CHAPTER

19 Enterobacteriaceae

Kimberly E. Walker, Connie R. Mahon, Donald Lehman, George Manuselis*

CHAPTER OUTLINE

OBJECTIVES

After reading and studying this chapter, you should be able to:

1. List the general characteristics of organisms that belong to the family Enterobacteriaceae.
2. Describe the antigenic structures of the family Enterobacteriaceae, and explain how these antigens are used for identification.
3. Compare the virulence factors of the *Escherichia coli* strains pathogenic for the gastrointestinal tract and the *E. coli* strains involved in extraintestinal diseases.
4. Compare the pathogenesis of the three species of *Yersinia* most often recovered from humans.
5. Describe the pathogenesis of the clinically relevant members of the family Enterobacteriaceae.
6. Given an organism's characteristic growth on nonselective and selective differential media, presumptively identify the isolate to the genus level.
7. Match the species of *Shigella* to their appropriate serogroup.
8. Given the key reactions for identification, place an unknown organism in its proper tribe, genus, and species.
9. Develop an algorithm using biochemical tests to presumptively identify clinically significant Enterobacteriaceae.

Case in Point

A 69-year-old diabetic woman hospitalized for an amputation complained of chest pain, a persistent cough, and shortness of breath. An expectorated sputum sample was plated. After 18 hours of incubation, a MacConkey (MAC) agar plate showed moderate growth of oxidase-negative, non–lactose-fermenting organisms. A sheep blood agar (SBA) plate showed very moist large isolated colonies. Biochemical tests to identify the isolate were performed with the following results: triple sugar iron, acid over acid with gas production; indole, methyl red, Voges-Proskauer, citrate (IMViC) reactions were – – + +; urea was hydrolyzed; lysine was decarboxylated; malonate was utilized; the organism was nonmotile.

*My comments are my own and do not represent the view of Health Resources and Services Administration of the Department of Health and Human Services.

Issues to Consider

After reading the patient's case history, consider:

- The significance of this patient's health status and medical history
- The colony morphology feature that provides clues about the identity of the organism
- The biochemical tests that are the most specific for identification of this organism

Key Terms

Buboes
Diffusely adherent *Escherichia coli* (DAEC)
Enterics
Enteroaggregative *Escherichia coli* (EAEC)
Enterohemorrhagic *Escherichia coli* (EHEC)
Enteroinvasive *Escherichia coli* (EIEC)
Enteropathogenic *Escherichia coli* (EPEC)
Enterotoxigenic *Escherichia coli* (ETEC)
H antigen
K antigen
O antigen
Shiga toxin (Stx)
Traveler's diarrhea
Verotoxin
Vi antigen

The family Enterobacteriaceae includes many genera and species. The last edition of *Bergey's Manual of Systematic Bacteriology* (Vol. 2) describes 176 named species among 44 different genera; however, clinical isolates in general acute-care facilities consist primarily of *Escherichia coli, Klebsiella pneumoniae,* and *Proteus mirabilis*. It is nonetheless important to be aware of the other species because they also cause significant infectious diseases.

This chapter is divided into three major areas: (1) clinically significant enteric species that cause opportunistic infections, (2) primary intestinal pathogens and their related human infections, and (3) methods of identification of these organisms. Among the many organisms in the family Enterobacteriaceae, this chapter focuses only on the members that have been associated with human diseases.

General Characteristics

The family Enterobacteriaceae, often referred to as **enterics**, consists of numerous diverse organisms. The Enterobacteriaceae have several key laboratory features in common, but as DNA studies on each of these organisms progress, classification of the members changes. Some organisms are being added (e.g., *Plesiomonas*), and some may eventually be removed from the family. Unlike other members of the family Enterobacteriaceae, *Plesiomonas* is oxidase-positive. This genus is discussed in Chapter 20 with the biochemically similar genera *Vibrio* and *Aeromonas*. Currently, several characteristics are still used to place an organism in the family Enterobacteriaceae (Box 19-1).

BOX 19-1 Key Characteristics of the Family Enterobacteriaceae

- They are gram-negative bacilli and coccobacilli.
- They do not produce cytochrome oxidase except for *Plesiomonas* (see Chapter 20).
- They all ferment glucose.
- They reduce nitrate to nitrite except for *Photorhabdus* and *Xenorhabdus*.
- They are motile at body temperatures except for *Klebsiella*, *Shigella*, and *Yersinia*.
- Except for *Klebsiella*, *Proteus*, and some *Enterobacter* isolates, none has remarkable colony morphology on laboratory media. They appear large, moist, and gray on sheep blood agar (SBA), chocolate (CHOC) agar, and most nonselective media.

Microscopic and Colony Morphology

Members of the family Enterobacteriaceae are gram-negative, non–spore-forming, facultatively anaerobic bacilli. On Gram-stained smears, they can appear as coccobacilli or as straight rods. Colony morphology on nonselective media, such as SBA or chocolate (CHOC) agar, is of little value in initial identification. With the exception of certain members (e.g., *Klebsiella* and sometimes *Enterobacter*) that produce characteristically large and very mucoid colonies, members of this family produce large, moist, gray colonies on nonselective media and are indistinguishable. However, many isolates of *E. coli* are β-hemolytic.

A wide variety of differential and selective media, such as eosin-methylene blue (EMB) and MAC agars, and highly selective media, such as Hektoen enteric (HE) and xylose-lysine-desoxycholate (XLD) agars, are available for presumptive identification of enteric pathogens. These media contain one or more carbohydrates, such as lactose and sucrose, which show the ability of the species to ferment specific carbohydrates. Fermentation is indicated by a color change on the medium, which results from a decrease in pH detected by a pH indicator incorporated into the medium. Nonfermenting species are differentiated by lack of color change, and colonies retain the original color of the medium. Species that produce hydrogen sulfide (H_2S) may be readily distinguished when placed on HE or XLD agar. HE and XLD agars contain sodium thiosulfate and ferric ammonium citrate, which produce blackening of H_2S-producing colonies. These features have been used initially to differentiate and characterize certain genera. Definitive identification depends on the biochemical reactions and serologic antigenic structures demonstrated by the particular species.

Classification

Members of the family are also subcategorized into numerous tribes based on biochemical characteristics. The use of tribes in classifying the members in this family was proposed by Ewing in 1963 and has been continued and extended. In classifying species into tribes, Ewing grouped bacterial species with similar biochemical characteristics. Within the tribes, organisms are classified further into genera and species. Differentiation of each genus and definitive identification of species are based on biochemical characteristics and DNA homology. Table 19-1 lists the

TABLE 19-1 Classification of Selected Species within the Family Enterobacteriaceae

Tribe	Genus	Species
I. Escherichieae	*Escherichia*	*coli*
		albertii
		blattae
		vulneris
		fergusonii
		hermanii
	Shigella	*dysenteriae*
		flexneri
		boydii
		sonnei
II. Edwardsielleae	*Edwardsiella*	*tarda*
		liquefaciens
		hoshinae
		ictaluri
III. Salmonelleae	*Salmonella*	*enterica*
		bongori
IV. Citrobacteriaceae	*Citrobacter*	*freundii*
		koseri (*C. diversus*)
		amalonaticus
		youngae
		braakii
		farmeri
V. Klebsielleae	*Klebsiella*	*pneumoniae* subsp. *pneumoniae*
		pneumoniae subsp. *ozaenae*
		pneumoniae subsp. *rhinoscleromatis*
		varicola
		ornitholytica
V. Klebsielleae, cont'd	*Enterobacter*	*aerogenes*
		cloacae
		gergoviae
		cancerogenus (*taylorae*)
		hormaechei
	Pantoea	*agglomerans*
	Cronobacter	*sakazakii*
	Hafnia	*alvei*
	Serratia	*marcescens*
		liquefaciens
		rubidaea
		fonticola
		odorifera
		plymuthica
VI. Proteeae	*Proteus*	*mirabilis*
		vulgaris
		penneri
		hauseri
		myxofaciens
	Morganella	*morganii*
	Providencia	*alcalifaciens*
		rettgeri
		stuartii
VII. Yersinieae	*Yersinia*	*pseudotuberculosis*
		pestis
		enterocolitica
		frederiksenii
		kristensenii
		intermedia
		ruckeri

Modified and revised from Ewing WH: *Edwards and Ewing's identification of Enterobacteriaceae*, ed 4, East Norwalk, CT, 1986, Appleton & Lange.

bacterial species in the family Enterobacteriaceae and their respective tribes; Table 19-2 shows the biochemical features that differentiate the tribes. The concept of using tribes in the classification of bacteria has been an effective way of placing species in groups based on similar biochemical features and is employed throughout this chapter.

Virulence and Antigenic Factors

The virulence of the Enterobacteriaceae genera is affected by many factors, such as the ability to adhere, colonize, produce toxins, and invade tissue. Some species harbor plasmids that can provide antimicrobial resistance genes. For example, an increasing number of *E. coli*, *K. pneumoniae*, and *Klebsiella oxytoca* clinical strains produce plasmid-mediated extended-spectrum β-lactamases (ESBLs) including carbapenemases, cephalosporinases, or metallo-β-lactamases, which can inactivate extended-spectrum cephalosporins (e.g., cefotaxime), penicillins, and aztreonam. Strains harboring these plasmids have been found in healthy volunteers and hospitalized patients. The *K. pneumoniae* carbapenemase, found in *Klebsiella* spp., *Enterobacter* spp., *Serratia marcescens*, and other genera, is the most common. Testing procedures for the detection of ESBLs are described in Chapter 13.

Many members of this family possess antigens that can be used in the identification of different serologic groups. These antigens include the following:

- **O antigen**, or somatic antigen—this is a heat-stable antigen located on the cell wall.
- **H antigen**, or flagellar antigen—this is a heat-labile antigen found on the surface of flagella, structures responsible for motility.
- **K antigen**, or capsular antigen—this is a heat-labile polysaccharide found only in certain encapsulated species. Examples are the K1 antigen of *E. coli* and the **Vi antigen** of *Salmonella enterica* subsp. *enterica* serotype Typhi.

Clinical Significance

Members of the family Enterobacteriaceae are ubiquitous in nature. Additionally, the Enterobacteriaceae, with few exceptions, share a common niche; they reside in the gastrointestinal (GI) tract. Except for *Salmonella*, *Shigella*, and *Yersinia*, they can be resident microbiota if confined to their natural environment. Paradoxically, they are often commensals, causing no harm, and yet they can be responsible for a large number of opportunistic infections when introduced into inappropriate body sites. Some species exist as free-living

TABLE 19-2 Biochemical Characteristics of Tribes of Enterobacteriaceae

Tests or Substrate	Escherichieae	Edwardsielleae	Citrobacteriaceae	Salmonelleae*	Klebsiellae	Proteeae†	Yersiniae
H_2S (TSI agar)	–	+	+ or –	+	–	+ or –	–
Urease	–	–	(+^{w}) or –	–	– or (+)	+ or –	+
Indole	+ or –	+	– or +	–	–	+ or –	+ or –
Methyl red	+	+	+	+	–	+	+
Voges-Proskauer	–	–	–	–	+	–	–
Citrate (Simmons)	–	–	+	+	+	d	–
KCN	–	–	+ or –	–	+	+	–
Phenylalanine deaminase	–	–	–	–	–	+	–
Mucate	d	–		d	+ or –	–	
Mannitol	+ or –	–	+	+	+	– or +	+

Modified from Ewing WH: *Edwards and Ewing's identification of Enterobacteriaceae*, ed 4, East Norwalk, CT, 1986, Appleton & Lange.
H_2S, Hydrogen sulfide; *KCN,* potassium cyanide; *TSI,* triple sugar iron; *+,* ≥90% positive within 1 or 2 days; *(+),* positive reaction after ≥3 days (decarboxylase tests: 3 or 4 days); *–,* ≥90% no reaction in 30 days; *+ or –,* most cultures positive, some strains negative; *– or +,* most strains negative, some cultures positive; *d,* different reactions, +, (+), –; +^{w}, weakly positive reaction.
**Salmonella* serovars Typhi and Paratyphi and some rare serovars fail to use citrate in Simmons medium. Cultures of serovar Paratyphi and some rare serotypes may fail to produce H_2S.
†Some cultures of *Proteus mirabilis* may yield positive Voges-Proskauer tests.

organisms in water, soil, or sewage, and some are plant pathogens.

Based on the clinical infections that they produce, members of the family Enterobacteriaceae may be divided into two broad categories: (1) opportunistic pathogens and (2) primary pathogens. The opportunistic pathogens are often a part of the usual intestinal microbiota of both humans and animals. However, outside their normal body sites, these organisms can produce serious extraintestinal, opportunistic infections, many of which are described in this chapter. For example, *E. coli*, one of the best-studied members of the Enterobacteriaceae, is a member of the normal bowel biota but can cause urinary tract infections (UTIs), septicemia, wound infections in healthy individuals, and meningitis in neonates.

Other organisms can be equally devastating in immunocompromised hosts or when introduced to wounds from contaminated soil or water. The primary pathogens, which include *S. enterica*, *Shigella* spp., and *Yersinia* spp., are considered true pathogens; that is, they are not present as commensal biota in the GI tract of humans. These organisms produce infections resulting from ingestion of contaminated food or water or from other sources, which are discussed in this chapter. Table 19-3 lists some diseases associated with members of the family Enterobacteriaceae.

TABLE 19-3 Bacterial Species and Infections They Commonly Produce

Bacterial Species	Diseases
Escherichia coli	Bacteriuria, septicemia, neonatal sepsis, meningitis, diarrheal syndrome
Shigella spp.	Diarrhea, dysentery
Edwardsiella spp.	Diarrhea, wound infection, septicemia, meningitis, enteric fever
Salmonella spp.	Septicemia, enteric fever, diarrhea
Citrobacter spp.	Opportunistic and hospital-acquired infections (wound, urinary)
Klebsiella spp.	Bacteriuria, pneumonia, septicemia
Enterobacter spp.	Opportunistic and hospital-acquired infection, wound infections, septicemia, bacteriuria
Serratia spp.	Opportunistic and hospital-acquired infection, wound infections, septicemia, bacteriuria
Proteus spp.	Bacteriuria, wound infection, septicemia
Providencia spp.	Opportunistic and hospital-acquired infection, wound infections, septicemia, bacteriuria
Morganella spp.	Opportunistic and hospital-acquired infections
Yersinia	
Y. pestis	Plague
Y. pseudotuberculosis	Mesenteric adenitis, diarrhea
Y. enterocolitica	Mesenteric adenitis, diarrhea
Erwinia spp.	Wounds contaminated with soil or vegetation
Pectobacterium spp.	Wounds contaminated with soil or vegetation

Modified from Washington J: *Laboratory procedures in clinical microbiology*, ed 2, New York, 1981, Springer-Verlag.

Opportunistic Members of the Family Enterobacteriaceae and Associated Infections

Escherichia coli

E. coli, the most significant species in the genus *Escherichia*, was first described by Escherich in 1885. *E. coli* was initially considered a harmless member of the colon resident biota. It is now recognized as an important human pathogen associated with a wide range of clinical syndromes, including UTIs, diarrheal diseases, and central nervous system infections. It is so commonly

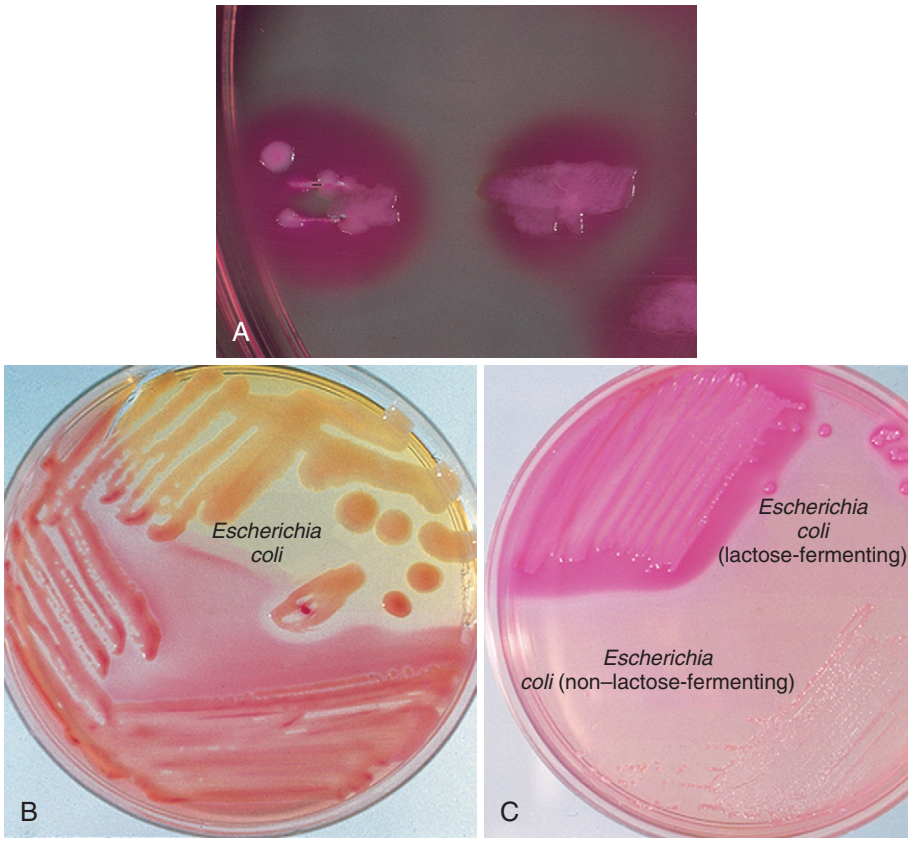

FIGURE 19-1 **A,** Typical dry, lactose-positive *Escherichia coli* growing on MacConkey (MAC) agar. Note the pink precipitate surrounding the individual colonies. **B,** Mucoid colonies of *E. coli* growing on MAC agar. **C,** Non–lactose-fermenting *E. coli* compared with typical lactose-fermenting *E. coli* on MAC agar. (**B** and **C,** Courtesy Jean Barnishan.)

isolated from colon biota that *E. coli* is used as a primary marker of fecal contamination in water quality testing.

Most strains of *E. coli* are motile and generally possess adhesive fimbriae and sex pili and O, H, and K antigens. *E. coli* O groups have shown remarkable cross-reactivity with O antigens from other members of the Enterobacteriaceae, most notably *Shigella* spp. This is one of the reasons *Escherichia* and *Shigella* spp. are grouped together in the tribe Escherichiae. Serotyping for both O and H antigens is often useful in identification of strains, particularly strains associated with serious enteric disease. The K antigen often masks the O antigen during bacterial agglutination testing with specific antiserum. This phenomenon can make it difficult to determine the serotype.

On certain selective and differential media, such as MAC or EMB agars, *E. coli* has a distinctive morphology. It usually appears as a lactose-positive (pink) colony with a surrounding area of precipitated bile salts on MAC agar (Figure 19-1). On EMB agar, it has a green metallic sheen. *E. coli* is associated with the following properties:

- Fermentation of glucose, lactose, trehalose, and xylose
- Production of indole from tryptophan
- Glucose fermentation by the mixed acid pathway: methyl red-positive and Voges-Proskauer-negative
- Does not produce H_2S, DNase, urease, or phenylalanine deaminase
- Cannot use citrate as a sole carbon source

Uropathogenic *Escherichia coli*

E. coli is widely recognized as the most common cause of UTIs in humans. The *E. coli* strains that cause UTIs usually originate in the large intestine as resident biota and can exist either as the predominant *E. coli* population or as a small part of the *E. coli* strains in the large intestine. Strains causing lower UTIs and acute pyelonephritis in immunocompetent hosts are different from strains causing disease in the urinary tracts of individuals who are compromised either by urinary tract defects or by instrumentation such as placement of catheters. *E. coli* strains that cause acute pyelonephritis in immunocompetent hosts have been shown to be the dominant resident *E. coli* in the colon. They belong to a few serotypes and are resistant to the antibacterial activity of human serum. Conversely, isolates from immunocompromised hosts consist of a wide variety of strains.

Strains that cause UTIs produce factors that allow them to attach to the urinary epithelial mucosa. The primary virulence factor associated with the ability of *E. coli* to cause UTIs is the production of pili, which allow uropathogenic strains to adhere to epithelial cells and not be washed out with urine flow. Other factors also contribute to the virulence of uropathogenic *E. coli*, such as cytolysins and aerobactins. Cytolysins (also often characterized as hemolysins) can kill immune effector cells and inhibit phagocytosis and chemotaxis of certain white blood cells.

Aerobactin allows the bacterial cell to chelate iron; free iron is generally unavailable within the host for use by bacteria.

Gastrointestinal Pathogens

E. coli may cause several different GI syndromes (Table 19-4). Based on virulence factors, clinical manifestation, epidemiology, and different O and H serotypes, there are five major categories of diarrheogenic *E. coli*: **enterotoxigenic *Escherichia coli* (ETEC)**, **enteroinvasive *Escherichia coli* (EIEC)**, **enteropathogenic *Escherichia coli* (EPEC)**, **enterohemorrhagic *Escherichia coli* (EHEC)**, and enteroadherent, which includes **diffusely adherent *Escherichia coli* (DAEC)** and **enteroaggregative *Escherichia coli* (EAEC)**. These five categories are sometimes collectively referred to as enterovirulent *E. coli* or diarrheogenic *E. coli*. The serotypes associated with these categories and the features associated with the intestinal infections produced by these strains are summarized in Chapter 34.

Enterotoxigenic *Escherichia coli*. ETEC strains are associated with diarrhea of adults and especially children in tropical and subtropical climates, especially in developing countries, where it is one of the major causes of infant bacterial diarrhea. In the United States and other Western industrialized nations, ETEC diarrhea is the most common cause of a diarrheal disease sometimes referred to as **traveler's diarrhea**. Travelers from industrialized countries often become infected with ETEC when they visit developing nations. ETEC infection is spread commonly via consumption of contaminated food or water. Poor hygiene, reduced availability of sources of potable water, and inadequate sanitation are major contributing factors in the spread and transmission of this disease. A high infective dose (10^6 to 10^{10} organisms) is necessary to initiate disease in an immunocompetent host. Protective mechanisms such as stomach acidity have been described as inhibiting colonization and initiation of disease; individuals with *achlorhydria* (deficiency of

TABLE 19-4 Features of Pathogenic *Escherichia coli*

Type	Virulence Factors	Relevant Disease	Relevant Serotypes	Laboratory Tests
Uropathogenic *E. coli*				
UPEC	P pilus/*pap* pili, type 1 fimbriae	UTIs		
DAEC*	Afa/Dr adhesions	UTIs		
Enteric Pathogens				
EPEC	Pathogenicity islands	Infantile diarrhea	O55:NM O55:H6 O111:NM O111:H2 O114:NM O114:H2	HeLa cell adherence assay, DNA probes
EHEC	Shiga toxin/verotoxin	Hemorrhagic diarrhea, colitis, HUS	O157:H7 O157:NM O26:H11 O104:H21 O111:H2 O111:H8 O113:H21 O118:H2	SMAC plates, MUG
EIEC	Invasin	Dysentery	O124:H30 O143:NM O164:NM	DNA probes
ETEC	LT, ST	Traveler's diarrhea	O6:NM O6:H16 O8:H9 O25:NM O27:NM O63:H12	Immunoassays for LT or ST
Enteroadherent *E. coli*				
EAEC	AAF fimbriae Afa/Dr adhesions, AIDA-1, pathogenicity islands	Persistent pediatric diarrhea	O44:H18	
DAEC*		Pediatric diarrhea, UTIs		HeLa cell adherence assay, DNA probes
Extraintestinal Pathogens				
	Capsule	Septicemia and meningitis	K1	

DAEC, Diffusely adherent *E. coli*; *EAEC,* enteroaggregative *E. coli*; *EHEC,* enterohemorrhagic *E. coli*; *EIEC,* enteroinvasive *E. coli*; *EPEC,* enteropathogenic *E. coli*; *ETEC,* enterotoxigenic *E. coli*; *HUS,* hemolytic uremic syndrome; *LT,* labile toxin; *MUG,* 4-methylumbelliferyl β-D-glucuronide; *NM,* nonmotile; *SMAC,* MacConkey agar containing sorbitol; *ST,* stable toxin; *UPEC,* uropathogenic *E. coli*; *UTI,* urinary tract infection.
*DAEC causes both UTIs and gastrointestinal infections.

hydrochloric acid within the stomach) seem to be at higher risk than are normal individuals.

Colonization of ETEC on the proximal small intestine has been recognized as being mediated by fimbriae that permit ETEC to bind to specific receptors on the intestinal microvilli. Once ETEC strains are established, they can release one or both of two toxins into the small intestine. They produce a heat-labile toxin (LT), which is similar in action and amino acid sequence to cholera toxin from *Vibrio cholerae*. LT consists of two fragments (A and B), which follow the A/B model of bacterial toxins, where A is the enzymatically active portion. The B moiety, or binding portion, confers the specificity. The B portion binds to the GM_1 ganglioside of the intestinal mucosa, providing entry for the A portion.

During infection, the A portion activates cellular adenylate cyclase, causing an increase in the conversion of adenosine triphosphate to cyclic adenosine monophosphate (cAMP). The consequence of accumulation of cAMP is hypersecretion of both electrolytes and fluids into the intestinal lumen, resulting in watery diarrhea similar to cholera. In contrast, the heat-stable toxin (ST) stimulates guanylate cyclase, causing increased production of cyclic guanosine monophosphate, accumulation of which also causes hypersecretion.

The usually mild, self-limiting disease caused by ETEC is characterized by watery diarrhea, abdominal cramps, and sometimes nausea, usually with no vomiting or fever. Mucosal penetration and invasion do not appear to be part of ETEC disease. Diagnosis of ETEC infection is made primarily by the characteristic symptoms and the isolation of solely lactose-fermenting organisms on differential media. Testing for toxins or colonizing factors is performed by research and reference laboratories; its use is not justified in the clinical laboratory for diagnostic purposes. Enzyme-labeled oligonucleotide probes have been reported to detect ETEC in fecal specimens, but this method is undergoing further testing to determine its efficacy. ETEC infections must be differentiated from other diarrheal illnesses that may appear similar.

Enteropathogenic *Escherichia coli.* Although the EPEC strain has been known to cause infantile diarrhea since the 1940s, its pathogenic role has remained controversial over the last few decades. Certain O serogroups of EPEC were identified in the late 1960s and 1970s as a cause of diarrhea, but only certain H antigenic types within each O serogroup were connected to the intestinal infections. However, O serogrouping could not differentiate these *E. coli* strains from strains of normal biota. In 1978, Levine and colleagues attempted to settle the dispute concerning the pathogenic role of EPEC by challenging volunteers with EPEC strains that lacked the toxins of ETEC and the invasiveness of EIEC. The study showed that these EPEC strains caused distinct diarrhea. Subsequent studies showed the adhesive property of EPEC strains, a characteristic not seen in ETEC or EIEC strains.

Diarrheal outbreaks caused by EPEC have occurred in hospital nurseries and daycare centers, but cases in adults are rarely seen. The illness is characterized by low-grade fever, malaise, vomiting, and diarrhea. The stool typically contains large amounts of mucus, but apparent blood is not present. Detection of diarrheal illness attributable to EPEC depends primarily on the suspicion of the physician. In cases of severe diarrhea in children younger than 1 year, infection with EPEC should be suspected. Serologic typing with pooled antisera may be performed to identify EPEC serotypes, but this is generally used for epidemiologic studies rather than for diagnostic purposes.

Enteroinvasive *Escherichia coli.* EIEC differs greatly from EPEC and ETEC strains. EIEC infection is rare in the United States and seen less commonly in developing countries than ETEC or EPEC. Enteroinvasive strains produce dysentery with direct penetration, invasion, and destruction of the intestinal mucosa. This diarrheal illness is very similar to that produced by *Shigella* spp. EIEC infections seem to occur in adults and children alike. Direct transmission of EIEC from person to person via the fecal-oral route has been reported. The clinical infection is characterized by fever, severe abdominal cramps, malaise, and watery diarrhea.

The organisms might be easily misidentified because of their similarity to shigellae. EIEC strains can be nonmotile and generally do not ferment lactose; cross-reaction between shigellae and EIEC O antigens has been reported. EIEC isolates may be mistaken for nonpathogenic *E. coli*; although EIEC do not decarboxylate lysine, more than 80% of *E. coli* strains do decarboxylate lysine. For these reasons, cases of diarrheal illness resulting from EIEC might be underreported.

Although EIEC and *Shigella* spp. are morphologically similar and produce similar clinical disorders, the infective dose of EIEC necessary to produce disease is much higher (10^6) than that of shigellae (about 100 bacterial cells). The enteroinvasiveness of EIEC has to be demonstrated for definitive identification. The tests currently available to determine the invasive property of EIEC are not performed in most clinical microbiology laboratories. It is possible to detect invasiveness using monolayer cell cultures with human epithelial–2 (HEp-2) cells. DNA probes for EIEC more recently have become commercially available. These kits are used to screen stool samples, eliminating the need for other tests to identify EIEC.

Enterohemorrhagic *Escherichia coli.* In 1982, the O157:H7 strain of *E. coli* was first recognized during an outbreak of hemorrhagic diarrhea and colitis. The EHEC strain serotype O157:H7 has since been associated with hemorrhagic diarrhea, colitis, and hemolytic uremic syndrome (HUS). HUS is characterized by low platelet count, hemolytic anemia, and kidney failure.

The classic illness caused by EHEC produces a watery diarrhea that progresses to bloody diarrhea with abdominal cramps and low-grade fever or an absence of fever. The stool contains no leukocytes, which distinguishes it from dysentery caused by *Shigella* spp. or EIEC infections. The infection is potentially fatal, especially in young children and elderly adults in nursing homes. Processed meats such as undercooked hamburgers served at fast-food restaurants, unpasteurized dairy products and apple cider, bean sprouts, and spinach all have been implicated in the spread of infection. In 2009, EHEC was the cause of a multistate outbreak linked to prepackaged cookie dough. Approximately 80 people in 31 states were affected. Infection was associated with consumption of raw dough.

E. coli O157:H7 produces two cytotoxins: **verotoxin** I and verotoxin II. Verotoxin I is a phage-encoded cytotoxin identical to the **Shiga toxin (Stx)** produced by *Shigella dysenteriae* type I. This toxin produces damage to Vero cells (African green

monkey kidney cells)—hence the term *verotoxin*. It also reacts with and is neutralized by the antibody against Stx. In contrast, verotoxin II is not neutralized by antibody to Stx. Verotoxin II is biologically similar to, but immunologically different from, both Stx and verotoxin I. These toxins have also been reported under the term *Shiga-like toxins* but are most likely to be found in the literature as Shiga toxin I (Stx1) and Shiga toxin 2 (Stx2); *E. coli* strains that produce these toxins are also called Shiga toxin–producing *E. coli* (STEC). Several different STEC strains have been identified; O157:H7 is only the first to have been widely reported. Any of the STEC serotypes can cause clinical syndromes similar to that produced by O157:H7 *E. coli*. Table 19-4 lists non-O157:H7 EHEC/STEC isolated from patients with bloody diarrhea, hemorrhagic colitis, or HUS. It is estimated that 73,000 infections and 60 deaths are caused by STEC annually in the United States.

In the laboratory, verotoxin-producing *E. coli* may be identified by one of three methods:

- Stool culture on highly differential medium, with subsequent serotyping
- Detecting the verotoxin in stool filtrates
- Demonstration of a fourfold or greater increase in verotoxin-neutralizing antibody titer

Stool culture for *E. coli* O157:H7 may be performed using MAC agar containing sorbitol (SMAC) instead of lactose. *E. coli* O157:H7 does not ferment sorbitol in 48 hours, a characteristic that differentiates it from most other *E. coli* strains. *E. coli* O157:H7 appears colorless on SMAC agar, whereas most other strains produce pink colonies for sorbitol fermentation. The use of this differential medium facilitates the primary screening of *E. coli* O157:H7, which ordinarily would not be distinguishable from other *E. coli* strains on lactose-containing MAC or other routine enteric agar. Although isolation of other non–sorbitol-fermenting organisms may occur in 15% of cultures, *E. coli* O157:H7, when present, produces heavy growth. An emergent phenotype, the sorbitol-fermenting, nonmotile *E. coli* O157:NM (NM indicates nonmotile) has been seen increasingly in European outbreaks, so relying on sorbitol as a single test to identify O157 strains is unwise.

In addition to sorbitol fermentation, the commercially available 4-methylumbelliferyl-β-D-glucuronide (MUG) assay is a biochemical test used to screen isolates for *E. coli* O157:H7. *E. coli* O157:H7 rarely produces the enzyme β-glucuronidase, whereas 92% of other strains do produce it. If the enzyme is present, MUG is cleaved, and a fluorescent product is formed. Sorbitol-negative and MUG-negative colonies are subsequently subcultured for serotyping using *E. coli* O157:H7 antiserum.

Enzyme-linked immunosorbent assay (ELISA) or latex agglutination can be used to detect the O157 antigen. In the latex agglutination assay, isolates must be tested with the negative control to detect nonspecific agglutination. The O157 somatic antigen, which is usually the target in the commercial assays, can present a problem with regard to specificity because other enteric bacteria produce false-positive results. It is also important to confirm biochemically the identification of MUG-negative or sorbitol-negative colonies as *E. coli* isolates. A latex test to detect H7 antigen is available as well. When testing colonies taken directly from the SMAC plate, the test for the H7 antigen may be initially negative. It is helpful to grow these isolates in motility media first to enhance flagella production and agglutination with the latex particles.

After serotyping, isolates are tested for the presence of Stx. Reports have shown that all *E. coli* O157:H7 strains produce high levels of cytotoxins, and STEC strains may be detected using cell culture assays with Vero cells. Because other toxins present in diarrhea stools can produce similar cytopathic effects, this test must be verified with specific antitoxins to Stx1 and Stx2. Free verotoxins present in stool specimens have been detected in samples that yielded negative culture results. It was previously reported that patients with hemorrhagic colitis shed the organisms for only brief periods; nevertheless, verotoxins may still be detected in the stool. An approved ELISA test from Meridian Diagnostics, Inc (Cincinnati, OH) is able to detect Stx in bloody stools, although not all patients have bloody stools. Gene amplification assays such as the GeneGen EHEC Detection Kit available in Europe from SY-LAB (Geräte GmbH, Austria) may be useful in detecting STEC strains. A fourfold increase in verotoxin-neutralizing antibody titer has been demonstrated in patients with HUS and in whom verotoxin or verotoxin-producing *E. coli* has been detected.

Enteroadherent *Escherichia coli*. Enteroadherent *E. coli* strains are generally associated with two kinds of human disease: diarrheal syndromes and UTIs. The two types of enteroadherent *E. coli* are DAEC and EAEC. DAEC may be associated with UTIs and diarrheal disease. Uropathogenic DAEC strains are closely associated with cystitis in children and acute pyelonephritis in pregnant women. They also seem to be associated with chronic or recurring UTIs. Several strains of DAEC have been associated with pediatric diarrheal disease, particularly in developing nations. However, other studies with adult volunteers did not demonstrate diarrheal disease.

EAEC causes diarrhea by adhering to the surface of the intestinal mucosa. These strains are found to adhere to HEp2 cells, packed in an aggregative "stacked-brick" pattern on the cells and between the cells by means of fimbriae. These organisms produce watery diarrhea, vomiting, dehydration, and occasionally abdominal pain, mostly in children. White blood cells and red blood cells are typically absent from the stool. The symptoms typically persist for at least 2 or more weeks. EAEC may be an important cause of diarrhea in infants in the United States and should be considered a cause of diarrhea in patients with human immunodeficiency virus infection. In 2011, an outbreak in Europe caused by STEC (O104:H4) that also had virulence related to EAEC resulted in more than 3400 total infections and about 850 cases of HUS with 32 HUS-related deaths. Most of the cases occurred in Germany, and trace-back studies indicated that fresh sprouts produced by a farm in Lower Saxony were responsible for the outbreak. At least two cases were reported in the United States in individuals who had recently traveled to Germany.

Extraintestinal Infections

E. coli remains one of the most common causes of septicemia and meningitis among neonates, accounting for about 40% of the cases of gram-negative meningitis. Similar infections resulting from this organism are uncommon in older children. A newborn usually acquires the infection in the birth canal just before or during delivery, when the mother's vagina is heavily colonized.

Infection may also result if contamination of the amniotic fluid occurs.

The strains associated with diarrheal disease appear to be distinct from strains associated with neonatal sepsis or meningitis. The capsular antigen K1 present in certain strains of *E. coli* is the most documented virulence factor associated with neonatal meningeal infections. *E. coli* K1 capsule is immunochemically identical to the capsular antigen of *N. meningitidis* group B. The association of K1 antigen was established when *E. coli* strains possessing the capsular K1 antigen were isolated from neonates with septicemia or meningitis. Fatality rates for infants with meningitis caused by *E. coli* K1 were higher than the fatality rates for infants infected with non-K1 strains. In addition to the neonatal population, *E. coli* remains as a clinically significant isolate in blood cultures from adults. *E. coli* bacteremia in adults may result primarily from a urogenital tract infection or from a GI source.

Other *Escherichia* Species

There are currently six recognized species within the genus *Escherichia*. *Escherichia hermannii* is a yellow-pigmented organism that has been isolated from cerebrospinal fluid (CSF), wounds, and blood. Reports of isolating *E. hermannii* from foodstuffs such as raw milk and beef, the same sources as *E. coli* O157:H7, have been published. However, its clinical significance is not yet established.

Escherichia vulneris has been isolated from humans with infected wounds. More than half of the strains of *E. vulneris* also produce yellow-pigmented colonies. Figure 19-2 compares the colony morphology of *E. hermannii* with *E. vulneris*. The newest species added to this genus, *Escherichia albertii*, is associated with diarrheal disease in children. The other members of the tribe Escherichiae, the shigellae, are discussed in the section on enteric pathogens of this chapter.

Klebsiella and *Raoutella*

Members of the genera *Klebsiella*, *Enterobacter*, *Serratia*, *Pantoea*, *Cronobacter*, and *Hafnia* belong to the tribe Klebsielleae. Members of these genera are usually found in the intestinal tract of humans and animals or free-living in soil, water, and on plants. These microorganisms have been associated with various opportunistic and hospital-acquired infections, particularly pneumonia, wound infections, and UTIs. Members of these genera demonstrate variable biochemical reactions. Common characteristics include the following:

- Most grow on Simmons citrate and in potassium cyanide broth.
- None produce H_2S.
- A few hydrolyze urea slowly.
- All give a negative reaction with the methyl red test and a positive reaction with the Voges-Proskauer test.
- With a few exceptions, no indole is produced from tryptophan.
- Motility is variable.

✓ Case Check 19-1

The organism described in the Case in Point at the beginning of the chapter showed morphologic and biochemical characteristics of the Klebsielleae. The polysaccharide capsule of *K. pneumoniae*, a distinctive feature, accounts for the moist, mucoid colonies observed in this species.

Usually found in the GI tract of humans and animals, the genus *Klebsiella* consists of several species, including *K. pneumoniae* subsp. *pneumoniae*, *K. oxytoca*, *K. pneumoniae* subsp. *ozaenae*, *K. pneumoniae* subsp. *rhinoscleromatis*, *K. ornitholytica*, *K. planticola*, and *K. terrigena*. The absence of motility distinguishes *Klebsiella* spp. from most other members of the family Enterobacteriaceae. Differential features of *Klebsiella* spp. are shown in Table 19-5. *K. pneumoniae* is the most commonly isolated species and has the distinct feature of possessing a large polysaccharide capsule. The capsule offers the organism protection against phagocytosis and antimicrobial absorption, contributing to its virulence. The capsule is also responsible for the moist, mucoid colonies characteristic of *K. pneumoniae*. Occasionally evident in direct smears from clinical materials, this capsule is sometimes helpful in providing a presumptive identification. Figure 19-3 illustrates the mucoid appearance of *K. pneumoniae* on MAC agar.

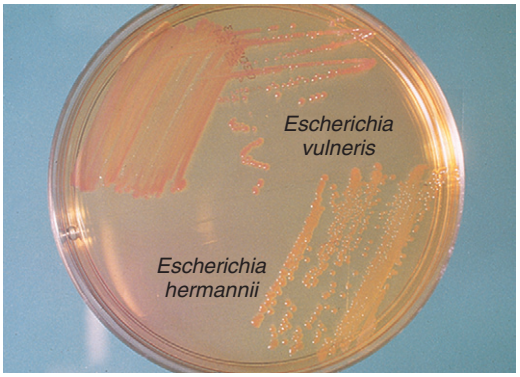

FIGURE 19-2 Comparison of the colony morphology of *Escherichia vulneris* and a yellow-pigmented *Escherichia hermannii* on MacConkey (MAC) agar. *Escherichia vulneris* may also produce a yellow-pigmented colony, but the yellow is more prevalent in *E. hermannii.* (Courtesy Jean Barnishan.)

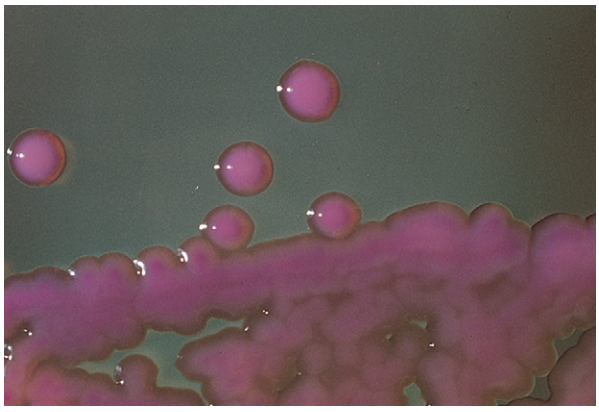

FIGURE 19-3 Mucoid appearance of *Klebsiella pneumoniae* on MacConkey (MAC) agar.

TABLE 19-5 Differentiation of Common Species within the Genus *Klebsiella*

	K. pneumoniae subsp. *pneumoniae*			*K. oxytoca*			*K. pneumoniae* subsp. *ozaenae*		
Test or Substrate	**Sign**	**% +**	**(% +)**	**Sign**	**% +**	**(% +)**	**Sign**	**% +**	**(% +)**
Urease	+	95.4	(0.1)	+	90		d	0	(14.8)
Indole	–	0		+	99		–	0	
Methyl red	– or +	10		–	20		+	97.7	
Voges-Proskauer	+	98		+	96		–	0	
Citrate (Simmons)	+	98	(0.6)	+	95		d	30	(32.4)
Gelatin (22°C)	–	0	(0.2)	–	0		–	0	
Lysine decarboxylase	+	98	(0.1)	+	99		– or +	40	(6.3)
Malonate	+	92.5		+	98		–	6	
Mucate	+	90		+	93		– or +	25	
Sodium alginate (utilization)	+ or (+)	88.5	(9.2)	nd			– or (+)	0	(11)
Gas from glucose	+	96		+	97		d	50	(9.4)
Lactose	+	98.7	(1)	+	100		d	30	(61.3)
Dulcitol	– or +	30		+or–	55		–	0	
Organic acid media									
Citrate	+ or –	64.4		nd			– or +	18	
D-Tartrate	+ or –	67.1		nd			– or +	39	

Modified from Ewing WH: *Edwards and Ewing's identification of Enterobacteriaceae*, ed 4, East Norwalk, CT, 1986, Appleton and Lange.
+, ≥90% positive within 1 or 2 days; *(+)*, positive reaction after ≥3 days (decarboxylase tests: 3 or 4 days); *–*, ≥90% no reaction in 30 days; *+ or –*, most cultures positive, some strains negative; *– or +*, most strains negative, some cultures positive; *d*, different reactions, +, (+), –; *nd*, no data.

Colonization of gram-negative bacilli in the respiratory tracts of hospitalized patients, particularly by *K. pneumoniae*, increases with the length of hospital stay. *K. pneumoniae* is a frequent cause of lower respiratory tract infections among hospitalized patients and in immunocompromised hosts such as newborns, elderly patients, and seriously ill patients on respirators.

✓ Case Check 19-2

The Case in Point illustrates typical predisposing risk factors for increased colonization of gram-negative bacilli in the respiratory tract of hospitalized patients. These include length of hospital stay, weakened immune response of elderly patients, and underlying serious illnesses. *K. pneumoniae* is among the most commonly recovered isolates from respiratory tract samples of hospitalized patients.

Other infections commonly associated with *K. pneumoniae* involving immunocompromised hosts are wound infections, UTIs, liver abscesses, and bacteremia. Reports describe hospital-acquired outbreaks of *Klebsiella* resistant to multiple antimicrobial agents in newborn nurseries. These outbreaks have been attributed to the plasmid transfer of antimicrobial resistance. Although antimicrobial resistance has been increasing within the family Enterobacteriaceae, it is probably most severe with *K. pneumoniae* because of the presence of the *K. pneumoniae* carbapenemase.

Other *Klebsiella* spp. have been associated with numerous infections. *K. oxytoca* produces infections similar to those caused by *K. pneumoniae*. In addition, isolates have been linked to antibiotic-associated hemorrhagic colitis. Biochemically, *K. oxytoca* is identical to *K. pneumoniae* except for its production of indole, and there are reports of ornithine-positive isolates as well. *K. pneumoniae* subsp. *ozaenae* has been isolated from nasal secretions and cerebral abscesses. This organism causes atrophic rhinitis, a tissue-destructive disease restricted to the nose. As previously mentioned, *K. pneumoniae* subsp. *ozaenae* is highly associated with the presence of plasmid-mediated ESBLs, contributing to the large numbers of antimicrobial-resistant hospital-acquired infections seen today.

K. pneumoniae subsp. *rhinoscleromatis* has been isolated from patients with rhinoscleroma, an infection of the nasal cavity that manifests as an intense swelling and malformation of the entire face and neck. Cases of rhinoscleroma have been reported in Africa and South America. Originally, both *K. ozaenae* and *K. rhinoscleromatis* were considered true species. Based on nucleic acid studies, they have been reclassified as subspecies of *K. pneumoniae*. Neither subspecies is isolated from the environment or GI tract; both are more commonly seen in tropical regions. *Raoutella* (*Klebsiella*) *ornithinolytica* (indole and ornithine decarboxylase-positive) and *Raoutella* (*Klebsiella*) *planticola* have been isolated from the urine, respiratory tracts, and blood of humans. *R. planticola* is difficult to distinguish from *K. pneumoniae*. *K. variicola* has been isolated from primarily sterile sites.

Enterobacter, Cronobacter, and *Pantoea*

The genus *Enterobacter* is composed of at least 12 species. Clinically significant *Enterobacter* spp. that have been isolated from clinical samples include *Enterobacter cloacae*, *Enterobacter aerogenes*, *Enterobacter gergoviae*, and *Enterobacter hormaechei*. Members of this genus are motile. The colony morphology of many of the species resembles *Klebsiella* when growing on MAC agar. *Enterobacter* spp. grow on Simmons citrate medium and in potassium cyanide broth; the methyl red test is negative, and the Voges-Proskauer test is positive. However, in contrast

TABLE 19-6 Diagnostic Features of *Enterobacter cloacae*, *Enterobacter aerogenes*, and *Klebsiella pneumoniae* subsp. *pneumoniae*

	E. cloacae			*E. aerogenes*			*K. pneumoniae* subsp. *pneumoniae*		
Test or Substrate	**Sign**	**% +**	**(% +)**	**Sign**	**% +**	**(% +)**	**Sign**	**% +**	**(% +)**
Urease	+w or –	65		–	2		+	95.4	(0.1)
Motility	+	95		+	97		–	0	
Lysine decarboxylase	–	0		+	98		+	98	(6.3)
Arginine dihydrolase	+	97	(2)	–	0		–	0	
Ornithine decarboxylase	+	96	(1.3)	+	98	(0.8)	–	0	
Gelatin (22°C)	(+)	0	(94.2)	(+)or–	0	(61.2)	–	0	(0.2)
Adonitol, gas	– or +	21.7	(1.3)	+	94.2		d	84.4	(0.3)
Inositol									
Acid	d	13	(8)	+	96.7		+	97.2	(0.9)
Gas	–	4.1	(1.5)	+	93.4		+	92.5	(1.5)
D-tartrate, Jordan's	– or +	30		+	95		+	95	
Sodium alginate (utilization)	–	0		–	0		+ or (+)	88.9	(8.9)

Modified from Ewing WH: *Edwards and Ewing's identification of Enterobacteriaceae*, ed 4, East Norwalk, CT, 1986, Appleton & Lange.
+, ≥90% positive within 1 or 2 days; *(+)*, positive reaction after ≥3 days (decarboxylase tests: 3 or 4 days); *–*, ≥90% no reaction in 30 days; *+ or –*, most cultures positive, some strains negative; *– or +*, most strains negative, some cultures positive; *d*, different reactions, +, (+), –; *+w*, weakly positive reaction.

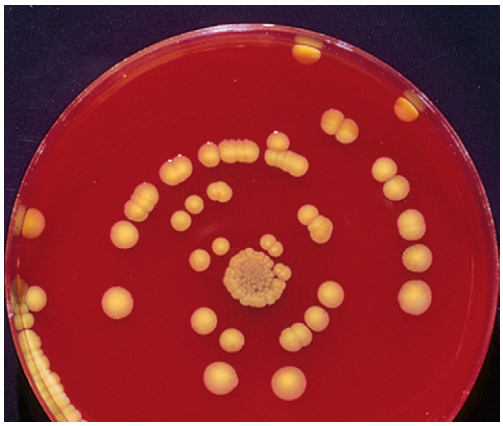

FIGURE 19-4 Yellow-pigmented *Pantoea agglomerans* on sheep blood agar (SBA). (Courtesy Jean Barnishan.)

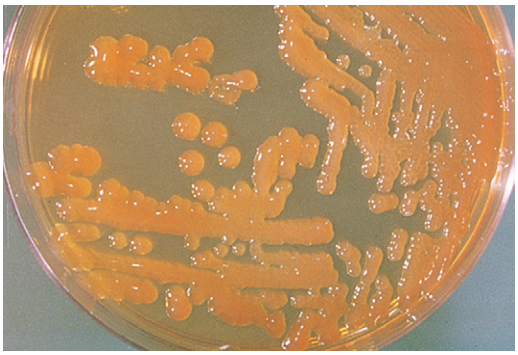

FIGURE 19-5 Mucoid, yellow-pigmented colonies of *Cronobacter sakazakii* growing on brain–heart infusion agar. (Courtesy Jean Barnishan.)

to *Klebsiella*, *Enterobacter* spp. usually produce ornithine decarboxylase; lysine decarboxylase is produced by most species but not by *E. gergoviae* or *E. cloacae*.

E. cloacae and *E. aerogenes* are the two most common isolates from this group. These two species have been isolated from wounds, urine, blood, and CSF. Distinguishing characteristics among *E. cloacae*, *E. aerogenes*, and *K. pneumoniae* are shown in Table 19-6. *Pantoea* (*Enterobacter*) *agglomerans* gained notoriety with a nationwide outbreak of septicemia resulting from contaminated intravenous fluids. Designated early on as *E. agglomerans* complex, it includes members that are lysine-, ornithine-, and arginine-negative or "triple decarboxylases-negative." More than 13 hybridization groups (HGs) have been described in this complex. *P. agglomerans* HG XIII, which may produce a yellow pigment, is primarily a plant pathogen. Figure 19-4 depicts a yellow-pigmented *P. agglomerans*.

E. gergoviae is found in respiratory samples and is rarely isolated from blood cultures. *Cronobacter* (*Enterobacter*) *sakazakii* typically produces a yellow pigment and has been documented as a pathogen in neonates causing meningitis and bacteremia, often coming from powdered infant formula. It has also been isolated from cultures taken from brain abscesses and respiratory and wound infections. Figure 19-5 illustrates the colony morphology of *C. sakazakii*. *E. hormaechei* has been isolated from human sources such as blood, wounds, and sputum. *E. asburiae* is similar biochemically to *E. cloacae* and has been isolated from blood, urine, feces, sputum, and wounds. *Enterobacter dissolvens* and *Enterobacter nimipressuralis* are newly recognized species with unknown clinical significance. *E. cancerogenus* (formerly *E. taylorae*) has been associated with osteomyelitis after traumatic wounds.

Serratia

The genus *Serratia* comprises *S. marcescens*, *S. liquefaciens*, *S. rubidaea*, *S. odorifera*, *S. plymuthica*, *S. ficaria*,

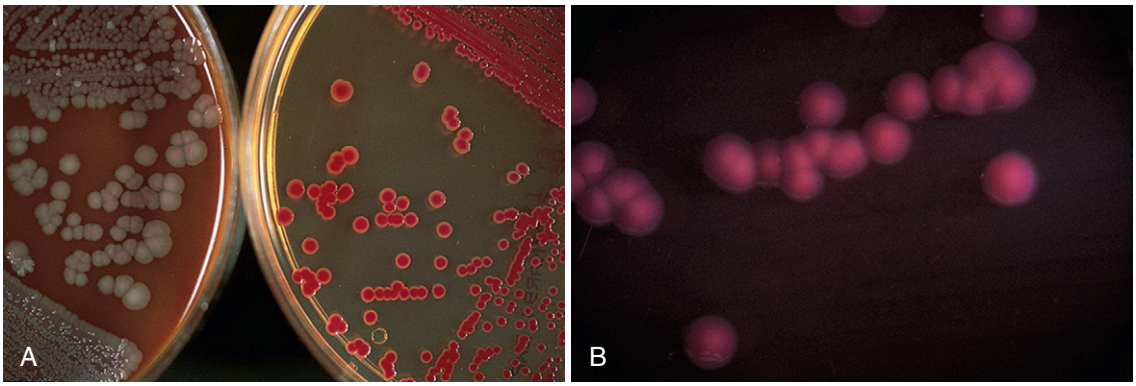

FIGURE 19-6 **A,** *Serratia marcescens* growing on CHOC agar *(left)* and showing brick-red pigment when grown on MacConkey (MAC) agar *(right).* **B,** Pinkish red pigmentation of *Serratia rubidaea* growing on MAC agar.

S. entomophila, and *S. fonticola*. *Serratia* spp. are opportunistic pathogens associated with outbreaks in health care settings. With the exception of *S. fonticola*, *Serratia* spp. ferment lactose slowly and are positive for the *o*-nitrophenyl-β-D-galactopyranoside (ONPG) test. They are differentiated from other members of the tribe by their ability to produce extracellular DNase. *Serratia* spp. are also known for their resistance to a wide range of antimicrobials. Susceptibility tests must be performed on each isolate to determine appropriate antimicrobial therapy.

S. marcescens, *S. rubidaea*, and *S. plymuthica* often produce a characteristic pink-to-red pigment, prodigiosin, especially when the cultures are incubated at room temperature. Pigment production is typically a characteristic in those strains of environmental origin. Figure 19-6 illustrates the pigmentation of *S. marcescens* and *S. rubidaea*. *S. marcescens* is the species considered most significant clinically. It has been found frequently in hospital acquired infections of the urinary or respiratory tract and in bacteremic outbreaks in nurseries and cardiac surgery and burn units. Contamination of antiseptic solution used for joint injections has resulted in an epidemic of septic arthritis.

S. odorifera contains two biogroups. As the species name implies, it emits a dirty, musty odor resembling that of rotten potatoes. *S. odorifera* biogroup 1 is isolated predominantly from the respiratory tract and is positive for sucrose, raffinose, and ornithine. In addition, biogroup 1 may be indole-positive (60%). *S. odorifera* biogroup 2 is negative for sucrose, raffinose, and ornithine and has been isolated from blood and CSF. Biogroup 2 may also be indole-positive (50%). *S. liquefaciens*, *S. rubidaea*, and *S. fonticola* have also been isolated from human sources.

Hafnia

The genus *Hafnia* is composed of one species, *H. alvei*. However, two distinct biotypes are recognized: *H. alvei* and *H. alvei* biotype 1. Biotype 1 grows in the beer wort of breweries and has not been isolated clinically. *Hafnia* has been isolated from many anatomic sites in humans and in the environment. *Hafnia* has been linked to gastroenteritis and is occasionally isolated from stool cultures. A delayed positive citrate reaction is a major characteristic of *Hafnia.*

Proteus

The genera *Proteus*, *Morganella*, and *Providencia* belong to the tribe Proteeae. They are widely disseminated in the environment, are normal intestinal microbiota, and are recognized as opportunistic pathogens. *Proteus mirabilis* is the most common clinical isolate. The tribe Proteeae is distinguished from the other members of the Enterobacteriaceae by virtue of the ability to deaminate the amino acid phenylalanine. Virtually no other members of the Enterobacteriaceae synthesize the required enzyme, phenylalanine deaminase. None of the members of this tribe ferment lactose.

The genus *Proteus* consists of at least four species. *P. mirabilis* and *P. vulgaris* are widely recognized human pathogens. Both species have been isolated from urine, wounds, and ear and bacteremic infections. *Proteus* spp. are responsible for 3% of all hospital-acquired infections in the United States, particularly UTIs. They ascend the urinary tract, causing infections in both the lower and the upper urinary tract. They can infect the proximal kidney tubules and can cause acute glomerulonephritis, particularly in patients with urinary tract defects or catheterization. The urease activity of *P. mirabilis* can lead to struvite kidney stones (calculi).

P. mirabilis and *P. vulgaris* are easily identified in the clinical laboratory because of their characteristic colony morphology. Both species, particularly *P. mirabilis,* can produce "swarming" colonies on nonselective media, such as SBA (Figure 19-7). This characteristic swarming is a result of a tightly regulated cycle of differentiation from standard vegetative cells (swimmers) to hyperflagellated, elongated, polyploid cells (swarmers) capable of coordinated surface movement. These swarmer cells also produce the distinct odor associated with *Proteus* colonies, sometimes described as "burnt chocolate," and are thought to play a role in the ascending nature of *Proteus*-associated UTIs. Both species hydrolyze urea and produce H_2S, although some strains of *P. vulgaris* are negative for H_2S. *P. mirabilis* is differentiated from *P. vulgaris* by the indole and ornithine decarboxylase tests.

TABLE 19-7 Differentiating Characteristics of Selected Species of *Proteus*, *Providencia*, and *Morganella*

Test	*Proteus penneri*	*Proteus mirabilis*	*Proteus vulgaris*	*Providencia alcalifaciens*	*Providencia stuartii*	*Providencia rettgeri*	*Morganella morganii*
Indole	–		+	+	+	+	+
Methyl red	+	+	+	+	+	+	+
Voges-Proskauer	–	– or +	–	–	–	–	–
Simmons citrate	–	+ or (+)	d	+	+	+	–
Christensen urea	+	+ or (+)	+	–	– or +	+	+
H_2S (TSI)	– (70%)	+	+	–	–	–	–
Ornithine decarboxylase	–	+	–	–	–	–	+
Phenylalanine deaminase	+	+	+	+	+	+	+
Acid produced from							
Sucrose	+	d	+	d	d	d	–
Mannitol	–	–	–	–	d	+	–
Salicin	–	–	d	–	–	d	–
Adonitol	–	–	–	+	–	+	–
Rhamnose	–	–	–	–	–	+ or –	–
Maltose	+	–	+	–	–	–	–
Xylose	+	+	+ or (+)	–	–	– or +	–
Arabitol	–	–	–	–	–	+	–
Swarms	+	+	+	–	–	–	–

Modified from Washington J: *Laboratory procedures in clinical microbiology*, ed 2, New York, 1981, Springer-Verlag.
H_2S, Hydrogen sulfide; *TSI,* triple-sugar iron agar; *+,* ≥90% positive reaction within 1 or 2 days; *–,* no reaction (≥90%) in 30 days; *– or +,* most strains negative, some cultures positive; *+ or (+),* most reactions occur within 1 or 2 days, some are delayed; *d,* different reactions, + or –, most cultures positive, some strains negative.

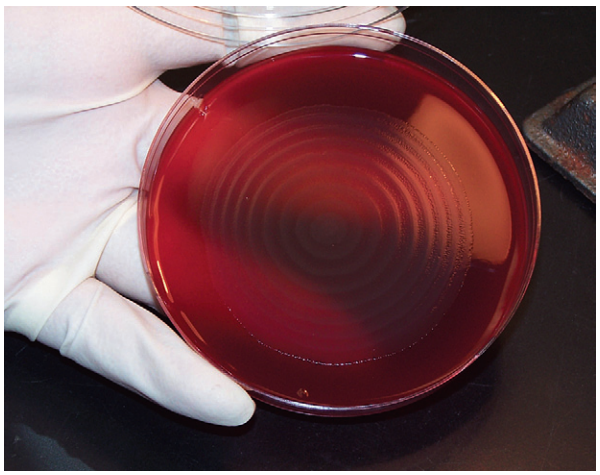

FIGURE 19-7 Example of *Proteus mirabilis* swarming on sheep blood agar (SBA). (Courtesy Kimberly Walker and R. Abe Baalness.)

P. mirabilis does not produce indole from tryptophan and is ornithine-positive, whereas *P. vulgaris* produces indole and is ornithine-negative. *P. vulgaris* ferments sucrose and gives an acid/acid reaction in triple sugar iron (TSI) agar.

P. penneri can also swarm on nonselective media. *P. penneri* has been isolated from patients with diarrhea, although the organism's role in disease has not been proven.

Morganella

The genus *Morganella* has only one species, *M. morganii*, with two subspecies: *M. morganii* subsp. *morganii* and *M. morganii* subsp. *sibonii*. Neither *M. morganii* subsp. *morganii* nor *M. morganii* subsp. *sibonii* has been implicated in diarrheal illness, but the role these subspecies might play as an etiologic agent of diarrheal disease has not been fully examined. However, *M. morganii* is a documented cause of UTI. It has also been identified as a cause of neonatal sepsis. *Morganella* is motile but does not swarm. Key identifying characteristics are listed in Table 19-7.

Providencia

The genus *Providencia* consists of five species: *P. alcalifaciens*, *P. stuartii*, *P. rettgeri*, *P. rustigianii*, and *P. heimbachae*. *P. rettgeri* is a documented pathogen of the urinary tract and has caused occasional outbreaks in health care settings. It has also been implicated in diarrheal disease among travelers. Similarly, *P. stuartii* has been implicated in outbreaks in burn units and has been isolated from urine cultures. Infections caused by *P. stuartii* and *P. rettgeri*, especially in immunocompromised patients, are particularly difficult to treat because of their resistance to antimicrobials. *P. alcalifaciens* is most commonly found in the feces of children with diarrhea; however, its role as a cause of diarrhea has not been proven. *P. rustigianii*, formerly identified as a strain of *P. alcalifaciens*, is rarely isolated, and its pathogenicity also remains unproven, whereas *P. heimbachae* has yet to be isolated from any clinical specimens. Table 19-7 shows the differentiating characteristics of medically important *Proteus*, *Providencia*, and *Morganella*.

Edwardsiella

The genus *Edwardsiella* is composed of three species: *E. tarda*, *E. hoshinae*, and *E. ictaluri*. *E. tarda* is the only recognized human pathogen. Members of this genus are negative for urea and positive for lysine decarboxylase, H_2S, and indole and do

not grow on Simmons citrate. *E. tarda* is an opportunist, causing bacteremia and wound infections. Its pathogenic role in cases of diarrhea is controversial. *E. hoshinae* has been isolated from snakes, birds, and water. *E. ictaluri* causes enteric septicemia in fish.

Erwinia and *Pectobacterium*

Both *Erwinia* and *Pectobacterium* spp. are plant pathogens and are not significant in human infections. *Erwinia* organisms grow poorly at 37° C and fail to grow on selective media, such as EMB and MAC, and other differential media typically used for the isolation of enterics. Identification of these organisms is more for academic interest than for the evaluation of their significance as causative agents of infection. Most *Erwinia* spp. have been placed in other genera and given new designations.

Citrobacter

Earlier classifications of the family Enterobacteriaceae included the genus *Citrobacter* within the tribe Salmonelleae, which formerly consisted of the genera *Salmonella*, *Citrobacter*, and *Arizona*. However, changes in the classification and nomenclature of bacterial species belonging to the tribe Salmonellae have caused the reclassification of the genus *Citrobacter* into its own tribe, Citrobacteriaceae, and of *Arizona* as a subspecies of *Salmonella*. The genus *Citrobacter* consists of at least 11 species that all have been isolated from clinical specimens. Most *Citrobacter* spp. hydrolyze urea slowly and ferment lactose, producing colonies on MAC agar that resemble those of *E. coli*. All species grow on Simmons citrate medium (hence the genus name) and give positive reactions in the methyl red test.

The citrobacters are considered inhabitants of the GI tract and are associated with hospital-acquired infections, most frequently UTIs. The three species most often isolated are *C. freundii*, *C. koseri*, and *C. braakii*. *C. freundii* can be isolated in diarrheal stool cultures, and although it is a known extraintestinal pathogen, its pathogenic role in intestinal disease is not established. *C. freundii* has been associated with infectious diseases acquired in hospital settings; UTIs, pneumonias, and intraabdominal abscesses have been reported. In addition, *C. freundii* has been associated with endocarditis in intravenous drug abusers. One reported case of *C. freundii* endocarditis required aortic valve replacement when antimicrobial therapy failed.

Because most (80%) *C. freundii* produce H_2S and some strains (50%) fail to ferment lactose, the colony morphology of *C. freundii* on primary selective media can be mistaken for *Salmonella* when isolated from stool cultures. Because of the pathogenic potential, it is important to differentiate *C. freundii* from *Salmonella*. Differentiation can be accomplished by using a minimal number of biochemical tests, such as urea hydrolysis and lysine decarboxylase. Most (70%) *C. freundii* hydrolyze urea, but all fail to decarboxylate lysine, whereas *Salmonella* fails to hydrolyze urea, and most isolates decarboxylate lysine.

C. koseri is a pathogen documented as the cause of nursery outbreaks of neonatal meningitis and brain abscesses. *C. amalonaticus* is frequently found in feces, but no evidence has been found that it is a causative agent of diarrhea. It has been isolated from sites of extraintestinal infections, such as blood and wounds. *C. gillenii* and *C. murliniae* have also been isolated from human specimens.

Primary Intestinal Pathogens of the Family Enterobacteriaceae

Salmonella and *Shigella* organisms produce GI illnesses in humans; neither is considered normal biota of the human intestinal tract. Salmonellae inhabit the GI tracts of animals. Humans acquire the infection by ingesting the organisms in contaminated animal food products or insufficiently cooked poultry, milk, eggs, and dairy products. Some *Salmonella* infections are transmitted by human carriers.

Infections caused by *Shigella* spp. are associated with human carriers responsible for spreading the disease; no animal reservoir has yet been identified. *Shigella* dysentery usually indicates improper sanitary conditions and poor personal hygiene. Infections caused by *Yersinia* spp. are transmitted by a wide variety of wild and domestic animals. *Yersinia* infections include GI disease, mediastinal lymphadenitis, fulminant septicemia, and pneumonia.

Salmonella

Members of the genus *Salmonella* produce significant infections in humans and in certain animals. Many *Salmonella* serotypes are typically found in cold-blooded animals as well as in rodents and birds, which serve as their natural hosts. The U.S. Centers for Disease Control and Prevention (CDC) reported 241 cases of human salmonellosis caused by *Salmonella* Typhimurium in 42 states by contact with African dwarf frogs in 2010-2011. Salmonellae are gram-negative, facultatively anaerobic bacilli that morphologically resemble other enteric bacteria. On selective and differential media used primarily to isolate enteric pathogens (e.g., MAC), salmonellae produce clear, colorless, non–lactose-fermenting colonies; colonies with black centers are seen if the media (e.g., HE or XLD) contain indicators for H_2S production. The biochemical features for the genus include the following:

- In almost every case, they do not ferment lactose.
- They are negative for indole, the Voges-Proskauer test, phenylalanine deaminase, and urease.
- Most produce H_2S; a major exception is *Salmonella* Paratyphi A, which does not produce H_2S.
- They do not grow in medium containing potassium cyanide.

Classification

Previously, the genus *Salmonella* comprised three biochemically discrete species: *S. enteritidis*, *S. choleraesuis*, and *S. typhi*. However, genetic studies showed that bacterial species in the genus *Salmonella* are very closely related and that only two species, *S. enterica* (the type species of the genus) and *S. bongori*, should be designated. *S. bongori* is a rarely isolated species that is named after the town of Bongor in Chad, Africa, and was initially isolated in 1966 from a lizard. It is usually isolated from cold-blooded animals and the environment, but there was a report of 18 cases of human enteritis caused by *S. bongori* in Sicily during the period 1984-1997.

Within the species *S. enterica* are six subspecies: *S. enterica* subsp. *enterica* (also called subspecies I), *S. enterica* subsp. *salamae* (subspecies II), *S. enterica* subsp. *arizonae* (subspecies IIIa), *S. enterica* subsp. *diarizonae* (subspecies IIIb), *S. enterica* subsp. *houtenae* (subspecies IV), and *S. enterica* subsp. *indica*

TABLE 19-8 Biochemical Differentiation of Selected Members of the Genus *Salmonella*

Test	*S.* serotype Choleraesuis	*S.* serotype Paratyphi	*S.* serotype Typhi	Other*
Arabinose fermentation	–	+	–	+
Citrate utilization	V	–	–	+
Glucose gas production	+	+	–	+
H_2S (TSI)	V	–	+	+
Lysine decarboxylase	+	–	+	+
Ornithine decarboxylase	+	+	–	+
Rhamnose fermentation	+	+	–	+
Trehalose fermentation	–	+	+	+

Data from Farmer JJ, et al: Enterobacteriaceae: introduction and identification. In Murray PR, et al, editors: *Manual of clinical microbiology*, ed 9, Washington, DC, 2007, ASM Press.
H_2S, Hydrogen sulfide; *TSI,* triple sugar iron agar; –, ≤9% of strains positive; +, ≥90% of strains positive; *V,* 10% to 89% of strains positive.
*Typical strains in serogroups A through E.

(subspecies VI). Nearly all former *Salmonella* spp. have been placed as serotypes below the level of *S. enterica* subsp. *enterica* (e.g., *S. enterica* subsp. *enterica* serotype Typhi); this is often more simply written as *Salmonella* Typhi (serotype is capitalized and not italicized). Table 19-8 shows the characteristic features of *Salmonella* serotype Typhi, *Salmonella* serotype Choleraesuis, and *Salmonella* serotype Paratyphi. Members of the former genus *Arizona*, now subspecies IIIa of S. *enterica*, are found in infections with symptoms identical to those of *Salmonella* infections and may be transmitted to humans from pet turtles, snakes, and fish.

Virulence Factors

Factors responsible for the virulence of salmonellae have been the subject of speculation and are still being investigated. The role of fimbriae in adherence in initiating intestinal infection has been cited. Another factor that contributes to the virulence of salmonellae is their ability to traverse intestinal mucosa. Enterotoxin produced by certain *Salmonella* strains that cause gastroenteritis has been implicated as a significant virulence factor.

Antigenic Structures

Salmonellae possess antigens similar to antigens of other enterobacteria. The somatic O antigens and flagellar H antigens are the primary antigenic structures used in serologic grouping of salmonellae. A few strains may possess capsular (K) antigens, designated Vi antigen. The serologic identification of the Vi antigen is important in identifying *Salmonella* serotype Typhi. Figure 19-8 shows the antigenic structures used in serologic grouping and their locations. The heat-stable O antigen of salmonellae, as is the case with other enteric bacteria, is the lipopolysaccharide located in the outer membrane of the cell wall. Many different O antigens are present among the subspecies of *Salmonella*; more than one O antigen can also be found in a particular strain. The O antigens are designated by Arabic numbers.

In contrast to the O antigens, flagellar antigen proteins are heat labile. The H antigens of salmonellae occur in one of two phases: phase 1, the specific phase, and phase 2, the nonspecific phase. Phase 1 flagellar antigens occur only in a few serotypes and determine the immunologic identity of the particular serotype. Phase 1 antigens agglutinate only with homologous

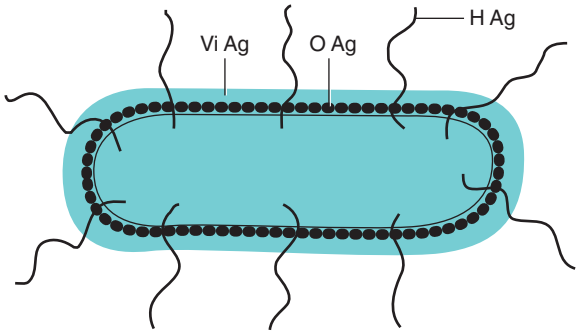

FIGURE 19-8 Antigenic structures of salmonellae used in serologic typing.

antisera. Phase 2 flagellar antigens occur among several strains. Shared by numerous serotypes, phase 2 antigens react with heterologous antisera. The heat-labile Vi (from the term ***vi****rulence*) antigen is a surface polysaccharide capsular antigen found in *Salmonella* serotype Typhi and a few strains of *Salmonella* serotype Choleraesuis. The capsular antigen plays a significant role in preventing phagocytosis of the organism. The Vi antigen often blocks the O antigen during serologic typing but may be removed by heating.

Clinical Infections

In humans, salmonellosis may occur in several forms, as follows:

- Acute gastroenteritis or food poisoning characterized by vomiting and diarrhea
- Typhoid fever, the most severe form of enteric fever, caused by *Salmonella* serotype Typhi, and enteric fevers caused by other *Salmonella* serotypes (e.g., *Salmonella* Paratyphi and Choleraesuis)
- Nontyphoidal bacteremia
- Carrier state following *Salmonella* infection

Humans acquire the infection by ingesting the organisms in food, water, and milk contaminated with human or animal excreta. With the exception of *Salmonella* Typhi and *Salmonella* Paratyphi, salmonellae organisms infect various animals that serve as reservoirs and sources of human infections. *Salmonella* serotypes Typhi and Paratyphi have no known animal reservoirs,

and infections seem to occur only in humans. Carriers are often the source of infection.

Gastroenteritis. One of the most common forms of "food poisoning," GI infection caused by salmonellae results from the ingestion of the organisms through contaminated food. Numerous outbreaks have also been linked to calves and poultry at petting zoos. The *Salmonella* strains associated with gastroenteritis are usually strains found in animals; most such strains in the United States are members of *S. enterica* subsp. *enterica.* The source of the infection has been attributed primarily to poultry, milk, eggs, and egg products as well as to handling pets. Insufficiently cooked eggs and domestic fowl, such as chicken, turkey, and duck, are common sources of infection. More recently in the United States, there has been a series of outbreaks by various *Salmonella* serovars related to the ingestion of foodstuffs such as peanut butter, cantaloupe, puffed rice and wheat cereals, corn and vegetable coated snacks, and raw tomatoes. In 2012, 49 cases, 47 in the United States and 2 in Canada, of human infections caused by *Salmonella* Infantis from dry dog food were reported. In 2011, there were 190 cases from broiled chicken livers, 136 from ground turkey, 106 from imported papaya, and 43 from pine nuts. Also from August 2010 through June 2011, the CDC documented 109 cases of *Salmonella* Typhimurium from clinical and teaching laboratories. Infected individuals included students in microbiology teaching laboratories and employees in clinical microbiology laboratories and their household contacts. Salmonellosis is a common cause of GI tract infections; 51,887 cases of salmonellosis were reported to the CDC in 2011, with an estimated incidence of 1.4 million.

Cooking utensils, such as knives, pans, and cutting boards used in preparing the contaminated meat, can spread the bacteria to other food. Direct transmission from person to person has been reported in institutions. *Salmonella* gastroenteritis occurs when a sufficient number of organisms contaminate food that is maintained under inadequate refrigeration, allowing growth and multiplication of the organisms. The infective dose necessary to initiate the disease, 10^6 bacteria, is much higher than the dose required for shigellosis. Infections resulting from lower infective doses have been reported.

The symptoms of intestinal salmonellosis, which may appear 8 to 36 hours after ingestion of contaminated food, include nausea, vomiting, fever, and chills, accompanied by watery diarrhea and abdominal pain. Most cases of *Salmonella* gastroenteritis are self-limiting. Symptoms usually disappear within a few days, with few or no complications. Patients with sickle cell disease and other hemolytic disorders, ulcerative colitis, and malignancy seem to be more susceptible to *Salmonella* spp. infection. The infection may be more severe in very young children, elderly adults, and patients with other underlying disease. Dissemination can occasionally occur; in such cases, antimicrobial therapy is required.

The antimicrobials of choice include chloramphenicol, ampicillin, and trimethoprim-sulfamethoxazole. Nevertheless, susceptibility testing must be performed. Antimicrobial therapy is usually not indicated in uncomplicated cases. Antimicrobial therapy is believed to prolong the carrier state. Antidiarrheal agents are also restricted in cases of salmonellosis because these agents may encourage adherence and further invasion. In cases of dehydration, fluid replacement therapy may be indicated.

Enteric Fevers. The clinical features of enteric fevers include:

- Prolonged fever
- Bacteremia
- Involvement of the reticuloendothelial system, particularly the liver, spleen, intestines, and mesentery
- Dissemination to multiple organs

Enteric fever caused by *Salmonella* Typhi is known as *typhoid fever*, a febrile disease that results from the ingestion of food contaminated with the organisms originating from infected individuals or carriers. *Salmonella* Typhi does not have a known animal reservoir; humans are the only known source of infection. Other enteric fevers include paratyphoid fevers, which may be due to *Salmonella* serotypes Paratyphi A, B, and C and *Salmonella* serotype Choleraesuis. The clinical manifestations of paratyphoid fevers are similar to typhoid fever but are less severe, and the fatality rate is lower.

Typhoid fever occurs more often in tropical and subtropical areas, where international travelers are more likely to acquire the infection. Improper disposal of sewage, poor sanitation, and lack of a modern potable water system have caused outbreaks of typhoid fever when the organisms reach a water source. This situation is uncommon in the United States and other developed countries, where water is purified and treated, and handling of waste is standardized. Carriers, particularly food handlers, are important sources of infection anywhere in the world. Direct transmission through fomites is also possible. Laboratory workers in the microbiology laboratory have contracted typhoid fever while working with the organisms.

Typhoid fever develops approximately 9 to 14 days after ingestion of the organisms. The onset of symptoms depends on the number of organisms ingested; the larger the inoculum, the shorter the incubation period. Characteristically, during the first week of the disease, the patient develops a fever accompanied by malaise, anorexia, lethargy, myalgia, and a continuous dull frontal headache.

When the organisms are ingested, they seem to be resistant to gastric acids and, on reaching the proximal end of the small intestine, subsequently invade and penetrate the intestinal mucosa. At this time, the patient experiences constipation rather than diarrhea. The organisms gain entrance into the lymphatic system and are sustained in the mesenteric lymph nodes. They eventually reach the bloodstream and spread further to the liver, spleen, and bone marrow, where they are immediately engulfed by mononuclear phagocytes. The organisms multiply intracellularly; later they are released into the bloodstream for the second time. The febrile episode becomes more evident during this release of the organisms into the circulatory system. At this time, the organisms may be isolated easily from the blood. Figure 19-9 shows the course of typhoid fever.

During the second and third weeks of the disease, the patient generally experiences sustained fever with prolonged bacteremia. The organisms invade the gallbladder and Peyer's patches of the bowel. They also reach the intestinal tract via the biliary tract. "Rose spots" (blanching, rose-colored papules around the umbilical region) appear during the second week of fever. Involvement of biliary system sites initiates GI symptoms as the organisms reinfect the intestinal tract. The organism now exists in large numbers in the bowel and may be isolated from the stool. The

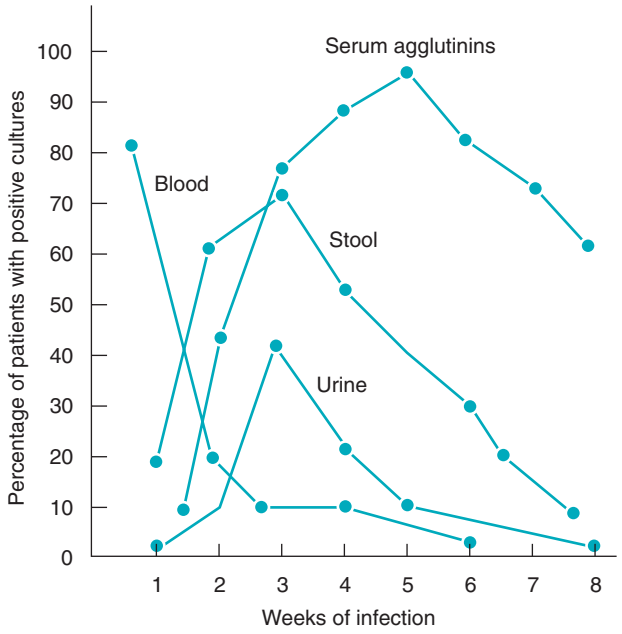

FIGURE 19-9 Culture and serologic diagnosis of typhoid fever. (Modified from Koneman E, et al: *Color atlas and textbook of diagnostic microbiology*, ed 4, Philadelphia, 1992, Lippincott.)

gallbladder becomes the foci of long-term carriage of the organism, occasionally reseeding the intestinal tract and shedding the organisms in the feces. Necrosis in the gallbladder leading to necrotizing cholecystitis and necrosis of the Peyer's patches leading to hemorrhage and perforation of the bowel may occur as serious complications. Other complications that occur in typhoid fever include pneumonia, thrombophlebitis, meningitis, osteomyelitis, endocarditis, and abscesses.

Bacteremia. *Salmonella* bacteremia, with and without extraintestinal foci of infection caused by nontyphoidal *Salmonella*, is characterized primarily by prolonged fever and intermittent bacteremia. The serotypes most commonly associated with bacteremia are Typhimurium, Paratyphi, and Choleraesuis. *Salmonella* infection has been observed among two different groups: (1) young children, who experience fever and gastroenteritis with brief episodes of bacteremia, and (2) adults, who experience transient bacteremia during episodes of gastroenteritis or develop symptoms of septicemia without gastroenteritis. The latter manifestations were observed among patients who had underlying illnesses, such as malignancies and liver disease. The risk of metastatic complications could be more severe than the bacteremia itself, even in individuals who do not have underlying diseases. Cases of septic arthritis can also occur in patients who had asymptomatic salmonellosis.

Carrier State. Individuals who recover from infection may harbor the organisms in the gallbladder, which becomes the site of chronic carriage. Such individuals excrete the organisms in their feces either continuously or intermittently; nevertheless, they become an important source of infection for susceptible persons. The carrier state may be terminated by antimicrobial therapy if gallbladder infection is not evident. Otherwise, cholecystectomy has been the only solution to the chronic state of enteric carriers.

TABLE 19-9 Biochemical and Serologic Differentiation of *Shigella* Species

Test	*S. dysenteriae*	*S. flexneri*	*S. boydii*	*S. sonnei*
Mannitol fermentation	–	+	+	+
ONPG	V	–	V	+
Ornithine decarboxylase	–	–	–	+
Serogroup	A	B	C	D

From Farmer JJ, et al: Enterobacteriaceae: introduction and identification. In Murray PR, et al, editors: *Manual of clinical microbiology*, ed 9, Washington, DC, 2007, ASM Press.
ONPG, O-nitrophenyl-β-D-galactopyranoside; –, ≤9% of strains positive; +, ≥90% of strains positive; *V,* 10% to 89% of strains positive.

Shigella

The genera *Shigella* and *Escherichia* are so closely related according to molecular analyses that they should be a single genus. However, for medical purposes, and because of the useful association of the genus epithet with the distinct disease shigellosis or bacillary dysentery, they remain as separate genera. Both genera belong to the tribe Escherichieae. However, *Shigella* spp. are not members of the normal GI microbiota, and all *Shigella* spp. can cause bacillary dysentery. The genus *Shigella* is named after the Japanese microbiologist Kiyoshi Shiga, who first isolated the organism in 1896. The organism, descriptively named *Shigella dysenteriae*, caused the enteric disease bacillary dysentery. Dysentery was characterized by the presence of blood, mucus, and pus in the stool. Characteristics of *Shigella* spp. include the following:

- They are nonmotile.
- Except for certain types of *S. flexneri*, they do not produce gas from glucose.
- They do not hydrolyze urea.
- They do not produce H_2S.
- They do not decarboxylate lysine.

In contrast to *Escherichia* spp., *Shigella* spp. do not use acetate or mucate as a source of carbon. Table 19-9 shows the biochemical characteristics of *Shigella* spp. *S. sonnei* is unique in its ability to decarboxylate ornithine. It slowly ferments lactose, producing a "delayed" positive fermentation of lactose with the formation of pink colonies on MAC agar only after 48 hours of incubation. *S. sonnei* is also ONPG-positive. These two key positive reactions help to distinguish it from the other three species. Figure 19-10 illustrates the growth of *S. sonnei* on MAC after 24 hours and 48 hours of incubation. On differential and selective media used primarily to isolate intestinal pathogens, shigellae generally appear as clear, non–lactose-fermenting colonies. Shigellae are fragile organisms. They are susceptible to the various effects of physical and chemical agents, such as disinfectants and high concentrations of acids and bile. Because they are susceptible to the acid pH of stool, feces suspected of containing *Shigella* organisms should be plated immediately onto laboratory media to increase recovery of the organism.

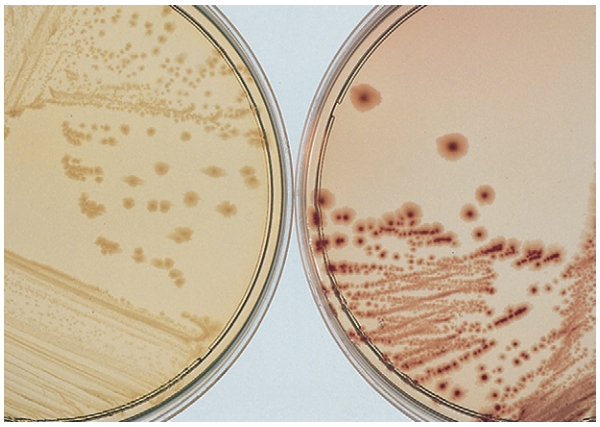

FIGURE 19-10 *Left,* Lactose-negative appearance of *Shigella sonnei* growing on MacConkey (MAC) agar at 18 to 24 hours of incubation. *Right,* Lactose-positive appearance of *S. sonnei* growing on MAC agar after 48 hours of incubation.

Antigenic Structures

The genus consists of four species that are biochemically similar. *Shigella* spp. are also divided into four major O antigen groups and must be identified by serologic grouping. The four species and their respective serologic groups are depicted in Table 19-9. Several serotypes exist within each species, with the exception of *S. sonnei*, which has only one serotype. All *Shigella* spp. possess O antigens, and certain strains can possess K antigens. *Shigella* K antigens, when present, interfere with the detection of the O antigen during serologic grouping. The K antigen is heat labile and may be removed by boiling a cell suspension. The shigellae are nonmotile; therefore they lack H antigens.

Clinical Infections

Although all *Shigella* spp. can cause dysentery, species vary in epidemiology, mortality rate, and severity of disease. In the United States, *S. sonnei* is the predominant isolate, followed by *S. flexneri*. In the United States and other industrialized countries, shigellosis is probably underreported because most patients are not hospitalized and usually recover from the infection without culture to identify the etiologic agent. *S. sonnei* infection is usually a short, self-limiting disease characterized by fever and watery diarrhea.

The demographics of *S. flexneri* infection have changed during recent years, from a disease affecting mostly young children to one producing infections in young adults (approximately 25 years old). This observation was made simultaneously with the recognition of gastroenteritis in men who have sex with men, in which *S. flexneri* has been the leading isolate. Conversely, in developing countries, *S. dysenteriae* type 1 and *S. boydii* are the most common isolates. *S. dysenteriae* type 1 remains the most virulent species, with significant morbidity and high mortality. Reports exist of mortality rates of 5% to 10%, and perhaps even higher, resulting from *S. dysenteriae* type 1, particularly among undernourished children during epidemic outbreaks.

Humans are the only known reservoir of *Shigella* spp. Transmission can occur by direct person-to-person contact, and spread can take place via the fecal-oral route, with carriers as the source. Shigellae may also be transmitted by flies, fingers, and food or water contaminated by infected persons. Personal hygiene plays a major role in the transmission of *Shigella* spp., and certain groups are affected more than others. Young children in daycare centers, particularly infants younger than 1 year of age, are the most susceptible. Most disturbing are the reports of multidrug-resistant *S. sonnei* outbreaks in daycare centers in several states. *Shigella* is also seen in people living in crowded and inadequate housing and in people who participate in anal-oral sexual activity. Multiple *Shigella* outbreaks associated with passengers on cruise ships from various cruise lines have also been reported.

Because of the low infective dose required to produce the disease, shigellosis is highly communicable. It has been reported that less than 100 bacilli are needed to initiate the disease in some healthy individuals. Bacillary dysentery caused by *Shigella* spp. is marked by penetration of intestinal epithelial cells after attachment of the organisms to mucosal surfaces, local inflammation, shedding of the intestinal lining, and formation of ulcers after epithelial penetration.

The clinical manifestations of shigellosis vary from asymptomatic to severe forms of the disease. The initial symptoms, marked by high fever, chills, abdominal cramps, and pain accompanied by tenesmus, appear approximately 24 to 48 hours after ingestion of the organisms. The organisms, which originally multiply in the small intestine, move toward the colon, where they may be isolated 1 to 3 days after the infection develops. Bloody stools containing mucus and numerous leukocytes follow the watery diarrhea, as the organisms invade the colonic tissues and cause an inflammatory reaction.

In dysentery caused by *S. dysenteriae* type 1, patients experience more severe symptoms. Bloody diarrhea that progresses to dysentery may appear within a few hours to a few days. Patients experience extremely painful bowel movements, which contain predominantly mucus and blood. In young children, abdominal pain is quite intense, and rectal prolapse may result from excessive straining. Severe cases of shigellosis may become life-threatening as extraintestinal complications develop. One of the most serious complications is ileus, an obstruction of the intestines, with marked abdominal dilation, possibly leading to toxic megacolon.

Although *Shigella* spp. infrequently penetrate the intestinal mucosa and disseminate to other body sites, it has been reported that 4% of severely ill hospitalized patients in Bangladesh have bacteremia caused by *S. dysenteriae* type 1. *S. flexneri* bacteremia and bacteremia resulting from other enteric organisms occur, presumably predisposed by ulcers initiated by the shigellae. Other complications of shigellosis include seizures, which may occur during any *Shigella* sp. infection, and HUS, a complication among the shigellae exclusively associated with *S. dysenteriae* type 1 shigellosis. EHEC causes about 80% of the cases of HUS in the United States. The effects of shigella toxin have been implicated as the mechanism responsible for the signs of disease, and it has been reported that the detectable toxin levels produced by *S. dysenteriae* type 1 are higher than those produced by other *Shigella* spp.

Yersinia

The genus *Yersinia* currently consists of 14 named species; most are considered environmental species. Although many have been isolated from humans, only three species are considered human pathogens. *Y. pestis* is the causative agent of plague, a disease primarily of rodents transmitted to humans by fleas. *Y. pseudotuberculosis* and *Y. enterocolitica* have caused sporadic cases of mesenteric lymphadenitis in humans, especially in children, and generalized septicemic infections in immunocompromised hosts. The DNA relatedness between *Y. pestis* and *Y. pseudotuberculosis* is about 90%. *Y. enterocolitica* produces an infection that mimics appendicitis. It has also been found to be the cause of diarrhea in numerous community outbreaks. The other members of the genus *Yersinia* are found in water, soil, and lower animals; isolates occasionally have been found in wounds and the urine of humans. Evidence that other species, in addition to *Y. enterocolitica*, have caused intestinal disease has not been found.

Yersinia pestis

The causative agent of the ancient disease plague still exists in areas where reservoir hosts are found. Plague is a disease primarily of rodents. It is transmitted to humans by bites of fleas, which are its most common and effective vectors. In humans, plague can occur in three forms: the bubonic, or glandular, form; the septicemic form; and the pneumonic form. The bubonic form, the most common, usually results from the bite of an infected flea. Characteristic symptoms appear 2 to 5 days after infection. The symptoms include high fever with painful regional lymph nodes known as **buboes** (swollen lymph nodes) begin to appear. The septicemic form occurs when the bacteria spread to the bloodstream. Pneumonic plague occurs secondary to bubonic plague or the septicemic form when organisms proliferate in the bloodstream and respiratory tract. Pneumonic plague can be a primary infection if the bacteria are inhaled. Subsequent epidemic outbreaks can arise from the respiratory transmission of the organisms. The fatality rate in pneumonic plague is high—essentially 100%—in untreated patients.

Y. pestis is a gram-negative, short, plump bacillus. When stained with methylene blue or Wayson stain, it shows intense staining at each end of the bacillus, referred to as *bipolar staining*, which gives it a "safety-pin" appearance. *Y. pestis* may be isolated on routine culture medium. Although it grows at 37° C, it has a preferential growth temperature of 25° C to 30° C. A *Y. pestis*–specific DNA probe for plague surveillance has been studied. If this DNA probe is proven successful, it may be applicable for laboratory diagnostic testing. *Y. pestis* is a class A bioterrorism agent; further information relating to this classification can be found in Chapter 30.

Yersinia enterocolitica

Human infections resulting from *Y. enterocolitica* have occurred worldwide, predominantly in Europe, although cases in the northeastern United States and Canada have been reported. It is the most commonly isolated species of *Yersinia.* The organisms have been found in a wide variety of animals, including domestic swine, cats, and dogs. The infection can be acquired from contact with household pets. The role of pigs as a natural reservoir has been greatly emphasized in Europe. Other animal reservoirs have also been identified, and cultures from environmental reservoirs, such as water from streams, have yielded the organism.

Human infections most often occur after the ingestion of contaminated food, often pork, and vacuum-packed deli meat, beef, lamb, chicken, and possibly chocolate milk and water. There are several reports of gastroenteritis, especially in infants who were infected by caretakers who had improperly handled raw pork chitterlings (pork intestines) during food preparation procedures. A major concern regarding the potential risk of transmitting this organism is its ability to survive in cold temperatures; food refrigeration becomes an ineffective preventive measure. In addition, *Y. enterocolitica* sepsis associated with the transfusion of contaminated packed red blood cells has been reported.

Y. enterocolitica infections manifest in several forms: an acute enteritis, an appendicitis-like syndrome, arthritis, and erythema nodosum. The incidence of generalized infection is higher among adults with underlying diseases, such as liver cirrhosis, diabetes, acquired immunodeficiency syndrome, leukemia, aplastic anemia, and other hematologic conditions. Cases of liver abscess and acute infective endocarditis caused by *Y. enterocolitica* have also been reported. Acute enteritis, the most common form of the infection, is characterized by acute gastroenteritis with fever accompanied by headaches, abdominal pain, nausea, and diarrhea. Stools may contain blood. This form of infection, which often affects infants and young children between the ages of 1 and 5 years, is usually mild and self-limiting.

The clinical form that mimics acute appendicitis occurs primarily in older children and adults. Patients present with severe abdominal pain and fever; the abdominal pain is concentrated in the right lower quadrant. Enlarged mesenteric lymph nodes and inflamed ileum and appendix are common findings in cases of *Y. enterocolitica* infections. Arthritis is a common extraintestinal form of *Y. enterocolitica* infection, usually following a GI episode or an appendicitis-like syndrome. This form of yersiniosis has been reported more often in adults than in children. The arthritic form resembles the arthritis seen in other bacterial infections—that is, in infections with *Shigella* spp., *Salmonella* spp., and *Neisseria gonorrhoeae* and in acute rheumatic fever. Erythema nodosum is an inflammatory reaction caused by *Y. enterocolitica* characterized by tender, red nodules that may be accompanied by itching and burning. The areas involved include the anterior portion of the legs; some patients have reported nodules on their arms. Reported cases have shown the syndrome to be more common in female patients compared with male patients.

Y. enterocolitica morphologically resembles other *Yersinia* spp., appearing as gram-negative coccobacilli with bipolar staining. The organism also grows on routine isolation media, such as SBA and MAC agar. It has an optimal growth temperature of 25° C to 30° C. *Y. enterocolitica* grows better with cold enrichment, and motility is clearly noted at 25° C but not at 35° C. Appropriate cultures on a specific *Yersinia* medium at 25° C should be performed in diarrheal outbreaks of unknown etiology. For cold enrichment, fecal samples suspected of containing this organism are inoculated into isotonic saline and kept at 4° C for 1 to 3 weeks, with weekly subculturing to selective agar for *Yersinia*.

Cefsulodin-irgasan-novobiocin (CIN) agar, a selective medium to detect the presence of *Y. enterocolitica*, incorporates

TABLE 19-10 Differentiation of Selected Species within the Genus *Yersinia*

Test	*Y. pestis*	*Y. enterocolitica*	*Y. pseudotuberculosis*
Indole	–	d	–
Methyl red	+	+	+
Voges-Proskauer			
25°C	–	d	–
37°C	–	–	–
Motility			
25°C	–	+	+
37°C	–	–	–
β-Galactosidase	+	+	+
Christensen urea	–	+	+
Phenylalanine deaminase	–	–	–
Ornithine decarboxylase	–	+	–
Acid produced from			
Sucrose	–	+	–
Lactose	–	–	–
Rhamnose	–	– or +*	+
Melibiose	–	– or +*	+
Trehalose	–	+ or –	+
Cellobiose	–	+	–

Modified from Washington J: *Laboratory procedures in clinical microbiology*, ed 2, New York, 1985, Springer-Verlag.
+, ≥90% positive reaction within 1 or 2 days; –, no reaction (≥90%) in 30 days; *– or +,* most strains negative, some cultures positive; *+ or (+),* most reactions occur within 1 or 2 days, some are delayed; *d,* different reactions; *+ or –,* most cultures positive, some strains negative.
*Test results at 25°C.

cefsulodin, irgasan, novobiocin, bile salts, and crystal violet as inhibitory agents. This medium, which inhibits normal colon microbiota better than MAC agar, provides better opportunities to recover *Y. enterocolitica* from feces. This selective medium has been modified, and manufacturers such as BD Diagnostic Systems (Sparks, MD) have added a differential property (mannitol) to the medium, named *Yersinia*-selective agar (YSA) base. Fermentation of mannitol results in a decrease in pH around the colony, causing the pH indicator, neutral red, to turn red at the center of the colony and the bile to precipitate. Nonfermentation of mannitol produces a colorless, translucent colony. A slightly modified formulation of the original CIN medium, known as CIN II, can be used to isolate simultaneously most *Aeromonas* spp. from stool samples.

Yersinia pseudotuberculosis

Yersinia pseudotuberculosis, similar to *Y. pestis*, is a pathogen primarily of rodents, particularly guinea pigs. In addition to farm and domestic animals, birds are natural reservoirs; turkeys, geese, pigeons, doves, and canaries have yielded positive cultures for this organism. *Y. pseudotuberculosis* causes a disease characterized by caseous swellings called *pseudotubercles*. The disease is often fatal in animals.

Human infections, which are rare, are associated with close contact with infected animals or their fecal material or ingestion of contaminated drink and foodstuff. When the organisms are ingested, they spread to the mesenteric lymph nodes, producing a generalized infection that is usually self-limiting. The clinical manifestations can include septicemia accompanied by mesenteric lymphadenitis, a presentation similar to appendicitis. *Y. pseudotuberculosis* appears as a typical-looking plague bacillus. It can be differentiated from *Y. pestis* by its motility at 18°C to 22°C, production of urease, and ability to ferment rhamnose. Table 19-10 shows differentiating characteristics among *Yersinia* spp.

Other Genera of the Family Enterobacteriaceae

Budivicia

Based on DNA hybridization, *Budivicia aquatica* is a group of closely related organisms. They are not as closely related to the other members of Enterobacteriaceae, but they do qualify as members of the family. These organisms are usually found in water; however, they occasionally occur in clinical specimens.

Buttiauxella

The genus *Buttiauxella* consists of seven species isolated from water. Only *B. agrestis* and *B. noackiae* have been isolated from human specimens. Biochemically, these organisms are similar to both *Citrobacter* and *Kluyvera* species, but DNA hybridization distinctly differentiates *Buttiauxella* from both genera.

Cedecea

The genus *Cedecea* is composed of five species: *C. davisae*, *C. lapagei*, *C. neteri*, and *Cedecea* species types 3 and 5. Most have been recovered from sputum, blood, and wounds. Of the five, *C. davisae* is the most commonly isolated species.

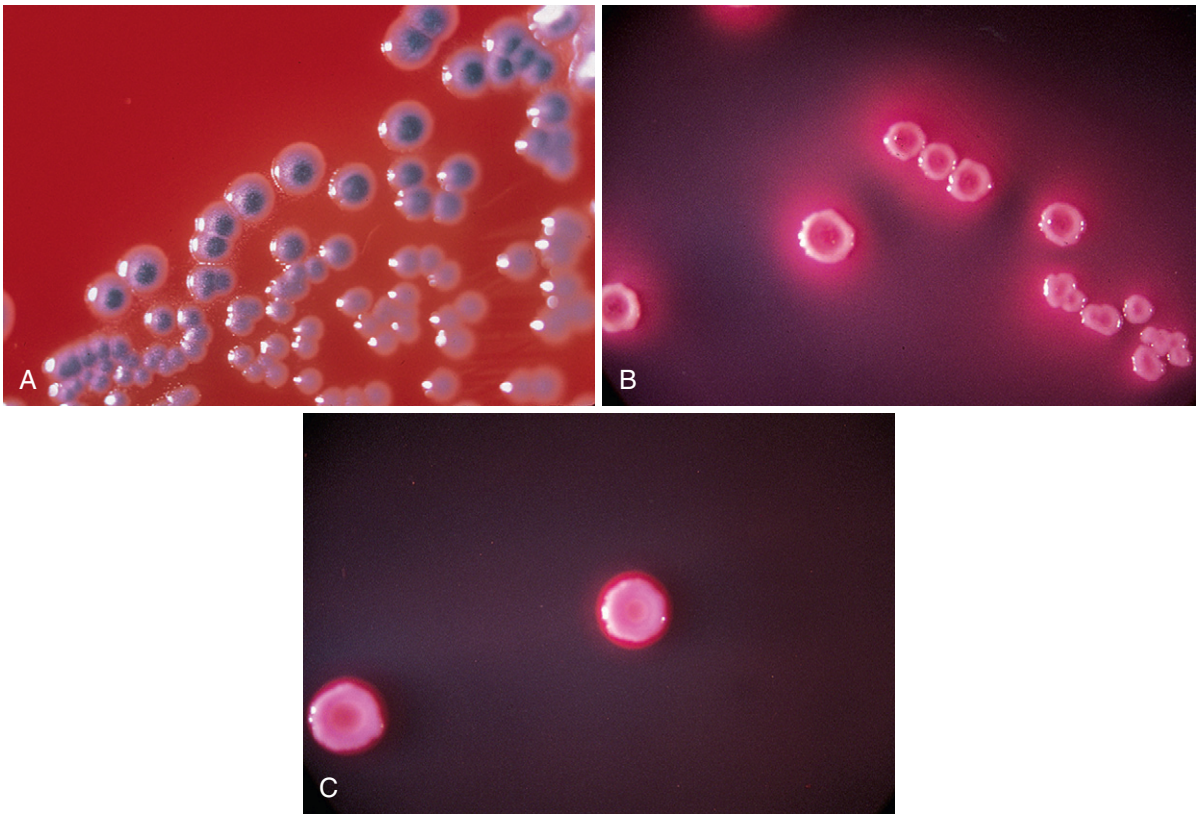

FIGURE 19-11 **A,** Blue-violet pigment of *Kluyvera* spp. growing on sheep blood agar (SBA). This *Kluyvera* species resembles the colony morphology of *Escherichia coli* growing on MacConkey (MAC) agar. **B,** Appearance of *K. cryocrescens* growing on MAC agar. **C,** Appearance of *K. ascorbata* growing on MAC agar.

Ewingella

Ewingella americana is the only species of the genus *Ewingella*. Most isolates have come from human blood cultures or respiratory specimens and exhibit resistance to multiple antimicrobial agetns. *Ewingella* was first thought to be related to *Cedecea*; however, DNA hybridization confirmed the placement of these organisms in separate genera.

Kluyvera

The genus *Kluyvera* is composed of three closely related species: *K. ascorbata* (the most common clinical isolate), *K. cryocrescens*, and *K. georgiana*. They have been found in respiratory, urine, and blood cultures. Most strains are nonpigmented, but occasional isolates may produce a reddish-blue or violet pigment. All species resemble *E. coli* colonies growing on MAC agar. Figure 19-11 illustrates the colony characteristics of *Kluyvera* spp. Cephalothin and carbenicillin disk susceptibility tests separate the first two species; *K. cryocrescens* shows large zones of inhibition, and *K. ascorbata* has small zones. In addition, *K. ascorbata* does not ferment glucose at 5° C, whereas *K. cryocrescens* ferments glucose at this temperature.

Leclercia

The name *Leclercia* was proposed in 1986 for 58 isolates from human clinical specimens, including blood, urine, sputum, and feces and 27 isolates from nonhuman sources. It has been isolated more recently in pure culture from a septicemia and wounds. The single species is *L. adecarboxylata*, which can have a yellow pigment but only on initial isolation. Although it has similar IMViC reactions to *E. coli*, it is negative for lysine and ornithine decarboxylase and arginine dihydrolase (i.e., triple decarboxylase-negative).

Leminorella

Leminorella was proposed as a genus for the Enteric Group 57, with two species: *L. grimontii* and *L. richardii*. These organisms produce H_2S and have shown weak reactions with *Salmonella* antisera. However, complete biochemical testing differentiates *Leminorella* from *Salmonella*; *Leminorella* spp. are relatively inactive. The clinical significance of these organisms is unknown; however, they have been isolated from patients with hospital-acquired infections.

Moellerella

The genus *Moellerella* contains one species, *M. wisconsensis*. *Moellerella* is positive for citrate, methyl red, lactose, and sucrose. It is negative for lysine, ornithine, arginine decarboxylase, and indole, and it resembles *E. coli* growing on enteric media. The clinical significance of this organism has not been established, although it has been isolated from feces in two cases of diarrhea, infected gallbladders, and a bronchial aspirate.

Obesumbacterium

Obesumbacterium proteus biogroup 2 is more closely related to *Escherichia blattae* than to other members of the family Enterobacteriaceae. These isolates are fastidious, slow-growing organisms at 37° C and have not been found in human specimens.

Photorhabdus

The genus *Photorhabdus* includes three species: *P. luminescens*, with subspecies *luminescens*, *akhurstii*, and *laumondii*; *P. asymbiotica*; and *P. temperate*. Their natural habitat is the lumen of entomopathogenic nematodes, but strains have occasionally been isolated from human specimens. They occur in two phases with the property of luminescence in phase 1 only. Most strains produce pink, red, orange, yellow, or green pigmented colonies on nutrient agar and especially on nutrient-rich media, such as trypticase soy agar and egg yolk agar. They are also negative for nitrate reduction.

Rahnella

Rahnella aquatilis is the name given to a group of water bacteria that are psychrotolerant, growing at 4° C. These organisms have no single characteristic that distinguishes them from the other members of the Enterobacteriaceae. They resemble *E. agglomerans*; however, they can be distinguished by a weak phenylalanine deaminase reaction; the fact that they are negative for potassium cyanide (KCN), gelatin, lysine, ornithine, and motility; and their lack of yellow pigmentation. They have been occasionally isolated from human clinical specimens, including wound infections, bacteremias, feces from patients with acute gastroenteritis, and septicemia, especially from immunocompromised patients.

Tatumella

Tatumella ptyseos is the only species of the genus *Tatumella*. This organism is unusual for the family Enterobacteriaceae in several ways: stock cultures can be kept frozen in sheep red blood cells or freeze-dried, but they die in a few weeks on agar slants; they show more biochemical reactions at 25° C than at 35° C; they are motile at 25° C but not at 35° C; and they demonstrate large 15- to 36-mm zones of inhibition around penicillin disks. In addition, *Tatumella* isolates are slow growing, produce tiny colonies, and are relatively nonreactive in laboratory media. These organisms have been isolated from human sources, especially sputum, and may be a rare cause of infection.

Trabulsiella

Trabulsiella guamensis is the only species in this genus, and although it is very rarely isolated, it is biochemically similar to *Salmonella*. It was formerly called Enteric Group 90. The type strain was isolated from vacuum-cleaner contents on the island of Guam when environmental indoor dirt samples were being collected. It has been isolated from human diarrheal samples as well, but its role in disease is unknown.

Yokenella

Yokenella regensburgei was first thought to be another species of *Hafnia*, but DNA hybridization showed a 15% relatedness, which was not sufficient to include these organisms in that genus. They are biochemically similar to *Hafnia* but differ primarily by yielding negative Voges-Proskauer test results. *Yokenella* strains have been isolated from human specimens, but further study is required to determine their significance in human disease.

Laboratory Diagnosis of Enterobacteriaceae

Specimen Collection and Transport

Members of the family Enterobacteriaceae can be isolated from a wide variety of clinical samples. Most often these bacterial species are isolated with other organisms, including more fastidious pathogens. To ensure isolation of both opportunistic and fastidious pathogens, laboratories must provide appropriate transport media, such as Cary-Blair, Amies, or Stuart media. Microbiology personnel must encourage immediate transport of clinical samples to the laboratory for processing, regardless of the source of the clinical specimen.

Isolation and Identification

To determine the clinical significance of the isolate, the microbiologist must consider the site of origin. Generally, enteric opportunistic organisms isolated from sites that are normally sterile are highly significant. However, careful examination is critical of organisms recovered from, for example, the respiratory tract, urogenital tract, stool, and wounds in open sites that are inhabited by other endogenous microbiota. Members of the family Enterobacteriaceae are routinely isolated from stool cultures; complete identification should be directed only toward true intestinal pathogens. Sputum cultures from hospitalized patients may contain enteric organisms that may require complete identification.

Direct Microscopic Examination

In contrast to gram-positive bacteria, in which microscopic morphology may help provide a presumptive identification, the microscopic characteristics of enterics are indistinguishable from other gram-negative bacilli. However, smears prepared directly from CSF, blood, and other body fluids or exudates from an uncontaminated site can be examined microscopically for the presence of gram-negative bacteria. Although this examination is nonspecific for enteric organisms, this presumptive result may aid the clinician in the preliminary diagnosis of the infection, and appropriate therapy can be instituted immediately.

Direct smears prepared from samples, such as sputum, that contain endogenous microbiota do not provide valuable information because their significance cannot be fully assessed unless the gram-negative bacteria are prevalent and endogenous inhabitants are absent. Direct smear examination of stool samples is not particularly helpful in identifying enteric pathogens but may reveal the presence of inflammatory cells. This information may be helpful in determining whether a GI disease is toxin-mediated or an invasive process.

Culture

Members of the family Enterobacteriaceae are facultative anaerobes, and most clinically significant species grow at an optimal temperature of 35° C to 37° C. Certain species can grow at low

temperatures (1° C to 5° C, such as *Serratia* and *Yersinia*) or tolerate high temperatures (45° C to 50° C, such as *E. coli*). Colonies become visible on nonselective and differential media after 18 to 24 hours of incubation. Most laboratories use a wide variety of nonselective media, such as SBA and CHOC agar, as well as selective media, such as MAC, to recover enteric organisms from wounds, respiratory tract secretions, urine, and sterile body fluids. On CHOC agar or SBA plates, enteric bacteria produce large, grayish, smooth colonies. On SBA, colonies may be β-hemolytic or nonhemolytic.

Screening Stool Cultures for Pathogens

Because of the mixed microbial biota of fecal specimens, efficient screening methods should be used for the recovery and identification of enteric pathogens including, *Salmonella*, *Shigella*, *Yersinia*, *E. coli* O157:H7, *Aeromonas*, *Campylobacter*, *Vibrio*, and *Plesiomonas shigelloides*. All fecal specimens should be routinely screened for *Salmonella*, *Shigella*, and *Campylobacter* (see Chapter 20). In addition, many laboratories screen for *E. coli* O157:H7. Screening routinely for the remaining organisms may not be cost-effective; these organisms should be addressed on the basis of patient history (e.g., travel near coastal areas where certain organisms are endemic) and gross description of the specimen (bloody or watery).

Stool specimens contain enteric organisms as normal colon microbiota; in processing stool samples, laboratories may develop their own protocol for the maximum recovery of enteric pathogens. Fecal pathogens are generally nonlactose fermenters (NLFs). These organisms appear as clear or colorless and translucent colonies on MAC agar. However, many of the bacteria that compose common fecal microbiota also appear as NLFs, for example, *Proteus*, *Providencia*, and *Pseudomonas*, as well as delayed lactose-fermenting organisms (e.g., *Serratia* and *Citrobacter*). For this reason, it is necessary to set up screening tests to differentiate these organisms from stool pathogens. An easy approach is to take a well-isolated, NLF colony and perform a screening battery of tests consisting first of an oxidase test and the inoculation of lysine iron agar (LIA) and TSI agar slants (Table 19-11). If the screening tests identify a group of organisms that are nonpathogens, the process is complete, and the culture is discarded (after 48 hours of incubation).

Most clinical microbiologists inoculate stool samples on highly selective media, such as HE or XLD agars, in addition to regular MAC and SMAC agar for *E. coli* O157:H7. An enrichment broth (e.g., selenite) has been traditionally inoculated to enhance recovery; however, laboratories are starting to discontinue this practice. As previously mentioned, CIN agar can serve the dual purpose of screening for both *Y. enterocolitica* and most *Aeromonas* spp., but one must remember that *Yersinia* is oxidase-negative, *Aeromonas* is oxidase-positive, and testing for this trait must be done on SBA, not on selective or differential media.

On HE agar, lactose-fermenting species produce yellow colonies, whereas NLFs such as *Shigella* spp. produce green colonies (Figure 19-12). However, *Proteus* spp. are NLFs, and species that produce H_2S also appear green with black centers on HE agar. *C. freundii* usually produces yellow colonies with black centers. NLF species such as *Salmonella enterica*, which produces lysine decarboxylase, produce red colonies with black centers on XLD medium (Figure 19-13). Another selective and differential medium is SS (*Salmonella-Shigella*) agar, a light straw-colored medium where *Salmonella* colonies appear colorless with dark black centers from the production of H_2S and *Shigella* colonies

TABLE 19-11 Stool Culture Screening for Enteric Pathogens Using Triple Sugar Iron and Lysine-Iron Agar in Combination

	TSI Reactions							
LIA Reactions	**K/A H_2S**	**K/AG H_2S**	**K/AG**	**K/A**	**A/A H_2S**	**A/AG**	**A/A**	**K/K**
R/A		*P. vulgaris* *P. mirabilis*	*M. morganii* *Providencia*	*M. morganii* *Providencia*	*P. vulgaris* *P. mirabilis*	—	*Providencia*	—
K/K H_2S	*Salmonella** *Edwardsiella*	*Salmonella** *Edwardsiella**	*Salmonella**	*Salmonella**	—	—	—	—
K/K	*Salmonella*	—	*Hafnia* *Klebsiella* *Serratia*	*Salmonella** *Plesiomonas*† *Hafnia*	—	*Klebsiella* *Enterobacter* *E. coli*	*Serratia*	*Pseudomonas*†
K/A H_2S	—	*Salmonella**	—	*Serratia*	—	—	—	—
K/A	—	*Citrobacter*	*Salmonella** *Shigella* *Aeromonas*† *E. coli* *Enterobacter* *Citrobacter*	*Shigella** *Yersinia* *Aeromonas*† *E. coli* *Enterobacter*	*Citrobacter*	*Aeromonas**† *E. coli* *Citrobacter* *Enterobacter*	*Aeromonas**† *Yersinia* *Citrobacter* *Enterobacter*	—

Data from Microbiology Laboratory, The Ohio State University Hospitals and Maureta Ott, Columbus, OH.
A, Acid; *G,* gas; *H_2S,* hydrogen sulfide; *K,* alkaline; *LIA,* Lysine-iron agar; *R,* deamination (red slant); *TSI,* triple sugar iron.
*Results of TSI and LIA reactions in this category indicate a potential pathogen; additional tests must be performed.
†Oxidase-positive.

FIGURE 19-12 Clear, green colonies of *Shigella* growing on Hektoen enteric (HE) agar. (Courtesy R. Abe Baalness.)

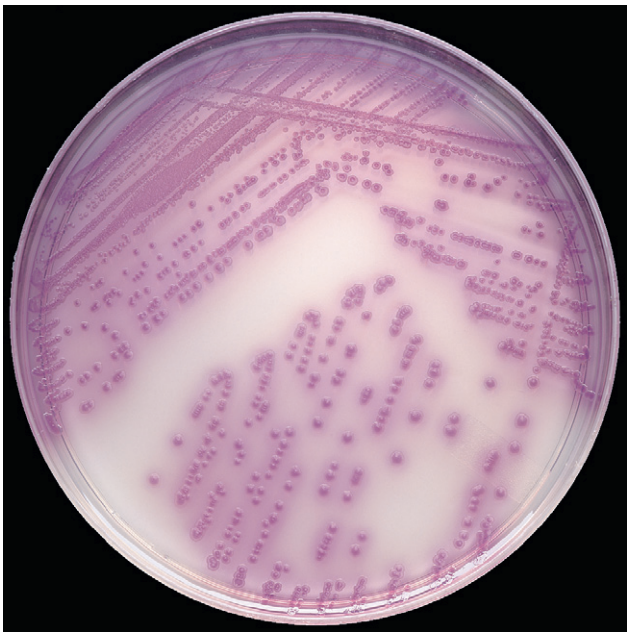

FIGURE 19-14 *Salmonella* growing on CHROMagar Salmonella differential agar. (Courtesy BD Diagnostic Systems, Sparks, MD.)

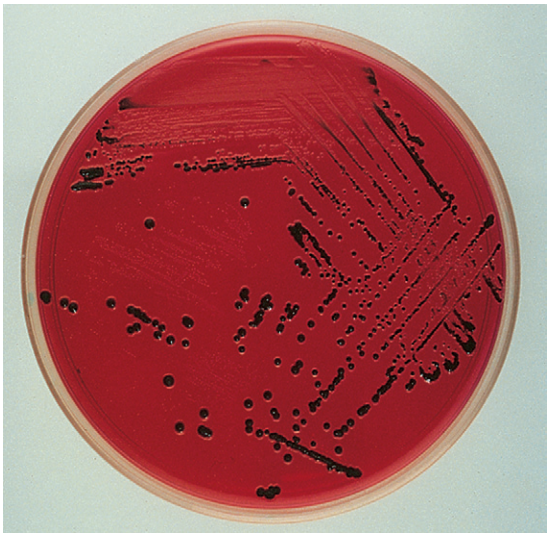

FIGURE 19-13 H_2S–producing colonies of salmonellae growing on xylose-lysine-desoxycholate (XLD) agar. (Courtesy American Society for Clinical Laboratory Science, Education and Research Fund, Inc, 1982.)

appear as colorless colonies only. On CHROMagar Salmonella (CHROMagar Co, Paris, France), *Salmonella* isolates produce mauve-colored colonies (Figure 19-14) owing to the activity of an esterase on a patented substrate. Other members of the family Enterobacteriaceae produce blue or white colonies.

Identification

The identification of members of the family Enterobacteriaceae can be accomplished in several ways. Certain laboratories choose to use conventional biochemical tests in tubes, whereas others may prefer miniaturized or automated commercial identification systems. See Chapter 9 for a thorough description of the currently available rapid and automated commercial identification systems. The use of conventional biochemical tests in tubes is cumbersome to test isolates with all the biochemical tests available. Most clinical laboratories develop identification tables and protocols using a limited number of tests that suit their needs and capabilities. These tables are based on the key features necessary to identify each particular genus and clinically relevant species. Figure 19-15 shows an example of a schematic diagram for the identification of commonly isolated enterics using conventional biochemical tests. Table 19-12 shows the differentiating characteristics of the species, biogroups, and enteric groups of the Enterobacteriaceae.

To identify an isolate, the clinical microbiologist first must determine whether the isolate belongs to the family Enterobacteriaceae. All members of the family: (1) are oxidase-negative except for *Plesiomonas shigelloides* (see Chapter 20), (2) ferment glucose, and (3) reduce nitrate to nitrite except *Photorhabdus* and *Xenorhabdus* (an environmental isolate). Gram-negative isolates, especially NLFs, should be tested for cytochrome oxidase production. The oxidase test should always be performed using young growth from an SBA plate. Testing colonies from highly selective media such as CIN can give a false-negative reaction, whereas MAC and EMB agars may give the appearance of a false-positive reaction from the pH indicators and dyes present in the media.

Regardless of the identification system used, the clinical microbiologist may presumptively determine utilization of carbohydrates by observing the colony morphology of the isolate on differential or selective media such as MAC, SMAC, CIN, HE, or XLD. The traditional biochemical tests to perform for identification include the following:

Text continued on p. 452

TABLE 19-12 Biochemical Reactions of Named Species and Unnamed Groups of the Family Enterobacteriaceae*

Organism	Indole Production	Methyl Red	Voges-Proskauer	Citrate (Simmons)	H_2S (TSI)	Urea Hydrolysis	Phenylalanine Deaminase	Lysine Decarboxylase	Arginine Dihydrolase	Ornithine Decarboxylase	Motility	Gelatin Hydrolysis (22° C)	Growth in KCN	Malonate Utilization	D-Glucose, Acid	D-Glucose, Gas	Lactose Fermentation	Sucrose Fermentation	D-Mannitol Fermentation	Dulcitol Fermentation	Salicin Fermentation	Adonitol Fermentation
Budvicia aquatic	0	93	0	0	80	33	0	0	0	0	27	0	0	0	100	53	87	0	60	0	0	0
Buttiauxella agrestis	0	100	0	100	0	0	0	0	0	100	100	0	80	60	100	100	100	0	100	0	100	0
Buttiauxella brennerae	0	100	0	0	0	0	0	0	0	33	100	0	100	100	100	100	67	0	100	0	100	67
Buttiauxella ferragutiae	0	100	0	0	0	0	0	100	0	80	60	0	40	0	100	100	0	0	100	0	100	0
Buttiauxella gaviniae	0	100	0	20	0	0	0	0	20	0	80	0	60	100	100	40	60	0	100	0	100	100
Buttiauxella izardii	0	100	0	0	0	0	0	0	0	100	100	0	67	100	100	100	100	0	100	0	100	0
Buttiauxella noackiae	33	100	0	33	0	0	100	0	67	0	100	0	100	100	100	100	0	0	100	0	100	0
Buttiauxella warmboldiae	0	100	0	33	0	0	100	0	0	0	100	0	33	100	100	100	0	0	100	0	100	0
Cedecea davisae	0	100	50	95	0	0	0	0	50	95	95	0	86	91	100	70	19	100	100	0	99	0
Cedecea lapagei	0	40	80	99	0	0	0	0	80	0	80	0	100	99	100	100	60	0	100	0	100	0
Cedecea neteri	0	100	50	100	0	0	0	0	100	0	100	0	65	100	100	100	35	100	100	0	100	0
Cedecea species 3	0	100	50	100	0	0	0	0	100	0	100	0	100	0	100	100	0	50	100	0	100	0
Cedecea species 5	0	100	50	100	0	0	0	0	50	50	100	0	100	0	100	100	0	100	100	0	100	0
Citrobacter amalonaticus	100	100	0	95	5	85	0	0	85	95	95	0	99	1	100	97	35	9	100	1	30	0
Citrobacter braakii	33	100	0	87	60	47	0	0	67	93	87	0	100	0	100	93	80	7	100	33	0	0
Citrobacter farmeri	100	100	0	10	0	59	0	0	85	100	97	0	93	0	100	96	15	100	100	2	9	0
Citrobacter freundii	33	100	0	78	78	44	0	0	67	0	89	0	89	11	100	89	78	89	100	11	0	0
Citrobacter gillenii	0	100	0	33	67	0	0	0	33	0	67	0	100	100	100	100	67	33	100	0	0	0
Citrobacter koseri (*C. diversus*)	99	100	0	99	0	75	0	0	80	99	95	0	0	95	100	98	50	40	99	40	15	99
Citrobacter murliniae	100	100	0	100	67	67	0	0	67	0	100	0	100	0	100	100	67	33	100	100	33	0
Citrobacter rodentium	0	100	0	0	0	100	0	0	0	100	0	0	0	100	100	100	100	0	100	0	0	0
Citrobacter sedlakii	83	100	0	83	0	100	0	0	100	100	100	0	100	100	100	100	100	0	100	100	17	0
Citrobacter werkmanii	0	100	0	100	100	100	0	0	100	0	100	0	100	100	100	100	17	0	100	0	0	0
Citrobacter youngae	15	100	0	75	65	80	0	0	50	5	95	0	95	5	100	75	25	20	100	85	10	0
Cronobacter sakazakii	11	5	100	99	0	1	50	0	99	91	96	0	99	18	100	98	99	100	100	5	99	0
Enteric group 137 (5 strains)	100	100	0	0	0	70	0	0	20	100	100	0	100	0	100	0	100	100	100	0	100	0
Edwardsiella hoshinae	50	100	0	0	0	0	0	100	0	95	100	0	0	100	100	35	0	100	100	0	50	0
Edwardsiella ictaluri	0	0	0	0	0	0	0	100	0	65	0	0	0	0	100	50	0	0	0	0	0	0
Edwardsiella tarda	99	100	0	1	100	0	0	100	0	100	98	0	0	0	100	100	0	0	0	0	0	0
Edwardsiella tarda biogroup 1	100	100	0	0	0	0	0	100	0	100	100	0	0	0	100	50	0	100	100	0	0	0
Enterobacter aerogenes	0	5	98	95	0	2	0	98	0	98	97	0	98	95	100	100	95	100	100	5	100	98
Enterobacter amnigenus biogroup 1	0	7	100	70	0	0	0	0	9	55	92	0	100	91	100	100	70	100	100	0	91	0
Enterobacter amnigenus biogroup 2	0	65	100	100	0	0	0	0	35	100	100	0	100	100	100	100	35	0	100	0	100	0
Enterobacter asburiae	0	100	2	100	0	60	0	0	21	95	0	0	97	3	100	95	75	100	100	0	100	0

From the Centers for Disease Control and Prevention, Atlanta, GA.
H_2S, Hydrogen sulfide; *KCN,* Potassium cyanide; *ONPG,* *o*-nitrophenyl-β-D-galactopyranoside; *TSI,* triple sugar iron.
*Each number is the percentage of positive reactions after 2 days of incubation at 36°C unless noted otherwise. Most of these positive reactions occur within 24 hours. Reactions that become positive after 2 days are not considered.

myo-Inositol Fermentation	D-Sorbitol Fermentation	L-Arabinose Fermentation	Raffinose Fermentation	L-Rhamnose Fermentation	Maltose Fermentation	D-Xylose Fermentation	Trehalose Fermentation	Cellobiose Fermentation	α-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 (25° C)	Nitrate Nitrite	Oxidase, Kovacs'	ONPG Test	Yellow Pigment	D-Mannose Fermentation	Tyrosine Utilization	D-Galactose	Citrate, Christensen's
0	0	80	0	100	0	93	0	0	0	0	0	0	27	0	20	27	0	0	0	100	0	93	0	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			
0	0	100	100	33	100	100	100	100	0	0	100	100	67	67	67	0	0	0	0	100	0	100	0	100			
0	100	100	0	100	100	100	100	100	40	0	100	0	0	0	60	0	0	0	0	100	0	100	0	100			
0	0	100	0	100	60	100	100	100	0	0	100	0	80	0	80	40	0	0	0	100	0	100	0	100			
0	0	100	33	100	100	100	100	100	0	0	100	67	0	33	100	67	0	0	0	100	0	100	0	100			
0	0	100	0	100	100	100	100	100	33	0	100	0	0	0	100	100	0	0	0	100	0	100	0	100			
67	0	100	0	100	100	100	100	100	0	0	100	0	0	0	0	0	0	0	0	100	0	100	0	100			
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			
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			
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			
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			
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			
0	99	99	5	100	99	99	100	100	2	0	5	0	0	60	96	96	86	0	0	99	0	97	0	100			
0	100	100	7	100	100	100	100	73	33	0	0	80	0	87	100	93	53	0	0	100	0	80	0	100			
0	98	100	100	100	100	100	100	100	75	0	0	100	0	65	100	93	80	0	0	100	0	100	0	100			
0	100	100	44	100	100	89	100	44	11	0	0	100	0	100	100	100	44	0	0	100	0	89	0	100			
0	100	100	0	100	100	100	100	67	0	0	0	67	0	67	67	100	0	0	0	100	0	67	0	100			
0	99	99	0	99	100	100	100	99	40	0	1	0	98	99	95	90	75	0	0	100	0	99	0	100			
0	100	100	33	100	100	100	100	100	0	0	0	33	0	100	100	100	33	0	0	100	0	100	0	100			
0	100	100	0	100	100	100	100	100	0	0	0	0	0	0	100	100	0	0	0	100	0	100	0	100			
0	100	100	0	100	100	100	100	100	0	0	17	100	0	83	100	100	83	0	0	100	0	100	0	100			
0	100	100	0	100	100	100	100	0	0	0	0	0	0	100	100	100	100	0	0	100	0	100	0	100			
5	100	100	10	100	95	100	100	45	0	0	5	10	5	90	100	100	65	0	0	85	0	90	0	100			
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			
0	100	100	100	100	100	100	100	100	80	0	100	100	0	100	100	50	100	0	0	100	0	100	0	100	0	100	90
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	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0	100			
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			
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			
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			
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			
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			
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			

Continued

TABLE 19-12 Biochemical Reactions of Named Species and Unnamed Groups of the Family Enterobacteriaceae—cont'd

Organism	Indole Production	Methyl Red	Voges-Proskauer	Citrate (Simmons)	H_2S (TSI)	Urea Hydrolysis	Phenylalanine Deaminase	Lysine Decarboxylase	Arginine Dihydrolase	Ornithine Decarboxylase	Motility	Gelatin Hydrolysis (22° C)	Growth in KCN	Malonate Utilization	D-Glucose, Acid	D-Glucose, Gas	Lactose Fermentation	Sucrose Fermentation	D-Mannitol Fermentation	Dulcitol Fermentation	Salicin Fermentation	Adonitol Fermentation
Enterobacter cancerogenus (*E. taylorae*)	0	5	100	100	0	1	0	0	94	99	99	0	98	100	100	100	10	0	100	0	92	0
Enterobacter cloacae	0	5	100	100	0	65	0	0	97	96	95	0	98	75	100	100	93	97	100	15	75	25
Enterobacter dissolvens	0	0	100	100	0	100	0	0	100	100	0	0	100	100	100	100	0	100	100	0	100	0
Enterobacter gergoviae	0	5	100	99	0	93	0	90	0	100	90	0	0	96	100	98	55	98	99	0	99	0
Enterobacter hormaechei	0	57	100	96	0	87	4	0	78	91	52	0	100	100	100	83	9	100	100	87	44	0
Enterobacter intermedium	0	100	100	65	0	0	0	0	0	89	89	0	65	100	100	100	100	65	100	100	100	0
Enterobacter nimipressuralis	0	100	100	0	0	0	0	0	0	100	0	0	100	100	100	100	0	0	100	0	100	0
Enterobacter pyrinus	0	29	86	0	0	86	0	100	0	100	43	0	0	86	100	100	14	100	100	0	100	0
Escherichia albertii	0		0	0	0	0	0	100	0	100	0	0	0	0	100	100	0	0	100	0	0	0
Escherichia blattae	0	100	0	50	0	0	0	100	0	100	0	0	0	100	100	100	0	0	0	0	0	0
Escherichia coli	98	99	0	1	1	1	0	90	17	65	95	0	3	0	100	95	95	50	98	60	40	5
Escherichia coli, inactive	80	95	0	1	1	1	0	40	3	20	5	0	1	0	100	5	25	15	93	40	10	3
Escherichia fergusonii	98	100	0	17	0	0	0	95	5	100	93	0	0	35	100	95	0	0	98	60	65	98
Escherichia hermannii	99	100	0	1	0	0	0	6	0	100	99	0	94	0	100	97	45	45	100	19	40	0
Escherichia vulneris	0	100	0	0	0	0	0	85	30	0	100	0	15	85	100	97	15	8	100	0	30	0
Ewingella americana	0	84	95	95	0	0	0	0	0	0	60	0	5	0	100	0	70	0	100	0	80	0
Hafnia alvei	0	40	85	10	0	4	0	100	6	98	85	0	95	50	100	98	5	10	99	0	13	0
Hafnia alvei biogroup 1	0	85	70	0	0	0	0	100	0	45	0	0	0	45	100	0	0	0	55	0	55	0
Klebsiella oxytoca	99	20	95	95	0	90	1	99	0	0	0	0	97	98	100	97	100	100	99	55	100	99
Klebsiella ornithinolytica	100	96	70	100	0	100	0	100	0	100	0	0	100	100	100	100	100	100	100	10	100	100
Klebsiella planticola	20	100	98	100	0	98	0	100	0	0	0	0	100	100	100	100	100	100	100	15	100	100
Klebsiella pneumoniae subsp. *ozaenae*	0	98	0	30	0	10	0	40	6	3	0	0	88	3	100	50	30	20	100	2	97	97
Klebsiella pneumoniae subsp. *pneumoniae*	0	10	98	98	0	95	0	98	0	0	0	0	98	93	100	97	98	99	99	30	99	90
Klebsiella pneumoniae subsp. *rhinoscleromatis*	0	100	0	0	0	0	0	0	0	0	0	0	80	95	100	0	0	75	100	0	98	100
Klebsiella terrigena	0	60	100	40	0	0	0	100	0	20	0	0	100	100	100	80	100	100	100	20	100	100
Kluyvera ascorbata	92	100	0	96	0	0	0	97	0	100	98	0	92	96	100	93	98	98	100	25	100	0
Kluyvera cryocrescens	90	100	0	80	0	0	0	23	0	100	90	0	86	86	100	95	95	81	95	0	100	0
Kluyvera georgiana	100	100	0	100	0	0	0	100	0	100	100	0	83	50	100	17	83	100	100	33	100	0
Leclercia adecarboxylata	100	100	0	0	0	48	0	0	0	0	79	0	97	93	100	97	93	66	100	86	100	93
Leminorella grimontii	0	100	0	100	100	0	0	0	0	0	0	0	0	0	100	33	0	0	0	83	0	0
Leminorella richardii	0	0	0	0	100	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0	0
Moellerella wisconsensis	0	100	0	80	0	0	0	0	0	0	0	0	70	0	100	0	100	100	60	0	0	100

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H_2S, Hydrogen sulfide; *KCN,* Potassium cyanide; *ONPG,* *o*-nitrophenyl-β-D-galactopyranoside; *TSI,* triple sugar iron.
*Each number is the percentage of positive reactions after 2 days of incubation at 36°C unless noted otherwise. Most of these positive reactions occur within 24 hours. Reactions that become positive after 2 days are not considered.

myo-Inositol Fermentation	D-Sorbitol Fermentation	L-Arabinose Fermentation	Raffinose Fermentation	L-Rhamnose Fermentation	Maltose Fermentation	D-Xylose Fermentation	Trehalose Fermentation	Cellobiose Fermentation	α-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 (25°C)	Nitrate Nitrite	Oxidase, Kovacs'	ONPG Test	Yellow Pigment	D-Mannose Fermentation	Tyrosine Utilization	D-Galactose	Citrate, Christensen's
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			
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			
0	100	100	100	100	100	100	100	100	100	0	100	100	0	0	100	0	100	0	0	100	0	100	0	100			
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			
0	0	100	0	100	100	96	100	100	83	0	0	0	0	4	96	13	74	0	0	100	0	95	0	100			
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			
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	0	100	0	100	100	0	100	100	0	0	100	0	0	0	0	0	0	0	0	100	0	100	0	100			
0	0	100	0	0	60	0	60	0	0	0	20	0	0	100	0		100	0	0	100	0	100	0	100			
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			
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			
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			
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			
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			
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	23	16	13	99	10	0	0	50	0	99	24	0	35	10	0	0	97	0	85	0	99			
0	0	95	2	97	100	98	95	15	0	0	7	0	0	95	0	70	15	0	0	100	0	90	0	100			
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			
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			
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	92	100	100	100	100	100	100	100	100	0	100	100	100	100	100	100	62	0	0	100	0	100	1	100			
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			
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			
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			
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			
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			
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			
0	0	100	100	83	100	100	100	100	100	0	100	100	0	33	83	50	83	0	0	100	0	100	0	100			
0	0	100	66	100	100	100	100	100	0	0	100	100	96	3	93	83	28	0	0	100	0	100	37	100			
0	0	100	0	0	0	83	0	0	0	0	0	0	0	17	100	100	0	0	0	100	0	0	0	0			
0	0	100	0	0	0	100	0	0	0	0	0	0	0	0	50	100	0	0	0	100	0	0	0	0			
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			

Continued

TABLE 19-12 Biochemical Reactions of Named Species and Unnamed Groups of the Family Enterobacteriaceae—cont'd

Organism	Indole Production	Methyl Red	Voges-Proskauer	Citrate (Simmons)	H_2S (TSI)	Urea Hydrolysis	Phenylalanine Deaminase	Lysine Decarboxylase	Arginine Dihydrolase	Ornithine Decarboxylase	Motility	Gelatin Hydrolysis (22° C)	Growth in KCN	Malonate Utilization	D-Glucose, Acid	D-Glucose, Gas	Lactose Fermentation	Sucrose Fermentation	D-Mannitol Fermentation	Dulcitol Fermentation	Salicin Fermentation	Adonitol Fermentation
Morganella morganii subsp. *morganii*	95	95	0	0	20	95	95	1	0	95	95	0	98	1	99	90	1	0	0	0	0	0
Morganella morganii subsp. *sibonii*	50	86	0	0	7	100	93	29	0	64	79	0	79	0	100	86	0	7	0	0	0	0
Morganella morganii biogroup 1	100	95	0	0	15	100	100	100	0	80	0	0	90	5	100	93	0	0	0	0	0	0
Obesumbacterium proteus biogroup 2	0	15	0	0	0	0	0	100	0	100	0	0	0	0	100	0	0	0	0	0	0	0
Pantoea agglomerans	20	50	70	50	0	20	20	0	0	0	85	2	35	65	100	20	40	75	100	15	65	7
Pantoea dispersa	0	82	64	100	0	0	9	0	0	0	100	0	82	9	100	0	0	1	100	0	0	0
Photorhabdus luminescens (all tests at 25°C)	50	0	0	50	0	25	0	0	0	0	100	50	0	0	75	0	0	0	0	0	0	0
Photorhabdus DNA hybridization group 5	0	0	0	20	0	60	0	0	0	0	100	80	20	0	100	0	0	0	0	0	0	0
Pragia fontium	0	100	0	89	89	0	22	0	0	0	100	0	0	0	100	0	0	0	0	0	78	0
Proteus mirabilis	2	97	50	65	98	98	98	0	0	99	95	90	98	2	100	96	2	15	0	0	0	0
Proteus myxofaciens	0	100	100	50	0	100	100	0	0	0	100	100	100	0	100	100	0	100	0	0	0	0
Proteus penneri	0	100	0	0	30	100	99	0	0	0	85	50	99	0	100	45	1	100	0	0	0	0
Proteus vulgaris	98	95	0	15	95	95	99	0	0	0	95	91	99	0	100	85	2	97	0	0	50	0
Providencia alcalifaciens	99	99	0	98	0	0	98	0	0	1	96	0	100	0	100	85	0	15	2	0	1	98
Providencia heimbachae	0	85	0	0	0	0	100	0	0	0	46	0	8	0	100	0	0	0	0	0	0	92
Providencia rettgeri	99	93	0	95	0	98	98	0	0	0	94	0	97	0	100	10	5	15	100	0	50	100
Providencia rustigianii	98	65	0	15	0	0	100	0	0	0	30	0	100	0	100	35	0	35	0	0	0	0
Providencia stuartii	98	100	0	93	0	30	95	0	0	0	85	0	100	0	100	0	2	50	10	0	2	5
Rahnella aquatilis	0	88	100	94	0	0	95	0	0	0	6	0	0	100	100	98	100	100	100	88	100	0
Salmonella bongori	0	100	0	94	100	0	0	100	94	100	100	0	100	0	100	94	0	0	100	94	0	0
Salmonella enterica subsp. *arizonae*	1	100	0	99	99	0	0	99	70	99	99	0	1	95	100	99	15	1	100	0	0	0
Salmonella enterica subsp. *diarizonae*	2	100	0	98	99	0	0	99	70	99	99	0	1	95	100	99	85	5	100	1	0	0
Salmonella enterica subsp. *enterica*	1	100	0	95	95	1	0	98	70	97	95	0	0	0	100	96	1	1	100	96	0	0
Salmonella enterica subsp. *houtenae*	0	100	0	98	100	2	0	100	70	100	98	0	95	0	100	100	0	0	98	0	60	5
Salmonella enterica subsp. *indica*	0	100	0	89	100	0	0	100	67	100	100	0	0	0	100	100	22	0	100	67	0	0
Salmonella enterica subsp. *salamae*	2	100	0	100	100	0	0	100	90	100	98	2	0	95	100	100	1	1	100	90	5	0
Salmonella serotype Choleraesuis	0	100	0	25	50	0	0	95	55	100	95	0	0	0	100	95	0	0	98	5	0	0
Salmonella serotype Gallinarum	0	100	0	0	100	0	0	90	10	1	0	0	0	0	100	0	0	0	100	90	0	0
Salmonella serotype Paratyphi A	0	100	0	0	10	0	0	0	15	95	95	0	0	0	100	99	0	0	100	90	0	0

From the Centers for Disease Control and Prevention, Atlanta, GA.
H_2S, Hydrogen sulfide; *KCN,* Potassium cyanide; *ONPG,* *o*-nitrophenyl-β-D-galactopyranoside; *TSI,* triple sugar iron.
*Each number is the percentage of positive reactions after 2 days of incubation at 36°C unless noted otherwise. Most of these positive reactions occur within 24 hours. Reactions that become positive after 2 days are not considered.

myo-Inositol Fermentation	D-Sorbitol Fermentation	L-Arabinose Fermentation	Raffinose Fermentation	L-Rhamnose Fermentation	Maltose Fermentation	D-Xylose Fermentation	Trehalose Fermentation	Cellobiose Fermentation	α-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 (25° C)	Nitrate Nitrite	Oxidase, Kovacs'	ONPG Test	Yellow Pigment	D-Mannose Fermentation	Tyrosine Utilization	D-Galactose	Citrate, Christensen's
0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	95	0	0	0	90	0	10	0	98			
0	0	0	0	0	0	0	100	0	0	0	0	0	0	7	7	100	0	0	0	100	0	0	0	100			
0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	0	100	0	0	0	90	0	20	0	100			
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			
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			
0	0	100	0	91	82	100	100	55	0	0	0	0	100	27	0	9	100	0	0	91	0	91	27	100			
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	60	20	0	0	0	0	0	60	100			
0	0	0	0	0	0	0	0	0	0	0	78	0	0	0	0	0	0	0	0	100	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	0	0	0	100	0	100	0	100	0	0	0	0	100	0	100	0	100	50	100	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	1	5	97	95	30	0	60	1	50	0	0	60	0	80	25	80	80	98	0	1	0	0			
1	1	1	1	0	1	1	2	0	0	0	0	0	0	15	0	90	40	0	0	100	0	1	0	100			
46	0	0	0	100	54	8	0	0	0	0	0	0	92	0	0	69	0	0	0	100	0	0	0	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			
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			
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			
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			
0	100	94	0	88	100	100	100	0	0	0	0	94	0	0	88	0	100	0	0	100	0	94	0	100			
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			
0	99	99	1	99	98	100	99	1	1	0	1	95	1	10	30	20	75	0	2	100	0	92	0	100			
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			
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			
0	0	100	0	100	100	100	100	0	0	0	0	89	0	33	89	100	89	0	0	100	0	44	0	100			
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			
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			
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			
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			

Continued

TABLE 19-12 Biochemical Reactions of Named Species and Unnamed Groups of the Family Enterobacteriaceae—cont'd

Organism	Indole Production	Methyl Red	Voges-Proskauer	Citrate (Simmons)	H_2S (TSI)	Urea Hydrolysis	Phenylalanine Deaminase	Lysine Decarboxylase	Arginine Dihydrolase	Ornithine Decarboxylase	Motility	Gelatin Hydrolysis (22° C)	Growth in KCN	Malonate Utilization	D-Glucose, Acid	D-Glucose, Gas	Lactose Fermentation	Sucrose Fermentation	D-Mannitol Fermentation	Dulcitol Fermentation	Salicin Fermentation	Adonitol Fermentation
Salmonella serotype Pullorum	0	90	0	0	90	0	0	100	10	95	0	0	0	0	100	90	0	0	100	0	0	0
Salmonella serotype Typhi	0	100	0	0	97	0	0	98	3	0	97	0	0	0	100	0	1	0	100	0	0	0
Serratia entomophila	0	20	100	100	0	0	0	0	0	0	100	100	100	0	100	0	0	100	100	0	100	0
Serratia ficaria	0	75	75	100	0	0	0	0	0	0	100	100	55	0	100	0	15	100	100	0	100	0
Serratia fonticola	0	100	9	91	0	13	0	100	0	97	91	0	70	88	100	79	97	21	100	91	100	100
Serratia liquefaciens	1	93	93	90	0	3	0	95	0	95	95	90	90	2	100	75	10	98	100	0	97	5
Serratia marcescens	1	20	98	98	0	15	0	99	0	99	97	90	95	3	100	55	2	99	99	0	95	40
Serratia marcescens biogroup 1	0	100	60	30	0	0	0	55	4	65	17	30	70	0	100	0	4	100	96	0	92	30
Serratia odorifera biogroup 1	60	100	50	100	0	5	0	100	0	100	100	95	60	0	100	0	70	100	100	0	98	50
Serratia odorifera biogroup 2	50	60	100	97	0	0	0	94	0	0	100	94	19	0	100	13	97	0	97	0	45	55
Serratia plymuthica	0	94	80	75	0	0	0	0	0	0	50	60	30	0	100	40	80	100	100	0	94	0
Serratia rubidaea	0	20	100	95	0	2	0	55	0	0	85	90	25	94	100	30	100	99	100	0	99	99
Shigella boydii	25	100	0	0	0	0	0	0	18	2	0	0	0	0	100	0	1	0	97	5	0	0
Shigella dysenteriae	45	99	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	1	100	0	0	0
Shigella flexneri	50	100	0	0	0	0	0	0	5	0	0	0	100	0	100	3	1	1	95	1	0	0
Shigella sonnei	0	100	0	0	0	0	0	0	2	98	0	0	0	0	100	0	2	1	99	0	0	0
Tatumella ptyseos	0	0	5	2	0	0	90	0	0	0	0	0	0	0	100	0	0	98	0	0	55	0
Trabulsiella guamensis	40	100	0	88	100	0	0	100	50	100	100	0	100	0	100	100	0	0	100	0	13	0
Xenorhabdus nematophilus	40	0	0	0	0	0	0	0	0	0	100	80	0	0	80	0	0	0	0	0	0	0
Yersinia aldovae	0	80	0	0	0	60	0	0	0	40	0	0	0	0	100	0	0	20	80	0	0	0
Yersinia bercovieri	0	100	0	0	0	60	0	0	0	80	0	0	0	0	100	0	20	100	100	0	20	0
Yersinia enterocolitica	50	97	2	0	0	75	0	0	0	95	2	0	2	0	100	5	5	95	98	0	20	0
Yersinia frederiksenii	100	100	0	15	0	70	0	0	0	95	5	0	0	0	100	40	40	100	100	0	92	0
Yersinia intermedia	100	100	5	5	0	80	0	0	0	100	5	0	10	5	100	18	35	100	100	0	100	0
Yersinia kristensenii	30	92	0	0	0	77	0	0	0	92	5	0	0	0	100	23	8	0	100	0	15	0
Yersinia mollaretii	0	100	0	0	0	20	0	0	0	80	0	0	0	0	100	0	40	100	100	0	20	0
Yersinia pestis	0	80	0	0	0	5	0	0	0	0	0	0	0	0	100	0	0	0	97	0	70	0
Yersinia pseudotuberculosis	0	100	0	0	0	95	0	0	0	0	0	0	0	0	100	0	0	0	100	0	25	0
Yersinia rohdei	0	62	0	0	0	62	0	0	0	25	0	0	0	0	100	0	0	100	100	0	0	0
Yersinia ruckeri	0	97	10	0	0	0	0	50	5	100	0	30	15	0	100	5	0	0	100	0	0	0
Yokenella regensburgei (*Koserella trabulsii*)	0	100	0	92	0	0	0	100	8	100	100	0	92	0	100	100	0	0	100	0	8	0
Enteric group 58	0	100	0	85	0	70	0	100	0	85	100	0	100	85	100	85	30	0	100	85	100	0
Enteric group 59	10	100	0	100	0	0	30	0	60	0	100	0	80	90	100	100	80	0	100	0	100	0
Enteric group 60	0	100	0	0	0	50	0	0	0	100	75	0	0	100	100	100	0	0	50	0	0	0
Enteric group 68	0	100	50	0	0	0	0	0	0	0	0	0	100	0	100	0	0	100	100	0	50	0
Enteric group 69	0	0	100	100	0	0	0	0	100	100	100	0	100	100	100	100	100	25	100	100	100	0

From the Centers for Disease Control and Prevention, Atlanta, GA.
H_2S, Hydrogen sulfide; *KCN,* Potassium cyanide; *ONPG,* *o*-nitrophenyl-β-D-galactopyranoside; *TSI,* triple sugar iron.
*Each number is the percentage of positive reactions after 2 days of incubation at 36° C unless noted otherwise. Most of these positive reactions occur within 24 hours. Reactions that become positive after 2 days are not considered.

myo-Inositol Fermentation	D-Sorbitol Fermentation	L-Arabinose Fermentation	Raffinose Fermentation	L-Rhamnose Fermentation	Maltose Fermentation	D-Xylose Fermentation	Trehalose Fermentation	Cellobiose Fermentation	α-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 (25°C)	Nitrate Nitrite	Oxidase, Kovacs'	ONPG Test	Yellow Pigment	D-Mannose Fermentation	Tyrosine Utilization	D-Galactose	Citrate, Christensen's
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			
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			
0	0	0	0	0	100	40	100	0	0	0	100	0	60	0	0	100	80	20	100	100	0	100	0	100			
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			
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			
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			
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			
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	100	100	100	95	100	100	100	100	0	0	95	100	0	40	5	100	60	35	100	100	0	100	0	100			
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			
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			
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			
0	43	94	0	1	20	11	85	0	0	0	0	15	0	50	0	50	0	0	0	100	0	10	0	100			
0	30	45	0	30	15	4	90	0	0	0	0	0	0	10	0	75	0	0	0	99	0	30	0	100			
0	29	60	40	5	30	2	65	0	0	0	0	55	1	10	0	30	8	0	0	99	0	1	0	100			
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			
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			
0	100	100	0	100	100	100	100	100	0	0	40	0	0	0	100	50	88	0	0	100	0	100	0	100			
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			
0	60	60	0	0	0	40	80	0	0	0	0	0	0	0	0	100	0	0	0	100	0	0	0	100			
0	100	100	0	0	100	100	100	100	0	0	20	0	0	0	0	100	0	0	0	100	0	80	0	100			
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			
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			
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			
15	100	77	0	0	100	85	100	100	0	0	0	0	45	70	0	40	8	0	0	100	0	70	0	100			
0	100	100	0	0	60	60	100	100	0	0	0	0	0	20	0	100	0	0	0	100	0	20	0	100			
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			
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			
0	100	100	62	0	0	38	100	25	0	0	0	50	0	38	0	100	0	0	0	88	0	50	0	100			
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	100	25	100	100	100	100	100	0	0	67	92	0	0	0	0	25	0	0	100	0	100	0	100			
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			
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			
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			
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			
0	100	100	100	100	100	100	100	100	100	0	100	100	0	0	100	0	25	0	0	100	0	100	0	100			

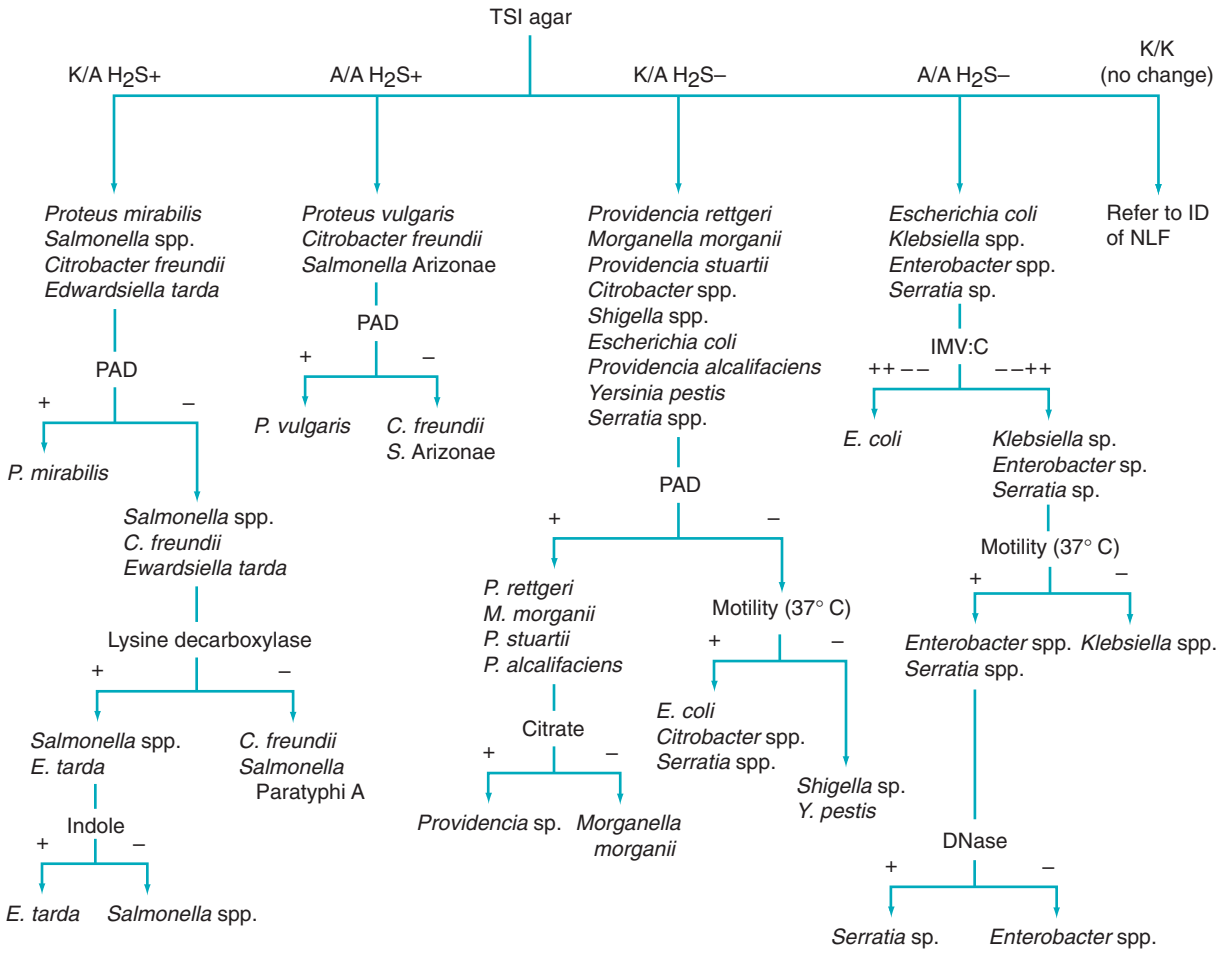

FIGURE 19-15 Flow chart for the presumptive identification of commonly encountered Enterobacteriaceae on triple sugar iron (TSI) agar. *A,* acid; *IMV:C,* indole, methyl red, Voges-Proskauer, citrate; *K,* alkaline; *NLF,* nonlactose fermenter; *PAD,* phenylalanine deaminase. (Data from Koneman E, et al: *Color atlas in diagnostic microbiology*, ed 5, Philadelphia, 1997, Lippincott-Raven.)

- TSI agar or Kligler iron agar (KIA) to determine glucose and lactose or sucrose utilization (sucrose in TSI only) and H_2S production
- LIA to determine lysine decarboxylase activity
- Urease test to determine hydrolysis of urea
- Simmons citrate to determine the ability to use citrate as the sole carbon source
- Semisolid motility agar
- Sulfide, indole, motility (SIM) or motility, indole, and ornithine (MIO) media
- Carbohydrate fermentation

The Clinical and Laboratory Standards Institute abbreviated identification of *E. coli* is a gram-negative bacillus forming nonswarming, β-hemolytic colonies and spot indole-positive. Occasional isolates that are not β-hemolytic must be indole-positive, lactose-positive on MAC or EMB, and must exhibit a negative pyrrolidonyl arylamidase (PYR) test to be identified as *E. coli.*

Serologic Grouping

When an isolate is biochemically identified as *Salmonella* or *Shigella*, serologic grouping of the isolate for the O serogroups (somatic antigen) must be performed for confirmation. Serologic grouping is also important in the identification of enterovirulent *E. coli.*

Salmonella

Based on the common O antigens, *Salmonella* may be placed into major groups designated by capital letters. Approximately 60 somatic O antigenic groups exist; however, 95% of *Salmonella* isolates from humans belong to serogroups A through E1. Laboratories should have agglutinating sera for these groups and report isolates as *Salmonella* groups (A through E1). If the isolate fails to agglutinate in the sera, it can be sent to a state or reference laboratory for additional serogrouping and serotyping. If an isolate is suspected of being one of these three serotypes, *Salmonella* Typhi, *Salmonella* Paratyphi, or *Salmonella* Choleraesuis, because of the medical implications it must be biochemically identified and serologically confirmed. Identification is very important in providing proper therapy for the patient and in limiting any possible complications that might develop.

It is imperative for any laboratory performing bacteriology to be able to identify serologically *Salmonella* serotype Typhi particularly and other members of *Salmonella* in O groups

A through G. Other isolates can be identified as "biochemically compatible with *Salmonella*" and submitted to a reference laboratory for further testing. To perform serologic grouping by slide technique, a suspension from a pure culture of the organism is prepared in sterile saline. Serologic typing is best performed on a colony taken from a pure culture growing on nonselective media, such as an SBA plate, although TSI or MAC can be used for presumptive serologic identification.

A slide with wells is easy to use for the agglutination test. A regular microscope slide may be used by marking separate circles with a wax pencil. The laboratory scientist places one drop of antisera on the appropriately labeled slide. One drop of bacterial suspension is added to each drop of antisera for a direct agglutination assay. Antisera kits usually consist of a polyvalent A through G, Vi, and individual antisera for serogroups A, B, C1, C2, D, E, and G. Latex agglutination kits offer improved visibility of agglutination reactions; this is an example of reverse passive agglutination.

In the event of a positive agglutination in the Vi antisera with no agglutination in the other groups, the suspension should be heated to 100°C for 10 minutes to inactivate the capsular Vi antigen. The Vi antigen is found on *Salmonella* Typhi and occasionally other serotypes and can mask the O antigens. The suspension is then cooled and retested with antisera A to G. Larger laboratories usually maintain antisera to serogroup salmonellae for all the somatic types. H antigen or flagella typing is usually performed in a state or reference laboratory that provides epidemiologic information in outbreaks.

Shigella

Similar serogrouping procedures may be used in the serologic testing for *Shigella*. Serologic grouping of *Shigella* is also based on the somatic O antigen. *Shigella* spp. belong to one of four serogroups: A (*S. dysenteriae*), B (*S. flexneri*), C (*S. boydii*), and D (*S. sonnei*). The O antisera used for serogrouping are polyvalent, containing several serotypes within each group (with the exception of group D, which contains only a single serotype). If agglutination fails, the suspension must be heated to remove the capsular antigen that may be present, and subsequently the agglutination test procedure is repeated.

Points to Remember

- Nearly any of the genera discussed in this chapter could conceivably be isolated from almost any clinical specimen, especially when dealing with immunocompromised patients.
- Although most isolates of *E. coli* are considered normal fecal microbiota, several strains (diarrheogenic *E. coli*) are known to cause intestinal tract infections. *E. coli* is the most significant cause of UTIs.
- *Salmonella* and *Shigella* are enteric pathogens and are not considered normal fecal biota.
- *Y. pestis*, one of the most virulent species in the family Enterobacteriaceae, causes the extraintestinal infection plague.
- A good patient history combined with proper selective screening agar (e.g., HE, XLD, and SMAC agars) can be very helpful in a timely and accurate identification of enteric pathogens associated with diarrheal disease.
- The use of an initial battery of selective agar media and key biochemical tests such as TSI, urea, and motility agars can usually result in a presumptive genus identification that can be confirmed with either conventional biochemical test or with the use of one of several multitest or rapid and automated identification systems.
- There are notable exceptions to the Enterobacteriaceae family "gold standard" definition of glucose fermenters that are able to reduce nitrate to nitrite and are oxidase-negative.

Learning Assessment Questions

1. What are the three general characteristics a gram-negative bacillus must possess to belong to the family Enterobacteriaceae with a few exceptions?
2. Match the *Shigella* spp. with the corresponding group antigen: A, B, C, and D.
   a. *S. sonnei*
   b. *S. boydii*
   c. *S. dysenteriae*
   d. *S. flexneri*
3. Which of the following test results is most helpful in categorizing an isolate as a member of the tribe Proteeae?
   a. Positive Voges-Proskauer
   b. Positive urea
   c. Positive phenylalanine deaminase
   d. Positive lactose fermentation
4. The causative agent of plague is:
   a. *Yersinia pestis*
   b. *Klebsiella rhinoscleromatis*
   c. *Citrobacter freundii*
   d. *Serratia marcescens*
5. A 47-year-old patient who had just returned from Mexico was admitted to the hospital with a 3-day history of vomiting and diarrhea, without fever, and no fecal leukocytes were found in the stool. When he was admitted to the hospital, a stool culture grew an organism identified as *Escherichia coli*. Which of the following strains is the most likely cause of the infection?
   a. EPEC
   b. ETEC
   c. EHEC
   d. EIEC
6. A gram-negative, oxidase-negative coccobacillus was isolated from the cerebrospinal fluid of an infant in the newborn nursery. The organism produced dark pink colonies on MAC agar and had the following biochemical results: triple sugar iron, acid over acid with gas; phenylalanine deaminase-negative; sulfide-indole-motility agar, H_2S-negative, indole-positive, and motile; urease-negative; and citrate-negative. The most probable identity of this organism is:
   a. *Escherichia coli*
   b. *Enterobacter aerogenes*
   c. *Klebsiella pneumoniae*
   d. *Serratia marcescens*
7. What organism is often associated with lobar pneumonia in elderly hospitalized patients?
   a. *Shigella* spp.
   b. *Proteus vulgaris*
   c. *Escherichia coli*
   d. *Klebsiella pneumoniae*
8. The most common cause of community-acquired UTIs is:
   a. *Klebsiella pneumoniae*
   b. *Escherichia coli*
   c. *Providencia stuartii*
   d. *Citrobacter freundii*

9. Which organism is an opportunistic pathogen that causes wound and urinary tract infections and may cause the production of kidney stones?
   a. *Yersinia enterocolitica*
   b. *Citrobacter freundii*
   c. *Proteus mirabilis*
   d. *Enterobacter cloacae*
10. An enteric organism that is acquired by eating improperly prepared and cooked or preserved contaminated food and produces dysentery is:
   a. *P. vulgaris*
   b. *Y. enterocolitica*
   c. *S. marcescens*
   d. *Shigella* spp.

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