

17 Typhoid

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Abstract

Typhoid fever is a severe systemic infection caused by the human-adapted *Salmonella enterica* serovar Typhi (*S. Typhi*). Paratyphoid fever is indistinguishable from typhoid fever in its clinical presentation but is associated with other typhoidal *Salmonella* serovars, including the human-adapted *S. enterica* serovars Paratyphi A, Paratyphi B, Paratyphi C, or Sendai. Although eradicated in most developed countries, these illnesses continue to be a major contributor to morbidity and mortality worldwide, and the emergence of antibiotic resistance is beginning to limit treatment options. Sanitation of drinking water is effective in reducing transmission of typhoid and paratyphoid fever, but eradication of these diseases also requires management of human carriers. Typhoidal *Salmonella* serovars are closely related genetically to nontyphoidal *Salmonella* serovars, which are associated with a localized gastroenteritis in humans. Recent studies suggest that differences in the clinical presentation of typhoid fever and gastroenteritis can be attributed to virulence mechanisms that enable typhoidal *Salmonella* serovars to evade innate immunity but that are absent from nontyphoidal *Salmonella* serovars. One such factor is the virulence capsular polysaccharide (Vi antigen) of *S. Typhi* and *S. Paratyphi C*. Studies on immunity to typhoid fever have resulted in licensing of a killed whole-cell parenteral typhoid vaccine, a live-attenuated oral typhoid vaccine, and a parenteral vaccine consisting of purified Vi antigen. Our entry will review basic and applied research on this enigmatic human disease.

History

Typhoid fever has been an important disease throughout history. A prominent typhoid fever victim in antiquity might have been Alexander the Great, who died from a febrile illness in Babylon on June 10, 323 BC (Oldach et al. 1998). Although the disease has likely contributed to morbidity and mortality for millennia, the nonspecific symptoms make it difficult to distinguish from other illnesses based on historic accounts. The term typhous (τυφωδης) fever, meaning smoky or hazy fever, was applied broadly since the time of Hippocrates (460 BC–ca. 370 BC) to describe continued febrile illnesses associated with delirium or stupor which likely included, but were not limited to, typhoid fever.

The first accurate account of the symptoms associated with typhoid fever is that of “putrid fever” described by the British physician Thomas Willis in 1659 (Willis 1682). However, an

influential treatise on classifying diseases published in 1763 by François Boissier de Sauvages de la Croix continued to apply the term typhus broadly to continued fevers (Sauvages de la Croix 1763). Pierre Charles Alexandre Louis was the first to define typhoid fever as a distinct disease entity in 1829. He used analytical methods to differentiate François Boissier de Sauvages de la Croix's term typhus into two distinct diseases, typhus and typhoid fever, the latter being characterized by lesions in the Peyer's patches and mesenteric lymph nodes (Louis 1836). Subsequent work in the 1830s by William Wood Gerhard, a disciple of Pierre Charles Alexandre Louis, helped establish the concept that typhoid fever is an independent disease entity in the United States (US). It was not until 1849, through case reports published by Sir William Jenner, that the concept of typhoid fever being distinct from typhus became accepted in the United Kingdom (Jenner 1849).

In 1856, the British physician William Budd published a pioneering epidemiological study in which he demonstrated the communicable nature of typhoid fever and its spread through contagious dejecta (Budd 1856). William Budd's contribution was remarkable as it preceded identification of the causative agent by decades and helped erect the modern discipline of epidemiology. Furthermore, the finding that typhoid fever spreads by fecal-oral transmission led William Budd to propose water chlorination as a preventive strategy. However, this idea was initially met with skepticism by those in the medical community, who remained convinced that typhoid fever spreads through the gases rising from putrid matter (miasma) rather than by contagion (germ theory). Eventually, William Budd's recommendations were heeded with the passing of the British Public Health Act in 1875, and this resulted in improved sanitary practices.

In 1880, the German pathologist Carl Joseph Eberth provided direct support for the theory that contagious germs cause typhoid fever by visualizing the causative agent, termed typhoid bacillus, in histological sections (Eberth 1880). In 1884, the typhoid bacillus was isolated and grown in pure culture by the German physician Georg Theodor August Gaffky, who worked under Robert Koch at the Imperial Health Office (Kaiserliches Gesundheitsamt) in Berlin (Gaffky 1884). One year after Georg Theodor August Gaffky's characterization of the typhoid bacillus, the bacteriologist Theobald Smith isolated *Bacterium cholerae-suis* from a pig while working under Daniel Elmer Salmon at the Veterinary Division of the United States Department of Agriculture (USDA) (Salmon and Smith 1885). In honor of Daniel Elmer Salmon, the genus *Salmonella* was erected in 1900 with *Salmonella cholerae-suis* as the type species (Salmonella-Subcommittee 1934). In 1904, the British physician Albert Sidney Grünbaum fulfilled the last Koch's postulates by demonstrating that experimental oral infection of chimpanzees with the typhoid bacillus produces a typhoid-like disease with the characteristic intestinal lesions (Grünbaum 1904). In 1918, the typhoid bacillus was renamed *Eberthella typhi* to recognize Carl Joseph Eberth's contribution to its discovery (Buchanan 1918). In 1933, the designation *Eberthella typhi* was changed to *Salmonella typhi*

(Salmonella-Subcommittee 1934). At this time, each species within the genus *Salmonella* was defined based on its fermentative and serological characteristics and was referred to by a Latin binomial (Kelterborn 1967). This nomenclature was eventually abandoned when it became clear that members of the genus *Salmonella* are closely related genetically (Crosa et al. 1973). Current nomenclature distinguishes only two species within the genus, *Salmonella enterica* (Le Minor and Popoff 1987) and *Salmonella bongori* (Reeves et al. 1989). All other former *Salmonella* species are now considered to be serovars (also known as serotypes). For example, the causative agent of typhoid fever is now regarded as a serovar belonging to the species *S. enterica*, and it is correctly referred to as *S. enterica* serovar Typhi (*S. Typhi*) (Brenner et al. 2000).

The identification of the causative agent of typhoid fever by Carl Joseph Eberth and Georg Theodor August Gaffky in the 1880s (Eberth 1880; Gaffky 1884) not only helped to fortify the concept that typhoid fever spreads by contagious germs, but it also spawned important discoveries related to diagnosis and prevention. For example, the ability to grow the organism in pure culture enabled Georges-Fernand-Isidor Widal to demonstrate in 1896 that serum from typhoid fever patients could agglutinate *S. Typhi* (Widal 1896). This serological test for typhoid fever was termed the Widal test and is still in use today. In the same year, bacteria isolated from two individuals with symptoms of typhoid fever that tested negative in the Widal reaction were termed *Bacille paratyphique* (Achard and Bensaude 1896), a pathogen now known as *S. enterica* serovar Paratyphi B (*S. Paratyphi B*).

Cultivation of *S. Typhi* also paved the way in 1896 for the development of the first heat-killed parenteral vaccine (Groschel and Hornick 1981), a strategy pursued in parallel by the British pathologist Sir Almroth Edward Wright at the Army Medical College in Netley (Wright 1896) and the German bacteriologist Richard Pfeiffer, who worked under Robert Koch at the Imperial Health Office in Berlin (Pfeiffer and Kolle 1896). Identification of the virulence (Vi) antigen, a capsular polysaccharide of *S. Typhi*, in 1934 (Felix and Pitt 1934) set the stage for the development of a purified Vi polysaccharide parenteral vaccine (Robbins and Robbins 1984). Finally, René Germanier and Emil FÜRer from the Swiss Serum and Vaccine Institute introduced a third immunization strategy in 1975 by developing the first live attenuated oral typhoid vaccine strain (Ty21a) (Germanier and Fuer 1975).

In 1903, Robert Koch gave a lecture at a conference in Kassel in which he proposed the new concept that typhoid fever spreads solely through human-to-human transmission and that healthy humans that chronically shed *S. Typhi* could serve as a reservoir. This presentation initiated measures to improve sanitary practices in Germany and launched public health measures to identify and isolate human carriers of the disease (Drigalski 1904). These public health measures for managing carriers proved essential for eradicating typhoid fever in North America and Europe. While it was established at the time that *S. Typhi* is regularly present in the gallbladder (Anton and Fütterer 1888), Robert Koch's lecture sparked renewed interest, and soon

persistence in the gallbladder was proposed as a mechanism for chronic human carriage (Kelly 1906; Porster 1906). In 1906, Heinrich Kayser, who worked under Robert Koch at the Imperial Health Office in Berlin, estimated that 13.5% of typhoid fever cases could be traced to apparently healthy human carriers (Kayser 1906). Perhaps the best-known case of chronic carriage was the one identified by the American sanitary engineer George Soper. In his 1907 report, he traced an outbreak of typhoid fever in the state of New York back to a cook named Mary Mallon, better known as typhoid Mary (Soper 1907).

Before improved sanitary practices were implemented, the annual incidence of typhoid fever remained as high as 500 cases per 100,000 people in the nineteenth century in Europe and North America. The discovery by William Budd that typhoid fever is transmissible led to improved sanitation of drinking water, while identification of the causative agent by Carl Joseph Eberth and Georg Theodor August Gaffky set the stage for vaccination and the identification and isolation of human carriers. Together, these public health measures reduced the incidence of typhoid fever in Europe and North America throughout the first half of the twentieth century to their current level of less than 1 case per 100,000 people.

Taxonomy

Salmonella Species

The genus *Salmonella* belongs to the phylum Proteobacteria, the class Gammaproteobacteria, the order Enterobacteriales, and the family Enterobacteriaceae. The genus consists of two species, *Salmonella enterica* (Le Minor and Popoff 1987) and *Salmonella bongori* (Reeves et al. 1989). The species *S. enterica* is further subdivided into six subspecies, which can be referred to either by roman numerals (subspecies I, II, IIIa, IIIb, IV, and VI) or by names (subspecies *enterica*, *salamae*, *arizonae*, *diarizonae*, *houtenae*, and *indica*, respectively) (Brenner et al. 2000; Tindall et al. 2005). All *Salmonella* serovars that are associated with

typhoid fever or paratyphoid fever in humans belong to *S. enterica* subspecies *enterica* (*S. enterica* subspecies I). However, the subspecies designation is commonly omitted when referring to a serovar (e.g., *S. enterica* serovar Typhi instead of *S. enterica* subspecies *enterica* serovar Typhi), and, in order to shorten reports, this designation is often further abbreviated (e.g., *S. Typhi*).

Salmonella Serovars

Each *Salmonella* serovar is defined by an antigen formula, which lists O factors, H1 factors, and H2 factors separated by colons (● Table 17.1). O factors (also known as O antigens or somatic antigens) correspond to antigenic determinants present in the oligosaccharide repeat units of lipopolysaccharide (LPS). Capsule expression is uncommon in the genus *Salmonella* but occurs in *S. Typhi* and *S. enterica* serovar Paratyphi C, which express the Vi antigen. Expression of the Vi antigen is indicated along with O factors before the first colon in the antigen formula. H factors (H antigens) represent antigenic determinants present in flagellin, the major protein subunit of flagella. The majority of *S. enterica* serovars express two flagellins, termed FliC (H1 factor) and FljB (H2 factor), respectively. Flagellin expression oscillates between states characterized by exclusive production of FliC (H1-phase flagellin) and exclusive production of FljB (H2-phase flagellin), a phenomenon known as flagellar phase variation (Andrews 1922). Sequence variants of FliC and FljB proteins can be distinguished serologically, and their reactivity with typing sera is indicated in the antigen formula of a serovar. Flagellar phase variation is mediated by a heritable molecular switching mechanism composed of an invertible promoter element controlling expression of the *fljBA* genes (Silverman and Simon 1980). If the invertible promoter element is oriented such that it drives expression of *fljBA* (phase H2 orientation), the cell produces FljB (H2-phase flagellin) and FljA. In turn, FljA prevents the production of H1-phase flagellin by binding to the 5'-untranslated region of the *fliC*

■ Table 17.1
Typhoidal *Salmonella* serovars and related nontyphoidal serovars

<i>S. enterica</i> serotype	O antigen : H1-phase : H2-phase	Other	Serogroup (immunodominant O factor)	H ₂ S production	D-tartrate utilization	Citrate utilization
Typhi	9, 12, [Vi] : d : -	[Z ₆₆]	D1 (O : 9)	+/-	+	+/-
Paratyphi A	1, 2, 12 : a : -		A (O : 2)	+/-	-	-
Paratyphi B	1, 4, [5], 12 : b : 1, 2		B (O : 4)	+	-	+/-
Paratyphi B var. Java (nontyphoidal)	1, 4, [5], 12 : b : 1, 2		B (O : 4)	+	+	+
Paratyphi C	6, 7, Vi : c : 1, 5		C1 (O : 7)	+	+	+
Sendai	1, 9, 12 : a : 1, 5		D1 (O : 9)	+/-	-	-
Miami (nontyphoidal)	1, 9, 12 : a : 1, 5		D1 (O : 9)	+	x	+

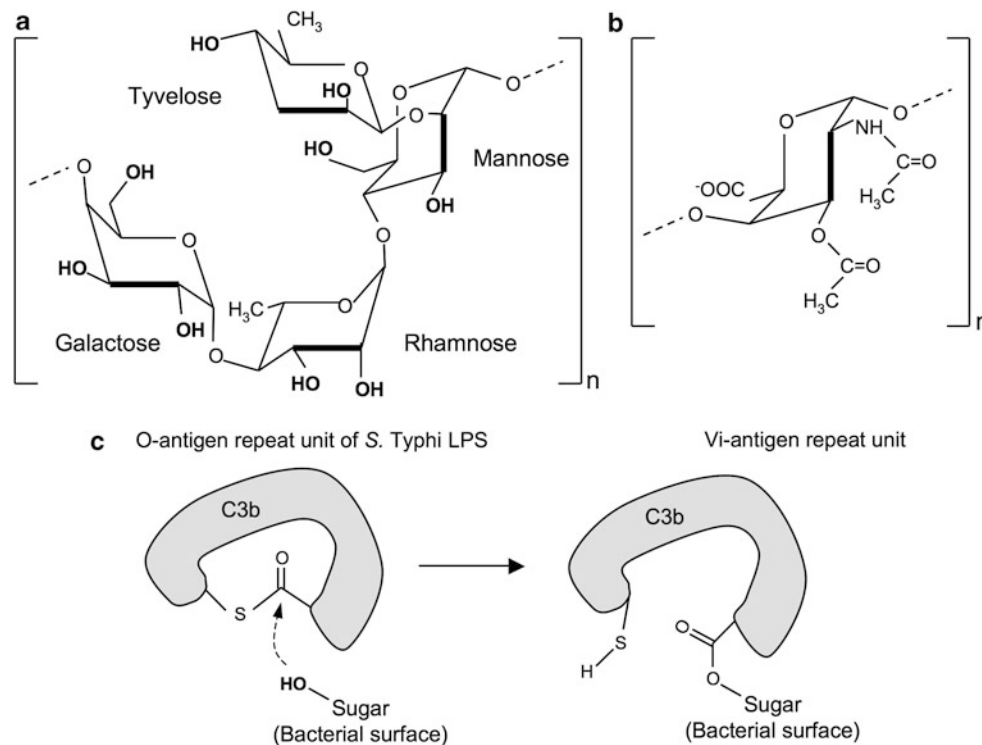
[] can be absent in isolates from this serotype, 1 the O factor 1 can be present due to phage conversion, : the H2 factor is absent, x weakly positive, +/- reaction can be positive or negative

transcripts (Bonifield and Hughes 2003), thereby blocking its translation (Yamamoto and Kutsukake 2006). Phase variation requires inversion of the controlling DNA element (phase H1 orientation), thereby silencing *fljBA*, which relieves the translational block of *fliC* mRNA and allows the cell to produce H1-phase flagellin (Silverman and Simon 1980).

S. Typhi has the O antigen formula 9, 12, [Vi] : d : -, which is a numeric representation of four antigenic epitopes: O factor 9, O factor 12, the Vi antigen, and H1 factor d (► Table 17.1). O factor 9 corresponds to a tyvelose branch that is α -(1,3)-linked to D-mannose in the trisaccharide backbone of the O antigen and represents the immunodominant epitope in the LPS of *S. Typhi*. O factor 12 corresponds to the trisaccharide backbone of the O antigen repeat unit consisting of α -D-mannose-(1,4)- α -L-rhamnose-(1,3)- α -D-galactose (► Fig. 17.1a). The Vi antigen is a homopolymer composed of (1,4)-2-acetamido-3-O-acetyl-2-deoxy- α -D-galacturonic acid that forms a capsular polysaccharide on the bacterial surface (Heyns and Kiessling 1967) (► Fig. 17.1b). Expression of the Vi antigen prevents agglutination of *S. Typhi* with serum against O factors 9 and 12 (Felix et al. 1934; Felix and Pitt 1934). The parenthesis in the antigen formula indicates that the Vi antigen is encoded on a genetically

unstable DNA region that can be lost upon laboratory passage of *S. Typhi* (Bueno et al. 2004; Nair et al. 2004). H1 factor d indicates that the FliC protein of *S. Typhi* reacts with H1 typing serum d. Finally, the absence of an H2 factor (-) indicates that *S. Typhi* is a monophasic serovar in which the *fljBA* genes have been lost by deletion. An exception to the monophasic phenotype of *S. Typhi* has been described for some Indonesian isolates which carry a *fljBA*-like operon on a linear plasmid that encodes the H : z₆₆ antigen (Huang et al. 2004; Baker et al. 2007).

Typhoid fever caused by *S. Typhi* is indistinguishable in its symptoms from paratyphoid fever, which can be caused by *S. enterica* serovar Paratyphi A (*S. Paratyphi* A), *S. Paratyphi* B, *S. Paratyphi* C, or *S. enterica* serovar Sendai (*S. Sendai*) (Kelterborn 1967). Pathogens causing typhoid fever or paratyphoid fever are collectively referred to as typhoidal *Salmonella* serovars (► Table 17.1). *S. Sendai* expresses O factors 9 and 12 but lacks the Vi antigen. *S. Paratyphi* A and *S. Paratyphi* B possess the same trisaccharide repeating unit, O factor 12, but differ from *S. Typhi* and *S. Sendai* in their immunodominant branching carbohydrate moieties. *S. Paratyphi* A has paratose α -(1,3)-linked to D-mannose (O factor 2), while the branching carbohydrate moiety of



■ Fig. 17.1

O antigen, Vi capsule, and complement deposition. (a) Chemical composition of the *S. Typhi* O antigen repeat unit (reproduced from (Wilson et al. 2011) with permission). Free hydroxyl groups available for complement deposition are indicated in bold font. (b) Chemical composition of the Vi antigen repeat unit (reproduced from (Wilson et al. 2011) with permission). Note the absence of free hydroxyl groups in the Vi antigen. (c) Mechanism of complement deposition (opsonization). An intramolecular thioester bond within C3b reacts with free hydroxyl groups in sugar moieties of the O antigen to form an ester bond that covalently attaches complement to the bacterial surface

S. Paratyphi B is abequose (O factor 4). In some isolates of *S. Paratyphi B*, the abequose branch can be acetylated, giving rise to O factor 5. O factor 12 serves as a receptor for P22 and related bacteriophages. Lysogenic conversion by these phages can result in expression of an additional epitope, O factor 1 (Zinder 1958), which represents a glucosyl branch linked to the galactose residue in the trisaccharide repeat unit. Finally, *S. Paratyphi C* (O antigen formula 6, 7, Vi) expresses the Vi antigen and an O antigen with pentasaccharide repeat units that are composed of β -D-mannose-(1,2)- α -D-mannose-(1,2)- β -D-mannose-(1,3)- β -N-acetyl-D-glucosamine (Lindberg et al. 1988). While *S. Paratyphi B*, *S. Paratyphi C*, and *S. Sendai* are biphasic (i.e., expressing H1 and H2 factors), *S. Paratyphi A* is a monophasic serovar due to mutations in the *hin* and *fin* genes (Kutsukake et al. 2006), which encode proteins that control inversion of the *fljBA* promoter element (Silverman and Simon 1980). As a result, the invertible *fljBA* promoter element is locked in the H1 orientation in *S. Paratyphi A*, resulting in exclusive expression of H1-phase flagellin (McClelland et al. 2004).

Salmonella Serogroups

Salmonella serovars that express identical immunodominant O factors can be further organized into serogroups (► Table 17.1). For example, *S. Typhi* and *S. Sendai* both express the immunodominant O factor 9 and thus belong to the same serogroup, designated D1. The majority (95.7%) of *Salmonella* serovars isolated from humans or food animals belong to only five serogroups, including B (O factor 4), C1 (O factor 7), C2 (O factor 8), D1 (O factor 9), and E1 (O factor 10) (Kelterborn 1967).

S. Typhi Phage Types

Phage typing is a method to distinguish different isolates of the same serovar for epidemiological purposes. The method is based on differences in the susceptibility of isolates to a set of bacteriophages (typing phages). Phage typing schemes are available for two typhoidal *Salmonella* serovars, *S. Typhi* and *S. Paratyphi B* (Anderson and Williams 1956; Craigie and Felix 1947). *S. Typhi* typing phages are derived from a bacteriophage isolated by Craigie and Yen in 1938, which is specific for the Vi antigen and is termed the type II Vi phage (CRAIGIE and YEN 1938). Derivatives of the type II Vi phage can be generated by sequential adaptation to growth on specific *S. Typhi* host strains. An adapted phage can yield progeny that forms more plaques on the parent strain than on genetically distinct *S. Typhi* isolates, and the respective phage stock can thus be used as a typing phage. Differences in the infectivity of these typing phages for different *S. Typhi* isolates are largely based on the restriction/modification systems encoded by the *S. Typhi* host strain used for sequential adaptation of the respective type II Vi phage stock (Pickard et al. 2008).

Isolation and Identification

Isolation

Salmonella serovars can be isolated from stool by streaking samples on low selective agar plates containing bile salts to inhibit growth of Gram-positive bacteria (e.g., MacConkey agar or deoxycholate agar) and on intermediate-selective agar plates containing bile salts, citrate, and a low concentration of Brilliant green (0.0003 g/l) to inhibit growth of Gram-positive bacteria and most coliform bacteria (e.g., *Salmonella-Shigella* agar). This method is commonly used for stool samples from febrile individuals with diarrhea because it screens for two possible causes, *Shigella* species and *Salmonella* serovars.

Isolation of *S. Typhi* from individuals with typhoid fever is best achieved using their bone marrow cultures, followed by duodenal content cultures, while blood cultures, stool cultures, or urine cultures are less reliable (Khourieh et al. 1989; Avendano et al. 1986; Gilman et al. 1975; Gilman and Hornick 1976; Benavente et al. 1984; Hoffman et al. 1984; Farooqui et al. 1991). A combination of these methods succeeds in isolating typhoidal *Salmonella* serovars from more than 90% of typhoid fever patients. However, due to the discomfort associated with bone marrow aspirate cultures and duodenal string-capsule cultures, isolation frequently relies on blood cultures, which reduces sensitivity to 40–70% (Hoffman et al. 1984; Benavente et al. 1984; Gilman et al. 1975; Farooqui et al. 1991).

A medium that is highly selective for *Salmonella* serovars is Brilliant green agar, which contains a high concentration of Brilliant green (12.5 g/l) to inhibit growth of Gram-positive bacteria and most Gram-negative bacilli (Kauffmann 1935). However, typhoidal *Salmonella* serovars do not adequately grow on Brilliant green agar. The medium of choice for isolating typhoidal *Salmonella* serovars is bismuth sulfite agar, which contains an intermediate concentration of Brilliant green (0.025 g/l) to inhibit growth of coliforms and Gram-positive bacteria (Wilson and Blair 1927, 1931). Bismuth sulfite agar contains ferrous sulfate (FeSO_4), which can be reduced to hydrogen sulfide (H_2S) by proteins encoded in the *phsABC* operon (Heinzinger et al. 1995) and the *asrABC* operon (Huang and Barrett 1991) of *Salmonella* serovars. Hydrogen sulfide (H_2S) reacts with the bismuth sulfite indicator ($\text{Bi}_2[\text{SO}_3]_3$) in the medium to produce bismuth sulfide (Bi_2S_3), a black insoluble precipitate that produces a characteristic black to brown coloration of colonies. H_2S production in typhoidal *Salmonella* serovars is variable and can be absent (► Table 17.1).

Enrichment can be performed for samples containing a low number of bacteria prior to streaking samples on selective agar plates. A common enrichment method for *Salmonella* serovars is static growth in tetrathionate broth (Muller 1923) or in derivatives of this medium. The *ttrRS ttrBAC* gene cluster (Hensel et al. 1995) enables *Salmonella* serovars to utilize tetrathionate ($\text{S}_4\text{O}_6^{2-}$) as a terminal respiratory electron acceptor (Barrett and Clark 1987), thereby promoting their outgrowth over competing microbes in tetrathionate broth under microaerophilic conditions.

Identification

Salmonella serovars are Gram-negative, rod-shaped, and commonly peritrichously flagellated bacteria. They can be distinguished based on their biochemical properties from closely related members of the Enterobacteriaceae that can grow on selective media used for isolation of *Salmonella* serovars. For instance, *Escherichia coli* and *Citrobacter* species utilize lactose, a property absent in *Shigella* species and most *Salmonella* serovars. However, some serovars of *S. enterica* subspecies *arizonae* and most serovars of *S. enterica* subspecies *diarizonae* can ferment lactose and therefore form pink colonies on MacConkey agar plates, which contain lactose and a pH indicator to visualize fermentation of this carbon source. *Citrobacter* species and most *E. coli* isolates can ferment sucrose, while *Salmonella* serovars are negative for this reaction. *Citrobacter* species and *Salmonella* serovars can reduce ferrous sulfate (FeSO_4) to hydrogen sulfide (H_2S), which distinguishes them from *E. coli* and *Shigella* species.

The above biochemical properties can be assessed, for example, by using triple sugar iron (TSI) agar slants, a medium containing lactose (1%), sucrose (1%), a small amount of glucose (0.1%), ferrous sulfate (FeSO_4), and the pH indicator phenol red (Russell 1911). A TSI agar slant is inoculated by streaking a pure culture over the surface, followed by stabbing the inoculum deep into the agar. An organism that can ferment lactose and/or sucrose, such as *E. coli* or *Citrobacter* species, will turn the medium yellow after incubation for 24 h. *Citrobacter* species produce H_2S , which forms a black ferrous sulfide (FeS) precipitate in TSI agar, with yellow-colored agar remaining visible at the top of the agar slant. No black precipitate is visible with slants inoculated with *Escherichia coli* and *Shigella* species. Since *Salmonella* serovars only ferment the small amount of glucose present in the medium, the top of the TSI agar slant remains red while H_2S production turns the agar black. It should be mentioned that H_2S production can be absent in typhoidal *Salmonella* serovars (► Table 17.1).

A quick test of whether a colony isolated on selective media represents a *Salmonella* serovar is slide agglutination with polyvalent O antiserum, which allows a rapid detection of the most common *Salmonella* serogroups. *Salmonella* serovars can then be assigned to *S. bongori* or to one of the subspecies of *S. enterica* using biochemical criteria. Most isolates belonging to *S. enterica* subspecies *enterica* can utilize tartrate as a carbon source, a property absent from *S. bongori* and other *S. enterica* subspecies. However, tartrate utilization is commonly absent in typhoidal *Salmonella* serovars, which are all members of *S. enterica* subspecies *enterica* (● Table 17.1).

Traditionally, the most common method for subdividing members of the genus *Salmonella* is serotyping. This is still the gold standard, but the procedure is time consuming and requires the availability of a collection of typing sera. A colony isolated on selective media can be immediately typed by slide-agglutination for its O factor and its H1 factor, but not for its H2 factor, because *Salmonella* serovars consist predominantly of bacteria in the H1-phase after in vitro passage (Stocker 1949). Isolation of H2-phase

bacteria is performed by stabbing a culture into motility agar supplemented with antiserum against its H1 factor. Since H1-phase bacteria become entrapped in the agar by antibodies, halos formed after overnight incubation consist of H2-phase bacteria, which can then be isolated to type their H2 factor.

In some cases, biochemical characterization is performed to distinguish biotypes within a serovar. For example, *S. Paratyphi* B isolates from patients with paratyphoid fever are generally tartrate utilization-negative, while isolates from individuals with gastroenteritis are commonly tartrate utilization-positive, and the latter biotype is referred to as *S. Paratyphi* B variety Java (► Table 17.1) (Kauffmann 1955; Ezquerro et al. 1993; Malorny et al. 2003). *S. Sendai* isolates associated with paratyphoid fever do not produce hydrogen sulfide (H_2S) and/or cannot grow on citrate, which distinguishes them from gastroenteritis isolates that share the same antigen formula but represent a different biotype classified as *S. Miami* (► Table 17.1) (Edwards and Moran 1945; Chau and Huang 1979).

Epidemiology

Habitat

Humans are the only known reservoir for *S. Typhi*, *S. Paratyphi* A, *S. Paratyphi* C, and for biotypes of *S. Sendai* and *S. Paratyphi* B that are associated with paratyphoid fever. Individuals with active typhoid fever commonly shed the organism with their feces or urine. During convalescence, at the time of hospital discharge, approximately 20% of individuals still excrete *S. Typhi* (Gould and Qualls 1912). This number declines to approximately 4% of individuals who develop chronic carriage in the gallbladder or, less frequently, in the urinary bladder (Stone 1912). Chronic carriage in the gallbladder leads to intermittent release of *S. Typhi* into the intestine, resulting in fecal shedding. In the absence of adequate treatment, this state can persist for the remainder of an individual's life (Gregg 1908). Contact carriers represent another important reservoir for excretion of *S. Typhi*. Contact carriers have no history of typhoid fever but shed *S. Typhi*, presumably because they developed a subclinical infection, commonly through contact with a typhoid fever patient, a condition that can arise in hospital workers (Stone 1912).

Routes of Transmission

Typhoid fever spreads by means of water, milk, and food products that become contaminated by individuals excreting *S. Typhi* (Stone 1912). Carriers handling water, milk, or food can introduce the contamination directly into these products. Infections can also occur remotely, as *S. Typhi* can survive outside its host and contaminate water supplies or spread through products distributed by trade.

Observations made during the eradication of typhoid fever in the United States illustrate the relative importance of different

routes of transmission. Typhoid fever was an important cause of death in major US cities when monitoring began. Typhoid fever death rates per 100,000 people were 65 for Boston in 1865, 73 for Chicago in 1867, 62 for Baltimore in 1875, 66 for Buffalo in 1882, 42 for Providence in 1884, and 43 for New Orleans in 1895 (Putnam 1927). A crucial initial step in the eradication of typhoid fever was improved sanitation of drinking water either by filtration or by chlorination (Howard 1920; Putnam 1927; Ferrie and Troesken 2008). The incidence of typhoid fever in the USA declined following the 1870s, but records during the initial period are sparse (Ausubel et al. 2001; Putnam 1927). Concurrently with the implementation of improved drinking water sanitation, the annual typhoid fever death rates in the USA dropped from an average of 31.3 per 100,000 people in 1900 to 3.8 per 100,000 people in 1925 (Putnam 1927). These data suggest that contaminated drinking water was the most important vehicle for typhoid fever transmission in the USA at the beginning of the twentieth century.

Given that 14.5% of typhoid fever cases had a fatal outcome in the USA during the first decades of the twentieth century (Graham 1916), a death rate of 3.8 per 100,000 people reported in 1925 (Putnam 1927) suggests an incidence of approximately 26 illnesses per 100,000 people, which is more than 100-fold higher than the current annual incidence of 0.2 illnesses per 100,000 people (Scallan et al. 2011). Thus, while sanitation of drinking water markedly reduced the incidence of typhoid fever, it did not result in an eradication of the disease (Leavitt 1992). This does not suggest that drinking water was not successfully treated because the measures taken were sufficient to eradicate the water-borne disease cholera in the USA by 1911 (Weber et al. 1994). The fact that typhoid fever remained endemic in the USA, even after the broad implementation of drinking water sanitation by 1925, has been attributed to healthy human carriers (Putnam 1927; Leavitt 1992). Chronic carriage of *Vibrio cholerae* does not develop in individuals that recover from an episode of cholera, which made it possible to eradicate the disease solely by providing access to safe drinking water. In contrast, the eradication of typhoid fever ultimately requires the identification and management of healthy human carriers in addition to improving water sanitation.

After drinking water in the USA became safe, the discovery and control of healthy typhoid carriers became an increasingly important goal in the effort to eradicate typhoid fever. For example, starting in the second and third decades of the twentieth century, confirmed chronic typhoid carriers in New York were placed under supervision by the local or state health department, which involved quarterly visits. Chronic typhoid carriers were not permitted to engage in any occupation involving the handling of food for other than members of their own household and were not permitted to reside on farms on which cows were kept. Furthermore, contacts of chronic carriers were advised to be vaccinated against typhoid fever. Chronic carriers remained under supervision by the local or state health department for the remainder of their lives, unless the carrier state was resolved by cholecystectomy, a procedure associated with 15% mortality in the 1920s and 1930s (Senftner and

Coughlin 1933). Although chloramphenicol therapy did not succeed in clearing *S. Typhi* carriage (Woodward et al. 1950), the advent of antibiotic therapy in the 1940s made cholecystectomy a safe procedure to cure chronic carriers (Freitag 1964). Eventually, these public health measures led to an eradication of typhoid fever in the USA by the second half of the twentieth century (Armstrong et al. 1999).

The Global Burden of Typhoid Fever

The most recent estimate suggests that 21,650,974 illnesses of typhoid fever occurred worldwide during the year 2000 (Crump et al. 2004). The incidence of typhoid fever is highest in south-central and Southeast Asia with more than 100 illnesses per 100,000 people each year. For comparison, the annual incidence in the USA at the beginning of the twentieth century was approximately 216 illnesses per 100,000 people (Putnam 1927). Typhoid fever is also endemic in the rest of Asia, Africa, Latin America, the Caribbean, and Oceania (with the exception of Australia and New Zealand) where it is responsible for between 10 and 100 illnesses per 100,000 people each year (Crump et al. 2004). For comparison, the incidence of typhoid fever in the USA was within this range in 1925, with 26 illnesses per 100,000 people, after improved sanitation of drinking water had been implemented, but before the problem of chronic carriage had been resolved (Putnam 1927). Currently, the disease is essentially eradicated in North America, Europe, and other developed countries. The Centers for Disease Control and Prevention (CDC) estimates 433 cases of typhoid fever occur each year in the USA (Scallan et al. 2011), and most of these cases can be traced back to foreign travel into endemic areas (Lynch et al. 2009).

Paratyphoid fever was responsible for an estimated 5,412,744 illnesses worldwide during the year 2000 (Crump et al. 2004). Globally, *S. Paratyphi A* is responsible for most episodes of paratyphoid fever. In southeast China, *S. Paratyphi A* has recently surpassed *S. Typhi* as the most common typhoidal serovar isolated from blood cultures (Ochiai et al. 2005). This might be due in part to successes in typhoid fever vaccination, which does not protect against *S. Paratyphi A* (Simanjuntak et al. 1991).

Using the conservative estimate of 1% for the case fatality derived from hospital-based studies and the estimate that 21,650,974 illnesses occurred worldwide in the year 2000, it has been approximated that typhoid fever was responsible for 216,510 deaths that year (Crump et al. 2004). A limitation of this estimate is that case fatality rate data from population-based studies in areas with a high incidence of the disease are lacking. A more recent meta-analysis of hospital-based studies conducted between 1984 and 2005 in Africa, Asia, Latin America, and Oceania suggests that 2% is a more realistic estimate for the current median case fatality rate of typhoid fever (Crump et al. 2008). When this more recent approximation for the case fatality rate is applied to predict the global death toll, it can be estimated that *S. Typhi* was responsible for 433,020 deaths in the year 2000.

Antibiotic Resistance

In 1948, chloramphenicol was identified as the first antibiotic to be effective in the treatment of typhoid fever (Woodward et al. 1948), revolutionizing management of the disease and quickly becoming the drug of choice in its treatment (Rosove et al. 1950). However, *S. Typhi* strains carrying plasmid-encoded resistance against chloramphenicol emerged in the early 1970s in Latin America and Asia (Anderson and Smith 1972), which led to a search for alternative drugs.

Amoxicillin/ampicillin and oral cotrimoxazole (trimethoprim/sulfamethoxazole) therapy surfaced as an effective alternative for treatment of typhoid fever in the 1960s and 1970s (Uwaydah and Shammaa 1964; Calderon 1974; Snyder et al. 1976). However, multidrug-resistant (MDR) *S. Typhi* strains carrying plasmid-encoded resistance to chloramphenicol, ampicillin, and trimethoprim-sulfamethoxazole appeared in the late 1980s in Asia (Anand et al. 1990). MDR *S. Typhi* currently comprises 7% of isolates from India, 22% of isolates from Vietnam, and 65% of isolates from Pakistan (Parry and Threlfall 2008).

In the 1980s and 1990s, new antibiotics for the treatment of MDR *S. Typhi* were introduced, which included extended spectrum cephalosporins (Soe and Overturf 1987; Islam et al. 1988) and fluoroquinolones (Wang et al. 1989; Dutta et al. 1993). Partial or full resistance to fluoroquinolones emerged in Vietnam in 1993 (decreased ciprofloxacin susceptible [DCS] *S. Typhi*), which has begun to limit treatment options (Wain et al. 1997; Chinh et al. 2000; Parry 2004). A recent survey suggests that 44% of typhoid fever isolates from Vietnam, 57% of isolates from India, and 59% of isolates from Pakistan represent DCS *S. Typhi* (Parry and Threlfall 2008). Current treatment recommendations for typhoid fever include the extended spectrum cephalosporin antibiotic ceftriaxone, the macrolide antibiotic azithromycin, the fluoroquinolone antibiotic gatifloxacin, and, in areas with low prevalence of plasmid-mediated resistance, chloramphenicol (Butler 2011).

Disease

Distribution of Bacteria

Typhoid fever is an infection characterized by systemic bacterial dissemination in immunocompetent individuals. Upon ingestion of contaminated food, milk, or water, *S. Typhi* enters the ileal mucosa at areas of Peyer's patches. From here, the pathogen passes through the mesenteric lymph nodes and the efferent lymphatics into the blood stream, from which it disseminates to internal organs. Postmortem examination reveals *S. Typhi* to be present more commonly in the ileum and less frequently in the colon (Tonney et al. 1916). In the absence of antibiotic treatment, *S. Typhi* is isolated consistently from the Peyer's patches, the mesenteric lymph nodes, the liver and the spleen, with great frequency from the bone marrow and the gallbladder, in about half of the cases from blood, and not commonly from

the kidneys and the lungs (Horton-Smith 1900; Levy and Gaetgens 1908). Bacteriuria occurs in approximately 22% of individuals toward the end of the disease or during convalescence, which is commonly associated with bacterial numbers high enough to turn the urine turbid (Richardson 1903; Horton-Smith 1900).

With the advent of antibiotic therapy, bacteriuria caused by *S. Typhi* has become rare (Mathai et al. 1995). However, *S. Typhi* can be isolated from rectal swab culture or stool culture in 26–37% of typhoid fever patients treated with antibiotics (Gilman et al. 1975; Hoffman et al. 1984; Benavente et al. 1984). Chronic gallbladder carriage develops in approximately 3% of patients despite antibiotic therapy (Huang and DuPont 2005; Gilman 1989).

Clinical Presentation

One striking feature of typhoid fever is that after ingestion of contaminated food, milk or water, the initial spread of *S. Typhi* through the Peyer's patches and mesenteric lymph nodes into the circulation does not evoke overt host responses, as indicated by the fact that the average incubation period of the disease is 2 weeks (Olsen et al. 2003). After this incubation period, the disease presents with nonspecific symptoms, most commonly including fever and a relative slowing of the heart rate (bradycardia) (Nasrallah and Nassar 1978). Splenomegaly, hepatomegaly, or rose spots on the skin are encountered less frequently (Nasrallah and Nassar 1978). Less than half of typhoid patients present with neurological symptoms, cough, abdominal pain, or headache (Secmeer et al. 1995; Chow et al. 1989). Typhoid fever is not considered a diarrheal disease as this symptom develops in only a fraction (approximately one third) of typhoid fever patients, while the remaining individuals remain either diarrhea-free or become constipated (Chow et al. 1989; Yap and Puthuchearry 1998). Paratyphoid fever is indistinguishable in its symptoms from typhoid fever but milder in its course.

Histopathology

The pathology of typhoid fever is characterized by the predominance of mononuclear phagocytes (macrophages and/or dendritic cells) and a paucity of neutrophils (Mallory 1898; Hornick et al. 1970). An exception is the accumulation of neutrophils that is occasionally seen in typhoidal meningitis, typhoidal osteomyelitis, or typhoidal pneumonia. However, neutrophils are typically scarce in typhoid fever lesions and the pathology consists of clusters of mononuclear phagocytes and lymphocytes, termed typhoid nodules, which are observed with variable frequency and size depending upon the severity of the condition (Nasrallah and Nassar 1978; Shin et al. 1994). These histopathological changes can be seen as early as 3 days after oral infection of volunteers in biopsies collected from the small intestinal mucosa, which exhibit granulomatous inflammatory lesions formed largely by mononuclear phagocytes (Sprinz et al. 1966). The characteristic swelling

of the Peyer's patches, the mesenteric lymph nodes, and the spleen during typhoid fever is due largely to the formation of mononuclear infiltrates (Mallory 1898). Mononuclear phagocytes can also be present in the urine (Pepper 1920) or in stool samples (Harris et al. 1972; Alvarado 1983).

Mononuclear phagocytes containing erythrocytes are a common finding in typhoid lesions (Shin et al. 1994; Ponfick 1872). These so-called typhoid cells represent hemophagocytic macrophages that engulf neutrophils, red blood cells, and platelets (Mallouh and Sa'di 1987). A general increase in the phagocytic activity of macrophages in the reticuloendothelial system is suggested by an increased clearance of tagged albumen particles from the blood of typhoid fever patients (Greisman et al. 1964). Hemophagocytosis is thought to be a mechanism contributing to complete blood count changes that can accompany typhoid fever, including neutropenia, anemia, and thrombocytopenia (Serefhanoglu et al. 2003; Khosla et al. 1995; Abdool Gaffar et al. 1992; Butler et al. 1978; Houston 1901).

Complications

A relapse occurs in approximately 8–10% of patients receiving no antibiotic therapy and in 15–20% of patients treated with chloramphenicol (Hornick et al. 1970). Relapses in typhoid fever represent a complete renewal of the primary disease, producing the same symptoms but commonly being of shorter duration (Horton-Smith 1900).

Intestinal perforation develops in approximately 5% of typhoid fever patients (Butler et al. 1985). Typhoid intestinal perforation is a significant cause of mortality from typhoid fever affecting both children and adults (Uba et al. 2007; Bitar and Tarpley 1985). The mononuclear cell infiltrate in ileal lymphoid tissue can be associated with fibrin deposition in capillaries, resulting in capillary thrombosis and ulceration at areas of Peyer's patches, usually observed in the second week of fever. Continuous ulceration can produce hemorrhage and intestinal perforation associated with peritonitis, which usually occurs in the third week of fever (Bitar and Tarpley 1985). Infiltrates observed in histological studies from perforated small intestines remain mononuclear while neutrophils are scarce (Mukawi 1978; Kraus et al. 1999).

Several complications of the hepatobiliary system have been described. Typhoid nodules may coalesce to form a liver abscess or hepatic necrosis or produce typhoid hepatitis with jaundice (Durrani 1995; Nasrallah and Nassar 1978). Jaundice can also be associated with cholecystitis, which develops in some individuals (Morison 1913). Spontaneous splenic rupture has also been reported (Huang and DuPont 2005). Finally, chronic gallbladder carriage is a risk factor for developing gallbladder cancer (Caygill et al. 1995; Caygill et al. 1994; Welton et al. 1979).

Approximately 1–6% of typhoid fever patients develop pneumonia, which is commonly associated with underlying abnormalities, such as immunosuppression (Dutta et al. 2001). Other atypical manifestations include osteomyelitis, arthritis, meningitis, and cardiac abnormalities (Huang and DuPont 2005).

Differences Between Typhoid Fever and Gastroenteritis

Typhoidal *Salmonella* serovars are closely related genetically to nontyphoidal *Salmonella* serovars (Selander et al. 1990) but cause strikingly different diseases in humans (Tsolis et al. 2008). Nontyphoidal *Salmonella* serovars are commonly associated with gastroenteritis in immunocompetent individuals (NTS gastroenteritis), with *S. enterica* serovars Typhimurium and Enteritidis being isolated most frequently (Rabsch et al. 2001). NTS gastroenteritis is a typical diarrheal disease that manifests as a localized infection of the terminal ileum, mesenteric lymph nodes, and less frequently the colon. Common symptoms include, in order of frequency, diarrhea, abdominal pain, fever, headache, muscle pains, chills, and vomiting. NTS gastroenteritis has a short incubation period, which is less than 1 day on an average (Glynn and Palmer 1992). The histopathological hallmark of NTS gastroenteritis is acute intestinal inflammation with infiltrates that are dominated by neutrophils (McGovern and Slavutin 1979; Murphy and Gorbach 1982). Similarly, fecal leukocytes from NTS gastroenteritis patients are dominated by neutrophils (Harris et al. 1972; Alvarado 1983). The disease is self-limited and symptoms subside within less than 10 days after onset (Glynn and Palmer 1992). Thus, the characteristics of NTS gastroenteritis differ markedly from the clinical features of typhoid fever (Table 17.2).

Infections with nontyphoidal *Salmonella* serovars can be complicated by bacteremia in immunocompromised individuals (NTS bacteremia) (Gordon 2008; Reddy et al. 2010), a condition accompanied by neutrophilia (Saballs et al. 1993; Fleisher 1991), an antibacterial response triggered by an interleukin (IL)-1 β -mediated release of neutrophils from bone marrow stores (Tewari et al. 1990). In contrast, neutrophilia does not develop during *S. Typhi* bacteremia. Instead, 25% of individuals with typhoid fever develop neutropenia (Khosla et al. 1995; Abdool Gaffar et al. 1992). Leukocytes in the blood respond to NTS bacteremia by mounting a respiratory burst, and the resulting production of reactive oxygen intermediates can be visualized by an increased ability of blood samples to reduce nitroblue tetrazolium (NBT) (Miller et al. 1976). In contrast, typhoid fever is associated with a negative NBT blood test, indicating a markedly reduced oxidative response in the blood of patients infected with *S. Typhi* (Miller et al. 1976). Collectively, these observations suggest that nontyphoidal *Salmonella* serovars trigger some innate antibacterial responses in the human host, which are not observed in individuals with typhoid fever.

Phylogeny and Evolution

Evolutionary History of the Genus *Salmonella*

The genus *Salmonella* is a clonal lineage, which is closely related to *E. coli*, *Shigella* species, and *Citrobacter* species (Fig. 17.2) (Petty et al. 2010). It has been estimated that the point of divergence between the genus *Salmonella* and the genus

Table 17.2
Differences between typhoid fever and NTS gastroenteritis

	Typhoid fever	NTS gastroenteritis
Etiology	<i>S. Typhi</i>	Nontyphoidal <i>Salmonella</i> serovars (e.g., <i>S. Typhimurium</i> , <i>S. Enteritidis</i>)
Distribution of bacteria in immunocompetent host	Systemic infection	Infection remains localized to intestine and mesenteric lymph nodes
Incubation period	14 days	<1 day
Common symptoms	Fever, relative bradycardia	Diarrhea, abdominal pain, fever, headache, muscle pains
Duration of symptoms	3 weeks	<10 days
Predominant cell type in intestinal infiltrates	Mononuclear cells and lymphocytes	Neutrophils
Fecal leukocytes	Mononuclear cells	Neutrophils

Escherichia was approximately 100 Ma ago (Ochman and Wilson 1987). *S. bongori* and the different *S. enterica* subspecies form a hierarchical population structure (▶ Fig. 17.3) (McQuiston et al. 2008). A low rate of recombination between these lineages is inferred from sequence data of housekeeping genes, which differ between 2.8% and 4.4% of nucleotides between species and subspecies (Nelson et al. 1991; Nelson and Selander 1992; Boyd et al. 1994). It has been estimated that *S. enterica* diverged from *S. bongori* during the Eocene period between 40 and 63 Ma ago, while *S. enterica* subspecies *enterica* emerged as a separate lineage 4 to 6 Ma ago (McQuiston et al. 2008). While other lineages within the genus *Salmonella* are largely reptile-associated, *S. enterica* subspecies *enterica* is commonly isolated from mammals and birds, contains the largest number of serovars (▶ Fig. 17.3), and is responsible for more than 99% of human clinical isolates (Aleksic et al. 1996; Bäumlér 1997). Recombination within *S. enterica* subspecies *enterica* is not uncommon (Lan et al. 2009; Didelot et al. 2007; Li et al. 1994; Smith et al. 1990).

Evolutionary History of Typhoidal *Salmonella* Serovars

The vast majority of *S. enterica* subspecies *enterica* serovars are associated with gastroenteritis in humans (Aleksic et al. 1996). Within this group, typhoidal *Salmonella* serovars developed in four phylogenetically unrelated clonal lineages, presumably from ancestral organisms that were originally associated with gastroenteritis (Selander et al. 1990). *S. Typhi* isolates, *S. Paratyphi C* isolates, and *S. Paratyphi B* isolates each form one of these distinct lineages. The fourth lineage is formed by isolates of *S. Paratyphi A* and *S. Sendai*. These observations suggest that *S. Sendai* evolved recently from *S. Paratyphi A* by acquiring an *rfb* biosynthesis gene cluster through horizontal gene transfer that encodes an O antigen of serogroup D1 (▶ Table 17.1) (Selander et al. 1990).

S. Typhi represents a clonal lineage within *S. enterica* subspecies *enterica* that likely emerged after human migrations

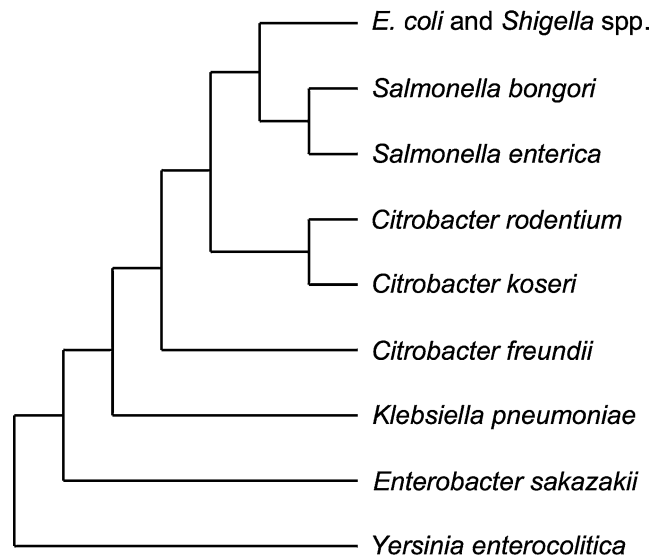
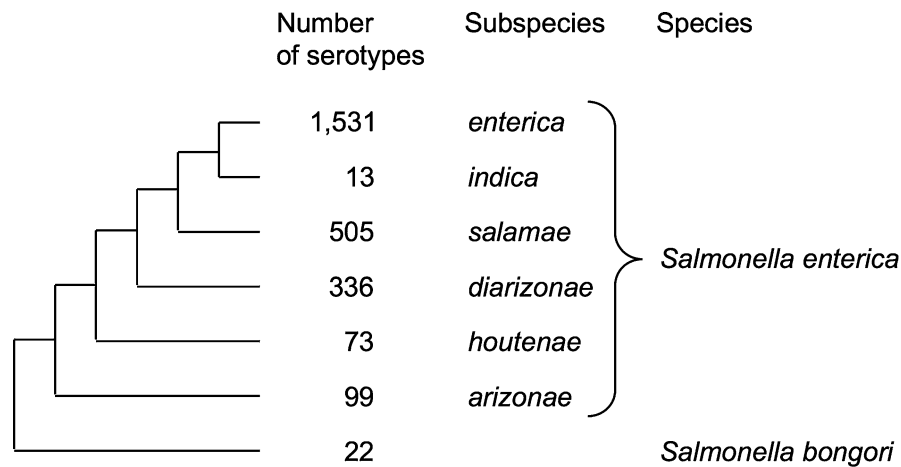


Fig. 17.2
Phylogenetic connection of *S. enterica* and *S. bongori* to related species within the Enterobacteriaceae. The tree is not drawn to scale. Phylogenetic relationships are based on genome analysis (Petty et al. 2010)

out of Africa but before the agricultural revolution of the Neolithic age (Roumagnac et al. 2006; Kidgell et al. 2002). This scenario is supported by sequence analysis of seven housekeeping genes from different *S. Typhi* isolates, which indicates that the lineage is between 15,000 and 150,000 years old (Kidgell et al. 2002). A more recent single-nucleotide polymorphism (SNP) analysis of 200 *S. Typhi* genes suggests a similar time of origin for the most recent common ancestor of the lineage, which lived an estimated 10,000–71,000 years ago (Roumagnac et al. 2006).

Although typhoidal *Salmonella* serovars form four phylogenetically distant lineages, evolution of the typhoidal lifestyle may have not occurred four times independently. For instance, the



■ Fig. 17.3

The genus *Salmonella*. The phylogenetic tree shown on the left is not drawn to scale and is based on sequence analysis of housekeeping genes (McQuiston et al. 2008). The number of serovars present in each species and subspecies has been reported recently (Grimont and Weill 2007). The classification into species and subspecies has been reviewed previously (Brenner et al. 2000)

viaB region encoding production of the Vi antigen is present in *S. Typhi* and *S. Paratyphi C*, suggesting that convergence by horizontal transfer of this DNA region occurred in their evolutionary history (Selander et al. 1990). Furthermore, horizontal gene transfer between *S. Typhi* and *S. Paratyphi A/S. Sendai* lineage has occurred on a scale so massive (Didelot et al. 2007) that it blurs the evolutionary relationship of their lineages (Chan et al. 2003). This large-scale recombination between the lineages of *S. Typhi* and *S. Paratyphi A/S. Sendai* occurred before the common ancestors of their contemporary clinical isolates emerged. During this phase, both lineages exchanged an astonishing 23% of their genome by horizontal gene transfer, presumably during coexistence in a shared human reservoir (Didelot et al. 2007).

It has been speculated that extensive horizontal gene transfer between *S. Typhi* and *S. Paratyphi A* might have conferred new genetic traits required for causing disseminated infections (Holt et al. 2008), and this event could thus mark the origin of typhoid and paratyphoid fever. Large-scale recombination with *S. Paratyphi A* occurred after the *S. Typhi* lineage had already passed through much (approximately 75%) of its evolutionary history (Holt et al. 2008). The clock rate and age of the *S. Typhi* lineage estimated by Achtman and coworkers (Roumagnac et al. 2006) would suggest that this large-scale exchange took place between 1,500 and 10,700 years ago. Typhoid fever might thus have its origin somewhere between antiquity and the agricultural revolution of the Neolithic age.

Subsequent to the large-scale recombination between *S. Typhi* and *S. Paratyphi A*, both lineages became isolated again, which was accompanied by the formation of pseudogenes at an accelerated rate, a process that is still ongoing (Holt et al. 2008). Many pseudogenes of typhoidal *Salmonella* serovars encode functions required for the gastrointestinal lifestyle of nontyphoidal *Salmonella* serovars, suggesting that the phase of isolation and accelerated pseudogene formation might represent the transition to chronic gallbladder carriage, a route of

transmission that became accessible to *S. Typhi* after it acquired the ability to cause disseminated infections. The transition to gallbladder carriage would have made functions obsolete that are required for transmission during gastroenteritis. Inactivation of such functions through pseudogene formation might have been accelerated because the lineage passed through bottlenecks as it became host-adapted (Holt et al. 2008). Finally, the lineage diversified to give rise to all contemporary *S. Typhi* isolates (Roumagnac et al. 2006).

Analysis of the contemporary population structure using SNP analysis suggests that extant *S. Typhi* lineages arose by clonal diversification from a monophasic, antibiotic-sensitive ancestral organism (Lan et al. 2009). A relatively recently emerged clonal lineage (Lan et al. 2009) acquired a linear plasmid (Baker et al. 2007) carrying a *fljBA*-like operon that encodes the H:z₆₆ antigen (Roumagnac et al. 2006; Octavia and Lan 2007). Isolates from this clonal lineage are biphasic and currently circulate in Indonesia (Huang et al. 2004). Analysis of the variation within the contemporary *S. Typhi* population does not reveal evidence for adaptive selection (Holt et al. 2008), with the exception of mutations in *gyrA*, which confer resistance to ciprofloxacin (Wain et al. 1997). Separate clonal lineages emerged in response to antibiotic selection either by acquisition of plasmid-encoded antibiotic resistance determinants or by gaining ciprofloxacin resistance through mutations in the chromosomal *gyrA* gene (Holt et al. 2010; Kariuki et al. 2010; Octavia and Lan 2009; Lan et al. 2009; Baker et al. 2008; Octavia and Lan 2007; Wain et al. 1997; Roumagnac et al. 2006). The absence of adaptive selection, other than that imposed by antibiotic resistance, might be explained by small population size and genetic drift, a scenario consistent with the proposed key role of chronic *S. Typhi* carriers as the main reservoir of this pathogen during evolution (Holt et al. 2008). Furthermore, an absence of adaptive selection suggests that *S. Typhi* is not under strong selective pressure from the host immune system in the niche the pathogen occupies during chronic carriage (Holt et al. 2008).

Pathogenesis and Genetics

Animal Models for Typhoid Fever

Oral inoculation of chimpanzees with *S. Typhi* produces an illness that closely mimics typhoid fever in its clinical course and its pathological alterations (Engelhardt 1972; Gaines et al. 1968; Edsall et al. 1960; Grunbaum 1904). However, lower primates, such as rhesus macaques, are resistant to experimental *S. Typhi* infection (Gaines et al. 1968; Grunbaum 1904). Similarly, nonprimate vertebrate animals generally do not show signs of infection upon ingesting *S. Typhi*. Historically, virulence of *S. Typhi* has been assessed by intraperitoneal or intravenous inoculation of mice, sometimes after administration of iron (Findlay 1951; Carter and Collins 1974; Powell et al. 1980; O'Brien 1982; Furman et al. 1994), but these models have fallen out of favor because they only poorly reproduce the course of infection and pathology characteristic of typhoid fever. More recently, *S. Typhi* has been shown to grow in the liver and spleen of humanized mice, which represents a promising new animal model for studying aspects of typhoid fever pathogenesis (Libby et al. 2010; Song et al. 2010; Firoz Mian et al. 2011).

Analysis of its phylogenetic history suggests that *S. Typhi* evolved from an ancestral nontyphoidal organism associated with gastroenteritis (Selander et al. 1990). Some virulence mechanisms inherited from this ancestral organism are likely shared with nontyphoidal *Salmonella* serovars, such as *S. Typhimurium*. This assumption provides the principle rationale for using *S. Typhimurium* infection in the mouse as an animal model for studying *S. Typhi* infection in humans. Mice ingesting *S. Typhimurium* develop a systemic illness characterized by bacterial multiplication in the liver, spleen, mesenteric lymph nodes, and Peyer's patches (Loeffler 1892; Müller 1912; Ørskov and Moltke 1929). This animal model has been used extensively to study the pathogenesis of typhoid fever (mouse typhoid model) (reviewed in (Tsolis et al. 1999b; Santos et al. 2001)). Work using the mouse typhoid model provides important insights into the basic pathogenic strategies deployed by nontyphoidal *Salmonella* serovars and into the functions of major virulence factors shared by typhoidal and nontyphoidal *Salmonella* serovars.

Ancestral Virulence Mechanisms Shared by Typhoidal and Nontyphoidal *Salmonella* Serovars

One virulence strategy conserved among typhoidal and nontyphoidal *Salmonella* serovars is the ability to invade the intestinal epithelium. Flagella contribute to epithelial invasion by increasing bacterial contact with host cells. Flagella-mediated motility contributes markedly to the efficiency of epithelial invasion observed *in vitro* for *S. Typhimurium* (Jones et al. 1992; Khoramian-Falsafi et al. 1990) as well as *S. Typhi* (Winter et al. 2009b; Liu et al. 1988). A second virulence factor required for invasion is the invasion-associated type III secretion system (T3SS-1) of *S. Typhi* (Elsinghorst et al. 1989) and *S. Typhimurium* (Galán & Curtiss 1989). The T3SS-1 apparatus is encoded by

Salmonella pathogenicity island 1 (SPI1) (Mills et al. 1995), a 40-kb DNA region conserved among isolates of *S. bongori* and *S. enterica* but absent from closely related bacterial species such as *E. coli* (Li et al. 1995). The T3SS-1 functions as a molecular syringe that injects proteins, termed effectors, into the host cell cytosol (Fu and Galan 1998). The T3SS-1 effector proteins SipA, SipC (SspC), SopA, SopB (SigD), SopD, SopE, and SopE2 cooperate in inducing actin rearrangements in the host cell cytosol, thereby promoting bacterial entry into the epithelium (Hong and Miller 1998; Hardt et al. 1998; Hayward and Koronakis 1999; Zhou et al. 1999; Friebel et al. 2001; Jepson et al. 2001; McGhie et al. 2001; Raffatellu et al. 2005b).

A second virulence strategy shared by typhoidal and nontyphoidal *Salmonella* serovars is the ability to survive in macrophages. Macrophage survival of *S. Typhimurium* (Ochman et al. 1996) and *S. Typhi* (Forest et al. 2010) requires the action of a second type III secretion system (T3SS-2) encoded by SPI2. SPI2 is a DNA region that is absent from *S. bongori* but highly conserved within *S. enterica* (Ochman and Groisman 1996; Hensel et al. 1997). Acting as a molecular syringe, the T3SS-2 injects the effector proteins SpiC, SseF, SseG, SlrP, SspH1, SspH2, SifA, SifB, SseI, SseJ, PipB, PipB2, SseK1, SseK2, GogB, and SopD2 into the macrophage cytosol (reviewed in (Abrahams and Hensel 2006)). One function of the T3SS-2 is to manipulate vesicular trafficking events in macrophages (Vazquez-Torres et al. 2000; Uchiya et al. 1999; Beuzon et al. 2000), but it remains unclear in most cases how individual effector proteins contribute to T3SS-2-mediated macrophage survival. Inactivation of T3SS-2 in *S. Typhimurium* attenuates virulence in the mouse typhoid model 10,000-fold (Hensel et al. 1995), suggesting that this virulence factor is important in the setting of a disseminated infection.

Induction of Innate Immunity During Gastroenteritis

To cause gastroenteritis in a calf model, *S. Typhimurium* uses flagella and T3SS-1 to invade the intestinal mucosa and T3SS-2 to survive in tissue (Tsolis et al. 1999a, c; Schmitt et al. 2001; Zhang et al. 2002; Winter et al. 2009a). The presence of *S. Typhimurium* in tissue is sensed by the innate immune system through a multitude of pathways, including the detection of cytosolic access by T3SS-1 through NLRC4 (NOD-like receptor [NLR] family caspase-associated recruitment domain [CARD]-containing protein 4) (Miao et al. 2006, 2010b; Franchi et al. 2006), the activation of NLRP3 (NLR family pyrin domain-containing protein 3) by an unknown mechanism (Broz et al. 2010), the detection of cell wall fragments by nucleotide-binding oligomerization domain (NOD)1 and NOD2 (Geddes et al. 2010, 2011; Le Bourhis et al. 2009), the detection of flagellin by TLR5 (Gewirtz et al. 2001), the detection of curli amyloid fibrils through TLR1/TLR2 (Tukel et al. 2005, 2009, 2010), and the detection of the lipid A moiety of LPS by TLR4 (Vazquez-Torres et al. 2004). Finally, the O antigen moiety of LPS is detected through complement, which results in the deposition of C3b,

a cleavage product of complement component 3 (C3), on the bacterial surface (Joiner et al. 1989) through covalent attachment of C3b by an ester bond formed with free hydroxyl groups in LPS sugar moieties (Sahu et al. 1994) (● Fig. 17.1c).

Detection of *S. Typhimurium* through these pathways triggers a cytokine storm in the intestinal mucosa that helps to orchestrate antibacterial responses, including macrophage activation, neutrophil recruitment, and the epithelial release of antimicrobials (reviewed in (Winter et al. 2010a; Santos et al. 2009)). Orchestration of these innate immune responses occurs rapidly, which explains the short incubation period of gastroenteritis (● Table 17.2). Infected macrophages sense T3SS-mediated cytosolic access and respond by releasing *S. Typhimurium* through pyroptosis, which renders the pathogen vulnerable to neutrophil attack (Miao et al. 2010a). Neutrophils are important for preventing spread of the pathogen beyond the mesenteric lymph node in humans (Noriega et al. 1994; Tumbarello et al. 1995) because C3-deposition on *S. Typhimurium* facilitates complement receptor 3 (CR3)-mediated phagocytosis (Joiner et al. 1989), an uptake mechanism that is linked to a respiratory burst in neutrophils (Joiner et al. 1989). Eventually, the pathogen is cleared from intestinal tissues through these mechanisms without transmitting to a susceptible host, which suggests that localization in tissue represents a dead end for *S. Typhimurium*.

However, growth of *S. Typhimurium* in the intestinal lumen is enhanced during intestinal inflammation (Stecher et al. 2007; Barman et al. 2008). Neutrophils migrate into the intestinal lumen, which gives rise to fecal leukocyte populations that are dominated by neutrophils, one of the characteristic clinical features of gastroenteritis (● Table 17.2) (Harris et al. 1972; Alvarado 1983). Neutrophil transepithelial migration promotes luminal growth of *S. Typhimurium* (Sekirov et al. 2010) because a by-product of the phagocyte respiratory burst is the generation in the intestine of a new respiratory electron acceptor, tetrathionate ($S_4O_6^{2-}$) (Winter et al. 2010b). Tetrathionate is a chemical long used for the enrichment of *Salmonella* serovars in samples containing competing microbes (Muller 1923). Growth by tetrathionate respiration in the inflamed intestine enables *S. Typhimurium* to outgrow competing microbes (Winter et al. 2010b) by using nutrients, such as ethanolamine, that do not support growth by fermentation (Thiennimitr et al. 2011). The resulting outgrowth of *S. Typhimurium* in the intestinal lumen promotes transmission of the pathogen to the next susceptible host through the fecal oral route (Lawley et al. 2008).

In conclusion, the principal virulence strategy of nontyphoidal *Salmonella* serovars is to use their virulence factors, flagella, T3SS-1, and T3SS-2, to attract neutrophils into the intestinal lumen, thereby gaining a luminal growth advantage that ensures their transmission (reviewed in (Santos et al. 2009)).

Evasion of Innate Immunity During Typhoid Fever

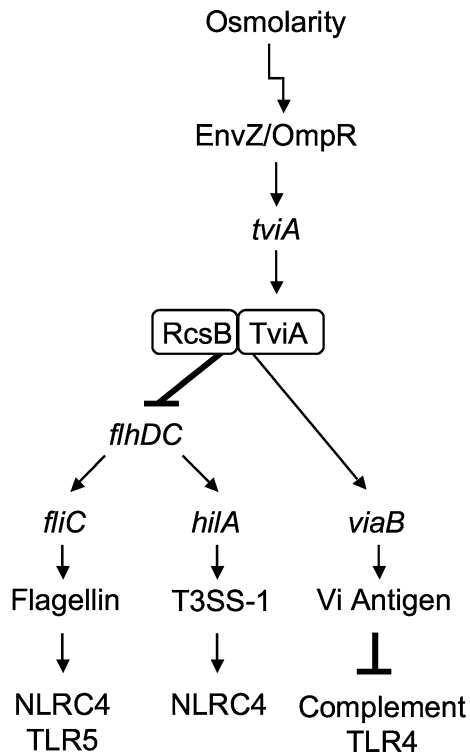
While nontyphoidal and typhoidal *Salmonella* serovars share flagella, T3SS-1, and T3SS-2, the former group is associated

with gastroenteritis in humans while the latter group causes typhoid and paratyphoid fever (● Table 17.2). This clinical correlation suggests that some of the genes that enable *S. Typhi* to cause typhoid fever in humans are not present in *S. Typhimurium*. It follows that the virulence mechanisms responsible for the differences between gastroenteritis and typhoid fever (● Table 17.2) cannot be interrogated by studying *S. Typhimurium* infection in mice (Tsolis et al. 2011). Since the vast majority of investigators studying typhoid fever pathogenesis rely on *S. Typhimurium* infection in the mouse as a model, the differences between gastroenteritis and typhoid fever remain less studied than the virulence mechanisms that are shared by nontyphoidal and typhoidal *Salmonella* serovars.

One striking difference between gastroenteritis and typhoid fever is that the latter has a very long incubation period (● Table 17.2), which illustrates that flagella/T3SS-1-mediated epithelial invasion and T3SS-2-mediated survival in macrophages are not accompanied by host responses severe enough to elicit symptoms within the first 2 weeks of an *S. Typhi* infection. Since the acute onset of intestinal inflammation that produces symptoms of gastroenteritis within less than 1 day is triggered by innate immune recognition of *S. Typhimurium*, the long incubation period of typhoid fever suggests that *S. Typhi* evades or prevents detection by the innate immune surveillance system. A DNA region involved in this process is the *viaB* locus of *S. Typhi* (Hirose et al. 1997; Sharma and Qadri 2004; Raffatellu et al. 2005a, 2007).

In addition to genes for the biosynthesis (*tviBCDE*) and the export (*vexABCDE*) of the Vi antigen, the *viaB* locus contains *tviA*, which encodes a positive regulator of capsule expression (Virlogeux et al. 1995). Expression of *tviA* is induced at tissue osmolarity by the two-component system EnvZ/OmpR, while high osmolarity encountered in the intestinal lumen prevents *tviA* expression (● Fig. 17.4) (Winter et al. 2009b, 2010c). TviA changes gene expression by forming heterodimers with the response regulator RcsB (Virlogeux et al. 1996), a repressor of the *flhDC* genes (Cano et al. 2002). In turn, FlhDC acts as a positive regulator for expression of flagella and T3SS-1 invasion genes (Frye et al. 2006). As a result, *S. Typhi* remains invasive, motile, and noncapsulated while it resides in the high-osmolarity environment of the intestinal lumen. However, expression of TviA is induced during the transit though the intestinal epithelium, resulting in a rapid repression of flagellin and T3SS-1 expression (Winter et al. 2010c), while expression of the Vi antigen, a capsular polysaccharide, is induced (Tran et al. 2010).

TviA regulation has two important consequences. First, some of the signals that enable the innate immune system to detect *S. Typhimurium* in tissue, such as cytosolic access by the T3SS-1 sensed through NLRC4 (Miao et al. 2006, 2010b; Franchi et al. 2006) and the presence of flagellin sensed through TLR5 (Gewirtz et al. 2001), are rapidly turned off by TviA when *S. Typhi* enters the intestinal mucosa (● Fig. 17.4). Second, the Vi antigen is expressed when *S. Typhi* transits through the intestinal epithelium (Tran et al. 2010), which ensures that the



■ Fig. 17.4

***TviA*-mediated evasion of innate immunity.** The EnvZ/OmpR two-component system activates *tvIA* expression in response to changes in osmolarity that *S. Typhi* encounters when it transits from the intestinal lumen into tissue. TviA forms heterodimers with RcsB, which in turn represses *flhDC* expression. Through this mechanism, TviA prevents innate immune recognition of flagella and T3SS-1 through TLR5 and NLRC4. Furthermore, TviA/RcsB activates genes in the *viaB* locus which are involved in the biosynthesis of the Vi antigen, a surface structure preventing complement activation and recognition through TLR4

pathogen is encapsulated by the time it encounters complement. Expression of the Vi antigen inhibits complement deposition (Wilson et al. 2011; Looney and Steigbigel 1986) because this capsular polysaccharide does not contain free hydroxyl groups available for ester formation with C3b (► Fig. 17.1b). The complement fragments C3a and C5a generated during complement deposition are also known as the anaphylatoxins due to their potency in inducing inflammatory responses (reviewed in (Haas and van Strijp 2007)). C5a synergizes with TLR4 to generate responses against LPS (Li et al. 2005; Riedemann et al. 2003), which might explain why expression of the Vi antigen enables *S. Typhi* to evade detection through TLR4 (Hirose et al. 1997; Wilson et al. 2008; Jansen et al. 2011). Thus, TviA regulation enables *S. Typhi* to evade detection through multiple innate immune sensors by inducing Vi antigen expression and repressing motility and T3SS-1 (► Fig. 17.4). These innate immune evasion mechanisms contribute to the scarcity of neutrophils in lesions of typhoid fever (Jansen et al. 2011;

Haneda et al. 2009; Raffatellu et al. 2007) and might help explain the long incubation period of typhoid fever (► Table 17.2).

Inhibition of complement deposition by the Vi antigen also has consequences for the interaction of *S. Typhi* with neutrophils. CR3-mediated phagocytosis by neutrophils is coupled to a respiratory burst (Joiner et al. 1989). Inhibition of complement deposition by the Vi antigen prevents CR3-mediated phagocytosis (Looney and Steigbigel 1986; Wilson et al. 2011) and the generation of a respiratory burst during the interaction of *S. Typhi* with neutrophils (Miller et al. 1972; Kossack et al. 1981). The inhibition of a respiratory burst in neutrophils helps explain why *S. Typhi* bacteremia is associated with a negative NBT blood test (Miller et al. 1976). Collectively, these properties might aid *S. Typhi* in overcoming a neutrophil barrier and spread beyond the mesenteric lymph node to cause a disseminated infection in humans (► Table 17.2).

Importantly, the ability to disseminate throughout the host and take residence in internal organs is a prerequisite for transmission through gallbladder carriage. Similar to *Mycobacterium tuberculosis*, *S. Typhi* is able to persist in tissue in a state of dormancy, a property that is poorly studied but likely important for developing chronic carriage. Persistence in tissue may occur in hemophagocytic macrophages, or typhoid cells (Silva-Herzog and Detweiler 2010; Nix et al. 2007), that are commonly observed in typhoid lesions (Shin et al. 1994; Ponfick 1872). Finally, persistence in the gallbladder can be accompanied by the formation of *S. Typhi* biofilms on the surface of gallstones (Crawford et al. 2010). Formation of these biofilms requires an O antigen capsule that is distinct from the Vi antigen (Crawford et al. 2008).

In conclusion, the principal virulence strategy of *S. Typhi* is based on virulence mechanisms shared with nontyphoidal *Salmonella* serovars. These include flagella/T3SS-1-mediated epithelial invasion and T3SS-2-mediated macrophage survival. However, while nontyphoidal *Salmonella* serovars stimulate innate immunity to promote their growth in the intestinal lumen, *S. Typhi* evades innate immunity to disseminate systemically and ensure its transmission through chronic gallbladder carriage (reviewed in (Raffatellu et al. 2006; Tsolis et al. 2008)).

Genomic Rearrangements

The gene order on the chromosomes of *E. coli* and *S. Typhimurium* is highly conserved (Riley and Anilionis 1978) and very few inversions are observed between their sequenced genomes (Rocha 2004), although both lineages diverged approximately 100 Ma ago (Ochman and Wilson 1987). This high degree of conservation suggests that the gene order may be under selection. One effect commonly associated with genome rearrangements is a lower growth rate because inversions may alter gene dosage, gene strand bias, or chromosome symmetry (Rebollo et al. 1988; Hill and Gray 1988; Campo et al. 2004). It is therefore thought that any advantage gained by multiplying at a maximum growth rate places the gene

order on the chromosome under selection. In the case of *S. Typhimurium*, selective pressure arises from the need to transmit from an infected host to a new susceptible host (Kingsley and Bäumlér 2000). Transmission requires outgrowth of competing microbes in the gut (Lawley et al. 2008), which likely necessitates multiplication at a maximum growth rate. Thus, the maximum growth rate needed for transmission of *Salmonella* serovars associated with gastroenteritis likely imposes selective constraints that prevent chromosomal rearrangements.

In contrast to nontyphoidal *Salmonella* serovars, transmission of *S. Typhi* no longer depends on being able to multiply at a maximum growth rate to successfully compete with other microbes residing in the intestine. Instead, transmission is likely aided by a state of dormancy or low growth rate in tissue of chronic carriers. This would suggest that selective constraints that prevent chromosomal rearrangements no longer apply to *S. Typhi*. Consistent with this idea, major genomic rearrangements due to homologous recombination between the *rrn* operons, leading to inversions and translocations, are commonly detected in genomes of *S. Typhi* isolates (Kothapalli et al. 2005; Liu and Sanderson 1995, 1996). Similar genome rearrangements are also common in *S. Paratyphi C* (Liu and Sanderson 1998). Recombination between *rrn* operons occurs with equal frequency in *S. Typhi* and *S. Typhimurium* in the laboratory, but *S. Typhimurium* strains carrying rearrangements have never been isolated from nature (Kothapalli et al. 2005). In contrast, analysis of *S. Typhi* isolated from human carriers over multiple years shows that chromosome rearrangements occur within the host over time (Matthews et al. 2011). The finding that gene order on the *S. Typhi* chromosome is not under strong selective pressure in the niche the pathogen occupies during chronic carriage supports the concept that multiplication at a maximum growth rate is not needed for transmission of typhoid fever.

Pseudogene Formation in Typhoidal *Salmonella* Serovars

Typhoidal serovars contain substantially more pseudogenes (genes with inactivating mutations) than their nontyphoidal relatives (► Fig. 17.5). Indeed, this accumulation of pseudogenes in typhoidal serovars is hypothesized to be both a cause and a consequence of their human host restriction, extraintestinal tissue residence and shift in disease manifestation. This process is not unique to human typhoidal isolates, however. Genome sequencing reveals substantial pseudogene accumulation has occurred independently in a variety of systemic, host-restricted pathogens (Chiu et al. 2004; Thomson et al. 2008; McClelland et al. 2004; Liu et al. 2009; Parkhill et al. 2001; Deng et al. 2003; Jarvik et al. 2010; Holt et al. 2009). Direct gene-for-gene comparisons between *S. Typhi* and *S. Paratyphi A* have revealed the two serovars share 25 independently evolved and 44 inherited or recombined pseudogenes (Holt et al. 2009). While understanding the initial changes that lead a *Salmonella* strain

down the path of typhoidal evolution can provide insight into host specificity and disease, every alteration in this process can be informative for the pathogenesis of both typhoidal and nontyphoidal serovars: While genes that have been inactivated in typhoidal serovars can be seen as dispensable for an extraintestinal lifestyle, these genes likely persist in nontyphoidal serovars because they aid in intestinal growth and transmission during gastroenteritis.

For example, *S. Typhi* strain CT18 contains pseudogenes in 7 of its 11 fimbrial operons of the chaperone usher assembly class (Townsend et al. 2001), which encode intestinal colonization factors (Weening et al. 2005). The *shdA* and *misL* genes encode nonfimbrial adhesins that enhance intestinal colonization of *S. Typhimurium* (Dorsey et al. 2005; Kingsley et al. 2000, 2002). Pseudogene formation in typhoidal serotypes led to inactivation of both *shdA* (*S. Typhi* and *S. Paratyphi A*) and *misL* (*S. Typhi*) (Parkhill et al. 2001; McClelland et al. 2004). Another pseudogene in *S. Typhi* strain CT18 is *ttrS* (Parkhill et al. 2001), which is required by *S. Typhimurium* to outgrow competing microbes in the inflamed intestine by tetrathionate respiration (Winter et al. 2010b). Tetrathionate respiration supports growth of *S. Typhimurium* on ethanolamine as a carbon source in the lumen of the inflamed gut (Thiennimitr et al. 2011). Growth on ethanolamine under anaerobic conditions necessitates biosynthesis of vitamin B12 by the *cob/cbi* gene cluster (Roof and Roth 1988), which carries pseudogenes in *S. Typhi* (*cbiM*, *cbiK*, *cbiJ*, and *cbiC*) (Parkhill et al. 2001) and *S. Paratyphi A* (*cbiA*) (McClelland et al. 2004). Additional pathways with shared pseudogenes in *S. Typhi* and *S. Paratyphi A* include iron uptake and chemotaxis (Holt et al. 2009). Interestingly, iron acquisition and chemotaxis are both required by *S. Typhimurium* to edge out competing microbes in the gut lumen during gastroenteritis (Raffatellu et al. 2009; Stecher et al. 2008). In conclusion, many of the pseudogenes found in the genomes of typhoidal *Salmonella* serovars encode functions that aid intestinal colonization and competition with the microbiota during gastroenteritis, which in turn enhances transmission of nontyphoidal *Salmonella* serovars by the fecal oral route (Lawley et al. 2008). Thus, one driving force responsible for pseudogene formation in typhoidal *Salmonella* serovars might be that functions required for gaining an advantage during growth competition with the resident microbiota are no longer under selection, presumably because the main route of transmission changed to chronic gallbladder carriage during evolution of the typhoidal lifestyle.

One group of genes affected by pseudogene formation in *S. Typhi* and *S. Paratyphi A* are those encoding pathogenicity-associated T3SS effector proteins. While the stable effectors in nontyphoidal serovars can be seen as relatively constant, with each serovar differing in status between four and five loci, the typhoidal effector repertoire appears to be more plastic, differing between nine and fifteen loci (► Fig. 17.5). This observation suggests that some T3SS effectors play a greater role in the development of gastroenteritis than they do in enabling a typhoidal lifestyle.

	Typhoidal			Non-Typhoidal	
Serovar	Paratyphi A	Paratyphi C	Typhi	Enteritidis	Typhimurium
Strain(s)	AKU_12601 ATCC 9150	RKS4594	CT18 Ty2	P125109	14028s SL1344
Chromosome statistics					
Chromosome size (bp)	4581797 / 4585229	4833080	4809037 / 4791961	4685848	4857432 **
Predicted CDS	4285 / 4263	4578	4395 / 4339	4318	4450 **
Pseudogenes	204 / 173	149	204 / 206	113	39 **
SPI-1 T3SS Effectors					
AvrA	-	-	-	■	□ / ■
SipA (SspA)	■	■	■	■	■
SipB (SspB)	■	■	■	■	■
SipC (SspC)	■	■	■	■	■
SopA	□	□	□	■	■
SopB (SigD)	■	■	■	■	■
SopD	■	■	■	■	■
SopE	■	-	■	■	- / ■
SopE2	■	■	□	■	■
SptP	■	■	■	■	■
SPI-2 T3SS Effectors					
GogB	-	■	-	-	■
PipB	■	■	■	■	■
PipB2	■	■	■	■	■
SifA	■	■	■	■	■
SifB	□	■	■	■	■
SopD2	□	■	□	■	■
SpiC (SsaB)	■	■	■	■	■
SpvB	-	■	-	-	■ / -
SseF	■	■	■	■	■
SseG	■	■	■	■	■
Ssel (SrfH)	-	□	-	■	■
SseJ	-	■	□	■	■
SseK1	-	■	-	■	■
SseK2	□	□	□	□	■
SseL	■	■	■	■	■
SspH2	-	□	■	■	■
SteA	■	■	■	■	■
SteB	-	■	-	■	■
SteC	□	□	■	■	■
Effectors of Both					
SlrP	□	□	□	□	■
SspH1	-	-	-	-	■ / -

■ Fig. 17.5

Chromosome statistics and type III secretion system effector gene status. Chromosomal gene and pseudogene content for typhoidal and nontyphoidal serovars is provided (** Typhimurium strain LT2). Genes encoding effector proteins secreted by T3SS-1, T3SS-2, and by both systems are represented in the table as follows: black box, intact; white box, pseudogene; and dash, absent. Human typhoidal isolates are listed in white cells while nontyphoidal isolates are listed in gray cells for comparison. Differing results from multiple isolates of a serovar are presented in respective order separated by a forward slash. For more information on these effector proteins, please consult the review by Haraga et al. (2008)

Vaccination

Killed Parenteral Vaccines

The first killed parenteral typhoid vaccines were developed independently by Sir Almroth Edward Wright in England and Richard Pfeiffer in Germany (Wright 1896; Pfeiffer and Kolle 1896). Killed parenteral typhoid vaccines are effective against a low-dose challenge, which might result from a waterborne exposure, but offer little protection against high-dose challenge (Ashcroft et al. 1964; Hornick and Woodward 1967). Killed parenteral vaccines have a high reactogenicity, and their use has therefore been discontinued in most countries.

Live Attenuated Oral Vaccines

René Germanier and colleagues used the mouse typhoid model to develop the first live attenuated oral typhoid vaccine strain. *S. Typhimurium* mutants defective for the biosynthesis of uridine-5'-diphosphate galactose epimerase (*galE*) are deficient in the biosynthesis of the outer core of LPS when grown in the absence of galactose and are avirulent and immunogenic for mice (Germanier 1970, 1972). Oral vaccination with a *S. Typhimurium galE* mutant confers better protection in the mouse typhoid model than parenteral vaccination with a killed *S. Typhimurium* vaccine (Germanier 1972). Using chemical mutagenesis, a *galE* mutant of *S. Typhi* strain Ty2 was derived (Ty21a) (Germanier and Fuer 1975) and shown to be protective as live attenuated oral vaccine in field trials (Wahdan et al. 1982; Black et al. 1983, 1990).

The *galE* mutation is likely not the sole reason for the attenuation of Ty21a because introduction of a defined mutation in *galE* does not render *S. Typhi* strain Ty2 avirulent for humans (Hone et al. 1988). Therefore, it remains unknown which chemically induced mutations contribute to the attenuation of the vaccine strain Ty21a. Subsequent studies have demonstrated that *S. Typhi* strains attenuated by defined mutations in *cya crp* (χ 3927) (Tacket et al. 1992a), *cya crp cdt* (χ 4073) (Tacket et al. 1992a), *aroC aroD* (CVD908) (Tacket et al. 1992b), *aroC aroD htrA* (CVD908-*htrA*) (Tacket et al. 1997, 2000), *phoPQ* (Ty800) (Hohmann et al. 1996), or *aroC ssaV* (ZH9) (Hindle et al. 2002) are immunogenic in humans. Nonetheless, Ty21a currently remains the only available live attenuated oral typhoid vaccine.

Vi Parenteral Vaccines

Antibodies against the Vi antigen are not elicited by immunization with the live attenuated oral typhoid vaccine because the vaccine strain Ty21a carries a mutation in the *viaB* locus (Germanier and Fuer 1983). However, antibodies against the Vi antigen are present in the serum of typhoid fever patients (Felix et al. 1935). Early studies show that parenteral administration of purified Vi antigen confers protection in mouse

models (Landy and Webster 1952; Landy 1954, 1957). Subsequent reports indicated variability in the efficacy by which purified Vi antigen conferred protection against *S. Typhi* (Gaines et al. 1961; Hornick et al. 1966). This variability in protection has been attributed to removal of O- and N-acetyl moieties from the Vi antigen during purification (Fig. 17.1b), which alters antigenic properties of the polysaccharide (Robbins and Robbins 1984). Purification of the Vi antigen by methods that do not remove its O- and N-acetyl moieties yields capsular polysaccharide that confers protection in animals and humans (Wong et al. 1974; Levin et al. 1975; Robbins and Robbins 1984). Vi antigen prepared by this method proved efficacious in clinical trials (Acharya et al. 1987; Klugman et al. 1987) and is now a licensed parenteral typhoid fever vaccine (Klugman et al. 1996; Hessel et al. 1999).

Antibodies elicited by immunization with purified Vi antigen are strictly T cell independent, and the vaccine therefore does not induce increased antibody titers to a recall injection (Landy 1954). Conjugation of the Vi antigen with a recombinant, nontoxic, exotoxin A from *Pseudomonas aeruginosa* (rEPA) yields a vaccine that elicits T cell-dependent responses and confers superior protection in children (Thiem et al. 2011; Canh et al. 2004; Lin et al. 2001; Szu et al. 1994). These data suggest that licensing of the Vi-rEPA vaccine could improve typhoid fever vaccination in children in the future.

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