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Escherichia vulneris: a New Species of *Enterobacteriaceae* Associated with Human Wounds

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The name *Escherichia vulneris* sp. nov. (formerly called Alma group 1 and Enteric group 1 by the Centers for Disease Control and API group 2 by Analytab Products, Inc.) is proposed for a group of isolates from the United States and Canada, 74% of which were from human wounds. *E. vulneris* is a gram-negative, oxidase-negative, fermentative, motile rod with the characteristics of the family *Enterobacteriaceae*. Biochemical reactions characteristic of 61 *E. vulneris* strains were positive tests for methyl red, malonate, and lysine decarboxylase; a delayed positive test for arginine dihydrolase; acid production from D-mannitol, L-arabinose, raffinose, L-rhamnose, D-xylose, trehalose, cellobiose, and melibiose; negative tests for Voges-Proskauer, indole, urea, H_2S, citrate, ornithine decarboxylase, phenylalanine deaminase, and DNase; and no acid from dulcitol, adonitol, *myo*-inositol, and D-sorbitol. Two-thirds of the strains produced yellow pigment. Most strains gave negative or delayed positive reactions in tests for lactose, sucrose, and KCN. The *E. vulneris* strains tested were resistant to penicillin and clindamycin, were resistant or showed intermediate zones of inhibition to carbenicillin and erythromycin, and were susceptible to 14 other antibiotics. DNA relatedness of 15 *E. vulneris* strains to the type strain averaged 75% in reactions at 60°C and 69% in reactions at 75°C, indicating that they comprise a separate species. DNA relatedness to other species in the family *Enterobacteriaceae* was 6 to 39%, an indication that this new species belongs in the family. *E. vulneris* showed the highest relatedness to species of *Escherichia* (25 to 39%) and *Enterobacter* (24 to 35%). On the basis of biochemical similarity, the new species was placed in the genus *Escherichia*. The type strain of *E. vulneris* is ATCC 33821 (CDC 875-72).

Between 1969 and 1981 the Enteric Section at the Centers for Disease Control received a total of 61 biochemically similar strains that did not belong to any described species of *Enterobacteriaceae*. These strains, most of which were isolated from human wound infections, were given the vernacular names Alma group 1 and then Enteric group 1 at the Centers for Disease Control. They have recently been called API group 2 by Analytab Products, Inc. Enteric group 1 initially seemed similar to *Enterobacter agglomerans* on the basis of overall biochemical reactions and because more than one-half of the Enteric group 1 strains produced a yellow pigment. Enteric group 1 differed from *E. agglomerans* by its positive reaction for lysine decarboxylase and its positive or delayed positive reaction for arginine dihydrolase.

Upon closer examination, Enteric group 1 was most similar phenotypically to members of the genus *Escherichia*, especially to the newly described species *Escherichia hermannii* (5). DNA hybridization studies showed that Enteric group 1 strains were a single new species in the family *Enterobacteriaceae*. The name *Escherichia vulneris* sp. nov. is proposed for this organism. In this paper, *E. vulneris* is characterized biochemically and genetically, and data are presented on its source of isolation.

(Parts of this study were done in partial fulfillment of the requirements for a doctoral degree in the School of Public Health at the University of North Carolina, Chapel Hill.)

MATERIALS AND METHODS

Nomenclature. With the exceptions of *Citrobacter amalonaticus* (*Levinea amalonatica*) and *Escherichia hermannii*, all bacterial names used have standing in nomenclature (21). The classification in the eighth edition of *Bergey's Manual of Determinative Bacteriology* (11) was used with the following exceptions. *Serratia liquefaciens* was not included in the eighth edition of *Bergey's Manual*. Some, but not all, of the

strains called *Enterobacter agglomerans* are undoubtedly synonymous to *Erwinia stewartii*, *Erwinia herbicola*, and *Erwinia uredovora*. Since we could not identify the synonymous strains and since the thrust of this paper was clinical, they were all called *E. agglomerans*. The names *Citrobacter diversus*, *Citrobacter amalonaticus*, *Morganella morganii*, *Providencia rettgeri*, *Providencia alcalifaciens*, and *Providencia stuartii* were used rather than *Citrobacter intermedius* biotype b, *Citrobacter intermedius* biotype 2, *Proteus morganii*, *Proteus rettgeri*, *Proteus inconstans* subgroup A, and *Proteus inconstans* subgroup B, respectively (the latter names appear in the eighth edition of *Bergey's Manual*).

Strains. The *Escherichia vulneris* strains used in DNA relatedness studies are listed in Table 1. Biochemical and source data were obtained from a total of 61 strains that are not listed individually. All *E. vulneris* strains were sent to the Enteric Reference Laboratory (formerly the Enteric Section) from state and federal laboratories between 1969 and 1981.

Preparation of unlabeled DNA. Cells were grown with shaking at 36 ± 1°C to stationary phase in 1,500-ml portions of brain heart infusion (BHI) broth. The cells were sedimented by centrifugation for 30 min at 8,000 rpm in the HG-4L rotor of a Sorvall model RC-3 centrifuge. The procedures for the extraction and purification of DNA are modifications of those of Marmur (18), Berns and Thomas (2), and Brenner et al. (7). The cells were suspended in a lysing solution (250 ml for each portion of cells sedimented from 1,500 ml of BHI broth) containing 0.05 M EDTA (pH 8.5), 0.05 M Tris-hydrochloride (Tris buffer, pH 8.1), and 0.1 M NaCl. Lysis was accomplished by the addition of 0.5% sodium dodecyl sulfate (SDS) and 50 μg of pronase per ml. Before use, pronase was self-digested at 37°C for 2 h to remove any contaminating DNases. The suspension was incubated overnight in a water bath at 37°C to maximize cell lysis. The SDS concentration was then adjusted to 1.0%, and an equal volume of phenol was added to the cell lysate. The mixture was shaken to equilibrate the phenol and aqueous phases, which were then separated by centrifugation for about 5 min at 4,000 rpm. At this point, the lower phenolic phase contained most of the cell proteins; cell wall debris and lipopolysaccharides were present in the SDS interphase. The upper, aqueous phase that contained the nucleic acids was carefully decanted. Sodium perchlorate was added to 1.0 M to help dissociate protein from nucleic acids. The aqueous phase was then extracted twice with an equal volume of chloroform to remove additional protein and lipopolysaccharide material as well as phenol. Two volumes of cold 95% ethanol were added to the aqueous phase to precipitate nucleic acids. The DNA precipitate was spooled on a glass rod and suspended in distilled water. A drop of chloroform was added to suppress microbial growth. After the addition of NaCl to 0.1 M, the DNA was reprecipitated twice with cold 95% ethanol and resuspended in distilled water. The DNA solution was then made 0.05 M with respect to both EDTA and Tris buffer and 0.1 M with respect to NaCl. Pancreatic RNase, B grade (preheated at 90°C for 10 min to inactivate any contaminating DNase), was added to a concentration of 50 μg/ml, and the DNA was incubated at 60°C in a water bath for 1 h. SDS (0.5% final concentration) and pronase (50 μg/ml final concentration) were added, and the solution was incubated overnight at 37°C. The SDS concentration was then increased to 1.0%, and the DNA was again extracted once with phenol and twice with chloroform, followed by three precipitations with ethoxyethanol. The suspension was made 0.1 M with respect to NaCl before each precipitation. DNAs were sheared by sonification at 4°C to a double-stranded molecular weight of 2.5×10^5 to 3.5×10^5 (14). The purity and concentration of the DNAs were assayed spectrophotometrically. Samples were diluted in distilled water to an optical density of between 0.20 and 1.0 at 260 nm, and their UV absorption spectra were determined between 300 and 220 nm. The equation used to determine the concentration of the DNA was:

(optical density at 260 nm − optical density at 300 nm) (dilution factor)/24 = milligrams of DNA per milliliter (1)

TABLE 1. Strains of *E. vulneris* used in DNA relatedness studies

Strain	Source	Sender
2524-69	Bird, intestine	Ahmed Radwan, Michigan
4774-70	Human, wound	Washington SHD[a]
5814-70	Rabbit	California SHD
2954-71	Human	Iowa SHD
4821-71	Human, wound	Tennessee SHD
875-72	Human	National Institutes of Health
3455-72	Human, wound	Wisconsin SHD
3763-72	Human, wound	North Carolina SHD
5641-72	Human, blood	Florida SHD
5907-72	Unknown	North Carolina SHD
1544-73	Human, wound	Wisconsin SHD
1659-73	Human, wound	Indiana SHD
2898-73	Human, wound	Louisiana SHD
3979-73	Human, wound	Wisconsin SHD
4121-73	Human	Hawaii SHD
4220-72	Human, wound	Alabama SHD

[a] SHD, State Health Department.

Preparation of labeled DNA. For labeling with ^{32}P, cells were grown to log phase in BHI broth, harvested by centrifugation, and suspended in 500 ml of Tris-buffered glucose-salt medium lacking phosphate but containing 0.5 to 1.0% BHI broth. Carrier-free ^{32}P (5 to 10 mCi) was added, and the cultures were incubated overnight at 37°C. Cells were then treated by the same procedures used for the preparation of unlabeled DNA. After sonification, the DNA was denatured in a boiling water bath for 4 to 5 min, cooled quickly by immersion in an ice bath, and passed over a hydroxyapatite (HA) column held at 60°C and equilibrated in 0.14 M phosphate buffer (PB, an equimolar mixture of NaH_2PO_4 and Na_2HPO_4 [pH 6.8])–0.4% SDS to remove cross-linked DNA and other contaminants that bind to HA. The single-stranded labeled DNA fragments that did not bind to HA in 0.14 M PB were collected, diluted 1:20 with 0.14 M PB–0.4% SDS, and assayed for radioactivity by Cerenkov counting (12) in a liquid scintillation spectrometer. The specific activity of the labeled DNA was determined as follows:

TABLE 2. Intraspecies DNA relatedness of *E. vulneris*[a]

Source of unlabeled DNA (strain)	Source of labeled DNA				
	E. vulneris 875-72			*E. vulneris* 2898-73	
	RBR (%), 60°C	% Divergence	RBR (%), 75°C	RBR (%), 60°C	% Divergence
875-72	100	0.0	100	67	1.5
2898-73	74	3.5	72	100	0.0
2524-69	86	1.0	74	86	2.0
4774-70	67	3.5	61	76	1.0
5814-70	70	3.5	69	79	1.0
2954-71	74	3.5	66	87	0.0
4821-71	72	4.0	61	71	0.5
3455-72	73	3.5	72	82	1.0
3763-72	79	5.5	78	74	5.5
4220-72	79	4.5	72	78	1.0
5641-72	74	4.0	67	NT[b]	NT
5907-72	73	3.5	69	69	0.5
1544-73	66	4.0	60	74	0.5
1659-73	78	4.0	69	77	1.0
3979-73	82	3.5	68	75	0.5
4121-73	81	4.0	70	88	0.0

[a] Divergence was calculated to the nearest 0.5%. The values shown are averages. All percentage reactions were done three or more times. Before normalization to 100% the percentage of DNA bound to HA in homologous reactions was 58 to 83. The amount of labeled DNA that bound to HA in control reactions that did not contain unlabeled DNA was 6 to 10% at 60°C and 11 to 13% at 75°C. These control values were subtracted from all reassociation reactions before normalization.

[b] NT, Not tested.

counts per minute per 1.0 ml of DNA/concentration of DNA in micrograms per milliliter = counts per minute per microgram of DNA (2)

For labeling with [^{3}H]thymidine, approximately 20 ml of log-phase cells in BHI broth was inoculated into 500 ml of Kahn-Helinski medium (17). The cultures were incubated with shaking at 37°C for 1 h or until a turbidity of about 10^8 organisms per ml was reached. [^{3}H]thymidine (2 mCi) was added to each 500 ml of culture. The cultures were reincubated with shaking at 37°C for 2 h or until the cell concentration reached about 10^9/ml. Cells were extracted, and the DNA was purified and treated by the same procedures used for [^{32}P]DNA.

DNA reassociation. Labeled, sheared DNA from reference strains at a concentration of 0.1 μg/ml (specific activity between 1×10^3 and 4×10^4 cpm/μg) was added to unlabeled DNA (at a concentration of 150 μg/ml) from the homologous strain and from other strains of interest. A "label only" control tube, containing only 0.1 μg of labeled DNA per ml and no unlabeled DNA, was used to control self-reaction of labeled DNA. The DNA mixtures were denatured by heating in a boiling water bath for 3 to 4 min and immediately quenched in an ice bath. The samples were incubated in 0.28 M PB at 60 or 75°C for 16 h. These reassociation criteria allowed the reaction for unlabeled DNA to reach 100 C_0t's (10), which is sufficient for almost complete reassociation of labeled with unlabeled DNA. Reassociation occurred ca. 3.5 times faster in 0.28 M PB than in 0.12 M PB (10). The C_0t for the unlabeled DNA was calculated as follows:

C_0t = (DNA concentration) (absorbance per microgram at 260 nm) (incubation time in hours)/2 (3)

$$C_0t \text{ (0.12 M PB)} = (150)\ (0.024)\ (16)/2 = 28.8 \quad (4)$$

$$C_0t \text{ (0.28 M PB)} = C_0t \text{ (0.12 M PB)} \times 3.5 = 28.8 \times 3.5 = 100.8 \quad (5)$$

The C_0t for labeled DNA was calculated as follows:

$$C_0t \text{ (0.12 M PB)} = (0.1)\ (0.024)\ (16)/2 = 0.019 \quad (6)$$

$$C_0t \text{ (0.28 M PB)} = 0.019 \times 3.5 = 0.067 \quad (7)$$

The C_0t for the labeled DNA was small enough to preclude significant self-reassociation of label. After incubation, the reassociation mixtures were diluted to 0.14 M PB in a volume of 15 ml, and reassociated DNA was separated from single-stranded DNA by passage through HA equilibrated with 0.14 M PB–0.4% SDS and kept at the temperature (60 or 75°C) at which the mixtures were incubated (6). At this criterion, double-stranded DNA binds to HA, and single-stranded DNA is eluted. After the sample was passed through, the HA was washed with four 15-ml portions of 0.14 M PB–0.4% SDS and then with four 15-ml portions of 0.4 M PB to elute double-stranded DNA. All eluates were placed directly into counting vials and assayed by the Cerenkov counting method. For ^{3}H-labeled reference strains, eluates were precipitated in 5% trichloroacetic acid in the presence of 0.05 ml of calf thymus DNA carrier (1 mg/ml). The precipitates were collected on 0.45-μm membrane filters, placed in counting vials, and dried. A 15-ml amount of scintillation fluid was added to all samples, which were then assayed for radioactivity. DNA relatedness was conveniently expressed as the relative binding ratio (RBR), which was obtained as follows. The percentage of DNA bound to HA in label only control reactions (usually 1

TABLE 3. DNA relatedness of *E. vulneris* strain 875-72 to species of *Enterobacteriaceae*

Source of unlabeled DNA	RBR (%), 60°C
Escherichia coli 09	39
E. coli 0143	31
E. blattae 541 IIA	32
E. blattae 9005-74	25
E. hermannii 980-73	29
Enteric group 10	33
Enterobacter gergoviae 604-77	35
E. cloacae 1347-71	32
E. agglomerans 2780-70	29
E. agglomerans 1600-71	26
E. amnigenus 1325-79	25
E. amnigenus 1319-79	24
Salmonella typhimurium LT2	31
Erwinia carotovora 495	33
E. cypripedii EC 155	23
E. rhapontici ER 106	22
E. nigrifluens EN 104	21
E. mallotivora 2851	19
E. amylovora EA 178	16
E. quercina EQ 102	16
Klebsiella oxytoca 13182	28
K. pneumoniae 2	27
Kluyvera cryocrescens 409-78	28
K. ascorbata 408-78	27
Cedecea davisae 3278-77	28
Cedecea lapagei 485-75	26
Cedecea sp. 3699-73	24
Cedecea sp. 4853-73	21
Citrobacter freundii 460-61	23
Serratia marcescens 868-57	22
S. ficaria 1165-77	18
S. fonticola 4556-71	17
Obesumbacterium proteus 4302-74	17
O. proteus 4296-74	16
Rahnella aquatilis 1327-79	17
Edwardsiella tarda 3592-64	16
Hafnia alvei 5632-72	13
Yersinia enterocolitica 497-70	13
Y. kristensenii 1474	13
Y. ruckeri 4535-69	12
Y. pseudotuberculosis P62	10
Morganella morganii 25830	11
Providencia rettgeri 1163	9
P. alcalifaciens 3370-67	8
P. stuartii 2896-68	6
Proteus vulgaris PR1	6

to 5%) was subtracted from the percentage of DNA bound to HA obtained in all homologous and heterologous DNA reassociation reactions. The percentage of DNA bound to HA in heterologous (labeled and unlabeled DNA from different strains) reactions was then normalized to that bound to HA in homologous reactions (labeled and unlabeled DNA from the same strain) to obtain the RBR. Before normalization, 50 to 80% of the DNA binds to HA in homologous reactions. Each reaction is done two or more times, and the mean value is given as the RBR. DNA relatedness is often mistakenly equated with homology or perfect pairing between DNA sequences. Related DNA sequences formed between strains of the same species can contain up to 6% unpaired nucleotide bases. In interspecies reactions, the amount of unpaired bases within related sequences can be as high as 20%. Unpaired bases within related nucleotide sequences cause decreased thermal stability. An index of relative thermal stability was obtained by thermal elution profiles. In this procedure, increased temperature, rather than 0.4 M PB, was used to elute the double-stranded DNA bound to HA. HA was washed with 0.14 M PB at increasing increments of 5°C up to 100°C to denature the DNA and elute it from the HA as single strands. The thermal elution midpoint, that temperature at which 50% of the DNA bound to HA is eluted, was calculated for homologous and heterologous reactions. Each decrease of 1°C in thermal stability of a heterologous DNA duplex is due to approximately 1% unpaired bases within related DNA (3). We therefore express the instability as percent divergence. A thermal elution midpoint decrease of 7°C is equal to a divergence of 7%.

G+C content. The guanine plus cytosine (G+C) content of DNA was determined optically by thermal denaturation (19).

Biochemical tests and antimicrobial susceptibility tests. Media and reaction conditions for biochemical tests were recently described (13, 16). Antibiograms were done on Mueller-Hinton agar by the disk method of Bauer et al. (1) as modified by Thornsberry (22). Zone sizes were designated as susceptible, intermediate, or resistant according to the recommendations of the National Committee for Clinical Laboratory Standards (20).

RESULTS

DNA relatedness. DNAs from two Enteric group 1 strains were labeled with either ^{32}P or ^{3}H and tested for relatedness to other Enteric group 1 strains and to representative species of *Enterobacteriaceae* (Tables 2 and 3). Data obtained with DNA labeled with either isotope were comparable and were therefore combined. Labeled DNA from strain 875-72 (subsequently designated as the type strain) showed an average of 75% relatedness to 15 other Enteric group 1 strains (range, 66 to 86%) in reactions at 60°C. The percent divergence in related DNA sequences was between 1 and 5.5. In reactions at 75°C, labeled 875-72 DNA showed an average of 69% relatedness (range, 60 to 78%). Similar results were obtained with labeled DNA from Enteric group 1 strain 2898-73. In this case, relatedness in reactions at 60°C averaged 77% (range, 67 to 88%), and the percent divergence was 0 to 5.5.

Strain 875-72 DNA was 6 to 35% related to other *Enterobacteriaceae*. Relatedness of 25% or more occurred with species of *Escherichia, Enterobacter, Salmonella, Klebsiella, Kluyvera*, and *Cedecea*. Relatedness was above 30% to *Erwinia carotovora* but less than 25% to other *Erwinia* species.

TABLE 4. Biochemical reactions of *E. vulneris*

Test[a]	% of 61 strains that are positive[b]	Reactions for type strain[c]
Indole	0	−
Methyl red	100	+
Voges-Proskauer	0	−
Citrate, Simmons	0	−
H_2S on TSI	0	−
Urease, Christensen's	0 (2)	−
Phenylalanine deaminase	0	−
Lysine decarboxylase	89 (2)	+
Arginine dihydrolase	28 (59)	(+)
Ornithine decarboxylase	0	−
Motility	100	+
Gelatin liquefaction (22°C)	0	−
KCN, growth in	15 (28)	+
Malonate utilization	85	+
D-Glucose, acid	100	+
D-Glucose, gas	97	+
Lactose	13 (64)	(+)
Sucrose	8 (26)	(+)
D-Mannitol	100	+
Dulcitol	0	−
Salicin	28 (69)	(+)
Adonitol	0	−
myo-Inositol	0	−
D-Sorbitol	0	−
L-Arabinose	100	+
Raffinose	100	+
L-Rhamnose	93 (3)	+
Maltose	100	+
D-Xylose	100	+
Trehalose	100	+
Cellobiose	100	+
α-Methyl-D-glucoside	26 (2)	−
Esculin hydrolysis	16 (70)	(+)
Melibiose	100	+
D-Arabitol	0	−
Mucate	77 (15)	+
Lipase, corn oil	0	−
DNase (25°C)	0	−
$NO_3^- \rightarrow NO_2^-$	100	+
Oxidase, Kovacs'	0	−
ONPG	100	+
Yellow pigment	56 (10)	+
D-Mannose	100	+
Erythritol	0	−
Glycerol	18 (36)	−
Jordan's tartrate	0	−
Acetate	20 (13)	(+)
Citrate, Christensen's	0 (2)	−
H_2S in PIA	0	−

[a] Abbreviations: TSI, triple sugar iron agar; ONPG, *o*-nitrophenyl-β-D-galactopyranoside; PIA, peptone iron agar.

[b] The values given are for 48 h of incubation (except for oxidase) at 36 ± 1°C, unless otherwise indicated. The values in parentheses are delayed reactions that became positive between 3 and 7 days.

[c] +, Positive reaction within 48 h; (+), positive reaction in 3 to 7 days; −, negative reaction after 7 days.

G+C content. The G+C contents of DNAs from three strains of *E. vulneris* were each determined at least four times spectrophotometrically, by thermal denaturation. The ratios obtained were between 58.5 and 58.7 mol% of G+C, well within the range for *Enterobacteriaceae*.

Biochemical reactions and description of *E. vulneris*. Biochemical test reactions for 61 *E. vulneris* strains and for the type strain are shown in Table 4. *E. vulneris* is a gram-negative, oxidase-negative, catalase-positive, nonsporeforming rod. It is motile, with peritrichous flagella (Fig. 1), reduces nitrate to nitrite, ferments glucose and other carbohydrates with the production of acid and gas, has 58 to 59 mol% G+C in its DNA, and is isolated from wounds and other human clinical specimens. *E. vulneris* strains are positive in the methyl red test, and most utilize malonate. They give a negative Voges-Proskauer reaction, do not produce indole, urea, or H_2S, and do not utilize citrate. They ferment D-mannitol, L-arabinose, raffinose, L-rhamnose, D-xylose, trehalose, cellobiose, and melibiose. They do not ferment dulcitol, adonitol, *myo*-inositol, D-sorbitol, erythritol, or D-arabitol. Strains give variable reactions for the fermentation of lactose, sucrose, and salicin, with many being delayed (3 to 7 days) positive. They are negative in tests for phenylalanine deaminase and ornithine decarboxylase, usually positive for lysine decarboxylase, and delayed positive for arginine dihydrolase. They are negative in tests for corn oil, DNase, and gelatin liquefaction. More than one-half of *E. vulneris* strains produce a yellow pigment. A further description of *E. vulneris* is found in the tables and text. The type strain (holotype) is ATCC 33821 (CDC 875-72), isolated from the intestine of a cowbird in Michigan. We propose *Escherichia vulneris* as a new species (vul.ner'is. L.n. *vulnus* a wound; L. gen. n. *vulneris* of a wound; *Escherichia vulneris* the

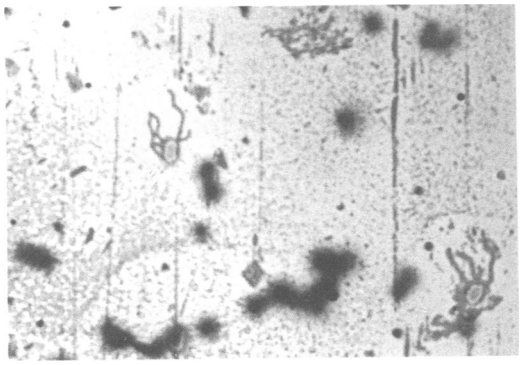

FIG. 1. Photomicrograph of *E. vulneris* showing flagella. ×1,000.

TABLE 5. Tests of value in differentiating *E. vulneris* from other *Enterobacteriaceae*

Test[a]	Reactions[b] for:										
	Shigellae	*Escherichia coli*	*Escherichia hermannii*	*Escherichia vulneris*[c]	*Enterobacter aerogenes*	*Enterobacter agglomerans*	*Enterobacter cloacae*	*Enterobacter gergoviae*	*Enterobacter sakazakii*	*Enterobacter amnigenus* biogroup	
										1	2
Indole	V	+	+	0	−	[−]	−	−	−	−	−
Methyl red	+	+	+	100	−	V	−	−	−	−	V
Voges-Proskauer	−	−	−	0	+	V	+	+	+	+	+
Citrate, Simmons'	−	−	−	0	[+]	[+]	+	+	+	V	+
Lysine decarboxylase	−	[+]	[−]	89 (2)	+	−	−	+	−	−	−
Arginine dihydrolase	−	[−]	−	28 (59)	−	−	[+]	−	+	−	V
Ornithine decarboxylase	V	V	+	0	+	−	+	+	[+]	V	+
KCN, growth in	−	−	+	15 (28)	+	V	+	−	+	+	+
Malonate utilization	−	−	−	85	+	V	V	+	[−]	+	+
Lactose	−	+	[−]	13 (64)	+	V	V	V	+	V	V
Sucrose	−	V	[−]	8 (26)	+	[+]	+	+	+	+	−
Cellobiose	−	−	+	100	+	[+]	+	+	+	+	+
Melibiose	V	[+]	−	100	+	V	+	+	+	+	+
Yellow pigment	−	−	+	56 (10)	−	[+]	−	−	+	−	−

[a] Test conditions are given in footnote *b*, Table 4.

[b] Symbols: +, 90 to 100% positive; [+], 75 to 89% positive; V, 26 to 74% positive; [−], 11 to 25% positive; −, 0 to 10% positive.

[c] The numbers not in parentheses represent the percentage of 61 *E. vulneris* strains that gave positive reactions, whereas the numbers in parentheses represent delayed reactions that became positive after 3 to 7 days (see Table 4).

Escherichia of a wound).

Differentiation of *E. vulneris* from other *Enterobacteriaceae*. Tests useful in differentiating *E. vulneris* from *Escherichia, Shigella*, and *Enterobacter* species are shown in Table 5. Its positive methyl red reaction and negative reactions for Voges-Proskauer and Simmons citrate separate *E. vulneris* from all *Enterobacter* species except for some strains of *Enterobacter agglomerans*. Most strains of *E. vulneris* give positive lysine decarboxylase reactions and positive or delayed positive arginine dihydrolase reactions; *E. agglomerans* is negative in both of these tests. KCN, lactose, and sucrose reactions, for which most *E. vulneris* strains give negative or delayed positive reactions, are also helpful in separating this organism from *Enterobacter* species. Yellow pigment production is a characteristic of about two-thirds of *E. vulneris* strains. *E. vulneris* is indole negative and ornithine decarboxylase negative, characteristics that separate it from other *Escherichia* species and from *Shigella* species. Reactions for arginine dihydrolase, KCN, malonate, cellobiose, and yellow pigment production are also extremely helpful in differentiating *E. vulneris* from shigellae and *Escherichia coli*. Positive melibiose and malonate (85%) reactions, as well as reactions in the decarboxylase tests, serve to separate *E. vulneris* from *E. hermannii*, which is its nearest phenotypic relative.

Antimicrobial susceptibility. Sixteen *E. vulneris* strains were tested for susceptibility to 18 antibiotics (Table 6). All strains were resistant to clindamycin, and 15 were resistant to penicillin. All strains either were resistant or gave intermediate zones against carbenicillin and erythromycin. Single strains were resistant to chloramphenicol and nalidixic acid. *E. vulneris* strains were uniformly susceptible to all other antibiotics tested except for intermediate zones of three strains to nitrofurantoin and of two strains to polymyxin B.

Origin, source, and clinical information. The 61 strains of *E. vulneris* were isolated from 24 states and from Canada. Of these, 56 strains were isolated from humans, 2 were isolated from animals, 1 was isolated from the environment, and 2 were of unknown origin. Where the sex of the patient was given, 29 of 48 isolates were from men. Eight isolates were from patients less than 10 years of age, five were from patients between the ages of 10 and 19, eight were from adults between the ages of 20 and 49, and nine were from patients 50 years or older. The source of isolation was given for 50 of the 56 human isolates; 37 were from wounds, at least 28 of which were arm or leg wounds. Five isolates were from throat or sputum cultures, four were from blood, and there was one isolate each from the vagina, urine, the stool, and the lymph nodes.

TABLE 6. Antimicrobial susceptibility pattern of 16 *E. vulneris* strains[a]

Antimicrobial agent (disk content)[b]	No. of strains that were:		
	Resistant	Intermediate	Susceptible
Amikacin (10)	0	0	16
Ampicillin (10)	0	0	16
Carbenicillin (100)	5	11	0
Cefamandole (30)	0	0	16
Cefoxitin (30)	0	0	16
Cephalothin (30)	0	0	16
Chloramphenicol (30)	1	0	15
Clindamycin (2)	16	0	0
Erythromycin (15)	8	8	0
Gentamicin (10)	0	0	16
Kanamycin (30)	0	0	16
Nalidixic acid (30)	1	0	15
Nitrofurantoin (300)	0	3	13
Penicillin G (10 U)	15	0	1
Polymyxin B (300 U)	0	2	14
Tetracycline (30)	0	0	16
Tobramycin (10)	0	0	16
Trimethoprim-sulfamethoxazole (1.25 + 23.75)	0	0	16

[a] Zone sizes were interpreted as susceptible, intermediate, or resistant according to the recommendations of the National Committee for Clinical Laboratory Standards (20).

[b] All disk contents are in micrograms unless otherwise indicated.

DISCUSSION

Taxonomic interpretation of DNA relatedness data. Five parameters can be used to genetically define a species: (i) relatedness at conditions optimal for DNA reassociation, (ii) relatedness at conditions less than optimal for DNA reassociation (at which only highly complementary sequences can reassociate), (iii) divergence in related nucleotide sequences, (iv) genome size, and (v) G+C content of DNA. The first three parameters are exclusive; e.g., if strains of a given species are 90% interrelated, they cannot be equally related to any other species, or if their related sequences show 2% divergence, they must exhibit a greater level of divergence to all other species. The last two parameters are not exclusive. A strain can be excluded from a species if its DNA has a totally different genome size or G+C content, but a strain cannot be included in a species solely because of similarities in genome size or G+C content (e.g., DNAs from *Bacillus subtilis* and humans have a similar G+C content). Experience has shown that, with very few exceptions, a species consists of strains whose DNAs are 70% or more related at optimal conditions and 55% or more related at less than optimal conditions and whose DNAs contain 6% or less divergence in related nucleotide sequences (4). DNA relatedness data are sufficient to identify bacteria to the species level, even in the absence of phenotypic data. Having said this, it is important to rapidly qualify this sweeping statement. The proper way to characterize a potentially new species involves three steps. The strains should first be grouped biochemically. The second step is to determine whether they constitute one or more unique DNA relatedness groups (separable from all described species on the basis of DNA relatedness). The third step is to choose those biochemical reactions that are useful in phenotypically separating a new DNA relatedness group from all described species. If no good routine tests serve this purpose, additional tests must be evaluated for this purpose (e.g., ascorbate utilization and growth at 5°C were necessary to separate the new species *Kluyvera ascorbata* and *Kluyvera cryocrescens*) (15). If biochemical tests cannot be found that correlate with DNA relatedness groups, it is wise, in our opinion, not to create new species. Of what use is a species that cannot be identified phenotypically? Phenotypic data are also of primary importance at the genus level. A genetic genus should ideally consist of a group of phenotypically similar species that are 40 to 65% related (just below the 70% or more relatedness found in strains of a single species). Unfortunately, this ideal genus does not often exist. The alternatives are to capriciously create new genera or to give either genetic or phenotypic similarity priority in establishing new genera or in assigning new species to existing genera. We believe that a genus is a somewhat artificial taxon and, as such, cannot be strictly defined genetically. When phenotypic and genetic data do not agree with respect to classification at the genus level, we lean towards a phenotypic genus, a group of species that share key biochemical characteristics and that must be separated from one another biochemically. This is a practical approach to classification at the genus level. This argument has been presented with specific examples in the families *Enterobacteriaceae* and *Legionellaceae* (8, 9).

Phenotypic and DNA relatedness data indicate that *E. vulneris* is a new species. Its distinctive biochemical profile poses no problem in identification for the diagnostic laboratory; however, it should be noted that several tests may become positive only after 3 to 7 days of incubation (arginine dihydrolase, KCN, lactose, sucrose, salicin, esculin, and mucate).

E. vulneris strains form a single genetic species. Although the relatedness of some strains to labeled DNA from each of two reference strains was slightly less than 70% in optimal DNA

reassociation reactions, divergence was never more than 5.5%, and relatedness in reactions at 75°C remained high (60% or more).

The type strain of *E. vulneris* was 39% or less related to other species of *Enterobacteriaceae*. The highest levels of relatedness were to species of *Enterobacter, Escherichia*, and *Salmonella*. These genera belong to the so-called "core" of the family *Enterobacteriaceae*, whose DNAs are 40 to 50% interrelated. Solely on the basis of DNA relatedness, *E. vulneris* could have been assigned to a new genus or to the genus *Enterobacter, Salmonella*, or *Escherichia*. When phenotypic characteristics were considered, *Salmonella* was eliminated, but the other choices remained.

Similar choices existed for the classification of *Escherichia hermannii*, another recently reported species (5). *E. hermannii* showed 40 to 50% relatedness to species of *Enterobacter* and *Escherichia* but differed from each of these genera in key diagnostic reactions. It was more similar to *Escherichia*, and we therefore decided to place it in *Escherichia* rather than in *Enterobacter* despite the fact that its G+C content (53 to 58%) was somewhat closer to the G+C contents of *Enterobacter* species than to those of *Escherichia* species. A new genus was considered but rejected because we were hesitant to create a new genus for a single species that was significantly related to existing genera and that would not pose identification problems if included in *Escherichia*. The same reasoning was used in assigning *E. vulneris* to *Escherichia* rather than to *Enterobacter* or to a new genus as a species that is methyl red positive, citrate negative, and Voges-Proskauer negative. *E. vulneris* phenotypically fits *Escherichia* better than *Enterobacter*, despite its having a G+C content more like *Enterobacter* than *Escherichia*. We again hesitated to create a new genus for a single species that can be readily identified within the confines of an existing genus.

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