DOMAIN 8 PATHOGENESIS

Invasive Nontyphoidal *Salmonella* Disease in Africa

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ABSTRACT Nontyphoidal salmonellae (NTS) are a major cause of invasive (iNTS) disease in sub-Saharan Africa, manifesting as bacteremia and meningitis. Available epidemiological data indicate that iNTS disease is endemic in much of the region. Antimicrobial resistance is common and case fatality rates are high. There are well-characterized clinical associations with iNTS disease, including young age, HIV infection, malaria, malnutrition, anemia, and sickle cell disease. However, the clinical presentation of iNTS disease is often with fever alone, so clinical diagnosis is impossible without blood culture confirmation. No vaccine is currently available, making this a priority area for global health research. Over the past ten years, it has emerged that iNTS disease in Africa is caused by distinct pathovars of *Salmonella* Typhimurium, belonging to sequence type ST313, and *Salmonella* Enteritidis. These are characterized by genome degradation and appear to be adapting to an invasive lifestyle. Investigation of rare patients with primary immunodeficiencies has suggested a key role for interferon gamma-mediated immunity in host defense against NTS. This concept has been supported by recent population-based host genetic studies in African children. In contrast, immunoepidemiological studies from Africa indicate an important role for antibody for protective immunity, supporting the development of antibody-inducing vaccines against iNTS disease. With candidate O-antigen-based vaccines due to enter clinical trials in the near future, research efforts should focus on understanding the relative contributions of antibody and cell-mediated immunity to protection against iNTS disease in humans.

INTRODUCTION

Salmonellae are motile, Gram-negative, facultative anaerobes, belonging to the family *Enterobacteriaceae*. The genus *Salmonella* is composed of two species, *Salmonella bongori* and *Salmonella enterica*, and is estimated to have diverged from *Escherichia coli* approximately 100 million years ago (1). *S. bongori* principally causes disease in reptiles, and has only rarely been reported to be associated with disease in humans (2). By contrast, the species *S. enterica* is composed of thousands of human disease-causing serovars.

S. enterica serovars can be broadly divided into two groups: typhoidal and nontyphoidal strains. The typhoidal serovars, *Salmonella enterica* serovar Typhi (*S. Typhi*) and *Salmonella enterica* serovars Paratyphi A, B, and C (*S. Paratyphi* A, B, and C), are host-adapted, human-restricted organisms,

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causing a single clinical syndrome, enteric fever, in immunocompetent individuals. Enteric fever, also known as typhoid fever, is characterized by a systemic, febrile, influenza-like illness, in which gastrointestinal symptoms (while common), are often not a cardinal presenting feature (3).

Nontyphoidal *Salmonella* (NTS) serovars have a broad vertebrate host range and cause a self-limiting enterocolitis in the majority of immunocompetent individuals in developed settings (4). In these individuals, secondary bacteremia is thought to be uncommon in the absence of immunocompromise, and estimated case fatality rates are low (0.0003–0.003%) (5, 6). However, in sub-Saharan Africa, among HIV-infected individuals and young children, NTS causes invasive disease, most commonly characterized by primary bacteremia and often in the absence of features of gastrointestinal infection. The emergence of invasive NTS (iNTS) lineages with expanded multidrug resistance, the lack of a pathognomonic clinical presentation alongside an absence of timely and accurate diagnostic tests for iNTS disease, combined with the lack of an available NTS vaccine for use in humans, all contribute to a substantial burden of morbidity and mortality in Africa from iNTS disease.

In this review, we present the current estimates of burden of disease from iNTS disease in Africa; risk factors for iNTS disease; our current understanding of the microbiology and transmission of iNTS disease in African populations; the clinical presentation, diagnosis, and management of iNTS disease in Africa; and, finally, the immunology and host genetics of iNTS disease susceptibility in African populations.

EPIDEMIOLOGY OF INTS DISEASE IN AFRICA

Burden of iNTS Disease

Sub-Saharan Africa is the principal region affected by iNTS disease (Fig. 1). iNTS disease in Africa is predominantly bacteremia without focal infection, but NTS is also an important cause of meningitis in African populations. In a meta-analysis of the etiologies of bacteremia identified in prospective, hospital-based studies in Africa, NTS is the leading cause of community-acquired bacteremia in African adults, and the second most commonly identified pathogen in African children (7). In 2010, of an estimated 3.4 million global cases of iNTS disease, 1.9 million were estimated to have occurred in sub-Saharan Africa (8).

Figure 1 Global distribution of invasive nontyphoidal *Salmonella* disease. High burden of disease is defined as >100 episodes per 100,000 person-years, and medium burden as 10 to 100 per 100,000 person-years. Reproduced from reference 135: MacLennan CA, Martin LB, Micoli F. 2014. *Hum Vaccin Immunother* 10:1478–1493, with permission.

iNTS disease in African populations primarily affects HIV-infected adults and young children. In the limited number of African studies with defined denominator populations (9–15), the median estimated incidence of iNTS disease in children is 195 cases (range 26 to 1,870) per 100,000 person-years of observation (Table 1). Among African adults, population-based surveillance data estimated the incidence of iNTS disease to be between 0 and 54 cases per 100,000 person-years of observation in 13 surveillance sites across sub-Saharan Africa (16). Among HIV-infected African adults, the estimated incidence of iNTS disease is considerably higher, with an estimated incidence of 7,500 per 100,000 person-years of observation in Ugandan HIV-infected adults with CD4 counts <200 cells/μl (17). It should be noted that there are significant limitations to estimates of iNTS disease burden in Africa. Available estimates of iNTS disease incidence are limited by a bias toward studies with hospital-based

ascertainment of disease, and to the few African settings with ready availability of bacterial culture services.

Mortality Secondary to iNTS Disease

Mortality secondary to iNTS disease in both African adults and children is high. Case fatality rates for iNTS disease in African adults appear to be higher than those seen in children (18), with a 37% case fatality in Kenyan adults (9) and 47% reported in Malawian adults (19). These high rates have decreased, in parallel with the falling incidence of iNTS disease, among antiretroviral therapy (ART)-treated African adults. Provision of ART treatment programs in Malawi resulted in the NTS-associated case fatality rate in Malawian adults declining to 11% (20). In studies reporting case fatality rates in African children (Table 2), the median case fatality is 12% (range 3 to 24%) (9, 12, 13, 15, 21). However, adopting a consensus

Table 1 Incidence estimates for iNTS disease in African children^a

Country	Region	Dates	Crude incidence per 100,000 person-years	Adjusted incidence estimates	Changing incidence over study period, IRR	Reference
Kenya	Kilifi	1998–2014	25.6 (22.0–29.5)	36.4 (35.6–37.1)	0.84 (0.81–0.86)	9
Kenya	Lwak	2006–2009	206 (138–298)	2085 (1181–2990)	2.69 (1.11–8.50) ^b	10
Kenya	Lwak	2009–2014	501.8 (411.6–611.6)	3914.3 (3646.5–4201.9)	0.73 (0.56–0.93) ^b	11
Kenya	Kibera	2006–2009	52 (17–122)	260 (102–419)	ND	10
Kenya	Kibera	2009–2014	254.9 (192.1–338.2)	997.9 (864.9–1151.3)	0.81 (0.68–0.97) ^b	11
Kenya	Western Kenya	2009–2013	1870 (1540–2280)	ND	ND	12
Mozambique	Manhica	2001–2014	184.5 (164.5–207)	ND	0.82 (0.79–0.85) ^b	13
Tanzania	Muheza	2006–2010	29.7 (25.3–34.9)	ND	0.41 (0.22–0.67) ^b	14
Gambia	Upper and Central River Divisions	2000–2004	300 (222–397)	ND	ND	15
Malawi	Blantyre	2008–2010	158 (145.2–172.1)	ND	0.58 (0.41–0.80) ^b	– ^c
South Africa	Gauteng	2003–2013	10.9 (10.3–11.5)	ND	0.95 (0.93–0.96)	44
Burkina Faso	Nioko II	2010–2014	9	35 (13–96)	ND	16
Burkina Faso	Polesgo	2010–2014	75	255 (138–470)	ND	16
Guinea-Bissau	Bandim	2010–2014	40	116 (69–161)	ND	16
Ghana	Asante Akim North	2010–2014	301	742 (631–873)	ND	16
Sudan	East Wad Medani	2010–2014	0	0	ND	16
Tanzania	Moshi Rural	2010–2014	0	0	ND	16
Tanzania	Moshi Urban	2010–2014	20	26 (8–88)	ND	16
Kenya	Kibera	2010–2014	31	31 (10–95)	ND	16
Madagascar	Imerintsiasosika	2010–2014	14	18 (3–99)	ND	16
Madagascar	Isotry	2010–2014	0	0	ND	16

^aND, no data; IRR, incidence rate ratio.

^bWhere not reported, annualized IRRs are calculated from incidence rates by Poisson regression in R.

^cCalculated from annual paediatric cases in Feasey et al. (45) and Blantyre urban population estimates from 2008 census (www.nsomalwi.mw/2008-population-and-housing-census).

Table 2 Clinical features and outcome of African children with invasive nontyphoidal *Salmonella* disease^a

	Muthumbi et al., 2015 (9)	Oneko et al., 2015 (12)	Mandomando et al., 2015 (13)	Enwere et al., 2006 (15)	Graham et al., 2000 (21)	Falay et al., 2016 (32)	MacLennan et al., 2017 (48)
Country	Kenya	Kenya	Mozambique	Gambia	Malawi	DRC	Malawi
Region	Kilifi	Western Kenya	Manhica	Upper & Central River Divisions	Blantyre	Oriental	Blantyre
Dates	1998–2014	2009–2013	2001–2014	2000–2004	1996–1998	2009–2014	2006
Cases	351	94	670	92	299	108	263
Age, months (median)	15	17.5	21.5	20	14	24	16
Male, %	55.7	44.7	54.6	52.2	59.1	58.3	54.8
Clinical practices at presentation, %							
Fever	74.4	99.0	97.0	100.0	96.6	NR	94.2
Tachycardia	NR	51.0	NR	NR	NR	NR	68.7
Tachypnea	NR	68.8	NR	77.2	72.1	NR	65.8
Diarrhea	29.9	45.8	33.0	37.8	47.5	28.0	31.1
Vomiting	32.0	NR	30.0	62.2	NR	45.3	37.8
Clinical ARI	17.1	NR	35.0	NR	NR	15.7	42.1
Hepatomegaly	17.6	NR	NR	NR	37.1%	18.8	22.4
Splenomegaly	19.0	NR	36.0	NR	44.7%	26.1	26.8
Mortality, %	22.1	3.1	10.3	5.8	23.8%	13.3	20.3

^aARI, acute respiratory infection; NR, not recorded; DRC, Democratic Republic of Congo.

case fatality rate of 20% for iNTS disease, global iNTS disease-related mortality was estimated at 680,000 deaths in 2010, of which 390,000 occurred in Africa (8).

While these rates of iNTS-related mortality represent the most robust estimates generated to date, there remains considerable uncertainty regarding their accuracy. It is noteworthy that the two lowest estimates of case fatality (3.1% and 5.8%) were reported in studies performing blood cultures for fever surveillance as part of vaccine trials (12, 15) including children managed in an outpatient setting, while the three highest case fatality estimates (20.3%, 22.1%, and 23.8%) were reported in studies undertaken exclusively in hospital settings (9, 21). Some of these differences will be secondary to differential availability of ART between study populations, and differential rates of mortality-associated comorbidities. Nevertheless, some of these differences seem likely to be secondary to ascertainment bias, with hospital-based studies resulting in estimated mortality rates not representative of those in the general population.

NTS Meningitis

The majority of iNTS disease encountered in African children is bacteremia without focal infection. However,

a small, but significant, proportion of iNTS disease in African children is NTS meningitis (22), commonly complicated by bacteremia. Among Kenyan children with iNTS disease, 22 of 321 (6.9%) had NTS meningitis (9). African children with NTS meningitis are typically younger than children with nonfocal bacteremic disease, with a median age of 7 months in one Malawian series (23). Eight of 22 Kenyan children with NTS meningitis in another study were aged less than 7 days (9). Mortality among children with NTS meningitis is particularly high, with case fatality rates of approximately 50% (9, 23), with neurological sequelae reported in 56% of survivors. These rates of mortality and neurological sequelae are unaffected by adjunctive steroid treatment (24).

Acquired Risk Factors for iNTS Disease

iNTS disease in African adults is overwhelmingly HIV-associated, with rates of HIV coinfection in excess of 95% among those with NTS bacteremia (19, 25). The role of HIV in the emergence of NTS bacteremia in Africa has been further clarified by whole-genome sequencing-based phylogenetic analysis of *S. Typhimurium* isolates causing invasive infection over several decades. In a seminal study (26), *S. Typhimurium* strains causing invasive disease were composed of two highly related lineages

that are genetically distinct from gastroenteritis-causing strains. The emergence of the first lineage in each country is temporally associated with the emergence of the HIV pandemic.

In African children, risk factors for iNTS disease are more diverse, with HIV infection, malnutrition (27), and malaria (28) all predisposing to disease. The relationship between iNTS disease and malaria is particularly complex, with concurrent parasitemia (29), recent malaria (30), and severe malarial anemia (31) all being associated with disease. The prevalence of these key risk factors among cases of pediatric iNTS disease in several African settings (9–13, 15, 18, 21, 32) is summarized in Table 3. Importantly, these risk factors for iNTS disease in African children do not operate independently of each other. For example, HIV-infected South African children with NTS bacteremia are more likely to be malnourished than their HIV-uninfected counterparts (18).

In high-income settings, iNTS disease is an uncommon manifestation of NTS infection (33). When it does occur, iNTS disease is typically restricted to young children (34), the elderly (35–41), and individuals with chronic disease (36), hypochlorhydria (36), and immunodeficiency states (40–42). These data are summarized in Table 4. The common immunodeficiency states described in association with iNTS disease in these settings are secondary to immunosuppressive drugs (corticosteroids and cytotoxic chemotherapy) and malignancies. iNTS disease remains

an important cause of invasive infection in HIV-infected individuals (43), although delivery of ART has made HIV an uncommon underlying cause of iNTS disease in high-income settings. Individuals with rare, inherited primary immunodeficiencies are also at substantially increased risk of iNTS disease. The association between monogenic immunodeficiencies and iNTS disease has been extremely instructive in terms of the immunobiology of NTS infection (discussed later). However, the rarity of these conditions makes their impact on iNTS disease risk at the population level negligible.

Changing Rates of iNTS Disease Over Time

Interpopulation differences in the rates of major determinants of iNTS disease susceptibility (HIV, malaria, and malnutrition) are likely to explain, in part, the wide variation in rates of iNTS disease between African regions (Table 1). Similarly, changes in the prevalence of major NTS-associated risk factors are likely to influence rates of iNTS disease in African populations over time. In African adults, the rates of iNTS disease have been observed to decline with increasing availability of ART. The minimum incidence of iNTS disease among ART-naïve, HIV-infected Malawian adults is estimated at 93 per 100,000 person-years (20). Following a transient rise to 757 per 100,000 person-years two months after ART initiation, incidence falls to 38 per 100,000 person-years after 9 months of ART (N. Feasey, personal communication), parallel to a similar fall in the incidence of

Table 3 Risk factors for invasive nontyphoidal *Salmonella* disease in African children^a

Study	Country	Region	Dates	HIV, %	Malnutrition, %	Malaria, %	Severe anemia, %
Muthumbi et al., 2015 (9)	Kenya	Kilifi	1998–2014	24.1	31.4	28.1	NR
Tabu et al., 2012 (10)	Kenya	Lwak	2006–2009	20.0	NR	NR	NR
Verani et al., 2015 (11)	Kenya	Lwak	2009–2014	28.6	NR	28.6	NR
Verani et al., 2015 (11)	Kenya	Kibera	2009–2014	NR	NR	8.3	NR
Oneko et al., 2015 (12)	Kenya	Western Kenya	2009–2013	16.7	8.3	58 ^b	32.2 ^c
Mandomando et al., 2015 (13)	Mozambique	Manhica	2001–2014	NR	31.0	43.0	19.0
Enwere et al., 2006 (15)	Gambia	Upper & Central River Divisions	2000–2004	NR	32.6	24.7	54.4 ^c
Graham et al., 2000 (21)	Malawi	Blantyre	1996–1998	19.7 ^d	14.5	31.4	26.8
Falay et al., 2016 (32)	DRC	Oriental	2009–2014	NR	15.9	69.3	29.7
Keddy et al., 2017 (18)	South Africa	Gauteng	2003–2013	63.9	19.9	NR	NR
MacLennan et al., 2017	Malawi	Blantyre	2006	43.2	25.0	15.3	19.3

^aNR, not recorded.

^bIncludes malaria up to 2 weeks prior to iNTS disease.

^cHemoglobin <8 g/dl.

^dClinical diagnosis.

Table 4 Risk factors for invasive nontyphoidal *Salmonella* disease in high-income settings^a

Study	Country	Population	Dates	Demographics		Chronic disease				Immunosuppressive drugs	
				Age >65 years	Male sex	Unspecified	Hypochlorhydria	Hematological malignancy	Solid-organ malignancy	Cytotoxic chemotherapy	Corticosteroids
Angelo et al., 2016 (35)	USA	National antimicrobial resistance surveillance	2003–2013	2.04 (1.73–2.41)	1.23 (1.09–1.39)	NR	NR	NR	NR	NR	NR
Parry et al., 2013 (36)	UK	Hospitalized adults (single hospital)	1982–2006	3.86 (2.15–6.93)	1.11 (0.65–1.92)	2.20 (1.26–3.83)	2.75 (1.32–5.71)	NR	NR	NR	NR
Varma et al., 2005 (37)	USA	National antimicrobial resistance surveillance	1996–2001	2.1 (1.5–2.9)	1.3 (1.1–1.6)	NR	NR	NR	NR	NR	NR
Mandal et al., 1988 (38)	UK	Hospitalized adults and children (single hospital)	1968–1983	1.63 (2.32–3.30) ^b	NR	NR	NR	NR	NR	NR	NR
Shimoni et al., 1999 (42)	Israel	Hospitalized adults and children (single hospital)	1987–1996	NR	NR	NR	NR	5.52 (2.09–14.54) ^c	NR	5.02 (2.06–12.22) ^c	3.75 (1.56–9.04) ^c
Laupland et al., 2010 (39)	Finland, Denmark, Australia, Canada	Multinational bacteremia surveillance network	2000–2007	– ^d	1.62 (1.35–1.96) ^e	NR	NR	NR	NR	NR	NR
Hsu et al., 2003 (40)	Taiwan	Hospitalized adults and children (single hospital)	1995–2001	– ^d	1.13 (0.68–1.88)	NR	NR	1.42 (0.71–2.84)	8.18 (3.66–18.28)	5.87 (3.35–10.27)	5.87 (3.35–10.27)
Fisker et al., 2003 (41)	Denmark	County-level surveillance	1991–1999	9.9 (5.8–17.0)	1.3 (0.9–2.0)	NR	1.5 (0.6–3.4)	NR	NR	3.4 (1.8–6.4)	3.4 (1.8–6.4)

^aEffects are calculated as odds ratios (95% confidence intervals) of iNTS disease compared with NTS enterocolitis. Significant associations are highlighted in bold. NR, not recorded.

^bAge >70 years.

^cOdds ratios compare cases of NTS bacteremia with cases of non-*Salmonella* Gram-negative bacteremia.

^dAssociated with increasing age, numbers not reported.

^eRisk ratio.

all-cause bacteremia. Similarly, declining rates of iNTS disease in South African adults between 2003 and 2013 are associated with increased access to ART in adults and children over the age of 5 years (44).

African centers reporting longitudinal surveillance (9, 11, 13, 14, 44, 45) have found a decline in pediatric iNTS disease incidence (Fig. 2). The median incidence rate ratio (IRR) per year during surveillance was 0.81 (range 0.41 to 2.69), suggesting an annual reduction of 19% in pediatric iNTS disease incidence. In coastal Kenyan and Gambian settings, this has been shown to be associated with waning malaria transmission (28, 46). The role of

falling malaria transmission in declining iNTS disease rates is supported by a study modeling observed changes in incidence in Malawian children (45). However, the same study also found evidence that changing rates of HIV and malnutrition have significant effects on iNTS disease incidence.

CLINICAL ASPECTS OF INTS DISEASE

Clinical Presentation

The clinical presentation of African children with iNTS disease is strikingly consistent (Table 2; references 9, 12, 13, 15, 21, and 32). Disease is predominantly seen in

Figure 2 Variation in invasive nontyphoidal *Salmonella* disease incidence in Africa over time. Variation in iNTS disease incidence in African children at seven sites between 2008 and 2014 (9, 11, 13, 14, 44, 45). Incidence per 100,000 person-years observation in children under 5 years.

young infants (study median age, 19 months; range, 14 to 24 months). Fever at presentation is common (study median, 97%; range 74 to 100%), but other clinical features are highly variable. Among Malawian adults with NTS bacteremia, fever and splenomegaly at presentation are predictive of NTS bacteremia (47). One-third of adults with iNTS disease have clinical features consistent with a respiratory infection at presentation (19).

Despite causing diarrheal disease in a range of settings, diarrhea in children and adults with iNTS disease is uncommon (study median in children, 35% [9, 12, 13, 15, 21, 32]; in Malawian adults, 46% [19]). The lack of reported diarrhea may represent attenuated host immune responses in a susceptible and immunosuppressed population, and/or pathogen-directed attenuation of intestinal inflammatory responses by invasive African strains of NTS (for a discussion of both of these features, see below). Blunted enteric inflammatory responses may well facilitate dissemination, enhancing the likelihood of systemic infection.

A clinical diagnosis of pneumonia at admission is made in a median of 19% of cases (range, 16 to 35%) (9, 13, 32, 48). On examination, children are commonly tachypneic (12, 15, 21, 48) (study median, 72%; range, 66 to 77%), and, where reported, tachycardic (12, 48) (prevalence in two studies, 51% and 69%). The observed tachypnea is likely secondary to acidosis, but may lead to a diagnosis of community-acquired pneumonia. Splenomegaly is also commonly reported (9, 13, 21, 32, 48) (study median, 31%; range, 19 to 45%), which is likely to represent recent malaria infection.

Clinical characteristics at presentation can predict outcome in iNTS disease. Among Malawian children, HIV coinfection, age at presentation under 6 months, or respiratory distress at presentation, are independent risk factors for inpatient mortality (48). Among South Africans (children and adults) with iNTS disease, those with HIV coinfection and individuals with severe illness at presentation are all at increased risk of death (18).

Diagnosis

The lack of a pathognomonic clinical presentation makes clinical diagnosis of iNTS disease impossible. There are no validated point-of-care biomarkers of NTS infection, and no available rapid diagnostic serological or PCR-based tools. This means that bacterial culture of blood or

cerebrospinal fluid is the only available method for diagnosis. In Africa, where blood culture facilities are not commonly available, the lack of diagnostic tests compromises clinical care and the ability to determine robust epidemiological estimates for iNTS disease burden.

Management

The effective clinical management of iNTS disease is focused on supportive care, and delivery of antimicrobial agents with adequate anti-*Salmonella* activity and penetration into tissues harboring salmonellae. Even where bacterial culture services are available, definitive microbiological diagnosis is delayed following admission. African children treated for iNTS disease will be initially managed with empirical antibiotics. Therefore, prescribing guidelines need to reflect the local burden and antibiotic resistance profile of NTS. Expanding antibiotic resistance (see below) has meant that empirical antibiotic guidelines for African children commonly fail to provide adequate *Salmonella* cover. For instance, chloramphenicol and gentamicin have, until recently, been used as empirical antibiotic treatment for suspected sepsis in Malawian children. The majority of invasive NTS isolates have chloramphenicol resistance, while gentamicin lacks intracellular penetration, limiting its effectiveness. Widespread use of penicillins for children with iNTS disease initially diagnosed as community-acquired pneumonia will lead to inadequate anti-*Salmonella* treatment. As with chloramphenicol, the majority of iNTS isolates are resistant to penicillins.

MICROBIOLOGY OF INTS DISEASE IN AFRICA

In the United Kingdom, where national surveillance of *Salmonella* infections has been in place since 1945, epidemics of NTS diarrhea have been caused most commonly by serovars Typhimurium and Enteritidis. Significant epidemics have also been caused by serovars Hadar and Agona (49). NTS serovars causing invasive disease in Africa are diverse, but, in common with diarrheal disease, the large majority are either *S. enterica* serovars Typhimurium or Enteritidis (Table 5).

Multidrug-Resistant NTS

Invasive multidrug-resistant (MDR) strains of NTS are resistant to ampicillin, cotrimoxazole, and chloramphenicol, and are widespread in Africa (Table 5). The emergence of these strains represents a major public health concern for a region in which these cheap and widely

Table 5 Nontyphoidal *Salmonella*/a isolates causing invasive disease in Africa^a

Study	Muthumbi et al., 2015 (9)	Tabu et al., 2012 (10)	Tabu et al., 2012 (10)	Onoko et al., 2015 (12)	Mandomando et al., 2015 (13)	Kalonji et al., 2015 (52)	Feasey et al., 2015 (50)	Feasey et al., 2010 (136)	Keddy et al., 2017 (18)
Country	Kenya	Kenya	Kenya	Kenya	Mozambique	DRC	Malawi	South Africa	South Africa
Region	Kilifi	Lwak	Kibera	Western Kenya	Manhica	4 sites	Blantyre	NRU	Gauteng
Dates	1998–2014	2006–2009	2006–2009	2009–2013	2001–2014	2011–2014	1998–2014	2003–2004	2003–2013
MDR, %	23.9	80.0 ^b	NR	52.1	NR	84.9	65.1	NR	23.8
Third-generation cephalosporin resistance, %	6.0	0.0	NR	16.7	0.0	0.0	0.2	NR	9.0
Decreased ciprofloxacin sensitivity, %	NR	NR	NR	1.1	0.0	1.9	0.2	NR	1.9
<i>S. Typhimurium</i> , %	43.3	88.3	85.7	70.6	68.9	49.2	79.0	67.0	55.7
<i>S. Enteritidis</i> , %	45.6	10.0	14.3	29.4	18.6	49.9	16.0	10.0	26.4
Nontypable/other, %	11.1	1.7	0.0	0.0	12.4	0.9	5.1	23.0	17.9

^aMDR, multidrug resistance (resistance to cotrimoxazole, chloramphenicol, ampicillin); DRC, Democratic Republic of Congo; NRU, national reference unit; NR, not recorded.^b*S. Typhimurium* isolates only.

available antibiotics are commonly used to treat clinical syndromes indistinguishable from iNTS disease. The prevalence of MDR strains among iNTS isolates in Africa has varied considerably (9, 18, 50). In Kenya and Malawi, there was a temporal relationship between the emergence of MDR among *S. Typhimurium*, and replacement of *S. Enteritidis* by *S. Typhimurium* as the dominant etiology of iNTS disease (9, 25). Similarly, declining MDR rates among *S. Enteritidis* isolates are significantly correlated with falling incidence of *S. Enteritidis* disease (Poisson regression $P = 1.9 \times 10^{-4}$; Figure 3).

High prevalence of MDR iNTS isolates has led to the use of ceftriaxone for treatment of undifferentiated sepsis in Africa (51). Along with ciprofloxacin for microbiologically confirmed iNTS disease, these antimicrobials now form the mainstay of anti-*Salmonella* antimicrobial therapy in Africa. Therefore, the emergence of isolates resistant to third-generation cephalosporins with decreased ciprofloxacin susceptibility in Africa is of considerable concern (Table 5) (9, 12, 18, 52). Whereas the emergence of MDR ST313 isolates (see below) did not have an appreciable impact on case fatality rate, third-generation cephalosporin-resistant ST313 isolates have increased associated mortality (9). Of particular concern is the detection of an ST313 isolate in a Malawian HIV-infected adult patient resistant to ciprofloxacin and ceftriaxone (51). This combination of antibiotic resistance would make the isolate untreatable in many African settings.

S. Typhimurium ST313

Whole-genome sequencing and phylogenetic analysis of African MDR *S. Typhimurium* has identified a dominant *S. Typhimurium* multilocus sequence type pathovar (ST313) (53). Invasive ST313 in Africa appears to have emerged as two distinct lineages, with the second replacing the first following the acquisition of chloramphenicol resistance in the context of considerable chloramphenicol use (26). Estimates of the emergence of the first lineage in several African settings suggests coincident emergence with the African HIV pandemic. *S. Typhimurium* ST313 causes invasive disease in African children and adults (54), resulting in both bacteremia and meningitis (22). Importantly, *S. Typhimurium* ST313 strains are also a cause of diarrhea in Africa (55).

S. Typhimurium ST313 has several distinct genomic features as compared with noninvasive *S. Typhimurium* serovars, including five prophage-like elements (BTP1

Figure 3 Multidrug resistance and incidence of invasive nontyphoidal *Salmonella* disease. The relationship between the annual frequency of invasive disease caused by *S. Enteritidis* and the rate of *S. Enteritidis* multidrug resistance. Poisson regression, curve plotted in blue, demonstrates significant positive correlation between MDR rates among *S. Enteritidis* isolates and the incidence of *S. Enteritidis* disease ($P = 1.9 \times 10^{-4}$). Data extracted from reference [50](#).

through BTP5), and a distinct pattern of genomic degradation ([53](#)). ST313 isolate D23580 has four plasmids, three with no assigned phenotype (pBT1 to pBT3) and a fourth, pSLT-BT. pSLT-BT has high levels of homology with pSLT, a virulence-associated plasmid encoding the *spv* (*Salmonella* plasmid virulence) operon ([56](#)). pSLT carriage in *S. Typhimurium* strains is variable, and data from developed settings suggest an overrepresentation of pSLT-positive isolates causing bacteremia as compared with isolates causing diarrhea ([57](#)). This concept is consistent with the hypothesis that pSLT carriage is a determinant of invasiveness in human infection. In ST313 isolates, pSLT-BT harbors a transposable element containing genes responsible for the ST313 MDR phenotype. Both documented ST313 lineages carry pSLT-BT ([26](#)).

Pathogenic Features of African NTS Isolates Causing Invasive Disease

Following the identification of a clearly defined and homogeneous genetic architecture of African invasive *S. Typhimurium* ST313 isolates, there has been considerable interest in defining phenotypic correlates of the ST313 invasive lineage. The BTP1 prophage encodes a glycosyltransferase (*gtr*) operon, which modifies lipopolysaccharide (LPS) chain length ([58](#)). While it has not been established that this is a correlate of virulence, this possibility seems plausible, given that extensive LPS modification directed by multiple *Salmonella* virulence factors determines extracellular and intramacrophage survival.

Comparisons of single ST313 (D23580) and ST19 (SL1344) isolates suggest phenotypic differences in invasive potential

(59, 60) and acid tolerance (59), with the ST313 strain exhibiting greater invasiveness and acid tolerance. Comparison of ST313 and non-ST313 (ST19) infection in the mouse streptomycin pretreated and the calf ligated ileal loop models of *Salmonella*-induced colitis demonstrates reduced inflammation in ST313 infections, with reduced neutrophil recruitment (61). *In vitro* models of epithelial and phagocytic infection demonstrate reduced SPI-1-mediated epithelial invasion in parallel with reduced expression of the SPI-1 effector SopE2 (62), and reduced inflammatory responses in phagocytic infection, in parallel with reduced flagellin expression in ST313 isolates (62). However, this phenotype of reduced epithelial invasiveness and attenuated intestinal inflammation in ST313 infection has been inconsistently observed in the murine and rhesus macaque model of *Salmonella* infection (63). These discrepancies may be secondary to use of different comparator strains, and findings will need to be replicated using a range of ST313 and non-ST313 isolates.

Combining insights from comparative genomics and phenotypic characterization of ST313 strains is, however, yielding mechanistic insights into the increased ability of ST313 isolates to cause invasive infection. Genomic and transcriptomic comparisons of ST313 and ST19 strains led to the identification of a *pgtE* promoter single-nucleotide polymorphism, associated with increased *pgtE* expression in ST313 isolates (64). PgtE is an outer membrane protease which facilitates degradation of host antimicrobial proteins deposited on the bacterial surface, including complement proteins. Increased PgtE protein levels result in increased resistance to complement deposition and thus serum killing of ST313 isolates: a phenotype that mediates hyperinvasiveness in the chicken model of invasive salmonellosis (64). *SseI*, which encodes the SPI-2 effector SseI, is pseudogenized in ST313 isolates. Loss of *SseI* underlies increased systemic dissemination of ST313 bacteria, as compared with ST19 isolates, from the gut (65). *SseI* in ST19 bacteria inhibits migration of *Salmonella*-infected dendritic cells from the gut to the mesenteric lymph nodes. The increased invasiveness of African ST313 isolates is thus a complex phenotype, with data suggesting that ST313 lineages have enhanced capacity to disseminate from the gut and survive extracellularly (Fig. 4).

Distinct *S. Enteritidis* Lineages Causing iNTS Disease

Whole-genome sequencing and phylogenetic analysis of African MDR *S. Enteritidis* strains have defined two

genetically distinct *S. Enteritidis* lineages causing invasive infection (66). Similar to the scenario with ST313, the *S. Enteritidis* virulence plasmid pSENV present in diverse *S. Enteritidis* strains is associated with antimicrobial resistance among African lineages. In addition, African *S. Enteritidis* isolates have a distinct pattern of genome degradation.

There are limited data describing phenotypic differences between invasive African strains of *S. Enteritidis* and strains causing enterocolitis in settings outside Africa. Invasive African isolates appear to exhibit a distinct metabolic profile, compared with isolates causing enterocolitis (66). Interestingly, African *S. Enteritidis* isolates appear to display reduced invasive potential compared with noninvasive strains in an avian infection model of salmonellosis. Defining the phenotypic characteristics determining invasive potential in *S. Enteritidis* isolates will require replication and validation of these findings in a range of invasive and noninvasive *S. Enteritidis* isolates.

Transmission of iNTS Disease in Africa

There are limited data describing the sources and routes of transmission of invasive NTS isolates in Africa. As mentioned, ST313 Typhimurium isolates exhibit marked genomic degradation (53), as do African *S. Enteritidis* isolates causing invasive disease (66). Genomic degradation has been observed to be associated with host adaption in host-restricted *Salmonella* serovars (e.g., *S. Typhi*, *S. Paratyphi A* [67] and *S. Gallinarum* [68]), and in other human-restricted pathogens that evolved from an ancestral pathogen with greater host promiscuity (e.g., *Mycobacterium leprae* [69] and *Yersinia pestis* [70]). Moreover, 11 of the 77 observed pseudogenes in the ST313 isolate D23580 have also been lost by pseudogene formation in *S. Typhi*. This observation has led to the hypothesis that ST313 isolates may be restricted, or be in the process of becoming restricted, to humans.

The suggestion that invasive *Salmonella* disease-causing isolates may, in large part, be restricted to humans is supported by studies comparing the epidemiology of NTS isolates in human and animal populations in African settings. In those studies, isolated *Salmonella* strains in humans and animals were distinct (71–73). This is in contrast to the genetic epidemiology of NTS isolates causing invasive disease in Malawian children and adults, which has demonstrated that, despite divergent risk factors for NTS disease in the adult and pediatric popula-

Figure 4 Proposed effects of ST313 phenotypes on the pathogenesis of an invasive NTS infection. Numbered steps in the progression of disseminated infection are as follows: 1, epithelial invasion of luminal bacteria; 2, infection of submucosal tissue phagocytes; 3, proinflammatory cytokine-mediated recruitment of neutrophils to the infected gut; 4, migration of infected phagocytes to the mesenteric lymph nodes; 5, systemic dissemination takes place with intracellular and extracellular bacteremia; 6, establishment of new foci of infection systemically, in particular, in the reticuloendothelial system; 7, periodic recirculation, establishing new infectious foci. Text boxes highlight phenotypes thought to be associated with the ST313 pathovar that may enhance invasiveness. Figure reproduced from reference 77: Gilchrist JJ, MacLennan CA, Hill AVS. 2015. *Nat Rev Immunol* 15:452–463, with permission.

tion, they appear to share a common infectious source (54).

The limited phenotypic characterization undertaken to date indicates that ST313 strains lack catalase activity (63) and have reduced ability to form biofilms (63, 74). Both characteristics suggest that ST313 may have reduced capacity for environmental persistence, as compared with gastroenteritis-causing ST19 strains. This phenotype may be consistent with increased anthro-

notic transmission. While epidemiological, genetic, and phenotypic data do appear to suggest increased host restriction, ST313 isolates retain the ability to cause systemic infection in birds and mammals other than humans (60, 61).

HIV-infected African adults can carry invasive NTS isolates for many months, resulting in multiple recurrent infections with the same organism (19, 75). This observation, considered together with the emergence of ST313

lineages alongside the HIV pandemic in Africa, supports the hypothesis that the introduction of a permissive host environment resulted in the emergence of an increasingly host-adapted organism causing epidemics of invasive disease in a susceptible population. This hypothesis has parallels to the recently described in-host evolution of an NTS isolate from an individual with a primary immune defect (IL-12R β 1 deficiency) associated with extreme susceptibility to iNTS disease (76).

GENETIC AND IMMUNOLOGICAL CORRELATES OF INTS DISEASE SUSCEPTIBILITY IN HUMANS

Primary Immunodeficiencies Predisposing to iNTS Disease

As well as causing invasive disease in Africans, NTS is an important cause of invasive disease among individuals with congenital and acquired primary immunodeficiency in high-income settings (77). While this burden of disease is modest in comparison with that in Africa, the study of these infections has helped define key features of anti-*Salmonella* immunity in humans. Studies defining immunological determinants of invasive *Salmonella* infection in the mouse model have been extremely informative, defining nonredundant roles in anti-*Salmonella* immunity for innate anti-*Salmonella* pathogen-associated molecular patterns (PAMP) recognition (78, 79); intracellular antibacterial effector mechanisms (80–82) (e.g., oxidative burst and metabolite restriction); antibody and complement as mediators of extracellular bacterial killing (83); and T-cell immunity as a facilitator of both intracellular bacterial killing (84) and mucosal immunity (85).

The complementary nature of insights from human studies, epidemiological data defining acquired risk factors, and studies in the mouse model is striking. Together, such studies have produced a highly detailed model of NTS immunobiology. The relative frequencies of NTS infections within these immunodeficiency states are extremely informative. Individuals with Mendelian susceptibility to mycobacterial disease (MSMD) and sickle cell disease are highly enriched for NTS as a causative agent of disease, whereas individuals with TLR pathway defects are susceptible to invasive *Salmonella* infection as part of a much broader immunodeficiency.

Mendelian Susceptibility to Mycobacterial Disease

MSMD is a genetically heterogeneous group of primary, monogenic immunodeficiencies, characterized by

extreme susceptibility to poorly pathogenic mycobacterial and NTS infections. To date, defects causing MSMD have been identified in ten genes, eight of which are autosomal (*IFNGR1*, *IFNGR2*, *STAT1*, *IRF8*, *ISG15*, *IL12B*, *IL12RB1*, and *TYK2*) and two X-linked (*CYBB* and *IKBKG*). The immunological roles of these genes are highly related, all encoding products that function to mediate either IL-12-dependent interferon gamma (IFN γ) production or IFN γ -stimulated effector functions (Fig. 5). iNTS disease has been described in patients with MSMD caused by mutations in *IFNGR1* (86, 87), *IL12B* (88), *IL12RB1* (89), *STAT1* (90), *TYK2* (91), and *IKBKG* (92).

These studies have established the importance of IFN γ -mediated immunity in host control of NTS infections. The distribution of NTS infections observed in these patients also highlights the importance of T_H17-mediated immunity in NTS host defense. Individuals with MSMD caused by mutations in the IL-12p40 subunit or the IL-12R β 1 receptor chain are at far greater risk of NTS disease than are individuals with mutations in genes directly affecting IFN γ signaling (93). This may be secondary to the IL-12p40 and IL-12R β 1 cytokine and receptor subunits contributing to IL-23 signaling and thus T_H17 immunity (Fig. 5).

Primary Immunodeficiencies Affecting Toll-Like Receptor Signaling

Toll-like receptors (TLRs) are membrane-expressed, innate pattern recognition receptors that function to detect *Salmonella* via ligation of a range of PAMPs. In keeping with findings from mice deficient in TLR-signaling molecules (78), iNTS disease has been described in patients with immunodeficiencies caused by mutations in TLR-signaling molecules. Loss-of-function mutations in *IKBKG* (encoding nuclear factor- κ B essential modulator, NEMO) cause anhydrotic ectodermal dysplasia with immunodeficiency (EDA-ID). The immunodeficiency associated with EDA-ID is characterized by susceptibility to invasive bacterial infection, including NTS in 4% of cases (94). Patients with immunodeficiencies secondary to MYD88 deficiency are susceptible to pyogenic bacterial infections, with NTS infections being reported in 7% of cases (95).

Chronic Granulomatous Disease

Individuals with chronic granulomatous disease (CGD), a primary immunodeficiency characterized by defective oxidative burst (96), are at increased risk of iNTS disease. Patients with CGD are susceptible to invasive infections,

Figure 5 Cell-mediated immunity to intracellular nontyphoidal *Salmonella* infections. Following uptake of nontyphoidal *Salmonella* (NTS) by a host phagocyte, IL-12, and IL-23 (heterodimeric cytokines with one shared and one distinct subunit) are released. In the case of IL-12, signaling occurs via the IL-12 receptor complex on natural killer (NK) cells and CD4⁺ T cells, resulting in T_H1 polarization and IFN γ release. IFN γ signaling at the IFN γ receptor complex on infected phagocytes results in STAT1 homodimerization and nuclear translocation, leading to phagocyte activation and control of the intracellular NTS infection. In the case of IL-23 signaling, signaling via the IL-23 receptor complex on CD4⁺ T cells results in T_H17 polarization, facilitating gut mucosal immunity. Genes for which there is host genetic evidence for acting as a determinant of NTS susceptibility are colored according to whether that evidence is derived from the mouse model, human studies, or both. Reproduced from reference 77, *Nature Reviews Immunology*, 15:452–463, by Nature Publishing Group.

commonly complicated by abscess formation, with catalase-positive organisms (*Staphylococcus* and *Aspergillus* species being most common) (97). However, NTS is the most common cause of bacteremia in patients with CGD (97).

Sickle Cell Disease

NTS is a common cause of invasive infection, especially osteomyelitis, in the context of sickle cell disease (98). Sickle cell disease is also a highly significant risk factor for iNTS disease in African children (99). The immune dysfunction associated with sickle cell disease is multifaceted, involving functional asplenia (100), defective opsonophagocytosis and serum bactericidal activity secondary

to alternative pathway complement deficiency (101), and impaired granulocyte oxidative burst secondary to hemolysis (102). Impaired complement-mediated lysis and neutrophil oxidative burst are likely to be responsible for a significant portion of the NTS susceptibility seen in sickle cell disease, both being predicted to compromise the host's ability to clear extracellular NTS.

Immunodeficiencies Characterized by T Cell, Antibody, and Complement Deficiency

In contrast to the mouse model and the extreme NTS susceptibility seen in HIV-infected patients, T-cell immunodeficiency states are not typically characterized by iNTS

disease. There are no reports of NTS infection in severe-combined immunodeficiency or complete DiGeorge syndrome, although patients with these conditions present in early infancy with invasive infections requiring early hematopoietic stem cell transplant, and so their window for *Salmonella* exposure is restricted. Nevertheless, milder T-cell immunodeficiency states are also not commonly associated with iNTS disease, with NTS reported in only 3 of 259 cases of idiopathic CD4⁺ T-cell lymphopenia (103). NTS infections may be more common in MHC class II deficiency, with NTS infection reported in 7 of 30 cases (although it is unclear whether these were invasive disease) (104).

Despite the clear role of antibody acquisition in protecting African children against iNTS disease (discussed later), NTS infections are unusual complications of antibody deficiency states. NTS infection is described rarely in common variable immunodeficiency (105), X-linked agammaglobulinemia (106), and X-linked hyper-IgM syndrome (107). When NTS does cause disease, it normally manifests as chronic or recurrent diarrhea without systemic disease.

While defective complement-mediated lysis has been identified as a contributory factor underlying several well-established risk factors for iNTS disease (e.g., sickle cell disease, malaria, HIV infection), isolated complement deficiency states are again rarely associated with iNTS disease. In a large series of patients with complement deficiency, iNTS disease was described in only 2 of 242 patients (108), suggesting that, while complement dysfunction can contribute to NTS susceptibility, in isolation it appears not to represent a dominant determinant of disease.

Acquired Impairment of Anti-NTS Immunity

Effects of HIV coinfection on anti-NTS immunity

Loss of CD4⁺ T cells is the key laboratory indicator of HIV infection progression and increased immunodeficiency, with increased risk of opportunistic infection. In African adults with NTS bacteremia, HIV infection is the dominant risk factor for disease, with the majority of these individuals demonstrating significant immunosuppression (median CD4⁺ T-cell count at presentation: 99 cells/ μ l, range 6 to 313) (19). While HIV coinfection in African children with NTS bacteremia is less frequent than in adults, it remains an important risk factor, with a median study prevalence of 20% (Table 3).

The immune defects in HIV infection contribute to NTS susceptibility in several ways. First, HIV-induced depletion of the CD4⁺ T cell compartment leads to the loss of T_H1 cells (109), and loss of *Salmonella*-specific IFN γ responses. Diminished IFN γ production capacity leads to inadequate induction of antibacterial effector mechanisms in *Salmonella*-infected phagocytes, resulting in failure to control intracellular infections. This has two consequences: first, a greatly increased risk of developing an episode of iNTS disease; second, a tendency toward recurrent infections, with recrudescence hypothesized to occur from intracellular sanctuary sites (75). In addition, gut mucosal T_H17 cells are preferentially depleted in early HIV infection (110). In the primate model of *Salmonella* infection, simian immunodeficiency virus (SIV)-infected macaques demonstrated attenuated gut mucosal T_H17 responses (85). This impairs intestinal recruitment of neutrophils, facilitating bacterial dissemination and establishment of systemic disease. Attenuated T_H17 responses, with diminished inflammatory cell recruitment to the infected gut, may explain why diarrhea is uncommon among Africans with iNTS disease (Table 2).

In addition to impaired cell-mediated immunity, antibody dysfunction in the context of HIV infection is well described. HIV-infected African adults generate high titers of anti-LPS IgG, which is able to fix complement, but is not bactericidal when present in high concentrations, with assembled membrane-attack complexes (MACs) unable to insert into the bacterial outer membrane (111).

Effects of malaria on anti-NTS immunity

Malaria impairs host ability to control intracellular and extracellular *Salmonella*. Macrophage ingestion of malaria pigment impairs oxidative burst capacity (112), which impairs intracellular control of coexisting NTS infection.

Complement consumption has been documented in malaria (113), which impairs bactericidal killing of extracellular NTS, and may interfere with the induction of neutrophil oxidative burst (114). In addition to its effects on phagocyte function, malaria results in impaired recruitment of phagocytes to mucosal and systemic sites of NTS infection, via the induction of the anti-inflammatory cytokine IL-10 (115, 116). Malaria coinfection also results in an increased intramacrophage bacterial burden, suggesting that malaria-dependent IL-10 induction may act to facilitate intracellular persistence of NTS (115). It has been suggested that malaria-derived IL-10 limits malaria-

induced immunopathology (117), and a consequence of this may be to increase susceptibility to iNTS disease.

Hemolysis regardless of its etiology (but including secondary to malaria infection), via induction of heme oxygenase-1 drives the egress of an immature population of oxidative burst-deficient neutrophils from the bone marrow, impairing neutrophil-mediated control of extracellular NTS (102, 118). This association with hemolysis, rather than malaria per se, may in part explain the increased association of NTS with severe malarial anemia (31), and the greater association between NTS and recent malaria than with concurrent malaria (30).

These data define both humoral and cell-mediated defects during malaria infection and convalescence compromising anti-NTS immunity. In keeping with this, Malawian children with febrile malaria have recently been shown to exhibit decreased anti-NTS bactericidal activity in serum, with an associated reduction in bacterial complement deposition, as compared with children with nonfebrile malaria (119). In addition, the same study demonstrated decreased anti-NTS oxidative burst, with a consequent reduction in whole-blood anti-NTS killing, in children with febrile malaria.

Effects of malnutrition on anti-NTS immunity

Undernutrition has pleiotropic effects on the immune system that will impair the ability of the host to control *Salmonella*, both within the gut and during systemic disease, for both intracellular and extracellular infection. Epithelial barrier function is impaired in malnourished children, facilitating translocation of enteric bacteria into blood and systemic bacterial dissemination (120). Children with malnutrition have reduced levels of complement components (particularly C3) (121), and neutrophils from undernourished children have diminished microbicidal capacity (122), both of which will impair clearance of extracellular NTS.

Intracellular control of NTS infection is also likely to be deficient in malnourished children. T_H1 cytokines are reduced (in favor of a T_H2 response), which will impair the ability of *Salmonella*-infected macrophages to clear intracellular bacteria (121). The reduced circulating B-cell numbers seen in malnourished children may also impair the host's ability to resolve NTS infection (123). Because reduced B-cell numbers appear not to be accompanied by either reduced antibody levels or altered vaccine responses (124), any effect of reduced B-cell numbers appears likely

independent of antibody. Data derived from the mouse model of invasive *Salmonella* infection suggest an antibody-independent role for B cells in anti-*Salmonella* immunity, which is dependent on T_H1 responses (125). Reduced B-cell numbers in the context of malnutrition may thus impair anti-*Salmonella* immunity through a reduction in T_H1 responses to *Salmonella* antigens.

Adult-onset, acquired immunodeficiency with invasive NTS infection

A group of patients with an adult-onset, acquired immunodeficiency, in the absence of HIV infection, has recently been described, presenting with iNTS disease and poorly pathogenic mycobacterial infection (126). The immune defect is due to the presence of autoantibodies against IFN γ . While anticytokine antibodies are unlikely to represent a common cause of iNTS disease in African children, the observation is informative, because it underscores the importance of IFN γ -mediated immunity in host control of NTS.

A further patient group, with considerable potential to provide insights into immunity to invasive infection, comprise individuals with autoimmune disease receiving targeted immunosuppressive agents. Tumor necrosis factor (TNF)-deficient mice have increased susceptibility to invasive *Salmonella* infection (127), and patients treated with anti-TNF agents would be predicted to have increased risk of iNTS disease. In a Spanish cohort of anti-TNF-treated individuals with rheumatoid arthritis, NTS infections were not found to be significantly more frequent than in patients not treated with anti-TNF agents. Nevertheless, the rates of invasive disease among anti-TNF-treated patients were substantial, with 7 of 17 developing bacteremia (128).

Genetic and Immunological Correlates of Anti-*Salmonella* Immunity in African Children

Many African children with iNTS disease lack defined, acquired risk factors for disease susceptibility. This has motivated efforts to define immunological correlates of iNTS disease susceptibility among populations of African children. Acquisition of anti-*Salmonella*, complement-fixing, bactericidal antibody is associated with the decline in NTS susceptibility as Malawian children approach the age of 2 years (129), suggesting an important role for antibodies. These anti-*Salmonella* antibodies induce bactericidal activity directed against extracellular bacteria, but also support anti-*Salmonella* cell-mediated immu-

nity, with *Salmonella*-specific antibody being required for optimal phagocytosis and oxidative burst by leukocytes in African children (114). The acquisition of bactericidal antibody is associated with the acquisition of antibody directed against LPS (130). Interestingly, *Salmonella*-specific CD4⁺ T-cell responses are detectable in early infancy in African children, but appear to be insufficient to confer anti-*Salmonella* immunity prior to development of protective antibody (130).

More recently, a genome-wide association study (GWAS) of NTS bacteremia in African children identified genetic variation in the *STAT4* gene as being associated with iNTS disease (131). *STAT4* is a member of the STAT family of transcription factors (132). In NK cells and CD4⁺ T cells, *STAT4* phosphorylation in response to IL-12 signaling results in T_H1 differentiation and IFN γ production (Fig. 5). The NTS-associated genetic variant is functional, in that it is associated with *STAT4* RNA expression levels in stimulated leukocytes, and with IFN γ protein production in both stimulated natural killer cells and Malawian children with acute iNTS disease. These data establish IFN γ production capacity as a key determinant of susceptibility to iNTS disease in African children, and highlight the overlap between the determinants of *Salmonella* susceptibility in individuals with rare primary immunodeficiencies (namely MSMD) and susceptibility at the population level.

In addition, an enrichment analysis of NTS bacteremia GWAS data in Kenyan children revealed a significant association between genetic variation in genes encoding enzymes of the methionine salvage pathway and disease (133). In the same study, the substrate of the methionine salvage pathway, methylthioadenosine (MTA), was shown to be associated with pyroptosis and predicted survival in adult patients with sepsis. This suggests that the methionine salvage pathway and its metabolites act as a key regulator of the inflammatory response in individuals with iNTS disease, and with sepsis more broadly.

While there has been significant progress in defining the genetic and immunological determinants of iNTS disease susceptibility in African children, the biological correlates of iNTS-associated mortality are less well described. Among Malawian children with iNTS disease, a cytokine signature at presentation suggestive of increased neutrophil migration and activation is predictive of inpatient mortality (134). Neutrophil-associated cytokines are well established as predictors of sepsis-related mortality in

other settings, and suggest a degree of overlap between the immunological predictors of mortality secondary to iNTS disease in children and mortality in undifferentiated sepsis.

CONCLUSIONS

NTS is an important cause of invasive infection in HIV-infected African adults and African children. There is no anti-NTS vaccine available for use in humans (135), and there are currently no vaccine candidates in human clinical trials. The considerable burden of NTS-associated morbidity and mortality, the lack of cheap and timely diagnostic tests, and expanding MDR in African NTS isolates make the development of an anti-NTS vaccine for use in African populations a research priority.

Despite the limited availability of robust epidemiological data describing the incidence of invasive bacterial disease in African children, in African settings where data are available, NTS consistently causes substantial morbidity and mortality. The importance of NTS as a cause of invasive bacterial infection, in African children in particular, seems likely to increase as access to available antibacterial vaccines (in particular, pneumococcal and *Haemophilus influenzae* type b vaccines) improves in Africa, and anti-*Salmonella* vaccines remain only available for typhoid fever (135).

Studies of the clinical features of African children and adults with iNTS disease consistently describe a clinical presentation characterized by fever with no pathognomonic signs or symptoms. Diagnosis is limited to blood and/or CSF culture, which, in the rare African setting in which bacterial culture is available, delays diagnosis. The lack of diagnostic features or tests available at presentation, combined with high rates of MDR among African NTS isolates, all contribute to the high rates of NTS-associated mortality borne by African children and adults.

Epidemiological data have identified both heritable and acquired risk factors for disease in African populations including sickle cell disease, HIV infection, current or recent malaria infection, and malnutrition. In addition, NTS serovars causing invasive disease in Africa are distinct, with a dominant sequence type of *S. Typhimurium* (ST313), responsible for much of the epidemic of invasive disease, rarely encountered outside Africa. A distinct pattern of genomic degradation seen in these isolates suggests that they may be in the process of becoming

more host restricted, although they currently retain the ability to cause invasive disease in species other than humans. It remains unclear whether there are phenotypic correlates of the ST313 pathovar that contribute to its invasive potential in African populations.

Studies of human primary immunodeficiencies have established the key role of IFN γ -mediated immunity, in particular, in the host control of *Salmonella* infection. Population-based host genetic studies have expanded these findings to unselected populations of African children, underlining the key role of cell-mediated immunity in iNTS disease. Importantly, however, immunoepidemiological studies have established a role for antibody in the acquisition of protective anti-NTS immunity in African children. The apparent requirement for both effective antibody and cell-mediated immunity to produce robust anti-NTS immunity in African populations has important implications for the design and evaluation of vaccines against NTS in African populations. This issue is further complicated by the observation that, in HIV-infected African adults, high levels of anti-NTS antibody are paradoxically associated with inhibition of serum bactericidal activity. Further defining the biological determinants of iNTS disease in African populations will facilitate the delivery of a much-needed vaccine for NTS in Africa.

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