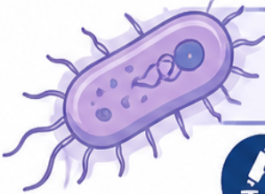


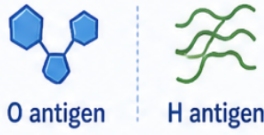
# DIARRHEAGENIC *E. COLI*



## DIAGNOSIS OF EPEC & ETEC

### EPEC

- EPEC strains are identified by **O antigen** and occasionally by **H antigen typing**




- A two-stage infection model using **HEp-2 or HeLa cells** also can be performed (in reference labs)



### ETEC

- Assays for **ETEC heat-labile toxin (LT)**:

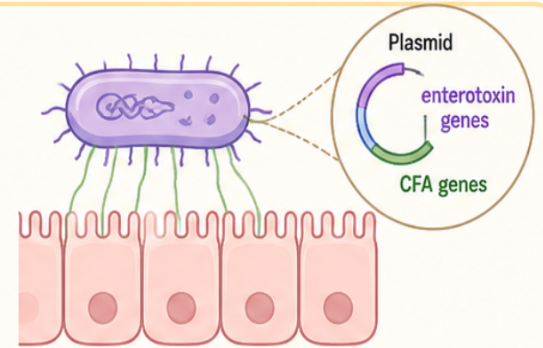
- ✓ cell cultures 
- ✓ immunologic assays 
- ✓ gene detection 
- Used in reference labs

### IMPORTANT

- The **plasmids** carrying the genes for **enterotoxins** may also carry genes for:

- colonization factor antigens (CFAs)**

→ facilitate attachment of *E. coli* strains to intestinal epithelium.



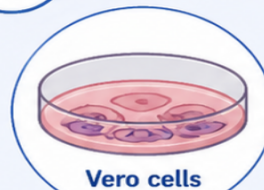
## DIAGNOSIS OF STEC, EIEC, EAEC

### STEC (O157:H7)

- ✓ Does not use **sorbitol**
- ✓ Negative (clear colonies) on **SMAC**
- ✓ Negative for  **$\beta$ -glucuronidase (MUG test)**
- ✓ Identified by:
  - specific antisera
  - enzyme immunoassays (EIAs) for **Shiga toxins**



### Other STEC tests



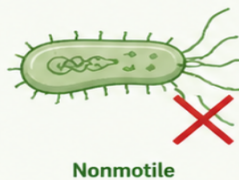
cell culture  
cytotoxin testing  
(Vero cells)






PCR for toxin genes  
(from stool)  
(directly from stool samples)

### EIEC

- ✓ Similar to **Shigella**
- ✓ Non-lactose or late lactose fermenters
- ✓ Nonmotile



### EAEC

- Can be suspected clinically 
- Requires tissue culture adhesion assays, which are 
- Not readily available in most labs 



# Treatment and Prevention of Diarrheagenic *E. coli*



## TREATMENT

### EPEC



- The duration of **EPEC** diarrhea can be shortened and the chronic diarrhea cured by antibiotic treatment.

### ETEC



- 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.



## PREVENTION



- 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**.



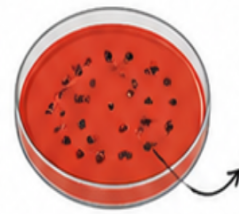
## KEY MESSAGE

Antibiotics shorten disease duration in EPEC and ETEC infections.  
Prevention through safe food, clean water, and proper cooking is crucial.





# SALMONELLA



Salmonella colonies on XLD agar

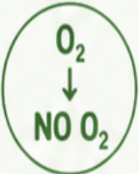
## MICROBIOLOGICAL & BIOCHEMICAL FEATURES



### MICROBIOLOGICAL FEATURES



Non-spore-forming



Facultative anaerobic



Gram-negative bacilli



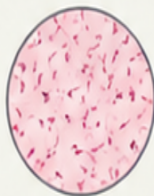
Most isolates are  
**Motile** with  
(peritrichous flagella)



Grow on simple agar

### SHAPE & APPEARANCE

Slender, rod-shaped bacilli  
(0.7–1.5  $\mu\text{m}$   $\times$  2–5  $\mu\text{m}$ )



### BIOCHEMICAL FEATURES



#### UTILIZE:

- citrate (carbon source)
- lysine (nitrogen source)



#### ALMOST NEVER FERMENT:

- lactose
- sucrose



Oxidase negative



#### FERMENT:

- glucose
- mannose  $\rightarrow$  acid  $\pm$  gas

*They form acid and sometimes gas from fermentation of glucose and mannose*

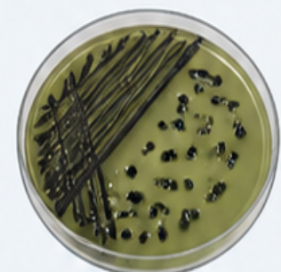


Usually produce  $\text{H}_2\text{S}$



### KEY POINTS

- ✓ Non-lactose fermenting, motile Gram-negative bacilli.
- ✓ Utilize citrate and lysine.
- ✓ Ferment glucose and mannose with acid  $\pm$  gas.
- ✓ Usually produce  $\text{H}_2\text{S}$  (black colonies on  $\text{H}_2\text{S}$ -containing media).

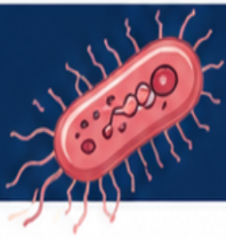


Black colonies on Bismuth sulfite agar

# CLINICAL DISEASES INDUCED BY **SALMONELLAE**



FEATURE	 <b>ENTERIC FEVER</b>	 <b>SEPTICEMIAS</b>	 <b>ENTEROCOLITIS</b>
 Incubation period	7–20 days	Variable	8–48 hours
 Onset	Insidious	Abrupt	Abrupt
 Fever	Gradual then high plateau with typhoid state	Rapid rise then spiking septic temperature	Usually low
 Duration of disease	Several weeks	Variable	2–5 days
 GI symptoms	Often early constipation later bloody diarrhea	Often none	Nausea, vomiting, diarrhea at onset
 Blood culture	Positive in first to second weeks of disease	Positive during high fever	Negative
 Stool culture results	Positive from second week on; negative earlier in disease	Infrequently positive	Positive soon after onset



# DIAGNOSTIC SPECIMENS



## 1 NONTYPHOIDAL SALMONELLA



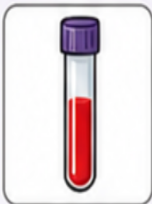
- Freshly passed stool is the preferred specimen for diagnosis of nontyphoidal *Salmonella*.
- Specimens collected during the early stages of enteric illness have the highest yield.
- Collection of multiple stool specimens may enhance recovery of *Salmonella* and other enteric pathogens (eg, *Shigella*).

## 2 ENTERIC FEVER – DIAGNOSIS



- For definitive diagnosis of enteric fever, *Salmonella Typhi* or *Salmonella Paratyphi* must be isolated in culture.

## 3 ENTERIC FEVER – SPECIMENS



Blood  
(most commonly used)



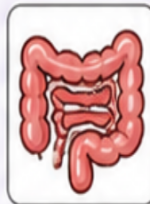
Bone marrow



Other sterile sites



Urine



Intestinal secretions

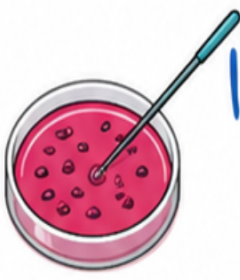
- Appropriate specimens for enteric fever include blood (most commonly used), bone marrow, other sterile sites, urine, or intestinal secretions.

## 4 CULTURE RESULTS (TIMING & YIELD)



- 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.

# CULTURE AND LABORATORY IDENTIFICATION



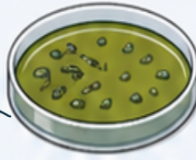
## 1 DIFFERENTIAL MEDIA (RAPID DETECTION)



EMB  
(eosin methylene blue)



MacConkey



Deoxycholate  
medium



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

## 2 BISMUTH SULFITE MEDIUM



H<sub>2</sub>S  
production



Bismuth sulfite medium permits rapid detection of salmonellae, which form black colonies because of H<sub>2</sub>S production.

## 3 SELECTIVE MEDIA



- 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.



Salmonella-  
shigella agar



Hektoen  
enteric agar



Xylose-lysine  
desoxycholate  
agar



Desoxycholate-  
citrate agar

## 4 CHROMOGENIC MEDIA



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

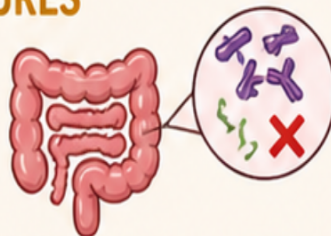
## 5 ENRICHMENT CULTURES



Selenite F  
broth



Tetrathionate  
broth

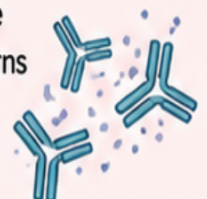


Enrichment cultures: Stool specimens can also be placed into selenite F or tetrathionate broth, which inhibit normal intestinal bacteria and permit multiplication of salmonellae.

## 6 IDENTIFICATION OF COLONIES

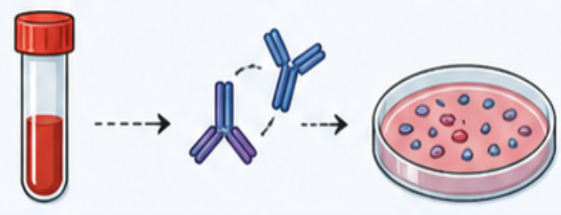


Suspect colonies from solid media are identified by biochemical reaction patterns and slide agglutination tests with specific sera.



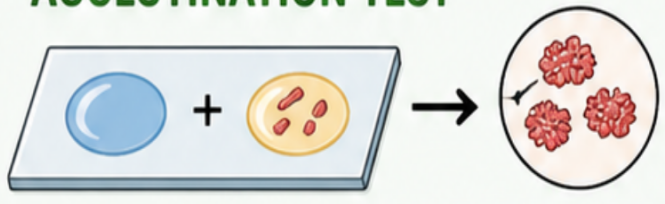
# SEROLOGY AND MOLECULAR DIAGNOSIS

## 1 SEROLOGIC TECHNIQUES (GENERAL)



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

## 2 AGGLUTINATION TEST



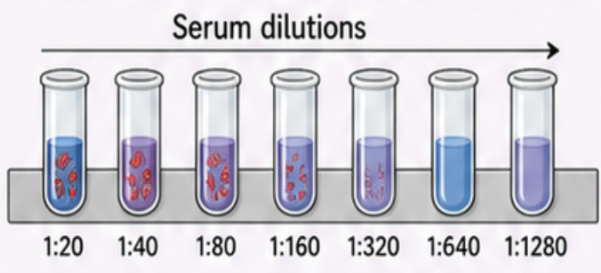
In the agglutination test, known sera and unknown culture are mixed on a slide, and clumping can be observed within a few minutes.

## 3 COMMERCIAL KITS (SEROGROUPING)



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

## 4 WIDAL TEST (TUBE DILUTION AGGLUTINATION TEST)



- Tube dilution agglutination test (the Widal test) detects antibodies against O and H antigens in suspected *S. Typhi* infection.
- At least two serum specimens, obtained 7–10 days apart, are needed to prove a rise in antibody titer.

## 5 LIMITATIONS OF SEROLOGY



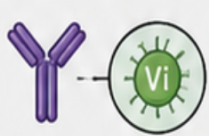
False-positive and false-negative results occur, so serologic tests cannot be relied upon to establish a definitive diagnosis of typhoid fever.

## 6 POSITIVE TITERS



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.

## 7 SPECIAL NOTE



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

## 8 MOLECULAR DIAGNOSIS



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

**TABLE 15–3: Representative Antigenic Formulas of *Salmonellae***

O Group	Serotype	Antigenic Formula
D	<i>Salmonella Typhi</i>	<b>9, 12</b> (Vi): d: —
A	<i>Salmonella Paratyphi A</i>	<b>1, 2, 12a</b> : —
C <sub>1</sub>	<i>Salmonella Choleraesuis</i>	<b>6, 7</b> : c: 1,5
B	<i>Salmonella Typhimurium</i>	<b>1, 4, 5, 12</b> : i: 1,2
D	<i>Salmonella Enteritidis</i>	<b>1, 9, 12</b> : g, m: —

**Notes:**

- O antigens: **boldface numerals**
- (Vi): Vi antigen if present
- Phase 1 H antigen: **lowercase letter**
- Phase 2 H antigen: **numeral**



# IMMUNITY



## 1 Immunity after infection



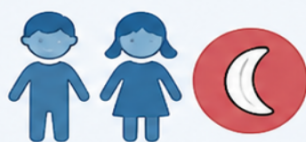
- ✓ Infections with *Salmonella Typhi* or *Salmonella Paratyphi* usually confer a certain degree of immunity.
- ✓ While reinfection may occur, it is often milder than the initial infection.

## 2 Antibodies & relapse



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

## 3 High-risk group



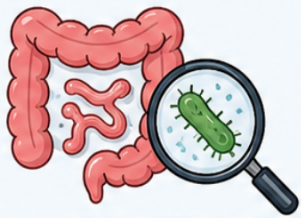
- Children, with sickle cell disease or sickle cell trait are more susceptible to *Salmonella* infections particularly to bacteremia and its complications (eg, osteomyelitis).



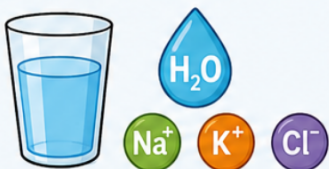
# TREATMENT OF **SALMONELLA** INFECTIONS



## 1 Non-typhoidal *Salmonella* (Gastroenteritis)



- 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.

## 2 When to give antibiotics



- Antimicrobial treatment of *Salmonella* gastroenteritis should be considered in neonates, immunosuppressed patients, and patients older than 50 years with suspected or confirmed vascular disease.

## 3 Drug options (if susceptible)

For susceptible organisms, oral therapy with:



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

## 4 Enteric fever

- ✓ **Uncomplicated enteric fever** can be managed with **oral azithromycin**



- ⚠ **Patients with complications** should be hospitalized and treated with a **parenteral third-generation cephalosporin** or **fluoroquinolone**



# 5 Special cases ☆



CHRONIC CARRIER



AMPICILLIN



CHOLECYSTECTOMY

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

- Non-typhoidal *Salmonella* bacteremia should be empirically treated with:

THIRD-GENERATION CEPHALOSPORIN (such as ceftriaxone)

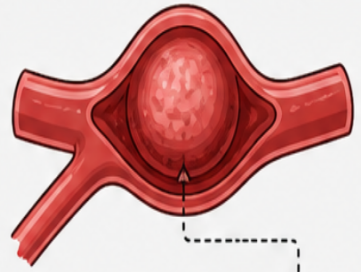


FLUOROQUINOLONE



until antimicrobial susceptibility testing results are available.

# 6 Severe infection ⚠️



ENDOVASCULAR INFECTION (eg, infected aneurysm)

- Endovascular infection (eg, infected aneurysm) should be treated with intravenous:

CEFTRIAXONE



OR

AMPICILLIN



OR

FLUOROQUINOLONE



- followed by

ORAL THERAPY



- early **surgical resection** of an infected aneurysm is recommended.





# SHIGELLAE MICROBIOLOGICAL, BIOCHEMICAL, AND IDENTIFICATION FEATURES

## 1 General characteristics



Slender, nonmotile Gram-negative rods

Coccobacillary forms occur in young cultures.

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

- Shigellae are facultative anaerobes but grow best aerobically.



## 2 Fermentation



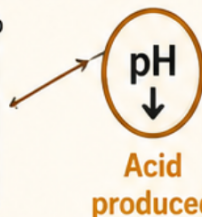
Glucose  
 $C_6H_{12}O_6$

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

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

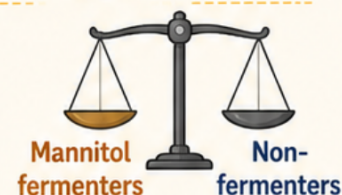


## 3 Biochemical features



- 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.



## 4 Pathogenic species



- The pathogenic species are:

- *S. sonnei*
- *S. flexneri*
- *S. dysenteriae*
- *S. boydii*



### TABLE 15–2: Pathogenic *Shigella* Species

Present Designation	Group and Type	Mannitol	Ornithine Decarboxylase
<i>Shigella dysenteriae</i>	<b>A</b>	–	–
<i>Shigella flexneri</i>	<b>B</b>	+	–
<i>Shigella boydii</i>	<b>C</b>	+	–
<i>Shigella sonnei</i>	<b>D</b>	+	+

# 1. Specimen collection



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

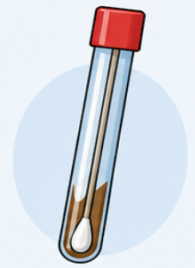


Specimens include:

- fresh stool
- mucus flecks
- 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*.



# 2. Culture media

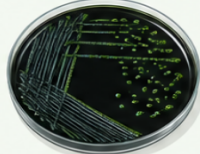


The materials are streaked on:

- differential media such as **MacConkey** or **EMB agar**



MacConkey agar



EMB agar

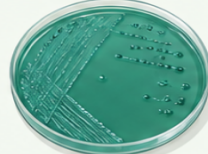
- and on selective media such as

**Hektoen Enteric agar** or

**xylose-lysine-deoxycholate agar**



Hektoen Enteric agar



xylose-lysine-deoxycholate agar



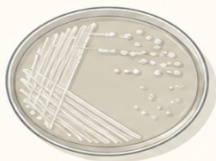
These selective media:

- suppress other *Enterobacteriaceae*
- and Gram-positive organisms

# 3. Colony identification (TSI step)

Colorless (lactose-negative) colonies are:

- inoculated into a **TSI** agar slant



Colorless (lactose-negative) colonies



TSI agar slant

Nonmotile organisms that:

- fail to produce **H<sub>2</sub>S**
- that produce **acid** but not **gas** in the butt
- and an **alkaline** slant in **TSI** agar medium

Alkaline (slant)

Acid (butt)



No H<sub>2</sub>S (no blackening)

No gas (no cracks or bubbles in the butt)

should be subjected to:

- slide agglutination by specific *Shigella* antisera

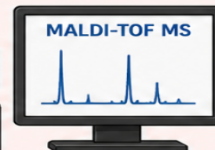


Positive agglutination

# 4. Important lab note

It should be noted that:

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



Cannot be reliably differentiated

# 5. Molecular tests

There are several commercial **NAATs** that:

- ✓ directly detect *shigellae* in fecal samples
- ✓ along with some of the other major enteric pathogens

NAAT (Multiplex PCR) Panel



- *Shigella* spp.
- *Salmonella* spp.
- *Campylobacter* spp.
- Others

# 6. Serology

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

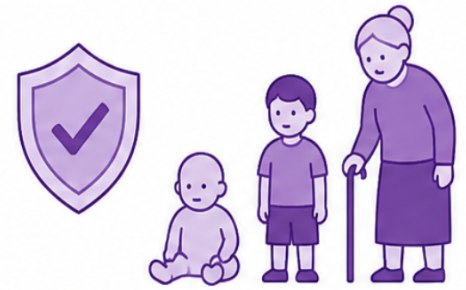


# Treatment of Shigellosis



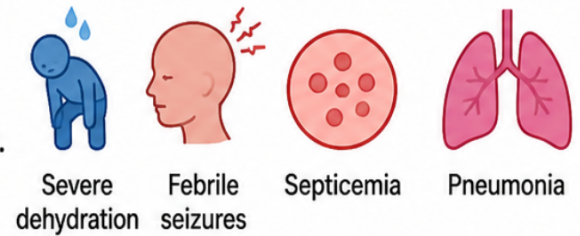
## General

- 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.



## Complications

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



## Fluid Replacement

- 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.



Oral fluid replacement

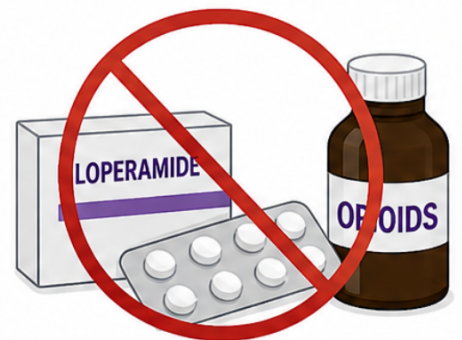


Intravenous fluid replacement



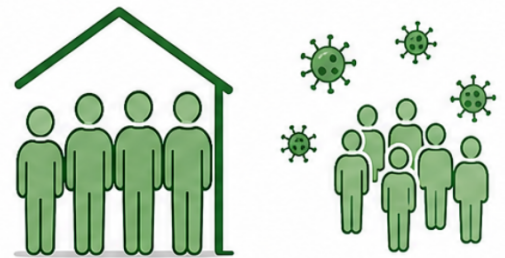
## Antidiarrheal Drugs

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



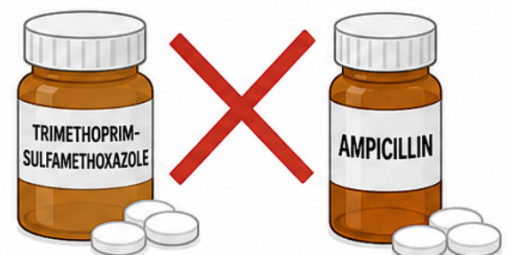
## Antibiotics – Indications

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



## Antibiotics – Resistance

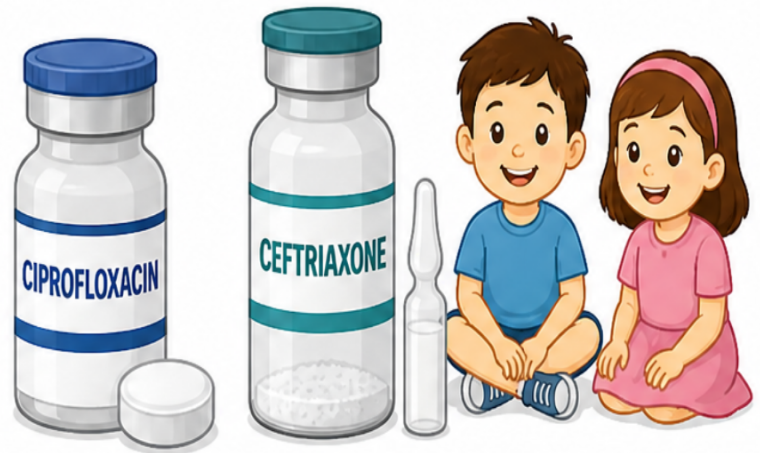
- Because of widespread resistance, trimethoprim-sulfamethoxazole and ampicillin are no longer recommended as first-line agents for treatment of shigellosis.





## Antibiotics – Drugs of Choice

- Ciprofloxacin and ceftriaxone are effective antibiotics of choice.
- Ceftriaxone is commonly used for treatment of children with shigellosis.



## Alternative Antibiotic

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

