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Phylogenetic Analysis of the Antibiotic Resistance Genes in *Salmonella* Species *in silico*

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Abstract

Antibiotic resistance is an emerging problem in both developed and developing countries. It has been responsible for 700,000 deaths worldwide. Some genotypes of bacteria are sensitive to certain antibiotics than others. Hence by conducting phylogenetic analysis of bacteria and detecting the presence of resistance genes in each genotype, we can select the antibiotic that would be most effective for the bacteria in that certain genotype. A total of forty-five *Salmonella* species were investigated for the presence of antibiotic resistance genes through *in silico* PCR (polymerase chain reaction) amplification and PFGE (pulsed-field gel electrophoresis) analysis was conducted to assess the phylogenetic relationship. Total twenty-eight antibiotic resistance genes were selected for screening the isolates and seventeen antibiotic resistance genes among the *Salmonella* strains were found. Almost all the isolates (n=43) exhibited PCR amplification product for *gyrA* genes while fluoroquinolone resistance *gyrB* (66.67%), *parC* (68.89%) and *parE* (15.56%) genes were also present. About 15.56% and 11.11% isolates were found to harbor adenylyltransferase gene, *aadA1* and *aadA2*, respectively while phosphotransferase gene was detected in only one isolate. Two isolates expressed both chloramphenicol acetyltransferase genes, *cat1* and *cat2*. Three isolates (6.67%) harbored chloramphenicol resistance gene *cmlA* gene while two isolates (4.44%) expressed florfenicol resistance gene, *floR*. Tetracycline resistance gene, *tetA* was more prevalent (8.89%) than *tetG* genes (2.22%). *Salmonella* harbored all three sulfonamide resistance genes while *sulII* was more prevalent (17.78%). Genotype 2 contained fifteen antibiotic resistance genes while genotype 3 contained only one antibiotic resistance genes. These investigations used a computer aided approach to genotype isolates and assess the difference in antibiotic resistance profile of *Salmonella* species based on genotype. This data helps to predict antibiotic resistance genes that might be present for an isolate of known genotype and select antibiotic for the treatment of *Salmonella* infections based on their phylogenetic group.

Keywords: *Salmonella; In silico;* Antibiotic resistance genes; Polymerase chain reaction; Pulsed-field gel electrophoresis; Genotype

Introduction

Zoonotic bacterium *Salmonella* colonizes on the intestinal tract. Humans and animals are affected by many diseases caused by *Salmonella* such as acute gastroenteritis, bacteremia and many other extraintestinal localized infections. So, rapid identification of *Salmonella* is needed to prevent the spread of the diseases [1]. Poultry products are the potential source of *Salmonella* infections [2,3] that cause significant economic loss in the poultry industry [1,4].

Animals that contribute to food production are treated with antimicrobials for therapeutics or production purposes. These antimicrobials improved animal health and their growth rate or feed conservation was reported by one study [5]. This overuse of antimicrobials also contributed to the development of multidrug-resistant bacteria including zoonotic pathogen *Salmonella* [3]. Multidrug-resistant (MDR) *Salmonella* has been increased worldwide due to overuse of antibiotics in humans and animal's infections. One study documented that seafood, chickens and fishes were considered as the source of *Salmonella* infections [6]. Another investigation screened *Salmonella* collected from food handler and animal isolates that showed same RAPD fingerprinting patterns. So, the animal was the root of the source for food handler infections as food handlers used these collected samples [7].

Deaths due to drug-resistant infections are estimated to increase from 700,000 to 10 million annually by 2050, and the financial burden because of this might be as high as US$100 trillion worldwide [8]. In developing countries, antimicrobials are used inappropriately in farming practices and this is contributed to the development of multidrug-resistant bacteria [9]. Non typhoidal *Salmonella enterica* was responsible for 56,969 deaths globally in 2010 [10]. Typhoidal *Salmonella* was responsible for 210,000 deaths worldwide in 2000 [11]. In Nigeria, multidrug-resistant (MDG) *Salmonella* is of important concern as it was responsible for bacteremia in children [12].

Recently one group found that third-generation fluoroquinolones were effective for the treatment of adult patients [13]. World Health Organization listed fluoroquinolones as an important antibiotic and its use for the treatment of children was reported by one group [14]. However, a study found a *Salmonella* serotype from a human source that showed a reduction in fluoroquinolone susceptibility [15]. One study found that a single mutation in DNA topoisomerase gene was responsible for the development of fluoroquinolone resistant *Salmonella enterica* [16]. Presence of *gyrA* mutation is an indicator of fluoroquinolone resistance gene and hence fluoroquinolones cannot be prescribed for treating the infection [17]. Mutations in DNA gyrase, *gyrA* genes were usually restricted to clinical human and veterinary samples [16,18-20].

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One study reported that chloramphenicol acetyltransferases (CAT), a plasmid-borne enzyme, was responsible for chloramphenicol resistance [21]. Another study documented nonenzymatic chloramphenicol resistance gene, *cmlA*, also conferred chloramphenicol resistance in *Salmonella* species [22]. *Salmonella* was also seen to harbour florfenicol resistance gene and it also showed cross-resistance to chloramphenicol [23].

In Iran, poultry originated *Salmonella* developed tetracycline resistance was reported by several authors [1,24-26]. Several reports found that *tetA* was the most common tetracycline resistance gene found in poultry [3,4,27]. One study reported that *tetA* and *tetB* genes both were present in *Salmonella* collected from human samples [28]. Another group found *tetD* resistance gene in *Salmonella* [29]. Other studies found tetracycline resistant *Salmonella typhimurium* that harbored *tetG* gene [30,31].

The *sulI* and *sulII* genes, encoding different forms of dihydropteroate synthase, are responsible for sulfonamide resistance [32]. Several studies documented that the *sulI* gene was linked to other resistance genes in class 1 integrons, while *sulII* gene was located on small nonconjugative plasmids [33] or large transmissible multi resistance plasmids [32]. Another study found sulfonamide resistance gene due to *sulIII* [34].

Salmonella usually develops their resistance mechanism by an enzymatic modification of the target compounds while other bacteria uses active efflux pump or enzymatic modification of 16S rRNA subunit to develop their resistance mechanism [35]. For strains isolated in USA, acetyltransferases, phosphotransferases, and nucleotidyltransferases genes modified and inactivated the aminoglycoside antibiotics and conferred their resistance [36,37].

The present study investigated the resistance genes profile of forty-five *Salmonella* species through *in silico* PCR amplification to determine the MDR gene profile and also identified the distribution pattern of the resistance genes within the genotypes by *in silico* PFGE analysis.

Materials and Methods

Strains used in the study

Strains used in the study are summarized in Table 1.

Primers used in the study

Primers used for detection of antibiotic resistance genes are summarized in Table 2 [38].

PCR amplification

In silico PCR amplification was performed on an online software http://insilico.ehu.eus/PCR/ [39,40] and resulting PCR product is computed automatically with desired band size of a specific gene [40].

PFGE digestion

PFGE digestion and construction of the dendrogram was done in the website http://insilico.ehu.es/digest/. The *Xba*I restriction enzyme was used that recognized the restriction sequence [39,40].

Results and Discussion

Genetic diversity of studied isolates

Genetic diversity of *Salmonella* species is determined by pulsed-field gel electrophoresis (PFGE) analysis. The *Xba*I was chosen as a restriction enzyme that recognizes T'CTAG_A sequence and different banding patterns were observed upon gel electrophoresis. Dendrogram was constructed in the website (Figure 1). This *in silico* PFGE analysis divided 45 isolates into five genotypes at 80% cutoff value.

Genotypic distribution of aminoglycosides resistance genes

The gene products of *aadA1* and *aadA2* confers resistance to streptomycin and spectinomycin. The *aadA1* gene was present in 15.56% (n=7) of the isolates and gave 497 bp gene product (Figure 2). The *aadA2* gene was detected in 11.11% (n=5) of the isolates with 470 bp gene product (Figure 3). The *aadB* gene cassette confers resistance to tobramycin, gentamicin and kanamycin [41]. The gene *aadD* confers resistance to kanamycin and neomycin [42] as well as tobramycin [43]. The primer for *aadB* and *aadD* genes [38] didn't give any amplicon in any of the isolates (not shown). The *aph(3)-IIa* specifies resistance to neomycin, ribostamycin, butirosin, paromomycin and kanamycin. One isolate was found to harbour phosphotransferase gene, *aph (3')-IIa* with 582 bp gene product (Figure 4). The *aac(3)IIa* gene mediates alteration of dibekacin, kanamycin, gentamicin, netimicin, tobramycin [37]. The primer of *aac(3)IIa* gene (Ma et la., 2007) [38] didn't give any amplicon. One study reported that phosphotransferase gene *aph(3)-IIa* genes were detected in 10 (1.4%) isolates while 57% (n=8) isolates had the acetyltransferase gene *aac(3)IIa* [13]. Based on our data, treatment of *Salmonella* infection is going to have a better prognosis if tobramycin, gentamicin, kanamycin, neomycin, ribostamycin, butirosin, paromomycin are used instead of other aminoglycosides such as streptomycin and spectinomycin.

Genotype 1 contained all three aminoglycoside positive genes while genotype 2 and 3 contained adenylyltransferase genes *aadA1* and *aadA2*. About 22.22% isolates present in genotype 1 carried *aadA1* gene while 11.11% isolates present in genotype 1 carried both *aadA2* and *aph(3')-IIa* genes. Phosphotransferase gene *aph(3')-IIa* was present in only genotype 1. Genotype 3 contained no aminoglycosides or chloramphenicol resistance genes. About 11.76% and 17.65% isolates present in genotype 2 carried *aadA1* and *aadA2* genes while *aadA1* was encountered in higher frequency (27.27%) in genotype 5 as compared to *aadA2* genes (9.09%). Isolates belonging to genotype 3,4 are unlikely to be resistant to streptomycin and spectinomycin and infections caused these genotype can be tackled with these antibiotics. All of the isolates are sensitive to dibekacin, gentamicin, netimicin, tobramycin. Isolates in genotype 3 are sensitive to all aminoglycosides.

Genotypic distribution of chloramphenicol resistance genes

The *cat* genes encode chloramphenicol acetyltransferase which detoxifies choramphenicol and is responsible for chloramphenicol resistance in bacteria [44]. Only two isolates harbored the *cat1* gene and a 683 bp gene product was seen (Figure 5). These two isolates were also found to express the *cat2* gene and produced a 547 bp gene product (Figure 6) while the primer for *cat3* gene [38] didn't yield any amplicon (not shown). The *cml* and *floR* genes confer resistance to chloramphenicol and florfenicol by efflux of the antibiotics [45]. The *cmlA* gene was seen to be harbored in three isolates with an approximate length of 683 bp gene product (Figure 7) while the primer for *cmlB* gene [38] failed to detect any amplicon (not shown). The florfenicol resistance gene, *floR* was present in two isolates and gave 1213 bp gene product (Figure 8). One study has been documented that chloramphenicol resistance gene was found in six isolates while more (10 of the 14 multidrug-resistant) isolates were found to express the *floR* and *cat2* genes [13]. Two isolates harboured *cat3* and about 61% and 69% isolates expressed *cmlA* and *cmlB* genes, respectively [13].

Serial number	Isolate
1	NC_021870 *Salmonella bongori* N268-08
2	NC_015761 *Salmonella bongori* NCTC 12419
3	NC_010067 *Salmonella enterica* subsp. *arizonae* serovar 62:z4, z23:--
4	NC_021818 *Salmonella enterica* subsp. *enterica* serovar Cubana str. CFSAN002050
5	NC_022991 *Salmonella enterica* subsp. *enterica* serovarAgona str. 24249
6	NC_011149 *Salmonella enterica* subsp. *enterica* serovar Agona str. SL483
7	NC_021844 *Salmonella enterica* subsp. *enterica* serovar Bareilly str. CFSAN000189
8	NC_022241 *Salmonella enterica* subsp. *enterica* serovar Bovismorbificans str. 3114
9	NC_006905 *Salmonella enterica* subsp. *enterica* serovar Choleraesuis str. SC-B67
10	NC_011205 *Salmonella enterica* subsp. *enterica* serovar Dublin str. CT_02021853
11	NC_011294 *Salmonella enterica* subsp. *enterica* serovar Enteritidis str. P125109
12	NC_011274 *Salmonella enterica* subsp. *enterica* serovar Gallinarum str. 287/91
13	NC_022221 *Salmonella enterica* subsp. *enterica* serovar Gallinarum/pullorum str. CDC1983-67
14	NC_016831 *Salmonella enterica* subsp. *enterica* serovar Gallinarum/pullorum str. RKS5078
15	NC_021810 *Salmonella enterica* subsp. *enterica* serovar Heidelberg str. 41578
16	NC_017623 *Salmonella enterica* subsp. *enterica* serovar Heidelberg str. B182
17	NC_021812 *Salmonella enterica* subsp. *enterica* serovar Heidelberg str. CFSAN002069
18	NC_011083 *Salmonella enterica* subsp. *enterica* serovar Heidelberg str. SL476
19	NC_020307 *Salmonella enterica* subsp. *enterica* serovar Javiana str. CFSAN001992
20	NC_011080 *Salmonella enterica* subsp. *enterica* serovar Newport str. SL254
21	NC_021902 *Salmonella enterica* subsp. *enterica* serovar Newport str. USMARC-S3124.1
22	NC_011147 *Salmonella enterica* subsp. *enterica* serovar Paratyphi A str. AKU_12601
23	NC_006511 *Salmonella enterica* subsp. *enterica* serovar Paratyphi A str. ATCC 9150
24	NC_010102 *Salmonella enterica* subsp. *enterica* serovar Paratyphi B str. SPB7
25	NC_012125 *Salmonella enterica* subsp. *enterica* serovar Paratyphi C strain RKS4594
26	NC_021984 *Salmonella enterica* subsp. *enterica* serovar Pullorum str. S06004
27	NC_011094 *Salmonella enterica* subsp. *enterica* serovar Schwarzengrund str. CVM19633
28	NC_022525 *Salmonella enterica* subsp. *enterica* serovar Thompson str. RM6836
29	NC_003198 *Salmonella enterica* subsp. *enterica* serovar Typhi
30	NC_004631 *Salmonella enterica* subsp. *enterica* serovar Typhi Ty2
31	NC_016832 *Salmonella enterica* subsp. *enterica* serovar Typhi str. P-stx-12
32	NC_021176 *Salmonella enterica* subsp. *enterica* serovar Typhi str. Ty21a
33	NC_022569 *Salmonella enterica* subsp. *enterica* serovar Typhimurium DT104
34	NC_003197 *Salmonella enterica* subsp. *enterica* serovar Typhimurium LT2
35	NC_021820 *Salmonella enterica* subsp. *enterica* serovar Typhimurium str. 08-1736
36	NC_016856 *Salmonella enterica* subsp. *enterica* serovar Typhimurium str. 14028S
37	NC_017046 *Salmonella enterica* subsp. *enterica* serovar Typhimurium str. 798
38	NC_016854 *Salmonella enterica* subsp. *enterica* serovar Typhimurium str. D23580
39	NC_022544 *Salmonella enterica* subsp. *enterica* serovar Typhimurium str. DT2
40	NC_016810 *Salmonella enterica* subsp. *enterica* serovar Typhimurium str. SL1344
41	NC_016857 *Salmonella enterica* subsp. *enterica* serovar Typhimurium str. ST4/74
42	NC_016860 *Salmonella enterica* subsp. *enterica* serovar Typhimurium str. T000240
43	NC_021151 *Salmonella enterica* subsp. *enterica* serovar Typhimurium str. U288
44	NC_016863 *Salmonella enterica* subsp. *enterica* serovar Typhimurium str. UK-1
45	NC_021814 *Salmonella enterica* subsp. *enterica* serovar Typhimurium var. 5- str. CFSAN001921

Table 1: Name of the isolates.

The *cmlA* and *floR* both genes were present in the same number in genotype 1 (11.11%) (Figure 9). Genotype 2 contained four chloramphenicol resistance genes (except *cmlB* gene) and about 5.88% isolates present in genotype 2 expressed all four chloramphenicol resistance genes. Twenty-five percent isolates present in genotype 4 harboured *cat1* and *cat2* genes while genotype 5 contained only *cmlA* genes (9.09%). Isolates in genotype 1 and 5 are likely to be resistant to chloramphenicol by enzyme detoxification rather than efflux while the reverse is true for genotype 2 and 4. All isolates of genotype 3 will be sensitive to chloramphenicol while all isolates in genotype 3-5 will be sensitive to florfenicol [46].

Genotypic distribution of fluoroquinolone resistance genes

Mutations in *gyrA, gyrB* regions of DNA gyrase and *parC* and *parE* regions of DNA topoisomerase IV have been responsible for fluoroquinolone resistance [47]. The *gyrA* gene was found in 43 positive isolates out of 45 isolates studied here and gave 251 bp gene products (Figure 10). Thirty isolates (66.67%) were found to possess *gyrB* gene and produced 172 bp gene products (Figure 11). Hence the mutations in the *gyrA* subunit are more likely to contribute to resistance when compared to *gyrB*. Thirty-one (68.89%) isolates were found to express topoisomerase IV, *parC* gene with 262 bp gene

Gene	Primer sequence (5'-3')	Amplicon size bp	References
aadA1	TTTGCTGGTTACGGTGAC GCTCCATTGCCCAGTCG	497	[38]
aadA2	GGTGCTAAGCGTCATTGAGC GCTTCAAGGTTTCCCTCAGC	470	[38]
aph (3')-IIa	TCTGAAACATGGCAAAGGTAG AGCCGTTTCTGTAATGAAGGA	582	[38]
cat1	AACCAGACCGTTCAGCTGGAT CCTGCCACTCATCGCAGTAC	550	[38]
cat2	AACGGCATGATGAACCTGAA ATCCCAATGGCATCGTAAAG	547	[38]
cmlA	GGCCTCGCTCTTACGTCATC GCGACACCAATACCCACTAGC	662	[38]
floR	ATGACCACCACACGCCCCG AGACGACTGGCGACTTCTCG	1213	[38]
gyrA	CGTTGGTGACGTAATCGG CCGTACCGTCATAGTTAT	251	[31]
gyrB	GCGCTGTCCGAACTGTACCT CGGTGATCAGCGTCGCCACTTCC	172	[31]
parC	CTATGCGATGTCAGAGCTGG TAACAGCAGCTCGGCGTATT	262	[31]
parE	TCTCTTCCGATGAAGTGCTG ATACGGTATAGCGGCGGTAG	238	[31]
tetA	TTGGCATTCTGCATTCACTC GTATAGCTTGCCGGAAGTCG	494	[38]
tetG	GCTCGGTGGTATCTCTGCTC CAAAGCCCCTTGCTTGTTAC	550	[38]
sulI	TTTCCTGACCCTGCGCTCTAT GTGCGGACGTAGTCAGCGCCA	425	[38]
sulII	CCTGTTTCGTCCGACACAGA GAAGCGCAGCCGCAATTCAT	435	[38]
sulIII	ATGAGCAAGATTTTTGGAATCGTAA CTAACCTAGGGCTTTGGATATTT	792	[38]

Table 2: Primers for antibiotic resistance genes detection.

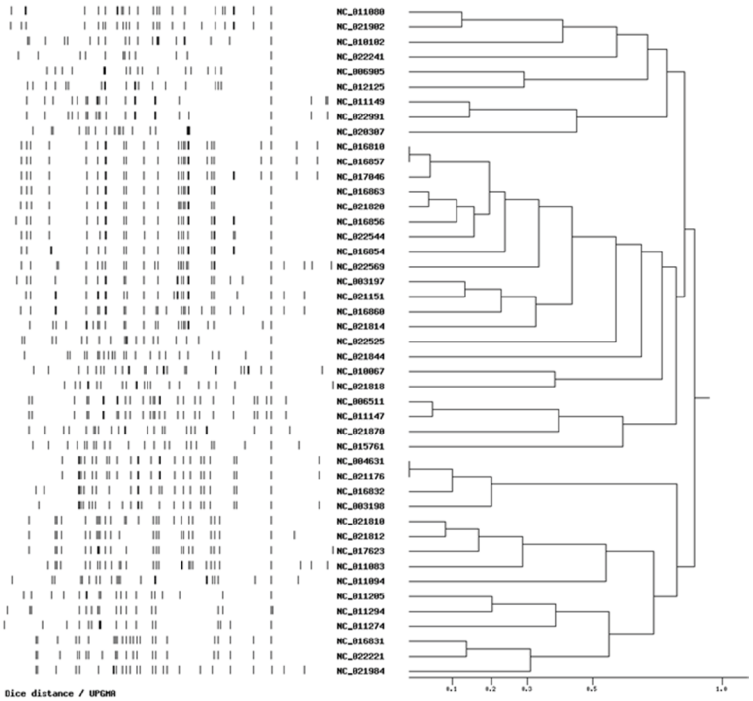

Figure 1: Phylogenetic diversity of *Salmonella* species identified by PFGE analysis.

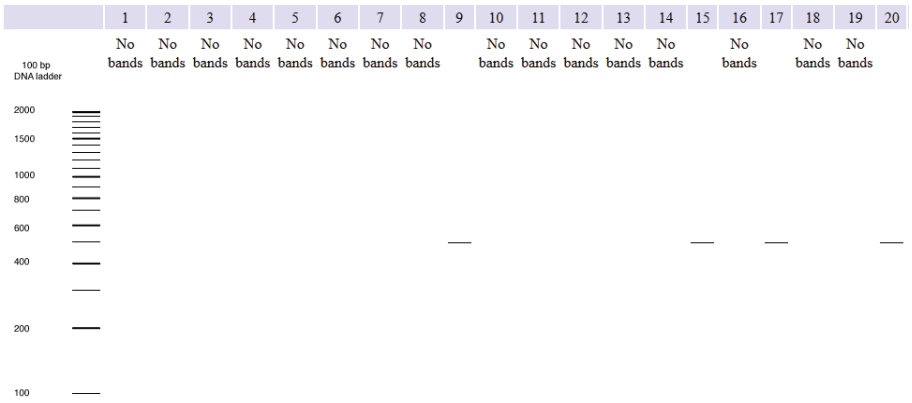

Figure 2: Detection of *aadA1* gene in *Salmonella* isolates. Isolates harbouring the gene gives a 497 bp amplicon.

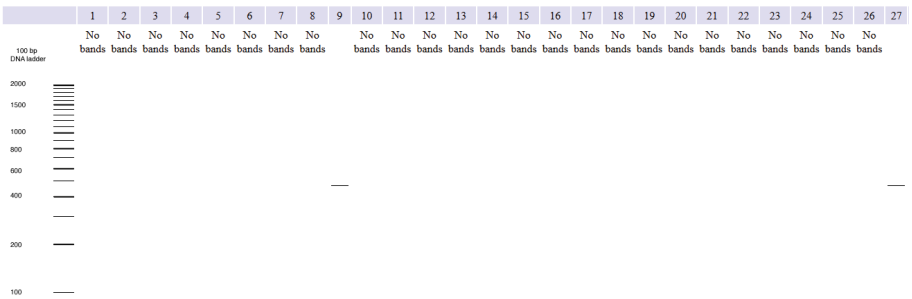

Figure 3: Detection of *aadA2* gene in *Salmonella* isolates. Isolates harbouring the gene gives a 470 bp amplicon.

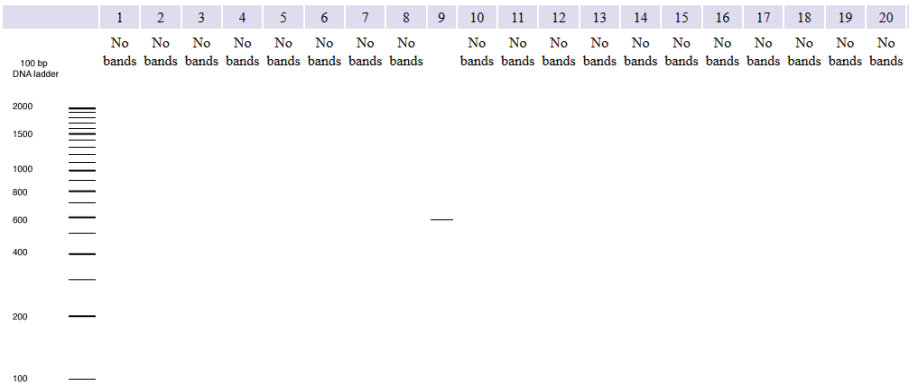

Figure 4: Detection of *aph (3'):IIa* gene in *Salmonella* isolates. Isolates harbouring the gene gives a 582 bp amplicon.

product (Figure 12) while *parE* gene was present in 15.56% (n=7) of the isolates with an approximate length of 238 bp gene product (Figure 13). Our data suggests mutations in DNA gyrase are more likely the reason of resistance in comparison to DNA topoisomerase IV. Genotype 2 and 5 carried all four fluoroquinolone resistance genes (Figure 14). All the isolates present in genotype 5 carried *gyrA* and *parC* genes (100%) while about 90.91% and 54.55% isolates present in genotype 5 expressed *gyrB* and *parE* genes. All the isolates present in genotype 4 carried all resistance genes except *parE*. The *gyrA* was more prevalent in genotype 1 (100%) while Genotype 3 harboured only *gyrA* gene (75%). Because of the high prevalence of alteast one gene responsible for fluoroquinolone resistance through all genotypes, eradicating *Salmonella* with fluoroquinolone is unlikely to yield positive results. Fluoroquinolones are the most commonly used antibiotic in the poultry industry [47] where *Salmonella* is frequently isolated. Hence it is no surprise that the excessive use of fluoroquinolones have contributed to the widespread resistance.

Genotypic distribution of tetracycline resistance genes

The *tetA* and *tetG* both encode efflux proteins associated with pumping out tetracyclines from the cytosol to the extracellular environment [48]. Tetracycline resistance gene, *tetA* was detected in

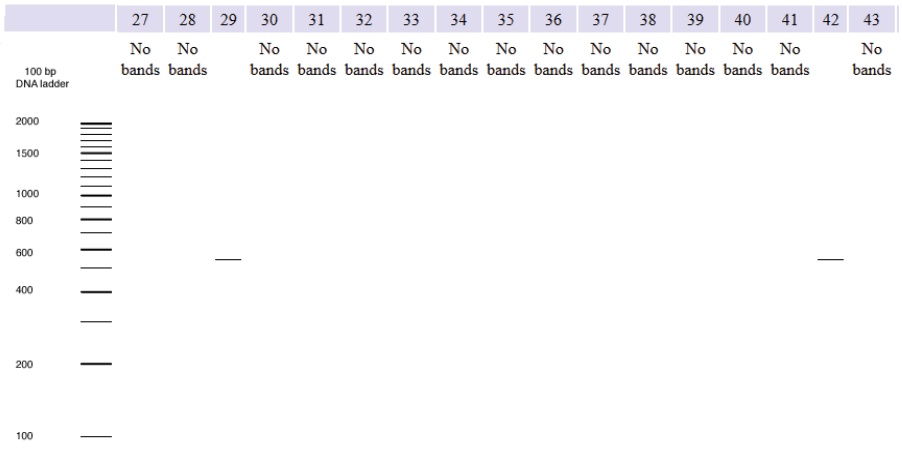

Figure 5: Detection of *cat1* gene in *Salmonella* isolates. Isolates harbouring the gene gives a 550 bp amplicon.

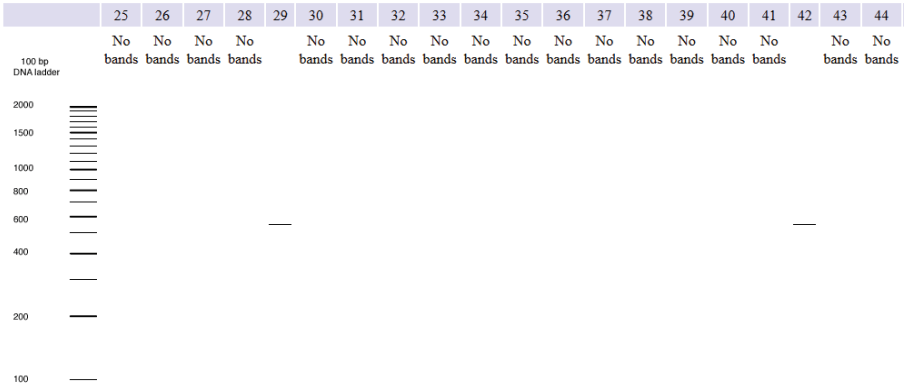

Figure 6: Detection of *cat2* gene in *Salmonella* isolates. Isolates harbouring the gene gives a 547 bp amplicon.

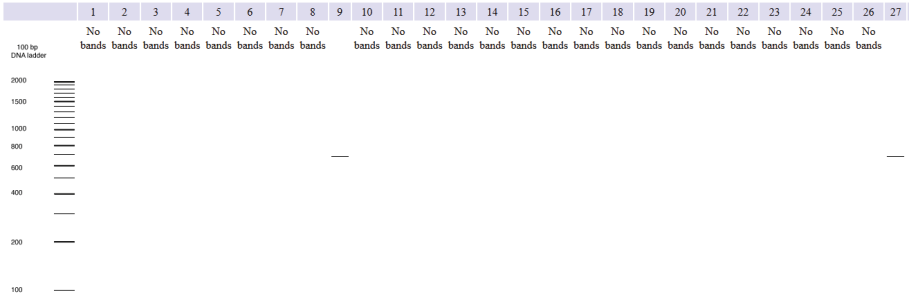

Figure 7: Detection of *cml* gene in *Salmonella* isolates. Isolates harbouring the gene gives a 662 bp amplicon.

8.89% (n=4) of the isolates with 494 bp gene product (Figure 15) while *tetG* gene was found in only one isolate (*Salmonella enterica* subsp. *enterica* serovar Typhimurium DT104) with an approximate length of 550 bp PCR product (Figure 16). Hence the tetA efflux protein is more common than tetG efflux protein. Resistance genes such as *tetM, tetO, tetS* confer resistance by ribosomal protection whereas *tetX* encodes proteins responsible for enzymatic alteration [48]. The primer for other tetracycline resistance genes [38] failed to give any amplicon product (not shown). The *tetA* gene was found in genotype 1, 2 and 5. Hence the isolates in other genotypes are unlikely to be tetracycline resistant because of *tetA* gene. About 11.11% and 18.18% isolates present in genotype 1 and 5 carried the *tetA* genes. About 5.88% isolates present in genotype 2 expressed both *tetA* and *tetG* genes. Genotype 3 contained no tetracycline resistance genes and hence any isolate belonging to this genotype will be sensitive to tetracycline. Other than genotype 2, isolates belonging to other genotypes are unlikely to resistant to tetracyline due to the efflux protein *tetG*.

Genotypic distribution of sulfonamide resistance genes

Sulfonamide resistance gene, *sulI* was detected in 7 isolates (15.56%) with 425 bp gene product (Figure 17) while 8 isolates (17.78%) gave 435 bp PCR products for *sulII* gene (Figure 18). The *sulIII* gene was present

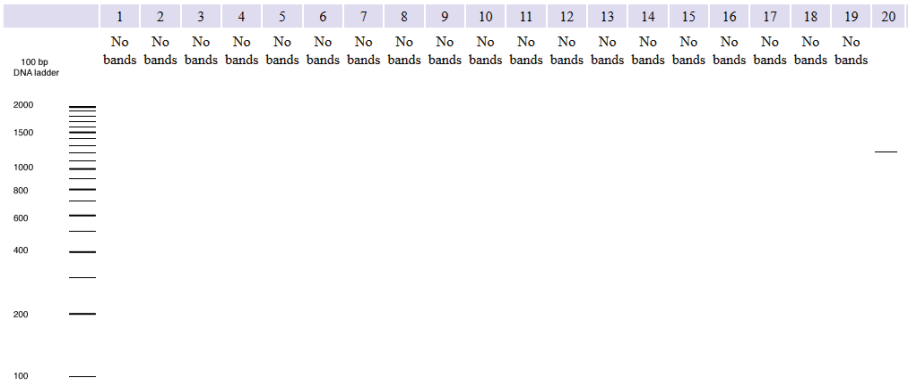

Figure 8: Detection of *floR* gene in *Salmonella* isolates. Isolates harbouring the gene gives a 1213 bp amplicon.

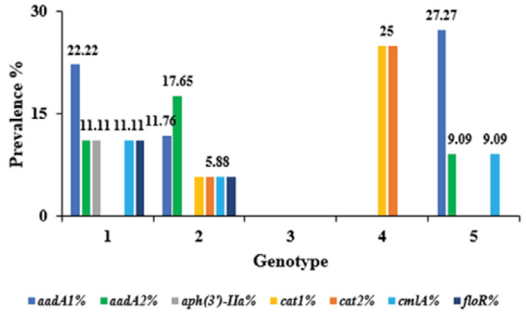

Figure 9: Genotypic distribution of aminoglycosides and chloramphenicol resistance genes. Genes encoding resistance proteins are as follows: *aadA1*: Aminoglycoside Adenyltransferase A1; *aadA2*: Aminoglycoside Adenyltransferase A2; *aph (3'): IIa*: Aminoglycoside O: Phosphotransferase IIa; *cat1*: Chloramphenicol Acetyltransferase 1; *cat2*: Chloramphenicol Acetyltransferase 2; *cmlA*: Non-Enzymatic Chloramphenicol Resistance A; *floR*: Florfenicol Resistance.

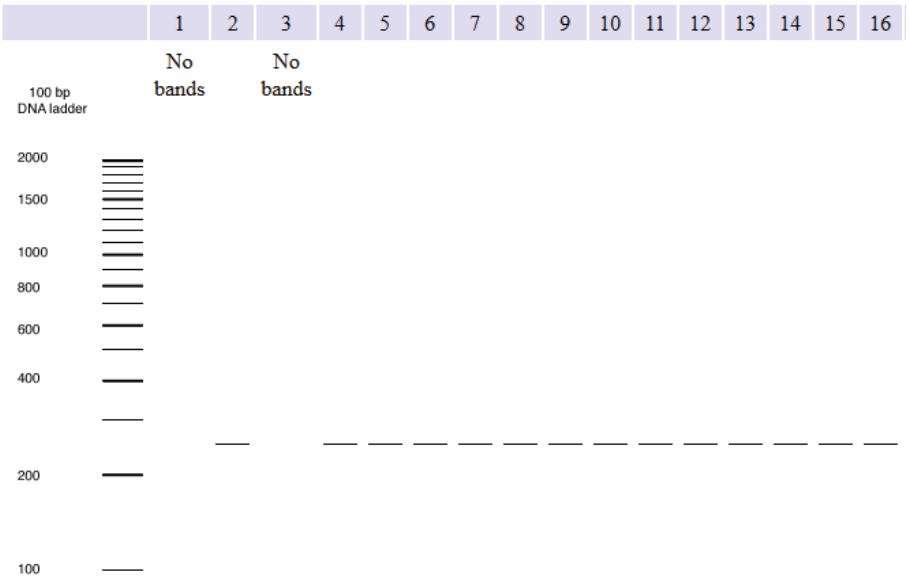

Figure 10: Detection of *gyrA* gene in *Salmonella* isolates. Isolates harbouring the gene gives a 251 bp amplicon.

in only three isolates and produced 792 bp gene products (Figure 19). Genotype 2 contained all five tetracycline and sulfonamide resistance genes (Figure 20). Genotype 1, 2 and 5 carried all three sulfonamide resistance genes. About 33.33% isolates present in genotype 1 harbored *sulI* gene while 11.11% isolates in genotype 1 carried both *sulII* and *sulIII* genes. Twenty-five percent isolates in genotype 4 expressed *sulII* genes. Genotype 3 contained no sulfonamide resistance genes and hence any isolate from this genotype will be sensitive to sulfonamides.

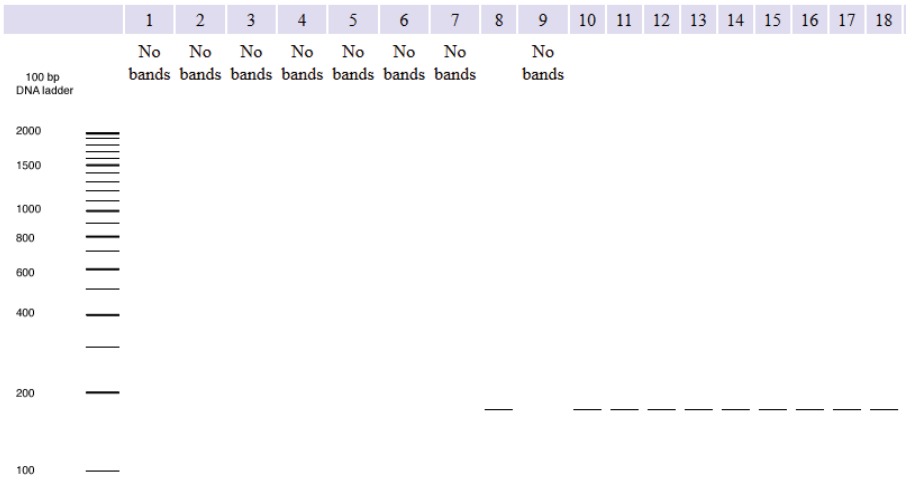

Figure 11: Detection of *gyrB* gene in *Salmonella* isolates. Isolates harbouring the gene gives a 172 bp amplicon.

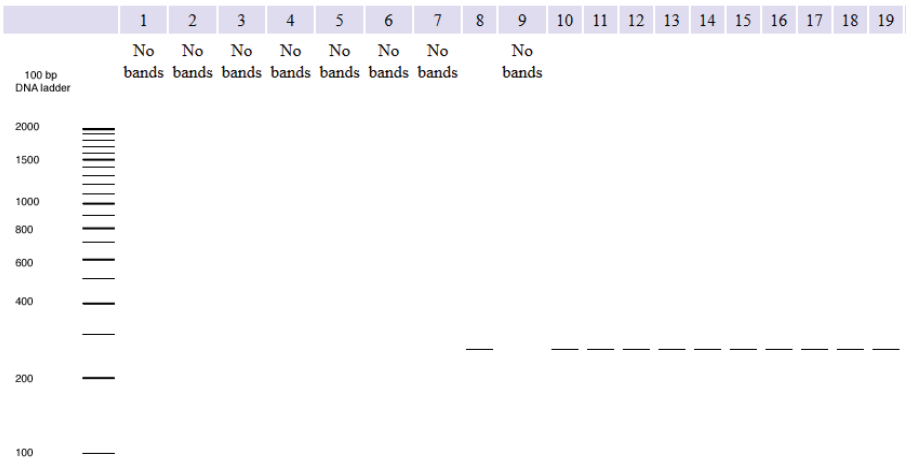

Figure 12: Detection of *parC* gene in *Salmonella* isolates. Isolates harbouring the gene gives a 262 bp amplicon.

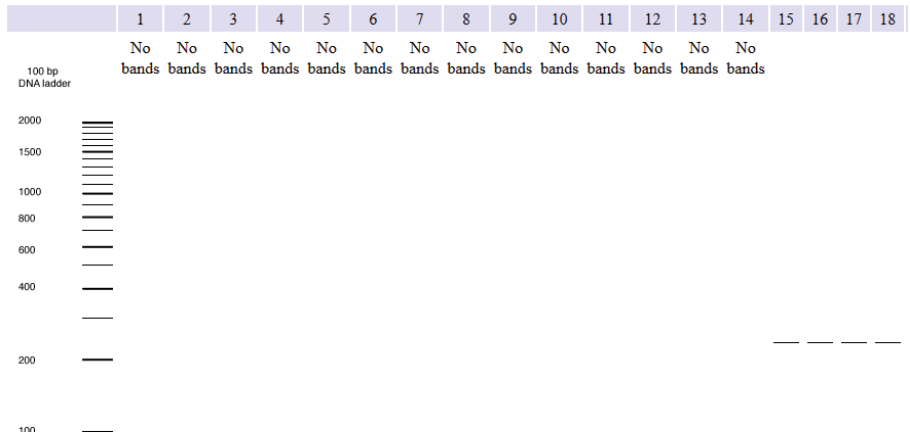

Figure 13: Detection of *parE* gene in *Salmonella* isolates. Isolates harbouring the gene gives a 238 bp amplicon.

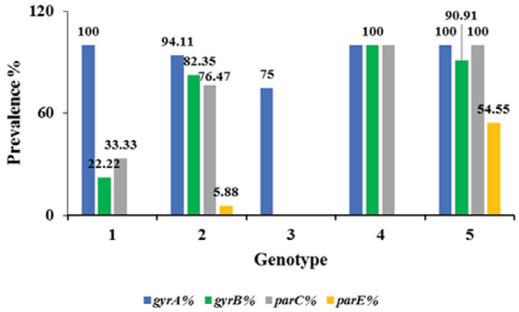

Figure 14: Genotypic distribution of fluoroquinolone resistance genes. Genes encoding resistance proteins are as follows: *gyrA*: gyrA Subunit of DNA Gyrase; *gyrB*: gyrB Subunit of DNA Gyrase; *parC*: parC Subunit of DNA Topoisomerase IV; *pare*: parE Subunit of DNA Topoisomerase IV.

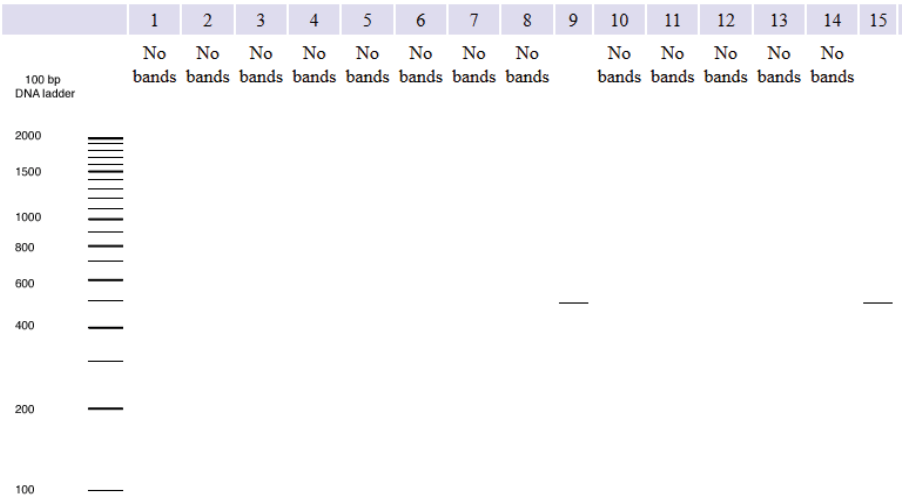

Figure 15: Detection of *tetA* in *Salmonella* isolates. Isolates harbouring the gene gives a 494 bp amplicon.

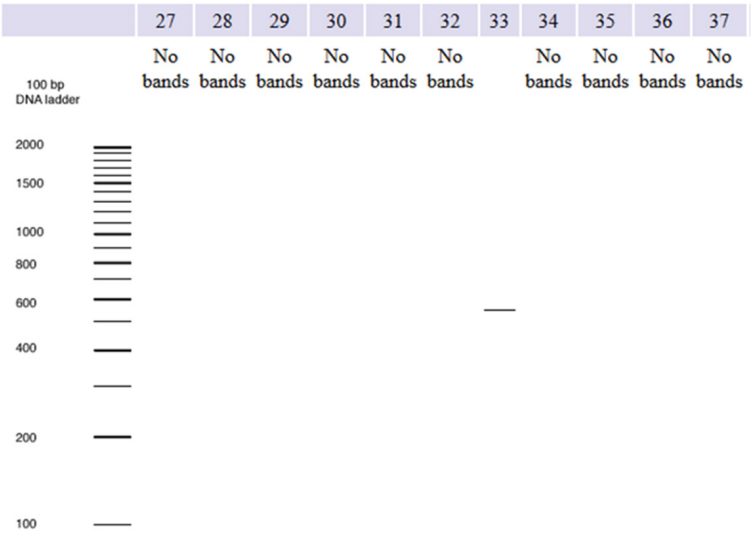

Figure 16: Detection of *tetG* in *Salmonella* isolates. Isolates harbouring the gene gives a 550 bp amplicon.

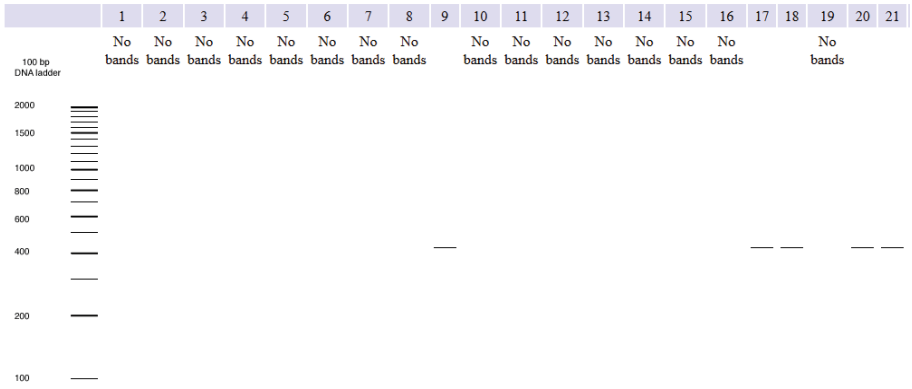

Figure 17: Detection of *sulI* gene is *Salmonella.* Isolates harbouring the gene gives a 425 bp amplicon.

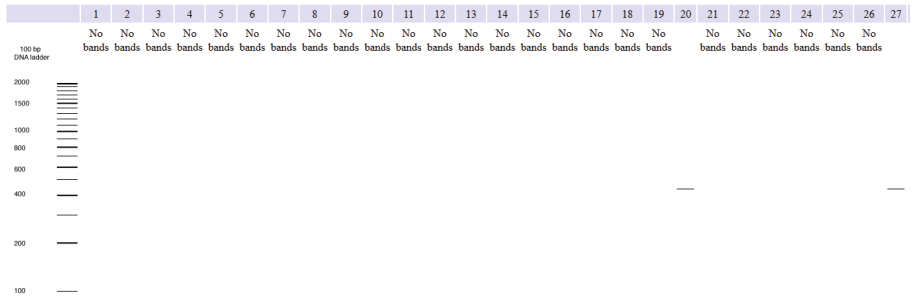

Figure 18: Detection of *sulII* gene is *Salmonella.* Isolates harbouring the gene gives a 435 bp amplicon.

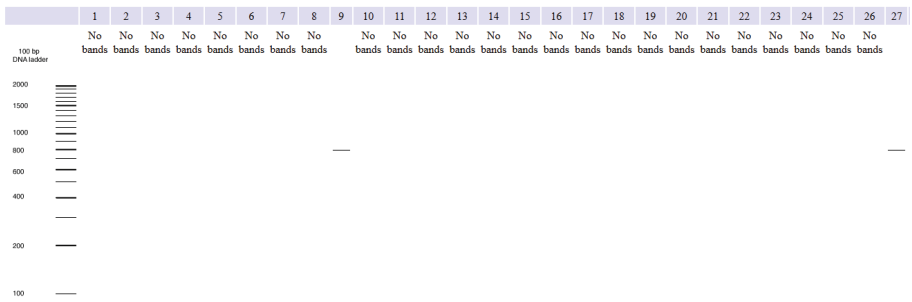

Figure 19: Detection of *sulIII* gene is *Salmonella.* Isolates harbouring the gene gives a 792 bp amplicon.

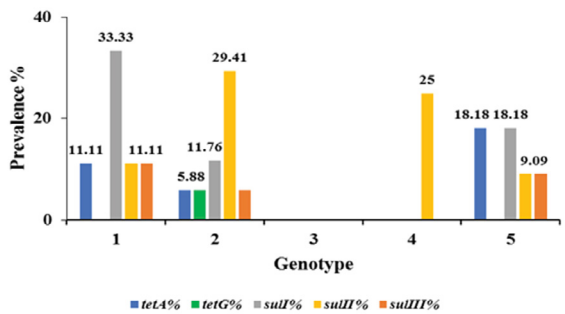

Figure 20: Genotypic distribution of tetracycline and sulfonamide resistance genes. Genes encoding resistance are as follows: *tetA*: Tetracycline Resistance Protein A; *tetG*: Tetracycline Resistance Protein G; *sulI:* Sulfonamide Resistance Gene I; *sulII:* Sulfonamide Resistance Gene II; *sulIII:* Sulfonamide Resistance Gene III.

Conclusion

Our study used a computer aided approach to genotype and detects antibiotic resistance genes and assesses the how the prevalence of these genes varies across the genotypes. Our data suggests that the resistance profile of *Salmonella* as well as the mechanism of resistance varies across genotypes. Genotype 3 was sensitive to all antibiotics except the fluroquinolone family. The present study found that therapeutic value of fluoroquinolone antibiotic is limited since *Salmonella* strains since resistance genes were present across all genotypes. However, prevalence of resistance genes in genotype 3 was lower. Isolates in genotype 1 and 5 were resistant to chloramphenicol by enzyme detoxification rather than efflux while the reverse is true for genotype 2 and 4. Mutations in *gyrA, gyrB* regions of DNA gyrase was more prevalent and hence has a greater contribution to fluoroquinolone resistance rather than mutations in *parC* and *parE* regions of DNA topoisomerase IV. Resistance due tetA efflux pump was more common than tetG pump and was only found in genotype 1, 2 and 5. Tetracyline resistance due to ribosomal protection or enyme modification in *Salmonella* was not seen. Resistance due to sulfonamide was primarily due to *sulII* followed by *sulI* and *sulIII*. Treatment of *Salmonella* infection is going to have a better prognosis if tobramycin, gentamicin, kanamycin, neomycin, ribostamycin, butirosin, paromomycin are used instead of other aminoglycosides such as streptomycin and spectinomycin because resistance genes for these were not present. It can be concluded that treatment process of *Salmonella* infections is difficult since *Salmonella* strains harboured many antibiotic resistance genes. A collaborative scheme was to be setup to supervise the antibiotic administration in animals to prevent the antimicrobial resistance and also improved its therapeutic efficacy.

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