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Enterobacter hormaechei subsp. *oharae* subsp. nov., *E. hormaechei* subsp. *hormaechei* comb. nov., and *E. hormaechei* subsp. *steigerwaltii* subsp. nov., Three New Subspecies of Clinical Importance

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Six species and six additional genovars are combined within the so-called *Enterobacter cloacae* complex, with one of them being the species *Enterobacter hormaechei*. In a recent population genetic study, two genetic clusters were found in close phylogenetic proximity to the genetic cluster of *E. hormaechei*. In order to prove the hypothesis that these three genetic clusters belong to the same species, we performed cross-hybridization experiments in microplates with DNAs of representatives of each genetic cluster. The close phylogenetic relationship among the clusters was reflected by their relatively low ΔT_m values, ranging from 0.3 to 4.8, confirming the hypothesis that the clusters are parts of the same species. These clusters can be distinguished from the other species of the *E. cloacae* complex, which have ΔT_m values of 5.6 to 10.3. Forty-eight *E. hormaechei* strains from the different genetic clusters were phenotypically characterized with 129 biochemical tests. In this way, *E. hormaechei* could be differentiated from the other species of the *E. cloacae* complex because it tests negative in the 3-hydroxy-butyrate test. The three genetic clusters of *E. hormaechei* could also be differentiated from each other by using phenotypic tests. Hence, we propose three new subspecies of *E. hormaechei* corresponding to genetic clusters VI, VII, and VIII of the *E. cloacae* complex. *E. hormaechei* subsp. *hormaechei* comb. nov. corresponds to the original species description, as it gives negative results for the adonitol, D-arabitol, D-sorbitol, and D-melibiose tests and a positive result for the dulcitol test. *E. hormaechei* subsp. *oharae* subsp. nov. gives negative results for the dulcitol, adonitol, and D-arabitol tests and positive results for the D-sorbitol and D-melibiose tests. *E. hormaechei* subsp. *steigerwaltii* subsp. nov. gives a negative result for the dulcitol test and positive results for the adonitol, D-arabitol, D-sorbitol, and D-melibiose tests. Among the members of the *E. cloacae* complex, *E. hormaechei* seems to be the species most frequently recovered from clinical specimens.

The genus *Enterobacter* was first described by Hormaeche and Edwards (9). Since the transfer of *Enterobacter agglomerans* to the genus *Pantoea* (5), 14 species are included in the genus (1), including *Enterobacter aerogenes*, which is considered a homotypic synonym of *Klebsiella mobilis* because it has the same type strain (13). Around *Enterobacter cloacae*, six genetically related and phenotypically similar species have been combined within the so-called *E. cloacae* complex (7), i.e., *E. cloacae*, *Enterobacter asburiae*, *Enterobacter dissolvens*, *Enterobacter hormaechei*, *Enterobacter kobei*, and *Enterobacter nimipressuralis*. In addition to these species, at least six genetic clusters are phylogenetically delineated within the complex (7). Two of the most prominent clusters (clusters VI and VIII) are closely related to the species *E. hormaechei*, together forming the so-called *E. hormaechei* metacluster. They displayed 98 to 99% sequence identity in an analysis of three housekeeping genes and showed nearly identical restriction patterns of their *ampC* genes, which chromosomally code for a Bush class 1 beta-lactamase (7).

E. hormaechei was first described on the basis of 23 isolates sent to the Centers for Disease Control and Prevention (Atlanta, Ga.) for identification. At that time, they could not be assigned to a species, since they were negative in D-sorbitol and melibiose tests and did not fit the biochemical profile of any established *Enterobacter* species. Later, this preselected set of isolates turned out to be genetically closely related to each other. The species *E. hormaechei* was proposed to be lactose, D-sorbitol, raffinose, melibiose, and esculin negative and 87% dulcitol positive (12). Subsequently, a clinical outbreak was observed that was caused by a different biotype of *E. hormaechei*, challenging the original species description (4). In a hy-

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TABLE 1. Strains used for this study

Cluster[a]	Strain	Species[b]	Source material[c]	Origin
VI	EN-18	ENOH	Stool	Munich, Germany
	EN-190	ENOH	Trachea	Munich, Germany
	EN-210	ENOH	BAL	Munich, Germany
	EN-218	ENOH	Ear	Munich, Germany
	EN-232	ENOH	Throat	Munich, Germany
	EN-248	ENOH	Sputum	Munich, Germany
	EN-260	ENOH	Trachea	Munich, Germany
	EN-276	ENOH	Blood	Munich, Germany
	EN-277	ENOH	Blood	Munich, Germany
	EN-312	ENOH	NS	Hannover, Germany
	EN-314^T	ENOH	Mouth (2-yr-old infant)	Hannover, Germany
	EN-332	ENOH	BAL	Gelsenkirchen, Germany
	EN-334	ENOH	Urine	Gelsenkirchen, Germany
	EN-351	ENOH	Throat	Jena, Germany
	EN-366	ENOH	Abscess	Lausanne, Switzerland
VII	ATCC 49162^T	ENHO	Sputum	California
	EN-280	ENHO	Sputum	Munich, Germany
	EN-291	ENHO	Throat	Regensburg, Germany
	EN-449	ENHO	Blood	Munich, Germany
	EN-670	ENHO	Groin	Munich, Germany
	EN-673	ENHO	Stool	Munich, Germany
VIII	EN-30	ENST	Trachea	Munich, Germany
	EN-285	ENST	Wound	Regensburg, Germany
	EN-288	ENST	Wound	Regensburg, Germany
	EN-305	ENST	Urine	Växjö, Schweden
	EN-311	ENST	NS	Hannover, Germany
	EN-315	ENST	NS	Hannover, Germany
	EN-320	ENST	Wound	Stockholm, Sweden
	EN-323	ENST	Wound	Stockholm, Sweden
	EN-325	ENST	Urine	Stockholm, Sweden
	EN-331	ENST	BAL	Gelsenkirchen, Germany
	EN-349	ENST	Urine	Jena, Germany
	EN-352	ENST	Sputum	Jena, Germany
	EN-359	ENST	Urine	Jena, Germany
	EN-365	ENST	Puncture fluid	Lausanne, Switzerland
	EN-369	ENST	Blood	Mallorca, Spain
	EN-370	ENST	Blood	Mallorca, Spain
	EN-371	ENST	Blood	Mallorca, Spain
	EN-380	ENST	NS	Marseille, France
	EN-384	ENST	Urine	Berlin, Germany
	EN-400	ENST	Throat	Frankfurt, Germany
	EN-403	ENST	Skin	Frankfurt, Germany
	EN-407	ENST	Wound	Frankfurt, Germany
	EN-466	ENST	Trachea	Kiel, Germany
	EN-468	ENST	Central venous line	Kiel, Germany
	EN-474	ENST	Vagina	Kiel, Germany
	EN-490	ENST	Lung biopsy	Tübingen, Germany
	EN-562^T	ENST	Wound	Brüssel, Belgium
I	ATCC 35953^T	ENAS	Lochia exudate	United States
II	CDC 1347-71^R		Blood	United States
	ATCC BAA-260^T	ENKO	Blood	Kobe City, Japan
XI	ATCC 13047^T	ENCL	Cerebrospinal fluid	United States
XII	ATCC 23373^T	ENDI	Maize	United States
	ATCC 33241^T	ENCA	Plant	Czech Republic
X	ATCC 9912^T	ENNI	Elm tree	United States

[a] Genetic cluster denominations according to a recent population genetic study (7).
[b] ENHO, *Enterobacter hormaechei* subsp. *hormaechei*; ENOH, *Enterobacter hormaechei* subsp. *oharae*; ENST, *Enterobacter hormaechei* subsp. *steigerwaltii*; ENAS, *Enterobacter asburiae*; ENKO, *Enterobacter kobei*; ENCL, *Enterobacter cloacae*; ENDI, *Enterobacter dissolvens*; ENCA, *Enterobacter cancerogenus*; ENNI, *Enterobacter nimipressuralis.*
[c] NS, not specified; BAL, bronchoalveolar lavage fluid.

bridization study of clinical isolates of the *E. cloacae* complex performed by Grimont and Grimont (6), *E. hormaechei* represented the most prominent proportion of all isolates studied (33%). Similarly, the *E. hormaechei* metacluster comprised 44% of all strains in our recent population genetic study (7). Because of its apparently highly underestimated clinical relevance (6, 7), we studied the *E. hormaechei* metacluster further, concluding that it consists of three different subspecies, for which we propose the names *E. hormaechei* subsp. *oharae*, *E. hormaechei* subsp. *hormaechei*, and *E. hormaechei* subsp. *steigerwaltii*.

TABLE 2. DNA relatedness of representatives of the species and subspecies of the *E. cloacae* complex

Cluster or species[a]	Strain[b]	ΔT_m for hybridization with labeled DNA[c]		
		ENOH EN-314	ENHO ATCC 49162^T	ENST EN-562
Cluster VI (ENOH)	EN314^T	0	3.2	1.8
	EN18	0.3	3.2	1.0
Cluster VII (ENHO)	ATCC 49162^T	4.0	0	4.3
	EN280^T	4.8	0	4.3
Cluster VIII (ENST)	EN562	2.2	3.7	0
	EN331	4.0	4.8	2.7
	EN30	2.2	3.6	1.3
E. cloacae	ATCC 13047^T	5.9	5.8*	7.7
E. dissolvens	ATCC 23373^T	6.0	6.0*	8.0
E. kobei	ATCC BAA260^T	6.6	6.7*	6.6
	CDC 1347-71^R	7.3	5.7*	7.5
E. asburiae	ATCC 35953^T	5.6	5.7*	6.2
E. cancerogenus	ATCC 33241^T	7.0	6.6*	6.8
E. nimipressuralis	ATCC 9912^T	9.2	10.3*	8.5

[a] ENOH, *E. hormaechei* subsp. *oharae* subsp. nov.; ENHO, *E. hormaechei* subsp. *hormaechei* comb. nov.; ENST, *E. hormaechei* subsp. *steigerwaltii* subsp. nov.
[b] T, type strain; R, reference strain.
[c] *, arithmetic mean from two hybridizations.

MATERIALS AND METHODS

Forty-eight strains and seven type strains were included in this study (Table 1). They were assigned to their respective genetic clusters of the *E. cloacae* complex by partial sequence comparisons of their *hsp60* genes as previously described (7). Type strains were purchased from the American Type Culture Collection (ATCC) or the Collection de l'Institut Pasteur (CIP).

Bacterial strains were cultured aerobically on Columbia agar with 5% sheep blood and in Luria-Bertani broth at 37°C for 18 to 24 h. They underwent phenotypic testing with the API20E and Biotype 100 systems (bioMérieux, Marcy l'Etoile, France) and additional tests, i.e., motility testing, testing of acid production from mucate, and growth in medium containing potassium cyanide (KCN). The motility test was performed with SIM-agar (Becton Dickinson, Sparks, Md.) and the mucate fermentation test was performed with mucate broth (Fluka-Sigma-Aldrich, Steinheim, Switzerland), with both tests being performed according to the manufacturers' instructions. The ability to grow in the presence of KCN was tested in a peptone broth (1% peptone, 0.5% NaCl, 22.5‰ KH_2PO_4, 0.5% $Na_2HPO_4 \cdot H_2O$) at pH 7.6 containing 75‰ KCN. Arginine dihydrolase and ornithine decarboxylase activities were tested in Moeller's broth (pH 6.5), consisting of 0.5% peptone, 0.5% meat extract, 0.05% glucose, 0.5% pyridoxal, bromcresol purple, cresol red, and 1% respective amino acids (2). The esculin hydrolase test was performed with a broth containing 0.3% NaCl, 0.2% K_2HP_4, 0.3% Lab-Lemco medium, 1% peptone, and 0.1% esculin (2). Citrate activity was tested on Simmon's agar (Oxoid, Basingstoke, Hampshire, United Kingdom). The Voges-Proskauer test was performed according to the guidelines given by Chapin and Lauderdale (3). Urease activity was tested in a broth (pH 7.1) consisting of 0.5% NaCl, 0.2% KH_2PO_4, 0.1% glucose, 0.1% peptone, phenol red, and 2% urea (2). Additionally, urease activity was tested on Christensen's agar.

Antimicrobial susceptibilities to ampicillin, amoxicillin plus clavulanic acid, piperacillin, piperacillin plus tazobactam, cefoxitin, ceftazidime, cefotaxime, cefepime, meropenem, ciprofloxacin, gentamicin, and trimethoprim plus sulfamethoxazole were determined by disk diffusion tests on Mueller-Hinton agar on the basis of the quantitative interpretation criteria recommended by the Clinical and Laboratory Standards Institute (11). All phenotypic and susceptibility tests were performed at 37°C in ambient air. DNA preparations and DNA-DNA hybridizations in microplates were performed as described by Mehlen et al. (10).

Nucleotide sequence accession numbers. Nucleotide sequence data are available at the EMBL/GenBank/DDBJ database under accession numbers AJ853889 and AJ853890 for 16S rRNA genes and under accession numbers AJ417108, AJ417124, AJ417129, AJ417141, AJ417142, AJ417143, AJ543761, AJ543765, AJ543766, AJ543771, AJ543777, AJ543779, AJ543782, AJ543783, AJ543788, AJ543790, AJ543791, AJ543795, AJ543796, AJ543798, AJ543810, AJ543811, AJ543815, AJ543821, AJ543822, AJ543825, AJ543826, AJ543827, AJ543835, AJ543836, AJ543846, AJ543849, AJ543851, AJ543854, AJ543863, AJ543908, AJ567895, AJ567899, AJ567900, AJ862841, AJ862866, and AJ862867 for *hsp60* genes.

RESULTS AND DISCUSSION

In order to analyze the systematics of the *E. hormaechei* metacluster (7), we performed cross-hybridization experiments. The DNAs of strains EN-314 of genetic cluster VI, EN-562 of genetic cluster VIII, and *E. hormaechei* ATCC 49162^T of genetic cluster VII were labeled and hybridized with two or three strains from each of the clusters as well as with the type strains of the other species of the *E. cloacae* complex (Table 2). In our recent phylogenetic analysis (7), the within-group sequence divergences of clusters VI and VII approximated zero, while that of cluster VIII was a bit higher (0.6 ± 0.4). The within-group heterogeneities were considered during the selection of strains for DNA-DNA hybridizations. The ΔT_m values resulting from the hybridizations are presented in Table 2. The close DNA-DNA relatedness within clusters VI and VII was reflected by ΔT_m values below 0.5. The relatively higher heterogeneity of cluster VIII was indicated by higher within-group ΔT_m values of up to 2.7. By evaluating the DNA relatedness among the clusters, we found that clusters VI and VIII are closely related (mean ΔT_m value = 2.2), while a relatively longer distance for *E. hormaechei* cluster VII from the members of clusters VI and VIII was indicated by the mean ΔT_m value of 4.0. However, all three genetic clusters could still be assigned to the same species (14). They could be genetically distinguished from the other species of the *E. cloacae* complex, which had ΔT_m values of 5.6 to 10.3 (Table 2). Phenotypic characterizations allowed a clear distinction of the *E. hormaechei* metacluster from the rest of the *E. cloacae* complex, e.g., by the lack of growth on 3-hydroxy-butyrate (Table 3). Similar to the original species description, the six *E. hormaechei* strains of cluster VII were negative in the esculin (0%), D-sorbitol (17%), and α-D-melibiose (17%) tests and positive in the dulcitol test (100%). However, some strains grew on raffinose (50%) and lactose (67%), which was inconsistent with the results of the study set used for the original species description, as none of those strains were raffinose positive and only 9% were lactose positive. Genetic clusters VI, VII, and VIII were

TABLE 3. Biochemical reactions of 48 strains in the API20E system, the Biotype 100 system, and conventional tests[a]

Test	% Positive isolates (cumulative)[b]					
	E. hormaechei subsp. *oharae* (*n* = 15)		*E. hormaechei* subsp. *hormaechei* (*n* = 6)		*E. hormaechei* subsp. *steigerwaltii* (*n* = 27)	
	48 h	7 days	48 h	7 days	48 h	7 days
Voges-Proskauer (37°C)[c]	100		100		100	
Voges-Proskauer (37°C)[d]	100		100		100	
Motility in SIM-agar at 37°C[d]	90		83		88	
Growth in KCN[d]	30	40	67		19	38
Mucate acid production[d]	100		100		89	96
β-Galactosidase (ONPG)[c]	100		100		100	
Arginine dihydrolase[c]	100		100		100	
Arginine dihydrolase[d]	100		100		100	
Ornithine decarboxylase[c]	100		100		100	
Ornithine decarboxylase[d]	100		100		100	
Lysine decarboxylase[c]	0		0		0	
H_2S production[c]	0		0		0	
Urease (API20E and broth)[c]	0		0		0	
Urease (Christensen's agar)[d]	100		83	100	81	
Indole production[c]	0		0		0	
Citrate[c]	100		100		100	
Citrate (Simmons agar)[d]	100		100		100	
Gelatinase[c]	0		0		0	
Esculine hydrolysation[d]	0	27	0	0	7	
α-Methyl-D-gycoside, acid production[d]	80	90	100		77	100
Growth on substrates						
1-0-Methyl-α-D-glucopyranoside	53	100	100		52	100
1-0-Methyl-α-galactopyranoside	93	100	0	0	93	100
1-0-Methyl-β-D-glucopyranoside	93	100	100		100	
1-0-Methyl-β-galactopyranoside	93	100	100		85	93
3-0-Methyl-D-glucopyranose	0	7	67		89	93
3-Hydroxybutyrate	0	0	0	0	0	0
5-Keto-D-gluconate	0	0	17		0	0
α-D-Fucose	46		50		75	80
α-D-Fucose (acid from)[d]	50		50		79	
α-D-Melibiose	100		17		100	
α-D-Melibiose (acid from)[d]	100		50		100	
Adonitol	13		17		93	
Adonitol (acid from)[d]	0		0		100	
α-Ketoglutarate	0	7	17	35	7	26
α-Lactose	80	100	50	67	63	89
α-L-Fucose	67	73	100		96	100
α-L-Fucose (acid from)[d]	30		50		98	
α-L-Rhamnose	93		100		100	
α-L-Rhamnose (acid from)[d]	100		100		100	
β-D-Fructose	100		100		100	
β-Gentiobiose	100		100		100	
Caprate	0	0	0	0	0	0
Caprylate	0	0	0	0	0	0
cis-Aconitate	100		67	83	100	
D(−)-Ribose	100		100		100	
D(−)-Tartrate	0	0	0	17	0	0
D(+)-Arabitol	7		17		96	
D(+)-Cellobiose	100		100		100	
D(+)-Galactose	100		100		100	
D(+)-Malate	0	7	0	0	7	15
D(+)-Mannose	100		100		100	
D(+)-Trehalose	100		100		100	
D(+)-Turanose	27		17		0	19
D(+)-Xylose	93	100	100		100	
D-Galacturonate	100		100		96	
D-Glucosamine	100		100		96	100
D-Glucose	100		100		100	
D-Glucuronate	100		100		96	100
DL-Glycerate	0	0	0	33	0	0
D-Lyxose	93		67		89	93
D-Mannitol	100		100		100	
D-Saccharate	87		100		89	93
D-Sorbitol	87	93	17		96	
D-Tagatose	0	7	0	0	0	0

Continued on following page

TABLE 3—*Continued*

Test	% Positive isolates (cumulative)[b]					
	E. hormaechei subsp. *oharae* (n = 15)		*E. hormaechei* subsp. *hormaechei* (n = 6)		*E. hormaechei* subsp. *steigerwaltii* (n = 27)	
	48 h	7 days	48 h	7 days	48 h	7 days
Dulcitol	0	0	100		0	4
Fumarate	100		100		100	
Glycerol	100		100		100	
i-Erythritol	7		17		0	
L(+)-Arabinose	100		100		100	
L(+)-Tartrate	0	0	0	0	0	0
Lactulose	47	53	33		37	41
L-Alanine	100		100		100	
Malonate	0	13	0	17	0	19
Maltitol	80	100	100		67	93
Maltose	100		100		100	
meso-Tartrate	0	0	17		0	0
myo-Inositol	7	20	0		7	15
Palatinose	100		100		96	
Phenylacetate	100		100		78	93
Protocatechuate	0	0	0	0	0	4
Putrescine	0	0	0	0	0	4
Raffinose	93		50		96	
Succinate	100		100		96	100
Sucrose	93		100		100	
trans-Aconitate	100		67	83	100	
Xylitol	0	7	0	0	22	63

[a] The following tests were 100% positive for all strains analyzed at 24 h: growth on 4-aminobutyrate, 5-aminovalerate, L(−)-arabitol, benzoate, betain, *m*-coumarate, ethanolamine, gentisate, glutarate, histamine, L-histidine, *m*-hydroxybenzoate, *p*-hydroxybenzoate, hydroxyquinoline-*b*-glucuronide, itaconate, D(+)-melezitose, 3-phenylpropionate, propionate, (−)quinate, L(+)-sorbose, L-tryptophan, L-tyrosine, tricaballylate, trigonelline, tryptamine, and xylotol. The following tests were 100% negative for all strains analyzed at 24 h: growth on D-alanine, L-aspartate, D-gluconat, L-glutamate, 2-keto-D-gluconate, DL-lactate, L(−)-malate, maltotriose, *N*-acetyl-D-glucosamine, L-proline, and L-serine. If not otherwise stated, the Biotype 100 system was used.

[b] A blank space indicates that the test was not read or that the results did not change at this time period.

[c] API20E system.

[d] Conventional test.

differentiable from each other by their growth on dulcitol and adonitol and by other test results (Table 3).

Our data correspond well to those reported by Grimont and Grimont (6), who found a group with DNA relatedness (DNA-relatedness group) around the *E. hormaechei* type strain, which they described as being "slightly heterogeneous with ΔT_m values ranging from 0.0 to 4.0." The authors observed seven biogroups within the DNA-relatedness group. Two of them were positive for growth on adonitol, D-arabitol, and D-sorbitol, corresponding to phylogenetic cluster VIII. Four were negative for growth on adonitol and D-arabitol but positive for growth on D-sorbitol, corresponding to phylogenetic cluster VI, and one was negative in all three tests, corresponding to *E. hormaechei* cluster VII. Davin-Regli et al. (4) reported an outbreak with an "*E. cloacae* strain with the *E. hormaechei* genotype" but an aberrant biotype. The strain exhibited all of the characteristics of *E. hormaechei* and was 80% related to the type strain in DNA-DNA reassociation experiments but was positive for growth on D-sorbitol and α-D-melibiose. Obviously, this outbreak was caused by a strain of genetic cluster VI. Hence, these studies are in agreement with our observation that genetic clusters VI and VIII belong to the species *E. hormaechei* (4, 6). Therefore, we propose that these clusters are new subspecies of *E. hormaechei* and we consequently reassign the species itself to *E. hormaechei* subsp. *hormaechei* comb. nov., which more or less keeps the original characteristics of the species.

Emended description of *Enterobacter hormaechei* O'Hara et al. 1989. *Enterobacter hormaechei* (hor.maé′che.i. N.L. gen. m. *Hormaeche*, after Estenio Hormaeche, a Uruguayan microbiologist, who, together with P. D. Edwards, proposed and defined the genus *Enterobacter* [9]).

This emended description is based on phylogenetic sequence data and DNA-DNA hybridization data collected from 48 strains during the course of the present study and a previous population genetic study (7). Phenotypic characterization was performed by using the API20E and Biotype 100 systems and a series of conventional tests performed during the course of the present study. *E. hormaechei* strains are gram-negative rods which are 83% motile, catalase positive, oxidase and DNase negative, fermentative, and nonpigmented and exhibit the general characteristics of the family *Enterobacteriaceae*, the genus *Enterobacter*, and the *E. cloacae* complex. Growth occurs as nonpigmented colonies after 18 to 24 h at 15 to 42°C, with an optimum at 36°C, on all nonselective media, such as Colombia agar with 5% sheep blood, chocolate agar, tryptic soy agar, Luria-Bertani agar, and brain heart infusion agar, as well as on semiselective media such as MacConkey and ENDO agar. A detailed biochemical profiling of the species is given in Table 3. Growth on the following substances is subspecies specific: D-sorbitol, D-fucose, α-D-melibiose, 1-0-methyl-α-galactopyranoside, D-arabitol, dulcitol, D-(+)-raffinose, adonitol, and 3-methyl-D-glucopyranose. Table 4 shows the tests used for the differentiation of *E. hormaechei* and its subspecies from the

TABLE 4. Biochemical differentiation of *E. hormaechei* subspecies from other clinically relevant species of the *E. cloacae* complex

Species or subspecies	Biochemical test result[a]												
	D-Sorbitol	D-Fucose	L-Fucose	α-D-Melibiose	1-0-Methyl-α-galactopyranoside	Esculin	α-Lactose	D-Arabitol	Dulcitol	D(+)-Raffinose	Adonitol	3-Methyl-D-gluco-pyranose	3-Hydroxy-butyrate
E. hormaechei subsp. *oharae*	+	V	V	+	+	V	+⁻	−	−	+	−⁺	−	−
E. hormaechei subsp. *hormaechei*	−⁺	V	+	−⁺	−	−	V	−⁺	+	V	−⁺	V	−
E. hormaechei subsp. *steigerwaltii*	+	V	+	+	+	V	V	+	+	+	+	+	−
E. asburiae[b]	+	−	−	+⁻	+⁻	+	+⁻	−	−	+	−	−	+
E. kobei[b]	+	−	−	+	+	−	+⁻	−	V	+	−	−	+⁻
E. cloacae[b]	+	−	−	+	+	−	V	−	−⁺	+	−	−	+
E. dissolvens[b]	+	V	V	+	+	+	+	−	−	+	V	−	+

[a] Incubation was done at 36°C. Symbols: −, 0 to 10%; −⁺, 10 to 20%; v, 20 to 80%; +⁻, 80 to 90%; +, 90 to 100%.
[b] Results are according to reference 8.

other species of the genus. The G+C content of the DNA is 58.3 ± 0.3 mol% (T_m).

The type strain is ATCC 49162 (equivalent to CIP 103441^T and CCUG 27126^T).

Description of *Enterobacter hormaechei* subsp. *hormaechei* comb. nov (12). The description of *Enterobacter hormaechei* subsp. *hormaechei* comb. nov is based on the particular properties given in Table 3. *E. hormaechei* subsp. *hormaechei* corresponds to genetic cluster VII of the population structure of the *E. cloacae* complex which was presented recently (7). The biochemical tests used for the differentiation of this subspecies from the other *E. hormaechei* subspecies and from other *Enterobacter* species are subsumed in Table 4. All strains produced a Bush class 1 beta-lactamase at a low level, conferring resistance to ampicillin, amoxicillin plus clavulanic acid, and cefoxitin, but not to cefotaxime, ceftazidime, and cefepime, in agar diffusion tests. All strains were susceptible to meropenem, ciprofloxacin, trimethoprim plus sulfamethoxazole, and gentamicin.

The type strain, ATCC 49162 (equivalent to CIP 103441^T and CCUG 27126^T), was isolated from the sputum of a male patient from California (12).

Description of *Enterobacter hormaechei* subsp. *oharae* subsp. nov. *E. hormaechei* subsp. *oharae* (o.há'rae. N.L. gen. f. *Ohara*, in honor of Caroline M. O'Hara, an American microbiologist who is affiliated at this time with the Centers for Disease Control, Atlanta, Ga., and who originally described the species *E. hormaechei* [12] and has contributed greatly to the taxonomy of various *Enterobacteriaceae*).

This description is based on the particular properties given in Table 3. *E. hormaechei* subsp. *oharae* corresponds to genetic cluster VI of the population structure of the *E. cloacae* complex which was presented recently (7). The biochemical tests used for the differentiation of this subspecies from the other *E. hormaechei* subspecies and from other *Enterobacter* species are subsumed in Table 4. All strains produced a Bush class 1 beta-lactamase (AmpC), with 69% producing the enzyme at a low level, conferring resistance to ampicillin, amoxicillin plus clavulanic acid, and cefoxitin, but not to cefotaxime, ceftazidime, and cefepime, in agar diffusion tests. Twenty-five percent of the strains hyperproduced the AmpC protein, conferring additional resistance to piperacillin, piperacillin plus tazobactam, cefotaxime, and ceftazidime, but not to cefepime, gentamicin, and trimethoprim plus sulfamethoxazole. One strain (EN-312) produced an extended-spectrum beta-lactamase and was resistant to all beta-lactam antibiotics, cephalosporins, and trimethoprim plus sulfamethoxazole. All strains were susceptible to meropenem and ciprofloxacin.

The type strain is EN-314, which is available at the German Collection of Microorganisms and Cell Cultures (DSMZ 16687^T) and the Collection de l'Institut Pasteur (CIP 108490^T). It was isolated from a mouth swab of a 2-year-old infant. The GenBank accession number for its 16S rRNA gene sequence is AJ853889.

Description of *Enterobacter hormaechei* subsp. *steigerwaltii* subsp. nov. *E. hormaechei* subsp. *steigerwaltii* (stei.ger.wál'ti.i. N.L. gen. m. *Steigerwalt*, in honor of Arnold G. Steigerwalt, an American microbiologist who contributed to the species descriptions of *E. asburiae* and *E. hormaechei*).

This description is based on the particular properties given

in Table 3. *E. hormaechei* subsp. *steigerwaltii* corresponds to genetic cluster VIII of the population structure of the *E. cloacae* complex which was presented recently (7). The biochemical tests used for the differentiation of this subspecies from the other *E. hormaechei* subspecies and from other species of the *E. cloacae* complex are subsumed in Table 4. All strains produced a Bush class 1 beta-lactamase (AmpC), with 54% producing the enzyme at a low level, conferring resistance to ampicillin, amoxicillin plus clavulanic acid, and cefoxitin, but not to the rest of the antibiotics tested. Forty-two percent of the strains hyperproduced the AmpC protein, conferring additional resistance to piperacillin, piperacillin plus tazobactam, cefotaxime, and ceftazidime, but not to cefepime, gentamicin, and trimethoprim plus sulfamethoxazole. One strain (EN-331) produced an extended-spectrum beta-lactamase and was resistant to all beta-lactam antibiotics, cephalosporins, and trimethoprim plus sulfamethoxazole. All strains were susceptible to meropenem and ciprofloxacin.

The type strain is EN-562, which is available at the German Type Cell Collection (DSMZ 16691^T) and the Collection de l'Institut Pasteur (CIP 108489^T). It was recovered from an infected surgical skin wound of a 49-year-old patient with tonsillar carcinoma. The GenBank accession number for its 16S rRNA gene sequence is AJ853890.

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