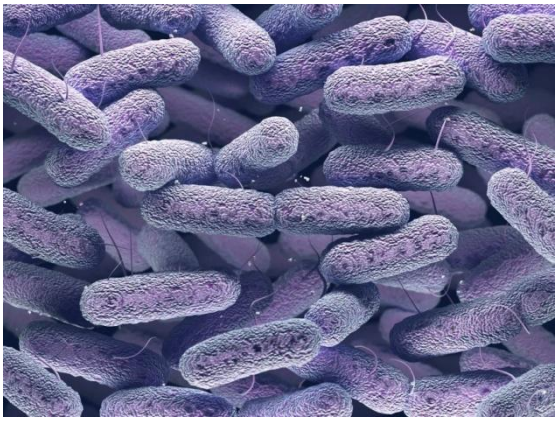
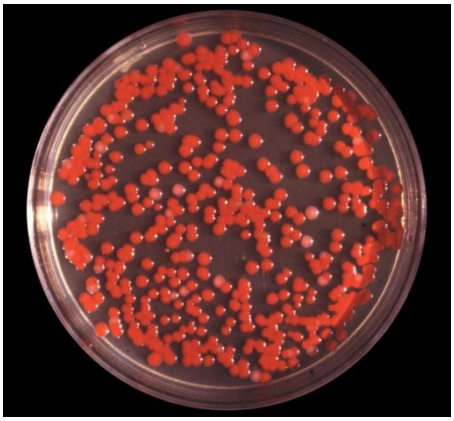
Pathogenic Bacteria

4th Level

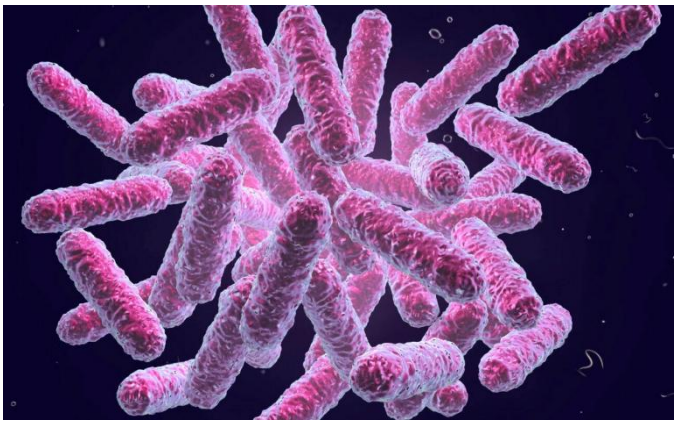

TOPICS	
Lab 1	*Escherichia coli, Klebsiella pneumonia, Enterobacter, Serratia*
Lab 2	*Proteus, Providencia rettgri, Morganella morganii*
Lab 3	*Salmonella, Shigella*
Lab 4	*Campylobacter, Helicobacter pylori, Haemophilus*
Lab 5	*Pseudomonas, Acinteobacter*
Lab 6	*Vibrio cholera, Vibrio parahaemolyticus*
Lab 7	*Staphylococcus aureus, Staphylococcus epidermidis.*
Lab 8	*Streptococcus* spp.
Lab 9	*Clostridium* spp.

First Exam	
Second Exam	

By: Assis. Lec. Ammar B. Al-Asadi

Family: Enterobacteriacea

Genus:



1. *Escherichia coli*
2. *Klebsiella pneumonia*
   K. oxytoca
   K. mobilis
   K. ornitholytica
3. *Enterobacter*
4. *Serratia*

General characteristics:

G-ve bacilli or coccobacilli, catalase +ve, oxidase –ve, capsulated or non-capsulated, motile or non-motile, non-spore former, aerobic or facultative anaerobic, pathogenic for human, animal and plants, intestinal parasite, normal flora, reduce nitrate to nitrite.

Classification:

1. Serological classification upon antigens (O-Ag, H-Ag, K-Ag).
2. Biochemical classification and sugar fermentation.
3. DNA-DNA hybridization / G:C ratio.

1- Escherichia coli.

G-ve coccobacilli or bacilli, non-spore former, motile, facultative anaerobes. Can cause:

1- Infants epidemic diarrhea, indicate fecal contamination.
2- Urinary tract infection (UTI).
3- Wound infection.
4- Bloody diarrhea.

2- Klebsiella:

G-ve bacilli, capsulated, the polysaccharide is very thick (show mucoid appearance of the colonies), virulent, resistant to phagocytosis, produce enterotoxin cause diarrhea also cause respiratory tract infection and septicemia as well as pneumoniae.

3- Serratia:

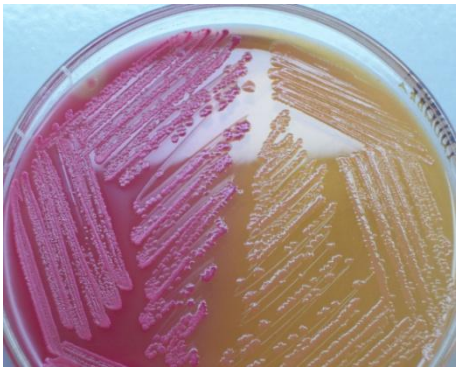
Normally non-pathogenic, opportunistic.

4- Enterobacter:

Mostly are non-pathogenic, they are very similar to *Klebsiella* in biochemical reactions but different in motility, *Klebsiella* is non-motile while *Enterobacter* is motile, it may cause UTI.

Lab diagnostic tests:

1- **Gram stain**: G-ve bacilli or coccobacilli.
2- **MacConkey agar**: selective or differential.



Selective:

- Crystal violet (inhibits G+ve)
- Bile salts (inhibits G-ve other than enteric bacteria.

Differential:

Differentiated between lactose fermenter (pink colony) and non lactose fermenter (pale colony).
Indicator: neutral red

- Yellow in alkaline pH.
- Pink in acid pH.

3- Eosin Methylene Blue (EMB).

Selective for Enterobacteriace (contain methylene blue inhibits G+ve).

Differential for *E. coli* (green metallic sheen).

Lactose fermentation ⟶ Alcohol + Eosin ⟶ green metallic sheen

4- Triple Sugar Iron Agar (TSI):

Contain glucose 1%, sucrose and lactose 10%, used for sugar fermentation, H_2S production.

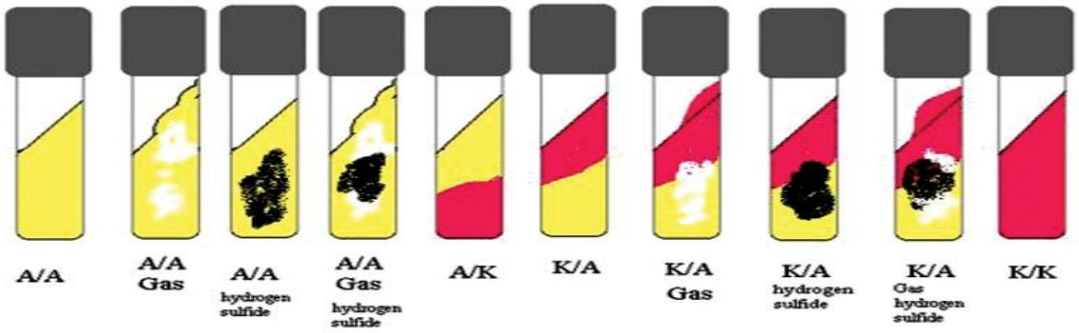
Indicator: phenol red.

- Yellow in acid pH.
- Pink in alkaline pH.

Gas ⟶ bubbles if fermentation occur (CO_2).

Na – thiosulfate + H_2 ⟶ H_2S

$H_2S + FeSO_4$ ⟶ FeS ↓ black precipitate



5- IMViC test (Indol, Methyl red, Voges-Proskauer, Citrate utilization).

- **Indol test:** deamination of tryptophane to pyruvic acid.

  Medium: peptone water.

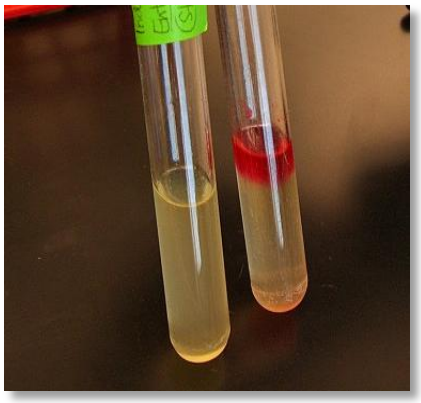
  Substrate: tryptophane.

  Indicator: Kovac's or Erlich reagaent

  (paradimethyl amino benzaldehyde)

  (+): red violet ring.

  (-): no change.



- **Methyl red, Voges-Proskauer:**

  Methyl red (MR): glucose full fermentation.

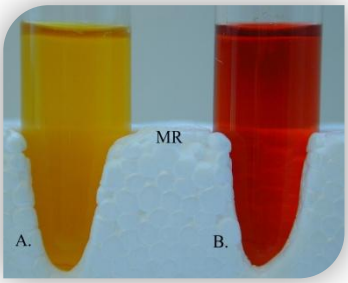
  Voges-Proskauer (VP): glucose partial fermentation.

Medium: MRVP

Substrate: glucose.

Glucose full fermentation (pyruvic acid + lactic acid + formic acid + succinic acid + acetic acid).

Indicator: MR 2 drops + 0.5 ml culture.



Red: pH< 5 (full fermentation).

Yellow: pH > 5.8

Glucose + H_2O → { lactic acid, acetic acid, formic acid } → $CO_2 + H_2$ → methyl red indicator (red color)

Glucose partial fermentation (acetyl methyl carbinol + acetone) pH 4.4

Indicator: 40% KOH (3 drops) + - nephthol (6 drops) to 1 ml culture

Glucose + $^1/_2\ O_2$ → acetic acid → $CO_2 + H_2$ + 2,3 butane diol + acetyl methyl carcinol $\xrightarrow{\text{indicator}}$ pink-red color (turbid)

- **Citrate utilization:**

Ability to utilize Na-citrate as the only carbon source in the medium.

Indicator: bromothymol blue, if utilized, pH increases (basal alkaline) the color change to blue.

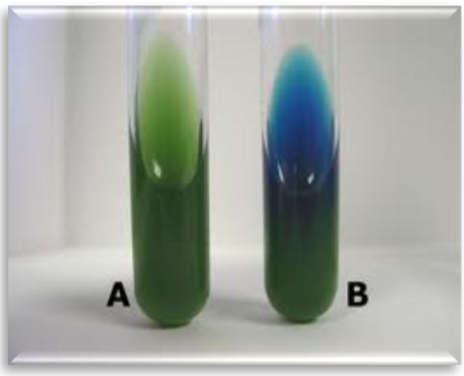
The bacteria contain a transport system which is begin with utilizing the citrate molecule by citrase enzyme (citrate lyase enzyme) and then make it possible to enter the bacterial cell wall by permease enzyme.

Citric acid $\xrightarrow{\text{urease}}$ oxaloacetic acid + acetic acid → pyruvid acid + excess in Na ions + excess of CO_2

Medium: Simmon citrate.

Substrate: citrate.

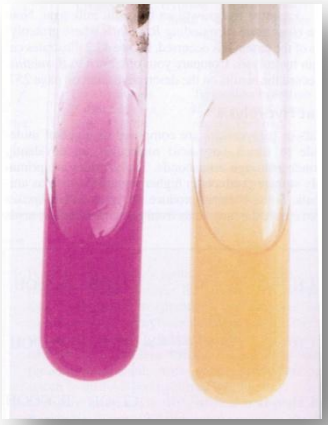
Indicator: bromothymol blue.



6- **Motility test:** stab method in semisolid media.

7- Urease test:

Production of ammonia from utilized urea by urease enzyme which increase the pH and convert the color from yellow to pink.



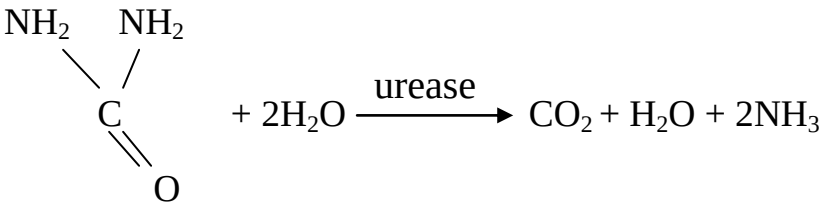
Medium: urea agar.

Substrate: urea.

Indicator: phenol red (pH: 6.8-8)

(+): pink in alkaline.

(-): yellow in acid.



8- **Sensitivity test:**

Medium: Muller-Hinton Agar.

Sensitive to aminoglycosides, cephalosporins, chloramphenicol, piperacillin.

Test	*E. coli*	*K. pneumoniae*	*Enterobacter*
Indol	+	-	-
MR	+	-	-
VP	-	+	+
Citrate	-	+	+
Urease	-	+	-
Motility	+	-	+
TSI	A/A + -	A/A + -	A/A + -
MacConkey agar	Pink colony, smooth, tough colony	Larger than *E. coli*, pink mucoid colony	Pink colony
EMB	Green metallic sheen	No green metallic sheen, large colony	Pink colony

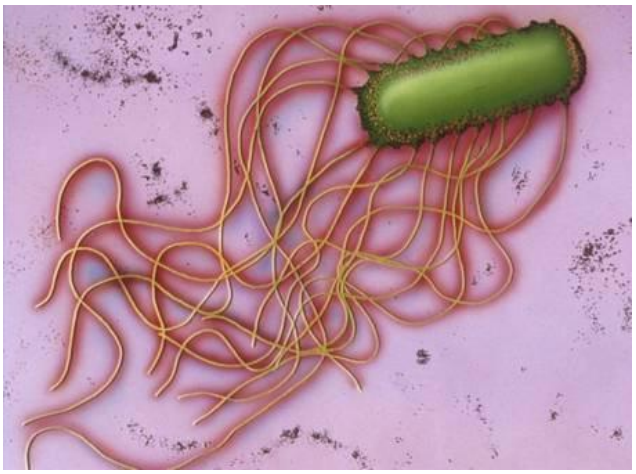
Family: Enterobacteriacea

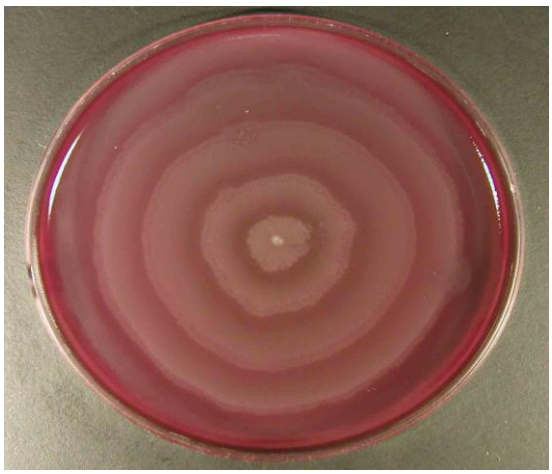
Genus:

1) *Proteus*
   a) *Proteus vulgaris* (UTI, wound infection)
   b) *Proteus mirabilis* (UTI, wound infection, nosocomial infection)
   c) *Proteus myxofaciens* (non pathogenic)
   d) *Proteus penneri* (UTI)
2) *Providencia rettgri*
3) *Morganella morganii* (cause summer diarrhea)

General characteristics:

G-ve bacilli or coccobacilli, lactose non fermenter, pleomorphic, actively motile with peritrichous flagella, **swarming on agar**, facultative anaerobic, non capsulated, non spore former. Natural habitat: some are free living in water, sewage, soil and even vegetables, some are normal intestinal flora, pathogenic strains cause mainly UTI, otitis media, wound infection, summer diarrhea in infants, one of the most causative agent of nosocomial infection.





Urease activity is an important factor determining its pathogenicity in UTI, forming of NH_3 from urea makes urine alkaline which affect the kidney causing kidney infection, NH_3 inactivates complement (C4+), alkalinity causes deposition of phosphate stone in bladder and ureter.

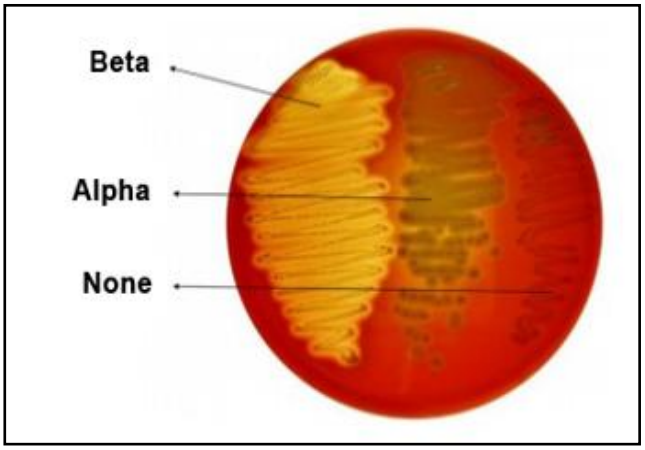
Serological classification is not dependable because of the cross reactivity with *Rikettsia* (typhus fever). Differentiation among 4 biotypes of *Proteus* (*mirabilis, vulgaris, myxofaciens*, *penneri*) by carbohydrate fermentation.

Enzymes produced by *Proteus*:

Proteolytic enzymes which are protease include gelatinase (liquification of gelatine), phenylalanine deaminase, urease and hemolysin.

Types of hemolysis:

1) α- hemolysis: partial hemolysis appear as a green zone around the colony.
2) β- hemolysis: complete hemolysis appear as a clear zone around the colony.
3) γ- hemolysis: no hemolysis.



Highly sensitive to piperacillin, cefotaxime and gentamycin.

Drug of choice: piperacillin.

Some factors inhibits the swarming phenomena:

1. percentage or agar concentration (4%).
2. Presence of bile salts (MacConkey).
3. Anaerobic conditions.

Lab diagnostic tests:

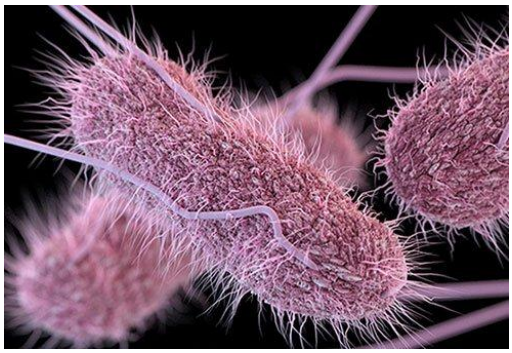
1. Gram stain: G-ve bacilli or coccobacilli, pleomorphic.
2. MacConkey agar: lactose non fermenter.
3. Blood agar: swarming and hemolysis.
4. TSI.
5. Urease test.
6. IMViC.
7. Gelatin liquification.

8. Phenylalanine deaminase.
9. Maltose (differentiation by fermentation).
10. Glucose (differentiation by fermentation).
11. Sensitivity test.

Test	Proteus vulgaris	Proteus mirabilis
MacConkey agar	L.N.F	L.N.F.
Blood agar	Swarming +, hemolysis	Swarming +, hemolysis
TSI	A/A ++, k/A ++	A/A ++, k/A ++
Urease	+	+
Indol	+	-
MR	+	+
VP	-	-
Citrate	+	+
Gelatin	+	+
Phenylalanine	+	+
Maltose	+	-
Glucose	+	+
Motility	+	+

Family: Enterobacteriacea

Genus:



1. ***Salmonella***
   a) *Salmonella typhi*
   b) *Salmonella paratyphi A*
   c) *Salmonella paratyphi B*
   d) *Salmonella typhimurium*
   e) *Salmonella enteritidis*

General characteristics:

G-ve bacilli, non spore former, non capsulated, motile except *S. gallinarum*, facultative anaerobic, lactose non fermenter, urease –ve, citrate utilizer, H_2S producers, grows well on synthetic media does not need complex requirements, mostly growth temperature as other Enterobacteriaceae from 4-40˚C, optimum 37 ^{o}C, biochemical identification is not dependable but serotyping is used (H & O). they are resistant to some chemicals (brilliant green, Na-deoxycholate), therefore it is useful for inclusion in media to isolate *Salmonella* from feces.

Sources of contamination:

Considered as intestinal parasite found in human, animals, birds and reptiles, transferred by direct contact as well as contaminated food and water causing gastroenteritis and food poisoning by *S. typhimurium* and *S. enteritidis* systemic infection and enteric fever.

Specimens for isolation: faeces, urine, blood and serum for serotyping.

Diseases:

1. Acute gastroenteritis, caused by *S. typhimurium* and *S. typhi*.
2. Septicemia and complex local infection by *Salmonella* spp.
3. Enteric fever e.g. typhoid and paratyphoid fever.

Diagnosis:

1. Biochemically: is not dependable because it shows variations.
2. Phage typing.

3. Serologically:

Widal test:

Is a classic serologic test used in diagnosis of Salmonella infection. O Ag and H Ag prepared from bacteria species used in this test to detect Abs in patient's serum. This test is agglutination test (tube or slide).

a) Rapid slide test: known sera and unknown culture are mixed on a slide. Clumping can be observed within 2 mins.
b) Tube dilution agglutination test:
Patient's sera diluted and mixed with known O or H Salmonella Ags during the second and third weeks to estimate the titer of Abs.

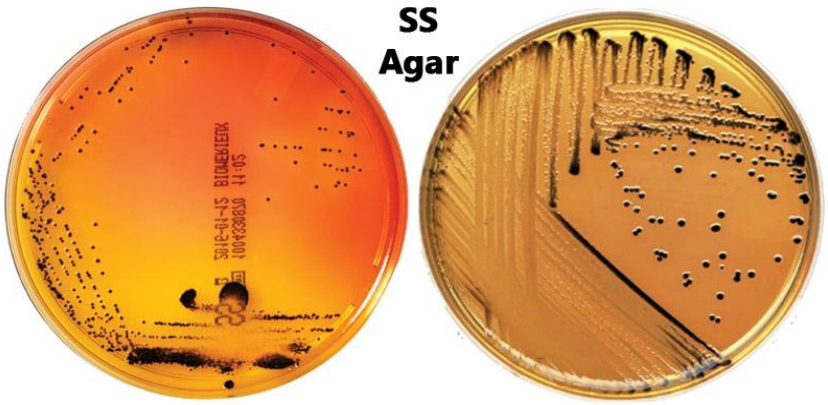
Conclusion of results:

1. High titer of O > 1:160 indicate active infection.
2. High titer of H > 1:160 suggest past immunization or past infection.
3. High titer of Abs to Ags occurs in some carriers.

Drug of choice: ciprofloxacin.

Biochemical lab tests:

1. **Gram stain**: G-ve bacilli.
2. **S.S. agar**: selective and differential.
Selective: because it contains bile salts for G+ve inhibition and G-ve other than enterobacteriaceae.
Differential: because it contains Na-thiosulfate and ferric citrate for H_2S production. Indicator is Neutral red. Does not need autoclaving because it includes inhibitors.



3. **MacConkey agar:** L.N.F.
4. **Brilliant green:** (lactose, sucrose, phenol red, brilliant green), selective: inhibits coliform, differential: *S. typhi, S. paratyphi* A,B and *Shigella.*
5. **Bismuth sulfite agar**: for isolation of *S. typhi* which appears as dark colonies.
6. **XLD**: (xylose lysine deoxycholate) contains xylose, lactose, sucrose, phenol red (indicator), differential for *Salmonella, Shigella* and coliform for H_2S production.
7. **DCA**: (deoxycholate citrate agar) contains lactose, neutral red (indicator). Selective and differential medium for *Salmonella* and *Shigella*, contains Na-deoxycholate, Na-citrate.
8. IMViC.
9. Motility.
10. TSI.
11. Mannitol.
12. Glucose.

Salmonella usually grown in selective & enrichment broth such as selenite broth or tetrathionate broth if isolation from stool is derived.

Test	S. typhi	S. paratyphi A	S. paratyphi B	S. typhimurium
TSI	K/A++	K/A++	K/A++	K/A++
Indol	-	-	-	-
MR	+	+	+	+
VP	-	-	-	-
SC	-	+	+	+
Glucose	+, no gas	+, gas	+, gas	+, gas
Mannitol	+. No gas	+, gas	+, gas	+, gas
motility	+	+	+	+
Urease	-	-	-	-
S.S. agar	L.N.F., H_2S	L.N.F., H_2S	L.N.F., H_2S	L.N.F., H_2S
MacConkey agar	L.N.F.	L.N.F.	L.N.F.	L.N.F.

Family: Enterobacteriacea

Genus: *Shigella*

a. *Shigella dysenteriae*
b. *Shigella flexneri*
c. *Shigella boydii*
d. *Shigella sonnei*

General characteristics:

G-ve bacilli, non-motile, non-capsulated, non-spore former, lactose non fermenter (colonies are pale on MacConkey agar), facultative anaerobic, normal human flora in small numbers, less than 10^3 cells can cause infection. Humans are the only host, infection caused by contaminated food. According to O-Ag there are more than 40 serotypes, they don't have K or H Ag. Growth temperature 10-40° C, optimum temperature 37°C. Does not produce H_2S.

Natural habitat is intestinal tract and the main disease is bacillary dysentery caused by *Shigella dysenteraie*, all species cause enteritis.

Isolation samples:

Fresh stool is preferable for direct diagnosis of dysentery. WBCs and RBCs may also seen in smear, then cultured on appropriate media.

Drug of choice: cephalosporin, tetracycline and chloramphenicol.

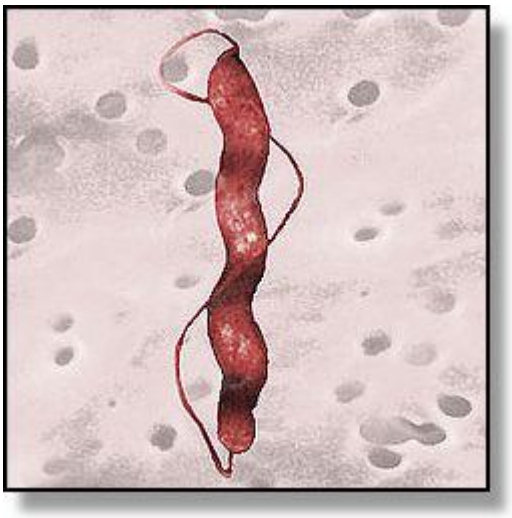
Diagnostic lab tets:

1. Gram stain: G-ve bacilli.
2. MacConkey agar: lactose non fermenter.
3. S.S. agar: L.N.F, transparent.
4. XLD: transparent.
5. DCA.
6. IMViC.
7. TSI
8. Motility: non motile.
9. Glucose.
10. Mannitol.
11. Urease.

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

Family: Campylobacteriaceae

Genus:



1. *Campylobacter.*
   a) *Campylobacter jejuni.*
   b) *Campylobacter coli.*
   c) *Campylobacter fetus.*
   d) *Campylobacter lari.*

2. *Helicobacter pylori*
3. *Archobacter.*

Campylobacter:

General characteristics:

Campylobacters are G-ve spiral bacteria. They are motile with either unipolar or bipolar flagella and do not form spores, they are oxidase +ve. *Campylobacter* produces two types of colonies:

Type 1: large, flat, mucoid, translucent, grayish and has an irregular edges.

Type 2: small, raised (convex), grayish-brown, smooth and glistening. (both colony types may appear on one agar plate).

The optimum growth temperature is 42˚C, the organism required selective media for isolation. e.g: Cary-Blair medium or Campy blood agar which contain 5 antimicrobial agents such as cephalothin, polymyxin B, vancomycin, amphotericin B and trimethoprime and supplemented with 5-10% sheep or horse blood. This media selective for intestinal isolation of *C. jejuni* and inhibits the *C. fetus* which is rarely responsible for enteric infections. They are microaerophilc, high concentrations of O_2 are toxic to these organisms (atmosphere of 5% O_2 with 10% CO_2 is optimal for their growth).

Pathogenicity:

C. jejuni cause enteric infection such as severe diarrhea or bloody diarrhea, they may cause symptoms similar to that of Salmonellosis or Shigellosis or food poisoning by *Staphylococcus*. In contrast with *Salmonella* or *Staph.* the *Campylobacter* does not multiply in food.

The infection is acquired by the oral route from food, drink or contact with infected animals. *Campylobacter* can be isolated from diarrhea and from blood.

Lab diagnostic tests:

1. **Isolation from feces:**

Samples should be transported to laboratory directly or transport media should be used (Cary-Blair medium). Samples can be refrigerated for 24 hrs because *Camp.* Is resistant to cold, the stool should be cultured on the selective agar and incubated at 42˚C in a microaerobic atmosphere for 7 days. The plate should be examined after 48 hrs to 5 days for characteristic colonies which are gray-brown or translucent gray with slightly mucoid looking.

2. **Gram stain:** G-ve with gull-wing shape.
3. **Oxidase test:** reduces the colorless oxidase reagent (1% tetramethyl paraphenyl diamine dihydrochloride) to dark blue-purple in 3-5 seconds.
4. **Catalase test:** an enzyme produced by bacteria which convert organic peroxide or hydrogen peroxide to water and O_2 so that the bacteria can neutralizing the toxic effect of peroxides.

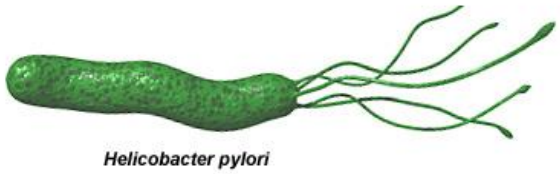
$$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2 \text{ as bubbles}$$

5. **TSI agar:** produce H_2S.
6. **Nitrate reduction test:** reduce NO_3 to NO_2 or N_2 (gas).

Genus:

Helicobacter pylori

(causative agent of gastric ulcer)



Helicobacter pylori

General characteristics:

G-ve spiral shape bacterium, fastidious, requires 3-7 days of incubation in microaerophilic conditions (5% O_2 + 10% CO_2) at 35-37˚C. motile with polar flagella. Good growth of *H. pylori* may be obtained in the presence of starch, charcoal and sodium pyruvate, for example of selective media:

1. Columbia blood agar.
2. Muller-Hinton agar.
3. *Brucella* agar.

For the detection of the presence of *H. pylori*, the tissue biopsy material should be transported to the lab and the culture incubated for 7 days in 37˚C, after that the colonies appear small, translucent, yellowish and raised.

Pathogenicity:

H. pylori found in the stomach and is associated with gastritis, gastric ulcer and duodenal ulcer. The mechanism of pathogenicity has been identified by production of cytotoxin and also produce large amount of urease, probably a useful strategy for survival in the acid environment of the gastric mucosa.

Lab diagnosis:

1. Gram stain: G-ve spiral bacterium.
2. Catalase +ve, Oxidase +ve.
3. Urease +ve.

Family: Pasteurellaceae

Genus: *Haemophilus*

1. *H. influenzae.*
2. *H. parainfluenzae.*
3. *H. haemolyticus.*
4. *H. parahaemolyticus.*
5. *H. ducreyi.*

General characteristics:

In specimens from acute infections, the organisms are short coccobacilli, sometimes occurring in pairs or short chains. In cultures, the morphology depends both on age and on the medium. At 6-8 hours in rich medium usually containing blood, the small coccobacillary forms predominate. Later there are longer rods and very pleomorphic forms. They are capsulated or non-capsulated, the capsule is the antigen used for typing. *H. influenza* type B is an important human pathogen causes meningitis in children and occasionally causes respiratory tract infection in children and adults. *H. ducreyi* is a sexually transmitted pathogen causes chancroid (genital ulceration).

Growth characteristics:

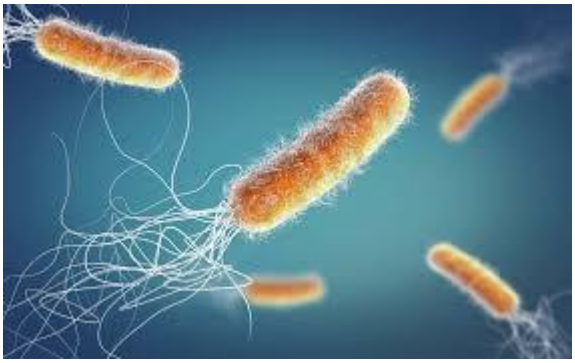
Identification of organisms of the *Haemophilus* group depends in part upon demonstrating the need for certain growth factors, hemin (factor X) and/or nicotinamide adenine dinuclutide (NAD) (factor V). The requirements for X and V factors of various *Haemophilus* species are listed in table below:

Species	Requires	
	X	V
H. influenzae	+	+
H. parainfluenzae	-	+
H. ducreyi	+	-

Family: Pseudomonadaceae

Genus:

1. *Pseudomonas.*



Fluorescent group:

a) *Pseudomonas aeruginosa.*
b) *Pseudomonas fluorescens.*
c) *Pseudomonas putida.*

Non fluorescent group:

d) *Pseudomonas stutzeri.*

2. *Acinteobacter.*

General characteristics:

G-ve bacilli, motile with polar flagella (monotrichous or polytrichous) and some of them are non-motile, catalse +ve, oxidase +ve, non spore former, non-capsulated, strict aerobic but can utilize nitrate as a source of respiration. They found in soil and any moist area like water. They present in small numbers of normal intestinal flora and skin, they are characterized by extracellular pigments, the color of this pigments differ according to the spp., *P. aeruginosa* produces bluish pigment called **pyocyanin**, other *Pseudomonas* spp. do not produce pyocyanin. *P. aeruginosa* and many other species produces the fluorescent pigment **pyoveridin**, which give greenish color to the agar, some strain produce the dark red pigment **pyorubin** or the black pigment called **pyomelanin**.

Pathogenicity:

P. aeruginosa is the most important species, it is invasive and toxigenic produce infection in patients with abnormal host defense and is an important nosocomial pathogen, they cause UTI and otitis media, the main infection of *Pseudomonas* is burn infection and wound infection.

They may found in antiseptic solution, eye drops, grows well in dettol, heating 55°C/1hr kill Pseudomonas, so it could survive in detergents.

Enzymes and toxins:

They are extracellular, include hemolysin, lipase, collagenase, protease. The most important toxin is exotoxin A which cause blockage of protein synthesis which leads to tissue necrosis.

Specimens: skin lesion, pus, urine, spinal fluid and sputum.

Classification:

1. Biochemical.
2. Serological (O-Ag, H-Ag).
3. Pyocin typing. *Pseudomonas* produces pyocin which is an antimicrobial agent.
4. Phage typing.
5. Sensitivity to antibiotics.

Treatment and drug of choice: piperacillin and cefotaxin.

Lab diagnostic tests:

1. Gram stain: G-ve bacilli.
2. Milk agar: for pigmentation.
3. Blood agar: for hemolysis.
4. King A, King B (selective & differential).
5. MacConkey agar.

6. TSI.
7. IMViC.
8. Motility.
9. OF (Oxidation Fermentation) contain glucose 1%, bromothymol blue, adding paraffin on the surface to produce anaerobic condition, inoculation by stabbing, the color change to yellow refer to acid production.
10. Nitrate broth.
11. Oxidase, Catalse.

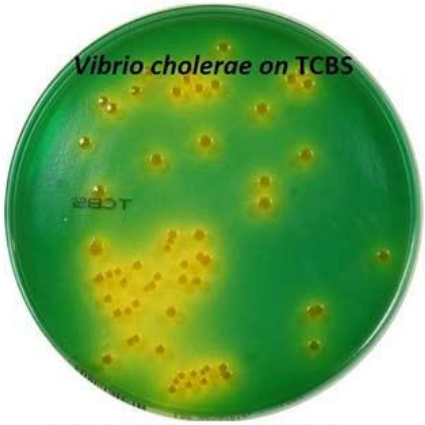
Test	*Pseudomonas aeruginosa*	*Pseudomonas fluorescens*
Indol	-	-
MR	-	-
VP	-	-
SC	+	+
TSI	K/K - -	K/K - -
Nitrate	+	+
Motility	+	+
Growth at 42°C	+	-
Growth at 4°C	-	+
King A	+, pyocyanin	+, no pigment
King B	+ fluorescein	+ fluorescein
MacConkey	L.N.F. transparence, irregular	L.N.F. transparence, irregular
Oxidase	+	+
Catalase	+	+
OF medium	Oxidation (+), ferm (-)	Oxidation (+), ferm (-)

Family: Vibrionaceae

Genus: *Vibrio*

General characteristics:

G-ve curved (comma shape), facultative anaerobic, motile with single polar flagellum, found in single or cluster, non spore former. *Vibrio* found in nature mostly in surface waters worldwide. Produce convex, smooth and round colonies, they are oxidase +ve, most vibrio grow well at 37° C on media containing mineral salts and asparagines as a source of carbon and nitrogen. *Vibrio cholerae* grows well on TCBS agar (thiosulfate-citrate-bile-sucrose). *Vibrio* grow at alkaline pH (8.5-9.5) and are rapidly killed by acid.



Vibrio cholerae on TCBS Agar

Vibrio parahaemolyticus on TCBS Agar

The medically important Vibrios are listed in table below:

Organism	Human disease
V. cholerae serogroups O1 and O139	Epidemic and pandemic cholera
V. cholerae serogroups non- O1/non-O139	Cholera-like diarrhea, mild diarrhea, extraintestinal infection (rarely)
V. parahaemolyticus	Gastroenteritis, perhaps extraintestinal infection
Others: *V. mimicus, V. vulnificus, V. fluvialis*	Ear, wound, soft tissues and other extraintestinal infection, all uncommon

V. cholerae serogroup O1 have 2 biotypes defined classical and El Tor.

El Tor	Classical
Haemolytic	Non haemolytic
Resistant to polymyxin B	Sensitive
Cause haemoagglutination of sheep RBCs	Does not

Toxins and pathogenesis:

1. *Vibrio choleare*:
   - Produce enterotoxin which is heat labile.
   - Some produce soluble hemolysin.
   - Mucinase (self protecting enzyme).
2. *Vibrio parahaemolyticus*:

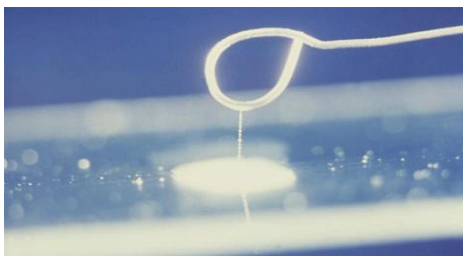
Halophilic bacterium that causes acute gastroenteritis following ingestion of contaminated sea food. After an incubation period 12-24 hr, nausea, vomiting, abdominal cramps, fever and watery to bloody diarrhea occur.

Treatment:

Rehydration with fluids and tetracycline.

Lab diagnostic tests:

1. Gram stain: G-ve, comma shaped.
2. String test: a loopful of growth is mixed with a drop of 0.5% Na-deoxycholate on a slide. If the mixture loses its turbidity, becomes mucoid and form a string when the loop is drawn away slowly, the test is positive.

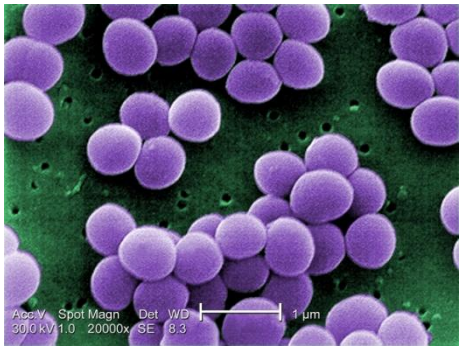


3. IMViC.
4. TSI.
5. Nitrate reduction.
6. Peptone water.
7. Motility.
8. TCBS agar.

Test	*V. cholera*	*V. parahaemolyticus*
Indol	+	+
MR	+ weak	-
VP	-	-
SC	+/-	+/-
Peptone w. + 7% NaCl	-	+
Peptone w. + 0% NaCl	+	-
TSI	A/A - -	K/A - -
Motility	+	+
String test	+	+
TCBS agar	Yellow colonies	Green colonies
Mannitol	+ weak	+ weak
Catalase	+	+
Oxidase	+	+
NO_3 reduction	+	+

Family: Staphylococcaceae

Genus: *Staphylococcus*



Spp.:

a) Staphylococcus aureus.
b) Staphylococcus epidermidis.
c) *Staphylococcus saprophyticus*.

General characteristics:

G +ve cocci (spherical), aerobic or microaerophilic, arranged as clusters but sometimes in pairs or single, non-motile, non-spore former, mostly non-capsulated, invasive M.O., resist high concentrations of NaCl (9%), drugs and dryness, capable to survive outside the body for extend periods.

They are endogenous to skin surface and mucous membranes of the upper respiratory tract, any break or injury in the skin or mucous membrane may lead to infection.

Isolation and pathogenicity:

The three major species include *S. aureus*, *S. epidermidis, S. saprophyticus*.

- *S. epidermidis*: may be the etiological agent for skin lesion and endocarditis.
- *S. saprophyticus*: may cause UTI.
- *S. aureus* are often responsible for:

1. Skin infection.
2. Abscess formation.
3. Boils, acne, impetigo.

Infection of deeper tissues lead to pneumonia, osteomylitis, endocarditis, cystitis, staphylococcal enteritis due to enterotoxin contamination of food.

Clinical samples:

1. Pus (abscesses).
2. Sputum (respiratory tract infection).
3. Stool (enteritis).

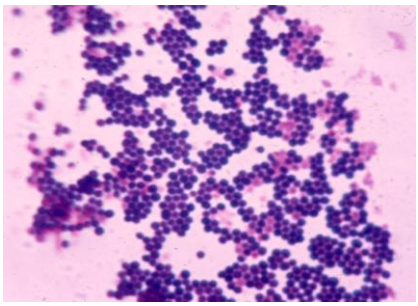
4. Blood (bacteremia).
5. Carriers.

Toxins & Enzymes:

1. **Catalase.**
2. **Coagulase:** *S. aureus* produces coagulase, an enzyme that clots plasma. Coagulase binds to prothrombin, together they become enzymatically active and initiate fibrin.

$$\text{Fibrinogen} \xrightarrow[\text{activation}]{\text{Coagulase + prothrombin}} \text{fibrin clot}$$

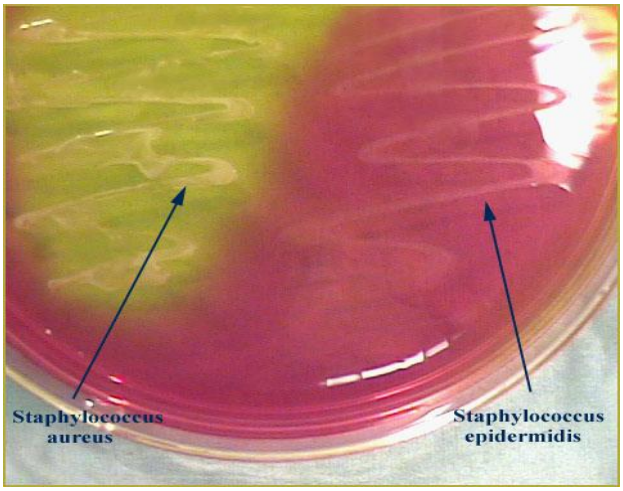
3. **Clumping factor:** (fibrinogen binding protein) is a surface *S. aureus* compound that is responsible for adherence of the organism to fibrinogen and fibrin.
4. **Nuclease:** extracellular enzyme which hydrolyse DNA or RNA to nucleotides which dissolve acid in acid. This enzyme acts on phosphodiester bonds.
5. **Leucocidin**: lysis or kill W.B.C.s.
6. **Haemolysin.**
7. **Proteinase.**
8. **Lipase.**
9. **B-lactamase.**
10. **Enterotoxin**: enteritis, food poisoning, the symptoms appear after 3-4 hrs after ingestion of contaminated, lead to vomiting and diarrhea. It is self limiting takes not more than 3 days.



Lab diagnostic tests:

1. Gram stain: G +ve cocci (grape-like cluster).
2. Blood agar (haemolysis).
3. Milk agar: for pigments.
4. Staph 110 (tolerance to 7.5% NaCl) selective media for *Staph.*
5. Mannitol salt agar (selective & differential): the indicator is phenol red.

   Selective for *Staph.* because it contains 7.5% NaCl. Differential for *S. aureus* because it ferments mannitol.

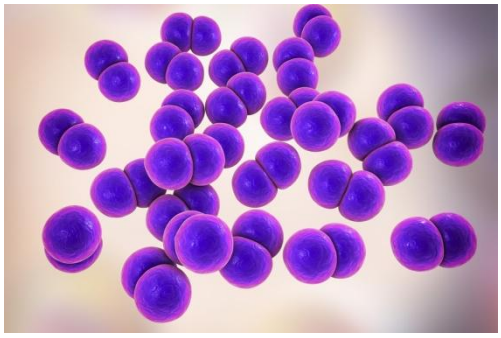


6. Gelatin: liquification of gelatin.
7. Coagulase test: adding few colonies of *Staph.* to human or rabbit plasma, incubated in 37˚C. if clots form in 1-4 hrs, the test is positive.
8. Catalse test.

Test		*S. aureus*	*S. epidermidis*	*S. saprophyticus*
Pigments		Golden	White	Light yellow
Mannitol salt agar	**Growth**	+	+	+
	Fermentation	+	-	-
Coagulase		+	-	-
DNase		+	-	-
Catalase		+	+	+
Gelatin liquification		+	+	+
Staph 110		+	+	+

Family: Streptococcaceae

Genus: *Streptococcus*



Spp.:

A. *Streptococcus pyogenes* (impetigo, rheumatic fever, glomerulonephritis).
B. *Streptococcus agalactiae* (puerperal fever).
C. *Streptococcus zooepidemicus*.
D. *Enterococcus faecalis* (UTI).

General characteristics:

G+ve cocci, arranged in chains, many of them fastidious, requiring enrichment media for growth such blood agar. Growth temperatures (15-45˚C), most *Strept.* Are facultative anaerobes, microaerophilic. *S. pneumoniae* arranged in pairs called *Diplococcus* and they are capsulated, mainly cause pneumonia, they are differ from other *Strept.* In some biological and chemical characters.

Classification of *Strept.*:

1. Type of hemolysis.
2. Serological classification.
3. Biochemical reactions and resistance to chemical factors.

Lab diagnostic tests:

1. Gram stain: G +ve, cocci.
2. Blood agar: type of hemolysis.
3. Bile solubility: in presence of bile salt (Na-deoxy cholate) the surface tension of this salts cause release of autolytic enzyme cause lysis of the cells
   (+) of this test is cell lysis (no growth)
   (-) of this test (growth of cells)

4- Streptokinase test: indicator for this test is the lysis of the plasma clot (inverse coagulation), we make clot in plasma by adding $CaCl_2$ & we add growth to see streptokinase production. All species of *Strept.* Produce streptokinase except *Strept. pneumonia* (-) .

This differentiate between *Strept.* & *Staph.* beside coagulation

(+) no clot

(-) clot

5- Carbohydrate fermentation: to differentiate among *Strept.* spp because *strept.* are fastidious, muller hinton agar with carbohydrate is used for fermentation, the indicator is bromothymol blue, better than phenol red, the sugar are glucose, inulin, mannitol & lactose.

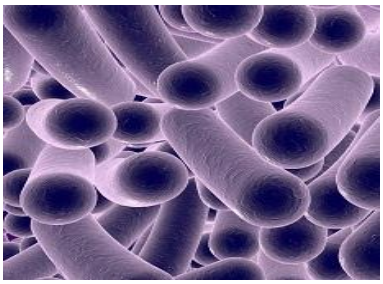
6- Bacitracin & Optochin: 2 kind of antibiotics are used in media

Strept. pyogenes (bacitracin + ve) *Strept. pnumoniae* (optochin + ve)

Tests	*S. pyogenes*	*E. faecalis*	*S. pneumonia*
Optochin	-	-	+
Bacitracin	+	-	-
Hemolysis	β	α	α
Inulin	-	-	+
Lactose	+ no gas	+	-
Mannitol	+ no gas	+	-
Glucose	+ no gas	+	-
Bile salt solubility	(-) growth	(-) growth	(+) no growth
Growth at 6.5% NaCl	+/-	(+) growth	(-) no growth
Streptokinase	+	+	-

Family: Bacillaceae

Genus : *Clostridium*



a) *C. tetani* (tetanus)
b) *C. perifringens* (gas gangrene)
c) *C. botulinum* (food poisoning)
d) *C. difficili* (sever diarrhea)

All spp. Are human pathogens and cause severe infection.

General characteristics:

G +ve anaerobic, spore former, large bacilli, saprophytes in the external environment (soil), although some are part of the intestinal flora of human & animals, spore are heat resistant & other physical agent, they produce exotoxin & enzyme , vegetative forms are slightly motile , spore are not motile, spore can resist oxygen except *C. tetani* which strict anaerobes, opt.temp. 37 C^{o} , few of them are capsulated, colony morphology is variable, hemolysis on blood agar is frequent. They lack catalase peroxidase as other anaerobic bacteria.

Specimens collection:

Samples must be carefully collected and transport in gassed out (evacuated) tube, inoculated on appropriated media, incubated anaerobically (gas pack and jars) use steuard transport medium which is colorless in reduced, the indicator is methylene blue.

Oxidase ⟶ blue

Reduction ⟶ colorless

Pathogenicity and Diseases:

1- *C. botulinum* : Result from ingestion of food contaminated by performed botulinum toxin, rather than form multiplication of *C. botulinum* in the

gastrointestinal tract, in this respect, it resemble *staphylococcal* food poisoning, the sample is stool.

2- *C. perifringens* : Form gas gangrene (lethal infection), the hemolysis of blood is by producing an exotoxin called α-toxin cause stormy fermentation of milk

3- *C. tetani* : strict anaerobes, capsulated from terminal spore appear as (tennis rackets) cause tetans which is a fatal disease caused by neurotoxin (tetanospasmin).

4- *C. difficili* : Cause diarrhea in children (sever summer diarrhea)

Drug of choice:

Vancomycin , Penicillin G.

Alternative drug:

Bcitracin , Chloramphenicol , Clindamycin