**Differentiation of gram-negative rods in general**

- Utilization of glucose
  
  ferment “F”, oxidize “O”, non-fermenter & oxidizer “N” or inactive “I”

- Cytochrome oxidase reaction (negative or positive)

- Growth on MacConkey agar (growth or no growth)

**Enterobacteriaceae Introduction**

- Most commonly encountered GNR’s in clinical specimens

- Widely dispersed in nature – soil, water, plants, normal flora of intestinal & genitourinary tracts of humans and animals

- Certain species are endemic to a particular hospital environment (i.e., *Enterobacter* sp., *Klebsiella* sp., *Serratia* sp.)

- May be considered a pathogen in virtually any infectious disease & potentially recovered from any clinical specimen
  
  - Colonization of skin and mucous membranes: hospitalized patients quickly (within days) become colonized with enterics endemic to hospital (may not be causing an infectious process but potential source for nosocomial infections)
  
  - Overt or primary pathogens (usually intestinal pathogens): always considered pathogenic or source of infection for others (i.e., *Shigella* sp., *Salmonella* sp., *Yersinia* sp., *E. coli* O157:H7)
  
  - Opportunistic pathogens (i.e., *Citrobacter* sp., *Enterobacter* sp., *Escherichia coli*, *Klebsiella* sp., *Morganella* sp., *Proteus* sp., *Providencia* sp., *Serratia* sp., etc.)
    
    - Immunosuppressed, debilitated
    - Can be passed from person to person
    - Endogenous strains (patient’s own bacterial strains) establish infections in a normally sterile body site
    - Nosocomial infections due to colonization or invasive procedure (catheterization, bronchoscopy, biopsies, etc.)

  - Endotoxic shock: potentially lethal manifestation of infection with gram-negative bacteria
    
    - Endotoxin – lipopolysaccharide contained within cell wall
    - Causes fever, leukopenia, capillary hemorrhage, hypotension, circulatory collapse
    - “Septic shock” due to gram-negative sepsis

  - Types of opportunistic infections: respiratory, urinary tract (UTI), septicemia, wounds, sterile body sites (pleural, peritoneal, etc.)
Isolation

- Growth on primary isolation media (BAP, CHOC) 24 hours of incubation at 35°C in ambient air or CO₂.
  Facultative anaerobes (can grow anaerobically)

- **In general**, colony morphology = large, gray, smooth; beta or nonhemolytic
  - *Klebsiella* sp. and *Enterobacter* sp. – can produce a mucoid colony due to capsule production
  - *Proteus* sp. – can produce a swarming colony on non-inhibitory medium

- Since many clinical specimens contain several species of bacteria, in addition to the primary non-selective media, both selective and differential media are routinely used to recover and isolate species of medical importance. (i.e., MAC, HE, SS, XLD, EMB Appendix A – p. 976 of text)

MacConkey (MAC) – selective/differential
- Lactose fermenter = pink colony
- Nonlactose fermenter = clear colony

Eosin-methylene blue (EMB) – selective/differential
- Lactose &/or sucrose fermenter = pink colony
- *Escherichia coli* = blue/black with metallic green sheen
- Lactose & sucrose nonfermenter = translucent (amber colored or colorless)

Salmonella-Shigella (SS) – selective/differential: used to select for *Salmonella* and most *Shigella* strains in stool specimen
- Lactose fermenter = pink/red colony
- Lactose nonfermenter = colorless colony
- H₂S producer = colony with black ppt.

Hektoen enteric (HE) – selective/differential, used to select for *Salmonella* and *Shigella* sp.
- Lactose &/or sucrose fermenter = pink/red colony
- Lactose & sucrose nonfermenter = colorless colony (sometimes described as green/blue green)
- H₂S producer = colony with black ppt.

Xylose lysine & desoxycholate (XLD) – selective/differential, used to select for *Salmonella* and *Shigella* sp. in stool specimen (contains lactose, sucrose, and xylose in a lower concentration)
- Excess carbohydrate fermenters regardless of lysine reaction = yellow (*E. coli*)
- Xylose fermenter only, lysine decarboxylase negative = yellow
- Xylose nonfermenter, lysine decarboxylase positive = red
- Lysine decarboxylase positive only = red
- Lactose, sucrose, xylose nonfermenter, lysine decarboxylase negative = colorless
- H₂S producer = colony with black ppt.
  - *Salmonella* sp. = red colony with black center
  - *Shigella* sp. = colorless colony

MacConkey Sorbitol – selective/differential, used to isolate *E. coli* O157:H7 in stool specimens
- Same components as MAC except D-sorbitol is substituted for lactose
- Sorbitol nonfermenter = clear colonies (i.e., possible *E. coli* O157:H7)
- Sorbitol fermenter = pink colonies (i.e., rules out *E. coli* O157:H7)
Cefsulodin-inrgasan-novobiocin or Yersinia selective agar – selective/differential: used to recover primarily \textit{Yersinia enterocolitica}
  
  Mannitol fermenters = pink (\textit{Yersinia} “bull’s-eyes” colonies w/pink center & clear periphery)
  Mannitol nonfermenter = clear

Gram-negative broth – selective/enrichment broth: used to enhance recovery of \textit{Salmonella} & \textit{Shigella}
from fecal specimens. At 6-8 hrs & 18-24 hours of incubation, inoculated broth should be subcultured
to selective/differential medium; use with latex agglutination detection of enteric pathogens

Phosphate buffered (or isotonic ) saline – utilized for cold enrichment to enhance recovery of \textit{Yersinia enterocolitica}
from stool specimens. Inoculated broth is subcultured to CIN or Yersinia selective agar after incubation at 4°C.

\textbf{Identification - Main Characteristics of all Enterobacteriaceae}

- \textbf{Glucose = “F”}
- \textbf{Oxidase = negative (except \textit{Plesiomonas})}: perform test on isolates from BAP or nutrient agar (agar
  must not have a pink or purple color)
- \textbf{Nitrate to nitrite = positive (reducers)} – rare strains negative
- Facultative anaerobes
- MacConkey agar = growth
  
  Pink colonies = lactose fermenter (LF, LFGNR)
  Clear colonies = lactose nonfermenter (NLF, NLFGNR)
- Motility – if motile have peritrichous (flagella around entire organism)
- Catalase positive (\textit{most gram-negative organisms are catalase positive so this test isn’t}
  routinely performed on gram-negatives as it doesn’t provide differential information for
  identification)

\textbf{Identification – Biochemical Tests}

\textbf{Incubation non-CO\textsubscript{2}, 18-24 hours unless otherwise noted}

\textbf{A. Carbohydrate Utilization}

1. Principle
   a. The action of many species on carbohydrate substrates results in acidification of the medium
   b. Detected by visually observing color change of pH indicator in the media

2. \textbf{Glucose Fermentation}
   a. Via the Embden Meyerhoff Pathway (EMP)
   b. Glucose --> pyruvic acid + lactic acid
   c. OF media
      
      Glucose fermenter – Glucose “F”:
      - oil overlayed well/tube = acid produced & open well/tube = acid produced
        or entire semi-solid agar deep = acid produced
      Glucose oxidizer – Glucose “O”
      - Oil overlayed well/tube = no acid produced & open well/tube = acid produced
        or upper 1/5 to 1/3 of tube = acid produced
      Glucose “inert, inactive, asaccharolytic” – Glucose “N” or Glucose “I”
      - Oil overlayed well/tube = no acid produced & open well/tube = no acid produced
        or upper 1/5 to 1/3 of tube alkaline due to peptone degradation or no color change
   d. \textit{Can also determine glucose fermentation using KIA, TSI agars}
3. **Lactose Fermentation (MacConkey, KIA, TSI agars)**
   a. Lactose is a disaccharide composed of glucose and galactose
   b. Two enzymes required for lactose to be utilized by bacteria
      - Beta-galactoside (lactose) permease
        o Allows penetration of lactose molecule into bacterial cell
      - Beta-galactosidase
        o Hydrolyzes beta-galactoside (lactose) once within the bacterial cell wall resulting in formation of glucose + galactose
        o Glucose is then degraded via EMP
   c. Lactose fermenters have both enzymes
   d. Non-lactose fermenters lack both enzymes and are incapable of producing acid from lactose
   e. Slow-lactose fermenters lack permease enzyme or have sluggish permease activity
      - Does possess beta-galactosidase, permitting rapid ID as a lactose fermenter (see ONPG Activity)

4. **Kligler’s Iron Agar (KIA) – glucose & lactose fermentation, H₂S production**
   a. Medium in a tube contains
      - Two sugars – lactose and glucose (concentration is 1/10 the lactose concentration allowing detection of glucose fermentation alone)
      - Phenol red indicator – indicates fermentation by turning from red to yellow
      - Ferrous sulfate – demonstrates H₂S production by turning black in butt of tube, H₂S production requires an acid environment
   b. Glucose fermentation results in acid production, changing indicator from red to yellow
      - Since slant portion of medium is under aerobic conditions, the acid in the slant becomes oxidized, reverting it to alkaline pH, changing indicator from yellow back to red
      - Since butt portion of medium is under anaerobic conditions, the acid present can not be oxidized, and thus, no reversion to alkaline pH take place (remains yellow)
   c. Lactose fermentation results in acid production, changing indicator from red to yellow
      - Since the concentration of lactose is 10 times greater than glucose, lactose fermentation will cause entire tube (slant and butt) to be yellow
   d. Interpretation of results after 24-hour incubation (slant/butt)
      - Red/Red (K/K) = No carbohydrate fermentation
        o I.e., *Pseudomonas aeruginosa*, other nonfermentative bacteria
      - Red/Yellow (K/A) = Only Glucose is fermented
        o I.e., *Shigella* species, other non-lactose fermenters
      - Red/Yellow with black in butt = Only Glucose is fermented with H₂S production
        o I.e., *Salmonella* species, *Citrobacter* species, some *Proteus* species, other non-lactose fermenting, H₂S producers
      - Yellow/Yellow (A/A) = Both glucose and lactose fermented
        o I.e., *Escherichia coli*, *Klebsiella-Enterobacter* group, other lactose fermenters
5. **Triple Sugar Iron Agar (TSI) glucose, sucrose & lactose fermentation, H₂S production**
   a. Same principle as KIA with the exception of the incorporation of another carbohydrate, sucrose, in the medium
   
   b. Glucose concentration is 1/10 that of lactose and sucrose
   
   c. If organism ferments lactose, it should also ferment sucrose, and thus allows for the more rapid detection of slow lactose fermenters
   
   d. Very useful to screen out *Salmonella* and *Shigella*, since neither (except for rare strains) utilizes lactose or sucrose

6. **Other Carbohydrates**
   a. Ability of organisms to utilize carbohydrates other than glucose, lactose and sucrose provides additional characteristics for identification of *Enterobacteriaceae*
   
   b. Examples include: mannitol, dulcitol, sorbitol, arabinose, raffinose, rhamnose and melibiose

**B. Ortho-nitro-phenyl beta-d-galactopyranoside (ONPG) Activity**

1. **Principle**
   
   Detects the enzyme beta-galactosidase
   
   - ONPG = compound similar to lactose but doesn’t require permease enzyme to enter cell
   - ONPG ----->H₂O and beta-galactosidase------> galactose + orthonitrophenol
     
   (colorless)                     (from organism)                                    (yellow)

2. Helpful in identifying slow lactose fermenters deficient in permease enzyme

3. Note - all lactose fermenters (with the permease enzyme) will be ONPG +

**C. Indole Test**

1. Conventional (incubation) method - principle
   
   - Tryptophan ----tryptophanase -------> indole + pyruvic acid + NH₃
     (amino acid)     (from organism)

   - Indole + p-dimethylaminobenzaldehyde ---> colored complex
     (yellow, clear)        (Ehrlich’s or Kovac’s Reagent)              (pink-red)

   Note
   
   - Ehrlich’s – more sensitive, must first add xylene to reaction tube
   - Kovac’s – less sensitive

2. Spot Indole Test (rapid test) – colony from 18-24 hour culture plate
   
   - Place 2-3 drops of spot indole rgt (para-dimethylaminocinnamaldehyde) on laboratory grade filter paper
   
   - Using a wooden stick or bacteriological loop, rub a portion of a colony from sheep blood agar onto a small area of the moistened filter paper. The growth medium must contain an adequate amount of tryptophan for optimal production of tryptophanase
   
   - Positive = Blue to blue green color development within 10 seconds
   
   - Not as sensitive as conventional indole reaction
D. Voges-Proskauer (VP) Test (detects specific pyruvic acid degradation pathway)

**Principle**
- Dextrose $\rightarrow$ pyruvic acid $\rightarrow$ acetyl-methyl carbinol (AMC or acetoin)

AMC $\rightarrow$ 40% KOH $\rightarrow$ diacetyl $\rightarrow$ alpha-naphtol $\rightarrow$ colored complex
(colorless) $\rightarrow$ (red)

E. Citrate Utilization

**Principle**
- Detects the organism’s ability to use citrate as its sole source of carbon
- Some bacteria can obtain energy in a manner other than carbohydrate fermentation
- Organisms utilizing citrate will grow on the medium and alkalinate it, changing the bromthymol blue indicator from green to blue

F. Urease Production

**Principle**
- Urea $\rightarrow$ CO$_2$ + H$_2$O + ammonia (ammonia carbonate)

(colorless yellow) $\rightarrow$ (pink-red color)

- Indicator is phenol red

G. Decarboxylation (or hydrolysis) of Lysine, Ornithine, and Arginine (formation of alkaline amines)

**Moeller Base – medium is acidic so enzymes are active**

1. Arginine Dihydrolase (ADH)
   - Arginine $\rightarrow$ citrulline
     (yellow) $\rightarrow$ (alkaline - purple)

2. Lysine Decarboxylase (LDC)
   - Lysine $\rightarrow$ cardaverine
     (yellow) $\rightarrow$ (alkaline - purple)

3. Ornithine Decarboxylase (ODC)
   - Ornithine $\rightarrow$ putrescine
     (yellow) $\rightarrow$ (alkaline - purple)

4. Indicator is bromcresol purple – as pH increases color change from yellow to purple.

5. Moeller based broths must be overlayed with sterile mineral oil
H. Deaminase Activity - Normally, if organism can deaminate amino acids, it can deaminate multiple amino acids...only perform one of the following

1. Phenylalanine Deaminase Production (PDA)
   a. Principle
      Phenylalanine -----phenylalanine deaminase-----> phenyl pyruvic acid
         (from organism)                          (colorless)
      phenyl pyruvic acid -----10% ferric chloride-----> colored complex (green)

2. Tryptophane Deaminase Production (TDA)
   a. Principle
      Tryptophane -----tryptophane deaminase-----> indole pyruvic acid
         (from organism)                          (colorless)
      indole pyruvic acid -----10% ferric chloride-----> colored complex (brownish-red)

3. Lysine Deaminase Production (Lysine Iron Agar – LIA) – detection of LDC or LDA enzymes & H₂S production
   a. Principle
      Lysine ------ ---lysine deaminase----------
         (from organism)
         
      burgundy/red wine color in slant with yellow butt

   Organisms:
   No production of deaminases or decarboxylases
   Or Production of decarboxylases
   Or Production of deaminases
   But not both

I. Lysine Iron Agar (LIA) - detection of LDC or LDA enzymes & H₂S production
   Principle – organism must ferment glucose to utilize this medium. The fermentation of glucose in the agar decreases the pH. If LDC or LDA enzyme is produced by the organism, it is able to decarboxylate or deaminate lysine. Production of H₂S produces a black precipitate (not as sensitive as KIA for H₂S production).

   Agar starts out purple/purple
   LDC + = purple/purple or K/K
   LDA + = red/yellow or red/A or R/A
   LDC & LDA (-) = purple/yellow or K/A (the only thing that happened is fermentation of glucose)
   H₂S + = H₂S written as part of tube interpretation (i.e., K/K H₂S = LDC + with H₂S production)

J. Hydrogen Sulfide Production (KIA, LIA, TSI, HE, SS, XLD)
   1. Principle
      a. Cysteine and/or thiosulfate ----enzymes-----> H₂S ----heavy metal salts----> precipitate
         (in medium)                          (from organism)                          (black)
K. Motility – Semisolid medium

Principle
a. Motile organisms are able to move throughout the semisolid medium, whereas nonmotile organisms grow just along the stab line

b. Motile Enterobacteriaceae (all genera except Klebsiella and Shigella) possess peritrichous flagella (however some strains of species that are usually motile may be non-motile)

c. Motile Pseudomonadaceae possess polar flagella (may not be detected by semisolid medium)

L. Gelatin

Principle
a. Detection of proteolytic enzyme, gelatinase, which breaks down the protein molecule gelatin
b. Various methods

Kohn charcoal gelatin: charcoal particles are held together with gelatin
    positive = charcoal particles disperse throughout tube/cupule
    negative = charcoal particles remain held together at bottom of tube/cupule

M. Nitrate reduction

Principle
a. Nitrate (NO$_3$) reduced to nitrite (NO$_2$) or reduced to gaseous end products such as (N$_2$↑)

b. Nitrate reagent: sulfanilic acid & N,N-dimethyl-1-naphthylamine

c. Nitrate reagent + NO$_2$ = red diazo dye

d. Zinc will reduce NO$_3$ to NO$_2$

1) Inoculate nitrate broth with organism, incubate overnight, add reagent, red color = NO$_3$ to NO$_2$ (Positive rxn)

2) Inoculate nitrate broth with organism, incubate, add rgt, no color, add zinc, red color developed = NO$_3$ not reduced (Negative rxn)

3) Inoculate nitrate broth with organism, incubate, add rgt, no color, add zinc, no color developed = NO$_3$ reduced to N$_2$↑ (Positive rxn)
N. Oxidase Test (rapid test)
1. Principle: The cytochromes are iron-containing hemoproteins that act as the last link in the chain of aerobic respiration by transferring electrons (hydrogen) to oxygen, with the formation of water. The cytochrome system is found in aerobic, microaerophilic and facultatively anaerobic organisms. In the oxidase test, a reagent dye substitutes for oxygen as an artificial electron acceptor. In the reduced state, the dye is colorless. In the presence of cytochrome oxidase and atmospheric oxygen the dye is oxidized thus forming a blue color within 10 seconds.

\[
\text{Tetramethyl-p-phenylenediamine dihydrochloride + O}_2 \rightarrow \text{indophenol blue & H}_2\text{O}
\]

2. Procedure
Drop reagent on filter paper and rub organism (<24 hours old from non-selective medium) into rgt with sterile stick or appropriate loop (platinum, plastic)
Can’t utilize stainless steel or nichrome inoculating loops as they cause false positives
Or
Touch an isolated colony to be tested with a sterile swab to obtain organism on the swab.
Place a free falling drop of oxidase reagent on the organism on the swab (do not touch dropper to the swab)

Blue color development 0-10 seconds = positive
No color development 0-10 seconds= negative

Enterobacteriaceae Antigens
A. “O” Antigen
1. Somatic antigen of the cell wall
2. Produced by all bacteria
3. Polysaccharide
4. Stimulates early antibody production

B. “K” Antigen
1. Envelop or capsular antigen surrounding the “O” antigen of the cell wall
2. Produced by some bacteria
3. Heat labile
4. “Vi” antigen = envelop or capsular antigen of Salmonella typhi

C. “H” Antigen
1. Flagellar antigen
2. Located in flagella of bacteria possessing flagella
3. Heat labile
4. Protein
5. Stimulates late antibody production
Organisms – *Enterobacteriaceae* Family

A. Genus Escherichia

1. *Escherichia coli*
   a. Characteristics
      - Most common facultative organism in stool
      - Includes the “inert group” which is nonmotile, can be biochemically mistaken for *Shigella* sp.
      - Colony morphology on EMB = green metallic sheen
      - Can be beta-hemolytic
      - Colonies on MAC = dark pink colonies with pink diffusing into agar around colony
   
   b. Disease states in addition to opportunistic infections
      - Nephropathogenic: # 1 etiologic agent of urinary tract infection (UTI)
      - Neonatal meningitis: 0-3 month age group
      - Enterohemorrhagic (EHEC) produces Shiga toxin/verotoxin: *E. coli* O157:H7 and others
        - Ingestion of undercooked hamburger, unpasteurized apple juice & milk, leaf lettuce
        - Bloody diarrhea
        - May progress to hemolytic uremic syndrome (HUS) especially in children
        - sorbitol negative (clear colonies on sorbitol MAC plate...SMAC)
      - Other causes Gastroenteritis – 4 other distinct syndromes caused by 4 other distinct syndromes – usually not diagnosed by culture
   
   c. Identification – Key biochemical reactions
      - MAC pink precipitate around individual colonies or green sheen on EMB
      - KIA = A/A with gas (H₂S negative) = lactose fermenter
      - Indole = positive
      - Citrate = negative

      **Abbreviated Identification** (can’t perform on blood, stool or GI isolates)
      - Gram-negative rod, oxidase negative, spot indole positive, beta-hemolytic, non-swarming, lactose-fermenting (MAC or EMB)
      - Gram-negative rod, oxidase negative, spot indole-positive, non-hemolytic on blood agar and lactose-positive (MAC or EMB), PYR negative
B. Genus *Edwardsiella* – opportunistic pathogen
   1. *Edwardsiella tarda* is the only human pathogen; septicemia & wound infections
      a. Identification – Key biochemical reactions
         - KIA = K/A H\(_2\)S+ 
         - LDC = positive 
         - Indole = positive 
         - Citrate = negative

C. Genus *Shigella* - overt or primary pathogen, transmission only human to human
   1. *Shigella dysenteriae* subgroup A (most severe)
      Disease state
      - Agent of bacillary dysentery or shigellosis
      - Produces an endotoxin and an exotoxin (shiga toxin: enterotoxin & neurotoxin) responsible for pathogenicity by acting on intestinal walls and possibly the brain leading to coma & death
      - Spreads rapidly in conditions of overcrowding or poor sanitation (rendered entire armies temporarily unfit for combat)
   2. *Shigella flexneri* subgroup B
      - Found in the U.S.
   3. *Shigella boydii* - Subgroup C
      - Found in the U.S.
   4. *Shigella sonnei* - Subgroup D (least severe)
      - Most predominant species in the U.S.
   5. Disease state
      - Unlike Salmonella, Shigella usually confined to GI tract
      - Septicemia rarely occurs
      - Disease usually self-limiting
   6. Identification
      a. Key biochemical reactions (very inert):

<table>
<thead>
<tr>
<th>Biochemical tests</th>
<th><em>Shigella</em> species subgroups A, B, C</th>
<th><em>Shigella sonnei</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>KIA</td>
<td>K/A (H(_2)S negative)</td>
<td>K/A (H(_2)S negative)</td>
</tr>
<tr>
<td>ONPG</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>LDC</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>ODC</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>Motility</td>
<td>Nonmotile</td>
<td>Nonmotile</td>
</tr>
</tbody>
</table>

b. All *Shigella* isolates must be serotyped.
   - Latex agglutination test for “O” somatic antigen detection
   - Screen initially using polyvalent typing antisera (contains groups A, B, C, D)
   - Identify species using monovalent typing antisera
   - NOTE: some *Shigella* species contain the “K” capsular antigen that may mask the “O” somatic antigen, resulting in no agglutination. Test suspension should be boiled at 100\(^\circ\)C for 30-60 minutes and retested (boiling destroys heat labile "K" antigen).
D. Genus Citrobacter – opportunistic pathogen
   a. Key biochemical reactions:

<table>
<thead>
<tr>
<th>Biochemical tests</th>
<th><em>Citrobacter freundii</em></th>
<th><em>Citrobacter koseri</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>KIA</td>
<td>K/A or A/A (75%)</td>
<td>K/A or A/A (50%)</td>
</tr>
<tr>
<td></td>
<td>(78% H$_2$S positive)</td>
<td>(H$_2$S negative)</td>
</tr>
<tr>
<td>LDC</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>ODC</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>Indole</td>
<td>Negative (60%)</td>
<td>Positive</td>
</tr>
<tr>
<td>Citrate</td>
<td>Positive (78%)</td>
<td>Positive</td>
</tr>
</tbody>
</table>

E. Genus Salmonella – overt or primary pathogen
   - Widely distributed throughout nature – humans and animals
   - Two species: *S. enterica* and *S. bongori* (rarely isolated, seen in Africa)
   - Most complex group with more than 2200 serotypes
   - Salmonella infections result in varying degrees of gastroenteritis
     - Most commonly caused by ingestion of contaminated food (poultry, eggs, milk, peanuts), water, handling of pets
     - Carriers (those individuals with previous infection) harbor the organism asymptotically in the gall bladder

1. *Salmonella* serotype Typhi – typhoid fever, only infects humans
   - Most pathogenic of Salmonella species
     - Early period of fever and constipation, rose spots on trunk, Peyer’s patches
     - Later period of severe bloody diarrhea
     - **Blood cultures positive initially, followed by positive stool and urine cultures**

2. *Salmonella* serotype Paratyphi – paratyphoid fever, milder disease than typhoid fever

   - Mild to fulminant diarrhea accompanied by low-grade fever, nausea, and diarrhea
   - No invasion of blood stream

   a. Identification - Key biochemical reactions:

<table>
<thead>
<tr>
<th>Biochemical tests</th>
<th><em>Salmonella</em> sp. (enterica)</th>
<th><em>Salmonella Typhi</em></th>
<th><em>Salmonella Paratyphi</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>KIA</td>
<td>K/A with H$_2$S</td>
<td>K/A trace amts H$_2$S</td>
<td>K/A (only 10% produce H$_2$S)</td>
</tr>
<tr>
<td>LDC</td>
<td>Positive</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>ODC</td>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>Indole</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Citrate</td>
<td>Positive (95%)</td>
<td>Negative</td>
<td>Negative</td>
</tr>
</tbody>
</table>
b. All *Salmonella* isolates must be serotyped
   - Latex agglutination test for “O” somatic antigen detection
   - Screen initially using 2 polyvalent typing antisera
     o Polyvalent antisera containing A-E and Vi
     o Polyvalent antisera containing serogroups F-I
   - Identify species using monovalent typing antisera
   - NOTE: some *Salmonella* species contain the “K” capsular antigen that may mask the “O” somatic antigen, resulting in no agglutination with monovalent antisera. Test suspension should be boiled at 100°C for 30-60 minutes and retested (boiling destroys heat labile “K” antigen).

F. **Genus Klebsiella**
   1. *Klebsiella pneumoniae*
      a. Disease states
         - opportunistic
         - destructive pneumonia with necrosis and hemorrhage (sputum red or “currant jelly-like”)
   2. *Klebsiella oxytoca* - opportunistic
   3. Identification
      a. Key biochemical reactions:
         
         | Biochemical tests | *Klebsiella pneumoniae* | *Klebsiella oxytoca* |
         |-------------------|-------------------------|----------------------|
         | KIA               | A/A with gas (H₂S negative) | A/A with gas (H₂S negative) |
         | LDC               | Positive                 | Positive              |
         | Indole            | Negative                 | Positive              |
         | Citrate           | Positive                 | Positive              |
         | Motility          | Nonmotile                | Nonmotile             |
         | VP                | Positive                 | Positive              |

      b. Colony morphology can be very mucoid due to presence of a capsule
      c. MAC: colonies light pink, mucoid
      d. Resistant to ampicillin

G. **Genus Enterobacter** - opportunistic
   Identification - Key biochemical reactions:

<table>
<thead>
<tr>
<th>Biochemical tests</th>
<th><em>Enterobacter cloacae</em></th>
<th><em>Enterobacter aerogenes</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>KIA</td>
<td>A/A with gas (H₂S negative)</td>
<td>A/A with gas (H₂S negative)</td>
</tr>
<tr>
<td>LDC</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>ODC</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Indole</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Citrate</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>VP</td>
<td>Positive</td>
<td>Positive</td>
</tr>
</tbody>
</table>

b. Colony morphology can be very mucoid due to presence of a capsule
   c. MAC: colonies light pink, mucoid
   d. Resistant to ampicillin & first generation cephalosporins (cefazolin, cephalothin)
H. Genus *Serratia* - opportunistic
   1. *Serratia marcescens*
      - Often colonizes hospitals
      - Some strains are chromogenic producing a red pigment at room temperature
      a. Identification – Key biochemical reactions
         - KIA = K/A (H₂S negative)
         - LDC = positive
         - ONPG = positive
         - ODC = positive
         - Indole = negative
         - Citrate = positive
         - VP = positive
      b. Resistant to ampicillin & first generation cephalosporins (cefazolin, cephalothin)

I. Genus *Proteus* – opportunistic
   - UTI - Strong hydrolysis of urea = ↑ pH of urine in bladder = ppt. cations (i.e., Ca⁺⁺) to form renal calculi/kidney stones
   a. Identification - Key biochemical reactions:
      | Biochemical tests | *Proteus mirabilis* | *Proteus vulgaris* |
      |-------------------|--------------------|-------------------|
      | KIA               | K/A with H₂S       | K/A with H₂S      |
      | LDA               | Positive           | Positive          |
      | ODC               | Positive           | Negative          |
      | Indole            | Negative           | Positive          |
      | Urea              | Strongly Positive  | Strongly Positive |
   b. Swarming motility: “swarming” colony morphology on non-inhibitory media (BAP, Choc, etc.) – wavelike spreading across the entire surface of the agar
      - Swarming should be inhibited on MAC, EMB
   c. Abbreviated identification – swarming on sheep blood agar:
      - spot indole negative, ampicillin susceptible = *Proteus mirabilis*
      - spot indole negative, ampicillin resistant:
        - ornithine positive (maltose negative) = *Proteus mirabilis*
        - ornithine negative (maltose positive) = *Proteus penneri*
      - spot indole positive = *Proteus vulgaris*
   d. Resistant to tetracycline

J. Genus *Providencia* - opportunistic
   a. Identification - Key biochemical reactions:
      | Biochemical tests | *Providencia rettgeri* | *Providencia species* |
      |-------------------|------------------------|-----------------------|
      | KIA               | K/A (H₂S negative)     | K/A (H₂S negative)    |
      | LDA               | Positive               | Positive              |
      | ODC               | Negative               | Negative              |
      | Indole            | Positive               | Positive              |
      | Urea              | Positive               | Negative              |

K. Genus *Morganella* - opportunistic
   1. *Morganella morganii*
      a. Identification – Key biochemical reactions
         - KIA = K/A (H₂S negative)
         - LDA = positive (very weak, delayed reaction at 24 hours)
         - ODC = positive
         - Indole = positive
         - Urea = positive
L. **Genus Yersinia** – overt or primary pathogen

1. *Yersinia pestis* – plague (3 forms)
   - Bubonic – painful “bubo” (inflammatory swelling of the lymph node)
   - Pneumonic (can be transmitted person to person)
   - Septicemic
     - Vector is rat flees, carried by rodents such as rats, prairie dog, certain types of squirrels
     - Man is accidental host (transmitted by infected rat flees)

2. *Yersinia enterocolitica*
   a. Disease states
      - May cause gastroenteritis (more common in children), bacteremia, septicemia

3. Identification
   a. Key biochemical reactions:
      - *Yersinia* sp. react best at room temperature. If incubated at 35-37°C, reactions will be delayed.
      - Due to delayed glucose fermentation:
        - KIA may appear orange at the bottom
        - LIA may appear grayish or dirty yellow

<table>
<thead>
<tr>
<th>Biochemical tests</th>
<th><em>Yersinia pestis</em></th>
<th><em>Yersinia enterocolitica</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>KIA</td>
<td>K/orange (H₂S negative)</td>
<td>K/orange (H₂S negative)</td>
</tr>
<tr>
<td>ONPG</td>
<td>Positive (slow)</td>
<td>Positive (slow)</td>
</tr>
<tr>
<td>LDC</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>LDA</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>ODC</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>Urea</td>
<td>Negative</td>
<td>Positive (75%) - rapid</td>
</tr>
<tr>
<td>Motility</td>
<td>Motile at room temp Nonmotile at 37°C</td>
<td>Motile at room temp Nonmotile at 37°C</td>
</tr>
</tbody>
</table>

b. Gram stain
   - Large, gram negative coccobacilli, with bipolar staining (‘safety pin’)

c. Yersinia Selective Agar = CIN agar (Cefsulodin, Irgason, Novobiocin)
   - Selective for *Y. enterocolitica*
   - Differential reaction for mannitol fermentation – colonies with deep red centers with sharp borders, surrounded by an outer translucent zone (Bull’s eye)

d. *Yersinia* sp. grow best at 25-30°C, cultures are incubated at room temperature

**Identification clues:**
- Lactose “F” think *E. coli, Klebsiella, Enterobacter* and possibly *Citrobacter*
- Lactose “F” w/mucoid colony think *Klebsiella* and *Enterobacter*
- H₂S + think *Proteus* sp., *Salmonella* sp., *Citrobacter freundii* and possibly *Edwardsiella tarda*
- Non-motile think *Klebsiella* and *Shigella*
- Deamination + think *Proteus, Providencia, Morganella*
- Voges-Proskauer (VP) + think *Klebsiella, Enterobacter, Serratia*