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SUPPLEMENT ARTICLE

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*.

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), Imerintsiatosika 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 Oxoid 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.

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Table 1. Study Profile and Pathogens Isolated From Blood Culture, Typhoid Fever Surveillance in Africa Program Study Patients, 2010–2013

Characteristic	Burkina Faso	Ghana	Guinea-Bissau	Madagascar	Senegal	Tanzania	Total
Patients recruited, No.	1674	3726	1021	2477	1058	680	10 636
Age, y, median (IQR)	6 (2–17)	2 (0–5)	3 (1–7)	24 (14–37)	22 (14–32)	10 (1–34)	. . .
Total pathogen isolated, No. (% of blood culture)	58 (3)	242 (6)	30 (3)	26 (1)	30 (3)	25 (4)	411 (4)
Salmonella Typhi, No. (% of pathogen)	15 (26)	47 (20)	3 (10)	8 (31)	7 (23)	9 (36)	89 (22)
Salmonella Typhimurium, No. (% of pathogen)	6 (10)	36 (16)	5 (17)	0 (0)	1 (3)	2 (8)	50 (12)
Salmonella Dublin, No. (% of pathogen)	1 (2)	14 (6)	0 (0)	1 (4)	0 (0)	0 (0)	16 (4)
Salmonella Enteritidis, No. (% of pathogen)	2 (3)	13 (5)	0 (0)	0 (0)	0 (0)	0 (0)	15 (4)
Salmonella enterica, No. (% of pathogen)	0 (0)	0 (0)	2 (7)	0 (0)	2 (7)	0 (0)	4 (1)

Abbreviation: IQR, interquartile range.

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* 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, 7157 (67%) were children <15 years of age, 5382 (51%) were female, and 4521 (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 salmonellae (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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