# *Enteric gram-negative bacterial infections of the Gastrointestinal tract 2*

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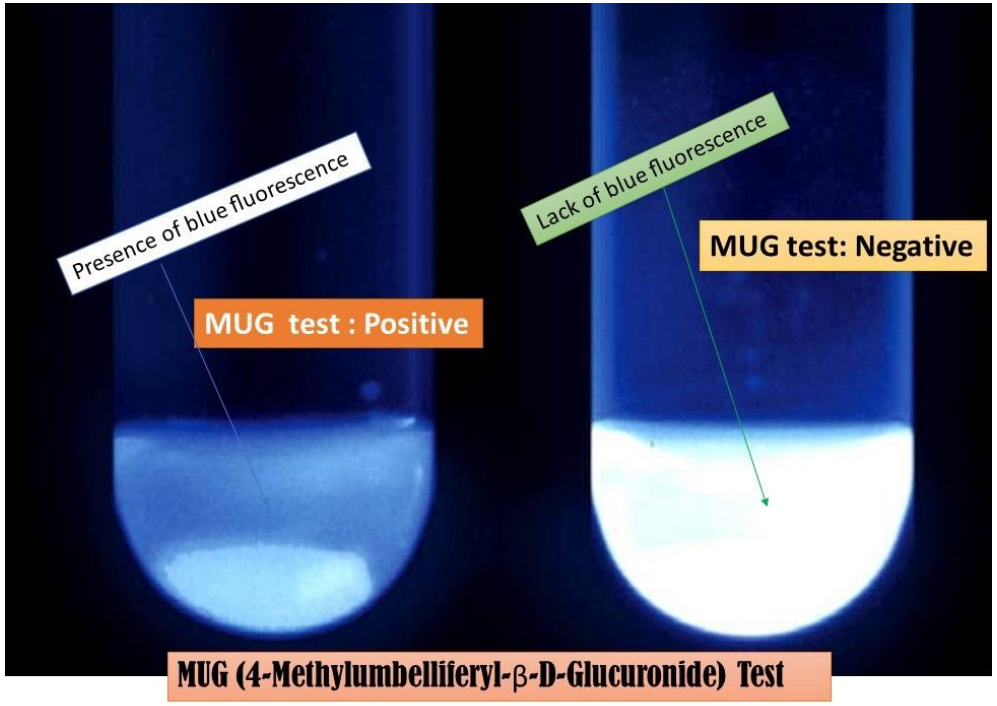
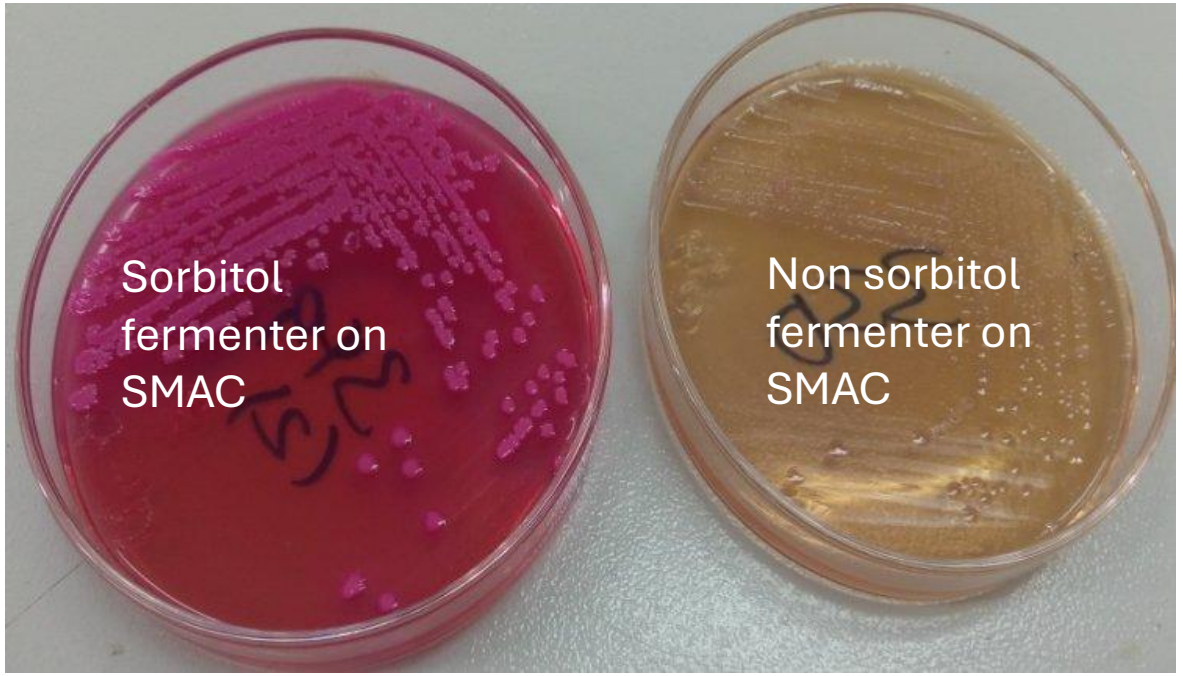
# Diagnosis of Diarrheagenic *E. coli*: EPEC and ETEC

- **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).
- 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.

# Diagnosis of STEC, EIEC, and EAEC

- **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.


Presence of blue fluorescence
MUG test : Positive
Lack of blue fluorescence
MUG test: Negative
MUG (4-Methylumbelliferyl-β-D-Glucuronide) Test


Sorbitol fermenter on SMAC
Non sorbitol fermenter on SMAC

- **EIEC** strains are similar to *Shigella* and are **non-lactose or late lactose fermenters** and are **nonmotile**.
- **EAEC** can be suspected clinically but requires confirmation by **tissue culture adhesion assays**, which are **not readily available in most clinical laboratories**.

# 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**.

# *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 **$H_2S$**.

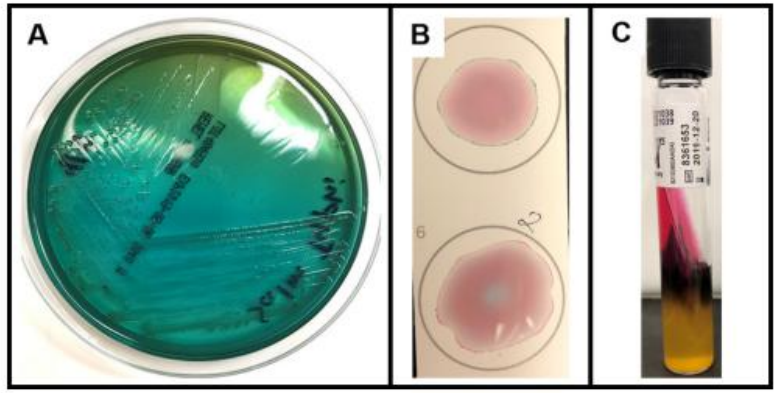



**FIG 1** Growth and biochemical characteristics of *Salmonella enterica* subsp. *enterica* serovar Typhi. (A) Growth on Hektoen enteric agar (non-lactose fermenter, slight $H_2S$ production). (B) Serological reactivity to Remel Wellcolex Color *Salmonella* test reagents 1 (top: green agglutination with red background = serogroup D) and 2 (bottom: red agglutination with blue background = Vi antigen), consistent with *S.* Typhi. (C) Growth on a triple sugar iron agar slant (K/A with slight $H_2S$ production).

https://journals.asm.org/doi/pdf/10.1128/jcm.01359-20

## TABLE 15-4 Clinical Diseases Induced by Salmonellae

| | Enteric Fevers | Septicemias | Enterocolitis |
|---|---|---|---|
| Incubation period | 7–20 days | Variable | 8–48 hours |
| Onset | Insidious | Abrupt | Abrupt |
| Fever | Gradual; then high plateau with "typhoidal" state | Rapid rise; then spiking "septic" temperature | Usually low |
| Duration of disease | Several weeks | Variable | 2–5 days |
| Gastrointestinal symptoms | Often early constipation; later, bloody diarrhea | Often none | Nausea, vomiting, diarrhea at onset |
| Blood culture results | 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

- **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.

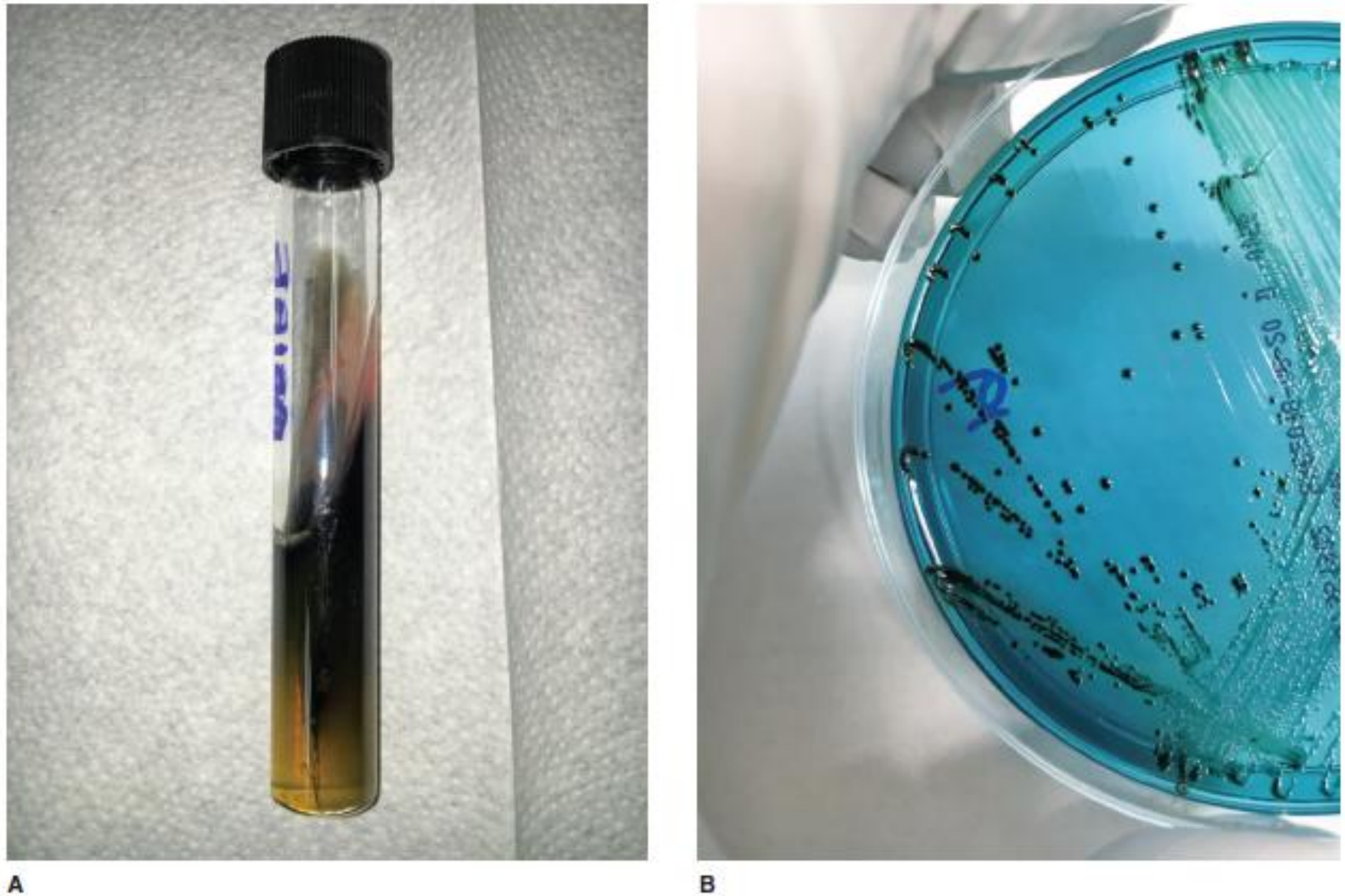
- Appropriate specimens for enteric fever include **blood (most commonly used), bone marrow, other sterile sites, urine, or intestinal secretions**.
- Blood cultures are often positive in the **first week** of enteric fever and septicemia.
- **Bone marrow cultures** have the highest sensitivity, **80% to 95%**, but are clinically less practical.
- Urine culture may become positive after the **second week** of illness.

# Culture and Laboratory Identification

- 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 $H_2S$ 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**.


FIG. 1. *Salmonella enterica*. Note the black center of the transparent colonies indicating $H_2S$ production in the absence of carbohydrate utilization. All serotypes of *Salmonella* have this appearance on HE agar except for serotype Typhi, which is a weak $H_2S$ producer, and rare strains of lactose-fermenting *Salmonella*.

https://asm.org/asm/media/protocol-images/hektoen-enteric-agar-protocol.pdf?ext=.pdf



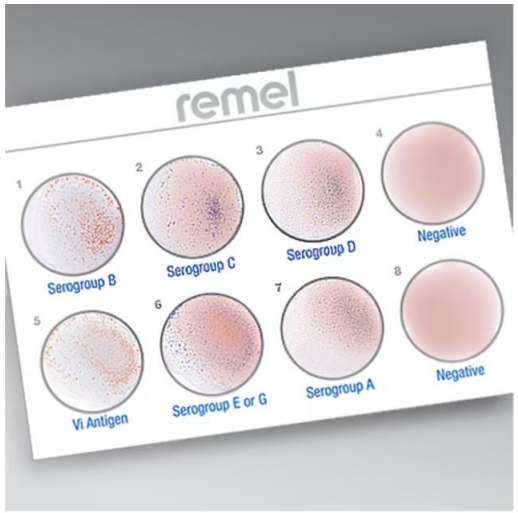
A B

**FIGURE 15-4** **A:** *Salmonella* species on TSI agar slant. **B:** *Salmonella* species on HE agar. *Salmonella* spp. do not ferment the carbohydrates present in the HE agar; however, the organism produces $H_2S$, and the ferric ammonium citrate in the HE agar result in the *Salmonella* colonies to appear black. On TSI agar slant, *Salmonella* spp. ferment glucose, but not lactose; they produce $H_2S$ and gas [K/A,G $H_2S^+$]. (Courtesy of S. Riedel.)

- **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**.



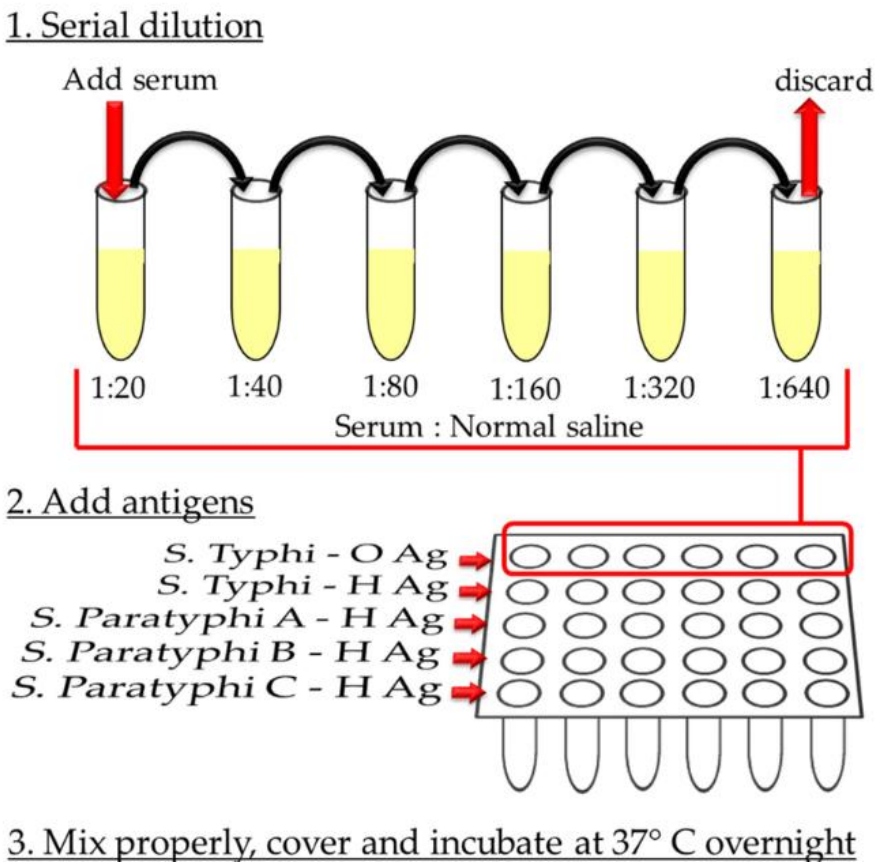

Wellcolex™ Color Salmonella Rapid Latex Agglutination Test Kit

**TABLE 15-3 Representative Antigenic Formulas of Salmonellae**

| O Group | Serotype | Antigenic Formula* |
|---|---|---|
| D | *Salmonella* Typhi | 9, 12 (Vi):d:— |
| A | *Salmonella* Paratyphi A | 1, 2, 12:a— |
| $C_1$ | *Salmonella* Choleraesuis | 6, 7:c:1,5 |
| B | *Salmonella* Typhimurium | 1, 4, 5, 12:i:1, 2 |
| D | *Salmonella* Enteritidis | 1, 9, 12:g, m:— |

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

- **Tube dilution agglutination test (t**he **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.
- 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.
- Several commercial **NAATs** are available for direct detection of salmonellae in fecal samples of patients with **acute diarrhea**.




Laboratory Diagnosis of Paratyphoid Fever: Opportunity of Surface Plasmon Resonance - Scientific Figure on ResearchGate. Available from: https://www.researchgate.net/figure/The-general-procedure-for-the-Widal-test-tube-method-44-1-An-essential-step-in-any_fig1_342526528

# 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).

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

- **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**.
- 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.

# 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**.

Pleural Empyema Due to Salmonella typhi - Scientific Figure on ResearchGate. Available from: https://www.researchgate.net/figure/Non-lactose-fermenting-colonies-on-MacConkey-agar_fig2_233875609

- 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***.

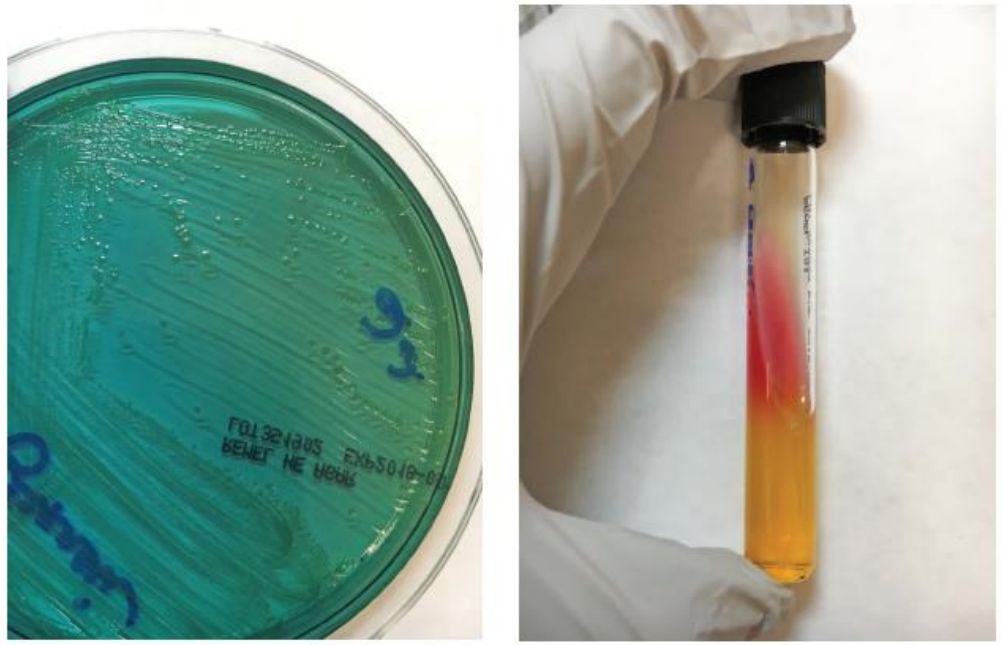
**TABLE 15-2** Pathogenic *Shigella* Species

| Present Designation | Group and Type | Mannitol | Ornithine Decarboxylase |
|---|---|---|---|
| *Shigella dysenteriae* | A | – | – |
| *Shigella flexneri* | B | + | – |
| *Shigella boydii* | C | + | – |
| *Shigella sonnei* | D | + | + |

# 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**.

- **Colorless (lactose-negative) colonies** are inoculated into a **TSI agar slant**.
- **Nonmotile organisms** that fail to produce **$H_2S$**, 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**.



A B

**FIGURE 15-3** **A:** *Shigella* species on Hektoen Enteric (HE) agar. **B:** *Shigella* species on TSI agar slant. *Shigella* spp. do not ferment lactose, salici or sucrose, and therefore appear as translucent, colorless colonies on HE agar. *Shigella* spp. ferment glucose, but not lactose and sucrose present i the TSI agar slant. Therefore, they produce an alkaline over acid (K/A) reaction and do not produce $H_2S$ or gas. (Courtesy of S. Riedel.)

# 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.

- **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.

- Riedel, S., et al. (Eds.). (2026). *Jawetz, Melnick, & Adelberg's Medical Microbiology* (29th ed.). McGraw Hill –chapter 15
- Ryan, K. J. (Ed.). (2022). Sherris & Ryan's Medical Microbiology (8th ed.). McGraw Hill- chapter 33