Validation and Identification of Invasive Salmonella Serotypes in Sub-Saharan Africa by Multiplex Polymerase Chain Reaction

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Salmonella enterica serovar Typhi and nontyphoidal Salmonella (NTS) cause the majority of bloodstream infections in sub-Saharan Africa; however, serotyping is rarely performed. We validated a multiplex polymerase chain reaction (PCR) assay with the White–Kauffmann–Le Minor (WKLM) scheme of serotyping using 110 Salmonella isolates from blood cultures of febrile children in Ghana and applied the method in other Typhoid Fever Surveillance in Africa Program study sites. In Ghana, 47 (43%) S. Typhi, 36 (33%) Salmonella enterica serovar Typhimurium, 14 (13%) Salmonella enterica serovar Dublin, and 13 (12%) Salmonella enterica serovar Enteritidis were identified by both multiplex PCR and the WKLM scheme separately. Using the validated multiplex PCR assay, we identified 42 (66%) S. Typhi, 14 (22%) S. Typhimurium, 2 (3%) S. Dublin, 2 (3%) S. Enteritidis, and 4 (6%) other Salmonella species from the febrile patients in Burkina Faso, Guinea-Bissau, Madagascar, Senegal, and Tanzania. Application of this multiplex PCR assay in sub-Saharan Africa could advance the knowledge of serotype distribution of Salmonella in the region.

Keywords. sub-Saharan Africa; Salmonella spp; serotyping; PCR.

Salmonella enterica serovar Typhi and nontyphoidal Salmonella (NTS) are the predominant cause of bacteremia both in adults and children in sub-Saharan Africa [1–3]. One prevention strategy to control these infections is effective immunization against predominant Salmonella serotypes. However, region-specific serotype data needed for the development of effective vaccine candidates and to decide on vaccine implementation are lacking. The standard serotyping method of Salmonella, the White–Kauffmann–Le Minor (WKLM) scheme, is costly, time consuming, and labor intensive [4] and thus is rarely applied in laboratories with limited resources, as in many African countries. Recently, polymerase chain reaction (PCR) methods have been established as a cost-effective and less laborious alternative to obtain serotyping-equivalent data of Salmonella strains [5]. In this study, we aim to validate and apply a multiplex PCR assay to identify common invasive Salmonella serotypes from sub-Saharan Africa.

This study was a part of the multicenter, multicountry Typhoid Fever Surveillance in Africa Program (TSAP) conducted in 9 healthcare facilities, located in Nikoko and Polesgo (Burkina Faso), Agogo (Ghana), Bandim (Guinea-Bissau), Imerintsiaka and Isotry (Madagascar), Pikine (Senegal), and 2 hospitals in Moshi (Tanzania) [6]. Patients with fever ≥38°C (tympanic temperature) were recruited from inpatient wards and/or outpatient departments.

Blood samples from study patients were collected for bacterial culture using automated blood culture systems (BACTEC, Becton Dickinson and Company, Gauteng, South Africa; or BacT/ALERT, bioMérieux, Zaltbommel, the Netherlands). Broth from positive blood culture bottles was plated on MacConkey agar, Columbia agar enriched with 5% sheep blood, and chocolate agar (Oxoid, Hampshire, United Kingdom). For the identification of Salmonella enterica, the Analytical Profile Index (API 20E) test (bioMérieux, Durham, North Carolina) was performed and confirmed by the Oxiid Salmonella Latex Test (Oxoid, Hampshire, United Kingdom). All Salmonella isolates were stored at −80°C until transported on dry ice to the Bernhard Nocht Institute for Tropical Medicine in Hamburg, Germany, and stored at −80°C until further processing.
DNA from the *Salmonella* isolates was extracted using the QIAamp DNA mini kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. All *Salmonella* isolates were tested by a multiplex PCR assay with previously described primers [5, 7–9] targeting *Salmonella enterica*, S. Typhi, *Salmonella enterica* serovar Typhimurium, *Salmonella enterica* serovar Enteritidis, and *Salmonella enterica* serovar Dublin using the Qiagen multiplex PCR kit (Qiagen, Hilden, Germany) as described by the manufacturer. The PCR conditions were adapted to 30 cycles of denaturation (94°C for 30 seconds), annealing (57°C for 90 seconds), and extension (72°C for 45 seconds), followed by a final extension of 10 minutes.

The multiplex PCR was validated using the blood culture–positive *Salmonella* isolates from Agogo, Ghana. On these isolates, the WKLM scheme of serotyping was also performed [10], which was used as the conventional standard method. In other TSAP study sites, the serotypes of *Salmonella* isolates were identified by the multiplex PCR. Serotypes that could not be identified by multiplex PCR were identified using the WKLM scheme of serotyping.

In total, 10,636 patients from 9 study sites were recruited. Among them, 7,157 (67%) were children <15 years of age, 5,382 (51%) were female, and 4,521 (43%) were admitted to the hospital. The local study site laboratories identified 181 *Salmonella* isolates using classical biochemical methods (API 20E), of which 7 (4%) isolates were lost during culture and could not be used for further analyses. From the remaining 174 isolates, API 20E presumptively identified 87 (50%) S. Typhi, 7 (4%) *Salmonella enterica* serovar Paratyphi B, 3 (2%) *Salmonella enterica* serovar Paratyphi A, and 77 (44%) NTS.

From Ghana, serotyping data of *Salmonella* were available from both the WKLM scheme and multiplex PCR method. Both methods separately identified the same serotypes among 110 *Salmonella* isolates. Of these, 47 (43%) were S. Typhi, 36 (33%) were S. Typhimurium, 14 (13%) were S. Dublin, and 13 (12%) were S. Enteritidis (Table 1).

The multiplex PCR assay identified the following serotype distribution among 64 *Salmonella* isolates at the other study sites: 42 (66%) S. Typhi, 14 (22%) S. Typhimurium, 2 (3%) S. Dublin, 2 (3%) S. Enteritidis, and 4 (6%) *Salmonella* species (Table 1). Of those 4 *Salmonella* species, 2 (3%) were S. Paratyphi A and 2 (3%) were *Salmonella enterica* serovar Choleraesuis, identified by the conventional standard serotyping method. Both *Salmonella* Paratyphi A were isolated from Senegal and both S. Choleraesuis were isolated from Guinea-Bissau.

In previous studies, it has been shown that the API 20E biochemical test efficiently identifies *Salmonella* species, but is not able to identify the serotypes accurately [11]. Data on the serotype distribution are of utmost importance for vaccine research to target predominant serotypes. However, sufficient laboratory capacity to monitor *Salmonella* serotype distribution by PCR is lacking in many countries in sub-Saharan Africa [12]. The multiplex PCR method offers the opportunity to apply regular surveillance on those *Salmonella* serotype distributions throughout countries to generate evidence for vaccine development.

In studies conducted in Mali and Chile, a sequential multiplex PCR for the identification of S. Typhi and S. Paratyphi was performed with 100% sensitivity and specificity [13]. The same study team described another series of multiplex PCR assays to detect S. Typhimurium, S. Enteritidis, S. Dublin, and S. Stanleyville [5], and most of the invasive nontyphoidal *Salmonella* (85%–95%) currently isolated in sub-Saharan Africa belong to those serotypes [13, 14].

In the present study, the multiplex PCR was not designed to identify serotypes other than S. Typhi, S. Typhimurium, S. Enteritidis, and S. Dublin. However, these serotypes cover 98% of the *Salmonella* serotypes currently circulating in sub-Saharan Africa. Thus, with regard to invasive *Salmonella* surveillance in those regions, this multiplex PCR method can be considered as an alternative to the WKLM scheme for typing the majority of *Salmonella* isolates.

**Notes**

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