Shigella species

Shigella spp. are bacteria that cause shigellosis, also known as bacillary dysentery. They are a highly infectious organism, with foodborne outbreaks often involving infected food handlers. Unlike other common foodborne pathogens, humans are the only natural hosts of *Shigella* spp.

Description of the organism

Shigella spp. are Gram-negative, non-spore forming rod-shaped bacteria and are members of the family Enterobacteriaceae. The genus *Shigella* is divided into four species based on their O antigen type and biochemical characteristics: *S. dysenteriae* (comprising 15 serotypes), *S. flexneri* (comprising 14 serotypes), *S. boydii* (comprising 20 serotypes) and *S. sonnei* (1 serotype) (Lampel and Maurelli 2003; Levine et al. 2007).

The most severe form of shigellosis is caused by *S. dysenteriae* serotype 1. *S. sonnei* causes the mildest form of disease, while *S. flexneri* and *S. boydii* can cause either severe or mild illness (FDA 2012). In Australia, *S. sonnei* was the most frequently reported species in 2010, representing 55.6% of all notified *Shigella* infections (OzFoodNet 2012). *S. dysenteriae* serotype 1 is very rare in Australia, with all reported cases acquired overseas (Lightfoot 2003).

Growth and survival characteristics

The growth and survival of *Shigella* spp. in foods is influenced by a number of factors such as temperature, pH, salt content and the presence of preservatives (refer to Table 1). For example, survival of *S. flexneri* has been shown to increase with: decreasing temperature, increasing pH, and decreasing NaCl concentration (Zaika and Phillips 2005).

The temperature range for growth of *Shigella* spp. is 6–8 to 45–47°C (ICMSF 1996). Rapid inactivation occurs at temperatures around 65°C. In contrast, under frozen (-20°C) or refrigerated (4°C) conditions *Shigella* spp. can survive for extended periods of time (Lightfoot 2003; Warren et al. 2006).

Shigella spp. grow in a pH range of 5–9 (ICMSF 1996). Zaika (2001) demonstrated that *S. flexneri* is tolerant to acid and can survive at pH 4 for 5 days in broth when incubated at 28°C. *Shigella* spp. are better able to survive lower pH conditions at reduced temperatures, with *S. flexneri* and *S. sonnei* able to survive for 14 days in tomato juice (pH 3.9–4.1) and apple juice (pH 3.3–3.4) stored at 7°C (Bagamboula et al. 2002).

S. flexneri is salt tolerant and is able to grow in media containing 7% NaCl at 28°C (Zaika 2002a). It is sensitive to organic acids typically used to preserve food. For example, lactic acid has been demonstrated to be effective at inhibiting *S. flexneri* growth, followed in order by acetic acid, citric acid, malic acid and tartaric acid (Zaika 2002b).

Shigella spp. have been shown to survive on various surfaces. *S. sonnei* has been isolated and cultured from fingers several hours after hand contamination (Christie 1968). A study by Nakamura (1962) demonstrated that *S. sonnei* was able to survive on cotton, glass, wood, paper and metal with survival times ranging from 2 days on metal to 28 days on paper at 15°C. *S. dysenteriae* serotype 1 has also been shown to survive on surfaces including plastic, glass, aluminium, wood and cloth (Islam et al. 2001).

S. sonnei, S. flexneri and *S. dysenteriae* serotype 1 can take on a viable but non-culturable (VBNC) state when exposed to various environmental conditions. These VBNC cells are able to survive in a dormant state while culturable cells die off (Colwell et al. 1985; Islam et al. 2001). A study by Nicolo et al. (2011) demonstrated that *S. flexneri* lost culturability when inoculated into grapefruit juice, however, when the VBNC *S. flexneri* was inoculated into resuscitating media it was able to grow again. As the VBNC cells are potentially still virulent and able to be resuscitated, they may be involved in shigellosis transmission (Colwell et al. 1985; Islam et al. 2001; Nicolo et al. 2011).

Table 1: Limits for growth of *Shigella* spp. when other conditions are near optimum (ICMSF 1996; Lightfoot 2003)

	Minimum	Optimum	Maximum
Temperature (°C)	6–8	-	45–47
pH	5	6–8	9
NaCl (%)	-	-	4–5

Symptoms of disease

The clinical symptoms of shigellosis range from mild diarrhoea to severe dysentery, depending on the *Shigella* serotype causing infection, dose and the immunity and age of the host. The incubation period is 1–7 days (usually 3 days) and symptoms typically last for 1–2 weeks (Lampel and Maurelli 2007). Initial symptoms include watery diarrhoea, fever and fatigue. In more severe cases, as is the case for *S. dysenteriae* serotype 1 infection, patients can develop dysentery (characterised by frequent, painful stools containing blood and mucus), abdominal cramps, nausea and vomiting (Niyogi 2005; Nygren et al. 2012). All *Shigella* spp. can cause acute bloody diarrhoea (FDA 2012).

For most *Shigella* serotypes illness is generally self-limiting and fatality is very rare, however, the fatality rate for *S. dysenteriae* serotype 1 can be as high as 20% (Lampel and Maurelli 2003). Cases may develop long-term sequelae such as Reiter's syndrome (reactive arthritis) following *S. flexneri* infection, and haemolytic uremic syndrome following *S. dysenteriae* serotype 1 infection (Warren et al. 2006).

Shigella spp. are shed in large numbers (10^3–10^9 cfu/g of stool) during the acute phase of infection and to a lesser extent (10^2–10^3 cfu/g of stool) in convalescing patients. Adults who live in areas where shigellosis is endemic may become asymptomatic carriers (continue to shed the bacteria but show no sign of infection) (Lampel and Maurelli 2003).

Virulence and infectivity

Once ingested, *Shigella* spp. must survive the acidic environment of the stomach and invade the epithelial cells of the colon to enable infection. *Shigella* spp. multiply inside the colonic epithelial cells and spread to adjacent cells, leading to the death of the infected cells. The colon becomes inflamed and ulcerated and the dead mucoid cells are shed, resulting in the bloody mucoid diarrhoea often characteristic of *Shigella* infection (Lightfoot 2003; Montville and Matthews 2005; Warren et al. 2006).

Shigella spp. have a virulence plasmid that encodes genes involved in the invasion process and intra- and inter-cellular spread. Other genes involved in the invasion process are located

on the chromosome (Warren et al. 2006). *S. flexneri* 2a produce the chromosome encoded *shigella* enterotoxin 1, while most *Shigella* serotypes produce the virulence plasmid encoded *shigella* enterotoxin 2. *S. dysenteriae* serotype 1 strains produce the potent Shiga toxin. Shiga toxin is chromosomally encoded and has cytotoxic, enterotoxic and neurotoxic effects (Niyogi 2005; Warren et al. 2006).

Mode of transmission

Shigella spp. are transmitted by the faecal-oral route by either person-to-person contact, or consumption of contaminated food or water (Nygren et al. 2012).

Nygren et al. (2012) analysed 120 reported foodborne shigellosis outbreaks in the United States (US) between 1998–2008. The contributing factors identified in these outbreaks included infected food handlers (58%), bare-handed contact of the food handler with ready-to-eat food (38%), inadequate cold-holding temperatures (15%), and inadequate cleaning of food preparation equipment (15%). It should be noted that more than one factor can be involved in an outbreak.

Contaminated water is another vehicle for transmission of *Shigella* spp. This can occur due to inadequately treated contaminated water being used for drinking and food preparation, seepage of sewage through the earth, or faecal contamination of recreational water (Lightfoot 2003).

Incidence of illness and outbreak data

Shigellosis is a notifiable disease in all Australian states and territories. The incidence of shigellosis in Australia in 2012 was 2.4 cases per 100,000 population (549 cases), which includes both foodborne and non-foodborne cases. This was a decrease from the previous 5 year mean of 2.8 cases per 100,000 population per year (ranging from 2.2–3.9 cases per 100,000 population per year) (NNDSS 2013).

The Northern Territory had the highest notification rate in 2012 with 46.9 cases per 100,000 population (NNDSS 2013). This was a significant reduction from the 2005–2009 average annual notification rate of 70.1 cases per 100,000 population. The decline in cases may be attributed to a marketing campaign to raise awareness about the importance of hand washing implemented in 2007/2008 targeting both Indigenous and non-Indigenous people, including remote communities (OzFoodNet 2012).

Children between 0–4 years had the highest notification rate in 2010, with 7.5 and 8.3 notifications per 100,000 population for males and females, respectively (OzFoodNet 2012). The higher rate of notified cases in this age group could be due to increased susceptibility or may be the result of other factors such as reduced personal hygiene practices, an increased likelihood of exposure and increased likelihood to seek medical care.

The notification rate for shigellosis in New Zealand in 2011 was 2.3 cases per 100,000 population (101 cases). This was similar to the 2010 rate of 2.4 cases per 100,000 population (Lim et al. 2012).

In the US, 4.82 cases of shigellosis were notified per 100,000 population in 2010. This was a slight decrease from the 2009 rate of 5.24 cases per 100,000 population (CDC 2012). In the European Union there was three strong evidence foodborne outbreaks of shigellosis in 2011 and one outbreak reported in 2010 (EFSA 2012; EFSA 2013).

Foods generally associated with outbreaks of *Shigella* spp. are those that are consumed raw or ready-to-eat foods that have substantial handling during production, such as salads (refer to Table 2).

Table 2: Selected major foodborne outbreaks associated with *Shigella* spp. (>50 cases and/or ≥1 fatality)

Year	Strain	Total no. cases	Food	Country	Comments	Reference
2007	*S. sonnei*	270	Baby corn	Australia and Denmark	Corn from a common packing shed in Thailand, sub-hygienic practices at collection houses and packing shed	(Lewis et al. 2009)
2004	*S. sonnei*	163	Raw carrot	US	Food hygiene deficiencies of caterer, chlorine vegetable sanitiser malfunctioning	(Gaynor et al. 2009)
2001	*S. flexneri* 2a	886	Tomatoes	US	Contamination likely to have occurred during hand sorting of tomatoes	(Reller et al. 2006)
2000	*S. sonnei*	406	Commercially prepared dip	US	Contamination thought to be from infected employee at the production facility	(Kimura et al. 2004)
1998	*S. sonnei*	486	Parsley	US and Canada	Most parsley sourced from a common farm in Mexico which used inadequately chlorinated water that was vulnerable to contamination	(Naimi et al. 2003)
1995–1996	*S. sonnei*	279	Fresh cheese made from pasteurised milk	Spain	Infected food handler at the cheese factory, unhygienic practices at the factory	(Garcia-Fulgueiras et al. 2001)
1988	*S. sonnei*	3175 (estimated)	Uncooked tofu salad	US	Food handlers recently had shigellosis	(Lee et al. 1991)

Occurrence in food

There is very little published surveillance data on the presence of *Shigella* in food. Some international surveys have been performed in which *Shigella* spp. have been found in a range in foods. For example, Ghosh et al. (2007) isolated *Shigella* spp. from 15% of coconut slices (n=150), 9% of ready-to-eat salads (n=150) and 7% of samples of coriander sauces (n=150) from Indian street vendors. *Shigella* spp. have also been detected in 11% of raw meat samples (n=250) from retail outlets in Pakistan (Hassan Ali et al. 2010). In Mexico, *Shigella* spp. have been isolated from 6% of freshly squeezed orange juice samples (n=100) and from the surface of 17% of oranges sampled (n=75). All four *Shigella* spp. were isolated from the surface of the oranges, whereas only *S. sonnei* and *S. dysenteriae* were isolated from the orange juice samples (Castillo et al. 2006).

Although *Shigella* can be isolated from a range of food, outbreaks often occur due to an infected food handler contaminating food that is served cold or raw. A study of foodborne shigellosis outbreaks in the US demonstrated that 20% of outbreaks were due to exclusively raw food (e.g. lettuce based salads) and 30% of outbreaks were from partially raw food (e.g. potato salad) (Nygren et al. 2012).

Host factors that influence disease

People of all ages are susceptible to *Shigella* spp. infection. However, infants, the elderly and immunocompromised individuals are most at risk (FDA 2012).

Protective immunity against *Shigella* infection can occur as a result of repeated exposure to the organism (Barnoy et al. 2010). A study by Ferreccio et al. (1991) tracked shigellosis in a cohort of children in Chile over 30 months. A previous case of shigellosis was found to confer 72% protection against illness with the same *Shigella* serotype. However, prior infection did not protect against illness due to other *Shigella* serotypes. This serotype-specific immunity is mediated, at least in part, by antibodies directed at the O antigen of the lipopolysaccharide that forms part of the bacterial cell wall. As the O antigen varies between serotypes, the immunity is serotype-specific (Levine et al. 2007; Kweon 2008).

Research into candidate vaccines against shigellosis has been performed for many years. Various live attenuated *S. flexneri* 2a vaccines have been trialled in animals and humans, and whilst shown to protect vaccinated individuals from *S. flexneri* 2a infection, immunity appears to be serotype-specific (Mel et al. 1965; Coster et al. 1999; Ranallo et al. 2012). Non-replicating vaccines including inactivated whole cell and subunit vaccines have also been trialled (Kaminski and Oaks 2009). A *S. sonnei* conjugate vaccine provided significant protection against shigellosis in the field; however it was only effective against *S. sonnei* infection (Cohen et al. 1997).

An experimental trivalent vaccine has been constructed which expressed the O antigens of *S. flexneri 2a and S. sonnei* and the *Vibrio cholera* toxin B subunit antigen. The trivalent vaccine was able to protect mice and rhesus monkeys from infection with *S. flexneri* 2a and *S. sonnei* (Wang et al. 2002). A pentavalent vaccine has been proposed consisting of *S. flexneri* 2a and 3a (cross-protection between most *S. flexneri* serotypes has been achieved in guinea pigs due to a common O antigen carbohydrate backbone), *S. flexneri* 6 (which does not cross-react with the other *S. flexneri* serotypes), *S. dysenteriae* serotype 1 and *S. sonnei.* Hypothetically, this could protect against the majority of the causes of shigellosis in the world (Noreiga et al. 1999; Levine et al. 2007).

Dose response

Very little data is available on the dose-response relationship for *Shigella* spp. During the 1960s and 1970s, human feeding trials using strains of *S. dysenteriae* serotype 1, *S. flexneri*, and *S. sonnei* were performed to determine the dose required to cause shigellosis. The dose response varied between strains; illness was caused by *S. dysenteriae* serotype 1, *S. flexneri*, or *S. sonnei* with ingestion of 10, 100 and 500 organisms, respectively (DuPont et al. 1989).

Recommended reading and useful links

FDA (2012) Bad bug book: Foodborne pathogenic microorganisms and natural toxins handbook, 2nd ed, US Food and Drug Administration, Silver Spring, p. 25–28. http://www.fda.gov/Food/FoodborneIllnessContaminants/CausesOfIllnessBadBugBook/ucm2006773.htm

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Last updated December 2013