Dijlah Journal of Medical Sciences (DJMS) Vol. 1, No. 2, January, 2025, pp. 38-62
P-ISSN: 3078-3178, E-ISSN: 3078-8625, Paper ID: 05

Sequencing and Phylogenetic Tree in Cat and Tet B Gene in Patients with Salmonella Typhi

Inas Sattar Abd*, Hiba Nabil Abbas
Al-Karkh University of Science, Baghdad, Iraq

Article Info

Article history:

Received September, 10, 2024
Revised October, 20, 2024
Accepted October, 29, 2024

Keywords:

Antibiotic Resistance,
Sequencing,
Phylogenetic tree,
Salmonella Enterica Serovar,
Typhi

ABSTRACT

Salmonella typhi is resistant to numerous antibiotics because recurrently treated per antibiotic. on bases the positive and strong isolates, we selected six isolates for Tet B gene and three isolates for Cat for sequencing analysis . This study detected genetic sequencing and phylogenetic relationships of Tet B and cat genes from patients infected with Salmonella typhi. during specific gene analysis , which detected present two mutations (silent) were identified in gene (cat) that that are protein remain same , while 14 mutation occur in Tet B gene were silent and another were missenses mutation. Phylogenetic analysis revealed that Salmonella typhi was similar to other salmonella on cat gene and tet B gene.

Aim of the study to appreciate Slamonella typhi role in antibiotic resistance mechanisms through detected mutation that occur in cat and tet B gene and their evolution within numerous strains of Salmonella typhi..

Corresponding Author:

*Inas sattar Abd
Al-Karkh University of Science, Baghdad, Iraq
Inasabd4@gmail.com

1- INTRODUCTION

Salmonella typhi is flagellate bacteria ,Gram-negative and facultative anaerobic when enter to healthy human by eating contaminated food or drinking contaminated water result in high fever, belly pain , nausea and belly bowel movement [1, 2]. Humans gain MDR strains from these contaminated aided by exchange in plasmid, transposon, and assistance the circulation of MDR strains about the universal population [3].

Antibiotic a material, created by a microorganism or from source of biology, which at concentration small can avert the growth of, or are fatal to other microorganism [4]. Chloramphenicol was considered “gold standard” agent for treatment of Salmonella typhi but chloramphenicol-resistant emergence suitable alternative is trimethoprim [5].

Salmonella Typhi has succeeded resistance toward antibiotic by attaining single or multiple foreign DNA elements, segment of gene or plasmid encoding antibiotic-modifying enzyme otherwise through prompting specific mutation in dissimilar loci of its chromosomal gene. In addition, gain foreign DNA element, plasmid by horizontal gene transfer, conjugation, transformation [6].
The bioinformatics analysis can use for taxonomic identification, antibiotic gene analysis and the capability for completely genomic sequencing [7].

Tet B and Cat genes are genes found in some bacteria, including Salmonella enterica serovar typhi These two genes are important because they often give bacteria resistance to a type of antibiotic called tetracycline and chloramphenicol.

Therefore, a patient infected with Salmonella typhi and taking tetracycline and chloramphenicol treatment may not affect the bacteria. For this reason, our study focused on these two genes, Tet B and Cat, which are present in these bacteria are warning sign.

Aim of the study to appreciate Slamonella typhi role in antibiotic resistance mechanisms through detected mutation that occur in cat and tet B gene and their evolution within numerous strains of Salmonella typhi.

2- METHOD

20 samples were collected from different age group among blood patients with Salmonella typhi . Taking blood sample in inoculated bottle and examined daily to notice changing in turbidity, hemolysis and gas formation. Then incubation lasts for 7 a day with constant shaking each day streaked by sterile loop on blood agar, and MacConkey agar.

According to the clinical laboratory standers institute this test was performed by the method of Kirby-Bauer (disk diffusion) technique using Muller-Hinton agar and different single antibiotic disc supplied commercially (CLSI 2020).

The identification of S. typhi was confirmed using VITEK2- Compact system. The Gram negative card was used for this purpose for S. typhi Identification, and Antibiotic sensitivity test (AST-GN76) for testing the antibiotic susceptibility of those isolates as this card detect MIC of S. typhi isolates.

The process of extraction was done according to the company's instructions for all (20) isolate, six isolates for Salmonella typhi were separated and the done Sequencing for tet B and three isolate for Cat gene that performed by macrogen korea, by BLAST program which was accessible at (NCBI) online at (http://www.ncbi.nlm.nih.gov). Then done phylogenetic tree for three isolated from all genes.

3- RESULTS

Detection of mutation of amino acid in Cat and Tet B gene of S. typhi

The positive and strongest isolates were selected that three isolate for Cat gene and six isolate for Tet B gene then sent to Korea to make the sequencing. Sequencing technique that used to determine sequence of amino acids, where an analysis of the amino acids of the Cat gene of S. typhi ID: AL513383.1 Length (218160), Number of Matches [1] ,Range 1: 154617 to 154846. It was performed to find out the chance of vital mutations of the amino acid group according to the Gen Bank Graphics from NCBI.

The results presented that group of amino acids of the Cat gene in two isolates is identical is about (99%) to the international strains that were compared with it, which verified a mutation (**silent mutation**) of the amino acid (**A\G)**. which occurred between the sequences(121-154796) of the amino acid sequences(table1) and with one isolate without mutation(Figure1,2&3).

Salmonella enterica subsp. enterica serovar Typhi str. CT18 plasmid pHCM1
Sequence ID: AL513383.1Length: 218160Number of Matches: 1
Range 1: 154617 to 154846GenBankGraphicsNext MatchPrevious Match

Score	Expect	Identities	Gaps	Strand
437 bits(227)	3e-121	229/230(99%)	0/230(0%)	Plus/Plus

```
Query  1       TGTACCTATAACCAGACCGTTCAGCTGGATATTACGGCCTTTTTAAAGACCGTAAAGAAA  60

Sbjct  154617  ............................................................  154676

Query  61      AATAAGCACAAGTTTTATCCGGCCTTTATTCACATTCTTGCCCGCCTGATGAATGCTCAT  120

Sbjct  154677  ............................................................  154736

Query  121     CCGGAGTTCCGTATGGCAATGAAAGACGGTGAGCTGGTGATATGGGATAGTGTTCACCCT  180

Sbjct  154737  .....A......................................................  154796

Query  181     TGTTACACCGTTTTCCATGAGCAAACTGAAACGTTTTCATCGCTCTGGAG  230

Sbjct  154797  ..................................................  154846
```

Figure 1: The sequences of amino acid in Cat gene of S. typi of ID. A

phi of ID: AL513383.1 in isolate no.1 (presence of silent mutation).

Salmonella enterica subsp. enterica serovar Typhi str. CT18 plasmid pHCM1

Sequence ID: AL513383.1Length: 218160Number of Matches: 1

Range 1: 154617 to 154846GenBankGraphicsNext MatchPrevious Match

Score	Expect	Identities	Gaps	Strand
442 bits(230)	6e-123	230/230(100%)	0/230(0%)	Plus/Plus

Query 1 TGTACCTATAACCAGACCGTTCAGCTGGATATTACGGCCTTTTTAAAGACCGTAAAGAAA 60

Sbjct 154617 154676

Query 61 AATAAGCACAAGTTTTATCCGGCCTTTATTCACATTCTTGCCCGCCTGATGAATGCTCAT 120

Sbjct 154677 154736

Query 121 CCGGAATTCCGTATGGCAATGAAAGACGGTGAGCTGGTGATATGGGATAGTGTTCACCCT 180

Sbjct 154737 154796

Query 181 TGTTACACCGTTTTCCATGAGCAAACTGAAACGTTTTCATCGCTCTGGAG 230

Sbjct 154797 154846

Figure 2: The sequences of amino acid in Cat gene of S. typhi of ID: AL513383.1 in isolate no.2 (no mutation).

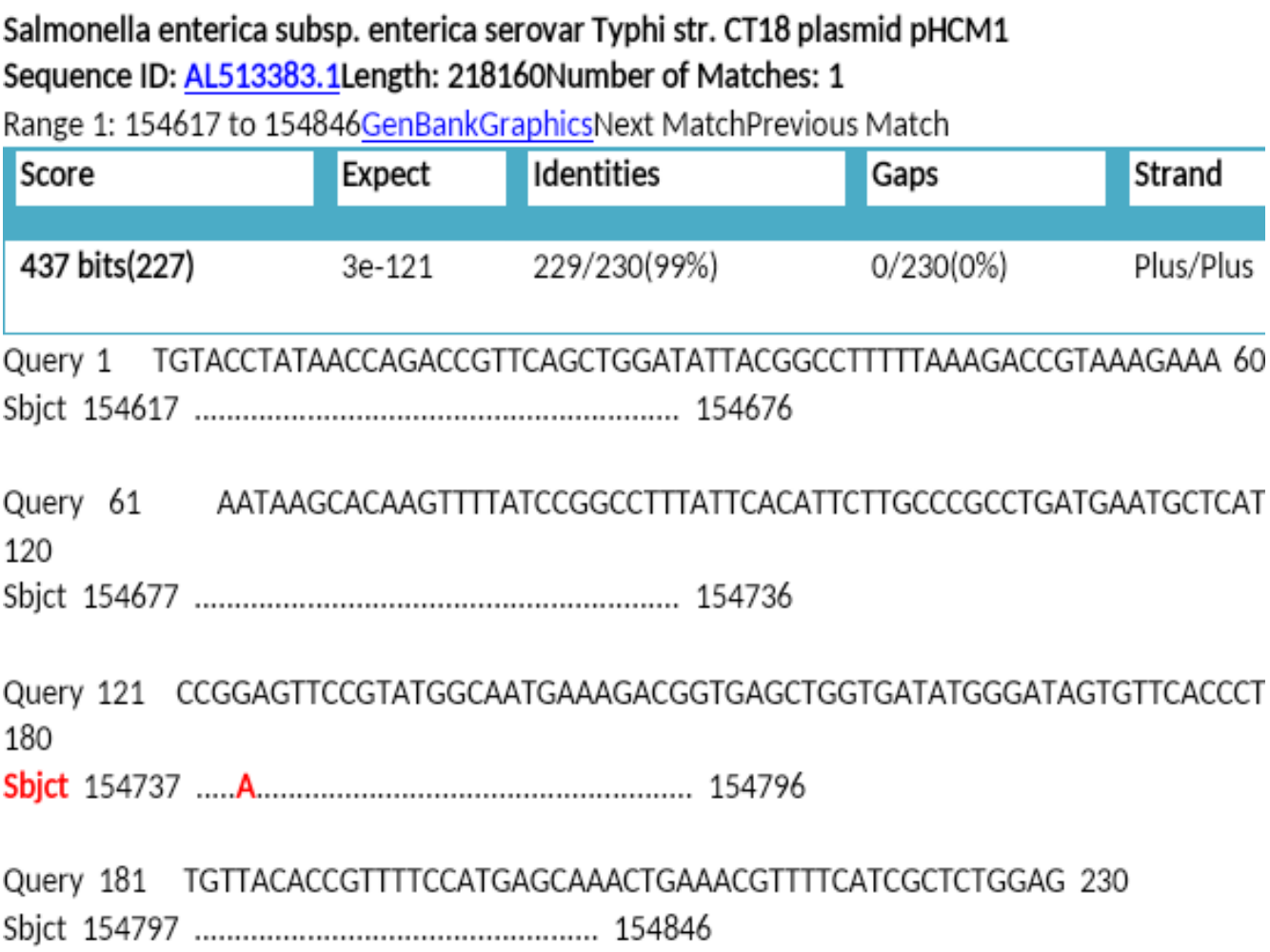

Figure 3: The sequences of amino acid in Cat gene of S. typhi of ID: AL513383.1 in isolate no.3 (presence of silent mutation).

For the detection of mutations in tet B gene, it also used the technique of sequencing the amino acids ID: CP029645.1Length (216963), Number of Matches [1], Range 1: 169137 to 169613Gen Bank Graphics from NCBI. It is revealed the presence of 14 mutations of amino acids, all of which met within the sequences that appeared in figure [4,5,6,7,8&9] of the amino acids within this strain. There are genetic alteration occur in particular base pair substitution modified code of gene that produce an amino acid which change from the typical amino acid at these position. These substitutions are two types: the first type was transitions are one ring pyrimidines (C ↔ T) occur at position of (169159 ,169411, 169477 & 169541) , where amino acid change from serine to leucine, alanine to valine, valine to alanine and cysteine to arginine respectively and mutation are point mutation (missense). In other hand at position (169286) this mutation was silent mutation which did not change in protein (amino acid) the phenylalanine remain phenylalanine the mutation have no effect. While occur interchange of two ring purine (G ↔ A) at position (169265, 169286 &169334) also not convert in protein (amino acid) the Alanine remain alanine, Leucine remain Leucine and Glutamine remain Glutamine the mutation have no effect this mutation were silent.

Interchanges of bases occur at position (169188, 169354, 169488) where nucleotide (G → C) and amino acid change from Valine to Leucine , Alanine to Glycine and Histidine to Aspartic acid and the mutation have effect this mutation were missense (point mutation). Moreover at position (169455 &169220) the nucleotide (A → C) the type of substation were transversion the mutation have effect when isoleucine change to Leucine at position (169455) missense mutation and no effect at position (169220) silent mutation. While at position (169421) nucleotide (A → T), the amino acid Glycine remain same.

Salmonella enterica subsp. enterica serovar Typhi strain 311189_217186 plasmid pHCM1, complete sequence
Sequence ID: CP029645.1 Length: 216963 Number of Matches: 1
Range 1: 169137 to 169613 GenBank Graphics Next Match Previous Match

Score	Expect	Identities	Gaps	Strand
852 bits(944)	0.0	475/477(99%)	0/477(0%)	Plus/Plus

```
Query  1       AATAGCATGATGGTTGGCTTTTTATTAGCGGGTCTTGGTCTTTTACACTCACTATTCCAA  60
Sbjct  169137  ......................C.............................G........  169196

Query  61      GCCTTTGTGGCAGGAAGAATAGCCACTAAATGGGGCGAAAAAACGGCAGTACTGCTCGGA  120
Sbjct  169197  ............................................................  169256

Query  121     TTTATTGCAGATAGTAGTGCATTTGCCTTTTTAGCGTTTATATCTGAAGGTTGGTTAGTT  180
Sbjct  169257  ............................................................  169316

Query  181     TTCCCTGTTTTAATTTTATTGGCTGGTGGTGGGATCGCTTTACCTGCATTACAGGGAGTG  240
Sbjct  169317  ............................................................  169376

Query  241     ATGTCTATCCAAACAAAGAGTCATCAGCAAGGTGCTTTACAGGGATTATTGGTGAGCCTT  300
Sbjct  169377  ............................................................  169436

Query  301     ACCAATGCAACCGGTGTTATTGGCCCATTACTGTTTGCTGTTATTTATAATCATTCACTA  360
Sbjct  169437  ............................................................  169496

Query  361     CCAATTTGGGATGGCTGGATTTGGATTATTGGTTTAGCGTTTTACTGTATTATTATCCTG  420
Sbjct  169497  ............................................................  169556

Query  421     CTATCGATGACCTTCATGTTAACCCCTCAAGCTCAGGGGAGTAAACAGGAGACAAGT  477
```

Figure 4: The sequences of amino acid in tet B gene of S. typhi of ID: CP029645.1 in isolate no.1 (presence of point mutation).

Salmonella enterica subsp. enterica serovar Typhi strain 311189_217186 plasmid pHCM1, complete sequence
Sequence ID: CP029645.1 Length: 216963 Number of Matches: 1
Range 1: 169137 to 169613 GenBank Graphics Next Match Previous Match

Score	Expect	Identities	Gaps	Strand
852 bits(944)	0.0	475/477(99%)	0/477(0%)	Plus/Plus

```
Query  1       AATAGCATGATGGTTGGCTTTTCATTAGCGGGTCTTGGTCTTTTACACTCAGTATTCCAA  60
Sbjct  169137  ............................................................  169196

Query  61      GCCTTTGTGGCAGGAAGAATAGCAACTAAATGGGGCGAAAAAACGGCAGTACTGCTCGGA  120
Sbjct  169197  ......................C.......................................  169256

Query  121     TTTATTGCGGATAGTAGTGCATTTGCCTTTTTAGCGTTTATATCTGAAGGTTGGTTAGTT  180
Sbjct  169257  ........A...................................................  169316

Query  181     TTCCCTGTTTTAATTTTATTGGCTGGTGGTGGGATCGCTTTACCTGCATTACAGGGAGTG  240
Sbjct  169317  ............................................................  169376

Query  241     ATGTCTATCCAAACAAAGAGTCATCAGCAAGGTGCTTTACAGGGATTATTGGTGAGCCTT  300
Sbjct  169377  ............................................................  169436

Query  301     ACCAATGCAACCGGTGTTATTGGCCCATTACTGTTTGCTGTTATTTATAATCATTCACTA  360
Sbjct  169437  ............................................................  169496

Query  361     CCAATTTGGGATGGCTGGATTTGGATTATTGGTTTAGCGTTTTACTGTATTATTATCCTG  420
Sbjct  169497  ............................................................  169556
```

Figure 5: The sequences of amino acid in tet B gene of S. typhi of ID: CP029645.1 in isolate no.2 (presence of silent mutation).

Figure 6: The sequences of amino acid in tet B gene of S. typhi of ID: CP029645.1 in isolate no.3 (presence of silent mutation).

Salmonella enterica subsp. enterica serovar Typhi strain 311189_217186 plasmid pHCM1, complete sequence
Sequence ID: CP029645.1 Length: 216963 Number of Matches: 1
Range 1: 169137 to 169613 GenBank Graphics Next Match Previous Match

Score	Expect	Identities	Gaps	Strand
847 bits(939)	0.0	474/477(99%)	0/477(0%)	Plus/Plus

```
Query  1       AATAGCATGATGGTTGGCTTTTCATTAGCGGGTCTTGGTCTTTTACACTCAGTATTCCAA  60
Sbjct  169137  ............................................................  169196

Query  61      GCCTTTGTGGCAGGAAGAATAGCCACTAAATGGGGCGAAAAAACGGCAGTACTGCTCGGA  120
Sbjct  169197  ............................................................  169256

Query  121     TTTATTGCAGATAGTAGTGCATTTGCCTTTTTAGCGTTTATATCTGAAGGTTGGTTAGTT  180
Sbjct  169257  ............................................................  169316

Query  181     TTCCCTGTTTTAATTTTATTGGCTGGTGGTGGGATCGGTTTACCTGCATTACAGGGAGTG  240
Sbjct  169317  ..........................................C.................  169376

Query  241     ATGTCTATCCAAACAAAGAGTCATCAACAAGGTGTTTTACAGGGATTATTGGTGAGCCTT  300
Sbjct  169377  ..........................G.....C.........................  169436

Query  301     ACCAATGCAACCGGTGTTATTGGCCCATTACTGTTTGCTGTTATTTATAATCATTCACTA  360
Sbjct  169437  ............................................................  169496

Query  361     CCAATTTGGGATGGCTGGATTTGGATTATTGGTTTAGCGTTTTACTGTATTATTATCCTG  420
Sbjct  169497  ............................................................  169556

Query  421     CTATCGATGACCTTCATGTTAACCCCTCAAGCTCAGGGGAGTAAACAGGAGACAAGT    477
Sbjct  169557  ............................................................  169613
```

Figure 7: The sequences of amino acid in tet B gene of S. typhi of ID: CP029645.1 in isolate no.4 (presence of point & silent mutation).

Salmonella enterica subsp. enterica serovar Typhi strain 311189_217186 plasmid pHCM1, complete sequence
Sequence ID: CP029645.1 Length: 216963 Number of Matches: 1
Range 1: 169137 to 169613 GenBank Graphics Next Match Previous Match

Score	Expect	Identities	Gaps	Strand
847 bits(939)	0.0	474/477(99%)	0/477(0%)	Plus/Plus

Query	1	AATAGCATGATGGTTGGCTTTTCATTAGCGGGTCTTGGTCTTTTACACTCAGTATTCCAA	60
Sbjct	169137	.....	169196
Query	61	GCCTTTGTGGCAGGAAGAATAGCCACTAAATGGGGCGAAAAAACGGCAGTACTGCTCGGA	120
Sbjct	169197	.....	169256
Query	121	TTTATTGCAGATAGTAGTGCATTTGCCTTTTTAGCGTTTATATCTGAAGGTTGGTTAGTT	180
Sbjct	169257	.....	169316
Query	181	TTCCCTGTTTTAATTTTATTGGCTGGTGGTGGGATCGCTTTACCTGCATTACAGGGAGTG	240
Sbjct	169317	.....	169376
Query	241	ATGTCTATCCAAACAAAGAGTCATCAGCAAGGTGCTTTACAGGGTTTATTGGTGAGCCTT	300
Sbjct	169377	.....A.....	169436
Query	301	ACCAATGCAACCGGTGTTCTTGGCCCATTACTGTTTGCTGCTATTTATAATCATTCACTA	360
Sbjct	169437	.....A.....T.....	169496
Query	361	CCAATTTGGGATGGCTGGATTTGGATTATTGGTTTAGCGTTTTACTGTATTATTATCCTG	420
Sbjct	169497	.....	169556
Query	421	CTATCGATGACCTTCATGTTAACCCCTCAAGCTCAGGGGAGTAAACAGGAGACAAGT	477
Sbjct	169557	.....	169613

Figure 8: The sequences of amino acid in tet B gene of S. typhi of ID: CP029645.1 in isolate no.5 (presence of silent & point mutation).

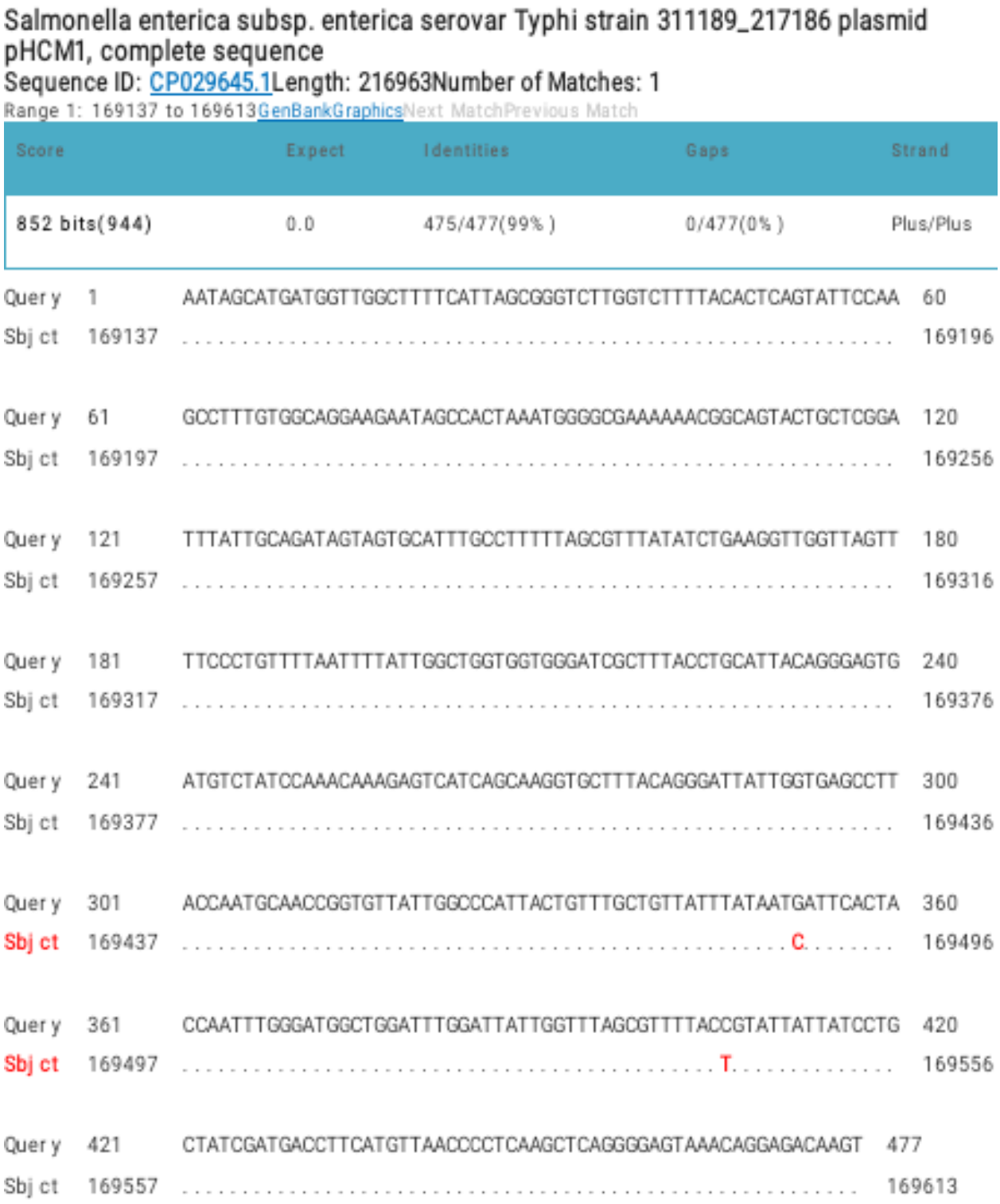

Figure 9: The sequences of amino acid in tet B gene of S. typhi of ID: CP029645.1 in isolate no.6 (presence of point mutation).

Table (1): Alteration of nucleotide substitution of amino acid and type mutation in Cat and tet B gene

	No. of isolate	Type of substitution	Location	Nucleotide	Nucleotide change	change in Amino acid	mutation	Identities
Cat	**1**	**Transition**	**154742**	**A\G**	**GAA\GAG**	**Glu→ Glu**	Silent	**99%**
	2	**---------**	------	**------**	**---------**	------	**------**	**100%**
	3	**Transition**	**154742**	**A\G**	**GAA\GAG**	**Glu→ Glu**	Silent	**99%**
Tet B	**1**	Transition	169159	**C\T**	**TCA\TTA**	**S → L**	Missense	**99%**
		Transvertion	169188	**G\C**	**GTA\CTA**	**V→ L**	Missense	
	2	Transvertion	169220	**C\A**	**GCC\GCA**	**Ala→ Ala**	Silent	**99%**
		Transition	169265	**A\G**	**GCA\GCG**	**Ala→ Ala**	Silent	
	3	Transition	169286	**T\C**	**TTT\TTC**	**Ph → Ph**	Silent	**99%**
		Transition	169334	**A\G**	**TTA\TTG**	**L → L**	Silent	
	4	Transvertion	169354	**C\G**	**GCT\GGT**	**Ala→ Gly**	Missense	**99%**
		Transition	169403	**G\A**	**CAG\CAA**	**Glu→ Glu**	Silent	
		Transition	169411	**C\T**	**GCT\GTT**	**Ala → V**	Missense	
	5	Transvertion	169421	**A\T**	**GGA\GGT**	**Gly→Gly**	Silent	**99%**
		Transvertion	169455	**A\C**	**ATT\CTT**	**I → L**	Missense	
		Transition	169477	**T\C**	**GTT\GCT**	**V → Ala**	Missense	
	6	Transvertion	169488	**C\G**	**CAT\GAT**	**H → Asp**	Missense	**99%**
		Transition	169541	**T\C**	**TGT\GT**	**C →Arg**	Missense	

Glu:Glutamic acid, S:Serine,L: Leucine, V:Valine,Ala: Alanine , Ph:Phenylalanine ,Gly: Glycine , Arg: Arginine, H: Histidine, Asp: Aspartic acid.

Phylogenetic tree of *Salmonella typhi*

The phylogenetic tree analysis was based on the molecular sequence of the Cat and tet B gene. It was used to detect S. typhi using UPGMA method identified two *S.* typhi [8] ideal tree with the summation of branch length equal (0.00438742) was appeared. To gather phylogenetic tree, tree was drawn at a specific scale and use branch lengths in equal units of evolutionary distances. By use of maximum composite Likelihood method to calculate, evaluation distance [9]. The analyses encompass 12 nucleotide sequences. Positions of codon comprised were (first +second +third + non coding). Wholly position having gaps and lost data were reduced. There were 230 positions in the last dataset. Analyses of evolution were directed in MEGA6 (10).

Three *Salmonella typhi* isolate [1, 2 & 3] (**AL513383.1)** were complete identical from the others established on the phylogenetic tree of gene (*Cat)* and the percentage of compatibility between them were 100% (figure 10,11,12,13) The second isolated was similar to the isolate of each of the countries UK (AL513383.1), India (CP046429.1), USA (CP044007.1), Denmark (CP040575.1), Brazil (CP029645.1), United Kingdom(LT904892.1),China (CP118537.1),

Taiwan(KM023773.1) and Brazil (CP029953.1) the percentage of similarity between them at 99% while the first and third isolated the percentage of compatibility between them and others country were 99%.

In contrast with Salmonella typhi isolate (**CP029645.1)** was identical to the isolates of each of the countries Brazil (CP029645.1), Brazil (CP029953.1), United Kingdom (LT906491.1), United Kingdom (LT905061.1), Congo (CP141260.1), India (CP003279.1), Chile (AY150213.1), USA (AF250878.1), Cambridge (AL513383.1) and USA(AF223162.1) the percentage of compatibility between them and others country were 99% (figure 14,15,16&17).

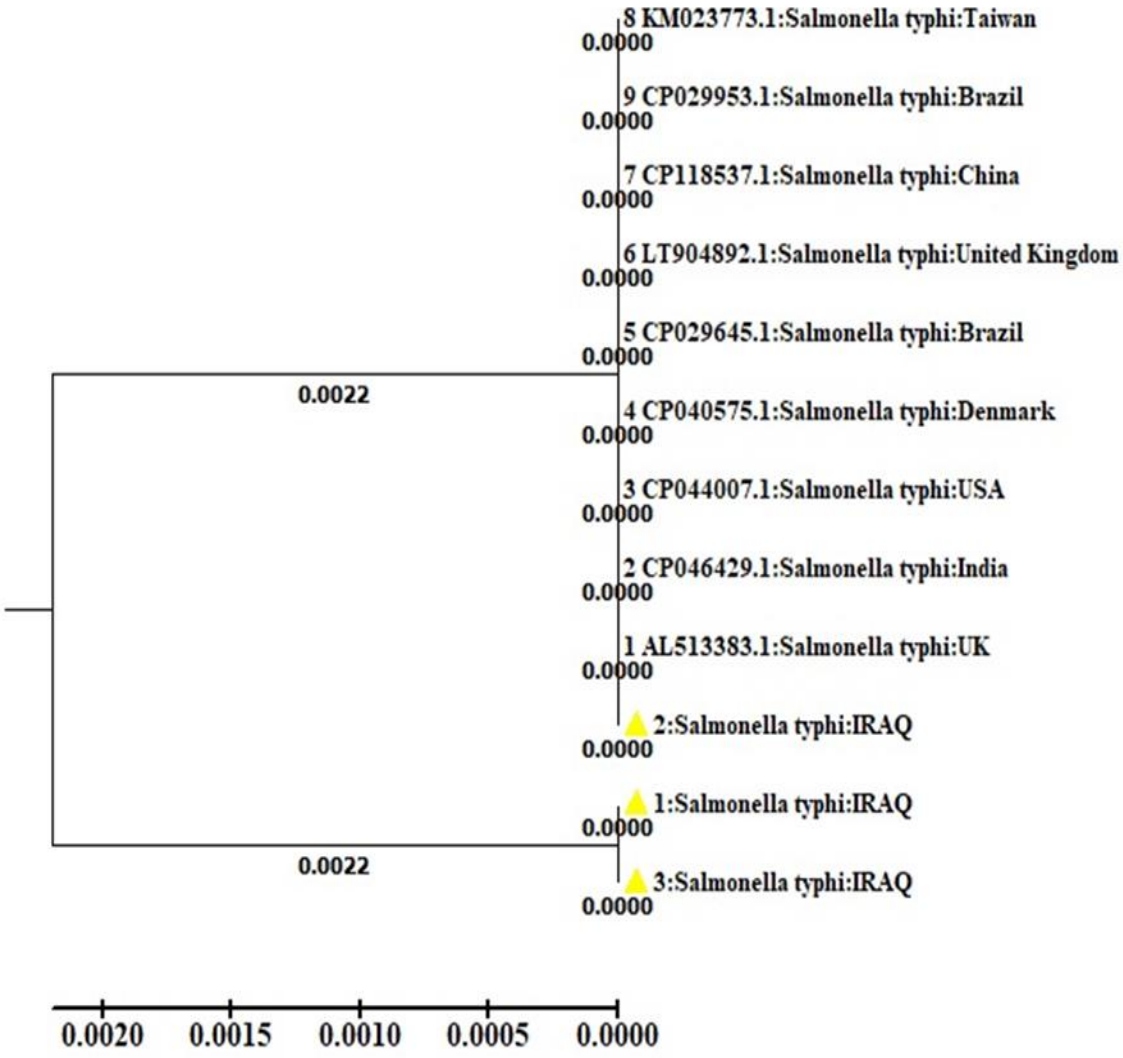

Figure (10): (isolate no.1)Phylogenetic tree analysis based on cat gene in S. typhi, which exposed the proximity of local & global isolates

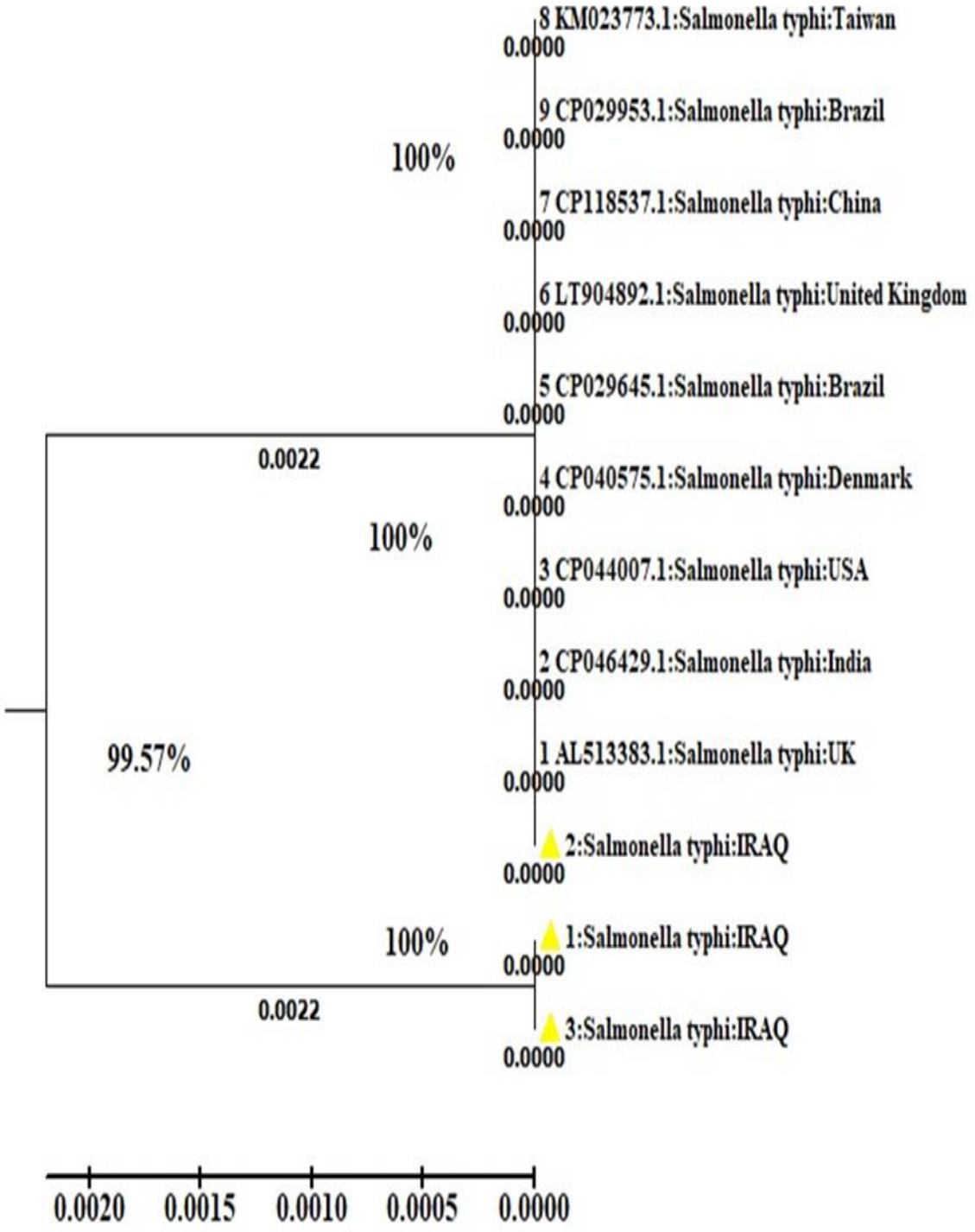

Figure (11): (isolate no.2)Phylogenetic tree analysis based on cat gene in S. typhi, which exposed the proximity of local & global isolates

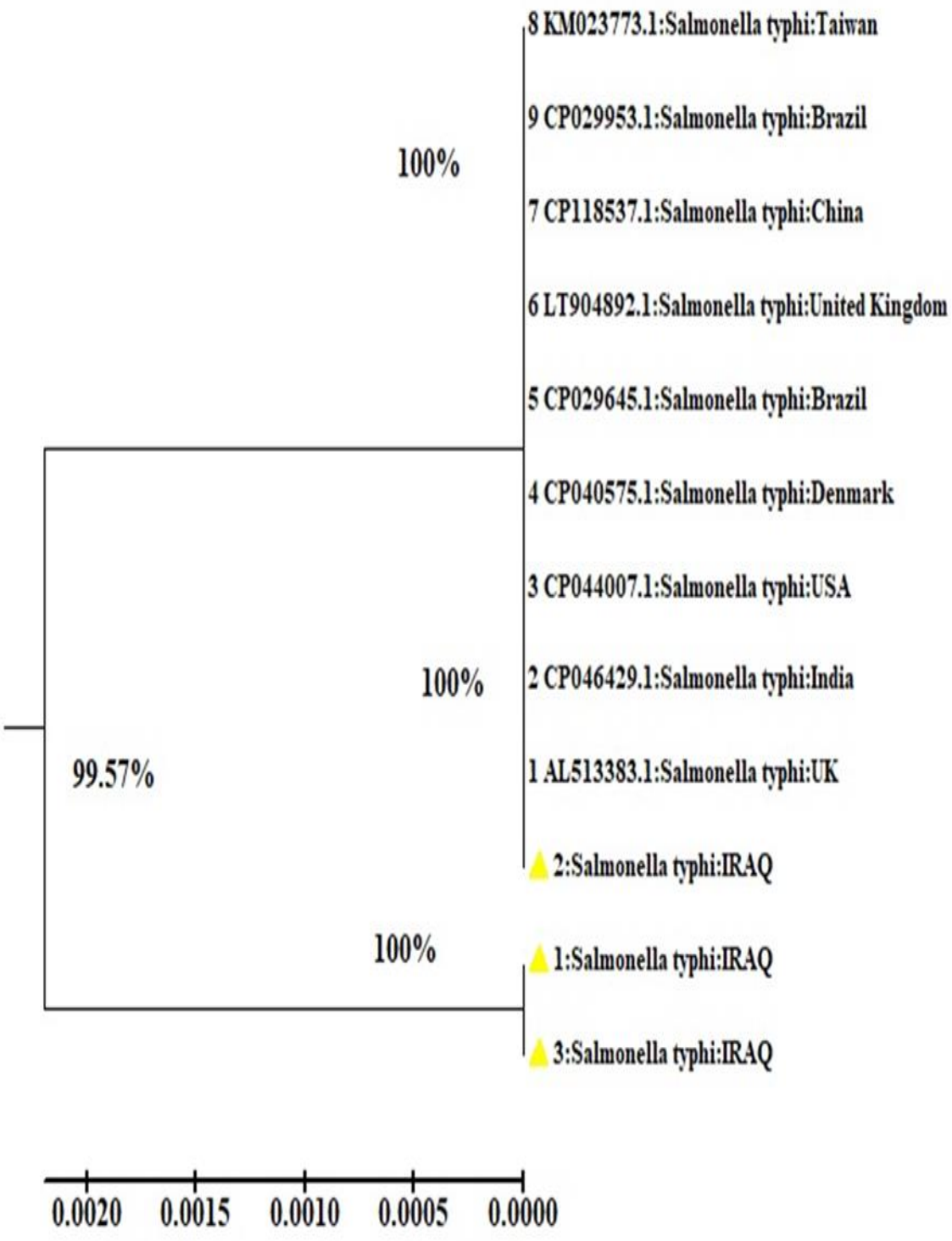

Figure (12): (isolate no.3)Phylogenetic tree analysis based on cat gene in S. typhi, which exposed the proximity of local & global isolates

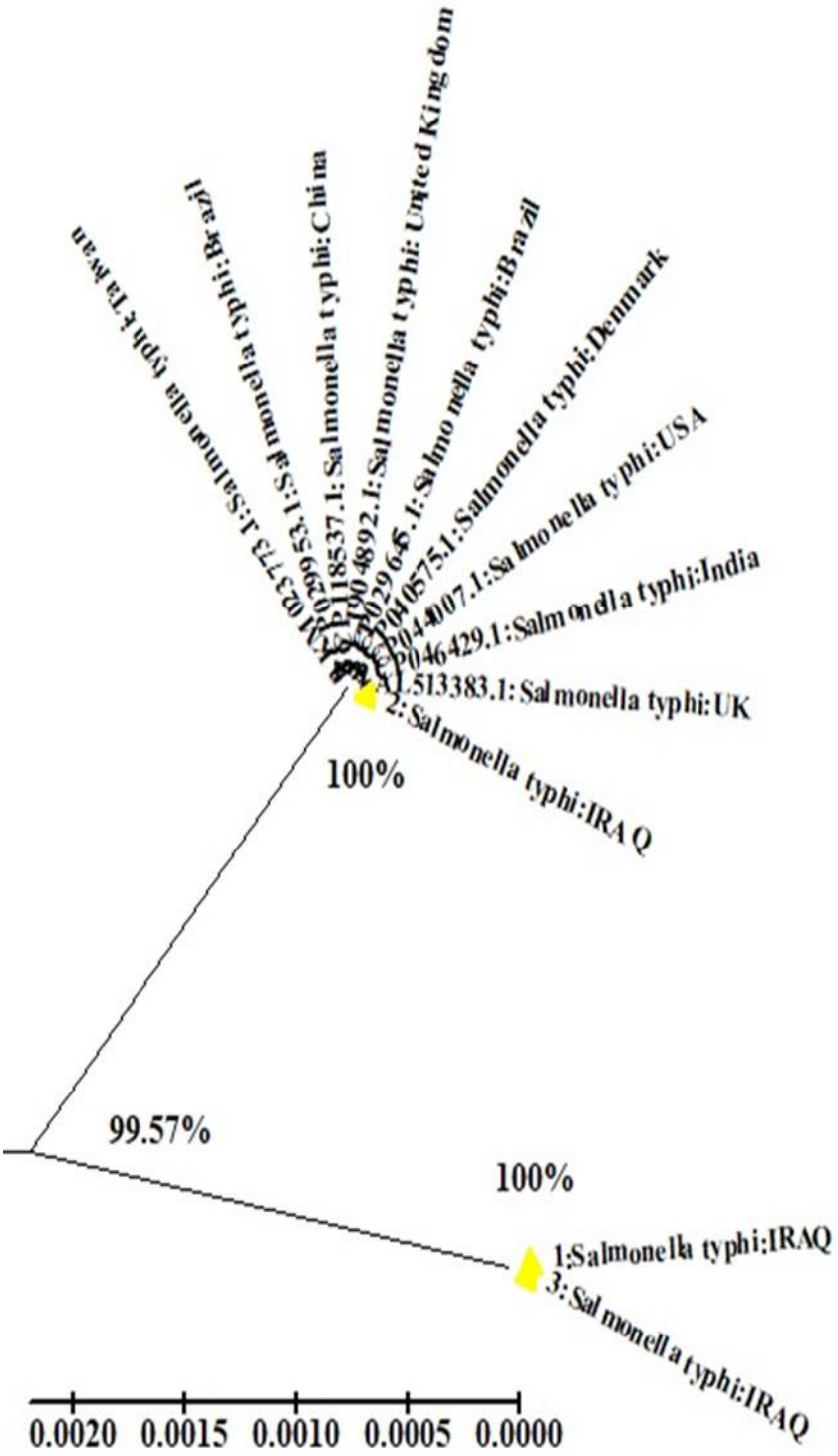

Figure (13): Phylogenetic tree analysis based on cat gene in S. typhi, which exposed the proximity of local & global isolates

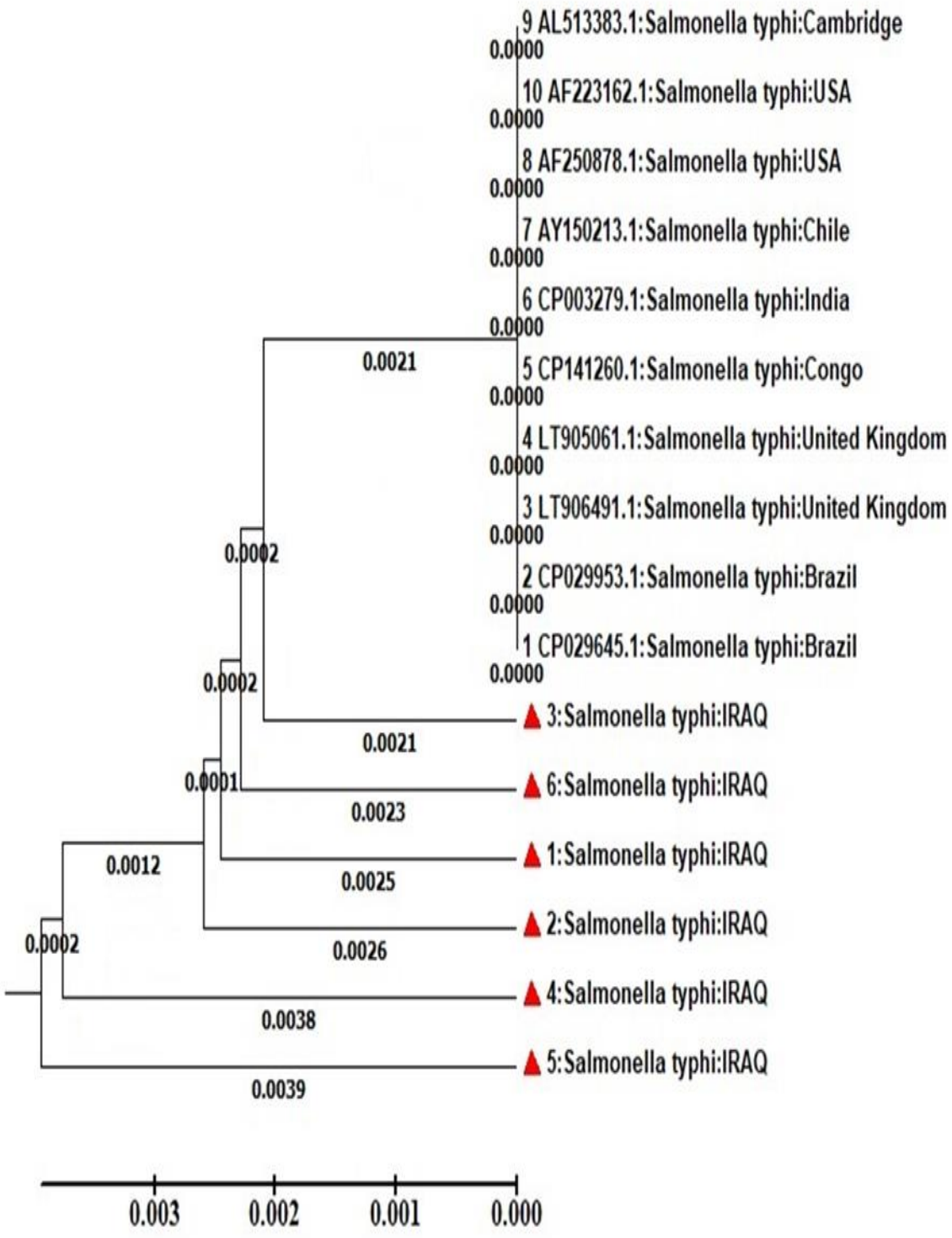

Figure (14): isolate no.1)Phylogenetic tree analysis based on tet B gene in S. typhi, which exposed the proximity of local & global isolates.

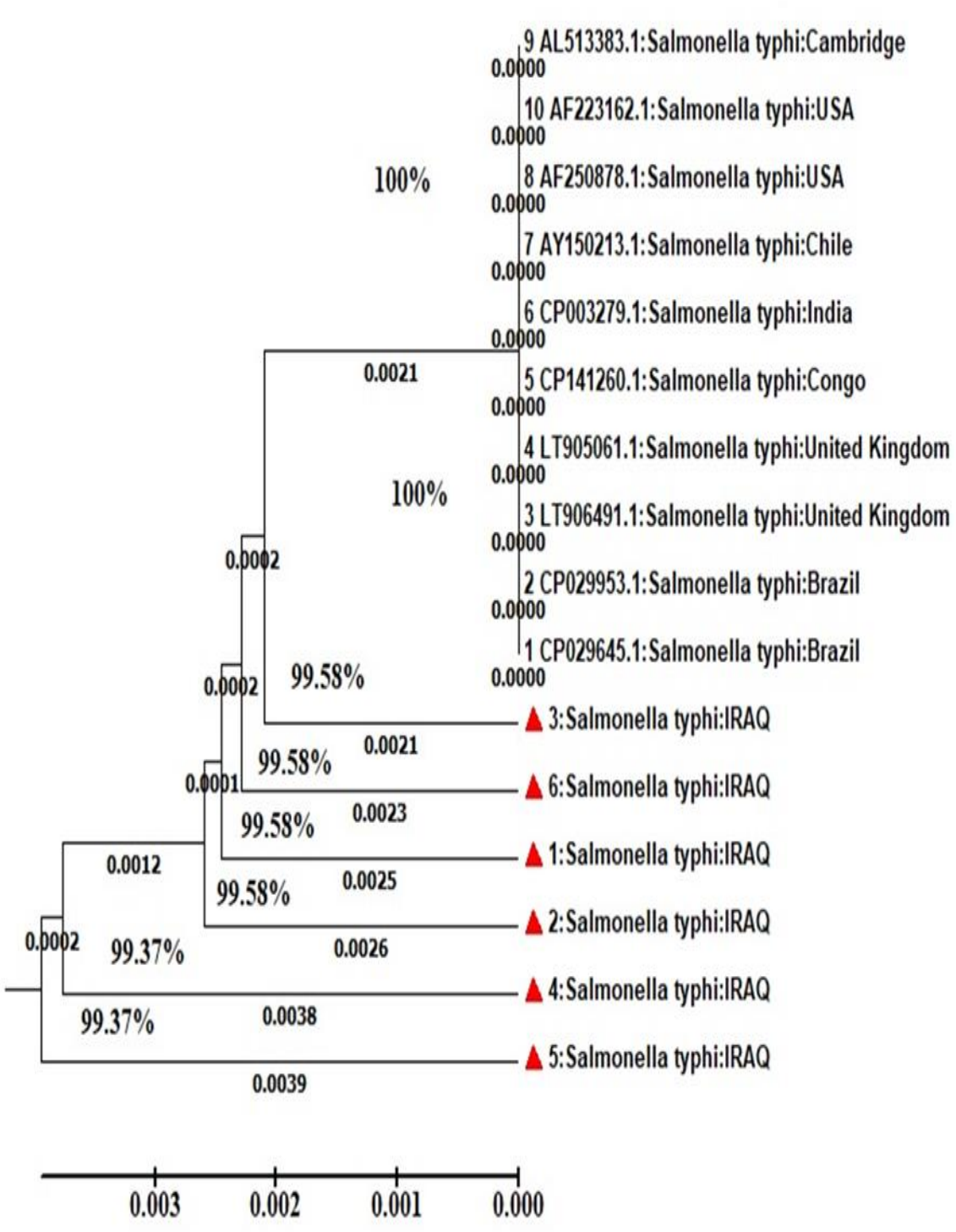

Figure (15): (isolate no.2)Phylogenetic tree analysis based on tet B gene in S. typhi, which exposed the proximity of local & global isolates.

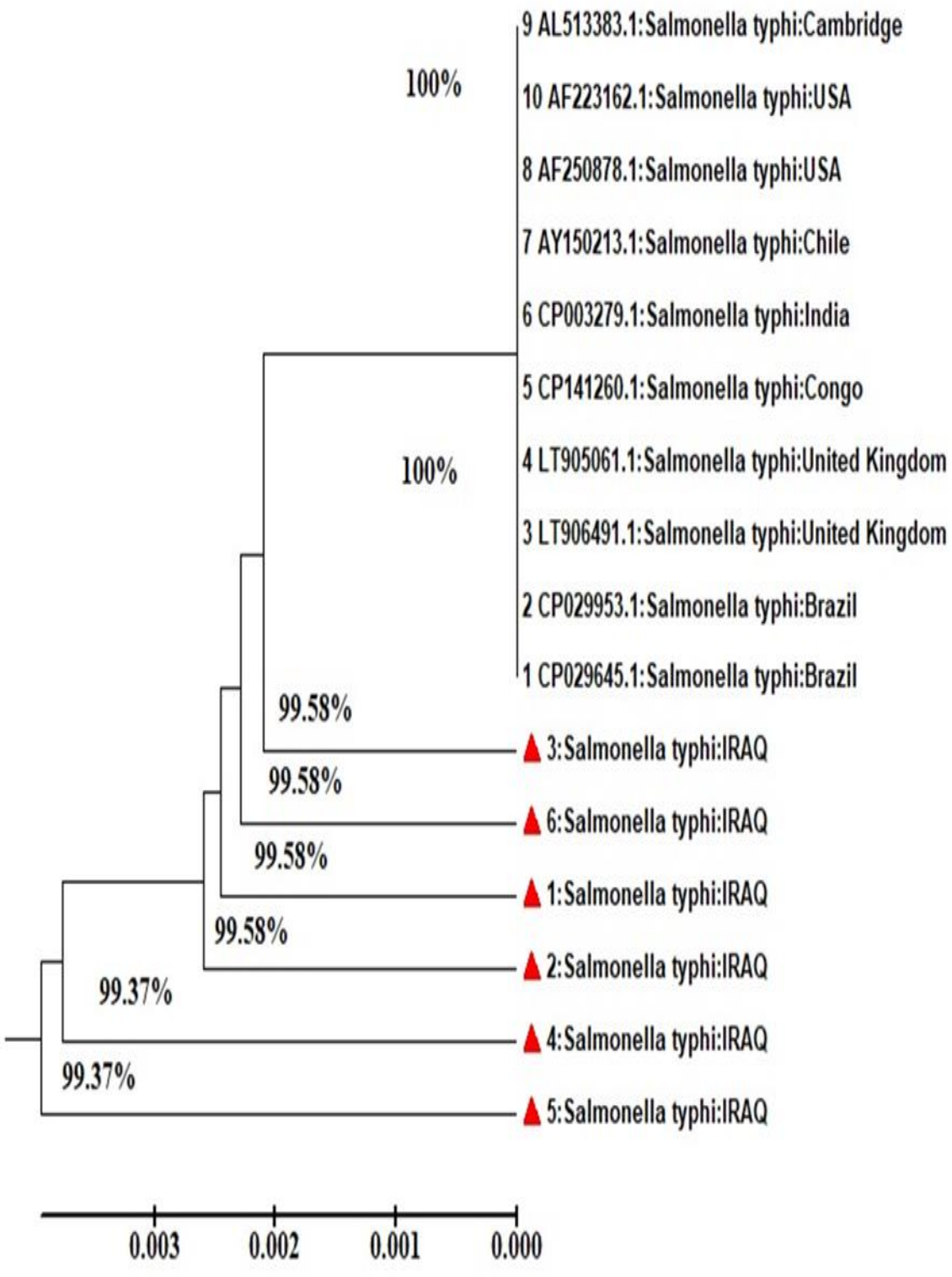

Figure (16): (isolate no.3)Phylogenetic tree analysis based on tet B gene in S. typhi, which exposed the proximity of local & global isolates.

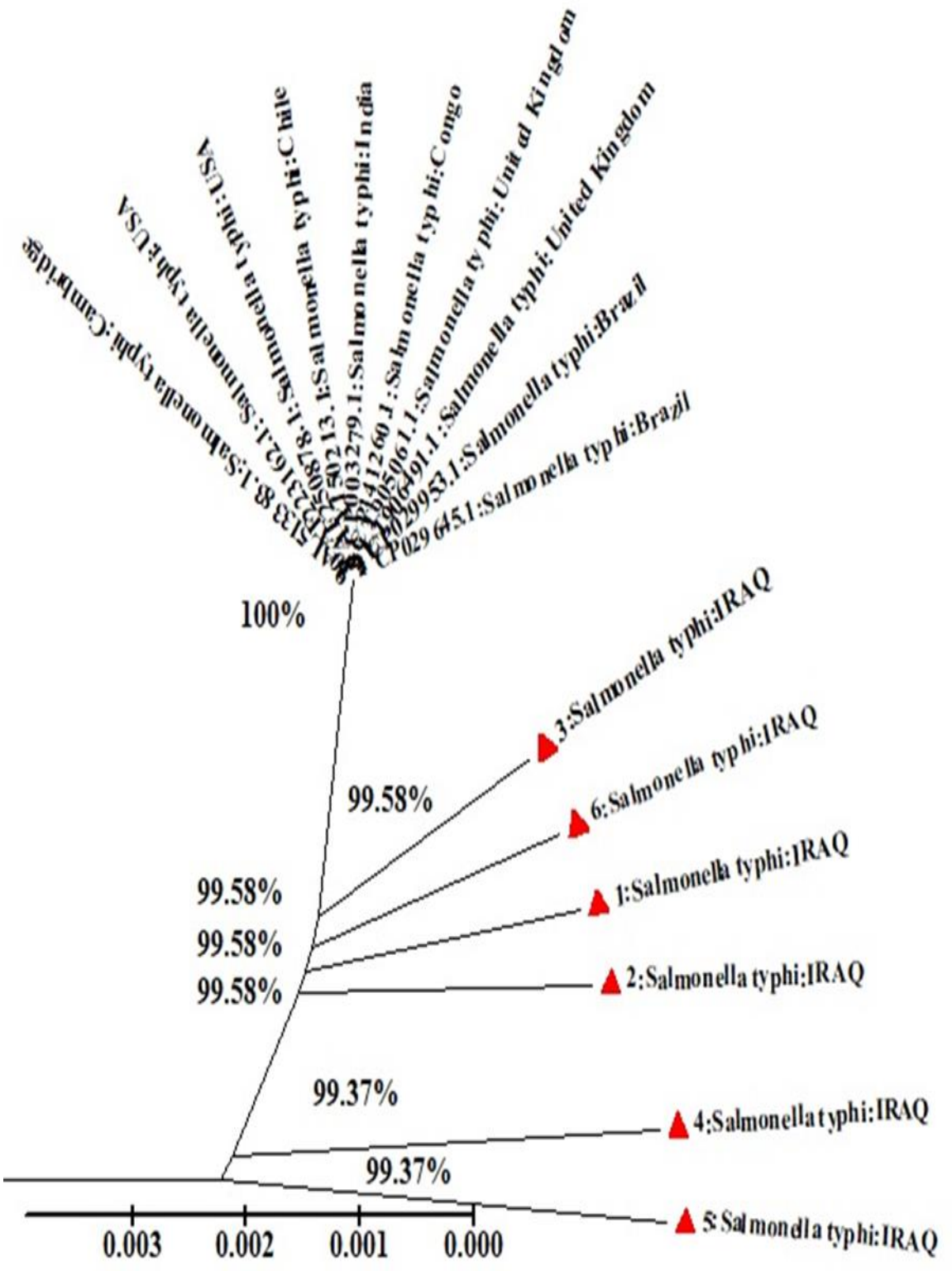

Figure (16): (isolate no.3)Phylogenetic tree analysis based on tet B gene in S. typhi, which exposed the proximity of local & global isolates

Submission of local Iraq isolate in NCBI.

The *Salmonella enterica subsp. enterica serovar Typhi* for (*cat*) gene three isolate ID: **AL513383.1** and tet B gene three isolate ID: CP029645.1 were sample were registered after the correspondence of NCBI and obtained accession number and became a reference to Iraq and the Middle East and the world

4- DISCUSSION

Sequence and phylogenetic trees are crucial material in studying bacteria such as Salmonella typhi that help appreciate how bacteria function and develop. The Tet B and Cat genes exhibit how bacteria can persist toward antibiotics, making it essential to study disease trends.

Salmonella typhi is deleterious bacteria can cause grave illness. Infection occurs as a result consumes contaminated water and food. Lead to patient with fever, stomach pain and diarrhea. *Salmonella typhi* are suitable progressively resistant to several antibiotic because recurrently treated per the identical antibiotic [11, 12]. In the last years, these bacteria have developed superior resistance to numerous antibiotics, comprising chloramphenicol, ciprofloxacin, ampicillin and levofloxacin thus referred to as multidrug-resistant (MDR) [13].

In current study during sequencing cat gene that detected the presence two mutation in both three isolates that protein remain same and mutation were silent mutation which reveals important evidence about capacity of bacteria and persist in challenging environments and it guards them from ruthless conditions.

The mechanisms can award resistance include mutation in gene or efflux pump present and a diminution in acetyl-CoA concentration be able to inhibit activity of catA1 and produce a vulnerability phenotype [14].

Tetracyclines are antibiotic used against most gram positive and negative bacteria in addition to aerobic and anaerobic bacteria therefore consider broad spectrum antibiotic, the action of these antibiotic include inhibit synthesis of protein through evading the link between RNA molecules and the 30S of ribosomal of bacteria, consequently averting the addition of amino acids and, subsequently, synthesis of protein [15, 20].

Tetracycline consider solitary of the greatest widely used antibiotic in production of animal associated to other antibiotic, which may be relate with the recurrent incidence of tet-B gene in isolate of *Salmonella typhi* [21].

The present study showed there 14 mutation in tet B gene some are point mutation and other are silent mutation. tet B gene helps bacteria resist antibiotics and helps patient acquire recovering through sequence tet B gene notice how Salmonella typhi can stampede from treatment It helps them decipher how patients treatment excellently. The resistance is due to the gaining of mobile genetic elements, ribosomal binding site mutations and chromosomal mutations leading to rise expression of resistance mechanism intrinsic. Resistance mechanisms include protection of ribosome, inactivation of enzymes in tetracycline and efflux pumps [22, 23].

Sequencing cat and tet B genes help patients infected with Salmonella typhi to obtain information from the, so they can know which antibiotics will work best, which helps patients recover faster and prevents the spread of bacteria.
Numerous diverse tet genes have been designated as conferring resistance to tetracycline in Salmonella. The greatest common types of tet genes belong to A, B, C, D and G class [24] in addition these genes are answerable for encoding tetracycline efflux pumps [25, 26].

The progress of AMR in S. typhi can arise instinctively through mutation [27]. Besides, point mutations in QRDR (quinolone resistance-determining region) concealing the genes for DNA gyrase (A, B) and topoisomerase IV (parC & pare) result in S. typhi quinolone-resistant [28]. In addition, S. Typhi acquire AMR gene from nonrelatives on mobile genetic elements for instance plasmid and transposon. This horizontal gene transfer consents the AMR gene to be move out between diverse species of bacteria [29].

A huge amount of differences concerning isolate of *Salmonella* point to that this isolate has numerous diverse sources include food and water contamination, exclusively firm food that health checks and cannot control in Iraq. Furthermore, constant movement of arrivals and free access from different countries of the world. Therefore, controlling the disease is more dangerous, especially since each clone has its own genetic characteristics (resistance). Therefore, requires the use of several treatments to attain positive grades [30].

By sequencing different strains of Salmonella typhi, we can see the changes. If strain have Tet B and Cat genes, we can suggestion it hind on phylogenetic tree to see if it comes from same line or completely diverse family.

These trees serve as maps of bacteria, showing how different bacteria are related. For example, Salmonella typhi can have strain in different parts of the world and we can trace back their family trees to determine where they derived from.

In current study phylogenetic analysis revealed that, *Salmonella* typhi was similar to other salmonella on cat gene and tet B gene. Understanding phylogenetic tree can help societies preclude disease tack place. By knowing how bacteria spread, strategies can be developed to retain safety of water and food.

5- CONCLUSION

When genotyping the isolates, the study detected to identify two (silent) mutations in the cat gene that protein remain same while 14 mutations occur in the Tet B gene some are silent mutation and other were missense mutation therefore there are significance of sequencing tet B and cat genes. It assistances to treat infections improved and ends the bacteria in its roads.

Phylogenetic analysis revealed that *Salmonella* typhi was similar to other salmonella on cat gene and tet B gene. Through these mapping out phylogenetic tree that create a family history for bacteria. This acquaintance helps us recognize how to competition these delicate aggressors.

REFERENCES

[1] Nazari Moghadam, M., Rahimi, E., Shakerian, A., & Momtaz, H. (2023). Prevalence of Salmonella Typhimurium and Salmonella Enteritidis isolated from poultry meat: Virulence and antimicrobial-resistant genes. BMC Microbiology, 23(1), 168. https://doi.org/10.1186/s12866-023-02908-8

[2] Masuet-Aumatell, C., & Atouguia, J. (2021). Typhoid fever infection, antibiotic resistance, and vaccination strategies: A narrative review. Travel Medicine and Infectious Disease, 40, 101946. https://doi.org/10.1016/j.tmaid.2021.101946

[3] Zhang, L., Fu, Y., Xiong, Z., et al. (2018). Highly prevalent multidrug-resistant Salmonella from chicken and pork meat at retail markets in Guangdong, China. Frontiers in Microbiology, 9, 2104. https://doi.org/10.3389/fmicb.2018.02104

[4] Popoola, O. O., Adepitan, D. S., Adeyemi, A. S., Oladeru, O. F., & Yusuff, S. Y. (2024). A national survey of the antibiotic use, self-medication practices, and knowledge of antibiotic resistance among graduates of tertiary institutions in Nigeria. Scientific African, 23, e01978. https://doi.org/10.1016/j.sciaf.2023.e01978

[5] Shanahan, P. M. A., Jesudason, M. V., Thomson, C. J., & Amyes, S. G. B. (1998). Molecular analysis of and identification of antibiotic resistance genes in clinical isolates of Salmonella Typhi from India. Journal of Clinical Microbiology, 36(6), 1595–1600. https://doi.org/10.1128/JCM.36.6.1595-1600.1998

[6] Chowdhury, A. R., Mukherjee, D., Chatterjee, R., & Chakravortty, D. (2024). Defying the odds: Determinants of the antimicrobial response of Salmonella Typhi and their interplay. Molecular Microbiology, 121(2). https://doi.org/10.1111/mmi.14870

[7] Abu Thahir, S. S., Rajendiran, S., Shaharudin, R., & Veloo, Y. (2023). Multidrug-resistant Salmonella species and their mobile genetic elements from poultry farm environments in Malaysia. Antibiotics, 12(6), 901. https://doi.org/10.3390/antibiotics12060901

[8] Sneath, P. H. A., & Sokal, R. R. (1973). Numerical taxonomy. Freeman.

[9] Tamura, K., Nei, M., & Kumar, S. (2004). Prospects for inferring very large phylogenies by using the neighbor-joining method. Proceedings of the National Academy of Sciences, 101(30), 11030–11035. https://doi.org/10.1073/pnas.0404206101

[10] Tamura, K., Stecher, G., Peterson, D., Filipski, A., & Kumar, S. (2013). MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Molecular Biology and Evolution, 30(12), 2725–2729. https://doi.org/10.1093/molbev/mst197

[11] Dutta, S., Das, S., Mitra, U., Jain, P., Roy, I., Ganguly, S. S., et al. (2014). Antimicrobial resistance, virulence profiles, and molecular subtypes of Salmonella enterica serovars Typhi and Paratyphi A blood isolates from Kolkata, India during 2009–2013. PLoS ONE, 9(8), e101347. https://doi.org/10.1371/journal.pone.0101347

[12] Wong, V. K., Baker, S., Pickard, D. J., Parkhill, J., Page, A. J., Feasey, N. A., et al. (2015). Phylogeographical analysis of the dominant multidrug-resistant H58 clade of Salmonella Typhi identifies inter-and intracontinental transmission events. Nature Genetics, 47(6), 632–639. https://doi.org/10.1038/ng.3281

[13] Das, S., Samajpati, S., Ray, U., Roy, I., & Dutta, S. (2017). Antimicrobial resistance and molecular subtypes of Salmonella enterica serovar Typhi isolates from Kolkata, India over a 15-year period 1998–2012. International Journal of Medical Microbiology, 307(1), 28–36. https://doi.org/10.1016/j.ijmm.2016.10.004

[14] Lima, N. C. B., Tanmoy, A. M., Westeel, E., Almeida, L. G. P., Rajoharison, A., Islam, M., Endtz, H. P., Saha, S. K., Vasconcelos, A. T. R., & Komurian-Pradel, F. (2019). Analysis of isolates from Bangladesh highlights multiple ways to carry resistance genes in Salmonella Typhi. BMC Genomics, 20, 530. https://doi.org/10.1186/s12864-019-5890-0

[15] Adesoji, A. T., Ogunjobi, A. A., Olatoye, I. O., & Douglas, D. R. (2015). Prevalence of tetracycline resistance genes among multi-drug resistant bacteria from selected water distribution systems in southwestern Nigeria. Annals of Clinical Microbiology and Antimicrobials, 14(1), 2–8. https://doi.org/10.1186/s12941-015-0076-9

[16] Marosevic, D., Kaevska, M., & Jaglic, Z. (2017). Resistance to the tetracyclines and macrolide-lincosamide-streptogramin group of antibiotics and its genetic linkage: A review. Annals of Agricultural and Environmental Medicine, 24(3), 338–344. https://doi.org/10.26444/aaem/74613

[17] Sheykhsaran, E., Baghi, H. B., Soroush, M. H., & Ghotaslou, R. (2019). An overview of tetracyclines and related resistance mechanisms. Reviews in Medical Microbiology, 30(2), 69–75. https://doi.org/10.1097/MRM.0000000000000154

[18] Grossman, T. H. (2016). Tetracycline antibiotics and resistance. Cold Spring Harbor Perspectives in Medicine, 6(4), a025387. https://doi.org/10.1101/cshperspect.a025387

[19] Roberts, M. C., & Schwarz, S. (2017). Tetracycline and chloramphenicol resistance mechanisms. In D. Meyers, J. Sobel, M. Ouellette, K. Kaye, & D. Marchaim (Eds.), Antimicrobial Drug Resistance (pp. 231–242). Springer. https://doi.org/10.1007/978-3-319-68797-3_18

[20] Hussain, T., Jamal, M., Nighat, F., & Andleeb, S. (2014). Broad spectrum antibiotics and resistance in nontarget bacteria: An example from tetracycline. Journal of Pure and Applied Microbiology, 8(4), 2667–2671. https://doi.org/10.22207/JPAM.08.04.37

[21] Chee-Sanford, J. C., Mackie, R. I., Koike, S., Krapac, I. G., Lin, Y.-F., Yannarell, A. C., Maxwell, S., & Aminov, R. I. (2009). Fate and transport of antibiotic residues and antibiotic resistance genes following land application of manure waste. Journal of Environmental Quality, 38(3), 1086–1108. https://doi.org/10.2134/jeq2008.0128

[22] Nguyen, F., Starosta, A. L., Arenz, S., Sohmen, D., Dönhöfer, A., & Wilson, D. N. (2014). Tetracycline antibiotics and resistance mechanisms. Biological Chemistry, 395(5), 1–24. https://doi.org/10.1515/hsz-2013-0292

[23] Roberts, M. C., & Schwarz, S. (2016). Tetracycline and phenicol resistance genes and mechanisms: Importance for agriculture, the environment, and humans. Journal of Environmental Quality, 45(2), 576–592. https://doi.org/10.2134/jeq2015.05.0251

[24] Xu, X., Biswas, S., Gu, G., Elbediwi, M., Li, Y., & Yue, M. (2020). Characterization of multidrug resistance patterns of emerging Salmonella enterica serovar Rissen along the food chain in China. Antibiotics, 9(11), 660. https://doi.org/10.3390/antibiotics9110660

[25] Dessie, H. K., Bae, D. H., & Lee, Y. J. (2013). Characterization of integrons and their cassettes in Escherichia coli and Salmonella isolates from poultry in Korea. Poultry Science, 92(11), 3036–3043. https://doi.org/10.3382/ps.2013-03271

[26] Mąka, Ł., & Popowska, M. (2016). Antimicrobial resistance of Salmonella spp. isolated from food. Rocznik Państwowego Zakładu Higieny, 67(4), 343–358.

[27] Ventola, C. L. (2015). The antibiotic resistance crisis: Part 1: Causes and threats. Pharmacy and Therapeutics, 40(4), 277–283.

[28] Makhtar, W. R. W. W., Bharudin, I., Samsulrizal, N. H., & Yusof, N. Y. (2021). Whole genome sequencing analysis of Salmonella enterica serovar Typhi: History and current approaches. Microorganisms, 9(10), 2155. https://doi.org/10.3390/microorganisms9102155

[29] Munita, J. M., & Arias, C. A. (2016). Mechanisms of antibiotic resistance. Microbiology Spectrum, 4(2), 119–127. https://doi.org/10.1128/microbiolspec.VMBF-0016-2015

[30] Al-Muhannak, F. H. N., & Mansour, A. O. (2020). Detection of tetracycline resistance genes A and B among clinical isolates of Salmonella Typhi from blood samples in Al-Najaf hospitals. International Journal of Multidisciplinary and Current Research, 8, 708. https://doi.org/10.14741/ijmcr/v.8.4

Dijlah Journal of Medical Sciences (DJMS) Vol. 1, No. 2, January, 2025, pp. 38-62
P-ISSN: 3078-3178, E-ISSN: 3078-8625, Paper ID: 05

التسلسل وشجرة النشوء والتطور في جين Cat وTet B في المرضى الذين يعانون من السالمونيلا التيفية

الخلاصة

تعرف السالمونيلا تايفي بكونها مقاومة للعديد من المضادات الحيوية لأنها تعالج بشكل متكرر بالمضادات الحيوية, وبإستعمال على أساس العزلات الإيجابية والقوية تم إختيار ست عزلات لجين (tetB) وثلاث عزلات لجين (Cat) لتحليل التسلسل.

كشفت الدراسة عن التسلسل الجيني والعلاقات التطورية لجينات (tet B) وجينات (cat) من المرضى المصابين بالسالمونيلا التيفوئيد. خلال التحليل الجيني النوعي الذي تم الكشف عن وجود طفرتين (صامتتين) في جين (cat) و تبقى البروتين كما هو، في حين حدثت 14 طفرة في جين (Tet B) كانت صامتة وأخرى كانت طفرة خطأ. كشف التحليل الوراثي أن السالمونيلا التيفوئيد كانت مشابهة للسالمونيلا الأخرى في جين (cat) وجين (tet B).

هدفت الدراسة إلى تقدير دور السالمونيلا التيفوئيد في آليات مقاومة المضادات الحيوية من خلال الطفرة المكتشفة التي تحدث في جين cat وtet B وتطورها داخل سلالات عديدة من سالمونيلا تايفي.