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NOTES

Biochemical Characteristics and Fatty Acid Composition of Gilardi Rod Group 1 Bacteria

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Fifteen strains of eugonic, nonoxidative, gram-negative rods isolated primarily from human wounds of the extremities and blood formed a distinct group which was designated Gilardi rod group 1. The phenotypic characteristics of Gilardi rod group 1 were most similar to those of CDC group M-5, with the major difference that nitrite reduction was observed with CDC group M-5. All 15 strains of Gilardi rod group 1 possessed a distinct fatty acid profile which was characterized by large amounts (>15%) of *cis*-vaccenic (18:1ω7c), palmitic (16:0), myristic (14:0), and lactobacillic (19:0 cyc^{11,12}) acids and moderate amounts (3 to 5%) of lauric (12:0), 3-hydroxylauric (3-OH-12:0), and palmitoleic (16:1ω7c) acids. This fatty acid profile is unique compared with the profiles of CDC group M-5 and other bacteria we have tested and is useful for the rapid identification of Gilardi rod group 1 isolates.

The Special Bacteriology Reference Laboratory of the Centers for Disease Control (CDC) receives bacterial isolates for identification and classification from reference and clinical laboratories throughout the United States and some foreign countries. In 1985, six gram-negative, rod-shaped isolates were received by the Special Bacteriology Reference Laboratory from G. L. Gilardi for comparative studies. The cultural and biochemical characteristics of these isolates, which were later designated by Gilardi as unnamed rod group 1 (2), were most similar to some CDC group M-5 organisms, but their fatty acid compositions were different from those of M-5 and all other organisms studied to date. Since 1985, we have received nine other isolates with biochemical characteristics identical to those of the original six Gilardi isolates. In this report, we describe the cultural and biochemical characteristics and cellular fatty acid (CFA) composition of these 15 isolates, which we designate as Gilardi rod group 1. With these data, clinical laboratories can recognize and distinguish Gilardi rod group 1 organisms from CDC group M-5 and other bacteria.

The cultural and biochemical characteristics of each Gilardi rod group 1 isolate and 20 CDC group M-5 strains were determined by previously described methods, except for the nitrite test, which was done with both 0.1 and 0.01% concentrations of potassium nitrite (1). Cells for fatty acid analysis were grown for 24 to 48 h at 35°C on heart infusion agar supplemented with 5% rabbit blood (BHIA; BBL, Cockeysville, Md.). The CFA were processed and analyzed as methyl esters with capillary gas-liquid chromatography as described previously (5). The identities of individual fatty acids were confirmed by mass spectrometry.

With the exception of nitrite reduction, the biochemical reactions and cultural characteristics of Gilardi rod group 1 and CDC group M-5 isolates were very similar (Table 1). None of the Gilardi rod group 1 strains reduced either 0.1 or 0.01% nitrite after incubation at 35°C for 48 h or 7 days, whereas all 20 CDC group M-5 strains were positive at 48 h in 0.01% nitrite broth. The cells of Gilardi rod group 1 were oval, medium-length, medium- to wide-width, and sometimes pleomorphic gram-negative rods with some biopolar staining, whereas CDC group M-5 cells were short- to medium-length and slender- to medium-width gram-negative rods. Strains of both groups grew well on BHIA, producing 0.5- to 1.5-mm-diameter colonies in 24 to 48 h. A narrow, translucent fringe which was not present around the CDC group M-5 colonies often was observed around the periphery of the Gilardi rod group 1 colonies. All Gilardi rod group 1 strains were strongly positive in the phenylalanine deaminase reaction, producing a deep green color in the agar slant, while the CDC group M-5 isolates, when positive, gave a weak to moderate reaction, producing a light to moderate green color. Both groups were considered negative for indole production, although several of the Gilardi rod group 1 isolates produced a light orange to salmon pink color, which increased in intensity with incubation longer than 48 h. However, the reduction of 0.01% nitrite by CDC group M-5 was the major biochemical test for distinguishing it from Gilardi rod group 1 organisms, which were negative in this test. In the description of CDC group M-5 by Clark et al. (1), 5 (16%) of 32 strains were negative for the reduction of nitrite when tested in 0.1% potassium nitrite. When we later tested these five strains in 0.01% potassium nitrite, they were positive for nitrite reduction (unpublished data). Gilardi rod group 1 isolates were susceptible to a variety of antimicrobial agents including various penicillins, cephalothin, and chloramphenicol (2).

Some other organisms with phenotypic characteristics similar to those of Gilardi rod group 1 are *Oligella urethralis*, *Moraxella osloensis*, and *Moraxella atlantae*. However, *O. urethralis* has a small coccoid cellular morphology and can be differentiated from both CDC group M-5 and Gilardi rod

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TABLE 1. Cultural characteristics of Gilardi rod group 1 and CDC group M-5[a]

Test	Gilardi rod group 1 (n = 15)	CDC group M-5 (n = 20)
Action on blood	20 gr + br, 20 gr, 13 lav + br, 7 ly	40 ly, 10 LG, 5 LG + br, 5 gr + ft br, 5 br, 5 lav
Oxidase	100	100
Acid from (OF base):		
D-Glucose, D-xylose	0	0
D-Mannitol, lactose	0	0
Sucrose, maltose	0	0
Growth on:		
MacConkey agar	91	15, 30 W (10)
SS agar	80	0
Catalase	100	100
Nitrate reduction	0	0
Nitrite reduction, 0.01%	0	100
Simmons citrate	0	0
Urea, Christensen	0	0
Phenylalanine deaminase	100	55
Litmus milk	20 K	15 K
Motility	0	0
Pigment		
Soluble	67 amber, 3 yel-tan, 13 yel-br, 7 tan	50 sl yel-yel
Nonsoluble	60 amber, 7 caramel, 7 apricot	10 yel, 5 amber
Nutrient broth		
0% NaCl	100	80 (15)
6% NaCl	7 (13)	25
Growth at 25, 35, and 42°C	100, 100, 47; 33 W	90, 100, 25; 25 W
H_2S (TSI agar)	0	0
Esculin hydrolysis	0	0
Gelatin hydrolysis	0	0

[a] Data are expressed as percent positive at 48 h; numbers in parentheses are late reactions, i.e., 3 to 7 days. Symbols and abbreviations: W, weak; K, alkaline; yel, yellow; br, brown; sl, slight; gr, green; ly, lysis; lav, lavender; LG, lavender green; ft, faint; n, number of strains examined; OF, oxidation-fermentation; SS, salmonella-shigella; TSI, triple sugar iron.

group 1 by the reduction of nitrite with formation of gas. Gas has not been detected with the reduction of nitrite by CDC group M-5. Some strains of *O. urethralis* alkalinize Simmons citrate, while all strains of CDC group M-5 and Gilardi rod group 1 have been negative. *M. osloensis* is a coccoid to short broad rod that can be distinguished from Gilardi rod group 1 by a positive reaction in the *M. osloensis* transformation test. Strains of *M. osloensis* are often phenylalanine negative. *M. atlantae* also is a coccoid to short broad rod and can be differentiated by being fastidious and phenyalanine negative.

TABLE 2. Cellular fatty acid compositions of Gilardi rod group 1 and CDC group M-5

Fatty acid[b]	Gilardi rod group 1[a] (n = 15)	CDC group M-5[a] (n = 20)
12:0	5	6
3-OH-12:0	3	4
14:0	18	6
3-OH-14:0	1	1
15:0	1	—
16:1ω7c	3	26
16:0	17	26
18:2	1	2
18:1ω9c	—	2
18:1ω7c	33	24
18:0	1	1
19:0 cyc^{11,12}	16	—

[a] n, number of strains examined. Values are percentages of total fatty acids and are arithmetic means; —, not detected.

[b] The number before the colon is the number of carbon atoms, and the number after the colon is the number of double bonds; 3-OH indicates a hydroxyl group at the 3 carbon; ω is the double-bond position from the hydrocarbon end of chain; c is the *cis* isomer; cyc indicates a cyclopropane ring at the 11,12 carbon atom.

The CFA composition of Gilardi rod group 1 differs from that of *O. urethralis*, *M. osloensis*, *M. atlantae*, and CDC group M-5 (5) (Table 2). Gilardi rod group 1 isolates were characterized by large amounts (>15%) of *cis*-vaccenic (18:1ω7c), palmitic (16:0), myristic (14:0), and lactobacillic (19:0 cyc^{11,12}) acids (Table 2). The isolates also contained moderate amounts (3 to 5%) of lauric (12:0), 3-hydroxylauric (3-OH-12:0), and palmitoleic (16:1ω7c) acids. The presence and relative concentrations of these acids constitute a unique CFA profile for Gilardi rod group 1. Particularly noteworthy is the large amount of 14:0, in combination with large amounts of 19:0 cyc^{11,12} and 18:1ω7c, and moderate amounts of 16:1ω7c. Other organisms known to contain large amounts of 14:0 include *Kingella*, *Actinobacillus*, *Pasteurella*, and *Cardiobacterium hominis* isolates, but none of these contain 19:0 cyc^{11,12} (6, 7). Some *Campylobacter* species contain large amounts of both 14:0 and 19:0 cyc^{11,12}, but these organisms also contain 3-hydroxymyristic (3-OH-14:0), 3-hydroxypalmitic (3-OH-16:0), or 3-hydroxystearic (3-OH-18:0) acids, which are absent in Gilardi rod group 1 (4) (Table 2).

The CFA profile of CDC group M-5 clearly differs from that of Gilardi rod group 1 by a larger amount of 16:1ω7c (26 versus 3%), smaller amounts of 14:0 (6 versus 18%), and the absence of 19:0 cyc^{11,12} (Table 2). The overall CFA profile of CDC group M-5 organisms is similar to that of *Neisseria elongata* subsp. *nitroreducens* (formerly CDC group M-6) (3) and other *Neisseria* spp. (5; unpublished data). Evaluation of this apparent relationship of CDC group M-5 with *Neisseria*

spp. as well as determination of the taxonomic status of Gilardi rod group 1 will require genetic studies.

The 15 cultures of Gilardi rod group 1 were isolated from a variety of human sources including leg, arm, and foot wounds, an oral lesion, and urine. Four strains were isolated from blood. Little clinical information on these isolates was available, and their pathogenic potential has yet to be determined. Recognition and identification of these organisms in microbiology laboratories will provide valuable information to evaluate their true incidence and clinical significance. The CFA data and results from conventional tests described in this report provide a means for identification of Gilardi rod group 1 isolates.

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