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 *aadA1* and *aadA2*, respectively while phosphotransferase gene was detected in only one isolate. Two isolates expressed both chloramphenicol acetyltransferase genes, *catA* and *cat2*. Three isolates (6.67%) harbored chloramphenicol resistance gene *cmrA* 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 *sulI* 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 for food handler infections as food handlers used these collected source for food handler infections as food handlers used these collected

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 gyrA, gyrB 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 sulIII 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 sulIII 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 nucleotidylyltransferases 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.eus/digest/. The XbaI 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 XbaI 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)-Ia specifies resistance to neomycin, ribostamycin, butirosin, paromomycin and kanamycin. One isolate was found to harbour phosphotransferase gene, aph (3’)-Ila with 582 bp gene product (Figure 4). The aac(3)Ia gene mediates alteration of dibekacin, kanamycin, gentamicin, neticin, tobramycin [37]. The primer of aac(3)Ia gene (Ma et al., 2007) [38] didn’t give any amplicon. One study reported that phosphotransferase gene aph(3’)-Ila genes were detected in 10 (1.4%) isolates while 57% (n=8) isolates had the acetyltransferase gene aac(3)Ia [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’)-Ila genes. Phosphotransferase gene aph(3’)-Ila was present only in genotype 1. Genotype 3 contained no aminoglycosides or chloramphenicol resistance genes. About 11.76% and 17.65% isolates in only 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 and 5 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, neticin, tobramycin. Isolates in genotype 3 are sensitive to all aminoglycosides.

Genotypic distribution of chloramphenicol resistance genes

The cat genes encode chloramphenicol acetyltransferase which detoxifies chloramphenicol 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].
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 2 expressed all four chloramphenicol sensitive to florfenicol [46]. Twenty-five percent isolates present in genotype 4 expressed all four chloramphenicol sensitive to chloramphenicol while all isolates in genotype 3-5 will be chloramphenicol by enzyme detoxification rather than efflux while the genes (9.09%). Isolates in genotype 1 and 5 are likely to be resistant to chloramphenicol while genotype 5 contained only **cmlA** 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 fleroxacin [46].

**Table 1**: Name of the isolates.

| 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: -23:--: |
| 4             | **NC_021818** Salmonella enterica subsp. enterica serovar Cubana str. CFSAN002050 |
| 5             | **NC_022991** Salmonella enterica subsp. enterica serovar Agona str. 24249 |
| 6             | **NC_011149** Salmonella enterica subsp. enterica serovar Agona str. SL483 |
| 7             | **NC_021844** Salmonella enterica subsp. enterica serovar Bareilly str. CFSAN00189 |
| 8             | **NC_022241** Salmonella enterica subsp. enterica serovar Bovismorbificans str. 3114 |
| 9             | **NC_006905** Salmonella enterica subsp. enterica serovar Choleraeuis 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. SL478 |
| 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. 140285 |
| 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 |

**Genotypic distribution of fluoroquinolone resistance genes**

Mutations in gyrA, gyrB regions of DNA gyrase 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 products (Figure 12).
| Gene     | Primer sequence (5’-3’) | Amplicon size bp | References |
|----------|-------------------------|------------------|------------|
| aadA1    | TTTGCTGTTTACGGTGAC      | 497              | [38]       |
| aadA2    | GGTGCTAGCGTCAGTACG      | 470              | [38]       |
| aph (3')-Ila | TCTGAAACATGCGAAAGTGAG | 582              | [38]       |
| cat1     | AACGACGCTCGGAGCGCTAC    | 550              | [38]       |
| cat2     | AAGGACGCTGCGAGCGAGTA    | 547              | [38]       |
| cmlA     | GGCCTGCTTACGCTATC       | 662              | [38]       |
| floR     | ATGACCGCTACGCCGCCG      | 1213             | [38]       |
| gyrA     | CGGCTGGTGACGTAACCGG     | 251              | [31]       |
| gyrB     | CGGCGTCCGAGCTATCC       | 172              | [31]       |
| parC     | CTATGCGGTAGTCAGGCTG     | 262              | [31]       |
| parE     | TACTCCGCGAGCTAGGCTG     | 238              | [31]       |
| tetA     | TTGGCGATTTCGATTATC      | 494              | [38]       |
| tetG     | GCTCGGTTATCTCTGCTG      | 550              | [38]       |
| sulI     | TTTCTGACCCTGGCGCTATC    | 425              | [38]       |
| sulII    | CGGCTGGTGACGTAACCGG     | 435              | [38]       |
| sulIII   | ATGACGACTTACGTTGGAAGTT | 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.

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 atleast 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
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 be resistant to tetracycline 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
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 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 fluoroquinolone 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. Tetracycline resistance due to ribosomal protection or enzyme modification in Salmonella was not seen. Resistance due to sulfonamide was primarily due to sul1 followed by sul2 and sul3. 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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