Original Article

Antimicrobial profile of *Salmonella enterica* serotype Choleraesuis from free-range swine in Kakamega fish market, western Kenya

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Abstract

Introduction: *Salmonella enterica* subspecies *enterica* serovar Choleraesuis is a host-adapted, facultative, intracellular pathogen that causes swine paratyphoid. Its antimicrobial resistance presents a challenge to feed manufacturing industries. However, stopping antibiotics in animal feed would have economic implications for the industry.
Methodology: Conventional microbial methods for isolation and identification of *S.* Choleraesuis were employed. The isolates were subjected to screening against 17 antimicrobial agents and genotyping of resistance markers by PCR. The data were then analyzed and presented in percentages.
Results: Phenotypically, 43 out of 95 isolates showed multidrug resistance. Among the 17 antibiotics tested, resistance was observed as follows: sulphonamides (45.2%), nalidixic acid (44.25%), tetracycline (42%), ampicillin (36.8%), erythromycin (34.7%), carbenicillin (31.5%), chrolamphenical (28.4%), gentamicin (27.3%), kanamycin (24.2%), spectinomycin (21%), sulfamethoxazole-trimethoprim (16.8%), streptomycin (12.6%), cephalothion (8.4%), ofloxacin (5.2%), ciprofloxacin (4.2%), and norfloxacin (4.2%). Fifty-two isolates were susceptible to the antimicrobial agents tested. A total of 3.1% of the isolates had the integron gene pattern combination of *dfrA2-aadA2* (2100 bp), *dfrA12* (2100 bp); 4.2% had *dfrA12-aadA2-sulI* (2100 bp); 2.1% had *dfrA12-aadA2* (2100 bp); and 1% had *dfrA2-aadA2-sulI* (2100 bp), *oxa1-aadA2* (1500 bp), *dfrA12-aadA2-sulI,* and bla_{PSE} (2100 bp).
Conclusions: The isolated *S.* Choleraesuis were resistant to more than 10% of the antimicrobial agents used in this study. Appropriate surveillance is warranted to gain more information about the epidemiology, as stopping antibiotics in animal feed would have economic implications for the industry.

Key words: antimicrobial resistance; integron; swine; *Salmonella Choleraesuis.*

J Infect Dev Ctries 2014; 8(11):1381-1390. doi:10.3855/jidc.3861

(Received 10 June 2013 – Accepted 11 August 2014)

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Introduction

Swine are prone to infection by a variety of serotypes of *Salmonella*. Some infected swine display clinical symptoms of salmonellosis, while others are asymptomatic. However, even in the absence of symptoms, contamination of pork products can occur [1], causing a public health risk when pork is consumed. *Salmonella* outbreaks of a common single foodborne origin have been reported. It has been reported that, as a result of a high degree of genetic diversity, all *Salmonella* isolates can be classified into a single species (formally called *S.* Choleraesuis), which can be sub-classified into seven subgroups based on DNA similarity and host range. Subgroup 1 contains all the serotypes pathogenic for humans [2], except for rare human infections with group IIIa and IIIb, formally designated *S. arizonae*. Here we use the more accepted nomenclature *S. enterica* subspecies *enterica* rather than *S.* Choleraesuis subgroup 1 serovar Choleraesuis (*S.* Choleraesuis) [3]. The most common serovars in swine are *S. enterica* subspecies *enterica* serovar Typhimurium (*S.* Typhimurium), *S.* Derby, and *S.* Choleraesuis [3-5]. *S.* Choleraesuis has been reported to be host-adapted and most commonly isolated from swine [4]. According to Berends *et al*. [6], there is a significant correlation between the number of swine harboring *Salmonella* while alive and the number of *Salmonella*-infected carcasses. Studies have shown that most *S.* Choleraesuis isolated from humans and swine exhibit the same or similar DNA fingerprints, an indication of cross-infection from pigs to humans through food, water, or eating of pigs' offal by the local population. Studies in Canada and the United Kingdom show that serotype Choleraesuis is infrequently isolated from human sources [7-9]. However, in Thailand between 1988 and 1993,

serotype Choleraesuis was the tenth most common serotype that caused salmonellosis in humans and was the second most common in Taiwan [10-12]. Two biotypes of *S.* Choleraesuis have been reported based on their biochemical activity on triple sugar iron (TSI) agar; these are *Choleraesuis sensu stricto*, which is H_2S negative, and *Kunzendorf*, which is H_2S positive. Choleraesuis incidence is increasing in other parts of the continent, *e.g.*, in Japan, Taiwan, and Turkey [13]. Recent reports suggest that critical forms of antibiotic resistance arise in human pathogens as a result of certain antibiotics being used in food-producing animals, and that such resistance can be transmitted to humans via the food chain [14-20]. As evidenced by reports from other studies, the importance of the use of antimicrobials in swine, poultry, and beef cannot be underestimated and presents considerable financial constraints to the gross domestic product (GDP) of affected countries [19-21]. However, despite the restriction against the use of antimicrobial agents in agriculture [22], resistance genes have been reported [21,22], a fact supported by studies conducted at the genome level, which indicates that even if antimicrobials are withdrawn from use, the disappearance of resistant variants from the population might be slower than anticipated [21]. This could be linked to compensatory mutations conferring fitness to resistant organisms [23]. Though there are a multitude of studies describing the frequency of antimicrobial resistance to different classes of antimicrobial agents, information on the association of resistance with antimicrobial use in food animals is often controversial. Nonetheless, it is believed that risk factors due to *Salmonella* infection both clinically or environmentally are associated with temperature variability, poor hygienic practices, and above-median feed conversion efficiency [24]. Besides antimicrobial resistance due to mutation of chromosomally located genes, acquired resistance genes might also be integrated into the chromosome, presenting one of the major emerging problems among foodborne bacterial pathogens [25]. On the other hand, 95% of antimicrobial resistance genes are encoded on plasmids, which facilitate horizontal transfer of genes within microorganisms that harbor integration systems [26]. Class 1 integrons make up most of the integrons found in clinical isolates and are strongly associated with multiple-antibiotic resistance. By the end of 1995, this aspect of resistance was found to point to various class 1 integron genes [16,25,27,28]. An efficient route of acquisition and vertical and horizontal dissemination of resistance determination is through mobile elements including plasmids, transposons, and gene cassettes in integrons [29,30]. It has been reported that clonal expansion of integron-bearing *S. enterica* would account for the occurrence of a particular genetic lineage with a specific integron in a variety of regions, while horizontal gene transfer would account for the existence of identical integrons in isolates of different genetic lineages [31-34]. Over 70 different gene cassette encoding proteins that confer resistance to many antibiotic families have been characterized within integrons, including enzymes that inactivate antibiotics (β-lactams, aminoglycosides, chloramphenicol), metabolic by-passes (trimethoprim), and efflux systems (chloramphenicol) [29,35]. Most of the *attC* sites of integron-associated resistance gene cassettes identified to date share only slight homologies. Thus, regular surveillance of *S.* Choleraesuis in domestic animals could be helpful for human health. To explore the roles of clonal expansion and horizontal gene transfer in the dissemination of antimicrobial drug resistance caused by class 1 integrons, we investigated the integron structure and serotypes of 95 MDR non-typhoidal *S. enterica* isolates from swine in western Kenya.

Methodology

Samples

Approximately 200 fecal samples from individual swine described as free-range were obtained from a fish market in Kakamega municipality, western Kenya (0°17′N 34°45′E) between 2009 and 2010. During collection, the fecal samples were picked with sterilized wooden spatulas; sterile swabs were used to collect fecal samples. A small piece (≈ 1gm) was enriched in 10 mL of tetrathionate broth (Himedia Labs, Mumbai, India) and rappaport-vassiliadis (RV) (Himedia, India) and incubated at 37°C for tetrathionate broth and 42°C for rappaport vassiliadis for 20 hours under anaerobic conditions in the Molecular Microbiology and Biotechnology Laboratory of Maseno University. After incubation, a loopful of broth culture was streaked across brilliant green (BG) agar (Himedia, India) and bismuth sulphite (BS) agar and later incubated at 37°C for 12 hours under aerobic conditions. Due to the prevalence of *S. Typhimurium* previously collected in a nearby hospital in this region [35], this study only concentrated on the isolation of *S.* Choleraesuis for the purpose of profiling its existence as a result of data patterns obtained in the previous study [35]. The presumptive *Salmonella* colonies in BG and BS agars were selected and subcultured onto triptic soy agar (TSA) for 12

hours at 37°C under aerobic conditions. The colonies were further confirmed by API (BioMerieux, Marcy l'Etoile, France), followed by serotyping using commercial antiserum (Sigma-Aldrich, St. Louis, USA), and the resulting serotype was identified using the Kauffman-White serotyping scheme. The isolates were classified into biotypes by confirming their H_2S production capacity in sulfide-indole-motility (SIM) media (Fluka, Sigma-Aldrich).

Antimicrobial susceptibility of the S. Choleraesuis isolates

Antimicrobial susceptibility of the isolates (95 *S.* Choleraesuis) was examined by the Clinical and Laboratory Standards Institute (CLSI) disk diffusion method [36,37]. The isolates were spread on Mueller-Hinton agar (Fluka, Sigma-Aldrich) plates using a dry-heat sterilized chrome wire loop [38]. Each isolate was first tested against a panel of 17 antimicrobial agents. The antimicrobials concentrations were ampicillin (10 µg), amoxicillin-clavulanic acid (25 µg), gentamicin (10 µg), cephalothin (30 µg), chloramphenicol (30 µg), erythromycin (15 µg), tetracycline (30 µg), sulfamethoxaeole-trimethopmin (23.75 µg), norfloxacin (10 µg), ciprofloxacin (5 µg), ofloxacin (5 µg), nalidixic acid (30 µg), sulfonamides (300 µg), strepctinomycin (10 µg), carbenicillin (100 µg), kanamycin (30 µg), and streptomycin (10 µg). *Escherichia coli* ATCC 25922 was used as a control strain. The breakpoint for antimicrobial drugs was based on the guidelines provided by the CLSI [38]. Isolates with intermediate values were considered susceptible for the purposes of this study, so as not to overestimate the extent of resistance.

Genotypic isolation and analysis of antibiotic resistance genes

Discreet *Salmonella* colonies isolated from overnight cultures at 37°C were transferred into 3 mL TSA tubes and incubated for eight hours. A total of 10 µL of the bacterial cell dilution (approximately 10^5 CFU/10 µL) was lysed by heating at 100ºC for 10 minutes in a water bath. Next, 5 µL of the bacterial cell dilution (approximately 10^5 CFU/10 µL) was aliquoted and mixed with 10 µL PCR buffer (10 mM Tris-HCl, pH 8.8; 1.5 mM $MgCl_2$; 50 mM KCl; and 0.1% Triton X-100), 200 pmol each of dATP, dGTP, dCTP, dTTP (Boeheringer Mannheim, Germany), 50 pmol each of the PCR primer, and 0.2 U ProTaq polymerase. The cycling conditions were as follows: 94°C for 5 minutes followed by 35 cycles of 94°C for 30 seconds, 60°C for 30 seconds, 72°C for 30 seconds, and a final extension step at 72°C for 10 minutes. The antibiotic resistance genes under investigation were the *pse* group (ampicillin), *dfrA2* (sulfamethoxaeole-trimethopmin or trimethoprim), *oxa1* (ampicillin), *aadA1a* (streptomycin and spectinomycin), *aphA* (kanamycin), *cmlA* (chloramphenicol), *sul* (sulfonamide), *aadB* (gentamicin), and *cat* (chloramphenicol) (Table 1). Amplicons were then loaded onto a casted 2% agarose gel (Eurobio, Les Ulis, France) and run in tris-borate-EDTA buffer (89 mmol 1^{-1} Tris pH 8.3, 89 mmol 1^{-1} borate and 2 mmol 1^{-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 UV (Daiham Scientific, Wonju, Korea)

Integron analysis

Resistance to sulfonamides is commonly carried on class 1 integrons, suggesting that some or all of the resistance alleles may be integron encoded. The integrons in *Salmonella* strains were detected using primers 5'CS-3'CS and intI1F-intI1R (Table 1) that were specific for integron conserved segments and the class 1 integronase gene. For the primers 5'CS-3'CS, DNA from target bacteria was amplified during 35 cycles of 94°C for 30 seconds, 55°C for 30 seconds, and a final extension step at 72°C for 2 minutes. PCR conditions for primers intI1F-intI1R were the same as those for the analysis of antibiotic resistance genes. The amplicons were analyzed as previously described.

Results

Phenotypic antibiogram screening of the isolates

A cross-sectional study of antimicrobial resistance among *S.* Choleraesuis and other *Salmonella* isolates (published data elsewhere) was performed within the market study area of Kakamega municipality. The resistance was categorized according to the number of resistant antibiotics hereby presented in categories A through M (Table 2). Category E had the highest number of strains that displayed a broad antimicrobial resistance spectrum; these strains were resistant to tetracycline, sulfamethoxazole-trimethoprim, norfloxacin, ciprofloxacin, ofloxacin, nalidixic acid, and sulfonamides (n = 8). This represented 41.1% of the resistant antimicrobial spectrum.

Table 1. Oligonucleotide sequences used in the amplification of integron and antimicrobial resistance genes in *S.* Choleraesuis

Primers	Sequence	Targeted gene	Specific antibiotic resistance	Product size (bp)	Accession no.	Reference
5'CS	GGC ATC CAA GCA GCA AGC	integron	-	—	M73819	[64]
3'CS	AAG CAG ACT TGA CCT GAT					
intI1F	CCC TCC CGC ACG ATG ATC	integrase I	-	280	M73819	[65]
intI1R	TCC ACG CAT CGT CAG GC					
PSE G1	ACC GTA TTG AGC CTG ATT TA	PSE group (PSE-1,	Ampicillin	321		[66]
PSE G2	ATT GAA GCC TGT GTT TGA GC	PSE-4,CARB-3)				
dfr12-F	ACT CGG AAT CAG TAC GCA	*dfrA*12	Trimethoprim	462	AF175203	[20]
dfr12-B	GTG TAC GGA ATT ACA GCT					
oxa1-F	AGC AGC GCC AGT GCA TCA	*oxa1*	Ampicillin	708	AJ009819	[67]
oxa1-R	ATT CGA CCC CAA GTT TCC					
ant-3'-1aF	GTG GAT GGC GGC CTG AAG CC	*aadA1a*	Streptomycin,	527	M10241	[67]
ant-3'-1aB	ATT GCC CAG TCG GCA GCG		Spectinomycin			
aphA-1L	TTA TGC CTC TTC CGA CCA TC	*aphA*	Kanamycin	489	U63147	[68]
aphA-1R	GAG AAA ACT CAC CGA GGC AG					
cat-F	CCT GCC ACT CAT CGC AGT	*cat*	Chloramphenicol	632	U46780	[20]
cat-B	CCA CCG TTG ATA TAT CCC					
aadB-F	GAG CGA AAT CTG CCG CTC TGG	*aadB*	Gentamicin	310	AF078527	[69]
aadB-B	CTG TTA CAA CGG ACT GGC CGC					
cmlA-F	TGTCATTTACGGCATACTCG	*cmlA*	Chloramphenicol	435	M64556	[20]
cmlA-B	ATCAGGCATCCC ATT CCC AT					
sul1-F	CCT CGA TGA GAG CCG GCG GC	*Sul*	Sulfonamide	437		[67]
sul1-R	GCA AGG CGG AAA CCC GCG CC					
QS-1	ATG AAA GGC TGG CTT TTT CTTG	*qac/sul1*		850		[70]
QS-2	TGA GTG CAT AAC CAC CAG CC					

Table 2. Antiograms of *Salmonella* Choleraesuis isolates from swine in Kakamega municipality, western Kenya

Antibiogram tested (N = 17)	No. in the category	No. of resistant antibiograms (%)	Categories
ENaSu	1	3 (17.6%)	A
ETNaSu	1	4 (23.5%)	B
ETNaSus	5	5 (29.4%)	C
AmpGmENaSu	3	6 (35.3%)	D
TSxtNorCipOflNaSu	8	7 (41.1%)	E
GmCETNaSuCbK	4	8 (47.1%)	F
AmpGmETNaSuSCbK	4	9 (52.9%)	G
AmpGmCfCETNaSuScbK	5	10 (58.8%)	H
AmpGmCfCETNaSuSCbK	5	11 (64.7%)	I
AmpGmCfCETSxtNaSuSpSCb	3	12 (70.6%)	J
AmpGmCfCETSxtNaSuSpSCbK	1	13 (76.5%)	K
AmpGmCTSxtNorCipOflNaSuSpSCbK	1	14 (82.4%)	L
AmpGmCfCETSxtNorCipOflNaSuSpSCb	2	15 (88.2%)	M

Amp: ampicillin; Gm: gentamicin; Cf: cephalothion; C: chloramphenicol; E: erythromycin; T: tetracycline; Sxt: sulfamethoxazole-trimethoprim; Nor: norfloxacin; Cip: ciprofloxacin; Ofl: ofloxacin; Na: nalidixic acid, Su: sulfonamides; Sp: streptomycin; S: spectinomycin; Cb: carbenicillin; K: kanamycin

Table 3. Phenotypes of antimicrobial resistance analysis for *S.* Choleraesuis isolates

Antimicrobial class	% of resistant strains (n = 95)
Penicillins	
Ampicillin	89.5%
Amoxicillin-clavulanic acid	0%
Carbenicillin	87.4%
Aminoglycosides	
Gentamicin	67.4%
Streptomycin	70.6%
Kanamycin	80%
Cephalosporin I	
Cephalothin	7.4%
Phenicols	
Chloramphenicol	75.8%
Tetracycline	93.7%
Sulfonamides and potentiated sulfonamides	
Trimethoprim-sulfamethoxazole	32.6%
Sulfonamides	100%
Quinolones and fluoroquinolones	
Nalidixic acid	100%
Ciprofloxacin	21.1%
Norfloxacin	21.1%
Ofloxacin	24.2%
Macrolides	
Erythromycin	80%
Aminocyclitols	
Spectinomycin	31.6%

Category I had five isolates exhibiting resistance to ampicillin, gentamicin, cephalothion, chloramphenicol, erythromycin, tetracycline, nalidixic acid, sulfonamides, stretionmycin, carbencillin, and kanamycin (64.7%); category H had five isolates resistant to ampicillin, gentamicin, cephalothion, chloramphenicol, erythromycin, tetracycline, nalidixic acid, sulfonamides, stretionmycin, carbencillin, and kanamycin (58.8%); category C had five isolates resistant to erythromycin, tetracycline, nalidixic acid, sulfonamides, and stretionmycin (29.4%); and category G had four isolates exhibiting resistance to ampicillin, gentamicin, erythromycin, tetracycline, nalidixic acid, sulfonamides, stretionmycin, carbencillin, and kanamycin (52.9%) (Table 2). There were a number of isolates resistant to a combination of ampicillin, kanamycin, streptomycin, sulfamethoxazole, and tetracycline. Among these isolates, resistance was highest to nalidixic acid (100%), followed by sulfonamides (100%), tetracycline (93.7%), ampicillin 89.5%), carbenicillin (87.4%), kanamycin (80%), erythromycin (80%), chloramphenical (75.8%), streptomycin (70.6%), and gentamicin (67.4%) (Table 3). Though the frequency of resistance is low, resistance to these antimicrobial agents, mainly cephalosporins and trimethoprim/sulfamethoxazole, is significant since these are some of the last-resort agents for human infection [39].

Integron detection and characterization

It should be noted that due to financial constraints of the project, the team could not proceed with the study of the *spv* virulence genes in the isolates to confirm the presence of a plasmid; this will be pursued in future studies. In order to confirm the phenotypic basis of resistance, a number of resistance gene were sorted; among them were those for beta-lactam inhibitors ($bla_{\text{PSE-1}}$, bla_{TEM} and *oxa1*), sulphonamide resistance (*sul1* and *sul2*), chloramphenicol acetyltransferase (*cat* and *cmlA*), kanamycin resistance (*aphA*), aminoglycoside adenyltransferase (*aadA1a*), and gentamicin (*aadB*) (Table 1). The isolates displayed a myriad of resistance gene patterns; 31.6% (30/95) displayed the presence of *dfrA12* gene, 1.1% (1/95) displayed the $bla_{PSE\text{-}1}$ gene, 30.5% (29/95) the *aadA1a* gene, 86.3% (82/95) the *aphA* gene, 1.1% (1/95) the *oxa1* gene, 30.5% (29/95) the *cmlA* gene, while 27.4% (26/95) displayed the *qac*/*sul1* gene. Integrons ranging between 650 bp and 2100 bp and integrase 1 genes of 280 bp were amplified in 32.6% (31/95) of isolates, with the 1500 bp integron size being encoded for streptomycin-spectinomycin and β-lactam resistance (*i.e.*, with the *oxa1-aadA1a* gene cassettes), while the rest were of 2100 bp size, which encode for the resistance to trimethoprim-sulfonamides and β-lactams.

Discussion

S. Choleraesuis is reported to have evolved through gene deletion and sequence alternations to become a very efficient and successful pathogen among non-typhoidal *Salmonella*, particularly with regard to its pathogenicity and antimicrobial resistance [3,29]. These deletions and sequence alterations bring out ESBL and *ampC* genes, and *qnr*-related resistance due to their mobile nature, making the treatment for invasive *Salmonella* infections challenging [40]. The abundant use of antibiotics in human and veterinary medicine and in food production has led to antimicrobial drug resistance [41]. Several authors have implicated the misuse of antimicrobials or the mobile genetic elements of bacteria (plasmids, transposons, and integrons) as the cause of widespread antimicrobial resistance [42-44], but this has now changed due to the generic nature by which different strains acquire the resistance genes. The ability of integrons to excise and integrate resistance gene cassettes from the environment or other bacteria have given them a cardinal role in antimicrobial resistance in bacteria [45-48,23]. Integrons have been identified among clinical isolates, in farm animals, and in aquatic environments [49]. In this regard, in order to better understand antimicrobial drug resistance gene mechanisms in *S.* Choleraesuis, this study characterized the class 1 integrons and genetic mechanisms associated with 43 out of 95 *Salmonella*-positive isolates obtained from within the surrounding fish market in Kakamega municipality, western Kenya. Out of 95 *Salmonella* isolates, 43 were found to be multidrug resistant, with the rest having single or double drug resistance (unpublished data). Other authors [50,51] have reported in Taiwan the existence of *S.* Choleraesuis that is resistant to multiple antibiotics, including ciprofloxacin and ceftriaxone, through various mechanisms. Apart from the different mechanisms – production of hydrolytic enzymes to destroy the antibiotic surrounding the environment, mutations in the specific genes to escape from the action of antibiotics, decreased outer membrane permeability to prevent antibiotics from entering the bacterial cell itself, and efflux systems to exclude antibiotics before they become effective – other mechanisms have been described [51,52]. Production

of enzymes due to extended-spectrum cephalosporinases has been observed in *S*. Choleraesuis resistance, and most of the isolates in this study were found to display this property with cephalothin (Table 3) [51]. This is an indication that the isolates could be harboring cefotaxime-hydrolyzing β-lactamases (CTX-M types) or CMY-2 AmpC β-lactamase that could hydrolyze cephalosporins normally transferred through conjugative plasmids, transposons, or integrons [52,53]. This is supported by the fact that resistance to β-lactams in *S. enterica* is primarily caused by the production of acquired β-lactamases [54], with TEM-1, PSE-1, and OXA-1 having been described as the enzymes most frequently related to ampicillin and amoxicillin/clavulanate resistance. Thus, the resistance pattern of amoxicillin-clavulanic acid, carbenicillin, and ampicillin observed in this study resulted from the β-lactamase enzyme [55]. The spread of resistant determinant genes that are clonally disseminated and horizontally transferred was also observed in β-lactam penicillins (ampicillin, amoxicillin-clavulanic acid, carbenicillin), to which over 80% of the isolates showed resistance, except amocxicillin-clavulanic acid, to which the isolates did not have any resistance. This is an indication of the existence of bla_{PSE1}, bla_{TEM}, and *oxa1* in the group of $bla_{\mathrm{CMY\text{-}2}}$ that are plasmid oriented (Table 3) [56,57]. This is an indication for the presence of plasmid-mediated β-lactamases including TEM-type penicillinase, inhibitor-resistant TEM (IRT) β-lactamase, and oxacillinase-1 (OXA-1) β-lactamase [57]. Resistance to quinolones and fluoroquinolones (nalidixic acid, ciprofloxacin, norfloxacin, and ofloxacin) observed in these isolates was indicative of a quinolone resistance gene being displayed (Table 3). This finding is supported by that of Ricci *et al*. [58], which found that *Salmonella* resistance to fluoroquinolones in most cases involved mutations in the quinolone resistance-determining regions of the DNA genes, active efflux (AcrAB efflux), and decreased outer membrane permeability strains [58,59]. In this study, the isolates could have harbored either *gyrA*, *gyrB*, *parC*, or *parE* genes as a result of point mutation in fluoroquinolones, though all these genes were not studied due to restricted funding; however, equal resistance was observed to ciprofloxacin, which has been shown to be anchored in mutations that give rise to the substitution of phenylalanine for serine at position 83 and asparagine for aspartic acid at position 87 in *gyrA* as documented by other authors [50,51,60-62]. The same trend of resistance has also been observed within sulfonamides and potentiated sulfonamides, with sulfonamides being the most resistant [60-62]. This was based on the amplification of *dfr12*, *qac*/*sul1*, and *sul1*, which are among the Tn21 class 1 integron gene cassettes [63]. These translated to streptomycin and sulfamethoxazole phenotypic resistance. The *cmlA* responsible for chloramphenicol resistance found in Tn1696 was also amplified in the isolates [60]. Overall, the most potent drug of choice in the management of *S.* Choleraesuis was amoxiclillin-clavulanic acid. The plasmid-encoding kanamycin resistance was through the aminoglycosides phosphotransferases *aphA1a* gene, which was detected in 82/95 isolates.

In conclusion, the emergence of *S.* Choleraesuis that is resistant to ampicillin, chloramphenicol, trimethoprim-sulfamethoxazole, tetracycline, gentamicin, and, notably, fluoroquinolones, was observed in this busy cosmopolitan marketplace where traders converge to sell their merchandise. Concern has been raised about the use of these agents for the empirical treatment of systemic infection caused by this organism in both humans and animals. In view of the serious implications of this situation, the chain of transmission and mechanism of resistance should be studied further to discern the possible resistance genes circulating within this environment in order to assist in clinical management of *S*. Choleraesuis in the region. It would be also important to obtain an aerial view of genes possessed by this particular serotype of *S*. Choleraesuis to corroborate the findings with those of human clinical studies within the region and to solve the worrying clinical problems by genotyping.

Acknowledgements

We acknowledge the municipal council authorities of Kakamega County for allowing us to collect pig droppings (stools) from the market and its surrounding areas. We also thank the public health department of the country for the assistance they gave us towards realizing our goal for the project. We thank Maseno University for giving us the necessary support needed within the university premises.
Lastly we thank Prof. Dr. David M. Onyango for allowing us to use his laboratory reagents and supplies from his many funded projects.

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Conflict of interests: No conflict of interests is declared.