Rapid molecular diagnostics for extensively drug-resistant *Salmonella* Typhi

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Background: *Salmonella* Typhi infections are a major problem globally, with over 20 million cases of typhoid diagnosed every year, disproportionately affecting children in low-income settings. This pathogen has become remarkably resistant to many antibiotics, complicating the treatment of these potentially life-threatening infections. Increasing reports of multi-drug resistant and extensively drug-resistant (XDR) *Salmonella* Typhi have escalated this pathogen to "high" on the WHO priority pathogens list. The "gold standard" for diagnosis of Salmonella infections in high income settings is blood culture, which often takes >48 hours for disease identification and determination of antimicrobial resistance. Diagnostic methods are at best limited or otherwise not available in low resource settings. Enhanced diagnostics that can simultaneously diagnose *Salmonella* Typhi infections alongside antimicrobial resistance determinants are urgently needed.

Methods: We developed a range of rapid diagnostic tests to detect XDR *Salmonella* Typhi alongside key markers for ciprofloxacin (*qnrS*) and ceftriaxone (*bla*$_{CTX-M}$) resistance. Clinical XDR *Salmonella* Typhi isolates were tested via real-time PCR and isothermal amplification (RPA and LAMP) detection assays, with validation using targeted amplicon sequencing and whole-genome sequencing.

Results: All XDR *Salmonella* Typhi isolates (16/16) were positive for two Typhi-specific detection genes, as well as a Typhi H58 clade-specific target, while antimicrobial resistance genes for ciprofloxacin (*qnrS)* and ceftriaxone *(bla*$_{CTX-M}$) could be detected in 75% of isolates (12/16). Negligible cross reactivity was observed in non-typhoidal Salmonella (n=68), and other pathogen (n=22) clinical isolates. Whole genome sequencing confirmed the *Salmonella* Typhi isolates as members of the currently disseminating H58 clade that displays extensive antimicrobial resistance, confirmed by the presence of a clade-specific deletion and ciprofloxacin and ceftriaxone antimicrobial resistance determinants.

Conclusion: These assays will enable the rapid detection of XDR *Salmonella* Typhi in laboratories and closer to the point of care, and empower clinicians to select the most appropriate antimicrobial treatment thereby improving patient-centered care.

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