Review Article

Invasive salmonellosis in Malawi

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Abstract

The incidence of invasive salmonellosis has increased among children and HIV-infected adults in Malawi. This has been associated with the emergence of drug resistance in the non-typhoidal *Salmonella* serovars Enteritidis and Typhimurium. In contrast, *S.* Typhi isolates have remained fully sensitive to commonly used antibiotics and the estimated incidence of typhoid fever, although still present, has fallen slightly among both adults and children. Infection with *S.* Typhi is not closely associated with underlying immunosuppression but it is possible that the non-typhoidal *Salmonellae* have adapted to the person-person human transmission niche in this frequently immunosuppressed population. The huge burden of invasive salmonellosis in Malawi, the high associated mortality, and the recent emergence of drug resistance emphasise the need for a better understanding of the epidemiology and the need for vaccine development.

Key Words: salmonellosis, Malawi, HIV, immunosuppression.

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Introduction

Clinical syndromes in humans caused by infection with *Salmonella enterica* are divided into typhoid fever, caused by *S. enterica* serovars Typhi and Paratyphi, and a range of clinical syndromes, including diarrhoeal disease, caused by the non-typhoid salmonellae (NTS) of which there are around 2,500 serovars. Typhoid fever is a human-restricted and highly adapted invasive systemic disease of adults and children that shows little association with immunosuppression [1]. In contrast, NTS have a broad vertebrate host range and epidemiology that often involves food animals, at least in industrialised countries where it usually presents as gastroenteritis. Severe, invasive disease due to NTS is usually associated with the immunocompromised state common in HIV-infected adults [1]. Invasive NTS disease is also common in young African children with co-morbidities such as severe anaemia, malnutrition and HIV infection [2].

Malawi is one of the most densely populated countries in Africa [3,4]. In the 10 years from 1994 to 2004, the infant mortality rate was 112 / 1,000 live births; the under-5 mortality rate was 187 / 1000; and life expectancy from birth was 41 to 45 years [5]. Malawi has been severely affected by the HIV/AIDS pandemic that has spread throughout sub-Saharan Africa. Sentinel surveillance site testing suggests that HIV prevalence increased rapidly from the late 1980s to the early 1990s and stabilised in the mid-1990s, but has not greatly changed to date; HIV seroprevalence among antenatal women in Blantyre was 2% in 1985 and rose to 32.8% by 1996 [6]. The 2004 Demographic and Health Survey found that HIV seroprevalence among 15- to 49-year-olds in Malawi was 12% overall, but was higher in the Southern Region (17.6%) particularly in Blantyre District (22.3%). Seroprevalence in Malawi is higher in urban compared to rural populations and higher with increasing level of education, increasing wealth, and among those with paid employment. Women become infected at a younger age than men, with seroprevalence being 3.7% among 15- to 19-year-old girls compared to 0.4% of boys the same age, and remaining higher among women until age 30 to 34 [5]. Access to free antiretrovirals has been increasingly available in Malawi since 2004.

Monitoring of invasive NTS disease in Malawi

Invasive bacterial disease has been continuously monitored for over a decade at Queen Elizabeth Central Hospital (QECH) in Blantyre, Malawi's largest city,

since the establishment of the Wellcome Trust-funded microbiology laboratory in 1996. NTS have consistently been the most common blood culture isolates observed among adults [7-10] and children [11,12] throughout this period; they were also a common cause of neonatal sepsis [13] and of meningitis in children under 2 years [14,15]. More restricted blood culture studies of febrile patients admitted to the central hospital in Lilongwe, the other major city in Malawi, report a similar pattern of bacteraemia [16,17] and NTS bacteraemia has also been described in children living in rural Malawi [18]. Most cases of invasive NTS disease in adults and children are caused by *S.* Typhimurium and *S.* Enteritidis (approximately 75% and 20% respectively in Blantyre), which is consistent with the pattern seen elsewhere in Africa.

Invasive NTS disease in Blantyre among children

There were 2,517 cases of NTS bacteraemia identified in children admitted to QECH in Blantyre over a 7-year period. This represents 45% of all paediatric blood isolates and compares to 48 (1%) isolates of *S.* Typhi over the same period. NTS bacteraemia is more common during the rainy seasons and most cases occur in children aged between 6 months and 3 years [11,12]. In contrast to other common causes of invasive bacterial disease in children such as pneumococcus, NTS is associated with malaria and anaemia [12]. This association has been described elsewhere in tropical Africa and is partly seasonal [2,19,20]. The clinical association with severe malaria is in the context of severe malarial anaemia [21]. Prospective studies in Blantyre of children with severe malaria and severe anaemia found that approximately 10% of children with severe anaemia or severe malarial anaemia have NTS bacteraemia, compared to 1% in controls without severe anaemia or with cerebral malaria [18,21]. Invasive NTS disease has also been noted to present after a recent history of hospitalisation for severe anaemia [22]. Similar to other common bacterial pathogens in children, NTS bacteraemia is also associated with severe malnutrition and HIV infection [23]. NTS accounts for over 50% of blood isolates in children with bacteraemia admitted with severe malnutrition in Blantyre (SM Graham: unpublished data), a pattern similar to that recently described in Uganda [24]. In a community-based cohort study of HIV-infected children of 2 years and older in Blantyre, the incidence of NTS septicaemia was 15 per 100 person-years of observation [25].

Two prospective studies of the aetiology of acute severe pneumonia in children over 2 months of age presenting to QECH, one published [26] and another not yet published (SM Graham; unpublished data), found that NTS was the second most common isolate, after pneumococcus, from blood culture. Although proven by lung aspiration in one case in Blantyre [22], NTS are unlikely to be a common cause of pneumonia. Rather, there is likely to be common clinical overlap between NTS septicaemia, anaemia and clinical signs that define severe pneumonia such as tachypnoea and chest indrawing [12,19,20].

NTS are also a common cause of meningitis [14,15]. Most cases occur in children younger than 2 years; the case-fatality rate is high; and neurological sequelae are common among survivors. NTS are a common cause of neonatal sepsis or meningitis in Blantyre, second to Group B streptococcus [13]. NTS are also a common cause of septic arthritis, usually presenting in the shoulder joints of infants, for which outcome is good [27].

The clinical presentation of NTS bacteraemia is well described and features observed in Malawi are consistent with those described elsewhere in tropical Africa [2,12,19]. Presenting features are usually of a non-specific febrile illness in a sick, young child. Diagnosis may be delayed if a malaria smear is positive. Cough is a feature in more than half of the cases and is more common than diarrhoea. Tachypnoea is also common, which means that there is often overlap with clinical presentation of pneumonia and anaemia [12,19].

The mortality associated with invasive NTS disease in Malawian children is high. Case-fatality rate is over 50% in children of any age with NTS meningitis and in neonates with septicaemia due to NTS [13,14].The case-fatality rate was 24% overall for children with NTS bacteraemia and risk factors for death included younger age, severe malnutrition and symptomatic HIV infection [12]. It is difficult to determine the impact of NTS bacteraemia alone on mortality when there are often co-morbidities.

Invasive NTS disease in Blantyre among adults

Among febrile adult admissions in Blantyre, there were 2,439 cases of NTS bacteraemia identified over the same 7-year period, representing 49% of all adult blood isolates. Over the same period there were 57 (1%) isolates of *S.* Typhi from adults. Among adults, NTS bacteraemia is overwhelmingly associated with advanced HIV disease; 92-96% of febrile admissions with NTS bacteraemia are HIV infected and 80% have

peripheral blood CD4 < 200 cell/μl. The incidence of bacteraemia in HIV-infected adults in Blantyre prior to the use of cotrimoxazole preventive therapy and ART was estimated from a community-based cohort study to be 16 events per 100 person years of observation; the majority was due to NTS [28]. The median age of adults with NTS bacteraemia was 30 years in women and 34 years in men, in keeping with the known epidemiology of HIV. Despite the association with HIV, a clear seasonal pattern is observed, which exactly parallels that seen in children, with peak incidence during and following the rainy season. Mortality is high, even after treatment with appropriate antibiotics. Mortality was observed as 33-47% in separate Blantyre cohorts [7,29], in keeping with the high mortality observed elsewhere, for example in Bangkok where mortality associated with adult NTS bacteraemia was 36% overall, but 59% among HIV positive adults [30]. Mortality in Blantyre was, however, noted to fall significantly from 29% to 20% over a sustained period of observation [10], suggesting, as has been seen in Kenya, that improved health seeking behaviour, clinician awareness and initiation of appropriate basic management can reduce in-patient mortality to approximately 20% [31].

The clinical features of NTS bacteraemia in HIV-infected adults in Malawi are in some respects reminiscent of typhoid fever [29]. Fever with no apparent focus is frequent and an initial diagnosis of malaria may be entertained. In many cases an absence of diarrhoea or abdominal features is seen; a lack of diarrhoea in NTS bacteraemia is known to be a marker of underlying immune suppression [32,33]. Hepato- or splenomegaly occurs in 40% of cases, and rose spots are sometimes seen [29]. In the context of a high prevalence of HIV in Africa, splenomegaly is a useful marker of NTS bacteraemia among febrile admissions and is more predictive of NTS than of TB or malaria [9]. Patients with advanced HIV and NTS bacteraemia frequently have chest signs and an abnormal chest radiograph. While focal pulmonary and thoracic NTS infection does occur with HIV [34-37], these focal clinical features are more often caused by co-infection with a second pathogen such as TB or *Streptococcus pneumoniae*, and empiric treatment plans should reflect this likelihood [29]. Unlike typhoid fever, however, gall-bladder involvement appears to be very rare and the late complications of upper GI bleed or haemorrhage, which are seen in typhoid, are not described in invasive NTS disease [1]. It is important to note that while typhoid fever mainly involves the small bowel, causing enteritis, NTS disease appears to primarily involve the large bowel. The wide variety of clinical presentations and lack of gastrointestinal features makes empirical diagnosis and treatment of NTS bacteraemia extremely challenging when blood cultures are not available [9].

Another important feature of NTS bacteraemia in HIV is a high rate of recrudescence and relapse of bacteraemia. We have observed this in 43% of cases treated with chloramphenicol, even when all NTS strains were fully susceptible [29], and in approximately 25% treated with ciprofloxacin (MA Gordon, unpublished data). Relapse typically occurs within 6 months and most commonly at 2 to 3 months, with a strain of NTS indistinguishable from the original infection [29]. This may be because the original infecting organisms find an intracellular sanctuary site; a dysregulated cytokine milieu in HIV may permit NTS to persist within macrophages [38]. Secondary antibiotic prophylaxis is recommended until such time as the patient is fully established on antiretroviral treatment, but there are few data to establish the effectiveness of antibiotic regimes.

Antibiotic resistance and epidemic outbreaks

Over the last ten years, we have observed the rapid emergence of high levels (over 90%) of *in vitro* antibiotic resistance to chloramphenicol, ampicillin and cotrimoxazole among NTS serovars in Blantyre. This situation poses a challenge for clinical management, as alternative antibiotics such as ceftriaxone and ciprofloxacin, to which NTS remain susceptible *in vitro*, are not readily available. These are more costly and are not currently recommended as first-line therapy for suspected invasive bacterial disease in adults or children in Malawi. The emergence of MDR was not associated with an increased case-fatality rate in Blantyre [10]. However, this may not be representative of elsewhere in Malawi as alternative antibiotics (ceftriaxone and ciprofloxacin) were available and antibiotic usage at QECH changed in response to the *in vitro* susceptibility data. The frequent problem of co-morbidities and their independent impact on outcome means that randomised trials are needed to compare the impact of different antibiotic regimes on outcome. More widespread use of third-generation cephalosporins and fluoroquinolones will almost inevitably lead to emergence of further MDR as has happened with *S*. Typhi in Asia [2].

A particularly interesting feature of the emergence of multidrug resistance (MDR) has been that, as individual NTS serovars acquired MDR, there was at the same time an epidemic increase in the prevalence of

that serovar. This was observed sequentially for *S.* Enteritidis then *S.* Typhimurium and suggests that acquisition of MDR may also confer advantages in transmission [10]. The molecular mechanisms of antibiotic resistance in these strains are under investigation and may help with better understanding the epidemiology of NTS transmission. Much remains to be understood about the relationship between stool carriage, diarrhoeal disease, and invasive disease in both adults and children in Africa.

It is also interesting to note that while the incidence of invasive NTS has increased among children and HIV-infected adults in Malawi alongside the emergence of MDR, in contrast, *S.* Typhi isolates have remained fully sensitive to commonly used antibiotics. The incidence of enteric fever caused by *S.* Typhi has also fallen slightly among both adults and children [10]. *S.* Typhi is not closely associated with underlying immunosuppression [1] and NTS may have successfully competed with *S.* Typhi for a person-person human transmission niche in this frequently-immunosuppressed population [39].

Finally, vaccine potential has been highlighted by a recent study from Blantyre that describes the importance of humoral immunity in protecting children from invasive NTS disease [40]. The huge burden of invasive NTS disease in Malawi, the high associated case-fatality rate, and the recent emergence of MDR emphasise the need for a better understanding of the epidemiology of NTS and the possibility of vaccine development [2].

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