A COMPARATIVE STUDY OF THE PERFORMANCE OF TYPHOID DIAGNOSTIC TESTS AMONG SUSPECTED PATIENTS AT THE UNIVERSITY OF CAPE COAST HOSPITAL, GHANA

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Abstract

Introduction: the World Health Organization recommends that the gold standard for diagnosing typhoid fever is the use of blood cultures. However, under real-world conditions in low-resource settings, there are lack of culture facilities as well as expertise, and usually, patients report late after prior antibiotic exposure. This study sought to evaluate the performance of typhoid rapid diagnostic tests, blood, and stool cultures as well as polymerase chain reaction (PCR) under such settings.

Methods: This cross-sectional study involved the use of blood and stool samples from 400 consenting outpatients suspected of typhoid fever by attending clinicians at the University of Cape Coast Hospital, Ghana.

Results: out of the 400 participants, 171 (42.8%) tested positive to at least one of the RDTs. Even though many of the participants had a history of fever, all of them were afebrile as none presented with a temperature of 38^0C and above. There was a high (48.0%) history of antibiotic use within two weeks before presentation. No participant was a culture or PCR positive for *S. typhi and paratyphi*. However, in 7(1.8%) participants, other salmonella species were detected by PCR. Also, *citrobacter* and *proteus vulgaris* were cultured in 13(3.3%) and 10(2.5%) participants respectively using stool samples. In blood samples, there was a 7(1.8%) growth of *Staphylococcus aureus*.

Conclusion: under real-world conditions where patients usually present without typical symptoms such as high temperatures probably after prior antibiotic use, cultures and PCR perform poorly. There is therefore a need for further studies aimed at improving the yields of these important diagnostic tests in such settings.

Keywords: typhoid fever, Ghana, rapid diagnostic test, afebrile

Background

Typhoid and paratyphoid (enteric) fevers continue to be major causes of mortality and morbidity globally and especially in the developing world [1]. They are therefore diseases of public health concern. The causative agents for typhoid and paratyphoid fevers are *Salmonella enterica* serovar Typhi and Paratyphi A respectively. Typhoid, the more common

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infection, is an important infectious disease in low- and middle-income countries [2]. The main risk factors associated with the occurrence of enteric fever are inadequate sanitation and hygiene, particularly regarding food, water, and disposal of human excreta [3]. Globally, in 2017, over 14.3 million cases of typhoid and paratyphoid fevers occurred resulting in an estimated 135.9 thousand mortalities [4]. Due to the lack of diagnostic laboratories in many parts of sub-Saharan Africa, the prevalence of *Salmonella* infection is largely unknown. Many fatal *Salmonella* infections are usually mistaken for malaria [5]. Over the years, Ghana has recorded high numbers of cases of typhoid fever which is ranked among the top twenty causes of outpatient morbidities and accounted for 1.2%, 1.7%, and 1.3% of hospital admissions respectively in 2015, 2016, and 2017 [6].

Salmonella Typhi and Paratyphi are transmitted by the faecal-oral route when a person ingests infected faecal matter in food or water. Chronic human carriers or convalescents are the most important reservoirs of infection. The most important known sources of transmission are carriers who handle food [7].

The organisms colonize the small intestine following ingestion. They then invade the gastrointestinal mucosal surface and spread throughout the body through the reticuloendothelial system especially the liver, spleen, and bone marrow [8]. Clinical disease manifestation correlates with multiplication and subsequent bacteraemia. Long-term chronic faecal shedding of the organisms occurs as a result of harbouring of the organism in the gall bladder [9]. The clinical symptoms of typhoid fever include sustained fever, severe headache, malaise, anorexia, non-productive cough, and abdominal symptoms, which include diarrhoea, nausea, vomiting, and abdominal pain. Typically, the abdominal pain is diffuse and poorly localised but may become occasionally intense in the right iliac fossa, mimicking appendicitis. [10]. The disease is usually complicated by encephalopathy, intestinal perforation, and gastrointestinal haemorrhage [2].

There are several approaches to the diagnosis of typhoid fever. These are aimed at either directly detecting bacteria or products of the bacteria such as their antigens or measuring the host immune response to the infection [11, 12]. Diagnoses of typhoid fever are usually done using clinical signs and symptoms, serological markers, bacterial culture, and DNA amplification (PCR) [13]. Diagnoses of typhoid fever are usually done using clinical signs and symptoms, serological markers, bacterial culture, and DNA amplification (PCR) [13]. Diagnosis of typhoid fever on clinical presentations alone is difficult, as the disease presentation is similar to that of diseases such as malaria and is easily misdiagnosed without

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laboratory confirmation, especially during the first week of the infection [14]. World Health Organization recommends that the gold standard for the diagnosis of typhoid fever is the isolation of *Salmonella* Typhi or Paratyphi from blood, stool, or bone marrow samples from patients suspected of having infection before treatment is commenced. However, in settings with limited resources that lack basic laboratory facilities for culture, this may pose a major challenge. Additionally, culturing is time-consuming, and requires several days for the causative organisms to be isolated and identified [11]. Even though blood culture is the mainstay of typhoid fever diagnosis, it is known to have low sensitivity, being positive in approximately 40–60% of suspected cases [15, 16]. Thus, a rapid and sensitive diagnostic test for the detection of Salmonella Typhi would be very helpful in the diagnosis and management of the disease.

Rationale

Over the years, many serology-based rapid diagnostic tests for typhoid fever have been manufactured and made commercially available. Several studies have been conducted to evaluate these commercial RDTs for their performance. Interestingly, none of these tests yielded satisfactory results when validated in different endemic settings [17]. Also, most studies conducted so far on the performance of typhoid RDTs have used carefully selected participants who meet well-defined criteria suggestive of the disease. However, in real-world conditions, many patients present without the classical symptoms and are usually referred to the laboratory for a rapid diagnostic test. There is therefore the need to evaluate the performance of newer RDTs that are commonly being used in an endemic setting such as Ghana.

Aim

This study thus sought to evaluate the performance of three of such new RDTs on the Ghanaian market among participants suspected of typhoid fever by attending clinicians.

Objectives

Specifically, the study had objectives that included determining the diagnostic values of three different typhoid RDT kits (Go Check, Jus Chek, and Green Life) using blood culture as the gold standard and assessing the potential risk factors for acquiring typhoid fever among participants.

METHODOLOGY

Study Design

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This was a comparative cross-sectional hospital-based study conducted from January 2021 to June 2021.

Setting

The study was conducted at the University of Cape Coast (UCC) Hospital which is located in the northern part of Cape Coast, in the Central Region of Ghana. UCC Hospital receives patients from Cape Coast and other parts of the Central Region of Ghana.

Participants

The study involved patients who visited the out-patient department (OPD) of UCC and were referred to the laboratory unit by the attending clinician on suspicion of having enteric fever.

Inclusion criteria

All patients 18 years old and above who have been suspected of enteric fever by an attending clinician and asked to conduct typhoid rapid diagnostic tests were included in the study.

Variables

The main outcome variable is typhoid positivity. The exposure variables include age, sex, educational level, marital status, source of drinking water, habits of eating raw meat, drinking raw milk, eating raw vegetables, and proper hand hygiene.

Quantitative variables

Age was the only quantitative variable included in this study. Age was conveniently grouped into three categories. These were 18 to 30 years, 31 to 60 years, and more than 60 years.

Bias

In order to reduce the possible coercive influence of clinicians on patients' acceptance to be part of the study, the recruitment was done by 2 trained research assistants who approached the patients whilst they were waiting for their turn at the laboratory.

Study Size

The sample size was calculated using the formula described for a single population proportion for cross-sectional studies. The prevalence estimate of the sample size was assumed to be 50%. Using a 95% confidence level and a 5% margin of error, the minimum sample size was 384 participants.

Sampling technique and Recruitment

Since about 15 patients report every day for typhoid test at the laboratory, a systematic random sampling technique was used to recruit 5 consented patients into the study using a sampling interval of 3 every day. Upon consent, participants were interviewed using a structured questionnaire before entering the laboratory for their samples to be taken.

Data Sources / Measurements

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Data was obtained through the administering of a questionnaire and the performance of rapid diagnostic tests, blood and stool cultures, blood film for malaria parasites, and PCR.

Questionnaire survey

Participants of the study were interviewed by trained research assistants using a structured questionnaire. The data collection instrument consisted of four sections. Section A aimed to collect data on participants' socio-demographic factors such as age, sex, level of education, family size, marital status, and residence. Section B collected data on possible risk factors for typhoid such as hand washing practice after visiting the bathroom (yes/no), use of toilet (yes/no), source of drinking water (pipe, stream/river), the habit of drinking raw milk (yes/no), the habit of eating raw meat (yes/no), and habit of eating raw vegetables (yes/no). Section C collected data on patients' symptoms whilst Section D was used to record the results of the various laboratory investigations performed.

Sample Collection and Analysis

About 7 ml of venous blood sample was collected from each patient for the performance of rapid diagnostic tests (all the three RDT kits were used to test each sample), blood culture, and polymerase chain reaction. Out of the 7 ml of blood collected, 3 ml was used for blood cultures, 2 ml was used for DNA extraction, and 1 ml for the RDT tests. Also, about 1 g of fresh stool specimen was obtained from each participant. The rapid diagnostic tests, and blood and stool cultures were conducted at the University of Cape Coast Hospital's laboratory by trained laboratory technologists. The PCR was conducted at the laboratory of the Department of Medical Laboratory Science of the University of Cape Coast by an expert in molecular diagnosis. Blood samples for PCR were frozen prior to transportation to the laboratory.

Rapid diagnostic testing

Rapid diagnostic tests were performed using Go Check, Jus Chek, and Green Life Typhoid RDT kits. These are the main RDT kits being used by health facilities in Cape Coast in the diagnosing of typhoid fever. The rapid diagnostic tests were performed per the manufacturer's instructions.

Stool Culture

Participants were provided with screw-capped containers for fresh stool specimens. Stool samples were inoculated onto *Salmonella Shigella* Agar (SSA). SSA was used for the isolation, cultivation, and differentiation of Gram-negative enteric microorganisms from both

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clinical specimens. The plates were incubated at 37°C for 24 hours. Isolation of *Salmonella* Typhi in stool culture will indicate an infection. The presence of growth was followed by a motility test to detect the presence of motile rods. Then, a Grams stain procedure was performed to detect the presence of Gram-negative bacilli (rods). This was followed by a biochemical test using Kligler iron agar (KIA) to read acid production of the slant, gas, and H_2S production. The presence of a red slant, yellow butt, weak H_2S reaction (black colony centers), and no gas indicated a positive KIA test.

Blood Culture

Venous blood (3 ml) drawn aseptically from each participant into the blood culture tube was inoculated into 30 ml of brain heart infusion broth (Oxoid, Basingstoke, United Kingdom) containing 0.05% sodium polyanetholesulfonate (Sigma, Poole, United Kingdom). A minimum blood-to-broth ratio of 1 to 10 was maintained. Blood culture broths were then incubated for 7 days, and subcultures were performed at 24 hours and after 7 days. All bottles were examined daily, and if a bottle showed visible signs of growth, subculture onto sheep blood agar, McConkey agar, and Salmonella or Shigella agar were performed. Bacterial isolates were identified by standard biochemical tests (glucose, lactose fermentation and production of gas, indole and H_2S production, citrate utilization, motility, and ability to split urea).

DNA extraction

Pure cultures of *Salmonella* Typhi were used for the standardization of PCR. These were sub-cultured on nutrient agar and verified by biochemical and serological tests. DNA was extracted from the cultures using the chloroform: isoamyl alcohol isolation method as described by Prabagaran, Kalaiselvi et al. (2017) [18].

Polymerase Chain Reaction

Touch-down PCR was carried out using *Salmonella* Typhi-specific primer pairs (TATGCCGCTACATATGATGAG and TTAACGCAGTAAAGAGAG) and primer pairs that amplify *Salmonella enterica* isolates (GTGAAATTATCGCCACGTTCGGGCAA and TCATCGCACCGTCAAAGGAACC) in a total reaction volume of 20.0 µl containing 3.0 µl DNA template and 9.0 µl of Tag 2X master mix (New England Biolabs), 0.5 µl MgCl2, 0.7 µl of 10 µmol l-1 forward primer, 0.7 µL of µmol l-1 reverse primer, and 6.1 µl ddH2O. The reaction mixture was subjected to 25 cycles of 2 min denaturation at 94 ^{o}C, annealing at 65 ^{o}C for 1 min 15 sec and elongation at 72 ^{o}C for 2 min followed by 20 cycles of initial denaturation of 94 ^{o}C of 2 min, annealing at 55 ^{o}C for 1 min and elongation at 72 ^{o}C for 2

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min. A final elongation of 10 min duration was done at 72 ^{o}C. Amplified products were detected by electrophoresis using 1.5% agarose gel (Sangon Biotech, PRC) in TAE buffer and visualized with ethidium bromide staining [18].

Microscopy for malaria parasites

About 1mL of participants' blood was used to prepare thick and thin films which were stained with Giemsa.

Statistical Methods

Quantitative data generated from questionnaire survey and laboratory investigation were entered into Excel spread sheet (Microsoft Corporation). Then coded data was analyzed using SPSS version 25. The data was summarized using descriptive statistics. The prevalence of enteric fever was calculated for Go Check, Jus Chek, and Green Life Typhoid RDT kits. Associations between categorical variables such as sex, educational levels, and typhoid positivity by RDTs were assessed using Pearson's Chi-square. All Pearson's Chi-square tests were considered significant if the P-value is ≤0.05 at a confidence interval of 95%.

Ethical consideration

Ethical clearance was obtained from the Institutional Review Board of University of Cape Coast (UCCIRB). Permission was obtained from the management of the University of Cape Coast Hospital before sampling began. Samples collected from participants were handled solely by trained laboratory technologists. Left over samples were autoclaved at 121°C for 15 minutes and then buried.

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The study posed minimal health risks to participants except for transient pain that was felt during blood collection. Sterile techniques and disposable, single-use materials were used at all times to avoid any infections. Written informed consent was obtained from each participant after an explanation was given by data collectors regarding the purpose of the study. All members of the research team had no conflict of interest in relation to the study.

RESULTS

Participants

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A total of 400 patients suspected of typhoid fever by attending physicians with requested typhoid tests were involved in the study.

Descriptive data on participants

Out of this number, 258 (64.5%) were females. Half of the participants (50.0%) were in the 18-30 years age group, followed by the 31-60 years age group (42.0%). Majority, 214 (53.5%) of the participants were single with about 160 (40.0%) being married. Many, 254 (63.5%) of the participants had received tertiary education with only 15 (3.8%) having no formal education. Table 1 shows the distribution of the demographic characteristics of the participants.

Table 1. Distribution of the demographic characteristics of participants.

Characteristic	*Frequency*	*Percentage*
Age group (years)		
18-30	200	50
31-60	168	42
>60	32	8
Sex		
Male	142	35.5
Female	258	64.5
Educational level		
Basic	44	11
Secondary	79	19.8
Tertiary	254	63.5
Vocational	8	2
No formal	15	3.7
Marital status		
Single	214	53.5
Married	160	40
Widowed	17	4.3
Divorced	9	2.2

Outcome Data

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Stool and Blood Culture

There was no growth for *Salmonella* and *Shigella* species when blood and stool of all participants were cultured. However, *Citrobacter* and *Proteus vulgaris* were cultured in 13(3.3%) and 10(2.5%) of participants respectively using stool samples. In blood samples, there was a 7(1.8%) growth of *Staphylococcus aureus*.

Polymerase Chain Reaction

No *Salmonella* Typhi were detected in the blood of participants. However, in 7(1.8%) participants, other *Salmonella* species were detected.

Participants who tested positive to at least one RDT were 171. This gives an overall prevalence of enteric fever among the study population using all RDTs as a composite tool as 42.8%.

Typhoid RDTs

All participants were tested using each of the RDTs. A positive test was defined as detecting the presence of any of the following: IgG, IgM or both. The Go Check RDT gave the highest positive proportion of 27.8%, followed by Jus Chek (25.0%) and Green Life (22.0%). Table 2 shows the distribution of the test results using the three RDTs among participants.

Table 2: Distribution of the various sources of drinking water among respondents by sex

Source of drinking water	*Male*	*Female*	*Total*
Pipe borne	28	34	62
Stream / river	2	3	5
Bore hole / Well water	0	4	4
Packaged water	94	189	285
Pipe borne and packaged water	18	28	46
Total	142	258	400

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Blood film for malaria parasites

No malaria parasites were detected in any of the blood samples using microscopy.

A chi-square test of independence was performed to examine the relation between test results produced by the different RDTs. There were significant differences between the results; Go Check and Jus Chek (X^2=39.10, p<.001), Go Check and Green Life (X^2=125.62, p<.001), Jus Chek and Green Life (X^2=44.75, p<.001).

Kappa Coefficient was used to measure the level of agreement between the various RDTs. Generally, all the RDTs showed weak levels of agreement with each other; Go Check versus Jus Chek (Kappa- 0.32), Go Check versus Green Life (Kappa-0.55) and Green Life versus Jus Chek (Kappa- 0.33).

Chi-square tests of independence as were performed to ascertain the relationships between several independent variables and the dependent variable of obtaining a positive test result using all three RDTs as a composite tool. The overall prevalence was found to be significantly associated with marital status (X^2=10.82, p=.013) and age group (X^2=13.4, p=0.001). The sex (X^2=0.075, p=0.78) and educational level (X^2=3.69, p=0.46) of participants were not significantly associated with positive test results. The following exposures were all not significantly associated with a positive test result; source of drinking water (X^2=7.27, p=0.122), the habit of eating raw vegetables (X^2=1.52, p=0.217), the habit of eating raw meat (X^2=1.75, p=0.185), the habit of drinking raw milk (X^2=0.709, p=0.265), and habit of proper hand hygiene (X^2=0.265, p=0.606).

When used in pairs in the diagnosis of enteric fever, Jus Chek and Go Check gave the highest prevalence of 39.8%, followed by Jus Chek and Green Life (35.0%) and Go Check and Green Life (33.0%) as shown in Table 3.

Table 3. Distribution of the test results using the three RDTs among participants

Typhoid	*Positive*			*Negative*
RDT	IgG	IgM	IgG +IgM	
Go Check	21	68	22	289
Jus Chek	2	57	41	300
Green	28	50	10	312

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The source of drinking water for majority of respondents was commercially sold packaged water, 283 (70.8%), followed by pipe borne water, 62(15.5%). Only 1.0% of respondents used Bore hole/Well water as their main source of drinking water. Table 4 shows the distribution of the various sources of drinking water among respondents by sex. There was no significant difference (X^2=5.7, p=.224) between the choice of drinking water among males and females.

Table 4. prevalence of enteric fever among participants using different combinations of RDTs

Typhoid RDTs	Positive	Negative
Jus Chek and Go Check	159	241
Jus Chek and Green Life	140	260
Go Check and Green Life	132	268

When asked if they have the habit of eating raw vegetables, majority, 241 (60.3%) answered yes. Only 29(7.2%) said they have the habit of eating raw meat. Most respondents 359(89.8%) did not have the habit of drinking raw milk. When asked if they regularly practiced proper hand hygiene, the majority of participants 342 (85.5%) answered yes.
Even though only 51 (12.8%) reported taking antibiotics within a week before presentation, as many as 192 (48.0%) of respondents had taken antibiotics within two weeks prior to the study.

Other Analyses

Symptoms Profile

Most participants 107 (26.8%) presented with a combination of fever, body pains, headache, chills and abdominal pain. When temperatures were measured none of the participants had a temperature of 38^0C and above. As many as 21 (5.3%) who presented for typhoid tests did not have any complaints. Further checks in their hospital records revealed that those tests were due to patients requests and, in some cases, clinicians wanted to find out if patients had recovered from earlier typhoid infections.

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DISCUSSION

Typhoid fever continues to be responsible for a high incidence of morbidity and mortality globally. In developing countries like Ghana, enteric fever remains a public health challenge because of poor sanitation issues. Early and accurate diagnosis of typhoid fever still remains a challenge in developing countries due to resource constraints. Accurate diagnosis of enteric fever at an early stage also helps to identify individuals who may serve as potential carriers, who may be responsible for acute enteric fever outbreaks [10].

This research sought to evaluate and compare three commonly used commercial serological immunochromatographic test (ICT) kits among patients suspected of enteric fever by attending physicians. Key results in this study were that even though a number of participants tested positive for typhoid RDTs, none was positive on blood and stool culture as well as using PCR. The finding of no participant testing positive for *Salmonella* Typhi and *Salmonella* Paratyphi on blood and stool culture is rare and may be due to several reasons. It may simply mean that none of the participants actually had active typhoid fever. Unlike most studies, this study did not use participants who were selected based on meeting well-defined clinical criteria. Participants were recruited based on their being suspected by the attending physicians of having typhoid fever. When their presenting symptoms were reviewed, none presented with the classical symptom of a high fever. This may be due to a prior antibiotic intake and or the use of antipyretics. Because of the non-specific ways typhoid fever presents, clinicians in endemic areas like Ghana do not always rely on the typical symptoms [10]. Studies have shown that maximum blood culture yields are obtained if samples are taken when bacteremia is high usually in the first three weeks of infection [19]. Another factor known to affect culture results is the use of antibiotics prior to sample collection [20]. Many (48.0%) participants had antibiotic exposure within two weeks before presentation. This high antibiotic use among the respondents might have influenced the outcome of blood culture, as indiscriminate antibiotic use has been well documented amongst Ghanaians by several publications [21, 22]. These factors commonly occur under real-world conditions. Bousefield and colleagues also found that blood culture performed poorly under real-world conditions and concluded that it cannot be considered a suitable gold standard [23]. Some other similar studies have recorded a low prevalence of typhoid fever using blood culture, especially in patients who may have been on antibiotics. A study in Pakistan by Munir et al recorded only 1% sensitivity among participants who were on antibiotics [24]. Similarly, population-based

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studies in South Asia and Africa have reported typhoid blood culture positivity rates between 2.6% and 6.4% [25, 26].

Blood culture sometimes does not identify the bacteria even if it exists in the blood because of many procedural and technical issues. The use of blood culture as a gold standard for the diagnosis of enteric fever is limited in utility due to its low sensitivity even when there is no prior exposure to antibiotics [14]. Additionally, the type of media used in culturing the bacteria is known also to influence the results. Some studies have found the addition of Ox Bile, 2.4% in enrichment media very useful in releasing bacteria from the blood intracellular compartment and has been shown to produce an almost two-fold rise in bacteria numbers. [27]. Some commercial culture bottles may contain resins and other antibiotic neutralizing substances (beads), thereby increasing the pathogen recovery in patients on antibiotic therapy compared with non-resin base culture media. The growth of bacteria in our study might be inhibited probably due to the absence of the antibiotic-binding substances in the media used, the volume of blood inoculated and the body temperature of participants, and the duration of illness when sampling was done. This drawback, however, could have been negated had the research employed the use of automated blood culture systems whose culture media possess additives that inactivate any antimicrobials that are present in the blood specimens added to them. These culture media are however not routinely used in resource-constrained settings.

Again, systematic reviews have demonstrated blood volume to be an independent predictor for blood culture positivity, with the positivity of blood culture test increasing by 3% for every additional milliliter of blood inoculated [26]. Other studies also indicated that blood culture yield could be improved to the equivalence of that of a bone marrow culture with a 90% sensitivity when about 10 mL or more of blood is inoculated [20]. Due to ethical reasons only 3mls of blood was used in this study and this may have affected the yields recorded with the blood cultures.

A number of serological test kits that detect antibodies against *Salmonella typhi* antigens in the blood are available with sensitivities reported to vary between 43% and 100% and specificity of 58-100% [23]. The commercially available typhoid serological tests commonly evaluated in published studies detect antibodies in enzyme-linked immunosorbent assay (ELISA) format. This study however focused on three commonly used typhoid test kits in Ghana that are based on immunochromatographic lateral flow assay format.

Despite our study having none of the samples giving a positive *Salmonella* Typhi result on blood culture, the three RDTs yielded between them, 51 positives for IgG only, 175 positives

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for IgM only and 73 positives for both IgG and IgM. Rapid diagnostic tests are known to have several limitations and must therefore be interpreted cautiously especially in typhoid endemic areas. This is because, false positives could arise due to the presence of background agglutinins and antibodies to *Salmonella* Typhi antigens [28]. Studies have shown that the presence of antibodies in blood does not always correlate with bacteraemia. Antibodies are known to persist in the body for several months, even years, posing a challenge in distinguishing between acute and chronic infections [29]. A study that looked at antibody decay profiles, found that IgG antibodies could persist in blood for over a year, whilst IgM levels appeared to decline after 3 to 4 months [30]. This therefore means that probably all those found to be antibody positive in this study may have been infected sometime in the past and may not have the bacteria present at all or in volumes inadequate to be cultured.

Since the use of blood culture as the gold standard for diagnosis of typhoid fever and the evaluation of rapid diagnostic test kits may be problematic [3, 31], a negative blood culture, therefore, does not exclude enteric fever [10]. A result that may seem false positive using blood culture as the gold standard may actually be a true positive result. This assertion is collaborated by a study by Oslen and his colleagues [32]. Similarly, in a study by Mogasale and his colleagues, it was noticed that 2 out of 5 people infected with *Salmonella* Typhi would remain undiagnosed if blood culture was deployed as the only diagnostic test [33]. This has led some authors to conclude that it is possible that RDTs may be more sensitive than blood cultures [14].

The use of PCR for the early diagnosis of typhoid fever is still a grey area which requires further studies. In our study, no *Salmonella* Typhi was detected by PCR even though other serotypes of non-typhoidal Salmonella were detected. The low positivity of the PCR used could be attributed, among other reasons, to the time of sampling that may affect the number of bacteria present in blood, the type of PCR used, since Monoplex PCR have been identified to yield high positivity rate than multiplex ones and the possible presence of PCR inhibiting factors. Several studies have shown that PCR results are dependent on the number of bacteria present in samples [34]. An earlier study showed that, PCR performs best in the first few days of fever [35]. A similar observation was made in a study conducted in Cambodia [36]. Since most of the participants in this study did not have a fever and most probably convalesced, it is likely that there will not be enough bacteria genetic material present if any to be detected through PCR. Regarding the type of PCR used, studies have shown that Real-

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Time PCR is more robust than Conventional PCR carrying the advantages of inducing less variability in assays and interpretations [37]. Also, many studies including this current study, have used nested primers in an attempt to improve sensitivity, yet nested PCR has known inherent problems such as unspecified applications and contaminations [37].

Our study also revealed weak levels of agreement between the various diagnostic tests used. A few head-to-head evaluations of comparative performance of diagnostic testing actually exist and just as was established in our study, previous reviews of diagnostic tests have also found a high level of variation in testing methods for typhoid fever globally and a lack of a single applicable gold standard is a challenge that is particularly acute given the low sensitivity of the most common reference standard, blood culture.

Many (171) participants in our study tested positive for at least one of the diagnostic test kits used. Hence, using all three RDTs as a composite test, an overall prevalence of 42.8% was realized. When used in pairs, the prevalence of 39.8%, 35.0%, and 33.05% were realized for Jus Check and Go Check, Jus Chek, and Green Life, and Go Check and Green Life respectively. A review conducted by Arora and colleagues found that diagnostic performance of typhoid tests improved when performed in combinations [38]. Storey, asserted that if no single reference standard test exists, using a composite reference standard could improve diagnostic test accuracy estimation [39]. The findings of this study suggest that when two or more rapid diagnostic tests are combined into a composite test, they may increase the likelihood of diagnosing typhoid among suspected cases.

This study did not find any significant difference among the sexes with regard to typhoid positivity. This is contrary to findings in an earlier Ghanaian study by Fusheni and Gyaw [40] where they picked females to be the most susceptible group (55.6%) in their study. That notwithstanding, a study done in the Kasese District of Uganda from 2008 to 2009 found that prevalence was higher (59.0%) among males [41]. Also, the study of Ousenu and his colleagues [42] found typhoid infection to be more prevalent in males (18.1%) than females (10.4%). The disparate prevalence in the various sex could be due to differences in proportion of sexes in the various studies. Differences in the diagnostic tests used, and recruitment criteria among others could also account for the variation in results.

Limitations of study

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The study was limited by the use of patients suspected of typhoid fever by clinicians instead of patients who met a clear clinical algorithm for typhoid fever. Also, the finding of no blood culture confirmed cases meant no gold standard was available to assess the performance of the various RDT kits used in the study. The findings of this study suggests that in the absence of confirmatory tests like blood cultures, two or more rapid diagnostic tests when combined into a composite test, may increase the likelihood of diagnosing typhoid among suspected cases.

Generalisability

Even though the study involved participants in the Cape Coast metropolis, it involved only the University of Cape Coast Hospital, and therefore the findings cannot be generalized to what pertains in other health care facilities in Cape Coast and in Ghana as a whole.

CONCLUSION

This study has shown that under real-world conditions where patients present late without classical symptoms and signs and would have taken antibiotics, blood culture and PCR might not be reliable in the diagnosis of typhoid fever. In routine clinical practice, the use of typhoid RDTs especially in combinations could be helpful in diagnosing enteric fever.

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Recommendation

Diagnosing typhoid fever in such settings among suspected cases could be improved by combining more than one typhoid RDT kit and the use of automated blood culture systems whose culture media possess additives that inactivate any antimicrobials that are present in the blood specimens added to them. Future studies should focus on the detection of antigens rather than antibodies. A rapid diagnostic test that is able to detect antigens of Salmonella Typhi in clinical specimens such as blood and stool could provide direct evidence of an active disease.

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