BMC Infectious Diseases

RESEARCH

Open Access

Diagnostic performance of Typhidot RDT in diagnosis of typhoid fever and antibiotic resistance characterisation in a cross-sectional study in Southern Ghana

Emmanuel Kweku Sam[1,2], Johnson Alagbo[1], Avis Asamoah[1], Felix Ansah[1], Kwesi Zandoh Tandoh[1,3], Lucas N. Amenga-Etego[1] and Samuel Duodu[1,2*]

Abstract

Background Typhoid fever remains a significant public health problem contributing to significant misapplication of antibiotics in Ghana. However, there is little data on the accuracy of the commonly used serology based rapid diagnostic Typhidot test kit (Typhidot RDT) for confirming typhoid fever.

Methods We conducted a study to assess the diagnostic accuracy of Typhidot RDT in seven clinical facilities across five regions in Southern Ghana. A total of 258 participants, clinically diagnosed with typhoid fever, were enrolled in this study. Blood and stool samples were obtained for culture, Typhidot and PCR assays. Disc diffusion antibiotic sensitivity was performed to determine the resistance pattern of *Salmonella enterica* isolates from positive blood and stool cultures.

Results Recovery of *S. enterica* isolates was higher from stool samples (14.7%) in comparison to blood samples (1.6%). The sensitivity and specificity of Typhidot compared to blood and stool cultures was 35% (19.94%—52.65%) and 45% (38.67%—51.45%), respectively. Compared to PCR, the Typhidot had a sensitivity and a specificity of 61% and 53%, respectively. Resistance phenotyping of isolates showed broad sensitivity to the front-line antibiotics used. Resistance to ampicillin (10%), cotrimoxazole (7%), azithromycin and ciprofloxacin (< 5%) was found in some isolates.

Conclusions These findings suggest sub-optimal performance of the Typhidot RDT for diagnosis of typhoid in Ghana with a higher chance for misdiagnosis and misapplication of antibiotics. The high proportion of isolates recovered from stool culture is consistent with the pathophysiology of bacterial shedding during the acute phase of infection, which provides a window of opportunity to control typhoid transmission.

Keywords Typhoid diagnostics, *Salmonella*, Ghana, Antibiotics resistance

*Correspondence:
Samuel Duodu
saduodu@ug.edu.gh
Full list of author information is available at the end of the article

Background

Typhoid fever, a systemic bacterial infection caused by *Salmonella enterica* serovar Typhi (*S.* Typhi) remains an important public health challenge in Ghana, contributing to antibiotic resistance due to frequent inaccurate diagnosis. It is a common disease in countries with insufficient clean water supply, poor hygiene and sanitation standards [1]. People contract the infection when they ingest water or food contaminated with infected human stool. Globally, there is an estimated burden of about 14.3 million cases with approximately 135.9 thousand deaths [2]. In the Ghanian typhoid epidemiological landscape, estimating accurate incidence is difficult, due to the poor laboratory-based surveillance and disease diagnostic challenges [3]. Though the case fatality rate is not high, the morbidity associated with the disease results in loss of productivity due to hospitalization and long periods of recovery and out of pocket payment for treatment and management of associated symptoms [4].

In Ghana, the current treatment policies for typhoid involves the use of antibiotic such as cephalosporins and fluoroquinolones. The macrolide antibiotic, azithromycin has also gained popularity as a treatment option for suspected resistant strains [5]. In a subset of *S.* Typhi infected individuals, the bacteria may persist long after symptoms have resolved [6]. In these chronic carriers, *S.* Typhi infects and establishes in the gall bladder forming biofilms that confer resistance to a range of antimicrobials [7]. The emergence of multidrug resistance (MDR) in *Salmonella* has made treatment of the disease more difficult and complicated. Several genes and plasmids have been implicated in the MDR of *S.* Typhi [8]. There are known variants of *Salmonella* that are likely to retain their drug resistant genes even in the absence of drug pressure [9]. Thus, infections would be more associated with frequent complications and treatment failures.

One area of great importance in typhoid management and control of MDR *S.* Typhi is the improvement of diagnostic methods. Inaccurate diagnosis has dire consequences for infected individuals, with increased risks of complications due to inaccurate treatment. Occurrence of disease symptoms must be confirmed by the isolation of the pathogen through culture, which remains the gold standard for typhoid diagnosis. The sensitivity of the standard test not only decreases with increased duration of fever, but is also time consuming and expensive for patients and clinics in resource limited settings where culture facilities may not be readily available [10, 11]. In most typhoid endemic regions, many still relied on the nineteenth century Widal serological tests for diagnosis, which uses antibody titres against *Salmonella* specific O (somatic) and H (flagellar) antigens in patient serum to detect acute infection. This is because it is relatively cheaper, easy to perform and requires minimal training and low sophisticated equipment [12]. However, such test is difficult to interpret due to cross reactivities, the time lag between infection and production of antibodies, and the persistence of target antibodies long after treatment with very low correlation with active disease [13]. In this regard, many studies suggest that the Typhidot assay based on the detection of IgM and IgG antibodies against the outer membrane protein antigen of *Salmonella enterica* is a viable alternative and has a better performance characteristic than Widal test with a reported sensitivity of about 75–97% [14, 15]. Nevertheless, variations in diagnostic sensitivity and specificity of the Typhidot assay have been noted among adults and children presenting with typhoid fever [13]. Different geographical settings have also reported variations in performance of this assay [16].

Most healthcare facilities in Ghana are transitioning from the use of Widal test to the use of Typhidot and PCR as diagnostic tools for suspected typhoid fever. Accurate diagnosis is essential for effective antibiotic treatment to prevent development of severe disease and further transmission. A major factor contributing to high antibiotic resistance in our study settings is the misapplication and unregulated use of antibiotics, suggesting that the typhoid diagnostic landscape could be an important contributor to the problem of antimicrobial resistance in endemic settings [17]. In this study, the diagnostic accuracy of the Typhidot RDT was evaluated in seven clinical facilities across five regions in southern Ghana to provide evidence-based data for improved interpretation of Typhidot RDT test results and management of typhoid fever.

Methods

Study design, participants, study sites and sampling

This was a cross-sectional study conducted between 2022 and 2023, during which samples were collected from patients with clinical symptoms suggestive of typhoid fever such as high fever, headaches, abdominal pain, weakness, and fatigue. The study included participants of all ages, encompassing both inpatients and outpatients, to capture a broad representation of individuals affected by typhoid fever. Potential study participants were counselled about the study parameters and informed consent documented. Recruitment was conducted consecutively, ensuring a systematic and unbiased selection process. A total of 258 participants consented and were recruited into the study. Participants were recruited from seven tertiary health facilities namely (Ho Government Hospital, Holy Spirit Clinic, Mercy Women's Catholic Hospital, Saltpond Government Hospital, St. Luke Catholic Hospital, Apam, Legon Hospital and Akyem Municipal

Hospital). These facilities are in the Volta, Eastern, Central, Greater Accra and Ashanti regions of Ghana, respectively (Fig. 1). In terms of capacity, the facilities have about 50–300 beds and are made up of clinical and non-clinical directorates with several departments. A volume of 5–10 mL of venous blood samples was collected aseptically from clinically diagnosed patients with the help of laboratory scientists and distributed into BD Vacutainer K2E-EDTA (1–2 mL) and BD blood culture bottle (4–8 mL). The closed EDTA tube was carefully inverted severally to ensure proper mixing of anticoagulant. Blood samples containing EDTA anticoagulant were transported and stored at a temperature of 2°C-10°C in the Microbiology Laboratory at the Department of Biochemistry, Cell and Molecular Biology, University of Ghana. Blood culture samples were transported at room temperature and cultures were set up upon arrival at the lab. About 5 g stool sample was collected into a sterile stool container and inoculated into Selenite F broth. Analysis of stool samples was performed at the Microbiology Department of the University of Ghana Medical Centre. Clinical and demographic information including age, gender, axillary temperature, signs and symptoms and prescribed treatment regimen were obtained from participants through an administered questionnaire completed by patients and clinicians (Supplementary Material 1).

Diagnosis of typhoid fever

Serological diagnosis by TyphiDot

Specimen and the TyphiDot RDT test kit stored at 2–8°C were brought to room temperature. The device was removed and placed on a clean flat surface before labelling with specimen identification number. The test buffer bottle was correctly opened by breaking the seal of the cap and piecing nozzle as per manufacturers recommendation. An aliquot of plasma (25 μL) was then pipetted into the sample well making sure that there were no air bubbles, and a drop of the assay buffer was added to the sample well. Results were read in 15–20 min. Negative or non-reactive results occur if the control (C) band is present and no burgundy color was observed in both test bands (M and G), indicating detection of no anti-S. Typhi or Paratyphi antibodies in the specimen. Positive or reactive results occur when, in addition to the C band,

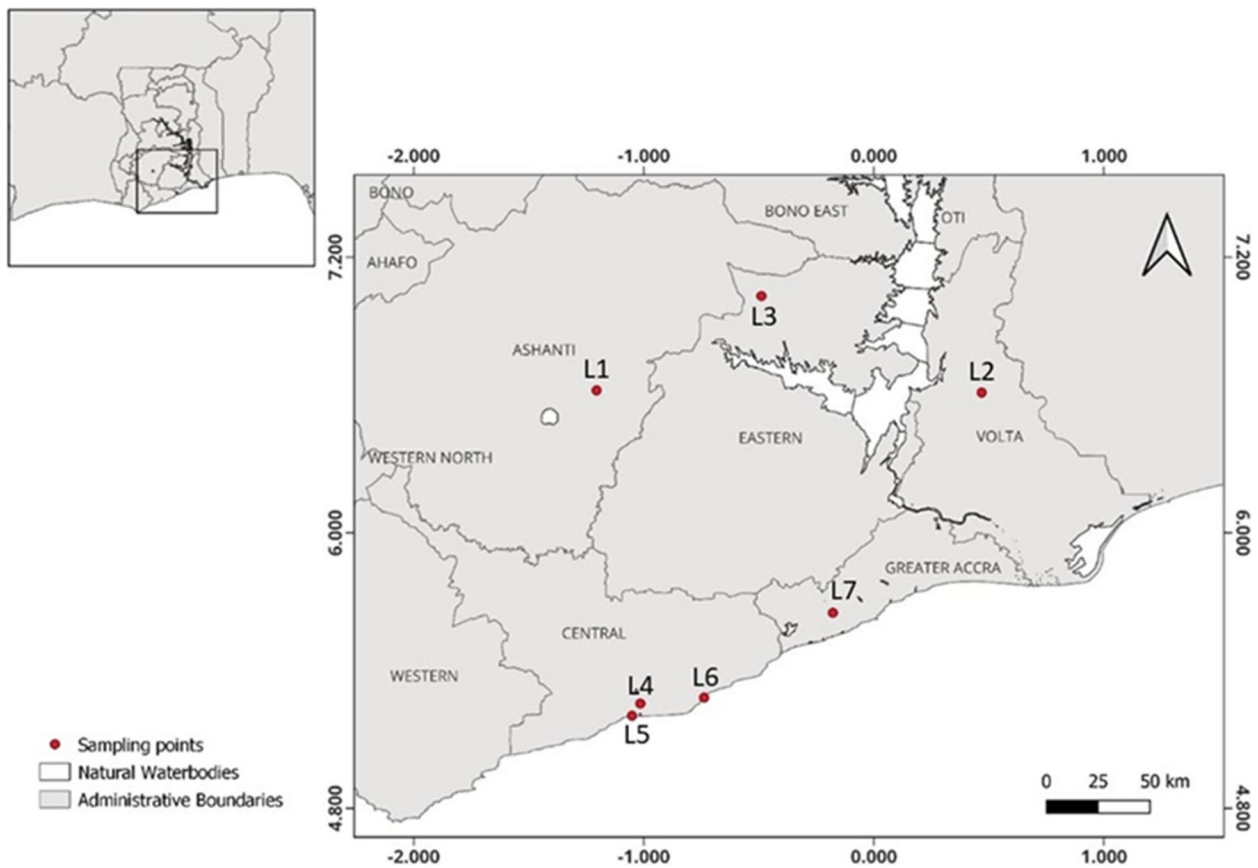

Fig. 1 Map showing the geo-spatial locations of the sampling sites. L1- Asante Akyem Municipal hospital, L2-Ho Municipal Hospital, L3- Holy Spirit Clinic, L4-Mercy Women's Catholic Hospital, L5-Saltpond Municipal Hospital, L6-St. Luke's Catholic Hospital, L7-University Hospital, Legon

there is the development of a burgundy color for M band, G band or both, indicating the presence of anti-S. Typhi or Paratyphi IgM antibodies, IgG antibodies or both, respectively. Invalid results are reported if there is no C-band development regardless of burgundy color in the test bands. Patients were classified based on the results of their serological testing. Those with positive IgM or both IgM/IgG were diagnosed with active typhoid infection and considered for treatment. Patients showing only IgG positivity were identified as having past exposure while individuals negative for both IgM and IgG were categorized as negative for typhoid infection.

PCR diagnosis of Salmonella enterica infection

DNA extraction from blood samples was performed using the QIAamp DNA Mini Kit (Qiagen), according to the manufacturer's instruction with some slight modification. Briefly 200µL each of blood sample and 200µL of buffer AL were added to 5 µL of Proteinase K (Qiagen) in a 1.5 mL microcentrifuge tube and mixed by pulse-vortexing for 15 s. The resulting mixture was incubated at 56 °C for 10 min. Absolute ethanol (200µL) was added, and the DNA precipitated and washed sequentially with 500 µL of Buffer AW1 and Buffer AW2. DNA was eluted with 100 µL of Buffer AE and stored -20 °C. Molecular confirmation of typhoid fever clinical diagnosis was done using conventional PCR method based on the protocol of Zhuang et al. and Zendrini et al., with slight modifications[18, 19]. F3 and B3 primer pair, which amplifies a 217-bp fragment of the STY 2021 gene, was used in the PCR amplification. Primers were synthesized commercially by Inqaba (Biotech Industries Pty, SA). The sequences for the forward and reverse primers are as follows: Forward primer (F3) CCGGACAAACGATTCTGG TA, Reverse primer (B3): CCGACATCGGCATTATCC G. Amplification was performed in 25 µL reaction volume consisting of Master Mix reagents (Go Taq 2x)- 12.5 µL, nuclease-free water- 6.0 µL, F3 and B3 primers (0.5 µM)-0.75µL each, and 5 µL of DNA template. The PCR reactions included pre-denaturation step at 95°C for 5 min; followed by 35 cycles of denaturation at 95°C for 1 min, annealing at 56°C for 1 min, extension at 72°C for 1 min; and a final elongation step at 72°C for 10 min. The PCR products were subsequently visualized by agarose gel electrophoreses.

Isolation and characterization of bacteria

Blood culture

Samples in blood culture bottles were incubated in the Bactec FX 40 system (Becton Dickinson Blood Culture System Diagnostics, USA). Positive cultures (per the Bactec flag) were sub-cultured unto chocolate, blood, and MacConkey agars. Plates were incubated at 37 °C for 24 h. Negative cultures were further incubated for 5 days until they were flagged as no bacterial growth.

Stool culture

A gram of stool sample was collected into a container and inoculated into a sterile Selenite F broth. Inoculum was incubated at 37°C and sub-cultured unto MacConkey with crystal violet agar, Salmonella Shigella (SS) agar and Xylose Lysine Deoxycholate (XLD) agar. Culture plates were incubated under aerobic conditions at 37 °C for 24 h.

Presumptive bacterial identification and API confirmation

Non-lactose fermenting isolates from MacConkey agar cultures were picked for spot indole and oxidase tests using the RapID Spot Indole Reagent (R8309002, LOT-3328215) and Gordon-McLeod Reagent (R026-100ML), respectively. Indole and oxidase-negative isolates were further tested using Triple Sugar Iron (TSI), citrate, urea, and motility agars for preliminary identification of *Salmonella enterica* and other gastrointestinal pathogens. For confirmatory identification, biochemical testing was performed using the API 20E test strip (Biomerieux, USA) following the manufacturer's instructions. Results were interpreted using the API catalog and software to confirm *Salmonella enterica* isolates.

Determination of antibiotic resistance phenotypes

The Kirby-Bauer disk diffusion method was employed to assess antibiotic susceptibility of *Salmonella enterica* isolates, following the Clinical and Laboratory Standards Institute (CLSI) guidelines for antimicrobial susceptibility testing (CLSI, 2020). In brief, bacterial suspensions were prepared to a 0.5 McFarland standard and inoculated onto Mueller–Hinton agar plates. Disks containing antibiotics, including ciprofloxacin, amoxicillin/clavulanate, ceftriaxone, cefuroxime, piperacillin/tazobactam, ampicillin, azithromycin, cefoxitin, and trimethoprim-sulfamethoxazole, were placed on the agar surface. Plates were incubated at 37°C for 24 h, and results were interpreted based on the CLSI 2020 guidelines. *Salmonella enterica* serovar Typhi ATCC 6539 was used as the reference strain for quality control.

Statistical analysis

STATA 15.1 (version 15.1) was used to perform all statistical analyses. Descriptive statistics were used to summarize categorical variables. The performance matrices (sensitivity, specificity, positive predictive value, negative predictive value, efficacy) determined based on confusion matrix and receiver operating characteristic (ROC) curve analysis were used to validate the Typhidot test in relation to culture (gold standard) and PCR.

Results

Demographic and clinical characterization of participants

A total of 258 participants clinically diagnosed with typhoid were recruited into the study. Most patients were adults between 18 and 60 years followed by children and adolescents between 5 and 17 years and the elderly above 61 years of age (Fig. 2 panel A). Of the total number of patients recruited, 62.0% ($n=160$) were females (Fig. 2 panel B). Common signs and symptoms patients presented with were fever, malaise, weakness, abdominal and general body pains, gastroenteritis, anemia, tiredness, vomiting with axillary temperature around 39°C (Figure S1).

Serological testing

Two hundred and fifty-eight blood samples were screened for typhoid IgG/IgM antibodies using Typhidot RDT. Overall, 45% ($n=116$) samples tested negative for both IgM and IgG antibodies, whereas 20.5% ($n=53$) samples tested positive for both IgM and IgG. 31.8% ($n=82$) of samples tested positive for IgM alone, whilst 2.7% ($n=7$) samples tested positive for IgG alone. Thus, given an overall 52.3% ($n=135$) positive cases by serology (Fig. 3). Based on the Typhidot test results, antibiotics were prescribed for 97% of the patients ($n=131$) while 2.2% ($n=3$) received a variety of analgesics and 0.7% antimalarials as part of the treatment by clinicians.

Culture isolation and identification of isolates

In all, 95.3% ($n=246$) of blood samples did not show growth after 5 days of incubation using the BACTEC FX

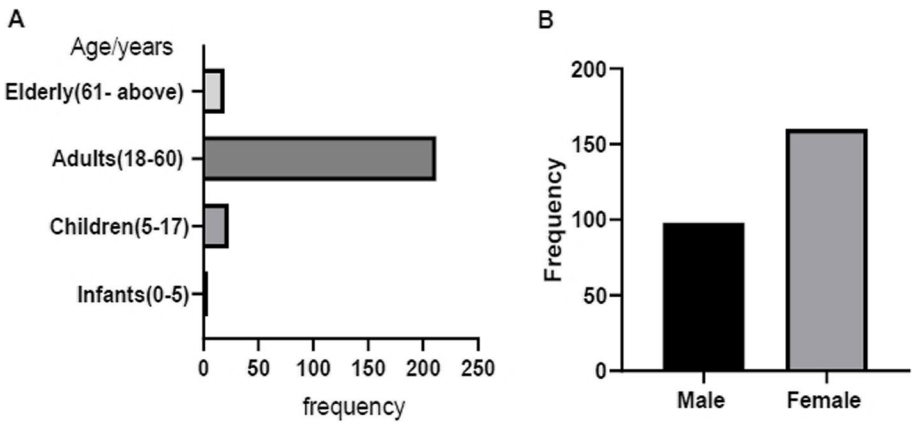

Fig. 2 Age and gender distribution of study participants (**A**) Age groups (**B**) Gender

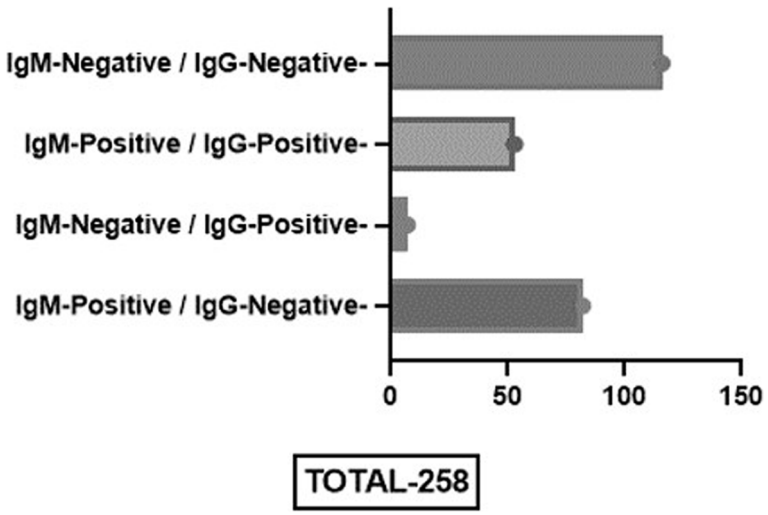

Fig. 3 Serological diagnosis using Typhidot test kit

40 blood culture system. Among the 12 samples that were flagged positive, seven (7) gram-positive cocci and five (5) gram-negative bacilli isolates were identified. Catalase and coagulase tests on the gram-positive isolates resulted in the identification of five (5) *Staphylococcus epidermidis,* which was regarded as contaminants and a single organism each of *S. aureus and S. saprophyticus.* For the gram-negative blood isolates, four (4) were identified as *Salmonella enterica* while a single *Pseudomonas aeruginosa* isolate was recovered. Therefore, prevalence of *S. enterica* from blood was 1.6%. Out of the 169 stool samples cultured, 25 *Salmonella enterica* isolates were confirmed, representing a prevalence of 14.8%. These isolates were identified as non-lactose fermenters (NLFs) and further confirmed through biochemical tests and API 20E.

Diagnostic sensitivity and specificity of Typhidot RDT

Typhidot IgM/IgG serological screening of blood samples resulted in 52.3% confirmed cases. Patients were regarded as having typhoid once IgM or IgM/IgG was positive. IgG positive alone was regarded as exposure to previous infections which does not warrant treatment. IgM/IgG negative patients were considered negative for typhoid. Using culture as standard, disease was present in 11.2% ($n=29$) and absent in 88.8% ($n=229$) of the collected samples (Table 1). Among the positive tests by culture, the Typhidot test detected 34.5% ($n=10$) positives and 65.5% ($n=19$) negatives. For culture negative samples, 55.0% false positives and 45.0% true negatives were recorded by RDT. An estimated sensitivity of 34.5% and specificity of 45.0% was obtained for the Typhidot RDT against culture diagnostic method (Table 1).

With the use of PCR-based diagnosis, disease was present in 40% ($n=103$) of the samples. Among these, 60.2% ($n=62$) were positive using Typhidot RDT with 39.8% ($n=41$) being negative. The RDT reported 47.1% ($n=73$) positives and 52.95% ($n=82$) negatives among the 155 samples that were negative by PCR amplification (Table 2). The area under the curve (AUC) ROC plots for Typhidot test efficacy was 0.5655 (data not shown). The performance of the Typhidot assay against PCR generated a sensitivity of 60.8% and a specificity of 52.9% (Table 2).

Antibiotic sensitivity testing

The cultured *S. enterica* pathogens were generally sensitive to all 8 antibiotics tested (Fig. 4). Among the isolates obtained from the blood culture samples ($n=4$), 25% ($n=1$) of isolates showed some level of resistance against ciprofloxacin, cotrimoxazole and ampicillin antibiotics (Fig. 4 & Figure S3). Few isolates from the stool culture ($n=25$) showed resistance to cotrimoxazole ($n=2$, 8%) and ampicillin ($n=3$, 12%). This suggests that isolates are not grossly resistant to common antibiotics used in the management of the disease.

Discussion

In Ghana, typhoid fever ranks among the leading five outpatient febrile illnesses alongside malaria, upper respiratory tract infections, diarrheal diseases, and intestinal worms [20]. Classification of suspected febrile illness patients into typhoid positive or otherwise is usually done by a combination of clinical signs and symptoms and laboratory test, mostly performed using Typhidot test kit or Widal indirect agglutination test. Though the Widal test is most used, particularly at the lower levels of the health infrastructure, lack of appreciation of the test procedure often leads to inaccurate results and over reporting of typhoid cases. This results in needless over prescription of antibiotics and exacerbation of the already precarious antibiotic resistance problem in Ghana. Thus, posing treatment challenges, reinfections, and the threat of *S.* Typhi superbugs that remain one of the most problematic bacteria linked to hospitalization, disease severity and mortality. This study examined the diagnostic efficacy of the Typhidot diagnostic method against culture as the gold standard in selected Ghanaian

Table 1 Diagnostic performance of Typhoid RDT against Culture

		Culture Diagnosis (Gold standard)	
		Disease Present (Positive)	**Disease Absent (Negative)**
Typhidot IgM/IgG	Positive	10	125
	Negative	19	104
Sensitivity (95% CI)	34.48% (19.94%—52.65%)		
Specificity (95% CI)	44.98% (38.67%—51.45%)		
PPV	7.35% (4.04%—13.01%)		
NPV	84.43% (76.95%—89.80%)		

Table 2 Diagnostic performance of Typhoid RDT against PCR

		PCR diagnosis	
		Disease Present (Positive)	**Disease Absent (Negative)**
Typhidot IgM/IgG	Positive	62	73
	Negative	41	82
Sensitivity (95% CI)	60.78% (54.82%-66.75%)		
Specificity (95% CI)	52.90% (46.80%-59.01%)		
PPV	45.93% (39.83%-52.02%)		
NPV	67.21% (61.47%-72.95%)		

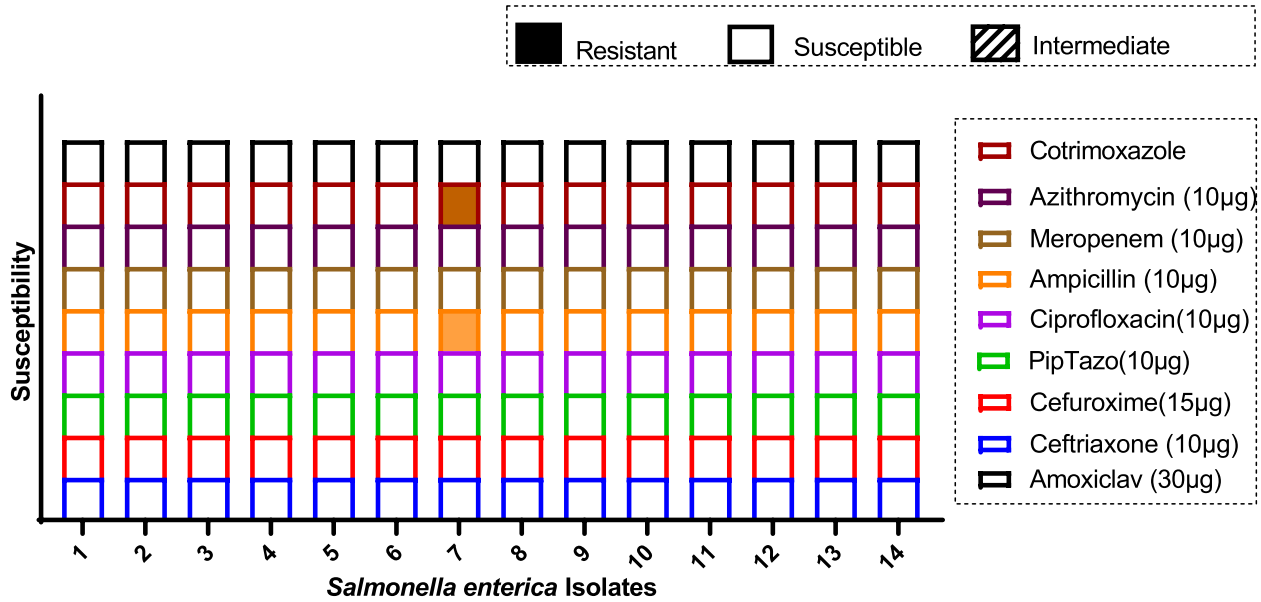

Fig. 4 Susceptibility pattern of commonly used antibiotics for treatment of typhoid infections in clinical facilities sampled

health facilities. Generally, patients diagnosed with Typhidot test assay presented with acute non-malarial fever (or history of fever within the past 24 h) with axillary temperatures ranging between 36.8 °C to 39 °C together with non-specific symptoms such as malaise, weakness, general body pains, vomiting and hypertension. Hence, highlighting the known overlap of clinical presentations for several co-circulating febrile pathogens, including endemic malaria.

The Typhidot test assay as a diagnostic tool is optimized by the manufacturer's to have a sensitivity and specificity of approximately 97% and 90%, respectively [21]. However, the level of sensitivity and specificity observed in this study suggests a sub-optimal performance with a sensitivity and specificity of 61% and 53%, respectively. The high rate of false positives in serology for culture-negative samples emphasizes the need for improved diagnostic accuracy and careful consideration in clinical decision-making to avoid misdiagnosis and unnecessary treatments. These findings are in tandem with reports from similar studies in other endemic regions in Africa and Asia [16, 22]. For instance, research from Bangladesh and India has demonstrated sensitivities as low as 50–70% in some cases, which poses a significant challenge for early detection and treatment [14]. Such variable performance may be attributed to several factors, including the quality of the Typhidot test kits used due to poor storage conditions, the intensity of infections and the like. For many years, isolation of *S.* Typhi from either the stool, blood or bone marrow is considered to be the diagnostic standard for typhoid fever [23]. Culture facilities are, however, non-existent in most health facilities in Africa including Ghana, and the bacterial recovery rate and turn-around time is poor. As observed in this study, only 4.6% (12/258) of isolates was recovered from blood cultures. This could be due to many factors, notably the observed low prevalence (1.6%) of typhoid fever in the populations sampled or antibiotic self-medication among patients seeking healthcare. Generally, larger volumes of blood in the range of 10- 20 mL are required to achieve 50% or more pathogen detection in typhoid fever [24]. Obtaining such volumes of blood from patients during this study was challenging and militated against patient's willingness to participate in the research. This technical limitation of the study might have affected the bacterial recovery rates of the blood culture assays done. On the other hand, stool culture resulted in about 21.9% NLFs bacterial recovery suggesting that participants were shedding bacteria into the environment. This is particularly worrying as the prevalence of *S. enterica* from stool samples was 14.8%, and open defecation remains high in the catchment communities of the health facilities sampled. However, these findings offer an opportunity for the formulation of WASH educational policies as targeted interventions aimed at preventing the transmission of these acute-on-chronic infections.

For typhoid detection in blood, PCR is generally not an ideal diagnostic tool due to the typically low pathogen loads in peripheral blood samples [4]. However, PCR seems to outperform culture for detection of *S. enterica* in the present study. One plausible explanation for this could be that the PCR assays used were optimized for

higher analytical sensitivity [25], allowing for the detection of low copies of bacterial DNA that might be missed by culture. Additionally, prior antibiotic use in patients could have suppressed bacterial growth, contributing to the lower culture sensitivity while PCR was still able to detect residual DNA.

Interestingly our data shows that most of these patients, regardless of Typhidot test and culture outcomes were treated with antibiotics including beta lactams (3rd generation cephalosporins), macrolides, nitroimidazoles and fluoroquinolones (Figure S2). However, contrary to reports from other typhoid endemic regions [26, 27], we did not observe high levels of resistance in the circulating strains isolated. Based on this data, we can assert that *Salmonella* organisms remain sensitive to existing antibiotics used for treatment as per the Ghana health services treatment guidelines. Although the low prevalence of antibiotic resistance observed across study sites is encouraging, it is important to interpret these findings with caution. The relatively small number of isolates tested may not fully capture the broader resistance landscape. As such, while these initial results offer an opportunity to enhance antibiotic stewardship and limit the selection of highly resistant strains, more comprehensive studies with larger sample sizes are needed to better understand the true prevalence of resistance. In addition, the timing of sampling is critical in bacterial detection. Sampling during the acute phase of infection may enhance *S. enterica* recovery rates in stool but limit the sensitivity of blood culture as observed in the current study.

Conclusions

The data presented showed that the diagnostic performance of the Typhidot test kit is not optimal as close to 50% of febrile cases could be misdiagnosed as typhoid. Even though such a high likelihood of typhoid misdiagnosis coupled with the high rate of empirical antibiotic treatment remains an important driver of bacterial antibiotic resistance, our study reported low levels of drug resistance among the circulating isolates. Overall, our findings underscore the need for further wider evaluation of the Typhidot test kit as a front-line diagnostic tool and highlight the pressing need for more sensitive alternative diagnostic tools.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12879-024-10160-2.

Supplementary Material 1: Typhoid Diagnosis Study Questionnaire.

Supplementary Material 2: Supplementary data Figure S1-S3.

Acknowledgements

We extend our sincere gratitude to the laboratory scientists, nurses, staff and study participants at St. Luke's Government Hospital, Mercy Women's Catholic Hospital, Saltpond Government Hospital, Asante Akyem Government Hospital, Holy Spirit Clinic, Ho Municipal Hospital, and University Hospital, Legon. Their expertise, dedication, tireless efforts and cooperation were crucial in the collection and processing of the samples for this study. We also thank Angela Ayiku at WACCBIP, University of Ghana for her helpful assistance.

Authors' contributions

S.D., K.T and L.A involved in conceptualization and study design; E.K.S., J.A., A.A and F.A. developed the methodology; E.K.S and L.A. used the software; S.D., F.A and L.A performed the validation; E.K.S did the formal analysis; E.K.S performed the investigation; S.D and L.A provided resources; S.D data curated; E.K.S and S.D wrote the original draft preparation; E.K.S., S.D., K. T and L.A reviewed and edited the draft; S.D and L.A did visualization of data; S.D and L.A supervised the work; S.D was the project administrator; S.D and L.A involved in fund acquisition. All authors have read and approved the final manuscript.

Funding

This research was supported by WACCBIP, University of Ghana, which receives funding from World Bank African Centre of Excellence Masters/PhD fellowship (ACE02-WACCBIP + NCDs, Awandare) and NIHR funded Global Health Research Group on Establishing Regional Hubs for Genomic Surveillance in West Africa grant reference number NIHR13471.

Data availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the College of Basic and Applied Sciences (ECBAS), University of Ghana with approval number ECBAS094/21–22 and the Ghana Health Service (GHS) Ethics Research Committee with approval number GHS-ERC:045/01/23**.** A written informed consent was obtained from all adult participants and/or parental (or legal guardian) consent for children involved in this study.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Author details

[1]West African Centre for Cell Biology of Infectious Pathogens, College of Basic and Applied Sciences, University of Ghana, Legon-Accra, Ghana. [2]Department of Biochemistry Cell and Molecular Biology, University of Ghana, Legon-Accra, Ghana. [3]Department of Epidemiology, Noguchi Memorial Institute for Medical Research, College of Health Sciences, University of Ghana, Legon-Accra, Ghana.

Received: 17 July 2024 Accepted: 30 October 2024
Published online: 07 November 2024

References

1. Mawazo A, Bwire GM, Matee MIN. Performance of Widal test and stool culture in the diagnosis of typhoid fever among suspected patients in Dar es Salaam. Tanzania BMC Res Notes. 2019;12(1):1–5. https://doi.org/10.1186/s13104-019-4340-y.
2. GBD 2017 Typhoid and Paratyphoid Collaborators. The global burden of typhoid and paratyphoid fevers: A systematic analysis for the Global Burden of Disease Study 2017. Lancet Infect Dis 2019;19(4):369–381. https://doi.org/10.1016/S1473-3099(18)30685-6.

3. Jeon HJ, Cho S, Lee HJ, et al. How can the typhoid fever surveillance in Africa and the severe typhoid fever in Africa programs contribute to the introduction of typhoid conjugate vaccines? Clin Infect Dis. 2019;69(Suppl 6):S417–21. https://doi.org/10.1093/cid/ciz629.
4. Von Kalckreuth V, Konings F, Aaby P, et al. The typhoid fever surveillance in Africa program (TSAP): Clinical, diagnostic, and epidemiological methodologies. Clin Infect Dis. 2016;62(Suppl 1):s9–16. https://doi.org/10.1093/cid/civ693.
5. Veeraraghavan B, Pragasam AK, Anandan S, et al. Evaluation of antimicrobial susceptibility profile in *Salmonella* Typhi and *Salmonella* Paratyphi A: Presenting the current scenario in India and strategy for future management. J Infect Dis. 2021;224(5):S502–16. https://doi.org/10.1093/infdis/jiab144.
6. Gonzalez-Escobedo G, Marshall JM, Gunn JS. Chronic and acute infection of the gall bladder by *Salmonella* Typhi: Understanding the carrier state. Nat Rev Microbiol. 2011;9(1):9–14. https://doi.org/10.1038/nrmicro2490.
7. Di Domenico EG, Cavallo I, Pontone M, Toma L, Ensoli F. Biofilm producing *Salmonella* Typhi: Chronic colonization and development of gallbladder cancer. Int J Mol Sci. 2017;18(9). https://doi.org/10.3390/ijms18091887.
8. Mina SA, Hasan MZ, Hossain AKMZ, Barua A, Mirjada MR, Chowdhury AMMA. The prevalence of multi-drug resistant *Salmonella* Typhi isolated from blood sample. Microbiol Insights. 2023;16:117863612211507. https://doi.org/10.1177/11786361221150760.
9. Mulvey MR, Boyd DA, Olson AB, Doublet B, Cloeckaert A. The genetics of *Salmonella* genomic island 1. Microbes Infect. 2006;8(7):1915–22. https://doi.org/10.1016/j.micinf.2005.12.028.
10. Ajibola O, Mshelia MB, Gulumbe BH, Eze AA. Typhoid fever diagnosis in endemic countries: A clog in the wheel of progress? Medicina. 2018;54(2):1–12. https://doi.org/10.3390/medicina54020023.
11. Manore C, Graham T, Carr A, et al. Modeling and cost benefit analysis to guide deployment of POC diagnostics for non-typhoidal *Salmonella* infections with antimicrobial resistance. Sci Rep. 2019;9:47359. https://doi.org/10.1038/s41598-019-47359-2.
12. Wasihun AG, Weldearegay GM, Gebretsadik D, et al. Diagnosis and treatment of typhoid fever and associated prevailing drug resistance in Northern Ethiopia. Int J Infect Dis. 2015;35:e96–102. https://doi.org/10.1016/j.ijid.2015.04.014.
13. Ousenu K, Ali IM, Sama LF, Ndam MN, Tchouangueu TF, Tume CB. A cross-sectional comparative study of the performance of the Widal test and the typhidot immunoassay for typhoid fever diagnosis in the West region of Cameroon. Can J Infect Dis Med Microbiol. 2021;2021:8279122. https://doi.org/10.1155/2021/8279122.
14. Dyson ZA, Klemm EJ, Palmer S, Dougan G. Antibiotic resistance and typhoid. Clin Infect Dis. 2019;68(Suppl 2):S165–70. https://doi.org/10.1093/cid/ciy1111.
15. Park SE, Pham DT, Boinett C, et al. The phylogeography and incidence of multi-drug resistant typhoid fever in sub-Saharan Africa. Nat Commun. 2018;9(1):5094. https://doi.org/10.1038/s41467-018-07370-z.
16. Sapkota J, Mohanan A, Parajuli K, et al. Comparative analysis of commercially available typhoid Point-of-Care tests: Results of a prospective and hybrid retrospective multicenter diagnostic accuracy study in Kenya and Pakistan. J Clin Microbiol. 2022;60(12):e01000-e1022. https://doi.org/10.1128/jcm.01000-22.
17. Wam EC, Arrey CN, Sama LF, Agyingi LA, Wam AN. Comparative study on the use of Widal test to stool culture in the laboratory diagnosis of typhoid fever in Holy Family Hospital Akum, Northwest Region of Cameroon. Open Microbiol J. 2019;13:73–80. https://doi.org/10.2174/1874285801913010073.
18. Zhuang L, Yu X, Liu S, et al. Detection of *Salmonella* spp. by a loop-mediated isothermal amplification (LAMP) method targeting *bcfD* gene. Lett Appl Microbiol. 2014;59(6):658–64. https://doi.org/10.1111/lam.12328.
19. Zendrini A, Cavallini N, Invernizzi F, et al. One-day molecular detection of *Salmonella* and *Campylobacter* in chicken meat: A pilot study. Foods. 2021;10(5):511. https://doi.org/10.3390/foods10051132.
20. Rufai T, Aninagyei E, Akuffo K, et al. Malaria and typhoid fever among patients presenting with febrile illnesses in Ga West Municipality, Ghana. PLoS ONE. 2023;18(5):e0267528. https://doi.org/10.1371/journal.pone.0267528.
21. Arora P, Thorlund K, Brenner DR, Andrews JR. Comparative accuracy of typhoid diagnostic tools: A Bayesian latent-class network analysis. PLoS Negl Trop Dis. 2019;13(5):e0007303. https://doi.org/10.1371/journal.pntd.0007303.
22. Wijedoru L, Mallett S, Parry CM. Rapid diagnostic tests for typhoid and paratyphoid (enteric) fever. Cochrane Database Syst Rev. 2017;5:CD008892. https://doi.org/10.1002/14651858.CD008892.pub2.
23. Mahmoud A, Ali S, Ezeonu CS, et al. Recent advances in the diagnosis and management of typhoid fever in Africa: A review. Int J Health Plann Manage. 2023;38(2):317–29. https://doi.org/10.1002/hpm.3599.
24. Kaur A, Kapil A, Elangovan R, et al. Highly-sensitive detection of *Salmonella* Typhi in clinical blood samples by magnetic nanoparticle-based enrichment and in-situ measurement of isothermal amplification of nucleic acids. PLoS ONE. 2018;13(9):e0203982. https://doi.org/10.1371/journal.pone.0203982.
25. Goay YX, Chin KL, Tan CLL, et al. Identification of Five Novel *Salmonella* Typhi-Specific Genes as Markers for Diagnosis of Typhoid Fever Using Single-Gene Target PCR Assays. BioMed Res Int. 2016;2016:8905675. https://doi.org/10.1155/2016/8905675.
26. Marchello CS, Carr SD, Crump JA. A systematic review on antimicrobial resistance among *Salmonella* Typhi worldwide. Am J Trop Med Hyg. 2020;103(6):2518–27. https://doi.org/10.4269/ajtmh.20-0258.
27. Yusof NY, Ariffin H, Sulaiman MR, et al. Prevalence of antimicrobial resistance genes in *Salmonella* Typhi: A systematic review and meta-analysis. Trop Med Infect Dis. 2022;7(10):271. https://doi.org/10.3390/tropicalmed7100271.

Publisher's Note