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Phylogenitic analysis of Biofilm Association Protein *(BapA)* amplicons in *Salmonella* Typhi Carrier in Gallbladder Diseases Patients in Thi-Qar Province/Iraq

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ABSTRACT: It is well known that many bacterial species exhibit noticeable genetic variations as a result of the differences in the clinical sources from which they were isolated. Within these bacterial species, Salmonella enterica serovar Typhi represents crucial bacterial organisms that can be used to adapt to the differences of the clinical sources by altering the genetic sequences of BapA (biofilm-associated protein A) locus. This study was conducted to identify the genetic polymorphisms Salmonella enterica serovar Typhi that were isolated from the gallbladder in Al-Nasiryia city. hospitals of. A genetic fragment of 651 bp length that partially covered a coding portion of the BapA gene within twenty-six bacterial samples (assigned S1 to S26) was amplified in this study. A direct sequencing strategy was performed for the observed PCR amplicons in the amplified genetic locus. Subsequently, a comprehensive phylogenetic tree was constructed in the observed variants to assess the accurate phylogenetic distances alongside other relative bacterial sequences. Our results indicated the presence of about 99% homology between our investigated samples with S. Typhi sequences. Six genetic variations were identified in this study, C114A, G173A, A190G, C209A, C210A, and C252T. These variations were variably distributed in the majority of the investigated samples. Meanwhile, S18 had not exhibited any detectable variation. According to this observation, the investigated bacterial samples were positioned in a particular phylogenetic position within the phylogenetic clade of this species in the currently generated comprehensive tree. It was shown that the investigated S. Typhi isolates having these variations were positioned into several adjacent phylogenetic clades, while S18 was taken another adjacent phylogenetic position in the same tree. Based on these identified BapA variations, S18 was positioned in the vicinity to multi-national strains of S. Typhi, while the other variations in the other samples were slightly tilted from the wild S18. Therefore, the utilization of PCR-sequencing strategy in all analyzed bacterial isolates has presented confirmed the presence of multiple sources of these isolates and showed a distinct pattern of their phylogenetic distribution within the S. Typhi sequences. In this study, the majority of identified variations have a missense effect on the BapA protein, which may suggest various degrees of sensitivity to the host immunity interaction. This observation may imply potential adaptation of the analyzed BapA locus to the host immunity. Thus, the majority of investigated isolates have different forms of

biologically altered BapA protein, which may show considerable alteration to innate host immunity.

Key words: - BapA amplicons, Salmonella Typhi, Gallbladder Chronic Infection

1. INTRODUCTION

Salmonella enterica consist of more than 2668 serovars. It is can cause disease in both human and animals (Saleh *et al*., 2015). It will invade the body by infected Food and water; the epithelium of the small intestine may be inserted within the intestines or penetrated to enter the bloodstream such that it may spread to In the liver, gallbladder, spleen, and other lungs, other organs settle and (Harvey *et al*., 2013). *Salmonella enterica* serovar Typhi (*S.* Typhi) is the etiologic agent for typhoid fever, causes about 20 million infections each year worldwide. (Dougan and Baker, 2014). The clinical symptom of typhoid fever is persistent fever, stomach pain, fatigue, and general lethargy. The involvement of distinct protective, as well as offensive virulence factors is consistent with the complex pathogenesis of systemic *Salmonella* infections. As an intracellular human pathogen, these factors contribute to its success and participate in multiple stages of invasion, intracellular reproduction, and survival within the host.When *S.* Typhi enters the gallbladder and causes an acute infection. amid cholecystitis, or act as an asymptomatic carrier state to mediate colonization in the gallbladder; by this mechanism utilized by the bacterium caused gallbladder abnormalities, especially gallstones (Crawford *et al.,* 2008). Colonization of the gallbladder and chronic presence of S. Typhi, there are the most effective on the surfaces of the gallstone, tends to be favored by biofilm formation. Biofilm production on gallstones, on the other hand, have also been frequently observed , bile and cholesterol have been showed to strengthen of the bacterial adhesion. (Gonzalez-Escobedo *et al.,* 2013). Characterizations of the molecular processes involvment in the creation of biofilms on biliary stones and the activity of S. Typhi remains to be further studied in the promotion of gallbladder inflammation and injury.Clinically prescribed antibiotics are usually ineffective against chronic bacterial contamination of the gallbladder breakdown In patients who have gallstones with both Salmonella Typhi and cholesterol within the gallbladder and are also at high risk of developing hepatobiliary carcinomas (Crawford *et al.,* 2008). Treatment with antibiotic were chosen based on the antimicrobial susceptibility test was always unsuccessful in eradicating biofilm-associated bacteria. (Zimmerli *et al.,* 2014). Once the biofilm is formed, there is an improved susceptibility of individual cells to antimicrobial agents, and antibiotic treatment alone is always insufficient. (Hengzhuang *et al.,* 2012). Aim of this study was making nucleotide sequence of the gene responsible for biofilm formation to do *S.* Typhi database in Thi-Qar province, and comparing sequences results of our study's isolates detected with previously described isolates from other regions of the world and then draw its phylogenetic tree.

2. METHODS

2.1 Genomic DNA extraction

The genomic DNA of *S.*Typhi was extracted by using Genaid Kit(UK) according to the manufacturer's instructions (Geneaid Biotech, Taiwan); a nanodrop were used for measurment the concentration and purity of DNA ; while the DNA integrity was checked by a standard 0.8% (w/v) agarose gel electrophoresis that is pre-stained with a higher concentration of ethidium bromide (0.7 μg/ml) in TBE buffer and using a 1 kb DNA ladder as a molecular

weight marker (Cat # D-1040, Bioneer, Daejeon, South Korea). The isolated DNA was used as a template for PCR.

2.2 PCR

BapA gene (table 1) amplification on the Thermocycler was done as in the table (2.1). By electrophoresis, the PCR products were visualized on 1.5 percent agarose gels in a 1X TBE buffer at 95 V for 45 minutes; table (2)

Table (1):- Primers sequences used for genes amplification

Gene name		**Sequence (5'-3')**	**size (bp) Product**
BapA	F	5'-CAAACGGCAGTATTACCAATC-3'	651
	R	5'-GTGTATTGTCGTCAGTGGTTG-3'	

Table (2):-PCR conditions to *Bap*A gene

St. No.		**Step**	**Temperature ^{o}C**	**Time**	**Number of Cycles**
I		Initial Denaturation	94	5min	1
II	I	Denaturation	94	30sec.	35
	II	Annealing	56	30sec.	
	III	Extension	72	60sec.	
III		Final Extension	72	5min.	1

2.3 DNA Sequencing of PCR amplicons

PCR products of the *BapA* gene were isolated by a 2% electrophoresis of agarose gel and visualized by exposure to ultraviolet light (302 nm) after red staining. Gene sequences have been conducted electronically by the National Environmental Management Instrumentation Center (nicem) at (http://nicem.snu.ac.kr/main/?en skin=index.html), Biotechnology Lab, DNA sequencer computer 3730XL, Applied Biosystem, Homology quest was performed using the Simple Local Alignment Search Tool (BLAST) software accessible online at the National Center for Biotechnology Information (NCBI) at http://www.ncbi.nlm.nih.gov and BioEdit.

2.4 Interpretation of sequencing data

The results of sequencing were edited, aligned and analyzed as long as with the respective sequences in the reference database by using BioEdit Sequence Alignment Editor Software Version 7.1 (DNASTAR, Madison, WI, USA). The nucleic acids observed were numbered in PCR amplicons as well as in their corresponding positions within the referring genome. Each detected variants within the *S.* Typhi genes was annotated by SnapGene Viewer ver. 4.0.4 (https://www.snapg ene.com).

2.5 Translation of nucleic acid variations into amino acid residues

The amino acid sequences of the targeted proteins were retrieved online from the protein data bank (http://www.ncbi.nlm.nih.gov). The variants observed in the coding portion was

translated into a reading frame corresponds to the referring amino acid residues by using the Expasy online program (Gasteiger *et al*., 2003).

2.6 Comprehensive phylogenetic tree construction

The specific phylogenetic tree was constructed in our study according to the cladogram construction showed by Hashim *et al.* (2020). The variants observed were compared with the neighbor homologous reference sequences by using the NCBI-BLASTn server (Zhang *et al*. 2000). Then, an inclusive tree, including the variant observed, was built by the neighbor-joining methods and visualized using iTOL suit to generate a traditional tool of clades construction (Letunic and Bork, 2019).

3. RESULTS

Within this locus, twenty-six samples were included, which showed about 651 bp amplicons length. Before sending these amplicons to sequencing, it was made sure that all the amplified amplicons had shown sharp, specific, and clean bands. The sequencing reactions indicated the confirmed identity of the amplified products by performing NCBI blastn. Concerning the 651 bp PCR amplicons of the currently targeted *BapA* sequences, the NCBI BLASTn engine showed a high sequence similarity between the sequenced samples and *Salmonella enterica* serovar Typhi sequences. NCBI BLASTn engine indicated the presence of about 99% of homology with the expected target that partially covered the coding portion of the *BapA* gene sequences. After positioning the 651 bp amplicons' sequences within the *BapA* sequences, the details of these sequences were highlighted within the amplified sequences. Alignment results of the 651 bp samples revealed the detection of six nucleic acid the variations compared with the corresponding *S.* Typhi referring sequences ; These sequence was prepared by aligning our investigated samples with the most relative sequences deposited in the NCBI database (GenBank acc. CP053702.1). These differences were observed in the currently observed nucleic acid sequences detected in the analyzed samples compared with the reference sequences. However, the sequencing chromatograms of the investigated sample, as well as its detailed annotations, were verified and documented, and the chromatograms of its sequences were shown according to their positions in the PCR amplicons .The presence of these variants was confirmed in its original chromatogram and the absence of any possible technical error was also confirmed. These detected nucleic acid substitutions were not found in the reference sequences of the *S.* Typhi. The observed variation was further analyzed to identify whether such nucleic acid substitutions induce a possible alteration in its corresponding position in the encoded amino acids from in the BapA protein. All nucleic acid sequences were translated to their corresponding amino acid sequences of the BapA protein using the expasy translate suite. It was found that four nucleic acid substitutions were found to exhibit four missense mutations in the amplified protein, (Fig. 4A). Since the selected 651 bp amplicon partially covered the coding portions of the BapA protein, the observed amino acid variations were repositioned according to their locations in the entire protein, namely C114A (p.1096 P>T), C209A (p.1128 P>T), C210A (p.1128 P>T), and C252T (p.1142P>S) (Fig. 4B). Whereas the other observed nucleic acid variations were just silent mutations without encoding any amino acid substitutions in the encoded BapA protein, namely G173A (p.1115 L=), A190G (p.1121 D=).To summarize all the results obtained from the sequenced 651 bp fragments, the exact positions of the observed variations were described in (Table 3).

Table 3. The pattern of the observed variations in the 651 bp of the *BapA* amplicons in comparison with the NCBI referring sequences (GenBank acc. no. CP053702.1).

Sample	**Variant**	**Position in the PCR fragment**	**Position in the reference genome**	**Amino acid position**	**Variant type**
S3, S4, S6, S10, S11, S12, S13, S14, S16, S17, S22, S23, S25, S26	C114A	114	3615172	P1096	Missense (p.1096 P>T)
S3, S4, S11, S12, S13, S16, S17	G173A	173	3615231	L1115	Silent (p.1115 L=)
S1, S2, S3, S5, S7, S8, S9, S10, S11, S14, S16, S20, S23, S25, S26	A190G	190	3615248	D1121	Silent (p.1121 D=)
S2, S3, S4, S5, S6, S7, S9, S10, S11, S12, S13, S15, S17, S19, S20, S21, S23, S24, S25, S26	C209A	209	3615267	S1127	Missense (p.1127S>R)
S2, S3, S4, S5, S6, S7, S10, S11, S13, S14, S15, S17, S22, S24, S25, S26	C210A	210	3615268	P1128	Missense (p.1128 P>T)
S7, S9, S11, S14, S15, S20, S23	C252T	252	3615310	P1142	Missense (p.1142P>S)

A comprehensive phylogenetic tree was generated, which was based on the investigated *BapA* nucleic acid sequences in the analyzed bacterial samples. Along with the other deposited DNA sequences, this phylogenetic tree contained the currently investigated samples (S1 to S26) aligned with its highly related sequences in a neighbour-joining mode. In the currently constructed tree, the total number of aligned nucleic acid sequences was 127 sequences. This comprehensive tree entailed the presence of only one species, *Salmonella enterica*, which represents the only, incorporated nucleic acid sequences within the tree. Based on the analyzed genetic sequences of *S.* Typhi, our analyzed *BapA* sequences were clustered into many adjacent phylogenetic clades, which entailed a wide range of diversity of this organism concerning our analyzed *BapA* sequences (Fig. 1). These adjacent groups were represented by *S.* Typhi, in which the currently investigated S1 – S26 samples were incorporated. In addition to *S.* serovar Typhi, other related serovars were also incorporated, including *S.* Weltevreden, *S.* Bareily, *S.* Typhimurium, *S.* Adjame, and *S.* Albany. However, all these subspecies were belonged to enterica serovar. Meanwhile, diarizonae was incorporated as outgroup subspecies within the main targeted bacterial serovar sequences.

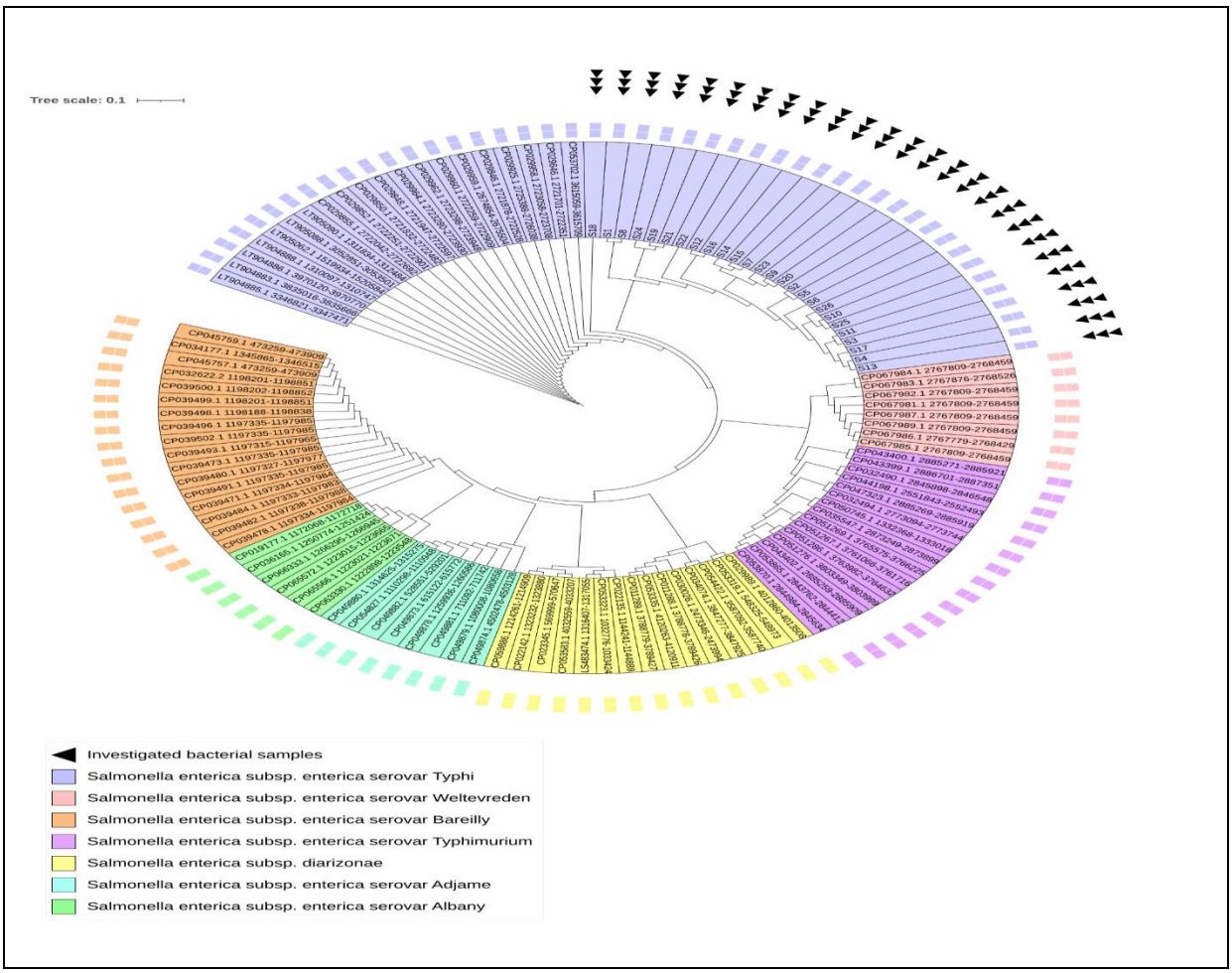

Fig. 1. The comprehensive phylogenetic tree of the 651 bp amplicons partially covered the coding portions of *BapA* sequences within *Salmonella enterica* serovar Typhi genomic sequences. The variable colors refer to the variable grouping of the analyzed variants, within their Genbank deposited sequences. The number "0.1" at the top left portion of the tree refer to the degree of scale range between the comprehensive tree categorized organisms. The described numbers in the tree refer to the degree of phylogenetic distances among the investigated bacterial organisms. The letter "S" refers to the code of the investigated samples in this study.

4. DISCUSSION

In this study, the genetic variations of various clinical samples of *S.* Typhi were investigated. To do so, the sequences of the *BapA* gene were screened in twenty-six bacterial samples (assigned S1 to S26) that were taken from gallbladder patients in Al-Nasiriya city. This gene encodes for a huge surface protein made of 3624 amino acids that is known as BapA protein. This protein has several roles correlated with the adhesion with cellular membranes and biofilm formation (Latasa *et al*., 2005). This protein plays a key essence role in the multilayer biofilm formation, and the deletion in the *BapA* can cause several undesired consequences represented by the loss of capacity to form the biofilm through which the pathogenic bacterial

species can adhere to host surfaces (Ibarra and Steele-Mortimer, 2009). The ability to form multilayer biofilm is an essential factor for the virulence of these bacterial species and has been shown to promote the survival of various *Salmonella* strains when they being exposed to host immunity or antibiotic treatment (Hamilton et al., 2009; Fàbrega and Vila, 2013). Based on the versatile role of the protein encoded by the *BapA* gene, it is interesting to assess the pattern of the *BapA* gene variations in the *S.* Typhi isolates of the investigated clinical samples. Thereafter, the subsequent evolutionary and clinical strategy of these bacterial samples would be evaluated according to the patterns of nucleic acid sequences observed in this targeted gene. Thus, it is extremely mandatory to explore the genetic variations of the *BapA* gene, phylogenetic positions of its consequent variations, and putative adaptation for the clinical samples of *S.* Typhi having these variations to communicate with the host immunity defense mechanisms. It was inferred from this tree that the most relative sequences to our investigated S1 – S26 samples were belonged to *S.* Typhi, while *S.* Weltevreden, *S.* Bareily, *S.* Typhimurium, *S.* diarizonae *S.* Adjame, and *S.* Albany were positioned in further phylogenetic places respectively. Based on the genetic analysis of the *BapA* genetic locus, several phylogenetic relationships between *S.* Typhi and other closely related serovars were inferred from this tree. These relations can be attributed to the ability of *BapA* sequences to reveal such close phylogenetic distances between *S.* Typhi from one side and the other related bacterial serovars from the other side. The closest serovar to *S.* Typhi belonged to *S.* Weltevreden (Makendi *et al*., 2016). This notion suggested that these serovars may exert the highest homology to the *S.* Typhi sequences. Beyond this serovar, several other closely related organisms were also suited, namely *S.* Bareily, *S.* Typhimurium, *S.* Adjame, and *S.* Albany respectively (Elgroud *et al*., 2017). The utilization of *BapA* amplicons revealed a significant genetic diversity of the serovar Typhi from these closely related counterparts (Okoro *et al*., 2015). However, all these serovars were belonged to enterica subspecies. Meanwhile, diarizonae was also incorporated within these serovars to give a close phylogenetic connection to these organisms (Giner-Lamia *et al*., 2019). However, all the incorporated *Salmonella* subspecies were commonly available in many human infection cases, which cause several invading grades on the clinical status of the infected persons (Joerger, 2020). Though our investigated samples resided in one of the major *S.* Typhi clades, two phylogenetic positions were observed from these samples. The observed variations in almost all samples caused these samples to take variable adjacent phylogenetic positions in the tree. These adjacent positions were attributed to the frequency of variations within these investigated samples, which induced variable distributions of these samples in these referred phylogenetic positions. In contrast to all investigated samples, S18 showed no mutation, and therefore it was localized in the immediate vicinity to the phylogenetic positions taken by the other bacterial samples belonging to the same serovar. It was inferred that S18 was suited in the vicinity of the GenBank accession number of CP053702.1, which was belonged to an Indian strain of *S.* Typhi sequences. However, it was also found that the S18 sample was also positioned in the vicinity to the GenBank accession numbers CP029646.1, CP029958.1, CP029925.1, CP029846.1, CP029959.1, CP029960.1, CP029962.1, CP029964.1, CP029848.1, CP02985.1, CP029850.1, CP029852.1, and CP029855.1 respectively, which were belonged to different *S.* Typhi strains isolated from Brazil. Furthermore, S18 sample was also suited in the vicinity of GenBank accession numbers of LT905090.1, LT90508.1, LT905088.1, LT905062.1, LT904888.1, LT904886.1, LT904883.1, LT904885.1 respectively, which were isolated from the United Kingdom. Thus, it is rational to say that our investigated samples - S18 or other investigated samples - were belonged to three multinational origins, whether being Asian, American, and European. Therefore, the

multinationalism of these samples was confirmed. Noteworthy, the observed phylogenetic distances among the incorporated organisms within this clade (tree scale 0.1), give an obvious indication of the presence of consecutive homology among the incorporated bacterial sequences. Accordingly, this high similarity between S1 – S26 and these *Salmonella enterica* serovars Typhi strains indicated the presence of multiple sources for our investigated samples. These sorts of S1 – S26 genetic distribution referred to the sensitivity of the utilized *BapA*-based 651 bp amplicons in the accurate discrimination among the investigated bacterial samples. Thus, the distinctive role of the generated phylogenetic tree in the detection of the currently analyzed samples could not be excluded from the explanation. Accordingly, this notion provides a further indication of these bacterial isolates and provides accurate genotyping phylogenetic distributions alongside highly relative sequences. Though several phylogenetic positions were observed from the analyzed samples of S1 – S17 and S19 – S26, only one clade was found for them. In the immediate vicinity to this clade, the S18 clade was originated. This close positioning may be attributed to the slight phylogenetic effect of the identified six variations in changing the positioning of *S.* Typhi samples. This finding entailed a possible role for these mutations in inducing a slight tilt inside the same serovar. Thus, these variations were only a biological diversity within the same Typhi serovar. However, the positioning of S1 – S17 and S19 – S26 samples have not occurred beside any related GenBank accession number within *S.* Typhi, which may have referred to the fact that these variations were not found in any serovar in Typhi. Consequently, the majority of our investigated samples resided in a unique clade within the *S.* Typhi. Further confirmation for this observation came from the positioning of S18. This sample exhibited a close positioning to many multinational *S.* Typhi strains, which however not found in the other samples due to their variations (C114A, G173A, A190G, C209A, C210A, and C252T). Though considerable diversity was observed within the majority of our samples, this diversity was only a minor deviation within the same serovar. Accordingly, the observed six nucleoids variations were in this study were not exceeded the serovar level of *S.* Typhi. Thus, this study revealed several nucleic acid substitutions that varied in their intensity in the majority of the investigated samples, which have occupied a particular phylogenetic position within the *S.* Typhi without being a part of any deviation from this serovar. Apart from the phylogenetic diversity, four out of six identified variations exhibited a missense effect on the final manifestation of protein. This notion suggested a direct relationship between the gallbladder environmental conditions and the adaptation mechanism through which these bacterial serovars behave to confront the host immunity. This observation may be applicable when a protein exerts several amino acid substitutions to conquer the host defense system or antibiotics treatment (Sarhan *et al*., 2019). Thus, the currently identified four amino acids substitutions (p.1096 P>T, p.1128 P>T, p.1128 P>T, and p.1142P>S) may be involved in these referred mechanisms, which may be related to particular roles for these missense mutations in altering the characteristics of BapA protein. It is well established that amino acid substitutions are the main mediators between bacterial – host communications, which may lead to different outcomes in infections (Ren *et al*., 2018). These amino acid substitutions may alter protein structure, function, and stability so that it may induce several alterations in the metabolic pathways in which the protein is involved (Albakri et al., 2020). Since BapA protein is one of the surface proteins for biofilm multilayer formation for *Salmonella* serovars (Latasa *et al*., 2005), any amino acid alteration in this protein may bring about several alterations in this process and may lead to a consequent modification for the adaptation of the altered *S.* Typhi toward host defense mechanisms. These modifications may lead to a consequent alteration in the adherence property of BapA protein to host surfaces (Silva-Hidalgo *et al*., 2016). This

modified adhesion may change the mechanism of communication between the mutant *S.* Typhi bacterial strains to gallbladder surfaces. Therefore, it can be stated that the majority of our investigated samples have adopted several missense mutations to meet up with the host by inducing four amino acid substitutions in the BapA protein. These alterations may also be involved in changing the adherence mechanism through which this involved to bind to undertake its scheduled role in the invasion of *S.* Typhi to the host.

5. CONCLUSION

The majority of identified variations have a missense effect on the BapA protein, which may suggest various degrees of presence in gallbladder,This observation may imply potential adaptation of the analyzed *BapA* locus to the antibiotic resistance.

6. REFERENCES

[1]. Albakri AH, Al-Shuhaib MB, Alwan SL, AbdulAzeez S, Borgio JF. Deleterious missense variants in the aflatoxin biosynthesis genes explain the low toxicity of Aspergillus flavus from infected rice. Microbial Pathogenesis. 2020; 7:104605.

[2]. Crawford RW, Gibson DL, Kay WW, Gunn JS 2008. Identification of a bile-induced exopolysaccharide required for *Salmonella* biofilm formation on gallstone surfaces. Infect Immun 76(11)::5341–5349

[3]. Dougan, G., and Baker, S. (2014). Salmonella enterica serovar Typhi and the pathogenesis of typhoid fever. Annual review of microbiology 68, 317-336.

[4]. Elgroud R, Granier SA, Marault M, Kerouanton A, Lezzar A, Bouzitouna-Bentchouala C, Brisabois A, Millemann Y. Contribution of avian Salmonella enterica isolates to human salmonellosis cases in Constantine (Algeria). BioMed research international. 2015, 12;2015.

[5]. Fàbrega A, Vila J. Salmonella enterica serovar Typhimurium skills to succeed in the host: virulence and regulation. Clinical microbiology reviews. 2013;1;26(2):308-41.

[6]. Gasteiger E., Gattiker A., Hoogland C., Ivanyi I., Appel R.D., Bairoch A. ExPASy: the proteomics server for in-depth protein knowledge and analysis. Nucleic Acids Res. 31:3784-3788(2003).

[7]. Giner-Lamia J, Vinuesa P, Betancor L, Silva C, Bisio J, Soleto L, Chabalgoity JA, Puente JL, Garcia-del Portillo F. Genome analysis of Salmonella enterica subsp. diarizonae isolates from invasive human infections reveals enrichment of virulence-related functions in lineage ST1256. BMC genomics. 2019;20(1):1-4.

[8]. Gonzalez-Escobedo, G.; Gunn, J.S. Identification of Salmonella enterica serovar Typhimurium genes regulated during biofilm formation on cholesterol gallstone surfaces. Infect. Immun. 2013, 81, 3770–3780.Hamilton S, Bongaerts RJ, Mulholland F, Cochrane B, Porter J, Lucchini S, Lappin-Scott HM, Hinton JC. 2009. The transcriptional programme of Salmonella enterica serovar Typhimurium reveals a key role for tryptophan metabolism in biofilms. BMC Genomics 10:599.

[9]. Harvey R.A., Cornelissen C.N. and Fisher, B.D .(2013). Lippincott's Illustrated Review of Microbiology .3th Edn ., Lippincott William & Wilkins , a Wolters Kluwer Health, China.

[10]. Hashim HO, Mohammed MK, Mousa MJ, Abdulameer HH, Alhassnawi AT, Hassan SA, Al-Shuhaib MB. Infection with different strains of SARS-CoV-2 in patients with COVID-19. *Archives of Biological Sciences*. 2020 Dec 25;72(4):575-85.

[11]. Hengzhuang, W.; Wu, H.; Ciofu, O.; Song, Z.; Høiby, N. In vivo pharmacokinetics/pharmacodynamics of colistin and imipenem in Pseudomonasaeruginosa biofilm infection. Antimicrob. AgentsChemother. 2012, 56, 2683–2690
[12]. Ibarra JA, Steele-Mortimer O. Salmonella–the ultimate insider. Salmonella virulence factors that modulate intracellular survival. Cellular microbiology. 2009 Nov;11(11):1579-86.
[13]. Joerger RD. Salmonella enterica's "Choice": Itaconic Acid Degradation or Bacteriocin Immunity Genes. Genes. 2020;11(7):797.
[14]. Latasa C, Roux A, Toledo-Arana A, Ghigo JM, Gamazo C, Penadés JR, Lasa I. BapA, a large secreted protein required for biofilm formation and host colonization of Salmonella enterica serovar Enteritidis. Mol Microbiol. 2005 Dec;58(5):1322-39.
[15]. Letunic I, Bork, P. 2019. Interactive Tree Of Life (iTOL) v4: recent updates and new developments. *Nucleic Acids Res* 2;47(W1): W256-W259.
[16]. Makendi C, Page AJ, Wren BW, Le Thi Phuong T, Clare S, Hale C, Goulding D, Klemm EJ, Pickard D, Okoro C, Hunt M. A phylogenetic and phenotypic analysis of Salmonella enterica serovar Weltevreden, an emerging agent of diarrheal disease in tropical regions. PLoS neglected tropical diseases. 2016;10(2):e0004446.
[17]. Okoro CK, Barquist L, Connor TR, Harris SR, Clare S, et al. (2015) Signatures of adaptation in human invasive Salmonella Typhimurium ST313 populations from sub-Saharan Africa. PLoS Negl Trop Dis 9: e0003611.
[18]. Ren W, Rajendran R, Zhao Y, Tan B, Wu G, Bazer FW, Zhu G, Peng Y, Huang X, Deng J, Yin Y. Amino acids as mediators of metabolic cross talk between host and pathogen. Front Immunol 9: 319.
[19]. Saleh B. Manal, Mezal H. Ezat , Hanan K. Zaman, 2016. Antimicrobial resistance, Virulence profiles of *Salmonella enterica* serovar Typhimurium isolated from diarrheal children in Thi-Qar province during 2015.Journal of Thi-Qar science ,No.1
[20]. Sarhan, R. S, Hashim, H.O., Al-Shuhaib, M.B.S. 2020. The Gly152Val mutation possibly confers resistance to beta-lactam antibiotics in ovine Staphylococcus aureus isolates. *Open Vet. J.* 9(4): 339-348.
[21]. Silva-Hidalgo G, López-Valenzuela M, Cárcamo-Aréchiga N, Cota-Guajardo S, López-Salazar M, Montiel-Vázquez E. Identification of bapA in strains of Salmonella enterica subsp. enterica Isolated from Wild Animals Kept in Captivity in Sinaloa, Mexico. Veterinary medicine international. 2016.
[22]. Zhang Z, Schwartz S, Wagner L, Miller W. 2000. A greedy algorithm for aligning DNA sequences. *J Comput Biol.* 7(1-2):203-14.
[23]. Zimmerli, W.; Trampuz, A.; Ochsner, P.E. Prosthetic-joint infections. N.Engl. J.Med. 2014, 351, 1645–1654.