

## MINIREVIEW

# Disseminated infections with antibiotic-resistant non-typhoidal *Salmonella* strains: contributions of host and pathogen factors

Kristen L. Lokken, Gregory T. Walker and Renée M. Tsois\*<sup>†</sup>

Medical Microbiology and Immunology, University of California, One Shields Avenue, Davis, CA 95616, USA

\*Corresponding author: Medical Microbiology and Immunology, University of California, One Shields Avenue, Davis, CA 95616, USA. Tel: +(530)-754-8497; E-mail: [rmtsolis@ucdavis.edu](mailto:rmtsolis@ucdavis.edu)

**One sentence summary:** Non-typhoidal *Salmonella* species normally cause a self-resolving gastroenteritis, but in individuals with concomitant HIV or malaria infection or certain comorbidities, these pathogens can cause disseminated infections; currently, they are a leading cause of childhood bloodstream infection in sub-Saharan Africa.

Editor: Brooke Napier

<sup>†</sup>Renée M. Tsois, <http://orcid.org/0000-0001-9131-6657>

## ABSTRACT

Non-typhoidal *Salmonella enterica* serovars (NTS) are generally associated with gastroenteritis; however, the very young and elderly, as well as individuals with compromised immunity, are at risk of developing disseminated infection that can manifest as bacteremia or focal infections at systemic sites. Disseminated NTS infections can be fatal and are responsible for over 600 000 deaths annually. Most of these deaths are in sub-Saharan Africa, where multidrug-resistant NTS clones are currently circulating in a population with a high proportion of individuals that are susceptible to disseminated disease. This review considers how genome degradation observed in African NTS isolates has resulted in phenotypic differences in traits related to environmental persistence and host–pathogen interactions. Further, it discusses host mechanisms promoting susceptibility to invasive infection with NTS in individuals with immunocompromising conditions. We conclude that mechanistic knowledge of how risk factors compromise immunity to disseminated NTS infection will be important for the design of interventions to protect against systemic disease.

**Keywords:** *Salmonella*; co-infection; malaria; bacteremia

## INTRODUCTION

*Salmonella enterica* serovars are associated with three major disease syndromes: typhoid fever, gastroenteritis and bacteremia. Typhoid fever, a disseminated infection caused by typhoidal *S. enterica* serovars in immunocompetent individuals and characterized by fever and abdominal pain, is associated with ~433 000 deaths annually (Crump, Luby and Mintz 2004; Crump et al. 2008). In immunocompetent individuals, non-typhoidal *S. enterica* serovars (NTS) are associated with gastroenteritis, a

localized infection of the terminal ileum and colon manifesting as diarrhea, vomiting and intestinal cramping, which is associated with an estimated 155 000 deaths annually (Majowicz et al. 2010). In immunocompromised individuals, including the very young, individuals undergoing cancer chemotherapy and those with inborn or acquired immunodeficiencies, NTS are associated with bacteremia, an extraintestinal infection responsible for ~681 000 deaths annually (Ao et al. 2015). The clinical presentation of NTS bacteremia is characterized by a non-specific

fever, while diarrhea is commonly absent (De Wit et al. 1988). These disseminated infections have also been referred to in the clinical/epidemiologic literature as ‘invasive NTS (iNTS) disease’ (Gordon 2011).

With a combined annual global death toll of 1.24 million, *S. enterica* serovars are one of the leading causes of human mortality worldwide. More than half of these deaths are attributable to NTS bacteremia, which is most prevalent in sub-Saharan Africa. Epidemiologic studies identified the principal underlying factors for NTS bacteremia in sub-Saharan Africa as very young age, malaria and malnutrition in children, and human immunodeficiency virus (HIV) infection in adults (summarized in Feasey et al. 2012). Notably, the age distribution for NTS bacteremia cases in children is quite different from that of typhoid, with NTS bacteremia affecting younger children (<5 years of age), and typhoid cases being identified in older children (Feasey et al. 2010). In this review, we will consider features of *S. enterica* serovar Typhimurium (*S. Typhimurium*) currently circulating in sub-Saharan Africa where disseminated NTS infections are highly prevalent. In addition, we will discuss insights into the pathogenesis of NTS bacteremia gleaned from clinical epidemiologic studies, from elegant whole genome analyses of epidemic clones, and from animal models investigating how underlying comorbidities compromise containment of NTS infection.

## PHENOTYPIC CHARACTERISTICS OF THE ST313 STRAINS

Sequence analysis of *Salmonella* Typhimurium blood isolates shows that NTS bacteremia in sub-Saharan Africa is associated with a clone, designated by multilocus sequence typing as sequence type (ST)313, which is rarely reported outside of Africa (Kingsley et al. 2009). In contrast, the most common sequence type reported in the rest of the world (including North America and Europe) is ST19. Whole genome sequencing of ST313 isolates identified a number of features that differentiate them from ST19 strains, including distinct repertoires of prophage elements and insertion sequences. In addition, evidence of genome degradation was noted, including pseudogene formation and chromosomal deletions, relative to reference genomes such as LT2 and SL1344 (Kingsley et al. 2009; Okoro et al. 2015). A more detailed characterization of degraded genome content in the ST313 isolates has revealed some notable differences to strains currently circulating outside sub-Saharan Africa, including predicted loss of functions associated with surface and exported proteins (Okoro et al. 2015), suggesting alterations in functions associated with host interaction. In addition, functions associated with utilization of two substrates available in the mammalian intestinal tract have been lost in ST313 strains: tartrate and allantoin (Okoro et al. 2015). Tartrate can replenish intermediates in the glyoxylate cycle (Nuccio and Baumler 2014). Allantoin is produced by mammalian gut bacteria via 5-hydroxyisourate hydrolases from uric acid, a product of mammalian purine metabolism (Lim et al. 2014). *Enterobacteriaceae* are able to utilize allantoin as a source of nitrogen and carbon (Cusa et al. 1999). Interestingly, increased intestinal allantoin content was reported to be a marker of intestinal inflammation in patients with inflammatory bowel disease (Schicho et al. 2012), suggesting that allantoin may provide a nutrient source for growth of *Enterobacteriaceae* in the inflamed intestine. Conversely, loss of allantoin metabolism in the ST313 strains may reduce their fitness in the inflamed intestine, as was observed

for a set of ST313 strains during infection of chickens (Parsons et al. 2013) (Table 1).

Two *in vitro* phenotypes observed for ST313 strains are related to multicellular behavior. One is the loss of the RDAR (red, dry and rough) colony phenotype on Congo red agar that is associated with biofilm formation (Simm et al. 2014). This phenotype is caused by defective cellulose production resulting from a nonsense mutation in the biosynthesis gene *bcsG* (Singletary et al. 2016), and was shown to be shared by a large panel of ST313 strains (Ramachandran et al. 2016). The second phenotype is loss of the stationary-phase catalase KatE, which is protective in high-density bacterial cultures. Together these features of ST313 strains suggest a reduced ability to survive in extreme environments or at high density (Singletary et al. 2016).

A further characteristic of the ST313 strains is reduced motility, which correlates with their reduced expression of flagellin compared to ST19 strains (Carden et al. 2015; Ramachandran et al. 2015). This is of particular interest, since invasive serovars of *Salmonella* such as *S. Typhi*, the causative agent of typhoid fever, downregulate flagellin rapidly on invasion of the epithelium to evade detection by TLR5 and NLRC4, as well as by flagellin-specific CD4 T cells (Winter et al. 2010, 2015; Atif et al. 2014). A similar pattern of reduced flagellin expression and motility was also observed for *S. Paratyphi A*, which causes a typhoid fever-like disease in humans (Elhadad et al. 2015). Further, loss of flagellin expression by *S. Gallinarum*, a poultry-adapted serovar causing fowl typhoid, is part of its adaptation to extraintestinal infection in chickens (de Freitas Neto et al. 2013). Consistent with the idea that reduced flagellin expression can promote invasive disease via immune evasion, ST313 strains were less inflammatory in cultured macrophages and induced less NLRC4-dependent pyroptotic cell death; these features may therefore reflect adaptation to disseminated infection (Carden et al. 2015).

Comparative studies on host responses to ST19 and ST313 strains in animal models have yielded some conflicting results, which may be attributed to the different animal models utilized and different ST19 strains (with different degrees of invasiveness) used for comparison. However, despite these differences an overall theme emerging from this work is that the ST313 strains are capable of invading the intestinal mucosa and eliciting intestinal inflammation in chicken, mouse, bovine and non-human primate models, findings that are consistent with isolation of ST313 strains from a subset of patients in sub-Saharan Africa with diarrheal disease (Paglietti et al. 2013; Kariuki and Onsare 2015). Therefore, while hospital-based studies have identified the ST313 strains as frequent causes of invasive disease, they have not lost their ability to cause intestinal inflammation and diarrhea—the absence of diarrhea in cases of invasive disease is likely to result from host factors that blunt intestinal inflammation (see below Raffatellu et al. 2008; Mooney et al. 2014). This being said, the ST313 strains appear to have a slightly reduced ability to cause intestinal inflammation in streptomycin-treated mice and bovine ligated ileal loops, compared to ST19 (Okoro et al. 2015). While the genetic basis for this phenotype is unknown, it is consistent with the reduced expression in ST313 strains of both flagellin and the Type III effector *sopE*, two proteins that contribute to intestinal inflammation in animal models (Hapfelmeier et al. 2004; Winter et al. 2009; Carden et al. 2015). It is not yet clear how this reduced inflammation observed in animal models with the ST313 strains relates to severity of diarrheal disease from salmonellosis in humans.

**Table 1.** Phenotypes distinguishing ST313 from ST19 strains.

Phenotype	Genetic difference	Reference
<i>In vitro phenotypes</i>		
Increased utilization of M-Tartrate and tricarballic acid	<i>ttdA</i> (tartrate dehydratase) pseudogene in ST313 strains	Okoro et al. (2015)
Decreased metabolism of L-Tartrate and dihydroxyacetone	<i>ttdA</i> pseudogene in ST313 strains	Okoro et al. (2015)
Decreased stationary-phase catalase production	KatE E117G mutation in D23580	Singletery et al. (2016)
Loss of RDAR colony formation	Nonsense mutation in <i>bcsG</i> in D23580	Ramachandran et al. (2016); Singletery et al. (2016)
Increased resistance to low pH	Unknown	Yang et al. (2015)
Decreased resistance to desiccation	Unknown	Ramachandran et al. (2016)
Reduced motility in agar	Unknown	Ramachandran et al. (2015)
Reduced expression of flagellin	Unknown	Carden et al. (2015); Ramachandran et al. (2015)
Acetylation of O antigen	Possibly insertion of BTP1 prophage	Micoli et al. (2014); Kintz et al. (2015); Onsare et al. (2015)
<i>Interactions in cellular infection models</i>		
Increased survival within macrophages	Unknown	Ramachandran et al. (2015)
Reduced NLR4 inflammasome activation	Reduced <i>fliC</i> expression	Carden et al. (2015)
Reduced death of infected macrophages	Reduced <i>fliC</i> expression	Carden et al. (2015); Ramachandran et al. (2015)
Reduced invasiveness for non-phagocytic cells	Reduced <i>sopE</i> expression	Carden et al. (2015); Okoro et al. (2015)
<i>Interactions in animal models</i>		
Increased invasiveness in chickens	Unknown	Parsons et al. (2013)
Reduced inflammatory changes in bovine ligated ileal loops and streptomycin-treated mice	Unknown	Okoro et al. (2015)
Equivalent levels of inflammation in rhesus macaque ligated ileal loops and streptomycin-treated mice	Unknown	Singletery et al. (2016)
More rapid dissemination to mesenteric lymph nodes in mice	Unknown	Singletery et al. (2016)
Increased colonization of liver and spleen in mice	Unknown	Yang et al. (2015); Singletery et al. (2016)

## ARE THE AFRICAN NTS ISOLATES MORE INVASIVE?

Epidemiologic evidence suggests that worldwide most NTS isolated from humans are able to cause bacteremia in a small fraction of individuals who are susceptible as a result of very young age or underlying immunodeficiency (Threlfall, Hall and Rowe 1992). *Salmonella* Typhimurium and *S. Enteritidis* are the serovars associated most frequently with human gastroenteritis (summarized in Rabsch, Tschape and Baumler 2001), and therefore, these pathogens are also the most common NTS cultured from blood of patients with bacteremia in England and Wales (Threlfall, Hall and Rowe 1992). Similarly, the pathogens most commonly associated with NTS bacteremia in Africa are *S. Typhimurium* and *S. Enteritidis* (reviewed in Reddy, Shaw and Crump 2010). However, while NTS bacteremia is rare in high-income countries, the large number of individuals in sub-Saharan Africa that are at risk for this complication due to advanced HIV disease, malnutrition or severe malarial anemia makes NTS bacteremia a major public health challenge in this region.

The finding that African ST313 isolates exhibit accelerated dissemination from the intestine to internal organs in chickens and in mouse infection models suggests that this lineage represents a distinct pathovariant of *S. Typhimurium* that has evolved, perhaps via genome degradation, to become more invasive (Parsons et al. 2013; Yang et al. 2015; Singletery et al. 2016).

Increased pseudogene formation is also evident in genomes of the *S. Typhimurium* clone DT2, which is associated with extraintestinal disease in pigeons (Kingsley et al. 2013). Increased accumulation of pseudogenes in *S. enterica* serovars that are exclusively associated with extraintestinal infections is likely a marker indicating a switch to invasive rather than enteric disease; however, it does not indicate an association with a specific host species (Nuccio and Baumler 2014; Langridge et al. 2015; Matthews et al. 2015). Therefore, while it has been hypothesized that pseudogene formation in ST313 occurred in a reservoir of immunocompromised humans, the possibility that this property was selected in a wild animal reservoir in which the pathogen causes invasive disease seems equally likely, and cannot be ruled out currently (Wain et al. 2013). However, it should be emphasized that in contrast to invasive serovars such as *S. Typhi* and *S. Paratyphi A*, the ability of the ST313 to cause enteric infection appears to be intact, because *S. Typhimurium* isolates from African children with diarrhea do not differ in their genotype from those associated with bacteremia (Kariuki et al. 2006; Kariuki and Onsare 2015), and ST313 strains have been isolated from cases of gastroenteritis in immunocompetent individuals (Paglietti et al. 2013).

The dominance of ST313 in sub-Saharan Africa raises the question of what selective forces were responsible for its epidemiologic success. An important factor contributing to the rise of epidemic *S. Typhimurium* clones in Europe and North

America is the acquisition of genes conferring resistance against antibiotics (reviewed in Rabsch, Tschape and Baumler 2001). The first recorded outbreak of drug-resistant *S. Typhimurium* affected cattle and humans in England and Wales in the early 1960s (Anderson 1968). The epidemic *S. Typhimurium* clone causing this outbreak was designated DT29 and carried resistance to ampicillin, tetracycline and streptomycin on conjugative plasmids (R-plasmids) (Anderson and Lewis 1965). Antibiotic resistance first emerged within African *S. Typhimurium* isolates around 1960, but this was only detected by a retrospective analysis performed more than 50 years later. Here, the emergence of antibiotic resistance involved insertion of a Tn21-like transposon element into the virulence plasmid (pSLT) of an *S. Typhimurium* clone designated as ST313 lineage I (Okoro et al. 2012). This Tn21-like element carries multidrug resistance determinants, suggesting that antibiotic resistance was likely an important factor in the subsequent epidemic spread of ST313 within sub-Saharan Africa.

Epidemiologic surveillance in Europe shows that after dominating in the 1960s, DT29 was replaced by a new multidrug-resistant *S. Typhimurium* clone, termed DT204, in the 1970s and 80s, which in turn was supplanted by *S. Typhimurium* clone DT104 in the 1990s (summarized in Rabsch, Tschape and Baumler 2001). Retrospective analysis suggests that a similar succession of epidemic multidrug-resistant *S. Typhimurium* clones also unfolded in sub-Saharan Africa. After its emergence in the 1960s, ST313 lineage I was initially the dominant *S. Typhimurium* clone in sub-Saharan Africa. However, a new clone, termed ST313 lineage II, emerged around 1975 and by 2006 it had completely replaced the preceding clone, ST313 lineage I (Okoro et al. 2012). The ST313 lineage II carries a chloramphenicol resistance determinant, which is absent from ST313 lineage I (Okoro et al. 2012). Since chloramphenicol was the drug of choice to treat severe bacterial infections in Africa at the time, it is likely that this resistance determinant drove replacement of ST313 lineage I by ST313 lineage II.

How does the current ST313 epidemic in sub-Saharan Africa affect the human population at risk for NTS bacteremia? The most important factor is likely the resistance of ST313 strains to multiple antibiotics, including ampicillin, chloramphenicol, streptomycin, sulfonamides, streptomycin and trimethoprim (Kingsley et al. 2009). More recent surveillance of antimicrobial resistance profiles of iNTS isolates in the Democratic Republic of the Congo and Kenya found these strains to have, in addition, decreased susceptibility to ciprofloxacin and third-generation cephalosporins (Lunguya et al. 2013; Kariuki et al. 2015); further spread of these strains will therefore curtail the already limited treatment options for disseminated infections with NTS. On the other hand, increased invasiveness of ST313 observed in animal models (Parsons et al. 2013; Yang et al. 2015; Singletary et al. 2016) is likely to be of minor importance in immunocompromised individuals. *Salmonella Typhimurium* clones circulating in Europe and North America were a cause of NTS bacteremia in patients with advanced HIV disease in the 1980s, before the advent of highly active antiretroviral therapy (Bottone, Wormser and Duncanson 1984; Jacobs et al. 1985; Nadelman et al. 1985; Fischl et al. 1986). Increases in the incidence of NTS bacteremia were noted on each of these continents during the first decade of the HIV pandemic (Levine et al. 1991; Pedro-Botet et al. 2002). These data show that *S. Typhimurium* isolates other than ST313 are fully capable of causing NTS bacteremia in immunocompromised individuals. Hence, factors compromising the host immune system are the main drivers of NTS bacteremia in individual patients, while differences in the virulence and other phenotypic charac-

teristics of different *S. Typhimurium* clones are likely to play a less prominent role in the overall outcome of the host-pathogen interaction. However, these phenotypic differences are of interest, because they shed light on the role of the degraded genome content in infection outcome.

## RISK FACTORS FOR DISSEMINATED NTS INFECTION

The very young and the elderly are at particular risk of developing bacteremic infection with NTS (Mandal and Brennan 1988). Further, NTS have long been recognized as a frequent cause of disseminated infection in individuals with compromised immune function (Table 2). In chronic granulomatous disease (CGD), a rare primary immunodeficiency caused by mutations in subunits of the NADPH oxidase, NTS is the most common cause of bacteremia (Winkelstein et al. 2000). In addition, individuals with genetic deficiencies in the IL-12/IFN- $\gamma$  pathways, a group of conditions known as 'Mendelian susceptibility to mycobacterial disease (MSMD)', are at increased risk of recurrent bacteremias with NTS (reviewed in Bustamante et al. 2014). A further set of genetic conditions that result in structural changes to hemoglobin, including sickle cell disease and the thalassemias, can lead to recurrent episodes of hemolytic anemia (Hook et al. 1957; Wanachiwanawin 2000; Williams et al. 2009; Richards, Howard and Klein 2011). For a more comprehensive review of genetic risk factors underlying NTS bacteremia, the reader is referred to an excellent recent article by Gilchrist and colleagues (Gilchrist, MacLennan and Hill 2015). Acquired immunodeficiencies can also predispose to invasive infections with NTS. These include HIV and immunosuppression secondary to corticosteroid treatment, as would occur with transplantation or autoinflammatory conditions. In fact, during the first years of the AIDS epidemic, NTS bacteremia was considered to be an early indicator of HIV infection (Bottone, Wormser and Duncanson 1984); however, with the availability of highly active antiretroviral therapy, it has become less common in North America.

In contrast to the situation in North America and Europe, disseminated NTS infections in sub-Saharan Africa have increased in prevalence in recent years, especially in the setting of HIV (Gordon et al. 2001, 2002). While HIV is the most common factor in adults that predisposes to NTS bacteremia in sub-Saharan Africa, in children the predisposing factors are very young age (<2 years old), *Plasmodium falciparum* malaria and malnutrition (Reddy, Shaw and Crump 2010). In Malawian children with exposure to NTS, an interesting correlation has been observed between acquisition of antibody responses around 2 years of age and declining incidence of invasive disease that suggests a role for antibodies in protection in this setting (Nyirenda et al. 2014). Epidemiological studies have noted a seasonal pattern in the frequency of bloodstream NTS infections in children, with maximal incidence following the rainy season, when the rates of both malaria and severe malnutrition are also greatest (O'Dempsey et al. 1994; Graham et al. 2000; Brent et al. 2006; Gordon et al. 2008). Several studies have found a strong association between severe childhood malnutrition and bloodstream infections with NTS (Bachou et al. 2006; Brent et al. 2006; Sigauque et al. 2009). However, despite substantial evidence linking malnutrition with extraintestinal NTS infection, it remains unclear how malnutrition compromises control of NTS infection. Furthermore, while a protein-deficient diet has been linked to increased severity of disease in mice (Peck, Babcock and Alexander 1992), other

**Table 2.** Epidemiologic factors associated with disseminated NTS infection.

Factor	References	Mechanism(s)	References
Young age	Muthumbi et al. (2015)	Possibly lack of specific antibodies	Nyirenda et al. (2014)
<i>Acquired immunodeficiencies</i>			
HIV infection	Bottone, Wormser and Duncanson (1984); Roberts et al. (1984); Israel and Plaisance (1991); Pitrak (1999); Feasey et al. (2012)	Th1/Th17 cell deficiency	Raffatellu et al. (2008)
Malnutrition	Brent et al. (2006); Feasey et al. (2015); Muthumbi et al. (2015); Shahunja et al. (2015)	Protein restriction	Peck, Babcock and Alexander (1992)
Neoplasia	Noriega et al. (1994); Beebe and Koneman (1995)	Unknown	–
Malaria	Park et al. (2016)	Deficiency in neutrophils	Cunnington et al. (2012b)
<i>Genetic immunodeficiencies</i>			
Sickle cell disease	Hook et al. (1957); Williams et al. (2009); Richards, Howard and Klein (2011)	Functional deficiency of neutrophils	Evans et al. (2015)
E-beta thalassemia	Ampel et al. (1989); Wanachiwanawin (2000)	Unknown	–
CGD	Winkelstein et al. (2000)	NADPH oxidase deficiency	Mastroeni et al. (2000); Vazquez-Torres et al. (2000)
Interferon- $\gamma$ /IL-12/IL-23 pathways (MSMD)	Bustamante et al. (2014)	Deficiency of Th1 cell and macrophage function	Nauciel and Espinasse-Maes (1992); Mastroeni et al. (1996)

micronutrient deficiencies that impair host defense against NTS are not known. Although our knowledge of causal relationships for any of these susceptibility factors is limited, studies in animal infection models (discussed below) are starting to unearth potential mechanisms.

## UNDERLYING IMMUNOLOGIC DEFICITS PREDISPOSE TO DISSEMINATED INFECTION

Clinical/epidemiologic associations illustrate that disruption of discrete immune functions can lead to NTS bacteremia, but the precise mechanisms by which these immune functions prevent bacterial dissemination has long remained elusive. Recent studies in animal models have begun to shed light on possible mechanisms underlying susceptibility to disseminated NTS infection in the context of HIV infection and *Plasmodium falciparum* malaria. A common mechanism that may impact both comorbidities is a reduced mucosal response to invasion of the epithelium by NTS. In a simian immunodeficiency virus (SIV) model of HIV/*Salmonella* Typhimurium co-infection utilizing rhesus macaques, SIV infection led to depletion of Th17 cells (a population of CD4 T cells) from the intestinal mucosa (Raffatellu et al. 2008), which corresponded with reduced mucosal inflammation in *S. Typhimurium*-infected ligated ileal loops and increased bacterial dissemination from the intestine to systemic sites. Th17 cells were subsequently shown in mouse models to be important for eliciting the neutrophil influx into the gut mucosa that is important for limiting systemic dissemination of NTS (Raffatellu et al. 2008). Reduced *Il17* expression and attenuated neutrophil infiltration into the ileal mucosa were also observed in a rhesus macaque model of malaria/NTS co-infection using *P. fragile* (Mooney et al. 2014). Follow-up studies in a mouse model of malaria/NTS co-infection showed that reduced intestinal inflammation in response to *S. Typhimurium* invasion of

the mucosa resulted from local induction of the immunoregulatory cytokine IL-10 by malaria parasites (Mooney et al. 2014). Hence, both HIV and malaria attenuate mucosal responses to *S. Typhimurium* invasion, but by different mechanisms—depletion of Th17 cells in the case of HIV vs. production of immunoregulatory cytokines in the case of malaria. Reduced intestinal inflammation observed during NTS co-infection with SIV or malaria in animal models may explain why diarrhea is commonly absent in patients with NTS bacteremia (De Wit et al. 1988).

In clinical malaria studies, deficits in both absorptive and barrier functions of the intestinal mucosa have been observed. Patients in the acute phase of malaria showed evidence of malabsorption and loss of epithelial tight junction integrity, which correlated with alterations to the intestinal architecture, including edema, mononuclear infiltrates and shortening and widening of the villi (Karney and Tong 1972; Molyneux et al. 1989). Similar pathologic features in the intestinal tract were also observed in murine malaria models (*P. berghei* and *P. yoelii*) in association with alterations to the colonic microbiota, most strikingly a reduction of members of the phylum Firmicutes (Mooney et al. 2015b; Taniguchi et al. 2015). Shifts in the microbiota composition of *P. yoelii*-infected mice promoted their colonization by *S. Typhimurium* and *Escherichia coli* (Mooney et al. 2015b). Notably, *P. yoelii* infection markedly reduced the implantation dose for *S. Typhimurium* in mice via the intragastric route, a finding that may be of significance during the initial exposure of children with malaria to contaminated food and water (Mooney et al. 2015b).

Loss of epithelial tight junction integrity during malaria was recapitulated in the *P. yoelii* murine malaria model, where it was linked to an increase in the abundance of mucosal mast cells—a feature that was also observed during *P. fragile* infection of rhesus macaques (Potts et al. 2016). While the precise role of mast cells in the intestinal pathology of malaria is not yet understood, blockade of histamine, a product of activated mast cells, in *P. yoelii*-infected mice increased detection of tight junction

complexes in the ileal epithelium, suggesting that histamine release from mast cells may contribute to increased intestinal permeability during malaria. Together, these studies suggest that multiple effects of HIV and malaria parasite infection on the gut mucosa may weaken intestinal barrier functions that limit systemic dissemination of NTS.

While effects of malaria and HIV on the intestinal mucosa may promote dissemination of NTS, it is important to note that NTS are capable of invading the intestinal epithelium and disseminating systemically in the absence of any underlying condition. However, once bacteria have disseminated from the intestinal tract, cells of the mononuclear phagocyte system—most importantly macrophages and neutrophils—limit the infection. In the case of malaria co-infection, murine models have provided insights into how underlying malaria can compromise control of systemic NTS infection by phagocytic cells. A study by Cunnington and colleagues demonstrated that malaria, via hemolysis of red blood cells and release of heme, impairs granulocyte maturation and oxidative burst capacity via activation of heme oxygenase (Cunnington *et al.* 2012a). As a consequence, immature granulocytes are released into the circulation of infected mice. These circulating immature cells harbor *S. Typhimurium*, suggesting that they may provide an intracellular niche in the bloodstream. Thus, heme oxygenase, which helps the host cope with elevated free heme during malaria, suppresses neutrophil development, exerting a detrimental effect on control of *S. Typhimurium*. Reduced neutrophil function secondary to hemolysis could also be an important determinant of susceptibility to disseminated NTS infection in the context of other hemolytic anemias such as sickle cell disease and the thalassemias. Taken together, data from mouse models suggest that malaria impacts neutrophil function in two ways: indirectly by blunting expression of inflammatory chemokines that recruit neutrophils to sites of epithelial invasion, and directly by reducing their maturation state and antibacterial activity. Considering that some genetic predispositions to disseminated NTS infection in humans, including sickle cell disease, and CGD involve a reduced function of neutrophils, these effects of malaria on neutrophil function are likely to be important in the context of human co-infection.

An additional immunocompromising consequence of malaria is suppression of macrophage function. During malaria, immunoregulatory responses such as IL-10 are induced to limit inflammatory pathology caused by the parasite infection. However, this response has negative consequences for the host response to NTS: by suppressing the ability of macrophages to respond to *S. Typhimurium* with proinflammatory cytokine production, IL-10 production blocks recruitment of neutrophils to sites of systemic infection, such as the liver (Lokken *et al.* 2014). Further, IL-10 produced by tissue macrophages in the liver acts locally on neighboring macrophages to drive development of an alternatively activated phenotype with reduced bactericidal function, and consequently, an inability to control bacteria that have disseminated from the intestine (Lokken *et al.* 2014). Another mechanism that may compromise macrophage function during malaria is phagocytosis of infected and uninfected red blood cells, a process that leads to malarial anemia (Facer and Brown 1981). Hemophagocytic macrophages also exhibit an alternatively activated phenotype, with reduction in both inflammatory responses to *S. Typhimurium* and bactericidal activity (Nix *et al.* 2007; McCoy, Moreland and Detweiler 2012). Since hemophagocytic macrophages and other alternatively activated macrophage populations are targeted by *S. Typhimurium* infection in mouse models, it is possible that

these cells may also harbor NTS in malaria patients, providing an intracellular niche for systemic infection (Nix *et al.* 2007; McCoy, Moreland and Detweiler 2012; Eisele *et al.* 2013). As a whole, these studies suggest that multiple components of the immune response to malaria, that are beneficial in the context of limiting parasite infection and its resulting immunopathology, serve to blunt responses that are critical to control of invasive bacterial infection.

## HOW CAN WE PREVENT DISSEMINATED INFECTION?

Given the devastating effects of disseminated NTS infections in sub-Saharan Africa, it is critical to develop strategies for reducing the burden of this disease. Epidemiologic evidence suggests that strategies for control of malaria impact NTS bacteremia by reducing the number of children with underlying malaria, as the prevalence of bacteremia cases has been shown to decline with decreasing malaria transmission (Scott *et al.* 2011; Verani *et al.* 2015). Another approach to prevention is the development of vaccines for use in susceptible populations (MacLennan, Martin and Micoli 2014). However, strategies will need to be targeted to the populations at risk, with consideration to the immunocompromised status of many of these individuals. Studies in animal models have shown that malaria causes transient suppression of both CD4 T cell responses and B cell responses conferred by vaccination against other infections (Ng *et al.* 2014; Mooney *et al.* 2015a), and to be effective at clearing NTS, circulating antibodies generated by subunit vaccines rely on functional neutrophils and macrophages, cells whose function is compromised during severe malaria. Suppression of existing adaptive immunity by acquired immune deficiencies may also explain in part the occurrence of disseminated NTS infection in a population in which immune responses to NTS are already detected in early childhood (Nyirenda *et al.* 2014). Therefore, incorporating these immunologic deficits found in the very young and in children with malnutrition, malaria and HIV into preclinical studies may aid in development of more efficacious vaccination strategies by identifying functional components of immunity that can be targeted by vaccines to enhance resistance to disseminated infection.

## ACKNOWLEDGEMENTS

We thank Annica Stull-Lane and Andreas Bäumlner for discussions and comments on this review.

## FUNDING

Our work on this topic was supported by Public Health Service grant AI098078.

**Conflict of interest.** None declared.

## REFERENCES

- Ampel NM, Van Wyck DB, Aguirre ML *et al.* Resistance to infection in murine beta-thalassemia. *Infect Immun* 1989;57:1011–7.
- Anderson ES. Drug resistance in *Salmonella typhimurium* and its implications. *Brit Med J* 1968;3:333–9.
- Anderson ES, Lewis MJ. Drug resistance and its transfer in *Salmonella typhimurium*. *Nature* 1965;206:579–83.

- Ao TT, Feasey NA, Gordon MA et al. Global burden of invasive nontyphoidal *Salmonella* disease, 2010. *Emerg Infect Dis* 2015;21:941–49.
- Atif SM, Winter SE, Winter MG et al. *Salmonella enterica* serovar Typhi impairs CD4 T cell responses by reducing antigen availability. *Infect Immun* 2014;82:2247–54.
- Bachou H, Tylleskar T, Kaddu-Mulindwa DH et al. Bacteraemia among severely malnourished children infected and uninfected with the human immunodeficiency virus-1 in Kampala, Uganda. *BMC Infect Dis* 2006;6:160.
- Beebe JL, Koneman EW. Recovery of uncommon bacteria from blood: association with neoplastic disease. *Clin Microbiol Rev* 1995;8:336–56.
- Bottone EJ, Wormser GP, Duncanson FP. Nontyphoidal *Salmonella* bacteremia as an early infection in acquired immunodeficiency syndrome. *Diagn Micr Infect Dis* 1984;2:247–50.
- Brent AJ, Oundo JO, Mwangi I et al. *Salmonella* bacteremia in Kenyan children. *Pediatr Infect Dis J* 2006;25:230–6.
- Bustamante J, Boisson-Dupuis S, Abel L et al. Mendelian susceptibility to mycobacterial disease: genetic, immunological, and clinical features of inborn errors of IFN-gamma immunity. *Semin Immunol* 2014;26:454–70.
- Carden S, Okoro C, Dougan G et al. Non-typhoidal *Salmonella* Typhimurium ST313 isolates that cause bacteremia in humans stimulate less inflammasome activation than ST19 isolates associated with gastroenteritis. *Pathog Dis* 2015;73.
- Crump JA, Luby SP, Mintz ED. The global burden of typhoid fever. *B World Health Organ* 2004;82:346–53.
- Crump JA, Ram PK, Gupta SK et al. Part I. Analysis of data gaps pertaining to *Salmonella enterica* serotype Typhi infections in low and medium human development index countries, 1984–2005. *Epidemiol Infect* 2008;136:436–48.
- Cunnington AJ, de Souza JB, Walther M et al. Malaria impairs resistance to *Salmonella* through heme- and heme oxygenase-dependent dysfunctional granulocyte mobilization. *Nat Med* 2012a;18:120–7.
- Cunnington AJ, Njie M, Correa S et al. Prolonged neutrophil dysfunction after *Plasmodium falciparum* malaria is related to hemolysis and heme oxygenase-1 induction. *J Immunol* 2012b;189:5336–46.
- Cusa E, Obradors N, Baldoma L et al. Genetic analysis of a chromosomal region containing genes required for assimilation of allantoin nitrogen and linked glyoxylate metabolism in *Escherichia coli*. *J Bacteriol* 1999;181:7479–84.
- de Freitas Neto OC, Setta A, Imre A et al. A flagellated motile *Salmonella Gallinarum* mutant (SG Fla+) elicits a pro-inflammatory response from avian epithelial cells and macrophages and is less virulent to chickens. *Vet Microbiol* 2013;165:425–33.
- De Wit S, Taelman H, Van de Perre P et al. *Salmonella* bacteremia in African patients with human immunodeficiency virus infection. *Eur J Clin Microbiol* 1988;7:45–7.
- Eisele NA, Ruby T, Jacobson A et al. *Salmonella* require the fatty acid regulator PPARdelta for the establishment of a metabolic environment essential for long-term persistence. *Cell Host Microbe* 2013;14:171–82.
- Elhadad D, Desai P, Rahav G et al. Flagellin is required for host cell invasion and normal *Salmonella* pathogenicity island 1 expression by *Salmonella enterica* serovar Paratyphi A. *Infect Immun* 2015;83:3355–68.
- Evans C, Orf K, Horvath E et al. Impairment of neutrophil oxidative burst in children with sickle cell disease is associated with heme oxygenase-1. *Haematologica* 2015;100:1508–16.
- Facer CA, Brown J. Monocyte erythrophagocytosis in falciparum malaria. *Lancet* 1981;1:897–8.
- Feasey NA, Archer BN, Heyderman RS et al. Typhoid fever and invasive nontyphoidal salmonellosis, Malawi and South Africa. *Emerg Infect Dis* 2010;16:1448–51.
- Feasey NA, Dougan G, Kingsley RA et al. Invasive non-typhoidal *Salmonella* disease: an emerging and neglected tropical disease in Africa. *Lancet* 2012;379:2489–99.
- Feasey NA, Everett D, Faragher EB et al. Modelling the contributions of malaria, HIV, malnutrition and rainfall to the decline in paediatric invasive non-typhoidal *Salmonella* disease in Malawi. *PLoS Neglect Trop D* 2015;9:e0003979.
- Fischl MA, Dickinson GM, Sinave C et al. *Salmonella* bacteremia as manifestation of acquired immunodeficiency syndrome. *Arch Intern Med* 1986;146:113–5.
- Gilchrist JJ, MacLennan CA, Hill AV. Genetic susceptibility to invasive *Salmonella* disease. *Nat Rev Immunol* 2015;15:452–63.
- Gordon MA. Invasive nontyphoidal *Salmonella* disease: epidemiology, pathogenesis and diagnosis. *Curr Opin Infect Dis* 2011;24:484–9.
- Gordon MA, Banda HT, Gondwe M et al. Non-typhoidal *Salmonella* bacteraemia among HIV-infected Malawian adults: high mortality and frequent recrudescence. *AIDS* 2002;16:1633–41.
- Gordon MA, Graham SM, Walsh AL et al. Epidemics of invasive *Salmonella enterica* serovar enteritidis and *S. enterica* Serovar typhimurium infection associated with multidrug resistance among adults and children in Malawi. *Clin Infect Dis* 2008;46:963–9.
- Gordon MA, Walsh AL, Chaponda M et al. Bacteraemia and mortality among adult medical admissions in Malawi—predominance of non-typhi salmonellae and *Streptococcus pneumoniae*. *J Infect* 2001;42:44–9.
- Graham SM, Walsh AL, Molyneux EM et al. Clinical presentation of non-typhoidal *Salmonella* bacteraemia in Malawian children. *T Roy Soc Trop Med H* 2000;94:310–4.
- Hapfelmeier S, Ehrbar K, Stecher B et al. Role of the *Salmonella* pathogenicity island 1 effector proteins SipA, SopB, SopE, and SopE2 in *Salmonella enterica* subspecies 1 serovar Typhimurium colitis in streptomycin-pretreated mice. *Infect Immun* 2004;72:795–809.
- Hook EW, Campbell CG, Weens HS et al. *Salmonella* osteomyelitis in patients with sickle-cell anemia. *New Engl J Med* 1957;257:403–7.
- Israel DS, Plaisance KI. Neutropenia in patients infected with human immunodeficiency virus. *Clin Pharmacy* 1991;10:268–79.
- Jacobs JL, Gold JW, Murray HW et al. *Salmonella* infections in patients with the acquired immunodeficiency syndrome. *Ann Intern Med* 1985;102:186–8.
- Kariuki S, Okoro C, Kiiru J et al. Ceftriaxone-resistant *Salmonella enterica* serotype typhimurium sequence type 313 from Kenyan patients is associated with the blaCTX-M-15 gene on a novel IncHI2 plasmid. *Antimicrob Agents Ch* 2015;59:3133–9.
- Kariuki S, Onsare RS. Epidemiology and genomics of invasive nontyphoidal *Salmonella* infections in Kenya. *Clin Infect Dis* 2015;61 (Suppl. 4):S317–24.
- Kariuki S, Revathi G, Kariuki N et al. Characterisation of community acquired non-typhoidal *Salmonella* from bacteraemia and diarrhoeal infections in children admitted to hospital in Nairobi, Kenya. *BMC Microbiol* 2006;6:101.
- Karney WW, Tong MJ. Malabsorption in *Plasmodium falciparum* malaria. *Am J Trop Med Hyg* 1972;21:1–5.
- Kingsley RA, Kay S, Connor T et al. Genome and transcriptome adaptation accompanying emergence of the definitive type 2 host-restricted *Salmonella enterica* serovar Typhimurium pathovar. *MBio* 2013;4:e00565–13.

- Kingsley RA, Msefula CL, Thomson NR et al. Epidemic multiple drug resistant *Salmonella* Typhimurium causing invasive disease in sub-Saharan Africa have a distinct genotype. *Genome Res* 2009;19:2279–87.
- Kintz E, Davies MR, Hammarlof DL et al. A BTP1 prophage gene present in invasive non-typhoidal *Salmonella* determines composition and length of the O-antigen of the lipopolysaccharide. *Mol Microbiol* 2015;96:263–75.
- Langridge GC, Fookes M, Connor TR et al. Patterns of genome evolution that have accompanied host adaptation in *Salmonella*. *P Natl Acad Sci USA* 2015;112:863–8.
- Levine WC, Buehler JW, Bean NH et al. Epidemiology of nontyphoidal *Salmonella* bacteremia during the human immunodeficiency virus epidemic. *J Infect Dis* 1991;164:81–7.
- Lim MY, Rho M, Song YM et al. Stability of gut enterotypes in Korean monozygotic twins and their association with biomarkers and diet. *Sci Rep* 2014;4:7348.
- Lokken KL, Mooney JP, Butler BP et al. Malaria parasite infection compromises control of concurrent systemic non-typhoidal *Salmonella* infection via IL-10-mediated alteration of myeloid cell function. *PLoS Pathog* 2014;10:e1004049.
- Lunguya O, Lejon V, Phoba MF et al. Antimicrobial resistance in invasive non-typhoid *Salmonella* from the Democratic Republic of the Congo: emergence of decreased fluoroquinolone susceptibility and extended-spectrum beta lactamases. *PLoS Neglect Trop D* 2013;7:e2103.
- MacLennan CA, Martin LB, Micoli F. Vaccines against invasive *Salmonella* disease: current status and future directions. *Hum Vaccin Immunother* 2014;10:1478–93.
- Majowicz SE, Musto J, Scallan E et al. The global burden of nontyphoidal *Salmonella* gastroenteritis. *Clin Infect Dis* 2010;50:882–9.
- Mandal BK, Brennand J. Bacteraemia in salmonellosis: a 15 year retrospective study from a regional infectious diseases unit. *BMJ* 1988;297:1242–3.
- Mastroeni P, Harrison JA, Chabalgoity JA et al. Effect of interleukin 12 neutralization on host resistance and gamma interferon production in mouse typhoid. *Infect Immun* 1996;64:189–96.
- Mastroeni P, Vazquez-Torres A, Fang FC et al. Antimicrobial actions of the NADPH phagocyte oxidase and inducible nitric oxide synthase in experimental salmonellosis. II. Effects on microbial proliferation and host survival *in vivo*. *J Exp Med* 2000;192:237–48.
- Matthews TD, Schmieder R, Silva GG et al. Genomic comparison of the closely-related *Salmonella enterica* serovars enteritidis, Dublin and Gallinarum. *PLoS One* 2015;10:e0126883.
- McCoy MW, Moreland SM, Detweiler CS. Hemophagocytic macrophages in murine typhoid fever have an anti-inflammatory phenotype. *Infect Immun* 2012;80:3642–9.
- Micoli F, Ravenscroft N, Cescutti P et al. Structural analysis of O-polysaccharide chains extracted from different *Salmonella* Typhimurium strains. *Carbohydr Res* 2014;385:1–8.
- Molyneux ME, Looareesuwan S, Menzies IS et al. Reduced hepatic blood flow and intestinal malabsorption in severe falciparum malaria. *Am J Trop Med Hyg* 1989;40:470–6.
- Mooney JP, Butler BP, Lokken KL et al. The mucosal inflammatory response to non-typhoidal *Salmonella* in the intestine is blunted by IL-10 during concurrent malaria parasite infection. *Mucosal Immunol* 2014;7:1302–11.
- Mooney JP, Lee SJ, Lokken KL et al. Transient loss of protection afforded by a live attenuated non-typhoidal *Salmonella* vaccine in mice co-infected with malaria. *PLoS Neglect Trop D* 2015a;9:e0004027.
- Mooney JP, Lokken KL, Byndloss MX et al. Inflammation-associated alterations to the intestinal microbiota reduce colonization resistance against non-typhoidal *Salmonella* during concurrent malaria parasite infection. *Sci Rep* 2015b;5:14603.
- Muthumbi E, Morpeth SC, Ooko M et al. Invasive Salmonellosis in Kilifi, Kenya. *Clin Infect Dis* 2015;61 (Suppl. 4):S290–301.
- Nadelman RB, Mathur-Wagh U, Yancovitz SR et al. *Salmonella* bacteremia associated with the acquired immunodeficiency syndrome (AIDS). *Arch Intern Med* 1985;145:1968–71.
- Nauciel C, Espinasse-Maes F. Role of gamma interferon and tumor necrosis factor alpha in resistance to *Salmonella* typhimurium infection. *Infect Immun* 1992;60:450–4.
- Ng DH, Skehel JJ, Kassiotis G et al. Recovery of an antiviral antibody response following attrition caused by unrelated infection. *PLoS Pathog* 2014;10:e1003843.
- Nix RN, Altschuler SE, Henson PM et al. Hemophagocytic macrophages harbor *Salmonella enterica* during persistent infection. *PLoS Pathog* 2007;3:e193.
- Noriega LM, Van der Auwera P, Daneau D et al. *Salmonella* infections in a cancer center. *Support Care Cancer* 1994;2:116–22.
- Nuccio SP, Baumler AJ. Comparative analysis of *Salmonella* genomes identifies a metabolic network for escalating growth in the inflamed gut. *MBio* 2014;5:e00929–14.
- Nyirenda TS, Gilchrist JJ, Feasey NA et al. Sequential acquisition of T cells and antibodies to nontyphoidal *Salmonella* in Malawian children. *J Infect Dis* 2014;210:56–64.
- O'Dempsey TJ, McArdle TF, Lloyd-Evans N et al. Importance of enteric bacteria as a cause of pneumonia, meningitis and septicemia among children in a rural community in The Gambia, West Africa. *Pediatr Infect Dis J* 1994;13:122–8.
- Okoro CK, Barquist L, Connor TR et al. Signatures of adaptation in human invasive *Salmonella* Typhimurium ST313 populations from sub-Saharan Africa. *PLoS Neglect Trop D* 2015;9:e0003611.
- Okoro CK, Kingsley RA, Connor TR et al. Intracontinental spread of human invasive *Salmonella* Typhimurium pathovariants in sub-Saharan Africa. *Nat Genet* 2012;44:1215–21.
- Onsare RS, Micoli F, Lanzilao L et al. Relationship between antibody susceptibility and lipopolysaccharide O-antigen characteristics of invasive and gastrointestinal nontyphoidal *Salmonellae* isolates from Kenya. *PLoS Neglect Trop D* 2015;9:e0003573.
- Paglietti B, Falchi G, Mason P et al. Diversity among human nontyphoidal salmonellae isolates from Zimbabwe. *T Roy Soc Trop Med H* 2013;107:487–92.
- Park SE, Pak GD, Aaby P et al. The relationship between invasive nontyphoidal *Salmonella* disease, other bacterial bloodstream infections, and malaria in Sub-Saharan Africa. *Clin Infect Dis* 2016;62 (Suppl. 1):S23–31.
- Parsons BN, Humphrey S, Salisbury AM et al. Invasive nontyphoidal *Salmonella typhimurium* ST313 are not host-restricted and have an invasive phenotype in experimentally infected chickens. *PLoS Neglect Trop D* 2013;7:e2487.
- Peck MD, Babcock GF, Alexander JW. The role of protein and calorie restriction in outcome from *Salmonella* infection in mice. *JPEN-Parenter Enter* 1992;16:561–5.
- Pedro-Botet ML, Modol JM, Valles X et al. Changes in bloodstream infections in HIV-positive patients in a university hospital in Spain (1995–1997). *Int J Infect Dis* 2002;6:17–22.
- Pitrak DL. Neutrophil deficiency and dysfunction in HIV-infected patients. *Am J Health-Syst Ph* 1999;56 (Suppl. 5):S9–16.
- Potts RA, Tiffany CM, Pakpour N et al. Mast cells and histamine alter intestinal permeability during malaria parasite infection. *Immunobiology* 2016;221:468–74.



- Rabsch W, Tschape H, Baumler AJ. Non-typhoidal salmonellosis: emerging problems. *Microbes Infect* 2001;**3**:237–47.
- Raffatellu M, Santos RL, Verhoeven DE et al. Simian immunodeficiency virus-induced mucosal interleukin-17 deficiency promotes *Salmonella* dissemination from the gut. *Nat Med* 2008;**14**:421–8.
- Ramachandran G, Aheto K, Shirliff ME et al. Poor biofilm-forming ability and long-term survival of invasive *Salmonella* Typhimurium ST313. *Pathog Dis* 2016;**74**.
- Ramachandran G, Perkins DJ, Schmidlein PJ et al. Invasive *Salmonella* Typhimurium ST313 with naturally attenuated flagellin elicits reduced inflammation and replicates within macrophages. *PLoS Neglect Trop D* 2015;**9**:e3394.
- Reddy EA, Shaw AV, Crump JA. Community-acquired bloodstream infections in Africa: a systematic review and meta-analysis. *Lancet Infect Dis* 2010;**10**:417–32.
- Richards LH, Howard J, Klein JL. Community-acquired *Salmonella* bacteraemia in patients with sickle-cell disease 1969–2008: a single centre study. *Scand J Infect Dis* 2011;**43**:89–94.
- Roberts RB, Murray HW, Rubin BY et al. Opportunistic infections and impaired cell-mediated immune responses in patients with the acquired immune deficiency syndrome. *T Am Clin Climatol Assoc* 1984;**95**:40–51.
- Schicho R, Shaykhtudinov R, Ngo J et al. Quantitative metabolomic profiling of serum, plasma, and urine by (1)H NMR spectroscopy discriminates between patients with inflammatory bowel disease and healthy individuals. *J Proteome Res* 2012;**11**:3344–57.
- Scott JA, Berkley JA, Mwangi I et al. Relation between falciparum malaria and bacteraemia in Kenyan children: a population-based, case-control study and a longitudinal study. *Lancet* 2011;**378**:1316–23.
- Shahunja KM, Leung DT, Ahmed T et al. Factors associated with non-typhoidal *Salmonella* bacteremia versus typhoidal *Salmonella* bacteremia in patients presenting for care in an urban diarrheal disease hospital in Bangladesh. *PLoS Neglect Trop D* 2015;**9**:e0004066.
- Sigauque B, Roca A, Mandomando I et al. Community-acquired bacteremia among children admitted to a rural hospital in Mozambique. *Pediatr Infect Dis J* 2009;**28**:108–13.
- Simm R, Ahmad I, Rhen M et al. Regulation of biofilm formation in *Salmonella enterica* serovar Typhimurium. *Future Microbiol* 2014;**9**:1261–82.
- Singletary LA, Karlinsey JE, Libby SJ et al. Loss of multicellular behavior in epidemic African nontyphoidal *Salmonella enterica* serovar Typhimurium ST313 strain D23580. *MBio* 2016;**7**:e02265.
- Taniguchi T, Miyauchi E, Nakamura S et al. *Plasmodium berghei* ANKA causes intestinal malaria associated with dysbiosis. *Sci Rep* 2015;**5**:15699.
- Threlfall EJ, Hall ML, Rowe B. *Salmonella* bacteraemia in England and Wales, 1981–1990. *J Clin Pathol* 1992;**45**:34–6.
- Vazquez-Torres A, Jones-Carson J, Mastroeni P et al. Antimicrobial actions of the NADPH phagocyte oxidase and inducible nitric oxide synthase in experimental salmonellosis. I. Effects on microbial killing by activated peritoneal macrophages *in vitro*. *J Exp Med* 2000;**192**:227–36.
- Verani JR, Toroitich S, Auko J et al. Burden of invasive nontyphoidal *Salmonella* disease in a rural and urban site in Kenya, 2009–2014. *Clin Infect Dis* 2015;**61** (Suppl. 4):S302–9.
- Wain J, Keddy KH, Hendriksen RS et al. Using next generation sequencing to tackle non-typhoidal *Salmonella* infections. *J Infect Dev Countries* 2013;**7**:1–5.
- Wanachiwanawin W. Infections in E-beta thalassemia. *J Pediatr Hematol Onc* 2000;**22**:581–7.
- Williams TN, Uyoga S, Macharia A et al. Bacteraemia in Kenyan children with sickle-cell anaemia: a retrospective cohort and case-control study. *Lancet* 2009;**374**:1364–70.
- Winkelstein JA, Marino MC, Johnston RB, Jr et al. Chronic granulomatous disease. Report on a national registry of 368 patients. *Medicine* 2000;**79**:155–69.
- Winter SE, Thiennimitr P, Nuccio SP et al. Contribution of flagellin pattern recognition to intestinal inflammation during *Salmonella enterica* serotype typhimurium infection. *Infect Immun* 2009;**77**:1904–16.
- Winter SE, Winter MG, Atluri V et al. The flagellar regulator TviA reduces pyroptosis by *Salmonella enterica* serovar Typhi. *Infect Immun* 2015;**83**:1546–55.
- Winter SE, Winter MG, Godinez I et al. A rapid change in virulence gene expression during the transition from the intestinal lumen into tissue promotes systemic dissemination of *Salmonella*. *PLoS Pathog* 2010;**6**:e1001060.
- Yang J, Barrila J, Roland KL et al. Characterization of the invasive, multidrug resistant non-typhoidal *Salmonella* Strain D23580 in a murine model of infection. *PLoS Neglect Trop D* 2015;**9**:e0003839.