Diagnosing *Salmonella enterica* Serovar Typhi Infections by Polymerase Chain Reaction Using EDTA Blood Samples of Febrile Patients From Burkina Faso

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**Background.** Globally, there are an estimated 22 million cases of *Salmonella enterica* serovar Typhi infection each year. However, this figure is likely to be an underestimate due to the low sensitivity of blood culture in *S.* Typhi diagnosis. The aim of this study was to diagnose *S.* Typhi by conventional polymerase chain reaction (PCR) using patient’s blood preserved with ethylenediamine tetraacetic acid (EDTA).

**Methods.** From April 2012 to September 2013, typhoid fever surveillance was conducted in Polesgo and Nioko, 2 dry slum areas in Ouagadougou, Burkina Faso. Blood culture was performed for febrile patients using an automated blood culture system. Additional blood was collected in EDTA tubes from those patients and preserved at −80°C. DNA was extracted from EDTA blood and PCR was performed to identify presence of *S.* Typhi. Randomly selected PCR products were further sequenced to identify *S.* Typhi–specific amplicons.

**Results.** Of 1674 patients, *S.* Typhi was isolated from 18 (1.1%) individuals by blood culture. EDTA blood was collected from 1578 patients, of which 298 EDTA samples were tested by PCR. *Salmonella* Typhi–specific DNA was identified in 44 (14.8%) samples. The sensitivity of *S.* Typhi–specific PCR from EDTA blood was 89% (74%–100%) among the blood culture–positive cases. Sixteen *S.* Typhi–positive PCR products were sequenced, and 13 retrieved the sequence of a *S.* Typhi–specific amplicon.

**Conclusions.** These findings suggest that blood culture–based diagnoses of *S.* Typhi underestimate the burden of typhoid fever in Burkina Faso. PCR could be considered as an alternative method for the identification and diagnosis of *S.* Typhi in blood samples.

**Keywords.** Burkina Faso; *Salmonella*; *Typhi*; PCR; sensitivity.

Globally, there were 22 million febrile cases attributed to infection by *Salmonella enterica* serovar Typhi that led to 217 000 deaths in 2000 [1]. This estimation was adjusted for blood culture sensitivity based on a conservative assumption of 50% [1]. The sensitivity of blood cultures for *S.* Typhi may be affected by several factors including antimicrobial pretreatment, the volume of blood collected [2, 3], the duration and conditions of sample transportation [4], laboratory facilities, and method of pathogen identification. Few alternative diagnostics for the detection of *S.* Typhi are available. The Widal test has been one of the most commonly used diagnostics to date despite its limited sensitivity of only 39% [5, 6]. Typhidot and Tubex, both serological assays, are also available for use [7, 8], and have a reported sensitivity of around 70% [9].

Recently, polymerase chain reaction (PCR) has shown great potential as a diagnostic tool for the detection of pathogens, as the method has a high degree of sensitivity and specificity [10–12]. However, the identification of bacterial DNA directly from blood samples has also been previously reported to show reduced sensitivity [13]. Several attempts have been made to improve the sensitivity of *S.* Typhi detection from blood. Use of ox bile for blood cell lysis, followed by micrococcus nuclease to remove human DNA, improves the detection of DNA from this intracellular bacteria [14]. An additional study has shown that use of erythrocyte lysis buffer before DNA extraction improves the detection rate of *S.* Typhi by both conventional PCR and real-time PCR [15]. However, both studies have demonstrated their methods on spiked blood samples rather than patient samples. Application of these methods may also face challenges in highly endemic areas and settings where laboratory capacity is limited. Feasibility issues are compounded by evidence that the presence of *S.* Typhi in the blood of most patients is very low [2]. As a result, the pretreatment of preserved blood samples prior to PCR amplification to decrease human DNA may also decrease the concentration of *S.* Typhi DNA. The objective of this study was to detect *S.* Typhi by conventional PCR using preserved ethylenediamine tetraacetic acid (EDTA) blood from febrile patients in Burkina Faso and to observe the
frequency of detected S. Typhi by PCR in comparison to blood culture.

METHODS

This study was conducted as part of the Typhoid Fever Surveillance in Africa Program (TSAP), a multicenter, multicountry surveillance network including 10 countries in sub-Saharan Africa [16]. This substudy included 2 sites in Burkina Faso, Pологe, and Niïko in the north of Ouagadougou. These sites form a component of the International Network for the Demographic Evaluation of Populations and Their Health in Developing Countries (INDEPTH) [17] and are supported by a comprehensive health and demographic surveillance system that is run by the University of Ouagadougou, Institut Supérieur des Sciences de la Population.

Blood Collection and Bacterial Culture

Blood was collected from all enrolled patients for bacterial culture and sample preservation. The volume of blood added to the blood culture bottle was 1–4 mL from the children and 8–10 mL from the adults. The remainder of blood (<1 mL), when available, was preserved in an EDTA tube. The collected specimens were transported to the Medical Center Protestant Schiphra, Ouagadougou, Burkina Faso, for further processing. EDTA blood was stored in a freezer at –80°C, and bacterial culture was performed using an automated blood culture system (BacTAlert, bioMérieux, Durham, North Carolina). Signal-positive samples were plated on MacConkey agar, Columbia agar enriched with 5% sheep blood, and chocolate agar (Oxoid, Hampshire, United Kingdom). For the identification of Salmonella enterica, API20E biochemical test (bioMérieux, Durham, North Carolina) and the Oxoid Salmonella Latex Test (Oxoid, Hampshire, United Kingdom) were performed in parallel. All isolates were stored at –80°C at the study laboratories. All EDTA blood tubes and bacterial isolates were transported on dry ice to the Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany. All S. Typhi isolates were confirmed by PCR [18].

EDTA Blood Samples

From all collected EDTA blood samples, 300 were selected randomly using a random number generator in Microsoft Excel (Microsoft Corporation, Redmond, Washington). Additionally, EDTA blood samples from all blood culture-positive S. Typhi cases were included for investigation in this study.

DNA Extraction

A QIAGEN FlexiGene DNA kit (Qiagen, Hilden, Germany) was used for the scalable extraction of DNA from 200–500 µL of EDTA blood. DNA was extracted following the manufacturer’s specifications with a minor modification to the DNA suspension step. In brief, 500 µL of EDTA blood was lysed with lysis buffer, and the proteins were digested with proteolytic enzyme. DNA was precipitated using isopropanol, washed in 70% ethanol, dried, and then resuspended in 100 µL of hydration buffer (Tris-Cl, pH 8.5). The volume of DNA hydration buffer was used depending on the available volume of EDTA blood for extraction (one-fifth blood volume) to keep the DNA suspension ratio the same. If the EDTA blood volume was <200 µL, the sample was excluded from further analysis in this study. The suspended DNA from all samples was then stored at –20°C until PCR amplification was performed.

DNA Amplification

The DNA extracts from EDTA blood were amplified with S. Typhi-specific primers and targeted the sty1599 gene, as described by Park et al [19]. QIAGEN multiplex PCR mastermix (Qiagen, Hilden, Germany) containing 3 mM of magnesium chloride and Q-solution was used to reduce nonspecific bands from highly concentrated human DNA. The final concentration of the primer pair was 0.10 µmol L⁻¹ in PCR reactions. Ten microliters of sample was added to each PCR mixture, with a total volume of 40 µL per reaction. The PCR conditions were denaturing (94°C for 30 seconds), annealing (58°C for 90 seconds), and extension (72°C for 45 seconds) for 35 cycles, followed by a final extension (72°C for 10 minutes).

Sensitivity of the PCR

All blood culture-positive S. Typhi cases were considered as true positives in this study. We considered only the blood culture-positive S. Typhi cases as reference, as the sensitivity of blood culture is low [1]. Therefore, the sensitivity of PCR was calculated among the cases where S. Typhi was isolated by blood culture.

Sequencing of the PCR Amplicons

Randomly selected PCR-positive products were sent to Eurofins Scientific (Hamburg, Germany) for purification and bidirectional

Table 1. Study Profile and Diagnosis of Salmonella Typhi, Burkina Faso, April 2012–September 2013

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Ouagadougou</th>
<th>Niïko</th>
<th>Pологe*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient demographics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients recruited</td>
<td>918</td>
<td>756</td>
<td></td>
</tr>
<tr>
<td>Children aged &lt;15 y, No. (%)</td>
<td>710 (77)</td>
<td>517 (68)</td>
<td></td>
</tr>
<tr>
<td>Median age, y (25th, 75th percentile)</td>
<td>4 (1, 12)</td>
<td>7 (3, 21)</td>
<td></td>
</tr>
<tr>
<td>Sex distribution, female, No. (%)</td>
<td>467 (51)</td>
<td>404 (53)</td>
<td></td>
</tr>
<tr>
<td>Inpatients, No. (% of total recruited)</td>
<td>66 (7)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Blood culture results</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total BC performed, No.</td>
<td>918</td>
<td>756</td>
<td></td>
</tr>
<tr>
<td>BC positive for Salmonella Typhi, No. (% of total BCs performed)</td>
<td>5 (0.5)</td>
<td>13 (1.7)</td>
<td></td>
</tr>
<tr>
<td>EDTA-PCR results</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Total PCR assays performed, No.</td>
<td>154</td>
<td>144</td>
<td></td>
</tr>
<tr>
<td>Total positive for Salmonella Typhi, No. (% of total PCR assays performed)</td>
<td>28 (18)</td>
<td>16 (11)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: BC, blood culture; EDTA, ethylenediamine tetraacetic acid; NA, not applicable; PCR, polymerase chain reaction.

*This site had no inpatient facility; recruitment was performed only in outpatient department.
sequencing. Forward and reverse sequences were assembled using Seqscape software version 2.1.1 (Applied Biosystems, Waltham, Massachusetts). Recovered sequences were screened for S. Typhi–specific amplicons (accession number NC_003198.1).

**Ethical Approval**
This substudy was embedded in the TSAP study [16]. The TSAP study protocol was approved by the institutional review board of the International Vaccine Institute and the national ethical review board in Burkina Faso. Ethical approval included the secondary analysis of patient samples.

**RESULTS**
A total of 1674 febrile patients were enrolled from Polegso and Nioko between April 2012 and September 2013. Among these, 1227 (73%) were <15 years of age and 871 (52%) were female. The median age of the enrolled patients was 7 years (interquartile range [IQR], 3–21 years) in Polegso and 4 years (IQR, 1–12 years) in Nioko (Table 1).

Blood culture was performed for all 1674 recruited patients, and S. Typhi was isolated in 18 samples (1.1%). EDTA blood samples were collected from 1578 (94%) patients. From the randomly selected 300 EDTA blood samples, 298 (99%) had a sufficient volume (≥200 µL) to extract DNA for PCR amplification. Forty-four of the 298 (14.8%) DNA samples were positive for S. Typhi by PCR (Figure 1) of which 4 samples were additionally blood culture positive. One blood culture–positive EDTA sample was found to be negative by PCR amplification. The remaining 253 samples were negative by both PCR and blood culture.

From 13 blood culture–positive S. Typhi cases, which were tested additionally, 12 were positive by PCR. In total, PCR positively detected S. Typhi in 16 of 18 blood culture–positive cases (Table 2). Of those 18 blood culture–positive cases, PCR sensitivity was 89% (95% confidence interval, 74%–100%).

![Figure 1](https://academic.oup.com/cid/article-abstract/62/suppl_1/S37/2566536)
Sixteen of 44 (36.4%) samples that tested positive for S. Typhi through PCR (4/16 blood culture positive) were sent for DNA sequencing. DNA sequences were recovered from 13 of 16 (81.2%) PCR-positive cases. All 13 retrieved DNA sequences were identified as S. Typhi-specific amplicons. The remaining 3 cases did not provide a sufficient signal to read the sequence data. Sequencing data were not used for diagnosis or for the calculation of disease prevalence or sensitivity.

**DISCUSSION**

This study demonstrated that the use of conventional PCR assays to diagnose S. Typhi infections uncovered a number of positive cases in Burkina Faso that were not identified by blood culture diagnoses. This molecular diagnostic method increased the frequency of identified S. Typhi among the febrile patients from 1.1% to 14.8%. The data confirm the low sensitivity of blood culture and suggest that blood culture for typhoid fever surveillance underestimates the disease burden, although it has been widely accepted as the standard diagnostic technique [1]. Accounting for potential false-negative results from blood culture, the total number of true S. Typhi–positive cases was unknown. Thus, the specificity of PCR could not be calculated in this study. From the known blood culture–positive cases, only the sensitivity of PCR (89%) was calculated. The sensitivity of blood culture was not compared with PCR as the gold-standard method for the diagnosis of S. Typhi, bone marrow culture, was not performed here. However, previous studies reported a sensitivity of 85%–99% for the detection of S. Typhi in blood samples by nested PCR, compared with 30%–62% sensitivity for blood cultures [6, 20].

The low sensitivity of blood culture has been shown to be associated with prior treatment with antimicrobials and the long duration between the onset of disease and sample collection [2]. Both procedures decrease the likelihood of growing S. Typhi in bacterial cultures. Use of low volume of blood for bacterial culture also affects sensitivity [3]. Bone marrow culture for the diagnosis of typhoid fever has been reported to have a higher sensitivity than blood culture [21]; however, the sample collection procedure is highly invasive and requires skilled medical personnel. Any culture-based diagnoses require a minimum of 2 days for isolation and identification of the pathogen. In contrast, PCR diagnosis can be completed in 7–8 hours. In addition, the volume of blood that is required for PCR is much less than what is recommended for blood culture. This is particularly important for low-income countries, where many children are often malnourished or underweight and reluctant to provide blood. In this study, 200–500 μL of blood was used for the DNA extraction and 3000–5000 μL of blood was drawn for blood culture in most cases. For each PCR reaction, 10 μL of sample DNA extracted from 50 μL of blood was used.

The study only analyzed 19% of EDTA samples collected in Burkina Faso. PCR of all EDTA samples may improve the precision of site-specific disease frequency estimates. The DNA sequence analysis was performed on only 36% of the PCR-positive amplicons. *Salmonella* Typhi–specific DNA was identified in all 13 sequence-recovered cases, indicating the presence of S. Typhi in PCR-positive samples.

The frequency of typhoid fever measured by conventional PCR was high among febrile patients (18% in Nioko and 11% in Polesgo), and low for blood cultures (0.5% in Nioko and 1.7% in Polesgo). Based on the blood culture results, annual incidence of S. Typhi calculated in the TSAP study (Marks et al, unpublished data) was 107 and 402 per 100 000 in Nioko and Polesgo, respectively. Despite this underestimation of incidence due to low sensitivity of blood cultures, the World Health Organization has classified Burkina Faso as a high-incidence region for S. Typhi [1]. The findings from this study suggest that the true burden of S. Typhi cannot be estimated by blood culture alone. We recommend using PCR in addition to standard blood culture for the routine diagnosis of suspected typhoid fever cases in hospitals, as well as extending this method to S. Typhi surveillance programs in other low-income countries.

**Notes**

*Disclaimer.* The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. The findings and conclusions contained within are those of the authors and do not necessarily reflect the positions or policies of the Bill & Melinda Gates Foundation.

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