CHAPTER 11

Isolation and Identification of *Shigella* spp.

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Introduction

Shigellosis, although commonly regarded as waterborne, is also a foodborne disease restricted primarily to higher primates, including humans. It is usually spread among humans by food handlers with poor personal hygiene. Foods most often incriminated in the transmission have been potato salad, shellfish, raw vegetables, and Mexican dishes. First discovered over 100 years ago by a Japanese scientist Kiyoshi Shiga. Shigellae are members of the family Enterobacteriaceae, and are genetically identical *to Escherichia coli* (*E. coli*) and are closely related to *Salmonella* and *Citrobacter* spp. The genus *Shigella* consists of four species: *S. dysenteriae* (subgroup A), *S. flexneri* (subgroup B), *S. boydii* (subgroup C), and *S. sonnei* (subgroup D). *Shigella* organisms may be very difficult to distinguish biochemically from *Escherichia coli.* Brenner considers *Shigella* organisms and *E. coli* to be a single species, based on DNA homology.

Shigella species are Gram-negative, facultatively anaerobic, nonsporulating, nonmotile rods (0.3 to 1 μm) in diameter and 1 to 6 μm in length, appearing singly, in pairs and in chains in the family *Enterobacteriaceae*. They do not decarboxylate lysine or ferment lactose within 2 days. They utilize glucose and other carbohydrates, producing acid but not gas. However, because of their affinity to *E. coli*, frequent exceptions may be encountered, e.g., some biotypes produce gas from glucose and mannitol. Neither citrate nor malonate is used as the sole carbon source for growth, and the organisms are inhibited by potassium cyanide. Typically, species of Shigella are oxidase negative, acetate and mucate negative, and do not produce gas from glucose. Additionally, Shigella are Voges-Proskauer negative and methyl-red positive, nor produce H_2S and are arginine dehydrolase and urease negative. *S. dysenteriae* type 1 strains produce a potent toxin known as Shiga toxin (STX). Three biological activities associated with STX are cytotoxicity, enterotoxicity, and neurotoxicity.

TABLE 1 Characteristics of *Shigella* spp.

Species	Serogroup	Serotypes	Geographic distribution	Distinguishing characteristics
S. dysenteriae	A	15	Indian subcontinent, Africa, Asia, Central America	Produce shiga toxin, causes most severe dysentery, high mortality rate if untreated
S. flexneri	B	6	Most common isolate in developing countries	Less severe dysentery
S. boydii	C	19	Indian subcontinent, rarely isolated in developed countries	Biochemically identical to *S. flexneri*, distinguished by serology
S. sonnei	D	1[a]	Most common isolate in developed countries	Mildest form of shigellosis

Enrichment

Two approaches are provided for the recovery of *Shigella*. The first approach is a conventional culture method that involves the use of a specially formulated medium, *Shigella* broth. Novobiocin is added to provide a selective environment. Sample enrichments are incubated as described below, and streaked to MacConkey agar. Typical colonies are biochemically and serologically confirmed as *Shigella* spp. The second approach uses DNA hybridization. The enzyme DNA gyrase induces negative supercoiling into closed circular DNA. It has been reported, however, that novobiocin inhibits DNA gyrase. Thus, the use of novobiocin in *Shigella* broth may cause this medium to be incompatible with DNA hybridization for detecting *Shigella*.

Conventional culture method

1. Enrichment of *Shigella sonnei*. Aseptically weigh 25 g sample into 225 ml *Shigella* broth to which novobiocin (0.5 µg/ml) has been added. Hold suspension 10 min at room temperature and shake periodically. Pour supernatant into sterile 500 ml Erlenmeyer flask. Adjust pH, if necessary, to 7.0 ± 0.2 with sterile 1 N NaOH or 1 N HCl. Place flask in anaerobic jar, insert anaerobic gas generating pouch/sachet (use number recommended by the anaerobic jar manufacturer, according to the volume of the jar), insert an anaerobic indicator, and tighten the lid. Incubate jars at 44.0°C in a forced air incubator for 20 h. Agitate enrichment culture suspension and streak on a MacConkey agar plate. Incubate 20 h at 35°C.
2. Enrichment of other *Shigella* species. Proceed as above, but use novobiocin at 3.0 µg/ml and incubate anaerobically at 42.0°C in a forced air incubator.

25 g sample into 225 ml *Shigella* broth with
3.0 ug/ml novobiocin (*S.sonnei*: 0.5 ug/ml)

↓ Incubate supernatant: 44°C for 20h, anaerobically

Streak enrichment culture onto
MacConkey agar plate

↓ Incubate 35°C for 20 h

Examine plates for characteristic *Shigella* colonies.
Pick suspect colonies and confirm with

↓ Biochemical test

↓ Serological test

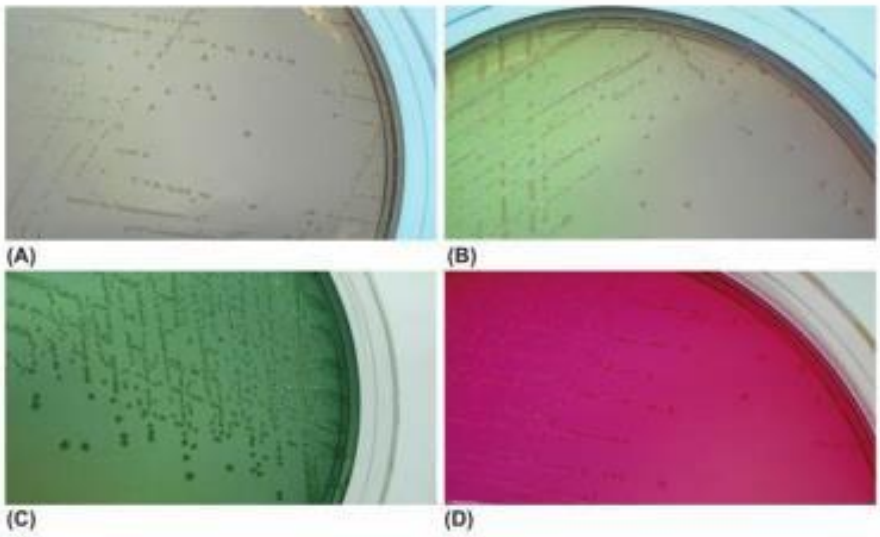
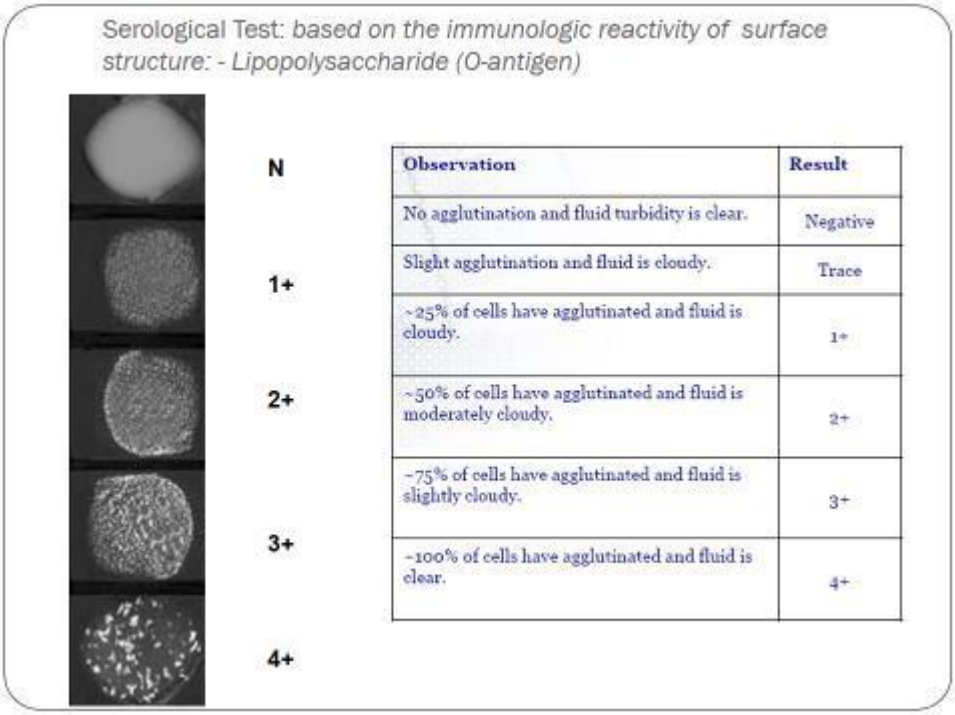


Figure 14.1 Appearance of *Shigella flexneri* colonies on: (A) MacConkey agar; (B) *Salmonella Shigella* (SS) agar; (C) Hektoen enteric (HE) agar; and (D) xylose lysine deoxycholate (XLD) agar plates.

Physiological characterization

Perform Gram stain and inoculate cultures giving satisfactory screening reactions to the other recommended biochemicals. The characteristics of *Shigella* are summarized as follows: Gram-negative rods; negative for H_2S, urease, glucose (gas), motility, lysine decarboxylase, sucrose, adonitol, inositol, lactose (2 days), KCN, malonate, citrate, and salicin; positive for methyl red. Pick isolates having positive reactions for *Shigella* to veal infusion agar slants. Use antisera for identification of serotype or compare with physiological behavior of the 32 serotypes. If serotype cannot be identified by these tests, two explanations are possible: 1) Several provisional serotypes have not been accepted by an international commission on the taxonomy of *Shigella* species. Proper interpretation of the mucate and acetate reactions should help. *Shigella* species tend to be negative in all these reactions, whereas anaerogenic *E. coli* tend to be positive in at least one of the reactions.



Serological Identification of shigella sp.