MICROBIOLOGY

Pathogenic Gram-Negative Bacilli (Enterobacteriaceae)
Gram-Negative Bacteria

Constitute the largest group of human pathogens

- Due in part to the presence of lipid A in the bacterial cell wall
  - Triggers fever, vasodilatation, inflammation, shock, and disseminated intravascular coagulation (blood clots within blood vessels)

Almost every Gram-negative bacterium that can breach the skin or mucous membranes, grow at 37°C, and evade the immune system can cause disease and death in humans
Diversity of Activities Associated with LPS
Members of the intestinal microbiota of most animals and humans

Ubiquitous in water, soil, and decaying vegetation

Enteric bacteria are the most common Gram-negative pathogens of humans
General Properties of Enterobacteriaceae

- Gram negative bacilli
- Aerobes and facultative anaerobes
- Grow on ordinary media
- Ferment glucose with production of acid or acid and gas
- Reduce nitrates to nitrites
- Catalase positive
- Oxidase negative
- Motile (peritrichous flagella) or non-motile
The Medium: Nitrate Broth
Glucose Fermentation
Antigens and virulence factors

- Outer membrane: (common antigen, O antigen, Lipid A)
- Capsular antigens (K; Vi in *Salmonella*)
- Flagellar antigens (H)

**Virulence Factors**
- Pilus
- Exotoxin
- Adhesin
- Plasmid (virulence genes)
- Iron-binding protein
- Hemolysin

**Plasmid**

Figure 20.8
Antigenic Structure of Enterobacteriaceae
Pili
(Fimbriae)

Flagellum
("H" Antigen)

Outer membrane
(Lipopoly saccharide,
lipoprotein)

Lipid

"O" Antigen
side chains

Solid membrane
(Murein layer, mucopeptide,
peptidoglycan)

Inner cytoplasmic membrane
(Plasma membrane)

Capsule: "K" Antigen of E. coli
"Vi" Antigen of S. typhi
Serotype antigen of K. pneumoniae
Diagnosis

- Enterobacteriaceae are cultured using selective and differential media
- Commercially available biochemical tests can rapidly identify enteric bacteria

Treatment

- Treatment of diarrhea involves treating the symptoms with fluid and electrolyte replacement
- Antimicrobial drugs are not usually needed since diarrhea is self-limited
Increasing concentration of drug
Prevention

Preventing enteric infections is almost impossible since they are a major component of the normal microbiota.

Good personal hygiene and proper sewage control are important in limiting the risk of infection.
Pathogenic Enterobacteriaceae are often classified into three groups

- Coliforms, which rapidly ferment lactose, are part of the normal microbiota, and may be opportunistic pathogens
- Noncoliform opportunists, which do not ferment lactose
- True pathogens
Incidence of Enterobacteriaceae Associated with Bacteremia

- 45% Escherichia
- 22% Klebsiella
- 20% Enterobacter
- 4% Serratia
- 4% Proteus
- 2% Citrobacter
- 3% Other
Aerobic or facultatively anaerobic, Gram-negative, rod-shaped bacteria

Commonly found in soil, on plants, and on decaying vegetation

Colonize the intestinal tracts of animals and humans

Examples: Escherichia, Klebsiella, Enterobacter, Citrobacter, Serratia

Presence of coliforms in water is indicative of impure water and of poor sewage treatment (i.e. one of the indicators of fecal pollution of water: *E. coli*, *Clostridium perfringens*, *Enterococcus fecalis*)
Indicator Concept

- Impossible to monitor for all pathogens
- Indicator organisms signal recent fecal contamination
- Indicator organisms always present in fecal material
- Indicator organism’s persistence similar to pathogens
- Detection methods relatively simple
Technical issues related to pathogen detection

- Low number of pathogens
- Human or non-human strains
- Pathogens difficult to detect
- Cannot determine viability
- Need large sample volumes
- Few water labs equipped or staffed
- Days to weeks for results
Bacteria in water
The most common and important of the coliforms (found in 100% of human intestines)

Virulent strains have genes located on virulence plasmids that allow the bacteria to colonize human tissue

Gastroenteritis is the most common disease associated with *E. coli* (enteropathogenic, enterotoxigenic and enteroinvasive strains)

- Often mediated by exotoxins that produce the symptoms associated with gastroenteritis

Most common cause of non-nosocomial urinary tract infections (cystitis & pyelonephritis)

Wound infections, meningitis in neonates
\textit{Escherichia coli} \\

E.coli O157:H7 is the most prevalent strain of pathogenic E.coli in developed countries (enterohemorrhagic)

- Causes diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome, a severe kidney disorder
- Most epidemics associated with undercooked ground beef or unpasteurized milk or juice
Nosocomial infections

Gram-positive bacteria

Gram-negative bacteria

Yeast

Enterobacteriaceae: coliforms:

Escherichia

Klebsiella

Serratia

Enterobacter

Citrobacter

Enterobacteriaceae: non-coliforms:

Proteus

Salmonella

Pseudomonas
(not in family Enterobacteriaceae)

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**Klebsiella**

- Found in the digestive and respiratory systems of humans and animals (*Klebsiella pneumoniae, aerogenes, ozaenae, rhinoscleromatis*)

- Can cause opportunistic infections

- No waterborne disease ever associated with Klebsiella in drinking water, all hospital acquired (nosocomial)

- Produce a capsule that protect the bacteria from phagocytosis (mucoid colonies)

- *K. pneumoniae* is the most commonly isolated pathogenic species
  - Causes pneumonia
  - May be involved in bacteremia, meningitis, wound infections, UTIs
The Methyl Red reagent.

MRVP Broth--The Methyl Red Test

Left: uninoculated control
Right: negative (copper color)

Left: uninoculated control
Right: positive (red color)

The VP reagents

MRVP broth--Voges-Proskauer Test
### Serratia

- **Produce a red pigment when grown at room temperature**
- **Can grow on catheters, in saline solutions, and other hospital supplies**
- **Can cause life-threatening opportunistic infections in the urinary and respiratory tracts of immunocompromised patients**
- **Difficult to treat due to resistance to various antimicrobial drugs**
Serratia marcescens
Enterobacter, Hafnia, and Citrobacter

- Found in soil, water, decaying vegetation, and sewage
- Reside in the digestive tracts of animals and humans
- All can be opportunistic pathogens
- Frequently involved in nosocomial infections of immunocompromised patients
- Difficult to treat due to resistance to various antimicrobial drugs
Enterobacter on MacConkey

Enterobacter on EMB
Include a number of opportunistic pathogens

**Proteus**

- Gram-negative, facultative anaerobe, urease positive
- *Proteus mirabilis* is the most common species associated with human disease
  - Can cause urinary tract infections in patients with long-term urinary catheters
  - Infection-induced kidney stones can develop
  - Resistant to many antimicrobial drugs
Proteus mirabilis is one of the common species of Enterobacteriaceae isolated in clinical laboratories.

It is well known as a pathogen in urinary tract infections and has been implicated in hospital outbreaks and cases of cross-infection.

The characteristic feature of Proteus in culture is “swarming”.

Methods to inhibit swarming

Diene’s phenomenon

Weil-Felix reaction: Proteus Ox19, Ox2, OxK & Rickettsia antibodies.
Morganella, Providencia, and Edwardsiella

- Cause nosocomial infections in immunocompromised patients
- Primarily involved in urinary tract infections