Detection of Fluoroquinolone and other Multi-drug Resistance Determinants in Multi-drug Resistant Non -Typhoidal *Salmonella* Isolated from Swine

*Janet A. Munyalo[1 2, 3], Wondwossen A. Gebreyes[1,4], Winnie Mutai[3], Omu Anzala[3], Artuso-ponte[1], Julius Merdadus[1], Samuel Kariuki[2, 4]

1. *Department of Veterinary Preventive Medicine, College of Veterinary Medicine, The Ohio State University, Columbus, OH.*
2. *Centre for Microbiology Research, Kenya Medical Research Institute (KEMRI), Nairobi, Kenya.*
3. *University of Nairobi, School of Medicine, College of Health Sciences, Faculty of Medical Microbiology.*
4. *VPH-Biotec Global Consortium.*

Corresponding Author: Janet A. Munyalo, Centre for Microbiology Research, Kenya Medical Research Institute (KEMRI), Nairobi, Kenya. E-mail: munyalojan@yahoo.com. Tel: + 254 790191488

Summary

BACKGROUND

Non-typhoidal *Salmonella* (NTS) is a significant pathogen that causes foodborne diseases in both humans and gastrointestinal illness in animals. The emergence of Multidrug Resistance among these strains is a public health concern.

AIM

The aim of this study was to determine the antibiotic and heavy metal resistance genes in Multi-drug resistant (MDR), Non-Typhoidal *Salmonella* of swine origin.

METHODOLOGY

In order to detect the determinants of multidrug and fluoroquinolone resistance, we tested for QRDR, Class I integrons and heavy metal tolerance genes in *Salmonella* isolates of swine originating from Kenya and Ethiopia. 121 *Salmonella* isolates collected from swine farms in Nairobi, (*n*=86) and Ethiopia (*n*=35) were characterized. Antibimicrobial susceptibility tests were done in all isolates against 12 antibiotics. Serogrouping PCR and DNA sequencing was performed for further identification. Minimum Inhibitory Concentration (MIC) of zinc-chloride and copper-sulfate was also done.

RESULTS

50 (41.3%) of the isolates showed fluoroquinolone resistance. Sequencing for quinolone resistance determining region (QRDR) showed point mutation in *gyr*A gene at positions Ser83 and Asp87. 87 (71.9%) isolates showed sulfamethoxazole resistance; 10 carried Class I integrons with predominant size being 1.5kb. Some of the integrons carried *aadA2*, *aadA7* and *dhfr7* gene cassettes. The isolates subjected to zinc and copper MIC determination, 98% (N=84) were susceptible. Zinc (*czc*D) and copper genes (*pco*A) were not detected.

CONCLUSION

Fluoroquinolone resistance predominated the MDR NTS isolates and therefore swine were potential reservoir of antimicrobial-resistant NTS posing a risk in food production chain. Even though all *Salmonella* serovars, antimicrobial resistance was not correlated with heavy metal tolerance, NTS pose a public health hazard in humans by contact as well.

***Keyword*; Multidrug resistant Non-typhoidal *Salmonella* , Fluoroquinolone resistance, Heavy metals, Swine**

[***Afr. J.*** Health Sci. 2020 33(2) : 1 - 11]

Introduction

Salmonella enterica is one of the leading causes of foodborne illnesses with an incidence of 48 million cases yearly. Between 1996-2010, in the United States alone, it was estimated to cause 128,000 cases of hospitalizations and 3,000 mortalities [1].

However, *Salmonella enterica* subspecies *enterica* had more than 2600 different serotypes. Serotypes *Typhi* (mainly limited to developing regions), *Enteritidis*, and *Typhimurium* were the most universal serotypes [2,3].

By 2010, Infections due to non-typhoidal *Salmonella* (NTS) had a high global human health impact with an incidence 93.8 million cases of which 80.3 million cases were estimated to be foodborne with the death rate of 155,000 cases each year [4]. Further, the recent rise and emergence of antibiotic resistance especially among foodborne pathogens has escalated the burden and treatment of infections associated with these pathogens [5, 6].

NTS had been isolated in various stages of swine production chain including primary production, transport of animals, the pre-slaughter and pre-evisceration steps especially during scalding, opening of the abdomen and withdrawing of the colon [7, 8]. In addition the use of antibiotics in swine production specifically as growth promoters provides a selective pressure for emergence of antibiotic resistant pathogens across the production steps outlined. Consequently posing a significant public health risk to consumers [9–11].

Despite use of antibiotics in animal husbandry, micronutrients such as copper and zinc among many others were included in swine and other livestock feed to achieve growth, promotion and increase feed efficiency [12]. The animals therefore, become reservoirs of antibiotic resistant pathogens and genetic determinants contributing to occurrence of phenotypes exhibiting both antibiotic resistance and tolerance to heavy metals through co-selection [13-14].

Previous studies done in developed countries in swine farms had reported the emergence of multidrug resistant (MDR) *Salmonella enterica* strains primarily linked to the routine practice of giving antimicrobials to swine and other domestic animals for prophylaxis and treating diseases [15-17]. This kind of practice enhances the subsequent transfer of resistant bacteria to human population following consumption [18].

Moreover, reports in the United States and China have documented the use of co-selective agents such as heavy metal ions (zinc and copper) in swine feed which could be the drivers of MDR pathogens [10,19].

Quinolones are currently recommended for treatment of *Salmonella* infections in animals as well as in humans posing a potential risk for development of resistance [20, 21]. Subsequently many studies have reported reduced susceptibility or resistance to these group of antibiotics in both animal and human NTS isolates [22-24]. Resistance was mainly mediated by mutations within the quinolone resistance-determining regions (QRDRs) of the chromosomal genes, *gyr*A and *gyr*B, that code for deoxyribonulcease *gyrase*, and cojointly *par*C and *par*E, that code for the *topoisomerase* IV [25]. Additionally, plasmid mediated mechanisms through *qnr* genes (*qnr*A, *qnr*B, and *qnr*S) may confer reduced susceptibility or low-level resistance of quinolones [26].

In order to detect the determinants of multidrug and fluoroquinolone resistance, we tested for QRDR, Class I integrons and heavy metal tolerance genes in *Salmonella* isolates of swine originating from Kenya and Ethiopia.

Materials and Methodology

Study Design and Sample Collection

The isolates originated from an ongoing project on the molecular epidemiology of foodborne pathogens in Eastern Africa, a collaborative partnership between Ohio State University, Kenya Medical Research Institute (KEMRI) and Addis Ababa University, Ethiopia.

A total of 121 isolates were selected based on three main criteria including:

1) Host origin (swine).
2) Phenotype showing MDR.
3) Geographic origin (Kenya and Ethiopia).

The number of isolates from each geographic region that fulfilled the criteria included 86 from Kenya and 35 from Ethiopia.

Serogrouping

We randomly selected 8 out of 86 isolates from Kenya and 35 isolates from Ethiopia for serogrouping. That was done at the National Veterinary Services Laboratories (Ames, Iowa). The isolates were typed using the classical serogrouping technique where the polyvalent and single factor *antisera* was applied to detect O and H antigens [42,43]. The *Salmonella* antigenic formulae was interpreted using the [44].

Antibiotic Susceptibility Testing

121 *Salmonella* isolates were tested against a panel of 11 antibiotic agents including:

a. Ampicillin (10 µg),
b. Amoxicillin-clavulanic acid (30 µg),
c. Cefotaxime (30 µg),
d. Chloramphenicol (30 µg),
e. Ciprofloxacin (5 µg),
f. Nalidixic acid (30ug),
g. Gentamicin (10 µg),
h. Streptomycin (10 µg),
i. Sulphamethoxazole (250 µg),
j. Trimethoprim (5 ug),
k. Sulphamethoxazole-trimethoprim(1.25/23.75 ug)
l. Tetracycline (30 µg)

by: Kirby-Bauer disc diffusion procedure.

Interpretation was based on the Clinical Laboratory Standards (CLSI) guidelines. *Escherichia coli* ATCC 25922 was used as standard control strain. *Salmonella* isolates showing resistance to two or more antibiotics were categorised as MDR, and those isolates showing intermediate resistance patterns were considered susceptible.

PCR for Quinolone Resistance Determining Region (QRDR) and Plasmid Mediated Quinolone Resistance (PMQR).

Considering that, the resistance was facilitated by mutation of chromosomal encoding genes or expression of PMQR genes, we selected a total of 50 isolates that exhibited resistance to ciprofloxacin for detection of QRDR and PMQR, *gyr*A and *qnr*A gene following the methodology described by Eaves et al (2004). Briefly, the isolates were cultured overnight in Luria bertani (LB) broth at 37^{o}C and the cells were harvested for subsequent extraction of DNA using DNeasy Blood and Tissue kit (Qiagen-GmbH, Hilden). Conventional PCR was used to amplify the QRDR for *gyr*A using primers

5'-CGTTGGTGACGTAATCGGTA-3'(forward) and *gyr*A 5'CCGTACCGTCATAGTTATCC-3'(reverse) and the primers used to amplify *qnr*A were 5'-ATTTCTCACGCCAGGATTTG-3' (forward) and 5-GATCGGCAAAGGTTAGGTCA-3' (reverse).

The primers were obtained from the Integrated DNA Technologies Coralville, Iowa). Thermocycling conditions were adopted from Eaves et al., (2004). The products were analyzed on a 1% gel electrophoresis. DNA ladder of 1- kb plus was used as the standard size marker.

PCR for Class I Integrin

A total of 87 *Salmonella* isolates were selected based on their resistance to sulfamethoxazole. Variable region of class I integrons were amplified using the following PCR primer sequences:

5'-GGCATCCAA GCAGCAAG-3'(forward)
5'-AAGCAGACTTGACCTGA-3' (reverse).

PCR conditions were adopted from [18].

DNA Sequencing for *gyr*A and Integrons

A total of 50 isolates were selected based on their resistance to ciprofloxacin. Premixed DNA was prepared by aliquoting 10µl PCR products into PCR tubes and sequencing primers were diluted to 5µM (pmol/µl) using PCR grade water. The preparations were then sent to GENEWIZ (South Plainfield, NJ) for DNA sequencing. The sequences were finaly analyzed using the BLAST and FASTA software (National Center for Biomedical Information (NCBI)). Accession numbers for the submitted sequences were assigned by NCBI as shown in ***Table 1 next page***.

Minimum Inhibitory Concentration (MIC) of Copper Sulfate and Zinc Chloride

All the *Salmonella* isolates (n=121) were also tested for tolerance against Copper Sulfate ($CuSO_4$) and Zinc Chloride ($ZnCl_2$) using agar plate-dilution method for MIC determination. Dilutions were prepared using Mueller-Hinton (MH)-II agar plates as

follows: $ZnCl_2$: 0µg/ml, 0.25µg/ml, 0.5µg/ml, 1µg/ml, 2µg/ml, 4µg/ml, 8µg/ml and 16mµg/ml and the pH of the medium was adjusted to 5.5. $CuSO_4$: 0µg/ml, 1µg/ml, 2µg/ml, 4µg/ml, 8µg/ml, 16µg/ml, 20µg/ml, 24µg/ml, 28µg/ml and 32µg/ml and the pH of the medium was adjusted to 7.2. 25 ml of MH agar was prepared and after solidifying using the replicator inoculum block 400 ul of bacterial suspension was inoculated. Bacterial suspension of 107CFU/ml was prepared by adding 100µl of each inoculum at 0.5 McFarland + 900µl of sterile 0.85% NaCl solutions. The plates were incubated at 37^0C for 16 to 18 hours. The MIC was defined as the lowest concentration that inhibits the visible growth of *Salmonella*. *Escherichia coli* 25922 was used as reference / control strains.

RESULTS

Antibiotic Susceptibility Testing

The majority 67.4% (n=58/86) of the isolates from Kenya showed resistance to sulphamethoxazole, tetracycline and amoxycillin, whereas those from Ethiopia showed resistance pattern to ciprofloxacin, nalidixic acid and nitrofurantoin 28.57% (n=10/35). Few isolates from Kenya were panSusceptibe whereas isolates from Ethiopia were all multidrug resistant as shown in ***Table 1.***

Table 1: *Antibiotic Pattern of the Isolates*

Country	Resistance pattern	Frequency (%)
Kenya	AmpStrSulCefCip	1 (0.8%)
	SulCip	19 (15.7%)
	PanS	6 (5%)
	SulTet	1 (0.8%)
	SulTetAmc	1 (0.8%)
	SulTetAmc	58 (47.9%)
Ethiopia	AmcAmpChlCefCipStrSulTet	3 (2.5%)
	AmcAmpChlCipNalStrSulTet	5 (4.1%)
	AmcAmpCipGenNalStrSulTet	1 (0.8%)
	AmcAmpCipNalStrSulTet	2 (1.7%)
	AmcAmpCipNal	1 (0.8%)
	AmpAmcStrTet	1 (0.8%)
	AmpStrSulTmp	1 (0.8%)
	AmpStrSulTetTmp	1 (0.8%)
	AmpStrSulSxlTetTmp	1 (0.8%)
	CipNalNit	10 (8.3%)
	CipGenNalStrSulTet	5 (4.1%)
	CipKanNalStrTet	4 (3.3%)

Key:
Amp - Ampicillin
Amc - Amoxycilin
Sul - *Sulphamethoxazole*
Tmp - *Trimethoprim*
Cef - *Cefotaxime*
Ci p- *Ciprofloxacin*
Tet - *Tetracycline*
Na - *Nalidixic acid*
Str - *Streptomycin*
Tet - *Tetracycline*
Chl - *Chloramphenicol*
Gen - *Gentamicin*
Nit - *Nitrofurantoin*
Sxl - *Trimethoprim-Sulphamethoxazole*

MDR *Salmonella* Serogroups

.From the MDR isolates selected for serougrouping, we identified 21 different serovars (6 from Kenya and 15 from Ethiopia) as shown on the ***Table 2*** in appendix 2. The predominant serotype from both regions was Kentucky (12 from Ethiopia and 2 from Kenya). Other serotypes determined included Hadar (n=5), Blockley (n=4) and Muenchen (n=2)

Genetic Basis of Quinolone Resistance (*gyr*A and *qnr* A).

Out of the fifty (50) isolates amplified thirty-five (35) isolates contained *gyr*A gene ***(Figure 1*** in appendix 1) and one (1) isolate contained *qnr*A gene. For the isolates that were sequenced, we observed mutation at codon *Ser*83 to *Phenylalanine* (*n* = 13)

Glycine	(*n* = 4)
Isoleucine	(*n* = 3)
Arg	(*n* = 1)
Aspar	(*n* = 4)
Cysteine	(*n* = 5)
Tyrosine	(*n* = 1)

Five isolates did not show any change at codon *Ser*83. There was observed amendment in sequence *Asp*87 to either Arginine (*n*=5), Lysine (*n*=2), Glycine (*n*=1) and Asn (*n*=2), two isolates did not show mutation of the codon at position *Asp*87. Selected amino acid substitutions are shown on the *Table 3* below. PMQR *qnr*A gene was detected in one isolate that was resistant to ciprofloxacin and with mutation in the QRDR *gyr*A gene at *Ser*83 position (substituted by Isoleucine) as shown in ***Table 3*** in appendix 3.

Class I Integron Profile

Class I integrons with varying sizes were detected from all geographic origins: Ethiopia (*n*=9) and Kenya (*n*=1). Class I integrons of 1.2kb was detected from one Kenyan isolate, while 9 isolates from Ethiopia harbored class I integrons of 1.5kb. Three isolates were selected to represent isolates with integrons for the detection of gene cassettes for further DNA sequence analysis. One of the two isolates of 1.5-kb Class 1 integrons comprised of one gene cassette, *aad*A1 (*aminoglycoside adenyltransferase gene*) and we also noted two isolates were incorporated and flanked *aad*A7 together with *dhfr*7 resistance gene cassettes into the same Class 1 integrons. These particular isolates were resistant to streptomycin and trimethoprim. We also noted two isolates are incorporated and flanked *aad*A7 together with *dhfr*7 resistance gene cassettes into the same Class 1 integrons, these particular isolates were resistant to streptomycin and trimethoprim as shown in ***Table 4***. These sequences were deposited in the NCBI Genbank (https://www.ncbi.nlm.nih.gov/nuccore/751372177/).

Table 4: *Class 1 Integron Gene Cassettes Sequences*

Isolate	Origin	Resistance pattern	Class1 integron size (kb) (gene cassettes)
S11589	Ethiopia	CipNalStrSulTet	1.5 [*aadA2*]
S11591	Ethiopia	AmpStrSulSxlTetTmp	1.5 [*dhfr 7-aadA7*]
S16612	Ethiopia	AmpStrSulSxlTetTmp	1.2 [*aadA7*]
S11579	Ethiopia	AmcAmpCipGenNalStrSulTet	0.7 [Negative]
S11581	Ethiopia	AmcAmpCipGenNalStrSulTet	1.5 [Negative]
S11583	Ethiopia	AmcAmpCipNalStrSulTet	1.5 [Negative]
S11584	Ethiopia	CipGenNalStrSulTet	1.5 [Negative]
S11586	Ethiopia	CipNalStrSulTet	1.5 [Negative]
S11588	Ethiopia	CipNalStrSulTet	1.5 [Negative]
S16579	Kenya	StrSuTet	1.2 [*aadA7*] [*dhfr 7*]

Heavy Metal Micronutrient Tolerance Detection

For the 86 isolates subjected to MIC determination for Zinc and Copper, 98% (*n*=84) had an MIC of 4µg/ml, one isolate (*n*=1) 0.5 µg/ml, and one isolate had MIC of 0.25 µg/ml for Zinc. Copper, 98% (*n*=84) had MIC of 8 µg/ml and additionally isolates exhibited 1.0 µg/ml (*n*=1) and 2.0 µg/ml (*n*=1) as shown in ***Table 5***.

Table 5: *Minimum Inhibitory Concentrations for Heavy Metals Copper and Zinc*

MIC(ug/ml)	0.25	0.5	1.0	2.0	4.0	8.0
ZnCl2	1 (1.2%)	1 (1.2%)	-	-	84 (98%)	-
$CuSO_4$	-	-	1 (1.2%)	1 (1.2%)	-	84 (98%)

Discussion

The study shows that, the predominant serotypes identified from both countries were *S. Kentucky* that are rarely observed among swine *Salmonella* serovars in Kenya. Previous studies on *Salmonella* isolates in swine from Kenya and Ethiopia supposedly reported a predominance of *S. Typhimurium, S. Enteritidids S. Saintpaul* and *S. Heidelberg* [27-31]. *S. Kentucky* had been isolated in poultry because they competitively adapt to the *caecum* of chicken compared to other *Salmonella* serovars (32).

As colonizers they could contaminate farm environments and animal processing facilities where they were capable of forming biofilms prompting their survival [33-34].

Domestic spread of this serovar within the farm environment could contribute to their transmission to other domestic animals including swine. This could explain the many cases of human *Salmonellosis* due to *S. Kentucky* being documented globally [35].

Other serotypes identified in this study included *S. Hadar, S. Enteritidis, S. Blockely* and *S .Muenchen*. Strain variation in the different studies could be driven by the type of samples analyzed as well as location and the study period.

Antibiotic resistance to ampicillin, amoxycillin-clavulanic acid, sulphamethoxazole - trimethoprim, ciprofloxacin and nalidixic acid was relatively high. These findings agree with studies in Kenya and Ethiopia which have reported similar antibiotic resistance trends. The high rate of ciprofloxacin and nalidixic acid resistance observed was due to point mutation of *chromosomal gry*A gene at QRDR. Point mutation of *Ser*83 to Phenylalanine accounted for the high number of quinolone resistant isolates comparable to what has been observed globally [[27,29,36,37].

Another important mutation observed was at position 87 where there was replacement of aspartic acid (asp-87) by glycine (gly) which has been linked to low/ reduced susceptibility to ciprofloxacin in NTS isolates from animals and humans as well [38, 39].

Majority of the isolates exhibited flouroquinolone resistance marked by mutation in *gyr*A gene but we were able to detect one Plasmid-mediated quinolone resistance (PMQR) expressed in the *qnr*A gene. Interestingly we observed one isolate that exhibited mutation on *gyr*A gene where there was replacement of serine with isoleucine. Additionally this isolate displayed a plasmid-mediated quinolone resistance (PMQR) *qnr*A gene. Both regions, that is the QRDR and PMQR encoded ciprofloxacin resistance.

However, the latter confers low level of fluoroquinolone resistance therefore, facilitated the selection of high level of chromosomal resistance [40]. The findings on the high number of resistance observed both for first line antibiotics used in treatment of NTS and quinolones was supported by indiscriminate use of these antibiotics in animal production for therapeutic use or as additives in animal feeds to promote growth [27].

Class1 Integrons in gram-negative bacteria play an major role in dissemination of antibiotic resistance. To note, integrons carry gene cassette that encode antibiotic resistance. However, [41] demonstrated that these gene cassettes are not always present in an integron and this explains their absence in some of the isolates that expressed class 1 integron in this study.

Among the ten isolates sequenced, all expressed Class 1 Integrons, despite only four isolates expressed gene cassettes that included *aadA*2, *aadA*7 and *dhfr*7 that mediated resistance to aminoglycosides (*aadA*2, *aadA*7) and trimethoprim (*dhfr*7).

Few studies have characterized antimicrobial resistance determinants and its correlation with heavy metal tolerance factors in *Salmonella* isolates of swine origin. In this study, the MICs of heavy metal micronutrients copper

*pco*A Cu^{+2} and *czc*D Zn^{+2} from the MDR isolates was very low (3%) and none of the tolerance marker genes was detected.

Conclusion

The high prevalence of MDR *Salmonella* isolated from swine in Kenya and Ethiopia indicated the potential significance of pigs as a reservoir of antimicrobial-resistant *Salmonell*a that could pose a public health hazard in humans by contact. However, for all *Salmonella* serovars, antimicrobial resistance was not correlated with heavy metal tolerance.

Acknowledgments

The authors thank the Kenya Medical Research Institute for employee support in the program and extend gratitude to the members of Infectious Diseases Molecular Epidemiology Laboratory (IDMEL) for the technical assistance.

Source of Financial Support

This work was funded by NHI-Fogarty Program "Molecular Epidemiology and Key Issues of Foodborne pathogens in Eastern Africa".

References

1. **Centers for Disease Control and Prevention (CDC).** Vital signs: incidence and trends of infection with pathogens transmitted commonly through food-foodborne diseases active surveillance network, 10 U.S. sites, 1996-2010. *MMWR Morb Mortal Wkly Rep [Internet].* 2011 Jun 10 [cited 2019 Oct 14];60(22):749–55. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21659984
2. **Galanis E, Wong DMALF, Patrick ME, Binsztein N, Cieslik A, Chalermchaikit T,** et al. Web-based Surveillance and Global *Salmonella* Distribution, 2000–2002. Emerg Infect Dis [Internet]. 2006 Mar [cited 2019 Oct 14];12(3):381–8. Available from: http://wwwnc.cdc.gov/eid/article/12/3/05-0854_article.htm
3. **Alghoribi MF, Doumith M, Alrodayyan M, Zayer M Al, Köster WL, Muhanna A,** et al. S. Enteritidis and *S. Typhimurium* harboring SPI-1 and SpI-2 are the predominant serotypes associated with human *salmonellosis* in Saudi Arabia. Front Cell Infect Microbiol. 2019;9(MAY).
4. **Majowicz SE, Musto J, Scallan E, Angulo FJ, Kirk M, O'Brien SJ,** et al. The Global Burden of Non-typhoidal *Salmonella* Gastroenteritis. Clin Infect Dis [Internet]. 2010;50(6):882–9. Available from: https://academic.oup.com/cid/article-lookup/doi/10.1086/650733
5. **CDC.** ANTIBIOTIC RESISTANCE THREATS in the United States. 2013.
6. **WHO.** WHO | Antimicrobial resistance in the food chain. WHO. 2017;
7. **Gomes-Neves E, Antunes P, Tavares A, Themudo P, Cardoso MF, Gärtner F,** et al. *Salmonella* cross-contamination in swine abattoirs in Portugal: Carcasses, meat and meat handlers. *Int J Food Microbiol.* 2012 Jun 15;157(1):82–7.
8. **Arguello H., Álvarez-Ordoñez A., Carvajal A., Rubio P., Prieto M.** Role of slaughtering in *Salmonella* spreading and control in pork production. *Vol. 76, Journal of Food Protection.* 2013. p. 899–911.
9. **Lekagul A, Tangcharoensathien V, Yeung S.** Patterns of antibiotic use in global pig production: A systematic review. *Vol. 7, Veterinary and Animal Science. Elsevier B.V.; 2019.*
10. **Yang H, Paruch L, Chen X, van Eerde A, Skomedal H, Wang Y, et al.** Antibiotic application and resistance in swine production in China: Current situation and future perspectives. Vol. 6, Frontiers in Veterinary Science. Frontiers Media S.A.; 2019.
11. **Landers TF, Cohen B, Wittum TE, Larson EL.** A review of antibiotic use in food animals: Perspective, policy, and potential. Vol. 127, *Public Health Reports. Association of Schools of Public Health;* 2012. p. 4–22.
12. **Medardus JJ, Molla BZ, Nicol M, Morrow WM, Rajala-Schultz PJ, Kazwala R**, et al. In-feed use of heavy metal micronutrients in U.S. swine production systems and its role in persistence of multidrug-resistant *salmonellae*. Appl Environ Microbiol. 2014;80(7):2317–25.
13. **Gómez-Sanz E, Kadlec K, Feßler AT, Zarazaga M, Torres C, Schwarz S.** Novel erm(T)-carrying multi-resistance plasmids from porcine and human isolates of methicillin-resistant Staphylococcus aureus ST398 that also harbor cadmium and copper resistance determinants. *Antimicrob*

Agents Chemother [Internet]. 2013 Jul [cited 2019 Oct 14];57(7):3275–82. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23629701.

14. **FAO**. DRIVERS, DYNAMICS AND EPIDEMIOLOGY OF [Internet]. 2016 [cited 2019 Oct 14]. Available from: www.fao.org/publications

15. **Gebreyes WA, Thakur S**. Multidrug-resistant *Salmonella enterica* serovar Muenchen from pigs and humans and potential interserovar transfer of antimicrobial resistance. *Antimicrob Agents Chemother*. 2005 Feb;49(2):503–11.

16. **Gong J, Zeng X, Zhang P, Zhang D, Wang C, Lin J.** Characterization of the emerging multidrug-resistant *Salmonella enterica serova*r Indiana strains in China. *Emerg Microbes Infect*. 2019 Jan 1;8(**1**):29–39.

17. **Elnekave E, Hong S, Mather AE, Boxrud D, Taylor AJ, Lappi V,** et al. *Salmonella enterica* Serotype 4,[5],12:i:-in Swine in the United States Midwest: An Emerging Multidrug-Resistant Clade. *Clin Infect Dis. 2018* Mar 5;66(6):877–85.

18. **Gebreyes WA, Davies PR, Turkson P-K, Morrow WEM, Funk JA, Altier C**, et al. Characterization of Antimicrobial-Resistant Phenotypes and Genotypes among *Salmonella enterica* Recovered from Pigs on Farms, from Transport Trucks, and from Pigs after Slaughter. *J. Food Pro*t [Internet]. 2004;67(4):698–705. Available from: http://www.ingentaconnect.com/content/iafp/jfp/2004/00000067/00000004/art00011.

19. **Wales AD, Davies RH.** Co-selection of resistance to antibiotics, biocides and heavy metals, and its relevance to foodborne pathogens. Antibiotics. 2015;4(**4**):567–604.

20. **WHO.** WHO / Use of quinolones in animal food and potential impact on human health. WHO. World Health Organization; 2014.

21. **Economou V, Gousia P.** Agriculture and food animals as a source of antimicrobial-resistant bacteria. *Vol. 8, Infection and Drug Resistance*. Dove Medical Press Ltd.; 2015. p. 49–61.

22. **Stevens A, Kaboré Y, Perrier-Gros-Claude J-D, Millemann Y, Brisabois A, Catteau M,** et al. Prevalence and antibiotic-resistance of Salmonella isolated from beef sampled from the slaughterhouse and from retailers in Dakar (Senegal). *Int J Food Microbiol* [Internet]. 2006 Jul 15 [cited 2019 Oct 15];110(2):178–86. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16774796.

23. **Raufu IA, Fashae K, Ameh JA, Ambali A, Ogunsola FT, Coker AO,** et al. Persistence of fluoroquinolone-resistant *Salmonella* enterica serovar Kentucky from poultry and poultry sources in Nigeria. *J. Infect Dev Ctries* [Internet]. 2014 Mar 13 [cited 2019 Oct; 15];8(3):384–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24619272.

24. **Chattaway M.A, Aboderin AO, Fashae K, Okoro CK, Opintan JA, Okeke IN.** Fluoroquinolone-resistant enteric bacteria in sub-saharan Africa: Clones, implications and research needs. Vol. 7, Frontiers in Microbiology. *Frontiers Media S.A.; 2016.*

25. **Yang H., Chen H., Yang Q., Chen M., Wang H.** High Prevalence of Plasmid-Mediated Quinolone Resistance Genes *qnr* and *aac* (6 J) - Ib-cr in Clinical Isolates of *Enterobacteriaceae* from Nine Teaching Hospitals in China . 2008;52(**12**):4268–73.

26. **Cattoir V, Weill F. X, Poirel L, Fabre L, Soussy C. J, Nordmann P.** Prevalence of *qnr* genes in *Salmonella* in France. *J Antimicrob Chemother. 2007*;59(4):751–4.

27. **Nyabundi D., Onkoba N., Kimathi R., Nyachieo A, Juma G, Kinyanjui P.** et al. Molecular characterization and antibiotic resistance profiles of *Salmonella* isolated from fecal matter of domestic animals and animal products in Nairobi. *Trop Dis Travel Med Vaccines* [Internet]. 2017;1–7. Available from: http://dx.doi.org/10.1186/s40794-016-0045-6

28. **Kikuvi G.M, Ombui J.N, Mitema E.S.** Serotypes and antimicrobial resistance profiles of **Salmonella** isolates from pigs at slaughter in Kenya. *J Infect Dev Ctries* [Internet]. 2010 Mar 9 [cited 2019 Oct 15];4(04):243–8. Available from: https://jidc.org/index.php/journal/article/view/20440063.

29. **Molla B., Berhanu A., Muckle A., Cole L., Wilkie E, Kleer J.,** et al. Multidrug Resistance and Distribution of *Salmonella* Serovars in Slaughtered Pigs. 2006;33:28–33.

30. **Wabeto W., Abraham Y., Anjulo A. A.** Detection and identification of antimicrobial-resistant *Salmonella* in raw beef at Wolaita Sodo municipal abattoir, Southern Ethiopia. J *Heal Popul Nutr. 2017*;36(1):1–7.

31. **Gebreyes W. A., Thakur S., Dorr P., Tadesse D. A., Post K., Wolf L..** Occurrence of spvA virulence gene and clinical significance for multidrug-resistant *salmonella* strains. *J Clin Microbiol.* 2009;47(3):777–80.

32. **Cheng Y, Pedroso A. A., Porwollik S., McClelland M., Lee M. D., Kwan T.,** et al. rpoS-regulated core genes involved in the competitive fitness of *Salmonella enterica* serovar kentucky in the intestines of chickens. *Appl Environ Microbiol.* 2015;81(**2**):502–14.

33. **Shi Z., Dittoe D. K., Ricke S. C.** Non-molecular characterization of pellicle formation by poultry *Salmonella Kentucky* strains and other poultry-associated *Salmonella* serovars in Luria Bertani broth. *J Environ Sci Health B* [Internet]. 2019 Sep 9 [cited 2019 Oct 16] ; 1–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/31496354

34. **Colello R., Ruiz M. J., Padín V. M., Rogé A. D., Leotta G., Padola N. L.,** et al. Detection and characterization of *Salmonella* Serotypes in the production chain of two pig farms in Buenos Aires Province, Argentina. *Front Microbiol. 2018* Jun 28;9(JUN).

35. **CDC.** National Enteric Disease Surveillance: *Salmonella* Annual Report, 2012 Human Surveillance Data: *Laboratory-based Enteric Disease Surveillance (LEDS)* [Internet]. 2012 [cited 2019 Oct 16]. Available from: http://www.cdc.gov/nationalsurveillance/PDFs/NationalSalmSurveillOverview_508.pdf.

36. **Kikuvi, J.N. O, E.S. M., S. S.** Antimicrobial resistance in *Salmonella* serotypes isolated from slaughter animals in Kenya. *East Afr Med J. 2007* May ; 84 (**5**) : 233 – 9.

37. **Tadesse G., Tessema T. S., Beyene G., Aseffa A.** Molecular epidemiology of fluoroquinolone resistant *Salmonella* in Africa: *A systematic review and meta-analysis. 2018* [cited 2019 Oct 18]; Available from: https://doi.org/10.1371/journal.pone.0192575.

38. **Adesiji Y. O., Shivakumaraswamy S. K., Deekshit V. K., Kallappa G. S., Karunasagar I.** Molecular characterization of antimicrobial multi-drug resistance in non-typhoidal *Salmonellae* from chicken and clam in Mangalore, India. *J Biomed Res.* 2018 ; 32(**3**) : 237 – 44.

39. **Aldrich C., Hartman H., Feasey N., Chattaway M. A, Dekker D., Al-Emran HM,** et al. Emergence of phylogenetically diverse and fluoroquinolone resistant *Salmonella Enteritidis* as a cause of invasive non-typhoidal *Salmonella* disease in Ghana. *PLoS Negl Trop. Dis. 2019* Jun 20 ; 13(**6**): e0007485.

40. **Bai L., Zhao J., Gan X., Wang J., Zhang X., Cui S,** et al. Emergence and diversity of *Salmonella enterica* serovar Indiana isolates with concurrent resistance to ciprofloxacin and cefotaxime from patients and food-producing animals in China. *Antimicrob Agents Chemother. 2016* Jun 1;60(6):3365–71.

41. **Stokes H. W, O'Gorman D. B, Recchia G. D, Parsekhian M., Hall R. M.** Structure and function of 59-base element recombination sites associated with mobile gene cassettes. *Mol Microbiol. 1997*;26(4):731–45.

42. **Mackie T. A, Barker D. K, Palmer E. A., Veterinary N., Laboratories S., Diseases Z. I.** et al. *Salmonella* Serotypes Isolated from Animals and Related Sources. 2016;13–6.

43. **Nash J.H.E.** Evaluation of Molecular Methods for Identification of. 2016 ; 54(**8**) : 1992–8.

44. **Euzéby J. P.** Revised *Salmonella* nomenclature: designation of *Salmonella enterica* (**ex Kauffmann and Edwards** 1952) Le Minor and Popoff 1987 sp. nov., nom. rev. as the neotype species of the genus *Salmonella Lignieres* 1900 (approved lists 1980), rejection of the name *Salmonella* choleraesuis (**Smith** 1894) **Weldin** 1927 (approved lists 1980), and conservation of the name *Salmonella typhi* (Schroeter 1886) **Warren** and **Scott** 1930 (approved lists 1980). Request for an opinion. *Int J Syst Bacteriol* [Internet]. 1999 Apr [cited 2019 Oct 14];49 Pt 2:927–30. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10319519.

Appendix 1.

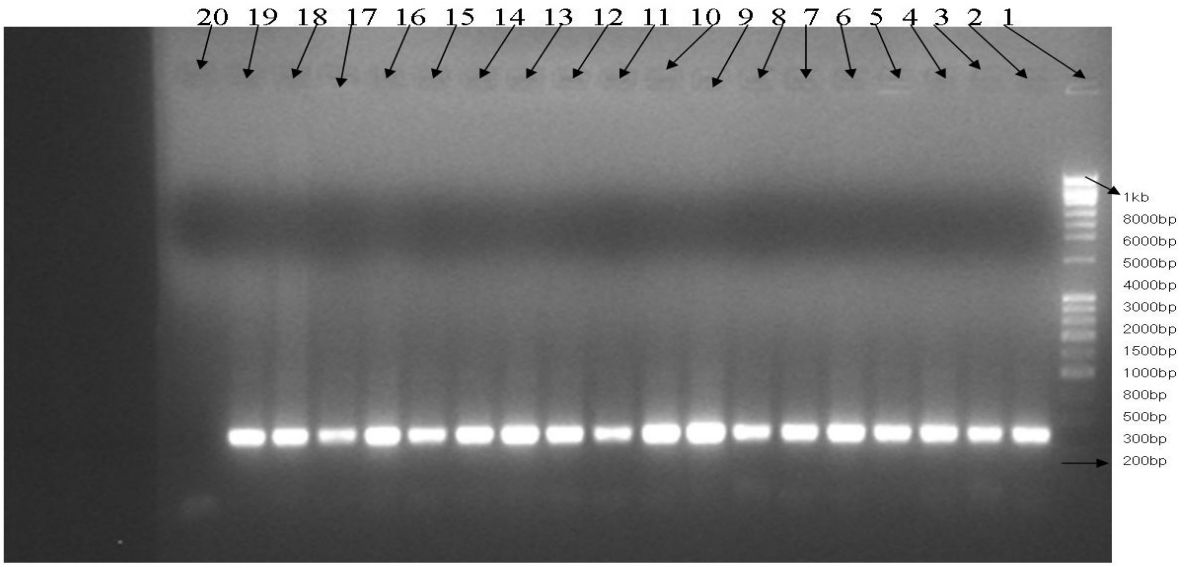

Figure 1: *PCR Amplification Plate for gyrA gene (251bp)*

Key: *Lane number 1kb- DNA ladder, Lanes 2 to 19 NTS DNA Template, Lane 20 negative Control (PCR water).*

Geographical location	Serotype (N)
Ethiopia	*1:6,8-enx (2)*
	1:6,8-Z10 (1)
	1:9,12 (2)
	Blockley (4)
	Braenderup (1)
	Dublin (1)
	Enteritidis PT 13a (1)
	Enteritidis Pt 8 (1)
	Enteritidis Pt911 (1)
	Hadar (5)
	Kentucky (12)
	Haifa (1)
	I: Rough-O (1)
	Kiambu (1)
	Typhimurium DT 104 (1)
Kenya	*Agona (1)*
	Blijdorp (1)
	Haifa (1)
	Kentucky (2)
	Muenchen (2)
	Stanley ville (1)

Appendix 3

Table 3: *Mutations of gyrA and qnrA Genea and Resulting Resistance Phenotype*

Resistant type	Numbers	*gyr*A mutation types		*qnr*A
		Ser 83	*Asp 87*	
AmcAmpChiCipFenNalSptStrSulTet	*1*	***Pheny (1)***	*Gly(1)*	*0*
AmcAmpCipGenNalSptStrSulTet	*2*	***Gly (1)***	*None*	*0*
		Lysine (1)		*0*
		Ser (1)		*0*
		a.a(1)		*0*
AmcAmpCipNalStrSulTet	*1*	***No.a.a (1)***	*Gly(1)*	*0*
AmcAmpCipNal	*1*	***Phenyl (1)***	*Asp(1)*	*0*
CipGenNalSptStrSulTet	*2*	***Iso (1)***		*1*
		Gly (1)		*0*
		Pheny (1)		*0*
CipKanNalNeoNitStrTet	*4*	***Pheny (3)***		*0*
		Ser (1)		*0*
		Arg(1)		*0*
CipNalNit	*5*	***Pheny (4)***		*0*
		Poor quality		*0*
SulCip	*19*	***Iso (2)***		*1*
		Aspar (2)		*0*
		Ser (2)		*0*
		Aspar (2)		*0*
		Gly (2)	*Lys(2)*	*0*
		Pheny (2)	*Asn(2)*	*0*
		Cysteine (5)	*Arg(5)*	*0*
		Pheny (1)	*N(1)*	*0*
		N (1)		*0*
		Tyrosine (1)		*0*
		Ser (1)		*0*

Key: *Pheny-phenylalanine,* ***Gly****-glycine,* ***Ser****-serine,* ***a.a****-amino acid,* ***Iso****-isoleucine,* ***Arg-****arginine,* ***Asp****-aspartic acid,* ***Aspar****-asparigine,* ***Lys****-lysine,* ***no.a.a*** *Amino acid,* ***N*** *base sequence the software was unable*