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Nontyphoidal salmonella disease: Current status of vaccine research and development

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ABSTRACT

Among more than 2500 nontyphoidal *Salmonella enterica* (NTS) serovars, *S. enterica* serovar Typhimurium and *S. enterica* serovar Enteritidis account for approximately fifty percent of all human isolates of NTS reported globally. The global incidence of NTS gastroenteritis in 2010 was estimated to be 93 million cases, approximately 80 million of which were contracted via food-borne transmission. It is estimated that 155,000 deaths resulted from NTS in 2010. NTS also causes severe, extra-intestinal, invasive bacteremia, referred to as invasive nontyphoidal *Salmonella* (iNTS) disease. iNTS disease usually presents as a febrile illness, frequently without gastrointestinal symptoms, in both adults and children. Symptoms of iNTS are similar to malaria, often including fever (>90%) and splenomegaly (>40%). The underlying reasons for the high rates of iNTS disease in Africa are still being elucidated. Evidence from animal and human studies supports the feasibility of developing a safe and effective vaccine against iNTS. Both antibodies and complement can kill *Salmonella* species *in vitro*. Proof-of-principle studies in animal models have demonstrated efficacy for live attenuated and subunit vaccines that target the O-antigens, flagellin proteins, and other outer membrane proteins of serovars Typhimurium and Enteritidis. More recently, a novel delivery strategy for NTS vaccines has been developed: the Generalized Modules for Membrane Antigens (GMMA) technology which presents surface polysaccharides and outer membrane proteins in their native conformation. GMMA technology is self-adjuvanting, as it delivers multiple pathogen-associated molecular pattern molecules. GMMA may be particularly relevant for low- and middle-income countries as it has the potential for high immunologic potency at a low cost and involves a relatively simple production process without the need for complex conjugation. Several vaccines for the predominant NTS serovars Typhimurium and Enteritidis, are currently under development.

The genus *Salmonella* belongs to the Enterobacteriaceae family and comprises Gram-negative, non-spore-forming, facultative anaerobic bacilli [1]. *Salmonella enterica* subspecies *enterica* serovar Typhi and *Salmonella* Paratyphi A and B cause enteric fever, a systemic febrile illness that only occurs in humans and is distinguished from the more common self-limited acute gastroenteritis caused by other *Salmonella* serotypes. Non-typhoidal *Salmonella* (NTS) infect a variety of hosts and are frequently zoonotic in origin [2]. Of the more than 2,500 NTS serovars, *Salmonella* Typhimurium and *Salmonella* Enteritidis account for approximately 50% of all human isolates of NTS reported globally. NTS has been recognized as a major cause of invasive bacterial infections in young children and HIV-infected individuals in sub-Saharan Africa as well as elderly and immunocompromised individuals worldwide [3,4].

The global incidence of NTS gastroenteritis was estimated to be 93 million cases in 2010; approximately 80 million contracted the infection via food-borne transmission [3]. It is estimated that 155,000 deaths resulted from NTS that year. NTS also causes severe, extra-intestinal, invasive bacteremia, referred to as invasive nontyphoidal *Salmonella* (iNTS) disease [2]. A recent estimate suggests that, globally, there are 49 cases (range of 30–94) of iNTS per

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100,000 population which means that 3.4 (range 2.1–6.5) million cases occur globally each year [5]. In Africa, the iNTS incidence is much higher (227 [range of 142–341] cases per 100,000). iNTS disease usually presents as a febrile illness, frequently without gastrointestinal symptoms, in both adults and children. Symptoms of iNTS are similar to malaria, often including fever (>90%) and splenomegaly (>40%). The underlying reason for the high rates of iNTS disease in Africa are still being elucidated; however, there are several established contributing factors that include increased invasiveness of distinct clades specific to Africa (*e.g. Salmonella* Typhimurium ST313), compromised host immunity in those with HIV infection, malaria, malnutrition, and increased opportunities for transmission (*e.g.*, through contaminated water supplies). NTS bacteremia is particularly virulent in HIV-infected African adults who have a mortality rate of 47 per cent and recurrence rate of 43 per cent [6]. As a whole, the iNTS case fatality rate is estimated to be ~20% translating into 681,316 (range of 415,164 to 1,301,520) deaths annually [5]. Although mortality is lower in high income countries, the economic burden of NTS in those countries is still significant. In the United States, NTS costs US$3.3 billion per year, with a loss of 17,000 quality-adjusted life years, the most of any food-borne pathogen [3]. iNTS disease has been overshadowed in the past by other diseases for which better data available, such as malaria, and HIV. The gaps in knowledge about the epidemiology of iNTS, however, are starting to close.

Because the typical clinical presentation of iNTS disease is non-specific, diagnosis is often difficult in resource-limited settings. Blood or bone-marrow culture may be used to diagnose cases of bacteremia. Polymerase chain reaction (PCR) on stool samples can potentially aid in the rapid diagnosis of *Salmonella* and multiplex PCR may serve as a means to identify specific invasive *Salmonella* serovars [7]. Serum ELISA is helpful in detecting past *Salmonella* infections, but is less useful for iNTS diagnosis for acute infections [8]. iNTS disease is primarily treated with antibiotics, whose class and duration are chosen on the basis of cost, availability, local patterns of resistance and treatment response. Treatment failure is of increasing concern in HIV-infected individuals and those infected with antibiotic-resistant strains (*e.g.* ST313). One approach toward overcoming these obstacles is to treat people with antibiotics that have optimal intracellular penetration, such as fluoroquinolones [9], although resistance to this class of antibiotics is increasing as well. The global burden of iNTS disease is likely to continue rising in absolute numbers and in the relative proportion of bacteremia cases, particularly as antimicrobial resistance becomes more prevalent and licensed vaccines reduce the incidence of other major causes of bacteremia, such as *Streptococcus pneumoniae* and *Haemophilus influenzae* b. As available tools for treatment become less effective, the development of effective vaccines will rise in priority for disease control efforts [2].

1. Biological feasibility for vaccine development

Effective *Salmonella* Typhi vaccines have been successfully licensed and administered to millions of people. Evidence from animal and human studies supports the feasibility of vaccine development against NTS as well. Both antibodies and complement can kill *Salmonella* species *in vitro*. Epidemiologic studies in sub-Saharan Africa have shown that the development of antibodies against NTS corresponds with a decrease in age-related incidence of iNTS disease, and that serum antibodies have corresponding *in vitro* bactericidal activity partly by mediating intracellular oxidation [9,10]. However, one African study found that high antibody titers against *Salmonella* lipopolysaccharide (LPS) O-antigen were associated with impaired *in vitro* serum killing of *Salmonella* Typhimurium in a proportion of HIV-infected Malawian adults [10]. The *in vivo* significance of this observation is not clear, as anti-LPS antibodies have bactericidal activity, protecting against NTS challenge in mouse models [9,10].

2. General approaches to vaccine development for low- and middle-income markets

Proof-of-principle studies have demonstrated efficacy, in animal models, of live-attenuated and subunit vaccines that target the O-antigens, flagellin proteins, and other outer membrane proteins of *Salmonella* Typhimurium and *Salmonella* Enteritidis. The relatively poor immunogenicity of purified O-antigens can be significantly enhanced through chemical linkage to carrier proteins. The subunit glycoconjugation approach specifically links LPS-derived O polysaccharide to carrier proteins and has been successful as, unlike *Salmonella* Typhi, the NTS are not encapsulated. More recently, a novel delivery strategy for NTS vaccines has been developed: the Generalized Modules for Membrane Antigens (GMMA) technology presents surface polysaccharides and outer membrane proteins in their native conformation. GMMA technology is self-adjuvanting, as it delivers multiple pathogen-associated molecular pattern molecules. GMMA may be particularly relevant for low- and middle-income countries as it has the potential for high immunologic potency at a low cost and involves a relatively simple production process without the need for complex conjugation.

The development of recombinant or purified protein vaccines based on surface or outer membrane protein antigens, such as flagellin and porins OmpC, F and D offer the potential for broad-spectrum coverage due to targeting of conserved antigens. A reverse vaccinology approach using bioinformatics analysis of whole genome sequences from clinically important serovars may facilitate identification of additional conserved antigens. However, manufacturing complexities in purifying outer membrane proteins with the appropriate conformation may obviate the utility of porins as immunogens. Other promising vaccine approaches against iNTS disease include live attenuated candidates, which can be delivered orally and induce robust mucosal and T cell immunity.

Vaccines for iNTS will also need to target 2-4 month old infants, before the peak incidence at age 12 months. Programmatic field implementation in children could integrate directly with existing Expanded Program on Immunization schedules, potentially at 6, 10, and 14 weeks, or at 9 months concomitant with measles vaccination. This schedule will allow for programmatic introduction, as well as will enable children to be protected at an earlier age when they are at higher risk of disease. Vaccine implementation would likely also include populations infected with HIV. In higher income countries, NTS vaccines could also target the elderly who experience a high case-fatality rate of up to 50%.

3. Technical and regulatory assessment

In 2013, the World Health Organization provided guidance on the regulation and prequalification of typhoid conjugate vaccines [11] [12]. Although no such pathway is available for NTS vaccines, the experience with typhoid vaccines may serve as a good model to adapt. There are relatively robust animal models that can be used to evaluate preclinical data. Mice, for example, are permissive to *Salmonella* Typhimurium and *Salmonella* Enteritidis systemic infection that begins via entry through the gut mucosa and spreads through the lymphatic system. In untreated mice, infection manifests as invasive disease without gastroenteritis. To produce an NTS enterocolitis infection, mice must be pre-treated with streptomycin or other antibiotics prior to bacterial challenge. There are important differences to consider between mouse and human infections,

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