Development of Real Time Polymerase Chain Reaction for the Detection of *Salmonella* in Stool Specimens

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Background: *Salmonellae* are among the leading cause of bacteraemia and death in sub-Saharan African children. The burden of *Salmonella* in Africa and the link between *Salmonella* exposure within the gastrointestinal tract and blood stream is poorly understood in part due to lack of reliable diagnostic test for detection of *Salmonella*. Stool culture, which is the gold standard for *Salmonella* detection, is less sensitive and time consuming. In this study, we aimed at validating Quantitative Real Time-Polymerase Chain Reaction (RT-PCR) test for the detection of *Salmonella* in stool specimens from a cohort of *Salmonella* asymptomatic children.

Methods: RT-PCR tests using primers from Tetrathionate (TTR) respiration gene and *Salmonella* Invasion gene A (InvA). TTR and InvA RT-PCR assays were tested for inclusivity using different *Salmonella* strains and exclusivity was tested using different gram positive and negative non *Salmonella* bacteria. PCR efficiency and limits of detection were determined using *Salmonella* Typhimurium D23580 reference strain. The primers were also validated against stool culture for *Salmonella*. *Salmonella* exposure events in 409 stool samples collected from a cohort of healthy children aged 6-18 months was also determined and sensitivity and specificity rates of the assays were calculated.

Results: Both TTR and InvA RT-PCR demonstrated 100% inclusivity and between 87% and 94% exclusivity rates. Both assays had superior limits of detection of up to 1 CFU/ml when sub cultured in selenite F broth with 98% PCR efficiency. Sensitivity and specificity of TTR was 73.91%, 91.3% and 96.89%, 95.08% and for InvA it was 78.26%, 82.61% and 92.49%, 90.41% for neat and selenite sub cultured stool samples respectively.

Conclusion: TTR and InvA RT-PCR assays demonstrated superior performance than stool culture. Selenite sub culturing of the samples improves performance and reduces cross reactivity. The two primers can be used together as a diagnostic tool for surveillance studies.