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Review

Diagnostics for invasive *Salmonella* infections: Current challenges and future directions

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ABSTRACT

Invasive Salmonellosis caused by *Salmonella enterica* serotype Typhi or Paratyphi A, B, C, or invasive non-typhoidal *Salmonella* serotypes, is an immensely important disease cluster for which reliable, rapid diagnostic tests are not available. Blood culture remains the gold standard but is insensitive, slow, and resource-intensive. Existing molecular diagnostics have poor sensitivity due to the low organism burden in bodily fluids. Commercially available serologic tests for typhoidal *Salmonella* have had limited sensitivity and specificity. In high burden, resource-limited settings, reliance on clinical diagnosis or inaccurate tests often results in frequent, unnecessary treatment, which contributes selective pressure for the emergence of antimicrobial resistance. This practice also results in inadequate therapy for other etiologies of acute febrile illnesses, including leptospirosis and rickettsial infections. A number of novel serologic, molecular, transcriptomic and metabolomic approaches to diagnostics are under development. Target product profiles that outline specific needs may focus development and investment, and establish benchmarks for accuracy, cost, speed, and portability of new diagnostics. Of note, a critical barrier to diagnostic assay rollout will be the low cost and low perceived harm of empiric therapy on behalf of providers and patients, which leaves few perceived incentives to utilize diagnostics. Approaches that align incentives with societal goals of limiting inappropriate antimicrobial use, such as subsidizing diagnostics, may be essential for stimulating development and uptake of such assays in resource-limited settings. New diagnostics for invasive Salmonellosis should be developed and deployed alongside diagnostics for alternative etiologies of acute febrile illnesses to improve targeted use of antibiotics.

1. Introduction

Invasive Salmonellosis is caused by *Salmonella enterica* serotype Typhi (*Salmonella*. Typhi) or Paratyphi A and B (*Salmonella*. Paratyphi), the causes of enteric fever, Salmonella Paratyphi C, which causes septicemia and metastatic purulent infections, or invasive non-typhoidal *Salmonella* (iNTS) serotypes, including *Salmonella*. Enteriditis and *Salmonella*. Typhimurium. Invasive non-typhoidal Salmonellosis has its highest burden among immunocompromised or malnourished individuals, especially children infected with HIV in resource-limited areas, among whom case fatality is high [1]. Similarly, enteric fever remains among the leading causes of disability from an infectious disease in the developing world [2]. Recent estimates of typhoidal *Salmonella* incidence have varied substantially [3–6], and iNTS estimates are sparse [7–9], in large part due to poor access to reliable diagnostics, particularly in low-resource outpatient settings where patients with these illnesses typically present for medical care. Measured by its burden and influence on antibiotic use, invasive Salmonellosis is perhaps the most important infectious disease cluster for which rapid and reliable (>90% sensitivity and specificity) diagnostics do not exist.

This diagnostic gap leads to under-diagnosis as well as inaccurate, over-diagnosis of enteric fever especially, the latter of which may lead to inappropriate and excessive antibiotic use. This results in selective pressure for the emergence of resistant bacteria, at a time in which highly resistant Gram-negative infections, including *Salmonella* [10–13], threaten to undermine reductions in case fatality rates for bacterial infections [14]. Additionally, inappropriate targeting of antibiotics for Salmonellosis results in inadequate therapy for other treatable infections, such as leptospirosis, rickettsia, and brucellosis. It also poses a challenge to the targeted rollout

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Table 1
Characteristics of currently available diagnostics for invasive Salmonellosis.

Diagnostic class	Sensitivity	Specificity	Time to result	Laboratory requirements	Comments
Blood culture	Low	Excellent	1–5 days	Moderate	Provides antibiotic susceptibility
Bone marrow cultures	High	Excellent	1–3 days	Moderate	Invasive; requires trained personnel
Rapid serology (Widal, Tubex, Typhidot, RTI)	Low–moderate	Moderate	<1 hour	Low	Only point-of-care tests available
Antigen	Moderate	Variable	1–3 hours	Moderate	Limited evidence on accuracy
Polymerase chain reaction (PCR)	Variable	Excellent	1–3 hours	High	Variable sens. among culture-negative

and evaluation of more effective, conjugated enteric fever vaccines, which are on the horizon [15,16]. A recent review (2011) of diagnostics for enteric fever provided a detailed summary of the state of existing diagnostics, with an emphasis on serologic assays and nucleic acid amplification-based tests [17]. Here, we briefly review the literature on currently available diagnostic approaches for both enteric fever and iNTS, and then provide an overview of diagnostic strategies under development, desirable test characteristics according to their utilization goal, and the development and implementation challenges for scale-up of new *Salmonella* diagnostics.

2. Available diagnostic approaches for enteric fever

Essentially all enteric fever diagnosis begins with evaluation of clinical signs and symptoms. For perhaps the majority of patients with suspected enteric fever worldwide, who live in settings where diagnostic microbiology is unavailable [18], this is also the end of the diagnostic algorithm, and a decision concerning empiric treatment is made at this juncture. Unfortunately, clinical diagnosis of typhoid is not reliable, as it is difficult to distinguish typhoid from other co-endemic acute febrile illnesses including influenza, dengue, leptospirosis, malaria, brucellosis, rickettsial infections, and other systemic infections. Fever and headache occur in the majority of patients, and a myriad of non-specific symptoms include abdominal pain, myalgias, chills, cough, sore throat, anorexia and nausea [19–25]. Diarrhea and constipation are both regularly reported in case series. Hepatomegaly, splenomegaly, and cervical lymphadenopathy are present in a minority of patients. Faget's sign (relative bradycardia in the presence of fever) occurs in less than half of patients and is not specific for enteric fever. Rose spots—a salmon-colored maculopapular eruption typically on the trunk—are seen in less than 30% of cases in most series [21], and are similarly not pathognomonic [26]. Laboratory abnormalities are also non-specific. Most patients have normal leukocyte counts, though leukopenia is present in a minority. Mild increases in hepatic transaminases, creatine kinase and lactic acid dehydrogenase have been reported but are also common to other infections in the differential diagnosis [19,21]. While *Salmonella* Paratyphi A has been considered to cause a more mild illness than typhoid, a recent large study from a co-endemic setting found these infections to be clinically indistinguishable [27]. Studies aiming to develop and validate prediction rules for enteric fever from clinical features and laboratory results have had very limited success [28–31].

After clinical diagnosis, the two most common diagnostic procedures for typhoid in use today were developed in the 19th century: bacterial culture and the Widal test. Since Eberth's discovery of the etiological agent of enteric fever in 1880 [32], culture has been the gold standard of enteric fever diagnosis. Whereas early culture techniques had low yield, Coleman and Buxton developed an improved approach by using larger quantities of blood (10 ml) and broth, and adding Ox-bile which lyses blood cells and inhibits antibacterial activity [33]. This approach, while effective, prevents isolation of many other important bacteria. Tryptone soy broths are among the most commonly used blood culture media today, with automated systems used in settings with sufficient resources. The majority of positive cultures are evident within 48 h, and nearly all are positive by five days (Table 1). Subculture, biochemical testing, and agglutination with specific antisera are typically performed to identify *Salmonella* serovars.

The sensitivity of culture varies substantially according to the specific fluid and volume assayed, age of the affected individual, prior antimicrobial use, and stage of the illness. Bone marrow cultures have the highest sensitivity (>80%) and are relatively unaffected by antibiotics [34,35]; however, this diagnostic procedure is not commonly performed in clinical settings where typhoid is endemic due to its invasive nature and the need for training and specialized, sterile equipment. The sensitivity of blood culture has been variably reported at 40–80%, with higher sensitivity in the first week of illness, when the bacterial concentration in blood is an order of magnitude higher than in subsequent weeks [34–36]. Unlike with bone marrow cultures, antibiotics substantially diminish the yield of blood cultures. Stool cultures and rectal swabs have lower sensitivity (<40%), though they can be enhanced by culturing three specimens or performing multiple cultures from a single stool specimen [37]. Duodenal string sampling and culture may provide a higher yield than stool or rectal swabs [38,39], but with all gastrointestinal site sampling, it should be recognized that positivity may reflect chronic carriage rather than invasive illness. Urine cultures have a low yield and are unlikely to provide an incremental benefit to diagnostic yield over blood culture. When present, rose spots can be cultured and provide some enhancement to diagnostic yield, particularly later in the course of illness [34].

The Widal agglutination test, in which killed *Salmonella* Typhi and Paratyphi A antigen is reacted with serum to measure agglutinating antibodies to the flagellar (H) and lipopolysaccharide (O) antigens, was developed in the 1890s [40], modified and standardized in the 1950s [41], and today remains in widespread use throughout typhoid-endemic settings. The simplicity and rapidity of the test enables its use in settings with minimal laboratory infrastructure, but misuse and misinterpretation of the results remains a critical problem. A single agglutination test has limited sensitivity and specificity, particularly early in the course of illness and in endemic settings [42,43]; comparison of acute and convalescent titers improves test accuracy but has limited utility in guiding clinical practice [44,45]. A number of ELISAs have been evaluated for typhoid diagnosis by targeting antibodies to the O, H and virulence polysaccharide (Vi) antigens; however, for diagnosis in the acute phase, these tests suffer from the same limitations as the Widal test [44,46,47].

Several serologic tests have been developed for point-of-care diagnosis of enteric fever. The two that have been most widely studied are TUBEX TF (IDL Biotech, Sweden) and Typhidot (Malaysian Biodiagnostic Research, Malaysia). TUBEX TF assays for antibodies to *Salmonella* Typhi LPS (O9) by quantifying inhibition of binding between O9 monoclonal antibodies and LPS-coupled magnetic particles [48]. Typhidot is a miniaturized dot-blot ELISA that detects IgM and IgG antibodies to a 50 kDa *Salmonella* Typhi outer membrane protein (OMP). Typhidot-M uses the same approach to detect IgM to OMP after removal of total serum IgG, to improve specificity for recent infection [49]. A recent systematic review and meta-analysis of TUBEX TF and Typhidot found sensitivity of 56–95% and 56–84%, respectively, with specificity of 72–95% and 31–97% [50]. Several assays based on antigen detection (O9, Vi,