Review

Shedding light on *Salmonella* carriers

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Host-to-host transmission in most *Salmonella* serovars occurs primarily via the fecal–oral route. *Salmonella enterica* serovar Typhi is a human host-adapted pathogen and some *S.* Typhi patients become asymptomatic carriers. These individuals excrete large numbers of the bacteria in their feces and transmit the pathogen by contaminating water or food sources. The carrier state has also been described in livestock animals and is responsible for food-borne epidemics. Identification and treatment of carriers are crucial for the control of disease outbreaks. In this review, we describe recent advances in molecular profiling of human carriers and the use of animal models to identify potential host and bacterial genes involved in the establishment of the carrier state.

Salmonella transmission

Salmonella enterica comprises generalist serovars such as *S. enterica* serovar Typhimurium (*S.* Typhimurium) and host-adapted strains such as *S. enterica* serovar Typhi (*S.* Typhi). *Salmonella* enters the host through the gastrointestinal tract and translocates by multiple mechanisms to systemic tissues. For the infection to extend beyond the intestinal mucosa and become systemic, *Salmonella* must survive and replicate in macrophages, a privileged niche that allows the bacterium to elude the adaptive immune response [1,2]. In many *S. enterica* serovars, host-to-host transmission occurs via the fecal–oral route. Transmission efficiency has been linked to increased levels of fecal bacteria, with high-shedding hosts (referred to as supershedders, Box 1) responsible for most of the transmission [3,4]. Some infected individuals become carriers and persistently shed *Salmonella* in their feces for long periods of time, thereby functioning as a reservoir for the pathogen. Epidemiological analysis has established that these infected individuals are a crucial target for disease control because they shed the pathogen in high enough numbers to transmit disease [4,5].

Intermittent shedding (referred to as showers of *S.* Typhi) is also a common occurrence in *Salmonella* infections, whereby systemic sites of the host are colonized but *Salmonella* is not detected in the feces. For the purposes of this review, we include these individuals as carriers because they periodically shed the bacteria (Box 2). The specific host and pathogen factors that facilitate the carrier state and enable host-to-host transmission are poorly understood. This area of study is relatively under-represented in microbial pathogenesis research. Recent advances in mammalian models of *Salmonella* infection, combined with transmission studies in livestock animals, have resulted in a new understanding of the *Salmonella* carrier state.

Much of what is known about the carrier state has been gleaned from studies of animal infections. In this review, we describe what is known about the development of the carrier state in humans and livestock (cows, pigs and chickens) following *Salmonella* infection. We highlight some of the known virulence factors that influence carriage and transmission, and review what is known about the host response. However, many questions remain unanswered. For example, how do we detect chronic *S.* Typhi carriers and how do we distinguish them from individuals who were infected and have cleared the pathogen? What factors determine whether an infected host will become a persistently infected carrier?

S. Typhi carriage in humans

Typhoid fever represents a serious global public health problem, with 16 million new cases and over 600 000 deaths per year [2]. One hallmark of *S.* Typhi pathogenicity is the ability to establish a persistent, usually asymptomatic, carrier state in some infected individuals. A significant percentage (1–6%) of typhoid patients become chronic carriers of *S.* Typhi [6,7]. These individuals serve as a reservoir, transmitting *Salmonella* to new hosts by contamination of food or water sources. The concept of an asymptomatic carrier, first described by Robert Koch [8], was brought to public light with two particularly infamous carriers, Typhoid Mary and Mr N. The former was Typhoid Mary, an immigrant cook, who infected dozens of people over the course of her lifetime. Her case posed a challenge for the nascent New York Public Health Department, because she was one of the first asymptomatic carriers studied [9]. Her contemporary in England, Mr N, was a milkman who is thought to have infected over 200 people [8]. The publicity surrounding these cases allowed doctors to begin tracing typhoid epidemics to their human sources and to identify carriers by obtaining both a medical history and a fecal sample.

The long-term persistence of *S.* Typhi in carriers explains why typhoid fever remains endemic in regions of the world with poor-quality drinking water and limited sewage treatment. Roumagnac *et al*. used molecular tools to investigate the evolutionary history of *S.* Typhi by analyzing mutations in specific genes across a global collection of isolates [10]. They found ancestral haplotypes in current-day isolates, which suggests that these strains persist in asymptomatic carriers.

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Box 1. Super-shedder carrier state

- Defined in mice infected with *S.* Typhimurium as individuals shedding over 10^8 cfu/gm of feces; might vary according to *Salmonella* serovar and infected host.
- In pigs, chickens and mice, high-shedding hosts had increased innate inflammatory responses (proinflammatory cytokines and granulocyte influx) [56,66–69].
- Specific host immune responses associated with this state must be identified, preferably those that can be measured from peripheral blood or fecal samples.

Despite the evolution of molecular methods of detection, identification of carriers remains incredibly difficult because they can shed the bacilli intermittently. The causes of this intermittent shedding or showers of *S.* Typhi remain unknown (Box 2). Retrospective studies of typhoid outbreaks have allowed us to understand some, but not all, of the risk factors associated with carriers. Several studies have shown an association between age and carrier status, with children more likely to be short-term carriers and adults, long-term carriers. Among adults, individuals aged 50 years and older are much more likely to become carriers [6,11]. *S.* Typhi carriers are also more likely to be female and to suffer from gallbladder problems, commonly gallstones [12,13]. In a 1964 study that tracked carriers in New York City, five out of 13 carriers had no previous history of fever. Some 60% of the carriers had detectable levels of *Salmonella* in their stool (at a detection limit of 500 bacteria) and 60% had gallstones [12]. The carrier state is thought to be related to bacterial biofilm formation on the surface of gallstones [14]. *Salmonella* biofilms have been found on gallstones in both *S.* Typhi patients and a mouse model of chronic *S.* Typhimurium infection [15].

Generalist serovars of *S. enterica* including *S.* Typhimurium typically cause self-limiting gastroenteritis in humans and are referred to as nontyphoidal *Salmonella* (NTS). Post-convalescent shedding of NTS has occasionally been observed, but at very low levels [16]. A study monitoring over 350 cases of travelers' diarrhea concluded that shedding was not detected after 1 year. Thus, screening for shedding of NTS was only recommended for hospital workers and food handlers to mitigate the risk of transmission to people susceptible to extremely low infective doses [17]. In the few cases in which person-to-person spread of *S.* Typhimurium has been described, transmission occurred at the peak of acute infection, typically caused by extended contact with patients who were very sick [18,19].

With recent increases in HIV–NTS co-infections, new strains of *S.* Typhimurium have emerged that disseminate beyond the gastrointestinal tract and cause bacteremia. Bacteremia is believed to occur because of HIV-induced ablation or dysregulation of multiple arms of the immune response [20,21]. These strains are an intriguing snapshot of the evolution of host-adapted microbes: of the 23 *S.* Typhimurium genes that are inactivated in *S.* Typhi, 11 are pseudogenes in these NTS isolates [22]. A recent study reported asymptomatic carriage of invasive NTS isolates in siblings or parents of the index patients, but it was unclear if these carriers had infected the index patient or vice versa. Moreover, bacterial shedding levels were not reported. However, these asymptomatic individuals could be potential carriers and as such, may play a significant role in transmission [23].

Box 2. What causes *S.* Typhi showers?

Asymptomatic carriers of *S.* Typhi periodically shed large numbers of this bacterial pathogen in their stools (showers of *S.* Typhi). Understanding the interactions between *Salmonella*, the host immune system and the host intestinal microbiota will be key to understanding the underlying mechanisms of the large fluctuations in shedding that occur in humans. For example, alterations in the composition of the intestinal microbiota that could be induced by changes in diet or antibiotic treatment might lead to an increase in the levels of *S.* Typhi in the intestine. In addition, alterations in the host immune status could lead to a perturbation in gut homeostasis, which influences the composition of the intestinal microbiota and the levels of *S.* Typhi. Future studies involving sequencing the microbiota and immune monitoring before and during *S.* Typhi showers may lead to novel treatments for carriers and decrease disease transmission.

How do we identify human carriers of *S.* Typhi?

Because of the hallmark showers of *S.* Typhi, carrier identification requires collection and culture of multiple fecal samples over the period of at least 1 year. However, this is inefficient and difficult to achieve. Carrier identification remains one of the best ways to prevent *Salmonella* epidemics, but there is a real dearth of inexpensive and efficient identification methods (Box 3).

One of the earliest *Salmonella* detection tests was designed by Widal, who observed agglutination of patient sera on addition to bacteria. Later, the test was refined to detect antibodies against *Salmonella* lipopolysaccharide (LPS) O antigen and flagellar H antigen. Studies have shown, however, that the test is more indicative of past and current *Salmonella* infection and not carrier status [24].

Whereas host-adapted *Salmonella* strains typically have increased pseudogenes compared to generalist species, *S.* Typhi contains an additional *Salmonella* pathogenicity island (SPI-7). SPI-7 encodes the Vi capsular polysaccharide, which is extremely immunogenic. Once it could be produced in large enough quantities, a serological test was then used to detect the origin of infection during outbreaks [25–27]. This technique was easier and less invasive than the previous standard, which involved the collection and culture of fecal samples and rectal swabs. Testing for Vi antibodies remained the primary

Box 3. Future direction

- Why do current techniques identify carriers poorly?
  - Culturing of fecal samples fails to identify carriers with intermittent fecal shedding as a result of *S.* Typhi showers.
  - Anti-O (LPS) and anti-H (flagellar) antibodies cannot distinguish between carriers and individuals who have cleared the disease.
  - Healthy individuals in Typhoid endemic areas have high levels of anti-Vi antibodies complicating carrier identification.
- Are there bacterial transcriptional profiles associated with the carrier state?
  Increased biofilm formation and increased expression of adherence factors are observed in patient fecal samples.
- Can host cytokine profiles be associated with carrier states? Increased inflammation is observed in both animal models and subsets of *S.* Typhi patients.

Table 1. Host specificity and transmission of *Salmonella* serovars

Animal	Serotype[a]	Shedding level	Transmission route[b]	Maximum shedding duration reported	Refs
Cattle	*S.* Typhimurium	Unknown	Seeder contact	71 days	[38]
	S. Dublin	10^3–10^5 CFU/g feces	Seeder contact, environment	400 days	[39]
Swine	*S.* Typhimurium	10^6 CFU/g feces for first 2 weeks, 10^4 CFU/g feces thereafter	Seeder contact, Environment	5 months	[47,48]
	S. Choleraesuis	Average pooled fecal shedding 10^2–10^3 CFU/g	Seeder contact	12 weeks	[44]
Chicken	*S.* Enteritidis	10^1–10^7 CFU/g cecum	Seeder contact, vertical transmission	24 weeks	[92,93]
	S. Gallinarum	Very low levels	Vertical transmission	___	[50,51]
	S. Typhimurium	10^1–10^5 CFU/g feces	Seeder contact, vertical transmission, environment	87 days	[50,94]
Mouse	*S.* Typhimurium	10^8–10^{10} CFU/g (super-shedder)	Seeder contact	125 days	[56]
Human	*S.* Typhi	10^6–10^{10} CFU/g	Seeder contact	40 years	[12]

[a]Bold type indicates a host-adapted strain of *Salmonella*.

[b]Seeder contact indicates that transmission was caused by contact with a single seeder individual or carrier. Contaminated food and water indicates that the carrier was shown to have contaminated the population's food and water source. Contamination of the environment is contact-independent and occurs through bacterial growth in the environment. Vertical transmission refers to transmission of *Salmonella* from a hen to a developing egg.

method of both diagnosis and carrier detection for the next few decades. Newer molecular techniques such as PCR and rapid immunoblots have had the same success rate as a Vi antigen test [13]. Unfortunately, people living in typhoid endemic regions have high background levels of anti-Vi antibodies. A recent study in Vietnam showed that rectal swabs from 107 people with high anti-Vi antibody titers were culture-negative for *S.* Typhi [28]. Although more specific methods of anti-Vi and anti-O antibody detection are now available [29,30], it has become clear that serology is not a reliable method of carrier detection, especially in endemic areas.

Treatment of *S.* Typhi carriers

First-line antibiotics used to treat typhoid fever in humans have historically included chloramphenicol and trimethoprim. However, multidrug-resistant (MDR) strains have emerged [2] and current treatment involves fluoroquinolones, including nalidixic acid and ciprofloxacin [31]. Unfortunately, fluoroquinolone resistance has also been reported in recent outbreaks in South Asia [32]. These drugs reversibly inhibit DNA replication by targeting proteins such as DNA gyrase. Reduced sensitivity is associated with point mutations in the *gyrA* genes [33], and infection with these strains increased median fever clearance time [32]. Unfortunately, carriers require longer treatment with larger doses, and side effects including gastrointestinal bleeding and gastric discomfort have been reported [31,34]. Because of the growth of *Salmonella* biofilms on gallstones and in bile [14], cholecystectomy (surgical removal of the gallbladder) has been used to treat human carriers. However, this does not always result in elimination of the pathogen [35,36]. In the future, it may be beneficial to treat *S.* Typhi carriers by more targeted treatment methods that reduce fecal shedding of *Salmonella* without the pleiotropic side effects of antibiotic treatment or invasive surgery. Various approaches have been explored in livestock and are discussed later in this review.

Carriage and transmission of *S. enterica* serovars in livestock

Salmonella infections are a major problem in livestock animals including cattle, pigs and chickens. In this section, we discuss transmission models and host susceptibility loci in these animals. Although *S.* Typhimurium can infect all three animals, specific serovars are associated with specific hosts, as outlined in Table 1. Contaminated meat and eggs make these animals an important reservoir for human disease. Prevention of human salmonellosis (NTS infections) depends on decreasing the prevalence of infections in livestock hosts and on identifying and intervening along key transmission routes. However, effective control strategies require an improved understanding of the dynamics of infection within host populations.

Experimental models of transmission dynamics have shown that to best fit the data observed for *Salmonella* outbreaks, the presence of high-shedding carriers (super-shedders) must be accounted for [37]. In this review, we advocate for the definition of a super-shedder carrier state to describe high-shedding individuals that are the main source of pathogen transmission within a herd (Box 1). Animal studies allow us the opportunity to quantify this state and examine the host pathological conditions associated with super-shedders. Table 1 summarizes the fecal shedding data available on various *Salmonella* serovars in infections. Whenever possible, we discuss the contribution of the super-shedder carrier state to *Salmonella* transmission in livestock animals.

Cattle

In an experimental evaluation of transmission in a herd, seeder calves (animals that are infected and then released into a herd for transmission studies) were able to transmit *S.* Typhimurium to up to 80% of naïve calves within 1 week; of these, 23% became asymptomatic carriers [38]. Similar transmission dynamics were also observed for animals infected with *Salmonella enterica* serovar Dublin [39],

demonstrating that the carrier state in cattle occurs across *Salmonella* serovars. These experiments highlight the importance of identifying individual carriers. However, to examine the contribution of super-shedders, experiments introducing model super-shedders to the herd must be conducted [40]. Although little is known about what induces a carrier state, transportation stress has been correlated with increases in fecal shedding [41]. However, experimental results attempting to model the effect of stress have been inconclusive [42].

The super-shedder carrier state in *Escherichia coli* O157 infections in cattle has been studied in more detail. Sustained fecal shedding was linked to colonization of the terminal rectal epithelia by *E. coli* O157, indicating that a localized chronic infection in the gastrointestinal tract contributed to fecal shedding. The super-shedder state was then defined as animals shedding greater than 10^4 CFU of *E. coli* O157 per gram of feces and displaying colonization of rectal epithelia [43]. In the future, it would be helpful to establish a similar standard for *Salmonella* infections (Box 1).

Pigs

In pigs, the host-adapted strain *Salmonella enterica* serovar Choleraesuis is thought to be transmitted primarily by carrier pigs, with both contact-dependent and fecal–oral routes of transmission being important [44]. Only relatively low levels of shedding have been detected for swine infected with *S.* Choleraesuis. However, *S.* Choleraesuis has been cultured from dried fecal pellets up to 13 months after collection, which indicates its ability to remain infective in the environment [45]. Multiple routes of infection have also been demonstrated for *S.* Typhimurium in swine, similar to *S.* Choleraesuis, although the relative importance of each is not yet fully understood [46,47]. Seeder pigs infected with varied doses of *S.* Typhimurium transmitted at similar levels, with the caveat that animals infected at lower doses had a delayed start in transmission [47,48]. In studies tracking fecal shedding in *S.* Typhimurium and *S.* Choleraesuis, shedding of both species was highest in the first 2 weeks of infection and declined rapidly thereafter [48,49]. However, a long-term experiment, in which the levels of *S.* Choleraesuis were followed from weaning to slaughter, showed that pigs can intermittently shed for up to 5 months after infection [49]. Unfortunately, most experiments following fecal shedding have involved culture of pooled fecal samples. Although this is informative for the overall infectious status of the herd, it fails to identify the individuals responsible for transmission and provides little ability to evaluate the contribution of the carrier state to infection dynamics and human disease transmission.

Chickens

Salmonella infection in chickens can be divided into host-specific and non-host-specific infections. *S. enterica* serovars Pullorum and Gallinarum are highly adapted to the host species and are of little public health concern [50]. Other serovars, including *S. enterica* serovar Enteritidis and *S.* Typhimurium, are non-host-specific and cause *Salmonella* food poisoning in humans. Unlike *Salmonella* infections in cattle and swine, *S.* Enteritidis can be transmitted vertically (bird to egg) in infected chickens. Chicks hatching from *Salmonella*-positive eggs spread the bacteria horizontally [50]. Horizontal transmission has been observed for three *Salmonella* serovars (Table 1). Similar to cattle and swine, a small percentage of infected birds become asymptomatic carriers and may excrete *Salmonella* continuously or intermittently [51,52]. However, it is important to note that detection of *Salmonella* shedding in chickens often utilizes cloacal swabs instead of fecal samples [53]. Despite this, the varied immunological and genetic tools available make the chicken model suitable for investigating factors that influence establishment of a carrier state. We discuss some of these findings later in this review.

Small mammals

Small mammals rarely transmit *Salmonella* to humans, but they can contribute to disease transmission among farm animals [50] and are a tractable model system for studying transmission and the carrier state. Petrie and O'Brien established that survivors of a natural *Salmonella* epidemic among laboratory guinea pigs continued to shed the bacteria despite appearing healthy [54]. A chronic mouse model of *Salmonella* infection was established by Monack *et al.* and has been used to demonstrate natural transmission [55,56]. Mice shedding over 10^8 CFU/g feces reliably transmitted the pathogen when placed in a cage of naïve mice. In addition, these super-shedder mice were characterized by gastrointestinal inflammation, particularly neutrophil influx.

Microbiota and carriers

The gut microbiota plays an important role in modulating the host mucosal immune response [57] and in the case of enteric diseases could interact directly with the pathogen. In the mouse model of persistent *S.* Typhimurium infection, the microbiota influences the levels of *S.* Typhimurium in the gastrointestinal tract and plays a crucial role in controlling transmission [56]. Importantly, a single dose of an antibiotic given to chronically infected mice (125 days post-infection) reactivated the super-shedder phenotype in mice that were not actively shedding detectable levels of *S.* Typhimurium. In another study using susceptible mice with low-complexity microbiota, an attenuated *S.* Typhimurium strain was shed asymptomatically for 40 days. Surprisingly, the gut microbiota was found to be necessary for mediating clearance [58]. These results indicate that antibiotic treatment, which can affect the intestinal microbiota composition, can increase fecal shedding and/or induce the carrier state. It has been shown that antibiotic treatment of human carriers slightly increases the duration, but not the quantity, of *S.* Typhimurium shedding [59]. The human gut microbiota contains over 10^{14} bacteria belonging to several families, and in super-shedders up to 10^{10} of these can be *Salmonella* [12]. To attain such high levels, *Salmonella* has to outcompete the other members of the microbiota.

In livestock, the reduction of *Salmonella* shedding in carriers is an area of active research. Computational models of *E. coli* transmission predicted that prevention of

infection in the 5% of animals with the highest infectiousness (super-shedders) could effectively control the spread of infection [60], suggesting that this approach might be successful in controlling outbreaks in livestock colonies. Recent advances in identifying microbiota–pathogen interactions have resulted in attempts to manipulate these relations to reduce fecal shedding of *Salmonella* and prevent transmission. In cattle, a probiotic *E. coli* strain was isolated from cows that remained uninfected despite exposure to *Salmonella* carriers in the herd. Addition of this *E. coli* strain, P8E5, to calves before or after infection with *S*. Typhimurium resulted in decreased fecal shedding, culminating in undetectable *Salmonella* levels at 2 weeks post-infection. Interestingly, P8E5 inhibited *Salmonella* growth in broth and resulted in downregulation of SPI-1-mediated invasion genes [61].

In chickens, addition of *Bacillus subtilis* spores before infection resulted in competitive exclusion of *Salmonella* from the gastrointestinal tract. In addition, treatment with *B. subtilis* post-infection reduced *Salmonella* shedding by a factor of 1000 [62,63]. Complex gut flora can further increase protection; a mixture of 29 microbial species obtained from the cecum of broiler chickens competitively excluded a number of enteric pathogens, including *S*. Enteriditis and *E. coli* [64]. These methods are examples of ways to target the carriers in an infected population, and possibly control spread of the infection and prevent the development of new carriers. However, much work remains to be done to determine ways in which *Salmonella* and members of the microbiota interact and to identify potential microbial targets that can outcompete *Salmonella* and reduce shedding.

Host immune response to *Salmonella*

Salmonella induces a strong mucosal antibody response, even in carriers [65]. However, this was found to be dispensable to the formation of the super-shedder state because IgA knockout mice showed no attenuation in cecal *Salmonella* loads compared to control mice [58].

While the T helper 1 response and increased Interferon-γ production are associated with all *Salmonella* infection models, neither has been linked to fecal shedding or development of the carrier state. However, several genetic loci responsible for the innate inflammatory response have been linked to increased fecal shedding of *Salmonella* in quantitative trait loci studies conducted in chickens [66,67] and in transcriptional immune response profiling conducted in pigs [68,69]. Convalescing typhoid patients (who have the potential to become carriers) have increased circulating inflammatory cytokines such as IL-6, TNFα and IL-1β [70,71].

Host genotype can play a major role in chronic *Salmonella* infections. Mice expressing a mutated copy of Nramp1 [72] (an ion transporter expressed on macrophages and dendritic cells) display increased susceptibility to intracellular pathogens including *Leishmania*, *Mycobacterium* and *Salmonella*. Persistent *Salmonella* infections can be established in mouse strains carrying wild-type Nramp1. However, this gene seems to be less important in chickens and pigs [67,73]. A recent human genome-wide study also failed to show any link between Nramp1 and susceptibility to *S*. Typhi [74].

Transcriptional profiling of a cohort of acute, convalescing and recovered typhoid patients uncovered specific neutrophil and lymphocyte gene expression sets associated with each of these stages [75]. Whereas 50% of the recovered patients had the same gene expression profile as the control group, an additional 25% displayed a transcriptional profile associated with a convalescing patient, a full 9 months after treatment. Stool samples from these patients tested negative for *S*. Typhi, but this data set supports the possibility of identifying immune responses in the peripheral blood that are specifically associated with a carrier state [75]. Similarly, in pigs infected with *S*. Typhimurium for 2 days, a subset had a transcriptional profile with higher innate inflammatory markers and these animals were subsequently found to be high shedders [68]. These studies underline the possibility of identifying a panel of peripheral blood markers that would allow for easy carrier identification. Peripheral blood is easy to obtain and analysis of multiple transcriptional responses may prove more useful than antibody titers in carrier identification, especially in endemic areas.

Virulence factors involved in shedding

Salmonella contains several pathogenicity islands, which encode virulence factors that are secreted through two type III secretion systems and induce host inflammation [76]. *Salmonella* is able to exploit this inflammation for nutrients and outcompete other bacterial species in the gut [77]. However, although the functional relevance of some virulence factors has been established in intracellular invasion and replication, few have been implicated in fecal shedding or the carrier state.

Adherence factors

Enteric pathogens express a set of proteins that adhere and bind to host cytoskeletal proteins and have been associated with increased gastrointestinal bacterial load These pathogens, including *Salmonella*, have varied adhesive mechanisms, including expression of fimbrae (proteinaceous extensions expressed on the bacterial surface), pili and secretion systems [78]. Genes encoding thin aggregative fimbrae – also called curli – are upregulated in bacteria from fecal samples and the gastrointestinal tract [79].

Secreted effectors

ShdA and MisL are two secreted effectors that are important for persistence in the mouse gastrointestinal tract. Both MisL and ShdA bind fibronectin but differ in their binding affinity for collagen. Bacterial mutants that are deficient for the genes encoding these proteins are attenuated in both feces and the gastrointestinal tract during persistent *S*. Typhimurium infection in mice [80,81]. Expression of ShdA and MisL genes was detected only *in vivo* and not in broth culture. *MisL* transcripts were also detected in blood from patients infected with *S. enterica* serovar Paratyphi [82]. MisL mutants were similarly attenuated in infection in chickens [83]. However, pigs infected with the *shdA* mutant showed no decrease in long-term shedding, suggesting that ShdA may have a host-specific effect on gastrointestinal colonization and fecal shedding [84].

Biofilm formation

Adhesins, flagellar proteins and capsular polysaccharides are involved in *Salmonella* biofilm formation [85,86]. FimH, a fimbrial protein, and FliC, a flagellar protein (both conserved across multiple *Salmonella* serovars), bind to cholesterol. Cholesterol binding is an important mechanism of biofilm formation on gallstones in both typhoid patients and *S.* Typhimurium-infected mice [14,15,78]. However, overexpression of fimbrial proteins inhibits flagellar-mediated cholesterol binding [87], revealing complex interactions in biofilm formation. Recent work examining *S.* Typhi isolates from patient stool samples has uncovered correlations between biofilm formation and shedding duration. Intriguingly, biofilm formation by these isolates was also associated with increased antibiotic resistance, with the best biofilm producers possessing multiple antibiotic-resistance cassettes [88]. Measurement of the ability of fecal bacterial isolates to form biofilms during the early onset of typhoid infection might be informative regarding both the duration and quantity of shedding.

Lipopolysaccharide

Salmonella LPS is anchored to the bacterial outer membrane by a lipid A tail and contains a sugar chain, the *O*-linked polysaccharide, which is highly immunogenic. Anti-O antibodies are a reliable readout for *Salmonella* infection, but not the super-shedder carrier state, in both people and animals [30,89]. A mutant with a mutation in *Salmonella* pathogenicity island SPI-16, which contains genes that are responsible for O antigen glycosylation, was outcompeted by wild-type bacteria in the gastrointestinal tract of infected mice, but not in systemic sites [90], suggesting that O antigen variation in *S.* Typhimurium is required for fecal shedding. In the future, transcriptional profiling of fecal isolates will be helpful in the identification of specific bacterial factors associated with carriers.

Concluding remarks

Transmission of *S.* Typhi is dependent on human carriers, yet little is known about the development of the carrier state or why it occurs only in specific individuals. Efficient and reliable identification of these carriers is currently problematic (Box 3). Recent studies identifying new antigens might result in serological tests that are potentially indicative of the carrier status [91]. In addition, identification of immune response parameters specifically associated with carriers that can be easily measured in peripheral blood (such as cytokine or transcriptional data) would be of great use [75].

We have also reviewed what is known about the *Salmonella* carrier state in livestock animals including cows, pigs and chickens. Work with herd animals helps in better modeling of population transmission dynamics, which highlights the need for better carrier detection and definition of a super-shedder carrier state. The recently established standards for detection of *E. coli* carrier states in cows may help to set similar standards for detection of *Salmonella* carriers [43]. Finally, the super-shedder state is most clearly defined in the mouse model of persistent *Salmonella* infection, making it an excellent platform for deciphering both the contributions of bacterial pathogenesis and the host immune response, as well as identifying specific members of the host microbiota involved in the development of the super-shedder carrier state.

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