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Biochemical Identification of New Species and Biogroups of *Enterobacteriaceae* Isolated from Clinical Specimens†‡

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In 1972 there were only 11 genera and 26 species in the family *Enterobacteriaceae*. Today there are 22 genera, 69 species, and 29 biogroups or Enteric Groups. This paper is a review of all of the new organisms. It has a series of differential charts to assist in identification and a large chart with the reactions of 98 different organisms for 47 tests often used in identification. A simplified version of this chart gives the most common species and tests most often used for identification. The sources of the new organisms are listed, and their role in human disease is discussed. Fourteen new groups of *Enterobacteriaceae* are described for the first time. These new groups are biochemically distinct from previously described species, biogroups, and Enteric Groups of *Enterobacteriaceae*. The new groups are *Citrobacter amalonaticus* biogroup 1, *Klebsiella* group 47 (indole positive, ornithine positive), *Serratia marcescens* biogroup 1, and unclassified Enteric Groups 17, 45, 57, 58, 59, 60, 63, 64, 68, and 69.

For over 30 years the Enteric Bacteriology Laboratories (recently reorganized into the following administrative units: Enteric Bacteriology Section, Nosocomial Infections Laboratory Branch, and Molecular Biology Laboratory) at the Centers for Disease Control (CDC) have received cultures of *Enterobacteriaceae* for identification (52). Many of these were from hospital and State Health Department laboratories in the United States, but others came from all parts of the world. Some of the cultures proved to be typical strains of existing species, but many were atypical strains of existing species, strains that were eventually assigned to new species or new "Enteric Groups" or strains which were reported simply as "unidentified." Traditionally a major mission of our laboratories has been to make these distinctions.

Until the early 1970s, identification was almost always based on visual analysis of results from biochemical and serological tests commonly used in enteric bacteriology. However, during the last 10 years several other techniques were introduced which have greatly increased the accuracy of identification. These included DNA-DNA hybridization, antibiotic susceptibility patterns, species or genus specific bacteriophages, and two different types of computer analysis (Fig. 1). These techniques have clearly shown that the number of genera, species, and biogroups of *Enterobacteriaceae* is much larger than previously recognized. The application of these techniques has resulted in the proposal of several new genera and species and in several changes in classification of *Enterobacteriaceae*. The extent of this new knowledge can be appreciated by comparing the 97 named organisms in Table 1 to the 26 named species described in the third edition of Edwards and Ewing's *Identification of Enterobacteriaceae* (33), which was published only 12 years ago.

Each week we receive inquiries about the new species of *Enterobacteriaceae*. Many of these come from clinical microbiologists who have received an identification, such as "*Serratia fonticola*," "*Cedecea neteri*," or "Enteric Group 17" from a commercial identification system. Manufacturers of commercial identification systems have obtained isolates of the new *Enterobacteriaceae*, determined their biochemical profiles, and then added them to the data base. Other inquiries have come from State Health Departments and reference centers in foreign countries, both of which must identify atypical or unusual isolates. Material describing all the species of *Enterobacteriaceae* is scattered throughout the literature, which has made it difficult to keep up with all these new organisms. For these reasons, we thought that it would be useful to have a single publication that would describe all the named species, biogroups, and Enteric Groups of the family *Enterobacteriaceae*. The purpose of this paper is to review these organisms. Many of the new organisms mentioned in this paper may never be seen in a given clinical microbiology laboratory, but will be encountered more frequently by reference laboratories. Thus, we urge clinical microbiologists to pick and choose the material that suits their particular needs. Some different approaches for identifying and reporting *Enterobacteriaceae* are given below. We emphasize that 80 to 95% of all isolates seen in a general hospital setting will be *Escherichia coli*, *Klebsiella pneumoniae*, or *Proteus mirabilis*. Over 99% of all clinical isolates will belong to only 23 species. The remaining 74 organisms will comprise less than 1% of *Enterobacteriaceae* found in clinical specimens. The reader is urged to keep this distribution in mind and not be overwhelmed with the large number of new species. This is best exemplified by the advice "When you hear hoofbeats, think horses, not zebras."

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† Dedicated to William H. Ewing on his 70th birthday.

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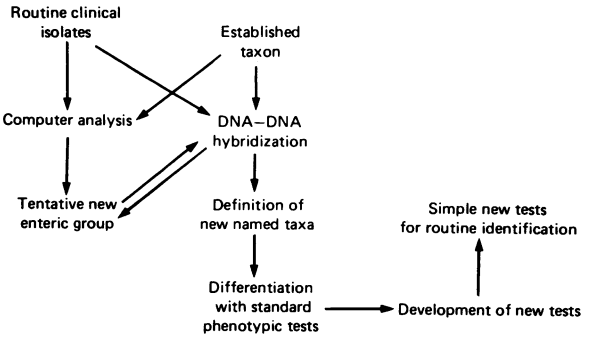

FIG. 1. DNA hybridization, computer analysis, and phenotypic properties in defining new groups of *Enterobacteriaceae*.

MATERIALS AND METHODS

Nomenclature. We use only names that have standing in nomenclature either by inclusion on the *Approved Lists of Bacterial Names* (124) or by publication or validation in the *International Journal of Systematic Bacteriology*. (The exceptions to this are two new species, *Escherichia fergusonii* and *Enterobacter taylorae*, which are described in this issue [47]. They will obtain standing in nomenclature as soon as they are validated in the *International Journal of Systematic Bacteriology*.) This nomenclature and classification is summarized in Table 1, which lists the genera, species, and biogroups. In this paper we describe the following Enteric Groups for the first time: Enteric Groups 17, 45, 57, 58, 59, 60, 61, 68, and 69. These Enteric Groups are defined as strains with similar biochemical reactions which are grouped together for convenience, until their classification can be studied, and a scientific name can be proposed.

General. Unless exceptions are given, the following statements hold throughout this paper: the temperature of incubation for biochemical tests was 36 ± 1°C (except for *Xenorhabdus* sp., which grows well at 25°C, but not at 37°C); water refers to glass-distilled water; commercial media were used whenever possible (the terms "from individual ingredients" or "was made with" appear when a commercial medium was not used); optical density was measured in a Bausch & Lomb Spectronic 20 spectrophotometer at 650 nm in 13- by 100-mm disposable glass tubes; filter sterilization was through a 0.22-μm nitrocellulose filter; refrigeration was at a temperature of 5 ± 1°C; all results are based on cultures picked from a single isolated colony; DNA hybridization refers to DNA-DNA hybridization; enzyme names should be understood to be within quotation marks since actual enzyme assays with cell-free extracts were not done.

Criteria for the definition of genus, species, and biogroup. The concepts of genus, species, and biogroup have been discussed in considerable detail previously (16, 20, 21, 46, 48). Briefly, we consider organisms to be of the same species if they are highly related by DNA hybridization. They are generally 70% or greater when hybridization is done at 60°C, and their percent relatedness drops only slightly when hybridization is done at the more stringent temperature, 75°C (16). In addition, values for delta T_m are usually less than 4. Organisms of the same species are usually similar biochemically and can be defined on this basis. They are biochemically different from related species in the same genus (Table 1). A genus is much harder to define. In some instances genera of *Enterobacteriaceae* represent natural evolutionary groups as defined by techniques that actually measure evolutionary distance. These natural groups share phenotypic similarities that differentiate them from other genera. An example of this type of genus is *Serratia*, which is a unique evolutionary group (65) and which is biochemically distinct from all other genera of *Enterobacteriaceae*. Another definition of a genus is "a group of species which are grouped together for convenience rather than because of a close evolutionary relationship" (17). *Enterobacter* is an example of this kind of genus. This type of genus is heterogeneous by phenotypic characteristics and by molecular techniques such as DNA hybridization. A biogroup (synonym, biovar) is defined to be a group of strains that have a common biochemical reaction pattern, which is often unusual for the particular species. Biogroups are usually defined to aid with identification (for examples, see the sections below on *Serratia marcescens* biogroup 1 and *Morganella morganis* biogroup 1).

Media and tests. Media preparation and biochemical testing were done by methods used in the Enteric Laboratories for many years. The exact methods used have been described in considerable detail elsewhere (43, 46, 73).

The data in Table 1 represent over a million biochemical reactions done over a period of 30 years by several dozen different microbiologists with the same media and procedures (43, 46, 73), which are generally available to clinical microbiology laboratories and reference laboratories. The purpose of Table 1 is to give all the species of *Enterobacteriaceae* (with the exception of *Erwinia*) and the biochemical test results that can be used to both define and to identify them. Many of the species and biogroups in Table 1 will be rarely, if ever, seen in many clinical laboratories. Table 2 gives the species of *Enterobacteriaceae* that should comprise over 99% of clinical isolates and lists the test most useful for identification. We suggest that clinical microbiologists use Table 2 as the first step in identification. Occasionally a clinical specimen will yield an isolate organism that will not be readily identified as one of the organisms listed. Table 1 can then be consulted along with the differential Tables 4 through 21. Table 1 should also be used for *Enterobacteriaceae* isolated from water, soil, food, the hospital environment, and similar sources. Organisms from these sources are frequently different species from the ones listed in Table 2. Table 1 should also be particularly useful for state and county health departments and national reference centers that must deal with all species of *Enterobacteriaceae*, not just those that occur primarily in clinical specimens.

Data tabulation for Table 1. The CDC publication *Differentiation of Enterobacteriaceae by Biochemical Reactions—Revised* (36) gives the biochemical reaction based on the species known and classification used in 1973. Beginning in 1972, all results for cultures reported by the Enteric Laboratories were coded and stored on magnetic tape for use in a large central computer. As taxonomic changes were made, the organism identification codes were simultaneously changed. This approach has allowed us to use the computer for tabulating data (with a computer program developed by C.E.) as changes are made in classification.

As others have pointed out, many of the cultures received by the Enteric Laboratories are atypical. For example, in the early 1970s the number of hydrogen sulfide-producing strains of *E. coli* was about 20% of the total number of *E. coli* received. However, in a large sample of *E. coli* strains isolated in clinical laboratories, this percentage of hydrogen sulfide-producing strains would be expected to be less than

TABLE 1. Biochemical reactions of the named species, biogroups, and Enteric Groups of the family *Enterobacteriaceae*[a]

Species	Indole production	Methyl red	Voges-Proskauer	Citrate (Simmons')	Hydrogen sulfide (TSI)	Urea hydrolysis	Phenylalanine deaminase	Lysine decarboxylase	Arginine dihydrolase	Ornithine decarboxylase	Motility (36°C)	Gelatin hydrolysis (22°C)	Growth in KCN	Malonate utilization	D-Glucose, acid	D-Glucose, Gas	Lactose fermentation	Sucrose fermentation
Buttiauxella																		
B. agrestis	0	100	0	100	0	0	0	0	0	100	100	0	80	60	100	100	100	0
Cedecea																		
C. davisae[b]	0	100	50	95	0	0	0	0	50	95	95	0	86	91	100	70	19	100
C. lapagei[b]	0	40	80	99	0	0	0	0	80	0	80	0	100	99	100	100	60	0
C. neteri[b]	0	100	50	100	0	0	0	0	100	0	100	0	65	100	100	100	35	100
Cedecea sp. 3[b]	0	100	50	100	0	0	0	0	100	0	100	0	100	0	100	100	0	50
Cedecea sp. 5[b]	0	100	50	100	0	0	0	0	50	50	100	0	100	0	100	100	0	100
Citrobacter																		
C. freundii[b]	5	100	0	95	80	70	0	0	65	20	95	0	96	15	100	95	50	30
C. diversus[b]	99	100	0	99	0	75	0	0	65	99	95	0	0	90	100	98	35	45
C. amalonaticus[b]	100	100	0	85	0	80	0	0	85	95	98	0	95	0	100	97	50	15
C. amalonaticus biogroup 1[b]	100	100	0	1	0	45	0	0	85	100	99	0	96	0	100	93	19	100
Edwardsiella																		
E. tarda[b]	99	100	0	1	100	0	0	100	0	100	98	0	0	0	100	100	0	0
E. tarda biogroup 1[b]	100	100	0	0	0	0	0	100	0	100	100	0	0	0	100	50	0	100
E. hoshinae	13	100	0	0	0	0	0	100	0	95	100	0	0	100	100	35	0	100
E. ictaluri	0	0	0	0	0	0	0	100	0	65	0	0	0	0	100	50	0	0
Enterobacter																		
E. aerogenes[b]	0	5	98	95	0	2	0	98	0	98	97	0	98	95	100	100	95	100
E. cloacea[b]	0	5	100	100	0	65	0	0	97	96	95	0	98	75	100	100	93	97
E. agglomerans[b]	20	50	70	50	0	20	20	0	0	0	85	2	35	65	100	20	40	75
E. gergoviae[b]	0	5	100	99	0	93	0	90	0	100	90	0	0	96	100	98	55	98
E. sakazakii[b]	11	5	100	99	0	1	50	0	99	91	96	0	99	18	100	98	99	100
E. taylorae[b]	0	5	100	100	0	1	0	0	94	99	99	0	98	100	100	100	10	0
E. amnigenus biogroup 1[b]	0	7	100	70	0	0	0	0	9	55	92	0	100	91	100	100	70	100
E. amnigenus biogroup 2	0	65	100	100	0	0	0	0	35	100	100	0	100	100	100	100	35	0
E. intermedium	0	100	100	65	0	0	0	0	0	89	89	0	65	100	100	100	100	65
Escherichia-Shigella																		
E. coli[b]	98	99	0	1	1	1	0	90	17	65	95	0	3	0	100	95	95	50
E. coli, inactive[b]	80	95	0	1	1	1	0	40	3	20	5	0	1	0	100	5	25	15
Shigella, serogroups A, B, and C[b]	50	100	0	0	0	0	0	0	5	1	0	0	0	0	100	2	0	0
S. sonnei[b]	0	100	0	0	0	0	0	0	2	98	0	0	0	0	100	0	2	1
E. fergusonii[b]	98	100	0	17	0	0	0	95	5	100	93	0	0	35	100	95	0	0
E. hermanii[b]	99	100	0	1	0	0	0	6	0	100	99	0	94	0	100	97	45	45
E. vulneris[b]	0	100	0	0	0	0	0	85	30	0	100	0	15	85	100	97	15	8
E. blattae	0	100	0	50	0	0	0	100	0	100	0	0	0	100	100	100	0	0
Ewingella																		
E. americana[b]	0	84	95	95	0	0	0	0	0	0	60	0	5	0	100	0	70	0
Hafnia																		
H. alvei[b]	0	40	85	10	0	4	0	100	6	98	85	0	95	50	100	98	5	10
H. alvei biogroup 1	0	85	70	0	0	0	0	100	0	45	0	0	0	45	100	0	0	0
Klebsiella																		
K. pneumoniae[b]	0	10	98	98	0	95	0	98	0	0	0	0	98	93	100	97	98	99
K. oxytoca[b]	99	20	95	95	0	90	1	99	0	0	0	0	97	98	100	97	100	100
Klebsiella group 47 indole positive, ornithine positive[b]	100	96	70	100	0	100	0	100	0	100	0	0	100	100	100	100	100	100
K. planticola[b]	20	100	98	100	0	98	0	100	0	0	0	0	100	100	100	100	100	100
K. ozaenae[b]	0	98	0	30	0	10	0	40	6	3	0	0	88	3	100	50	30	20
K. rhinoscleromatis[b]	0	100	0	0	0	0	0	0	0	0	0	0	80	95	100	0	0	75
K. terrigena	0	60	100	40	0	0	0	100	0	20	0	0	100	100	100	80	100	100

TABLE 1—*Continued*

D-Mannitol fermentation	Dulcitol fermentation	Salicin fermentation	Adonitol fermentation	*myo*-Inositol fermentation	D-Sorbitol fermentation	L-Arabinose fermentation	Raffinose fermentation	L-Rhamnose fermentation	Maltose fermentation	D-Xylose fermentation	Trehalose fermentation	Cellobiose fermentation	alpha-Methyl-D-glucoside fermentation	Erythritol fermentation	Esculin hydrolysis	Melibiose fermentation	D-Arabitol fermentation	Glycerol fermentation	Mucate fermentation	Tartrate, Jordan's	Acetate utilization	Lipase (corn oil)	DNase at 25°C	Nitrate → nitrite	Oxidase, Kovac's	ONPG	Yellow pigment	D-Mannose fermentation
100	0	100	0	0	0	100	100	100	100	100	100	100	0	0	100	100	0	60	100	60	0	0	0	100	0	100	0	100
100	0	99	0	0	0	0	10	0	100	100	100	100	5	0	45	0	100	0	0	0	0	91	0	100	0	90	0	100
100	0	100	0	0	0	0	0	0	100	0	100	100	0	0	100	0	100	0	0	0	60	100	0	100	0	99	0	100
100	0	100	0	0	100	0	0	0	100	100	100	100	0	0	100	0	100	0	0	0	0	100	0	100	0	100	0	100
100	0	100	0	0	0	0	100	0	100	100	100	100	50	0	100	100	100	0	0	0	50	100	0	100	0	100	0	100
100	0	100	0	0	100	0	100	0	100	100	100	100	0	0	100	100	100	0	0	0	50	50	0	100	0	100	0	100
99	55	5	0	3	98	100	30	99	99	99	99	55	5	0	0	50	0	98	95	90	80	0	0	99	0	95	0	100
100	50	20	98	0	99	100	0	100	100	100	100	99	40	0	2	0	100	98	93	75	75	0	0	100	0	96	0	100
100	0	40	0	0	100	100	5	99	99	99	100	100	5	0	10	5	0	70	98	85	75	0	0	99	0	100	0	100
100	4	0	0	0	100	100	100	100	100	100	100	100	70	0	0	100	0	55	100	93	82	0	0	100	0	100	0	100
0	0	0	0	0	0	9	0	0	100	0	0	0	0	0	0	0	0	30	0	25	0	0	0	100	0	0	0	100
100	0	0	0	0	0	100	0	0	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0	100
100	0	50	0	0	0	13	0	0	100	0	100	0	0	0	0	0	0	65	0	0	0	0	0	100	0	0	0	100
0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0	100
100	5	100	98	95	100	100	96	99	99	100	100	100	95	0	98	99	100	98	90	95	50	0	0	100	0	100	0	95
100	15	75	25	15	95	100	97	92	100	99	100	99	85	0	30	90	15	40	75	30	75	0	0	99	0	99	0	100
100	15	65	7	15	30	95	30	85	89	93	97	55	7	0	60	50	50	30	40	25	30	0	0	85	0	90	75	98
99	0	99	0	0	0	99	97	99	100	99	100	99	2	0	97	97	97	100	2	97	93	0	0	99	0	97	0	100
100	5	99	0	75	0	100	99	100	100	100	100	100	96	0	100	100	0	15	1	1	96	0	0	99	0	100	98	100
100	0	92	0	0	1	100	0	100	99	100	100	100	1	0	90	0	0	1	75	0	35	0	0	100	0	100	0	100
100	0	91	0	0	9	100	100	100	100	100	100	100	55	0	91	100	0	0	35	9	0	0	0	100	0	91	0	100
100	0	100	0	0	100	100	0	100	100	100	100	100	100	0	100	100	0	0	100	0	0	0	0	100	0	100	0	100
100	100	100	0	0	100	100	100	100	100	100	100	100	100	0	100	100	0	100	100	100	0	0	0	100	0	100	0	100
98	60	40	5	1	94	99	50	80	95	95	98	2	0	0	35	75	5	75	95	95	90	0	0	100	0	95	0	98
93	40	10	3	1	75	85	15	65	80	70	90	2	0	0	5	40	5	65	30	85	40	0	0	98	0	45	0	97
93	2	0	0	0	30	60	50	5	30	2	80	0	0	0	0	50	0	10	0	30	2	0	0	100	0	2	0	100
99	0	0	0	0	2	95	3	75	90	2	100	5	0	0	0	25	0	15	10	90	0	0	0	100	0	90	0	100
98	60	65	98	0	0	98	0	92	96	96	96	96	0	0	46	0	100	20	0	96	96	0	0	100	0	83	0	100
100	19	40	0	0	0	100	40	97	100	100	100	97	0	0	40	0	8	3	97	35	78	0	0	100	0	98	98	100
100	0	30	0	0	1	100	99	93	100	100	100	100	25	0	20	100	0	25	78	2	30	0	0	100	0	100	50	100
0	0	0	0	0	0	100	0	100	100	100	75	0	0	0	0	0	0	100	50	50	0	0	0	100	0	0	0	100
100	0	80	0	0	0	0	0	23	16	13	99	10	0	0	50	0	99	24	0	35	10	0	0	97	0	85	0	99
99	0	13	0	0	0	95	2	97	100	98	99	15	0	0	7	0	0	95	0	70	15	0	0	100	0	90	0	100
55	0	55	0	0	0	0	0	0	0	0	70	0	0	0	0	0	0	0	0	30	0	0	0	100	0	30	0	100
99	30	99	90	95	99	99	99	99	98	99	99	98	90	0	99	99	98	97	90	95	75	0	0	99	0	99	0	99
99	55	100	99	98	99	98	100	100	100	100	100	100	98	2	100	99	98	99	93	98	90	0	0	100	0	100	1	100
100	10	100	100	95	100	100	100	100	100	100	100	100	100	0	100	100	100	100	96	100	95	0	0	100	0	100	0	100
100	15	100	100	100	92	100	100	100	100	100	100	100	100	0	100	100	100	100	100	100	62	0	0	100	0	100	1	100
100	2	97	97	55	65	98	90	55	95	95	98	92	70	0	80	97	95	65	25	50	2	0	0	80	0	80	0	100
100	0	98	100	95	100	100	90	96	100	100	100	100	0	0	30	100	100	50	0	50	0	0	0	100	0	0	0	100
100	20	100	100	80	100	100	100	100	100	100	100	100	100	0	100	100	100	100	100	100	20	0	0	100	0	100	0	100

Continued on following page

TABLE 1—*Continued*

Species	Indole production	Methyl red	Voges-Proskauer	Citrate (Simmons')	Hydrogen sulfide (TSI)	Urea hydrolysis	Phenylalanine deaminase	Lysine decarboxylase	Arginine dihydrolase	Ornithine decarboxylase	Motility (36°C)	Gelatin hydrolysis (22°C)	Growth in KCN	Malonate utilization	D-Glucose, acid	D-Glucose, Gas	Lactose fermentation	Sucrose fermentation
Kluyvera																		
K. ascorbata[b]	92	100	0	96	0	0	0	97	0	100	98	0	92	96	100	93	98	98
K. cryocrescens[b]	90	100	0	80	0	0	0	23	0	100	90	0	86	86	100	95	95	81
Moellerella																		
M. wisconsensis	0	100	0	80	0	0	0	0	0	0	0	0	70	0	100	0	100	100
Morganella																		
M. morganii[b]	98	97	0	0	5	98	95	0	0	98	95	0	98	1	100	90	1	0
M. morganii biogroup 1[b]	100	95	0	0	41	100	100	100	0	95	0	0	91	5	100	91	0	0
Obesumbacterium																		
O. proteus biogroup 2	0	15	0	0	0	0	0	100	0	100	0	0	0	0	100	0	0	0
Proteus																		
P. mirabilis[b]	2	97	50	65	98	98	98	0	0	99	95	90	98	2	100	96	2	15
P. vulgaris[b]	98	95	0	15	95	95	99	0	0	0	95	91	99	0	100	85	2	97
P. penneri[b]	0	100	0	0	30	100	99	0	0	0	85	50	99	0	100	45	1	100
P. myxofaciens	0	100	100	50	0	100	100	0	0	0	100	100	100	0	100	100	0	100
Providencia																		
P. rettgeri[b]	99	93	0	95	0	98	98	0	0	0	94	0	97	0	100	10	5	15
P. stuartii[b]	98	100	0	93	0	30	95	0	0	0	85	0	100	0	100	0	2	50
P. alcalifaciens[b]	99	99	0	98	0	0	98	0	0	1	96	0	100	0	100	85	0	15
P. rustigianii[b]	98	65	0	15	0	0	100	0	0	0	30	0	100	0	100	35	0	35
Rhanella																		
R. aquatilis[b]	0	88	100	94	0	0	95	0	0	0	6	0	0	100	100	98	100	100
Salmonella																		
Subgroup 1 serotypes[b]—most	1	100	0	95	95	1	0	98	70	97	95	0	0	0	100	96	1	1
S. typhi[b]	0	100	0	0	97	0	0	98	3	0	97	0	0	0	100	0	1	0
S. choleraesius[b]	0	100	0	25	50	0	0	95	55	100	95	0	0	0	100	95	0	0
S. paratyphi A[b]	0	100	0	0	10	0	0	0	15	95	95	0	0	0	100	99	0	0
S. gallinarum[b]	0	100	0	0	100	0	0	90	10	1	0	0	0	0	100	0	0	0
S. pullorum[b]	0	90	0	0	90	0	0	100	10	95	0	0	0	0	100	90	0	0
Subgroup 2 strains[b]	2	100	0	100	100	0	0	100	90	100	98	2	0	95	100	100	1	1
Subgroup 3a strains[b] (*Arizona*)	1	100	0	99	99	0	0	99	70	99	99	0	1	95	100	99	15	1
Subgroup 3b strains[b] (*Arizona*)	2	100	0	98	99	0	0	99	70	99	99	0	1	95	100	99	85	5
Subgroup 4 strains[b]	0	100	0	98	100	2	0	100	70	100	98	0	95	0	100	100	0	0
Subgroup 5 strains[b]	0	100	0	100	100	0	0	100	100	100	100	0	100	0	100	80	0	0
Serratia																		
S. marcescens[b]	1	20	98	98	0	15	0	99	0	99	97	90	95	3	100	55	2	99
S. marcescens biogroup 1[b]	0	100	60	30	0	0	0	55	4	65	17	30	70	0	100	0	4	100
S. liquefaciens group[b]	1	93	93	90	0	3	0	95	0	95	95	90	90	2	100	75	10	98
S. rubidaea[b]	0	20	100	95	0	2	0	55	0	0	85	90	25	94	100	30	100	99
S. odorifera biogroup 1[b]	60	100	50	100	0	5	0	100	0	100	100	95	60	0	100	0	70	100
S. odorifera biogroup 2[b]	50	60	100	97	0	0	0	94	0	0	100	94	19	0	100	13	97	0
S. plymuthica[b]	0	94	80	75	0	0	0	0	0	0	50	60	30	0	100	40	80	100
S. ficaria[b]	0	75	75	100	0	0	0	0	0	0	100	100	55	0	100	0	15	100
"Serratia" fonticola[b]	0	100	9	91	0	13	0	100	0	97	91	0	70	88	100	79	97	21
Tatumella																		
T. ptyseos[b]	0	0	5	2	0	0	90	0	0	0	0	0	0	0	100	0	0	98
Yersinia																		
Y. enterocolitica[b]	50	97	2	0	0	75	0	0	0	95	2	0	2	0	100	5	5	95
Y. frederiksenii[b]	100	100	0	15	0	70	0	0	0	95	5	0	0	0	100	40	40	100

TABLE 1—*Continued*

D-Mannitol fermentation	Dulcitol fermentation	Salicin fermentation	Adonitol fermentation	*myo*-Inositol fermentation	D-Sorbitol fermentation	L-Arabinose fermentation	Raffinose fermentation	L-Rhamnose fermentation	Maltose fermentation	D-Xylose fermentation	Trehalose fermentation	Cellobiose fermentation	alpha-Methyl-D-glucoside fermentation	Erythritol fermentation	Esculin hydrolysis	Melibiose fermentation	D-Arabitol fermentation	Glycerol fermentation	Mucate fermentation	Tartrate, Jordan's	Acetate utilization	Lipase (corn oil)	DNase at 25°C	Nitrate → nitrite	Oxidase, Kovac's	ONPG[c]	Yellow pigment	D-Mannose fermentation
100	25	100	0	0	40	100	98	100	100	99	100	100	98	0	99	99	0	40	90	35	50	0	0	100	0	100	0	100
95	0	100	0	0	45	100	100	100	100	91	100	100	95	0	100	100	0	5	81	19	86	0	0	100	0	100	0	100
60	0	0	100	0	0	0	100	0	30	0	0	0	0	0	0	100	75	10	0	30	10	0	0	90	0	90	0	100
0	0	0	0	0	0	0	0	0	0	0	10	0	0	0	0	0	0	5	0	95	0	0	0	90	0	5	0	98
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	0	100	0	0	0	91	0	0	0	95
0	0	0	0	0	0	0	0	15	50	15	85	0	0	0	0	0	0	0	0	15	0	0	0	100	0	0	0	85
0	0	0	0	0	0	0	1	1	0	98	98	1	0	0	0	0	0	70	0	87	20	92	50	95	0	0	0	0
0	0	50	0	0	0	0	1	5	97	95	30	0	60	1	50	0	0	60	0	80	25	80	80	98	0	1	0	0
0	0	0	0	0	0	0	1	0	100	100	55	0	80	0	0	0	0	55	0	85	5	45	40	90	0	1	0	0
0	0	0	0	0	0	0	0	0	100	0	100	0	100	0	0	0	0	100	0	100	0	100	50	100	0	0	0	0
100	0	50	100	90	1	0	5	70	2	10	0	3	2	75	35	5	100	60	0	95	60	0	0	100	0	5	0	100
10	0	2	5	95	1	1	7	0	1	7	98	5	0	0	0	0	0	50	0	90	75	0	10	100	0	10	0	100
2	0	1	98	1	1	1	1	0	1	1	2	1	0	0	0	0	0	15	0	90	40	0	0	100	0	1	0	100
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	50	25	0	0	100	0	0	0	100
100	88	100	0	0	94	100	94	94	94	94	100	100	0	0	100	100	0	13	30	6	6	0	0	100	0	100	0	100
100	96	0	0	35	95	99	2	95	97	97	99	5	2	0	5	95	0	5	90	90	90	0	2	100	0	2	0	100
100	0	0	0	0	99	2	0	0	97	82	100	0	0	0	0	100	0	20	0	100	0	0	0	100	0	0	0	100
98	5	0	0	0	90	0	1	100	95	98	0	0	0	1	0	45	1	0	0	85	1	0	0	98	0	0	0	95
100	90	0	0	0	95	100	0	100	95	0	100	5	0	0	0	95	0	10	0	0	0	0	0	100	0	0	0	100
100	90	0	0	0	1	80	10	10	90	70	50	10	0	1	0	0	0	0	50	100	0	0	10	100	0	0	0	100
100	0	0	0	0	10	100	1	100	5	90	90	5	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0	100
100	90	5	0	5	100	100	0	100	100	100	100	0	8	0	15	8	0	25	96	50	95	0	0	100	0	15	0	95
100	0	0	0	0	99	99	1	99	98	100	99	1	1	0	1	95	1	10	90	5	90	0	2	100	0	100	0	100
100	1	0	0	0	99	99	1	99	98	100	99	1	1	0	1	95	1	10	30	20	75	0	2	100	0	100	0	100
98	0	60	5	0	100	100	0	98	100	100	100	50	0	0	0	100	5	0	0	65	70	0	0	100	0	0	0	100
100	100	0	0	0	100	100	0	100	100	100	100	0	0	0	0	75	0	0	100	0	100	0	0	100	0	100	0	100
99	0	95	40	75	99	0	2	0	96	7	99	5	0	1	95	0	0	95	0	75	50	98	98	98	0	95	0	99
96	0	92	30	30	92	0	0	0	70	0	100	4	0	0	96	0	0	92	0	50	4	75	82	83	0	75	0	100
100	0	97	5	60	95	98	85	15	98	100	100	5	5	0	97	75	0	95	0	75	40	85	85	100	0	93	0	100
100	0	99	99	20	1	100	99	1	99	99	100	94	1	0	94	99	85	20	0	70	80	99	99	100	0	100	0	100
100	0	98	50	100	100	100	100	95	100	100	100	100	0	0	95	100	0	40	5	100	60	35	100	100	0	100	0	100
97	0	45	55	100	100	100	7	94	100	100	100	100	0	7	40	96	0	50	0	100	65	65	100	100	0	100	0	100
100	0	94	0	50	65	100	94	0	94	94	100	88	70	0	81	93	0	50	0	100	55	70	100	100	0	70	0	100
100	0	100	0	55	100	100	70	35	100	100	100	100	8	0	100	40	100	0	0	17	40	77	100	92	8	100	0	100
100	91	100	100	30	100	100	100	76	97	85	100	6	91	0	100	98	100	88	0	58	15	0	0	100	0	100	0	100
0	0	55	0	0	0	0	11	0	0	9	93	0	0	0	0	25	0	7	0	0	0	0	0	98	0	0	0	100
98	0	20	0	30	99	98	5	1	75	70	98	75	0	0	25	1	40	90	0	85	15	55	5	98	0	95	0	100
100	0	92	0	20	100	100	30	99	100	100	100	100	0	0	85	0	100	85	5	55	15	55	0	100	0	100	0	100

Continued on following page

TABLE 1—*Continued*

Species	Indole production	Methyl red	Voges-Proskauer	Citrate (Simmons')	Hydrogen sulfide (TSI)	Urea hydrolysis	Phenylalanine deaminase	Lysine decarboxylase	Arginine dihydrolase	Ornithine decarboxylase	Motility (36°C)	Gelatin hydrolysis (22°C)	Growth in KCN	Malonate utilization	D-Glucose, acid	D-Glucose, Gas	Lactose fermentation	Sucrose fermentation
Y. intermedia[b]	100	100	5	5	0	80	0	0	0	100	5	0	10	5	100	18	35	100
Y. kristensenii[b]	30	92	0	0	0	77	0	0	0	92	5	0	0	0	100	23	8	0
Y. pestis[b]	0	80	0	0	0	5	0	0	0	0	0	0	0	0	100	0	0	0
Y. pseudotuberculosis[b]	0	100	0	0	0	95	0	0	0	0	0	0	0	0	100	0	0	0
"Yersinia" ruckeri	0	97	10	0	0	0	0	50	5	100	0	30	15	0	100	5	0	0
Xenorhabdus																		
X. luminescens (25°C)	50	0	0	50	0	25	0	0	0	0	100	50	0	0	75	0	0	0
X. nematophilus (25°C)	40	0	0	0	0	0	0	0	0	0	100	80	0	0	80	0	0	0
Enteric Group 17[b]	0	100	2	100	0	60	0	0	21	95	0	0	97	3	100	95	75	100
Enteric Group 41[b]	100	100	0	0	0	50	0	0	0	0	100	0	100	50	100	100	100	100
Enteric Group 45[b]	0	100	0	100	0	0	0	100	22	100	100	0	78	0	100	89	0	0
Enteric Group 57[b]	0	70	0	40	100	0	0	0	0	0	0	0	30	0	100	60	0	0
Enteric Group 58[b]	0	100	0	85	0	70	0	100	0	85	100	0	100	85	100	85	30	0
Enteric Group 59[b]	10	100	0	100	0	0	30	0	60	0	100	0	80	90	100	100	80	0
Enteric Group 60[b]	0	100	0	0	0	50	0	0	0	100	75	0	0	100	100	100	0	0
Enteric Group 63	0	100	0	0	0	0	0	100	0	100	65	0	0	0	100	100	0	0
Enteric Group 64	0	100	0	50	0	0	0	0	50	0	100	0	100	100	100	50	100	0
Enteric Group 68[b]	0	100	50	0	0	0	0	0	0	0	0	0	100	0	100	0	0	100
Enteric Group 69	0	0	100	100	0	0	0	0	100	100	100	0	100	100	100	100	100	25

[a] Each number gives the percentage of positive reactions after 2 days of incubation at 36°C (except *Xenorhabdus*, which was incubated at 25°C). The vast majority of these positive reactions occur within 24 h. Reactions that become positive after 2 days are not considered.
[b] Known to occur in clinical specimens.
[c] ONPG, *o*-Nitrophenyl-β-D-galactopyranoside.

0.1% (50). Thus in Table 1 the value for hydrogen sulfide production in *E. coli* was set at 1%. Similar adjustments were made in Table 1 so that the percentages better reflect the reactions expected in clinical laboratories. This was done by comparing our percentages with tabulations either based on a less biased sample published in the literature or based on earlier data previously tabulated at the CDC. This skewed distribution was not present for the new organisms. Thus, in Table 1 the final data should be similar to those obtained in primary clinical laboratories.

Case histories and clinical information. Often a short case history, clinical summary, or discharge summary was submitted along with the culture. This information was used as a first step in trying to establish the role of the new *Enterobacteriaceae* in human disease (Table 3). There are many problems with this approach, and prospective studies are needed to provide definitive data about the clinical significance of the new *Enterobacteriaceae* (6, 46, 51, 62). The isolation of these new *Enterobacteriaceae* from a clinical specimen does not imply an etiological or causal role in human disease. Clinical evidence and repeated isolation are needed to document colonization or infection, rather than a transient state.

Reporting of cultures sent for identification. In the Enteric Bacteriology Laboratories at the CDC this has been done in several ways. In most instances a species name is given as a definitive identification, but atypical reactions are listed, such as "*Enterobacter cloacae* arginine$^-$, D-sorbitol$^-$, L-arabinose$^-$." In other instances, the culture is reported as unidentified to the sender, but is coded in the computer and filed under a designation such as "*Citrobacter freundii*??," "new *Yersinia* species??," or "colistin-resistant group which is very mucoid and from sputum." Other cultures are simply filed as unidentified. Many attempts have been made over the years to form new groups from these unidentified cultures. Biochemical reactions can be compared manually to form new groups, but this has been a tedious process, with limited success. From 1970 to 1976 a number of computer programs were developed which have allowed systematic analysis of these biochemical data.

Computer programs. The first computer program is based on the normalized likelihood described in detail by Lapage and co-workers (88). A data matrix is developed (Table 1), and the biochemical reactions of an individual isolate are compared with each of the biochemically distinct groups in the matrix. This is done by a series of mathematical calculations (88), and the final result indicates how well the test strain fits each of the biochemical groups in the matrix. This computer program was also used to select strains to be included in DNA hybridization experiments (Fig. 1). Both typical and atypical strains were selected to better define the limits of the new species. The second computer program (strain matcher) was developed in our laboratory, and it compares the biochemical reactions of a test strain with the reactions of all individual strains previously reported and put into the computer. This is in contrast to the normalized likelihoods program, in which the test strain is compared with the average biochemical reaction of each named group. Figure 1 summarizes our approach in the study of the family.

Biochemical reactions of *Enterobacteriaceae*. Table 1 gives a complete listing of all the named groups of *Enterobacteriaceae*. All values are the percent positive for the biochemical

TABLE 1—*Continued*

D-Mannitol fermentation	Dulcitol fermentation	Salicin fermentation	Adonitol fermentation	*myo*-Inositol fermentation	D-Sorbitol fermentation	L-Arabinose fermentation	Raffinose fermentation	L-Rhamnose fermentation	Maltose fermentation	D-Xylose fermentation	Trehalose fermentation	Cellobiose fermentation	alpha-Methyl-D-glucoside fermentation	Erythritol fermentation	Esculin hydrolysis	Melibiose fermentation	D-Arabitol fermentation	Glycerol fermentation	Mucate fermentation	Tartrate, Jordan's	Acetate utilization	Lipase (corn oil)	DNase at 25°C	Nitrate → nitrite	Oxidase, Kovac's	*o*-Nitrophenyl-β-D-galactopyranoside	Yellow pigment	D-Mannose fermentation
100	0	100	0	15	100	100	45	100	100	100	100	96	77	0	100	80	45	60	6	88	18	12	0	94	0	90	0	100
100	0	15	0	15	10	77	0	0	100	85	100	100	0	0	0	0	45	70	0	40	8	0	0	100	0	70	0	100
97	0	70	0	0	50	100	0	1	80	90	100	0	0	0	50	20	0	50	0	0	0	0	0	85	0	50	0	100
100	0	25	0	0	0	50	15	70	95	100	100	0	0	0	95	70	0	50	0	50	0	0	0	95	0	70	0	100
100	0	0	0	0	50	5	5	0	95	0	95	5	0	0	0	0	0	30	0	30	0	30	0	75	0	50	0	100
0	0	0	0	0	0	0	0	0	25	0	0	0	0	0	0	0	0	0	0	50	0	0	0	0	0	0	50	100
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	60	0	0	20	20	0	0	60	80
100	0	100	0	0	100	100	70	5	100	97	100	100	95	0	95	0	0	11	21	30	87	0	0	100	0	100	0	100
100	100	100	100	0	0	100	100	100	100	100	100	100	0	0	100	100	100	0	100	100	0	0	0	100	0	100	100	100
100	0	11	0	0	0	100	22	100	100	100	100	100	0	0	55	80	0	0	0	13	55	0	0	89	0	80	0	100
0	50	0	0	0	0	90	0	0	0	90	0	0	0	0	0	0	0	0	60	100	0	0	0	100	0	0	0	0
100	85	100	0	0	100	100	0	100	100	100	100	100	55	0	0	0	0	30	0	60	45	0	0	100	0	100	0	100
100	0	100	0	0	0	100	0	100	100	100	100	100	10	0	100	0	10	10	60	50	50	0	0	100	0	100	25	100
50	0	0	0	0	0	25	0	75	0	0	100	0	0	0	0	0	0	75	0	75	0	0	0	100	0	100	0	100
100	0	100	0	0	100	100	0	100	100	100	100	100	65	0	100	0	0	0	65	0	0	0	0	100	0	100	0	100
100	0	100	100	0	0	100	0	100	100	100	100	100	0	0	100	0	100	0	100	50	0	0	0	100	0	100	0	100
100	0	50	0	0	0	0	0	0	50	0	100	0	0	0	0	0	0	50	0	0	0	0	100	100	0	0	0	100
100	100	100	0	0	100	100	100	100	100	100	100	100	100	0	100	100	0	0	100	0	25	0	0	100	0	100	100	100

reaction at 36°C after 2 days of incubation. Some biochemical reactions will become positive on further incubation, but these delayed reactions are not considered in Table 1. Similarly, some species are more active at 25 than at 36°C, but this is not considered in Table 1. Most positive test results are positive at the end of 24 h. Table 2 lists the species most commonly encountered in the clinical microbiology and the tests often used to identify them.

The sources of the cultures belonging to new *Enterobacteriaceae* groups are given in Table 3. Only the cultures actually studied at the CDC are included, but the text below often discusses cultures described in the literature. The sources given in Table 3 are taken from computer printouts of data furnished by the sender. More information is given about the new organisms in the sections below.

RESULTS

The genus *Buttiauxella*. Proposed by Ferragut and co-workers in 1981 (54), *Buttiauxella* is a new genus in the family *Enterobacteriaceae* with only one species, *Buttiauxella agrestis*. All of the strains were from water and previously had been called "group F" by Gavini et al. (60) and are not to be confused with "group F" of halophilic vibrios described by Furniss et al. (55). Originally Gavini et al. thought that these strains resembled *Citrobacter* because both groups are indole negative, methyl red positive, Voges-Proskauer negative, and citrate positive (60). However, the genus *Buttiauxella* is more similar to *Kluyvera* in its phenotypic properties (Table 4). By DNA hybridization, *Buttiauxella* and *Kluyvera* were only 30 to 36% related (58). There was also a difference in the guanine plus cytosine (G+C) content of DNA of the two genera; *Kluyvera* had 55 to 56% G+C, and *Buttiauxella* had 49% G+C (58). We have received five cultures of *B. agrestis* studied by Ferragut et al., and a tabulation of their biochemical reactions is given in Table 1. No strains of *B. agrestis* have been from humans.

Two other groups of strains studied by Ferragut et al. (54) may eventually be classified in the genus *Buttiauxella*. Strains CUETM 78-31 (CDC 1180-81), 78-35 (CDC 1182-80), and 78-50 (CDC 1183-81) are phenotypically similar to each other and were 62 to 66% related by DNA hybridization to *Buttiauxella* (54). We have arbitrarily called this group of strains Enteric Group 63. Similarly strain CUETM 77-159 (CDC 1175-81) was 62 to 66% related to *B. agrestus* by DNA hybridization, but phenotypically distinct from it and from Enteric Group 63. We have arbitrarily called it Enteric Group 64.

The genus *Cedecea*. The genus *Cedecea* was proposed in 1980 (J. J. Farmer III, P. A. D. Grimont, F. Grimont, and M. A. Asbury, Abstr. Annu. Meet. Soc. Microbiol. 1980, C123, p. 295) and was formally published in 1981 (66). *Cedecea davisae* and *C. lapagei* were named in 1981 (66), and *C. neteri* was named in 1982 (51). In addition, there are two unnamed species; *Cedecea* sp. 3 and *Cedecea* sp. 5 (66). All five species were defined on the basis of differences in phenotypic properties (Table 5) and DNA hybridization (66). *Cedecea* is phenotypically distinct from other genera in the family *Enterobacteriaceae* (Table 1). Cultures are usually lipase (corn oil) positive and resistant to colistin and cephalothin (66). These three properties are shared with *Serratia*, but *Cedecea* differs in being unable to hydrolyze gelatin or DNA. Strains of *Cedecea* have the acetoin pathway of glucose catabolism, but often are negative in our standard (O'Meara) Voges-Proskauer test because of the small amount of 2,3-butanediol produced (Table 1). *C. lapagei* is usually strongly Voges-Proskauer positive with the O'Meara methods, but the other species of *Cedecea* appear negative unless the test is held for several hours after the reagents are added. *Cedecea* has also been included in some of the commercial

TABLE 2. Biochemical reactions of the *Enterobacteriaceae* that are the most important in human infections or that are frequently isolated from clinical specimens[a]

Species	Indole production	Methyl red	Voges-Proskauer	Citrate (Simmons')	Hydrogen sulfide (TSI)	Urea hydrolysis	Phenylalanine deaminase	Lysine decarboxylase	Arginine dihydrolase	Ornithine decarboxylase	Motility (36°C)	Gelatin hydrolysis (22°C)	D-Glucose, gas	Lactose fermentation	Sucrose fermentation	D-Mannitol fermentation	Dulcitol fermentation	Adonitol fermentation	D-Sorbitol fermentation	L-Arabinose fermentation	Raffinose fermentation	L-Rhamnose fermentation	D-Xylose fermentation	Melibiose fermentation	DNase, 25°C	ONPG[b]
Escherichia coli	98	99	0	1	1	1	0	90	17	65	95	0	95	95	50	98	60	5	94	99	50	80	95	75	0	95
Shigella serogroups A, B, and C	50	100	0	0	0	0	0	0	5	1	0	0	2	0	0	93	2	0	30	60	50	5	2	50	0	2
Shigella sonnei	0	100	0	0	0	0	0	0	2	98	0	0	0	2	1	99	0	0	2	95	3	75	2	25	0	90
Salmonella, most serotypes	1	100	0	95	95	1	0	98	70	97	95	0	96	1	1	100	96	0	95	99	2	95	97	95	2	2
Salmonella typhi	0	100	0	0	97	0	0	98	3	0	97	0	0	1	0	100	0	0	99	2	0	0	82	100	0	0
Salmonella paratyphi A	0	100	0	0	10	0	0	0	15	95	95	0	99	0	0	100	90	0	95	100	0	100	0	95	0	0
Citrobacter freundii	5	100	0	95	80	70	0	0	65	20	95	0	95	50	30	99	55	0	98	100	30	99	99	50	0	95
Citrobacter diversus	99	100	0	99	0	75	0	0	65	99	95	0	98	35	45	100	50	98	99	100	0	100	100	0	0	96
Klebsiella pneumoniae	0	10	98	98	0	95	0	98	0	0	0	0	97	98	99	99	30	90	99	99	99	99	99	99	0	99
Klebsiella oxytoca	99	20	95	95	0	90	1	99	0	0	0	0	97	100	100	99	55	99	99	98	100	100	100	99	0	100
Enterobacter aerogenes	0	5	98	95	0	2	0	98	0	98	97	0	100	95	100	100	5	98	100	100	96	99	100	99	0	100
Enterobacter cloacae	0	5	100	100	0	65	0	0	97	96	95	0	100	93	97	100	15	25	95	100	97	92	99	90	0	99
Hafnia alvei	0	40	85	10	0	4	0	100	6	98	85	0	98	5	10	99	0	0	0	95	2	97	98	0	0	90
Serratia marcescens	1	20	98	98	0	15	0	99	0	99	97	90	55	2	99	99	0	40	99	0	2	0	7	0	98	95
Proteus mirabilis	2	97	50	65	98	98	98	0	0	99	95	90	96	2	15	0	0	0	0	0	1	1	98	0	50	0
Proteus vulgaris	98	95	0	15	95	95	99	0	0	0	95	91	85	2	97	0	0	0	0	0	1	5	95	0	80	1
Providencia rettgeri	99	93	0	95	0	98	98	0	0	0	94	0	10	5	15	100	0	100	1	0	5	70	10	5	0	5
Providencia stuartii	98	100	0	93	0	30	95	0	0	0	85	0	0	2	50	10	0	5	1	1	7	0	7	0	10	10
Providencia alcalifaciens	99	99	0	98	0	0	98	0	0	1	96	0	85	0	15	2	0	98	1	1	1	0	1	0	0	1
Morganella morganii	98	97	0	0	5	98	95	0	0	98	95	0	90	1	0	0	0	0	0	0	0	0	0	0	0	5
Yersinia enterocolitica	50	97	2	0	0	75	0	0	0	95	2	0	5	5	95	98	0	0	99	98	5	1	70	1	5	95
Yersinia pestis	0	80	0	0	0	5	0	0	0	0	0	0	0	0	0	97	0	0	50	100	0	1	90	20	0	50
Yersinia pseudotuberculosis	0	100	0	0	0	95	0	0	0	0	0	0	0	0	0	100	0	0	0	50	15	70	100	70	0	70

[a] Each number gives the percentage of positive reactions after 2 days of incubation at 36°C. The vast majority of these positive reactions occur within 24 h. Reactions that become positive after 2 days are not considered.
[b] ONPG, *o*-Nitrophenyl-β-D-galactopyranoside.

systems used for identification. Table 3 indicates that all of the isolates of *Cedecea* have come from clinical specimens, and the respiratory tract was the most common source.

C. davisae. *C. davisae* is the most common of the *Cedecea* species (66). Seventeen isolates have been studied (Table 3), and sputum was the most common source. Others were from gall bladder (one isolate), hand wounds (two isolates), and an eye swab of a 4-day-old infant. Some comments that accompanied cultures include the following: "finger wound which resulted from a power mower accident"; "sputum of an 89-year-old male with angina who died"; "exudate from finger following an insect bite"; "50 year-old male with pulmonary embolism; predominant organism isolated was *Serratia marcescens*, also a few *Haemophilus influenzae*"; and "from gall bladder which was probably infected during surgery." Two isolates of *C. davisae* were described by Bae et al. (6). These were obtained from two elderly patients with heart disease who possibly had pneumonia. In this report the isolates were referred to as "Enteric Group 15—Davis subgroup." These are now classified as *C. davisae*. Neither patient had bacteremia, but *C. davisae* was isolated as the predominant organism from sputum in both. One patient's report on a sputum culture stated "scant growth of *Klebsiella oxytoca*." Thus, *C. davisae* was not shown to be the cause of pneumonia. Because the two patients were hospitalized one room apart from each other at the time the organism was isolated and because the hospital had had no isolates of *Cedecea* before or after the two cases, it was concluded that a common source or cross-infection was involved. Bae and Sureka (5) recently reported a case in which they believed *C. davisae* caused or was involved in a scrotal abscess.

C. lapagei. The other *Cedecea* species, *C. lapagei*, was originally named in 1981 (66). We have had only five cultures, four from sputum and one from a throat culture. No information was included, which strongly suggested clinical significance.

TABLE 3. Sources of the new genera, species, biogroups, and Enteric Groups of *Enterobacteriaceae*

Species	Relative frequency[a]	Clinical significance		No. of isolates from the following sources:												
				Human sources							Other sources					
		Diarrhea[b]	Other[c]	Spinal fluid	Blood	Urine	Wounds	Respiratory tract	Stool	Other	Animals	Water	Soil	Environment	Food	Other, unknown
Buttiauxella																
B. agrestis	0	–	–	0	0	0	0	0	0	0	0	5	0	0	0	0
Cedecea																
C. davisea	2	–	(–)	0	0	1	4	11	2	4	0	0	0	0	0	0
C. lapagei	1	–	(–)	0	0	0	0	5	0	0	0	0	0	0	0	0
C. neteri	1	–	+	0	1	0	1	1	0	0	0	0	0	0	0	0
Cedecea sp. 3	1	–	(–)	0	1	0	1	1	0	0	0	0	0	0	0	0
Cedecea sp. 5	1	–	(–)	0	0	0	1	0	0	0	0	0	0	0	0	0
Citrobacter																
C. amalonaticus	5	–	+	0	3	1	2	1	21	5	1	0	0	0	0	8
C. amalonaticus biogroup 1	4	–	+	0	0	2	4	0	15	4	0	0	0	0	0	2
C. diversus	6	–	++	4	5	24	5	15	6	15	0	0	0	0	0	18
C. freundii	7	(+)	++	0	8	20	9	6	101	21	7	6	4	0	10	53
Edwardsiella																
E. hoshinae	1	–	–	0	0	0	0	0	0	0	7	1	0	0	0	0
E. ictaluri	0	–	–	0	0	0	0	0	0	0	9	0	0	0	0	0
E. tarda	4	(+)	+	0	3	1	3	0	18	3	21	8	0	0	1	7
E. tarda biogroup 1	0	–	–	0	0	0	0	0	0	0	7	0	0	0	0	0
Enterobacter																
E. aerogenes	7	–	++	1	2	6	9	6	1	8	1	0	0	0	0	1
E. agglomerans	6	–	+	1	11	16	15	19	6	18	15	0	0	1	7	24
E. amnigenus biogroup 1	1	–	–	0	0	0	1	2	1	0	0	7	0	0	0	0
E. amnigenus biogroup 2	0	–	–	0	0	0	0	0	0	0	0	3	0	0	0	0
E. cloacae	7	–	+++	3	23	21	16	12	30	7	1	4	0	1	2	18
E. gergoviae	3	–	+	0	5	2	6	15	0	8	0	0	0	1	0	36
E. intermedium	0	–	–	0	0	0	0	0	0	0	0	5	0	0	0	0
E. sakazakii	3	–	+	8	3	6	16	20	5	13	1	1	0	1	1	9
E. taylorae	2	–	(–)	0	0	1	14	5	6	5	3	1	0	0	0	1
Escherichia																
E. blattae	0	–	–	0	0	0	0	0	0	0	4	0	0	0	0	0
E. fergusonii	2	–	(–)	0	2	5	1	0	16	0	9	0	0	0	0	7
E. hermanii	2	–	(–)	1	2	2	19	5	15	10	0	0	0	0	3	7
E. vulneris	2	–	(–)	0	3	3	42	4	0	13	2	0	0	1	0	4
Ewingella																
E. americana	1	–	+	0	11	1	7	18	2	2	0	0	0	0	1	2
Hafnia																
H. alvei	6	(+)	+	1	1	3	4	16	0	1	0	0	0	0	0	3
H. alvei biogroup 1	0	–	–	0	0	0	0	0	0	0	0	0	0	0	0	4
Klebsiella																
K. oxytoca	7	(+)	+++	0	17	7	3	3	25	8	5	1	0	5	2	22
K. planticola	4	–	(–)	0	0	2	0	0	0	0	2	2	0	2	4	1
K. terrigena	0	–	–	0	0	0	0	0	0	0	0	5	0	0	0	0
Klebsiella group 47, indole positive, ornithine positive	2	–	(+)	0	2	2	4	16	0	1	0	0	0	0	0	3
Kluyvera																
K. ascorbata	4	(–)	(+)	0	4	14	7	47	16	13	1	1	0	0	1	19
K. cryocrescens	2	–	(–)	0	1	5	0	8	0	3	0	0	1	1	1	9
Moellerella																
M. wisconsensis	1	(–)	(–)	0	0	0	0	0	8	0	0	1	0	0	0	0
Morganella																
M. morganii biogroup 1	4	–	(+)	0	0	3	5	7	4	3	0	0	0	0	0	0

Continued on following page

TABLE 3—*Continued*

Species	Relative frequency[a]	Clinical significance		No. of isolates from the following sources:												
				Human sources							Other sources					
		Diarrhea[b]	Other[c]	Spinal fluid	Blood	Urine	Wounds	Respiratory tract	Stool	Other	Animals	Water	Soil	Environment	Food	Other, unknown
Obesumbacterium																
O. proteus biogroup 2	0	–	–	0	0	0	0	0	0	0	0	0	0	0	0	3
Proteus																
P. myxofaciens	0	–	–	0	0	0	0	0	0	0	1	0	0	0	0	0
P. penneri	3	–	+	0	1	9	1	1	4	0	0	0	0	0	0	4
Providencia																
P. rustigianii	3	–	(–)	0	0	0	0	0	3	0	0	0	0	0	0	8
Rahnella																
R. aquatilis	1	–	(–)	0	0	0	1	0	0	0	0	14	0	0	0	0
Serratia																
S. ficaria	1	–	(–)	0	0	0	1	1	0	1	2	0	0	0	8	0
S. fonticola	0	–	(–)	0	0	0	11	2	0	0	0	16	0	1	0	0
S. liquefaciens group	5	–	(–)	0	0	0	2	5	0	4	0	0	1	0	0	3
S. marcescens biogroup 1	5	–	+	0	0	16	0	2	0	3	0	0	0	0	0	3
S. odorifera biogroup 1	2	–	(–)	0	0	0	3	8	3	2	0	0	0	0	2	4
S. odorifera biogroup 2	3	–	(–)	1	5	1	5	11	3	1	0	0	0	1	0	3
S. plymuthica	2	–	(–)	0	0	0	0	5	0	0	0	2	0	0	0	9
S. rubidaea	4	–	(–)	0	2	1	3	5	1	5	1	1	0	0	0	2
Tatumella																
T. ptyseos	2	–	(+)	0	3	2	0	46	1	3	0	0	0	0	0	1
Yersinia																
Y. enterocolitica	6	++	++	1	12	5	9	6	104	17	13	11	0	1	1	11
Y. frederiksenii	3	–	(+)	0	0	0	1	4	2	2	0	1	0	1	0	2
Y. intermedia	3	–	(+)	0	0	1	3	0	3	2	0	2	0	0	1	5
Y. kristensenii	3	–	(+)	0	1	1	0	0	4	0	4	2	1	0	0	0
Y. ruckeri	1	–	–	0	0	0	0	0	0	0	0	0	0	0	0	0
Xenorhabdus																
X. luminescens	0	–	–	0	0	0	0	0	0	0	4	0	0	0	0	0
X. nematophilus	0	–	–	0	0	0	0	0	0	0	5	0	0	0	0	0
Enteric Group 17	2	–	(+)	0	2	11	4	8	4	8	0	1	0	0	0	2
Enteric Group 41	1	–	–	0	0	0	0	0	0	0	0	1	0	0	0	0
Enteric Group 45	1	–	(–)	0	0	0	5	2	1	1	0	1	0	0	0	0
Enteric Group 57	1	–	(–)	0	0	1	0	0	8	0	1	0	0	0	0	0
Enteric Group 58	1	–	(–)	0	0	0	5	0	1	0	0	0	0	0	0	0
Enteric Group 59	1	–	(–)	0	0	0	1	6	0	0	0	0	0	0	1	0
Enteric Group 60	1	–	(–)	0	0	3	0	1	0	0	0	0	0	0	0	0
Enteric Group 63	0	–	–	0	0	0	0	0	0	0	0	3	0	0	0	0
Enteric Group 64	0	–	–	0	0	0	0	0	0	0	0	1	0	0	0	0
Enteric Group 68	1	–	(–)	0	0	2	0	0	0	0	0	0	0	0	0	0
Enteric Group 69	0	–	–	0	0	0	0	0	0	0	4	0	0	0	0	0

[a] Definitions for relative frequency in clinical specimens (see text for more details): 10, most common by far; 8, extremely common; 7, common; 6, occasional; 5, uncommon; 4, rare; 3, very rare; 2, extremely rare; 1, only a few clinical isolates known; 0, not known to occur.

[b] Definitions for clinical significance in diarrhea: +++, intrinsic cause; ++, documented cause, but not all strains are able; (+), mentioned as a possible cause, but there is not universal agreement; (–), unlikely cause, but mentioned in literature; –, not a cause.

[c] Definitions for clinical significance: +++ and ++, clinically significant; +, reported to be clinically significant at least at some sites, may not be significant at other sites; (+), clinical significance at least suggested in the literature, but more data are needed; (–), no data at present to suggest significance, but significance can not be totally excluded because of occurrence in clinical specimens; –, not significant.

Cedecea neteri. The name *C. neteri* was recently proposed (51) for the organism originally called "*Cedecea* species 4" by Grimont and co-workers. The original two isolates were from sputum and a foot wound. However, a recent isolate of *C. neteri* was described in a case history (51) and is the first reported case of bacteremia caused by a strain of *Cedecea*. *C. neteri* was isolated from five different blood cultures from a 62-year-old patient with a history of valvular heart disease (51).

***Cedecea* sp. 3 and 5.** *Cedecea* sp. 3 and 5 were distinct from the three named species by both DNA hybridization and phenotype (66), but they were not given a scientific name because only one culture was available for each. In the original report by Grimont et al., *Cedecea* sp. 3 was referred

TABLE 4. Differentiation of *Buttiauxella*, *Kluyvera*, and two related Enteric Groups

Test	*Buttiauxella agrestis*	Enteric Group 63 (*Buttiauxella*?)	Enteric Group 64 (*Buttiauxella*?)	*Kluyvera ascorbata*	*Kluyvera cryocrescens*
Sucrose fermentation	−[a] (60)[b]	−	−	+	(+)
Ascorbate test (7 days)	−	+	−	+	−
D-Arabitol fermentation	−	−	+	−	−
Melibiose fermentation	+	− (67)	−	+	+
Raffinose fermentation	+	−	−	+	+
α-Methyl-D-glucoside fermentation	−	v (100)	− (100)	+	+
Citrate utilization (Simmohs')	+	− (67)	v (100)	+	(+)
Lysine decarboxylase	−	+	−	+	(−)
Lactose fermentation	+	−	+	+	+

[a] Symbols (all data are for reaction within 2 days unless otherwise specified): +, 90 to 100% positive; (+), 75 to 89.9% positive; v, 25.1 to 74.9% positive; (−), 10.1 to 25% positive; −, 0 to 10% postive.

[b] If delayed reactions are useful for differentiation, the percent positive within the time period 3 to 7 days is given within parentheses; otherwise no delayed reactions are given.

to as "*Cedecea* sp—strain 001," and *Cedecea* sp. 5 was referred to as "*Cedecea* sp—strain 012." The original isolate of *Cedecea* sp. 3 (4853-73) was from sputum. An isolate of *Cedecea* sp. 3 was recently reported by Mangum and Radisch (95). It was from heart blood at autopsy, but its clinical significance was doubtful since it was not isolated from blood cultures taken before death, and systemic infection with *Candida albicans* was also present. The original isolate (3699-73) of *Cedecea* sp. 5 was from the toe of a patient in Canada.

Recently we have been receiving cultures that appear different from the five described *Cedecea* species. These may represent additional species of this genus.

The genus *Citrobacter*. Table 1 shows that we recognize three named species and one biogroup in the genus *Citrobacter*. *Citrobacter freundii* has been known and studied (32) for many years as a possible cause of diarrhea and as a cause of extraintestinal infections (44). Many of the isolates of *C. freundii* referred to the CDC came with a note such as "*Salmonella*—unable to serotype." These were identified as typical strains of *C. freundii* which would have been easily recognized with a complete set of biochemical tests. *Citrobacter diversus* was originally described in 1932 by Werkman and Gillen, but the name has come into general use only since it was used by Ewing and Davis in 1972 (37). *C. diversus* (which is frequently called *Citrobacter koseri* in England and *Levinea malonatica* in France) is a documented pathogen, and in recent years it has attracted attention as an important cause of nursery outbreaks of neonatal meningitis and brain abscesses. Table 6 gives the differential characteristics for *Citrobacter*.

***Citrobacter amalonaticus*.** The classification of *C. amalonaticus* has been the cause of some confusion. In 1977 Brenner and colleagues (21) proposed that the organism originally named *Levinea amalonaticus* (143) be classified in the genus *Citrobacter* based on DNA hybridization and its G+C content of DNA. Although this proposal was generally accepted, the name *C. amalonaticus* only recently gained standing in nomenclature (44). Most strains of *C. amalonaticus* studied at the CDC have been from human clinical specimens, primarily feces (Table 3); however, three were from blood. *C. amalonaticus* is probably a cause of human infection, but there is no evidence that it can cause diarrhea.

***C. amalonaticus* biogroup 1.** We first used this name in 1979 for a group of indole-positive strains that resembled *C. amalonaticus*, but which were biochemically somewhat different. Most strains of *C. amalonaticus* biogroup 1 have come from human feces. Table 1 shows that *C. amalonaticus* biogroup 1 strains usually ferment sucrose, raffinose, α-methyl-D-glucoside, and melibiose and do not utilize citrate. Strains of *C. amalonaticus* usually have the opposite reactions. The classification of these strains, which we have listed as a biogroup of *C. amalonaticus*, requires further

TABLE 5. Differentiation within the genus *Cedecea*

Test	*C. davisae*	*C. lapagei*	*C. neteri*	*Cedecea* sp. 3	*Cedecea* sp. 5
Ornithine decarboxylase (Moeller's)	+[a]	−	−	−	v
Fermentation of:					
Sucrose	+	−	+	v	+
D-Sorbitol	−	−	+	−	+
Raffinose	−	−	−	+	+
D-Xylose	+	−	+	+	+
Melibiose	−	−	−	+	+
Malonate utilization	+	+	+	−	−

[a] For definitions of symbols, see footnote *a* of Table 4.

TABLE 6. Differentiation within the genus *Citrobacter*[a]

Test	*C. amalonaticus*	*C. freundii*	*C. diversus*
Biochemical tests			
Indole production	+[b]	−	+
H_2S production (TSI)	−	+	−
Malonate utilization	−	(−)	+
Growth in KCN	+	+	−
Tyrosine clearing	−	−	+
Adonitol fermentation	−	−	+
Antibiogram—zone sizes			
Cephalothin (30 μg)[c]	18.0 (2.0)[d]	10.9 (2.9)	23.5 (1.2)
Ampicillin (10 μg)	8.7 (2.4)	14.3 (3.1)	7.1 (1.2)
Carbenicillin (100 μg)	16.5 (2.4)	24.1 (0.9)	12.6 (1.6)

[a] Adapted from reference 44.

[b] For definitions of symbols, see footnote *a* of Table 4.

[c] The number within parentheses is the amount of the antibiotic in the disk.

[d] The first number is the mean and the number in parentheses is the standard deviation of the zones of inhibition (8). Data are based on 10 strains of each species. One strain was found to have multiple antibiotic resistance. It was excluded and replaced with a sensitive strain.

TABLE 7. Differentiation within the genus *Edwardsiella*

Test or property	*E. tarda*		*E. hoshinae*	*E. ictaluri*
	Most strains	Biogroup 1		
Indole production	+[a]	+	(−)	−
H_2S production (TSI)	+	−	−	−
Motility	+	+	+	−
Malonate utilization	−	−	+	−
Fermentation of:				
D-Mannitol	−	+	+	−
Sucrose	−	+	+	−
Trehalose	−	−	+	−
L-Arabinose	−	+	(−)	−
Tetrathionate reduction[b]	+	−	+	
Present in human clinical specimens	+	−	−	−

[a] For definitions of symbols, see footnote *a* of Table 4.
[b] The data for this test are based on the results of Grimont et al. (69).

study by DNA hybridization. Some strains of *Citrobacter* have biochemical reactions intermediate between these two named groups.

The genus *Edwardsiella*. Until recently, *Edwardsiella tarda* was the only species in the genus *Edwardsiella* (45). *E. tarda* is a documented cause of extraintestinal infections, but its role as a cause of diarrhea is still controversial (45).

Two new species of *Edwardsiella* and a new biogroup of *E. tarda* have been described since 1980. On the basis of DNA hybridization and phenotypic properties (Table 7), Grimont et al. described *Edwardsiella hoshinae* as a new species similar to, but distinct from, *E. tarda* (68). The isolates of *E. hoshinae* were from birds, reptiles, and water. Several recent isolates have been from human stool cultures, but there is no evidence that *E. hoshinae* can cause diarrhea. Thus far the evidence indicates that this is a very rare species not involved in human infections. Grimont et al. also described a biochemically distinct group of strains that were closely related to *E. tarda* by DNA hybridization (68). This new group was referred to as "*E. tarda* (atypical)" to distinguish it from "*E. tarda* (typical)." In this paper we refer to the latter group as *E. tarda* biogroup 1 (Table 7). All of the biogroup 1 strains were isolated from snakes (68). Apparently *E. tarda* biogroup 1 is a rare organism that has not been isolated from human clinical specimens. In 1981 Hawke and co-workers (72) described *Edwardsiella ictaluri*, a third species of *Edwardsiella*, which was distinct from *E. tarda* by DNA hybridization. *E. ictaluri* is also distinct in its phenotypic properties from all the other *Edwardsiella* species (Table 7). All isolates of *E. ictaluri* have been from white or channel catfish in which the organism causes "enteric septicemia" (72). There have been no human clinical isolates of *E. ictaluri*.

The genus *Enterobacter*. The third edition of Edwards and Ewing's book (33) recognized four species in *Enterobacter*: *E. cloacae* (the type species), *E. aerogenes*, *E. hafniae*, and *E. liquefaciens*. These latter two species have now since been classified in other genera, *E. hafniae* as *Hafnia alvei* and *E. liquefaciens* as *Serratia liquefaciens* (17). Table 1 shows that there are now eight species of *Enterobacter* including *E. taylorae* described in a companion paper (47) in this issue. Table 8 gives the tests for differentiating these species.

E. cloacae and *E. aerogenes* (17) are the established species in the genus, covered extensively in the literature. *Enterobacter agglomerans* is the name we used for a heterogenous group of *Enterobacteraceae* which has also been called *Erwinia herbicola*, *Erwinia stewartii*, *Erwinia uredovora*, *Erwinia ananas*, *Escherichia adecarboxylata*, or "*Erwinia* species" in the literature. *E. agglomerans* was redefined in 1972 by Ewing and Fife (40), and this name has gained some acceptance, particularly in clinical microbiology. *E. agglomerans* gained prominence because of its involvement in a nation-wide outbreak of septecemia due to commercial intravenous fluids that became contaminated after sterilization. Table 3 shows that our isolates have come from a wide variety of sources, including 3 from spinal fluid and 23 from blood. Table 1 also shows that *E. agglomerans* is very heterogeneous in its biochemical reactions, which makes precise identification difficult. This heterogeneity is not surprising, since Brenner and his colleagues (17) have shown that strains identified as *E. agglomerans* belong to over a dozen species (different DNA hybridization groups). Much work will be required before a satisfactory system of classification and nomenclature can be proposed. At present, cultures that are negative for lysine and ornithine decarboxylase and arginine dihydrolase (referred to as "triple decarboxylase negative") and that are yellow pigmented are

TABLE 8. Differentiation within the genus *Enterobacter*

Test or property	*E. aerogenes*	*E. agglomerans*	*E. amnigenus* biogroup:		*E. cloacae*	*E. gergoviae*	*E. intermedium*	*E. sakazakii*	*E. taylorae*
			1	2					
Lysine decarboxylase	+[a]	−	−	−	−	+	−	−	−
Arginine dihydrolase	−	−	−	v	+	−	−	+	+
Ornithine decarboxylase	+	−	v	+	+	+	(+)	+	+
Growth in KCN	+	v	+	+	+	−	v	+	+
Fermentation of:									
Sucrose	+	(+)	+	−	+	+	v	+	−
Dulcitol	−	(−)	−	−	(−)	−	+	−	−
Adonitol	+	−	−	−	(−)	−	−	−	−
D-Sorbitol	+	v	−	+	+	−	+	−	−
Raffinose	+	v	+	−	+	+	+	+	−
α-Methyl-D-glucoside	+	−	v	+	(+)	−	+	+	v
D-Arabitol	+	v	−	−	(−)	+	−	−	−
Yellow pigment	−	(+)	−	−	−	−	−	+	−
Present in human clinical specimens	+	+	−	−	+	+	−	+	+

[a] For definitions of symbols, see footnote *a* of Table 4.

usually reported as *E. agglomerans*. The classification of other triple decarboxylase-negative strains is more uncertain.

Enterobacter gergoviae. *E. gergoviae* was first described by Richard and co-workers (115) as a new group resembling *E. aerogenes*, but which was KCN negative, sorbitol positive, mucate negative, and gelatinase negative. The name *E. gergoviae* was proposed by Brenner et al. (23) 4 years later because this group of strains was distinct from other species of *Enterobacter* by DNA hybridization. *E. gergoviae* is a human pathogen that has caused at least one hospital outbreak of urinary tract infection (115). This particular outbreak strain was antibiotic resistant. In our series (Table 3) most of the strains were from the respiratory tract, but five were from blood.

Enterobacter sakazakii. *E. sakazakii* was named in 1980 (46), but two older case reports of neontal meningitis due to "yellow-pigmented *Enterobacteriaceae*" were later shown to be caused by *E. sakazakii*. Our series includes 71 human isolates. Eight were from spinal fluid, and three were from blood, but wounds and the respiratory tract were the most common sources. *E. sakazakii* is a documented pathogen which causes meningitis, brain abscesses, and bacteremia in neonates (100). It is of doubtful clinical significance when isolated from sites other than blood or spinal fluid, but this point requires further study. *E. sakazakii* can be recognized by its "tough" colonies (46), which are bright yellow when incubated at about 25°C (or room temperature). It is further characterized by its decarboxylase pattern (lysine negative, arginine positive, ornithine positive), negative sorbitol reaction, and delayed positive DNase reaction (46). We studied the organism named *Praschechia flavescens* by Weisglass and Krznaric-Sucic (140) and found it to be a typical strain (phenotypically) of *E. sakazakii*.

Enterobacter amnigenus. *E. amnigenus* was the name proposed in 1981 by Izard et al. (82) for a group of *Enterobacteriaceae* that they previously had called "group H3." All the strains were from drinking water, surface water, and soil. The DNA hybridization data of Izard et al. (81) indicated two distinct groups, but both were included in the definition of *E. amnigenus*. Because the biochemical reactions of the two groups are different, we have divided *E. amnigenus* into two biogroups, which should aid in identification (Table 1). We define *E. amnigenus* biogroup 1 for the strains that ferment sucrose and raffinose, but not D-sorbitol. Our strains of *E. amnigenus* biogroup 1 include the seven strains from water described by Izard et al. (82), but also include four strains isolated from human clinical specimens. Two were from the respiratory tract, one was from a wound, and one was from feces. We define *E. amnigenus* biogroup 2 for strains that ferment D-sorbitol, but not sucrose or raffinose. Our series includes only three strains, and all were from water and described by Izard et al. There is no evidence that *E. amnigenus* can cause human infection; however, it has occurred in clinical specimens.

Enterobacter intermedium. *E. intermedium* was proposed in 1980 by Izard and co-workers (80) for a new group of *Enterobacteriaceae* that they had previously named "group H1." The original isolates were from drinking water, surface water, and unpolluted soil (80). All of the cultures of *E. intermedium* in our series were from Izard et al., so there is no evidence that *E. intermedium* can cause human disease or even occur in human clinical specimens.

The genus *Erwinia*. *Erwina* as a separate genus (126, 127) has not been a useful concept in clinical microbiology. The "true *Erwinia* species" represented by *Erwinia amylovora* do not grow at 35 to 37°C and are biochemically inactive in the tests used to identify *Enterobacteriaceae*. Ewing and Fife (40) classified the "Herbicola-Lathyri group of *Erwinia*" in the genus *Enterobacter* as *E. agglomerans*. This group of organisms occurs occasionally in clinical specimens (61, 96, 117, 137). There are really many different species in what is now called *E. agglomerans* (16, 17). This is analogous to the situation with *Staphylococcus epidermidis*, which has been split into many additional species. Even with all these problems, *E. agglomerans* has proved to be a useful name for reporting clinical isolates, and we will continue to use it until a better classification becomes available. To

TABLE 9. Differentiation within *Escherichia-Shigella*

Test or property	*Shigella* serogroups A, B, and C	*S. sonnei*	*E. coli*		*E. blattae*	*E. fergusonii*	*E. hermannii*	*E. vulneris*
			Inactive	Normal				
Indole production	v[a]	−	(+)	+	−	+	+	−
Lysine decarboxylase	−	−	v	+	+	+	−	(+)
Ornithine decarboxylase	−	+	−	v	+	+	+	−
Motility	−	−	−	+	−	+	+	+
Gas produced during fermentation	−	−	−	+	+	+	+	+
Acetate utilization	−	−	v	+	−	+	(+)	v
Mucate fermentation	−	−	v	+	v	−	+	(+)
Lactose fermentation	−	−	(−)	+	−	−	v	(−)
Growth in KCN	−	−	−	−	−	−	+	(−)
Yellow pigment	−	−	−	−	−	−	+	v
Fermentation of:								
D-Mannitol	(+)	+	+	+	−	+	+	+
Adonitol	−	−	−	−	−	+	−	−
D-Sorbitol	v	−	(+)	+	−	−	−	−
Cellobiose	−	−	−	−	−	+	+	+
D-Arabitol	−	−	−	−	−	+	−	−
Present in human clinical specimens	+	+	+	+	−	+	+	+
Isolated from cockroaches	−	−	−	−	+	−	−	−

[a] For definitions of symbols, see footnote *a* of Table 4.

indicate that this group is heterogeneous, we now report "*Enterobacter agglomerans* group."

The genus *Escherichia*. *Escherichia* is the type genus for the family *Enterobacteriaceae* (89). The type species for the genus *Escherichia* is *E. coli*, which is the most studied of all living organisms and has been the subject of many reviews (29). The genus *Escherichia* includes four other species: *E. adecarboxylata*, *E. blattae*, *E. hermannii*, and *E. vulneris*. In addition, the new species *Escherichia fergusonii* is formally proposed in a companion paper in this issue (47). Table 9 gives the differential reactions for the *Escherichia-Shigella* group.

***Escherichia adecarboxylata* (Enteric Group 41).** *E. adecarboxylata* was named in 1962 by Leclerc (90) and has standing in nomenclature as a valid species (124). However, it has received little attention. Strains of *E. adecarboxylata* produce a yellow pigment and are indole and methyl red positive. They are negative for acetoin production, citrate utilization, and for lysine, arginine, and ornithine decarboxylase, but ferment most of the sugars and polyhydroxyl alcohols used in identification (90). Ewing and Fife studied the type strain and identified it as *Enterobacter agglomerans* biogroup G3 (40). We compared the type strain of *E. adecarboxylata* to all of our clinical strains and found 6 to 10 that were very similar. It is likely that *E. adecarboxylata* is part of the *Enterobacter agglomerans-Erwinia* group, since it is yellow pigmented and triple decarboxylase negative. Until all of these problems have been resolved and more authentic strains of *E. adecarboxylata* have been studied, we will report cultures as Enteric Group 41 rather than *E. adecarboxylata*. However, it should be emphasized that several cultures from clinical specimens are very similar to the type strain of *E. adecarboxylata*.

***Escherichia blattae*.** *E. blattae* is the name coined in 1973 by Burgess and co-workers (27) for the species whose ecological niche is the cockroach intestine. Table 1 gives the biochemical reactions for this species. These data were based on the type strains studied by Burgess et al. and strains isolated from cockroaches in Georgia, South Carolina, and Easter Island. The biochemical reactions of these four strains were compared with all our clinical isolates, but none could be definitely identified as *E. blattae*. Although people often have contact with several species of cockroaches (27), there is no evidence that *E. blattae* from cockroach feces is capable of causing human infections or occurring in clinical specimens.

***Escherichia hermannii*.** *E. hermannnii*, formerly known as Enteric Group 11, is a new species in the genus *Escherichia*. Brenner et al. (19) found that most of their strain which had been reported as "*Escherichia coli*—atypical" were highly related to *E. coli* strain K-12 by DNA hybridization. However, one group of the biochemically atypical strains was only 40 to 46% related and was defined to be a separate species. This group was designated Enteric Group 11 and was later named *E. hermannii* (19). *E. hermannii* strains produce yellow pigment, grow in the presence of cyanide (KCN test), and ferment cellobiose (Table 9). *E. hermannii* is ornithine decarboxylase positive, in contrast to *Enterobacter agglomerans*. Strains of *E. coli* usually have the opposite pattern. Wounds and feces were the most common sources of *E. hermannii*. There was one isolate from spinal fluid and two from blood, which indicate possible clinical significance.

***Escherichia vulneris*.** *E. vulneris* is a new species in the genus *Escherichia* (22). Before it was formally named *E. vulneris*, it was called "Enteric Group 1," "API group 2," and "Alma group 1." It was originally defined in 1976 as a group of strains which were similar to *Enterobacter agglomerans*, but which were positive (either rapidly or delayed) for lysine decarboxylase, arginine dihydrolase, or both. Over half the strains produced yellow pigment. By DNA hybridization, strains of *E. vulneris* were highly related to each other, but only 6 to 39% related to other species in the family *Enterobacteriaceae* (22). *E. vulneris* was about equally related to *Escherichia*, *Enterobacter*, and several *Erwinia* species, so based on its phenotypic properties (negative for the Voges-Proskauer test and citrate utilization), it was classified in the genus *Escherichia*. Most strains of *E. vulneris* have been from human wounds. The species name "*vulneris*" was coined from the latin word "vulnus" for a wound.

The genus *Ewingella*. *Ewingella* is a new genus that was recently proposed by Grimont and co-workers (63) for a group of organisms previously known as "Enteric Group 40." *Ewingella americana* is the only species in the genus. Grimont and co-workers were studying a group of strains that were initially thought to be related to the new genus *Cedecea*. However, by DNA hybridization the strains were less than 21% related to other named species of *Enterobacteriaceae*. Thus, a new genus was proposed. Table 1 gives the biochemical reactions for *E. americana*. Strains are negative for lysine, arginine, and ornithine and formerly may have been classified in the "*Enterobacter agglomerans* complex." Strains of *E. americana* varied in their reactions for L-rhamnose and D-xylose. Grimont et al. (63) reported that most strains are negative for these tests, but that strain 0679-79, which was positive, was less related to the other strains by DNA hybridization.

The sources of the 44 cultures of *E. americana* now in our collection are given in Table 3. Surprisingly, 11 were from blood; 5 of these were from a single outbreak of bacteremia (107). Two others were closely related in time at the same hospital and appeared to be a small cluster. The respiratory tract was also a common source; 14 were from sputum, and 4 were from throat. In six instances the culture was sent with a note saying that a commercial identification system had identified it as *Yersinia pestis*, but that this identification seemed very unlikely.

The genus *Klebsiella*. Until recently *Klebsiella* was considered as an uncomplicated genus composed of the ubiquitous species *Klebsiella pneumoniae* and the two very rare species

TABLE 10. Differentiation within the genus *Klebsiella*

Test	*K. pneumoniae*	*K. oxytoca*	*K. terrigena*	*K. planticola*[a]	*Klebsiella* group 47 indole positive ornithine positive
Indole production[b]	−[c]	+	−	v	+
Ornithine decarboxylase[b]	−	−	−	−	+
Growth and D-glucose fermentation at[d]:					
5°C	−	−	+	+	+
10°C	−	+	+	+	+
41°C	+	+	−	+	+
44.5°C	+	v	−	−	(−)

[a] The reactions of *K. trevisanni* (*Klebsiella* group K) are the same as *K. planticola*.

[b] Based on CDC data.

[c] For definitions of symbols, see footnote *a* of Table 4.

[d] Based on limited CDC data; compiled from the literature (53, 59, 79); 44.5°C is the temperature setting of water baths used for the fecal coliform test.

K. ozaenae and *K. rhinoscleromatis* (33). The DNA hybridization studies of Brenner et al. (24) showed that the latter two were not true species, but were biochemically inactive strains of *K. pneumoniae*. (In the 1984 *Bergey's Manual of Systematic Bacteriology*, *K. ozaenae* and *K. rhinoscleromatis* are classified as subspecies of *K. pneumoniae* [104].) For simplicity in reporting and because of the association with specific human disease, we will continue to classify and report them as *K. ozaenae* and *K. rhinoscleromatis*, realizing they are not true species, but adapted strains of *K. pneumoniae*. Recently four other *Klebsiella* species or biogroups have been described: *K. oxytoca*, *K. terrigena*, *K. planticola*, and *K. trevisanii*. In this paper we also describe a new group of *Klebsiella* strains that are indole positive and ornithine positive. Although the genus *Klebsiella* has become more complex, the species can usually be differentiated by simple phenotypic tests (Table 10).

Klebsiella oxytoca. Until recently, indole-positive strains of *Klebsiella* were considered to be an indole positive biogroup of *K. pneumoniae* (33). However, in 1974, Jain and co-workers (84) showed that the indole-positive strains were distinct from *K. pneumoniae* by DNA hybridization. This was later confirmed by other workers (21); thus *K. oxytoca* is now recognized as a separate species. The sources of our 98 strains of *K. oxytoca* are given in Table 3. Over half of the 63 human strains were from feces, but 17 were from blood.

Klebsiella planticola. *K. planticola* was named by Bagley et al. in 1981 (7). Earlier, Naemura et al. (101) and Woodward et al. (141) had studied a large collection of *Klebsiella* strains and found isolates that were distinct from the named species of *Klebsiella*. They referred to this new group of strain as "*Klebsiella* species 2," which Bagley and co-workers named *K. planticola* based on DNA hybridization and phenotypic analysis (7). The strains of *K. planticola* described by Bagley et al. came from vegetables (22 isolates from seeds, leaves, and tissue), redwood tanks or chips (5 isolates), pulp and paper mill effluent (5 isolates), drinking water (3 isolates), human clinical isolates (2 urine, 1 wound, 1 unspecified), and bovine mastitis (2 isolates). The biochemical reaction of four strains received from R. J. Seidler (102) are given in Table 3.

***Klebsiella* group 47—indole positive, ornithine negative.** Several years ago we began receiving cultures sent as "indole$^+$ ornithine$^+$ *Klebsiella*??" with a note that the sender had never seen an ornithine-positive *Klebsiella*. The biochemical reactions were tabulated, and a new group was formed called "Enteric Group 47," which was later changed to "*Klebsiella* group 47" when it became apparent that these belonged in *Klebsiella*. DNA hybridization data indicated that this group of strains is related to the new species *K. planticola*, but only a small percentage of the described strains of *K. planticola* have been indole positive, and none has been ornithine positive. It appears that *Klebsiella* group 47 may be a distinct biogroup of *K. planticola* which is indole positive and ornithine positve. Further study is required on this point. Most of our isolates (Table 3) have been from human clinical specimens, and over half were from the respiratory tract, but two were from blood.

***Klebsiella terrigena* ("group L" of Izard et al.).** The new species *K. terrigena* was proposed in 1981 by Izard et al. (79). Earlier Gavini et al. (59) had studied a collection of 122 strains belonging to or related to *Klebsiella*. They noted a group of 37 soil and water isolates that appeared to be distinct from the others and called these "group L." On the basis of differences by DNA hybridization, phenotype and protein electrophoresis patterns, they named group L as *K. terrigena*. The strains described by Izard et al. were from drinking water (25 isolates), surface waters (5 isolates), soil (5 isolates), and sewage (2 isolates). Table 1 gives the biochemical reactions of the five isolates of *K. terrigena* received from Gavini et al. There have been no reports of *K. terrigena* causing human disease or occurring in clinical specimens.

TABLE 11. Differentiation within the genus *Kluyvera*

Test	*K. ascorbata*	*K. cryocrescens*
Ascorbate test	+	−
D-Glucose fermentation at 5° C (21 days)	−	+
Zone sizes for cephalothin and carbenicillin (48)	Small[a]	Large[a]

[a] *K. ascorbata* has zone size of 17 mm or less around cephalothin; *K. cryocaescens* usually has larger zones.

***Klebsiella* "group K" of Izard et al.** "Group K" was also defined in addition to "group L" by Gavini et al. (59). The strains of group K described by Izard et al. were from sewage (5 isolates), soil (4 isolates), surface water (15 isolates), and drinking water (3 isolates). Group K was recently named *Klebsiella trevisanii* by Farragut et al. (53), but this species name may be a synonym of *K. planticola*, in which case the name *Klebsiella planticola* would be used rather than *Klebsiella trevisanii* because it was validly published first.

The genus *Kluyvera*. *Kluyvera* is a genus (86) with a turbulent history (48). Although the name has been used in the literature (1–4, 102, 103), the genus name and the two original species names, *K. citrophila* and *K. noncitrophila* (3), did not appear on the *Approved Lists of Bacterial Names* (124), perhaps because they were never considered. This nomenclatural problem was resolved in the proposal of a redefined genus *Kluyvera*, with two species—*Kluyvera ascorbata* and *K. cryocrescens* (48). A third group of strains, distinct by DNA hybridization, was named "*Kluyvera* sp. 3" (48). This latter group needs further study before a scientific name can be proposed.

Kluyvera occurs in clinical specimens and may cause human infections. Schwach (118) reported three isolates of *Kluyvera* (called Enteric Group 8 in the report), all from upper respiratory tract specimens, which were subsequently identified as *Kluyvera ascorbata*. Since the strains were in mixed culture and were not detected in subsequent specimens, the conclusion was that they were probably not clinically significant. Braunstein and co-workers (15) reported two patients who yielded isolates that we had originally identified as Enteric Group 8 (both are *K. ascorbata*). One of these was from the sputum of a 6-year-old boy with pulmonary tuberculosis. This isolate was not considered clinically significant. A second isolate was from gall bladder drainage fluid of a 63-year-old woman with acute pancreatitis. On the basis of chart review, this isolate was considered clinically significant (15). Of our series of 144 *Kluyvera* strains, none has been from spinal fluid, but five strains have been from blood; three strains (two from France) of *K. ascorbata*, one strain of *K. cryocrescens* (a 3-month-old, at autopsy), and one strain of *Kluyvera* sp. group 3. No other information was included to allow further evaluation of their clinical significance. These five blood isolates and the report of Braunstein and co-workers (15) suggest that *Kluyvera* may be able to cause human infections. The respiratory tract was the most common source for *Kluyvera*, but there is no strong evidence that it is clinically significant at this site (one isolate of *K. ascorbata* was, however, from a lung at

autopsy). The respiratory tract (particularly sputum) is notoriously difficult to evaluate for clinical significance, except in carefully designed prospective studies. The urinary tract was the next most common source. Feces were a common site of isolation, and food (85) is an obvious source of these isolates. The association of *Kluyvera* with diarrhea has been mentioned by Fainstein et al. (42), but no convincing evidence was presented that it was the actual cause.

The two named species of *Kluyvera*, *K. ascorbata* and *K. cryocrescens*, are very close biochemically, but they can be differentiated by simple tests (Table 11) and by differences in the zones of inhibition (8) around carbenicillin (100 μg/disk) and cephalothin (30 μg/disk). *K. cryocrescens* has big zones (usually 17 mm or more, sometimes with resistant colonies in the zones), but *K. ascorbata* has much smaller zones. We recommend that clinical laboratories report "*Kluyvera* sp." unless they actually use the test (Table 11) to differentiate these two closely related species.

The genus *Mollerella*. The new genus *Mollerella*, with only one species, *M. wisconsinsis*, was recently described by Hickman-Brenner et al. (77). Formerly it was known as Enteric Group 46. This group was recognized in 1980 from a series of cultures that had been sent from Wisconsin. All had been isolated from feces. After Enteric Group 46 was defined, a search of our records indicated that six cultures previously reported could be identified as Enteric Group 46. Five of these were from feces and had been isolated in Wisconsin from 1975 to 1979, and one was from Virginia. Additional isolates have now come from Wisconsin and New York. These had originally been reported as "unidentified" or "*Providencia* species." The first isolate (2252-80) reported as Enteric Group 46 had an interesting case history. A physician had a severe case of bloody diarrhea, fever, abdominal pain, and cramps 48 h after arriving in Peru. He was quite anxious to determine what made him ill. The laboratory noted that there was scant growth of *E. coli* when his feces was cultured, and Enteric Group 46 predominated on deoxycholate medium. The stool was negative for parasites, *Salmonella*, *Shigella*, *Yersinia*, *Campylobacter*, and *Aeromonas*. A second isolate of Enteric Group 46 (2397-81) was from a 19-year-old male with diarrhea and was isolated during a search for *Yersinia*. There is no evidence that *M. wisconsinsis* can actually cause diarrhea.

The genus *Morganella*. *Morganella morganii* is the name we now use for the organism previously known as *Proteus morganii*, which was originally known as "Morgan's bacterium 1." Its removal from the genus *Proteus* into a new genus *Morganella* was based on several lines of evidence (20). The G+C DNA content of *M. morganii* is 50% in contrast to the 39% content of the swarming *Proteus* species (*P. vulgaris*, *P. mirabilis*, and *P. myxofaciens*). In addition, by DNA hybridization *M. morganii* was less than 20% related to the species of *Proteus* and *Providencia* (20). *Morganella* is also different from both *Proteus* and *Providencia* by phenotypic tests (Table 12).

TABLE 12. Differentiation of the three genera in the *Proteus* group

Test or property	*Proteus*	*Providencia*	*Morganella*
Citrate utilization	v[a]	+	−
H_2S production (TSI)	+	−	−
Ornithine decarboxylase	v	−	+
Gelatin liquefaction	+	−	−
Lipase (corn oil)	+	−	−
D-Mannose fermentation	−	+	+
Swarming[b]	+	−	−

[a] For definitions of symbols, see footnote *a* of Table 4.
[b] On sheep blood agar on Trypticase soy agar.

TABLE 13. Differentiation within the genus *Proteus*

Test or property	*P. mirabilis*	*P. myxofaciens*	*P. penneri*	*P. vulgaris* biogroup	
				2	3
Indole production	−[a]	−	−	+	+
Ornithine decarboxylase	+	−	−	−	−
Maltose fermentation	−	+	+	+	+
D-Xylose fermentation	+	−	+	+	+
Salicin fermentation	−	−	−	+	−
Esculin hydrolysis	−	−	−	+	−
Chloramphenicol susceptibility[b]	S	S	R	V	S
Present in human clinical specimens	+	−	+	+	+
Occurs as a pathogen gypsy moth larvae	−	+	−	−	−

[a] For definitions of symbols, see footnote *a* of Table 4.
[b] S, Susceptible; R, resistant; V, variable.

***M. morganii*.** *M. morganii* is a species well known to microbiologists and was recently reviewed by Penner (105). Since its discovery in 1906 by Morgan, it has been implicated as a cause of diarrhea. This is a role that needs to be reexamined today. *M. morganii* is a documented cause of urinary tract infections and is cultured from many other body sites.

***M. morganii* biogroup 1 (lysine positive, nonmotile, glycerol positive).** We now use *M. morganii* biogroup 1 for reporting strains that are biochemically similar to *M. morganii*, but which are lysine positive and nonmotile and which ferment glycerol within 24 h (74). Most strains of *M. morganii* are lysine negative and motile and ferment glycerol very slowly or not at all. Hickman and colleagues (74) showed that strains of *M. morganii* biogroup 1 are highly related to each other and to typical strains of *M. morganii* by DNA hybridization. Thus this unique group was classified as a biogroup of *M. morganii* rather than as a distinct species. Three strains that were lysine positive, motile, and negative or slow for glycerol fermentation were also described, but these are now excluded from *M. morganii* biogroup 1 and are reported simply as "*M. morganii* lysine$^+$." The biochemical reactions of *M. morganii* biogroup 1 are given in Table 1 and are similar to those of *M. morganii*, except for lysine decarboxylase, motility, and glycerol fermentation.

The genus *Obesumbacterium*. The genus *Obesumbacterium* (121, 122) should be of little concern to the clinical microbiologist. The classification of *Obesumbacterium* has been very confused, but was clarified by Priest et al. (114) and Brenner et al. (16). In 1973, Priest and co-workers recognized two distinct groups of strains which they called *Obesumbacterium proteus* group 1 and *O. proteus* group 2. Both groups appear to have a unique ecological niche, beer breweries (28), where they are associated with the brewing yeast early in the wort fermentation (119–123). By DNA hybridication, *O. proteus* biogroup 1 was highly related to *Hafnia alvei*, but phenotypically it was less active biochemically (16). In addition, *O. proteus* biogroup 1 was lysed by the *Hafnia*-specific bacteriophage 1672 of Guinée and Valkenburg (71). Based on these data we propose that *O. proteus* biogroup 1 (with its type strain ATCC 12841) be classified in *H. alvei* as a distinct biogroup. We propose the name "*H.*

alvei biogroup 1" or "*H. alvei*—brewery biogroups" be used for these strains. *O. proteus* biogroup 2 was not closely related to *O. proteus* biogroup 1 by DNA hybridization and could not be assigned to any known species (16). Thus we will continue to use the name *O. proteus* biogroup 2. There is no evidence that the brewery biogroup of *H. alvei* (formerly known as *O. proteus* biogroup 1) or *O. proteus* biogroup 2 occur in human clinical specimens. They grow slowly (129, 130) and are fastidious when incubated at 36°C (136; H. J. J. van Vuurery Ph.D. thesis, Rijksuniversiteit Gent, Belgium, 1978), which makes it more difficult to identify them.

The genus *Proteus*. *P. mirabilis* and *P. vulgaris* are the two well-known species in the genus *Proteus* (34, 105). Two other species, *P. myxofaciens* (30) and *P. penneri* (75) are more recent additions to the genus (Table 13). *P. penneri* was not included in *Bergey's Manual of Systematic Bacteriology* (87) because it was published after the submission date for the chapter on *Proteus*.

***P. vulgaris* biogroups 2 and 3.** The DNA hybridization studies of Hickman et al. (75) indicated that strains identified as *P. vulgaris* could be divided into three groups. One group was indole negative, salicin negative, esculin negative, and resistant to chloramphenicol. This group was named *P. penneri* and is discussed below. The second DNA hybridization group was indole positive, salicin positive, and esculin positive, and the name *P. vulgaris* biogroup 2 will be used until a more definitive classification can be given. This group may be named as a new species in *Proteus* since it does not include the type strain of *P. vulgaris*. The third group is indole positive, salicin negative, and esculin negative and will be designated *P. vulgaris* biogroup 3 (Table 13). The type strain (ATCC 13315) of *P. vulgaris* does not fit into any of the above groups by DNA hybridization, but is biogroup 3 by phenotype.

***Proteus penneri*.** The new species *P. penneri* was proposed in 1982 by Hickman et al. (75) and was formerly called *P. vulgaris* biogroup 1. It was defined when DNA hybridization indicated that strains identified as *P. vulgaris* were not all highly related to each other. Further studies showed that the strains that are indole negative, salicin negative, esculin negative, and chloramphenical resistant were highly related to each other, but were more distantly related to *P. vulgaris*. This distinct group had originally been called *P. vulgaris* biogroup 1. Based on differences in DNA hybridization and phenotype, *P. penneri* was named as a separate species (75).

***Proteus myxofaciens*.** *P. myxofaciens* was described in 1966 by Cosenza and Podgwaite (30), but was not generally accepted as a new species at the time. In the eighth edition of *Bergey's Manual of Determinative Bacteriology* (26), the statement is made that it ". . . is probably not a *Proteus* species, but identical to *Erwinia herbicola*." The situation was clarified when Brenner et al. (20) showed that *P. myxofaciens* was related to *P. vulgaris* and *P. mirabilis* by DNA hybridization. Its phenotypic properties are also very similar to these two species. The most unusual property of *P. myxofaciens* is the large amount of slime it produces in broth media when grown at 25°C. When the contents of a Trypticase soy broth (BBL Microbiology Systems) tube culture are poured into a petri dish, they emerge as a plug presumably of slime material produced by *P. myxofaciens* during growth. The name myxofaciens was derived from "myxo," Greek for slime, and "faciens," Latin for producing. *P. myxofaciens* swarms when incubated at 25°C, but not when incubated at 36°C. Thus *P. myxofaciens* is a member of the "swarming proteus group" that is now defined to be the genus *Proteus*, since *Providencia* and *Morganella* have been removed. *P. myxofaciens* has not been isolated from a human clinical specimen. The only isolates of this rare species have come from living and dead gypsy moths collected in Glenville, N.Y., and in an outbreak of disease in gypsy moth larvae in Colchester, Conn. (30).

TABLE 14. Original definition (39) of biogroups in *Providencia*

Biogroup	Gas produced during fermentation	Adonitol	Inositol	Classified as:
1	+	+	−	*P. alcalifaciens*
2	−	+	−	*P. alcalifaciens*
3	+	−	−	*P. alcalifaciens*
4	−	−	−	*P. alcalifaciens*
5	−	−	+	*P. stuartii*
6	−	+	+	*P. stuartii*

The genus *Providencia*. Ewing and co-workers originally defined six biogroups of *Providencia* (39), which were based on the fermentation of adonitol and *myo*-inositol and on gas production (Table 14). However, DNA hybridization studies indicated that revision was needed in this schema (20). Table 1 shows that we recognize four species in the genus *Providencia*: *P. alcalifaciens*, *P. rustigianii* (formerly known as *P. alcalifaciens* biogroup 3), *P. rettgeri* (formerly known as *Proteus rettgeri*), and *P. stuartii*. This classification is based on data from DNA hybridization (20, 76), structural differences of specific proteins, and extensive phenotypic analysis. The evidence was summarized by Brenner et al. (20), more recently by Penner (105), and by Hickman-Brenner et al. (76). Table 15 gives the differentiation of the four species now recognized in the genus.

P. rettgeri and *P. stuartii* are documented pathogens of the urinary tract, where they have caused a number of nosocomial outbreaks (105). Both species have been isolated at other sites, but are rarely isolated from feces. *P. stuartii* has caused serious infections in burn patients as part of several nosocomial outbreaks. In contrast, *P. alcalifaciens* is usually isolated from feces. Over the years it has been frequently isolated from children with diarrhea-gastroenteritis, but its causative role, although suggestive, has not been established.

***Providencia rustigianii*.** The new species *P. rustigianii* was recently proposed by Hickman-Brenner et al. (76) for the group of strains formerly known as *P. alcalifaciens* biogroup 3 (34). In 1978, Brenner et al. (20) showed that biogroup 3 of *P. alcalifaciens* was distinct from biogroups 1 and 2 by DNA hybridization. They considered it a separate species, but did not name it because simple tests were not available to

TABLE 15. Differentiation within the genus *Providencia*

Test	*P. alcalifaciens*	*P. rustigianii*	*P. stuartii*	*P. rettgeri*
Urea hydrolysis	−[a]	−	v	+
Fermentation of:				
myo-Inositol	−	−	+[b]	+
Adonitol	+	−	−[b]	+
D-Arabitol	−	−	−	+
Trehalose	−	−	+	−
D-Galactose	−	+	+	+

[a] For definitions of symbols, see footnote *a* of Table 4.

[b] *P. stuartii* biogroup 4 strains are inositol negative, and biogroup 6 strains are adonitol positive; these two biogroups are rare.

differentiate it from other named *Providencia*. Hickman-Brenner et al. found simple tests to accomplish this (Table 15) and then proposed *P. rustigianii* as a new species. *P. rustigianii* is rare in clinical specimens and accounts for only about 2% of *Providencia* isolates (39). Most isolates have been from human feces. Although *P. alcalifaciens* has been implicated occasionally as a cause of diarrhea, there is no evidence at present implicating *P. rustigianii* as a cause; however, this point needs further study. The name *Providencia friedericiana* was recently proposed by Muller (99) for a large group of strains isolated from penguins. This new organism appears to be the same as *P. rustigianii*, in which case the name *P. rustigianii* would have priority.

The genus *Rahnella*. In 1976, Gavini et al. (57) defined a new group of *Enterobacteriaceae* and gave it the vernacular name "group H2." The original definition was based on clustering by numerical taxonomy and the phenotypic differences between group H2 and other *Enterobacteriaceae*. In 1979, Izard et al. (81) used DNA hybridization to compare strains of group H2 with each other and with named species of *Enterobacteriaceae*. On the basis of the close relatedness within group H2 and the low relatedness to other *Enterobacteriaceae*, they proposed the new genus *Rahnella* with one species, *Rahnella aquatilis*. All of the original strains of *R. aquatilis* were isolated from water. The names *Rahnella* and *R. aquatilis* were validly published, but were not validated in the International Journal of Systematic Bacteriology before January 1, 1980; thus they did not appear on the *Approved Lists of Bacterial Names*. However, both names have now been validly published and have standing in nomenclature (83).

Rahnella has no single distinguishing feature to differentiate it from other *Enterobacteriaceae* (Table 1). Strains of *Rahnella* are nonmotile at 36°C, but motile at 25°C, negative for lysine and ornithine decarboxylases and for arginine dihydrolase, and weakly positive (less so than the *Proteus* group) for phenylalanine deaminase and do not produce a yellow pigment. These properties differentiate *Rahnella* from the heterogeneous group of bacteria classified in the *Enterobacter agglomerans-Erwinia herbicola* complex. Strains now identified as *Rahnella* might have been identified in the past as *E. agglomerans* (40).

The natural habitat of *Rahnella* is water, and all of the isolates of Gavini and co-workers were from waters in France (60). In the United States we have identified several water isolates as *R. aquatilis*. One puddle of water standing over red-clay soil near Atlanta, Ga., had two different strains (distinct biogroups) of *R. aquatilis*. One strain in our series was from a human burn wound. Only a few *Rahnella* strains have been described, so much more study is needed to define its ecology and possible role in human disease.

The "*Salmonella-Arizona* group." We have adopted some changes in the nomenclature and classification of *Salmonella-Arizona*, and this is reflected in the way we now report cultures at the CDC (49). The classification of this group of *Enterobacteriaceae* has been the source of confusion for a number of years because of different names used in reporting. In the paragraphs that follow the reasons are given for the new reporting system. Table 16 summarizes some of the differences between the different subgroups of *Salmonella*.

The genus *Salmonella*. In 1972, the Enteric Reference Laboratories, Bureau of Laboratories at CDC, adopted a revised nomenclature and classification of the genus *Salmonella* (35). Only three species were recognized: *S. typhi*, *S. cholerae-suis*, and *S. enteritidis*. All of the other species or serotype names such as "*typhimurium*," "*anatum*,"

TABLE 16. Properties of the six subgroups within the genus *Salmonella* (the *Salmonella-Arizona* group)[a]

Property or test	Results in *Salmonella* subgroup:					
	1	2	3a	3b	4	5
DNA hybridization group of Crosa et al. (31)	1	2	3	4	5	Not studied
Genus according to Ewing (35)	*Salmonella*	*Salmonella*	*Arizona*	*Arizona*	*Salmonella*	*Salmonella*
Salmonella subgenus names formerly used	I	II	III	III	IV	V
Subspecies according to Le Minor et al. (94)	*cholerae-suis*	*salamae*	*arizonae*	*diarizonae*	*houtenae*	*bongori*
Usually monophasic (Mono) or diphasic (Di) flagella	Di	Di	Mono	Di	Mono	Mono
Usually isolated from humans and warm-blood animals	+	−	−	−	−	−
Usually isolated from cold-blooded animals and the environment	−	+	+	+	+	+
Pathogenic for humans	++++	+	+	+	+	+?
Tests						
Dulcitol fermentation	96[b]	90	0	1	0	100
Lactose fermentation	1	1	15	85	0	0
ONPG[c]	2	15	100	100	0	100
Malonate utilization	1	95	95	95	0	0
Growth in KCN medium	1	1	1	1	95	100
Mucate fermentation	90	96	90	30	0	100
Gelatin hydrolysis[d]	−	+	+	+	+	−
D-Galacturonate fermentation[d]	−	+	−	+	+	+
Lysis by O1 bacteriophage[d]	+	+	−	+	−	v

[a] Adapted from Le Minor et al. (93, 94) and Farmer et al. (49).

[b] The numbers give the percent positive for the tests after 2 days of incubation at 36°C; the results from the first six tests are based on CDC data. The vast majority of the positive tests occur within 24 h; reactions positive after 2 days are not considered.

[c] ONPG, *o*-Nitrophenyl-β-D-galactopyranoside.

[d] Based on the data of Le Minor et al. (93, 94): +, 90% or more positive; −, 10% or less positive; v, variable. The test for gelatin hydrolysis is the rapid film method at 36°C (almost all strains are negative by the tube method at 22°C within 2 days).

"*newport*" and over 1,500 others were defined as serotypes of the species *S. enteritidis*. In this nomenclature, a culture previously reported as "*Salmonella typhimurium*" was now reported "*Salmonella enteritidis* serotype *typhimurium*" (35). This nomenclature and classification was used by the National Salmonella Center at the Enteric Reference Laboratories, CDC, but it was not used in the Salmonella Surveillance Reports published by the Bureau of Epidemiology at CDC and was not adopted by most of the National Salmonella Centers in other countries. Thus, for many years in the United States there has been confusion in *Salmonella* nomenclature. The same organism was being reported as "*Salmonella enteritidis* serotype *typhimurium*" and "*Salmonella typhimurium*." In the nomenclature and classification proposed by Ewing (35), *Arizona* was recognized as a distinct genus, separate from *Salmonella*. "*Arizona*" cultures were reported as "*Arizona hinshawii*" with an accompanying antigenic formula from the antigenic schema for *Arizona*, which was separate from the *Salmonella* antigenic schema. This was in conflict with the system used by most of the other National Salmonella Laboratories under the World Health Organization's Collaborating Center for Reference and Research on *Salmonella*. Here, cultures of "*Arizona*" were reported as *Salmonella arizonae* with their *Salmonella* O and H antigens and with the equivalent antigens from the *Arizona* schema given within parentheses. From the above discussions it is clear that there has not been universal agreement on the way to identify, name, or report *Salmonella* and *Arizona* cultures.

Until the early 1970s, all of the classifications of *Salmonella-Arizona* were proposed without knowledge of their evolutionary relationships. However, in 1973 Crosa and co-workers (31) showed that representatives from four of the different subgroups (subgenera) of *Salmonella* and *Arizona* were very closely related by DNA hybridization. All of the strains tested were considered to belong to the same species in a genetic, phylogenetic, or evolutionary sense. They concluded that the differences in biochemical reactions, antigenic structures, host adaptations, and geographical distributions were due to divergence within a single species. They found five subgroups within *Salmonella-Arizona* based on a higher relatedness by DNA hybridization of strains within each subgroup. These subgroups corresponded almost exactly to the "subgenus concept" used by the World Health Organization Salmonella Centers. The one exception was that subgenus III (*Arizona*) could be further subdivided by DNA hybridization into two groups that correlated with whether the flagella antigens were monophasic or diphasic and with the speed of lactose fermentation (Table 16).

Based on all the above-mentioned factors, the Enteric Bacteriology Section at the CDC has made several changes in the way *Salmonella-Arizona* cultures are reported. These changes went into effect 1 June 1983.

Change 1. Discontinuation of the term "*Salmonella enteritidis* serotype _____." This term has perhaps been the greatest cause of confusion because of the similarity in the species name *Salmonella enteritidis*, and the serotype name *Salmonella enteritidis* (antigen formula, 1, 9, 12:*g*, *m*:−). We have returned to the system that reports *Salmonella* serotypes. Thus, we will report "*Salmonella* serotype typhimurium" rather than "*Salmonella enteritidis* serotype typhimurium." In laboratory reports, surveillance data, and scientific articles, it is often desirable to artificially treat *Salmonella* serotypes as if they are species (which they clearly are not). Thus, the names "*Salmonella* serotype typhimurium" and "*Salmonella typhimurium*" refer to the same organism.

TABLE 17. Comparison of our laboratories' old and new reports for *Salmonella-Arizona* (49)

Old report	New report
Salmonella enteritidis serotype typhimurium	*Salmonella* serotype *typhimurium*[a]
Salmonella enteritidis serotype agona	*Salmonella* serotype *agona*[a]
Salmonella enteritidis serotype hadar	*Salmonella* serotype *hadar*[a]
Salmonella enteritidis serotype enteritidis	*Salmonella* serotype *enteritidis*[a]
Salmonella typhi	*Salmonella* serotype *typhi*[a]
Salmonella cholerae-suis	*Salmonella* serotype *choleraesuis*[a]
Arizona hinshawii 23:24:31	*Salmonella* serotype 47:r:z*, *Formerly *Arizona hinshawii* 23:24:31
Arizona hinshawii 1,3:1,7,8	*Salmonella* serotype 44:z_4,z_{32}:−*, *Formerly *Arizona hinshawii* 1,2:1,7,8

[a] It is often convenient to artificially treat the serotype as species: *Salmonella typhimurium*, *Salmonella agona*, *Salmonella hadar*, *Salmonella enteritidis*, *Salmonella typhi*, etc.

Treating *Salmonella* serotypes as species is a convenient way to avoid long names such as "*Salmonella cholerae-suis* subsp. *cholerae-suis* seroytpe typhimurium." This system has been widely accepted in much of the world. The use of these "species" names provides the most valuable information for clinical and epidemiological purposes.

Change 2. *Arizona* is no longer classified as a separate genus. The studies of Crosa et al. (31) indicated that *Arizona* strains are very closely related to each other and to other strains of *Salmonella* and should be included in the same genus and species. Thus, we now classify "*Arizona*" cultures in the genus *Salmonella*. They will be reported as "*Salmonella* serotype _____" rather than *Arizona hinshawii*, and they will be reported with antigenic formulas taken from the *Salmonella* schema (142) rather than with the antigenic formulas of the *Arizona* schema previously used at the CDC. As a cross-reference, we will include the old "*Arizona* formula" for the strain. The correspondence of these formulas is given in the World Health Organization's *Salmonella* schema (142). Table 17 gives the new way *Salmonella* ("*Arizona*") cultures will be reported.

Table 1 gives the complete reactions of *Salmonella* groups that differ biochemically. The term "subgroup" refers to the subdivisions within *Salmonella* based on DNA hybridization (93, 94) and phenotypic analysis (Tables 1 and 16). Subgroup 1 (Table 16) refers to subspecies group 1 of Le Minor and co-worker (94) and also stands for DNA hybridization subgroup 1 and "old subgenus I." The vast majority of *Salmonella* cultures isolated from human clinical specimen belong to this first subgroup. This includes serotypes such as "typhimurium," "enteritidis," and "heidelberg." This group of *Salmonella* cultures is very uniform in its biochemical reactions and should not pose a problem in identification. However, within *Salmonella* subgroup 1, strains of *S. typhi*, *S. cholerae-suis*, and *S. paratyphi* A are less active biochemically and are listed separately in Table 1. These serotypes are important in human disease and are frequently isolated from blood cultures as well as from feces. *S. gallinarum* and *S. pullorum* are usually isolated from birds, but can occasionally occur in human clinical specimens. These two "fowl-adapted serotypes" are listed in Table 1 because they are distinct biochemically. The biochemical

reactions for *Salmonella* subgroup 2 (synonym, *Salmonella* subgenus II), *Salmonella* subgroup 3a (synonyms, *Arizona hinshawii* monophasic and *Salmonella* subgenus III), *Salmonella* subgroup 3b (synonyms, *Arizona hinshawii* diphasic and *Salmonella* subgenus III), *Salmonella* subgroup 4 (synonym, *Salmonella* subgenus IV), and *Salmonella* subgroup 5 (synonym, *Salmonella* subgenus V) are given in Tables 1 and 16. Cultures of *Salmonella* subgroups 2, 3a, 3b, 4, and 5 can occur in clinical specimens and should be considered as potential enteric pathogens when they are isolated from a diarrheal stool in the absence of other potential pathogens. However, they are more likely found in cold-blooded animals and the environment (Table 16).

The genus *Serratia*. *Serratia* is one of the best known of all the genera in the family *Enterobacteriaceae* and has been the subject of several recent reviews (64, 65), including a book (138). Through the years there have been many changes in the number of species recognized. Studies in the last 10 years based on DNA hybridizations have done much to clarify the status of cultures in the genera. Phenotypically, *Serratia* is one of the easiest genera to differentiate from others in the family *Enterobacteriaceae*. Strains of *Serratia* usually produce extracellular DNase, gelatinase, and lipase, and they are also usually resistant to the antibiotics colistin and cephalothin. This combination of properties is unique among *Enterobacteriaceae*. One species currently in the genus, *Serratia fonticola* (64, 65), does not share these properties (Table 18), and it will eventually be removed. Most clinical isolates of *Serratia* are *S. marcescens*, but the other species can occasionally occur. *S. marcescens* is a well-documented human pathogen, but the other species require more study to define their roles. Table 18 gives the differential reaction for *Serratia*.

***Serratia ficaria*.** *S. ficaria* was described in 1977 by Grimont and co-workers (69) in their study of *Serratia* strains associated with plants. The ecological niche of this species is the fig wasp (*Blastophaga psenes*)-fig ecosystem. Figs of the smyrna variety (Calimyrna variety in California) require pollination to ripen and become edible and are bacteriologically sterile until pollinated by the fig wasp (69). They then become colonized with *S. ficaria* from the fig wasp. Strains of *Serratia* isolated from figs and fig wasps were distinct from other species of *Serratia* by both phenotype and DNA hybridization, which led to the proposal of the name *S. ficaria*.

The original isolates of *S. ficaria* were from figs or fig wasps in California or Tunisia. The one exception was an isolate from a black ant (species not specified) in Bordeaux, France. We have studied 3 cultures isolated from human clinical specimens in addition to the 10 cultures furnished by Grimont and co-workers. One culture was from the sputum of a patient hospitalized for heart surgery. This case was reported (62), and the source of the culture was probably the figs that the patient frequently ate. Two other isolates were from Hawaii. One was from a patient with venous insufficiency and alcoholic cirrhosis whose purulent leg ulcer yielded heavy growth of *S. ficaria* along with heavy growth of three other organisms (106). The attending physician concluded that *S. ficaria* may have had an etiological role in the infection. The second isolate was from an "NGT tube drainage." These data suggest that *S. ficaria* is more widely distributed than just the fig-fig wasp ecosystem; however, there is no evidence that eating figs carries any human health hazard. *S. ficaria* is very rarely found in human clinical specimens, and there is no strong evidence that it is clinically significant.

***"Serratia" fonticola* (which is not really a *Serratia*).** In 1965, Leclerc and Buttiaux (91) were studying the genus *Citrobacter* and noticed a group of strains that were similar to *Citrobacter*, but were lysine positive. This group of strains was originally referred to as "class C." Crosa and colleagues (32) studied the strains of class C by DNA hybridization and found that they were closer to *Serratia* than *Citrobacter*. This was later confirmed by Gavini and co-workers (56), who showed that class C strains were 75 to 91% related to each other; 57% related to *S. marcescens*, the type species for the genus *Serratia*; and only 0 to 36% related to other species in the family *Enterobacteriaceae*. Based on these data they proposed class C as a new species and classified it in the genus *Serratia*. The new species was named *S. fonticola* (56). Our strains include some of those of Gavini et al. isolated from water, some additional strains isolated from water and originally identified as *Kluyvera* (133), and two strains isolated from well water in the state of Washington. Thus *S. fonticola* is widely distributed in water. One additional strain (originally identified as *Kluyvera*) was isolated from a plant leaf (9) and had the unique ability to grow on hammelose (2-*C*-hydroxymethyl-D-ribose), which is often found in plants (131). In addition to the environmental strains, we have studied 14 strains isolated from human clinical specimens (Table 3). The majority were from wounds, but their clinical significance is unknown.

***Serratia liquefaciens*.** *S. liquefaciens* is well known in microbiology, first as *Enterobacter liquefaciens* and for about the past 10 years as *S. liquefaciens*. Isolates have frequently come from water, plants, insects, food, and other

TABLE 18. Differentiation within the genus *Serratia*

Biochemical test	*S. ficaria*	*S. liquefaciens*	*S. marcescens*	*S. odorifera*	*S. plymuthica*	*S. rubidaea*	*"Serratia" fonticola*
DNase (25°C)	+[a]	(+)	+	+	+	+	−
Lipase (corn oil)	(+)	(+)	+	v	v	+	−
Gelatinase (22°C)	+	+	+	+	v	(+)	−
Lysine decarboxylase	−	+	+	+	−	v	+
Ornithine decarboxylase	−	+	+	v	−	−	+
Odor of *S. odorifera*	+	−	−	+	−	(−)	−
Red, pink, or orange pigment	−	−	v	−	v	v	−
Fermentation of:							
L-Arabinose	+	+	−	+	+	+	+
D-Arabitol	+	−	−	−	−	(+)	+
D-Sorbitol	+	+	+	+	v	−	+
Adonitol	−	−	v	v	−	+	+
Dulcitol	−	−	−	−	−	−	+

[a] For definitions of symbols, see footnote *a* of Table 4.

environmental sources. Grimont and Grimont (65) found that *S. liquefaciens* comprised 2.4% of their 1,107 clinical isolates of *Serratia* at Pellgrin Hospital, Bordeaux, France, from 1968 to 1975. Probably the best clinical information about *S. liquefaciens* is from Washington and co-workers (139) and was based on isolates from the Mayo Clinic Hospital and outpatient facilities. During the 35-month study period, there were 13 isolates from patients in two hospitals and 8 isolates from outpatients; 9 of the 13 former isolates were considered to have been hospital acquired. Isolates from all 21 cases were mixed with other organisms. The respiratory tract was the usual source, and the organism was considered as a commensal. In 15 cases, *S. liquefaciens* was considered as commensal, but in 3 cases it was thought to be a secondary invader; in one case it was considered primary. These data are typical for the United States, where *S. marcescens* is much more common than the other *Serratia* species. In contrast, the *Communicable Disease Report* for England, Scotland, and Wales often reports *S. liquefaciens* almost as commonly as *S. marcescens* in positive blood cultures. We originally suspected that the explanation for the unusually large number of *S. liquefaciens* was that they were really *S. marcescens*, but had been incorrectly identified because of a false-positive fermentation of L-arabinose (which is the main difference between the two species) in commercial identification systems. However, almost all of these cultures sent for confirmation to the Computer Identifications Laboratory, England's National Reference Laboratory, were L-arabinose negative and thus were really *S. liquefaciens*. Thus there is no explanation for why *S. liquefaciens* is more common in England. Table 3 shows that our series of *S. liquefaciens* is small. The above discussion applies to *S. liquefaciens* as it was defined until recently. In 1982 Grimont and co-workers (70) studied their collection of *S. liquefaciens* cultures and, based on phenotypic analysis and DNA hybridization, proposed that it be subdivided. They proposed two new species, *Serratia proteamaculans* and *Serratia grimesii*, both of which formerly had been classified as biogroups within the species *S. liquefaciens*. This confirmed the earlier DNA hybridization studies of Steigerwalt and colleagues (128), who showed that strains classified as *S. liquefaciens* belonged to different DNA hybridization groups.

Unfortunately, it is very difficult to differentiate these four named groups that were formerly all classified as *S. liquefaciens*. Even the specialized carbon source utilization tests do not adequately distinguish them. Thus, clinical laboratories will probably continue to report *S. liquefaciens* in a broad sense rather than trying to differentiate the four named species and subspecies. The terms "*Serratia liquefaciens* group," "*Serratia liquefaciens*-like," "*Serratia liquefaciens*—broad sense," or "*Serratia* sp. not *S. marcescens*" could be used in reporting.

Serratia grimesii. The strains of *S. grimesii* described by Grimont et al. were from water (two isolates), plants (two isolates), dairy products (two isolates), soil (one isolate), and animals (four isolates). Three isolates were from blood cultures from patients at two different hospitals in France. No further information was given to assist in evaluating the significance of these isolates.

Serratia proteamaculans. *S. proteamaculans* is not known to occur in clinical specimens, but this species complicates the identification of *Serratia* cultures.

Serratia marcescens. *S. marcescens* is the ubiquitous species in the genus and is widely distributed in the environment as well as being the most common *Serratia* in human infections (138). Most strains of *S. marcescens* are typical biochemically and are easy to identify, since *S. marcescens* does not ferment L-arabinose, but the other species do.

***Serratia marcescens* biogroup 1.** Strains of *S. marcescens* usually have the following characteristics: methyl red negative, Voges-Proskauer positive, citrate positive, lysine positive, ornithine positive, motile, gelatin positive, KCN positive, and gas positive. However, over the last 10 years we have received cultures that appear to be *S. marcescens* (they are L-arabinose negative), but which are atypical on three to eight of the above reactions. In our computer identification program based on normalized likelihoods, these strains had a low score as being *S. marcescens*, but they received a very high score as being in the genus *Serratia*. Previously, we reported these strains as "*Serratia* sp." However in 1981 we defined "*Serratia marcescens* biogroup 1" and added this to the identification program. When the strains reported as *Serratia* sp. were retested, they had a very high computer identification score as *S. marcescens* biogroup 1. We report a strain as *S. marcescens* biogroup 1 if it does not ferment L-arabinose, is typical of the genus *Serratia*, and has at least four of the properties listed in Table 19 for biogroup 1. Most of the 24 strains of *S. marcescens* biogroup 1 have been from urine, and our hypothesis is that this biogroup has lost some of its metabolic diversity through natural selection of strains pathogenic for the human urinary tract. This type of selection, with loss of biochemical activity, has been well documented in *Escherichia coli*, *Klebsiella pneumoniae*, and other *Enterobacteriaceae*. Strains of *Serratia* from human clinical specimens that are biochemically inactive are most likely to be *S. marcescens*. We hope the definition of *S. marcescens* biogroup 1 will aid in the identification of these strains.

Serratia odorifera. *S. odorifera* was described in 1978 by Grimont et al. (67) and was based on its differences in DNA hybridization and phenotypic properties from all of the named *Serratia* species. One unusual property of *S. odorifera* is the production of a pungent odor that has been described by various authors as "musty," "potato like," "like crushed wild poppies," or "vegetable like." Originally this was called "38-like odor" because *Serratia* strain 38 produced it. The odor is probably due to the production of 2-methoxy-3-isopropylpyrpyrazine (or a structurally similar compound) which was first noted to cause the unusual odor of *Pseudomonas perolens* (97). This odor is pungent and is often noted as soon as the incubator door is opened.

S. odorifera may really be two different species. By DNA hybridization, strains of *S. odorifera* fell into two groups. Biogroup 1 strains were 71 to 98% related to each other when hybridization was done at 60°C, and they were 85 to 115% related at 75°C. In contrast, strains of biogroup 2 were only 60 to 69% related to biogroup 1 at 60°C and 58 to 64% related at 75°C. On the basis of this difference and differences in biochemical reactions, Grimont et al. defined two biogroups of *S. odorifera*. However, the two biogroups could also be considered as two distinct species.

***Serratia odorifera* biogroup 1 (ornithine positive, sucrose positive, raffinose positive).** Strains of biogroup 1 are positive for ornithine decarboxylase and ferment sucrose and raffinose. We have studied 21 strains of biogroup 1, and the respiratory tract was the most common source. Three strains were from feces, and two were from food, which indicates a possible reservoir. None of the strains was accompanied with comments that might suggest a possible association with disease. One isolate from feces was sent with the comment, "obtained in pure culture from stool after cold

TABLE 19. Biochemical reactions of strains defined to be *Serratia marcescens* biogroup 1

Strain	Day the reaction became positive for:								
	Methyl red[a]	Voges-Proskauer	Citrate utilization	Lysine decarboxylase	Ornithine decarboxylase	Motility	Gelatin hydrolysis	Growth in KCN	D-Glucose, gas
0812-73	2[a]	2	0	1	1	0	7	1	0
2495-73	2	2	0	1	0	0	7	1	0
4548-73	2	2	0	1	1	0	1	1	0
2197-74	2		0	0	1	0	1	0	0
2207-74	2	2	0	1	1	1	7	0	0
0531-75	2	2	7	1	0	0	7	1	0
1397-75	2	2	1	1	0	0	1	0	0
1580-75	2	2	1	1	1	0	1	0	0
1074-76	2	2	1	1	0	0	1	0	0
1270-76	2	2	1	0	1	0	0	1	0
1560-76	2	2	7	1	1	1	8	1	0
1674-76	2	0	0	1	0	8	7	1	0
2221-76	2	0	0	1	0	1	7	1	0
1695-77	2	2	0	0	1	0	0	1	0
1965-77	2	2	0	5	1	1	0	0	0
0018-78	2	2	0	1	0	0	8	1	0
1947-78	2	0	0	0	1	0	8	1	0
0862-79	2	0	1	1	1	0	1	1	0
3063-79	2	0	1	0	0	0	0	2	0
0489-80	2	0	0	6	1	0	0	2	0
0583-80	2	0	1	0	0	0	0	2	0
0896R80	2	0	1	1	1	1	3	1	0
0942-80	2	0	3	0	1	0	0	0	0
1073-80	2	0	6	0	1	0	0	2	0

[a] The number gives the day the reaction became positive, "8" indicates a positive in 8 to 21 days. The methyl red and Voges-Proskauer tests were done only at day 2.

enrichment for *Yersinia*." This is not surprising since *S. odorifera* grows well at 5°C (67). There is doubt whether *S. odorifera* biogroup 1 can cause human infections; however, it occasionally occurs in clinical specimens.

***Serratia odorifera* biogroup 2 (ornithine negative, sucrose negative, raffinose negative).** Strains of biogroup 2 are negative for ornithine decarboxylase and do not ferment sucrose or raffinose. We have studied 31 strains of this biogroup. One culture was from spinal fluid, and five were from blood cultures (one from a fatal case). No additional information was available on these isolates from blood or spinal fluid, but these sources are more suggestive of a pathogenic role for biogroup 2 than for biogroup 1. Interestingly, one culture was originally thought to have been from spinal fluid, but the submitting laboratory found that the organism had contaminated the agar plate used in culturing the spinal fluid. Clearly more information is needed about the role of *S. odorifera* biogroups 1 and 2 as a cause of bacteremia or human infections.

***Serratia plymuthica*.** *S. plymuthica* is an old species originally described in 1896 by Lehmann and Newmann as *Bacterium plymuthicum* and placed in the genus *Serratia* is the 6th edition of *Bergey's Manual of Determinative Bacteriology*. *S. plymuthica* did not gain general acceptance as a *Serratia* species until Grimont et al. (67) showed that it was a distinct species of *Serratia*, recognizable by DNA hybridization and biochemical tests (Table 18). Most of our strains of *S. plymuthica* have come from culture collections or from others studying *Serratia* taxonomy. Our five clinical isolates were all from the respiratory tract. No additional information was included about these isolates. Grimont and Grimont found no isolates of *S. plymuthica* among 1,107 *Serratia* isolates at Pellegrin Hospital, Bordeaux, France, from 1968 to 1975 (65). The available data indicate that *S. plymuthica* is extremely rare in clinical specimens and is a doubtful cause of human infections. Future case reports are needed to clarify this point.

***Serratia rubidaea*.** *S. rubidaea* was originally described in 1940 by Stapp (125) as *Bacterium rubidaeum*, but the name remained obscure until Ewing and colleagues reclassified it in the genus *Serratia* as *S. rubidaea* (38). Independently, Grimont and Grimont (65) studied similar strains and compared them to *Serratia marinorubra*, which had been isolated from seawater by Zobell and Upham in 1944. For several years, *S. rubidaea* and *S. marinorubra* were used in the literature as names for the same organism. The controversy was settled when the *Approved Lists of Bacterial Names* listed both species (124). The older name *S. rubidaea* has priority because this synonym is 4 years older. Thus reports in the literature referring to *S. marinorubra* should be considered to refer to *S. rubridaea* in the currently accepted nomenclature.

Since 1972 we have studied 20 strains of *S. rubidaea*. Two were from blood (one at autopsy), but most were from the respiratory tract, wounds, or feces (Table 3). The role of *S. rubidaea* in human disease requires more study.

The genus *Shigella*. *Shigella* is an old genus that is well known to clinical microbiologists as the cause of bacillary dysentery. In Table 1, *Shigella* is listed with *Escherichia* because of their close relatedness. *Shigella dysenteriae* (*Shigella* serological group A), *S. flexneri* (group B), and *S. boydii* (group C) are all very similar biochemically and must be differentiated with serological methods. They are combined as "*Shigella*—serogroups A, B, C" in Table 1 because of this close biochemical similarity. *Shigella sonnei* is biochemically distinct and is unique among *Shigella* in that it is ornithine positive. *Shigella* is classified as a separate genus, but the DNA hybridization studies of Brenner (16) showed that strains of *Escherichia coli* and all four of the *Shigella* species are very closely related and could be considered as

the same species in an evolutionary sense. Because most strains of *Shigella* can cause bacillary dysentery and most strains of *E. coli* cannot, the separation of *Shigella* and *E. coli* has been maintained as a matter of convenience in spite of the genetic data.

The genus *Tatumella*. The name *Tatumella* was proposed by Hollis et al. (78) for the group of organisms that had previously been known as "group EF-9" by the Special Bacteriology Section at the CDC. This group had been known for many years, but its taxonomic position was only recently investigated. Twenty-seven strains originally thought to be group EF-9 were studied by DNA hybridization, biochemical reactions, and antibiotic susceptibility (78). Twenty-five of these strains were related by 85% or more by DNA hybridization to the type strain ATCC 33301, but one strain was not related. By DNA hybridization, other taxa in the family *Enterobacteriaceae* were related by 7 to 38%, including 25 to 30% relatedness of *Escherichia*, the type genus of the family. On the basis of these data, group EF-9 was proposed as a new genus, *Tatumella*, with *T. ptyseos* as the only species (78).

Tatumella shares many of the properties of *Enterobacteriaceae* (Table 1), but is unusual in several ways. Stock cultures may die within a few weeks on agar or in semisolid stock culture media. This is unlike most *Enterobacteriaceae*, which can be kept almost indefinitely in sealed tubes at room temperature. However, *Tatumella* cultures frozen in 5% rabbit blood and stored at −40°C remained viable after storage for up to 14 years. This latter method (or perhaps freeze-drying) should be used for long-term preservation. In contrast to most other *Enterobacteriaceae*, *Tatumella* has large zones of inhibition around 10-U penicillin G disks (range of 15 to 36 mm, mean of 24, standard deviation of 4.6 mm; method of Bauer et al. [8]). The flagellation of *Tatumella* was also unusual. Strains were nonmotile at 36°C, but 66% were motile at 25°C. No flagella were seen on most strains, but those flagella visible were polar, subpolar, or lateral (92), rather than peritrichous. Biochemically, *T. ptyseos* was more active at 25°C than at 36°C. The biochemical reactions are summarized in Table 1. All the strains of *T. ptyseos* were isolated from human clinical specimens, and 86% were from the respiratory tract. Three cultures were from blood, so it appears that *T. ptyseos* may be a rare cause of human infections.

The genus *Yersinia*. *Yersinia* is a recent addition to the family *Enterobacteriaceae* (11). Previously, the names *Pasteurella pestis* and *Pasteurella pseudotuberculosis* were used, but with their transfer to *Yersinia* they became *Yersinia pestis* and *Yersinia pseudotuberculosis*, respectively. Within the last 10 years *Yersinia enterocolitica* has become accepted as the third species in the genus. These three species are well known to clinical microbiologists and have been the subject of many reviews and several books (13, 14).

TABLE 20. Differentition of the four species formerly included in *Yersinia enterocolitica*

Fermentation test[a]	*Y. entero-colitica*	*Y. kristen-senii*	*Y. inter-media*	*Y. freder-iksenii*
Sucrose	+	−	+	+
L-Rhamnose	−	−	+	+
Raffinose	−	−	+	−
Melibiose	−	−	+	−

[a] These characteristic fermentation patterns occur rapidly at 25°C, but are sometimes delays 3 to 7 days at 36°C.

Most strains of *Yersinia* are more active biochemically at 25 to 30°C than at 35 to 37°C. The motile species are almost always motile only at the lower temperature. These facts can pose problems for routine identification since most incubators are set at 35 to 37°C.

In the late 1970s the DNA hybridization studies of Brenner and colleagues (10, 12, 18, 25, 134) showed that strains which were being called *Y. enterocolitica* were heterogeneous and could be divided into four major subgroups. These subgroups could also be differentiated on the basis of simple phenotypic tests (Table 20). In 1980 three new *Yersinia* species, *Y. frederiksenii* (134), *Y. intermedia* (18), and *Y. kristensenii* (12), were formally proposed. One species that had been classified in *Yersinia*, *Y. philomiragia*, was shown to distinct from *Yersinia*, and its removal from the genus was proposed by Ursing et al. (135).

***Yersinia frederiksenii*.** *Y. frederiksenii* was once classified as an "L-rhamnose-positive biogroup of *Y. enterocolitica*," but is now classified as a separate species (134). There are three subgroups among the rhamnose-positive strains, but they are all classified as *Y. frederiksenii*. The largest published series (201 strains) of *Y. frederiksenii* has been from the International *Yersinia* Center at the Pasteur Institute. The most common sources were water and sewage (53%), and fish made up an additional 7%. *Y. frederiksenii* was only occasionally found in human clinical specimens (23%), but almost all of these were stool isolates of doubtful clinical significance. They were obtained by cold enrichment and were rarely associated with a gastrointestinal disease typical of *Y. enterocolitica*. Two percent of the human isolates were from blood or sputum. Other sources of *Y. frederiksenii* included cattle and pigs (10%), wild rodents (2%), food (milk or sandwiches; 4%), and soil (1%).

Our series of *Y. frederiksenii* includes one wound and four respiratory tract isolates (Table 3) as well as isolates from more typical sources. *Y. frederiksenii* is widely distributed in the environment and occasionally occurs in clinical specimens. There is no evidence that it can cause diarrhea.

***Yersinia intermedia*.** *Y. intermedia* was formerly included in *Y. enterocolitica* as "atyical strains which fermented L-rhamnose, raffinose, and melibiose." *Y. intermedia* was proposed as a separate species in 1980, based on DNA hybridization and phenotypic differences from other species of *Yersinia* (18). The International *Yersinia* Center has described a large series of *Y. intermedia* strains (18). There were 321 strains from 15 different countries in many parts of the world. Fresh water and sewage isolates comprised 74% of the isolates, and aquatic animals such as fish, oysters, shrimp, or snails comprised an additional 8%. Human strains represented only 13% of the total. Eleven of the human strains were from feces, and most had been isolated by cold enrichment. These fecal isolates were probably not clinically significant. There were 14 blood isolates from children at the same hospital who had been admitted for various reasons. Their symptoms could not be related to isolation of *Y. intermedia*, so this may be another example of an outbreak of pseudobacteremia, in which the organism was not actually present in the patients' blood. Twelve of the 16 other strains from extraintestinal sources had been previously described by Bottone (13). Other nonhuman sources include wild rodents (4%) and food (milk, cream, or meat; 2%). It was postulated that the presence of *Y. intermedia* in food may be due to cold enrichment (by refrigeration) of strains that have been introduced from water.

Our series of *Y. intermedia* includes three isolates from stool, three isolates from wounds, and one isolate from

TABLE 21. Differentiation of the four sucrose-negative groups of *Yersinia*[a]

Test	*Y. enterocolitica* biogroup 5[b] I	Yersinia biogroup: *Y. kristensenii*	X1	X2
Sucrose fermentation	−[c]	−	−	−
Trehalose fermentation	−	+	+	+
Ornithine decarboxylase	v	+	−	+
L-Rhamnose fermentation	−	−	−	+
$NO_3^- \rightarrow NO_2^-$	−	+	+	+

[a] Adapted from Bercovier et al (10).
[b] Biogroup 5 includes sucrose positive and sucrose negative strains. These data are for the sucrose negative strains, which could be confused with *Y. kristensenii*.
[c] See footnote *a* of Table 4.

urine. The majority of the others were environmental or from culture collections. *Y. intermedia* appears to be an infrequent human pathogen (13), but is more likely to be isolated from the environment. When it occurs in feces, it is probably not a cuase of gastrointestinal illness.

Yersinia kristensenii. The new species *Y. kristensenii* was formerly classified as a sucrose-negative, trehalose-positive biogroup of *Y. enterocolitica*. However the DNA hybridization studies of Brenner et al. showed that it was distinct from *Y. enterocolitica*, so it was classified as a separate species (12).

The largest published series of *Y. kristensenii* has been from the International *Yersinia* Center (12). Of the 115 strains studied, 61% were from animals, 11% were from water, 6% were from soil, 4% were from vegetables, and 18% were from humans. The isolates were from six European countries, Japan, Australia, and the United States. Only two of the human isolates were stool isolates; the remainder were extraintestinal. *Y. kristensenii* also grows at 4°C, so it can be selected by cold enrichment. Our series includes six human isolates, four from stool, one from blood, and one from urine. The remainder were from animals, water, or soil. Bercovier et al. (12) showed that *Y. kristensenii* was commonly found in soil (70% of samples positive), but it is difficult to determine whether soil is the source of the animal isolates or whether animals are the source of the soil isolates. There is no evidence that *Y. kristensenii* can cause diarrhea, but it can occur in other clinical specimens, where it may have a pathogenic role.

Yersinia ruckerii. In 1966 Ross and co-workers (116) described a bacterium isolated many times from kidneys of rainbow trout (*Saliva gairdneri*) suffering from "red mouth" disease. This organism, known for many years as "the red mouth bacterium," was thought to belong in the family *Enterobacteriaceae*. Ewing et al. (41) showed that cultures of the "red mouth bacterium" were highly related to each other by DNA hybridization, but not closely related to other named species in the family. Although the "red mouth bacterium" was no more closely related to *Yersinia* than to other genera, the organism was named *Y. ruckerii* (41) because of its phenotypic similarity to *Yersinia* and G+C content, which was close to that of this genus. Both "*Serratia*" *fonticola* and "*Yersinia*" *ruckerii* are species searching for a better genus as a final home.

Y. ruckerii is well documented as a fish pathogen (116) that has been isolated from rainbow trout, steelhead trout, sockeye salmon, and Chinook salmon. Isolates have come from hatcheries in Alabama, Arizona, California, Idaho, Ohio, Tennessee, and Washington. One isolate identified as *Y. ruckerii* was from a human clinical specimen. This isolate, culture 0724-77, was from the bile of a Connecticut patient. No information was available about the clinical significance of this isolate. *Y. ruckerii* appears to be extremely rare in human clinical specimens, but since it usually grows poorly on plating media incubated at 36°C, isolates could possibly have been missed. Further case reports are needed.

***Yersinia* biogroups X1 and X2.** Biogroups X1 and X2 of *Yersinia* are mentioned by Bercovier et al. (10) in their study of *Y. enterocolitica* and similar strains. We have not studied any strains that resemble either of these *Yersinia* biogroups. Little information was given about them, and the classification was not given. Table 21 indicates that they are both sucrose negative and can be differentiated from the other sucrose-negative *Yersinia* groups. Eventually one or both of these may be named as a new species of *Yersinia*.

The genus *Xenorhabdus*. *Xenorhabdus* is a new genus proposed in 1979 by Thomas and Poinar (132). The two species of *Xenorhabdus*, *X. nematophilus* and *X. luminescens*, do not grow at 36°C. Both species are pathogenic for nematodes (98, 108, 113), but should be of little concern for the clinical microbiologist, since they have never been isolated from a clinical specimen (109–112).

Description of new Enteric Groups. In this paper we describe nine new Enteric Groups—Enteric Groups 17, 45, 57, 58, 59, 60, 61, 68, and 69. These are defined as groups containing biochemically similar strains whose classification needs further study. The term Enteric Group is merely a convenient name to use for these organisms until they can be given a scientific name. For example, *Kluyvera* was originally reported as Enteric Group 8. In the paragraphs that follow, information about each group is summarized. The biochemical reactions of the Enteric Groups are listed in Table 1.

Enteric Group 17. Enteric Group 17 was defined in 1978, and previously the strains had been reported as unidentified or as atypical strains of *Citrobacter* or *Enterobacter*. We have received 41 strains of Enteric Group 17; 38 of these were from humans, 1 was from water, and 2 had no information as to source included. Among the human clinical isolates, 3 were from blood, 11 were from urine, 4 were from wounds, 8 were from the respiratory tract, 4 were from stools, 2 were from skin, and 1 each was from lochia, gall bladder, penis, and Penrose drainage tube.

Enteric Group 41. See *Escherichia adecarboxylate*, above.

Enteric Group 45. Enteric Group 45 was first recognized when cultures reported as "atypical *Hafnia alvei*" were studied by DNA hybridization. This group was not highly related to *Hafnia alvei*, was not lysed by the *Hafnia*-specific bacteriophage, and was biochemically different. Before the name Enteric Group 45 was assigned to these organisms, they were thought of as "*Hafnia* species 3," and this name was used (17), although it was clear that they did not belong in *Hafnia*, but were closer to *Salmonella*, *Citrobacter*, *Escherichia*, and *Enterobacter*. The nine strains of Enteric Group 45 came from human clinical specimens. The sources were as follows: arm wound (two isolates), foot wound (two isolates), throat or sputum (two isolates), stool (one isolate), knee fluid (one isolate), and unknown (one isolate).

Enteric Group 57. Enteric Group 57 was first recognized in 1981. There was a group of nine similar strains that were all H_2S positive and that had been reported as unidentified. These cultures became Enteric Group 57. Strains of Enteric Group 57 have come from feces, except one which was from

urine. One of the cultures had been sent as *Salmonella* for confirmation. We obtained weak agglutination in antisera for *Salmonella* group B and agglutination in O-groups C2 and O27. However the culture was not lysed by the *Salmonella*-specific O1 bacteriophage. The exact taxonomic position of Enteric Group 57 is now being studied.

Enteric Group 58. Enteric Group 58 is a small group of strains that were first recognized in 1981. Four of the five isolates were from wounds (hip, leg, ankle, and foot), and the other isolate was from feces. The following comments accompanied two of the cultures: "32-year-old male with a hip wound infection"; "54-year-old male with an open fracture of the big toe."

Enteric Group 59. Enteric Group 59 was first recognized in 1981. Strains in this group are arginine dihydrolase positive within 4 days; otherwise they would probably have been identified as *Enterobacter agglomerans*. Thus one way of remembering Enteric group 59 is by thinking of it as an arginine-positive *E. agglomerans* group. We have studied eight isolates of Enteric group 59. Six were from sputum, one was from a foot wound, and one was from food (ham). No comments have accompanied any of these cultures.

Enteric Group 60. Enteric Group 60 was first recognized in 1981. The strain matcher program (see above) included three strains that were almost identical, and all had been reported as unidentified. Strains are inactive biochemically, and there is no hint of the correct taxonomic position of Enteric Group 60. The group was first thought to be inactive strains of *Morganella*, but they are sensitive to colistin and tyrosine negative, which tend to rule out *Morganella*. Three of the strains were from urine, and one was from sputum.

Enteric Group 63. See the discussion of *Buttiauxella*, above.

Enteric Group 64. See the discussion of *Buttiauxella*, above.

Enteric Group 68. Enteric Group 68 was first recognized in 1981. The group is positive for DNase, but is otherwise quite different from *Serratia* (Table 1). Both strains in the group were from urine.

Enteric Group 69. Enteric Group 69 was defined in 1981 for a group of four strains sent for confirmation as *Enterobacter sakazakii*. The strains had been isolated by Anders Ternstrom of the Swedish Meat Research Institute. About 50 similar strains had been isolated from the same slaughterhouse, and all were from beef muscle. The strains are phenotypically similar to *E. sakazakii* in most of their reactions and in yellow pigment production (Table 1); however, they ferment D-sorbitol rapidly and are DNase negative at the normal cutoff period (36°C, 7 days). They are weakly DNase positive with extended incubation. All four strains are almost identical in their biochemical properties. By DNA hybridization, Enteric Group 69 was only 22 to 43% related to labeled strains of *Enterobacter cloacae* and *E. sakazakii*. The strains of Enteric Group 69 grow slowly at 5°C, which may explain their presence on refrigerated beef carcasses.

DISCUSSION

The identification and reporting of *Enterobacteriaceae* have been debated and discussed for many years. The following two main questions seem to always result: "How far should we go in identifying cultures?," and "How should we word reports?" Since the majority of clinical microbiology laboratories now use commercial identification systems, the answer to the first question has been settled, since the test system usually has a fixed set of differential tests. The tests are run, and the resulting profile is checked in a code book or with a computer system. This final result is usually the name of an organism. For example, in the API 20E system (Analytab Products, Plainview, N.Y.), the biochemical profile 0 105 121 is found in the "Quick Index" to be *Cedecea davisae*, which is one of the new species of *Enterobacteriaceae*. We suggest that the laboratory report reflect this identification and give the scientific name, both genus and species. Unlike a report of *Escherichia coli* or *Salmonella typhi*, a report of *Cedecea davisae* will probably have little meaning to a physician or allied health professional who might receive it. Thus, an explanation is desirable, if not essential, as a comment on the report. The following is one suggested approach. "Heavy growth of *Cedecea davisae*. This is a new species of *Enterobacteriaceae* which occasionally occurs in clinical specimens. It is of questionable clinical significance at most sites, but there are three reports in the literature which have suggested possible clinical significance. Repeated isolation and the patient's status are essential for correct interpretation."

This explanation could be written on the report form, or it could be given in a number of other ways.

Another example is *Enterobacter sakazakii*, which can be a life-threatening pathogen, even though it is rare. A possible report follows. "Heavy growth of *Enterobacter sakazakii*. This is a new species of *Enterobacteriaceae* which is a documented cause of sporadic cases and small outbreaks of neonatal meningitis."

These comments should alert the reader about its clinical relevance and possible danger of spread to other babies in the nursery. A report of "*Enterobacter* sp." would clearly lack this inpact.

In recent years commercial companies have obtained strains of the new *Enterobacteriaceae* and added them to their data bases. A list of all the organisms included is usually given in the instructions or in a code book. These lists are revised as more data become available. Users of the commercial systems should have little difficulty in identification or reporting. Since most clinical laboratories use commercial systems, the vast majority of strains will be reported based on these methods. The data in Table 1 and the differential tables will probably be of more value to reference laboratories which use conventional biochemical tests.

The identification of *Enterobacteriaceae* can be simplified by taking advantage of the fact that three species comprise 80 to 95% of all isolates in clinical settings. These species are *Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis*. The first stage in identification would be to recognize these three common organisms. All three species have characteristic colonial morphology on McConkey or blood agar (or both) and have characteristic antibiotic susceptibility patterns. These facts have led to the suggestion that typical strains of these three species can be reported with no additional or a minimum of biochemical testing. This approach is very accurate most of the time, especially when practiced by an experienced microbiologist.

A second stage of identification would consider the other species isolated frequently from clinical specimens. This stage includes the 23 species given in Table 2. Identification can be accomplished with commercial identification systems or conventional biochemical tests. Over 99% of clinical isolates normally fall into this category. A good example of this simplified approach is the genus *Serratia*. Most clinical isolates of *Serratia* are *S. marcescens*. The other nine

species (including subspecies) comprise only a small percentage of clinical isolates. *S. marcescens* does not ferment L-arabinose, but all the other species do. This allows a simple method for reporting strain with typical properties of *Serratia*: to report the L-arabinose-negative cultures as "*Serratia marcescens*" and to report the L-arabinose-positive cultures as "*Serratia* sp., not *S. marcescens*." This type of approach can be devised for all the other genera with appropriate spot tests.

The third stage of identification would be for those rare isolates that do not fit one of the common patterns. These isolates can be compared to the reaction in Table 1 and to the differential tables. Frequently these isolates will simply be typical strains of rare species and can be reported as such. Other strains will not fit any of the patterns, and can be reported as unidentified. If there is sufficient interest, or if the isolate appeared to cause a serious infection, the strain can be referred to a reference laboratory for further study. Many of the new species were defined on the basis of a group of cultures referred in this manner. Table 1 and the differential tables should be especially helpful in the identification of these rare or unusual strains. The tables do not imply that every clinical isolate must be identified this completely.

It is an inescapable fact that knowledge accumulates as the result of scientific inquiry. Each new species of *Enterobacteriaceae* would previously have been called an "unidentified, gram-negative, fermentative rod." The first step in learning more about an organism is to give it a name. In some cases this is a vernacular (common) name such as Enteric Group 9. As the organism is studied further, it can be given a scientific name. Enteric Group 9 was later named *Enterobacter sakazakii*. This usually leads to further study and a better understanding of the organism (100). The naming and study of microorganisms is a continuing process that was begun over 100 years ago when the first bacterial causes of disease were discovered. All the species of *Enterobacteriaceae* are a culmination of this process. The purpose of our paper is to make clinical microbiologists aware of the new organisms. Isolates of these new species may never occur in a given laboratory, but if they do occur, we hope that this review will prove useful for their identification and for learning more about them.

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ADDENDUM IN PROOF

After this review was submitted, several new *Enterobacteriaceae* species were named and described. A report by Bercovier et al. (Int. J. Syst. Bacteriol. **34**:166–172, 1984) proposed the name *Yersinia aldovae* for the sucrose-negative *Yersinia* species that we referred to as *Yersinia* biogroup X2. *Y. aldovae* has been isolated from water and fish, but not from clinical specimens. A report by Aldová et al. (Zentralbl. Bakteriol. Parasitenkd. Infectionskr. Hyg. Abt. 1 Orig. Reihe A **254**:95–108, 1983) described *Budvicia* and *Budvicia aquatica*, a new species which produces hydrogen sulfide. All of the strains of *B. aquatica* described by Aldova et al. were isolated from water, but we have studied similar strains that had been isolated from human stool samples. There is no evidence at present that *B. aquatica* can cause diarrhea or extraintestinal infections. A report by Kasako et al. (Jpn. J. Med. Sci. Biol. **37**:117–124, 1984) described *Yokenella* and *Yokenella regensburgei*, new *Enterobacteriaceae* strains isolated from insects and human clinical specimens. We have not yet studied strains of *Y. regensburgei*, but the published biochemical reactions of this new species are very similar to those of Enteric Group 45 (Table 1), which we named as *Koserella trabulsii* in a separate paper in this issue (Hickman-Brenner et al., J. Clin. Microbiol. **21**:39–42). Strains of these two new genera need to be compared.

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