IDENTIFICATION OF BACTERIA

Characterisation of aerobic gram-negative bacteria from subgingival sites of dogs – potential bite wound pathogens

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Ninety-eight aerobic, gram-negative bacterial isolates from subgingival samples from family-owned dogs with naturally occurring periodontitis were characterised phenotypically by conventional biochemical testing, by cellular fatty acid profiling and by the use of commercial identification systems. The majority (48, 81%) of the fermentative isolates but only 18% of the non-fermenters were identified by conventional biochemical testing alone. With additional cellular fatty acid profiling, another 7 (12%) fermentative and 23 (59%) non-fermentative isolates were identified to genus or group level. Cellular fatty acid analysis was essential for the identification of most non-fermenters, many of which are difficult to identify due to a paucity of positive reactions in routine biochemical tests. Commercial identification systems were less useful and did not contribute to further identification of these problematic isolates. This study underlines the difficulties encountered in the identification of canine oral bacteria – a group of potential bite wound pathogens – and presents schemes for microbiology laboratories to characterise such isolates.

Introduction

Aerobic, gram-negative bacteria of the dog oral flora have been recognised as pathogens (e.g., *Pasteurella*, CDC group EF-4, *Neisseria weaveri*) in infected dog bite wounds in man [1, 2] and may also be involved in the initiation and development of periodontal disease in dogs [3]. At present scant information on the composition of the aerobic canine oral flora [3–5] and minimal information on the detailed phenotypic characteristics of the individual organisms is available. Routine clinical microbiology laboratories are not generally proficient in species-level identification of veterinary bacteria, as the identification methods available are primarily concerned with identification of human isolates.

The present study phenotypically characterised the aerobic gram-negative bacteria isolated from subgingi-

val sites of 16 pet dogs suffering from naturally occurring periodontitis. Special attention was paid to the identification of recently described or reclassified canine organisms, including *Moraxella canis* [6], *P. canis*, *P. dagmatis*, *P. stomatis* [7], CDC group NO-1 [8] and *N. weaveri* (formerly CDC group M-5) [9, 10]. The utility of conventional biochemical testing, cellular fatty acid profiling and commercial identification systems for identification of canine isolates was assessed with a goal to devise guidelines, for use by clinical and veterinary microbiology laboratories, to facilitate the recognition and identification of these bacteria.

Materials and methods

Bacterial isolates

Ninety-eight aerobic, gram-negative isolates from the Department of Bacteriology Culture Collection, National Public Health Institute (KTL), Helsinki, Finland, obtained from diseased (n = 32) and healthy (n = 16) subgingival sites from 16 pet dogs with naturally occurring periodontitis, were studied. Detailed information about the dogs included in the study and the methods used for bacteriological sampling and culture,

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as well as the criteria and parameters used for clinical and radiographic diagnosis of periodontitis have been presented previously [11].

Reference strains

The following reference strains were included in the study for comparison: *P. canis*, American Type Culture Collection (ATCC) 43326^T (Culture Collection, University of Göteborg (CCUG) 12400); *P. dagmatis*, ATCC 43325^T (CCUG 12397); *P. stomatis*, ATCC 43327^T (CCUG 17979); Bisgaard's taxon 16, CCUG 17204; *Cardiobacterium hominis*, ATCC 15826^T (CCUG 2711); CDC group EF-4a, ATCC 29858 (CCUG 11627); CDC group EF-4b, ATCC 29859 (CCUG 11628); CDC group NO-1, CDC G1448 (CCUG 31239); *M. canis*, Culture Collection, Laboratorium voor Microbiologie, Universiteit Gent, Ghent, Belgium (LMG) 11194^T (CCUG 8415); *M. catarrhalis*, ATCC 25238^T (CCUG 353); *N. elongata* subsp. *elongata*, ATCC 25295^T (CCUG 2130); *N. elongata* subsp. *glycolytica*, ATCC 29315^T (CCUG 6508); *N. elongata* subsp. *nitroreducens*, ATCC 49377^T (CCUG 30802); *N. weaveri*, ATCC 51410^T (CCUG 4007) and *Bergeyella zoohelcum*, ATCC 43767^T (CCUG 30535).

Conventional characterisation

Initial characterisation was based on colonial morphology, cellular morphology through Gram staining, spot oxidase reaction (Kovac's method) with the tetramethyl-*p*-phenylenediamine dihydrochloride reagent, production of catalase upon addition of H₂O₂ 3%, ability to grow on MacConkey agar (Difco Laboratories, Detroit, USA) and reaction in Hugh and Leifson's low-peptone oxidation-fermentation basal medium (enteric OF) containing glucose and bromothymol blue [12]. Additional tests included assessment of motility by hanging-drop preparations; production of indole by the spot test from Brucella agar plates [13, 14], and by xylene extraction from pre-reduced indole-nitrate broth medium [15] with Ehrlich's reagent; production of urease in Christensen's agar medium [12], which contained phenol red (6 mg/L) and was supplemented with neutralised liver digest (Oxoid) 100 mg/L, and glucose 0.1% w/v; hydrolysis of gelatin in Edelstein's gelatinase test medium [16] and nitrate reduction in pre-reduced nitrate broth medium [15]. The ability to ferment carbohydrates was determined in phenol red broth medium [12] supplemented with calf serum, except for maltose, which was supplemented with rabbit serum because of false positive reactions with the former [17]. Arginine dihydrolase (ADH), lysine and ornithine decarboxylase, β -galactosidase (ONPG) and γ -glutamyl-aminopeptidase activities, and aesculin and tributyrin hydrolysis were determined with diagnostic tablets (Rosco Diagnostica, Taastrup, Denmark). Arginine dihydrolase and ornithine decarboxylase activities were also determined in decarboxylase basal medium modified from Moeller

[16]. Production of gas from glucose and production of extracellular DNAase on DNAase test agar (Difco) was recorded when appropriate.

Tests for oxidation-fermentation, production of urease, gelatin hydrolysis and fermentation of carbohydrates were observed for up to 10 days.

Commercial identification systems

These were API 20 E and API 20 NE (bioMérieux sa, Marcy-l'Etoile, France) and RapID NF Plus and RapID NH (Remel, Norcross, GA, USA).

Cellular fatty acid profiling

Cellular fatty acids were extracted and analysed with the Microbial Identification System (MIS; Hewlett-Packard and MIDI, Newark, DE, USA) and the CLIN Library software, version 3.8 (MIDI). Isolates were subcultured on trypticase soy agar plates with sheep blood 5% and incubated in a CO₂ 5% atmosphere at 35°C for 24 h. The growth from the most dilute quadrant showing confluent growth was harvested. Saponification, methylation of fatty acids, extraction of the methyl ester derivatives and base wash were performed according to the manufacturer's instructions. The fatty acid methyl esters were separated on a fused silica capillary column (Ultra-2) with a Hewlett-Packard 5890 gas chromatograph equipped with a flame ionisation detector. Identifications were confirmed by mass spectrometry with a Hewlett-Packard 5971A mass selective detector connected to an HP 5890 gas chromatograph equipped with an HP-5 capillary column. Each identification proposed by the MIS was accompanied by a similarity index (SI) which is a numerical value (range 0.000–1.000) that indicates how closely the fatty acid composition of an unknown compares with the best matching library entry (entries).

Identification

The identification of each isolate was based on generally accepted criteria and on newly described characteristics [6–10, 18–23]. The flow chart used for identification is depicted in Table 1.

Results

The 98 canine isolates represented 20 different species or groups and included five unidentified isolates. Fifty-nine isolates (60%) were fermentative and 39 (40%) were non-fermentative. The majority (48, 81%) of the fermentative isolates were identified to species or group level by conventional biochemical testing and two isolates (enterobacteria) by the API 20 E system. Nine isolates (15%) required additional cellular fatty acid profiling, of which two remained unidentified. Only 18% of the non-fermentative isolates were identified by

Table 1. Flow chart for identification of gram-negative canine oral bacteria

Characteristic	Organism*
Gram-negative coccus	
Tributyrin +	
DNAase +	
γ -glutamylaminopeptidase +	<i>Moraxella canis</i>
γ -glutamylaminopeptidase –	<i>M. catarrhalis</i>
Gram-negative coccobacillus/gram-negative bacillus	
OF: F	
oxidase +	
catalase +, MCC –	
indole +	
ODC +	<i>P. canis</i> biotype 1
ODC –	<i>P. dagmatis</i> (urease +)
	<i>P. stomatis</i> (urease–, ONPG–)
	Bisgaard's taxon 16 (urease–, ONPG +)
	<i>Cardiobacterium hominis</i> , canine isolates (CFA)
	CDC group EF-4a
indole –	
catalase –	
indole +	<i>C. hominis</i> , ATCC 15826 ^T
oxidase –	
non-fastidious	Enterobacterium (API 20 E)
OF: NR	
oxidase +	
indole +	<i>Bergeyella zoohelcum</i> (urease +)
indole –	<i>Moraxella</i> spp. (CFA)
	<i>Neisseria</i> spp. (CFA)
oxidase –	CDC group NO-1 (nitrate reduction +, CFA)

F, fermentative in oxidation-fermentation medium (OF); MCC, MacConkey agar; ODC, ornithine decarboxylase; NR, non-reactive (non-fermentative, non-oxidative) in OF; CFA, cellular fatty acid analysis; ^T, type strain.

*Tests required for further identification are indicated in parentheses.

conventional biochemical testing alone. Most non-fermenters (82%) required extended characterisation by cellular fatty acid profiling, but nine isolates (23%) remained unidentified. By such profiling, most (87%) of the fermentative bacteria were identified to genus or group level, but most (as indicated above) did not require fatty acid analysis for identification. The detailed results obtained are compiled in Tables 2–9. The aetiological implications of the findings, with regard to canine periodontitis, will be presented in a separate paper.

Pasteurella and Bisgaard's taxon 16

Organisms belonging to the species *P. canis*, *P. dagmatis* and *P. stomatis* were identified by conventional biochemical testing by using the tests shown in Table 2. The ability to ferment maltose and trehalose varied within some of the species. Two isolates designated *Pasteurella*-like exhibited characteristics not consistent with the genus, including β -galactosidase activity (ONPG-positive) and acid production from lactose. However, the cellular fatty acid composition was consistent with that of the genus *Pasteurella* (Table 3). The most abundant fatty acids detected in all members of the genus were 14:0, 16:1 ω 7c, and 16:0, comprising c. 17, 35 and 29% of the total fatty acids, respectively. The only hydroxy fatty acid detected was 14:0(3OH) ($\approx$ 9%). Isolates identified as Bisgaard's taxon 16 were phenotypically similar to *P. stomatis* (Table 2). Differentiation was based on the β -galactosidase activity, present only in the former. The predominant cellular fatty acids of the Bisgaard's taxon

16 isolates were similar to those of the genus *Pasteurella* (Table 3).

Cardiobacterium and *enterobacteria*

The isolates designated *C. hominis*-like formed cigar-shaped cells with a cellular fatty acid profile most similar to that of the *C. hominis* type strain (Table 4). Major fatty acids detected were 14:0, 16:0, and 18:1, comprising c. 14, 24 and 41% of the total acids, respectively (Table 2). A small amount of 12:0(3OH) ($\approx$ 2%) was present only in the canine isolates, while 16:0(3OH) ($\approx$ 2%) was present only in the type strain (Table 4), which also produced acid from maltose, mannitol and sorbitol, a feature absent from the canine isolates (Table 2). The canine isolates also consistently produced catalase, a feature present in only 1% of human *C. hominis* isolates [18]. Greening of the agar around colonies was present on sheep blood agar. The enterobacteria *Morganella morganii* and *Proteus vulgaris* were identified with API 20 E (data not shown).

Unclassified groups

Within CDC group EF-4a, the ability to hydrolyse gelatin and the ADH activity varied (Table 2); 11 (50%) of the isolates were positive for both of these reactions, whereas 9 (41%) were negative. Two isolates hydrolysed gelatin but exhibited no ADH activity. All isolates fermented glucose and were, therefore, regardless of their ADH activity, assigned to bivar 'a', according to generally accepted criteria [20]. All isolates reduced nitrate. Two cellular fatty acid profiles,

Table 2. Phenotypic features of selected fermentative and non-fermentative canine isolates, comparison with reference strains*

Test or characteristic	<i>P. canis</i> biotype 1 (12/5) [†]	<i>P. canis</i> ATCC 43326 ^T	<i>P. dagmatis</i> (3/3)	<i>P. dagmatis</i> ATCC 43325 ^T	<i>P. stomatis</i> (6/6)	<i>P. stomatis</i> ATCC 43327 ^T	<i>Pasteurella</i> - like (2/1)	Bisgaard's taxon 16 (5/3)	Bisgaard's taxon 16 CCUG 17204	<i>C. hominis</i> - like (5/5)	<i>C. hominis</i> ATCC 15826 ^T	CDC group EF-4a (22/22)	CDC group EF-4a ATCC 29858
Oxidase	+	+	+	+	+	+	+	+	+	+	+	+	+
Catalase	+	+	+	+	+	+	+	+	+	+	+	+	+
Growth on MacConkey agar	—	—	—	—	—	—	—	—	—	—	—	—	—
Oxidation-fermentation	F	F	F	F	F	F	F	F	F	F	F	F	F
Motility	—	—	—	—	—	—	—	—	—	—	—	—	—
Indole	+	+	+	+	+	+	+	+	+	+	+	—	—
Urease	—	—	+	+	—	—	—	—	—	—	—	—	—
Hydrolysis of aesculin	—	—	—	—	—	—	—	—	—	—	—	—	—
gelatin	—	—	—	—	—	—	—	—	—	—	—	v 13/22	+
Nitrate reduction	+	+	+	+	+	+	+	+	+	—	—	+	+
Gas from glucose	—	—	+	—	—	—	—	—	—	—	—	—	—
Acid from arabinose	—	—	—	—	—	—	—	—	—	—	—	—	—
dulcitol	—	—	—	—	—	—	—	—	—	—	—	—	—
fructose	+	+	+	+	+	+	+	+	+	+	+	—	—
galactose	+	+	+	+	+	+	+	+	+	—	—	—	—
glucose	+	+	+	+	+	+	+	+	+	+	+	+	+
lactose	—	—	—	—	—	—	+	—	—	—	—	—	—
maltose	—	+	+	+	v 4/6	+	—	v 4/5	+	—	+	—	—
mannitol	—	—	—	—	—	—	—	—	—	—	+	—	—
mannose	+	+	+	+	+	+	+	+	+	+	+	—	—
salicin	—	—	—	—	—	—	—	—	—	—	—	—	—
sucrose	+	+	+	+	+	+	+	+	+	+	+	—	—
sorbitol	—	—	—	—	—	—	—	—	—	—	+	—	—
trehalose	v 8/12	—	v 1/3	+	v 3/6	+	—	+	+	—	—	—	—
xylose	—	—	—	—	—	—	—	—	—	—	—	—	—
Arginine dihydrolase [§]	—	—	—	—	—	—	—	—	—	—	—	v 11/22	+
Lysine decarboxylase	—	—	—	—	—	—	—	—	—	—	—	—	—
Ornithine decarboxylase [§]	+	+	—	—	—	—	—	—	—	—	—	—	—
ONPG	—	—	—	—	—	—	+	+	+	—	—	—	—
Major cellular fatty acids (%)	14:0 (15)	14:0 (15)	14:0 (18)	14:0 (22)	14:0 (16)	14:0 (10)	14:0 (20)	14:0 (11)	14:0 (11)	14:0 (14)	14:0 (16)	16:1 ω 7c (29)	16:1 ω 7c (29)
	16:1 ω 7c (37)	16:1 ω 7c (35)	16:1 ω 7c (36)	16:1 ω 7c (35)	16:1 ω 7c (36)	16:1 ω 7c (40)	16:1 ω 7c (31)	16:1 ω 7c (39)	16:1 ω 7c (37)	16:0 (24)	16:0 (20)	16:0 (23)	16:0 (25)
	16:0 (30)	16:0 (29)	16:0 (28)	16:0 (29)	16:0 (28)	16:0 (26)	16:0 (28)	16:0 (29)	16:0 (30)	18:1 (41)	18:1 (47)	18:1 (23)	18:1 (22)

Table 2. (continued)

Test or characteristic	CDC group EFp4b ATCC 29859	CDC group NO-1 (7/7)	CDC group NO-1 CDC G1448	<i>Moraxella</i> spp (5/1)	<i>N. weaveri</i> - like (3/3)	<i>N. weaveri</i> ATCC 51410 ^T	<i>Neisseria</i> spp (6/6)	<i>B. zoohelcum</i> (3/3)	<i>B. zoohelcum</i> ATCC 43767 ^T	Non-fermentative 'green' (6/6)
Oxidase	+	v 3/7 [‡]	—	+	+	+	+	+	+	+
Catalase	+	+	+	+	+	+	+	+	+	+
Growth on MacConkey agar	—	—	w	—	—	—	—	—	—	—
Oxidation-fermentation	F	NF	NF	NF	NF	NF	NF	NF	NF	NF
Motility	—	—	—	—	—	—	—	—	—	—
Indole	—	—	—	—	—	—	—	+	—	—
Urease	—	—	—	—	—	—	—	+	+	—
Hydrolysis of aesculin	—	—	—	—	—	—	—	—	—	—
gelatin	—	v 1/7	—	—	—	—	v 1/6	+	+	+
Nitrate reduction	—	+	+	—	v 1/3	—	v 3/6	—	—	—
Gas from glucose	—	NT	NT	NT	NT	NT	NT	NT	NT	NT
Acid from arabinose	—	NT	—	NT	NT	—	NT	NT	—	NT
dulcitol	—	NT	—	NT	NT	—	NT	NT	—	NT
fructose	—	NT	—	NT	NT	—	NT	NT	—	NT
galactose	—	NT	—	NT	NT	—	NT	NT	—	NT
glucose	+	—	—	—	—	—	—	—	—	—
lactose	—	—	—	—	—	—	—	—	—	—
maltose	—	—	—	—	—	—	—	—	—	—
mannitol	—	—	—	—	—	—	—	—	—	—
mannose	—	NT	—	NT	NT	—	NT	NT	—	NT
salicin	—	NT	—	NT	NT	—	NT	NT	—	NT
sucrose	—	—	—	—	—	—	—	—	—	—
sorbitol	—	NT	—	NT	NT	—	NT	NT	—	NT
trehalose	—	—	—	—	—	—	—	—	—	—
xylose	—	—	—	—	—	—	—	—	—	—
Arginine dehydrolase	—	—	—	—	—	—	—	+	+	—
Lysine decarboxylase	—	—	—	—	—	—	—	—	—	—
Ornithine decarboxylase	—	—	—	—	—	—	—	—	—	—
ONPG	—	—	—	—	—	—	—	—	—	—
Major cellular fatty acids (%)	16:1 ω 7c (29)	16:1 ω 7c (20)	16:1 ω 7c (20)	16:1 ω 7c (26)	16:1 ω 7c (29)	16:1 ω 7c (30)	16:1 ω 7c (18)	15:0iso (54)	15:0iso (58)	15:0iso (40)
	16:0 (28)	16:0 (35)	16:0 (37)	16:0 (15)	16:0 (23)	16:0 (24)	16:0 (44)	iso17:1 ω 9c (9)	iso17:1 ω 9c (10)	iso17:1 ω 9c (14)
	18:1 (23)	18:1 (27)	18:1 (25)	18:1 ω 9c (23)	18:1 (24)	18:1 (24)	—	17:0iso3OH (12)	17:0iso3OH (12)	—

*T, type strain; +, all isolates positive; —, all isolates negative; v, variable reaction; w, weak growth; F, fermentative; NF, non-fermentative; NT, not tested.

[†]Number of clinical isolates tested (biochemically/by cellular fatty acid analysis).

[‡]Number of isolates positive of all isolates tested.

[§]Both methods used (diagnostic tablets and broth) produced identical results.

^{||} β -Galactosidase.

Table 3. Cellular fatty acid composition of canine *Pasteurella* and Bisgaard's taxon 16 isolates, comparison with reference strains

Organism*	Percent (SD) of total fatty acids†													
	16:1iso‡	12:0	14:0	Δ² 14:1§	14:0(3OH)¶	16:1ω7c	16:0	17:1	18:2** ω6,9c	18:1ω9c	18:1†† ω7c/9t/12t	18:0	20:1ω9t	20:0
<i>P. canis</i> (5)	...	...	14.82 (1.08)	...	8.64 (0.72)	37.29 (1.21)	29.62 (1.48)	...	...	1.85 (0.27)	1.95 (0.42)	4.07 (0.55)	...	...
<i>P. canis</i> ATCC 43326 ^T	...	...	15.36	1.18	8.64	35.37	28.63	1.28	1.90	2.63	1.58	3.43	...	...
<i>P. dagmatis</i> (3)	...	...	17.88 (0.41)	1.03 (0.17)	8.79 (0.15)	36.39 (1.95)	28.27 (1.87)	...	...	1.43 (0.22)	1.76 (0.50)	3.39 (1.25)	...	...
<i>P. dagmatis</i> ATCC 43325 ^T	...	...	21.59	...	9.77	34.79	28.59	...	...	...	...	1.69	...	...
<i>P. stomatis</i> (6)	...	1.30 (1.45)	15.55 (3.81)	...	8.26 (1.24)	36.17 (6.01)	28.40 (2.03)	...	...	1.71 (0.74)	2.05 (1.07)	3.36 (1.54)	...	...
<i>P. stomatis</i> ATCC 43327 ^T	1.4	...	9.76	1.06	7.41	39.91	26.45	...	1.51	1.52	4.49	4.28	2.21	...
<i>Pasteurella</i> -like (1)	3.91	3.3	19.65	1.12	6.84	30.54	27.83	...	...	1.8	1.13	2.27	...	...
Bisgaard's taxon 16 (3)	...	...	10.80 (1.25)	...	8.39 (0.21)	38.53 (2.12)	28.78 (2.56)	...	...	...	4.01 (1.45)	5.09 (1.11)	1.99 (1.23)	...
Bisgaard's taxon 16 CCUG 17204	...	...	11.45	...	8.80	36.63	29.56	...	...	...	3.31	7.08	1.85	1.32

*Numbers in parentheses indicate number of strains analysed, ^T = type strain.
†Values are arithmetic means when more than one isolate was analysed, only values ≥1.00 are shown.
‡Summed feature 3 by the MIS.
§14:0(3OH) breakdown product, unknown 14.503 by the MIS.
||Summed feature 5 by the MIS.
**Summed feature 6 by the MIS.
††Summed feature 7 by the MIS.

Table 4. Cellular fatty acid composition of canine *C. hominis* and CDC group EF-4 isolates, comparison with reference strains

Organism*	Percent (SD) of total fatty acids†														
	12:0 (3OH)	12:0	14:0 (3OH)	14:0	15:0	14:0* (3OH)	16:1ω7c	16:1ω5c	16:0	17:1ω8c	17:1ω6c	16:0 (2OH)	16:0 (3OH)	18:1§ ω7c/9t/12t	18:0
<i>C. hominis</i> -like (5)	6.42 (1.31)	2.14 (0.23)	13.57 (2.14)	...	...	2.04 (0.20)	8.07 (2.96)	...	24.15 (3.90)	...	...	...	...	41.37 (3.16)	1.17 (0.32)
<i>C. hominis</i>	8.39	...	15.92	...	...	2.63	3.96	...	19.79	...	...	...	2.10	46.79	...
ATCC 15826 ^T	5.90 (0.34)	3.59 (0.34)	6.34 (0.52)	1.84 (0.66)	2.64 (0.30)	2.64 (0.30)	30.79 (1.43)	1.56 (0.13)	20.78 (1.48)	1.11 (0.41)	1.63 (0.70)	...	...	20.57 (1.86)	...
CDC group EF-4a, ADH + (11)	5.57 (0.19)	3.80 (0.28)	3.25 (0.44)	...	...	3.17 (0.41)	27.20 (1.51)	1.27 (0.50)	25.71 (2.15)	...	...	2.06 (0.58)	...	25.46 (3.03)	...
CDC group EF-4a, ADH - (11)	6.14	4.24	4.72	...	...	3.77	28.99	1.56	25.38	...	...	...	...	22.32	1.28
ATCC 29858 ^T	5.72	4.13	3.49	...	...	3.18	28.91	1.10	27.53	...	...	1.61	...	23.29	...
CDC group EF-4b															
ATCC 29859 ^T															

*Numbers in parentheses indicate number of strains analysed, [‡] = type strain.†Values are arithmetic means when more than one isolate was analysed, only values ≥ 1.00 are shown.[‡]Summed feature 3 by the MIS.[§]Summed feature 7 by the MIS.

which correlated with the ADH activity, were detected. The profiles were different by qualitative and quantitative analysis (Table 4). Fatty acids 15:0, 17:1ω8c and 17:1ω6c were detected only in the ADH-positive organisms, whereas 16:0(2OH) was detected only in the ADH-negative organisms, which also exhibited smaller quantities of 14:0. The predominant fatty acids detected in both groups were 16:1ω7c (≈29%), 16:0 (≈23%) and 18:1 (≈23%) (Table 2). The ADH-positive isolates showed a profile distinct from those of the CDC group EF-4a and CDC group EF-4b type strains, whereas the profile of the ADH-negative isolates was similar to that of the latter type strain. The cellular fatty acid profiles of the type strains were similar with only minor qualitative differences detected; fatty acid 18:0 was present only in CDC group EF-4a and 16:0(2OH) only in the CDC group EF-4b type strain. Also, both type strains fermented glucose but the CDC group EF-4b type strain was negative for nitrate reduction, gelatin hydrolysis and ADH activity.

Isolates designated CDC group NO-1-like most closely resembled the genus *Acinetobacter* by cellular morphology and by biochemical reactions. However, the ability to reduce nitrate and the cellular fatty acid profile, consistent with CDC group NO-1, distinguished them from this genus (Tables 2 and 5). The predominant fatty acids detected were 16:1ω7c, 16:0 and 18:1, which constituted *c.* 20, 35 and 27% of the total fatty acids, respectively (Table 2). Three isolates were oxidase positive; variability was also observed in the ability to hydrolyse gelatin (Table 2). Furthermore, the CDC group NO-1-like isolates were characterised by RapID NF Plus and API 20 NE; however, these systems do not include CDC group NO-1 in their databases. The profiles obtained are shown in Table 9.

Moraxella

The cells of *M. canis* and of the organism designated *M. catarrhalis*-like were cocci that occurred in pairs, the latter also in tetrads. Both organisms were non-fermentative and positive for catalase, oxidase and DNAase activity, and both hydrolysed tributyrin (data not shown). γ -Glutamyl-aminopeptidase activity was present only in *M. canis*. The cellular fatty acid profiles, characterised by large amounts of 18:1ω9c, were different by qualitative and quantitative means as outlined in Table 6. Additional characterisation with RapID NF Plus and RapID NH, which include *M. catarrhalis* but not *M. canis* in their profile indexes, generated profiles for which no valid identifications were obtained (Table 9). The type strains LMG 11194 (*M. canis*) and ATCC 25238 (*M. catarrhalis*) exhibited features (catalase, oxidase, DNAase, tributyrin, γ -glutamyl-aminopeptidase) identical to those of the canine isolates (data not shown). However, no identifications were obtained for the type strains with use of RapID NF Plus (Table 9) and RapID NH (data not shown). The cellular fatty acid profile of the canine *M.*

Table 5. Cellular fatty acid composition of canine CDC group NO-1 and non-fermentative *Neisseria* isolates, comparison with reference strains

Organism*	Percent (SD) of total fatty acids†													
	10:0 (3OH)	12:0	12:0 (3OH)	14:0	15:0	14:0‡ (3OH)	16:1ω7c	16:1ω5c	16:0	16:0 (3OH)	18:2§ ω6,9c	18:1ω9c	18:1 ω7c/9t/12t	18:0
CDC group	4.25 (0.49)	...	3.27 (0.31)	6.97 (1.22)	...	...	20.42 (4.51)	...	34.81 (2.88)	...	...	...	27.34 (5.80)	1.22 (0.49)
NO-1-like (7)														
CDC group	4.16	...	2.85	8.15	...	...	19.60	...	37.35	...	...	...	24.93	...
NO-1 CDC G1448														
<i>N. weaveri</i> -like (3)	...	5.51 (0.06)	3.53 (0.09)	6.08 (0.12)	...	2.71 (0.18)	29.48 (2.35)	1.96 (0.30)	22.68 (1.77)	...	...	1.08 (0.26)	24.35 (1.16)	1.14 (0.10)
<i>N. weaveri</i>	...	5.53	4.30	5.78	...	3.44	29.71	1.76	24.10	...	...	...	24.42	...
ATCC 51410 ^T														
<i>Neisseria</i> spp. (6)	...	6.74 (0.21)	7.45 (0.23)	7.65 (2.21)	...	...	18.42 (3.23)	...	43.54 (2.41)	1.25 (0.27)	1.81 (0.65)	3.75 (1.90)	7.19 (2.62)	1.15 (0.13)
<i>N. elongata</i> subsp.	...	5.50	4.64	4.99	1.46	3.92	32.38	...	33.85	1.22	...	...	10.84	1.20
<i>elongata</i> ATCC														
25295 ^T														
<i>N. elongata</i>	...	5.69	4.44	3.64	1.12	3.99	31.90	...	36.34	...	...	...	10.29	1.13
subsp. <i>glycolytica</i>														
ATCC 29315 ^T														
<i>N. elongata</i>	...	5.79	4.51	5.62	2.11	3.75	32.75	...	30.62	1.37	...	...	11.53	1.02
subsp. <i>nitroreducens</i>														
ATCC 49377 ^T														

*Numbers in parentheses indicate number of strains analysed, ^T = type strain.

†Values are arithmetic means when more than one isolate was analysed, only values ≥1.00 are shown.

‡Summed feature 3 by the MIS.

§Summed feature 6 by the MIS.

||Summed feature 7 by the MIS.

Table 6. Cellular fatty acid composition of canine *Moraxella* isolates, comparison with reference strains

Organism*	Percent (SD) of total fatty acids†							
	10:0	12:0	Δ^2 12:1‡	12:0 (3OH)	14:0	14:0 (3OH)§	16:0 Nalcohol	16:1 ω 9c
<i>M. canis</i> (3)	3.00 (0.29)	2.88 (0.29)	1.00 (0.02)	5.69 (0.55)	...	2.71 (0.33)	...	...
<i>M. canis</i> LMG 11194 ^T	2.38	2.80	...	4.34	...	2.12	...	...
<i>M. catarrhalis</i> -like (1)	1.44	10.04	1.30	10.45	2.60	...	...	2.61
<i>M. catarrhalis</i> ATCC 25238 ^T	3.33	2.5	...	4.04	...	...	...	...
<i>Moraxella</i> sp. (1)	7.11	...	...	9.59	2.20	...	1.61	...
<i>Moraxella</i> -like								
A	...	6.75	...	5.28	...	2.16	...	1.43
B	1.33	5.70	...	5.72	1.60	2.27	3.21	...

Table 6. Continued

Percent (SD) of total fatty acids†									
16:0	17:1 ω 8c	18:3 ω 6c	18:2 ω 6,9c	18:1 ω 9c	18:1 ω 7c/9t/12t**	18:0	summed feature 8††	20:4 ω 6, 9,12,15	
5.83 (2.43)	3.65 (0.18)	...	2.66 (0.43)	...	66.03 (2.21)	...	4.90 (0.87)	...	...
6.76	4.97	...	2.96	...	65.75	...	7.04	...	...
8.98	13.75	1.04	1.23	4.06	33.67	2.79	5.11	...	...
2.38	6.85	1.89	4.1	...	62.43	...	8.22	...	...
26.31	15.41	...	...	7.30	23.46	...	4.92	...	1.14
12.26	3.95	4.35	1.53	3.57	50.64	...	2.31	3.15	...
6.45	16.76	1.06	2.66	10.56	29.94	...	10.57	...	1.26

*Numbers in parentheses indicate number of strains analysed, ^T = type strain.

†Values are arithmetic means when more than one isolate was analysed, only values ≥ 1.00 are shown.

‡12:0 (3OH) breakdown product, unknown 12.486 by the MIS.

§Summed feature 3 by the MIS.

||Summed feature 6 by the MIS.

**Summed feature 7 by the MIS.

††Unknown 18.756 or fatty acid 19:1.

canis isolates and that of the *M. canis* type strain were essentially identical, whereas the profile of the canine *M. catarrhalis*-like isolate, although qualitatively similar, differed quantitatively from that of the *M. catarrhalis* type strain (Table 6).

Isolates designated *Moraxella* spp. were non-fermentative with filamentous ($>100 \mu\text{m}$), serpentine-like cellular morphology and they formed translucent colonies with a sweet caramel-like odour when grown on blood agar. The isolates were inert in the biochemical reactions tested, except for the oxidase and catalase reactions (Table 2). The cellular fatty acid analysis was suggestive of *Moraxella* spp. with the predominant fatty acids 16:1 ω 7c ($\approx 26\%$), 16:0 ($\approx 15\%$) and 18:1 ω 9c ($\approx 23\%$) (Tables 6 and 8). The profile obtained with RapID NF Plus did not correspond to a valid identification, whereas that obtained with API 20 NE also was suggestive of *Moraxella* spp. (Table 9). The isolates designated *Moraxella*-like (A and B) were similar with regard to cellular, colonial and biochemical features (data not shown) but exhibited cellular fatty acid profiles that were different by qualitative and quantitative means (Table 6). However, both profiles, characterised by large amounts of 18:1 ω 9c, were suggestive of the genus *Moraxella* as was the profile obtained with API

20 NE, whereas no valid identification was obtained with use of RapID NF Plus (Table 9).

Neisseria

Organisms designated *N. weaveri*-like were non-fermentative rods that phenotypically resembled the species *N. weaveri*. Greening of the agar around colonies was present on sheep blood agar. Major fatty acids detected were 16:1 ω 7c ($\approx 29\%$); 16:0 ($\approx 23\%$) and 18:1 ($\approx 24\%$) in concordance with the fatty acid profile of the *N. weaveri* type strain (Table 5). One isolate reduced nitrate in disagreement with features previously reported with regard to the species [9, 10]. No valid identification was obtained for the canine isolates or for the type strain with RapID NF Plus and API 20 NE (Table 9), of which only the former include *N. weaveri* (as CDC group M together with CDC group M-6) in its profile index. However, RapID NH yielded a profile (1010), for both the canine isolates and the *N. weaveri* type strain, that most closely resembled CDC group M-5 or *N. cinerea* (data not shown).

A group of non-fermentative rods (1–2 μm in length), that formed colonies with a slightly yellow pigment, was identified as *Neisseria* spp. The ability to hydrolyse gelatin and to reduce nitrate varied within

Table 7. Cellular fatty acid composition of canine *B. zooohelcum* and non-fermentative 'green' isolates, comparison with the *B. zooohelcum* type strain

Organism*	Percent (SD) of total fatty acids†											
	11:0iso	11:0iso3OH	13:0iso	unknown	14:0iso	14:0	15:1isoF	15:0iso	15:0anteiso	15:0	16:0iso	
<i>B. zooohelcum</i> (3)	...	...	2.21 (0.27)	13.566	...	...	...	53.86 (4.23)	1.11 (0.12)	...	...	
<i>B. zooohelcum</i> ATCC 43767‡	...	...	2.44	5.24 (1.61)	...	...	...	57.57	1.22	...	...	
Non-fermentative 'green' (6)	6.21 (1.08)	7.86 (1.28)	...	4.78	1.32 (0.94)	2.44 (1.18)	4.48 (2.27)	40.10 (3.28)	3.91 (2.66)	1.34 (0.46)	2.49 (1.05)	
	16:1ω7c	summed feature 4‡	16:0	15:0iso3OH	iso17:1ω9c	17:1§	unknown	17:0iso	18:1ω9c	17:0iso3OH		
<i>B. zooohelcum</i> (3)	...	6.18 (1.21)	1.17 (0.33)	3.27 (0.35)	9.05 (1.10)	...	16.580	...	1.43 (0.48)	12.45 (1.12)		
<i>B. zooohelcum</i> ATCC 43767‡	...	5.74	...	3.13	9.85	1.69	...	...	...	11.60		
Non-fermentative 'green' (6)	1.44 (0.43)	...	7.32 (1.57)	...	14.03 (3.28)	...	...	3.52 (1.62)	...	...		

*Numbers in parentheses indicate number of strains analysed, † = type strain.

†Values are arithmetic means when more than one isolate was analysed, only values ≥1.00 are shown.

‡Fatty acid 15:0iso2OH or 16:1ω7t

§Summed feature 5 by the MIS.

the group as indicated in Table 2. The cellular fatty acid profiles were suggestive of *Neisseria* spp. (Table 8), with the predominant fatty acid 16:0 comprising >40% of the total fatty acids (Table 5). The profiles were both qualitatively and quantitatively different from those of the reference strains of the rod-shaped *Neisseria* species *N. elongata* and *N. weaveri*. The profiles generated by RapID NF Plus and API 20 NE are indicated in Table 9.

Bergeyella

B. zooohelcum, a yellow-green pigment-producing non-fermenter with a sweet odour, resembling that of bitter almond, was identified by conventional biochemical testing and by RapID NF Plus (Tables 2 and 9). The organism produced indole and urease (rapid reaction), and hydrolysed gelatin and arginine. More than 80% of the total fatty acids were odd-numbered iso-branched acids with 15:0iso (≈54%), iso17:1ω9c (≈9%) and 17:0iso(3OH) (≈12%) as major compounds, in agreement with the fatty acid profile of the *B. zooohelcum* type strain (Table 7). This strain produced an API 20 NE profile identical to that of the canine isolates but a different RapID NF Plus profile for which no identification was obtained (Table 9).

Unidentified organisms

On the basis of the phenotypic tests that were used in the current study two fermentative and nine non-fermentative isolates could not be definitely assigned to a genus. However, six of the non-fermentative isolates, designated non-fermentative 'green' because of the intense green pigment which they produced when grown on blood agar, exhibited similar phenotypic features including cellular and colonial morphology, and cellular fatty acid profiles. The cells were short rods, 1.5 μm in length. Their cellular fatty acid profiles comprised almost 80% iso-branched, odd-numbered fatty acids (Table 7), a feature similar to that of *B. zooohelcum*. The most abundant of these was 15:0iso, which made up c. 40% of the total acids, followed by iso17:1ω9c (≈14%), 11:0iso(3OH) (≈8%), and 11:0iso (≈6%). Less than 15% of the total acids were straight chain acids, predominantly 16:0, which made up ≈7%. Further characterisation with RapID NF Plus and API 20 NE did not yield valid identifications (Table 9).

The remaining fermentative (A, B) and non-fermentative (C, D, E) isolates could not be assigned to any of the bacterial species or groups described above. They did not form uniform groups but exhibited distinct phenotypic characteristics. The unidentified fermentative isolates resembled the genera *Flavobacterium* (A) and *Pseudomonas* (B) by cellular fatty acid composition at low similarity index levels, whereas the cellular fatty acid compositions of the unidentified non-fermenters were unique (Table 8).

Table 8. Canine bacteria, identifications proposed by the MIS, comparison with reference strains

Organism*	Identification	Similarity index [†]
<i>P. canis</i> (5)	<i>P. multocida</i>	0.811
<i>P. canis</i> ATCC 43326 ^T	<i>P. multocida</i>	0.779
<i>P. dagmatis</i> (3)	<i>P. multocida</i> (1), <i>P. gallinarum</i> (2)	0.449 0.567
<i>P. dagmatis</i> ATCC 43325 ^T	<i>P. gallinarum</i>	0.774
<i>P. stomatis</i> (6)	<i>P. multocida</i> (3), <i>P. gallinarum</i> (3)	0.482 0.610
<i>P. stomatis</i> ATCC 43327 ^T	<i>P. multocida</i>	0.246
<i>Pasteurella</i> -like (1)	<i>P. gallinarum</i>	0.514
Bisgaard's taxon 16 (3)	<i>Haemophilus</i> (1), <i>P. multocida</i> (2)	0.229 0.507
Bisgaard's taxon 16, CCUG 17204	<i>P. multocida</i>	0.291
<i>C. hominis</i> -like (5)	<i>C. hominis</i> (4), no match (1)	0.280 ...
<i>C. hominis</i> ATCC 15826 ^T	<i>C. hominis</i>	0.880
CDC group EF-4a, ADH + (11)	CDC group EF-4	0.611
CDC group EF-4a, ADH - (11)	CDC group EF-4	0.637
CDC group EF-4a, ATCC 29858	CDC group EF-4	0.652
CDC group EF-4b, ATCC 29859	CDC group EF-4	0.583
Unidentified fermenter		
A (1)	<i>Flavobacterium</i>	0.204
B (1)	<i>Pseudomonas</i>	0.384
CDC group NO-1 (7)	<i>Campylobacter</i> (1), no match (6)	0.106 ...
CDC group NO-1, CDC G1448	No match	...
<i>M. canis</i> (3)	<i>M. lacunata</i> (2), <i>M. osloensis</i> (1)	0.212 0.233
<i>M. canis</i> LMG 11194 ^T	<i>M. osloensis</i>	0.227
<i>M. catarrhalis</i> -like (1)	No match	...
<i>M. catarrhalis</i> ATCC 25238 ^T	<i>Branhamella catarrhalis</i>	0.371
<i>Moraxella</i> sp (1)	No match	...
<i>Moraxella</i> -like A, B (2)	<i>M. lacunata</i>	0.150
<i>N. weaveri</i> -like (3)	CDC group EF-4 (2), <i>N. meningitidis</i> (1)	0.624 0.585
<i>N. weaveri</i> ATCC 51410 ^T	CDC group EF-4	0.585
<i>Neisseria</i> spp. (6)	<i>N. elongata</i> (1), <i>N. sicca</i> (4), no match (1)	0.288 0.129 ...
<i>N. elongata</i>		
subsp. <i>elongata</i> , ATCC 25295 ^T	<i>N. meningitidis</i>	0.665
subsp. <i>glycolytica</i> , ATCC 29315 ^T	<i>N. elongata</i>	0.606
subsp. <i>nitroreducens</i> , ATCC 49377 ^T	<i>N. sicca</i>	0.658
<i>B. zoohelcum</i> (3)	<i>W. zoohelcum</i>	0.592
<i>B. zoohelcum</i> , ATCC 43767 ^T	<i>W. zoohelcum</i>	0.659
Non-fermentative 'green' (6)	No match	...
Unidentified non-fermenter		
C (1)	No match	...
D (1)	No match	...
E (1)	No match	...

*Numbers in parentheses indicate number of isolates analysed, ^T = type strain.[†]Mean value when more than one isolate was analysed.

MIS

The identifications proposed by the MIS and the similarity index of each identification are indicated in Table 8. Most (33, 70%) fermentative canine isolates were identified to genus or group level with a similarity index ≥ 0.5 (indicating a good match), whereas eight isolates (17%) were identified to the genus with an SI < 0.5 (indicating a low match or likelihood). Three (9%) non-fermentative isolates were identified to species (*B. zoohelcum*) and one isolate (3%) to genus (*N. weaveri*) level with an SI ≥ 0.5 , whereas 10 isolates (29%) were identified to the genus with an SI < 0.5 . Of the total number of isolates analysed (n = 82) the MIS identified to species, genus or group level 37 isolates

(45%) with an SI ≥ 0.5 and 18 isolates (22%) with an SI < 0.5 . The cellular fatty acid profile of 19 isolates did not match any entry in the CLIN library database and six isolates were misidentified. The identifications proposed for two isolates were uncertain as the isolates could not be definitely assigned to the genera proposed by other methods. Of the 15 reference strains analysed three were identified to species and six to genus or group level with an SI ≥ 0.5 , whereas three strains were identified to the species (1 isolate) or genus (2 isolates) with an SI < 0.5 . Bisgaard's taxon 16, CDC group NO-1 and *N. weaveri*, which are not included in the library database, were either not identified (no match) or were misidentified.

Table 9. RapID NF Plus and API 20 NE profiles of canine nonfermenters, comparison with reference strains

Organism*	Numerical profile/identification	
	RapID NF Plus	API 20 NE
CDC group NO-1-like (7)	01 00 22/no ID 41 00 22/no ID 61 00 06/ <i>Alcaligenes faecalis/odorans</i> 61 00 22/no ID 63 04 22/no ID	100 000 0 (3) [†] / <i>Pasteurella</i> spp. 100 004 0 (1)/ID not valid 100 000 4 (3)/low discrimination: two genera
CDC group NO-1, CDC G 1448	63 04 26 (2)/no ID	
<i>M. canis</i> (3)	63 04 22/no ID 41 00 34 (2)/ <i>Pseudomonas cepacia</i> 41 00 36/no ID	100 000 0/ <i>Pasteurella</i> sp. NT [‡]
<i>M. canis</i> LMG 11194 ^T	01 40 36/no ID	NT
<i>M. catarrhalis</i> (1)	41 00 24/ <i>A. faecalis/odorans</i>	NT
<i>M. catarrhalis</i> ATCC 25238 ^T	61 02 26/no ID	NT
<i>Moraxella</i> spp. (5)	41 00 04/ <i>A. faecalis/odorans</i>	000 000 4/ <i>Moraxella</i> spp.
<i>Moraxella</i> -like (2)	41 02 24/ <i>P. cepacia</i>	000 000 4/ <i>Moraxella</i> spp.
<i>N. weaveri</i> -like (3)	22 02 04 (1)/no ID	000 004 4/ <i>Brevundimonas diminuta/Oligella urethralis</i>
<i>N. weaveri</i> ATCC 51410 ^T	22 02 24 (2)/no ID 23 02 24/no ID	002 004 4/ID not valid
<i>Neisseria</i> spp. (6)	40 02 14 (1)/ <i>P. cepacia</i> 60 02 16 (1)/ <i>P. aeruginosa</i> [§] 61 02 14 (1)/ <i>P. cepacia</i> 61 02 16 (2)/ <i>P. aeruginosa</i> 63 02 14 (1)/ <i>P. cepacia</i>	000 000 4 (2) <i>Moraxella</i> spp. 100 000 4 (4) low discrimination: two genera
<i>B. zoohelcum</i> (3)	11 42 35 (1)/ <i>Weeksella zoohelcum</i> 11 42 75 (2)/ <i>W. zoohelcum</i>	221 000 4/ <i>Myroides</i> sp.
<i>B. zoohelcum</i> ATCC 43767 ^T	13 42 75/no ID	221 000 4/ <i>Myroides</i> sp.
Non-fermentative 'green' (6)	41 06 74 (3)/no ID 43 06 74 (3)/ <i>P. putrefaciens</i> **	000 000 4 (5)/ <i>Moraxella</i> spp. 001 000 4 (1)/ <i>Weeksella virosa/Empedobacter brevis</i>
Unidentified non-fermenter		
C (1)	63 04 26/no ID	100 000 4/low discrimination: two genera
D (1)	41 00 24/ <i>A. faecalis/odorans</i>	000 000 4/ <i>Moraxella</i> sp.
E (1)	61 00 26/no ID	100 000 4/low discrimination: two genera

*Numbers in parentheses indicate number of strains analysed, ^T = type strain.[†]Number of isolates exhibiting the profile.[‡]Not tested.[§]Second choice *P. stutzeri*.^{||}Second choice *P. aeruginosa*, third choice *Xanthomonas maltophilia*.**Second choice *P. diminuta*.

Discussion

The 23% of canine isolates for which no identification (library match) was obtained by the MIS, and the relatively low number (35%) of isolates identified to species or group (with regard to unclassified organisms) level, was in part due to the fact that dog-derived organisms, such as CDC group NO-1, *M. canis* and *N. weaveri*, are not included in the CLIN Library database version 3.8. Nevertheless, it facilitated the identification of the canine organisms, particularly the non-fermenters, by generating fatty acid profiles which were comparable to profiles of previously described organisms [24].

The commercial identification systems API 20 E, API 20 NE, RapID NF Plus and RapID NH are commonly used by clinical microbiologists for rapid identification of human-derived isolates. Previously, RapID NF Plus was reported to yield excellent results when used for identification of gram-negative non-fermentative clinical isolates; 90.1% of the organisms studied were correctly identified without additional tests [25]. Recently, API 20 E was reported to identify equally well animal- and human-derived gram-negative bacter-

ia; 76% of the animal-derived organisms and 83% of the human-derived organisms were identified to at least genus or group level [26]. However, these systems were less useful for rapid identification of the canine isolates. Among those characterised (enterobacteria and non-fermenters), only the enterobacteria and the *B. zoohelcum* isolates were identified with API 20 E and RapID NF Plus, respectively. The other isolates were either not identified or yielded results in conflict with those obtained by conventional biochemical testing or by cellular fatty acid profiling. Moreover, different identification profiles were obtained for individual isolates encountered in the same species or group, even though distinct species or groups of organisms generated unique profiles with no overlap. In our experience, the manual identification systems, which have been designed primarily for identification of human-derived organisms, are useful for identification of bacteria common to both humans and dogs (e.g., enterobacteria) and for identification of non-fermentative organisms that are reasonably reactive in biochemical tests (e.g., *B. zoohelcum*), whereas organisms not routinely isolated from human samples, such as those from subgingival sites of dogs, are only preliminary subgroupable by these systems. The low

accuracy of identification is explained by the absence of profiles in the system databases for many organisms characterised in this study. Addition of dog-derived bacteria to the databases (including the CLIN Library) would probably permit rapid identification of these organisms as well.

The canine subgingival bacteria represent genera and groups of organisms that are also isolated from infected dog bite wounds in man [1, 2]. This is not unexpected as periodontitis is a common disease in dogs, and organisms present at infected sites may be transmitted by the biter to the victim. Detailed knowledge of the bacteriology of the canine mouth and specifically of the phenotypic features of the individual organisms, will facilitate clinical microbiology laboratories when establishing the bacteriology of infected dog bite wounds in man and eventually when deciding on accurate management with regard to antimicrobial therapy. The results of the present study indicate that most fermentative canine bacteria are identifiable by conventional biochemical testing, by tests normally available in the routine clinical or veterinary microbiology laboratory. However, the non-fermentative bacteria which, due to few positive reactions, are difficult to identify by conventional methods alone may be sent to a reference laboratory for additional cellular fatty acid profiling (if not available in the routine laboratory), when accurate identification is required.

Further studies based on 16S rRNA sequencing and DNA–DNA hybridisation are needed to assess whether the CDC group EF-4a subgroups detected represent distinct species and to assess how they relate genetically to the type strains. Genotypic studies are also required to determine whether, in fact, three groups of the CDC group EF-4 organisms exist, as is indicated by the cellular fatty acid profiles. Likewise, those non-fermenters – particularly the *Moraxella* isolates, the rod-shaped *Neisseria*-like organisms, and the non-fermentative ‘green’ isolates – which could not be speciated in the current study warrant further characterisation by 16S rRNA sequencing and DNA–DNA hybridisation to establish their taxonomic position. Even so, genotypic studies are needed to establish whether the canine *C. hominis*-like isolates are true *C. hominis* strains or whether they represent a distinct group of organisms, as suggested by the phenotypic dissimilarities that were found between the canine isolates and the type strain.

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