LAB 8:

FAMILY: ENTEROBACTERIACEAE

GENERA: SHIGELLA AND SALMONELLA

A- *Shigella:* *Shigella* species are classified in to four serogroups:

- Serogroup **A**: ***Sh****igella* ***dysenteriae*** (12 serotypes)
- Serogroup **B**: ***Sh****igella* ***flexneri*** (6 serotypes)
- Serogroup **C**: ***Sh****igella* ***boydii*** (23 serotypes)
- Serogroup **D**: ***Sh****igella* ***sonnei*** (1 serotype)

General characteristics

Gram negative, rod, cylindrical, non-motile, non-spore former, unencapsulated, 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.

Drug of choice: Severe dysentery can be treated with Ampicillin, Trimethoprim / Sulfamethoxazole (co-trimoxazole), fluoroquinolones such as ciprofloxacin.

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:

G -ve 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: 10^5- 10^8 cells will be caused by *S. typhi* and *S. typhimurium.*

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

C- Enteric fever (10^4-10^6cells) of *S. typhi*. or *S. parartyphi* A and B will cause infection.

Specimens:

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

Drug of choice:

Chloramphenicol and sulfa drug (Trimethoprim / Sulfamethoxazole)

** Biochemical test used to differentiate from Shigella.

*** 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(H_2S), 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*.

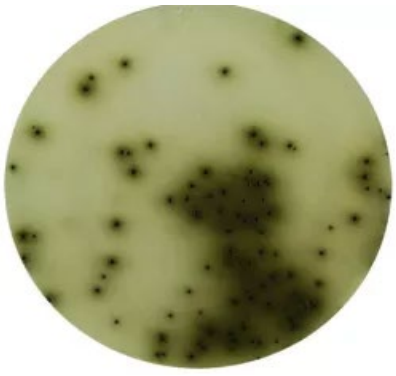
15- Bismuth sulfite agar is used to isolate *Salmonella* species. It uses glucose as a primary source of carbon and Bismuth inhibit the gram-positive growth. Bismuth sulfite agar are to tests the ability of utilizing the ferrous sulfate and convert it to hydrogen sulfide (*S. typhi* which appear as black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

<u>*Shigella* lab dignostic test:</u>

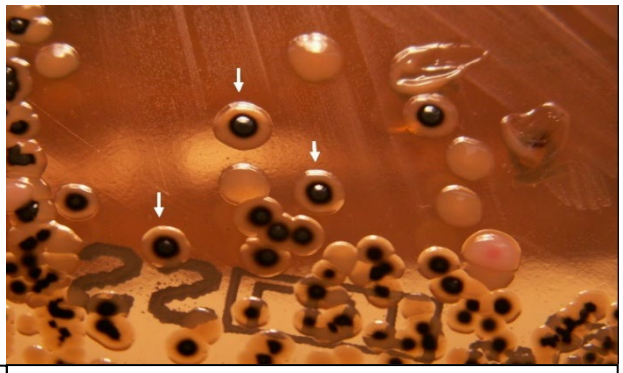
Test	*Sh,dysenteriae*	*Sh. flexneri*	*Sh. sonnei*	*Sh. boydii*
Indol	+/-	+/-	-	+/-
MR	+	+	+	+
VP	-	-	-	-
citrate	-	-	-	-
motility	-	-	-	-
glucose	+ no gas	-	+ no gas	+ no gas
manniltol	-	+	+	+
TSI	K/A - -	K/A - -	K/A - -	K/A - -
Phenylalanine	-	-	-	-
Gelatin	-	-	-	-
Urease	-	-	-	-
MacConkey	N. L.F.	N. L.F.	L.F.	N. L.F.
S-S agar	Pale colony	Pale colony	Pale colony	Pale colony

Salmonella **lab diagnostic test:**

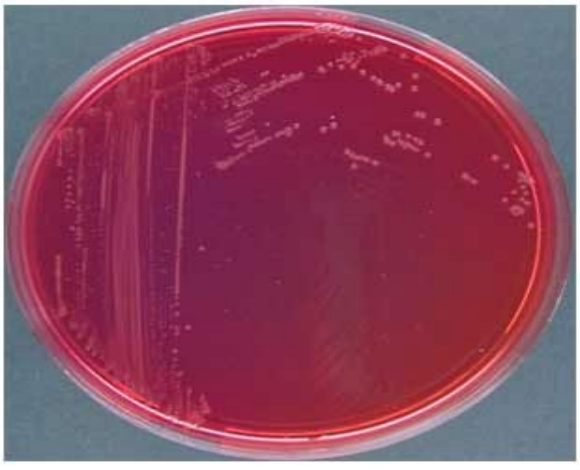
Test	*S. typhi*	*S. typhimurium*	*S. para typhi A*	*S. para typhi B*
TSI	K/A+ + gas weak	K/A + +	K/A + -	K/A + +
Indol	-	-	-	-
MR	+	+	+	+
VP	-	-	-	-
Sc	-	+	+	+
Mannitol	+gas	+gas	+gas	+gas
Motility	+	+	+	+
urease	-	-	-	-
S-S agar	Transparent + H_2S	Transparent ++ H_2S	Transparent	Transparent +++ H_2S
MacConkey agar	L.N.F.	L.N.F.	L.N.F.	L.N.F.
Brilliant green	- Pinkish white colony opaque	+	+	+
Bismuth sulfate agar	Black colonies	-	-	-



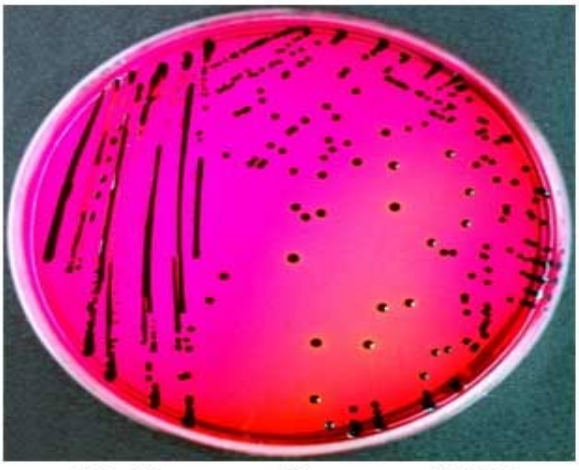
Salmonella on Bismuth sulfite agar



Salmonella on S. S. agar with black center



Shigella on XLD.



Salmonella on XLD.