pigmentation was looked for on all solid media after 1 to about 10 days (A, B), or on nutrient agar and casein agar after 5 days (C) at the temperatures indicated in "cell morphology." When it became apparent that there were discrepancies between the laboratories, the strains in question were reexamined several times in laboratory A on nutrient agar slants incubated at both temperatures (for revised results, see Table 3).

Motility. Motility was studied in semisolid motility agar at 35 C and at room temperature (A), or in hanging drops from overnight broth cultures at 22 C (C).

Twitching. Twitching was studied by plate micros-

TABLE 1. *Strains of Moraxella-like organisms included in this study*^a

Strain	Origin	Source	Strain	Origin	Source
WM17	Lautrop	Lymph node biopsy	A351	Thornley to Lautrop	Poultry (Phenon 3)
WM27	Lautrop	Venule	A353	Lautrop	Pus from abscess
WM28	Lautrop	Venule	A365	Lautrop	Guinea pig, autopsy
WM32	Lautrop	Venule	A366	Lautrop	Conjunctivitis
WM34	Lautrop	Venule	"New Mor- axella"	Bijsterveld to Henriksen	Conjunctivitis
A163	Lautrop	Plate contaminant	"New Mor- axella"	Sutton to Henriksen	Corneal abscess
A259	Lautrop	Urine	Pintér 16	Pintér to Shewan	Clinical material
A272	Lautrop	Venule	Pintér 21	Pintér to Shewan	Clinical material
A273	Lautrop	Venule	Henderson 55	Henderson to Shewan	Soil
A280	Lautrop	Pleural fluid	Henderson 69	Henderson to Shewan	Soil
A285	Lautrop	Venule	NCIB 9021	Shewan	Strain of Ferguson and Roberts
A317	Lautrop	Urine	NCIB 9300	Shewan	<i>A. metalcaligenes</i> from Brisou
A326	Lautrop	Venule	NCMB 98	Shewan	Lemon sole, gills
A331	Lautrop	Unknown	NCMB 131	Shewan	Arctic cod, skin
A332	Lautrop	Skin	NCMB 168	Shewan	Arctic cod, skin
A333	Lautrop	Unknown	NCMB 200	Shewan	Skate, skin
A338	Lautrop	Spinal fluid	NCMB 307	Shewan	Arctic cod, skin
A348	Thornley to Lautrop	Poultry (Phenon 3)	MJT/F4/5, 7	Thornley to Shewan	Chicken

^a Unless otherwise stated, all specimens of clinical material were of human origin.

copy of 6- to 8-, 24-, and 48-h-old cultures at 30 C and 22 C, incubated in a humid atmosphere on a medium consisting of 0.05% tryptone, 0.05% yeast extract, and 1% agar (B) (all from Difco).

Temperature. Effects of temperature were studied using the following methods: incubation in closed jars with water at the bottom at 35, 37, 40, and 42 C, and at room temperature (A); incubation at 37 and at 22 C (B); incubation overnight at 22, 30, 33, and 37 C, and at 5 and 45 C for 5 days (C). In all other tests (see below), strains failing to grow at 35 to 37 C were incubated at lower temperatures (22 C or room temperature).

Anaerobic growth. Anaerobic growth was examined after incubation in anaerobic jars in a hydrogen atmosphere for 3 days (A), or in 90% hydrogen and 10% CO₂ overnight (C).

Acid from carbohydrates. Tests were carried out with 1% solutions in Hugh and Leifson's O/F medium (glucose both with and without vaseline seal) (A, B, C), in peptone water (glucose, lactose, maltose, sucrose and mannitol), and in Mueller-Hinton broth (glucose, maltose and sucrose). The method of Pickett and Pedersen (16) was also tried without giving additional information (A). Glucose was also tested in ammonium salt medium and peptone water (C), and observed for 14 days.

Starch hydrolysis. For the starch hydrolysis test, 1% starch in nutrient agar was used and was flooded with iodine after 5 days (C).

Catalase. For testing catalase, three different proce-

dures were used. H₂O₂ (10%) was dropped on 20- to 24-h-old nutrient agar slope cultures (A). Growth from a 24-h-old nutrient agar culture was emulsified in a drop of 3% H₂O₂ on a slide with a glass rod (B). A loopful of growth from nutrient agar was emulsified in 6% H₂O₂ (C).

Oxidase. For the oxidase test, Kovacs' method (12) was used both with dimethyl- and tetramethyl-phenylenediamine, 1% solutions (A). Kovacs' method with the tetramethyl compound was also used, with 20 (B) and 10 (C) s as the limit for a positive reaction.

Nitrate reduction. Nitrate broth (0.2%) (11) was tested with sulfanilic acid and alpha-naphthylamine after 5 and 10 days, and with zinc powder in negative cases (A); 0.1% nitrate in peptone water was tested by the same method after 2 and 4 days (B); or 0.1% nitrate in peptone water was tested with sulfanilic acid and 1-naphthylamine-7-sulfonic acid after 5 days (C).

Nitrate reduction. Nitrite broth (0.001%) was tested after 5 days with sulfanilic acid and 1-naphthylamine-7-sulfonic acid (C).

Gelatin liquefaction. For the gelatin liquefaction test, culture in 15% gelatin in nutrient broth at room temperature was observed for 30 days (A). Stab cultures in FeCl₃-gelatin medium (11) were incubated at 22 C for 35 days (B). Nutrient gelatin stab cultures were observed for 28 days at 22 C (C).

Serum liquefaction. For serum liquefaction, Loeffler serum slopes were observed for 30 (A) or 28 days (C).

Indole. To test for indole reaction, casein digest

medium, incubated for 2, 5, and 10 days, was tested with Kovacs' reagent (A). Indole broth, incubated for 2 and 4 days, was tested with Ehrlich's reagent; the 96-h-old culture was shaken with 4 to 5 drops of xylene before adding the reagent (B). Nutrient broth, incubated for 5 and 10 days, was tested by Kovacs' and Ehrlich's methods (C).

Phenylalanine and tryptophan deamination. To test for phenylalanine and tryptophan deamination, a heavy suspension from blood agar cultures was made in 0.5 ml of 0.1% L-phenylalanine (or saturated solution of DL-tryptophan:0.2% DL-tryptophan did not quite dissolve) in water, preserved with 0.01% Merthiolate. The tubes were placed nearly horizontally at room temperature for 4 h, after which 4 to 5 drops of 10% FeCl_3 were added. Results (green or red color) were recorded as "−," no color, "w," weak greenish or reddish color, and "+," dark blackish-green or cherry-red color (A). Cultures from 2-day-old agar plates were suspended to a density of about 2.5×10^9 bacteria per ml in 0.5% phenylalanine solution. To 2.5 ml of suspension, 0.5 ml of borate-hydrochloric acid (pH 8) was added. Incubation was at 35 °C in a thin layer at the bottom of a flask; 0.3-ml samples were taken after 4 and 22 h, and 1 drop of 0.1 N HCl and then two drops of 2% FeCl_3 were added. A strong green color after 1 min was considered a positive reaction. The green color did not become as deep blackish-green as with *Proteus* strains (B). Malonate-phenylalanine broth, incubated for 2 days, was acidified with 0.1 N HCl and a few drops of FeCl_3 were added (C).

Urease. In the urease test, Christensen's medium (6) was observed for 3 (A) or 5 days (C). A very heavy suspension was made in urea (1%), KH_2PO_4 (0.1%), K_2HPO_4 (0.1%), 5 ml of phenol red per liter (1:500) (pH 7.1); readings were made after 2, 4, and 20 h. Only change within 4 h was recorded as a positive reaction (B).

ONPG. The method of Le Minor and Hamida (13) was used for testing o-nitrophenyl- β -D-galacto-pyranoside (A). The method of Bülow (2, 3) (B), with ONPG broth was used for 5 days (C).

H_2S . In testing for H_2S production, triple sugar iron medium was observed up to 10 days (A). Lead acetate paper over cysteine nutrient broth was observed for 5 days (C).

Citrate utilization. Koser's citrate medium was observed for 10 (A) or 5 days (C) in the citrate utilization test. Hutner's medium (see reference 20) was used with 0.1% citrate as the only carbon and energy source. There were three passages in fluid medium before definitive reading (B).

Poly-beta-hydroxybutyrate inclusions. The medium of Cohen-Bazire et al. (7) was used, with 0.5% beta-hydroxybutyrate, and checked for inclusions by phase-contrast microscopy and after staining by the method of Burdon (4) as soon as growth was apparent (A). Basal medium of Owens and Keddle (15) was used with 0.5% beta-hydroxybutyrate; after 2 days the cells were stained with Sudan Black B and examined microscopically for inclusions (C). A phase-contrast microscope was used to observe cultures in fluid Hutner's medium, with 0.1% beta-hydroxybutyrate as the only carbon and energy source (B).

Growth in mineral media. To test for growth in

mineral medium, Hutner's mineral base (20) was used with 0.1% acetate, butyrate, citrate, and ethanol as carbon source and 0.1% $(\text{NH}_4)_2\text{SO}_4$ as nitrogen source. Three passages were made in fluid medium before the final reading (B).

Decarboxylases. Decarboxylases were tested with arginine, lysine, and ornithine by the method of Richard (17). A few strains failed to grow in the medium. The method of Shaw and Clarke (18) was also tested, but it failed in some instances because the suspensions would not sediment well on centrifugation and, therefore, were too alkaline (A). Arginine, lysine, and ornithine were tested in Møller's medium using a heavy inoculum and incubating for 5 days (B and C). Arginine was also tested by Thornley's method (22) for 5 days (C).

Penicillin sensitivity. The filter paper disk method of Ericsson (8) was first used to determine penicillin sensitivity. When the results showed marked differences from those of the other laboratories, probably because this method is unsuitable for such slowly-growing and fastidious organisms as some of our strains, the tests were repeated in Todd-Hewitt broth tubes containing 1.0, 0.1, and 0.02 unit of penicillin G per ml (A). Tests were done by Alice Reyn on gonococcus resistance plates in a series with known concentrations of penicillin (B). Saline suspensions of bacteria were inoculated on 5% horse blood agar plates containing 1 and 0.1 unit of penicillin G per ml; they were incubated overnight (C).

Bile sensitivity. Overnight incubation was carried out on 5% horse blood agar ditch plates with 10% and 40% ox bile (C) to test bile sensitivity.

Phosphatase production. Phosphatase production was determined with 0.01% phenolphthalein phosphate in nutrient agar. After 2 days of incubation, it was exposed to ammonia (C).

Esculin hydrolysis. Esculin hydrolysis was tested on esculin/ferric chloride agar, with 10 days of incubation (C).

Casein digestion. Casein digestion was determined with 5% casein in nutrient agar plates, which were examined for clearing 5 days after inoculation (C).

Egg yolk opacity. Egg yolk opacity was tested with 1% egg yolk in nutrient agar after incubation for 5 days (C).

Tween hydrolysis. Tween hydrolysis was determined with 0.5% Tween in nutrient agar plus 0.01% CaCl_2 (20); the inoculated medium was examined for precipitation after 5 days (C).

RESULTS

Eight of the strains were oxidase negative, both with the dimethyl- and tetramethyl-*p*-phenylenediamine reagents. Other results obtained with these strains are shown in Table 2. The results are compatible with their classification as *Acinetobacter* strains, and therefore these strains were not studied further.

Results obtained with the remaining 28 strains are presented in Table 3. All of these strains gave positive oxidase reactions with both reagents. None of the strains attacked insulin or

TABLE 2. *Characters of oxidase-negative, gram-negative strains^a*

Characters	Strains							
	Pintér 16	Pintér 21	Henderson 55	Henderson 69	NCIB 9021	NCIB 9300	NCMB 168	MJT/F4/5, 7
Size	sl	p	s	p	s/p	s	s	s
Shape	r	o	r	o/r	O/r	r	c/r	c
Arrangement	wd	d	d	d	wd	-	d	d
Colony size	s	s	s	s	s	m/l	s	s
Phase (M, S)	S	S	S	S	S	S	S	S
Growth, room temp	+	+	+	+	+	+	+	+
Growth, 35 C	w	+	+	-	+	+	-	-
Glucose	-	-	+	-	-	+	w	-
O/F	-	O	-	-	-	O	O	-
Arabinose	-	w	+	-	-	+	-	-
Galactose	-	-	+	-	-	w	w	-
Xylose	-	-	+	-	-	w	w	-
Lactose	-	-	w	-	-	w	-	-
Urease	-	-	-	-	-	w	-	-
Nitrate	-	-	-	-	-	-	-	+
Citrate	-	-	+	-	+	+	w	-
Penicillin	M	M	R	M	M	M	M	S

^a Symbols for penicillin: S, sensitive; M, moderate to low sensitivity; R, resistant. Other symbols as in Table 3. The following reactions were uniformly negative: attack on sugars other than aldoses, indole, phenylalanine deaminase, ONPG, H₂S, gelatin liquefaction. The catalase reaction was uniformly positive.

inositol or decarboxylated amino acids. All strains grew at 30 C and none of them grew at 45 C. They were all nonmotile.

A striking feature of Table 3 is the considerable number of discrepancies between the results obtained in different laboratories. Some of these discrepancies, e.g., evaluation of size and shape of the bacterial cells and appearance of the colonies, can be explained by the purely subjective nature of the observations.

Other discrepancies are quite obviously due to differences in technique and/or media, e.g., the tests for H₂S production and phenylalanine deamination. The phenylalanine test showed the largest number of differences, mainly because the method used in laboratory A is more sensitive than those used in laboratories B and C. Even in laboratory A, however, most of the positive reactions were weak, far weaker than reactions given by *Proteus* strains, and probably of little significance in taxonomy. When the strains which gave weak positive reactions with phenylalanine were tested for deamination of tryptophan, they gave negative reactions. Only the strains A348, A351, A353, NCMB 307, NCMB 131, and NCMB 200 deaminated both amino acids, and with much stronger reactions than any of the other strains. When laboratories B and C reported negative results with these strains, it became necessary to investigate this matter further. In repeated tests with these two reactions under varied conditions, suspensions of these strains from

blood agar gave stronger reactions than suspensions from nutrient agar (Mueller-Hinton medium), which sometimes gave negative reactions. Suspensions from 2- to 3-day-old cultures gave stronger reactions than suspensions from 24-h-old cultures, which sometimes gave very weak or negative reactions. Finally, very heavy suspensions of the strains were needed to obtain strong positive results by the method used in laboratory A. When attention was paid to these experimental conditions, the more strongly positive strains mentioned above gave stronger deaminase reactions than the other strains, although not quite as strong as a *Proteus* control strain.

The discrepancies in the tests for anaerobic growth (a difference between no growth in one laboratory and minimal growth in another), gelatin liquefaction, and nitrate reduction can probably also be ascribed chiefly to differences in techniques and media.

The variation in assessment of pigment production may be due partly to different media and incubation times. When the discrepancies became known, tests for pigment production were repeated in laboratory A, using nutrient agar slopes and blood agar plates with incubation extended for 1 week or more. A few of the discrepancies were eliminated by this procedure. The repeated tests appeared to confirm pigment production by all strains which have received the symbols "+" or "+/-" in Table 3, except strain A365, in which case

TABLE 3. Characteristics of oxidase-positive, *Moraxella*-like, gram negative organisms^a

Characteristic	Participating laboratories	Yellow-pigmented strains										Unpigmented mesophilic strains				Unpigmented psychrophilic strains								Previously described strains							
		WM17 (A, C) ^b	A317 (A, C) ^b	WM27 (A, B) ^b	WM28 (A, B) ^b	WM34	A326	A331	A338	A163	A259	A285	A272	A273	WM32	A280	A332	A365	A333	A348	A351	A353	NCMB 98		NCMB 307	NCMB 131	NCMB 200	A366	Bljsterveld	Sutton	
Size	A, C	d	d	d	d	s	s/sl	s/sl	s	s/sl	s/sl	s/sl	s	s	s	s	s	s	s	c/o	c/o	c/o	c/o	c/r	c/o	d	s	d	sl		
Shape	All	c	c	r	r	r	r	r	d	d	d	d	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	r		
Arrangement	A	d	d	p	p	p	p	p	d	d	d	d	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	r	r		
Colony size	A, B	s	s	m	m	l	l	l	m	m	m	m	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	d	d		
Phase (M, S)	All	M	M	M	M	S	S	S	S/M	S/M	S/M	S/M	S	S	S	S	S	S	S	S/M	S	S	S	S	S	S	S	d	d		
Pigment	All	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	m/l	m/l		
Pitting	A, C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	M	M		
Twitching	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Growth, 5 C	C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Growth, 20 C	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Growth, 35-37 C	All	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Growth, 40 C	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Growth, 42 C	A	w	w	w	w	w	w	w	w	w	w	w	w	w	w	w	w	w	w	w	w	w	w	w	w	w	w	w	w	w	
Without O ₂	A, C	w	w	w	w	w	w	w	w	w	w	w	w	w	w	w	w	w	w	w	w	w	w	w	w	w	w	w	w	w	
Glucose	All	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
O/F	A, C	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
Mannose	A, B	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Galactose	A, B	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fructose	A, B	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Arabinose	A, B	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Xylose	A, B	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Rhamnose	B	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lactose	All	w/+	w/+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Maltose	All	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sucrose	All	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Melibiose	B	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cellobiose	B	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Melezitose	B	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trehalose	All	+	+	w	w	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Raffinose	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mannitol	All	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

pigment production was not detected. The strains WM17 and A317 showed marked lemon-yellow pigmentation after growth on blood agar in a humid atmosphere for 5 to 6 days. The many discrepancies between the results of fermentation tests may be due partly to the nutritional inadequacy of Hugh and Leifson's medium, partly to the use of additional media in some laboratories, and partly perhaps to the fact that some strains produced extremely small quantities of acid. Some discrepancies must, however, be attributed to technical errors or mistakes.

Strain A333 showed marked test differences between laboratories, especially in the production of acid from aldose sugars. Repeat tests showed that growth of this strain was much better at room temperature than at 35 or 37 C and that the strain produced acid from aldose sugars only at temperatures below 35 C.

The differences in the indole reactions observed are disturbing. These reactions were repeated several times in laboratory A, and positive reactions were obtained each time for those strains which showed differences between the laboratories in Table 3. The reactions were weak, however, and the color appeared more yellow than usual, although distinctly different from the negative control strains. The identity of the substance giving these color reactions has not been determined.

The first results for penicillin sensitivity (not shown in Table 3) were very inconsistent, and the results from laboratory A showed much higher sensitivities than those of the other laboratories. Repeated tests in laboratory A with fluid media containing penicillin instead of the filter paper disk method reduced or eliminated some of the discrepancies. Thus, the diffusion method did not appear to be suitable for such fastidious and slowly growing organisms as some of the strains in the collection.

DISCUSSION

The results indicate that many bacteriological tests are highly dependent upon the techniques used. This is particularly true of tests for production of H₂S and deamination of phenylalanine. In the original description of the phenylalanine test (9), certain modifications of the procedure were shown to result in weak positive reactions with some organisms which otherwise gave negative reactions. This is unimportant when the test is used for members of the family *Enterobacteriaceae*, where the test is most useful for taxonomic purposes and

where moderate variations of the technique do not appear to alter the results. But with organisms such as those studied in this work, the weak to moderate reactions are quite confusing. The results suggest that deamination of tryptophan, which does not appear to give such weak reactions, may be preferable or that both tests should be used in taxonomic work outside the family *Enterobacteriaceae*.

The strains listed in Table 3 can be subdivided into several groups which are more or less uniform. The strains WM17 and A317 are very similar. They are both plump cocci which do not produce rod-shaped cells even when growing in the presence of subinhibitory concentration of penicillin (10). Both produce mucoid colonies that, after growth on blood agar for several days, develop a lemon-yellow pigment. They oxidize a wide range of carbohydrates, including the alcohols sorbitol and dulcitol, split urea, and store poly-beta-hydroxybutyrate granules. These strains resemble neisseriae to some extent—in cell shape, pigment production, oxidase reaction, and oxidative attack on sugars—but they differ from *Neisseria* species in attack on sugar alcohols, urease production, and in storing poly-beta-hydroxybutyrate. It seems probable that they are not closely related to neisseriae.

The strains WM27, WM28, WM34, A326, A331, A338, A163, A259, A285, A272, and A273 appear to form a group with some common features. They are in general small, slender organisms which were found to produce pigment in at least two of the laboratories. With two exceptions (A331 and A338), they give color reactions with Kovacs' indole reagent (some discrepancies). But there are also marked differences between the strains, except for strains WM27 and WM28, which appear to be closely interrelated, and strains A272 and A273, which are nearly identical and appear to form another sub-group together with strain A285. The differences from moraxellae in pigmentation, cell size, biochemical reactions, and response to penicillin make it unlikely that these strains are closely related to moraxellae. More probably they could be fitted into the ill-defined genus *Flavobacterium*.

The unpigmented strains WM32, A280, A332, and A365 also have small cells which do not resemble moraxellae. Three of them (WM32, A280, and A332) are apparently closely interrelated, whereas the fourth (A365) may be different. It is unlikely that any of these strains will eventually prove to belong to the genus *Moraxella*. Possibly, they may also be found to fit into the genus *Flavobacterium* in spite of their lack of yellow pigment.

The unpigmented strains A333, A348, A351, A353, A366, NCMB 98, NCMB 307, NCMB 131, and NCMB 200 show very slight or no growth at 35 to 37 C. All grow well at room temperature and all except A333 and A366 grow at 5 C. With some exceptions, as indicated, the strains have the following main characteristics: plump cells in diplo-arrangement (A366 appeared to have somewhat smaller cells, laboratory A), urease production (except A333), fairly strong tryptophan deamination (except A333, NCMB 98, and A366), and reasonable sensitivity to penicillin (NCMB 200 and A366 slightly less sensitive). Strain A366 is exceptional in having markedly mucoid colonies and in storing poly-beta-hydroxybutyrate, and it appears different. Like acinetobacters, seven strains oxidized all the aldose sugars tested but did not attack any other sugars. These strains corresponded to Thornley's (23) Phenon 3 and appear to represent a rather homogeneous and well-defined group. The two remaining strains (NCMB 131 and NCMB 200) do not attack sugars and are the two strains in the collection showing the greatest resemblance to moraxellae. These psychrophilic strains may be related to *Acinetobacter*, as suggested by Thornley (23), or to *Moraxella*, or may represent an intermediate group between the two genera. Further taxonomic studies of these organisms are needed.

The strains of Bijsterveld (1) and of Sutton et al. (21) were both isolated from eye infections, and the results indicate that they are nearly identical. They differ from moraxellae in morphology, as they produce rather long slender rods, and by their attack on several sugars and production of indole, although they are sensitive to penicillin. These strains should not be classified as moraxella without genetic evidence, and may represent a new species, which might, perhaps, be classified in one of the genera of *Brucellaceae*, or possibly in a genus of their own.

The oxidase-negative strains were classified as acinetobacters and are not, therefore, further discussed in this paper.

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REPRINT REQUESTS

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