LAB: 7 **Practical Pathogenic bacteria**

Family: Enterobacteriaceae

Lactose none fermenting

Genus: *Proteus. spp*

Genus: *Shigella .spp*

Genus: *Salmonella .spp*

Taxonomy:

Kingdom: Bacteria

Phylum: Proteobacteria

Class: Gammaproteobacteria

Order: Enterobacteriales

Family: Enterobacteriaceae

Genus: Proteus

A) *Proteus vulgaris* (UTI, wound infection).

B) *Proteus mirabilis* (UTI, wound infection, nosocomial infections).

C) *Proteus penneri* (UTI, wound infection, nosocomial infections).

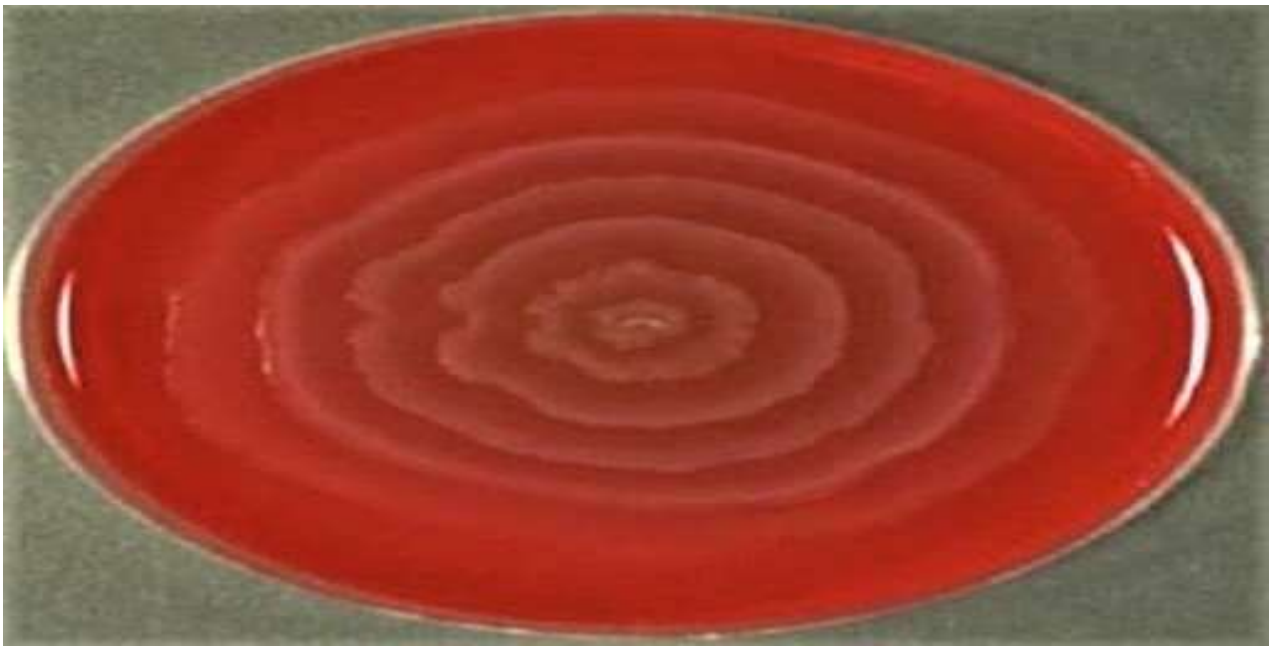
Distinguishing Features:

Gram negative, pleomorphic (bacilli or coccobacilli), actively motile with peritrichous flagella (see figure below), non-lactose fermenter, facultative anaerobes, non-capsulated, non-spore former, swarming on agar, growth at 25-37 C˚. Natural habitat: some are free living in water, sewage, soil and vegetable. some are normal intestinal flora. Proteus species produce infections in humans only when the bacteria leave the intestinal tract. They can cause urinary tract infections, bacteremia, pneumonia, and focal lesions in debilitated patients or those receiving contaminated intravenous infusions.

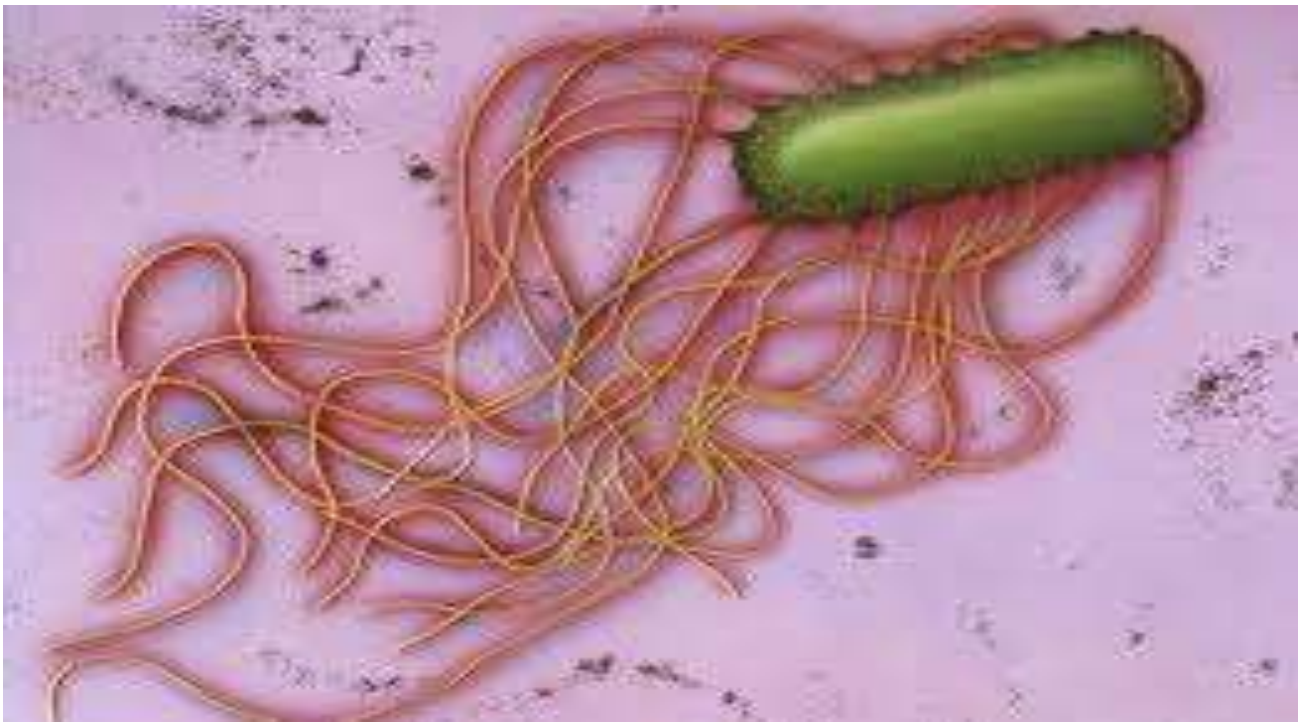
Proteus spp. are common causes of UTIs, occasionally in normal hosts and very commonly in those with indwelling catheters or anatomic or functional abnormalities of the urinary tract. UTIs caused by Proteus spp. tend to be more severe than those caused by E. coli. Pathogenesis: Proteus species produce urease, resulting rapid hydrolysis of urea with liberation of ammonia. Thus, in urinary tract infections with Proteus species, the urine becomes alkaline, promoting stone formation in bladder and ureters. Furthermore, Ammonia inactivate the complement system (C4+). - Motility may aid entry into bladder - Endotoxin causes fever and shock when septicemia occurs.

***Serological classification is not dependable due to cross reactivity with Rickettsia (Typhus fever), and for differentiation among different biotype of Proteus will be done by carbohydrate fermentation test. Enzymes produced by Proteus spp.: Proteolytic enzymes: Protease, Gelatinase, Phenylalanine deaminase, Urease and Hemolysin.**

****Highly sensitive to piperacillin, cefotaxime and Gentamycin, the drug of choice is piperacillin.**

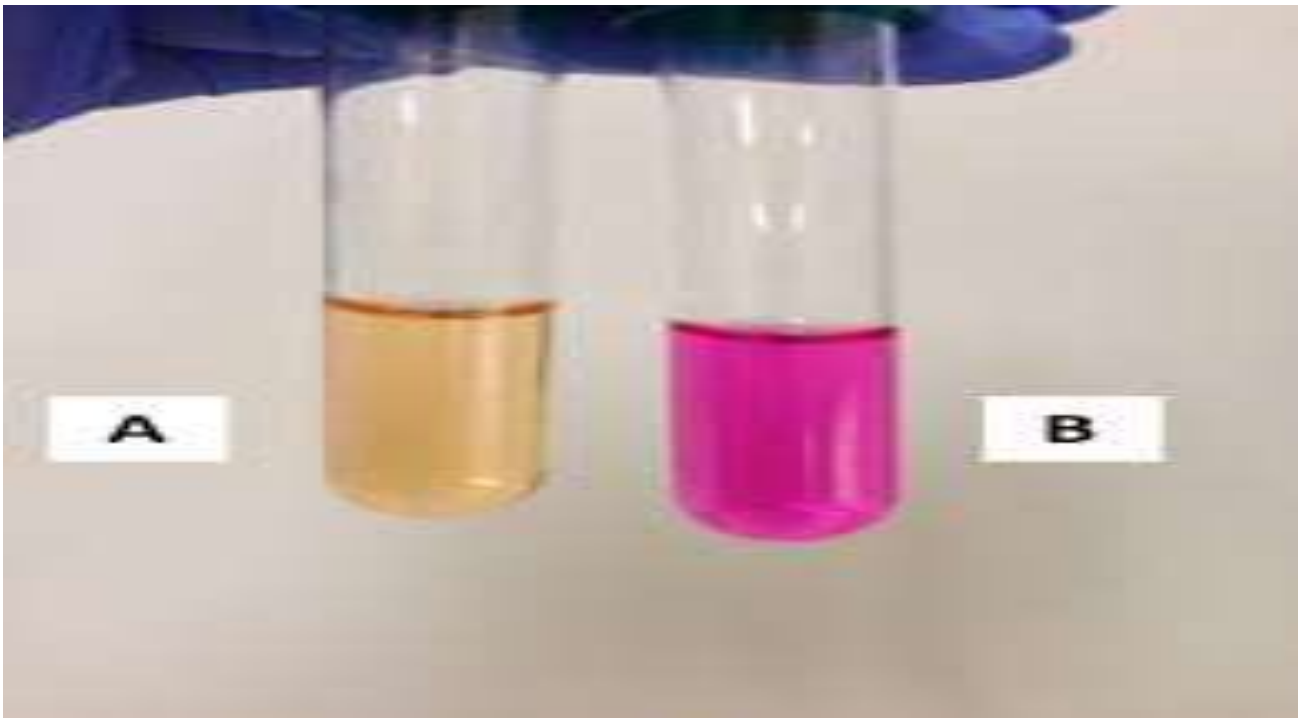


Proteus swarming on blood agar



Urease producing bacteria

1. Proteus
2. Klebsiella
3. Staphylococcus
4. Psuedomonas
5. Providentia
6. Ureaplasma



Proteus urease test positive (pink color).

Some factors inhibit the swarming phenomena:

1- Adding 4% agar to media.

2- Presence of bile salts (MacConkey agar).

3- Anaerobic conditions.

Diagnostic tests for proteus spp.:

1- Gram stain: Gram negative bacilli or coccobacilli (pleomorphic).

2- Inoculation MacConkey agar.

3- Blood agar (swarming and hemolysis)

4-TSI agar test.

5- Urease test.

6- IMViC test .

7- Gelatin liquefaction.

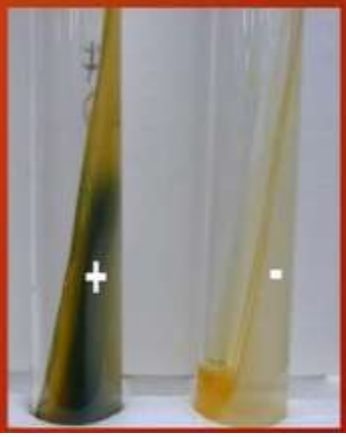
8- Phenylalanine deaminase test:

Also known as phenyl pyruvic acid (PPA) test is used to test the ability of an organism to produce enzyme deaminase. This enzyme removes the amine group from the amino acid phenylalanine and produces phenyl pyruvic acid (PPA) and ammonia. Phenyl pyruvic acid reacts with ferric salts to give a green color (see figure below). This test is useful in initial differentiation of Proteus, Morganella, and Providencia from the rest of the Enterobacteriaceae. Procedure: The long slant of the phenylalanine agar is inoculated with the tested organism. The tube is incubated at 37oC for 18- 24 hours. Four to five drops of 10% ferric chloride solution is allowed to run down over the slope. If the test is positive, a green color will develop in the fluid and in the slope.

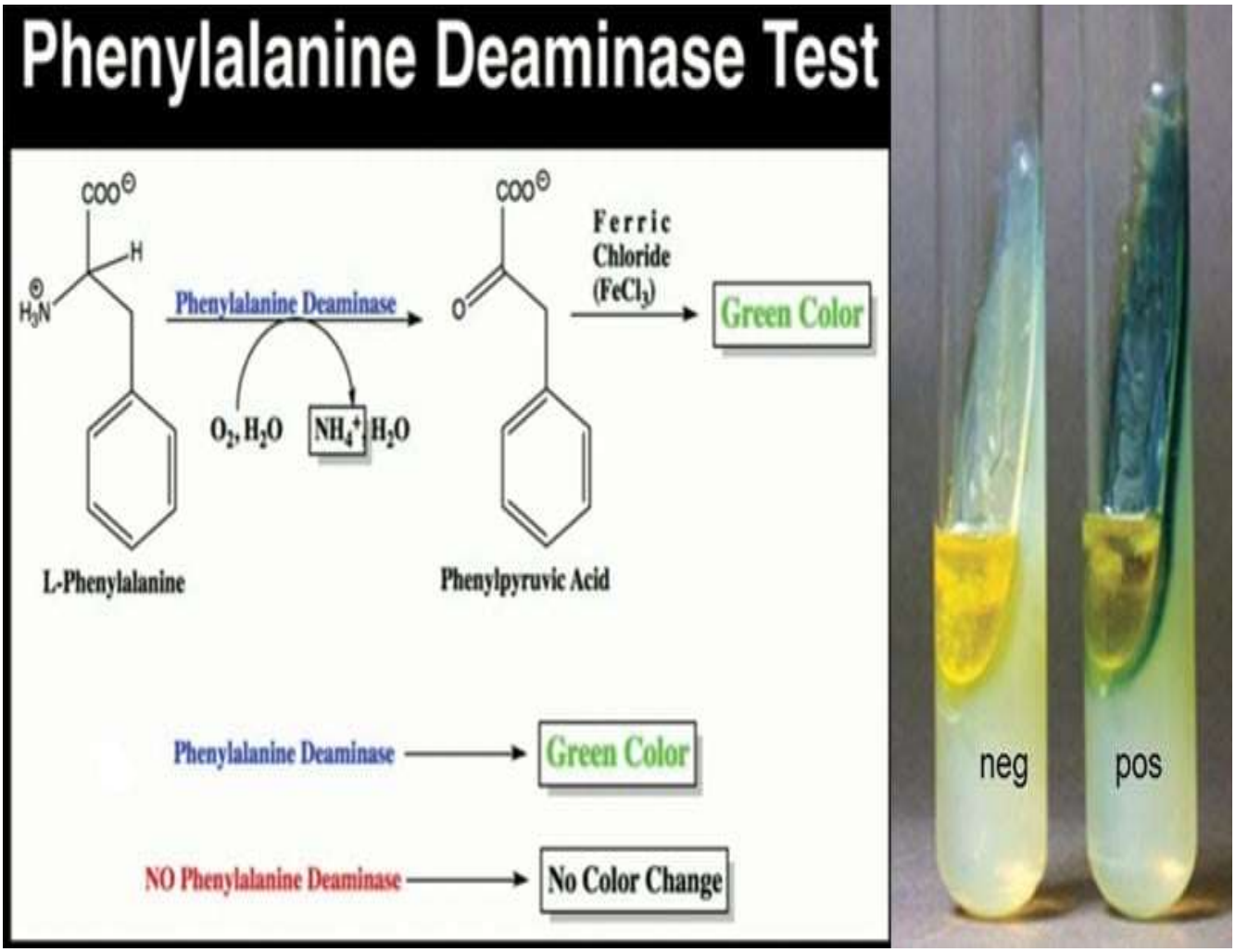
Phenylalanine Deaminase Test

13

- Used to identify bacteria possessing the enzyme phenylalanine deaminase
  - Phenylalanine deaminase converts phenylalanine to phenylpyruvic acid + NH_3
  - Ferric chloride ($FeCl_3$) reacts with the phenylpyruvic acid and changes color from yellow to green



Reagent = FeCl3 , positive result = green color



9 - Maltose (Differentiation and fermentation).

10 - Glucose (Differentiation and fermentation).

Some biochemical and culture characteristics of *Proteus spp*.

tests	*P.mirabilis*	*P.vulgaris*	*P. penneri*
IMViC	- , + , - ,v	+ , +, - ,v	- , + , - ,-
TSI	A/K CO2+ ,H2S +	A/KCO2+,H2S +	A/KCO2 +,H2S -
Catalase	+	+	+
Oxidase	-	-	-
Urease	+	+	+
Capsule	+	-	-
Swarming	+	+	+
H2S	+	+	-
Motility	+	+	+
MacConkey agar	L.N.F	L.N.F	L.N.F
Phenylalanine	+	+	+
Glucose	+readily	+ readily	+
Maltose	-	+readily	+
Sucrose	Ferment slowly	Ferment readily	+
Blood hemolysis	Non hemolysis	Non hemolysis	Beta
Gelatinase	+	+	+

Biochemical reactions of Proteus, Morganella, and Providencia species

Species	Urea	Cit	Ind	Suc	H_2S	PDA	GG
P.mirabilis	+	D	-	-	+	+	+
P.vulgaris	+	D	+	+	+	+	D
M.morgani	+	-	+	-	-	+	D
P.rettgeri	+	+	+	+	-	D	D
P.stuarti	-	+	+	+	-	+	-

Key: Urea = Urease test, Cit = Citrate test, Ind = Indole test, Suc = Sucrose fermentation, H_2S = Hydrogen sulphide production, PDA = Phenylalanine deaminase test, GG = Gas from glucose fermentation, D = Different strains give different results

Genera: Shigella and Salmonella

A- Shigella:

Shigella species are classified in to four serogroups:

- **Serogroup A: *Shigella dysenteriae* (12serotypes)**

Serogroup B: *Shigella flexneri* (6 serotypes)

- **Serogroup C: *Shigella boydii* (23 serotypes)**

Serogroup D: *Shigella sonnei* (1 serotype)

General characteristics

Gram negative, rod, cylindrical, non-motile, non-spore former, encapsulated, non-lactose fermenter, the colonies appear pale on MacConkey agar, facultative anaerobic, considered as intestinal normal flora of human (if present in small number), about 200 cells can pass to the intestine causing infection (highly virulent). The infection is caused by contaminated food with fecal materials. Growth temperature ranged between

(l0-42C˚) and the optimum temperature is 37C˚. Shigella causes dysentery and that lead to destruction of the epithelial cells of intestinal mucosa in the cecum and rectum. all Shigella spp. are ferment glucose without gas except Shigella flexneri, all Shigella spp. are non-lactose fermenter. Except Shigella sonnei which are Lactose fermenter .

Specimens:

Stool during 4-5 days after infection, mucous blood from the intestine or rectal swab for the detection of cells.

B- Salmonella:

Salmonella typhi, Salmonella paratyphi A, Salmonella paratyphi B, Salmonella typhimurium, Salmonella enteritidis, Salmonella ariwna, Salmonella choleraesuis, Salmonella gallinarium, Salmonella schottmuelleri para A

General characteristics

Gram negative bacilli , non-spore former, motile except S. gallinarium (cause acute enteritis),they are N. L. F., Urease negative, Citrate utilizer, H2S producer, growth temperature (4- 40C˚), Biochemical test are undependable in diagnosis but serotyping is used for identification, all Salmonella causes enteritis, Salmonella characterized by resistant to some chemical like brilliant green, Na-tetracholate and Na deoxycholate, therefore it is useful to add these chemical to the medium for Salmonella isolation and can be used without sterilization. Source of contamination with these bacteria by human feces, animals, birds and reptiles which transmitted directly through contact as well as contamination of food and water causing gastroenteritis and food poisoning.

Pathogenicity:

A- Acute gastroenteritis: 105- 108 cells will be caused by S. typhi and S. typhimurium.

B- Septicemia and complex local infection by all Salmonella spp.

C- Enteric fever (104 -106 cells) of S. typhi. or S. parartyphi A and B will cause infection.

Specimens:

For isolation: stool, urine, blood and serum for serological identification.

***** Serological diagnosis by widal test for somatic antigen (O-Ag) and Flagellar antigen (H-Ag) or by Phagtyping.**

Lab. Diagnostic tests:

1- Gram stain.

2- MacConkey agar (pale colonies)

3- S-S agar is a selective and differential medium for Salmonella and Shigella. The medium is differential for Shigella where colonies are appearing with pale color while Salmonella give black color in the center (see figure below), the media contain brilliant green as an inhibitor for the other group of Enterobacteriaceae and bile salt as an inhibitor for G +ve, G -ve, the indicator is Thiosulfate and Ferric citrate for the detection of H2S production.

4- IMVIC test

5- Motility

6- Glucose fermentation.

7- TSI test.

8- Mannitol

9- Gelatin.

10- Phenylalanine.

11- XLD agar (Xylose, Lysine Deoxycholate) is a selective media used for isolation of Salmonella and Shigella species from clinical specimen and also from food sample. It has a pH of approximately 7.4 which give the medium a bright pink or red appearance due to the indicator the phenol red. Sugar

fermentation decrease the pH and the phenol red indicator turned to yellow. Most gut bacteria, including Salmonella can ferment the sugar xylose to produce acid while Shigella cannot do this and therefore remain red. After exhausting the xylose supply Salmonella colonies will decarboxylate lysine, increasing the pH once again to alkaline and mimicking the red Shigella colonies. Salmonellae metabolize thiosulfate to produce hydrogen sulfide (H2S), which leads to the formation of colonies with black centers and allows them to be differentiated from the similarly colored Shigella colonies. It also contains the Lactose and sucrose.

12- Urease.

13- CDA (citrate deoxycholate agar) selective for Salmonella and Shigella.

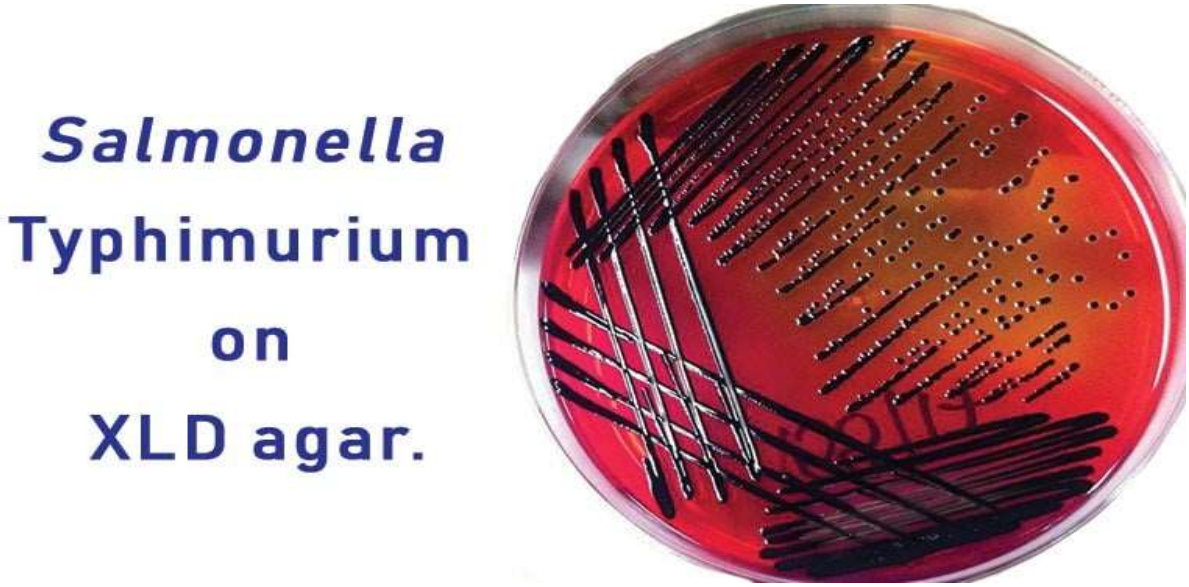
14- Brilliant green agar (selective and differential): it contain lactose, sucrose, phenol red, brilliant green. All Salmonella spp. grow except Salmonella typhi.

15- Bismuth sulfite agar is used to isolate Salmonella species. It uses glucose as a primary source of carbon and Bismuth inhibit the gram positive growth. Bismuth sulfite agar are to tests the ability of utilizing the ferrous sulfate and convert it to hydrogen sulfide (S. typhi which appear as black colonies while other doesn't grow).

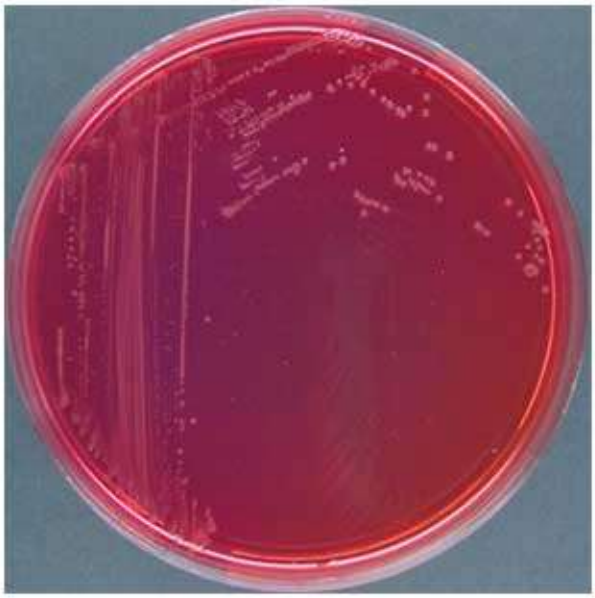
Figure : Salmonella on S-S agar (black colonies)

Figure: Shigella on S-S agar (pale colonies)

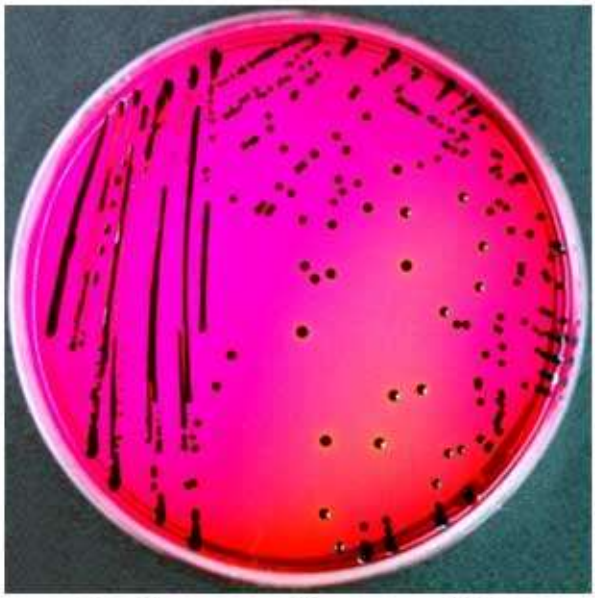
Figure : Salmonella on XLD agar (yellow colonies with black center)




XLD Agar media
Salmonella
Coliforms
Proteus
Citrobacter
Shigella

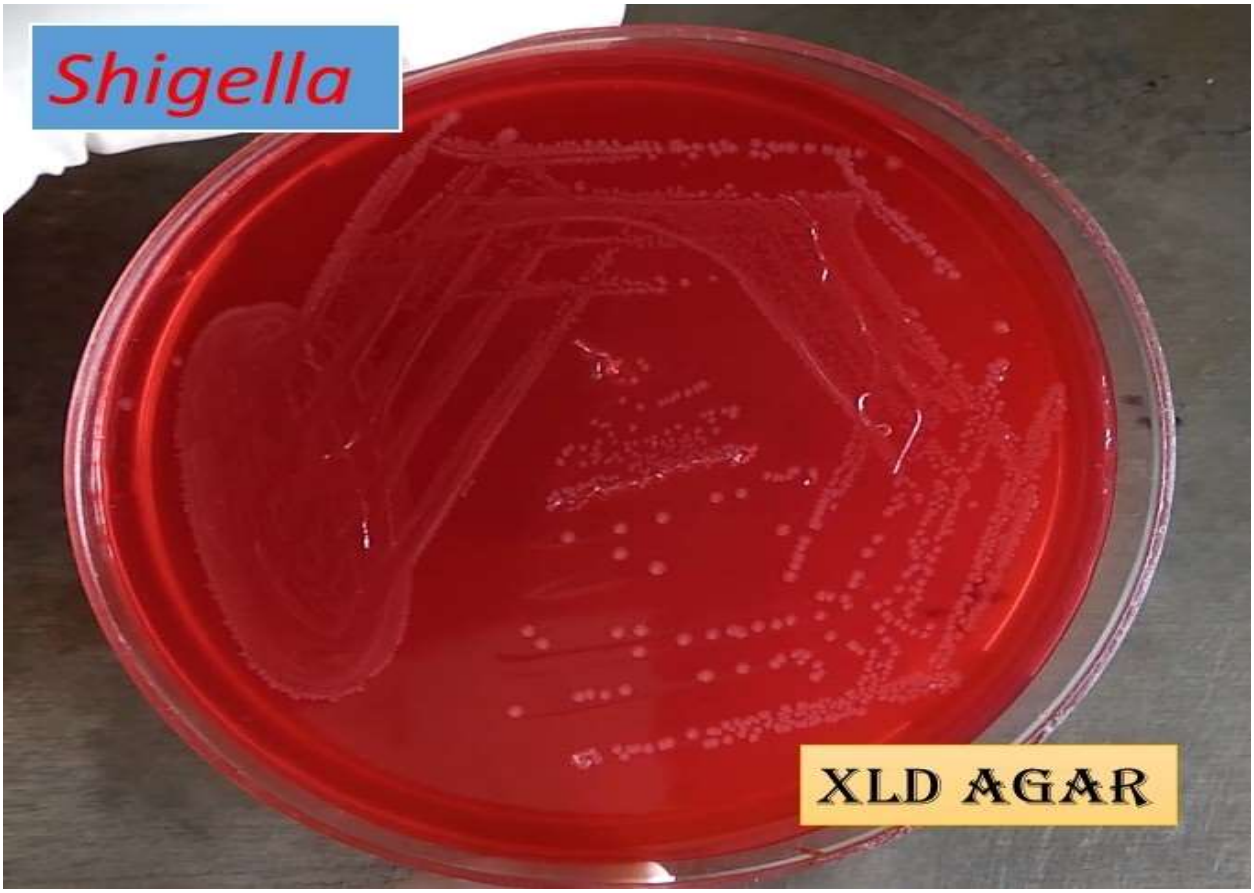


Shigella on XLD.



Salmonella on XLD.

Image Source: Faculty of Health and Medical Sciences - University of Copenhagen, Denmark



Colony characteristics on XLD agar

Organisms	Colony characteristics
Salmonella **H2S positive**	**Red colonies with black centers**
Shigella **spp.** **and** ***Salmonella*** **H2S negative**	**Red colonies**
E. coli	**Large, flat, yellow colonies**
Proteus **spp.**	**Red to Yellow colonies**
Enterobacter / Klebsiella	**Mucoid, yellow colonies**

Some biochemical and culture characteristics of *Shigella spp*.

tests	*Sh.dysenteriae*	*Sh.flexneri*	*Sh.boydii*	*Sh.sonnei*
IMViC	V , + , - ,-	V , +, - , -	V, + , - ,-	- ,+ ,- , -
TSI	A/K CO2- ,H2S -	A/KCO2-,H2S -	A/KCO2 -,H2S -	A/KCO2 -,H2S -
Catalase	+	+	+	+
Oxidase	-	-	-	-
Urease	N	N	N	N
H2S	N	N	N	N
Motility	N	N	N	N
MacConkey agar	L.N.F	L.N.F	L.N.F	
Phenylalanine	N	N	N	N
Glucose	+ NO gas	-	+ NO gas	+ NO gas
Mannitol	N	P	P	P
Gelatinase	N	N	N	N
S-S agar	Pale colony	Pale colony	Pale colony	Pale colony

P=positive, N=negative

Some biochemical and culture characteristics of *Salmonella spp*.

tests	*S.typhi*	*S.thyphimurium*
IMViC	- , + , - ,-	- , +, - ,+
TSI	A/K CO2 + ,H2S -	A/K CO2+ , H2S +
Catalase	+	+
Oxidase	-	-
Urease	_	_
H2S	-	+
Motility	+	+
MacConkey agar	L.N.F	L.N.F
Phenylalanine	_	_
Glucose	+	+
Maltose	+ gas	+ gas
S-S agar	Pale colony	Pale colony with black center