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Invasive Non-typhoidal Salmonella Disease – epidemiology, pathogenesis and diagnosis

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Abstract

Purpose of review—To highlight and discuss important publications over the past 12 months providing new insights on invasive non-typhoidal Salmonella disease (iNTS).

Recent findings—There have been informative new estimates of the burden of iNTS in Asia and in high-resource low-incidence settings. Important information has emerged in the last year about the relationships between HIV, malaria, iNTS and typhoid fever in adults and children in Africa. HIV causes susceptibility to iNTS disease, but has been shown to be protective against typhoid fever. Clinical guidelines for presumptive diagnosis frequently fail to identify iNTS disease in Africa, and there remains a need for improved diagnostic tools. Experimental studies in humans have helped us to understand the intracellular pathogenesis of iNTS and to direct the search for appropriate protein vaccine targets.

Summary—The most important remaining gap in our knowledge is probably an understanding of how NTS are transmitted, and the nature of the relationship between diarrhoeal disease, carriage and invasive disease in Africa, so that diagnostic and prevention tools can be appropriately directed.

Keywords

Salmonella; non-typhoid Salmonella; Salmonella Typhimurium; Salmonella Typhi; typhoid; Africa; malaria; HIV; blood stream infection

Introduction

Salmonella enterica causing human disease are divided into human-restricted typhoidal serovars (Typhi and Paratyphi) causing typhoid fever, and non-typhoidal Salmonella (NTS) serovars which have a broader host-range and are frequently zoonotic. NTS have emerged as an important cause of invasive bloodstream infection in sub-Saharan Africa, among young children with malaria and malnutrition, and among adults with HIV. Both groups present with a non-specific febrile illness (1-3), making presumptive diagnosis and treatment a challenge for the clinician in low-resource settings. Case fatality from iNTS in African adults and children is 22-25% (4).

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There are hundreds of NTS serovars that may cause invasive NTS (iNTS) human disease, with varying invasive virulence in humans (5). Although the NTS serovars Typhimurium and Enteritidis are not notably invasive in industrialised settings, they are the commonest cause of invasive NTS disease in sub-Saharan Africa. A novel pathovar of *S. Typhimurium*, ST313, has recently been reported. ST313, which is uniquely associated with iNTS in Africa, has become host-adapted to cause invasive disease in humans (6). Transmission of iNTS in Africa may be primarily human to human, rather than zoonotic (7), but little is known about the relationship of invasive disease to diarrhoeal Salmonella disease in Africa.

The immunology of Salmonella infections has been recently expertly reviewed (8), and a summary of the 3rd American Society of Microbiology Salmonella conference provides an overview of recent basic scientific research into salmonella biology and pathogenesis (9)

Global epidemiology and burden of iNTS disease

iNTS disease has an estimated annual incidence of 175-388/100,000 among young children (<5y) in Africa (10-12), and 1800-9000/100,000 per pyo among non-ART treated HIV-prevalent cohorts in Africa (13-15). Previous estimates of the burden of invasive Salmonella disease in Asia have focused only on typhoid fever (16;17), but a population-based fever surveillance study, conducted as part of a typhoid vaccine trial (17), has also reported only 6 cases of iNTS among 20,537 febrile events (18). The highest reported incidence, from Karachi, was 7.2 /100,000 among subjects aged 2-15 years. The Kolkata and Jakarta sites, which surveyed all ages, reported incidences of 1.8 and 1.0 / 100,000 respectively. Two sites did not survey children under 5y. The overall data, however, suggest that iNTS is a far less significant problem than typhoid fever in Asia. The reasons for this disparity between continents may include the high prevalence of falciparum malaria in Africa.

iNTS is also a relatively rare event in industrialised populations, and the International Bacteraemia Surveillance Collaborative, using national-level surveillance reporting systems, described the first population-based crude and age-and-gender standardised incidences of invasive Salmonella infections in Finland, Australia, Denmark and Canada from 2000-2007. The study reported an overall crude annual incidence of 1.02/100,000 population (19). The data, describing 622 incident events during 60 million combined years of surveillance, showed variation with region, and a gradual overall increase in iNTS over the period of study. A seasonal pattern was observed, with increased rates of iNTS in the autumn. 79% of isolates were non-typhoidal with *S. Enteritidis*, Heidelberg and Typhimurium being the commonest serovars. The strongest risk factors for iNTS were male gender and older age.

Although it has been shown that the initiation of ART in HIV-infected adults leads to a reduction in recurrent invasive NTS disease (20), there have previously been no data at population level to demonstrate the impact of antiretroviral treatment on the incidence of NTS disease. Larsen et al used the Danish national civil and health-care registration systems to demonstrate a markedly higher incidence of both NTS and Campylobacter infections among HIV-positive patients in the pre-HAART era, and a dramatic fall in the incidence of both infections following the introduction of HAART. Over the same period, incidence of these infections changed very little in the general Danish population. 26% of NTS infections in HIV-infected adults were invasive, but the proportion of invasive compared to diarrhoeal NTS events in the general population was not reported. The authors also reported that NTS and Campylobacter events occurred at higher CD4 count among HIV-infected patients in the post-HAART compared to the pre-HAART period, and that their incidence in HIV-infected patients remained slightly higher than the general population even in the post-HAART period, and suggested that iNTS remains a significant clinical problem among HIV-infected adults on HAART. There may, however, be significant ascertainment bias, with HIV-

positive patients being more likely to be microbiologically investigated, so these findings are less robust and need to be validated in other populations.

Invasive NTS among children in Africa

In African children, there is a well-established relationship of iNTS with malaria, including specific associations with severe malarial anaemia (3;21), recent malaria (22) and acute severe malaria (23). Two publications from Muheza, Tanzania further explore the relationship of iNTS with malaria, in an area of high malaria transmission. Nadjm et al (24) assessed the performance of the current WHO guidelines for first-referral level care, for detecting invasive bacterial disease and initiating appropriate presumptive antimicrobial therapy. Nearly 1/3 of children with invasive bacterial disease were not presumptively diagnosed using the guidelines. Over 50% of invasive bacterial events were caused by NTS, and mortality among children with invasive bacterial disease was significantly higher than among those without. The rate of detection of invasive bacterial disease could be significantly improved by the use of simple criteria such as “severe anaemia” and “prostration”. Graham and English have previously highlighted the similar difficulty caused by a clinical overlap of the features of iNTS with algorithms to identify lower respiratory tract infections in African children (25).

At the same site in Tanzania, but over a different time period, Mtove et al described the results of fever surveillance, enrolling 1502 children over 12 months (26). 10% of children had a pathogen identified in blood, of which 29% were NTS and 12% *S. Typhi*. The relatively high incidence of *S. Typhi*, not seen at most other sub-Saharan African sites, permitted the authors to comment on features that distinguished these two forms of invasive *Salmonella* disease in children. Children with typhoid fever were older and had a longer duration of fever, while children with iNTS were more likely to have had recent treatment for malaria, and to have anaemia, jaundice or hypoglycaemia, all of which were interpreted as being complications of malaria.

Because of the close association between malaria and iNTS in African children, there has been speculation that a reduction of malaria transmission might result in reduced rates of iNTS among children. Mackenzie et al reported a temporal association between historical reductions in malarial admissions and percentage of positive outpatient malaria slides, and reductions in estimated incidence of iNTS among children at 2 sites in the Gambia. Estimates of the incidence of invasive pneumococcal bacteraemia did not change over the same period (27). These observations were retrospective and largely based on point-estimates or short periods of data collection for disparate purposes, and hence the data are susceptible to biases and confounding factors. Prospectively or continuously collected data from other African sites will help to support or clarify this finding, which might have important implications for effective disease prevention.

iNTS most commonly occurs in children below the age of 2-3 years, but is relatively uncommon in the first 3-4 months of life (28). Despite this, NTS remain an important cause of neonatal sepsis in Africa. Milledge et al previously described iNTS as the second commonest cause of invasive bacterial disease in neonates in Malawi, carrying a 62% mortality (29). Talbert et al recently described invasive bacterial infections among neonates born in and out of hospital in Kilifi, Kenya (30). iNTS accounted for 5% of blood culture bacterial isolates and 10% of CSF isolates among 4467 children <60 days old born out of hospital, but no iNTS was identified among 2189 children born in hospital, suggesting that the domestic environment is important in transmission.

Invasive NTS and typhoid fever in Africa

The relationship between iNTS and typhoid fever in sub-Saharan Africa has been an area of uncertainty, and several new observations have been published. Feasey et al (31) reported on the demography of invasive typhoidal and NTS disease, which showed notable consistency between Malawi and South Africa. The case frequency of iNTS was overwhelmingly higher than that of *S. Typhi* at both sites, and there was a discrete bimodal age distribution of iNTS, with peaks occurring in children under 2 years and in adults aged 25-40 years. There was a significant gender difference in the age at which adults acquired iNTS, being a median of 5 years younger in women than men at both sites. HIV is the principal risk factor for iNTS, and this gender disparity would be consistent with the known younger acquisition of HIV among women at both sites. *S. Typhi*, by contrast, showed a single broad age peak in children of school age and younger adults, suggesting a different epidemiology. The relative sparing of pre-school children in typhoid fever is typical of a low-incidence area (16).

There are no new population-based data to assess the incidence of invasive *Salmonella* disease in Africa, but Reddy et al (32) have undertaken an important systematic review and meta-analysis of 22 studies reporting community-acquired blood stream infections in febrile adults and children admitted to hospital in Africa, which confirms the importance of *Salmonellae* as a cause of invasive disease across the continent. Among 58,296 patients studied (26% adults), *S. Enterica* were the commonest isolates both overall (29%) and among adults (42%), and the second commonest (21%) among children. Of *S. enterica*, 58% were non-typhoidal serovars (mainly Typhimurium and Enteritidis), and there was a striking disparity between Northern Africa, where *S. Typhi* accounted for 99% of *Salmonellae*, and other regions of Africa, where iNTS predominated. An analysis from 7 countries where HIV was reported showed a strong positive correlation of iNTS with HIV, and a negative correlation of HIV with *S. Typhi*.

A report of invasive bacterial disease among febrile adults in Moshi, Northern Tanzania, is notable because *S. Typhi*, rather than NTS, was the commonest isolate in this HIV-prevalent area, giving an important opportunity to study the interaction of *S. Typhi* with HIV. Previous single-centre studies from Africa and Asia have failed to demonstrate any definite interaction of typhoid fever with HIV (33). Crump et al (34) were able to clearly demonstrate that HIV was protective against *S. Typhi* bloodstream infection in their febrile adult cohort, in contrast to the strong positive association that has repeatedly been observed between HIV and iNTS disease. An accompanying commentary (35) postulated that this apparent negative association could be explained by a lack of cell-mediated immune response to *S. Typhi* in HIV-infected individuals, such that although infected, they do not manifest fever or other inflammatory features, and have intracellular, quiescent, sub-clinical disease. Although an attractive hypothesis, if this was the case one might expect that the availability and high uptake of free ART in Africa would result in clinically obvious *S. Typhi*-related disease manifesting during immune reconstitution as an IRIS syndrome, but this has not been reported in African ART cohorts. Other important differences in susceptibility to iNTS compared to *S. Typhi* may arise at gut mucosa in HIV at cellular level (36).

Although most sub-Saharan African sites have consistently reported *S. Typhimurium* and *S. Enteritidis* as the commonest iNTS isolates, a recent report from Ethiopia highlights *S. Concord* as the commonest isolate from diarrhoeal and blood cultures, and as having a particularly high rate of detection from blood (37).

Pathogenesis of iNTS in Africa

The high rate of bacteraemic recurrence of iNTS in HIV-infected adults (1) suggests intracellular persistence and recrudescence, and a study by Gordon et al, using quantitative culture of blood and bone marrow during iNTS events, has provided evidence that iNTS persist and replicate in the bone marrow following an index event, that iNTS infection in HIV has an intracellular component in both blood and bone marrow, and that recrudescence is associated with failure of immunological control of persisting intracellular bacteria (38). This reinforces the importance of treatment with antibiotics with optimal intracellular penetration, potentially favouring fluoroquinolones over 3rd generation cephalosporins. The emergence of regional pathovars which are potentially adapted to cause human invasive disease (6), and the immunological niche provided by HIV are both probably contributors to iNTS adopting this intracellular pathogenesis.

Other risk factors for susceptibility to invasive NTS among adults

Dadwal et al (39) described a retrospective case series of 18 recipients of allogeneic or autologous hematopoietic stem cell transplants, presenting over 15 years. They noted that diarrhoea was absent in 1/3 of cases of iNTS, as is commonly described in immunosuppressed groups. 67% of cases, however, did have diarrhoea, with 28% having diarrhoea as the predominant presenting clinical feature, and the authors highlight the potential diagnostic confusion which could arise in allogeneic HSCT patients in whom both GVHD and NTS could give rise to diarrhoea.

Studies of patients with genetic deficiencies in the γ IFN and IL-12/23 pathways have already demonstrated that these individuals are also susceptible to recurrent iNTS disease, and deficiencies of the IL-12/23 pathways appear to be more important in susceptibility to iNTS than those of γ IFN (40). An emerging and recently described acquired immunodeficiency, caused by autoantibodies against γ IFN, is already known to be associated with opportunistic mycobacterial infections. Tang et al describe iNTS, disseminated penicilliosis and burkholderiosis as possible further consequences of autoantibodies against γ IFN (41). A single patient with recurrent and focal iNTS disease and non-tuberculous mycobacteriosis was found to have antibodies against γ IFN. This patient, however, also had a background of quiescent SLE and was taking steroid treatment. Although they also found that 5/7 patients with a history of iNTS disease, but only 1/100 with SLE had autoantibodies against γ IFN, the potential for these autoantibodies to cause susceptibility to iNTS remains to be clarified.

Rapid diagnosis of iNTS

Current guidelines frequently fail to presumptively identify iNTS disease in African children (24;25), and clinical diagnosis is similarly difficult in HIV-infected adults (1;2), so there is an urgent need for improved diagnostic tools. PCR has recently been exploited for rapid diagnosis of *Salmonellae* from stool samples (42;43), and a multiplex PCR has been described that can reliably identify the commonest typhoidal and non-typhoidal *Salmonellae* causing invasive disease in West Africa (44). Although such methodology may be useful for epidemiological surveillance of isolates, iNTS has been shown to present with only 1 cfu/ml in blood (38), and it seems unlikely that PCR will be sensitive as a rapid diagnostic test without pre-test enrichment culture. Indeed, a multiplex PCR designed in Vietnam for detection of *S. Typhi* and *Paratyphi* had only 39-42% sensitivity for culture-positive blood samples, and a detection limit of only 100-200 organisms/ml in inoculated blood, a figure significantly above the median of 1 live organism / ml found in blood during typhoid fever by Wain et al (45). Similar limitations are likely to pertain in iNTS. A proof of concept study has, however, reported a novel methodology, based on fluorescent detection of the

Salmonella chromosomal locus *oriC*, called “MAMEF” (microwave-accelerated metal-enhanced fluorescence) which could be developed for rapid diagnosis of iNTS at these very low infective loads (46).

Prevention and vaccine development

Previous work from Malawi has already highlighted the potential importance of antibody in both serum killing and intracellular oxidative killing of iNTS in African children (28;47), making an antibody-inducing vaccine an attractive goal for prevention of iNTS in Africa. A study of humoral immunity in HIV-infected Malawian adults, however, made the counter-intuitive finding that impaired serum killing of local strains of *S. Typhimurium*, found in a proportion of Malawian HIV-infected adults, correlated with higher titres of antibodies directed against Salmonella LPS (48). These anti-LPS antibodies, rather than being protective, inhibited killing of bacteria, and were in competition with antibodies directed against Salmonella outer membrane proteins that mediated effective killing of *Salmonellae*. The clinical significance of these anti-LPS antibodies has not been proven in relation to disease susceptibility or clinical outcomes, nor is it clear how and when they may develop during the course of HIV disease. These findings have, however, been important in directing vaccine development effort away from an LPS-based conjugate vaccine, which could potentially cause harm, and towards protein targets that could be protective, such as outer-membrane proteins.

Conclusions

There have been informative new estimates of the burden of iNTS in Asia and in high-resource low-incidence settings. Important descriptions have been made of the relationships between HIV, malaria, iNTS and typhoid fever in adults and children in Africa. Experimental studies in humans have helped to understand the intracellular pathogenesis of iNTS, and to direct the search for appropriate vaccine targets. There remains a need for improved diagnostic tools. The most important gap in our knowledge is probably an understanding of how NTS are transmitted, and the nature of the relationship between diarrhoeal disease, carriage and invasive NTS disease in Africa, so that diagnostic and prevention tools can be appropriately directed.

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Key Points

- Both iNTS and typhoid fever are important causes of invasive bacterial disease in Africa, and they have discrete epidemiologies.
- iNTS has a strong association with malaria in children, and longitudinal changes in both diseases need to be better defined and understood in Africa
- HIV causes susceptibility to iNTS in African adults, but is apparently protective against typhoid fever
- NTS have adopted an intracellular invasive pathogenesis in adults with HIV
- Better diagnostic tools and algorithms for iNTS are needed in Africa
- Vaccines against iNTS in Africa are being developed against Salmonella protein targets, rather than LPS