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# Tetracycline Resistance Genes in *Salmonella enterica* Serovars With Animal and Human Origin

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**Keywords:** Tetracycline resistance genes, Salmonella serovars, PCR

**Abstract**

**Background:** Tetracycline is one of the important antibacterial agents which is used against various bacterial infections. Different bacterial species and strains convey various tetracycline resistance (*tet*$^r$) genes.

**Objective:** The present study was conducted to evaluate the occurrence of five *tet*$^r$ genes (*tetA*, *tetB*, *tetC*, *tetD*, and *tetM*) among *Salmonella* serovars obtained from humans and animals.

**Materials and Methods:** A total of 60 different *Salmonella* strains previously recovered from humans, poultry, and animals were subjected to polymerase chain reaction (PCR) and sequence analysis of the genes.

**Results:** In total, 6 strains were positive for the presence of *tetA* gene; three serotypes were also positive for the presence of *tetC* gene. The sequence analysis and phylogenetic tree showed similarities between the sequences of serovars in the present study and other *Salmonella* serovars and some other bacteria species in GenBank data.

**Conclusion:** The results showed the great distribution of tetracycline resistance genes among *Salmonella* serovars with different sources which could be the effect of widespread use of the antibiotic particularly in the animals breeding farms.

**Keywords:** Tetracycline resistance genes, *Salmonella* serovars, PCR

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## Background

*Salmonella enterica* serovars are gram-negative, rod-shaped, foodborne pathogenic bacteria which are responsible for some of the most important infections such as typhoid fever, gastroenteritis, and septicemia in humans and different levels of enteritis, abortion, and systemic infections in animals.[1,2] Different antibiotics can be useful for treatment of salmonellosis but it is not a matter of concern here. One of these antibiotics is tetracycline and some of the bacteria belonging to tetracycline producing streptomycetes or *Enterobacteriaceae* family such as *Salmonella* strains transport the genes of tetracycline resistance to other bacteria using various horizontal gene transfer mechanisms.[3] Tetracycline is one of the most consumable antibiotics in both medicine and veterinary therapy particularly in the case of *Mycoplasma* and *Chlamydia* infections all around the word, due to its low level of side effects and cost. However, wide ranges of bacterial strains including *Enterobacteriaceae* family or/and genus *Pseudomonas* carry plasmids which are responsible for tetracycline resistance.[3,4] Resistance to tetracycline is conducted by 36 currently described *tet* genes, which produce 3 mechanisms of resistance, a ribosomal protection, direct enzymatic inactivation of the antibiotic, and an efflux pump which is the most common mechanism among gram-negative species.[3] However, the presence of the genes is not the definite reason for the emergence of resistant strain and it seems that the variety of genes and the sequence of each gene can affect the tetracycline phenotype of the isolates.[5]

## Objective

The present study was conducted in order to compare the sequences of tetracycline resistance determinants among *Salmonella* serotypes with animal and human source and other sequences in bioinformatics data bases to evaluate the origin of the genes responsible for tetracycline resistance.

## Materials and Methods

### *Salmonella* Serovars and Template DNA

In overall, 60 *Salmonella* serotypes were used in the present study. These serotypes had previously been recovered from samples submitted to Microbiology Laboratory

of the School of Veterinary Medicine and Human Fecal Isolates obtained from a diagnostic laboratory during 2004 to 2008 in Shiraz (Table 1). In addition, strains had previously been identified by conventional methods and serotyped by the *Salmonella* reference center (Razi Institute, Iran). Total DNA of each serotype was extracted using boiling method according to the procedure of the Lin et al.[6]

## Detection of Tetracycline Resistance Genes

Five tetracycline resistance determinants including *tetA*, *tetB*, *tetC*, *tetD,* and *tetG* were subjected to PCR using specific oligonucleotides listed in Table 2. The PCR reaction mixtures included: 3 µL of DNA template, 2.5 µL 10x PCR buffer, (Takapuzist, Iran), 1.5 µL $MgCl_2$, (Takapuzist, Iran), 1.5 µL dNTPs (50 µM, Takapuzist, Iran), 1U Taq DNA polymerase (Takapuzist, Iran), and 1 µL (25 pmol) of both forward and reverse primers (Takapuzist, Iran); the volume of reaction mixture was completed to 25 µL using distilled deionized water. The PCR programs were set up on thermal cycler (MJ mini, BioRad Co, USA) according to the primer's annealing temperature (Table 2) followed by 94°C and 72°C for denaturation and extension phase of the reaction, respectively. The PCR products were separated in 1.5% agarose gel stained with ethidium bromide and visualized using a UV transilluminator (BTS-20, Japan). The 100 bp DNA marker (Bioneer, Nourth Korea) was used as molecular size indicator.

## Gel Extraction and Sequence Analyzing

The PCR products of the samples which were positive for the presence of tetracycline resistance genes were immediately extracted and purified from agarose gel using gel extraction kit (Takapuzist, Iran) according to the manufacturer instructions. Extracted products were sent to the Takapuzist Corporation for sequencing using Sanger method. Sequencing results were submitted to the GenBank database of National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov) and assigned specific accession number (Table 3). Then, the sequences were compared to the NCBI GenBank databases by the Basic Local Alignment Search Tool (BLAST) program using Molecular Evolutionary Genetics Analysis (MEGA) software, version 7; the phylogenetic tree was also drawn according to comparison of the sequences with other similar sequences in the GenBank.[11]

**Table 1.** Distribution of Tetracycline Resistance Genes in *Salmonella* Serotypes

| Source/Serotype | No. of Isolates | No. Positive for *tet* Genes (%) | | | | |
|---|---|---|---|---|---|---|
| | | ***tetA*** | ***tetB*** | ***tetC*** | ***tetG*** | ***tetM*** |
| Poultry | | | | | | |
| *S. enteritidis* | 15 | 0 | 0 | 0 | 0 | 0 |
| *S. typhimurium* | 9 | 1 | 0 | 0 | 0 | 0 |
| *S. infantis* | 5 | 2 | 0 | 1 | 0 | 0 |
| *S. colindal* | 1 | 1 | 0 | 1 | 0 | 0 |
| Animals | | | | | | |
| *S. abortusovis* | 12 | 2 | 0 | 0 | 0 | 0 |
| *S. enteritidis* | 7 | 0 | 0 | 0 | 0 | 0 |
| *S. typhimurium* | 5 | 0 | 0 | 0 | 0 | 0 |
| *S. virchow* | 1 | 0 | 0 | 0 | 0 | 0 |
| Human | | | | | | |
| *S. enteritidis* | 3 | 0 | 0 | 1 | 0 | 0 |
| *S. bardo* | 2 | 0 | 0 | 0 | 0 | 0 |
| Total | 60 | 6 (10) | 0 | **3** (5) | 0 | 0 |

**Table 2.** Nucleotide Sequences Used as Primers for PCR Detection of Tetracycline Resistance Determinants

| Target Gene | Primer Sequence (5′ to 3′) | Annealing Temperature | Product Size (bp) | Reference |
|---|---|---|---|---|
| *tetA* | Forward: GCT ACA TCC TGC TTG CCT TC<br>Reverse: CAT AGA TCG CCG TGA AGA GG | 50 | 210 | (7) |
| *tetB* | Forward: TTG GTT AGG GGC AAG TTT TG<br>Reverse: GTA ATG GGC CAA TAA CAC CG | 50 | 659 | (8) |
| *tetC* | Forward: CTT GAG AGC CTT CAA CCC AG<br>Reverse: ATG GTC GTC ATC TAC CTG CC | 49 | 418 | (8) |
| *tetG* | Forward: GCT CGG TGG TAT CTC TGC TC<br>Reverse: AGC AAC AGA ATC GGG AAC AC | 49 | 468 | (9) |
| *tetM* | Forward: GTG GAC AAA GGT ACA ACG AG<br>Reverse: CGG TAA AGT TCG TCA CAC AC | 46 | 406 | (10) |

## Results

Among 60 different *Salmonella* serotypes, 6 strains (including 2 *S. abortusovis* from animals, 2 *S. Infantis* from poultry, 1 *S. colindale* from poultry, and one *S. typhimurium* from poultry) were positive for the presence of *tetA* gene (Figure 1). Three serotypes (including 1 *S. infantis*, 1 *S. enteritidis*, and one *S. colindale*) were also positive for the presence of *tetC* gene (Figure 2). Other tetracycline resistance determinants including *tetB*, *tetG*, and *tetM* were not detected in *Salmonella* serovars. A *S. colindale* with poultry origin showed simultaneous presence of both *tetA* and *tetC* genes. Generally, 6 *tetA* and 1 *tetC* sequences of PCR products of the detected genes were deposited in GenBank and acquired accession numbers which are listed in Table 3. In addition, phylogenetic trees of the sequence results as compared with the other similar sequences of GenBank databases showed that *tetA* sequences of the S. abortusovis were similar to related sequences of *Acinetobacter baumannii* and *Klebsiella pneumonia* (Figure 3). Only the PCR product of the *S. enteritidis tetC* gene was sequenced while others were not subjected to sequencing. The sequence demonstrated high similarity with *S. typhimurium* and somehow with *Pseudomonas marginalis* (Figure 4).

## Discussion

In this study, tetracycline was used for treatment of some bacterial diseases and as food additive for growth promotion in animals.[12] According to previous studies, resistance to tetracycline among *Salmonella* serotypes was varied and closely associated with the source of the isolates, ranging between 0 to 73 percent.[13,14] The main purpose of the present study, regardless of the tetracycline phenotypic characteristics of the *Salmonella* serotypes, was the distribution and comparison of the nature of tetracycline resistance genes (*tet*$^r$) which are located on plasmids and transposons. These plasmid mediated determinants, according to some studies, can be transferable resistance elements between several bacterial strains and species.[3,15] Therefore, different *Salmonella* serotypes, species specific or/and non-host adapted, can transfer these determinants through various horizontal gene transfer mechanisms. Previous studies have indicated that bacterial species and also different serotypes of one species carry distinct *tet*$^r$ genes.[3,16] *Tet* resistance in *Salmonella* often occurs by efflux pumps that eliminate the tetracycline from the bacteria before it can stop the binding of tRNA to the A site of the 30S subunit of ribosome, thus preventing protein synthesis.[17] The genes responsible for this mechanism among *Salmonella* spp. are of various types including *tetA*, *tetB*, *tetC,* and *tetD* all of which code for energy-dependent membrane-associated proteins that export tetracycline out of the cell.[12] However, in the present study, only 2 of the most important resistance genes, that is, *tetA* and *tetC* were found. None of these 2 genes unlike *tetB* gene can cause resistance to other similar compounds such as minocycline.[18] The sequences of *tetA* gene are relatively similar. Some of the sequences showed similarity with the relative sequences of other species. Specifically, the MG283265 sequence of the *S. abortusovis* which was homologous with *Acinetobacter baumanni* related sequence was isolated from Greece and *Klebsiella pneumonia* was isolated from Australia while the other sequences of the present study were placed in one similar clade (Figure 3). Frech and Schwarz[16] previously reported *tetA* gene among *Salmonella* serovars including Typhimurium, Dublin and Choleraesuis. Using a hybridization assay, they showed that some of

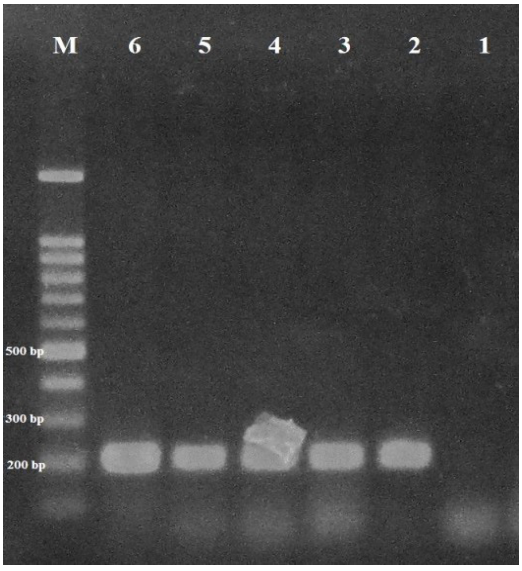


**Figure 1.** Agarose gel electrophoresis of the *tetA* PCR products; Lane 1: Negative control; Lane 2-6: positive samples; Lane M: 100 bp DNA ladder.

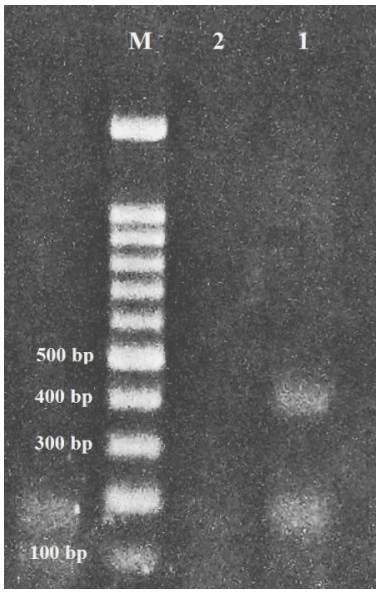


**Figure 2.** Agarose Gel Electrophoresis of the *tetC* PCR Products. Lane 1: positive sample; Lane 2: Negative control; Lane M: 100 bp DNA ladder

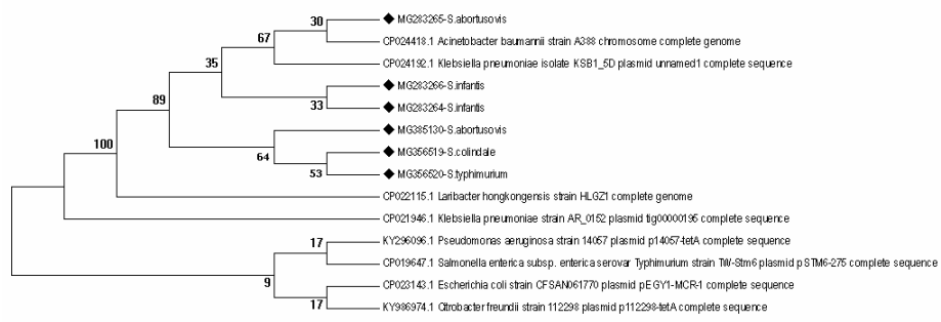


**Figure 3.** Phylogenetic tree of the *tetA* sequences comparison with other similar sequences in GenBank using MEGA software version 7.

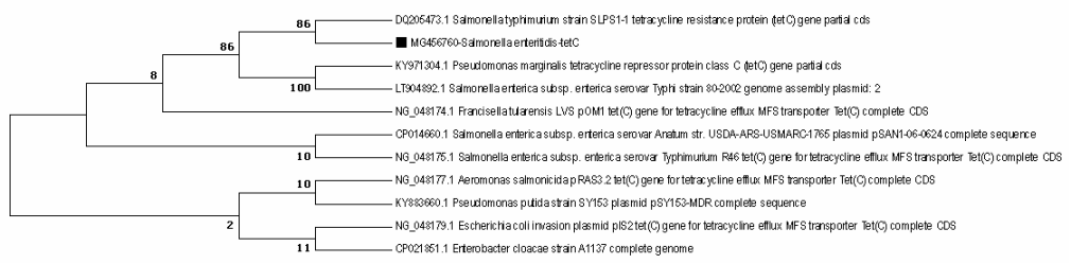


**Figure 4.** Phylogenetic Tree of the *tetC* Sequences Comparison With Other Similar Sequences in GenBank Using MEGA Software Version 7.

the *Tet* genes were located on the chromosomal DNA of the corresponding *Salmonella* serovars. Surprisingly, a similar sequence of *tetA* gene in GenBank database of *A. baumannii* was located on chromosomal DNA which could indicate the transferable nature of the tet$^r$ genes which could integrate through the main DNA. There was a different story about *tetC* gene sequences which showed more similarity between the present study *S. enteritidis* sequence and *S. typhimurium* isolated from swine in China and somewhat with *Pseudomonas marginalis* isolated from aquaculture in Turkey (Figure 2). Presence of these genes can affect the phenotypic characteristics of the microorganism and alter the level of resistance.[5] Nevertheless, the occurrence of more than one resistance gene did not cause significantly higher minimum inhibitory concentrations.[4] Today, it is known that outbreaks of *Salmonella* in some countries have been associated with food making from animal sources such as chicken, eggs, beef, and ground turkey.[19] As a result, organized programs for controlling the antibiotic administration that are used among these animal farms is a crucial management operation and the first one for controlling and preventing the infections in humans.

## Conclusion

In conclusion, based on the distribution of tetracycline resistance determinants among diverse *Salmonella* serovars from different sources, a footprint of the extensive use of the antibiotic in the animals particularly poultry farms can be inferred since the resistance genes among poultry isolates can be found more than other sources.

### Authors' Contributions

All authors have equal contribution in the research work.

### Ethical Approval

Not applicable.

### Conflict of Interest Disclosures

The authors declare that they have no conflict of interests.

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### References

1. Barlow M, Hall BG. Origin and evolution of the AmpC beta-lactamases of *Citrobacter freundii*. Antimicrob Agents Chemother. 2002;46(5):1190–1198. doi:10.1128/AAC.46.5.1190-1198.2002
2. Majowicz SE, Musto J, Scallan E, et al. The global burden of nontyphoidal *Salmonella* gastroenteritis. Clin Infect Dis. 2010; 50(6):882–889. doi:10.1086/650733.
3. Chopra I, and Roberts M. Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. Microbiol Mol Biol Rev. 2001;65(2):232-260. doi:10.1128/MMBR.65.2.232-260.2001.
4. Bryan A, Shapir N, Sadowsky MJ. Frequency and distribution of tetracycline resistance genes in genetically diverse, nonselected, and nonclinical *Escherichia coli* strains isolated from diverse human and animal sources. Appl Environ Microbiol. 2004;70(4):2503-2507. doi:10.1128/AEM.70.4.2503-2507.2004.
5. Seifi S, Khoshbakht R. Prevalence of tetracycline

resistance determinants in broiler isolated *Escherichia coli* in Iran. Br. Poult Sci. 2016;57(6):729-733. doi:10.1080/00071668.2016.1232478.

6. Lin AW, Usera MA, Barrett TJ, Goldsby RA. Application of random amplified polymorphic DNA analysis to differentiate strains of *Salmonella enteritidis*. J Clin Microbiol. 1996; 34(4): 870-876.
7. Mendez B, Tachibana C, Levy SB. Heterogeneity of tetracycline resistance determinants. Plasmid. 1980;3(2):99–108. doi:10.1016/0147-619X(80)90101-8.
8. Marshall B, Tachibana C, Levy SB. Frequency of tetracycline resistance determinant classes among lactose-fermenting coliforms. Antimicrob Agents Chemother. 1983; 24(6): 835–840. doi:10.1128/AAC.24.6.835.
9. Zhao J, Aoki T. Nucleotide sequence analysis of the class G tetracycline resistance determinant from *Vibrio anguillarum*. Microbiol and Immunol. 1992;36(10):1051–1060.
10. Warsa UC, Nonoyama M, Ida T, et al. Detection of *tet*(K) and *tet*(M) in *Staphylococcus aureus* of Asian countries by the polymerase chain reaction. J Antibiotic. 1996;49(11): 1127–1132. doi:10.7164/antibiotics.49.1127.
11. Kumar S, Stecher G, Tamura K. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Mol Biol Evol. 2016;33(7):1870-1874. doi:10.1093/molbev/msw054.
12. Roberts M. Tetracycline resistance determinants: mechanisms of action, regulation of expression, genetic mobility, and distribution. *FEMS Microbiol Rev*. 1996;19(1):1-24. doi:10.1111/j.1574-6976.1996.tb00251.x.
13. Hur J, Jawale C, Lee JH. Antimicrobial resistance of *Salmonella* isolated from food animals: A review. *Food Res Int*. 2012;45(2):819-830. doi:10.1016/j.foodres.2011.05.014.
14. Tessema K, Bedu H, Ejo M, Hiko A. Prevalence and Antibiotic Resistance of *Salmonella* Species Isolated from Chicken Eggs by Standard Bacteriological Method. *J Vet Sci Technol*. 2017;8:421. doi:10.4172/2157-7579.1000421.
15. Agerso Y, Sandvang D. Class 1 integrons and tetracycline resistance genes in *Alcaligenes*, *Arthrobacter*, and *Pseudomonas* spp. isolated from Pigsties and Manured soil. Appl Environ Microbiol. 2005;71(12):7941–7947. doi:10.1128/AEM.71.12.7941-7947.2005
16. Frech G, Schwarz S. Molecular analysis of tetracycline resistance in *Salmonella enterica* subsp. enterica serovars Typhimurium, Enteritidis, Dublin, Choleraesuis, Hadar and Saintpaul: construction and application of specific gene probes. J Appl Microbiol. 2000; 89(4):633-641. doi:10.1046/j.1365-2672.2000.01160.x.
17. Foley SL, Lynne AM. Food animal-associated challenges: Pathogenicity and antimicrobial resistance. J Animal Sci. 2008;86(14):E173-E187. doi:10.2527/jas.2007-0447.
18. Chopra I, Hawkey PM, Hinton M. Tetracyclines, molecular and clinical aspects. J Antimicrob Chemother. 1992;29(3):245-277. doi:10.1093/jac/29.3.245.
19. Scallan E, Hoekstra RM, Angulo FJ, et al. Foodborne illness acquired in the United States—major pathogens. Emerg Infect Dis. 2011;17(1):7-15. doi:10.3201/eid1701.P11101.