Journal of Infection (2017) **75**, 104–114

BIAM British Infection Association

www.elsevierhealth.com/journals/jinf

An evaluation of purified *Salmonella* Typhi protein antigens for the serological diagnosis of acute typhoid fever

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http://dx.doi.org/10.1016/j.jinf.2017.05.007

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Accepted 16 May 2017
Available online 25 May 2017

KEYWORDS
Typhoid fever;
Enteric fever;
Salmonella Typhi;
Diagnostics;
Bangladesh;
Vi polysaccharide;
IgM;
Febrile disease

Summary *Objectives:* The diagnosis of typhoid fever is a challenge. Aiming to develop a typhoid diagnostic we measured antibody responses against *Salmonella* Typhi (S. Typhi) protein antigens and the Vi polysaccharide in a cohort of Bangladeshi febrile patients.
Methods: IgM against 12 purified antigens and the Vi polysaccharide was measured by ELISA in plasma from patients with confirmed typhoid fever (n = 32), other confirmed infections (n = 17), and healthy controls (n = 40). ELISAs with the most specific antigens were performed on plasma from 243 patients with undiagnosed febrile disease.
Results: IgM against the S. Typhi protein antigens correlated with each other (rho > 0.8), but not against Vi (rho < 0.6). Typhoid patients exhibited higher IgM against 11/12 protein antigens and Vi than healthy controls and those with other infections. Vi, PilL, and CdtB exhibited the greatest sensitivity and specificity. Specificity and sensitivity was improved when Vi was combined with a protein antigen, generating sensitivities and specificities of 0.80 and >0.85, respectively. Applying a dynamic cut-off to patients with undiagnosed febrile disease suggested that 34–58% had an IgM response indicative of typhoid.
Conclusions: We evaluated the diagnostic potential of several S. Typhi antigens; our assays give good sensitivity and specificity, but require further assessment in differing patient populations.

Introduction

Enteric (typhoid) fever is a systemic infection caused by *Salmonella enterica* serovars Typhi (S. Typhi) and Paratyphi A (S. Paratyphi A).[1,2] There are an estimated 12 million cases of typhoid (S. Typhi only) worldwide annually leading to approximately 120,000 deaths.[3,4] The organisms are transmitted via the fecal-oral route and the disease remains common in low/middle income countries in South/Southeast Asia and sub-Saharan Africa.[5] Despite S. Paratyphi A being an emergent cause of enteric fever in parts of South and Southeast Asia,[6] S. Typhi remains the most commonly reported etiological agent of enteric fever in Asia and Africa.

Typhoid occurs only in humans, making it a disease that can technically be eradicated.[7] Indeed, typhoid has all but been eliminated from several countries in Southeast Asia where it was the most common cause of hospitalized febrile disease 20–30 years ago.[8,9] Elimination in these areas is generally attributed to extensive improvements in sanitation rather than widespread immunization schemes. The lack of data regarding the long-term impact of mass immunization for typhoid and the performance of licensed vaccines have hindered immunization as a sustainable typhoid control and elimination strategy. Future considerations for rational control measures for typhoid will rely on more accurately assessing disease burden, which requires a reliable diagnostic approach.[10]

All commonly used typhoid diagnostics perform poorly and are a roadblock for disease control efforts.[11,12] Currently, the only reliable method for the identification of febrile individuals with typhoid is the culture of a causative organism from a biological specimen.[12,13] However, this procedure is restricted to laboratories with adequate equipment and microbiology training, and the method has a limited sensitivity due to low concentrations of organisms in the peripheral circulation.[14–16] Low bacterial loads have a similar impact on other methods that rely on detecting the presence of the infecting organisms, such as antigen detection or nucleic acid amplification. These methods are often reported to be highly sensitive, but have unrealistic performances; pre-treatment with antimicrobials is likely to compound this issue further.[11,15] New typhoid diagnostics are a necessity and various approaches have been evaluated, including measurement of innate immune responses,[17,18] antibody in lymphocyte supernatants,[19,20] and the identification of metabolomic signatures.[21] However, these advances are still restricted to research laboratories and are not yet ready to be developed into simple, rapid diagnostic tests (RDTs).

We previously exploited a protein microarray to identify a multitude of immunogenic S. Typhi protein antigens to which an antibody response was generated during the early stages of typhoid.[22] With the aim of validating antigens that could be used in a diagnostic assay we expressed and purified several of these potentially serodiagnostic S. Typhi