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ORIGINAL PAPER

Evaluation of the detection of *staA*, *viaB* and *sopE* genes in *Salmonella* spp. using the polymerase chain reaction (PCR)

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Abstract

Typhoid fever is caused by the bacteria *Salmonella enterica* subspecies *enterica* serovar Typhi (*S*. Typhi) and remains a significant health problem in many developing countries. Lack of adequate diagnostic capabilities has contributed greatly in making typhoid fever endemic in these regions. Reliable and inexpensive diagnostic tests are needed to improve the management of this disease burden. We evaluated the ability of *staA, viaB* and *sopE* genes to detect and differentiate between the three most prevalent *Salmonella* spp. in Kenya (*S*. Typhi, *S*. Typhimurium and *S*. Enteritidis) using conventional polymerase chain reaction (PCR). The *staA* primers and *viaB* primers were found to be specific only for the different strains of *S*. Typhi, producing PCR products of 585 bp and 540 bp, respectively. The *sopE* primers was demonstrated to be specific for all *Salmonella* spp. producing a 465 bp PCR product with no amplification with *E. coli* and *S. boydii* bacterial strains.

Keywords *Salmonella* Typhi · Typhoid fever · Conventional PCR · Diagnosis

Introduction

Microbial foodborne diseases are widespread and have resulted in considerable economic losses in many parts of the world particularly the low-income developing countries of South East Asia, Africa, and Latin America (Edelman and Levine 1986; Parry et al. 2002). *Salmonella* is a major foodborne pathogen responsible for a large number of food-poisoning cases in humans in both the developed and developing countries. The most common symptoms associated with food poisoning are diarrhea, vomiting, nausea, fever or stomach upset. For example, *Salmonella* accounts for approximately 32% and 11% of food poisoning cases in France (Agbaje et al. 2011; Van Cauteren et al. 2015; Augustin et al. 2020) and the United States (Scallan et al. 2011; Hoffmann et al. 2012; Economic Research Service (ERS) and U.S. Department of Agriculture (USDA) 2019), respectively. Additionally, the costs associated with *Salmonella* infections are also remarkably high. For example, according to the World Health Organization (WHO), the *Salmonella* associated costs in the United States of America are estimated at US$ 3.6 billion annually (Economic Research Service (ERS) and U.S. Department of Agriculture (USDA) 2019). In the sub-Saharan Africa, the disease burden is high and the cost constraints prevent the creation of robust surveillance programs and the widespread use of newer, more effective but expensive diagnostic tools, thus making *Salmonella* incidences endemic (Pang 2008). Large outbreaks of *Salmonella* infections have been associated with poor sanitation and poor hygiene conditions (World Health Organization 2018). Transmission of *Salmonella* to humans has been linked to numerous sources, including contaminated and uncooked poultry and poultry products, meat, milk and other dairy products, pork, fresh vegetables and fruits as well as contaminated water and contact with infected animals (Buchholz et al. 2005; Sillankorva et al. 2010).

Typhoid fever is a bacterial disease, caused by the typhoidal *Salmonella* serovar *S*. Typhi and is transmitted through the ingestion of food or drink contaminated by the faeces or urine of infected people or carriers. It is a significant

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health problem in many developing countries. Worldwide, an estimated 17 million cases (WHO 2003) occur annually with most of the disease burden occurring among citizens of low-income countries, particular those in South East Asia, Africa, and Latin America (Edelman and Levine 1986). To avoid severe complications or even the loss of life because of *Salmonella* infections, definite and accurate diagnosis and treatment need to be initiated as soon as the onset symptoms of the infection begin to manifest. However, the lack of adequate diagnostic capabilities in poor resource settings common in most public health facilities in Kenya and Africa in general hinder prompt diagnosis of *Salmonella* infections particularly typhoid fever. This has often led to misdiagnosis of the disease, thereby delaying appropriate treatment and making typhoid fever endemic in most resource-poor areas.

To improve accurate and early detection of *Salmonella* Typhi (*S*. Typhi) in Kenya, we tested the ability of three pairs of primers targeting three different genes: SopE invasion-associated secreted protein (Gene ID: 1250812), ViaB region DNA for Vi antigen (GI: 426443) and StaA fimbrial protein (Gene ID: 1246701) to detect and differentiate *S*. Typhi from other *Salmonella* serovars and closely related disease-causing bacteria. These specific genes were selected because: *sopE* gene is involved in bacterial invasion of epithelial cells and plays a role in virulence (Bakshi et al. 2000; Parkhill et al. 2001), Vi antigen gene (*viaB)* is a virulence factor absent in non-typhoidal *Salmonella* that is induced when *S*. Typhi transits from the intestinal lumen into the illeal mucosa (Tran et al. 2010) and *staA* gene is a member of the fimbrial gene family specific to *S*. Typhi and has also been linked to *S*. Typhi pathogenicity (Pratap et al. 2013).

Materials and methods

Bacterial strains

Salmonella strains were obtained from the Centre for Microbiology Research (CMR) at the Kenya Medical Research Institute (KEMRI) (Kavai et al. 2018; Kariuki et al. 2019) and Kenyatta National Hospital (Getanda et al. 2015) in Nairobi, Kenya. The isolates were confirmed using slide agglutination techniques and conventional (standard/traditional) polymerase chain reaction using Salmonella 16S rRNA (Trkov 2003). Antimicrobial susceptibility testing against β-lactams and fluoroquinolones was done using the Kirby–Bauer disc diffusion technique (Bauer et al. 1966).

The three most common *Salmonella* serovars: *Salmonella* Typhi, *Salmonella* Typhimurium and *Salmonella* Enteritidis were used in this study with each serovar consisting of three subtypes in regards to their antimicrobial susceptibility: susceptible (S), intermediate (I) and resistant (R). Three standard organisms: *Escherichia* coli (*E. coli*) ATCC 25922, *Shigella* boydii (*S. boydii*) ATCC 9207 and *Salmonella* Typhimurium (*S*. Typhimurium) ATCC 14022 from the National Microbiology Reference laboratory (NMRL) at the National Public Health Laboratory (NPHL) in Nairobi, Kenya were also included in the study as control organisms (Table 1).

The bacteria stock cultures were maintained on a 5% nutrient broth agar slope at 4 °C. Bacterial cultures for genomic DNA extraction were cultured in Tryptic Soy Broth (TSB) for *Salmonella* bacteria and Luria–Bertani (LB) medium for non-*Salmonella* bacteria. A sample of each culture was also plated on MacConkey plates for *Salmonella* bacteria and LB agar plates for non-*Salmonella* bacteria.

DNA isolation

Genomic DNA was extracted from a pure culture following the standard phenol–chloroform method (Sambrook and Russell 2001). Quantification of DNA was done spectrophotometrically using a UV mini 1240 UV–Vis spectrophotometer (Shimadzu, Kyoto, Japan).

PCR primers

16S rRNA target primers (Trkov 2003) were used for *Salmonella* enterica bacteria confirmation. The *staA*, *viaB* and *sopE* primers were used to differentiate between different *Salmonella* serovars and also differentiate non-*Salmonella* bacteria. The primers were designed by targeting a reading frame within the gene that has the ability to be translated. The list of all the primers (Inqaba biotec, Pretoria, South Africa) is indicated in Table 2.

Conventional PCR amplification of *staA*, *viaB* and *sopE* gene

PCR amplification of the 16S rRNA, *staA, viaB* and *sopE* genes was carried out with 50 ng of purified genomic DNA (template DNA), 1 µM of upstream primer, 1 µM of downstream primer, 1X GoTaq Green Master Mix solution (Promega, Wisconsin, USA) and nuclease-free water (Promega,

Table 1 List of bacterial isolates used in the study

1	*E. coli* ATCC 25922	4	*S*. Typhi (S)	7	*S*. Typhimurium (S)	10	*S*. Enteritidis (S)
2	*S. boydii* ATCC 9207	5	*S*. Typhi (I)	8	*S*. Typhimurium (I)	11	*S*. Enteritidis (I)
3	*S*. Typhimurium ATCC 14022	6	*S*. Typhi (R)	9	*S*. Typhimurium (R)	12	*S*. Enteritidis (R)

Table 2 List of primer used in identification and differentiation of salmonella bacteria

Gene and primer	Length	Tm (°C)	Amplified fragment size (bp)	Source (GenBank Accession numbers)
16s rRNA				
MINf—forward (5′-ACGGTAACAGGAAGCAG-3′) MINr—reverse (5′-TATTAACCACAACACCT-3′)	17 17	51.7 44.4	402	J01859.1
viaB				
vi—forward (5′-ATG AGG TTT CAT TTC TGG CC-3′) vi—reverse (5′-TTA CAG TAA AGT AAC TGA ATC CGG C-3′)	23 25	55.6 54.9	540 (4,524,679–4,524,140)	NC_003198.1 (AL513382.1)
sopE				
SopE—forward (5′-ATG CTT CAA ACG CTC AAT GAT ATA G-3′) SopE—reverse (5′-TCA GGG AGT GTA TTG TAT ATA TTT ATT AGC-3′)	25 30	53.7 52.8	465 (4,482,059–4,482,523)	NC_003198.1 (AL513382.1)
staA				
StaA-forward (5′—ATG AAA GCG ATT TTA GCT GC-3′) StaA-reverse (5′-TTA CTG GTA AGT AAA GGT ATA CAT TGC-3′)	23 27	52.9 52.9	585 (217,411–216,827)	NC_003198.1 (AL513382.1)

Table 3 Temperature programme used in the PCR amplification of the 16S rRNA, *staA*, *viaB* and *sopE* genes

Cycle step	Temperature	Time	Number of cycles
Initial denaturation	95	2 min	1
Denaturation	95	1 min	
Annealing	43 °C (16S rRNA) 51 °C (*staA*) 53 °C (*viaB*) 51 °C (*sopE*)	45 s	35
Extension	72 °C	1 min	
Final extension	72 °C	10 min	1
Hold	4 °C	∞	1

Wisconsin, USA) to a final volume of 20 µl. The amplification reaction was performed on a Tprofessional thermal cycler (Biometra, Goettingen, Germany) with the following temperature and duration profile (Table 3): a volume of 10 µl of the PCR product was analyzed on a horizontal 1% (w/v) agarose gel and another 20 µl of the PCR product sent to a commercial vendor (Macrogen, Netherlands) for sequencing.

Results

Genomic products from DNA extraction

An overnight (16 h. at 37 °C) 5 ml culture of each of the bacterial isolates was harvested and DNA extracted using the standard phenol–chloroform method. The genomic DNA

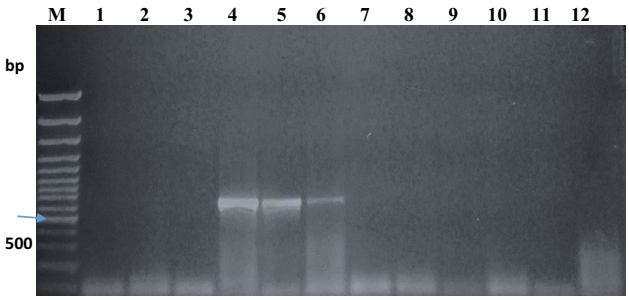

Fig. 1 1% agarose gel of the PCR products using *staA* target primers and DNA templates extracted from various bacterial isolates (expected product size is 585 bp). M (100 bp DNA ladder). Lane 1–12 (PCR product of various bacteria using *staA* target primers). 1—*E. coli*, 2—*S.* boydii, 3—*S.* Typhimurium, 4—*S.* Typhi (s), 5—*S.* Typhi (i), 6—*S.* Typhi (r), 7—*S.* Typhimurium (s), 8—*S.* Typhimurium (i), 9—*S.* Typhimurium (r), 10—*S.* Enteritidis (s), 11—*S.* Enteritidis (i), 12—*S.* Enteritidis (r)

products extracted from the 12 bacterial isolates, was analyzed and confirmed on a 1% agarose gel.

Evaluation of the primers

The specificity of each set of primers (Table 2) was tested against each of the bacterial isolates (Table 1). The 16S rRNA primers (Minf/Minr) was able to confirm the genus *Salmonella*. Amplification occurred for all the tested *Salmonella* spp. resulting in a 402 bp PCR product. No amplification was observed from the *E. coli* and *S. boydii* bacterial strains using the same primers.

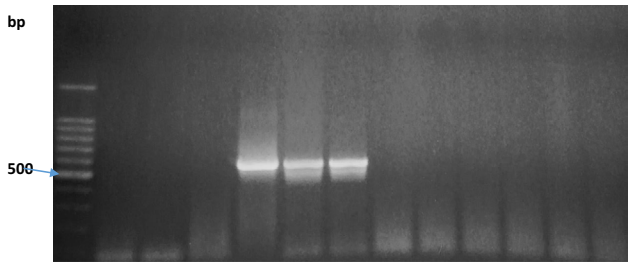

Fig. 2 1% Agarose gel of the PCR products using *viaB* target primers and DNA templates extracted from various bacterial isolates (expected product size is 540 bp). M (100 bp DNA ladder). Lane 1–12 (PCR product of various bacteria using *viaB* target primers). 1—*E. coli*, 2—*S.* boydii, 3—*S.* Typhimurium, 4—*S.* Typhi (s), 5—*S.* Typhi (i), 6—*S.* Typhi (r), 7—*S.* Typhimurium (s), 8—*S.* Typhimurium (i), 9—*S.* Typhimurium (r), 10—*S.* Enteritidis (s), 11—*S.* Enteritidis (i), 12—*S.* Enteritidis (r)

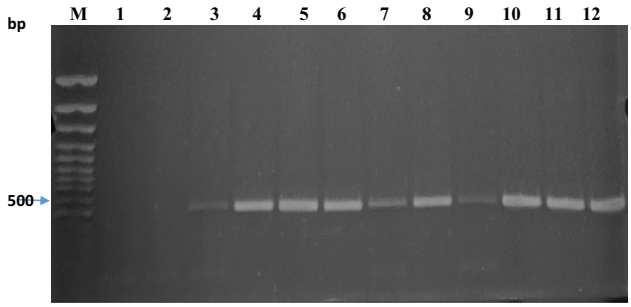

Fig. 3 1% agarose gel of the PCR products using s*opE* target primers and DNA templates extracted from various bacterial isolates (expected product size is 465 bp). M (100 bp DNA ladder). Lane 1–12 (PCR product of various bacteria using *sopE* target primers). 1—*E. coli*, 2—*S.* boydii, 3—*S.* Typhimurium, 4—*S.* Typhi (s), 5—*S.* Typhi (i), 6—*S.* Typhi (r), 7—*S.* Typhimurium (s), 8—*S.* Typhimurium (i), 9—*S.* Typhimurium (r), 10—*S.* Enteritidis (s), 11—*S.* Enteritidis (i), 12—*S.* Enteritidis (r)

The *staA* primers (StaA-forward/StaA-reverse) and *viaB* primers (vi—forward/vi—reverse) were found to be specific only for the different strains of *S.* Typhi, with amplification resulting in a 585 bp (Fig. 1) and 540 bp (Fig. 2) PCR product, respectively. No amplification was observed with *S.* Typhimurium, *S.* Enteritidis, *E. coli* and *S. boydii* bacterial strains.

The *sopE* primers (SopE—forward/SopE—reverse) was found to be specific for all *Salmonella* spp. with PCR amplification resulting in a 465 bp product (Fig. 3). No amplification was observed with the *E. coli* and *S. boydii* bacterial strains.

The PCR products of each amplification were sequenced (Macrogen, Netherlands) for confirmation of the related genes and the resulting sequences were identified as producing significant similarities to *Salmonella* spp. using The Basic Local Alignment Search Tool (BLAST). The BLASTN program was used to search nucleotide databases using the PCR nucleotide sequence as a query and the top nucleotide sequence accession numbers were found to be CP012151.1, CP002099.1 and CP012151.1 for the *staA*, *viaB* and *sopE* PCR products, respectively (Table 4). The BLASTX program was used search protein databases using the translated PCR nucleotide sequence as a query and the top protein sequence accession numbers were found to be WP_000709193.1, WP_001210944.1 and CIM07225.1 for the *staA*, *viaB* and *sopE* PCR products respectively (Table 5).

Discussion

The goal of this study was to analyze the ability of three different sets of primers (*staA, viaB* and *sopE)* to identify *S.* Typhi strains circulating in Kenya and examine their capacity to identify and differentiate commonly isolated *Salmonella* spp. and closely related bacteria. The results demonstrated the ability of the designed primers targeting the StaA fimbrial protein gene (*staA* gene), the ViaB region DNA for Vi antigen gene (*viaB* gene) and the SopE invasion-associated secreted protein gene (*sopE* gene) to detect *Salmonella*

Table 4 BLASTN analysis of sequencing results of the amplified PCR products

Query		Subject				Identities		
Name	Length	Accession number	Gene	Start	End	*E* value	Match	Pct (%)
170414-069_G03_Sta_1	585	CP012151.1	*S.* Typhi strain B/SF/13/03/195, complete genome	3920239	3919655	0.0	584	99
161212-075_I14_5_VIAR	540	CP002099.1	*S.* Typhi str. Ty21a, complete genome	4507486	4506946	0.0	540	100
170414-069_K01_Sop_3_1	465	CP012151.1	*S.* Typhi strain B/SF/13/03/195, complete genome	4463527	4463991	0.0	465	100

The top nucleotide sequence accession numbers from the BLASTN analysis of the *staA* (170414-069_G03_Sta_1), *viaB* (161212-075_I14_5_VIAR) and *sopE* (170414-069_K01_Sop_3_1) PCR amplified product sequences were: CP012151.1, CP002099.1 and CP012151.1 respectively, which all belong to *S.* Typhi

Table 5 BLASTX analysis of sequencing results of the amplified PCR products

Query		Subject				Identities		
Name	Length	Accession number	Gene	Start	End	*E* value	Match	Pct (%)
170414-069_G03_Sta_1	185	WP_000709193.1	Fimbrial protein [*Salmonella enterica*]	6	185	4E−108	180	100
161212-075_I14_5_VIAR	179	WP_001210944.1	Vi polysaccharide biosynthesis protein TviA [*Salmonella enterica*]	1	179	2.00E−122	179	100
170414-069_K01_Sop_3_1	154	CIM07225.1	guanine nucleotide exchange factor SopE [*S.* Typhi]	1	154	2E−104	154	100

The top protein sequence accession numbers from the BLASTX analysis of the *staA* (170414-069_G03_Sta_1), *viaB* (161212-075_I14_5_VIAR) and *sopE* (170414-069_K01_Sop_3_1) PCR amplified product sequences were: WP_000709193.1, WP_001210944.1 and CIM07225.1 respectively, that represents the *Salmonella* fimbrial protein, *Salmonella* Vi polysaccharide biosynthesis regulator TviA and the *Salmonella* guanine nucleotide exchange factor SopE proteins, respectively.

serovars isolated from different regions in Kenya using conventional (qualitative) PCR. The primers targeting the *staA* gene and the *viaB* gene were both found to be specific for only *S.* Typhi while the primers targeting the SopE gene were able to detect all tested *Salmonella* serovars but not any of the non-*Salmonella* bacteria.

It has been noted that there has been an increase in multidrug-resistant (MDR) *S.* Typhi isolated from patients in Kenya since 1997 and sporadic outbreaks have also been reported in resource-poor settings, especially in slum areas (Kariuki et al. 2000, 2004, 2006; Getanda et al. 2015). This very alarming trend will continue to burden the Kenyan health system especially with poor diagnostic capabilities, the increasing rate of over-the-counter sale without prescription of first-line antibiotics for typhoid fever and the continued overcrowding and population rise within the slum areas (Kariuki et al. 2010). The primers used in this study targeting the *staA* gene and the *viaB Salmonella* genes demonstrated their ability to only detect *S.* Typhi bacterial using conventional PCR. This may provide another tool in the accurate diagnosis of patients with typhoid fever in resource poor, endemic regions. Additionally, primers targeting the *SopE Salmonella* gene may be used to also identify human non-typhoidal *Salmonella* (NTS) infections in Kenya, that have increased markedly over the years with the two main serovars isolated from cases of bacteremia and gastroenteritis with high fatality, being *S.* Typhimurium and *S.* Enteritidis (Kariuki et al. 2006). With an increased prevalence of multidrug resistance among NTS serotypes (Kariuki et al. 2005), conventional PCR using primers targeting the *sopE Salmonella* gene used in this study can be applied to identify NTS when combined together with conventional PCR using *staA* or *viaB* primers used in this study.

The current diagnostic methods used at health facilities in Kenya are mainly the stool culture method and the Felix–Widal test. These methods are relatively inexpensive and readily available in resource poor areas. The culture method for *Salmonella* detection consists of a series of steps that include nonselective enrichment (LB media), selective enrichment [TSB, tryptic soy iron (TSI) broth], and selective/differential plating (nutrient agar in combination with Mac Conkey agar) and, finally, biochemical (Kligler iron agar, lysine iron agar, and motility-indole-ornithine medium) and serological confirmation (2003). Although the culture method, followed by microbiological identification is the gold standard of typhoid fever diagnosis, this procedure is labor-intensive and requires a minimum of 2–5 days to complete the analysis and identify any organism (Zhou and Pollard 2010). This delay in diagnosis makes it too late to initiate proper antibiotic therapy and thus serves no practical value in endemic areas where early diagnosis of the disease and prompt treatment are essential for optimal management (Zhou and Pollard 2010; Baker et al. 2010). The Felix–Widal test is based on measuring agglutinating antibody levels against O (somatic), H (flagellar) and Vi (capsular) antigens (2003; Wain and Hosoglu 2008; Baker et al. 2010). The Widal test is easier and less expensive to perform than the culture method and is widely available. However, it suffers from a lack of sensitivity and specificity, particularly when used as a single screening test for patients with fever. (Parry 2004; Zhou and Pollard 2010; Baker et al. 2010). Enzyme-linked immunosorbent assay (ELISA) based methods are and kits are also available for *Salmonella* diagnosis. Majority of these assays have been based on the detection of anti-lipopolysaccharide (anti-LPS) antibodies and have been reported to be more sensitive than widal tests. Recently, ELISAs for the detection of antiflagellum and anti-polysaccharide antibodies have also been developed (Jesudason et al. 1998; House et al. 2001; Gopalakrishnan et al. 2002; Fadeel et al. 2004). However, ELISA tests are often cumbersome and may not prove to be as advantageous in a hospital setting. Additionally, expensive equipment and reagents may not be affordable for in many endemic areas and developing countries (Barrett et al. 1982; Fadeel et al. 2004).

The *staA* primers (StaA-forward/StaA-reverse), and *viaB* primers (vi—forward/vi—reverse) used in this study have been shown to be specific only for the different strains of *S.* Typhi in Kenya and may be used as a diagnostic tool to

detect the presence of the organism in individuals or foods samples using conventional PCR methods. Additionally, the *sopE* primers (SopE- Forward/SopE- Reverse) in this study were proven specific for the three most common *Salmonella* spp. while discriminating the closely related bacteria *E. coli* and *S. boydii* bacterial strains. Further studies are to be carried out with actual food samples and human samples (blood, stool or saliva) to determine the effectiveness of conventional PCR using these *staA* primers, *viaB* primers and *sopE* primers in the detection of common *Salmonella* spp. in Kenya.

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Author contributions FK acquired, analyzed and interpreted the data, drafted and revised the manuscript. PG acquired the data, evaluated and interpreted antimicrobial resistance data. The concept and design of the study and manuscript revision were performed by FK, AN, GJ, PK, JK. All authors read and approved the manuscript.

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Availability of data and material All data generated or analyzed during this study are included in this published article.

Code availability Not applicable.

Declarations

Conflict of interest The authors declare that they have no competing interests.

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

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