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Full Length Research Paper

Integron-plasmid mediated antibiotic resistance and virulence factors in clinical *Salmonella enterica serovars* in rural Western Kenya

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Integron-mediated antibiotic resistance is common among diverse *Salmonella* serovars. Phenotypic antibiotic resistance was correlated with the presence of intergron–mediated genes in 65 *Salmonella typhimurium* isolates using conventional microbiological methods and PCR gene amplification. The resistance genes detected in the 20 *S. typhimurium* isolates were mainly those identified as part of the chromosomal multiresistance cluster *bal*$_{PSE-1}$, *bla*$_{TEM}$ and *aad*A. The plasmid encoded *sul* 1/ 2 and *aphA* - 1, *aac* (6) -1 located on the chromosome were not observed, however *str*B and *Grm* genes were observed. Genes coding for *PSE* -1 was predominant in the isolates as compared to *TEM*. The two genes were distributed differently between the various resistance phenotypes as could be observed of variability in *int* gene. Pathogenicity also varied in relation to individual isolate antimicrobial mechanism profile. These findings demonstrate integron mechanism as a means of antibiotic resistance in the isolates.

Key words: Intergron–mediated, *Salmonella typhimurium*, multiresistance, pathogenicity.

INTRODUCTION

Drugs for *Salmonella* infections include fluoroquinolones, ampicilin, trimethoprim-sulfamethoxazole or third generation cephalosporins (Hohmann et al., 2001). However, emergence of *Salmonella* species that are resistant to extended-spectrum cephalosporins (Herikstad et al., 1997; Threlfall et al., 1997) is cause for worldwide concern. In addition, *Salmonella* with plasmids conferring resistance to gentamicin, β-lactam has also been reported (Threlfall et al., 1997). For several decades, an increase in antibiotic resistance has been noted in *Salmonella enterica* (CDC, 2007). This has been as a result of mobile genetic elements such as plasmids, transposons and integrons, which disseminate antibiotic resistance genes by horizontal or vertical transfer as part that play an important role in the evolution and dissemination of multidrug resistance (Gomez, 1997; Liebert, 1999; Boyd, 2001; 2002). *Salmonella* antibiotic resistance mediated by integrons has been reported extensively (Hall et al., 1998; Carratoli et al., 2001). Integrals are frequently associated with plasmids and are therefore easily transferable among and between different bacteria (Carattoli, 2003). Integrons are common in *S. enterica* and make an important contribution to the extent of antimicrobial resistance in this species (Carratoli et al., 2001; 2003).

CTX-M ESBLs arise by plasmid acquisition of preexisting chromosomal ESBL genes. ESBL can be plasmid mediated and thus capable of spread (Babic, 2006). Class 1 integrons on plasmids are considered to be a main mechanism for the rapid spread of multidrug-resistant phenotypes among Gram negatives bacteria (Rowe- Magnus, 2002). Among the different classes of integrons described, class 1(*int*1) are the most frequently found in *Salmonella* and are known to harbor multiple resistance genes (Shaw et al., 1993). Of late, integrons

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have been found to consist of resistance cassettes built in between two conservative segments (5' - CS and 3' - CS) (Viktor et al., 2004), this has lead to four classes of integrons being described based on the similarities between them. Class 1 integrons carry the *aad*A_2, *bla* (*Carb*$_2$) or *pse*1 [*bla* (*Carb*$_2$) and *pse*1 cassettes (Randall et al., 2004) which are an integral part of the chromosome. The *flo*R gene and the *tet*R-*tet*(G) genes are bracketed by two class 1 integrons, one containing an *aad*A2 cassette and the other a *pse-1* cassette (Peters et al., 2001).

Most of these integrons are located within transposons that contribute to the vertical transmission, favouring their mobilization between plasmids and the bacterial chromosome by transposition events (Carattoli et al., 2001). Resistant genes are also known to be located on extra-chromosomal genetic elements or in segments inserted within the chromosome that originate from other genome. However, resistance genes encoded in plasmids are often located within genetic elements (Carattoli et al., 2003).

The horizontally transferred sulfonamide resistant genes are in several studies responsible for the majority of sulfonamide resistance studies in gram negative isolates. Only three sulfonamide genes are currently known that are still effectively spreading. The first two genes, *sul* -1 and -2 have been known to be plasmid-bone since the 1960s and were for a long time described to be equally distributed (Skold, 2000).

There are three types of chromosomally conferred resistance to trimethoprim; loss of thymidlylates synthetase activity making the dihydrofolate reductase redundant for the bacteria since it will depend on external supply of thymine, changes in the structure or expression of porins and through mutations in the *flo*A gene encoding the bacteria dihydrofolate reductase, DHFR, which lead to a lower affinity of the drug to the enzyme and thus to a lower level of enzyme inhibition (Skold, 2001). Since a resistant gene that has emerged on a plasmid, located within a transposon or an integron, may be transferred to other strains and species enabling it to penetrate into niches not accessible to its original host strain, surveillance and monitoring of antimicrobial-drug resistance, including screening of class 1 integrons as likely indicators of evolution of drug resistance mechanisms and acquisition of new resistance traits, are necessary steps in planning effective strategies for containing this phenomenon.

Several *S. enterica* isolates are characterized by the presence of host-adapted virulence plasmids encoding genes contributing to colonization and resistance to complement killing, such as the *spv*A, *spv*B and *spv* C (*Salmonella* plasmid virulence) and the *rck* (resistance to complement killing) genes (Guiney et al., 1994). Thus our underlying goal in this study was to unravel integron-plasmid mediated antibiotic resistance and virulence factors in clinical *Salmonella enterica* serovars in rural western Kenya.

MATERIALS AND METHODS

Bacteria strains

A total of sixty- five *Salmonella* isolates were obtained from the blood, pus, and diarrhoea stools from both in- and out- patients at Maseno and St. Elizabeth Mukumu Mission hospital in rural Western Kenya.

Isolation and identification of *Salmonella*

This was a cross sectional study carried out between February 2004 and June 2005. Patients with both fever (≥ 38°C) and diarrhea who presented at Maseno and St. Elizabeth Mukumu Mission Hospitals and consented to participate in the study were recruited.

Blood cultures were incubated in 5% CO_2 at 37°C for 24 h. The obtained isolates were then sub-cultured on Blood agar, MacCokey agar (Himedia Laboratories pvt Mumbai, India). The cultures were observed for signs of bacterial growth, including hydrogen sulfide production. The isolates were subcultured on Triple Sugar Iron (TSI), Brilliant Green Agar, and finally to XLD all from Himedia Laboratories Pvt Mumbai, India. The cultures were subjected to Gram-staining, motility, urease production, hydrogen sulphide production and citrate utilization tests. All Gram- negative, rod-shaped, motile, urease-negative isolates that produced on alkaline slant with acid butt on TSI were regarded as species of the genus *Salmonella* according to Cowan and Steel (1993). Stool samples were initially enriched on selenite F broth (Himedia Laboratories pvt Mumbai, India) prior to culture and biochemical test analysis.

Out of the viable isolates, sixty five *Salmonella* strains were obtained based on their phenotypic and genotypic characteristics. Strains were then selected with a bias for major serotypes prevalent in the region, that is, *Salmonella enterica* serovar *Typhimurium*, *S. enterica* serovar *enteritidis* and *S. enterica* serovar *typhi*.

Genotypic isolation and analysis of *Salmonella*

DNA was extracted from *Salmonella enterica Typhimurium* colonies using a QIAmp DNA mini kit (Qiagen, Germany). Analysis of *Salmonella* housekeeping gene was done according to Amavitsi et al. (2005) with minimum adjustments for confirmation of the phenotypically identified isolates. Plasmid DNA isolation from the 20 *S. Typhimurium* were extracted using plasmid Mini prep kit (Qiagen, Germany) according to manufacturer's instruction and analyzed according to Sambrook et al. (1989).

Preparation of antibiotics stock solutions

The antibiotics used in this study for MIC determination were donated by GlaxoSmithkline in powder form; while the sensitivity discs were commercially obtained from Himedia Laboratories pvt Mumbai, India. The antibiotic panel used in this study were those frequently used in the region, viz:- ampicillin, chloromphenicol, cotrimoxazole, streptomycin, tetracycline, sulfamethoxazole, nalidixic acid, and ciprofloxacin. However, additional antibiotics used for the management of Salmonellosis were also considered. The antibiotic powder was each dispensed in one liter glass bottles and appropriate amount of deionized double distilled water (ddH_2O) was added according to manufacturer's instruction. The mixtures were gently vortexed to allow for uniformity. The obtained drug solution was then filtered using a 0.45 ∝m Millipore (Bedford, Massachusetts 01730) diameter filter paper disc. This was labeled and then kept in the refrigerator at 4°C. For use, the working solutions were prepared from the stock solution by obtaining the appropriate amount of drug stock solution and diluted to the

required concentration (µg/ml) in sterile 250 mls glass bottles. These working solutions were again filtered using 0.45 µm Millipore (Bedford, Massachusetts 01730) for any possible solid impurities and other contaminants that could be present.

Antimicrobial assays

Antimicrobial minimum inhibition concentration (MIC) determination

The MICs of the five antimicrobial agents were determined by use of broth microdilution technique where two folds serial dilutions of antimicrobial agents in 100µl of antibiotic medium with an inoculum of 10^3 to 10^4 CFU as per 0.5 McFarland turbidity standards solutions for confluent growth (the correct density of the turbidity standard was further verified by using a spectrophotometer with a 1 cm light path and matched cuvette to determine the absorbance) of logarithmically grown cells were prepared. The MICs were determined by a standard tube dilution method. The bacteria isolates were emulsified in 0.9% sodium chloride solution and vortexed to obtain uniform mixture. The bacteria density suspension was adjusted to equal that of the freshly prepared 0.5 McFarland turbidity standards solutions (10^6 CFU) for a confluent growth.

The top of each well- isolated bacteria colonies on the XLD agar plate cultures (see Isolation and identification of *Salmonella*) after being incubated at 37ºC for 18 h were touched with sterile loop and transferred to a tube containing 5 ml of sterilized 0.9% normal physiological saline suspension and adjusted to the 0.5 McFarland turbidity standard, visually against a card with a white background. A sterile cotton swab was then dipped and rotated severally and pressed firmly on the side wall of the tube above the fluid level to remove excess inoculum from the swab into the adjusted suspendsion. This was then streaked onto the surface of a dried Mueller Hinton agar plate having respective antibiotic. Antibiotic susceptibilities of the isolates were thus determined according to the guidelines of National Committee for Clinical Laboratory Standards (NCCLS). For confirmation of the antimicrobial spectrum, the automated antimicrobial susceptibility system, VITEK R^2 (bioMeriux) for 11 antimicrobials that interpreted according to the National Committee Clinical Laboratory Standards (NCCLS) was used.

Polymerase chain reaction assays

The oligonucliotide primers for antibiotic resistance genes and for class 1 integrons are shown in Table 1, with respect to annealing temperatures and gene bank accession numbers where available. The integrons in *Salmonella* strains were detected using primers 5´ CS- 3´CS. This primer were specific for integron conserved segments and class I integronase gene. Class 1 integron gene cassettes were identified by PCR in a 50 µl final volumes containing 5 µl, 10 × Qiagen buffer, 10 µl, Q –buffer, 0.5 µl forward (*Int* 1F 5´ GGC ATC CAA GCA GCA AG – 3 ´) and reverse (*Int* 2R 5 ´- AAG CAG ACT TGA CCT GA- 3 ´), 2 µl dNTPs and 0.5 µl Hot start Taq poly Enzyme all from Qiagen (Qiagen, Germany). Five microliter QIAmp chromosomal DNA was used as a template. All the amplification reactions were performed using MJ Gradient Thermocycler (PTC – 225, Peltier Thermocycler BioEnzymes, Germany) using the following temperature profile; building up of Hot start temperature at 95°C for 15 min, denaturization temperature at 94°C for 1 min, for 40 cycles, annealing temperature of 55°C for 45 s, 72°C for 30 s, extension temperature at 72°C for 7 min then 4°C until it is removed. The PCR conditions also applied for the detection of *bla* PSE-1, *bla* TEM, *aphA1-lab*, *aac* (6)-1, *aad*A, *aad*B, *str*B, *sul* A, and *int* genes using specific respective primer sequences (Table 1). Each amplification reaction included a negative control, which contained all the PCR master mix reagents except 5 µg of targeted DNA segment sequence. The amplified amplicons were then separated by horizontal 1.5% agarose gel (1.5 g agarose powder + 100 ml of 1 × TBE buffer) electrophoresis with a gene marker of 100 bp, for 25 min at 135 V and visualized under ultraviolet (UV) light (Gel Logic 100 Imaging System, Kodak). The PFGE was used to compare DNA fingerprinting profiles of *Salmonella* isolates. The PFGE procedure was performed according to Threlfall et al. (2002) for phage typing.

DNA Sequencing of *Salmonella enterica Typhimurium* 16S RNA and antibiotic resistance gene

Sequencing of the PCR products was performed with the dideoxy-chain termination method. *S. enterica Typhimurium* antibiotic gene ladder of specific base pair on 1.5% gel were cut using a sharp razor on the Uv transilluminator and dispensed on 1.5 ml eppendorf tube. The DNA from the gel was extracted using QIAquick Gel Extraction Kit protocol (QIAmp Qiagen, Germany) and purification process performed using QIA quick PCR purification kit (Qiagen, Germany). The obtained DNA from the gel was labeled using POP4 –polymer and TAMRA- 500 sequencer dye. The mixture was injected in ABI PRISM -3100 Genetic Analyzer (ABI Biosystems, Foster City, Calif.) automated capillarity sequencer and run for 6 h. The nucleotide sequence analysis was done using Repeat Finder Genescan software version 3.1.

Determination of Salmonella pathogenecity (*spv*C1 and *spv*C2) and invasive genes (*In*V 1 and *In*V 2)

The *Salmonella* plasmid virulence factor (*spv*C $_1$ and *spv*C $_2$) and *Salmonella* invasive genes (*In*V 1 and *In*V 2) were also determined. The primer sequences were according to Jenikova et al. (2000) (Table 1). DNA was extracted from the *Salmonella* bacteria cells using QIAmp DNA mini kit (Qiagen, west Sussex, UK) and amplifications were performed in a total volume of 50 µl containing, 31.5 µl water, 5 µl of 10 × Qiagen buffer (Qiagen, west Sussex, UK), 0.75 µl each of both primer pairs (*spv*C_1, *spv*C_2, *in*V_1, *in*V_2), 3 µl dNTPs, 2 µl $MgCl_2$, 0.5 µl Qiagen *Taq* polymerase and 5 µl containing DNA. Conditions for cycling were according to Liu et al. (2003).

RESULTS

Varied antimicrobial MIC resistance profile was observed with the 20 *S. Typhimurium* isolates (Table 1).

Phage type and antimicrobial susceptibility

Out of 65 *Salmonella* isolates collected from clinical samples, 13 (20%, n = 65) were *S. typhi*, 20 (30.8%, n=65) *S. Typhimurium*, and 3 (4.6% n = 65) *S. enteritidis*. None of the 65 isolates displayed the presence of DT 104 despite having varied antimicrobial drug resistance.

Amplification of integrated gene cassettes by PCR

Purposively, all the 20 *S. Typhimurium* isolates that had shown antimicrobial phenotypic resistance (Onyango et al., 2009) were analyzed for the presence of

Table 1. PCR primer used in identification of antimicrobial resistance, *Salmonella* plasmid virulence (*spv*C_1) and invasive (*inv* 1 and 2) genes.

Gene	Oligonucleotide sequences	Expected product size (bp)	Drug	Annealing temp.ºC	Accession No. reference
aphA1-1ab	5′ AAA CGT CTT GCT CGA GGC 5′ CAA ACC GTT ATT CAT TCG TGA	500	Kanamycin	55	Gebreyes and Altier, 2002 U63147
aac(6)-1	5′ TGA GCA TGA CCT TGC GAT 5′ GAA CAG CAA CTC AAC CAG	337			
aadA,	5′ GTG GAT GGC GGC CTG AAG CC 5′ AAT GCC CAG TCG GCA GCG	528	Streptomycin	55	Gebreyes and Altier, 2002 M10241
aadB	5′ GAG CGA AAT CTG CCG CTC TTG 5′ CTG TTA CAA CGG ACT GGC CGC	310	Gentamicin		Frana et al., 2001 AF078527
strB	5′ ATC GTC AAG GGA TTG AAA CC 5′ GGA TCG TAG AAC ATA TTG GC	509			
sulA	5′ CAC TGC CAC AAG CCG TAA 5′ GTC CGC CTC AGC AAT ATC	360	Sulfamethoxazole	55	Gebreyes and Altier, 2002
int	5Cs′ GGC ATC CAA GCA GCA 3Cs′ AAG CAG ACT TGA CCT GAT AGC		Variable integron gene		Gebreyes and Altier, 2002M73819
spvC1	5´ ACT CCT TGC ACA ACC AAA TGC GGA				Jenikova et al., 2000
spvC2	5´ TGT CTC TGC ATT TCG CCA CCA TCA				
invA1	5´ ACA GTG CTC GTT TAC GAC CTG AAT				
inv A2	5´ AGA CGA CTG GTA CTG ATC GAT AAT				

antimicrobial resistance genes. Fifteen (75% n = 20) had ampicillin *bla* PSE1 β- lactamase, while 18 (90%, n = 20) had *bla*TEM, 20 (100%, n = 20) had streptomycin *aad*A aminoglycoside 3´ adenyltransferase, only 1 (5%, n = 20) isolate had tetracycline *tet*A gene, and 10 (50%, n = 20) had gentamicin *Grm* resistance gene. No kanamycin *Apha I-lab*, aminoglycoside phosphotransferase *aac* (6) -1 0(0%) and dihydropteroate synthase *Sul* 1/2, 0(0%) genes were observed. Ten isolates (50%, n = 20) displayed class 1 integron gene. The presence of *bla* PSE1 and *bla*TEM genes was not strongly associated with class 1 integron (Table 2).

However, all *bla* PSE1, genes were associated with *int* 1 except few cases where *int* 1 was negative but *bla*TEM positive. The isolates displayed varied sizes of PCR products of integron gene between 0.7 and 1.4 kb.

Six isolates positive of class 1 integron had a fragment of 1.2 Kb while 3 had fragments of 0.8 kb. Resistance gene to *aadB* 5% , *aac (6) - 1* 0% , *AphaI –lab* 0%, *aad*A 100%, *str*B 100%, *Sul* 1, 0% were also observed.

Table 2. Strains positive for class 1 integrons, approximate size of integron PCR products and resistance genes within or associated with integrons.

Serotype	Resistance profile	No. with profile	Resistance genes associated with resistance profile	Integron PCR size(Kb)
S. typhi	Amk, Amp, Cef, Cefu, Cef-Ax, Pip, Tob, TXM, Cefa	13	ND	ND
S. enteritidis	Amk, Amp, Cef, Cefu, Pip, Cefa, Tob,	03	*bla* $_{TEM}$, *bla* $_{PSE-1}$, *int*, *Sul* 2, *tet* A	0.8, 1.2, 1.4
S. typhimurium	Tet, Amp, Chlo, SXT, Cef, Cefo, Cef- Ax, Cefer,Tob,	10	*bla* $_{TEM}$, *bla* $_{PSE-1}$, *int, Sul* 1, *tet* A, aadA, *Sul* 2	0.8,1.2 , 1.4

Amp, Ampicilin; Amk, Amikacin, Cef = Cefalotin, Cefu = Cefuroxamine, Cef-Ax = Cefuroxamine Axetil, Pip = Piperacillin, Tob = Tobramycin, TXM = Trimethoxazole, Cefa = Cefaclor

Table 3. MIC range for strains with specific resistance genes.

Resistance gene (µg/L)	Mechanism of resistance	Resistance to	MIC range for organisms with gene
Int	Integrase		-
Bla (TEM)	β – lactamase	AMP	1-256
Bla (PSE-1)	β –lactamase	AMP	1-256
Apha I –lab	Aminoglycoside phosphotransferase	KAN	ND
*aad*B	Aminoglycoside phosphotransferase	GEN	ND
*aad*A	Streptomycin	STR	4 to > 1024
*Str*B	Streptomycin phosphotransferase	STR	4 to > 1024
Sul 1	Dihyropteroate synthase	TMP	0.5 - 256
Sul 2	Dihydropteroate synthase	TMP	0.5 - 256
aac(6)-1	Aminoglycoside phosphotransferase	KAN	ND

AMP, Ampicilin; GEN, Gentamicin; STR, Streptomycin; TMP, Sulfamethoxazole-trimethothrim, KAN, Kanamycin; ND, Not done.

Amplification of *Salmonella* plasmid virulence factor (*spv*) and invasive gene (*invC*$_1$)

Analysis of the 20 *Salmonella* isolates for s*pv* and *invC*$_1$ genes according to Jenikova et al. (2000) (Table 3) displayed a band of 571bp on agarose gel specific for *Salmonella* invasion factors was observed. However, the three *S. typhi* isolates that were tested for *Salmonella* invasion factors only showed one band of 244bp, as an indication of virulence gene without invasion activity.

DISCUSSION

Antimicrobial drug resistance can occur by point mutations in the bacterial genome or through horizontal transfer of genetic elements carrying resistance genes. Resistance may be disseminated through clonal expansion of drug-resistant strains or through horizontal transfer of genetic elements coding for resistance determinants. Class 1 integron gene cassettes were identified by PCR and the gene cassettes encoding resistance were phenotypically observed. In accordance to these, the Class 1 integron gene cassettes that contains *sul* 1 and 2 which encodes resistance to sulfonamides were lacking in the isolates; aminoglycosides adenyltransferase *aad*A and *aad*B, and [beta]-lactams (*bla* TEM/PSE) were however amplified. Beta lactamase genes are often integrated within mobile genetic elements such as transposons or plasmids and can therefore be transferred between bacteria. Their expression is often induced by β- lactam antibiotic

16S- 13 # 873 AAC TCA AAT G AAT TGA CGG G GGC CCC GCA C

16S- 002 # 901 AAC TCA AAT G AAT TGA CGG GG G : CCC GCA C

16S -21 # 870 AAC TCA AAA TG AAT TGA CGG GGG : CCC GCA C

16S- 450 #900 AAC TCA AAT G AAT TGA CGG GGG : CCC GCA C

16S – 13 #1383 GAG TGG GTT G CAA AAG AAG T AGG: TA : GCT

16S-002 #1411 GAG TGG GTT G CAA AAG AAG T AGG : TA : GCT

16S-21 #1380 GAG TGG GTT G CAA AAG AAG T AGG : TAT GCT

16S -450 #1410 GAG TGG GWT G CAA AAG AAG T AGG TTA

16S-13 #963 TCC ACA GAA GAA TCC AGA GAT GGA TTG GTG

16S-002 #991 TCC ACA GAA GAA TCC AGA GAT GGA TTG GTG

16S-21 #960 TCC ACA GAA GAA TCC AGA GAT GGA TTG GTG

16S-450 #990 TCC ACA GAA CTT TCC AGA GAT GGA TTG GTG

***Str*B-21 #481 TCT TCT ATA GGT TTC AAT CCC CTT GAC GAT**

***Str*B-37 #481 TCT TCT ATA GGT TTC AAT : CCC TTG ACG AT**

***bla* TEM -21 #31 GTT GGC AGC AGT GTT ATC ACT CAT GGT TAT**

***bla* TEM-37 #26 GTT GGC AGC AGT GTT ATC ACT CAT GGT TAT**

Figure 1. 16S RNA sequence, *bla* Tem and *Str* sequence for selected *S. enterica Typhimurium* isolates.

(Wilke, 2005). Thus the exposure of these patients to penicillin, ampicillin, amikacin, cefalotin, cefuroxamine, cefuroxamine axetil, piperacillin, tobramycin (penicillin, cephalosporins, monobactams and carbapenems) in the management of various illnesses within the region could have turned-on the expression of β- lactamase enzymes genes that hydrolyze the amide bond of the β- lactam ring of the antibiotic thus rendering the drug inactive before it reaches the penicillin-binding proteins (PBP). This was also seen as point mutations in sequenced gene of the specific resistant isolates (Figure 1). The integron amplified regions acted as regions that housed the

antimicrobial characteristics within the isolates. However, all *bla* PSE-1 genes were associated with *int* I except in few cases where *int* 1 was negative but *bla* TEM positive. In isolates where *bla*PSE1 was negative, *bla*TEM was positive and the isolates were resistant to trimethoprim. The two genes, *bla* PSE-1 and *bla* TEM were distributed differently between the various resistance phenotypes. Even though a chromosome located *Apha* -1 gene responsible for kanamycin resistance (Frech et al., 2003) was not detected in this study, other studies elsewhere have reported that genes coding for *bla* PSE1 and *bla* TEM are clearly predominant and present as the only β – lactamase gene in the absence of *Apha-1* gene. Initially *bla* PSE-1 accorded bacteria resistance to ampicillin, but of latel, *bla* TEM has also been observed to play the same role. The *bla* $_{TEM}$ genes are usually located on transposons; such as *Tn*3 (Krause et al., 1992) and it is a rare observation for both genes with same function to be expressed by a single isolate at the same time. Usually, isolates negative of *bla* PSE-1 are positive of *bla*TEM. Occasions where the two genes are expressed at the same time has only been observed in phage DT 104 (Randall et al., 2004).

Since in this study phage DT 104 was not identified, it is thought that independent acquisi-tion of transposons before the development of the chromosomal multi-resistance gene cluster might provide an explanation for the presence of more than one gene coding for similar type of resistance property in these strains (Frech et al., 2003). 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. However in some instances, strains negative for class 1 integrons also contained two different resistance genes for the same antibiotic (Randall et al., 2004). In light of this study, these genes were encoded in the class 1 integron region that was polymorphic within the plasmids. Ampicillin- resistant *S .Typhimurium* strains had an integron containing *bla* TEM and *bla* PSE1.

The *aad*A gene encoding streptomycin–spectinomycin resistance was also highly observed in the isolates and this could correlate to the dispersion of integron gene regions. The selection and dispersion of *aad*A genes in the integrons could be related to the extensive use of this antibiotic for therapeutic purposes. These phenotypic and genotypic patterns of resistance confirm those of Guerra et al. (2000) and Frech et al. (2003) where, multiresistant phenotypes which included ampicillin, chloramphenical, streptomycin–spectinomycin, sulfadiazine and tetracy-cline were observed. Phenotypic expression of plasmid–mediated β– lactamase– producing strains that is resistant to minopenicillin (ampicillin, piperacillin, peperacillin/taxobactam, amoxillin/clavilanic acid), carbo-xypenicillin and first–generation cephalosporin (cefalotin, cefofuroxime, cefuroxin axetil, ceferclor) was observed in this study (Wilke, 2005). These plasmid–mediated β–lactamase resistance pattern correlates well with those of plasmid profile in this study. In accordance with the plasmid profile amplification of *Salmonella* plasmid virulence factor (*spv*) and invasive gene (*inv*) for all strains, five plasmid profiles were obtained. The presence of this plasmid profile confirmed the amplification of *Salmonella* pathogenicity factor gene and *Salmonella* invasive factor gene.

The difference in plasmid size in these isolates correlated with the antimicrobial resistance in the isolates. The isolates also displayed the invasive genes thus depicting their invasive characteristic space. Most of the isolates had a plasmid size range between 0.7 to 1.4 kb and this correlated well with the variable integron cassette sizes that are involved in antimicrobial resistance. This was an indication that the *spv*R a regulatory loci gene and the four, *spv*A, *spv*B, *spv*C, and *spv*D structural genes were being displayed and thus the amplification of the locus (Jenikova et al., 2000). This finding supported those of Coynault et al. (1992), in which *spv* gene expression was found to be regulated by the growth phase of the bacterial culture *in vitro* with the genes being induced during post-exponential growth (Rhen et al., 1993).

However the expression of *spv* C gene is not always a presumptive identification of multi-drug–resistant *S. Typhimurium* DT 104 as documented by Lance et al. (1999). This data was partially in agreement to that of Soo et al. (2002) in which only 2 out of 22 identified phage type DT 104 were responsible to multidrug–resistance studied *S. Typhimurium* isolates. Twenty iso-lates lacked the phage type DT 104, a similar observation to this study (Onyango et al., 2009). To identify factors that generate polymorphism within *Salmonella* species, the 20 *Salmonella* isolates were analyzed for the presence of *Salmonella* invasion factor (*inv*C_1) according to Jenikova et al. (2000). The isolate in this study lacked the invasion gene operon, *in*vA reported by Lance et al. (1999) to be essential in *Salmonella* for full virulence where it is thought to trigger internalization required for invasion of deeper tissues (Gianella et al., 1973). They also lacked the invasiveness phenomenon characterized by the bacteria. This is because pathogenic *Salmonella* isolates display both the invasive and pathogenic gene used for intestinal cell adherence. We were able to isolate plasmids of different sizes and numbers and with different resistance gene contents from the strains in this study. Class 1 integrons containing the *aad*A gene was observed among the isolates, however *sul* 1 was not observed in any strain in this study. The plasmid encoded *sul* 2 that appears to associate with genes that confer resistance to streptomycin also was not observed but we were able to observe *str*B. The Kanamycin resistance *aphA* - 1 and *aac* (6) -1 located on the chromosome were not observed but *Grm* gene for gentamicin resistance was observed implying that the resistance is plasmid mediated. Genes coding for *PSE* -1 was clearly predominant in the isolates as compared to *TEM*. The two genes were distributed differently between the various resistance phenotypes.

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