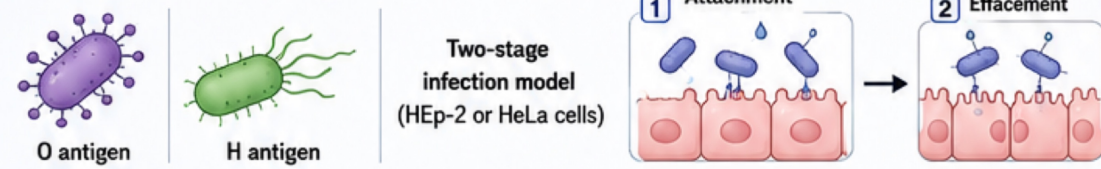


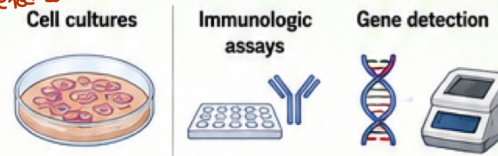
EPEC

- EPEC strains are identified by **O antigen** and occasionally by **H antigen typing**, and a **two-stage infection model** using **HEp-2 or HeLa cells** also can be performed for EPEC (in reference labs).

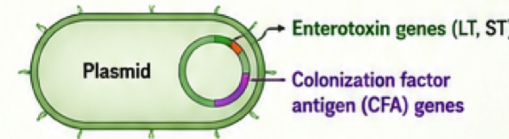


ETEC

- Assays for ETEC **heat-labile toxin (LT)**, such as **cell cultures**, **immunologic assays** and **gene detection**, are used in reference labs.



- The **plasmids** carrying the genes for **enterotoxins** may also carry genes for the **colonization factor antigens (CFAs)** that facilitate attachment of *E. coli* strains to intestinal epithelium.



MNEMONIC

EPEC = "O, H, 2 Cell"

- O** O antigen
- H** H antigen typing
- 2** Two-stage infection model
- HEp-2 or HeLa cells**

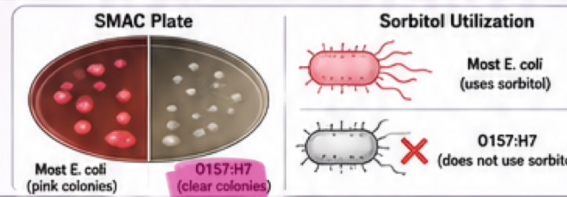
ETEC = "L, C, I, G, P"

- L** Heat-Labile toxin (LT)
- C** Cell cultures
- I** Immunologic assays
- G** Gene detection
- P** Plasmids with enterotoxin & CFA genes

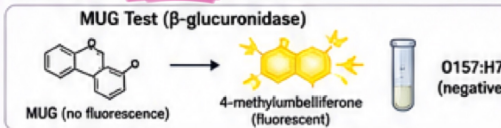
DIAGNOSIS OF STEC, EIEC, AND EAEC

STEC (Especially O157:H7)

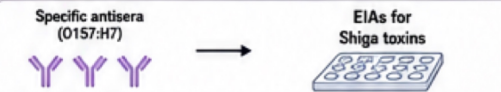
- STEC **O157:H7** does not use sorbitol, unlike most other *E. coli*, and is **negative** (clear colonies) on **sorbitol MacConkey agar (SMAC)**.



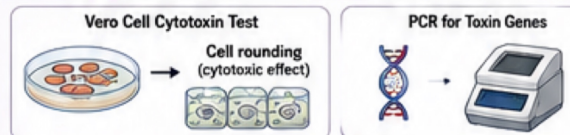
- O157:H7 strains also are **negative** for β -glucuronidase using the substrate 4-methylumbelliferyl- β -glucuronide (**MUG test**).



- Specific **antisera** are used to identify the O157:H7 strains, and **enzyme immunoassays (EIAs)** for **Shiga toxins** detection.



- Other tests for STEC: **cell culture** **cytotoxin testing** using **Vero cells** and **polymerase chain reaction** for **direct detection of toxin genes** directly from **stool** samples.

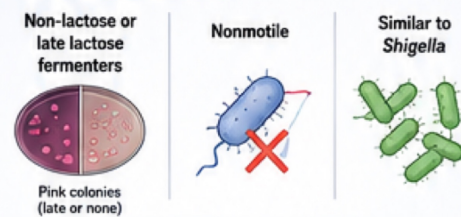


STEC O157:H7 MNEMONIC "S.O.R.B.I.T.O.L."

- S** Sorbitol - does NOT use sorbitol (clear on SMAC)
- O** O157:H7
- R** β -glucuronidase - negative (MUG test)
- B** Blue/clear (no color change in MUG)
- I** Immunoassay (EIAs for Shiga toxins)
- T** Toxin detection
- O** Other tests - Vero cell cytotoxin, PCR
- L** Look for toxin genes in stool (PCR)

EIEC

- EIEC strains are **similar to Shigella** and are **non-lactose or late lactose fermenters** and are **nonmotile**.

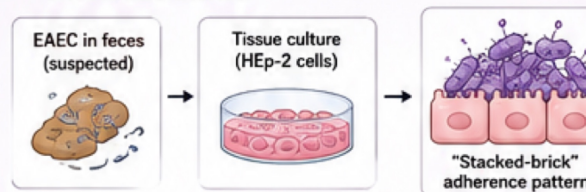


EIEC = "S.N.N."

- S** Similar to Shigella
- N** Non-lactose (or late lactose fermenters)
- N** Nonmotile

EAEC

- EAEC can be suspected clinically but requires confirmation by **tissue culture adhesion assays**, which are not readily available in most clinical laboratories.



QUICK SUMMARY TABLE

Pathotype	Key Identification Features	Key Tests
EPEC	Identified by O antigen and occasionally by H antigen typing, and a two-stage infection model using HEp-2 or HeLa cells also can be performed for EPEC (in reference labs).	O & H typing, two-stage infection model (HEp-2/HeLa cells)
ETEC	Assays for ETEC heat-labile toxin (LT), such as cell cultures, immunologic assays and gene detection, are used in reference labs. Plasmids may carry enterotoxin and CFA genes.	LT detection (cell culture, immunologic assays, gene detection), Plasmid analysis (enterotoxin & CFA genes)
STEC (O157:H7)	Does not use sorbitol (clear on SMAC), β -glucuronidase negative (MUG test). Identified by antisera (O157:H7) and EIAs for Shiga toxins. Other tests: Vero cell cytotoxin, PCR for toxin genes.	SMAC, MUG test, antisera, EIAs for Shiga toxins, Vero cell cytotoxin, PCR for toxin genes
EIEC	Similar to Shigella; non-lactose or late lactose fermenters; nonmotile.	Biochemical tests, motility test
EAEC	Can be suspected clinically but requires confirmation by tissue culture adhesion assays, which are not readily available in most clinical laboratories.	Tissue culture adhesion assays (not routinely available)

REMEMBER ALL TOGETHER!

- EPEC** sticks in 2 steps,
- ETEC** leaks with LT,
- STEC** brings Shiga toxins,
- EIEC** acts like Shigella,
- EAEC** stacks like bricks!

TREATMENT AND PREVENTION OF DIARRHEAGENIC E. COLI

- The duration of **EPEC** diarrhea can be **shortened** and the **chronic diarrhea** cured by **antibiotic treatment**.
- When **ETEC** diarrhea develops, antibiotic treatment effectively **shortens the duration of disease**.
- For **traveler's diarrhea**, antimicrobial **prophylaxis** can be **effective** but may result in **increased antibiotic resistance** and probably should not be uniformly recommended.
- It is widely recommended that **caution** be observed in regard to food and drink in areas where environmental sanitation is poor.
- Early and brief treatment, for example with **ciprofloxacin** or **trimethoprim-sulfamethoxazole**, should be substituted for prophylaxis of traveller's diarrhea.
- Many cases of hemorrhagic colitis and its associated complications can be prevented by **thoroughly cooking ground beef** and by **avoiding unpasteurized products** such as apple cider.

MNEMONIC: "E. COLI CARE"

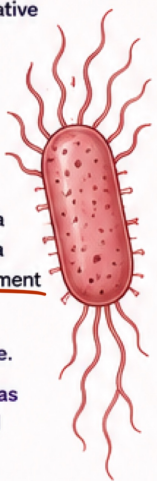
- C** Cure EPEC with antibiotics (shorten & cure)
- A** Antibiotics shorten ETEC diarrhea
- R** Resist overuse – avoid uniform antimicrobial prophylaxis
- E** Exercise caution with food and drink in poor sanitation areas
- C** Ciprofloxacin or trimethoprim-sulfamethoxazole for early & brief treatment
- R** Avoid unpasteurized products
- E** & cook ground beef thoroughly

Think: **CARE** for E. coli = Cure, Avoid, Resist, Exercise caution!

SALMONELLA MICROBIOLOGICAL AND BIOCHEMICAL FEATURES

- Salmonellae are non-spore-forming, facultative anaerobic, Gram-negative bacilli that **vary in length**.
- Most isolates are **motile** with **peritrichous flagella**.
- Salmonellae grow readily on **simple agar media**.
- They are able to **utilize citrate** as a sole carbon source and **lysine** as a nitrogen source, almost **never ferment lactose or sucrose**.
- Salmonellae are oxidase negative.
- They form acid and sometimes gas from **fermentation of glucose and mannose**.
- They usually **produce H₂S**.

all covered it



KEY FEATURES AT A GLANCE					
GRAM STAIN Gram-negative bacilli 	MOTILITY Motile with peritrichous flagella 	SPORES Non-spore-forming 			
OXYGEN REQUIREMENT Facultative anaerobic 	GROWTH Grows readily on simple agar media 	BIOCHEMICAL REACTIONS			
		Citrate (C source) Positive (utilized)	Lysine (N source) Positive (utilized)	Lactose Almost ferment	Sucrose Almost ferment
OXIDASE TEST Oxidase negative	CARBOHYDRATE FERMENTATION Acid and sometimes gas from fermentation of glucose and mannose 	H₂S PRODUCTION Usually produces H ₂ S 			

MNEMONIC: "Salmonella"

S Simple agar growth

A Anaerobic (facultative)

L Lysine utilized

M Motile (peritrichous flagella)

O Oxidase negative

N Non-spore-forming

E Employs citrate (as sole C source)

L Lactose not fermented

L Little to no sucrose fermentation

A Acid (± gas) from glucose & mannose; produces H₂S

Think: **SALMONELLA** has it **ALL** (features)!

DIAGNOSTIC SPECIMENS

- Freshly passed **stool** is the **preferred specimen** for diagnosis of **non-typhoidal Salmonella**.
- Specimens collected during the early stages of enteric illness have the highest yield.
- Collection of multiple stool specimens may enhance recovery of *Salmonella* and other enteric pathogens (eg, *Shigella*).
- For definitive diagnosis of **enteric fever**, *Salmonella Typhi* or *Salmonella Paratyphi* must be isolated in culture.

Appropriate specimens for enteric fever include blood (most commonly used), bone marrow, (stool), other sterile sites, urine, or intestinal secretions.

Blood (most commonly used)

Bone marrow

Urine

Intestinal secretions

Other sterile sites

- Blood** cultures are often positive in the **first week** of enteric fever and septicemia.
- Bone marrow** cultures have the **highest sensitivity**, 80% to 95%, but are clinically less practical.
- Urine** culture may become positive **after the second week** of illness.

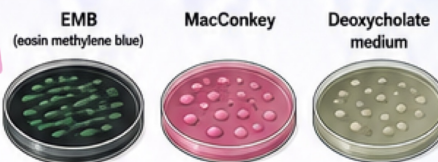
MNEMONIC: "B.B.U.I."

- B** Blood (early week)
- B** Bone marrow (highest yield)
- U** Urine (after 2nd week)
- I** Intestinal secretions

Think: **B.B.U.I.** = **Best specimens Used in illness!**

CULTURE AND LABORATORY IDENTIFICATION

- Specimens may be cultured on differential media, including **EMB (eosin methylene blue)**, **MacConkey**, or **deoxycholate medium**, which permit rapid detection of **lactose non-fermenters**.



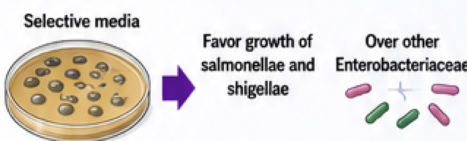
- Bismuth sulfite medium** permits rapid detection of **salmonellae**, which form **black colonies** because of **H₂S** production.



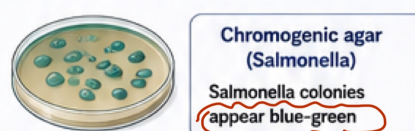
- Selective media** include **salmonella-shigella agar**, **Hektoen enteric agar**, **xylose-lysine desoxycholate agar**, and **desoxycholate-citrate agar**.



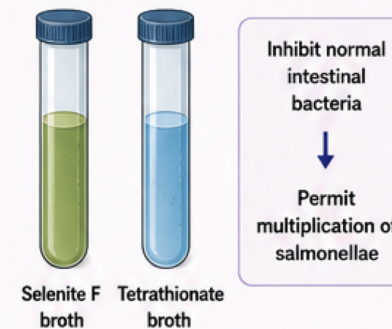
- These selective media **favor** growth of **salmonellae** and **shigellae** over other **Enterobacteriaceae**.



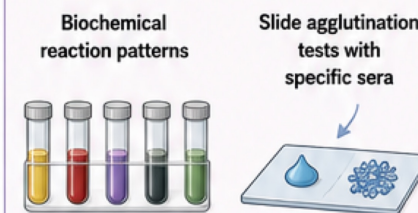
- Chromogenic agars** specifically for **Salmonella** recovery are also available.



- Enrichment cultures:** Stool specimens can also be placed into **selenite F** or **tetrathionate broth**, which inhibit normal intestinal bacteria and permit multiplication of **salmonellae**.

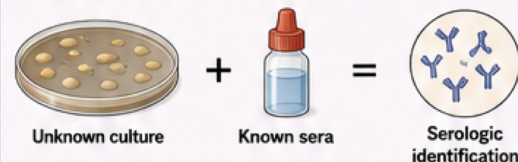


- Suspect colonies from solid media are identified by **biochemical reaction patterns** and **slide agglutination tests** with specific sera.

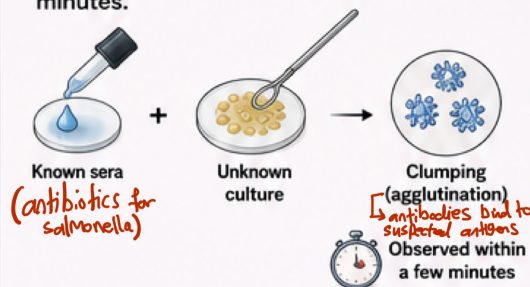


SEROLOGY AND MOLECULAR DIAGNOSIS

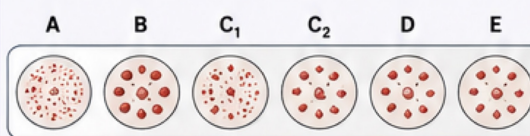
- Serologic techniques** are used to identify **unknown cultures** with **known sera**, but **antibody testing is not very useful** for diagnosis of **Salmonella** infections.



- In the **agglutination test**, known sera and unknown culture are mixed on a slide, and clumping can be observed within a few minutes.

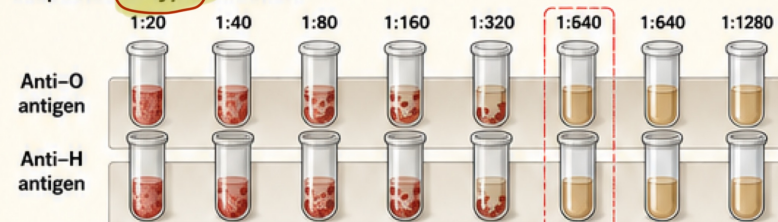


- Commercial kits are available to agglutinate and **serogroup salmonellae** by **O antigens: A, B, C₁, C₂, D, and E**



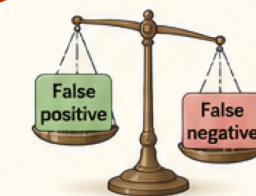
- Tube dilution agglutination test (the Widal test)** detects antibodies against **O and H antigens** in suspected **S. Typhi** infection.

For Salmonella, serology is less reliable than culture methods.



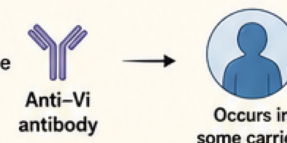
- At least **two serum specimens**, obtained **7-10 days** apart, are needed to prove a rise in antibody titer.

- False-positive and false-negative** results occur, so serologic tests **cannot be relied upon** to establish a definitive diagnosis of typhoid fever.



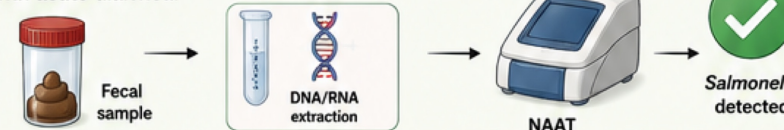
- A single titer against the **O antigen of greater than 1:320** and against the **H antigen of greater than 1:640** is considered positive.

- High titer of antibody to the **Vi antigen** occurs in some **carriers**.

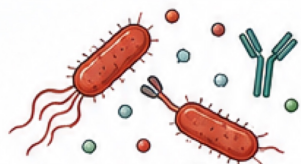


This means 2 specimens are taken to approve rising Antibody titer (needed for diagnosis)

- Several commercial **NAATs** are available for **direct detection** of salmonellae in **fecal** samples of patients with acute diarrhea.



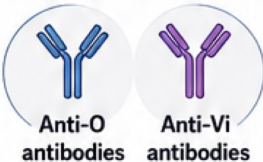
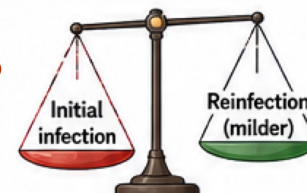
IMMUNITY



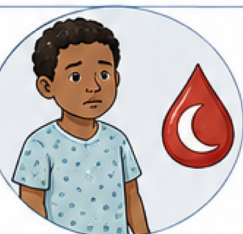
- Infections with *Salmonella Typhi* or *Salmonella Paratyphi* usually confer a certain degree of **immunity**.



- While **reinfection** may occur, it is often **milder** than the initial infection.



- Circulating antibodies to **O** and **Vi** are related to **resistance** to infection and disease, however, **relapses** may occur in **2-3 weeks**.



- Children, with **sickle cell disease** or sickle cell trait are more susceptible to *Salmonella* infections particularly to **bacteremia** and its complications (eg, **osteomyelitis**). *Sickle cells => Salmonella*



TREATMENT OF SALMONELLA INFECTIONS

- Non-typhoidal *Salmonella* gastroenteritis is typically **self-limited**; antimicrobial therapy is usually **not necessary** and **not recommended**.



- In severe diarrhea, replacement of **fluids and electrolytes** is essential.



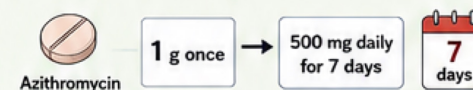
- Antimicrobial treatment of *Salmonella* gastroenteritis should be considered in **neonates**, **immunosuppressed patients**, and patients **older than 50 years** with suspected or confirmed **vascular disease**.
when??
because Salmonella can seed damaged vessels and cause aneurysms.



- For susceptible organisms, **oral** therapy with **amoxicillin**, **trimethoprim-sulfamethoxazole**, or a **fluoroquinolone** is appropriate.
(ciprofloxacin)



Uncomplicated enteric fever can be managed with **oral azithromycin**, 1 g once, followed by 500 mg daily for 7 days.



- Patients with complications should be **hospitalized** and treated with a **parenteral third-generation cephalosporin** or **fluoroquinolone** for **at least 10 days**.
(ceftriaxone)



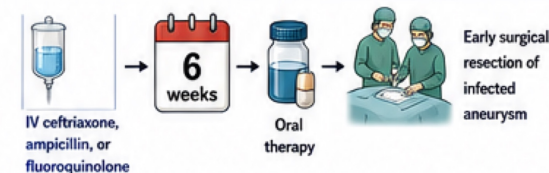
- In **chronic carriers**, **ampicillin** combined with **cholecystectomy** can be **therapeutic**.



- Non-typhoidal *Salmonella* bacteremia should be empirically treated with a third-generation cephalosporin, such as **ceftriaxone**, and a **fluoroquinolone** until antimicrobial susceptibility testing results are available.



- Endovascular infection (eg, infected aneurysm) should be treated with **intravenous ceftriaxone**, **ampicillin**, or a **fluoroquinolone** for **6 weeks**, followed by oral therapy; **early surgical resection** of an infected aneurysm is recommended.

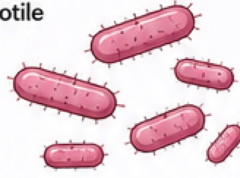


Summary:

Condition	Treatment
Mild non-typhoidal gastroenteritis	Fluids/electrolytes only
High-risk non-typhoidal cases	Amoxicillin, TMP-SMX, fluoroquinolone
Uncomplicated typhoid fever	Oral azithromycin
Complicated typhoid fever	IV ceftriaxone or fluoroquinolone
Chronic carrier	Ampicillin + cholecystectomy
Salmonella bacteremia	Ceftriaxone + fluoroquinolone
Endovascular infection	Long IV therapy + surgery

SHIGELLAE MICROBIOLOGICAL, BIOCHEMICAL, AND IDENTIFICATION FEATURES

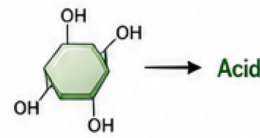
- Shigellae are slender, nonmotile Gram-negative rods; **coccobacillary** forms occur in **young cultures**.



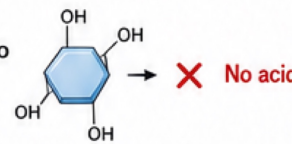
- Shigellae are facultative anaerobes but **grow best aerobically**.



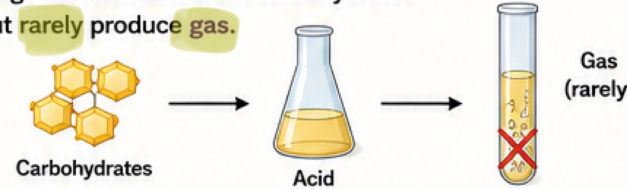
- All strains of *Shigella* species **ferment glucose**.



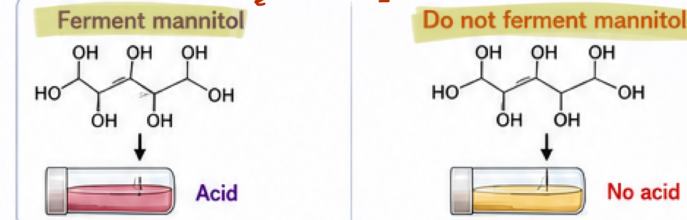
- With the exception of *Shigella sonnei*, they do not ferment **lactose**.



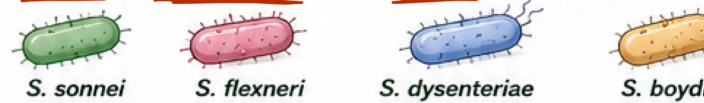
- Shigellae form **acid** from carbohydrates but **rarely produce gas**.



- They may also be divided into those organisms that ferment **mannitol** and those that do not.



- The **pathogenic species** are *S. sonnei*, *S. flexneri*, *S. dysenteriae*, and *S. boydii*.



DIAGNOSTIC LABORATORY TESTS AND IDENTIFICATION

- For **optimal organism recovery**, fecal specimens should be collected during the **early stages** of the illness.

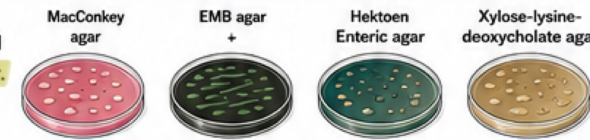


- Specimens include **fresh stool**, **mucus flecks**, and **rectal swabs** for culture.

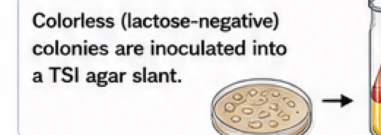
- While **whole stool** is usually the preferred specimen for laboratory workup of diarrhea, **rectal swabs with visible fecal staining** may be the preferred specimen for the isolation of shigellae.



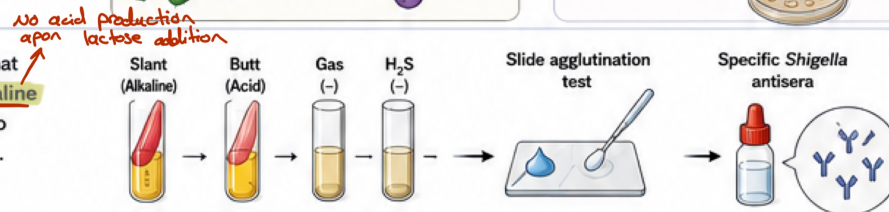
- The materials are streaked on differential media such as **MacConkey** or **EMB agar** and on selective media such as **Hektoen Enteric agar** or **xylose-lysine-deoxycholate agar**.



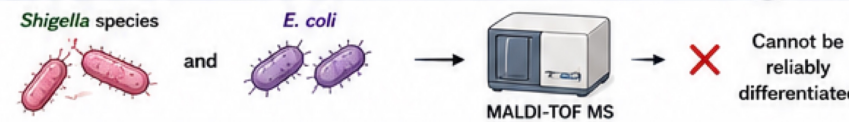
- These **selective media suppress other Enterobacteriaceae** and **Gram-positive organisms**.



- Nonmotile organisms that **fail to produce H₂S**, that produce **acid but not gas** in the butt and an **alkaline slant** in TSI agar medium, should be subjected to slide agglutination by specific *Shigella* antisera.



- It should be noted that *Shigella* species and *E. coli* cannot be reliably differentiated by **MALDI-TOF MS**.



- There are several commercial **NAATs** that directly detect shigellae in fecal samples along with some of the other major enteric pathogens.



- Serology is not used** to diagnose *Shigella* infections.

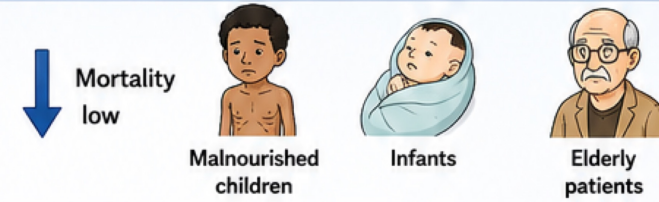


TREATMENT OF SHIGELLOSIS

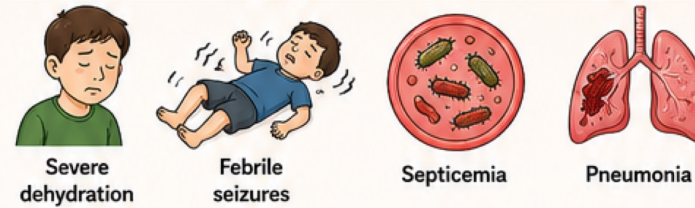
- In general, shigellosis is a **self-limited illness**, and many patients recover without treatment within **5-7 days**.



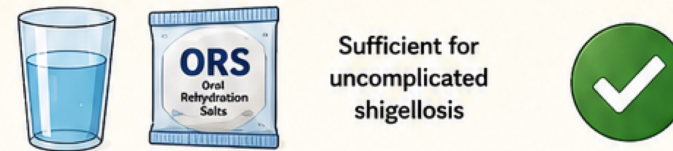
- Mortality** is generally **low** in shigellosis, **except** in **malnourished children, infants, and elderly patients**.



- Severe dehydration, febrile seizures, septicemia, and pneumonia** are **potential complications** of severe shigellosis.



- In general, **oral fluid replacement** is considered to be sufficient for treatment of **uncomplicated** shigellosis.



- In **high-risk patient populations**, **intravenous fluid replacement** may be required.



- Antidiarrheal** medications such as **loperamide** and **opioids** should be **avoided** in *Shigella* dysentery, as such medications may worsen the symptoms of the illness

حسبنا عنه بالفارما



Antibiotic treatment is recommended for the treatment of severe infections and to prevent secondary spread among people living in closed quarters or during outbreaks.

- Because of widespread **resistance**, **trimethoprim-sulfamethoxazole** and **ampicillin** are **no longer recommended** as first-line agents for treatment of shigellosis.



- Ciprofloxacin** and **ceftriaxone** are effective antibiotics of choice.



- Ceftriaxone** is commonly used for treatment of children with shigellosis.



- Azithromycin** has been shown as a useful antibiotic for treatment of **antibiotic-resistant Shigella** infections in adults and children

