Chapter 19

Enterobacterales

INTRODUCTION

CHAPTER NINETEEN

Setting the Stage

- Comprehensive genomic analysis with phylogenetic reconstructions of members of the heterogenous family Enterobacteriaceae resulted in the creation of a new order, Enterobacterales.
  - All members of the original family Enterobacteriaceae were placed in the new order

Setting the Stage (Cont.)

The order Enterobacterales was divided into nine families

Bruguierivoraceae	Morganellaceae
Budviciaceae	Pectobacteriaceae
Enterobacteriaceae	Thorselliaceae
Erwiniaceae	Yersiniaceae
Hafniaceae	

Setting the Stage (Cont.)

The family Enterobacteriaceae include the following genera:

Citrobacter	*Kluyvera*
Cronobacter	*Raoultella*
Enterobacter	*Salmonella*
Escherichia	*Shigella*
Klebsiella	Several others

What's Ahead?

- Clinically significant enteric species that cause opportunistic infections in *humans*
- Primary intestinal pathogens and their related *human* infections
- Methods of identification of these organisms

GENERAL CHARACTERISTICS

CHAPTER NINETEEN

Introduction

- The order Enterobacterales consists of numerous diverse organisms.
- They have several key laboratory features in common.

Key Characteristics of the Order Enterobacterales

BOX 19.1 Key characteristics of the order Enterobacterales

- They are gram-negative, facultatively anaerobic, non–spore-forming bacilli and coccobacilli.
- They do not produce cytochrome oxidase, except *Plesiomonas*.
- They ferment glucose.
- They reduce nitrate to nitrite, except *Photorhabdus* and *Xenorhabdus*.
- They are motile at 37° C, except *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 agar, and most nonselective media. Most are nonhemolytic on SBA, except *E. coli*.

Microscopic and Colony Morphology

- Gram-negative
- Non-spore-forming
- Facultatively anaerobic bacilli
- On Gram stain
  - Coccobacilli or as straight rods
- Colony morphology on nonselective media (i.e., chocolate or sheep blood agar)
  - Are of little value in initial identification

Tip to Remember

- To help remember key characteristics of the Enterobacteriaceae family, remember this fun statement:
  - GOFers move at nite—rite?
    - G: for gram negative bacilli/coccibacilli
    - O: for oxidase negative
    - F: for ferments glucose
    - Move: they are motile except for Klebsiella, Shigella, and Yersinia
    - Nite—rite: they reduce nitrate to nitrite

Microscopic and Colony Morphology (Cont.)

- General macroscopic morphology
  - Large moist, gray colonies on nonselective media and are indistinguishable
    - Many isolates of *E. coli* are β-hemolytic
  - Exception- certain members produce characteristically large and very mucoid colonies
    - *Klebsiella* and sometimes *Enterobacter*
- Selective and differential media are available for presumptive identification of clinical pathogens

Classification of Selected Species within the Order of Enterobacterales

Table 19.1 Classification of selected species within the order Enterobacterales

Family	Genus	Species
Enterobacteriaceae	*Escherichia*	*coli*
		albertii
		fergusonii
	Shigella	*dysenteriae*
		flexneri
		boydii
		sonnei
	Citrobacter	*freundii* complex
		koseri
		amalonaticus
	Cronobacter	*sakazakii*
		malonaticus
		farmeri
	Enterobacter	*cloacae* complex
	Klebsiella	*pneumoniae* subsp. *pneumoniae*
		pneumoniae subsp. *ozaenae*
		pneumoniae subsp. *rhinoscleromatis*
		aerogenes
		variicola
	Raoultella	*ornitholytica*
	Salmonella	*enterica*
		bongori
	Cronobacter	*sakazakii*
		malonaticus
Hafniaceae	*Edwardsiella*	*tarda*
		hoshinae
	Hafnia	*alvei*
		paralvei
Morganellaceae	*Morganella*	*morganii*
	Proteus	*mirabilis*
		vulgaris
		penneri
		myxofaciens
	Providencia	*alcalifaciens*
		rettgeri
		stuartii
Yersiniaceae	*Serratia*	*marcescens*
		liquefaciens
		rubidaea
		fonticola
		odorifera
		plymuthica
	Yersinia	*pestis*
		enterocolitica
		pseudotuberculosis
		frederiksenii
		kristensenii
		intermedia
Erwiniaceae	*Pantoea*	*agglomerans*

Modified from Schoch, C., et al. *NCBI taxonomy: a comprehensive update on curation, resources, and tools.* Database (Oxford). 2020: Baaa062.

Biochemical Characteristics of Select Genera of Enterobacterales

Table 19.2 Biochemical characteristics of select genera of Enterobacterales

Tests or substrate	*Escherichia and Shigella*	*Edwardsiella*	*Citrobacter*	*Salmonella*[a]	*Klebsiella, Enterobacter,* and *Serratia*	*Proteus, Morganella,* and *Providencia*[b]	*Yersinia*
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 +	+

[a]*Salmonella* serotypes Typhi and Paratyphi and some other rare serotypes fail to use citrate in Simmons medium. Cultures of serotype Paratyphi and some rare serotypes may fail to produce H_2S.

[b]Some cultures of *Proteus mirabilis* may yield positive Voges-Proskauer tests.

+, ≥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; *H_2S*, hydrogen sulfide; *KCN*, potassium cyanide; *TSI*, triple sugar iron.

Modified from Ewing, W. H. (1986). *Edwards and Ewing's identification of enterobacteriaceae* (4th ed). East Norwalk, CT: Appleton & Lange.

Virulence and Antigenic Factors

- The ability to
  - Adhere
  - Colonize
  - Produce toxins
  - Evade host defenses
- Many pathogenic species of this order possess antigens that can be used in the identification of different serologic groups
  - See list on next slide

Virulence and Antigenic Factors (Cont.)

- Antigen or somatic antigen
  - Heat-stable antigen located in the cell wall
- H antigen or flagellar antigen
  - Heat-labile antigen found on the surface of flagella
  - The structures are responsible for motility
- K antigen or capsular antigen
  - Heat-labile polysaccharide found only in certain encapsulated species
    - Examples: K1 antigen of *E. coli*, Vi antigen of *Salmonella typhi*

Virulence and Antigenic Factors (Cont.)

- Lipopolysaccharide – LPS
  - Consists of 3 regions
    1. Antigenic O-Specific polysaccharide
       - Provides attachment sites
       - Enhances attachment to host cells
    2. Core polysaccharide
    3. Inner lipid
       - Lipid A
         - Endotoxin
         - Fiver: Release of pyrogens from WBCs acts on hypothalamus
         - Hypotension: Release of vasoactive substances
           - Bradykinin
           - Serotonin

Virulence and Antigenic Factors (Cont.)

- Lipopolysaccharide – LPS
  - Lipid A
    - Endotoxin
    - Fever: Release of pyrogens from WBCs acts on hypothalamus
    - Hypotension: Release of vasoactive substances
      - Bradykinin
      - Serotonin
    - Activation of complement
      - Generates inflammatory factors
      - Consequences to cell membrane?
        - Activates to destroy cells in vivo
    - DIC
    - Leucopenia
    - Hypoglycemia

Clinical Significance

- Two major types
  - Opportunistic pathogens
    - Often part of usual intestinal microbiota in humans and animals
      - Best studied—member of the group—*E. coli* can cause, septicemia, wounds, urinary tract infections (UTIs), meningitis
    - Primary pathogens
      - *Salmonella* spp.
      - *Shigella* spp.
      - *Yersinia* spp.
    - Generally, from ingesting contaminated food and water

Bacterial Species and the Infections They Commonly Produce

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.

Antimicrobial Resistance in the Enterobacterales

- In 2019, the CDC listed 18 drug-resistant threats to the U.S. based on level of concern:
  - Urgent
    - CRE- Carbapenem-resistant Enterobacteriaceae

      referring to the original family of same name
  - Serious
  - Concerning

Antimicrobial Resistance in the Enterobacterales (Cont.)

- CRE definition
  - Resistance to imipenem, meropenem, doripenem, ore ertapenem
  - Documentation that the isolate possesses a carbapenemase
    - Real-time multiplex PCR assay rapidly detects 5 genes for carbapenemases
      - 100% sensitivity
      - >99% specificity
    - Screening of hospitalized patients is recommended to help contain the spread of carbapenemase-producing bacteria

Antimicrobial Resistance in the Enterobacterales (Cont.)

- 2016
  - Discovery of plasmid-transmissible colistin marker *mcr-1* in a CRE strain
- Antimicrobial resistance continues to grow more serious
- Example of clinical strains that produce plasmid-mediated extended-spectrum-β-lactamases
  - *E. coli, K. pneumoniae, K. oxytoca*

OPPORTUNISTIC MEMBERS OF THE ORDER ENTEROBACTERALES AND ASSOCIATED INFECTIONS

CHAPTER NINETEEN

E. coli Characteristics

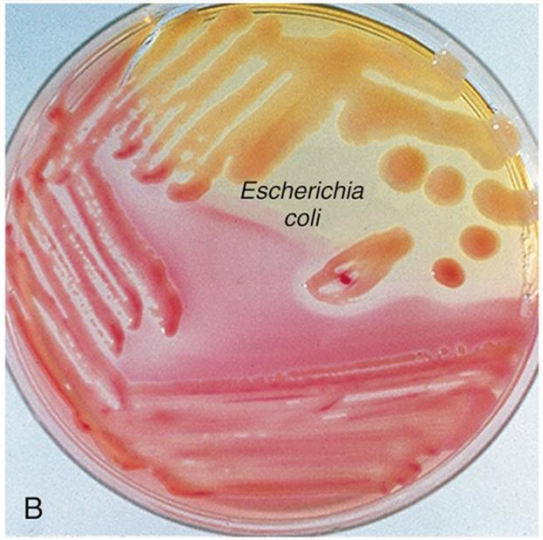
- Most strains
  - Lactose-positive(pink colonies with surrounding area of precipitated bile salts on MAC plates
  - β-hemolysis on blood agar plate (BAP)
  - Green metallic sheen on EMB
  - Motile and possess adhesive fimbriae and sex pili
  - Possess O, H, K antigens
- Commonly isolated from colon biota
  - Used as primary marker of fecal contamination in water quality testing

E. Coli Biochemical Reactions

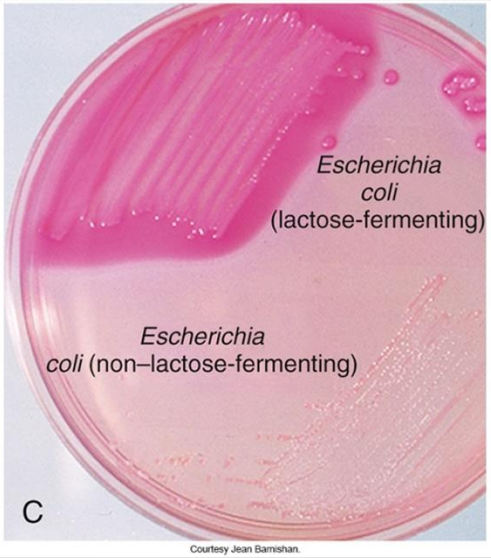
- Fermentation of glucose, lactose, trehalose, and xylose
- Indole production from tryptophan
- Glucose fermentation via mixed acid pathway
  - MR positive and VP negative
- Does not produce H_2S, DNase, urease, or phenylalanine deaminase (PAD)
- IMVC pattern

  positive; positive; negative; negative
- Cannot use citrate as sole carbon source

Colonies Growth of *E. coli*



Courtesy Jean Barnishan.



Courtesy Jean Barnishan.

Extraintestinal *E. coli* Infections

- One of the most common causes of septicemia and meningitis in neonates
  - Some strains have the capsular antigen K1
    - Documented virulence factor, high fatality rates
- Similar infections are uncommon in older children
- In adults
  - Bacteremia as a result of urogenital infection or from a GI source
- Other sources:
  - Colonization of birth canal just before or during delivery, contamination of the amniotic fluid

Extraintestinal Infections of *E. coli*

- Septicemia and meningitis
  - Most common in neonates and very young children
  - Gain infection just before or during delivery, and when there is contamination of amniotic fluid
  - Capsule antigen K1
    - Predisposition for meningitis

Uropathogenic *E. coli*

- Most common cause of UTIs in humans
- Primary virulence factor
  - Production of pili allow attachment to epithelial cells and washed out with urine flow
- Cytolysins (hemolysins) can kill immune cells and inhibit phagocytosis.
- Aerobactin allows bacteria to chelate iron.

Gastrointestinal Pathogenic *E. coli*

- Five major categories
  - Enterotoxigenic *E. coli* (ETEC)
  - Enteropathogenic *E. coli* (EPEC)
  - Enteroinvasive *E. coli* (EIEC)
  - Shiga toxin-producing *E. coli* (STEC)
  - Enteroaggregative *E. coli* (EAEC)
- These five categories are sometimes collectively referred to as enterovirulent or diarrheagenic *E. coli*
- It is often difficult to distinguish diarrheagenic strains from *E. coli* strains that are part of the normal microbiota by culture

Features of Pathogenic *E. coli*

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[a]	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[a]		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.
[a]DAEC causes both UTIs and gastrointestinal infections.

Enterotoxigenic *E. coli* (ETEC)

- Diarrhea in infants and adults in tropics and subtropics
- Traveler's diarrhea (requires large inoculum 10^6–10^{10} organisms) lasts 1 to 5 days.
- Transmitted via contaminated food and drink
- Organisms can colonize the small intestine.

ETEC (Cont.)

- Established organisms release toxins
  - Heat labile toxin (LT): A and B subunits; follow A/B model of bacterial toxins
    - A portion is the enzymatically active portion
    - B portion binds to GM_1 ganglioside of the intestinal mucosa, providing entry for the A portion
    - Heat-stable toxin (ST) stimulates guanylate cyclase, increasing cyclic guanosine monophosphate (cGMP), leading to accumulation which in turn causes hypersecretion.

ETEC (Cont.)

- Clinical manifestations
  - Mild, self-limiting
  - Watery diarrhea, abdominal cramps, occasional nausea
  - No vomiting or fever
  - Like other diarrheal illnesses but must be differentiated
- Typical diagnosis
  - Characteristic symptoms and isolation of solely lactose-fermenting organisms on differential media
  - Immunologic assays
  - Multiplex PCR

Enteropathogenic *E. coli* (EPEC) (Cont.)

- Associated with
  - Severe diarrhea in children younger than 1 year of age
  - Nurseries and daycares
- Rare in adults
- Symptoms
  - Low-grade fever
  - Malaise
  - Vomiting
  - Diarrhea
    - Persistent watery diarrhea
    - Large amounts of mucus without apparent blood
- Detection is based primarily on the suspicion of the physician

Enteroinvasive *E. coli* (EIEC)

- Affects adults and children
  - Rare in the United States
- Dysentery with direct penetration, invasion, and destruction of intestinal mucosa
- Very similar to *shigella* (coming up soon) but require higher amounts of inoculum

EIEC (Cont.)

- Clinical infection symptoms
  - Fever
  - Severe abdominal cramps
  - Malaise
- Generally, does not ferment lactose (thus very similar to shigellae)

Shiga Toxin-Producing *E. coli* (STEC)

- STEC refers to *E. coli* strains that produce Shiga toxin 1 (Stx1) and/or Shiga toxin 2 (Stx2).
  - Toxins are named because of their similarity to Shiga toxin produced by *Shigella dysenteriae* type 1
  - Stx is also referred to as verotoxin because this cytotoxin produces damage to Vero cells (African green monkey kidney cells)
  - Stx 1 and Stx 2 are immunologically distinct
  - Stx follows the A/B model of bacterial toxins

STEC (Cont.)

- Shiga toxins preferentially affect endothelial cells of the GI tract and renal glomeruli.
- In 1982, the O157:H7 strain of *E. coli* was first recognized during an outbreak of hemorrhagic diarrhea and colitis.
- These isolates are referred to as enterohemorrhagic *E. coli* (EHEC)-a subset of STEC
- EHEC is associated with
  - Hemorrhagic diarrhea
  - Colitis
  - Hemolytic uremic syndrome (HUS)-see next slide

STEC- EHEC

- Hemolytic uremic syndrome (HUS) is characterized by
  - Low platelet count
  - Hemolytic anemia
  - Kidney failure
- Classic illness caused by EHEC
  - Watery diarrhea that progresses to bloody diarrhea with abdominal cramps and low-grade fever
  - No leukocytes in stool samples
  - Symptoms range from mild to life-threatening HUS
  - Can be fatal in young children and older adults

STEC-EHEC (Cont.)

- Mode of transmission
  - Processed meats
    - Undercooked ground beef
    - Unpasteurized dairy products
    - Apple cider
    - Bean sprouts
    - Spinach
    - Romaine lettuce

STEC (Cont.)

- Laboratory diagnosis
  - Stool culture on selective and highly differential medium with subsequent serotyping
  - Screening stool filtrates for toxin
  - Demonstrate a fourfold increase in toxin-neutralizing antibody titer

STEC (Cont.)

- Culture media choices
  - Stool cultures should incorporate MAC containing sorbitol (SMAC) instead of lactose or SMAC with cefixime and tellurite (SMAC-CT)
  - Some strains are sensitive to tellurite
- *E. coli* O157:7 strain does not ferment sorbitol in 24 hours

STEC (Cont.)

- An emergent phenotype, the sorbitol-fermenting, nonmotile *E. coli* O157:NM (NM indicates nonmotile)
- Other laboratory diagnosis methods
  - ELISA
  - Latex agglutination
  - Testing for flagella antigens
  - After serotyping, isolates may be tested for Stx or *stx* genes
  - Cell cultures using Vero cells
  - EIA
  - Molecular diagnostic tests

Enteroaggregative *E. coli* (EAEC)

- EAEC cells stick together, cultured on cell monolayers, plastic surfaces, and the surface of the intestinal mucosa
  - "Stacked-brick" pattern on the cells and between the cells by means of fimbriae and can lead to biofilm formation
- Symptoms-mostly in children
  - Watery diarrhea, vomiting, dehydration, occasional abdominal pain
  - Persistent for at least 2 or more weeks

EAEC (Cont.)

- No WBCs or RBCs in the stool
- Important cause of diarrhea in infants
- Should be considered a cause of diarrhea in patients with HIV infection
- Stacked-brick growth pattern on monolayer cell cultures is suggestive of EAEC
  - DNA probes for virulence genes offer a definitive identification
- EAEC also contains several toxin-encoding genes, on plasmids and the chromosome, that can be found on other diarrheagenic *E. coli* strains.

Other *Escherichia* Species

- *Escherichia fergusonii*
  - Has been isolated from urine, blood, wounds, feces, and gallbladder
- *Escherichia albertii*
  - Associated with diarrheal disease
  - Some isolates care the *stx2f* gene, which codes for a Shiga-like toxin and the cdtB gene, which codes for a toxin found in *Clostridioides difficile.* These isolates can cause a condition resembling HUS

Klebsiella

- Found in the intestinal tract of humans as normal microbiota
- Associated with opportunistic and health-care associated infections, particularly
  - Pneumonia, wound infections, UTIs
- The genus *Klebsiella* comprises several species, including *K. aerogenes*, *K. michiganensis*, *K. oxytoca*, *K. pneumoniae* subsp. *ozaenae*, *K. pneumoniae* subsp. *pneumoniae*, *K. pneumoniae* subsp. *rhinoscleromatis*, and *K. variicola*.

Klebsiella (Cont.)

- Common characteristics include the following:
  - Most grow on Simmons citrate and in potassium cyanide (KCN) broth.
  - None produce H_2S.
  - A few hydrolyze urea slowly.
  - All are methyl red test negative and Voges-Proskauer positive.
  - With a few exceptions, no indole is produced from tryptophan.
  - Motility is variable

Differentiating Commonly Encountered *Klebsiella* Species

TABLE 19.5 Differentiation of Common Species Within the Genus *Klebsiella*

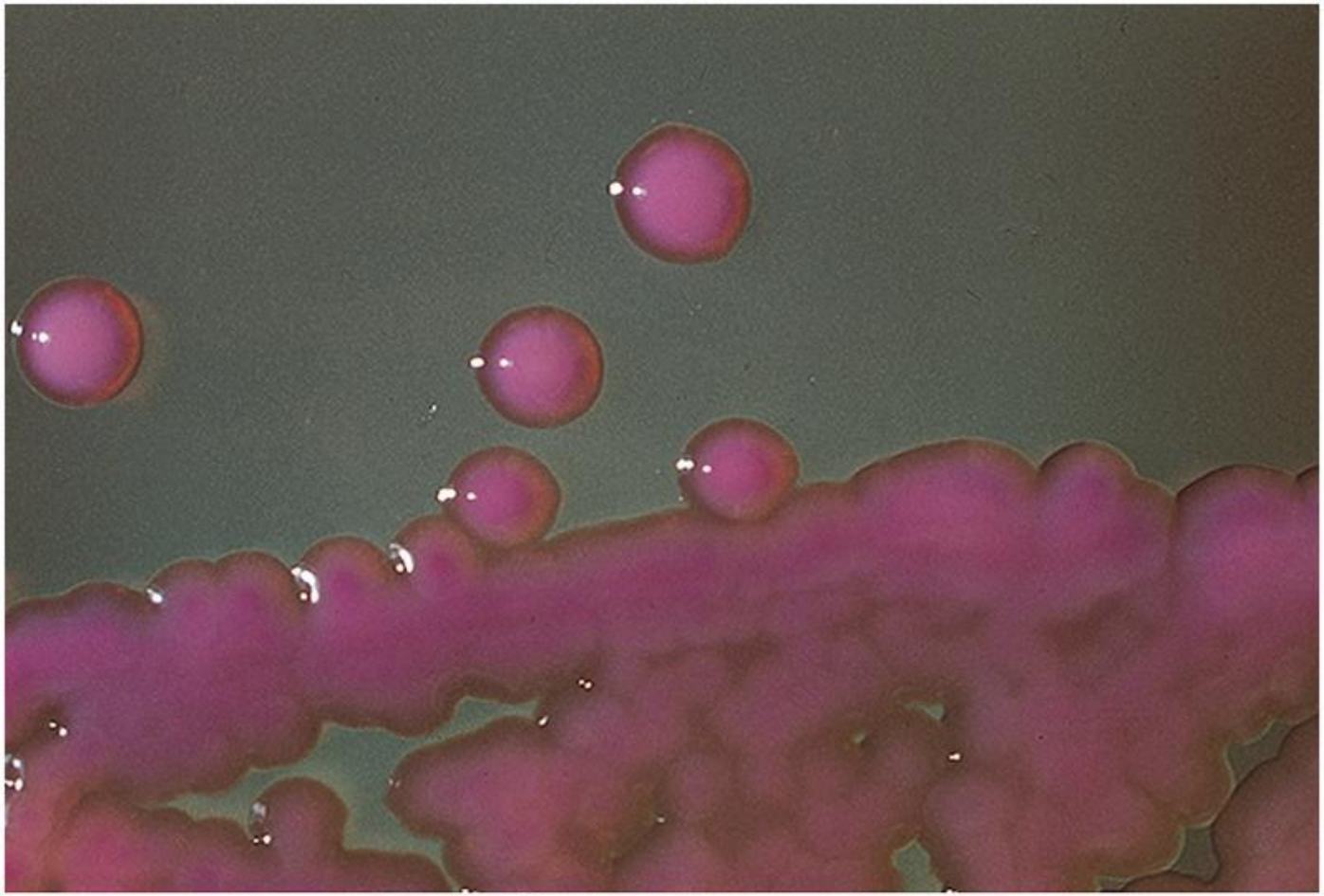
Test or Substrate	*K. pneumoniae* subsp. *pneumoniae*			*K. oxytoca*			*K. pneumoniae* subsp. *ozaenae*		
	Sign	% +	(% +)	Sign	% +	(% +)	Sign	% +	(% +)
Urease	+	95.4	(0.1)	+	90		d	0	(14.8)
Indole	–	0		+	99		–	0	
Methyl red	– or +	10		–	20		+	97.7	
Voges-Proskauer	+	98		+	96		–	0	
Citrate (Simmons)	+	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	

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

Klebsiella pneumoniae

- Most common isolate
  - Respiratory tract infection of hospitalized patients
  - Also wound, UTIs, liver abscesses, and bacteremia
    - Increased resistance, including carbapenemase
- Moist gray mucoid colonies
- Virulence factor
  - Polysaccharide capsule-responsible for mucoid colonies
    - Prevents phagocytosis and some antimicrobials

Colonies of *K. pneumoniae*



Other *Klebsiella* Species

- *K. oxytoca*
  - Similar to *K. pneumoniae* except indole positive
  - Affects similar sites
- *K. pneumonia* subsp. *ozaenae*
  - Isolated from nasal secretions and cerebral abscesses
    - Plasmid-mediated ESBLs
- *K. pneumoniae* subsp. *rhinoscleromatis*
  - Rhinoscleroma-infection of the nasal cavity
    - Intense swelling and malformation of the entire face and neck
  - Africa and South America

Other Species of *Klebsiella* (Cont.)

- *K. aerogenes* (formerly *Enterobacter aerogenes*)
  - Healthcare-associated infections such as respiratory infections, UTIs, sepsis, post-neurosurgical meningitis
  - Linked to poor clinical outcomes in patients with bloodstream infections relative to those caused by *Enterobacter* spp.
  - Organism as become a multidrug-resistant pathogen

Raoultella

- Reclassification of some *Klebsiella* spp. to *Raoultella* spp.
  - *R. ornithinolytica*
    - Indole and ornithine decarboxylase positive
  - *R. terrogena***
  - *R. planticola***

**Both species found in urine, respiratory tracts, and blood

Enterobacter and *Cronobacter*

- Clinically relevant species include
  - *E. asburiae***, *E. cancerogenus*, *E. cloacae*, *E. ludwigii*, and *E. hormaechei*
- Common healthcare-associated pathogens
  - Increasing multidrug resistance
- Infection sites-*E. asburiae*
  - Wound, urine, blood, feces
- Osteomyelitis after traumatic wounds- *E. cancerogenus*

Enterobacter Species Characteristics

- Citrate positive, potassium cyanide broth positive
- MR negative, VP positive
- Usually produce ornithine decarboxylase
  - Lysine decarboxylase produced by most (not *E. cloacae* or *E. gergoviae*)

Diagnostic Features of Select *Enterobacter* Species

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)

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

Cronobacter

- Most common isolates
  - *C. sakazakii*
    - Documented as a pathogen in neonates
    - Causes meningitis, bacteremia, necrotizing enterocolitis
    - Associated with powdered milk
    - Isolated from brain abscesses, respiratory and wound infections
    - Produces yellow pigment
  - *C. malonaticus*
    - Found in adults
- Most accurate diagnosis method is DNA sequencing of *fusA* and *rpoB* genes

Photograph of *C. sakazakii*

Courtesy Jean Bamishan.

Citrobacter

- Consists of at least 19 species
- Inhabitants of the GI tract
- Associated with infectious disease in healthcare-associated infections,
  - Most frequently causes UTIs
- The species most often isolated are
  - *C. freundii* complex (includes *C. freundii*, *C. braakii*, and *C. youngae*, among others) and *C. koseri*
    - Can be isolated in diarrheal stools

Citrobacter (Cont.)

- *C. freundii* has been associated with
  - UTIs, pneumonias, septicemia, intraabdominal abscesses, endocarditis in IV drug users
- It is difficult to speciate *Citrobacter*
  - Hence the term *Citrobacter freundii* complex
- Most species:
  - Hydrolyze urea slowly
  - Positive methyl red
  - Ferment lactose
  - Grow on Simmons citrate medium

Differential Tests to Identify *C. freundii* from *Salmonella*

	Citrobacter freundii	*Salmonella*
Colony morphology when isolated from stool cultures	On primary selective media may be mistaken for *Salmonella*	On primary selective media, resembles *Citrobacter*
Decarboxylate lysine	No	Yes (most strains)
Hydrolyze urea	70% yes	No

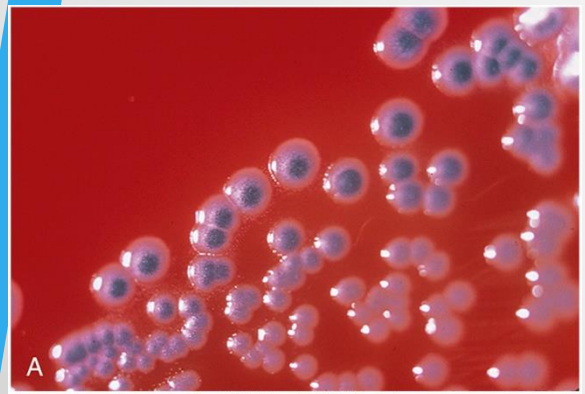
Citrobacter (Cont.)

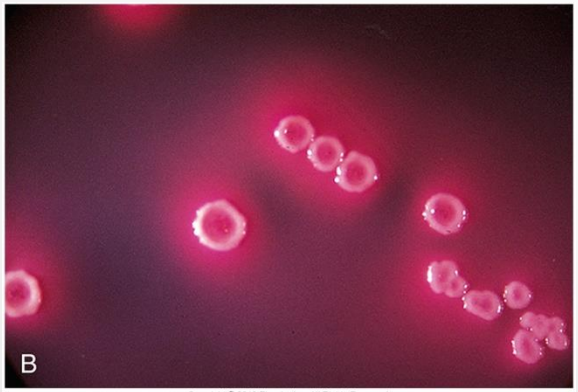
- *C. koseri*
  - Documented as the cause of nursery outbreaks of
    - Neonatal meningitis, pneumonia, brain abscesses
- *C. braakii*
  - Associated with community-acquired infections
    - Including septicemia in a patient with cervical cancer

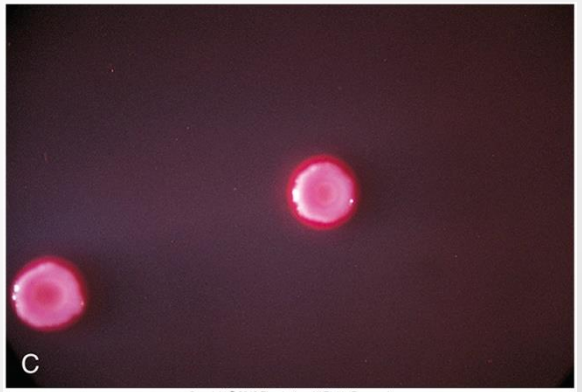
Kluyvera

- Five closely related species
  - *K. ascorbata, K. cryocrescens, K. georgiana, K. intermedia, and K. sichuanensis.*
- Found in respiratory, urine, CSF and blood cultures
- Most strains are nonpigmented

Kluyvera Colony Morphology







Plesiomonas

- Formerly in the family Vibrionaceae
- Oxidase-positive
- Glucose-fermenting
- Facultatively anaerobic
- Straight gram-negative bacilli
  - Singly, pairs, short chains, filamentous form
- No capsule formation

Plesiomonas (Cont.)

- The genera *Plesiomonas* and *Shigella* share
  - Biochemical and antigenic features.
- Plesiomonads often cross-agglutinate with
  - *Shigella sonnei*, *S. dysenteriae*, and even *S. boydii* antisera, hence the species name shigelloides.
- *P. shigelloides* possess a much lower virulence potential than *Shigella*, with a low symptomatic carriage rate among humans.

Plesiomonas (Cont.)

- *Plesiomonas* can be serotyped by somatic O antigens and their flagellar H antigen.
- *P. shigelloides*
  - Found in soil and aquatic environments
  - Fresh and estuarine waters of tropical and subtropical climates
  - Widely distributed among warm- and cold-blooded animals
  - Mode of transmission
    - Undercooked seafood, particularly shellfish
    - Untreated water

Plesiomonas (Cont.)

- Three major clinical types of gastroenteritis
  - The more common watery or secretory diarrhea
  - A subacute or chronic disease that lasts from 14 days to 2 to 3 months
  - A more invasive, dysenteric form that resembles colitis

Plesiomonas Epidemiology

- Found in soil and aquatic environments
  - Found in fresh and estuarine waters, subtropical climates
- Widely distributed among warm- and cold-blood animals

Plesiomonas

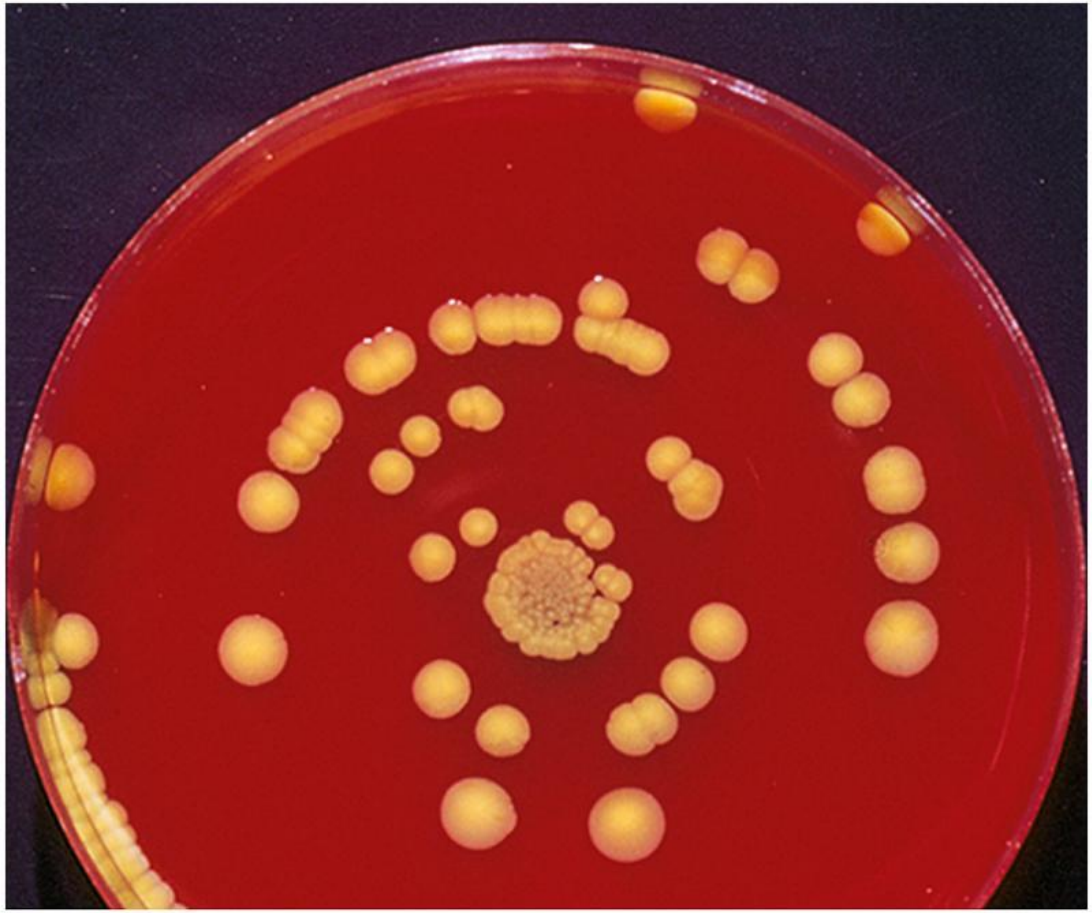
- 25% to 40% of all infected patients present with
  - Fever, vomiting, or both
  - Most common clinical symptom—abdominal pain
- Most cases self-limiting
- Antimicrobial therapy is indicated in severe and prolonged cases.

Erwiniacea

Pantoea

- A diverse group of about two dozen species of yellow-pigmented organisms containing many plant pathogens.
- Environmental isolates
- Rarely recovered in human specimens
- Several strains have been isolated from clinical specimens
  - Blood, CSF, sputum, urine
  - *P. agglomerans*-nationwide outbreak of septicemia resulting from contaminated IV fluids

Photograph of *P. agglomerans*



Courtesy Jean Barnishan.

Morganellaceae

- Consists of three genera
  - *Proteus*
  - *Morganella*
  - *Providencia*
- Widely disseminated in the environment, normal intestinal microbiota
- Recognized as opportunistic pathogens
- All genera are non-lactose fermenting
- All genera deaminate phenylalanine

Proteus

- At least 13 species
  - *P. mirabilis* and *P.vulgaris* widely recognized human pathogens
    - *P. mirabilis* is the more common isolate
- Both species have been isolated from
  - Urine, wounds, and ear and bacteremic infections
- The urease activity of *P. mirabilis* results in an increased urine pH and can lead to struvite kidney stones (calculi)

Swarming *Proteus*

Courtesy Kimberly Walker and R. Abe Baalness.

Differential Tests to Identify *P. mirabilis* and *P. vulgaris*

	Proteus mirabilis	*Proteus vulgaris*
Production of indole from tryptophan	No	Yes
Ornithine decarboxylase	Positive	Negative
Other tests of importance		Ferments sucrose Acid/acid in triple sugar iron (TSI) agar

Morganella

- *M. morganii*
  - Multidrug resistant, increased virulence
  - Healthcare-associated pathogen
  - UTIs, occasional wound infections and bacteremia
  - Non-swarmer
  - Motile

Providencia

- Four clinical notable species, two of which are below
  - *P. rettgeri*
    - Urinary tract pathogen
    - Occasionally nosocomial outbreaks
    - Implicated in diarrheal disease among travelers
  - *P. stuartii*
    - Nosocomial burn unit outbreaks
    - Also isolated in urine cultures
  - Both have high resistance to antimicrobials.

Differentiating Characteristics of *Proteus*, *Providencia*, and *Morganella*

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	+	+	+	–	–	–	–

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.

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

Hafniaceae

- Genus- *Hafnea*
  - Can produce a verocytotoxin
  - Linked to gastroenteritis
  - Occasionally isolated from stool cultures
  - Delayed citrate reaction
- *H. alvei* is more likely to be toxigenic, and have been linked to gastroenteritis and are occasionally isolated in stool cultures

Edwardsiella

- *E. tarda* is the only recognized pathogen.
  - Urea negative
  - Lysine decarboxylase positive
  - H_2S positive
  - Indole positive
  - No growth on Simmons citrate medium
- Clinical manifestations
  - Bacteremia, GI infections, wound infections
  - Reported to have significant mortality rate

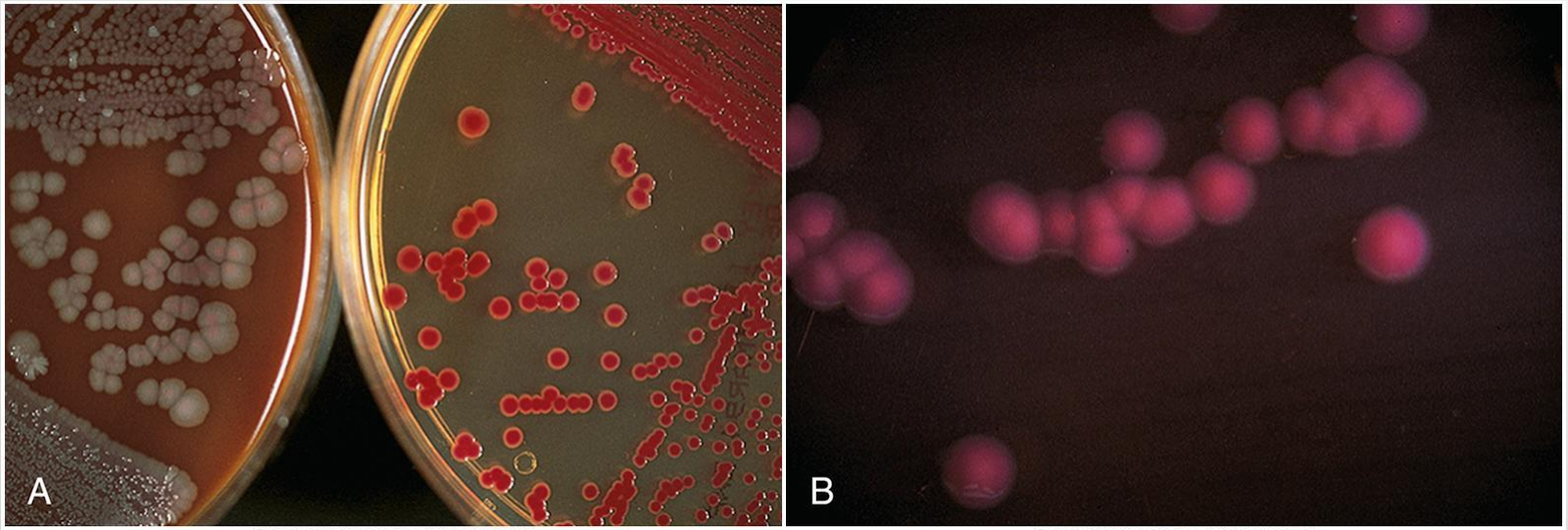
Serratia

- Diverse group with at least 25 named species
- Widespread in the environment
- Opportunistic pathogens isolated from outbreaks in health care settings.
- *S. marcescens*, *S. rubidaea*, and *S. plymuthica* are noted for
  - producing a characteristic pink-to-red pigment, prodigiosin, especially when the cultures are incubated at room temperature

Colonies of *Serratia*

Left: *S. marcescens* on chocolate (CHOC) agar

Right: *S. rubidaea* on MAC



Serratia marcescens

- Opportunistic pathogens associated with outbreaks in health care settings
  - Urinary and respiratory tract infections
  - Nursery, cardiac surgery, burn unit outbreaks
  - Contamination of antiseptic solution
    - Resulted in an epidemic of septic arthritis
  - Ophthalmic infections.

Serratia odorifera

- *S. odorifera*
  - Dirty, musty odor resembling that of rotten potatoes
- Isolated predominantly from
  - Sputum
  - Blood
  - Urine
- Other species that have been isolated in humans
  - *S. liquefaciens*, *S. rubidaea*, *S. ficaria*, *S. fonticola*, and *S. quinivorans*

Serratia Species

- General characteristics
  - Ferment lactose slowly
  - ONPG positive
  - Typically VP positive
  - Resistance to a wide range of antimicrobials
  - Susceptibility tests must be performed on each isolate to determine appropriate antimicrobial therapy

PRIMARY PATHOGENS IN THE ORDER ENTEROBACTERALES

CHAPTER NINETEEN

Overview

- Primary human intestinal pathogens that are *not* normal biota of the intestinal tract
  - *Salmonella*
    - Inhabit the gastrointestinal (GI) tracts of animals
    - Human infection occurs via contaminated or undercooked animal food products.
  - *Shigella*
    - Human carriers coupled with improper sanitary conditions and poor personal hygiene
  - *Yersinia*
    - Transmitted by wide variety of animals

Salmonella

- Gram-negative bacilli, facultatively anaerobic
- Clear, colorless, nonlactose-fermenting on MAC and colonies with black centers on HE or XLD
  - Negative for indole, phenyalanine deaminase, urease, and VP
  - Most produce H_2S
    - Exception: *Salmonella paratyphi A*
  - No growth in potassium cyanide

Salmonella (Cont.)

- Classification
  - Two species based on DNA homology and sequencing
  - *S. enterica*- accounts for most all human infections
    - Six subspecies
  - *S. bongori*-rarely isolated

Salmonellae Virulence Factors

- Capsule production
- Fimbriae used in adherence in initiating intestinal infection
- Ability to traverse intestinal mucosa and survive inside host cells
- Enterotoxins involved in gastroenteritis
  - Implicated as a significant virulence factor

Biochemical Differentiation of Select *Salmonella*

TABLE 19.8 Biochemical Differentiation of Selected Members of the Genus *Salmonella*

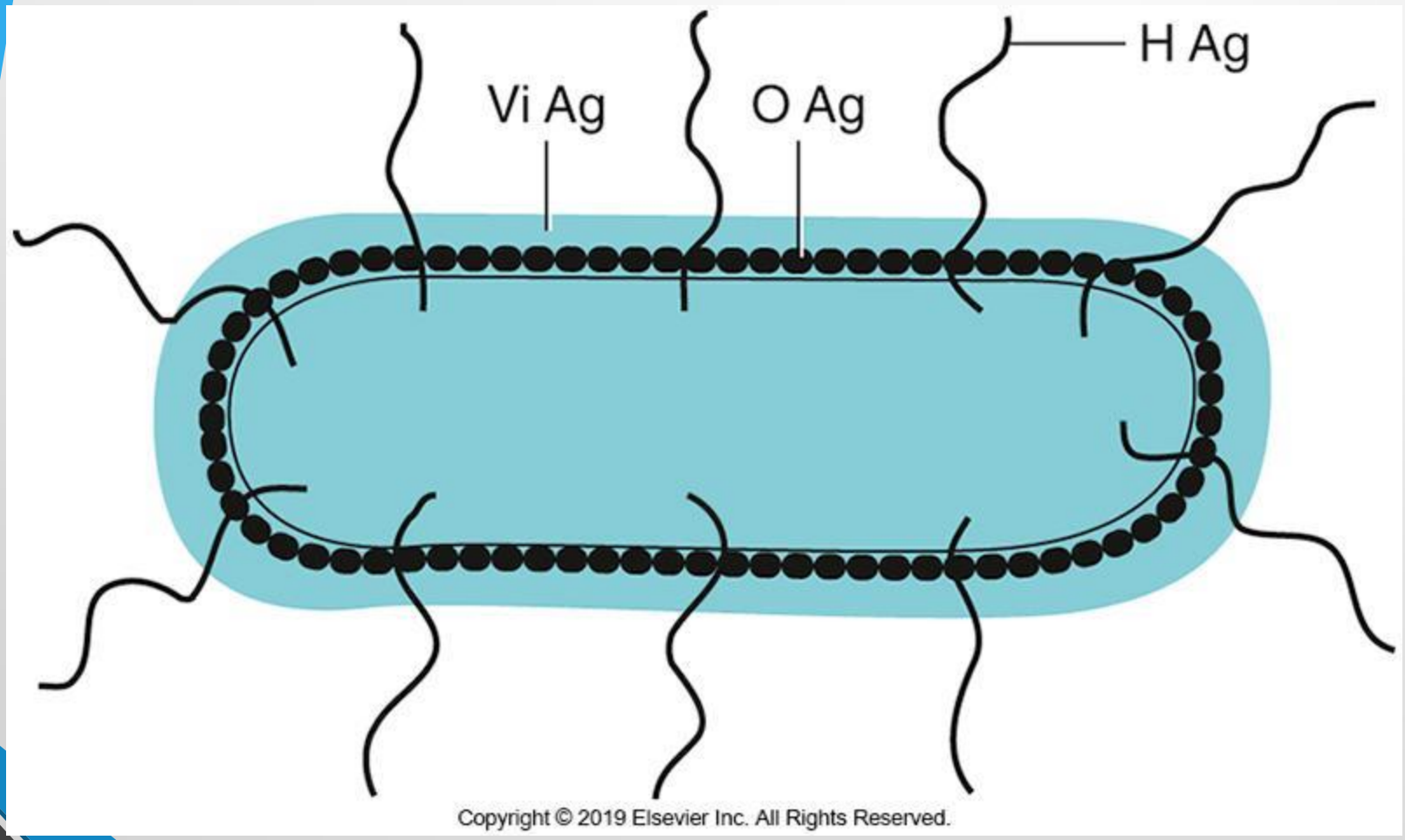
Test	*S.* serotype Choleraesuis	*S.* serotype Paratyphi	*S.* serotype Typhi	Other[a]
Arabinose fermentation	–	+	–	+
Citrate utilization	V	–	–	+
Glucose gas production	+	+	–	+
H_2S (TSI)	V	–	+	+
Lysine decarboxylase	+	–	+	+
Ornithine decarboxylase	+	+	–	+
Rhamnose fermentation	+	+	–	+
Trehalose fermentation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

H_2S, Hydrogen sulfide; *TSI,* triple sugar iron agar; –, ≥9% of strains positive; +, ≥90% of strains positive; *V,* 10% to 89% of strains positive.
[a]Typical strains in serogroups A through E.
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.

Antigenic Structures

- Primary
  - Somatic O and flagellar H antigens
  - O is heat-stable, H heat labile
- H antigens occur in 2 phases
  - The specific stage-determines the immunologic identity of a particular serotype- agglutinate only with homologous antisera
  - The nonspecific stage
- Other
  - Capsular (K), designated Vi antigen
  - Vi is important in identifying *Salmonella* serotype Typhi

Antigenic Structures of *Salmonellae* Used in Serologic Typing



Salmonella Clinical Infections

- Forms of salmonellosis
  - Acute gastroenteritis/food poisoning
    - Vomiting and diarrhea
  - Typhoid fever, most severe form of enteric fever
  - Nontyphoid bacteremia and other extraintestinal complicatons
  - Carrier state following *Salmonella* infection

Salmonella Gastroenteritis

- Food poisoning
- Sources of infection
  - Primarily poultry, eggs, milk, egg products, handling pets
  - Ingestion of foodstuffs
    - Examples- papayas, tahini, chicken, ground beef, cereal, peaches, mushrooms
  - Calves and poultry at petting zoos
  - Direct person to person
  - Cooking utensils

Salmonella Gastroenteritis (Cont.)

- Symptoms appear 8 to 36 hours after ingestion of contaminated food.
  - Nausea, vomiting, fever, chills
  - Accompanied by watery or blood-tinged diarrhea and abdominal pain
- Most infections are self-limiting.
- Antimicrobials of choice in most cases include
  - fluoroquinolone, azithromycin, and ceftriaxone

Enteric Fevers

- Clinical features
  - Prolonged fever
  - Bacteremia
  - Involvement of the reticuloendothelial system (RES), particularly the liver, spleen, intestines, and mesentery
  - Dissemination to multiple organs
- Enteric fever caused by *Salmonella typhi* is called typhoid fever.

Typhoid Fever
Transmission and Epidemiology

- Transmission
  - Improper disposal of sewage
  - Poor sanitation lack of a modern potable water system
- Epidemiology highlights
  - Tropical and subtropical areas where international travelers are more likely to acquire the infection

Typhoid Fever
Disease Course

- Symptoms develop 7 to 14 days after ingestion of organisms.
  - The larger the inoculum, the shorter the incubation time.
  - First week of disease patients typically develop
    - Sustained fever, malaise, anorexia, lethargy, myalgia, and a dull headache

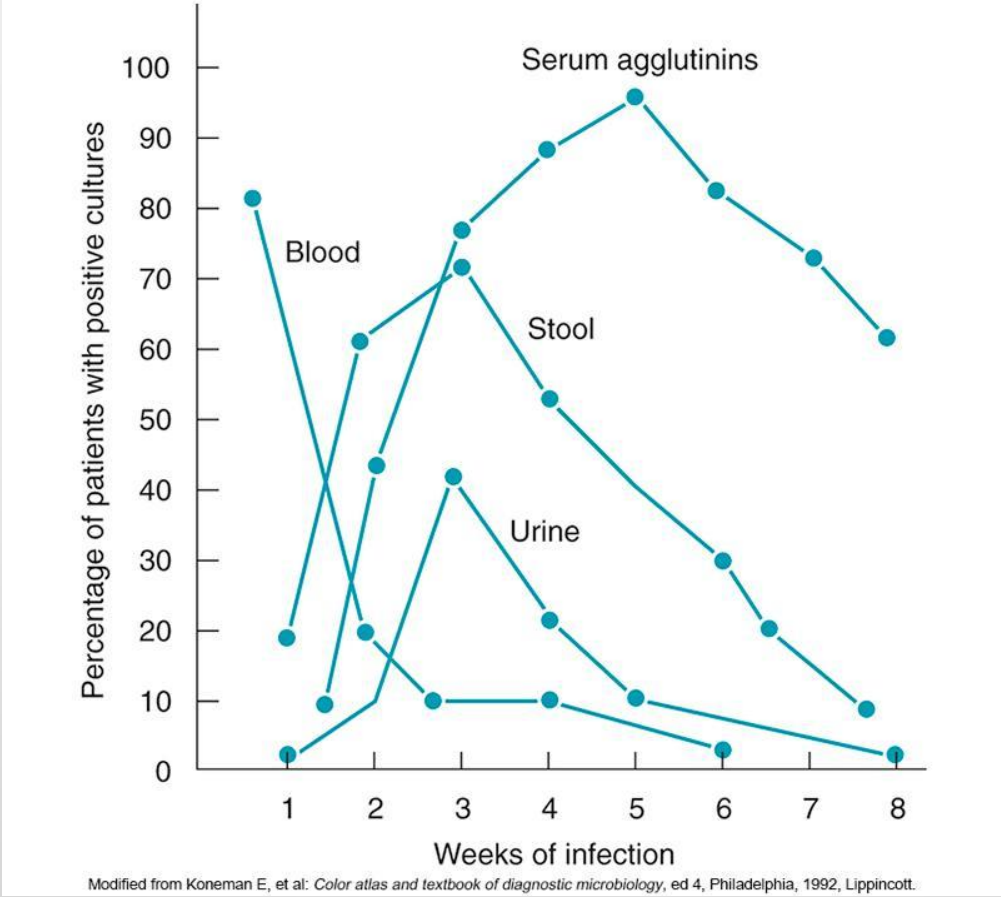
Typhoid Fever
Disease Course (Cont.)

- Organisms appear to be resistant to gastric acids.
  - Reach the small intestine, invade, and penetrate the intestinal mucosa
  - Leads to constipation
- Organisms gain access to the lymphatic system and are sustained in mesenteric lymph nodes.
- Organisms reach bloodstream and spread to liver, spleen, bone marrow
  - Immediately engulfed by mononuclear phagocytes

Typhoid Fever (Cont.)

- The organisms multiply intracellularly; later they are released into the bloodstream a 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.

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.

Typhoid Fever
Disease Course (Cont.)

- Weeks 2 and 3 of the disease (without treatment)
  - Patient experiences sustained fever with prolonged bacteremia.
  - Organisms invade the gallbladder and Peyer's patches of the bowel; they also reach the intestinal tract via the biliary tract.
  - "Rose spots" appear during week 2 of the fever.
  - Infection of the intestinal tract typically transpires resulting in large numbers of organisms.
    - May be isolated from the stool

Typhoid Fever
Disease Course (Cont.)

- Invasion of the gallbladder and Peyer's patches; release of bacteria into the bowel via the biliary duct
  - Gallbladder is the foci in long-term infections.
  - Severe infections can cause necrosis of gallbladder and/or Peyer's patches.
    - Hemorrhage and perforation of the bowel

Typhoid Fever
Disease Course (Cont.)

- The gallbladder becomes the foci of long-term carriage of the organism
  - Necrosis in the gallbladder may lead to necrotizing cholecystitis and necrosis of the Peyer's patches.
    - Hemorrhage and perforation of the bowel may occur as serious complications.
- Other complications that may occur
  - Pneumonia, thrombophlebitis, meningitis, osteomyelitis, endocarditis, abscesses

Salmonella Bacteremia

- Without extraintestinal foci of infection caused by nontyphoidal *Salmonella*
  - Characterized by prolonged fever and intermittent bacteremia
- Two groups at risk for developing infection
  - Young children
    - Fever and gastroenteritis with brief episodes of bacteremia
  - Adults
    - Transient bacteremia during episodes of gastroenteritis or develop symptoms of septicemia without gastroenteritis

Salmonella Carrier State

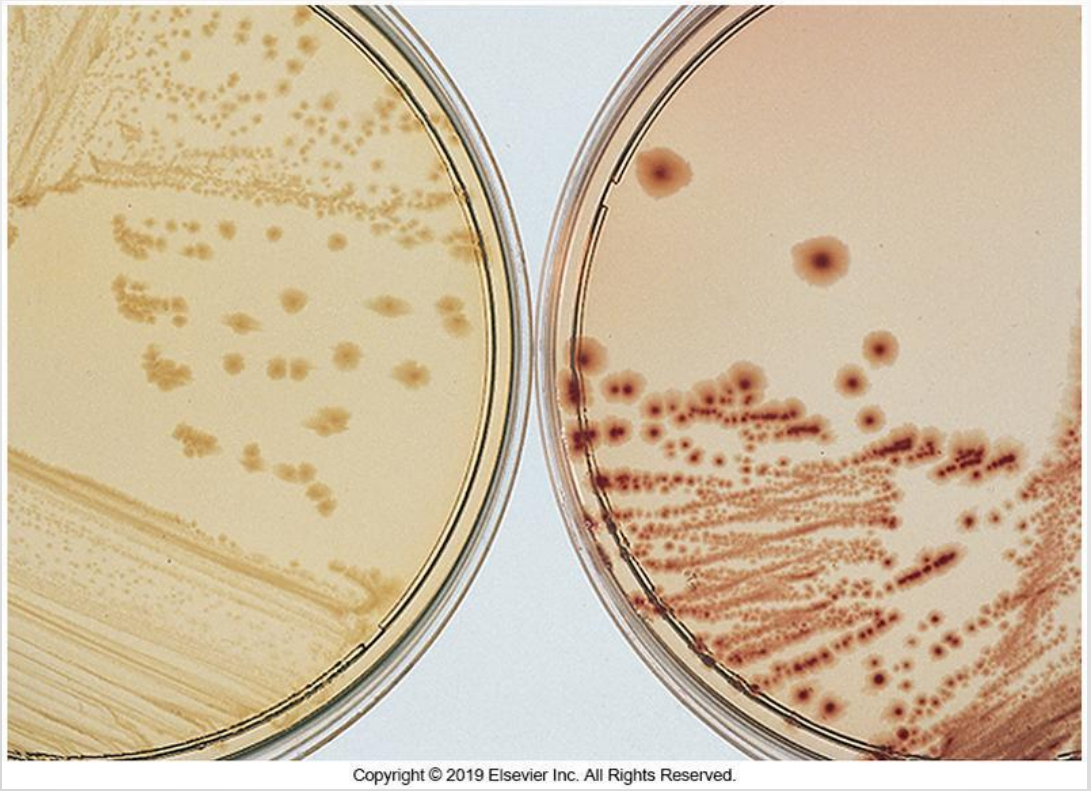
- Individuals who recover from infection may harbor the organisms in the gallbladder.
  - Gallbladder becomes the site of chronic carriage.
  - Organisms are excreted in the feces either continuously or intermittently.
- Carrier state may be terminated by antimicrobial therapy if gallbladder infection is not evident.
- Cholecystectomy has been the only solution to chronic state of enteric carriers.

Shigella Species Characteristics

- Nonmotile, most are slow lactose fermenters
- Generally do not produce gas from glucose
  - Except some types of *S. flexneri*
- No urease production
- No H_2S in TSI
- No decarboxylation of lysine
- Cannot utilize acetate or mucate as carbon source
- *S. sonnei* is positive for ONPG and ornithine decarboxylase.
- *S. flexneri* is negative for these tests.

Shigella Colony Morphology

Clear, nonlactose-fermenting colonies



Shigella Antigenic Structures

- O antigens are separated by serologic grouping.
- K antigens
  - Must be removed to type O antigen
  - Heat labile
- Lack H antigens
  - Nonmotile

Biochemical and Serologic Differentiation of *Shigella* Species

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

ONPG, *o*-Nitrophenyl-β-D-galactopyranoside; –, ≥9% of strains positive; +, ≥90% of strains positive; *V*, 10% to 89% of strains positive.
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.

Shigella Epidemiology

- Human and large primates are only known reservoirs.
- Transmission
  - Direct person-to-person
  - Fecal–oral route with carriers as the source
  - Transmitted by flies, fingers, and food or water contaminated by infected persons
  - Personal hygiene

Shigella Epidemiology (Cont.)

- Personal hygiene plays a major role in transmitting organisms.
- Groups at risk
  - Children in daycare centers (particularly infants younger than 1 year of age)
  - Individuals living in crowded and inadequate housing
  - Individuals who practice anal–oral sexual activity
  - Known to cause outbreaks on cruise ships

Shigella Clinical Infections

- Shigellosis
  - Important cause of bloody diarrhea worldwide
  - Responsible species vary in epidemiology, mortality rate, and severity of disease
  - Predominant isolate in the U.S. and Europe
    - *S. sonnei* followed by *S. flexneri*
  - *S. sonnei* characterized by fever and watery or bloody diarrhea
  - Initial symptoms
    - High fever, chills, abdominal cramps, pain accompanied by tenesmus 24 to 48 hours after ingestion of organisms

Shigella Clinical Infections (Cont.)

- Shigellosis
  - 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
  - The organisms invade the colonic tissues and cause an inflammatory response

Shigella
Clinical Infections (Cont.)

- Shigellosis
  - 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.
  - Clinical manifestations are caused by penetration of gastric epithelial cells and production of toxins.

Shigella
Clinical Infections (Cont.)

- Dysentery caused by *S. dysenteriae* type 1
  - Patients experience severe symptoms
    - Bloody diarrhea progresses to dysentery within a few hours to a few days
    - Painful bowel movements, which contain predominately mucus and blood
    - May become life-threatening as extraintestinal complications develop
      - Example- ileus, an obstruction of the intestines, with marked abdominal dilation, possibly leading to toxic megacolon

Yersinia

- 25 named species; most are considered environmental isolates
- 3 species are considered as human pathogens
  - *Y. pestis*- causative agent of the plague
  - *Y. pseudotuberculosis* and *Y. enterolitica*- known to cause sporadic cases gastroenteritis, mesenteric lymphadenitis (especially in children); generalized septicemia infections in immunocopromised hosts
  - All 3 species are zoonotic agents

Yersinia pestis

- *Y. pestis*
  - Causative agent of plague
    - Bubonic or glandular, septicemic, and pneumonic forms
  - Plague is transmitted through bite of infected fleas
  - Pneumonic plague transmitted through respiratory droplets
  - Gram-negative coccobacilli resembling a safety pin appearance (bipolar staining)
    - Preferential growth at 25 to 30° C
  - Class A bioterrorism agent

Y. Pestis Clinical Manifestations

- Symptoms of bubonic plague-most common
  - Sudden onset (2 to 6 days after infection) of high fever, chills, and headache with swollen, painful regional lymph nodes known as buboes
- Pneumonic plague
  - Can be a primary infection if bacteria are inhaled, or it can be secondary to bubonic plague
- Septicemic plague
  - Fever, chills, shock, extreme weakness, abdominal pain, sometimes bleeding leading to a rash

Y. pestis (Cont.)

- Nonmotile, gram-negative, short, plump bacillus
- Bipolar staining at each end of the bacillus that resembles a "safety pin" appearance
  - Methylene blue or Wayson stain
- Grows on routine media at optimal temperature of 25° to 30°C

Yersinia enterocolitica

- Mode of transmission
  - Contact with household pets
  - Fecal-oral route
  - After ingestion of contaminated food
    - Often pork, vacuum-packed deli meat, beef, lamb, chicken
  - Major concern- organism survives in cold temperatures; food refrigeration becomes an ineffective preventive measure
  - Associated with transfusion of contaminated packed red blood cells

Y. enterocolitica (Cont.)

- Most common forms of infection
  - Gastroenteritis
  - Appendicitis-like syndrome (mesenteric lymphadenitis)
  - Terminal ileitis
  - Septicemia
  - Arthritis
  - Erythema nodosum

*Tender red nodules with itching and burning on lower legs (shins)

Y. enterocolitica
Acute Enteritis

- Often affects infants and young children between the ages of 1 and 5 years
- Symptoms
  - Acute gastroenteritis with fever accompanied by headaches, abdominal pain, nausea, diarrhea
- Stools often contain blood.
- Usually mild and self-limiting

Y. enterocolitica Appendicitis-like Syndrome

- Occurs primarily in older children and adults
- Possible symptoms
  - Severe abdominal pain concentrated in right lower quadrant
  - Fever
  - Enlarged mesenteric lymph nodes
  - Inflamed ileum and appendix

Y. enterocolitica (Cont.)

- Arthritis form
  - Uncommon extraintestinal form
  - Usually after GI episode or an appendicitis-like syndrome
  - Reported more often in adults than in children
- Erythema nodosum
  - Inflammatory reaction caused by *Y. enterocolitica*
    - Red nodules that may be accompanied by itching and burning
    - Mainly affects anterior portion of the legs
    - More common in women than in men

Y. enterocolitica Appearance and Culture Media

- Gram-negative coccobacilli appearance (bipolar staining)
  - Preferential growth at 25 to 30° C
- Motile at 25°C but not at 35°C
- Cold enrichment can be used to increase recovery in fecal samples.
- Selective media available
  - Cefsulodin–irgasan–novobiocin (CIN)

Yersinia pseudotuberculosis

- Primary pathogen of rodents (especially guinea pigs), farm animals, birds
- Human infection rare
  - Associated with close contact with infected animals or their fecal material or ingestion of contaminated drink and foodstuff

Y. pseudotuberculosis Clinical Manifestations

- Ingested organisms spread to the mesenteric lymph nodes.
  - General infection that is usually self-limiting
- May include septicemia accompanied by mesenteric lymphadenitis
  - Presentation similar to appendicitis
- Infections most common in children and young adults

Y. pseudotuberculosis

- Typical-looking plague bacillus
- Motility at 18° to 22°C
- Produces urease
- Ferments rhamnose

Differentiation Within the Genus *Yersinia*

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 +[a]	+
Melibiose	–	– or +[a]	+
Trehalose	–	+ or –	+
Cellobiose	–	+	–

+, ≥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.
[a]Test results at 25° C.
Modified from Washington J: *Laboratory procedures in clinical microbiology,* ed 2, New York, 1985, Springer-Verlag.

OTHER GENERA OF THE ORDER ENTEROBACTERALES

CHAPTER NINETEEN

New Genera of Enterobacteriaceae

- *Budivicia*
- *Buttiauxella*
- *Cedecea*
- *Ewingella*
- *Leclercia*
- *Leminorella*

New Genera of Enterobacteriaceae (Cont.)

- *Moellerella*
- *Photorhabdus*
- *Rahnella*
- *Tatumella*

LABORATORY DIAGNOSIS OF ENTEROBACTERALES

CHAPTER NINETEEN

Specimen Collection and Transport

- Organisms can be isolated from a wide variety of clinical samples.
  - Most often isolated with other organisms including more fastidious pathogens
- Appropriate and immediate transport media important for isolation

Isolation and Identification

- Consider site of origin
- Generally enteric opportunistic organisms isolated from sites that are normally sterile are highly significant
- Complete identification should be directed only toward true intestinal pathogens.
- Multiplex PCR assays can detect multiple pathogens in clinical specimens
  - FilmArray can detect up to 22 common GI pathogens including bacteria, parasites and viruses in 1 hour(ish)

Direct Microscopic Examination

- Microscopic characteristics of enteric bacteria are indistinguishable from other gram-negative bacilli
- Sterile sites can be examined for the presence of gram-negative bacteria
- A presumptive results can aid the clinician in the preliminary diagnosis of the infection, appropriate empiric therapy can be instituted immediately

Direct Microscopic Examination (Cont.)

- Direct smears prepared from samples containing endogenous microbiota, such as sputum, do not provide valuable information.
- Direct smear examination of stool sample is not helpful either but may reveal the presence of inflammatory cells
  - May be helpful in determining whether a GI disease is ~~a~~ toxin-mediated or invasive process

Screening Stool Cultures for Pathogens

- Fecal samples
  - *Salmonella*, *Shigella*, STEC, and *Campylobacter*
  - Protocols and media should be available for
    - *Yersinia*, *Aeromonas*, *Vibrio*, *P. shigelloides*
  - Laboratories should develop protocols for the maximum recovery of enteric pathogens
  - Media to consider
    - MAC, SMAC, HE, XLD, CIN II, others as appropriate

Lysine Iron Agar (LIA) and TSI Screening

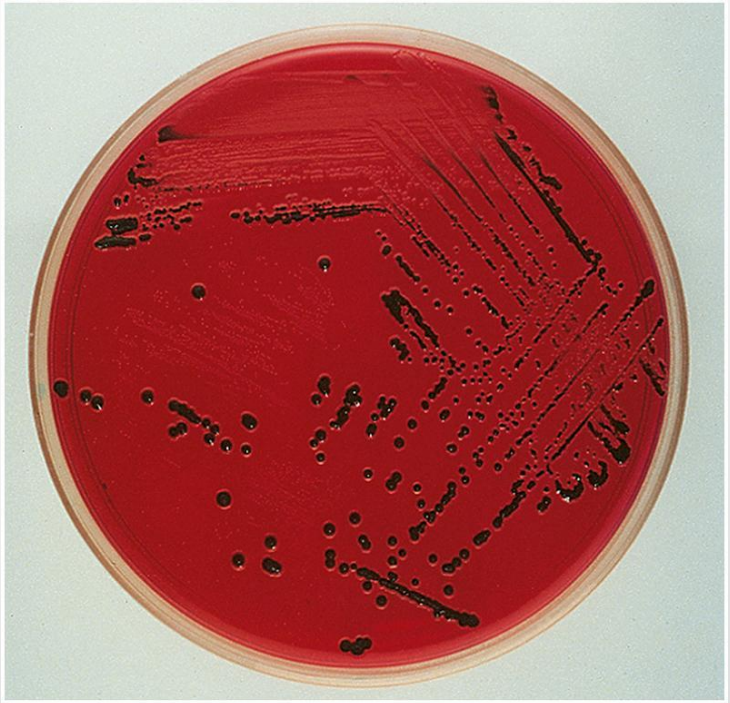
TABLE 19.11 Stool Culture Screening for Enteric Pathogens Using Triple Sugar Iron and Lysine-Iron Agar in Combination

	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*[a] *Edwardsiella*	*Salmonella*[a] *Edwardsiella*[a]	*Salmonella*[a]	*Salmonella*[a]	—	—	—	—
K/K	*Salmonella*	—	*Hafnia* *Klebsiella* *Serratia*	*Salmonella*[a] *Plesiomonas*[b] *Hafnia*	—	*Klebsiella* *Enterobacter* *E. coli*	*Serratia*	*Pseudomonas*[b]
K/A H_2S	—	*Salmonella*[a]	—	*Serratia*	—	—	—	—
K/A	—	*Citrobacter*	*Salmonella*[a] *Shigella* *Aeromonas*[b] *E. coli* *Enterobacter* *Citrobacter*	*Shigella*[a] *Yersinia* *Aeromonas*[b] *E. coli* *Enterobacter*	*Citrobacter*	*Aeromonas*[a,b] *E. coli* *Citrobacter* *Enterobacter*	*Aeromonas*[a,b] *Yersinia* *Citrobacter* *Enterobacter*	—

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

HE and XLD Agar

Courtesy R. Abe Baalness.



Courtesy American Society for Clinical Laboratory Science, Education and Research Fund, Inc., 1982.

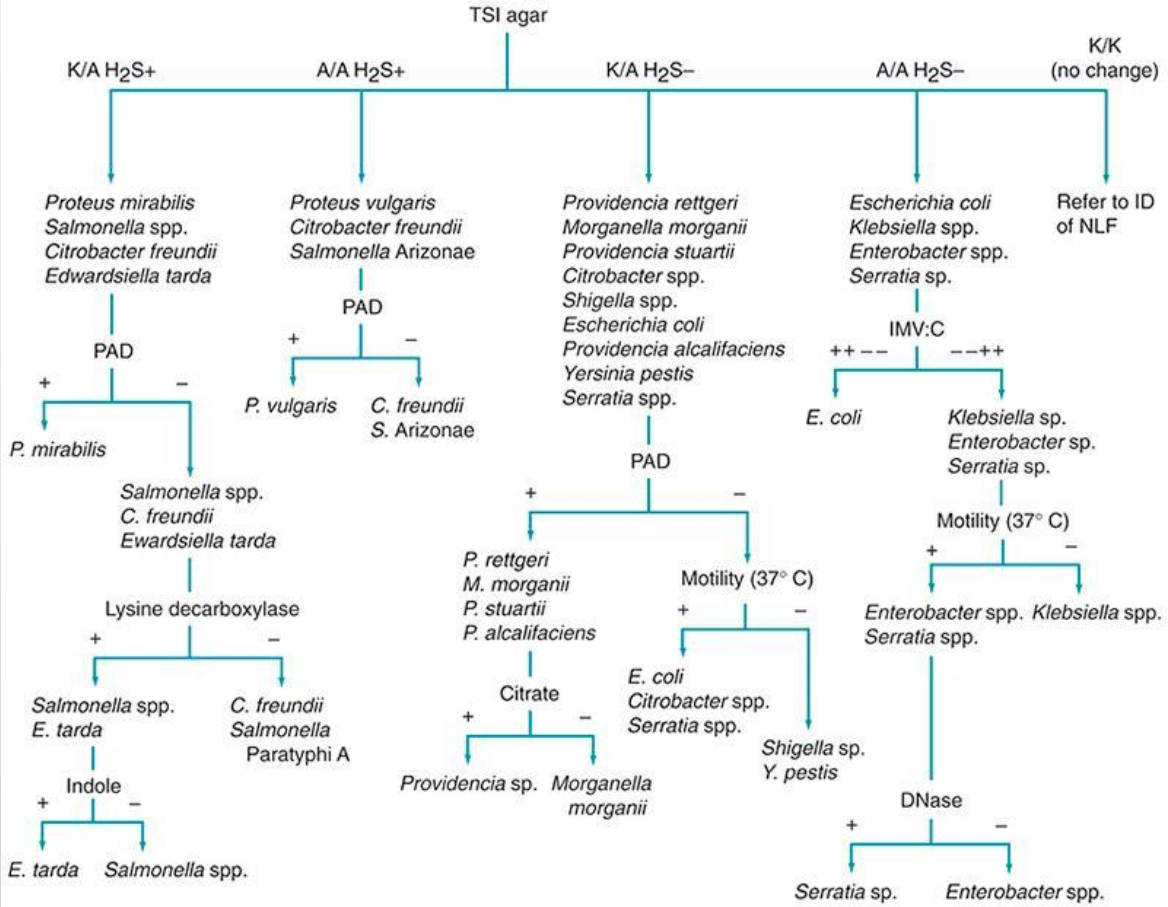
Salmonella on CHROMagar™

Courtesy BD Diagnostic Systems, Sparks, MD.

Identification

- Determine if the isolate belongs to the order Enterobacter
  - Gram-negative
  - Oxidase negative
    - Except for *P. shigelloides*
    - Always use young colonies from sheep blood agar (SBA) plates
  - Ferment glucose
  - Reduce nitrate to nitrite
    - Except *Photorhabdus* (rare human isolate) and *Xenorhabdus* (environmental isolate)
- Molecular biology assays now available

Schematic Diagram for Enterobacterales Identification



Data from Koneman E, et al: *Color atlas in diagnostic microbiology*, ed 5, Philadelphia, 1997, Lippincott-Raven.

Differentiating Characteristics of Enterobacterales

Table 19.12 Biochemical reactions of selected named species of the order Enterobacterales[a]

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
Budvicia aquatic	0	93	0	0	80	33	0	0	0	0	27	0	0	0	100	53	87	0	60	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
Buttiauxella noackiae	33	100	0	33	0	0	100	0	67	0	100	0	100	100	100	100	0	0	100	0	100
Cedecea davisae	0	100	50	95	0	0	0	0	50	95	95	0	86	91	100	70	19	100	100	0	99
Cedecea lapagei	0	40	80	99	0	0	0	0	80	0	80	0	100	99	100	100	60	0	100	0	100
Cedecea neteri	0	100	50	100	0	0	0	0	100	0	100	0	65	100	100	100	35	100	100	0	100
Citrobacter amalonaticus	100	100	0	95	5	85	0	0	85	95	95	0	99	1	100	97	35	9	100	1	30
Citrobacter braakii	33	100	0	87	60	47	0	0	67	93	87	0	100	0	100	93	80	7	100	33	0
Citrobacter farmeri	100	100	0	10	0	59	0	0	85	100	97	0	93	0	100	96	15	100	100	2	9
Citrobacter freundii	33	100	0	78	78	44	0	0	67	0	89	0	89	11	100	89	78	89	100	11	0
Citrobacter koseri	99	100	0	99	0	75	0	0	80	99	95	0	0	95	100	98	50	40	99	40	15
Citrobacter youngae	15	100	0	75	65	80	0	0	50	5	95	0	95	5	100	75	25	20	100	85	10
Cronobacter sakazakii	11	5	100	99	0	1	50	0	99	91	96	0	99	18	100	98	99	100	100	5	99
Edwardsiella hoshinae	50	100	0	0	0	0	0	100	0	95	100	0	0	100	100	35	0	100	100	0	50
Edwardsiella tarda	99	100	0	1	100	0	0	100	0	100	98	0	0	0	100	100	0	0	0	0	0
Enterobacter asburiae	0	100	2	100	0	60	0	0	21	95	0	0	97	3	100	95	75	100	100	0	100
Enterobacter cancerogenus	0	5	100	100	0	1	0	0	94	99	99	0	98	100	100	100	10	0	100	0	92
Enterobacter cloacae	0	5	100	100	0	65	0	0	97	96	95	0	98	75	100	100	93	97	100	15	75
Enterobacter hormaechei	0	57	100	96	0	87	4	0	78	91	52	0	100	100	100	83	9	100	100	87	44
Escherichia albertii	0		0	0	0	0	0	100	0	100	0	0	0	0	100	100	0	0	100	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
Escherichia fergusonii	98	100	0	17	0	0	0	95	5	100	93	0	0	35	100	95	0	0	98	60	65
Ewingella americana	0	84	95	95	0	0	0	0	0	0	60	0	5	0	100	0	70	0	100	0	80
Hafnia alvei	0	40	85	10	0	4	0	100	6	98	85	0	95	50	100	98	5	10	99	0	13
Klebsiella aerogenes	0	5	98	95	0	2	0	98	0	98	97	0	98	95	100	100	95	100	100	5	100
Klebsiella oxytoca	99	20	95	95	0	90	1	99	0	0	0	0	97	98	100	97	100	100	99	55	100

Serologic Grouping

- Serologic grouping
  - *Salmonella*
    - 60 types of O antigens
      - 95% are serogroups A through E1
    - Imperative for laboratories to be able to identify *Salmonella* serotype Typhi
    - Slide technique, agglutination test
  - *Shigella*
    - A through D serogroups
    - Agglutination test, procedure may need to be altered to ensure accurate results

Antigenic Formulae of the *Salmonella* Serovars

Table 19.13 Designation of *Salmonella* serogroups of most clinical significance

Old designation	New designation
A	2
B	4
C_1-C_4	6, 7
C_2-C_3	8
D_1	9
D_2	9, 46
D_3	9, 46, 27
E_1-E_2-E_3	3, 10
E_4	1, 3, 19
F	11
G_1-G_2	13

From Grimont, P. A. D., & Weill, F-X. (2007). *Antigenic formulae of the* Salmonella *serovars, 2007*. (9th ed.). Paris, France: WHO Collaborating Centre for Reference and Research on *Salmonella*.

POINTS TO REMEMBER

CHAPTER NINETEEN

Points to Remember

- Most of the genera discussed in this chapter could conceivably be isolated from almost any clinical specimen, especially when dealing with immunocompromised patients.
- ETEC strains can produce LT and/or ST.
- EIEC strains genetically and phenotypically resemble *Shigella* spp.

Points to Remember (Cont.)

- Although most isolates of *E. coli* are considered normal fecal microbiota, several strains (enterovirulent *E. coli*) are known to cause intestinal tract infections; in the United States STEC is the most notable.
- *E. coli* is the most significant cause of UTIs.

Points to Remember (Cont.)

- STEC strains produce Shiga toxin 1 and/or Shiga toxin 2. Some of these strains, referred to as EHEC, produce a more severe infection called HUS.
- *Salmonella* and *Shigella are enteric pathogens and are not considered n*ormal fecal biota.

Points to Remember (Cont.)

- *Yersinia pestis*, causes plague.
- A good patient history combined with proper selective screening agar (e.g., HE, XLD, and SMAC agars) can be very helpful in the timely and accurate identification of enteric pathogens associated with diarrheal disease.

Points to Remember (Cont.)

- Presumptive genus identification may include a battery of selective agar media and key biochemical tests
  - Oxidase test, TSI, urea, LIA, IMViC
  - Confirm with additional conventional biochemical tests, one of several multitest or rapid and automated identification systems, or a molecular biology assay.
- Serogrouping is an important aspect in the identification of enteric pathogens.