Enterobacteriacea

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Gram-Negative Rods Related to the Enteric Tract - Introduction

Overview

- Gram-negative rods are a diverse group of organisms.
- Divided based on clinical relevance and anatomical site:
  - Enteric tract
  - Respiratory tract
  - Animal sources

Classification of Enteric-Associated Gram-Negative Rods

Group	Description
1. Pathogens both within and outside the enteric tract	e.g. *Escherichia coli*, *Salmonella enterica*
2. Pathogens primarily within the enteric tract	e.g. *Shigella spp.*, *Vibrio cholerae*, *Campylobacter jejuni*

3. Pathogens outside the enteric tract	e.g. *Proteus spp., Klebsiella spp.*

📌 Explanation:

- Some bacteria like *E. coli* can cause both intestinal (diarrhea) and extraintestinal (UTIs, neonatal meningitis) diseases.
- Others, like *Shigella,* are strictly enteric pathogens, confined to the gastrointestinal tract.

Epidemiology in the United States

- Frequent GI pathogens:
  - *Salmonella*
  - *Shigella*
  - *Campylobacter*
- Less common in the U.S:
  - *Escherichia coli* (especially enterotoxigenic E. coli or ETEC)
  - *Vibrio spp.*
  - *Yersinia enterocolitica*

⚠ Note:

ETEC is common in developing countries, causing traveler's diarrhea, but is rare in the U.S.

Diseases Caused by Enteric Gram-Negative Rods

1. Diarrheal Diseases

- Common agents:
    - *Shigella spp.* – dysentery
    - *Salmonella spp.* – gastroenteritis, typhoid fever
    - *Campylobacter jejuni* – bacterial gastroenteritis
    - *Vibrio cholerae* – cholera
    - *ETEC (E. coli)* – traveler's diarrhea
    - *Yersinia enterocolitica* – pseudoappendicitis

2. Urinary Tract Infections (UTIs)

- Primary pathogen: *E. coli*

- Other less common pathogens:
    - *Proteus mirabilis*
    - *Klebsiella pneumoniae*
    - *Enterobacter spp.*

Associated Autoimmune Complications

- Certain enteric pathogens may trigger post-infectious autoimmune syndromes:
    - Reactive arthritis (ReA) – joint inflammation after GI or urogenital infections.
    - Reiter syndrome – classic triad: arthritis, urethritis, conjunctivitis.
        - Commonly associated with *Shigella*, *Salmonella*, *Yersinia*, and *Campylobacter*.
    - Guillain-Barré syndrome (GBS) – acute flaccid paralysis post *Campylobacter jejuni* infection.

The Enterobacteriaceae Family

- Many gram-negative rods of clinical importance belong to this family.
- Common features include:
    - Facultative anaerobes
    - Oxidase-negative

- Ferment glucose
- Often motile with peritrichous flagella (except *Shigella, Klebsiella* – non-motile)

Enterobacteriaceae & Related Organisms

General Overview

- A large family of Gram-negative rods, commonly found in the colon of humans and animals.
- Often part of normal intestinal flora.
- Represent the major facultative anaerobes of the large intestine.
    - Outnumbered by strict anaerobes like *Bacteroides spp.*

Key Characteristics of Enterobacteriaceae

📌 Common Features (Mnemonic: FOG-N)

All Enterobacteriaceae share four metabolic traits:

1. F – Facultative anaerobes

2. O – Oxidase-negative (lack cytochrome oxidase)

3. G – Glucose fermenters (may vary in fermenting other sugars)

4. N – Nitrate reducers (nitrate → nitrite)

✅ These traits distinguish them from non-fermenters like *Pseudomonas aeruginosa*.

Comparison: Enterobacteriaceae vs Pseudomonas aeruginosa

Feature	Enterobacteriaceae	Pseudomonas aeruginosa
Oxygen requirement	Facultative anaerobe	Strict aerobe
Glucose fermentation	Yes	No
Oxidase test	Negative	Positive
Nitrate	Yes (to nitrite)	No

reduction

Energy metabolism	Fermentation & respiration	Oxidative only
Clinical relevance	GI, UTIs, sepsis, pneumonia	Hospital-acquired UTIs, sepsis

🔎 *Pseudomonas* is not part of the Enterobacteriaceae despite being Gram-negative—it's a non-fermenter and behaves differently both metabolically and clinically.

Examples of Enterobacteriaceae & Their Associated Diseases

Organism	Associated Diseases
Escherichia coli	UTIs, neonatal meningitis, septicemia, diarrhea types (ETEC, EHEC, etc.)
Klebsiella pneumoniae	Pneumonia, UTIs, liver abscesses
Proteus	UTIs, struvite stones (urease-positive)

mirabilis

Salmonella enterica	Gastroenteritis, typhoid fever
Shigella spp.	Bacillary dysentery
Yersinia enterocolitica	Gastroenteritis, pseudoappendicitis
Enterobacter spp.	Opportunistic infections (UTIs, respiratory)
Serratia marcescens	Nosocomial infections, catheter-related sepsis

Pathogenesis

1. Endotoxin (Lipopolysaccharide - LPS)

- Common to all Gram-negative bacteria.
- Found in the outer membrane of the cell wall.
- Responsible for:

- Fever
- Hypotension
- Disseminated intravascular coagulation (DIC) in severe cases

2. Exotoxins

- Produced by some members:

Organism	Toxin	Effect
E. coli (ETEC)	Heat-labile & heat-stable enterotoxins	Activate adenylate/guanylate cyclase → watery diarrhea
E. coli O157:H7	Shiga-like toxin (Verotoxin)	Inhibits 60S ribosome → HUS (Hemolytic Uremic Syndrome)
Vibrio cholerae	Cholera toxin	Activates adenylate cyclase → profuse watery diarrhea

Related Non-Fermenting Gram-Negative Rods

(*Not part of Enterobacteriaceae, but important to remember*)

Genus	Infections
Pseudomonas aeruginosa	Nosocomial infections, burns, CF lungs
Acinetobacter	Respiratory & wound infections
Moraxella	Otitis media, sinusitis
Eikenella, Kingella	Endocarditis, dental infections
Achromobacter, Alcaligenes, Flavobacterium	Rare opportunistic pathogens

⚠ These are aerobic, oxidase-positive, and non-fermenters. Often resistant to multiple antibiotics.

✅ Summary Table: Enterobacteriaceae Essentials

Feature	Enterobacteriaceae
Gram stain	Negative rods
Habitat	Colon (mainly), environment
Respiration	Facultative anaerobes
Glucose fermentation	Yes
Oxidase	Negative
Nitrate reduction	Yes (to nitrite)
Endotoxin	Present
Exotoxin production	In some species
Pathologies	GI, UTI, pneumonia, sep

✅ Categories of Gram-Negative Rods

🗄 Source of Infection & Genus

Enteric Tract–Related Gram-Negative Rods

These organisms are categorized based on where they primarily cause disease:

1. Pathogens that cause infection both within and outside the enteric tract:

- Genus: *Escherichia* and *Salmonella*

2. Pathogens that primarily cause infection within the enteric tract:

- Genus: *Shigella, Vibrio, Campylobacter, Helicobacter*

3. Pathogens that cause infection outside the enteric tract (but are related to enteric flora):

- Genus:
    - *Klebsiella–Enterobacter–Serratia* group
    - *Proteus–Providencia–Morganella* group
    - *Pseudomonas*
    - *Bacteroides, Prevotella, Fusobacterium*

Respiratory Tract–Related Gram-Negative Rods

- Genus: *Haemophilus, Legionella, Bordetella*

Animal Source–Related Gram-Negative Rods

- Genus: *Brucella, Francisella, Pasteurella, Yersinia*

Frequency of Diseases Caused by Gram-Negative Rods in the U.S.

📌 This shows which organisms commonly cause infections in the United States, based on the site of infection.

1. Infections of the Enteric (Gastrointestinal) Tract

🔷 Frequently involved pathogens:

- *Salmonella*
- *Shigella*
- *Campylobacter*

🔷 Less frequently involved pathogens:

- *Escherichia*
- *Vibrio*
- *Yersinia*

2. Infections of the Urinary Tract

🔷 Frequent pathogen:

- *Escherichia* (especially *E. coli*)

🔷 Less frequent pathogens:

- *Enterobacter*
- *Klebsiella*
- *Proteus*
- *Pseudomonas*

✅ Gram-Negative Rods Causing Diarrhea

This classifies diarrhea-causing bacteria based on mechanism, presence of fever, leukocytes in stool, infective dose, and key features.

A. Enterotoxin-Mediated Diarrhea

These organisms produce toxins that increase fluid secretion → watery diarrhea, usually no fever or inflammation.

1. Escherichia coli (ETEC, etc.)

- Fever: Absent (–)
- Leukocytes in stool: Absent (–)
- Infective Dose: Unknown (?)
- Key Feature: Ferments lactose

2. Vibrio cholerae

- Fever: Absent (–)

- Leukocytes in stool: Absent (–)
- Infective Dose: High (10^7 organisms)
- Key Feature: Comma-shaped bacteria

B. Invasive-Inflammatory Diarrhea

These organisms invade mucosa → fever, leukocytes in stool, and often bloody diarrhea.

1. Salmonella (e.g., S. enterica)

- Fever: Present (+)
- Leukocytes in stool: Present (+)
- Infective Dose: Moderate (10^5 organisms)
- Key Feature: Does not ferment lactose

2. Shigella (e.g., S. dysenteriae)

- Fever: Present (+)
- Leukocytes in stool: Present (+)
- Infective Dose: Very low (10^2 organisms)
- Key Feature: Does not ferment lactose

3. Campylobacter jejuni

- Fever: Present (+)
- Leukocytes in stool: Present (+)

- Infective Dose: Low (10^4 organisms)
- Key Feature: Comma- or S-shaped bacteria; grows best at 42°C

4. Enteropathogenic E. coli (EPEC, etc.)

- Fever: Present (+)
- Leukocytes in stool: Present (+)
- Infective Dose: Unknown (?)
- Key Feature: Not specified, but known to cause infantile diarrhea

5. E. coli O157:H7 (EHEC)

- Fever: May be present or absent (+/—)
- Leukocytes in stool: Present (+)
- Infective Dose: Unknown (?)
- Key Feature: Transmitted by undercooked hamburger; causes hemolytic-uremic syndrome (HUS)

C. Those With Uncertain Mechanisms

May have mixed or unclear pathogenesis, sometimes involving enterotoxins.

1. Vibrio parahaemolyticus

- Fever: Present (+)
- Leukocytes in stool: Present (+)
- Infective Dose: Unknown (?)
- Key Feature: Transmitted via seafood

2. Yersinia enterocolitica

- Fever: Present (+)
- Leukocytes in stool: Present (+)
- Infective Dose: High (10^8 organisms)
- Key Feature: Usually transmitted from pets, especially puppies

🔎 Note: Some strains of Vibrio and Yersinia may produce enterotoxins, but their role is not clearly established.

✅ Gram-Negative Rods Causing UTI or Sepsis

💡 All these organisms are Gram-negative rods
🔬 UTI diagnosed by urine culture
💧 Sepsis diagnosed by blood or pus culture

Lactose-Fermenting Organisms (appear pink on MacConkey agar)

1. Escherichia coli

- Lactose fermentation: Positive (+)
- Feature: Colonies show green metallic sheen on EMB agar

2. Enterobacter cloacae

- Lactose fermentation: Positive (+)
- Feature: Causes nosocomial infections; often drug-resistant

3. Klebsiella pneumoniae

- Lactose fermentation: Positive (+)
- Feature: Large mucoid capsule, colonies appear viscous

Non-Lactose Fermenters (colorless colonies on MacConkey)

4. Serratia marcescens

- Lactose fermentation: Negative (–)
- Feature: Produces red pigment; nosocomial and drug-resistant

5. Proteus mirabilis

- Lactose fermentation: Negative (–)
- Feature: Swarming motility on agar; urease-producing

6. Pseudomonas aeruginosa

- Lactose fermentation: Negative (–)
- Feature: Produces blue-green pigment and fruity odor; causes nosocomial infections and is often drug-resistant

🧪 EMB agar = Eosin Methylene Blue agar — used to identify lactose fermenters like *E. coli*

✅ Diseases Caused by Enterobacteriaceae Members

1. Escherichia (E. coli)

- Diseases:
  - Urinary tract infection
  - Traveler's diarrhea
  - Neonatal meningitis

2. Shigella

- Disease:
  - Dysentery (bloody, mucus-filled diarrhea)

3. Salmonella

- Diseases:
    - Typhoid fever
    - Enterocolitis
- Minor related genera:
    - *Arizona*
    - *Citrobacter*
    - *Edwardsiella*

4. Klebsiella

- Diseases:
    - Pneumonia
    - Urinary tract infections

5. Enterobacter

- Diseases:
    - Pneumonia
    - UTIs

- Minor related genus: *Hafnia*

6. Serratia

- Diseases:
    - Pneumonia
    - UTIs

7. Proteus

- Disease:
    - Urinary tract infections
- Minor related genera:
    - *Providencia*
    - *Morganella*

8. Yersinia

- Diseases:
    - Plague (Y. pestis)

- Enterocolitis
- Mesenteric adenitis (can mimic appendicitis)

🔬 ANTIGENS of Enterobacteriaceae

Certain antigens are used for identification and serotyping in clinical and epidemiological investigations, particularly for *Salmonella* and *Shigella*.

🔶 1. O Antigen (Somatic or Cell Wall Antigen)

- Location: Outer polysaccharide part of lipopolysaccharide (LPS) on the bacterial outer membrane.

- Structure: Made of oligosaccharide repeating units (3–4 sugars repeated 15–20 times).

- Function:

  - Basis for serological classification of many enteric bacteria.
  - Highly variable:
    - ~1500 O types in *Salmonella*
    - ~150 O types in *E. coli*

- Clinical Importance:
    - Used in slide agglutination tests.
    - Heat-stable antigen.

✅ Mnemonic Tip: *"O is for Outer LPS and Ongoing diversity."*

🔷 2. H Antigen (Flagellar Antigen)

- Location: On the flagella of motile organisms.
- Present in: *E. coli, Salmonella,* etc.
- Absent in: Non-motile species like *Shigella, Klebsiella.*
- Special Feature in Salmonella:
    - Exists in two phases: Phase 1 and Phase 2.
    - Organisms switch between them to evade immune detection (Antigenic variation).

✅ Mnemonic Tip: *"H is for Hair-like Flagella and Hidden phases."*

🔷 3. K Antigen (Capsular Polysaccharide Antigen)

- Location: Surrounds the cell wall (capsule).
- Seen in: Prominent in heavily encapsulated organisms like *Klebsiella*.
- Detection:
    - Quellung reaction (capsular swelling seen under microscope with specific antisera).
- Clinical Use:
    - Used to serotype E. coli and Salmonella typhi.
    - In *S. typhi*, it is known as the Vi antigen (Vi = virulence antigen).

✅ Mnemonic Tip: *"K is for Klebsiella's Capsule and Kill-proof coat."*

🧫 LABORATORY DIAGNOSIS of Enterobacteriaceae

🔷 Specimen Inoculation

- Media Used:

1. Blood Agar: General purpose, non-selective.

2. Selective Differential Media:
   - MacConkey Agar
   - Eosin-Methylene Blue (EMB) Agar

MacConkey Agar

- Selective for: Gram-negative rods (due to bile salts and crystal violet inhibiting gram-positive growth).

- Differentiates:

  - Lactose fermenters → Pink colonies
  - Non-lactose fermenters → Colorless colonies

Organism	Colony Color on MacConkey
E. coli	Pink (lactose fermenter)
Shigella, *Salmonella*	Colorless (non-lactose fermenters)

EMB Agar

- Differentiates based on: Lactose fermentation.

- E. coli colonies show a green metallic sheen (classic diagnostic feature).

- Also selective against gram-positive bacteria.

🔷 Screening Biochemical Tests

Before final identification, some basic tests are run:

1. Triple Sugar Iron (TSI) Agar

    - Tests fermentation of glucose, lactose, and sucrose, and H_2S production.
    - Butt/Slant color, Gas bubbles, and Black precipitate (H_2S) are observed.

2. Urea Agar

    - Detects urease enzyme.
    - *Proteus* is strongly urease-positive (pink color).

🧪 Summary Table: Antigen Features

Antigen	Location	Found in	Special Feature	Clinical Use

O	Outer LPS	Most enterics	Highly variable	Serotyping
H	Flagella	Motile (e.g., *Salmonella*)	Phase variation	Serotyping
K	Capsule	Encapsulated (e.g., *Klebsiella, S. typhi*)	Vi antigen in *S. typhi*	Quellung test

📌 Lactose Fermentation in Enterobacteriaceae

Lactose Fermentation Organisms

Occurs (Fast Fermenters)	*Escherichia coli, Klebsiella, Enterobacter*
Does NOT Occur	*Shigella, Salmonella, Proteus, Pseudomonas*
Occurs Slowly	*Serratia, Vibrio*

◈ Clinical Relevance:

- Differentiates pathogens from normal flora and contaminants.
- Fast fermenters (like *E. coli*) are usually less virulent than non-fermenters (like *Salmonella, Shigella*).

Triple Sugar Iron (TSI) Agar Reactions

Composition:

- Sugars: Glucose (0.1%), Lactose (1%), Sucrose (1%)
- Indicators: Phenol red (pH), $FeSO_4$ (H_2S detection)
- Zones: Slant (aerobic), Butt (anaerobic)

Interpretation of Results:

Slant	Butt	Gas	H_2S	Representative Organisms
Acid	Acid	+ / −	−	*E. coli, Klebsiella, Enterobacter*
Alkalin	Acid	−	−	*Shigella, Serratia*

e				
Alkalin e	Acid	+	+	*Salmonella, Proteus*
Alkalin e	Alkalin e	–	–	*Pseudomonas* (non-fermenter)

🟤 Acid (A) = Yellow; 🟤 Alkaline (K) = Red
⚫ Black precipitate in butt = H_2S production

🔍 TSI Agar Explanation:

1. Fermentation of Lactose/Sucrose → Acid → Slant & Butt yellow (A/A)

2. Fermentation of Glucose only → Butt yellow, slant reverts red (K/A)

3. No fermentation → Both red (K/K)

4. H_2S production → Black butt

5. Gas → Cracks or bubbles in butt

🔑 Mnemonic for TSI:
"Yellow for Acid, Red for No Sugar, Black for H_2S"

🫓 Urea Agar Test

Test	Component	Indicator	Positive Test
Urease test	Urea	Phenol red	Pink (alkaline)

- Positive organisms: *Proteus* spp., *Klebsiella pneumoniae*
- Urease hydrolyzes urea → NH_3 → ↑pH → phenol red turns reddish-purple

💧 Coliforms & Public Health

- Coliforms = Gram-negative, lactose-fermenting rods (e.g., *E. coli*, *Enterobacter*, *Klebsiella*)
- Water Testing Indicator:
    - *E. coli* is the most reliable indicator of fecal contamination, because it's only found in the gut.
- Criteria for E. coli in water:

- Lactose fermenter → produces acid + gas
- Grows at 44.5°C
- Metallic green sheen on EMB agar

- Unsafe drinking water: > 4 E. coli/dL

- Prevention: Water chlorination → kills E. coli and other pathogens

✅ Public Health Note: Water disinfection is one of the greatest advancements of modern public health.

💊 Antibiotic Therapy for Enterobacteriaceae

🔰 Empirical treatment is often not reliable due to:

- Antibiotic resistance

- β-lactamase production (including ESBLs)

- Horizontal gene transfer via plasmids (R factors)

🔬 New Delhi Metallo-β-Lactamase (NDM):

- Confers resistance to:

  - Penicillins

- Cephalosporins
- Monobactams
- Carbapenems

◈ Effective classes (depending on sensitivity testing):

- Penicillins (only some)
- Cephalosporins
- Aminoglycosides
- Quinolones
- Tetracyclines
- Sulfonamides
- Chloramphenicol

🔘 Key Concept: Always base therapy on antibiotic susceptibility testing, not on general class efficacy.

📓 Summary Flash Table

Test/Feature	E. coli	Salmonella	Shigella	Proteus
Lactose Fermentation	+	–	–	–
Gas from Glucose	+	+	–	+

H_2S Production	–	+	–	+
Urease Test	–	–	–	+
TSI Reaction	A/A, gas	K/A, gas + H_2S	K/A	K/A, gas + H_2S
EMB Agar	Green sheen	Colorless	Colorless	Colorless