



TEAM443  
MICROBIOLOGY

# Shigella & Salmonella

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# Objectives



Develop an algorithm using biochemical tests to identify and classify Salmonella and Shigella



Describe the antigenic structures and virulence factors of Salmonella and Shigella



Compare the pathogenesis of various species of Salmonella and Shigella



Describe the clinical features and risk factors-4 for the infection with the two organisms



Describe the general concepts for the management of gastroenteritis caused by both organisms

Any future corrections will be in the editing file, so please check it frequently

## Color Index:

Main text

Important

Doctor Notes

Males slide

Females slide

Extra



# Salmonella

## General info

- ◆ **Gram negative, motile {6}** (they have flagella in their structural characteristics), facultative anaerobic bacilli, **Non lactose fermenting colonies**.
- ◆ Highest during the **rainy** season in **tropical climates** and during the warmer months in temperate climates (The earth is divided into climate regions or zones, tropical and temperate are part of them).



## Types of Salmonella species & subspecies

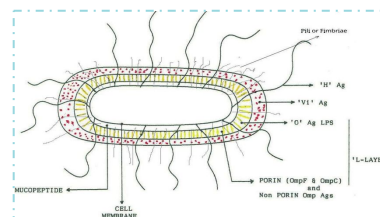
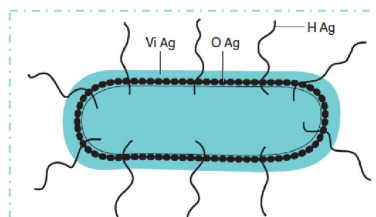
- ◆ Two species of **Salmonella**: **S. enterica** (six subspecies I, II, III, IV, V, VI) and **S. bongori** (rare).
- ◆ Found in cold **blooded animals: Turtles, Snakes and Fish**. / Warm blooded animals: Birds, Rodents.

Salmonella subspecies	No. of serotypes within subspecies	Usual habitat
<b>S. enterica subsp. enterica (I)</b>	1504	<b>Warm-blooded animals</b>
S. enterica subsp. salmae (II)	502	Cold-blooded animals and the environment
S. enterica subsp. arizonae (IIIa)	95	
S. enterica subsp. diarizonae (IIIb)	333	
S. enterica subsp. houtenae (IV)	72	
S. enterica subsp. indica (VI)	13	
S. bongori (V) rare	22	
Total	2541	-

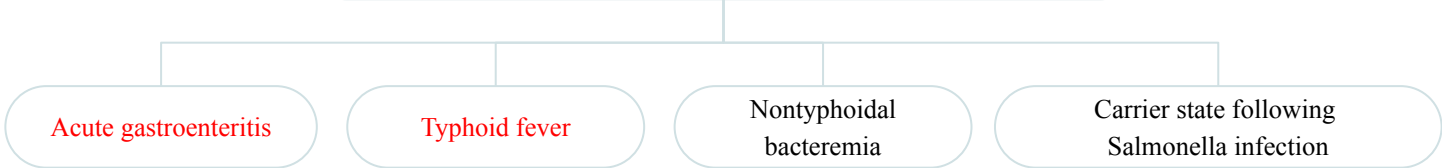


# Salmonella Virulence & Antigenic factors

Antigenic factor {5}	
O. Somatic antigen (Heat – stable)	A lipopolysaccharide in <b>the outer membrane</b> (A,B,C1,C2,D,E).
H. Flagellar antigen (Heat – Labile)	Found in flagella.
K. Capsular antigen	-
Vi antigen (Heat – Labile)	In Salmonella serotype typhi (virulence heat-labile capsular homopolymer of N-acetyl-galactosamino-uronic acid) vs phagocytosis Protects salmonella from phagocytosis by immune cells.
Virulence factors	
Fimbria (aka. pili)	Helps in <b>adherence</b> of bacteria on host cells.
Endocytosis	After adherence of bacteria, it triggers the host cell to engulf it: 1) SPI 1-T3SS 2) TLR.
Replication in macrophage	—
Enterotoxin	—



## Clinical diseases of salmonella



- ◆ Source:
  - 1) Contaminated water, milk and food
  - 2) Human or animal excreta.
- ◆ S.typhi and S.paratyphi: the source is **human**.



# Salmonella {3}




Very Important

## Salmonella Gastroenteritis

<b>Etiology</b>	<ul style="list-style-type: none"> <li>◦ S. enterica subsp. Enterica the common cause</li> <li>◦ <b>Infective dose: <math>10^6</math> bacteria</b></li> <li>◦ Incubation period : 8 – 36 hrs.</li> </ul>
<b>Source</b>	<ol style="list-style-type: none"> <li>1) Food poisoning through contaminated food.</li> <li>2) Source: poultry, milk, egg &amp; egg products and handling pets.</li> </ol>
<b>Symptoms</b>	<ul style="list-style-type: none"> <li>◦ Fever, chills, watery diarrhea and abdominal pain. Self limiting.</li> <li>◦ <b>In sickle cell, hemolytic disorders {7}</b>, ulcerative colitis, elderly or very young patients; <b>the infection may be very severe.</b></li> </ul>
<b>Treatment</b>	<ul style="list-style-type: none"> <li>◦ Uncomplicated cases require fluid and electrolyte replacement <u>only</u>.</li> <li>◦ Patients at high risk for dissemination and antimicrobial therapy is indicated.</li> </ul>

## Enteric (Typhoid) fever {4}

<b>Etiology</b>	<ul style="list-style-type: none"> <li>◦ Caused by Salmonella serotype typhi or S. paratyphi A, B and C (Less severe).</li> <li>◦ Incubation period: 9–14 days.</li> <li>◦ Bacteremia</li> <li>◦ Dissemination to multiple organs</li> </ul>
<b>Epidemiology {8}</b>	Common in tropical , subtropical countries, and travelers (sewage ,poor sanitation).
<b>Source</b>	<b>Ingestion of contaminated food by infected or carrier individual.</b>

<b>Pathogenesis {9}</b>	Early phase “First week”	Second phase “2nd & 3rd week”
	 <p><b>{10}</b></p> <ul style="list-style-type: none"> <li>◦ <b>Major symptoms:</b> fever, malaise, anorexia, myalgia and a continuous dull frontal headache then patient develops constipation</li> <li>◦ Ingestion of contaminated food → invasion of intestinal cells → Engulfment of Salmonella by mononuclear phagocytes and spread to mesenteric lymph node → Primary Bacteremia → Dissemination to multiple organ like: The reticuloendothelial system (liver, spleen, bone marrow) → Bacteria released into the bloodstream again and can lead to high fever (Secondary Bacteremia).</li> <li>◦ <b>Blood culture is positive.</b></li> </ul>	<ul style="list-style-type: none"> <li>◦ Sustained fever/prolonged fever &amp; prolonged bacteremia.</li> <li>◦ <b>Rash (Rose spots)</b> 2nd week of fever</li> <li>◦ Invade gallbladder and Payer's patches               <ul style="list-style-type: none"> <li>◦ Biliary tract → GIT</li> <li>◦ Organism isolated from stool</li> </ul> </li> </ul>

<b>Treatment {19}</b>	<ul style="list-style-type: none"> <li>◦ <b>Ceftriaxone or Ciprofloxacin</b></li> <li>◦ <b>Azithromycin (for uncomplicated).</b></li> <li>◦ <b>Azithromycin or Ceftriaxone for patients from India and SE Asia due to strains resistant to Ciprofloxacin. Ciprofloxacin can be used for patients from other areas</b></li> </ul>
<b>Complications of S. Typhi</b>	<ul style="list-style-type: none"> <li>◦ <b>Necrotizing cholecystitis.</b></li> <li>◦ <b>Bowel hemorrhage</b> and perforation</li> <li>◦ Pneumonia and thrombophlebitis</li> <li>◦ <b>Meningitis</b>, osteomyelitis, endocarditis and abscesses.</li> </ul>



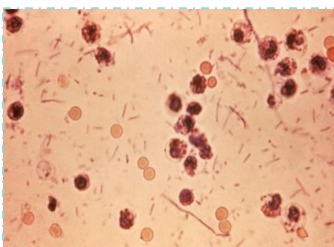
# Shigella {11}



Very  
Important

## Bacillary dysentery by shigella

<b>Overview</b>	<ul style="list-style-type: none"><li>◦ Shigella is <b>non lactose fermenting Gram negative</b> bacteria.</li><li>◦ It Causes: <b>bacillary dysentery</b> {12} (blood, mucus and pus in the stool) ◦ Incubation period: 24 - 48 hrs</li></ul>
<b>Etiology</b>	<ul style="list-style-type: none"><li>◦ <b>Human is the only reservoir</b>, Shigella has 4 species:<ol style="list-style-type: none"><li>1) S.sonnei (group D1) most predominant in USA ( fever, watery diarrhea)</li><li>2) S.flexneri (group B15) is the 2nd most common.</li><li>3) <b>S. dysenteriae</b> (group A 6 ) and S. boydii (group C 20) <b>are most common isolates in developing countries.</b></li><li>4) <b>S. dysenteriae type 1 associated with morbidity and mortality.</b></li></ol></li></ul>
<b>Antigenic factors</b>	<ul style="list-style-type: none"><li>◦ Shigella has 4 major O antigen groups.<ol style="list-style-type: none"><li>1) All Shigella species have O antigens</li><li>2) Some serotypes has K antigen (heat-labile, removed by boiling)</li><li>3) Shigella are non motile, <b>they lack H antigen.</b></li></ol></li></ul>
<b>Risk factors</b>	<ul style="list-style-type: none"><li>◦ Young children in daycare ◦ Young adult ( man who have sex with man) ◦ People in crowded area</li><li>◦ Anal oral sex in developed countries.</li></ul>
<b>Transmission</b>	<ol style="list-style-type: none"><li>1) <b>Food and water.</b> 2) Person to person through <b>fecal – oral route.</b></li><li>3) <b>Flies, fingers (have a role in spread).</b></li></ol>
<b>Pathogenesis</b>	<ul style="list-style-type: none"><li>◦ <b>Low infective dose &lt; 200 bacilli</b></li><li>◦ Penetrate epithelial cells, leads to local inflammation, shedding of intestinal lining and ulcer formation.</li></ul>
<b>Symptoms</b>	<ul style="list-style-type: none"><li>◦ High fever, chill, abdominal cramp and pain accompanied by <b>tenesmus , bloody stool with mucus &amp; leukocytes.</b></li><li>◦ Can lead to rectal prolapse in children</li></ul>
<b>Treatment</b> {13} Same as salmonella	<ul style="list-style-type: none"><li>◦ Antibiotic usage aiming to reduce duration of illness: - <b>Ceftriaxone or ciprofloxacin or Azithromycin or Trimethoprim-sulfamethaxazole or Ampicillin</b></li></ul>
<b>Complications</b>	<ul style="list-style-type: none"><li>◦ Ileus. ◦ <b>Obstruction dilatation and toxic megacolon</b> ◦ <b>Bacteremia in 4 % of severely ill patient.</b></li><li>◦ Seizures, <b>Hemolytic-uremic syndrome.</b></li></ul>





# Salmonella & Shigella {14},{15}

## ◆ Diagnosis Methods {16},{17}

### Stool culture :

On 1) selective selenite enrichment broth media 2) MAC 3) SS 4) XLD 5) **HEA** {18} 6) BS.

### Sero-grouping:

Based on O & H antigen

## ◆ Laboratory Findings

### Laboratory findings of salmonella and shigella in stool

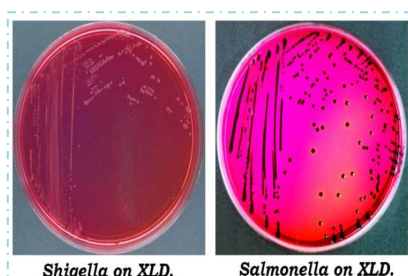
**Gram stain:** Both are gram negative bacilli

**Culture:** On Selective media (XLD) Salmonella produces black colonies due to H<sub>2</sub>S<sup>{1}</sup>

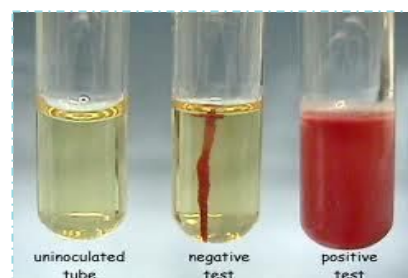
Biochemical test

**Motility tests**<sup>{2}</sup>

**Serology:** the final detection is by serotyping using agglutination Ag+Ab test for salmonella, shigella and E. coli



{1}



{2}



1. In this lecture we are going to discuss three infections:
  - food poisoning
  - gastroenteritis
  - typhoid fever
2. In general we have two classifications of diarrhea:
  - infectious diarrhea which caused by → Campylobacter , yersinia, Salmonella. They cause diarrhea for 3 days.
  - non infectious diarrhea
3. **infectious diarrhea:**
  - **Organisms:** Campylobacter, yersinia, salmonella
  - **Campylobacter:** causes diarrhea and it is self-limiting , usually we don't give the patient any treatment but in severe cases we do.
  - **Yersinia:** it mimics appendicitis but it is Mesenteric lymphadenitis(affect the small intestine) , **the patient will come suffering from right lower quadrant pain in abdomen**
  - **Salmonella:** it consists of two types →
    1. S.non typhi: it cause Gastroenteritis salmonellosi and if you treat the diarrhea that caused by it → you will increase the Carriage rate (without treatment the symptoms will carry in for two weeks, but with treatment it will last for a month. **So, it is better if we do not treat it**)
      - IC: 1-3 days.
    2. Salmonella typhi: it is a mild disease like a disease caused by campylobacter, but for some patients it causes severe fever and symptoms (for children, HIV patient, elderly patient, sickle- cell disease)
      - IC: longer 1-2 weeks or 5 days- 2 weeks
      - Salmonella typhi has two types: A- Salmonella typhi B- Salmonella paratyphi , and both of them cause typhoid fever.
4. **Salmonella typhi (المصيبة الكبيرة):**
  - it is not Gastroenteritis
  - the course of the disease is in stages and in each stage the symptoms differ
  - In the beginning it will be gastroenteritis but it will undergo dissemination of blood, lymph node , spleen, liver and again it will go to the blood then to the biliary tree then the biliary patches , and lead to rupture and complication.
  - Example: A nurse came from india and has been suffering from a fever for about 2-3 weeks , we treated her but still there is no response because she has resistance to antibiotics. we call it Enteric fever or we call it typhoid fever.





5. Why did we say that these disease are mild and others are severe? depending on the antigen that presents on the cell membrane of the bacteria, so that we classified the organisms depending on the antigens into:

- O → means somatic
- H → means flagella
- K → means capsule

So :

- Salmonella non-typhi has all these three antigens.
- Salmonella typhi → O,H,Vi, it has capsule but in order to differentiate between typhi and non typhi we named it as Vi ( which is highly virulent)

6. salmonella motile or nonmotile? motile because it has antigen H which refers to flagella

7. **Very important to know that sickle cell patient is highly susceptible to infections, like: streptococcus pneumoniae and salmonella**

8. S.typhi we mainly see it in foreign countries , for example: a saudi child came to the hospital suffering from typhi infection which was transmitted to him from his maid that came from india.

9. cycle of taphi:GI→ lymphatic→ macrophages → Blood→ short bacteremia→ goes to biliary tree , biliary patches→ stool.

10. one of the symptoms of Enteric fever is paradoxical bradycardia (55 for heart rate+ fever)

11. **Shigella:**

- incubation period : 1-2 days
- diarrhea→ small amount with blood+ mucus ( also known as dysentery )
- lower abdominal pain
- Infectious dose is 200 organisms (highly infectious)
- non motile → only O and K , No H

12. Shigella is not the only organism that causes dysentery , we have for example:

A. Amebic dysentery ( caused by protozoan parasite entamoeba histolytica)

B. Bacillary dysentery → they feel pain in the lower abdominal area (suprapubic region) and once they defecate they feel better

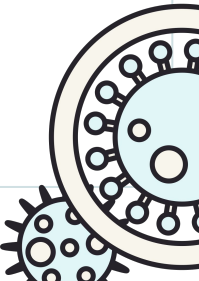
13. why is it important to treat shigella ?

- because even it causes a localized infection but has the ability to spread to other organs
- short IC → small dose of bacteria will lead to infection

14. **how do we differentiate between salmonella and shigella? by**

- motile or non motile
- Antigen on membrane
- Both of them are non lactose fermented

15. the similarities between salmonella and shigella : Diarrhea , non lactose fermented , oxidase negative.





## 16. Diagnosis of salmonella :

- S.non typhi → stool
- S.typhi → **blood culture** and stool
- why blood culture? because of the fever that is caused by bacteremia
- if we did serology we might find the organisms but blood culture is more important.
- H<sub>2</sub>S production( the one that produce black color dots in culture)
- morphology: colorless colonie with black dots in the center.

## 17. Diagnosis of shigella:

- stool → in liquid media(SB) → contain antibiotics that inhibit GI flora bacteria → then culture in HEA (selective media)
- morphology: only colorless colonies.

## 18. **the media we use to differentiate between salmonella and shigella is HEA.**

## 19. the drugs of the choice that is currently being used is ceftriaxone as an empirical treatment, but if there is resistance we will use Azithromycin →if there is resistance → we will use "الامبيبييا" عجزت القط وش يقول

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### General Notes

#### 1. **Food poisoning :organisms:** staphylococcus, clostridium perfringens, bacillus cereus (fried rice)

- **Symptoms:** vomiting , diarrhea(for 2-4 hours or for 6-12 hours)
- **Treatment:** Self-limiting
- **Incubation period:** half a day or one day
- **Type of diarrhea:** watery diarrhea
- **source :** uncooked food, like potato salad and sauce.

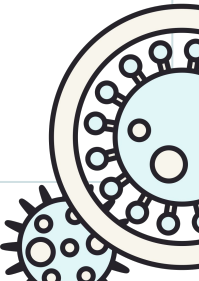
#### 2. **Sources :**

- Campylobacter → chicken
- yersinia → pork
- Shigella → human
- Salmonella has two sources :
  - o for non typhi → chickens mainly / reptiles / lizards / snakes
  - o for typhi → human (uncommon in Saudi Arabia ).

#### 3. another infection came from tropical & subtropical area which leads to death if left untreated: is malaria

#### 4. About the bacterial structural resistance :

Bacteria has a chromosome and sometimes a plasmid, the plasmid is the part that carries resistance (also called filmic of resistance), we noticed that if the organism holds mec resistance to ciprofloxacin, automatically it will hold mec resistance to ceftriaxone , So in other words, if the bacteria has resistance against ceftriaxone it will also have resistance against ciprofloxacin .





# Summary

## Salmonella

<b>General information</b>	<ul style="list-style-type: none"> <li>◦ Gram negative, motile, facultative anaerobic bacilli, Non lactose fermenting colonies.</li> <li>◦ It has Two species of Salmonella: <i>S. enterica</i> and <i>S. borgori</i> (rare).</li> </ul>
<b>Location</b>	Found in cold <b>blooded animals: Turtles, Snakes and Fish.</b> / Warm blooded animals: Birds, Rodents.
<b>Source</b>	<ul style="list-style-type: none"> <li>◦ <i>S. Non typhoid</i> : 1-Contaminated water, milk and food 2-Human or animal excreta.</li> <li>◦ <i>S.typhi</i> and <i>S.paratyphi</i>: the source is <b>human</b>.</li> </ul>
<b>Antigenic factor</b>	O. Somatic antigen   H. Flagellar antigen   K. Capsular antigen.   Vi. in Salmonella serotype typhi
<b>Virulence factors</b>	<ul style="list-style-type: none"> <li>◦ Fimbria - Adherence</li> <li>◦ Endocytosis.</li> <li>◦ Replication in macrophage</li> <li>◦ Enterotoxin</li> </ul>

## Clinical disease caused by Salmonella

1- Salmonella Gastroenteritis	
<b>Etiology</b>	◦ <b>Caused by Salmonella Non typhi</b> ◦ <i>S. enterica</i> subsp. ◦ <b>Infective dose: 10<sup>6</sup> bacteria</b>
<b>Source</b>	1) Food poisoning through contaminated food. 2) Source: poultry, milk, egg & egg products and handling pets.
<b>Symptoms</b>	<ul style="list-style-type: none"> <li>◦ Fever, chills, watery diarrhea and abdominal pain. Self limiting.</li> <li>◦ In sickle cell, hemolytic disorders the infection may be very severe.</li> </ul>
2- Enteric (Typhoid) fever	
<b>Etiology</b>	◦ Caused by Salmonella serotype typhi or <i>S. paratyphi</i>
<b>Epidemiology</b>	<i>S.typhi</i> we mainly see it in foreign countries , for example: a saudi child came to the hospital suffering from typhi infection which was transmitted to him from his maid that came from india.
<b>Source</b>	Ingestion of contaminated food by infected or carrier individual.
<b>Pathogenesis</b>	Early phase "First week"
	<ul style="list-style-type: none"> <li>◦ Ingestion of contaminated food → invasion of intestinal cells → Engulfment of Salmonella by mononuclear phagocytes and spread to mesenteric lymph node → Primary Bacteremia → Dissemination to multiple organ like: The reticuloendothelial system (liver, spleen, bone marrow) → Bacteria released into the bloodstream again and can lead to high fever (Secondary Bacteremia).</li> <li>◦ <b>Blood culture is positive.</b></li> </ul>
	Second phase "2nd & 3rd week"
	<ul style="list-style-type: none"> <li>◦ Sustained fever/prolonged fever &amp; prolonged bacteremia.</li> <li>◦ <b>Rash (Rose spots)</b> 2nd week of fever</li> <li>◦ Invade gallbladder and Payer's patches</li> <li>◦ Biliary tract → GIT</li> <li>◦ Organism isolated from stool</li> </ul>
<b>Treatment</b>	◦ <b>Ceftriaxone or Ciprofloxacin</b> ◦ <b>Azithromycin (for uncomplicated).</b>
<b>Complications of S. Typhi</b>	<ul style="list-style-type: none"> <li>◦ <b>Necrotizing cholecystitis.</b>   ◦ <b>Bowel hemorrhage</b> and perforation</li> <li>◦ Pneumonia and thrombophlebitis ◦ <b>Meningitis</b>, osteomyelitis, endocarditis and abscesses.</li> </ul>



# Summary

## Shigella

### Bacillary dysentery by shigella

<b>Overview</b>	<ul style="list-style-type: none"> <li>◦ Shigella is non lactose fermenting, non motile, Gram negative bacteria.</li> <li>◦ It Causes: bacillary dysentery ◦ Incubation period: 24 - 48 hrs, IC: 1-2day</li> </ul>
<b>Etiology</b>	◦ <b>Human is the only reservoir,</b>
<b>Antigenic factors</b>	1) All Shigella species have O antigens 2) Some serotypes has K antigen 3) <b>they lack H antigen.</b>
<b>Transmission</b>	1) <b>Food and water.</b> 2) Person to person through <b>fecal – oral route.</b>
<b>Pathogenesis</b>	<ul style="list-style-type: none"> <li>◦ <b>Low infective dose &lt; 200 bacilli</b></li> <li>◦ Penetrate epithelial cells, leads to local inflammation, shedding of intestinal lining and ulcer formation.</li> </ul>
<b>Symptoms</b>	<ul style="list-style-type: none"> <li>◦ High fever, chill, abdominal cramp and pain accompanied by <b>tenesmus , bloody stool with mucus &amp; leukocytes.</b></li> <li>◦ Can lead to rectal prolapse in children</li> </ul>
<b>Treatment</b>	<b>Ceftriaxone or ciprofloxacin or Azithromycin or Trimethoprim-sulfamethaxazole or Ampicillin</b>
<b>Complications</b>	<ul style="list-style-type: none"> <li>◦ Ileus. ◦ Obstruction dilatation and toxic megacolon ◦ Bacteremia in 4 % of severely ill patient.</li> <li>◦ Seizures, Hemolytic-uremic syndrome.</li> </ul>

## How to detect Shigelle & salmonella

<b>Diagnosis Methods</b>	<p><b>Stool culture :</b></p> <p>1) selective selenite enrichment broth media    2) MAC    3) SS    4) XLD</p> <p>5) <b>HEA</b>    6) BS.</p> <p><b>Sero-grouping:</b> Based on O &amp; H antigen</p>
<b>Laboratory Findings</b>	<p><b>Gram stain:</b> Both are gram negative bacilli</p> <p><b>Culture:</b> On Selective media (XLD) Salmonella produces black colonies due to H<sub>2</sub>S</p> <p><b>Biochemical test, Motility tests Serology</b></p>



# MCQs

Q1 - Which of the following is the primary mode of transmission of Shigella?			
A) Vector-borne	B) Air-borne	C) Fecal-oral	D) Blood-borne
Q2 - A 28-year-old male has been diagnosed with typhoid fever. What is the most common source of transmission for Salmonella typhi?			
A) Contaminated water	B) Mosquito bites	C) Person-to-person contact	D) Animal bites
Q3 - A child is brought to the emergency room with bloody diarrhea and dehydration. Shiga toxin is detected in the stool sample. What complication should be closely monitored in this patient?			
A) HUS	B) Neurological sequelae	C) Septic shock	D) Liver failure
Q4 - Which one of the following organisms is gram -ve, non-lactose fermenter, +ve O antigen and -ve Vi and H antigen?			
A) Shigella	B) Salmonella	C) Cholera	D) E. coli
Q5 - A 48-year-old patient presented with salmonella gastroenteritis. Which of the following morbidities requires mandatory antibiotic therapy?			
A) Rheumatoid arthritis	B) Heart disease	C) DM	D) Sickle cell anemia
Q6 - A patient came with abdominal cramps, fever and bloody diarrhea. Microbiology lab isolated an organism that was suspected as salmonella spp. or shigella spp. Which one of the following is a <u>differentiating test</u> between the two organisms?			
A) Oxidase test	B) Fermentation test	C) Production of H <sub>2</sub> S	D) Gram stain

Answer key: 1-C 2-A 3-A 4-A 5-D 6-C



# SAQ

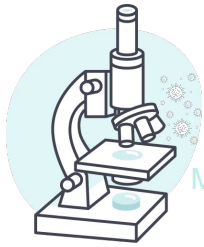
## Case 1

A 4 year old child presented with fever, diarrhea and skin rash her mother said that she has a fever, constipation and malaise last week. The doctor took stool and blood samples for culture ,gram stain and motility tests. The gram stain showed gram negative bacilli , the motility test was positive and the culture on XLD media showed the growth of black colonies.

- Q1: What is the most likely diagnosis?
- Q2: What is most likely the causative agent?
- Q3: What do you expect the blood culture to show?
- Q4: What caused the growth of black colonies on XLD media?
- Q5: Name 2 complications of this disease

### Answers

- A1:** Typhoid fever
- A2:** S.Typhi
- A3:** Positive for salmonella typhi
- A4:** H<sub>2</sub>S production by salmonella
- A5:** Meningitis, bowl hemorrhage, necrotizing polycystitis, and Pneumonia



TEAM 443  
MICROBIOLOGY

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Danah Almuhausen

Abdulrahman Almusallam

Remaz Almahmoud

Areej Alquraini

Zeyad Alotaibi

Aleen Alkulyah

Layan Al-Ruwaili

Luay Alhudaithy

Rafan Alhazzani

Haya Alzeer

Nazmi A Alqutub

Reuf Alahmari

Raseel Almutairi

Rahaf Alshowihi

Reena Alsadoni