Enterobacteriaceae: Introduction and Identification In the fifth edition of this Manual in 1991, Farmer and Kelly commented that it was becoming more difficult to cover the family Enterobacteriaceae in a single chapter. The family includes many important organisms (see Tables 1 to 7) such as the plague bacillus Yersinia pestis, the typhoid bacillus Salmonella enterica serotype Typhi (Salmonella typhi), four genera with species that often cause diarrhea and other intestinal infections, seven species that frequently cause nosocomial infections, many other organisms that occasionally cause human or animal infections, dozens of species that occasionally occur in human clinical specimens, and many other species that do not occur in human clinical specimens but can be contused with those that do. In the sixth edition, the material on Enterobacteriaceae was divided among three chapters: an introduction to the family that described the overall plan for isolation and identification; a chapter that covered Salmonella, Shigella, Escherichia coli, and Yersinia, the enteric pathogens; and a chapter that covered the remaining genera and species in the family. In rhc seventh	Enterobacteriaceae: Giới thiệu và định danh Trong lần tái bản thứ năm của Quyển sổ tay này xuất bản vào năm 1991, Farmer và Kelly thấy rằng việc gộp các nội dung liên quan đến vi khuẩn đường ruột vào một chương duy nhất có vẻ khó khăn. Họ bao gồm rất nhiều sinh vật quan trọng (xem Bảng 1-7) chẳng hạn như trực khuẩn bệnh dịch hạch Yersinia pestis, các trực khuẩn thương hàn Salmonella enterica serotype S. typhi (Salmonella typhi), bốn chi với các loài thường gây tiêu chảy và nhiễm trùng đường ruột khác, bảy loài thường xuyên gây nhiễm trùng bệnh viện, rất nhiều sinh vật khác đôi khi gây bệnh nhiễm trùng cho người hoặc động vật, hàng chục loài thỉnh thoảng xuất hiện trong các mẫu lâm sàng người, và nhiều loài khác không xuất hiện trong các mẫu lâm sàng người nhưng có thể bị nhầm lẫn.Trong lần tái bản thứ sáu, các nội dung liên quan đến vi khuẩn đường ruột được chia thành ba chương: giới thiệu về họ mô tả toàn bộ kế hoạch phân lập và định danh; một chương đề cập đến Salmonella, Shigella, Escherichia coli, và Yersinia, các tác nhân gây bệnh đường ruột; và một chương đề cập đến các chi còn lại và các loài trong họ. Trong lần tái bản thứ 7 của RHC, một chương thứ tư được	

edition, a fourth chapter was added that covered Klebsiella. Entenbacter, Citmbacter, and Scrrutid. In the eighth edition, there were also four chapters. However, Yersinia was assigned to its own chapter, and Klebsiella, Enterobacter, Citrobacter, Serratia and Plesumunuu (see this chapter) and the remaining Enterobacteriaceae were grouped together. This organization has been maintained for the ninth edition.	bổ sung vào đề cập đến Klebsiella.Entenbacter, Citmbacter, và Scrrutid. Trong lần tái bản thứ tám, cũng có bốn chương. Tuy nhiên, Yersinia được tách riêng ra một chương, và Klebsiella, Enterobacter, Citrobacter, Serratia và Plesumunuu (xem chương này) và các vi khuẩn đường ruột còn lại được gộp vào chung với nhau. Cách sắp xếp này vẫn duy trì trong lần tái bản thứ chín.	
Because of space limitations, many topics in the present chapter arc discussed briefly and only a few primary literature citations are given. Several books, reviews, and chapters are recommended for more detailed information (5, 12-14. 16. 24. 32, 37. 38. 43. 45. 49. 55. 63. 68. 69, 82, 90. 92).	Do hạn chế không gian, nhiều chủ đề trong Chương này được trình bày ngắn gọn và chúng tôi chỉ đưa ra một vài trích dẫn quan trọng. Để tạo điều kiện cho độc giả tìm hiểu sâu hơn vấn đề, chúng tôi giới thiệu một số tài liệu tham khảo như sách, các bài báo tổng quan, và các chương trong sách khác (5, 12-14. 16. 24. 32, 37. 38. 43, 45. 49. 55. 63. 68. 69, 82, 90. 92).	
NOMENCLATURE AND CLASSIFICATION	Thuật ngữ và Phân loại	
The nomenclature and classification of the genera, species, subspecies, biogroups, and serotypes of Enterobacteriaceae have always been topics for heated debate and differing opinions (12-14. 24. 31-34, 37, 38, 55, 63). Until recently, genera and species were defined by biochemical and antigenic analysis. Today, newer	[redacted]	

techniques such as nucleic acid hybridization and nucleic acid sequencing, which measure evolutionary distance (see chapters 16 and 19 in this Manual), have made it possible to determine the evolutionary relationships among organisms in the family (12-16, 21, 37. 38. 55). The use of these molecular techniques has led to the discovery of many new species and has resulted in the proposed reclassification of others (12-14. 37). This chapter includes the different names and classifications that clinical microbiologists are likely to encounter in the scientific literature and in material accompanying commercial products. I he nomenclature and classification given in Tables I to 5 are a compromise based on all available evidence. They include most of the genera, species, subspecies, biogroups, and unnamed Enteric Groups included in the family. If two names are widely used for the same organism, both are mentioned in this chapter with one in parentheses. Many of the “nonclinical" organisms in the family are also included, because there is a possibility that they will be isolated from a human clinical specimen in the future (12-14, 16, 37, 55).	[redacted] [redacted]	

Most of the newly described organisms arc very rarely found in clinical specimens (26. 32. 37). This is illustrated by the published listings of organisms that most often cause bacteremia, nosocomial infections, and infections of the gastrointestinal tract (see Tables 6 and 7). The National Library- of Medicine's Internet taxonomy database has a useful list of organisms in the family Enterobacteriaceae and its relatives (http://www.ncbi.nlm.nih.gov Taxonomy/Browser/ wwwtax.cgi.Hds5 543). This list may also be accessed through http: /, www.nch.nlm.nih.gm-entrer/query.fcgiMb=Taxonomy by selecting "Taxonomy" in the “Search" field and typing “Enterobacteriaceae" in the adjacent “for" field. The Internet site of J. P Euzeby (http://www.Kicterio.cict.fr) gives nomenclature, classifications, original literature citations, and other information for all of the genera and species in the family Enterobacteriaceae and its relatives. \ Unfortunately, the Euzeby site has no alphabetical list of the genera included in the family. New Species That Occur in Human Clinical Specimens Names of several new organisms	[redacted]	

and several "proposed alternative classifications" have been published since the previous edition of this Manual (Table 2), and several of the new organisms occur in human clinical specimens. It is becoming more and more difficult to update the biochemical reaction table (Table 3) of clinically important Enterobacteriaceae. For example, Klebsiella granudomatis (Cahrnmatnbacterium granuLirruiis) does not grow on most bacteriological media and lacks a type strain that can be grown and described based on	[redacted]	
TABLE I Genera and species of Enterobacteriaceae that cause, or are associated with, specific or unusual human disease, syndromes, or conditions Disease, syndrome, or condition Hemorrhagic colitis Histamine poisoning (scombrold poisoning) Intestinal infection preceded by Entamoeba Histolytica infection Meningitis and sepsis in neonates caused by ingesting contaminated infant formula Citrobacter diversus, Enterobnacter sakazakii Klebsiella granudomatis (Calymmatobactemim granudomatis)	[redacted]	

Escherichia coli O l57:H7 Proteus morganii, Klebsiella=Raoultella, others Edwardsiella tarda Enterobacter sakazakii	[redacted]	
Severe, often fatal disease in neonates, survives have severe mental impairment; cause outbreaks in hospital nurseries.	[redacted]	
One the most important human diarrheal diseases ("invasive” strain of Escherichia cok cause a similar but often milder disease)	[redacted]	
Chronic genital ulcerative disease; organism difficult to demonstrate microbiologically because it does not grow on laboratory media(see text).	[redacted]	
Other Shiga toxin-producing strains can also causes a similar, but often milder disease.	[redacted]	
Caused by bacteria that produce large amounts of histamine and histamine-like substances (scombrotoxin) when they multiply and spoil fish tissues (via bacterial histidine decarboxylase)	[redacted]	
Several interesting studies surest that the protozoan infection must precede Edwardsiella tarda in order for it to cause infection.	[redacted]	
Nursery outbreaks in which infants acquire the strain from dried infant formula that is	[redacted]	

contaminated with the bacterium; other coliform organisms have also been isolated from formula samples. Neutropenic patients—Initial fever and fever after empirical antibiotic treatment Ozena Paratyphoid fever Plague (pneumonic and bubonic plague) Pneumonia associated with alcoholism—"Friedlander's pneumonia" Reiters syndrome (reactive arthritis) Salmonellosis Shigellosis Tropical sprue/enteropathy Typhoid fever Escherichia coli, Klebsilla, Enterobacter Klebsilla ozacnae Salmonella serotypes Paratyphi A. B, C, and others Yersinia pestis Klebsiella pneumoniae Yersinia enterocolitica Klebsiella rhinoseletomatis Salmonella, Shigella, enteropathogenic strains of Yersinia enterocolitica Salmonella—any of the named or numbered serotypes Shigella—any of the named and provisional serotypes Klebsiella, Enterobacter, Hafnia, others	[redacted] [redacted] [redacted]	

Salmonella serotype Typhi	[redacted]	
Chronic atrophic rhinitis (ozena), foul smelling discharge from the nose: causative role is uncertain; it may be just colonization.	[redacted]	
Causation: paratyphoid fever is an enteric fever that is similar to typhoid fevers.	[redacted]	
	[redacted]	
One of the most important human diseases— the "Black Death” of the Middle Age	[redacted]	
Capsular types KI-K6 are most frequently isolated.	[redacted]	
The appendix is normal after surgical removal	[redacted]	
Chronic granulomatous infection of the nasal passages and respiratory tract; usually seen in the tropics	[redacted]	
Sometimes occurs after gastrointestinal infection; more common in patients with the HLA-B27 histocompatibility antigen	[redacted]	
One of the most important human diarrheal diseases	[redacted]	
One of the most important human diarrheal diseases	[redacted]	
“Syndrome of enigmatic origin" characterized by prolonged diarrhea and malabsorption by certain residents of the tropics (68); strong presumption for causation (68)	[redacted]	
One of the most important human diseases	[redacted]	
TABLE 2 Newly proposed genera, species, and subspecies of Enterobacteriaceae, including	[redacted]	

several “proposed alternative classifications" for previously described organisms Organism Occurrence in human clinical specimens Proposed as: commentsReference Averyyella dalhousiensis (formerly classified as Enteric Group 58) Yes New genus and new species that colonize or infect traumatic injuries; septicemia in a patient receiving total parenteral nutrition (TPN) through a subcutaneous port Citrobacter Group 139 Yes Proposed alternative classification for Enteric Group 139. which caused a small hospital outbreak 94 Enterobacter cloacae subsp.dissolvents Yes Proposed alternative classification for Enterobacter dissolvents; isolated from plants, blood, skin abscess, abdomen 51 Enterobacter biduigiil Yes New species. 16 clinical isolates from blood, urine, etc.; formerly included in Enterobacter cloacae; now part of this species complex 52 Enterobacter radicincitans No New species isolated from phyllosphere of winter wheat; fixes nitrogen,	[redacted] [redacted] [redacted] [redacted]	

promotes plant growth 59 Escherichia albertii Yes New spccics; originally misidentified as "Shiga toxin-producing Hafnia alvei" 53 Klebsiella singaporensis No New species represented by a single isolate from soil 67 Klebsiella variicolaYes New species that is phenotypically almost identical to Klebsiella pneumoniae; isolated from plants but also from human blood 81 Kluyvera intermedia No Proposed alternative classification for Enterobacteria intermedius 74 Photorhabdus asymbiotica subsp australis Yes New subspecies isolated from blood and wounds of patients in Australia 2 Phntorhabdus luminescent subsp. kayaii and subsp. thracensis ~~No Two new subspecies isolated from nematodes in Turke~~y 48 Salmonella enterica Yes An old species, but newly made legitimate (see text) 58 Samsonia erythrinae No New species isolated from diseased erythrina trees (Erythrina sp.) 89 Serratia marcescem subsp. sakuensis No New subspecies; reported to produce	[redacted] [redacted] [redacted] [redacted]	

endospoces; isolated from wastewater 1 Serratia quinivorans No Proposed alternative classification of Serratia proceamacudams subsp. quinivora; isolated from plants and insects 3	[redacted]	
Yersinia aleksiciae Yes (feces only) New species represented by only five strains, formerly included in Yersinia kristensenii serogroup 0:16; isolated from human feces, pork products, and rats/ moles 87 Yersinia entemcolitica subspecies palearctica Yes New subspecies that was proposed to include one of the three evolutionary groups in the species 72	[redacted]	
Xenorhabdus budapestensis, X. ehlersii, X.inexii. X szentirmali No Four new species that are symbiotic in nematodes of the genus Steinemema (family Sletnemematidae) and that are insect pathogens 65	[redacted]	
This table includes organisms that were not included in this chapter in the 8th edition of the Manual. This chapter should be cited as the reference for Citrobacter Group 139 as a promoted alternative classification for	[redacted]	

Enteric Group 139).

simple phenotypic methods. Another problem has been the unavailability of certain strains (62). Other new organisms are almost identical to older organisms in their phenotypic properties. For example, Klebsiella variicola will be very difficult to differentiate from K. pneumoniae and other Klebsiella species. All the newly described species of Enterobacteriaceae need to be characterized and added to Table 3.

Organisms That Do Not Occur in Human Clinical Specimens

New or unusual Enterobacteriaceae that do not occur in human clinical specimens are listed in Tables 1 to 5, and more information and literature citations can be found at the Internet sites previously cited and in the new edition of Bergey's Manual of Systematic Bacteriology (16). These new organisms should be characterized and added to Table 3.

The Expanding Number of Enterobactenaceac Species

How many species of Enterobacteriaceae are there? There are probably many hundreds, if not thousands. This is becoming more apparent as methods such as DNA-DNA hybridization and 16S rRNA

[redacted]

[redacted]

sequencing (14, 16) are being used to study strains isolated from human clinical specimens, plants, animals. …………………………………… …………………………………… ……	[redacted]	
TABLE 4 New, unusual, fastidious, or unculturable genera and species that have been classified" (14) in the family Enterobacteriaceae Human pathogen KLebsiella gramdomatic (Calymmatobacterium granudomatis)—causes donovanosis (granuloma inguinale) (see text) Associated with plants. But some species may occasionally cause or be associated with human clinical infections (see text) Pantoea, 7 species. 2 subspecies Pathogenic for or associated with plants; not isolated from human clinical specimens	[redacted] phân Gây bệnh cho thực vật [redacted]	
Brenneria, 6 species; causes a variety of diseases of deciduous trees and walnut tree. Dickeya, 6 species (4 new species plus 2 additional species from other genera)(83) ………………………. ………………………. …………. Two species are found only in nematode and insects that are infected by the	[redacted] lá rộng [redacted]	

nematode. One species, P.asymbiotica, causes bacteremia and wound infections in humans (see text). .... Xenorhabdus—several species; found only in nematodes and the insects that the nematodes infect; insect pathogens Associated with extreme environments, not isolated from human clinical specimens ……—habitat is coastal hot springs and the environment. One example is the study by Muller et al. (71), who found six new species of Buttiauxella and two new species of Kuyvera in a large collection of strains isolated from snails. Similarly, additional DNA-DNA hybridization subgroups, which are probably new species (sometimes called genomospecies), have been found in systematic studies of Enterobacter cloacae (12), Proteus vulgaris (73), Rahnella aqiuttilis (17), Klebsiella (67, 81), Enterobacter (48. 52.54.59), Yersinia (7, 8), atul Cttmboctcr (15. 18. 94). Most of the Enterobacteriaceae that clinical microbiologists encounter every day belong to just a few of the many species described (32). However, the expanding number of Enterobacteriaceae species is	[redacted]	

becoming a serious problem for reference laboratories and for commercial identification systems, whose identification methods are becoming inadequate for complete and accurate identification. When a commercial identification system gives an unusual organism for a final identification, there are several possibilities to consider (56): the identification is correct, just unusual; the identification is incorrect because another aerobic or anaerobic organism Ls present (42) and the biochemical profile is the result of the metabolic activities of the mixture; or a handling or coding error was made somewhere along the way. Before a final report of an unusual organism is issued, it is advisable to do as much checking as possible. This checking could include repeating the biochemical tests with the same commercial system after confirming the absence of a contaminating aerobic or anaerobic organism (42), testing the isolate with another commercial identification system (56) or with tube tests, and comparing the strain's antibiogiam with known patterns reported for this organism.	[redacted]	

II these steps do not resolve the problem, the state health department or a reference laboratory can be contacted for advice, and the culture will often be accepted for further study. Different commercial systems often give different identifications for the same strain. The "gold standard" for identification is DNA-DNA hybridization; however, it is unavailable except in a few research laboratories. A different standard is evolving that is based on 16S rRNA sequencing. Although less accurate, if is a readily available alternative, and unusual strains can he submitted to a commercial laboratory (Accugenix. Newark. Del. (http://www.accugenix.com] or Midi. Newark. Del. [http://www.midi-inc-coml) for a "fee-for-service" identification. Clinical isolates reported with results obtained with these commercial tests should be reported with a disclaimer to indicate their research ("non-Clinical Laboratory Improvement .Amendment (CL1A)") status. We suspect that a reference laboratory's identification based on phenotypic characteristics will be the final result for most difficult strains and will be done at state or national health	[redacted] đối chứng [redacted]	

departments or commercial reference laboratories. Changes in Classification: "Proposed Alternative Classifications" Contrary to popular opinion, there is no designated international body that considers every proposed change in classification and then issues an official classification. For almost 75 years, the Subcommittee on Enterobacteriaceae (http:// www.the-icsp.org/subcoms/Enterobactcrinccae.htm) of the International Committee on Prokaryotes (http://www.the-icsp.org/default.htm) has studied and discussed the nomenclature and classification of the family. When the Enterobacteriaceae Subcommittee studies a specific “proposed reclassification,” it can only make a recommendation, which can then be accepted or rejected by individuals in the scientific community. It should be emphasized that changes in classification are decided by usage, not by a judicial decision or action (see chapter 19 for further discussion). Sometimes two classifications are widely used, and both can be "correct." Classifications are correct if they	[redacted] đối chứng [redacted] Cần nhấn mạnh rằng những thay đổi trong phân loại được quyết định bằng thỏa thuận, chứ không bởi một hành động hay quyết định pháp lý nào (xem chương 19 để thảo luận thêm). Đôi khi cả hai cách phân loại đều được sử dụng rộng rãi, và cả hai đều có	

conform to all the	thể là "chính xác."	

TABLE 5 Enterobacteriaceae that are difficult to differentiate and identify completely; use of the term “complex” as a solution for reporting cultures	[redacted]
Vernacular name Organisms included, definition, and comment	[redacted]
Citrobacter complex In addition to C. freundii , this term includes C. braakii. C. gillenii. C. murliniae. C. rodentium, C sedlakii. C. werkmanii, and C. youngae, which are difficult to differentiate (15. 18).	[redacted]
Enterobacter agglomerans complex This term includes over 60 named organisms: over a dozen “Enterobacter agglomerans DNA-DNA hybridization groups," the species of Brenneria, Dickeya. Erwinia. Pectobacterium. Pantoea. and perhaps also Enterobacter cowanii, all of which are difficult or impossible to differentiate.	[redacted] [redacted]
Enterobacter cloacae complex E.i cloacae s made up of at least five DNA-DNA hybridization groups (12). The definition of the complex would include Enterobacter ludwigii plus these unnamed groups. For practical identification schemes, the term includes Enterobacter amnigenus and Enterobacter kobei ,which are difficult to differentiate.	[redacted]
Klebsiella pneumoniae complex In addition to K. pneumoniae the term includes the	[redacted]

closely related species (subspecies)	[redacted]
K. ozaenae and K. rhinoscleromatis and the new species K. ludwigii. For practical identification schemes, the term includes Klebsiella (Raoultella) planticola and K .terrigena, which are very difficult to differentiate. Klebsiella (Raoultella) ornithinolytica is ornithine and thus phenotypically distinct.	[redacted]
KIuyvera-Buttiauxella complex This complex includes two genera with almost a dozen species (Table 3) and now includes Kluyvera intermedia, formerly classified as Enterobacter intermedium	[redacted]
Proteus vulgaris complex P. vulgaris is made up of at least four DNA-DNA hybridization groups. The definition of the complex could be expanded to include the closely related species P.penneri and P.hauseri, which can often be differentiated.	[redacted]
Rahnella aquatilis complex R. aquatilis is made up of at least three DNA-DNA hybridization groups.	[redacted]
Serratia liquefaciens complex The term includes S. liquefaciens and three closely related species S. grimesii S. proteamaculans, and S. quinovorans, which are difficult to differenttiate.	[redacted]

Yersinia enterocolitica complex In addition to Y. enterocolitica, the term includes the closely related species Y. aldovae	[redacted]
V. bercovieri, Y. frederiksenii, Y. intermedia, Y. kristensenii, and Y. mollaretii which are difficult to differentiate.	[redacted]
…………………………….	[redacted]
Some of the Enterobacter agglomerans DNA-DNA hybridization groups can rately occur in human clinical speciemens.	[redacted]
rules in the Bacteriological Code (International Code of Nomenclature of Bacteria). However, classifications can be useful or not useful and can be frequently used in the literature or rarely used (14. 16. 38).	[redacted]
	[redacted]
Proposed Changes in Classification and Other Changes in Table 3	[redacted]
Several "alternative classifications" have been proposed in the literature. Some of these appear to be totally justified and have been incorporated into Table 3. However, others have not been fully discussed or widely accepted by the scientific community (28). Table 3 gives the nomenclature and	[redacted]

classification that one of us (J.J.K) has incorporated into tables, data matrices, and computer programs used to identify clinical and nonclinical isolates of Enterobacteriaceae. It will differ from other nomenclatures and classifications.

In the seventh edition, the genus Plesiomonas was classified in the family Vibrionaceae along with Aeromonas. Because Plesiomonas is closer to Enterobacteriaceae than to Vibrionaceae based on 16S rRNA sequencing and because it contains the enterobacterial common antigen, it was included in the family Enterobacteriaceae in the eighth edition, and this classification has been maintained in the ninth edition. However, Plesiomonas is oxidase positive, a characteristic not shared with other species of Enterobacteriaceae, and is a distant relative of E. coii. the type spccics of the type genus of Enterobacteriaceae (14). Thus, the classification of Plesiomonas in the family Enterobacteriaceae might best be viewed as tentative.

In Table 3, the organism originally classified (9. 40) as Xenorhabdus luminescens DNA hybridization group 5 is now classified as Photorhabdus asymbiotica (44). It has caused rare cases of bacteremia and wound infection in the United States (40 and Australia (75).

[redacted]

[redacted]

[redacted]

[redacted]

These Australian strains are distinct in some ways and have been proposed (Table 2) as Photorhabdus asymbiotica subspecies australis (2). The names Citrobacter diversus and Citrobacter koseri have both been used in the literature tor some time, but the name Citmbacter diversus has been used much more frequently. Many workers recognized the phenotypic similarity of these two organisms and thought that they might be the same. The species have different type strains, and so considering them to be the same will always be a subjective tnnttcr. They can be considered subjective synonyms but not objective synonyms (which must have the same type strain). the name Citrobacter diversus became the correct name for this organism on 1January 1980, when the Approved lists of Bacterial Names was issued, because under the laws of priority it was the older name. However, in 1993 the judicial Commission of the International Committee on Systematic Bacteriology issued an Opinion (57) that die name Citrobacter koseri should be conserved over the name. Citrobacter diversus even though the name Citrobacter diversus was the older name, was on the Approved lists of Bacterial	[redacted] [redacted] [redacted]

Names was the correct name under the Rules of the Bacteriological Code, and was the name used most frequently in the literature. This opinion needs much more discussion by the scientific community, which is beyond the scope of this chapter; therefore, both names are included in Table 3.	[redacted]
Phenotypic and 16S rRNA sequencing data indicate that Kluyvera cochleae is almost identical to Enterobacter intermedium, and the proposed reclassification of this organism as Kluyvera intermedia (74) appears to solve several problems (Tables 2 and 3).	[redacted]
Another change in Table 3 is that species in the same irenus are now grouped with their closest phenotypic and evolutionary relatives (14. 16, 38) rather than listed alphabetically. For example, three subgroups of the genus Citrobacter are defined as A. B. and C and listed together. In addition, we propose that Entenc Group 139 be reclassified as ~~Citrobacter group 139. A similar notation is used in Table 3 for other genera; Tables 2 and 4 and the text give explanations.~~	[redacted]
Other Proposed Changes in Nomenclature and Classification	[redacted]
Proposed Classification of Three Klebsiella Species in Raoultella	

In 2001, Drancourt et al. (28) proposed that Klebsiella planticola, K. ornithinolytica, and K. terrigena be classified in a new genus, Raoultella. as R. planticola, R. ornithinolytica, and R. terrigena. These three .species are extremely similar to Klebsiella pneumoniae in their phenotypic properties (37). making differentiation very difficult (Table 3). I bis proposed alternative classification needs further evaluation; however, we agree that these three species should be grouped together and have done this in Table 3.

Enterobacter agglomerans Group-Pantoea

The Enterobacter agglomerans - Pantoea complex is a confusing subject, and writers continue to make errors in the definition

TABLE 7 Shigella isolates in the United States tor 2003'

Rank	Serotype	Isolates
1	Shigella sonneti(serogroupD)	9.263
	Shigella flexneri (serogroup B)	1.660
3	Shigella boydii (serogroup C)	125

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

4 Shigella dysenteriae (serogroup A) 41	[redacted]
Not (completely) serotyped 463	[redacted]
Total Shigella isolates 11,552	[redacted]
	[redacted]
Data are from the Centers for disease control and prevention at http//wwww.cdc.gov /ncidod/dbmd/phlisdata/shigella.htm . Surveillance reports for previous years are also at this internet address. Note: the entire document for a given yearis typically very large so it maybe prudent to download individual tablets to avoid time and computer data storage problems ………………………………….	[redacted]
and circumscription (boundaries) of Pantoea agglomerans	[redacted]
	[redacted]
1972, Ewing and Fite redefined the name Entcrvbacter aggicms erans to include u wide variety of organisms known under many different names (32). These investigators also defined 11 different biogroups to recognize the phenotypic diversity of the many strains included in Enterobacter agglomerans. This name has become useful lor clinical microbiologists, and it has been used extensively in the literature. Systematic analysis by	[redacted]

Brenner and coworkers using DNA-DNA hybridization indicated that in Enterobacter agglomerans is very heterogeneous, with at least 14 DNA hybridisation groups (12). For this reason, the names " Enterobacter agglomerans complex" and " Enterobacter agglomerans group " (37) have been used to better indicate the heterogeneity ot this “species" (Tables 3 and 5). However, it has been very difficult to find simple tests to differentiate and identify all of the DNA hybridization groups (37). For this reason, workers have been reluctant to subdivide the Enterobacter agglomerans group until a definitive classification could Ixs proposed (37). Gavini et al. (46) took the first step toward more logical classification tor this complex group by proposing that the group of six strains defined by Brenner et al. as “DNA hybridization group 13 of the Enterobacter agglomerans be classified in a new genus. Pantoea, as P. agglomerans They also defines a new species in the genus, Pantoea dispersa (46). previously classified as Enterobacter agglomerans DNA hybridization gnwip 3 by Brenner (12). However, this new classification has caused communication problems. Some author' have	[redacted] [redacted] [redacted]

broadened the original definition of Gavini et al. for a Pantoea agglomerans to include organisms that are not phylogenerically related. Since DNA- DNA hybridization is not routinely done and since simple tests are not available to definitively identify strains to the level of DNA hybridization group, it seems prudent to retain the vernacular name “Enterobacter agglomerans complex" as a convenient name for clinical microbiologists to use in reporting clinical isolates (Tables 3 and 5). This term is defined biochemically in Table 3. and it should be emphasized that it is used merely for convenience because the name Enterobacter agglomerans is well understood and widely used in the literature. Eventually, this term will be replaced with a better classification. When definitive testing in a reference laboratory (usually including DNA hybridization) is done, more precise names can be used in reporting. Examples could include Pantoea agglomerans (limited to strains that fall into DNA hybridization group 13). Pantoea dispersa (limited to 'trains that fall into DNA hybridization group 3). and Enterobacter agglomerans DNA hybridization group 1, etc. Tables 3 and 5 use and define the	[redacted] [redacted]

vernacular name Enterobacter agglomerans complex, a term that may prove useful for reporting isolates in most microbiology laboratories because almost none can do DNA-DNA hybridization. A less desirable vernacular name for this group of organisms is the “Pantoea agglomerans complex."	[redacted]
Enterobacter taylorae-Enterobarter cancerogenus	[redacted]
Enterobocter taylorae and Enterobarter cancerogenus	[redacted]
may be two names for the same organism (47). However, they have different type strains; therefore, they are not objective synonyms under the rules of the Bacteriological Code. Until the identity of these two organisms is universally accepted, both names will be used (Table 3).	[redacted]
Nomenclature, Classification, and Reporting of the Genus Salmonella	[redacted]
Alter much study and a lengthy judicial process (58). there is now good agreement on many issues in the nomenclature and classification of the genus Salmonella (32. 41, 58, 70, 76, 78, 79). The recent decision of the Judicial Commission on the International Committee on Systematics of Prokaryotes (58)	[redacted]

to replace Salmonella choleraesuis with Salmonella enterica stabilizes this issue which has caused confusion and the use of illegitimate names in the literature for many years. In 2005 (58), Salmonella enterica finally became ihe legitimate species name to include most of the most important serotype names. Three 2005 references (58, 78, 91) and the Internet site of J. R Euzcby (http://www.bactcrio.cict.fr) provide additional historical insights and alternative perspectives on the issues.

Until the 1970s, the species concept in the genus Salmonella was based on epidemiology, host range, biochemical reactions, and antigenic structure (the O antigen, phases 1 and 2 of the H antigen, and the Vi antigen, if present), and strains that differed in one or all of these properties were given distinct names. Names such as Salmonella typhi. Salmonella cholerae-suis (originally some names such .is this one were written with a hyphen, which was eventually dropped because of changes in the bacteriological Code). Salmonella paratyphi A. Salmonella paratyphi A var. durazzo, Salmonella typhimirium. Salmonella typhimirium var.Copenhagen. Salmonella enteritidis. and Salmonella newport began to appear, and the

[redacted]

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[redacted]

list rapidly expanded to include hundreds of names. Some workers believed that these names really represented biological species, but others thought that they were antigenic and biochemical varieties with an uncertain evolutionary relationship. However, there was universal agreement that the names were an extremely useful way to communicate about the particular serotypes and the diseases they caused. Most authors wrote the serotype names in italics as a species in the genus Salmonella, for example. Salmonella typhimutiuym (32, 41). Several proposals to the Judicial Commission of the International Committee on Systematic Bacteriology have requested that important serotype names be preserved (31, 33, 34) to preserve stability in nomenclature, but it is not clear whether this is a matter that will Iv decided by judicial action or by usage. In 1973, Crosa et al. (25) used DNA-DNA hybridization to show that Salmonella strains could be grouped into five main evolutionary groups. Two (possibly three) additional groups are now known (11. 78, 79). The vast majority of strains that cause human infections occur in DNA hybridization group 1 (Salmonella group I). Strains isolated from	[redacted] [redacted]

animals and the environment clustered into the four other groups, designated DNA groups 2 (II). 3a (Ilia), 3b (111b), and 4 (IV). Over the years, different authors have used different terms to refer to these evolutionary groups: DNA»DNA hybridisation groups (25. 41), multilocus enzyme electrophoresis clusters (11, 79), subgenera, species (see the A/'fmxvJ lusts of liacteruil Xames and http://www.bacterio.cict.fr). and subspecies (70, 76. 77). Crosa et al. (25) showed that all five groups lASahrunidla were very lughh related gene tic all v. With the operational species definition usually used m DNA hvhndization. these five groups were considered to belong to the same species. Under the rules of the bacteriological Code, the name of this species had to be However, The species name is a cause of confusion, since Salmonella choleraesuis would have two total different meanings, a broad one .is a species and a narrow one as a serotype. There was support for making an exception to the rules of the Bacteriological Code and using a name that has never been used as a serotype name to avoid confusion. There was a formal proposal in 1999 to coin a new name, Salmonella eixterica (31), which would replace the name

[redacted]

Salmonella choleraesuis- as the species name to represent most of the serotypes of Salmonella.However, the proposal to replace the name Salmonella choleraesuis \ with Salmonella enterica was denied by the Judicial Commission ot the International Committee on Systematic Bacteriology. Thus, Salmonella choleraesuis remained the correct name until a variation of the original proposal to the Judicial Commission was approved in 2005 (58). Even though it was an illegitimate name, the name Salmonella enterica had already been used by the World Health Organization's International Center for Salmonella (76) and by many of the World Health Organization's national centers for Salmonella. The name had also been used widely in the literature. Fortunately, the 2C05 decision of the Judicial Commission has made Salmonella enterica the correct name, which is gaining universal acceptance. .Another point of confusion concerns the method of writing serotype names. For almost 100 years, serotype names have been written as species (the serotype-as-species nomenclature), for example. Salmonella enteritidis. The World Health Organization's International Center for Salmonella at the Institute	[redacted] [redacted]

Pasteur. Paris. France, introduced a different nomenclature in which the serotype name is capitalized and not written m italics. In this nomenclature, the name Salmonella enteritidis would be written in one of the following ways: “Salmonella enterica serovar enteritidis," Salmonella serovar enteritidis "," “Salmonella ser. enteritidis ," or “Salmonella Enteritidis." The nomenclature described by McWhorter-Murlin and Hickman-Brenner (70) is similar, but these authors use the term “serotype" instead of “serovar." The main advantage of these nomenclatures is that they do not artificially treat the serotypes as species. The main disadvantage is that they create a new nomenclature that differs from one that has been widely accepted and used for more than 70 years. There have been literally hundreds of thousands of uses of the serotype-as- species nomenclature in the literature. The International Center for Salmonella's nomenclature appears in the second edition of Bergey ‘s ,Manual (78) and is being used (sometimes with modifications) by the national centers for Salmonella (19, 70). However, many published articles and books continue to use the nomenclature. Since Salmonella names arc being written differently by different authors	[redacted]

and different national centers for Salmonella, it is not surprising that the literature is beginning to reflect this confusion. Recent examples of the way “serotype Typhimurium" is being written include Salmonella serotype Typhimurium. Salmonella ser. Typhimurium. Salmonella typhimurium. Salmonella Typhimurium. Salmonella typhimurium. Salmonella serovar Typhimurium. and Salmonella serovar Tjphmiwniiin or simply Typhimurium (omitting the genus name Salmonella entirely) (88). When the variations are combined with the four species and subspecies possibilities, i.e., Salmonella choleraesuis. Salmonella choleraesuis subspecies choleraesuis. Salmonella enterica, and Salmonella enterica subspecies enterica. the number of possible variations is multiplied considerably. One example of the almost endless possibilities is Salmonella enterica subspecies entenrica serovar Typhimurium.	[redacted]
The current disagreements in Salmonella nomenclature and classification include the use of the term "serotype" (19. 70) versus “serovar” (76, 77) (both terms are often abbreviated as “ser; the best way to write the names of the serotypes; the use of	[redacted]

names versus antigenic formulas for some of’ the serotypes; the argument over whether some well-known serotype names should be eliminated and combined with other serotypes (19,70,76,77); and the question of how to name the distinct DNA hybridization groups.	[redacted]
Most clinical microbiology laboratories identify Salmonella isolates with a commercial identification system and then with commercial salmonella "polyvalent grouping antisera," which will agglutinate only those strains with the O antigen groups contained in the polyvalent scrum (often only groups A through E). These two methods usually give definitive results, and a simple report can be issued such a' “ salmonella serogroup B," avoiding the problems described above. Abbreviating “serotype" and “serovar" as “ser." would be a further simplification and would avoid the disagreement over these two terms. Reference laboratories that do complete serotyping and biochemical testing can issue a definitive report such as “Salmonella serotype Typhimurium or “Salmonella enterica serotype Typliimurium.”	[redacted]
Nomenclature for Shiga Toxins/Verotoxins Produced by E. coli and Shigella	[redacted]

Several different names are being used in the literature for the cytotoxins produced by E. cub and Shigella. Tins topic is critical because of the importance of E. cou Ol 57 and other strains that produce these toxins (sec chapter of tins Manual). Several different commercial assays for these toxms arc being marketed; thcrcfi>rc, it is essential to read the package insert carefully to determine exactly which toxin(s) the kit is detecting and to word laboratory reports accordingly.

For almost 100 years, it has been known that Shigella dysenteriae serogroup O1 produces a potent cytotoxin known as Shiga toxin. More recently, it has been shown that certain strains of E. coli that cause intestinal infections produce a similar toxin, which was first detected because it was cytotoxic tor Vero cells in tissue culture. A number of recent studies have defined these proteins from S. dysenteriae O1 and E. coli. and there is agreement that they constitute a family of toxins. They are being referred to in the literature as Shiga toxin (ST), Shiga- like toxins (SLT), verocytotoxin(s), and verotoxin(s) (VT). and at least five different toxins are involved (20.86). This complex subject was recently reviewed by Scheutz and Strockbine (86). The

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E. coli strains that produce these toxins are often referred to as STEC and VTEC. Calderwood et al. (20) summarized the data available and proposed that strains of E. coli that produce these toxins be called “Shiga toxin-producing" E. coli. which would replace the previous term, “Shiga-like toxin producing" They also recommended that the new toxin name be cross-referenced with the corresponding verotoxin name. With this nomenclature, a laboratory rcpon for a stool culture might be worded. “Positive for E colt 0157:H7, which privlucc- Shiga toxins Stxl (VT1) anil Stx2 (VT2)." Hopefully, the differences between those using the two different nomenclatures will be resolved, resulting in a single nomenclature.	[redacted] [redacted]

Proposed Reclassification of Calymmatobacterium granulomatis as Klebsiella granulomatis Calymmatobacterium granulomatis has received little attention in industrialized countries. In the seventh edition of this Manual, Calymmatobacterium was mentioned only twice (pages 25 and 50). It was listed as an aerobic bacterium that can be found in the genital area, and under the topic “Specimen Management" it was mentioned under the disease granuloma inguinale, or ulcerative donovanosis. with the notes "mostly a tropical disease" and “culture is nonproductive." Calymmatobacterium granulomatis has been described as a highly pleomorphic gram-negative rod that does not grow on laboratory media. Diagnosis of granuloma inguinale has been based on showing the presence of “Donovan bodies" in Giemsa-stained smears of mononuclear cells or histiocytes from the patient's genital ulcers. It had been assumed for almost a century that Calymmatobacterium granulomatis has no relationship to the “easy-to-culture" organisms	[redacted] [redacted] [redacted] [redacted]

of the family Enterobacteriaceae. However, Carteret al. (21) proposed that Calymmatobacterium granulomatis be reclassified in the genus Klebsikella as Klebsiella granulomatis. This proposal was based both on nucleotide sequence relntedness and on disease similarity. Granuloma inguinale is a disease similar to rhinoscleroma, also a tropical disease (nasal infection) caused by (or associated with) Klebsiella rhinoscleromatis. While this alternative classification is being evaluated and tested, it would be helpful to write both scientific names, with the writer's preference listed first; "Klebsiella granulomatis (Calymmatobacterium granulomatis)" (which we prefer) or "Calymmatobacterium granulomatis • (Klebsiella granulomatis)." Other diseases of unknown etiology may be caused by unculturable Enterobacteriaceae	[redacted]
DESCRIPTION OF THE FAMILY ENTEROBACTERIACEAE	[redacted]
Most genera and species in the family Enterobacteriaceae share the following properties: they are gram negative and rod shaped; do not form spores; are motile with peritrichous flagella or are nonmotile; grow on peptone or meat extract media without the	[redacted]

addition of other supplements or sodium chloride; grow well on MacConkey agar; grow both aerobically and anaerobically; are often active biochemically; ferment (rather than oxidize) D-glucose and other sugars, often with gas production; are catalase positive and oxidase negative; reduce nitrate into nitrite; contain the enterobacterial common antigen; and have 39 to 59% guanine-plus- cytosine (G+C) contents in DNA (5. 12-14, 38, 55). Host-adapted species that are unculturable, difficult to culture, or slow growing appear to have evolved in some genera H4) (Table4).	[redacted]
When techniques that measure evolutionary distance are used, genera and species in the family should also be more closely related to E coli, the type species of the type genus of the family, than they are to organisms in other families (14. 38). Tables 1 to 5 expand on this- definition and give most of the exceptions.	[redacted]
NATURAL HABITATS Enterobacteriaceae are widely distributed on plants and in soil, water, and the intestines of humans and animals (5, 14. 43. 55). Some species occupy very limited ecological niches. Salmonella serotype Typhi causes typhoid fever and is found only in	[redacted] [redacted]

humans (50, 68). In contrast, strains of Klebsiella pneumoniae arc distributed widely in the environment and contribute to biochemical and geochemical processes (63). However, strains of K. pneumoniae also cause human infections. ranging from asymptomatic colonization of the intestinal. urinary, and respiratory tracts to fatal pneumonia, septicemia, and meningitis.

CLINICAL SIGNIFICANCE

Some Enterobacteriaceae are associated with or cause specific humans diseases tablet 1) (14. 55,68,69,82). Many cause, or are isolated from, abscesses, pneumonia, meningitis, septicemia, and infections of wounds, the urinary tract, and the intestine (68. 69). They are a major component of the normal intestinal flora of humans but are relatively uncommon as normal flora of other body sites. Several species of Enterobacteriaceae are very important causes of nosocomial infections (69). Enterobacteriaceae may account for 80% of clinically significant isolates of gram-negative bacilli and 50% of clinically significant bacteria in clinical microbiology laboratories (JO). They account for nearly 50% of septicemia cases, more than 70% of urinary

[redacted]

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[redacted]

tract infections, and a significant percentage of intestinal infections (68, 69).	[redacted]
Human Extraintestinal Infections	
Except for the species of Shigella, which rarely cause infections outside the gastrointestinal tract, many species of Enterobacteriaceae ommonly cause extraintestinal infections. However, a small number of species, i.e., E. coli, Klebsiella pneumoniae, Klebsiella oxytoca, Proteus mirabilis, Enterobacter aerogenes. the Enterobacter cloacae complex, and Serratia marcescens, account for most of these infections. Urinary tract infections, primarily cystitis, are the most common (85), followed by respiratory, wound, bloodstream (27), and central nervous system infections. Many of these infections, especially sepsis and meningitis, are life threatening and are often hospital acquired. Because of the severity of these infections, prompt isolation, identification, and susceptibility testing of Enterobacteriaceae solates arc essential.	[redacted]
Human Intestinal Infections	[redacted]
Several organisms in the family Enterobacteriaceae are also important causes (Tables 6 and 7) of intestinal infections of humans and animals worldwide. Although other species in the family have	[redacted]

been associated with diarrhea (93) or even implicated as causes of diarrhea, only organisms in four genera. Escherichia (29, 36, 55, 61), Salmonella (25,41,50,78), Shigella (32, 68), and Yersinia (7, 60, 68, 80), have been clearly documented as enteric pathogens. These four genera are discussed in chapters 43 and 44 of this Manual. Other Enterobacteriaceae such as Citrobacter, Edwardsiella, Hafhia, Morganella. Proteus, Klebsiella, Enterobacter, and Serratia may have an association with diarrhea in certain studies (39, 93), and some authors have gone as far as to implicate them as actually causing diarrhea (5, 93). Strains of these Enterobacteriaceae that produce “biologically active" com-pounds (often vastly overstated as being “enterotoxin- producing strains”) have been isolated from people with diarrhea (93), but the causal role of these strains in diarrhea is uncertain. One possible way to emphasize the drastic change in the stool flora would be to issue a report such as “Klebsiella pneumoniae isolated in essentially pure culture (10 of 10 colonics tested); please consult the laboratory to discuss possible significance." The patient's antibody response, or lack of one. would be a helpful way to assess the particular organism's causative role. There	[redacted]

is no evidence that strains of these other genera are important causes of diarrhea.	[redacted]
In contrast to the arguable role of the organisms listed above, the evidence for the causal role of Plesiomonas shigelloides (see chapter 45 in this Manual) in diarrhea is somewhat stronger. A safe generalization would be that “certain strains of P. shigelloides may cause diarrhea in certain people under certain conditions, but it is probably not an intrinsic pathogen.” For an intrinsic pathogen, most strains would cause diarrhea in most people, under most conditions (59).	[redacted]

Surveillance at the National and International Levels 11/7 Many countries provide surveillance data on the Internet for plague, typhoid fever, salmonellosis, shigellosis, diarrheagenic E. coli, institutional infections, bacteremia, meningitis, antibiotic resistance, and other enteric and nonenteric Infections. Often the word “infection" or a similar word is used when the term “clinical microbiology isolate" would be more appropriate. Care must be used in interpreting these data because "association" and “clinical microbiology isolate" do not equate with “causation” and “infection" in each instance (39), For example, few would argue with the use of the term "infection" for a clinical isolate of Salmonella serotype Typhi from the stool of a patient with typhoid fever. In contrast, the word "infection" would be an overstatement if used to describe a stool isolate of a nonenteropathogenic Yersinia enterocoliiica serotype (such as 010) or one of the other six species of the Y. enterocoliiica group (Table 3). Check to see if surveillance data make these important distinctions. SPECIMEN COLLECTION, TRANSPORT, AND PROCESSING	[redacted]

Extraintestinal Specimens	[redacted]
Enterobacteriaceae are recovered from infections at many different body sites, and normal practices (see chapters 5 and 20 of this Manual) for collecting blood, respiratory, wound, urine, and other specimens should be followed.	[redacted]
Intestinal Specimens	[redacted]
Stool cultures arc usually submitted to the laboratory with a request to isolate and identify the cause of a possible intestinal infection, usually manifested as diarrhea (see chapter 20). The groups of Enterobacteriaceae usually associated with diarrhea in the United States are Salmonella (22), Shigella (23), and certain pathogenic strains of E. coli and Yersinia enterocolitica	[redacted]
Stool specimens require special attention to both collection and transportation and should be obtained early in the course of illness, when the causative agent is likely to be present in the largest numbers in feces. At this stage, the use of enrichment broths should he unnecessary. If rapid processing (within 2 h of collection) is not possible, a small portion of feces or a swab coated with feces should be placed in transport medium, such as Stuart. Amies. Cary-Blair. or buffered glycerol	[redacted]

saline. Cary-Blair is probably the best overall transport medium for diarrheal stools. In cases of diarrhea that do not yield a causative agent, a tube of frozen stool can be invaluable for looking for new causative agents or for testing against the patient's convalescent serum.

More information about the isolation, identification, typing, and virulence testing of isolates of Salmonella. Shigella, E.coli, and Y. enteroco- litica is given in chapters 43 and 44.

Macroscopic and Microscopic Examination

Stool specimens should be examined visually for the presence of blood or mucus, but microscopic examination is less helpful because ot’ its lack of specificity (84). Although identification by fluorescent-antibody staining b theoretically possible for all enteric pathogens it has been of limited success because the method is difficult and there are many serological cross- among the species of Enterobacteriaceae (32). This technique was most often used to detect Salmonella strains (primarily in the food industry) and certain serogroups of E. coli and to aid in outbreak investigations.

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	[redacted]
ISOLATION Extraintestinal Specimens	[redacted]
Most strains of Enterobacteriaceae grow readily on the plating media commonly used in clinical microbiology laboratories (see chapter 20). MacConkcy agar, generally interchangeable with eosin methylene blue agar, is usually used, because it allows a preliminary grouping of enteric and other gram-negative bacteria. The most common isolates of Enterobacteriaceae have a characteristic appearance on blood agar and MacConkey agar that is useful for preliminary identification (Table 8). Broth enrichment can increase the isolation rate if small numbers of Enterobacteriaceae are present, but this step is not normally required.	[redacted]
Intestinal Specimens	[redacted]
Media that can be used routinely for intestinal specimens include a nonselective medium such .is blood agar, a differential medium of low to moderate selectivity such as MacConkey agar, and a more selective differential medium such as xylose-lysine-deoxycholatc (XLD) agar or Hektoen enteric agar (HE). A broth enrichment substance such as selenite (or GN (gram-negative	[redacted]

broth) or tetrathionate) can be included, particularly if the specimen is not optimal. A highly selective medium such .is brilliant green agar, bismuth sulfite, Rambach, or CHROM agar Salmonella (BD Diagnostics, Sparks, Md.) can also be included tor isolating strains ot Salmonella. A special plate, such as sorbitol-MacConkey agar (or one one of its modifications), can be added to enhance the isolation of Shiga toxin-producing strains of E. coli Ol57:H7. This medium should be used if the stool is frankly bloody or if the patient has a diagnosis of hemolytic- uremic syndrome, and it can be used for all fecal specimens if resources permit (see chapter 42). When the presence of Yersinia enterocolitica is suspected, a selective-differential medium, such as CIN (cefsulodin-lrgasan-novobiocin) agar (also called Yersinia selective agar), can be added (see chapter 44). A complete stool culture procedure should also include media for isolation of Campylobacter and possibly Vibrio strains in areas where cholera and other Vibrio infections are common. Several new plating media appear to be more sensitive or specific and are gaining in popularity (see	[redacted]

chapters 43 and 44).	[redacted]
IDENTIFICATION	[redacted]
There are many different approaches to identifying strains of Enterobacteriaceae (14. 37. 38).	[redacted]
Conventional Biochemical Tests in Tubes	[redacted]
Tube testing was once used by all clinical microbiology laboratories, and it is still widely used in reference and public health laboratories (32. 37). Although some laboratories pre-pare their own media from commercial dehydrated powders, most of the common media are also available commercially in glass tubes that are ready to use. Growth from a single colony is inoculated into each tube, and the tests are read at 24 h and usually also at 48 h. In many reference laboratories, most tests arc often kept for 7 days to detect delayed reactions. Unfortunately, the media and tests arc not completely standardized. and few laboratories use exactly the same formulations or procedures. Even with these variables, this approach usually results in correct identifications of the common species ot Enterobacteriaceae Table 3 gives	[redacted]

the results for Enterobacteriaceae in 48 tests (for the media and methods used to generate the data in this table, sec references 32. 35. and 37).	[redacted]
Computer Analysis To Assist in Identification Two microcomputer programs were developed in the 1980s at the Centers for Disease Control and Prevention (CDC)'s Enteric Reference Laboratories to assist with the identification of Enterobacteriaceae cultures. "George" and "Strain Matcher" were described in the 1985 review of the family (37). One of us (J.J.F.) plans to revise and update these programs to run on current operating systems and make them more available. These plans include modifying the Enterobacteriaceae data matrix in Table 3 and other data matrices to be compatible with the probabilistic identification program PIBWin that is free and can be downloaded from the Internet (http://www.som.soton.ac.uk/staff/tnb/pib.htm).	[redacted]

Screening Tests, Using All Information Available	[redacted]
Over the years, the Enteric Reference Laboratories at CDC have found that many genera, species, and serotypes can be tentatively Identified with a number of screening tests (Table 9). More precise identification can be made by using a complete set of tests of commercial identification systems. Because of the limited availability of certain reagents (bacteriophage O1 and Yersinia typing sera, etc.). these screening tests may be more useful in a reference or research laboratory.	[redacted]
Example 1. A urine isolate has the following properties: colonies on MacConkey agar are 2 to 3 mm in diameter, are bright red and nonmucoid, and have precipitated bile around them; it is indole positive and 4-methylumbelliferyl- P-D-glucuronidase (MUG) positive: it grows at 44.5 C; and it is antibiotic resistant. These results arc completely compatible with E. coli.	[redacted]
Example 2. An isolate from the feces of a diarrhea patient has the following properties: colonies on MacConkcy agar are 2 to 3 mm in diameter and colorless; colonics on XLD agar arc 2 to 3 mm and	[redacted]

black; the isolate agglutinates in Salmonella polyvalent O scrum and in O-group B scrum; the MUCAP test (hydrolysis of 4-methylumbelliferyl caprylate; Biolife, Milan. Italy) and lysis by bacteriophage O1 are positive; and it is antibiotic resistant. All these results arc compatible with Salmonella serogroup B.	[redacted]
Commercial "Kits" for Identification	[redacted]
A commercial kit Is defined as a panel of miniaturized or standardized tests that are available commercially. The tests incorporated in the kits are often a subset of those given in Table 3. The approach for using kits is similar to the conventional tube method, with the main differences being in the miniaturization, the number of tests available, the suspending medium, and the method of reading and interpreting results (sometimes by machine). Kits are now used by most American laboratories and are discussed in chapter 15. Kits often give the correct identification for the most common species of Enterobacteriaceae, but they may not be ns accurate for some of the	[redacted]

new species. It is important to check the instruction manual to determine which organisms have been included in the database and the number of strains that were used to define each organism. The main problem with kit-based identification is that the tests used (usually about 20 tests) are becoming inadequate to differentiate all of the current species of Enterobacteriaceae given in Tables 1 to 5. This is also becoming a problem with conventional tube tests, even when the 48 tests listed in Table 3 ate used. Unsual identifications or “no identification" obtained with a kit could be verified by other methods or approaches (56). but referral to a reference laboratory may be the best alternative. Other methods might	[redacted]
TABLE 9 Screening test tor the enteric pathogens Salmonella. Shigella, Escherichia coli. Yersinia, and for the other important Enterobacteriaceae and those most frequently isolated from human clinical specimens	[redacted]
Salmonella Lactose , sucrose,. HsS, O1 phage .MUCAPd+, agglutinates in polyvalent serum/ typical colonies on media selective /differential for	[redacted]

Salmonella (brilliant green agar. SS agar, Rambach agar. CHROM agar. etc.). lysed by the Salmonella-specific "bacteriophage O1. often antibiotic resistant	[redacted]
Salmonella typhi Ornithine . H_2S+ (trace amount only), L-rhamnose. no gas produced during fermentation, agglutinates in group D serum. Vi serum, and flagella ”d” serum	[redacted]
Shigella Nonmotile, lysine. gas,agglutinates in polyvalent serum biochemically inactive, often antibiotic , resistant. PhoE+ (molecular test)	[redacted]
Shigella dysenteria	[redacted]
Agglutinates in group A serum., D-mannitol-	[redacted]
Shigella dysenteria Ol Catalase -. agglutinates in O1 serum. Shiga toxin+	[redacted]
Shigella flexneri	[redacted]
Agglutinates in group B serum. D-mannitol+	[redacted]
Shigella boydii Agglutinates in group C serum. D-mannitol+	[redacted]
Shigella sonnei Agglutinates in groupD serum. D-mannitols, ornithine decarboxy lase +, lactose (delayed), characteristic	[redacted]

colony variation from smooth to rough	[redacted]
Escherichia coli Extremely variable biochemically, indole +. MUG+, grows at 44-5‘C, sometimes antibiotic resistant, PhoE + (mocular test)	[redacted]
Escherichia coli 0157:H7 Colorless colonies on sorbitol-MacConkey agar (SMAC, red colonies on MacConkey agar. MUG-.D-sorbitol - (or delayed), agglutinates in 0157 serum and H7 serum; many commercial media and tests are available (95)	[redacted]
Escherichia coli—invasive strains Many strains resemble Shigella because they are “inactive” biochemically: lactose -, nonmotile, lysine - O antigen groups O28, O29, O112, O124,O136, O143, O144, O152,O164,O167. and others;	[redacted]
no commercial assay or simple way to isolate and identify	[redacted]
Yersinia Grows on CIN agar, often more active biochemically at 25 than 36oC ; motile at 250C, nonmotile at 36°C, urea+	[redacted]
Yersinia enterocolitica	[redacted]
Smaller colonies pathogenic serotypes (often less than 1 mm)	[redacted]

than other Enterobacteriaceae species on enteric plating media, CR-MOX +. pyrazinamidase-. salicin-, esculin-, agglutinate in O-typing sera for "enteric pathogenic” serotypes: 3; 4.32; 5,27; 8; 9; 13a, 13b; 18; 20; or 21	[redacted]
Yersinia enterocolitica O3 (a pathogenic serotype)	[redacted]
D-Xylose-, agglutinates in O3 serum, tiny colonies at 24 h on plating media; in most countries it is the most frequently isolated pathogenic serotype	[redacted]
Yersinia enterocolitica nonpathogenic serotypes CR-MOX- . pyrarinamidase+, salicin+, esculin+". do not agglutinate in O-typing sera for “enteric pathogenic" serotypes: 3; 4.32; 5.27; 8; 9; 133.13b; 18; 20; or 21	[redacted]
Citrobacter citrate + , lysine decarboxylase - , often grows on CIN agar, strong characteristic odor	[redacted]
Enterobacter Variable biochemically, citrate+. VP+, resistant to cephalothin	[redacted]
Enterobacter sakazakii . Yellow colonies (more pigmented at 25 than 360C), often "tough as leather"; grout on several selective media designed for its isolation; D-sorbitol negative,	[redacted]

delayed positive DNase at 360C Hafnia Lysed by Hafnia-specific bacteriophage 1672/ often more active biochemically at 25 than 36'C	[redacted]
Klebsiella Mucoid colonies, encapsulated cells, nonmotile. Lysine+. very active biochemically,ferment most sugars. VP+. Malonate+. resistant to carbenicillin and ampicillin	[redacted]
Proteus-providencia-Morganella Phenylalanine+, tyrosine hydrolysis+, often urea+, resistant to colistin	[redacted]
Proteus Swarms on blood agar, pungent odor, H2S+, gelatin+. Lipase+	[redacted]
Proteus mirabilis Urea+. Indole-, ornithine+, maltose -	[redacted]
Proteus vulgaris Urea+, indole+, ornithine-, maltose+	[redacted]
Providencia No swarming, H2S-, ornithine-, gelatins, lipase-	[redacted]
Morganella Very inactive biochemically, no swarming, citrate-, H2S-. ornithine+, gelatin-, lipase-, urea+	[redacted]
Plesiomonas shigelloides Oxidase+. Lysine+, arginine +, ornithine +. mw-inositol+	[redacted]
Serratia DNase +, gelatinase+. Lipase+, resistant to	[redacted]

colistin and cephalothin	[redacted]
Serratia marcescens L-Arablnose-	[redacted]
Serratia,. other species L-Arabinose+	[redacted]
include a different kit (which will have similar limitations), a kit that contains more tests (such as those in 96-well plastic plates), or more expensive research techniques such as molecular tests or 16S rRNA sequencing (56).	[redacted]
Molecular Methods of Identification	[redacted]
Molecular methods have proved extremely useful for identification to the level of family, yen us, species, serotype, clone, and strain and for differentiating pathogenic from nonpathogenic strains (see chapter 16 of this Manual). For example, a PCR test for the phoE gene appears to be a sensitive and specific test tor determining if a strain belongs to the Escherichia- Shigella group (88). However, few if any of these molecular methods arc commercially available. In the United States, commercial diagnostic tests must often be approved by the Food and Drug Administration if they arc used on human clinical specimens. Regulatory and cost limitations have greatly restricted the use of molecular methods in clinical microbiology laboratories.	[redacted]

However, they have proved extremely useful in a research setting. In the United States, to conform to the CLIA regulation?, of 1988, also called CLIA '88, it is necessary to report these research results with a disclaimer unless all the CLIA requirements have been met.

Problem Strains

Most strains of Enterobacteriaceae grow rapidly on plating media and on media used lor biochemical identification, but occasionally a slow-growing or fastidious strain is encountered. Some strains grow poorly on blood agar but much better on chocolate agar incubated in a candle jar. This characteristic suggests a possible nutritional requirement or a mutation involving respiration.

There are slow-growing strains of E coli, Klebsiella pneumoniae, and Serratia marcesens, and typical biochemical reactions of these strains usually require extended incubation. Another type of problem organism is sometimes isolated from patients being treated with antimicrobial agents. Li et al. described such "pleiotropic" (having multiple phenotypic expression) mutants of S.marcescens (06) and salmonella after exposure to gentamicin. These strains react

[redacted]

atypicallv in many of the standard biochemical tests and are difficult to identify. A different type of pleiotropic mutant induced by chemical exposure was reported by Lintugan and Hussian (64). A Salmonella strain lost the ability to produce hydrogen sulfide, reduce nitrate to nitrite, and produce gas from glucose because of chlorate resistance acquired after exposure to Dakin's solution (a solution that contains chlorate and is found in hospitals). Similarly, “dwarfs colony forms of Salmonella serotype Typhi have been known tor many years. They are only 0.2 to 0.3 mm in diameter after 24 h of incubation but are normal size if the medium is supplemented with sulfite or thiosulfate. Some atypical and slowly growing strains become more typical and grow better after they have been transferred several times. Laboratories occasionally isolate strains that grow rapidly but have a biochemical reaction profile that does not fit (Table .3) any ol the described species, biogroups, or Enteric Groups of Enterobacteriaceae (56). At present, this type of culture can be reported only as "unidentified." It may be an atypical strain of one of the organisms listed in Tables I to 5, or it may belong to a new species that has not been described (37. 56. 94). Additional testing at a state, national, or

[redacted]

international reference laboratory can often answer the question about the culture’s identity and has led to the discovery of new causes of human infections (12-18, 37, 55, 56, 71, 94). Commercial Products and Services A wide variety of commercial products and diagnostic services are available for Enterobacteriaceae '. but availability is constantly changing. The best approach is to go to a suppliers' Internet site to check availability, technical information, and price. Products include routine and reference identification products and kits (with or without antimicrobial susceptibility tests), combination isolation-identification products, dehydrated media, ready-to-use media in tubes and plates, antisera. reagents, antibiotic products, cultures, and bacteriophages. Services include serodiagnosis, isolation. identification, antimicrobial susceptibility testing, molecular testing. serotyping. and subtyping. For more information, see chapters 15 and 16 of this Manual, the U. S. Food and Drug Administration's BAM Manual Online (http://www.cfean.fda.gov/~cb.irn ybam-toc. html), and references	[redacted]

55 and 92.	[redacted]
ANTIBIOTIC SUSCEPTIBILITY	[redacted]
Several methods are available for testing the antibiotic susceptibility of Enterobacteriaceae, but the most popular are disk diffusion (6) and broth dilution (sec chapters 17 and 70 to 78). Several textbook and infectious disease reviews describe antibiotic usage in clinical practice (4, 68, 69, 82).	[redacted]
When antibiotics were first introduced, there was only slight resistance among the species of Enterobacteriaceae,. Today, antibiotic resistance is much more common among strains isolated from humans and animals. Resistance patterns vary depending on the organism and its origin (4, 68,69, 82).	[redacted]
Intrinsic Resistance	[redacted]
Intrinsic resistance is a genetic property of most strains of a species and evolved long before the clinical use of antibiotics. For example, essentially all strains of Serratia marcescens have intrinsic resistance to penicillin G. colistin, and cephalothin. This evolution of resistance can best he shown by studying strains isolated and stored before the antibiotic era or by studying a large collection of strains from a wide variety of	[redacted]

sources including strains that have had little or no exposure to antibiotics. Table 10 lists some common Enterobacteriaceae and their intrinsic resistance patterns.	[redacted]
The Antibiogram as a Marker in Epidemiological Studies	[redacted]
Antibiotic susceptibility testing is usually done on isolates that are clinically significant and provides an antibiogram that is useful for comparing isolates in epidemiologic studies. When the selective ecological pressure of antibiotics i> changed, the resistance patterns of epidemic (or endemic) strains may also change (4, 68, 69, 82). These changes have been documented in outbreaks that have lasted for several months or longer. Even with these limitations in stability, the antibiogram is probably the most useful and practical laboratory marker for comparing strains and can be extremely helpful in recognizing and analyzing infection problems.	[redacted]
Use of Antibiograms for Identification	[redacted]
The antibiogram of a culture can be compared with those of known isolates (Table 10) to provide a different approach to identification. When the antibiogram and identification are	[redacted] Khi một kháng sinh đồ và kết quả định danh không

incompatible (for example, a strain of Klebsiella that is susceptible to ampicillin and carbcnicillin or a culture of Enterbacter that is susceptible to cephalothin). the culture should he streaked and checked for purity. In addition, both the identification and the antibiogram may have to be repeated.	tương thích với nhau (ví dụ, một dòng Klebsiella mẫn cảm với ampicillin và carbcnicillin hay một chủng Enterbacter mẫn cảm với cephalothin), chủng đó nên được rà soát và kiểm tra độ tinh khiết. Ngoài ra, cả kết quả định danh và kháng sinh đồ có thể phải được lặp lại.