Published in final edited form as:

Vaccine. 2015 June 19; 33(0 3): C21–C29. doi:10.1016/j.vaccine.2015.03.102.

Antimicrobial resistance and management of invasive *Salmonella* disease

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Abstract

Invasive *Salmonella* infections (typhoidal and non-typhoidal) cause a huge burden of illness estimated at nearly 3.4 million cases and over 600,000 deaths annually especially in resource-limited settings. Invasive non-typhoidal *Salmonella* (iNTS) infections are particularly important in immunosuppressed populations especially in sub-Saharan Africa, causing a mortality of 20–30% in vulnerable children below 5 years of age. In these settings, where routine surveillance for antimicrobial resistance is rare or non-existent, reports of 50–75% multidrug resistance (MDR) in NTS are common, including strains of NTS also resistant to fluoroquinolones and 3rd generation cephalosporins. Typhoid (enteric) fever caused by *Salmonella* Typhi and *Salmonella* Paratyphi A remains a major public health problem in many parts of Asia and Africa. Currently over a third of isolates in many endemic areas are MDR, and diminished susceptibility or resistance to fluoroquinolones, the drugs of choice for MDR cases over the last decade is an increasing problem. The situation is particularly worrying in resource-limited settings where the few remaining effective antimicrobials are either unavailable or altogether too expensive to be afforded by either the general public or by public health services. Although the prudent use of effective antimicrobials, improved hygiene and sanitation and the discovery of new antimicrobial

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agents may offer hope for the management of invasive salmonella infections, it is essential to consider other interventions including the wider use of WHO recommended typhoid vaccines and the acceleration of trials for novel iNTS vaccines. The main objective of this review is to describe existing data on the prevalence and epidemiology of antimicrobial resistant invasive *Salmonella* infections and how this affects the management of these infections, especially in endemic developing countries.

Keywords

invasive *Salmonella*; non-typhoidal salmonella; typhoid; epidemiology; antimicrobial resistant

Introduction

Invasive *Salmonella* infections (typhoid and non-typhoidal) are a leading cause of morbidity, with high rates of mortality, in resource-limited settings especially in sub-Saharan Africa (SSA) and parts of the Indian and Asian sub-continent. Specifically, invasive non-typhoidal *Salmonella* spp (iNTS) are a major cause of bloodstream infections in SSA, especially among children and HIV-infected adults who have low CD4 T-lymphocyte counts with mortality rates of nearly 10–30% [1,2]. Most pediatric cases of iNTS occur between ages 6 months and 3 years, an observation which supports the importance of passive and acquired humoral immunity in prevention of invasive disease [3–5]. Multidrug resistant iNTS disease poses a major challenge to the clinical management of infections in resource-limited settings especially as alternative more effective antibiotics are either unaffordable or simply unavailable for majority of patients. Furthermore, there are no published clinical trials to support treatment decisions in iNTS disease especially in endemic settings in SSA although a number of clinical handbooks give treatment recommendations. *Salmonella enterica* serovar Typhi (*Salmonella* Typhi), remains an important global public health problem, causing 22 million outbreak-associated and sporadic cases of typhoid and approximately 200,000 deaths annually worldwide [6]. It is primarily a rare imported disease in industrialized countries, since improved sanitation and water supply has eradicated endemic disease, but is endemic in South/South East/Central Asia and parts of SSA and still causes large outbreaks [7–10]. The true burden of typhoid fever is largely unknown in SSA because credible measures of disease incidence require a confirmed diagnosis based on blood or bone marrow culture. Laboratory facilities to make such a diagnosis are limited or non-existent in many potentially endemic SSA countries. This review looks at the current situation on epidemiology and burden of illness caused by invasive *Salmonella* infections (including iNTS and Typhoid fever), especially in endemic areas and further explores the problem of AMR in these infections, their clinical impact and management issues.

Invasive non-typhoidal *Salmonella* (iNTS) disease

Global Epidemiology

In industrialized countries, NTS predominantly cause non-invasive enteric diarrhoeal disease. The bacteria are transmitted by either infected animal products or by industrially-

produced food contaminated with infected animal faeces. NTS usually cause a self-limited enterocolitis with diarrhoea in immunocompetent humans, although individuals with immunocompromising conditions are susceptible to invasive bloodstream infection. Bloodstream infections (iNTS) occur in approximately 6% of laboratory confirmed patients with diarrheal enterocolitis, although this may be an underestimate as blood cultures are not always taken. Infants, young children, the elderly, and immunocompromised individuals are at particular risk for bacteremia, and multidrug resistant strains are also more likely to cause invasive disease [5,11,12].

Enteric NTS syndromes were globally estimated to cause 93.8 million illnesses and 155,000 deaths [13]. In further estimates, enteric NTS infections accounted for 4.8 million disability-adjusted life years [14] and 81,300 deaths [15]. In contrast to the picture in industrialized settings, in Africa NTS are associated with invasive disease without gastroenteritis as a prominent feature. The clinical features of iNTS disease are either nonspecific and similar to those of other common diseases such as malaria or may be focal or cause pneumonia or meningitis. This not only presents a diagnostic dilemma to health workers in a resource-limited setting but also makes burden of disease estimates uncertain in the absence of laboratory confirmation. The global burden of disease for iNTS disease has recently been estimated for the first time, and suggests that there is a huge and unrecognized burden of illness and mortality [Ao et al. In Press-EID]. There are an estimated 3.4 million cases globally, and assuming a case fatality of 20%, 681,316 deaths annually. Approximately two-thirds of this burden falls on children, and 55% in Africa. It is, however, important to note that it is still unknown to what extent the NTS strains that cause invasive disease are genomically or phenotypically different from NTS strains that cause enteric disease, so the term “iNTS” should be used with caution with reference to the micro-organisms. This is an important area of current research, as described below.

Salmonella Typhimurium and *Salmonella* Enteritidis are the most widely reported invasive serovars across SSA. Smaller more localized contributions or outbreaks in SSA are reported for *Salmonella* Concorde in Ethiopia [16], *S. Bovismorbificans* [17], *S. Stanleyville* and *S. Dublin* in Mali [18] and *S. Isangi* in South Africa [19]. Case fatality rates for iNTS vary according to the infecting serovar. Some serovars, *S. Newport* for example, are associated with a lower case fatality ratio (0.3%) when compared with *S. Typhimurium* [12,20].

Genomic adaptation of invasive *Salmonella*

Host restriction among *Salmonella* spp appears to be associated with a more specialized lifestyle involving an invasive pathogenesis and the loss of the ability to colonize and infect the gastrointestinal tract of multiple vertebrates. The genomic changes associated with host restriction and invasive disease in *Salmonella* spp are increasingly understood, having been first described in *Salmonella* Typhi strains [21,22]. They are typified by the loss or degradation of genes, including those associated with an enteric lifestyle [23], such as metabolic genes required for anaerobic survival in the inflamed intestinal lumen [23,24].

A novel *S. Typhimurium* multi-locus sequence type, ST313, has been described that accounts for a significant proportion of the invasive disease in sub-Saharan Africa. This sequence type has a unique prophage repertoire, and a degraded genome that shows some

convergence with *S. Typhi*, consistent with increased host specialization or invasiveness [25]. One putative virulence gene, *ST313td*, has been described in *S. Typhimurium* ST313 that is also present in *S. Dublin*, another pathovar which is invasive in humans [26]. Current genomic, transcriptomic and phenotypic investigations of *S. Typhimurium* ST313 and other iNTS pathovars from Africa, such as *S. Enteritidis*, promise further new insights.

Sources and modes of transmission

Epidemiologic studies of iNTS in endemic areas of sub-Saharan Africa are very limited. Although transmission by food contaminated with animal faeces must be considered, a greater role than in industrialized countries has been suggested for waterborne transmission, or transmission between people, independent of a non-human animal reservoir. Sub-genomic studies of iNTS strains from humans and those carried by animals found in the households of children with invasive disease in Africa have not suggested any domestic animal sources for transmission, whereas family members of cases have been found to have more closely related isolates [4]. Although it was hypothesized that the degraded genome of *S. Typhimurium* ST313 might reflect a reduced host range and human-restriction of the ST313 pathovar, recent studies have shown that ST313 strains also display a severe invasive phenotype in chickens with a reduced potential for cecal colonization [27]. In a study of the epidemiology of invasive *S. Typhimurium* strains using whole-genome sequence-based phylogenetic methods to define the population structure of these strains in SSA compared to global *S. Typhimurium* populations it was shown that the vast majority of SSA invasive *S. Typhimurium* ST313 pathovars fell within two closely related, highly clustered phylogenetic lineages that were estimated to have emerged independently ~52 and ~35 years ago in close temporal association with the current HIV pandemic. The emergence of the two distinct clades of *S. Typhimurium* ST313 pathovars across SSA also show important temporal relationships to acquired antimicrobial resistance determinants, particularly to the first-line antimicrobial chloramphenicol [28]. This suggests that transmission among humans may have exerted a significant genomic selection pressure.

Little is known about the spectrum of prevalent enteric NTS strains in Africa. NTS were not a common cause of moderate – severe diarrheal disease in the recent GEMS study in multiple African sites [29]. In contrast, asymptomatic carriage of NTS appears to be relatively common [4,29]. The contribution in Africa of *S. Typhimurium* ST313 or other invasive pathovars of NTS, to diarrhoeal illness or asymptomatic carriage is also still unclear, although there appears to be considerable diversity among NTS strains from enteric samples in Africa [30]. Taken together, these findings may suggest a role for humans as a symptomatic or asymptomatic reservoir for *S. Typhimurium* ST313 infection, similar to the pattern observed for *S. Typhi*. This possibility has yet to be studied using classical epidemiologic methods. It is not known whether stool shedding is relatively transient or whether there is significant long-term carriage of NTS serovars in some individuals and these remain important areas of uncertainty.

Host risk factors for iNTS disease

Multiple conditions have been identified which increase the risk of iNTS disease, in contrast to the situation with typhoid fever where few immunological host susceptibilities have been

identified. Achlorhydria or hypochlorhydria, and acid suppression increase the risk of *Salmonella* infection, and individuals at the extremes of age are also at increased risk of iNTS disease. In the case of older people, this may be because of multiple co-morbidities such as diabetes, renal disease, or iatrogenic immunosuppression.

In SSA, there is a clear bimodal age distribution in contrast to the continuous age distribution through childhood to young-adulthood of typhoid. Children aged from 6–18 months, and adults aged 25–40 years in whom HIV prevalence is also highest, show the highest incidences of iNTS disease in SSA [31]. Among children, there is a relatively low incidence of iNTS disease below the age of 6 months, likely arising from protection from transplacental transfer of protective IgG and from breastfeeding [32]. Neonatal iNTS disease is also described, particularly in community out-born children [33,34].

iNTS disease is also associated with specific forms of immunosuppression. These include chronic granulomatous disease (a defect of oxidative cellular killing [35], and inherited deficiencies of cytokines that are known to be critical for intracellular killing, particularly IL12 and IL23. Children homozygous for sickle cell disease are also extremely susceptible to iNTS infections [36,37], while heterozygosity for the sickle cell gene is protective against invasive Gram negative infections, including iNTS disease, most likely through a protective effect against malaria [38].

There is an overwhelming association of iNTS disease with advanced HIV disease among African adults, with >95% of cases being HIV infected [39]. In cohorts of African children with iNTS disease around 20% are typically HIV-infected [40], and among children, HIV is associated with a 3.2-fold increase in odds among children for presenting with iNTS [3]. Several immune defects have been described that could contribute to the marked susceptibility of adults with advanced HIV to recurrent iNTS disease. These include the loss of IL-17 producing CD4 cells in the gut mucosa permitting rapid invasion [41] and dysregulated excess production of anti-LPS IgG that inhibits serum killing of extracellular *Salmonella*, in a concentration-dependent fashion [42]. Once in the intracellular niche, reduction and dysregulation of pro-inflammatory cytokine responses in HIV-infected individuals [43] allows intracellular survival and persistence, leading to frequent recrudescence of bacteremia, with identical strains of NTS [28,44]. In contrast to the findings in adults with HIV, a lack of protective antibody is implicated in the susceptibility of African children to iNTS disease; antibody is likely to be important both for cellular and cell-free control of NTS infection in children [32,45,46].

Falciparum malaria is strongly associated with iNTS disease among children in Africa. Recent malaria [40], acute severe malaria [47] and severe malarial anaemia, but not cerebral malaria [48], have all been specifically described as risk factors for iNTS disease. Several groups have also described temporal-spatial relationships of malaria and iNTS disease among children [49–51]. However, this association is not specific just for iNTS disease; a strong association between malaria and all bacteremias has been noted in Kenyan children where the bacteremia incidence rate ratio associated with malaria parasitaemia was 6.69. A causative relationship with malaria was inferred by a reduced odds ratio for sickle cell trait (which is protective against malaria) among children with Gram-negative bacteraemia [38].

Severe malnutrition is also associated with iNTS disease in African children [40]. In rural Kenya, NTS bacteremia was associated with severe child malnutrition with an odds ratio of 1.68 [3]. iNTS disease is strongly seasonal among both adults and children, coinciding with the rainy season [5]. It is not clear whether this seasonality reflects food or environmental contamination leading to increased rates of disease transmission, seasonal malaria transmission during the rains, food scarcity and malnutrition that may happen at the onset of heavy rains. It is likely that these factors may be additive and mutually interact.

Management of iNTS disease

There are no clinical trials of treatment to provide an evidence base for management of iNTS, and little published clinical experience. In addition to a case fatality rate of 20% in adults and children a very high rate of recrudescence (20–40%) has been described among adults with advanced HIV in the pre-ART-era, likely attributable to intracellular persistence, suggesting that prolonged treatment or secondary antimicrobial prophylaxis is merited.

The Infection Diseases Society of America (IDSA) guidelines for prevention and treatment of opportunistic infections in HIV for adults and adolescents limits discussion to bloodstream infections in the context of enteric diarrhoeal disease, which is not a universal feature of iNTS disease in immune-compromised hosts. The adult and adolescent guidelines have recently been updated to strengthen the recommendation that treatment is given to all HIV-infected patients with enteric *Salmonella* infections, regardless of the presence of invasive blood stream infection, but there is no specific recommendation for iNTS disease without associated diarrhoea. Recommended treatment is with ciprofloxacin as first-line treatment, and levofloxacin, moxifloxacin, cotrimoxazole or the extended spectrum cephalosporins ceftriaxone or cefotaxime as alternatives. Recommended treatment duration for diarrhoeal disease alone is 7–14 days, for invasive disease 14 days, and for patients with $CD4 < 200$ cells/mm³ or with severe diarrhoea for 2–6 weeks. Since most patients with invasive disease present with $CD4 < 200$ cells/mm³, it would be important for prolonged use of this treatment to be confirmed in a clinical trial, especially since fluoroquinolones form part of the management strategy for MDR tuberculosis, and prolonged exposure on a co-infected patient could promote the development of resistant pathogens. Secondary prophylaxis is suggested for those with recurrence or $CD4 < 200$ cells/mm³.

The guidelines generally promote early initiation of ART, although there is no specific guidance for *Salmonella*, and it is suggested that secondary prophylaxis can be stopped once the patient has a sustained response to ART, with suppressed viral load and $CD4 < 200$ cells/mm³. IDSA paediatric guidance also encourages the early initiation of ART for non-CNS opportunistic infections but there is no specific discussion of iNTS infections in children in the guidelines, beyond the general principle that invasive infections in severely immunocompromised children must be treated with appropriately broad-spectrum antimicrobials covering a range of resistant organisms. Specific primary antimicrobial prophylaxis is not recommended, and there are no recommendations for secondary prophylaxis.

Antimicrobial Resistant iNTS and implications for treatment and management

Multidrug-resistant (MDR) NTS isolates are associated with increased morbidity compared to antibiotic-sensitive strains and are an important health and safety concern in both humans and animals [52,53]. Previously in countries in SSA where NTS is endemic the first line treatment choices for enteric and iNTS disease were co-trimoxazole, ampicillin or chloramphenicol. However, from the late 1980s due to an increasing prevalence of resistance to commonly available antibiotics, extended-spectrum cephalosporins and fluoroquinolones replaced these older agents for the management of iNTS disease (Figure) [54,55]. These alternative antimicrobials are less widely available and more expensive to use in resource-limited settings. For instance in Malawi [5,56] MDR *S. Typhimurium* account for 90% of all NTS isolated from blood. Recent studies in Kenya (Kariuki et al., In Press AAC 00078-15) and Malawi [61], showed that resistance to β -lactams, including ceftriaxone was associated with carriage of a combination of *bla*_{CTX-M-15}, *bla*_{OXA-1}. All the β -lactam-encoding genes were borne on a novel ca 300kb *incHI2* plasmid that harbored two class 1 integrons. The carriage of such an array of resistance genes on a mobile genetic element potentially increases rapid dissemination among enteric pathogens in the same environment.

Although ceftriaxone resistant *S. Typhimurium* have previously been reported in other countries in Europe, [57], Asia [58,59] and in the USA [55,60], until recently there was no data on this phenotype in SSA. It is of concern therefore that this phenotype has now been detected in Kenya (Kariuki et al. In press) and Malawi [61]. In Kenya the ceftriaxone resistant *S. Typhimurium* ST313 isolates came from adults and children who reported to a referral hospital with fever, with or without diarrhea and were initially treated with ceftriaxone and failed to respond. The spectrum of resistance in these isolates extends beyond the cephalosporins to include tetracyclines, co-trimoxazole, chloramphenicol, and aminoglycosides such as streptomycin. These isolates remained susceptible to carbapenems and fluoroquinolones, but rising MICs for the latter are being observed in recent NTS isolates. Fluoroquinolone resistance is also a growing problem in NTS isolates, with the first report of ciprofloxacin resistance in *Salmonella enterica* infection (eventually leading to treatment failure) being published in 1990 [62]. Since then, there have been reports of ciprofloxacin-resistant isolates being found in many countries, including India, Pakistan, Vietnam, Spain and Malawi [61,63].

In these studies [61] it was also shown that an increased incidence of bacteremia was temporally associated with the acquisition of multidrug resistance to ampicillin, co-trimoxazole, and chloramphenicol by each serovar and occurred while the incidence of infection due to other common bloodstream pathogens remained constant. In Burkina Faso, MDR was also common among invasive *Salmonella* spp. [64]. In addition, decreased ciprofloxacin susceptibility and extended-spectrum beta lactamase (ESBL) production was reported for one NTS isolate each.

Although we do not have a WHO recommended vaccine for prevention of iNTS disease there have been a number of initiatives that have led to the development of promising vaccine candidates that may play a major role in management and control of iNTS disease in endemic areas [65,66].

Enteric Fever

Epidemiology and management

Typhoid fever causes an estimated 22 million illnesses and >200 000 deaths worldwide each year with an additional 5.4 million cases due to paratyphoid fever [8,67,68]. Countries in Asia carry the highest burden [7]. In one study over five Asian countries the incidence ranged from 15 to 450/100,000 population/year [69]. The burden in Africa has been more difficult to determine as estimates, based on a handful of studies may have overestimated the number of cases [70]. Indeed in the 2010 study [8] advises caution in interpreting its estimates as one Kenyan study contributes disproportionately [10]. Recent reports of new epidemics of typhoid fever in sub-Saharan Africa suggest it is re-emerging as an important public health issue [71–73]. The results of the 10-country Typhoid Fever Surveillance in Africa study (TSAP: <http://www.ivi.int/web/www/home>) are keenly awaited.

One of the puzzling aspects of enteric fever is what determines the proportion of cases caused by Typhi or Paratyphi A in any individual area. Usually Typhi predominates but reports of an increasing proportion of cases caused by Paratyphi A have appeared from China, Nepal and most recently in south-east Asia [74–79]. In Africa it remains uncommon. The reasons for the increases seen in some parts of Asia have not been determined but might include the effects of increasing levels of typhoid vaccination, the differential effects of population antibiotic usage on serovars with different susceptibility patterns or different patterns of food or water-borne transmission [77]. Paratyphi A has been reported to cause a clinical syndrome of equal severity to Typhi [80] and unlike Typhi there is no vaccine available. A number of reports have suggested that Paratyphi A MICs are generally higher than those of Typhi across a range of antimicrobials [79].

Antimicrobial Resistance and Management of enteric fever

The management of patients with enteric fever requires appropriate attention to fluid balance, symptomatic treatment of fever and in severe patients appropriate intensive care and in some instances prompt surgery. Many patients can be successfully managed at home – hospital admission may be required if the patient has persistent vomiting and unable to keep down fluids, if they are severely toxic or if they have developed a specific complication. The early initiation of effective antimicrobial therapy shortens the duration of illness and reduces the risk of complications and death. Resistance to commonly used antimicrobials in Typhi and Paratyphi A is a widespread problem in endemic areas and returning travellers [7]. Some recent studies on resistance rates are summarised in Table 1. Such surveillance data must be interpreted with some caution as the laboratory capacity to conduct resistance surveillance is patchy and the results depend on isolation of the bacteria that may bias the results to more severe cases with higher levels of resistance. The data also depends on the willingness of investigators to publish the results collected. In spite of these caveats the table does illustrate the current resistance problems.

Large outbreaks of Typhi and Paratyphi in the late 1980s and 1990s in Asia were caused by multidrug resistant (MDR) strains resistant to the first-line antimicrobials chloramphenicol, ampicillin and co-trimoxazole [81]. Since that time the proportion of MDR strains has

declined in many areas of Asia such as India and Nepal. In contrast to the picture in Asia, recent emerging epidemics in Africa have been strongly associated with MDR infections [71–73]. Where MDR levels have fallen close to zero there has been a debate whether to return to using these antimicrobials as first-line agents [82,83]. The need for multiple daily dosing and two to three week treatment durations to prevent relapse are disadvantages of these agents. Some physicians are also wary about using chloramphenicol because of the risk of dose related and reversible bone marrow depression and the rare, unpredictable but usually fatal bone marrow failure [84]. Co-trimoxazole may be a suitable option as it is available and increasingly used in endemic areas although there have been no recent evaluations in randomised controlled trials. The genes responsible for the MDR phenotype have typically been borne on transmissible plasmids, in particular of the IncHI1 group [85]. Recent reports from Bangladesh and Zambia indicate that this block of resistance genes is now also found on the chromosome [71,86]. Re-introduction of the older drugs for treatment runs the risk of encouraging the re-emergence of strains with the MDR phenotype.

In the face of MDR infections, fluoroquinolones, such as ciprofloxacin and ofloxacin, have been widely used for treatment in the last twenty years. This has included use in children who form more than half the burden of enteric fever cases [87]. When isolates are fully susceptible (ciprofloxacin MIC < 0.06 µg/mL) these drugs have proved very effective [81,83,88]. They can be given orally and generic formulations are relatively affordable. With the widespread use of these agents resistance has emerged, initially low-level but later high-level. Low-level resistance, associated with a ciprofloxacin MIC of 0.1–0.5 µg/mL has also been known as nalidixic acid resistance, decreased ciprofloxacin susceptibility, and most recently has been re-categorised as intermediate susceptibility to ciprofloxacin [89]. Although these strains were initially thought susceptible it has become clear over time that infections with such isolates are associated with an impaired response to standard doses of ciprofloxacin and ofloxacin. Typically a slow resolution of fever and other symptoms have been described with an increased risk of clinical and microbiological failure and of complicated disease [84,90–92]. The later generation fluoroquinolone, gatifloxacin, is clinically more effective against infections with such isolates, probably because of slight differences in its topoisomerase target [82,92].

Isolates with intermediate susceptibility to ciprofloxacin are commonly characterized by point mutations in the *gyrA* gene, the principal target for ciprofloxacin and ofloxacin. Such strains have in recent years been associated with the H58 haplotype, now the dominant Typhi haplotype in much of Asia, and increasingly spreading into Africa [93,94]. Isolates with high-level fluoroquinolone resistance, an MIC ≥ 1.0 µg/mL, are associated with 2–3 mutations in the topoisomerase genes, and are now commonly described in the Indian sub-continent but are also appearing in Africa [9,60,91]. In addition plasmid mediated quinolone resistant *qnr* genes are being reported in Typhi [95]. In view of the high numbers of infections with fluoroquinolone non-susceptible isolates in many parts of Asia, this class of antimicrobials should be used with caution in this area in the absence of susceptibility data. Continued use of ciprofloxacin and ofloxacin in infections with intermediate susceptibility is likely to be driving the emergence of high-level resistant strains.

Azithromycin is another effective oral option for uncomplicated disease [83,88]. Unfortunately resistance to azithromycin has been difficult to monitor because of a lack of validated breakpoints. Current evidence, based on the epidemiological distribution of MIC values and randomised controlled trial data, suggests that an MIC of 16 µg/mL can be classified as susceptible and associated with a good response to azithromycin treatment. Typhi isolates with an MIC > 16 µg/mL are considered non-susceptible and most authorities setting breakpoints are adopting this cut-off. Although a number of reports suggest that most enteric fever isolates have an MIC 16 µg/mL [96], there are an increasing number of reports of Typhi and Paratyphi A isolates with azithromycin MIC > 16 µg/mL [96,97]. These higher MICs appear to be more common in Paratyphi A than Typhi isolates (Dutta, 2014; Parry CM, unpublished data). The clinical response to treatment of infection with isolates with an azithromycin MIC > 16 µg/mL is not yet defined. The extended spectrum cephalosporins, such as ceftriaxone and cefotaxime, have remained a reliable reserve antimicrobial particularly for hospital admitted cases [83]. The response to treatment with ceftriaxone can be slow and relapse a problem but resistance until recently has been rare. The sporadic reports of extended spectrum cephalosporin resistance in Typhi and Paratyphi A are therefore of serious concern (Table 2). Where the resistance has been characterized CTX-M has been the most common plasmid borne extended spectrum cephalosporin (ESBL) gene although AmpC genes have also been described. In a recent report from Nepal the proportion of 400 Typhi and Paratyphi A isolated between 2009 and 2011, 45% of strains were reported to be ESBL positive although the serovar involved was not specified and the strains were not studied further [98]. Cefixime, on oral third generation cephalosporin, is also recommended for enteric fever treatment [99]. Despite this recommendation the treatment response to cefixime was poor in two randomised controlled trials and the true effectiveness of this drug is at present unclear [100,101].

When selecting an antimicrobial in enteric fever, where there is uncertainty about the susceptibility of the strain or indeed the diagnosis, an extended spectrum cephalosporin such as ceftriaxone is the safest choice if the patient is admitted to hospital as resistance is currently unlikely. Where the diagnosis is suspected but not confirmed, for example in patients managed at home, knowledge of the prevailing strains in the area helps in the choice of oral treatment. In the absence of MDR strains co-trimoxazole or chloramphenicol are options. Azithromycin and possibly cefixime are alternatives if MDR infections are common and ciprofloxacin or ofloxacin can be used if it is known that ciprofloxacin non-susceptible strains are uncommon or absent which remains the case in much of sub-Saharan Africa. When choosing empiric therapy for undifferentiated fever other diseases that can mimic typhoid such as rickettsial infections may require specific additional treatment such as with doxycycline.

Where the diagnosis is subsequently confirmed, an oral fluoroquinolone, such as ciprofloxacin given in the maximum recommended dose for 7–10 days, is safe and appropriate if the strain is susceptible. If the strain has intermediate susceptibility or resistance to ciprofloxacin, oral azithromycin is an effective alternative, again given in the maximum possible dose for 7 days. If faced with an ESBL positive and MDR strain resistant to ciprofloxacin and azithromycin the options are extremely limited but might include expensive drugs such as the carbapenems or tigecycline.

Concluding remarks

Invasive *Salmonella* disease, whether caused by typhoidal or nontyphoidal serovars, causes a major burden of disease worldwide. The lack of robust diagnostic methods means that this burden is relatively neglected. Improvements in hygiene, sanitation and the availability of clean water are known to help in the prevention and control of enteric fever but the gaps in our understanding of the transmission of NTS in Africa is compromising our ability to devise control strategies to prevent iNTS disease. Data from regions where enteric fever and iNTS disease are endemic clearly show that antimicrobial resistance is a major public health challenge, with MDR the norm and evidence of emerging resistance to cephalosporins and the fluoroquinolones that render these conditions untreatable in resource limited settings. In addition to advocating for prudent use of available antimicrobials where they are still effective, improved sanitation to reduce burden of illness and the wider introduction of WHO recommended typhoid vaccines and the acceleration of trials for novel typhoid, paratyphoid and iNTS vaccines are further important steps that will play a major role in management and control of these infections.

Acknowledgements

SK is supported by National Institute of Allergy and Infectious Diseases (NIAID) of the National Institutes of Health under award number 1R01AI099525 and The Wellcome Trust. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. CMP is supported by an institutional grant by the Japanese Government to Nagasaki University.

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Reports of emergence MDR from some parts of Africa where NTS is a major cause of paediatric bacteraemia

Figure.

Map of Africa showing trends of antimicrobial resistance and areas where MDR and ESBL NTS isolates from invasive disease in children less than 5 years of age have been reported in sub-Saharan region.

Table 1

Antimicrobial resistance patterns of *Salmonella enterica* serovar Typhi and Paratyphi A isolated post 2007 in selected studies from Asia and Africa. Studies were selected where the number of isolates was at least 15 and where the resistance results could be divided by serovar

Country	Years	Serovar	No of isolates	% of isolates resistant to antimicrobial ^a				
				Chlor	Amp	Sxt	MDR	Cip ^{NSb} Cip ^c
India	2007–9	Typhi	191	-	-	-	14	73 9
India	2008–10	Typhi	257	0.4	68	2	-	100 25
India	2009–13	Typhi	77	22	18	23	18	99 20
India	2010–12	Typhi	266	10	13	5	3	96 35
India	2011	Typhi	61	20	8	15	-	5 -
Pakistan	2008–10	Typhi	131	72	99	47	-	17 -
Pakistan	2009–11	Typhi	2576	67	66	67	66	88 -
Nepal	2008	Typhi	29	3	7	7	-	66 3
Nepal	2011–12	Typhi	56	0	2	0	0	91 0
Bangladesh	2007	Typhi	38	58	68	58 ^e	-	82 40
Sri Lanka	2009–10	Typhi	19	28	28	25	-	- 50
Indonesia	2007–9	Typhi	55	4	2	2	-	2 0
Vietnam	2007–8	Typhi	51	80	80	80	-	20 0
Cambodia	2007–11	Typhi	20	-	-	-	75	90 0
Cambodia	2006–9	Typhi	41	56	56	56	56	81 0
Cambodia	2007–11	Typhi	148	86	85	85	85	90 0
Kenya	2001–8	Typhi	136	-	-	-	77	12 -
Uganda	2007–09	Typhi	27	5	76	76	0	0 0
Uganda	2011	Typhi	18	83	83	83	83	6 0
Tanzania	2007–08	Typhi	28	28	89	-	-	0 0
Tanzania ^f	2009–10	Typhi	46	22	23	22	19	1 0
Malawi-Moza mbique	2009	Typhi	46	100	100	100	100	10 0
Zambia	2010–12	Typhi	94	83	83	83	83	4 0
DRC	2007–11	Typhi	201	41	65	58	30	15 0

Country	Years	Serovar	No of isolates	% of isolates resistant to antimicrobial ^a				
				Chlor	Amp	Sxt	MDR	Cip ^c
DRC	2011–12	Typhi	18	33	72	72	33	0
Nigeria	2008–09	Typhi	21	42	41	52	-	0
Ghana	2007–8	Typhi	37	73	70	71	-	0
Ghana	2009	Typhi	15	100	100	100	100	0
Egypt	2011–12	Typhi	39	-	-	-	-	15
India	2008–10	Paratyphi A	45	2	49	4	-	100
India	2009–13	Paratyphi A	25	0	0	0	0	96
India	2010–12	Paratyphi A	77	0	3	0	0	100
Pakistan	2008–10	Paratyphi A	71	62	99	21	-	18
Pakistan	2009–11	Paratyphi A	1726	3	2	3	2	84
Nepal	2008	Paratyphi A	30	3	10	3	-	93
Nepal	2011–12	Paratyphi A	30	3	0	0	0	90
Sri Lanka	2009–10	Paratyphi A	73 ^g	0	22	0	0	-

• Chlor: chloramphenicol; Amp: ampicillin; Sxt: Trimethoprim-sulphamethoxazole; MDR: Multi-drug resistant; Cip^{NS} ciprofloxacin non-susceptible; Cip: Ciprofloxacin resistant; Cro; Ceftriaxone; Azm: azithromycin

• Ciprofloxacin non-susceptible: Based on ciprofloxacin MIC > 0.6 or nalidixic acid disc resistant and/or ciprofloxacin disc resistant if ciprofloxacin MIC not performed

• Ciprofloxacin resistant: Based on ciprofloxacin MIC 1.0 mg/L or ciprofloxacin disc resistant if MIC not performed

• Azithromycin non-susceptible: Based on azithromycin MIC > 16 mg/L

• Based on trimethoprim result alone

• Based on the previous ciprofloxacin breakpoint of MIC 4.0 mg/L

• Not all isolates tested: Chloramphenicol 44; Ampicillin 67; Trimethoprim-sulphamethoxazole 49; ciprofloxacin 71; ceftriaxone 63

Table 2

Published reports of extended spectrum cephalosporin resistant *Salmonella enterica* serovar Typhi or Paratyphi A

Country	Year	Serovar	Number (%) of isolates cephalosporin resistant	Resistance pattern ^a	Enzyme	Plamid
Bangladesh	1999	Typhi	1	AmpCro	Not determined	Not determined
Kuwait/UAE ^d	2003–6	Typhi	2	Not recorded	CTX-M-15	Not determined
Nepal	2004	Paratyphi A	3/288 (1)	Not recorded	Not determined	Not determined
Egypt	2002–7	Typhi	2/654 (0.3)	Not recorded	Not ESBL	Not determined
Bangladesh	2005–13	Typhi	1/404 ((0.2)	Not recorded	Not determined	Not determined
The Philippines	2007	Typhi	1	AmpSxtCtx	SHV-12	IncHI2
The Philippines	2007	Typhi	1	AmCroTm	SHV-12	IncHI2
Iraq	2008	Typhi	1	ChlorAmpSxtCtxCip ^b Azm ^c	CTX-M-15	IncN
Japan	2008	Typhi	1/48 (2)	AmpCroNalCip ^{NS}	CTX-M-15	Not determined
India	2009	Typhi	1	AmpCro	ACC-1 AmpC	Not determined
India	2009	Typhi	1	AmpCro	CMY-2 AmpC	IncA/
Pakistan	2009–11	Typhi	2/2576 (0.1)	Not recorded	Not determined	Not determined
Guatemala	2013	Typhi	1	AmpCtx	CTX-M-15	IncL/
Japan ^e	2013	Paratyphi A	1	AmpSxtCroNalCip ^{NS}	CTX-M-15	Not determined

• Chlor: chloramphenicol; Amp: ampicillin; Sxt: Trimethoprim-sulphamethoxazole; Nal: Nalidixic acid; Cip^{NS}: ciprofloxacin non-susceptible; Cip: Ciprofloxacin resistant; Cro: Ceftriaxone; Ctx: cefotaxime; Tm Trimethoprim; Azm: azithromycin

• Ciprofloxacin MIC 1.0 ng/L

• Azithromycin MIC not-stated

• Both patients were Indian nationals

• Patient had been travelling in India, China, Myanmar, Thailand and Nepal