Genetic markers associated with resistance to beta-lactam and quinolone antimicrobials in non-typhoidal Salmonella isolates from humans and animals in central Ethiopia

Citation for published version:

Digital Object Identifier (DOI):
10.1186/s13756-017-0171-6

Link:
Link to publication record in Edinburgh Research Explorer

Document Version:
Publisher's PDF, also known as Version of record

Published In:
Antimicrobial resistance and infection control

Publisher Rights Statement:
© The Author(s). 2017 Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated.

General rights
Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy
The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.
Genetic markers associated with resistance to beta-lactam and quinolone antimicrobials in non-typhoidal *Salmonella* isolates from humans and animals in central Ethiopia

Tadesse Eguale¹*, Josephine Birungi², Daniel Asrat³, Moses N. Njahira⁴, Joyce Njuguna², Wondwossen A. Gebreyes⁵, John S. Gunn⁶, Appolinaire Djikeng² and Ephrem Engidawork⁷

**Abstract**

**Background:** Beta-lactam and quinolone antimicrobials are commonly used for treatment of infections caused by non-typhoidal *Salmonella* (NTS) and other pathogens. Resistance to these classes of antimicrobials has increased significantly in the recent years. However, little is known on the genetic basis of resistance to these drugs in *Salmonella* isolates from Ethiopia.

**Methods:** *Salmonella* isolates with reduced susceptibility to beta-lactams (*n* = 43) were tested for genes encoding for beta-lactamase enzymes, and those resistant to quinolones (*n* = 29) for mutations in the quinolone resistance determining region (QRDR) as well as plasmid mediated quinolone resistance (PMQR) genes using PCR and sequencing.

**Results:** Beta-lactamase genes (*bla*) were detected in 34 (79.1%) of the isolates. The dominant *bla* gene was *bla*TEM, recovered from 33 (76.7%) of the isolates, majority being TEM-1 (24, 72.7%) followed by TEM-57, (10, 30.3%). The *bla*OXA-10 and *bla*CTX-M-15 were detected only in a single S. Concord human isolate. Double substitutions in *gyrA* (Ser83-Phe + Asp87-Gly) as well as *parC* (Thr57-Ser + Ser80-Ile) subunits of the quinolone resistance determining region (QRDR) were detected in all S. Kentucky isolates with high level resistance to both nalidixic acid and ciprofloxacin. Single amino acid substitutions, Ser83-Phe (*n* = 4) and Ser83-Tyr (*n* = 1) were also detected in the *gyrA* gene. An isolate of S. Miami susceptible to nalidixic acid but intermediately resistant to ciprofloxacin had Thr57-Ser and an additional novel mutation (Tyr83-Phe) in the *parC* gene. Plasmid mediated quinolone resistance (PMQR) genes investigated were not detected in any of the isolates. In some isolates with decreased susceptibility to ciprofloxacin and/or nalidixic acid, no mutations in QRDR or PMQR genes were detected. Over half of the quinolone resistant isolates in the current study 17 (58.6%) were also resistant to at least one of the beta-lactam antimicrobials.

**Conclusion:** Acquisition of *bla*TEM was the principal beta-lactamase resistance mechanism and mutations within QRDR of *gyrA* and *parC* were the primary mechanism for resistance to quinolones. Further study on extended spectrum beta-lactamase and quinolone resistance mechanisms in other gram negative pathogens is recommended.

**Keywords:** Non-typhoidal *Salmonella*, Antimicrobial resistance, Mechanisms of resistance, Beta-lactamase, Quinolone, Fluoroquinolone, Human strains, Animal strains, Ethiopia

* Correspondence: tadesse.eguale@aau.edu.et
1 Aklilu Lemma Institute of Pathobiology, Addis Ababa University, P.O. Box 1176, Addis Ababa, Ethiopia
Full list of author information is available at the end of the article

© The Author(s). 2017 Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated.
Background
Salmonellosis in humans is caused by several serovars belonging to *Salmonella enterica* subspecies enterica. Infection by *Salmonella* causes two forms of diseases; typhoid fever, a febrile illness caused by a few host specific serovars such as *Salmonella enterica* subspecies enterica serovar Typhi (S. Typhi), and S. Paratyphi A, while the majority of *Salmonella* serovars cause non-typhoidal salmonellosis characterized by self limiting gastroenteritis and occasional invasive salmonellosis in immunocompromised, young and elderly patients. Infection with non-typhoidal *Salmonella* (NTS) serovars is one of the leading causes of foodborne illnesses worldwide [1]. NTS infection is commonly associated with consumption of contaminated food of animal origin such as poultry products, beef and pork as well as contact with infected animals [2–4].

Antimicrobial treatment is usually not recommended due to the self-limiting nature of the disease. However, in cases of invasive complicated salmonellosis, treatment with beta-lactam antimicrobials such as ampicillin, ceftriaxone and quinolone drugs are employed as lifesaving agents [5]. Resistance to beta-lactam antimicrobials and quinolones has increased dramatically in NTS isolates from humans as well as food animals worldwide [6–9]. The common mechanism of resistance to beta-lactam antimicrobials is due to production of beta-lactamase enzymes with variable level of activity against different generations of beta-lactam antimicrobials. In addition to the first generation beta-lactamases: *blaTEM1, blaSHV1*, several extended spectrum *blaTEM* and *blaSHV* variants, other extended spectrum beta-lactamase enzymes such as *blaCTX-M, blaCMY, blaOXA* and AmpC have been reported in *Salmonella* serotypes from different parts of the world [10–13].

Resistance to quinolone drugs is primarily mediated by mutations in Quinolone Resistance Determining Region (QRDR) of *gyrA* and *parC* genes in *Salmonella* and other Gram-negative organisms. Specifically, high level resistance to ciprofloxacin is frequently attributed to double mutations in the *gyrA* gene and single or double mutation in the *parC* gene [14]. In addition to chromosomal mutations, other mechanisms such as activation of efflux pumps (multidrug efflux pump and quinolone specific plasmid mediated efflux pump encoded by *qep* genes), *qnr* (plasmid-mediated quinolone resistance), porins, and quinolone-modifying enzyme (*aac(6’)-Ib-cr*) have been associated with decreased susceptibility to quinolones [14]. Of particular concern is the occurrence, within the last few years in different parts of the world, of plasmid-mediated quinolone resistance encoded by several *qnr* genes. These genes encode for pentapeptide proteins that protect bacterial topoisomerases from the effect of quinolones. They do not induce high level resistance but their presence leads to mutation in the QRDR [15]. However, recent report from Senegal indicated the presence of *qnrB1* together with the quinolone modifying enzyme *aac(6’)-Ib-cr* in *Salmonella* associated with high level resistance to ciprofloxacin even in the absence of mutations in the QRDR [16]. These resistance determinants have been observed in various gram negative organisms including *Salmonella* [16, 17]. In recent years, the rate of resistance to ciprofloxacin has increased considerably in both clinical and food isolates of *Salmonella* [6, 18, 19].

In Ethiopia, reports revealed resistance to beta-lactam antimicrobials and quinolones in *Salmonella* isolates from human patients and food of animal origin [20, 21]. However, little data is available on the genetic basis of the observed phenotypic drug resistance. Multidrug resistant *S. Concord* isolates obtained from children adopted from Ethiopia in different European countries and USA were reported to harbor *blaCTX-M-15, blaTEM1, blaSHV-12* genes encoding for resistance to third generation cephalosporins, *qnrA* and *qnrB* encoding for reduced susceptibility to fluoroquinolones [22, 23]. The aim of this study was to investigate the genetic markers associated with resistance to beta-lactam and quinolone antimicrobials among NTS isolates collected from humans and animals in central Ethiopia.

Methods
Bacterial isolates
Non-typhoidal *Salmonella* strains investigated in the current study were isolated from feces of food animals (cattle *n* = 50, poultry *n* = 26, swine *n* = 8) in Addis Ababa and surrounding districts of Oromia region namely: Ada, Barake, Sebeta and Sululta. In addition, *Salmonella* isolates obtained from stool of temporally and spatially related diarrheic human patients from primary health centers and Tikur Anbessa Specialized Hospital in Addis Ababa (*n* = 68) were also included. All human and animal isolates were collected from 2013 to 2014.

Antimicrobial susceptibility testing, serotyping and phage typing
Susceptibility of each isolate to beta-lactam and quinolone antimicrobials was determined using disk diffusion method according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI). The interpretation of the categories of susceptible, intermediate or resistant was also based on the CLSI guidelines [24]. For purposes of analysis, all readings classified as intermediate were considered resistant unless otherwise mentioned. *Escherichia coli* ATCC 25922 was used as a quality control. *Salmonella* isolates were serotyped and phage-typed at the Public Health Agency of Canada, World Organization for Animal Health
Reference Laboratory for Salmonellosis, Guelph, Ontario, Canada as described previously [25].

Bacterial DNA extraction
Isolates were grown on Luria Bertani (LB) agar (37 °C, over night). A single colony was inoculated to 5 ml of LB broth and grown in a shaking incubator at 37 °C for 16–18 h. Genomic DNA was then extracted using the QIAGEN genomic DNA extraction kit (QIAGEN, USA) according to the manufacturer’s recommendation.

Detection and characterization of beta-lactamase enzymes
A total of 43 isolates, 12 from humans and 31 from animals, with reduced susceptibility to one or more of beta-lactam antimicrobials (ampicillin, amoxicillin + clavulanic acid, cephalothin, ceftriaxone) were tested for genes encoding for beta-lactamase enzymes. PCR and DNA sequencing were performed for the detection and characterization of beta-lactamase (bla) genes with oligonucleotide primers previously described for blaTEM, blaSHV, blaPER, blaPSE, blaOXA1, blaOXA4, blaOXA10, blaCMY, and blaCTX-M genes (Table 1). The PCR conditions for all reactions involved an initial denaturation for 3 min at 95 °C followed by 30 cycles of (95 °C for 30 s, specific annealing temperature for 1 min, and extension at 72 °C for 30 s) followed by a final extension at 72 °C for 5 min. Specific annealing temperature for each PCR reaction is shown in Table 1. Group specific primers were used to characterize blaCTX-M enzymes [26]. The PCR amplicons were purified using QIAGEN PCR purification kit (QIAGEN, USA) and sequenced with forward and reverse primers at Sequencing, Genotyping, Oligosynthesis and Proteomics (Segolip) unit of Biosciences eastern and central Africa (BecA). All amplicon sequences were assembled and translated to

Table 1 List of primers used for detection and characterization of beta-lactamases

<table>
<thead>
<tr>
<th>Gene/target</th>
<th>Primer</th>
<th>Sequence 5’-3’</th>
<th>Amplicon size</th>
<th>AT °C</th>
<th>Ref</th>
<th>Remark</th>
</tr>
</thead>
<tbody>
<tr>
<td>BLATem Gene</td>
<td>TEM-F1</td>
<td>ATGAGTATCCACATTTCCG</td>
<td>862-bp</td>
<td>55</td>
<td>[34]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TEM-R1</td>
<td>GACAGTACACATACTTCA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>blaTEM-F2</td>
<td>TAA CCA TGAATGATACCAT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>blaTEM-R2</td>
<td>GCGATGTT GTCGAAGTAA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BLA SHV gene</td>
<td>Bla SHV-F1</td>
<td>CTTCACGCCTTTATCG</td>
<td>827-bp</td>
<td>56</td>
<td>[34]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bla SHV-R1</td>
<td>TCCCCGC AGATAAATACCA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>blaSHV-F2</td>
<td>ACTGCGCTTTTTCGCAGAT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>blaSHV-R2</td>
<td>CAGTTCGTTTCCCCAGCGGT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bla OXA-1</td>
<td>OXA-1-F</td>
<td>ATGAAAAAACACACACACAT</td>
<td>755-bp</td>
<td>48</td>
<td>[13]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>OXA-1-R</td>
<td>TTTTCCGTAAGTGGCGACAC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bla OXA-4</td>
<td>OXA-4-F</td>
<td>TCAACAGATATCTTCTAGTG</td>
<td>216 bp</td>
<td>54</td>
<td>[13]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>OXA-4-R</td>
<td>TTTATCCCATTGGAATATG</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bla OXA-10</td>
<td>OXA-10-F</td>
<td>TCAACAAATGCCAGAGAAG</td>
<td>277 bp</td>
<td>57</td>
<td>[13]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>OXA-10-R</td>
<td>TCCCCACACAGAACACACAC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>bla PER</td>
<td>Per1-F</td>
<td>AATTGGGCTTATGCGGAGAAG</td>
<td>925 bp</td>
<td>55</td>
<td>[50]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Per1-R</td>
<td>ATGAATGTCATTATAAAAGC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>blaPSE</td>
<td>blaPSE-F</td>
<td>TGTCCTGCAACTATGCTAC</td>
<td></td>
<td></td>
<td></td>
<td>[42]</td>
</tr>
<tr>
<td></td>
<td>blaPSE-R</td>
<td>AGCTGGTGTGAGCTAGAT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>blaCMY</td>
<td>blaCMY2-F</td>
<td>TGCCCGTGGCGGTATCTAC</td>
<td>868</td>
<td>62</td>
<td>[38]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>blaCMY2-R</td>
<td>CCCGTTTTGACCGCAT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTX-M group I</td>
<td>CTXM1-F3</td>
<td>GACGATGTCAGCTGGCGAGC</td>
<td>499</td>
<td>55</td>
<td>[38]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CTXM1-R2</td>
<td>AGCCCGGCGCGCTAATACA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTX-M group II</td>
<td>TOHO1-2 F</td>
<td>GCGACGCTGTTTTACACCAATCC</td>
<td>351</td>
<td>55</td>
<td>[38]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TOHO1-1R</td>
<td>CGTAAGTATGCGCTAAGCC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTX-M group III</td>
<td>CTXM825F</td>
<td>CGTCTTGGCAATGCGACGACC</td>
<td>307</td>
<td>55</td>
<td>[38]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CTXM825R</td>
<td>GCT CATACGATCGCACCC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTX-M group IV</td>
<td>CTXM914F</td>
<td>GCTGGAGAAGGAGCGCGCGAG</td>
<td>474</td>
<td>62</td>
<td>[38]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CTXM914R</td>
<td>GTAAGCTGACGCAGCTCG</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
amino acid sequences using CLC Main Work Bench (Inqaba Biotechnical Industries, (Pty) Ltd, Denmark) and compared with protein sequences in the Genbank database. Classification of blaTEM enzymes was based on beta-lactamase classification database (https://www.lahey.org/studies/temtable.asp).

Investigation of quinolone resistance mechanism
Isolates with reduced susceptibility to nalidixic acid and/or ciprofloxacin (n = 29), three human isolates and 26 animal isolates were examined for the presence of known quinolone resistance determinants. Quinolone resistance determining region (QRDR): gyrA, gyrB, parC and parE genes were amplified using PCR. PCR was also used to examine for various plasmid mediated quinolone resistance genes: qnrA, qnrB, qnrD, qnrS, qepA, and aac(6′)-Ib-cr as described previously (Table 2). Similar PCR conditions described previously were used and annealing temperature for each primer set is presented in Table 2. PCR amplicons were purified using QIAGEN PCR purification kit and sequenced as previously described. Presence of mutation in the QRDR was examined by translating nucleotide sequences into proteins and aligning against reference sequence of S. Typhimurium strain LT2 on NCBI database (Accession Number AE006468).

Results
Resistance to beta-lactam antimicrobials and beta-lactamase genes in Salmonella isolates from animals and humans
Of the 20 different serotypes investigated in the current study, resistance to at least one beta-lactam antimicrobial was detected in nine serotypes and the bla gene was detected only in isolates belonging to six serotypes. Of the 43 isolates resistant to one or more beta-lactam antimicrobials (ampicillin, amoxicillin + clavulanic acid, cephalothin, ceftriaxone), bla genes were detected in 34/43 (79.1%) of the isolates. The dominant bla gene responsible for resistance to beta-lactam antimicrobials in the majority of Salmonella isolates, 33 (76.7%) was found to be variants of blaTEM gene. Most of these were TEM-1 type, 24 (72.7%) followed by TEM-57, 10 (30.3%). Both phenotypic resistance to beta-lactam antimicrobials and detection of bla genes was more common in isolates obtained from poultry compared to isolates from other sources (Table 3). In one of the human isolates of S. Concord, two bla genes (blaOXA-10 and blaCTX-M-15) were detected. Both of these genes

<table>
<thead>
<tr>
<th>Table 2</th>
<th>List of primers used for detection of quinolone resistance mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gene</td>
<td>Primer name</td>
</tr>
<tr>
<td>gyrA</td>
<td>GyrAFP</td>
</tr>
<tr>
<td></td>
<td>GyrARP</td>
</tr>
<tr>
<td>gyrB</td>
<td>GyrB FP</td>
</tr>
<tr>
<td></td>
<td>GyrB RP</td>
</tr>
<tr>
<td>parC</td>
<td>ParC FP</td>
</tr>
<tr>
<td></td>
<td>ParC RP</td>
</tr>
<tr>
<td>ParE</td>
<td>ParE FP</td>
</tr>
<tr>
<td></td>
<td>ParE RP</td>
</tr>
<tr>
<td>qnrA</td>
<td>qnrA FP</td>
</tr>
<tr>
<td></td>
<td>qnrA RP</td>
</tr>
<tr>
<td>qnrB</td>
<td>qnrB FP</td>
</tr>
<tr>
<td></td>
<td>qnrB RP</td>
</tr>
<tr>
<td>aac(6′)-Ib</td>
<td>aac(6′)-Ib FP</td>
</tr>
<tr>
<td></td>
<td>aac(6′)-Ib-RP</td>
</tr>
<tr>
<td></td>
<td>aac(6′)-Ib-cr-seq</td>
</tr>
<tr>
<td>qepA</td>
<td>QepA FP</td>
</tr>
<tr>
<td></td>
<td>QepA RP</td>
</tr>
<tr>
<td>QnrD</td>
<td>QnrD FP</td>
</tr>
<tr>
<td></td>
<td>QnrD RP</td>
</tr>
<tr>
<td>QnrS</td>
<td>QnrS FP</td>
</tr>
<tr>
<td></td>
<td>QnrS RP</td>
</tr>
</tbody>
</table>

FP forward primer, RP Reverse primer
encode for enzymes capable of extended spectrum beta-lactamase activity. This isolate was resistant to the third generation cephalosporin, ceftriaxone in addition to ampicillin, and cephalothin. In eight (18.6%) of the isolates, none of the tested bla genes were detected (Table 3).

Among the dominant serotypes, 66.7, 92.3, 50 and 100% of S. Typhimurium, S. Saintpaul, S. Virchow and S. Kentucky were positive for variants of the blaTEM gene, respectively. All of the 10 S. Kentucky isolates collected from cattle, poultry and humans were resistant to ampicillin, cephalothin and amoxicillin + clavulanic acid and were all positive for blaTEM-1 gene (Table 4).

Interestingly, all 10 blaTEM-57 were recovered from S. Saintpaul isolated from poultry, while those S. Saintpaul strains obtained from cattle and human were all TEM-1 type. Despite a change in amino-acid sequences, there was no distinct difference in phenotypic antimicrobial susceptibility pattern to beta-lactam antimicrobials among isolates carrying blaTEM-57 and blaTEM-1 enzymes.

Among eight isolates in which none of the tested bla genes were detected, most of them were susceptible to the major beta-lactams and were at the margin of susceptibility and intermediate; S. Dublin (n = 2) and S. Typhimurium (n = 2) only to cephalothin and S. Haifa to ampicillin (n = 1) On the other hand, three S. Virchow isolates and one S. V;ROUGH-O;:::- were completely resistant to ampicillin and cephalothin.

Mechanism of resistance to quinolone antimicrobials

Out of the 29 Salmonella isolates with reduced sensitivity to quinolones, high level resistance to both nalidixic acid and ciprofloxacin was observed in only 10 S. Kentucky isolates (34.5%) (Table 5). All of these S. Kentucky isolates had double mutations in gyrA (Ser83-Phe + Asp87-Gly) and parC (Thr57-Ser + Tyr83-Phe) genes. Single mutation in gyrA (Ser83-Phe) was observed in four isolates (S. Livingstone var.14+ (2), S. Virchow (1), S. L6;7,14:-1;w (1). All these isolates were resistant to nalidixic acid and intermediately resistant to ciprofloxacin. A single amino acid substitution in gyrA (Ser83-Tyr) was detected in one S. Haifa from poultry with an R-phenotype [resistant to nalidixic acid and intermediate resistant to ciprofloxacin]. Overall, double and single substitutions in gyrA were detected in 15 (51.7%) of the isolates. Double substitution in parC (Thr57-Ser + Tyr83-Phe) was detected in one S. Miami isolated from swine. This strain was sensitive to nalidixic acid and intermediately resistant to ciprofloxacin. The Tyr83-Phe is a novel mutation. A strain of S. Agona with only single substitution at Thr57-Ser was intermediately resistant to nalidixic acid but sensitive to ciprofloxacin. Double substitution in the gyrB gene (Val423-Gly + Asp459-His) was detected in two isolates; S. Mikawasima and S. Braenderup, the latter having additional substitution in parC gene (Thr57-Ser) associated with intermediate susceptibility to both nalidixic acid and ciprofloxacin, whereas the former with intermediate susceptibility only to nalidixic acid. A strain of serotype V:rough-O;:::- that was susceptible to nalidixic acid but intermediately resistant to ciprofloxacin had single substitution of Ser463-Ala on gyrB gene. Over half of the quinolone resistant isolates in the current study 17 (58.6%) were also resistant to at least one of the beta-lactam antimicrobials (Table 5) and all S. Kentucky isolates resistant to nalidixic acid and ciprofloxacin were also shown to be MDR to several antimicrobials in our previous works [27, 28]. No mutation was detected in parE gene in any of the isolates examined in the current study. Nine isolates with reduced sensitivity to nalidixic acid and or ciprofloxacin had no mutation in any of the QRDR (Table 5).

Plasmid mediated quinolone resistance

None of the tested plasmid mediated quinolone resistance genes were detected in the isolates examined in the current study. Seven isolates belonging to serotypes Saintpaul, Typhimurium, Aberdeen, Virchow and Haifa were susceptible to nalidixic acid but had shown reduced sensitivity to ciprofloxacin according to CLSI (2013) cut-off points, with zone of inhibition ranging from 25 to 28 mm. There appears to be other resistance mechanisms responsible for the observed decreased sensitivity.

Table 3 Occurrence of bla genes in Salmonella isolates from different sources with reduced susceptibility to beta-lactam antimicrobials

<table>
<thead>
<tr>
<th>Source</th>
<th>Total no. of isolates</th>
<th>resistant to at least one beta-lactam (%)</th>
<th>bla genes detected</th>
<th>Not detected</th>
<th>% of positive for bla genes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>blTEM</td>
<td>blaOXA10</td>
<td>blaCTX-M</td>
</tr>
<tr>
<td>Cattle</td>
<td>50</td>
<td>16(32)</td>
<td>12</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Poultry</td>
<td>26</td>
<td>13(50)</td>
<td>12</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Swine</td>
<td>8</td>
<td>2(25)</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Human</td>
<td>68</td>
<td>12(17.7)</td>
<td>9</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>152</td>
<td>43(28.3)</td>
<td>34</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Source</th>
<th>Total no. of isolates</th>
<th>resistant to at least one beta-lactam (%)</th>
<th>bla genes detected</th>
<th>Not detected</th>
<th>% of positive for bla genes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>blTEM</td>
<td>blaOXA10</td>
<td>blaCTX-M</td>
</tr>
<tr>
<td>Cattle</td>
<td>50</td>
<td>16(32)</td>
<td>12</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Poultry</td>
<td>26</td>
<td>13(50)</td>
<td>12</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Swine</td>
<td>8</td>
<td>2(25)</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Human</td>
<td>68</td>
<td>12(17.7)</td>
<td>9</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>152</td>
<td>43(28.3)</td>
<td>34</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

*Though all isolates were screened for blaSHV, blaOXA1, blaOXA4, blaPER, blaoPE and blaCMY2, none of them were positive for these genes
*blaOXA10 and blaCTX-M-15 were detected in a single isolate.
Discussion
Detection of a high resistance rate to beta-lactam antimicrobials (50%) and the presence of \( bla \) genes in isolates from poultry could be due to the fact that drugs like ampicillin and amoxicillin are frequently employed in poultry farms in Ethiopia leading to selection pressure. The dominant beta-lactamase genes detected in the current study were variants of \( bla\)TEM with the majority being \( bla\)TEM-1, which is concordant with the observed spectrum of resistance to only ampicillin and amoxicillin.
first generation cephalosporin in most of the isolates. In Africa, blaTEM-1 has been reported from Salmonella isolated from poultry in Egypt [29], from children adopted from Mali [30], and S. Enteritidies in Senegal [31]. All S. Saintpaul isolated from poultry in the current study carried blaTEM-57, while those from cattle and human carried blaTEM-1. This is probably due to mutation of a blaTEM gene in a strain of S. Saintpaul in one of the poultry farms and clonal spread of strain carrying this mutant gene to farms in the area. All poultry S. Saintpaul were isolated from farms in the Adaa district. Compared to TEM-1, TEM-57 has a substitution of Gly to Asp at position 92 of amino acid sequence, which was first reported from Proteus mirabilis [32] and later on from E. coli in China [33]. To our knowledge, this is the first report of detection of blaTEM-57 in Salmonella. Fortunately, this mutation was not associated with extended spectrum activity against second and third generation cephalosporins.

One of the human isolates, S. Concord, resistant to ampicillin, cephalexin, cefotaxim and ceftriaxone was shown to produce blaCTX-M-15 and blaOXA-10. Previous studies have also reported blaCTX-M-15 in S. Concord isolated from children adopted from Ethiopia to different European countries and USA [34, 35]. In fact, a separate study also showed that blaCTX-M-15 isolated from

---

**Table 5** Susceptibility of isolates to quinolone drugs and mutation in QRDR

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Zone of inhibition mm (susceptibility category)</th>
<th>R-pattern betalactams</th>
<th>Mutation in QRDR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Na</td>
<td>Cip</td>
<td>Amp</td>
</tr>
<tr>
<td>Cattle</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aberdeen</td>
<td>19(S)</td>
<td>27(I)</td>
<td>-</td>
</tr>
<tr>
<td>Virchow</td>
<td>20(S)</td>
<td>25(I)</td>
<td>-</td>
</tr>
<tr>
<td>Typhimurium PT 3</td>
<td>21(S)</td>
<td>25(I)</td>
<td>Amp</td>
</tr>
<tr>
<td>Typhimurium PT 4</td>
<td>22(S)</td>
<td>27(I)</td>
<td>Amp</td>
</tr>
<tr>
<td>Haifa</td>
<td>21(S)</td>
<td>25(I)</td>
<td>-</td>
</tr>
<tr>
<td>Saintpaul</td>
<td>20(S)</td>
<td>31(S)</td>
<td>Amc</td>
</tr>
<tr>
<td>Cattle</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saintpaul</td>
<td>17(I)</td>
<td>25(I)</td>
<td>Amp</td>
</tr>
<tr>
<td>Cattle</td>
<td>21(S)</td>
<td>27(I)</td>
<td>-</td>
</tr>
<tr>
<td>Saintpaul</td>
<td>20(S)</td>
<td>27(I)</td>
<td>-</td>
</tr>
<tr>
<td>Human VROUGH-O:-:</td>
<td>22(S)</td>
<td>28(I)</td>
<td>Amp</td>
</tr>
<tr>
<td>Cattle</td>
<td>20(S)</td>
<td>25(I)</td>
<td>-</td>
</tr>
<tr>
<td>Agona</td>
<td>17(I)</td>
<td>31(S)</td>
<td>-</td>
</tr>
<tr>
<td>Braenderup</td>
<td>17(I)</td>
<td>25(I)</td>
<td>-</td>
</tr>
<tr>
<td>Swine</td>
<td>22(S)</td>
<td>27(I)</td>
<td>-</td>
</tr>
<tr>
<td>Haifa</td>
<td>0(R)</td>
<td>25(I)</td>
<td>-</td>
</tr>
<tr>
<td>Virchow</td>
<td>0(R)</td>
<td>26(I)</td>
<td>Amp</td>
</tr>
<tr>
<td>Livingstone var.14+</td>
<td>0(R)</td>
<td>24(I)</td>
<td>-</td>
</tr>
<tr>
<td>t67,14:-,uW</td>
<td>7(R)</td>
<td>30(I)</td>
<td>Amp</td>
</tr>
<tr>
<td>Livingstone var.14+</td>
<td>0(R)</td>
<td>20(I)</td>
<td>-</td>
</tr>
<tr>
<td>Kentucky</td>
<td>0(R)</td>
<td>14(R)</td>
<td>Amp</td>
</tr>
<tr>
<td>Cattle</td>
<td>0(R)</td>
<td>12(R)</td>
<td>Amp</td>
</tr>
<tr>
<td>Kentucky</td>
<td>0(R)</td>
<td>11(R)</td>
<td>Amp</td>
</tr>
<tr>
<td>Kentucky</td>
<td>0(R)</td>
<td>9(R)</td>
<td>Amp</td>
</tr>
<tr>
<td>Kentucky</td>
<td>0(R)</td>
<td>12(R)</td>
<td>Amp</td>
</tr>
<tr>
<td>Kentucky</td>
<td>0(R)</td>
<td>10(R)</td>
<td>Amp</td>
</tr>
<tr>
<td>Kentucky</td>
<td>0(R)</td>
<td>9(R)</td>
<td>Amp</td>
</tr>
<tr>
<td>Human</td>
<td>0(R)</td>
<td>8(R)</td>
<td>Amp</td>
</tr>
<tr>
<td>Kentucky</td>
<td>0(R)</td>
<td>10(R)</td>
<td>Amp</td>
</tr>
<tr>
<td>Kentucky</td>
<td>0(R)</td>
<td>9(R)</td>
<td>Amp</td>
</tr>
<tr>
<td>Kentucky</td>
<td>0(R)</td>
<td>11(R)</td>
<td>Amp</td>
</tr>
</tbody>
</table>

PT Phagetype, Amp Ampicillin, Amc Amoxicillin and clavulanic acid, Cf Cephalexin, Cip Ciprofloxacin, Na- Nalidixic acid, S susceptible, I intermediately resistant, R resistant

*Resistance status, isolates were fully resistant to antimicrobials written in Bold and intermediately resistant to those written in italics*
S. Concord from Ethiopia was chromosomally encoded [35]. Nevertheless, the previous studies also showed production of blaSHV-12 in most of the S. Concord from Ethiopia, but OXA-10 was not reported. This is presumably due to loss of a plasmid encoding for SHV-12 and acquisition of OXA-10 in a new isolate from Ethiopia. During the last few years, CTX-M-15 and other related CTX-M enzymes have been widely reported from various Enterobacteriaceae including Salmonella in different African countries from both hospital and community settings [16, 36–39]. Oxacilinas including OXA-10 have also been commonly isolated from different enterobacteriaceae including Salmonella [40].

The possible reason for the absence of bla genes in a few isolates with reduced susceptibility in the current study despite testing for most of the known bla reported in Salmonella could be due to poor sensitivity of phenotypic resistance detection methods. In two S. Typhimurium and two S. Dublin intermediately resistant only to cephaplatin and one S. Haifa immediately resistant to ampicillin, the reading was at the margin of intermediate and susceptible. However, all the three S. Virchow were fully resistant to ampicillin and cephaplatin and intermediately resistant to amoxicillin + clavulanic acid. For these isolates, other resistance mechanisms not investigated in this study such as alterations in the beta-lactam targets (PBPs) [41], absence or down-regulation of the production of outer membrane porins [42], over expression of efflux pumps [43] and different ampC beta-lactamases [44] might be responsible for the observed reduction in susceptibility. In general, the rate of occurrence of extended spectrum beta-lactamases in Salmonella isolates in the current study is low. This could be due to the fact that most of the human isolates were obtained from primary health care centers and use of 2nd and 3rd generation cephalosporins is not a common practice in veterinary medicine [28, 45]. The single MDR S. Concord in the current study was isolated from hospitalized 1 year old child.

Amino acid substitutions at codon 83 and 87 of gyrA gene have been associated with high level fluoroquinolone resistance [46–49] whereas resistance to only nalidixic acid is associated with single or double mutation in parC gene in Salmonella and other Gram-negative pathogens [14, 50]. Detection of two amino acid substitutions in the gyrA gene at codon 83 and 87 and the parC gene at codon 57 and 80 in all S. Kentucky isolates with high level resistance to both nalidixic acid and ciprofloxacin obtained from humans and animals suggests the possibility of clonal spread of S. Kentucky strain in the human and animal population in the study area. Similar mutations in gyrA and parC genes were reported from S. Kentucky from French travelers returning from north east and eastern Africa [51]. Studies of S. Kentucky ST198 from different countries have also shown a similar substitution in gyrA at codon 83 (Ser83-Phe) for all isolates and substitution of aspartate at codon 87 with asparagine, tyrosine or glycine residues. S. Kentucky isolates in the current study also belonged to ST198 suggesting the clonal relatedness of our isolates to the internationally spreading clone of S. Kentucky (unpublished data). However, only single substitution in the parC gene at codon 80 (Ser80-Ile) was previously and none of them had substitution at codon 57 of parC gene [52]. Additional substitution at codon 57 of the parC gene in the Ethiopian isolates might have occurred separately. Contrary to these local and global spread of MDR fluoroquinolone resistant S. Kentucy, a previous study on S. Typhimurium showed that mutation based fluoroquinolone resistance is associated with fitness cost and resistant strains are less invasive [53]. This suggests that this internationally dispersed clone of S. Kentucky has unique mechanisms. Furthermore, we have previously shown that S. Kentucky strains from Ethiopia has strong biofilm forming ability which is one of the important traits for persistence of the organism in the host or the environment [54] that might have contributed to its dissemination.

Four of the Salmonella isolates resistant to nalidixic acid and intermediately resistant to ciprofloxacin had only a single substitution in the gyrA, Ser83-Phe, whereas one isolate S. Haifa from poultry had a Ser83-Tyr substitution. Previous studies have also shown that a single mutation in gyrA results only in resistance to nalidixic acid and not to ciprofloxacin [47, 53]. Although isolates with a single mutation in parC gene resulted only with reduced susceptibility to nalidixic acid, an S. Miami isolate with no mutation in gyrA gene but double substitution in parC gene: (Thr57-Ser) and a novel substitution (Tyr83-Phe) was fully susceptible to nalidixic acid and intermediately resistant to ciprofloxacin. This suggests that the novel mutation at codon 83 of parC gene might accentuate the activity of nalidixic acid and attenuate the activity of ciprofloxacin.

The observation of double substitution in gyrB gene (Val423-Gly + Asp459-His) associated with intermediate susceptibility only to nalidixic acid shows a minor contribution of mutation in gyrB compared to gyrA for development of resistance to quinolones. Interestingly, nine isolates with reduced sensitivity to ciprofloxacin and some to nalidixic acid had no mutation in QRDR. We have also not detected PMQR genes in any of the isolates. Other resistance mechanisms not tested in this study such as multidrug efflux pumps, other PMQR mechanisms recently described in Salmonella such as oqxAB efflux pump [19], and altered outer membrane porins might be involved [14].

**Conclusion**

Co-occurrence of beta-lactamases with ciprofloxacin resistant determinants in large proportion of isolates is a major threat. Occurrence of MDR S. Kentucky with high
level fluoroquinolone resistance mediated by double mutations in gyrA and parC genes in cattle, poultry, and human in the study area suggests clonal spread of this strain and the need for strict pathogen control strategies to hamper further spread of this pathogen. As the majority of the isolates in this study were from healthy animals at the farm level and human patients from primary health care centers, the data presented here may not represent the national status. Further studies on extended spectrum beta-lactamase and fluoroquinolone resistance mechanisms in Salmonella and other Gram-negative pathogens in hospital and community settings is recommended.

Abbreviations

blα: Beta-lactamase gene; CLSI: Clinical and Laboratory Standards Institute; MDR: Multi-drug resistance; NT: Non-typhoidal Salmonella; PMQR: Plasmid mediated quinolone resistance; QRDR: Quinolone resistance determining region

Acknowledgments

We are grateful to Dr Roger Johnson, Linda Cole, Shaun Kernaghan, Ketra Mistry, Ann Perret and Betty Willkie of the Public Health Agency of Canada, National Microbiology Laboratory at Guelph for serotyping and phageotyping of the Salmonella isolates. Technical assistance of Mr. Nega Nigusie and Mr. Halie Alemayehu during sample collection and laboratory isolation is highly appreciated.

Funding

This study was supported by the BecA-ILRI Hub through the Africa Biosciences Challenge Fund (ABCF) program. The ABCF Program is funded by the Australian Department for Foreign Affairs and Trade (DFAT) through the BecA-CSIRO partnership; the Syngenta Foundation for Sustainable Agriculture (SFSA); the Bill & Melinda Gates Foundation (BMGF); the UK Department for International Development (DFID) and; the Swedish International Development Cooperation Agency (SIDA). It was also supported by The National Institutes of Health (NIH) Fogarty International Center (grant 043TW008650) to W.A.G./J.S.G.

Availability of data and materials

All the data supporting the findings are presented in the manuscript.

Authors’ contributions

TE, EE, WG, JSG and DA JB, and AD, participated in conception of the study and contributions. TE was involved in sample collection laboratory work. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Ethics approval and consent to participate

Ethical clearance for the study was obtained from the National Research Ethics Review Committee, Ethiopia. Informed oral consent was obtained from the farm owners and patients from health centers and hospital at the time of sample collection.

Author details

1Addis Ababa University, P.O. Box 1176, Addis Ababa, Ethiopia. 2Biosciences Eastern and Central Africa-International Livestock Research Institute (BecA-ILRI) Hub, P O Box 30709, Nairobi, Kenya. 3Department of Microbiology, Immunology & Parasitology, School of Medicine, College of Health Sciences, Addis Ababa University, Churchill Avenue, P.O. Box 9086, Addis Ababa, Ethiopia. 4CIPE-African Insect Science for Food and Health, P.O. Box 30722-00100, Nairobi, Kenya. 5Department of Veterinary Preventive Medicine, The Ohio State University, 1920 Coffey Rd., Columbus, OH 43210, USA. 6Department of Microbial Infection and Immunity, Center for Microbial Interface Biology, The Ohio State University, Biomedical Research Tower, 460 West 12th, Columbus, OH 432101214, USA. 7Department of Pharmacology and Clinical Pharmacy, School of Pharmacy, College of Health Sciences, Addis Ababa University, Churchill Avenue, P.O. Box 1176, Addis Ababa, Ethiopia.

Received: 19 November 2016 Accepted: 5 January 2017

Published online: 17 January 2017

References


38. Kiiru J, Kariuki S, Goddeeris BM, Butaye P. Analysis of beta-lactamase genes in Salmonella enterica isolates from salmonell...


