Original Article

Phenotypic and genotypic analysis of clinical isolates Salmonella serovar Typhimurium in western Kenya

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Abstract

Background: *Salmonella* spp. are recognized as some of the most common causes of enteritis worldwide. This study aimed to identify clinically isolated *S.* Typhimurium in western Kenya and to assess antimicrobial resistance profiles and strain inter-relatedness.

Methods: The study was performed in rural Maseno, Nyanza province in Kenya, between February 2004 and June 2005. Sixty-three patients with diarrhoea and fever were recruited. *S.* Typhimurium isolates were confirmed using serotyping, biochemical testing, and 16S rRNA sequencing. Susceptibility to 20 antimicrobials was determined and specific resistance genes were identified by polymerase chain reaction (PCR). Strain diversity was further analyzed using pulsed-field gel electrophoresis (PFGE), fluorescence amplified fragment length polymorphism (fAFLP), and multi-locus-variable-number-tandem regions (MLVNTR).

Results: Twenty *S.* Typhimurium strains were isolated in the course of the study and their identity was confirmed by 16S rRNA gene sequencing. All 20 *S.* Typhimurium strains were resistant to ampicillin, streptomycin and sulfamethoxazole; ciprofloxacin resistance and phage DT104 were not detected. PFGE, plasmid profiling, and analysis of selected VNTR loci revealed further heterogeneity among the strains in the study.

Conclusion*: S.* Typhimurium was commonly isolated from patients with diarrhoea and fever in Maseno. Considerable phenotypic and genotypic diversity was observed among isolates, suggesting that strains belonging to multiple lineages are responsible for disease in the study region. Multiple resistance was common and mediated by a variety of resistance genes but not by phage DT104.

Key Words: *Salmonella*, PFGE, antibiotic resistance, VNTR, phage typing, strains.

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Introduction

Salmonella enterica are widespread in humans and animals worldwide and are of increasing public health concern as causative pathogens of food poisoning [1,2]. In many countries where sanitation is poor, typhoid and paratyphoid fevers, which are transmitted by the faecal-oral route, are major causes of gastric illness [3,4]. Sanitation and hygiene are difficult if not impossible to implement in many developing countries, and unfortunately, the effectiveness of antimicrobial chemotherapy is also being eroded by the emergence of antibiotic resistance [5]. Non-typhoidal human *Salmonella* diarrhoea does not warrant antimicrobial therapy; however, there are occasions when the infections can lead to life-threatening systemic infections that require effective chemotherapy [6]. Of increasing concern is the worldwide emergence of multidrug-resistant phenotypes among *Salmonella* serotypes, in particular *S.* Typhimurium [4,7], that express resistance to ampicillin, chloramphenicol, streptomycin, sulfamethoxazole and tetracycline [4,5]. In Kenya, multidrug resistance to commonly available antibiotics poses a major health concern, as alternative therapeutic choices are either unavailable or too expensive to be affordable for most patients [8]. Multidrug resistant *S.* Typhimurium has been reported to be the predominant cause of bacteraemic illness in children in Zaire and Rwanda, while in Kenya it was the main isolate in adults with *Salmonellae* bacteraemia [9]. Phenotypic identification of *Salmonella* by use of Kauffman's White Scheme, a classification system that depends on cell wall O antigens and the flagellar H antigen, has not been implemented in most laboratories due to different strains [10]. Therefore, the use of genotypic

identification typing methods based on the characterization of either plasmid or chromosomal DNA is encouraged and can help identify strains that may be clonal. The following methods, in particular, are being encouraged: plasmid profile typing, plasmid fingerprinting, determination of variable-number tandem repeats (VNTR), and the identification of plasmid mediated virulence genes [11]. However, reliable classification of clonal bacteria into genetically related groupings can be analyzed by sequence variation of multiple genes encoding housekeeping genes and the flanking region of the VNTR locus [12,13]. Currently, many laboratories use pulsed-field gel electrophoresis (PFGE) to determine strain relatedness, to confirm outbreaks of bacterial diseases, and to identify the source of a strain or outbreak.

Extensive use of antimicrobial agents in human and veterinary medicine has led to the increase of resistance among food-borne pathogens. Whereas antimicrobial drug resistance in zoonotic *Salmonella* may be associated with adverse consequences in several ways, treatment failures have been infrequently reported until recently [14,15,16], which may indicate an epidemic spread of multiresistant clones of particular serotypes of *Salmonella.* Not only do these clones have the potential to spread infection, but they also have the potential to develop additional resistance to the new antibiotics, which is the case for *S.* Typhimurium [17].

In Africa and most other developing regions, multidrug resistance, particularly to commonly available antibiotics, remains a major challenge for the health system [4]. Inadequate sanitation to prevent strain dissemination and over-the-counter distribution of antimicrobials can exacerbate *Salmonella* infection. In their study, Kariuki *et al.* documented multidrug-resistant *S.* Typhimurium as the predominant cause of community-acquired bacteraemic illness in both children and in adults. They observed non-typhoidal infection as well as multiple resistance to commonly available antibiotics, including ampicillin, chloramphenicol, cotrimoxazole and tetracycline [4].

Bacterial resistance to aminoglycoside antibiotics, such as kanamycin, amikacin and gentamicin, is often mediated by enzymes that modify those drugs by acetylation, adenylation, or phosphorylation [18,19]. The corresponding genes are often part of plasmids or transposons [18]. In 1995, there was a substantial increase in multiresistant DT 104, showing resistance to ampicillin (A), chloramphenicol (C), streptomycin (S), sulfonamide (Sul), and tetracycline (T) [(ACSSuT)] mediated by a bacteriophage with additional chromosomally encoded resistance to ciprofloxacin,the drug of choice for the treatment of invasive Salmonellosis in humans [20]. A study by Gorman *et al.* found that 98.5% of the *S.* Typhimurium isolated showed a high level of drug resistance, a result which correlated with those of investigations performed in the European Union [20]. Multidrug resistance was observed in 88% of *S.* Typhimurium strains and the resistance profile for 77.6% of these strains was ampicilin, choramphenicol, streptomycin, sulfamethoxazole, and tetracycline (ACSSuT) [21].

This antimicrobial resistance phenomenon, accompanied with a dearth of data from developing countries including Kenya, prompted this study on phenotypic and genotypic identification of *S.* Typhimurium clinical isolates. The study also aimed to assess their antimicrobial resistance profiles and gain insight to the mechanisms of resistance acquisition and spread.

Materials and Methods

Study site, design and patient inclusion criteria

The study was performed in a rural setting in Maseno, Nyanza province, Kenya. Consent to collect samples from the patients in the hospital was obtained from relevant authorities, namely, from Maseno University as well as from the Provincial Medical Officer and respective hospital superintendents. Between February 2004 and June 2005, patients with both fever ($\geq 38°C$) and diarrhoea who presented at Maseno and St. Elizabeth Mukumu Mission Hospitals and consented to participate in the study were recruited. In order to isolate as many *Salmonella* as possible, no age limit was set in the study. Blood, pus, cerebral spinal fluid, and stool samples were collected from a total of 63 patients who met the inclusion criteria.

Phenotypic isolation and identification of Salmonella *species in blood, cerebral spinal fluid (CSF) and stool*

Out of the 63 *Salmonella* samples collected, only 59 were viable when subcultured in Germany. Forty of the 59 samples were collected from stool and 12 were from blood. Four pus samples from peritoneum

tissue were also collected from four of the patients whose blood had been collected but who were experiencing swollen abdomen and flatulence, and three from patients who were anaemic and convulsing. The collected samples were inoculated in Selenite-F medium (HIMedia Laboratories Pvt. Ltd Mumbai, India) and incubated at 37°C for 18 hours for maximum recovery of the isolates. To analyze for the presence of *Salmonella*, inocula were obtained from Selenite-F medium using a sterile cotton swab onto MacConkey agar (Oxoid No.3 CM 115 Basingstoke, England) media and incubated into Deoxycholate Citrate Agar (DCA) (HIMedia Laboratories Pvt. Ltd Mumbai, India). The isolates were then subcultured in Kliger Iron Agar (KIA) (HIMedia Laboratories Pvt. Ltd Mumbai, India) and Simmon Iron Medium (SIM) (Oxoid Basingstoke, England), Peptone water (Oxoid Basingstoke, England), phenylalanine media, and lysine agar slants. These steps were followed by biochemical and serotyping identification. For all the culture media used, a *Salmonella*-positive control obtained from KEMRI/CDC (ATTC 14028- American Type Culture Collection) was used to compare the isolates. The *Salmonella*-positive specimens were then subcultured in nutrient broth and stored in the refrigerator at 8°C for antibiotic susceptibility testing.

Genotypic isolation and analysis of Salmonella

Discreet *S.* Typhimurium colonies were picked from the saturated solid agar-disk plates using a sterile plastic inoculation loop, and DNA extracted using a QIAmp DNA mini kit (Qiagen, west Sussex, UK). For the presence of malic dehydrogenase (*mdh*) and definitive Type 104 (DT 104), all amplifications were performed in a final volume of 50 µl containing 0.5 µl of both primers (tables 1 and 2) as well as 2 µl Qiagen dNTP, 5 µl of 10 × Buffer, 1 µl $MgCl_2$, 0.25 µl Qiagen *Taq* polymerase, 5 µg DNA containing sample and 36 µl water to make up the volume. The cycling conditions were as follows: 94°C for 5 minutes, followed by 35 cycles of 94°C for 25 seconds, 54°C for 45 seconds, 72°C for 45 seconds, and a final extension step at 72°C for 7 minutes. The amplicons were then loaded onto a casted 1.5% agarose gel (Eurobio, Les Ulis, France) run in TBE buffer (89 mmol l^{-1} Tris pH 8.3, 89 mmol l^{-1} borate and 2 mmol l^{-1} EDTA). The gel was stained with ethidium bromide solution (0.5 µg ml^{-1}) and run at 135 V for 25 minutes then photographed under ultraviolet light (Gel Logic 100 Imaging System, Kodak). A negative and a positive control were included in each PCR reaction.

Analysis of Variable Number Tandem Repeats (VNTR)

The amplifications were performed in a total volume of 50 µl containing, 5 µl of 10 × Qiagen buffer (Qiagen, west Sussex, UK), 0.5 µl of both primer pairs (table 1), 2 µl dNTP, 0.25 µl Qiagen *Taq* polymerase, 1 µl $MgCl_2$, and 5 µl containing DNA. Conditions for cycling were according to Liu *et al*. To compare the results, analysis of VNTR was also performed according to methods described by Lindstedt *et al*. Gel analysis was done as previously mentioned.

Pulsed-field gel electrophoresis of microrestricted chromosomal DNA

Twenty isolated *Salmonella* species were prepared for PFGE by individual suspension of the bacterial cells, grown on trypic soy agar at 37ºC. The cells were then transferred into a cell suspension buffer (100mM Tris-HCl, 100mM EDTA, pH 8.0) and measurements taken using spectrophotometer at an absorbance of 0.7 ± 0.05 at 612nm. Protease K (20 µl) was then added to 400 µl of the suspension along with 400 µl of molten (54ºC) 1% Seakem Gold Agar. These were mixed quickly, and approximately 300 µl were dispensed into prepared plug molds. Once solidified, the plugs were placed into 1.5 ml cell lysis buffer (50 mM Tris / HCl, 50 mM EDTA, pH 8.0, 1% Sarcosyl) and 40 µl of proteinase K and incubated for 1.5 hours at 54°C in a shaking water bath. The plugs were washed twice in ultrapure water for 15 minutes in a 50°C water bath followed by four washings in Tris – EDTA (TE) buffer (10 mM Tris – HCl, 1 mM EDTA, pH 8.0). The washed plugs were cut into 3mm × 9mm pieces and then digested in 173 µl of sterile water, 2 µl of bovine serum albumin, 20 µl of 10 × ReAct II buffer, and 5 µl of *Xba*I [New England Biolabs, Beverly, and Mass] (5' – TCTAGA -3') (10U/µl) at 37°C in a shaking water bath for 1.5 hours. The plugs were run in a 1% agarose gel using a CHEF III Pulsed-Field System (Bio-Rad) in 0.5% Tris – borate – EDTA buffer (Sigma) at 10°C. The parameters were set with the initial switch time at 2.2 seconds, the final switch time at 64 seconds, a voltage of 6 V/cm, and a duration of 21 hours. Included on the gel were *Xba* I-digested plugs of *S.* Typhimurium to be used as size standards. The gels were stained with ethidium bromide and recorded on a Gel Doc System (Bio-Rad Laboratories, Inc., Hercules, CA). The file images were processed by

Table 1. Primer sequences of various oligonucleotides used in the study.

Target	Oligonucleotide sequence	Amplicon size (bp) reference	Reference
mdh	F;5′ – TGC CAA CGG AAG TTG AAG TG – 3′ R; 5′ – CGC ATT CCA CCA CGC CCT TC - 3′	216	43
*fli*C	*Fli* 15 (length 22) – 5' – CGGTGTTGCCCAGGTTGGTAAT -3' *Tym* (length 22) - 5' – ACTCTTGCTGGCGGTGCGACTT 3'	559	30
ST 11- ST 15	ST 11(length 24) 5 ' – GCCAACCATTGCTAAATTGGCGCA-3' ST 15 (length 24) - 5' – GGTAGAAATTCCCAGCGGGTACTGG -3'	429	30
*Fli*15 & *Typ*04	Fli 15 (length 22) 5′-CGGTGTTGCCAGGTTGGTAAT - 3′ Tym (length 22) 5′ - ACTCTTGCTGGCGGTGCGACTT -3′	620	30
*sef*A	*sef* 167 (length 20) - 5' – AGGTTCAGGCAGCGGTTACT - 3'; *sef* 478 (length 20) - 5' – GGGACATTTAGCGTTTCTTG 3'	312	30
MLVNTR sequences	TR_1 F; 5′ -AGA ACC AGC AAT GCG CCA ACG A -3′ R; 5′ -CAA GAA GTG CGC ATA CTA CAC C - 3′	200	11
	TR_2 F; 5′ - CCC TGT TTT TCG TGC TGA TAC G - 3′ R; 5′ - CAG AGG ATA TCG CAA CAA TCG G - 3′	300	11
	TR_4 F; 5′- AAA AGC CCG TCT AGT CTT GCA G - 3′ R; 5′- ATC CTT CGG TAT CGG GGT ATC C - 3′	400	11
	TR_5 F; 5′- TGA AAA CCG GCT CGT AGC AGT G - 3′ R; 5′ - CAT ACG GTT ACT GCG GGA TTG G - 3′	200	11
DT 104	F 5′ – GTC AGC AGT GTA TGG AGC GA – 3′ R 5′ – AGT AGC GCC AGG ACT CGT TA - 3′	261,162	43

BioNumerics software (Applied Maths BVBA, Kortrijk, Belgium). All the isolates within a PFGE subtype had identical bands; therefore, one isolate from each PFGE subtype was randomly selected as a representative and cluster analysis was completed on the subtypes by Dice similarity coefficient and 0.8% band position tolerance as recommended by BioNumerics [31].

Phage typing, plasmid detection and sizing

Bacteria isolates confirmed as *S.* Typhimurium were sent to the Robert Koch's Institute, Enteric Pathogen Unit, Germany, for phage typing and processed according to Threlfall *et al*. [32] and Scalzo *et al*. [33]. Plasmid DNA extraction was performed using a plasmid Mini Prep Kit (Qiagen, west Sussex, UK) according to the manufacturer's instructions. Plasmids were separated by electrophoresis on horizontal 0.8% agarose gels at 100 V for two hours. Plasmid sizes were determined by co-electrophoresis with plasmids of known sizes from *E. coli* strains V157 (NCTC 50193) (53.7,7.2,5.6,3.9,3.0,2.7,2.1kb) and 39R861 (NCTC 50192) (147,63,43.5,6.9kb). DNA bands were visualized with an ultraviolet transilluminator (UVP Inc) after staining with 0.5 μgml^{-1}ethidium bromide.

fliC *and* sefA *gene typing*

ST 11- ST 15; *Sef* 167 –*Sef* 478; *Fli* 15-*Tym Salmonella* primers used were according to Soumet *et al.* [34] (table 1). Amplifications were conducted in a total volume of 50 µl, containing 0.5 µl of Hot start

Table 2. Number of *Salmonella* species using five primer pairs on different *Salmonella* strains.

Bacteria Strains	Number of strains showing positive results by PCR					
	N	216bp[a]	312bp[b]	429bp[c]	559bp[d]	620bp[e]
S. Enteritidis	4	4	4	4	0	4
S. Typhimurium	20	20	0	20	20	20
S. Typhi	33	33	0	33	0	0
Salmonella spp.	2	2	0	2	0	0

[a] The 216bp fragment was amplified with the primer *mdh*
[b] The 312bp fragment by the primer *sef* 167 and *sef* 478 (*sef* A)
[c] The 429bp fragment was obtained with primer ST 11 – ST 15
[d] The 559bp fragment was obtained with primers *Fli* 15 and *Tym*
. [e] The 620bp fragment was amplified with the primers *fli*C 15 and *Ty*04

Taq Polymerase (Qiagen, west Sussex, UK), 0.5 µl of each primer, 2 µl dNTP, 10 µl Q-Buffer (Qiagen, west Sussex, UK), 5 µl of 10×Buffer (Qiagen, west Sussex, UK) and 5 µl DNA. Reaction conditions were similar to those of *mdh* and DT 104.

Phenotypic and genotypic antimicrobial screening of the S. *Typhimurium isolates*

Antibiotic susceptibility was evaluated according to the National Committee for Clinical Laboratory Standards, 2000 and 2001. Commercial antibiotic disks (HIMedia Laboratories Pvt. Ltd Mumbai India) of different antibiotic concentrations [(ampicilin, (10 µg/ml), chloramphenicol (30 µg/ml), tetracycline (30 µg/ml), streptomycin (10 µg/ml), ciprofloxacin (5 µg/ml), nalidixic acid (30 µg/ml) and cefotaxime (30 µg/ml)] were used (table 1). The zone of inhibition diameter of any growth observed was measured by use of Vanier callipers and compared with that of the positive control organism obtained from the Kenya Medical Research Institute (KEMRI), CDC Microbiology Unit (CDC 6516-60), and the American Type Culture Collection (ATCC 14028). MIC determination by use of VITEK^R 2 [bioMeriux], a semi-automated system for 11 antimicrobials that works on MIC principles and can also distinguish between *S.* Typhi and non-Typhi isolates, was performed by obtaining the XDL bacteria growth with a sterile plastic loop and emulsified in 0.9% sodium chloride solution. DNA was extracted using a QIAmp DNA mini kit (Qiagen, west Sussex, UK) and amplified for various antimicrobial resistance genes using respective primers. Identification of class 1 integron gene cassettes was performed in 50-µl final volumes containing 5 µl, 10 × Qiagen buffer, 10 µl, Q-buffer, 0.5 µl primer, 2 µl dNTPs ,0.5 µl Hot start *Taq* poly, 5 µl DNA template and topped with 26.5 µl water. All the amplification reactions were performed under the following conditions: hot start temperature at 95°C for 15 minutes, followed by 40 cycles of 94°C for one minute, 55°C for 45 seconds, 72°C for 30 seconds, and a final extension step at 72° C for 5 minutes. The same procedure was applied to *bla* $_{PSE-1}$, *bla* $_{TEM}$, *aphA1-lab*, *aac* (6)-1, *aad*A, *aad*B, *str*B, *sul* A, and *int* genes. The gel was read as described earlier.

Results

Phenotypically isolated Salmonella *spp. from blood, cerebral spinal fluid and stool*

Twenty *S.* Typhimurium were recovered from 59 samples; all 20 (33.9%) were from stool. Out of the remaining 39 *Salmonella* samples, 33 (55.9%; 33/59) yielded *S.* Typhi of which 20 (33.9%, n = 59) were from stool, 12 (20.3%) from blood, and 2 (3.4%) from pus samples. One patient had *S.* Typhi both in stool and blood samples collected.

Molecular confirmation of S. *Typhimurium*

*fli*C gene analysis specific for phase 1 gene (flagellin phase – 1) and 2 flagellar (*rfb* cluster gene – phase 2) proteins (the H_1 and H_2 antigens respectively) observed during polyvalent "H" serotyping confirmed *Salmonella* species. Positive PCR results using the primer pair Fli 15 and Tym (ref. 35 table 1) confirmed the molecular identification of the 24 strains as *S.* Typhimurium (table 2). However, four of the 24 strains produced a 312 bp band suggesting *S.* Enteritidis. Amplification of *Salmonella* housekeeping gene, malic acid dehydrogenase (*mdh*) (GenBank accession no. X61029) from 20 of the *Salmonella* isolates gave a specific band of 261 bp for all 20 *S.* Typhimurium isolates (table 2). Four out of the 20 strains were then randomly selected and sequenced for 16S rRNA. The 16S rRNA of the strains was found to be 99% homologous to the *S.* Typhimurium LT2 sequence since they had ≥ 99% sequence homology to a sequence entry at the

Table 3. The most observed VNTR gene loci ($STTR_1$ – $STTR_8$) among *S.* Typhimurium isolates**.**

VNTR ($STTR_1$ – $STTR_8$)	Variable loci size (s)	Expected product	Responsible Gene
$STTR_1$	649, 713, 743	770	*tol*A
$STTR_2$	459, 638, 683	711	*ssp*H_2
$STTR_3$	159, 201, 222, 223, 238, 247, 257, 301, 405, 451	490	*big* A
$STTR_4$	> 1000 (1138)	1138	*Shd*A
$STTR_5$	223,259, 301	259	*Yoh*M
$STTR_6$	321, 333	342	
$STTR_7$	574, 611, 624	594	*fts*K
$STTR_8$	340, 347, 439,579	925	

BLAST search function located in the NCBI data base (accession AE008893 version AE 008893.1), but were not identical to each other. This acted as confirmation of the 16S rRNA (BLAST LT2), and phenotypic results obtained by serology, biochemical test and VITEK^R 2.

PFGE, plasmid profile, phage typing and variable number tandem repeat

Based on the Robert Koch's Institute (RKI-Berlin Germany) PFGE results, five PFGE patterns (A 80% [n = 16], B 5% [n = 1], C 5% [n = 1], D 5% [n = 1] and E 5% [n = 1] were identified (data not shown). Taking together the results for lysotyping, plasmid profile, and PFGE, it was concluded that *S.* Typhimurium isolates were RDNC (2) and (3) lsotypes that were very similar (table 3). The following four plasmid profiles were observed within the 20 isolates (table 4): (i) 70, 55, 1.8, 1.4Kb (n = 4); (ii) 70, 1.8, 1.4Kb (n = 10); (iii) 70, 4.6, Kb (n = 1); (iv) 60Kb (n = 2). Phage profiles were categorized as type 1 (n = 3), type 2 (n = 8) and type 3 (n = 5); three strains had a rough outer membrane surface and could not be phage typed. One strain was untypeable (table 4) [25, 26]. The VNTR analysis was performed using two different protocols. According to the protocol by Liu *et al*., polymorphisms were observed for TR_1, TR_2, TR4 and TR_5 loci but not for the TR_3 loci. However, the variability between the TR_1, TR_2, TR_4, TR_5 loci was not observed. These findings were later analyzed according to Lindstedt *et al*. (GenBank accession no. AE006468). *big*A gene ($STTR_3$) was the most variable, with repeat lengths of 33 bp (table 4). All 20 *S.* Typhimurium were *spv*C and *inv*A positive.

Phenotypic and genotypic antimicrobial resistance profile

S. Typhimurium antibiotic resistances were as follows: ampicilin 95% (n = 19); amikacin 95% (n = 19); streptomycin 95% (n = 19); chloramphenicol 85% (n = 17); kanamycin 85% (n = 17); cotrimoxazole 70% (n = 14); sulfamethoxazole trimethoprime 60% (n = 12); gentamicin 60% (n = 12); tetracycline 35% (n = 7); cefaclor 30% (n = 6); and none was resistant to ciprofloxacin. This resistance pattern was also observed in terms of respective resistance gene detection where *bla* $_{PSE-I}$ 75% (n = 15); *bla* $_{TEM}$ 90% (n = 18); *grm* 50% (n = 10); *aad*A 100% (n = 20); *aa*dA 5% (n = 1); *str*B 100% (n = 20*) tet* 5% (n = 1). We also identified *bla* $_{PSE1}$ 75%, *bla* $_{TEM}$ 90%, *aad*A 100%, *aad*B 5%, *str*B 100%, *aph*A1-lab 0%, *aac* (6)-1 0%, *Sul* A 0%, *Grm* 50%, *tet* 5%, and *Cip* 0% encoding for ampicilin and streptomycin. The cassette regions within Tn 7 or Tn 21of class 1 integrons ranged between 0.8 (n =3) and 1.2Kb (n = 6) respectively.

Discussion

These strains were identified as *S.* Typhimurium based on their O and H antigens, *fliC*, *mdh*, ST 11-ST 15, Fli 15 and Tym, Fli 15 and Ty04 genes (table 2). Thirty-nine other strains that did not conform to this test set were of the *S.* Typhi and *S.* Enteritidis groups [36, 37]. Molecular analysis by PFGE, MLVNTR, 16S rRNA and fAFLP demonstrated that the *S.* Typhimurium isolates were considerably heterogeneous (table 3). PFGE analysis showed that genetic variations are present among different phage types, suggesting diversification of serovar Typhimurium in Maseno and its environs. Similarly, the strains, which were multiply resistant, showed a broad range of antimicrobial resistance profiles. The presence of bla_{PSE-1} and bla_{TEM} gene was not strongly associated with class 1 integrons. In isolates where bla_{PSE-1} was negative, bla_{TEM} was positive and the isolates were resistant to trimethoprim. Class 1

Table 4. Phenotypic and genotypic antimicrobial resistance profile of clinically isolated *S.* Typhimurium.

***S.* Typhimurium Isolate**	**Resistance profile**	**Resistance genes detected**	**Plasmid profile (molecular weights, KDa)**	**Class 1 integron (size of variable cassette region)**	**Phage type**	**PFGE profile**	**VNTR loci (kb)**
13	Amk, Sul, S, Kan, Gent, A, Amox-clav, Pip, C,	*aad*B, *aad*A, *str*B, bla_{Tem}	70; 1.8; 1.4;65; 3.4	0bp	n.d	n.d	n.d
02	Amk, Sul, S, Kan, Gent, A, Amox-clav, Pip, C	*aad*B, *aad*A, *str*B, bla_{Tem}	70; 55; 1.8; 1.4	1.2kb	1	A	1.2
04	Sul, S, Kan, A, Amox-clav, Pip	*aad*B, *aad*A, *str*B, bla_{Tem}	70; 55; 1.8 ; 1.4	1.2kb	Ut	A	1.2
06	Amk, Sul, S, Kan, Gent, A, Amox-clav, Pip, C	*aad*B, *aad*A, *str*B, *grm*, bla_{PSE1}, bla_{Tem}	70 ; 1.8; 1.4	0bp	2	A	Neg
07	Amk, S, Kan, Gent, Pip	*aad*B, *aad*A, *str*B, bla_{PSE1}, bla_{Tem}	60	1.2kb	3	B	1.2
08	Amk, Sul, S, Kan, A, Amox-clav ,Pip, C,	*aad*B, *aad*A, *str*B, *grm*, bla_{Tem}	60	ND	2	E	n.d
10	Sul, S, A, Amox-clav, Pip, C,	*aad*B, *str*B, bla_{PSE1}, bla_{Tem}	70; 1.8; 1.4	1.2kb	3	A	1.2
16	Amk, Sul, S, Kan, A, Amox-clav, Pip, C,	*aad*B, *aad*A, *str*B, *grm*, bla_{PSE1}, bla_{Tem}	70 ; 1.8; 1.4	800bp	3	A	1.2
17	Amk, Sul, S, Kan, Gent, A, Amox-clav, Pip, C,	*aad*B, *aad*A, bla_{PSE1}, bla_{Tem}	70; 1.8; 1.4	800bp	3	C	0.8
18	S, Gent, A, Amox-clav, Pip, C,	*aad*B, *aad*A, *str*B, bla_{Tem}	70; 1.8; 1.4	0bp	2	A	Neg
19	Amk, Sul, S, Kan, Gent, A, Amox-clav, Pip, C,	*aad*B, *aad*A, bla_{PSE1}	70; 4.6	800bp	3	D	0.8
20	Amk, Sul, S, Kan, Gent, A, Amox-clav, Pip,	*aad*B, *aad*A, *str*B, *grm*, bla_{Tem}	70; 1.8; 1.4	0bp	2	A	Neg
27	Amk, S, Kan, A, Amox-clav, Pip, C,	*aad*B, *aad*A, *str*B, bla_{PSE1}, grm, bla_{Tem}	70; 1.8; 1.4	ND	2	A	1.2
28	Amk, Sul, S, Kan, A, Amox-clav, Pip, C,	*aad*B, *aad*A, *str*B, bla_{PSE1}, *grm*, bla_{Tem}	70; 1.8; 1.4	1.2kb	1	A	Neg
29	Gent, A, Amox-clav, Pip, C	*aad*B, *aad*A, *str*B, bla_{Tem}	70; 1.8; 1.4	0bp	1	A	Neg
33	Amk, Sul, S, Kan, Gent, A, Amox-clav, Pip	*aad*B, *aad*A, *str*B, bla_{PSE1}, *grm*, bla_{Tem}	70; 1.8; 1.4	0bp	2	A	n.d
36	Amk, Sul, S, Kan, A, Amox-clav, Pip, C,	*aad*B ,*aad*A, *str*B, bla_{PSE1}, *grm*, bla_{Tem}	70; 55; 1.8; 1.4	ND	2	A	Neg
37	Amk, Sul, S, Kan, A, Amox-clav, Pip	*aad*B, *aad*A, *str*B, bla_{PSE1}, *grm*, bla_{Tem}	70; 55 ; 1.8; 1.4	0bp	2	A	1.2
05	Amk, Sul, S, Kan, Gent, A, Amox-clav, Pip, C,	*aad*B, *aad*A, *str*B, bla_{PSE1}, bla_{Tem}	n.d	1.2kb	Serol-rough	A	Neg
30	Amk, Sul, S, Kan, Gent, A, Amox-clav, Pip, C,	*aad*B, *aad*A, *str*B, *grm*, bla_{Tem}	n.d	0bp	Serol-rough	Ut	Neg
35	Amk, Sul, S, Kan, A, Amox-clav, Pip, C	*aad*B, *aad*A, *str*B, bla_{PSE1}, bla_{Tem}	Ut	0bp	Serol-rough	A	Neg

Amk = Amikacin, Sul = Sulfametoxazole-trimetoprim, S = Streptomycin, Kan = Kanamycin, A = Ampicilin, Amox-clav = Amoxicilin-clavinon, Pip = Piperacin, C = Chloramphenical.

integrons are known to capture and express mobile genes, known as cassettes, which in most cases are antibiotic resistant genes; this group of genes were commonly observed in *S.* Typhimurium isolates [38] (table 4). The chromosome-located *apha*-1 gene responsible for kanamycin resistance [39] was not detected in this study, though investigations done elsewhere have reported that genes coding for bla_{PSE-1} and bla_{TEM} are clearly predominant and present as the only beta-lactamase gene in the absence of *Apha-1* gene. Initially bla_{PSE-1} accorded bacteria resistance to ampicillin / kanamicin, but lately bla_{TEM} has also been observed to play the same role.

Phage DT 104 was not identified in this study. However, DT104 was characterized by chromosomal resistance to ampicillin (A), chloramphenicol (C), streptomycin (S), sulfonamides (Sul), and tetracycline (T) and is commonly referred to as having resistance (R) type ACSSuT [40]. It is thought that independent acquisition of transposons before the development/acquisition of the chromosomal multi-resistance gene cluster ACSSuT might provide an explanation for the presence of more than one gene coding for the same resistance property in these strains, as documented by Frech *et al.* [37]. It is also possible that for class 1 integron-positive strains, one gene was associated with integron, but the strain also harboured a plasmid containing the other resistance gene. The *sul*1 gene was not detected in the study even though most strains were sulphonamide resistant, an indication that *sul*1 gene is not a consistent maker for the presence of class 1 integrons. The acquisition of a multiple antibiotic resistance plasmid observed by PFGE may have been favoured by the selective pressure of antibiotics in the environment. Asymptomatic carriers, who were unnoticed within the community set-up, could provide an explanation for the existence and dissemination of these heterogeneous strains in our geographical area. However, in some instances, strains negative for class 1 integrons also contained two different resistance genes for the same antibiotic.

Chloramphenicol resistance was observed in most strains by agar disk diffusion, an indication of the presence of chloramphenicol acetyl-transferase activity. Though the presence of chloramphenical acetyl-transferase activity was not determined in this study, resistance to chloramphenical may be with the expression of the chloramphenicol transferase *cat* gene as documented by Faldynova *et al*. The isolates were resistant to sulfamethoxazole conferred by genes other than *sul1*, potentially *sul2* or *sul3*, which were not tested in this study. The former is a likely possibility since, according to Guerra *et al*., the *sul* 2 gene often appears to be associated with genes that confer resistance to streptomycin (*str*A, *str*B) and all the isolates were positive for *str* B gene [42, 43].

Our data reveals that the tested isolates in this study did not show the presence of phage DT 104 that is responsible to drug resistance. We therefore conclude that *S.* Typhimurium strains from western Kenya show considerable diversity and are also different from strains which originate from other parts of Kenya (Nairobi, Kiambu and Rift Valley) and around the world [4-11].

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