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Leminorella, a New Genus of *Enterobacteriaceae*: Identification of *Leminorella grimontii* sp. nov. and *Leminorella richardii* sp. nov. Found in Clinical Specimens

F. W. HICKMAN-BRENNER,[1]* M. P. VOHRA,[2] G. P. HUNTLEY-CARTER,[1] G. R. FANNING,[3] V. A. LOWERY III,[1] DON J. BRENNER,[4] AND J. J. FARMER III[1]

Enteric Bacteriology Section[1] and Molecular Biology Laboratory,[4] Division of Bacterial Diseases, Centers for Disease Control, Atlanta, Georgia 30333; Department of Medical Microbiology, PostGraduate Institute of Medical Education and Research, Chandigarh-160012, India[2]; and Division of Biochemistry, Walter Reed Army Institute of Research, Washington, D.C. 20307[3]

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Leminorella* is proposed as a new genus for the group of *Enterobacteriaceae* formerly known as Enteric Group 57. Strains of *Leminorella* gave positive tests for H_2S production, acid production from L-arabinose and D-xylose, and tyrosine clearing; they were negative for indole production, Voges-Proskauer, urea hydrolysis, phenylalanine deaminase, motility, gelatin liquefaction, lysine and ornithine decarboxylases, arginine dihydrolase, growth in KCN, and acid production from adonitol, D-arabitol, cellobiose, erythritol, D-galactose, *myo*-inositol, lactose, maltose, D-mannitol, D-mannose, melibiose, α-CH_3-glucoside, raffinose, L-rhamnose, salicin, D-sorbitol, sucrose, and trehalose. By DNA hybridization, strains of *Leminorella* were only 3 to 16% related to other *Enterobacteriaceae* and were divided into three groups. *Leminorella grimontii* is proposed as the type species for the genus and strain CDC 1944-81, ATCC 33999, is designated as the type strain. There were four strains of *L. grimontii* from stool specimens and two from urine specimens. *L. richardii* is proposed as the name for the second species (type strain, CDC 0978-82, ATCC 33998). All four *L. richardii* strains were from stool specimens. *L. grimontii* can be distinguished from *L. richardii* because it produces gas from glucose (100%) and acid from dulcitol (83%) and is methyl red positive (100%). One strain, CDC 3346-72, was more related to *L. grimontii* by DNA hybridization than to *L. richardii*, but the lower relatedness to both of these species indicated that it may be a third species. Biochemically it could not be distinguished from *L. grimontii*. All *Leminorella* strains were resistant (no zone of inhibition) to ampicillin, carbenicillin, and cephalothin. Some of the *Leminorella* strains were sent to us for *Salmonella* serotyping, and two reacted weakly in *Salmonella* antisera. The clinical significance of *Leminorella* is unknown.

The vernacular name "Enteric Group 57" was first coined in 1981 when a diagnostic strain was being studied. A computer search of the records of the Enteric Bacteriology Section, Centers for Disease Control, revealed nine similar isolates (Table 1). An additional isolate was found in 1982. These isolates produced H_2S in triple sugar iron agar, but were otherwise relatively inactive biochemically (Table 2). Some of them had been sent to us as *Salmonella*, and two reacted weakly in *Salmonella* antisera (Table 1). The name Enteric Group 57 was used until these strains could be studied further and a better classification could be proposed. The purpose of this paper is to show that Enteric Group 57 strains belong in a new genus in the Family *Enterobacteriaceae*. We propose *Leminorella* as a new genus and *Leminorella grimontii* and *L. richardii* as two species.

MATERIALS AND METHODS

Bacterial strains. Eleven strains identified as Enteric Group 57 were studied (Table 1). All strains were maintained on semisolid Trypticase soy agar (BBL Microbiology Systems, Cockeysville, Md.) at room temperature (18 to 28°C) and were also frozen in 10% skim milk and maintained at −70°C. This semisolid medium contained the following: Trypticase peptone (BBL), 15 g; phytone peptone, 5 g; sodium chloride, 5 g; agar, 4 g; and distilled water, 1,000 ml. All incubations were at 36 ± 1°C unless otherwise noted.

Media and biochemical tests. Commercial media were used whenever possible. The biochemical tests listed in Table 2 were done by the methods of Edwards and Ewing (5), with some modifications as previously described (10, 11).

DNA relatedness. DNA relatedness was determined for the 11 Enteric Group 57 strains listed in Table 3. Unlabeled DNA was isolated and purified by methods previously described (3, 4). DNA from strain 1944-81 (ATCC 33999) was labeled first in vitro with $^{32}PO_4$ by nick translation essentially by the methods of Rigby et al. (19) and instructions furnished with a commercial nick translation reagent kit (catalog no. 8160; Bethesda Research Laboratories, Inc., Gaithersburg, Md.). The relatedness of strain 1944-81 to unlabeled DNA from the other 10 Enteric Group 57 strains was determined on hydroxyapatite by the methods of Brenner et al. (4). Since these 10 strains fell into two DNA hybridization groups, DNA from strain 0978-82 (ATCC 33998) in the second group was then labeled and tested against unlabeled DNA from all strains. Labeled DNAs from both 1944-81 and 0978-82 were tested against stock DNAs from 56 named and unnamed species of *Enterobacteriaceae* (Table 4) chosen to rule out species-level relatedness to all members of the family. DNA from *Budivicia aquatica* (strain 0440-84), another H_2S-positive *Enterobacteriaceae* that was

* Corresponding author.

TABLE 1. List of *Leminorella* strains studied

Species	Strain no.	Location of sender	Source	Other clinical information
L. grimontii	1362-73	Florida	Stool	5.5-month-old female
	3244-76	Hawaii	Urine	80-year-old female with urinary tract infection
	3257-76	California	Stool	10-year-old male, community illness in children's home
	3595-77	Pennsylvania	Urine	9-year-old female with suspected salmonellosis; culture agglutinated weakly in *Salmonella* antisera
	0301-79	Maryland	Stool	Mouse
	1944-81 (ATCC 33999)[a]	Hawaii	Stool	7-month-old female with gastroenteritis
L. richardii	0598-78	Indiana	Stool	Male, culture sent in as *Salmonella*
	2209-80	Pennsylvania	Stool	47-year-old male with fever of unknown origin
	2502-80	Texas	Stool	2-month-old male
	0978-82 (ATCC 33998)[a]	Texas	Stool	Female with diarrhea on corticosteroid therapy; no enteric pathogens isolated
Leminorella sp. 3	3346-72	Georgia	Stool	10-month-old female, culture agglutinated weakly in *Salmonella* antisera

[a] Type strain for the species.

recently described (1), was labeled and tested against DNA from *Leminorella* strains 1944-81, 0978-82, and 3346-72.

Antimicrobial susceptibility tests. Antimicrobial susceptibility was determined for all strains by the disk method of Bauer et al. (2). The antibiotics and the concentrations used are listed in Table 5.

RESULTS AND DISCUSSION

DNA hybridization. Labeled DNA from strain 1944-81 was 77 to 97% related to five other Enteric Group 57 strains in 60°C reactions (Table 3). There was 0.0 to 0.5% divergence in the related sequences, and relatedness remained high in 75°C reactions. These six strains represent *L. grimontii*. Labeled DNA from strain 0978-82 was more than 90% related to three other Enteric Group 57 strains in both 60 and 75°C reactions. Divergence within these related sequences was 0.5 to 1.0%. These four strains represent *L. richardii*. DNA relatedness between *L. grimontii* and *L. richardii* in reciprocal 60°C reactions was 32 to 49% with 11.0 to 13.5% divergence, and 11 to 25% in reciprocal 75°C reactions. DNA relatedness between *Leminorella* species and other representative *Enterobacteriaceae* was 3 to 16% at 60°C, confirming the uniqueness of these two new species. One Enteric Group 57 strain (3346-72) was more related to *L. grimontii* than to *L. richardii*, but the low level of relatedness suggested that it may be a third species in the genus. Until further strains are available for study, we call this strain *Leminorella* sp. 3. Labeled DNA from *B. aquatica* strain 0440-84 was 11% related at 60°C to DNA from the type strains of *L. grimontii* and *L. richardii* and from strain 3346-72.

Description of *Leminorella*. We propose the genus name *Leminorella* for the group of strains formerly referred to as Enteric Group 57. The generic name is derived from the surname of Leon Le Minor, a French microbiologist, and was chosen to honor him for his many contributions to enteric bacteriology. At the Pasteur Institute, Paris, he has worked to clarify the nomenclature and serology of *Salmonella*, has described many new serotypes, and has done studies on a wide variety of topics in enteric bacteriology including lysogeny, metabolic plasmids, and new and rapid biochemical tests (12–14). *Leminorella* also honors Simone Le Minor, wife and colleague of Leon Le Minor, who has also made many contributions to enteric bacteriology as head of the National *Salmonella* Centre of France and with her research on *Serratia* serology (13, 15). *Leminorella* (pronounced Lə̄-mean-nohr-rel'-la) is a neo (modern) Latin feminine noun formed by adding the diminutive ending "-ella" to the noun Le Minor. Strains of *Leminorella* have the general properties of the family *Enterobacteriaceae* (Table 2). They are unique in that they give positive tests for H_2S production, acid production from L-arabinose and D-xylose, and tyrosine clearing, but are otherwise rather inactive (Table 2). They are resistant (no zone of inhibition) to ampicillin, carbenicillin, and cephalothin. A complete description of the genus and its species is given in Tables 2 through 6.

Description of *L. grimontii*. The name *L. grimontii* (pronounced gree-mohn'-tee-eye) is proposed for the first DNA hybridization group and is proposed as the type species for the genus. This species name is derived from the surname of Patrick Grimont, a French microbiologist at the Pasteur Institute, and for his wife and colleague, Francine Grimont. They have done definitive work on the genus *Serratia*, described new species of *Enterobacteriaceae*, and studied enteric bacteria phenetically and genetically (6–9). The species name is treated as a modern Latin genitive noun meaning "of Grimont." The type strain for this species is designated as CDC 1944-81 (ATCC 33999).

Description of *L. richardii*. The name *L. richardii* (pronounced ri-shar'-dee-eye) is proposed for the second DNA hybridization group. This name was chosen to honor Claude Richard, who is also a French microbiologist at Pasteur Institute, for his studies on the biochemical characterization of *Enterobacteriaceae* and the description of new species (16–18). This species name is also treated as a modern Latin genitive noun meaning "of Richard." Strain 0978-82 (ATCC 33998) is designated as the type strain. *L. grimontii* can be differentiated from *L. richardii* because it produces gas from D-glucose (100%) and acid from dulcitol (83%) and is methyl red positive (100%).

***Leminorella* sp. 3.** Based on DNA hybridization studies, we have placed strain 3346-72 in a third species. Biochemically, however, it was identical to the *L. grimontii* strains (Table 2).

Clinical information. Very little clinical information is available for the 11 *Leminorella* strains (Table 1). Two of the

TABLE 2. Biochemical reactions of six *L. grimontii* strains, four *L. richardii* strains, their type strains, and *Leminorella* sp. 3 strain 3346-72[a]

Test	*L. grimontii* (6 strains)				*L. richardii* (4 strains)				Reaction for *Leminorella* sp. 3 strain 3346-72[b]
	Cumulative % positive on day:			Reaction for type strain ATCC 33999[b]	Cumulative % positive on day:			Reaction for type strain ATCC 33998[b]	
	1	2	7		1	2	7		
Indole production		0		−		0		−	−
Methyl red		100		+		0		−	+
Voges-Proskauer		0		−		0		−	−
Citrate (Simmons)	0	100	100	+[2]	0	0	0	−	+[3]
H_2S on triple sugar iron	100	100	100	+	100	100	100	+	+
H_2S on PIA[c]	17	100	100	+[2]	0	100	100	+[2]	+
Urea hydrolysis	0	0	0	−	0	0	0	−	−
Phenylalanine	0	0	0	−	0	0	0	−	−
Lysine (Moeller)	0	0	0	−	0	0	0	−	−
Arginine (Moeller)	0	0	0	−	0	0	0	−	−
Ornithine (Moeller)	0	0	0	−	0	0	0	−	−
Motility	0	0	0	−	0	0	0	−	−
Gelatin hydrolysis (22°C)	0	0	0	−	0	0	0	−	−
KCN, growth in	0	0	0	−	0	0	0	−	−
Malonate utilization	0	0	0	−	0	0	0	−	−
D-Glucose									
Acid	86	100	100	+	0	100	100	+[2]	+
Gas	0	33	100	+[2]	0	0	0	−	+[2]
Acid from:									
Adonitol	0	0	0	−	0	0	0	−	−
L-Arabinose	100	100	100	+	100	100	100	+	+
D-Arabitol	0	0	0	−	0	0	0	−	−
Cellobiose	0	0	0	−	0	0	0	−	−
Dulcitol	50	83	83	+	0	0	0	−	+[4]
Erythritol	0	0	0	−	0	0	0	−	−
D-Galactose	0	0	0	−	0	0	0	−	−
Glycerol	0	17	33	−	0	0	0	−	−
myo-Inositol	0	0	0	−	0	0	0	−	−
Lactose	0	0	0	−	0	0	0	−	−
Maltose	0	0	0	−	0	0	0	−	−
D-Mannitol	0	0	0	−	0	0	0	−	−
D-Mannose	0	0	0	−	0	0	0	−	−
Melibiose	0	0	0	−	0	0	0	−	−
α-CH_3-glucoside	0	0	0	−	0	0	0	−	−
Raffinose	0	0	0	−	0	0	0	−	−
L-Rhamnose	0	0	0	−	0	0	0	−	−
Salicin	0	0	0	−	0	0	0	−	−
D-Sorbitol	0	0	0	−	0	0	0	−	−
Sucrose	0	0	0	−	0	0	0	−	−
Trehalose	0	0	0	−	0	0	0	−	−
D-Xylose	83	83	83[d]	+	100	100	100	+	+
Esculin	0	0	0	−	0	0	0	−	−
Mucate-acid from	17	100	100	+[2]	0	50	75	+[2]	+[2]
Tartrate (Jordan)	100	100	100	+	100	100	100	+	+
Acetate utilization	0	0	0	−	0	0	0	−	−
Lipase (corn oil)	0	0	0	−	0	0	0	−	−
DNase (25°C)	0	0	0	−	0	0	0	−	−
DNase (36°C)	0	0	0	−	0	0	0	−	−
$NO_3^- \rightarrow NO_2^-$	100			+	100			+	+
Oxidase	0			−	0			−	−
ONPG test	0	0	0	−	0	0	0	−	−
Citrate (Christensen)	33	33	50	+	0	50	50	−	+[2]
Tyrosine clearing	50	83	100	+	0	75	100	+[2]	+

[a] A blank space indicates not determined.
[b] Symbols: −, negative at end of incubation period; +, positive at 24 h or time of test. Superscript numbers indicate the day the reaction became positive.
[c] PIA, Peptone-iron agar.
[d] Test was 100% positive in 10 days.

L. grimontii strains were isolated from urine specimens; the remaining isolates were from stool specimens. Five of the strains were sent to us as *Salmonella* or with a diagnosis of suspected salmonellosis or diarrhea; two were sent as possible *Citrobacter* and one as an H_2S-positive *Shigella*.

Practical identification of *Leminorella*. At 36°C the *L. richardii* strains and one of the *L. grimontii* did not produce

TABLE 3. DNA relatedness between *L. grimontii* and *L. richardii*

Source of unlabeled DNA	Source of labeled DNA					
	L. grimontii ATCC 33999			*L. richardii* ATCC 33998		
	RBR[a] at 60°C	*D*[b]	RBR at 75°C	RBR at 60°C	*D*	RBR at 75°C
L. grimontii 1944-81 (ATCC 33999)	100	0.0	100	49	11.5	23
L. grimontii 3244-79	97	0.5	99	49	11.0	24
L. grimontii 3257-76	83	0.5	85	39	12.5	22
L. grimontii 3595-77	83	0.0	84	42	12.5	21
L. grimontii 1362-73	81	0.0	82	42	12.5	20
L. grimontii 0301-79	77	0.5	78	42	12.0	20
L. richardii 0978-82 (ATCC 33998)	44	13.5	23	100	0.0	100
L. richardii 0598-78	43	11.5	25	94	0.5	97
L. richardii 2502-80	43	13.0	23	94	0.5	97
L. richardii 2209-80	32	11.5	11	93	1.0	96
Leminorella sp. 3 3346-72	60	8.0	51	40	12.5	21

[a] RBR, Relative binding ratio, expressed as a percentage.
[b] *D*, Percentage of divergence within related sequences, expressed to the nearest 0.5% (4).

acid from D-glucose until 48 h. In addition, some of the strains grew poorly when taken from stock cultures. We therefore recommend freezing these strains for long-term maintenance. When biochemical tests were repeated on the two type strains and 3346-72 at 25°C, the reactions were not improved; some were even weaker. They were not motile at 25°C.

Strains of *Leminorella* should be easy to recognize. They are H_2S positive, D-mannose negative, and tyrosine positive much like *Proteus* species, but they are also urea negative, phenylalanine negative, and L-arabinose positive. They are susceptible to colistin, but have no zone of inhibition around penicillin, ampicillin, carbenicillin, and cephalothin. On primary plating media, *Leminorella* strains appear as lactose-negative colonies. Within 48 h in triple sugar iron agar, they give an alkaline slant and a weak acid reaction in the butt with H_2S production. Gas production in D-glucose, a positive methyl red test, and acid production in dulcitol by *L. grimontii* separate it from *L. richardii*. We hope this paper will stimulate others to isolate and identify these organisms.

We are sometimes asked the question, "Why name a new genus or species on the basis of a few strains of unknown clinical significance?" The first step in learning more about an organism is to give it a scientific name. Whenever possible, new organisms are classified in existing genera; however, many of the newly discovered organisms have been different from all existing genera both by phenotype and by molecular techniques (3, 8). Our purpose in naming a new organism is not to create a burden for clinical microbiologists, but to provide a simple means of communicating about an entity found in the laboratory. The best method of

TABLE 4. DNA relatedness of *L. grimontii* and *L. richardii* strains to other *Enterobacteriaceae*

Source of unlabeled DNA	% Relatedness to labeled DNA of *L. grimontii* ATCC 33999 at:		% Relatedness to labeled DNA of *L. richardii* ATCC 33998 at:	
	60°C	75°C	60°C	75°C
Buttiauxella agrestis 1176-81	6	ND[a]	10	ND
Cedecea davisae 3278-77	12	ND	13	ND
Cedecea sp. 3 4853-73	13	5	14	4
Citrobacter freundii 0460-61	8	3	11	3
Edwardsiella tarda 3592-64	7	3	9	2
Enterobacter aerogenes 1627-66	12	5	14	2
Enterobacter agglomerans 3123-70	9	5	12	3
E. agglomerans 0219-71	12	4	13	3
E. agglomerans 1600-71	6	ND	8	ND
E. agglomerans 1741-71	9	ND	11	ND
E. agglomerans 6003-71	10	ND	10	ND
Enterobacter cloacae 1347-71	10	ND	12	ND
Enterobacter amnigenus 1325-79	11	5	13	3
Enterobacter gergoviae 7601	13	ND	12	ND
Erwinia amylovora EA 178	9	3	10	2
Erwinia carotovora 495	10	2	11	2
Erwinia mallotivora 2851	8	ND	8	ND
Erwinia nigrifluens EN 104	11	4	15	3
Erwinia quercina EQ 102	9	ND	8	ND
Erwinia rhapontici ER 106	8	ND	9	ND
Erwinia salicis ES 102	9	ND	8	ND
Escherichia blattae 9005-74	13	ND	10	ND
Escherichia coli K-12	8	3	11	2
Escherichia hermannii 0980-72	8	ND	9	ND
Escherichia vulneris 2898-73	12	3	14	2
Ewingella americana 1468-78	10	3	13	3
Hafnia alvei I 5632-72	8	ND	11	ND
H. alvei II 4510-75	8	2	11	2
Klebsiella oxytoca 13182	9	ND	12	ND
Klebsiella planticola 4245-72	11	4	12	3
Klebsiella pneumoniae 2	8	3	11	3
Klebsiella terrigena 9001-81	10	ND	12	ND
Klebsiella trevisanii 9009-82	9	3	14	3
Kluyvera ascorbata 0408-78	10	ND	10	ND
Moellerella wisconsensis 3349-72	12	ND	13	ND
Morganella morganii 25830	9	2	10	1
Obesumbacterium proteus 4296-74	9	ND	10	ND
O. proteus 4302-74	5	3	8	2
Proteus mirabilis PR 14	3	ND	4	ND
Providencia alcalifaciens 3370-67	4	ND	5	ND
Providencia rettgeri 1163	4	ND	6	ND
Profidencia rustigianii 0132-68	4	ND	4	ND
Providencia stuartii 2896-68	4	ND	6	ND
Rahnella aquatilis 1327-79	10	ND	9	ND
Salmonella typhimurium LT2	11	4	13	2
Serratia ficaria 1165-77	10	ND	10	ND
Serratia fonticola 4556-71	11	4	16	2
Serratia marcescens 0868-57	16	6	16	3
Shigella boydii C13	8	2	11	3
Shigella flexneri 24570	11	ND	11	ND
Tatumella ptyseos 9591-78	3	ND	6	ND
Xenorhabdus nematophilus 9012-80	4	ND	5	ND
Xenorhabdus sp. 1426-81	4	ND	5	ND
Yersinia enterocolitica 0497-70	8	3	12	2
Yersinia pseudotuberculosis P62	6	2	10	2
Yersinia ruckeri 4535-69	7	ND	8	ND

[a] ND, Not determined.

TABLE 5. Susceptibility of *L. grimontii*, *L. richardii*, and *Leminorella* sp. 3 by agar diffusion

Antimicrobial agent	*L. grimontii*										*L. richardii*								*Leminorella* sp. 3 strain 3346-72	
	Zone size (mm)					Range	Mean	SD	% Susceptible		Zone size (mm)				Range	Mean	SD	% Susceptible	Zone size (mm)	% Susceptible
	1362-73	3244-76	3257-76	3595-77	0301-79	1944-81					0598-78	2209-80	2502-80	0978-82						
Colistin (10 μg)	14	14	13	14	14	14	13 to 14	14	0.4	100	15	15	14	15	14 to 15	15	0.5	100	14	100
Nalidixic acid (30 μg)	26	27	24	23	24	26	23 to 27	25	1.6	100	25	27	24	22	22 to 27	24	2.1	100	24	100
Sulfadiazine (250 μg)	6	38	34	33	38	31	6 to 38	30	12.1	86	38	40	38	38	38 to 40	38	1.0	100	36	100
Gentamicin (10 μg)	20	22	19	17	19	17	17 to 22	19	1.9	100	20	22	21	21	20 to 22	21	0.8	100	20	100
Streptomycin (10 μg)	14	13	12	11	12	10	10 to 14	12	1.4	0	14	14	14	14	14	14	0	0	13	0
Kanamycin (30 μg)	20	20	18	15	19	16	15 to 20	18	2.1	71	20	21	21	20	20 to 21	20	0.6	100	20	100
Tetracycline (30 μg)	6	24	22	21	22	25	6 to 25	20	7.0	67	24	24	24	23	23 to 24	24	0.5	100	21	100
Chloramphenicol (30 μg)	25	25	24	23	25	27	23 to 27	25	1.3	100	24	24	23	22	22 to 24	23	1.0	100	25	100
Penicillin (10 U)	6	6	6	6	6	6	6	6	0	0	6	6	6	6	6	6	0	0	6	0
Ampicillin (10 μg)	6	6	6	6	6	6	6	6	0	0	6	6	6	6	6	6	0	0	6	0
Carbenicillin (100 μg)	6	6	6	6	6	6	6	6	0	0	6	6	6	6	6	6	0	0	6	0
Cephalothin (30 μg)	6	6	6	6	6	6	6	6	0	0	6	6	6	6	6	6	0	0	6	0

TABLE 6. Differentiation of *L. grimontii* and *L. richardii*

Test	% positive for:		
	L. grimontii		*L. richardii*
	1 to 2 days	3 to 7 days	7 days
Methyl red	100		0
Citrate (Simmons)	100	100	0
Gas from D-glucose	33	100	0
Acid from dulcitol	50	83	0

communication is a genus name plus species name. Until recently, a strain of *Leminorella* would probably have been reported as "unidentified gram-negative rod which . . ."

There is no current evidence to indicate that *Leminorella* is important in human disease. However, it is now possible for physicians, clinical microbiologists, epidemiologists, and allied health professionals to contribute knowledge about this new organism. Previously this was impossible. Clinical microbiologists do not have to identify or report *Leminorella*, but that option is now available. Previously, experience with *Enterobacter sakazakii*, *Vibrio vulnificus*, *Legionella*, and several other new organisms indicates that some will be important in human disease. However, it is also important to know that an organism is not clinically significant in certain segments of the human population or at certain body sites. It is too early to evaluate the clinical significance of *Leminorella*. We hope that this report will begin the process of learning more about it.

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