Family: Enterobacteriaceae

Lactose non-fermenter Enterobacteriaceae

Genus: *Proteus*

- ***Proteus vulgaris***
- ***P. mirabilis***
- ***P. penneri***

General Characteristics ***:*** gram-negative rods, pleomorphic, lactose non-fermenter, non capsulated, *Proteus* species move very actively by means of peritrichous flagella, resulting in "swarming" on solid media unless the swarming is inhibited by chemicals, eg, phenylethyl alcohol or CLED (cystine-lactose-electrolyte-deficient) medium, non capsulated, *Proteus* species are urease-positive . *Proteus* species produce urease, resulting in rapid hydrolysis of urea with liberation of ammonia. Thus, in urinary tract infections with *proteus*, the urine becomes alkaline, promoting stone formation and making acidification virtually impossible. The rapid motility of *proteus* may contribute to its invasion of the urinary tract. Strains of proteus vary greatly in antibiotic sensitivity. *P mirabilis* is often inhibited by penicillins; the most active antibiotics for other members of the group are aminoglycosides and cephalosporins.

Proteus species produce infections in humans only when the bacteria leave the intestinal tract.
They are cause:

1. urinary tract infections
2. bacteremia
3. pneumonia
4. and focal lesions in debilitated patients or those receiving intravenous infusions.
5. ***P. mirabilis*** is the major cause of human urinary tract infections and occasionally other infections.
6. ***P. vulgaris*** is important nosocomial pathogens.

Specimens: Specimens included urine, blood, pus, spinal fluid, sputum, or other material, as indicated by the localization of the disease process. Specimens are plated on both blood agar and differential media.

Laboratory diagnostic tests:

1. **Gram stain:** gram-negative rods, pleomorphic.
2. **Inoculation on selective and differential media e.g. MacConkey agar (Lactose non-fermenter)**
3. **Blood agar: to test swarming and haemolysis.**
4. **TSI.**
5. **IMViC test.**
6. **Urease test (positive): members of the genus *Proteus* can be distinguished from other enteric non lactose-fermenting bacteria (*Salmonella, Shigella*) by their fast urease activity.**
7. **Gelatin liquefaction tset.**
8. **Phenylalanine Deamination test.**
9. **Maltose and glucose fermentation.**

Gelatin liquefaction test: When boiled in water, the connective tissue collagen (which is stringy, insoluble, and indigestible) changes into elatine, a soluble mixture of polypeptides. Certain bacteria are able to hydrolyze elatine by secreting a proteolytic enzyme called gelatinase. The resulting amino acids can then be used as nutrients by the bacteria. The ability of some bacteria to digest elatine is an important characteristic in their differentiation Gelatin hydrolysis can also be used to assess the pathogenicity of certain bacteria. The production of gelatinase can often be correlated with the ability of a bacterium to break down tissue collagen and spread throughout the body of a host.

Gelatin liquefaction (the formation of a liquid) can be tested by:

1. Stabbing nutrient elatine deep tubes.
2. Following incubation, the cultures are placed in a refrigerator or ice bath at 4°C until the bottom resolidifies.
3. If elatine has been hydrolyzed, the medium will remain liquid after refrigeration. If elatine has not been hydrolyzed, the medium will resolidify during the time it is in the refrigerator.

Nutrient elatine may require up to a 14-day incubation period for positive results.

Phenylalanine Deamination test: Phenylalanine deaminase catalyzes the removal of the amino group (NH3+) from phenylalanine. This test can be used to differentiate among enteric bacteria such as *E. coli* (-) and *P. vulgaris.*(+) *P. vulgaris* produces the enzyme phenylalanine deaminase, which deaminates phenylalanine, producing phenylpyruvic acid. When ferric chloride is added to the medium, it reacts with phenylpyruvic acid, forming a green compound (figure 1).

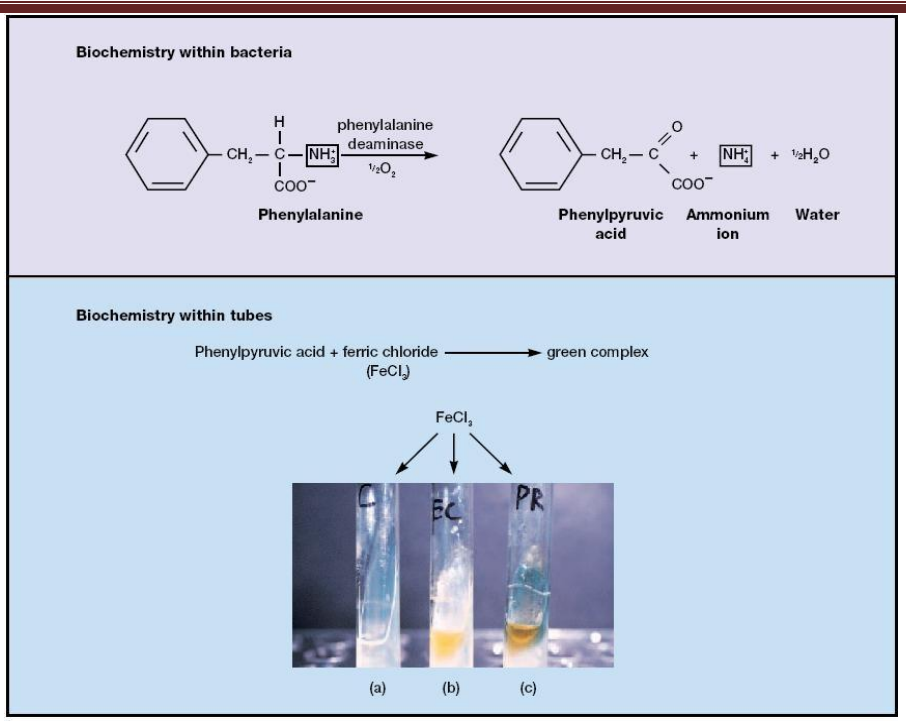
Medium: phenylalanine deaminase agar slants

Substrate: phenylalanine

pH Indicator: bromothymol blue

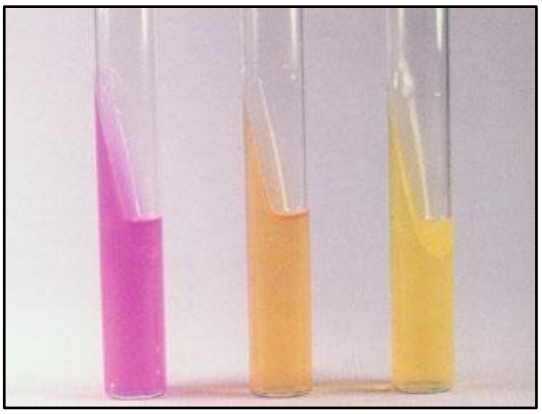
Reagent: ferric chloride (10% $FeCl_3$)

Test	*P. vulgaris*	*P. mirabilis*
Urease	**+**	**+**
TSI	**A/A + +** **K/A + +**	**A/A + +** **K/A + +**
MacConkey agar	**L.N.F.**	**L.N.F.**
Indol	**+**	**-**
MR	**+**	**+**
VP	**-**	**-**
Citrate	**+**	**+**
Gelatin liquefaction	**+**	**+**
Phenylalanine deaminase	**+**	**+**
Maltose	**+**	**-**
Glucose	**+**	**+**
Motility	**+**	**+**
Blood agar	**Swarming, haemolysis**	**Swarming, haemolysis**



Phenylalanine Deamination. (a) Uninoculated control. (b) Phenylalanine negative. (c) Phenylalanine positive.

Gelatin liquefaction tset



+ control -

Urease test

Proteus spp. on MacConkey agar (Lactose non-fermenter)

Proteus spp. on Blood agar (swarming)

Family: Enterobacteriaceae

Lactose non-fermenter Enterobacteriaceae

Genus: *Salmonellae*

***Salmonella enterica* subspecies *enterica* serotype Typhimurium (*Salmonella* Typhimurium)**
***Salmonella* Typhi**
***Salmonella* Paratyphi A**
***Salmonella* Paratyphi B**
***Salmonella* Choleraesuis**
***Salmonella* Enteritidis**

General Characteristics **:** gram-negative rods, lactose non-fermenter, non capsulated, *Salmonellae* vary in length. Most isolates are motile with peritrichous flagella. *Salmonellae* grow readily on simple media, but they almost never ferment lactose or sucrose. They form acid and sometimes gas from glucose and mannose. They usually produce H_2S. urease negative. They survive freezing in water for long periods. *Salmonellae* are resistant to certain chemicals (eg, brilliant green, sodium tetrathionate, sodium deoxycholate) that inhibit other enteric bacteria; such compounds are therefore useful for inclusion in media to isolate *salmonellae* from feces.

Salmonellae are often pathogenic for humans or animals when acquired by the oral route. They are transmitted from animals and animal products to humans, where ***they cause***:

1. **The "Enteric Fevers" (Typhoid Fever):** Four serotypes of *salmonellae* that cause enteric fever can be identified in the clinical laboratory by biochemical and serologic tests. They are as follows: *Salmonella* Paratyphi A, *Salmonella* Paratyphi B, *Salmonella* Choleraesuis, and *Salmonella* Typhi.
2. **Bacteremia with Focal Lesions:** This is associated commonly with *S choleraesuis* but may be caused by any salmonella serotype.
3. **Enterocolitis.**

Specimens: included faeces (stool), urine, blood.

Laboratory diagnostic tests:

1. **Bacteriologic Methods for Isolation of *Salmonellae***

   Selective and Differential Medium Cultures: EMB, **MacConkey's** and **deoxycholate medium** permits rapid detection of lactose non-fermenters (not only *salmonellae* and *shigellae* but also *proteus, serratia, pseudomonas*, etc). Also the specimen is plated on **salmonella-shigella agar (S.S agar) : *Selective*** because it contains bile salts for G+ve bacteria inhibition and G-ve other than Enterobacteriaceae and brilliant green for the inhibition of coliform and other Enterobacteriaceae. ***Differential*** because it contains Na-thiosulfate, ferric citrate for H_2S production and neutral red as indicator (dose not need autoclaving because it includes inhibitors)**, Hektoen enteric agar, XLD (xylose lysine deoxycholate)** contain **(**xylose, lactose, sucrose and phenol red as indicator), **DCA** (**deoxycholate-citrate agar)** which favor growth of *salmonellae* and *shigellae* over othe

Enterobacteriaceae. **Bismuth sulfite medium** permits rapid detection of *salmonellae* which form black colonies because of H_2S production.

Enrichment Cultures: The specimen (usually stool) also is put into **selenite F** or **tetrathionate broth**, both of which inhibit replication of normal intestinal bacteria and permit multiplication of *salmonellae*. After incubation for 1–2 days, this is plated on differential and selective media.

2. **Gram stain:** gram-negative rods
3. **TSI.**
4. **IMViC test.**
5. **Urease test**
6. **Mannitol and glucose fermentation**
7. **Serologic Methods**

Agglutination Test: In this test, known sera (commercial kits) and unknown culture are mixed on a slide. Clumping, when it occurs, can be observed within a few minutes. This test is particularly useful for rapid identification of cultures.

Tube Dilution Agglutination Test (Widal Test): Serum agglutinins rise sharply during the second and third weeks of *Salmonella* Typhi infection. The Widal test to detect these antibodies against the O and H antigens has been in use for decades. Serial dilutions of unknown sera are tested against known antigens from representative *salmonellae*. A titer against the O antigen of > 1:320 and against the H antigen of > 1:640 is considered positive. High titer of antibody to the Vi antigen occurs in some carriers.

Test	*S.* Typhi	*S.* Paratyphi A	*S.* Paratyphi B	*S.* Typhimurium
TSI	**K/A + +**	**K/A + +**	**K/A + +**	**K/A + +**
MacConkey agar	**L.N.F.**	**L.N.F.**	**L.N.F.**	**L.N.F.**
Indol	**-**	**-**	**-**	**-**
MR	**+**	**+**	**+**	**+**
VP	**-**	**-**	**-**	**-**
Citrate	**-**	**+**	**+**	**+**
Glucose	**+ no gas**	**+ no gas**	**+ no gas**	**+ no gas**
Mannitol	**+ no gas**	**+ with gas**	**+ with gas**	**+ with gas**
Motility	**+**	**+**	**+**	**+**
Urease	**-**	**-**	**-**	**-**
S.S. agar	**L.N.F. (colorness) with H_2S**	**L.N.F. (colorness) with H_2S**	**L.N.F. (colorness) with H_2S**	**L.N.F. (colorness) with H_2S**

Genus: *Shigellae*

S. dysenteriae
S. flexneri
S. boydii
S. sonnei

General Characteristics : *Shigellae* are slender gram-negative rods; coccobacillary forms occur in young cultures, non-motile, do not produce H_2S, lactose non-fermenter, non capsulated. Facultative anaerobes but grow best aerobically. Convex, circular, transparent colonies with intact edges reach a diameter of about 2 mm in 24 hours. All shigellae ferment glucose, with the exception of *Shigella sonnei. Shigellae* form acid from carbohydrates but rarely produce gas. They may also be divided into those that ferment mannitol and those that do not. Urease negative. The natural habitat of *shigellae* is limited to the intestinal tracts of humans and other primates. ***They cause:***

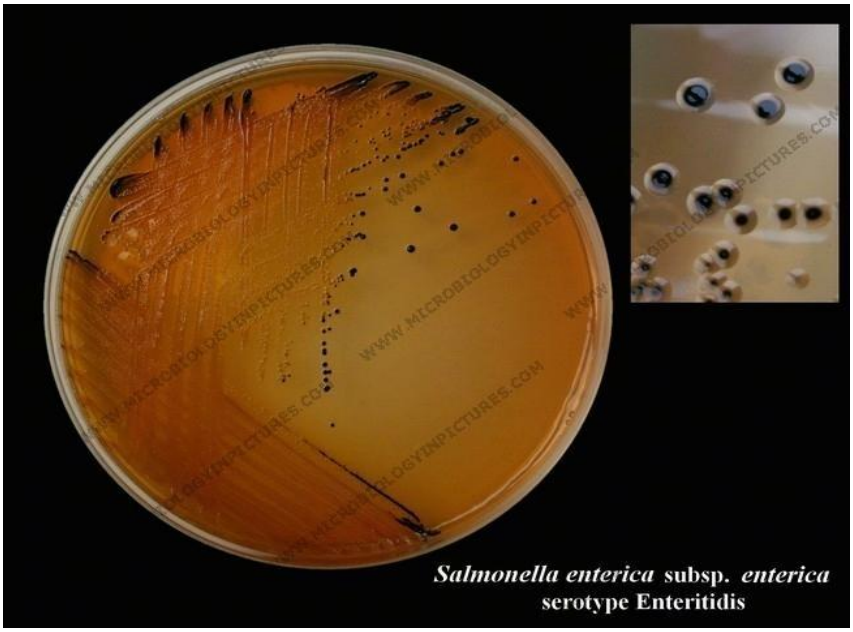
Bacillary dysentery.
Shigella infections are almost always limited to the gastrointestinal tract; bloodstream invasion is quite rare.

Specimens: included fresh stool, mucus flecks, and rectal swabs for culture. Large numbers of fecal leukocytes and some red blood cells often are seen microscopically.

Laboratory diagnostic tests:

1. **Selective and Differential Medium Cultures:** (eg, MacConkey's or EMB agar, Hektoen enteric agar or salmonella-shigella agar, XLD, DCA)
2. **Gram stain:** gram-negative rods
3. **TSI.**
4. **IMViC test.**
5. **Urease test**
6. **Motility test (non-motile).**

Test	*S.dysenteriae*	*S. flexneri*	*S. sonnei*	*S. boydii*
TSI	**K/A - -**	**K/A - -**	**K/A - -**	**K/A - -**
MacConkey agar	**L.N.F.**	**L.N.F.**	**L.N.F.**	**L.N.F.**
Indol	**+/-**	**+/-**	**-**	**+/-**
MR	**+**	**+**	**+**	**+**
VP	**-**	**-**	**-**	**-**
Citrate	**-**	**-**	**-**	**-**
Glucose	**+ no gas**	**+ no gas**	**-**	**+ no gas**
Mannitol	**-**	**+ no gas**	**+ no gas**	**+ no gas**
Motility	**-**	**-**	**-**	**-**
Urease	**-**	**-**	**-**	**-**
S.S. agar	**L.N.F. transparent**	**L.N.F. transparent**	**L.N.F. transparent**	**L.N.F. transparent**



***Salmonella enterica* on Salmonella-Shigella agar (S.S. agar)**

Salmonella-Shigella agar (S.S. agar)