Enterobacter gergoviae sp. nov.: a New Species of *Enterobacteriaceae* Found in Clinical Specimens and the Environment

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The name *Enterobacter gergoviae* sp. nov. is proposed for a group of organisms isolated from various clinical sources in France, Africa, and the United States and from environmental sources. *E. gergoviae* deoxyribonucleic acid contains 60% guanine plus cytosine. Deoxyribonucleic acid relatedness studies showed *E. gergoviae* strains to be very highly related, as is characteristic of strains of a single species. The biochemical profiles of *E. gergoviae* strains were extremely similar. Phenotypically, *E. gergoviae* belongs to the family *Enterobacteriaceae* and is most similar to *Enterobacter aerogenes*. *E. gergoviae* is distinguishable from *E. aerogenes* by its positive urea reaction and negative reactions for KCN, sorbitol, mucate, and gelatinase. Strain CIP 76.01 (= ATCC 33028 = CDC 604-77) is the type strain of this new species.

Richard et al. (8) described a new group of organisms that was biochemically most similar to *Enterobacter aerogenes*. The 30 strains comprising this group were distinguishable from *E. aerogenes* by their positive urea reaction and negative reactions for KCN, sorbitol, mucate, gelatinase, β -xylosidase, and α -methylglucoside. These strains were isolated from a variety of clinical and environmental sources in France and Africa. Ten strains isolated from urines during a hospital outbreak were multiply drug resistant.

Since the initial description of this group (8), 40 additional strains were received at the Center for Disease Control, and 11 additional strains were received at the Institut Pasteur. We now report characterization of this group by biochemical tests, antibiotic susceptibility, and deoxyribonucleic acid (DNA) relatedness. On the basis of these data, we propose the name *Enterobacter gergoviae* sp. nov. for these organisms and designate strain CIP 76.01 (= Center for Disease Control [CDC] 604-77 = American Type Culture Collection [ATCC] 33028) as the type strain.

MATERIALS AND METHODS

The strains used in this study are listed in Table 1. Biochemical tests were done essentially as previously described by Edwards and Ewing (5) and Richard et al. (7). Antibiotic susceptibility was assayed with the standardized disk method of Bauer et al. (1).

The preparation, isolation, and purification of labeled and unlabeled DNA, the method used for DNA reassociation, and the method used to separate single-

and double-stranded DNA on hydroxyapatite have been described elsewhere (3).

Guanine plus cytosine (G+C) composition was determined by CsCl buoyant density ultracentrifugation (6). Samples were analyzed twice in the presence of reference DNA from phage 2C of density 1.742 g/cm³, with reference to DNA from *Escherichia coli* K-12 taken to be 1.710 g/cm³.

RESULTS AND DISCUSSION

Biochemical tests. The biochemical reactions of 81 strains of *E. gergoviae* are presented in Table 2. Thus far, test results have shown these organisms to be homogenous biochemically. The only test in which 90% or more of the strains failed to give a similar (+ or -) reaction within 48 h was acid from lactose. In this test, 58% of the strains were positive within 48 h, and an additional 40% gave delayed positive reactions (3 to 7 days).

E. gergoviae is a gram-negative, oxidase-negative, nitrate-positive, fermentative, rod-shaped organism that is peritrichous when motile. As such, it conforms to the definition of *Enterobacteriaceae*.

The biochemical profile of *E. gergoviae* is most similar to those of *Enterobacter* species. *E. gergoviae* is perhaps most similar to *E. aerogenes*, but these two species can be separated by their reactions in tests for KCN, sorbitol, mucate, and urease (Table 3). The tests of use in differentiating *E. gergoviae* from other species of *Enterobacter*, *Klebsiella pneumoniae*, and *Hafnia alvei* (*Enterobacter hafniae*) are shown in Table 3.

TABLE 1. *Bacterial strains used in this study*

Strain	Source and history	
<i>Edwardsiella tarda</i>	3592-64	CDC
<i>Enterobacter aerogenes</i>	1627-66	CDC
<i>Enterobacter agglomerans</i>	2780-70	CDC
<i>Enterobacter agglomerans</i>	1429-71	CDC
<i>Enterobacter agglomerans</i>	1645-71	CDC
<i>Enterobacter agglomerans</i>	3482-71	CDC
<i>Enterobacter agglomerans</i>	4388-71	CDC
<i>Enterobacter agglomerans</i>	5378-71	CDC
<i>Enterobacter cloacae</i>	1347-71	CDC
<i>Enterobacter gergoviae</i>	76.01 (CDC 604-77)	IP ^a , urine, France
<i>Enterobacter gergoviae</i>	76.02 (CDC 605-77)	IP, urine, France
<i>Enterobacter gergoviae</i>	1/77 (CDC 606-77)	IP, cosmetic, France
<i>Enterobacter gergoviae</i>	12/76 (CDC 607-77)	IP, pus, France
<i>Enterobacter gergoviae</i>	11/76 (CDC 608-77)	IP, environment, France
<i>Enterobacter gergoviae</i>	5/76 (CDC 609-77)	IP, urine, France
<i>Enterobacter gergoviae</i>	20/75 (CDC 610-77)	IP, urine, France
<i>Enterobacter gergoviae</i>	2656-76	CDC, blood, United States
<i>Enterobacter gergoviae</i>	2787-76	CDC, sputum, United States
<i>Enterobacter gergoviae</i>	2863-76	CDC, abdominal wound, United States
<i>Enterobacter gergoviae</i>	3782-76	CDC, abscess, United States
<i>Enterobacter gergoviae</i>	3866-76	CDC, sputum, United States
<i>Enterobacter gergoviae</i>	569-77	CDC, abscess, United States
<i>Enterobacter gergoviae</i>	1004-77	CDC, sputum, United States
<i>Enterobacter gergoviae</i>	1007-77	CDC, sputum, United States
<i>Enterobacter gergoviae</i>	1061-77	CDC, ?, United States
<i>Enterobacter gergoviae</i>	1062-77	CDC, leg wound, United States
<i>Enterobacter gergoviae</i>	1063-77	CDC, urine, United States
<i>Enterobacter gergoviae</i>	1064-77	CDC, leg wound, United States
<i>Enterobacter gergoviae</i>	1065-77	CDC, hand, United States
<i>Enterobacter gergoviae</i>	1067-77	CDC, hand wound, United States
<i>Enterobacter gergoviae</i>	1068-77	CDC, blood, United States
<i>Enterobacter gergoviae</i>	1069-77	CDC, ?, United States
<i>Enterobacter gergoviae</i>	1070-77	CDC, ?, United States
<i>Enterobacter gergoviae</i>	1071-77	CDC, water, United States
<i>Enterobacter gergoviae</i>	1072-77	CDC, blood, United States
<i>Enterobacter gergoviae</i>	1073-77	CDC, blood, United States
<i>Enterobacter gergoviae</i>	1404-77	CDC, oral abscess, United States
<i>Enterobacter gergoviae</i>	1448-77	CDC, urine, United States
<i>Enterobacter gergoviae</i>	2460-77	CDC, ?, United States
<i>Enterobacter gergoviae</i>	2461-77	CDC, ?, United States
<i>Enterobacter gergoviae</i>	2462-77	CDC, ?, United States
<i>Enterobacter gergoviae</i>	2463-77	CDC, ?, United States
<i>Enterobacter gergoviae</i>	2464-77	CDC, leg, United States
<i>Enterobacter gergoviae</i>	2465-77	CDC, ?, United States
<i>Enterobacter gergoviae</i>	2466-77	CDC, sputum, United States
<i>Enterobacter gergoviae</i>	2467-77	CDC, sputum, United States
<i>Enterobacter gergoviae</i>	2529-77	CDC, urine, United States
<i>Enterobacter gergoviae</i>	2530-77	CDC, ?, United States
<i>Enterobacter gergoviae</i>	2531-77	CDC, sputum, United States
<i>Enterobacter gergoviae</i>	2532-77	CDC, kidney, United States
<i>Enterobacter gergoviae</i>	2533-77	CDC, cyst, United States
<i>Enterobacter gergoviae</i>	2534-77	CDC, ?, United States
<i>Enterobacter gergoviae</i>	2535-77	CDC, ?, United States
<i>Enterobacter gergoviae</i>	2781-77	CDC, ear drainage, United States
<i>Enterobacter gergoviae</i>	3439-77	CDC, lung, United States
<i>Enterobacter gergoviae</i>	3548-77	CDC, sputum, United States
<i>Enterobacter gergoviae</i>	3597-77	CDC, vagina, United States
<i>Enterobacter gergoviae</i>	439-78	CDC, cosmetic, United States
<i>Enterobacter gergoviae</i>	513-78	CDC, sputum, United States
<i>Enterobacter sakazakii</i>	4562-70	CDC

TABLE 1.—*continued*

Strain	Source and history	
<i>Escherichia coli</i>	K-12	University of Washington
<i>Hafnia alvei</i>	4510-72	CDC
<i>Hafnia alvei</i>	5632-72	CDC
<i>Klebsiella oxytoca</i>	13182	ATCC
<i>Klebsiella pneumoniae</i>	2	CDC
<i>Proteus mirabilis</i>	PR14	CDC
<i>Proteus vulgaris</i>	PR1	CDC
<i>Providencia alcalifaciens</i>	26240	CDC
<i>Providencia rettgeri</i>	1163	CDC
<i>Providencia stuartii</i>	2896-69	CDC
<i>Salmonella typhi-murium</i>	LT2	T. Theodore
<i>Serratia liquefaciens</i>	446-68	CDC
<i>Serratia marcescens</i>	868-57	CDC
<i>Serratia rubidaea</i>	934-72	CDC
<i>Yersinia enterocolitica</i>	497-70	IP, H.H. Mollaret
<i>Yersinia ruckeri</i>	4535-69	CDC

^a IP, Institut Pasteur.

TABLE 2. *Biochemical reactions of E. gergoviae*

Test	Reaction ^a	% Positive ^b	Reaction of type strain
Indole	—	0	—
Methyl red	v	18	—
Voges-Proskauer	+	99	+
Citrate (Simmons)	+	98	+
H ₂ S (triple sugar iron)	—	0	—
Urease	+	100	+
Phenylalanine deaminase	—	0	—
Lysine decarboxylase	v	80 (19)	+
Arginine dihydrolase	—	0	—
Ornithine decarboxylase	+	100	+
Motility	+	94	+
Gelatin (22°C)	—	0	—
KCN (growth)	—	0	—
Malonate	+	100	+
D-Glucose (acid)	+	100	+
D-Glucose (gas)	+	97	+
Acid from:			
Lactose	v	54 (29)	—
Sucrose	+	100	+
D-Mannitol	+	100	+
Dulcitol	—	0	—
Salicin	+	98	+
Adonitol	—	0	—
i-Inositol	—	11 (16)	—
D-Sorbitol	—	0	—
Sorbitol	—	0	—
L-Arabinose	+	99	+
Raffinose	+	100	+
L-Rhamnose	+	100	+
Maltose	+	98	+
D-Xylose	+	99	+
Trehalose	+	100	+
Cellobiose	+	98	+
α-CH ₃ -glucoside	—	1	—
Erythritol	—	0	—
Mannose	+	98	+
Melibiose	+	99 (1)	+

TABLE 2.—*continued*

Test	Reaction ^a	% Positive ^b	Reaction of type strain
D-Arabitol	+	96 (2)	+
Glycerol	+	100	+
ONPG	+	99	+
Esculin	+	98	+
Mucate	—	0	—
Tartrate (Jordan)	+	100	+
Acetate	+	98 (2)	+
Lipase (corn oil)	—	0	—
Deoxyribonuclease (25°C)	—	0	—
β -Xylosidase	—	0	—
Tetrathionate reductase	—	0	—
Nitrate to nitrite	+	98	+
Oxidase	—	0	—
Yellow pigment	—	0	—
Lysed by Guinee's	—	0	—
<i>Hafnia</i> -specific phage	—	0	—

^a Reactions obtained within 48 h at 36 $\pm$ 1°C unless specified; +, 90% or more positive; —, 0 to 9.9% positive; v, variable, 10.0 to 89.9% positive.

^b The figures in parentheses are percentages of delayed positive reactions (3 to 7 days).

^c ONPG, *o*-nitrophenyl- β -D-galactopyroside.

TABLE 3. *Differentiation of Enterobacter species from each other, Klebsiella pneumoniae, and Hafnia alvei (4)^a*

Test	<i>E. cloacae</i>	<i>E. aerogenes</i>	<i>E. agglomerans</i>	<i>E. sakazakii</i>	<i>E. gergoviae</i>	<i>K. pneumoniae</i>	<i>H. alvei</i>
Motility	+	+	v	+	+	—	v
Lysine decarboxylase	—	+	—	—	+ or (+)	+	+
Arginine dihydrolase	+	—	—	+	—	—	v
Ornithine decarboxylase	+	+	—	+	+	—	+
Urease	v	—	v	—	+	+	—
Methyl red	—	—	v	v	—	v	v
Voges-Proskauer	+	+	v	+	+	+	v
Citrate (Simmons)	+	+	v	+	+	+	v
KCN (growth)	+	+	v	+	—	+	+
Adonitol	v	+	v	—	—	v	—
<i>i</i> -Inositol	v	+	v	v	—	+	—
D-Sorbitol	+	+	v	—	—	+	—
Mucate	v	+	v	—	—	+	—
Deoxyribonuclease 25°C	—	—	—	+ or (+)	—	—	—
Yellow pigment	—	—	v	+	—	—	—
G+C (%)	53	54–56	52–58	56–60	60	54–56	48–52

^a Reactions were obtained at 36 $\pm$ 1°C (except for deoxyribonuclease, 25°C). The positive urease activity in *E. gergoviae* and *K. pneumoniae* is significantly weaker than that seen typically in species of *Proteus* and *Yersinia*. +, 90% or more positive within 48 h; (+), delayed positive, 3 to 7 days; —, 0 to 9.9% positive within 48 h; v, variable, 10.0 to 89.9% positive within 48 h.

Antibiotic susceptibility. Susceptibility of 25 strains to antibiotics was determined by the disk method of Bauer et al. (1). All strains were resistant to penicillin (Table 4). Most strains were susceptible to the other antibiotics tested. Multiple resistance was found in six strains. A strain isolated in France was resistant to penicillin, ampicillin, and cephalothin. In addition to being resistant to penicillin, one strain isolated in the United States was resistant to cephalothin, and one was resistant to nalidixic acid. Three strains isolated from an outbreak in France (8) were resistant to penicillin, nalidixic acid, and cephalothin. Two of these were also resistant to sulfadiazine, kanamycin, and chloramphenicol.

G+C content and DNA hybridization. Buoyant density ultracentrifugation in CsCl was used to determine the G+C content of DNA from *E. gergoviae* strains 76.01 (604-77) and 11/76 (608-77). The buoyant density of both strains was 1.719 g/cm³, which corresponds to 60 mol% G+C.

³²PO₄-labeled DNA from *E. gergoviae* strain 76.01 was used in DNA hybridization experiments. DNAs from 10 strains of *E. gergoviae* were tested for relatedness with the labeled DNA (Table 5). In 60°C reactions, *E. gergoviae*

strains were at least 75% related (75 to 97%). The thermal stability of DNA duplexes formed in homologous *E. gergoviae* 76.01 reactions at 60°C was approximately 94°C. The average thermal stability of heterologous *E. gergoviae* DNA duplexes was only 1°C lower than that of homologous duplexes. Thus, almost no divergence has occurred in DNA sequences held in common between strains of *E. gergoviae*. The 60°C incubation temperature is close to optimal for DNA reassociation. When DNA is hybridized at 75°C, only closely related DNA sequences can reassociate. On the basis of the very low percentage of divergence of related *E. gergoviae* DNA in 60°C reactions, one would predict that almost all of these duplexes would be stable at 75°C. This is indeed the case (Table 5). Thus, biochemical homogeneity in *E. gergoviae* is reflected by a high level of genetic relatedness in strains from France, Africa, and several states in the United States.

E. gergoviae is most similar biochemically to the genus *Enterobacter*. DNA relatedness of *E. gergoviae* to members of *Enterobacteriaceae* is highest with *E. aerogenes*, *E. cloacae*, two of the hybridization groups in *E. agglomerans*, and *Klebsiellae* (35 to 40%). *E. gergoviae* is 7 to 15% related to *Hafnia*, *Yersinia*, and *proteus*, and 20 to 30% related to other genera.

E. gergoviae is distinct both biochemically and genetically from the presently described species of *Enterobacteriaceae*. The G+C value of 60 mol% is compatible with findings for species of *Serratia*, *Klebsiella*, and *Enterobacter*. *E. gergoviae* is closest genetically to *Enterobacter* and *Klebsiella*. Biochemically it is closest to *Enterobacter* on the basis of its motility and positive reaction for ornithine decarboxylase.

The name *Enterobacter gergoviae* sp. nov. is proposed for this organism. The specific epithet *gergoviae* indicates that the type strain was isolated from samples taken during a urinary infection outbreak in Clermont-Ferrand University Hospital near Gergovie Highland in France. The suggested pronunciation is ger (rhymes with her) gó vee ē.

Strain CIP 76.01 (= ATCC 33028 = CDC 604-77) is the type strain of the species.

The clinical significance of *E. gergoviae* is not yet known. Some 80 sporadic isolates have been identified in our laboratories since 1976, and the organism has been implicated in a nosocomial outbreak of urinary tract disease (8). This already relatively large number of isolates will undoubtedly increase as laboratories become familiar with the characteristics of *E. gergoviae*.

REPRINT REQUESTS

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TABLE 4. Antibiotic susceptibility profiles for 25 strains of *E. gergoviae*

Antimicrobial agent	No. of strains		
	Susceptible	Intermediate	Resistant
10 ^a Colistin (8, 11) ^b	25	0	0
30 Nalidixic acid (13, 19)	9	12	4
250 Sulfadiazine (12, 17)	22	1	2
10 Gentamicin (12, 13)	25	0	0
10 Streptomycin (11, 15)	21	4	0
30 Kanamycin (13, 18)	23	0	2
30 Tetracycline (14, 19)	24	0	1
30 Chloramphenicol (12, 18)	20	2	3
10 Penicillin (11, 22)	0	0	25
10 Ampicillin (11, 14)	21	3	1
100 Carbenicillin (17, 24)	16	9	0
30 Cephalothin (14, 18)	18	3	3

^a The number preceding the name of the antimicrobial is the disk concentration in milligrams.

^b The first number in parentheses is the largest zone of inhibition (in millimeters) whereby the organism is considered resistant to the antibiotic. The second number in parentheses is the smallest zone of inhibition (in millimeters) whereby the organism is considered susceptible to the antibiotic. Zone sizes between these values are considered to indicate intermediate resistance. For instance, for colistin, with a zone of 8 mm or less the organism is considered resistant; with a zone of 11 mm or more, the organism is considered susceptible, and with a zone of 9 to 10 mm, the organism is considered intermediate.

TABLE 5. DNA relatedness of *E. gergoviae* 76.01 to *E. gergoviae* strains and members of Enterobacteriaceae

Source of unlabeled DNA		RBR ^a		
		60°C	75°C	%D ^b , 60°C
<i>Enterobacter gergoviae</i>	76.01 (604-77)	100	100	0.0
<i>E. gergoviae</i>	76.02 (605-77)	86	94	
<i>E. gergoviae</i>	1/77 (606-77)	77	86	0.1
<i>E. gergoviae</i>	12/76 (607-77)	75	82	0.6
<i>E. gergoviae</i>	11/76 (608-77)	77	92	
<i>E. gergoviae</i>	20/75 (610-77)	97	100	0.7
<i>E. gergoviae</i>	569-77	97	99	
<i>E. gergoviae</i>	2656-76	76	81	1.5
<i>E. gergoviae</i>	2787-76	88	92	
<i>E. gergoviae</i>	2863-76	88	86	
<i>E. gergoviae</i>	3782-76	89	93	2.1
<i>Enterobacter aerogenes</i>	1627-66	40	15	
<i>Enterobacter cloacae</i>	1347-71	39	15	12.6
<i>Enterobacter sakazakii</i>	4562-70	28	9	
<i>Klebsiella pneumoniae</i>	2	38	16	12.8
<i>Klebsiella oxytoca</i>	13182	36	19	
<i>Enterobacter agglomerans</i>	2780-70	26		
<i>E. agglomerans</i>	1429-71	23		
<i>E. agglomerans</i>	3482-71	17	3	
<i>E. agglomerans</i>	4388-71	40	13	
<i>E. agglomerans</i>	5378-71	40	12	
<i>E. agglomerans</i>	1645-71	22		
<i>H. alvei</i>	4510-72	14		
<i>H. alvei</i>	5632-72	13		
<i>Serratia marcescens</i>	868-57	19	11	13.8
<i>S. liquefaciens</i>	446-68	24		
<i>S. rubidaea</i>	934-72	23	3	
<i>E. coli</i>	K-12	33	9	14.0
<i>Salmonella typhi-murium</i>	LT2	30	9	14.7
<i>Edwardsiella tarda</i>	3592-64	20		
<i>Yersinia enterocolitica</i>	497-70	15		
<i>Yersinia ruckeri</i>	4535-69	15	1	
<i>Proteus mirabilis</i>	PR14	7		
<i>Proteus vulgaris</i>	PR1	10	0	
<i>Providencia rettgeri</i>	1163	10	0	
<i>Providencia alcalifaciens</i>	26240	7	0	
<i>Providencia stuartii</i>	2896-69	9		

^a RBR, Relative binding ratio; a convenient way to express percent DNA relatedness. RBR = % heterologous DNA reaction/% homologous DNA reaction × 100.

^b %D, Percentage of divergence; calculated on the assumption that each 1°C in thermal stability of a DNA duplex is caused by 1% of unpaired bases within that duplex (2). For example, consider organisms A and B that are 50% related. An A-A duplex has a mean thermal stability of 90°C, and an A-B duplex has a mean thermal stability of 80°C. The D in related DNA is 10%.

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