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RESEARCH ARTICLE

Molecular Detection of Some Virulence Genes of *Salmonella Typhymurium* and *Salmonella Enteritidis* Isolated from Patients Suffering Watery Diarrhea

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Abstract

Salmonella enterica virulence factors can be divided into chromosomal, plasmid and bacteriophages encoded virulence factors. Many of the *Salmonella* virulence factors, such as adhesion, invasion, and toxin genes are clustered in certain areas of the chromosome known as "*Salmonella* pathogenicity islands" (SPI). A total of 200 clinical stool samples were collected during this study which obtained from patient Suffering from watery diarrhea who a dmintted to three main hospitals of Babylon Governorate: Merjan Medical city, Al-Hillah Surgical Teaching Hospital and Babil Teaching Hospital for Women and Children during a period of three months (from july 2018 to September 2018). Out of 200 specimens 35(17.5%) were detected by culture and vitek2 compact, 24 (12%) of them were confirmed by PCR using target gene, (17) *Salmonella typhimurium* and (7) *Salmonella enteritidis*. PCR was conducted to determine the some virulence genes of the isolates by using primers *inv A, sdiA* and *sifA*. The PCR amplification products were visualized by electrophoresis on 1% agarose gels for 35min at 70v. The sizes of the amplicons were determined by comparison to the 200 bp allelic ladder. Among isolates studies it was found that *invA* gene present in 13(54)% isolates of *S. enterica* ,(9) isolates of *S. typhimurium* and (4) isolates of *S. enteritidis* , *sdiA* gene it was found in all isolates (100)% while *sifA* gene it was found in all isolates only isolate number 18 not contained this gene 23(96)%.

Keywords: *Salmonella typhimurium, S. enteritidis, invA, sdiA and sifA.*

Introduction

Salmonella enterica infections remain a major public health concern worldwide, contributing to the economic burden of both industrialized and under developed countries through the costs associated with surveillance, prevention and treatment of disease [1]. *Salmonella enterica* is a Gram-negative, facultative rod- shaped bacterium belonging to the family *Enterobacteriaceae* [2].

Members of this genus are generally motile with peritrichous flagella, aerogenic, non-lactose fermenting, oxidase-negative, urease-negative, citrate-utilizing, acetyl methyl carbinol-negative and potassium cyanide-negative [3]. The invA gene usually codes for protein in the inner bacterial membrane that is responsible for invasion of intestinal cells of the host [4]. The invA gene contains unique sequences specific to the genus *Salmonella* and has been proved as a specific PCR target with important diagnostic applications [5]. The invA target gene of *Salmonella* is located on the pathogenicity island 1 (SPI-1), it is important for the invasion of host epithelial cells. This gene is highly specific in most *Salmonella* serotypes and has been used as an important target for detection of *Salmonella* [6].

In *Salmonella enterica* serovar Typhimurium, SdiA positively regulates two loci, (1) the *rck* (resistance to complement killing)operon located on the virulence plasmid, pSLT [7] and (2) *srgE* (*sdiA*-regulated gene), a single gene horizontal acquisition that encodes an effector protein

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that is secreted by type III secretion system 2 (T3SS2) [8]. Quorum sensing (QS) is a density dependent regulatory mechanism mediated by the accumulation of signaling molecules produced by bacteria and is related to transcriptional regulation of several genes, including those involved in biofilm formation, bacterial adhesion, host colonization and virulence factors [9].One of the most significant regulators of bacterial pathogenicity and especially of Salmonella intestinal virulence seems to be QS[10], and SdiA as a QS system product is the sole LuxR-type receptor in S. enterica [11].

The potential roles of sdiA may be construed from the recognized functions of genes known to be regulated by SdiA [11]. Existence and expression of the sdiA and sdiA-regulated genes would be beneficial to Salmonella within the gastrointestinal environment, and the observed upregulation of sdiA in the population brings the relevance of the differentiated state closer to the milieu of the host environment [12].The T3SS-2 effector protein SifA [13] plays a significant role in *Salmonella* virulence and several phenotypes are linked to the translocation of SifA.

SifA is required to maintain the integrity of the *Salmonella*-containing vacuole (SCV) [14] and in epithelial cells promotes the formation of tubular membranous structures connected to SCVs, which have been named *Salmonella*-induced filaments (Sifs) [15,16].In absence of SifA, the molecular motor kinesin-1, which is directly recruited by the T3SS-2 effector PipB2 [17,18], accumulates on the SCV. This accumulation on the SCV is also visible for PipB2 and other membrane bound T3SS-2 effectors such as SifA or SseJ. We have suggested that these accumulations result from a slow formation of SCV-derived vesicles [19].

Materials and Methods

Samples Collection

Two hundred fecal samples were collected from Persons suffering from diarrhea, from both sexes in Merjan Medical city, Al-Hillah Surgical Teaching Hospital and Babel Teaching Hospital for Women and Children in Babylon province during the period from july 2018 to September 2018. Fecal samples (1gm) were put immediately in a sterile tube contained buffered peptone water, and transmitted immediately to the laboratory of Collage of Medicine in Babylon university with cooling box [20].

Culturing of Samples

Spread a 10 µl(loop full) from the inoculated and incubate tetrathionate broth on XLD ,S.S. Agar and on BGA agar plates and incubate at 37°C overnight (18-24 hours) and read the XLD plates and BGA plate *Salmonella* suspect colonies on XLD ,S.S. Agar and BGA agar onto non-selective media, (nutrient agar)plates for morphology and biochemical confirmation of *Salmonella* .After culturing of sample used vitek 2 compact system to detection of *Salmonella enterica*.

DNA Extraction for Gram Negative Bacteria

DNA extraction was carried out according to the genomic DNA purification kit supplemented by manufactured company (Gene aid, UK).

Confirmed Detection of *S. typhimurium* and *S. enteritidis* by Multiplex PCR using Specific Primer

For *Salmonella enterica* ser. Enteritidis specific motifs were found in the target gene SEN1383 - hypothetical protein, the primers flanking portion length 304 bp were selected. The target gene STM0159 - restriction endonuclease demonstrated specificity for *Salmonella enterica* ser. Typhimurium, the primers flanking region 224 bp were choose [21].

Molecular Detection of *Salmonella Typhimurium* and *Salmonella Enteritidis* Group using Multiplex PCR

PCR mixture was prepared by adding 12.5 µl of Green master mix (2x) promaga, 2.5 µl template DNA, 1.5 µl from forward primer and 1.5 µl from each four revers primer, final volume was completed to 25 µl by adding nucleuse free water.

Table 1: The primer sequences and PCR conditions of *S.typhimurium* and *S.enteritidis*

Genes	P	Primer sequence (5′-3′)	bp	PCR condition	Reference
Salmonella enterica ser.	F	TGTGTTTTATCTGATGCAAGAGG	304	95 °C 5min	[21]
	R	TGAACTACGTTCGTTCTTCTGG		95 °C 60Sec	

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Enteritidis				57 °C 60Sec **30x**	
Salmonella enterica ser. Typhimurium	F	ATGATGCCTTTTGCTGCTTT	224	72 °C 5min 72 °C 7min	
	R	TCCCAGCTCATCCAAAAA			

Detection of Some Virulence Gene Markers by PCR

The primers and PCR conditions used to amplify genes encoding virulence factors with PCR are listed in Table (2). The primer includes *invA* gene, *sdiA* gene and *sifA* gene.

Each 25µl of PCR reaction contained 5µl of each upstream and downstream primer, 5 µl of free nuclease water, 2.5µl of DNA extraction and 12.5 µl of master mix. The PCR amplification products were visualized by electrophoresis on 1% agarose ladder (promega, USA).

Table 2: Virulence factor primers sequences with their amplicon size Base pair (bp) and their condition

Genes	Primer sequence (5'-3')	Size bp	PCR condition	Reference
Sdi A1 Sdi A2	AATATCGCTTCGTACCAC GTAGGTAAACGAGGAGCAG	**274**	**94 °C 5min 94 °C 30Sec 52 °C 40Sec 30x 72 °C 60Sec 72 °C 7min**	[22]
Inv F Inv R	ACAGTGCTCGTTTACGACC TGAAT AGACGACTGGTACTGATCG ATAAT	244	**94 °C 3min 94 °C 60Sec 58 °C 60Sec 30x 72 °C 60Sec 72 °C 10min**	[23]
SifA F SifA R	TTTGCCGAACGCGCCCC CACACG GTTGCCTTTTCTTGCGCT TTCCACCCATCT	449	**95 °C 5min 95 °C 60Sec 60 °C 40Sec 35x 72 °C 60Sec 72 °C 10min**	[24]

Results and Discussion

Molecular Detection of *invA* gene, *sdiA* gene and *sifA* gene in Salmonella enterica:

To confirmation of diagnosis for *Salmonella enterica ser.typhimurium* depend on STM0159- restriction endonuclease and *Salmonella enterica ser. enteritidis* depend on SEN1383- hypothetical protein in Multiplex PCR, the results revealed that only 24(12) % out of 35 were positive for culture and biochiemical test, these 24 isolates were 17 (48.57) % S. *typhimurium* and 7 (20) % S. *enteritidis* as show in Figure (1). The results was obtained in this study by molecular methods were 24 (12) % out of 35 samples was positive results and this study agreement with results obtained by [25] who found that (10%) were identified as carriers of *Salmonella typhimurium* and *Salmonella enteritidis* on the basis of the molecular methods .Multiplex PCR is considered as a rapid molecular approach for simultaneous detection of several targets a single amplification reaction. This technique is frequently evaluated in order to assess the possible presence of microbial pathogens causing foodborne diseases [26].

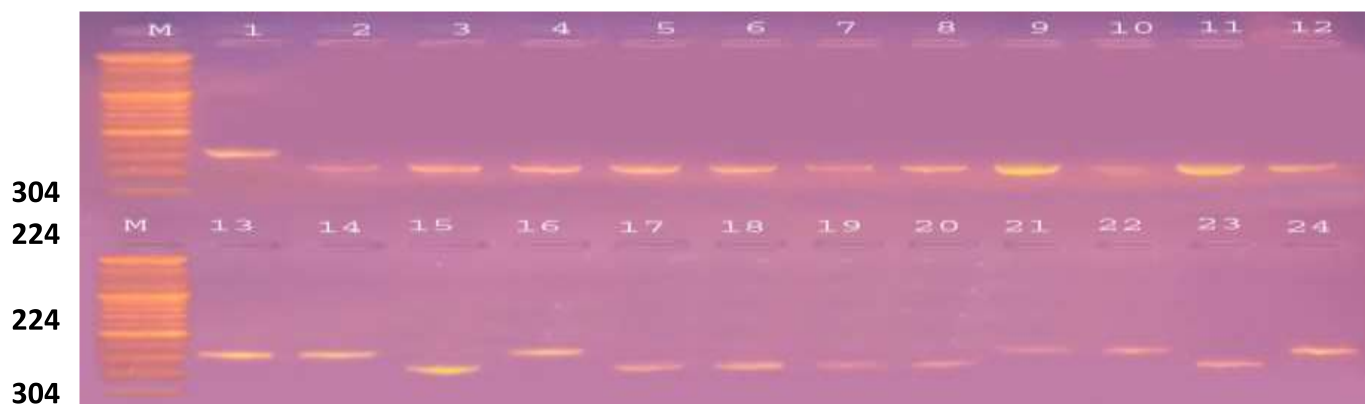

Figure 1: 1% Agarose gel electrophoresis at 70 volt for 30 min for SEN1383 - hypothetical protein and STM0159 - restriction endonuclease *PCR* products visualized under U.V light at 280 nm after staining with ethidium bromide. L: 1500 bp ladder;lane(1-24) were positive for these genes, the size of product is 304 bp for SEN1383 target gene*(S. enteritidis*) and 224 bp for STM0159 - restriction endonuclease target gene (*S. typhimurium*)

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Molecular detection of *invA* gene was done by using specific primer. It was found that *invA* gene observed in 13 isolates (54%) of *S. enterica* strains with long length (244 bp) as shown in Figure (2). The result of this study was dis agreement with the result obtained by [27] and [28] who were found that the percentage of invA gene in *Salmonella* is 100%.

This gene encodes a protein in the inner membrane of bacteria which is responsible for invasion to the epithelial cells of the host [29].The gene may not also always be present in all *Salmonella* spp. While it may be possible to state that the species that showed the *invA* band may be virulent, penetrate and cause infection in host cells. The detection of the gene in the *Salmonella* isolated implies the organisms are virulent and will be able to penetrate host epithelia cells, causing infection.

Absence of the gene in the confirmed *Salmonella* isolates can lead to lack of invasiveness by those isolates [30]. However, our study, this gene cannot be used for diagnosis because some isolates do not have this gene, the differences between presence the virulence genes in *S. enterica* isolates in our study and other studies may be influence of geographic conditions, dietary factors, movement the virulence genes by transposon and integron in addition to plasmids are a major mechanism for the spread of virulence genes in bacterial populations by conjugation, and/or host genetic factors. Other studies showed that the failure of the PCR detection was due to naturally occurring deletions in the centisome 63 PAI. Interestingly, the strains that were not associated with disease were shown to lack invA sequences [31].

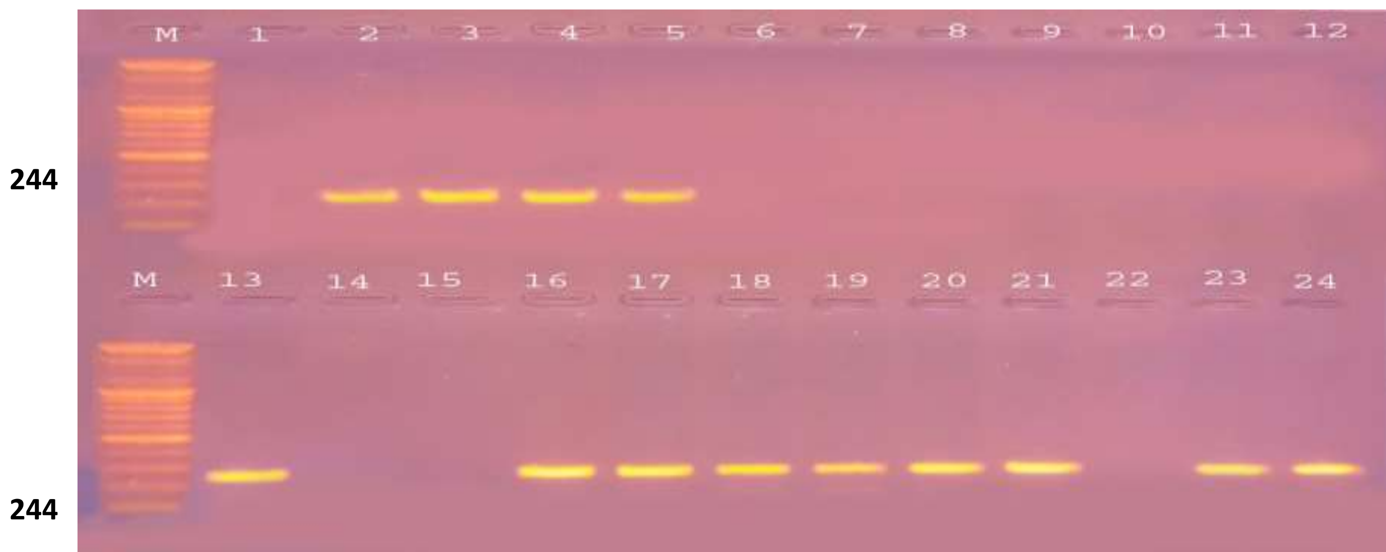

Figure 2: 1% Agarose gel electrophoresis at 70 volt for 30 min for *invA PCR* products visualized under U.V light at 280 nm after staining with ethidium bromide. L: 1500 bp ladder; lane(2,3,4,5,17,18,19,20,23) *S. typhimurium*(13,16,21,24) *S. enteritidis* for this gene, the size of product is 244 bp for *invA* gene

The quorum sensing gene (*sdiA)* was investigated by PCR technique using specific primers for this gene. The results of this experiment indicate for positive amplification as shown in figure (3). It was found that sdiA marker was observed in 24 isolates, (100%). This result was correlated with the results obtained by [22], [32] who were found that *sdiA* gene at similar frequencies 100% respectively. [32] Were showed that the sdiA quorum sensing gene can be used as a target gene for detection of Salmonella spp. by PCR. Quorum sensing (QS) is a density dependent regulatory mechanism mediated by the accumulation of signaling molecules produced by bacteria and is related to transcriptional regulation of several genes, including those involved in biofilm formation, bacterial adhesion, host colonization and virulence factors [9]. Signaling mechanisms, like sdiA in Salmonella, control those pathways which are responsible for expressing various virulence factors. There are over 2,600 serovars of S. enterica, and it is likely that the SdiA regulon is different among the serovars. SdiA regulates accessory factors that may contribute to intestinal survival or colonization [33]. For example, the sdiA gene of S. typhimurium regulates two loci, the rck operon and the srgE gene [34].

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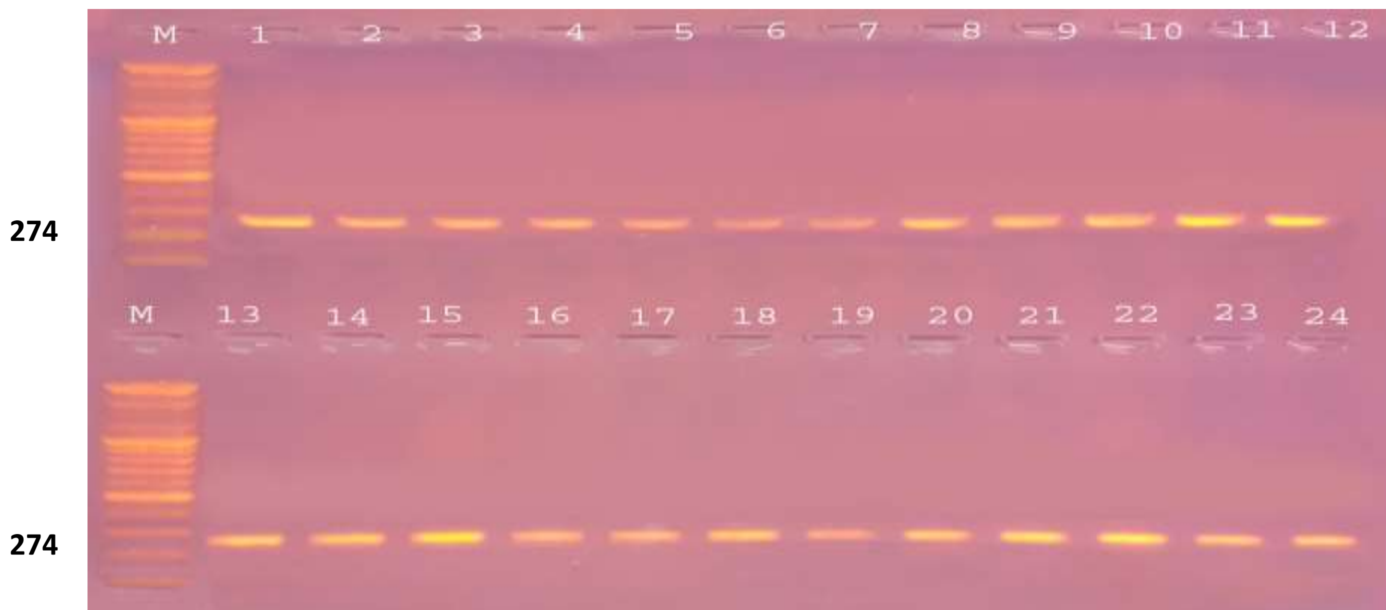

Figure 3: 1% Agarose gel electrophoresis at 70 volt for 30 min for sdiA PCR products visualized under U.V light at 280 nm after staining with ethidium bromide. L: 1500 bp ladder;lane(1-24) were positive for this gene, the size of product is 274 bp for sdiA gene

Salmonella-induced filament (*sifA)* gene was investigated by PCR technique using specific primers for this gene. The results of this experiment indicate for positive amplification as shown in figure (4). It was found that sifA marker was observed in 23 isolates only isolate number 18 not contained this gene, (96%).

Figure 4: 1% Agarose gel electrophoresis at 70 volt for 30 min for *sifA* PCR products visualized under U.V light at 280 nm after staining with ethidium bromide. L: 1500 bp ladder;lane(1-24) only isolate No. 18 not contained for this gene, the size of product is 449 bp for *sifA* gene

This result was closly correlated with the results obtained by [24], [35] who were found that *sifA* gene at similar frequencies 100% respectively. SifA is a *Salmonella typhimurium* effector protein that is translocated across the membrane of the *Salmonella*-containing vacuole by the *Salmonella* pathogenicity island 2-encoded type III secretion system. SifA is necessary for the formation of *Salmonella* induced filaments and for the maintenance of the vacuolar membrane enclosing the pathogen [36]. SIF are tubular aggregations of late endosomal/lysosomal vesicles. Presence of lysosomal glycoproteins (lgp) such as lysosome-associated membrane protein 1 (LAMP1) is characteristic for SIF membranes. The Salmonella SPI2-T3SS effector protein SifA is crucial for formation of SIF [37] and stability of SCV during intracellular replication [38]. The T3SS-2 effector protein SifA3 plays a significant role in *Salmonella* virulence and several cellular phenotypes are linked to its translocation.

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SifA is required to maintain the integrity of the *Salmonella*-containing vacuole (SCV) [38]. It promotes the formation of tubular membranous structures connected to SCVs that are named *Salmonella*-induced tubules [39].

References

1. Lee KM, Runyon M, Herrman TJ, Phillips R, Hsieh J (2015) Review of Salmonella detection and identification methods: Aspects of rapid emergency response and food safety. Food Control, 47: 264-276.
2. Williams KP, Gillespie JJ, Sobral BW, Nordberg EK, Snyder EE, Shallom JM, Dickerman, AW (2010) Phylogeny of gammaproteobacteria. Journal of bacteriology, 192(9): 2305-2314.
3. Agbaje M, Begum RH, Oyekunle MA, Ojo OE, Adenubi OT (2011) Evolution of Salmonella nomenclature: A critical note. Folia Microbiologica, 56(6): 497-503.https://doi.org/10.1007/s12223-011-0075-4
4. Sharma I, Das K (2016) Detection of invA Gene in Isolated Salmonella from Marketed Poultry Meat by PCR Assay. Journal of Food Processing & Technology, 7: 1-9 https://doi.org/10.4172/2157-7110.1000564
5. Malorny B, Hoorfar J, Bunge C, Helmuth R (2003) Multicenter validation of the analytical accuracy of salmonella PCR: Towards an international standard. Applied and Environmental Microbiology, 69(1): 290-296. https://doi.org/10.1128/AEM.69.1.290-296.2003
6. Karmi M (2013) Detection of Virulence Gene (inva) in Salmonella Isolated from Meat and Poultry Products. International Journal of Genetics, 29(11): 1928-1940. https://doi.org/10.5829/idosi.ijg.2013.3.2.82204.
7. Abed N, Grépinet O, Canepa S, Hurtado-Escobar GA, Guichard N, Wiedemann A, Virlogeux-Payant I (2014) Direct regulation of the pefI-srgC operon encoding the Rck invasin by the quorum-sensing regulator SdiA in SalmonellaTyphimurium. Molecular Microbiology, 94(2): 254-271. https://doi.org/10.1111/mmi.12738
8. Habyarimana F, Sabag-Daigle A, Ahmer BMM (2014) The SdiA-regulated gene srgE encodes a type III secreted effector. Journal of Bacteriology, 52(4):933-945. https://doi.org/10.1128/JB.01602-14
9. Antunes LCM et al (2010) ‘Quorum sensing in bacterial virulence’, Microbiology. Microbiology Society, 156(8): 2271-2282.
10. Schmidt H, Hensel M (2004) Pathogenicity islands in bacterial pathogenesis. Clinical Microbiology Reviews, 17(1): 14-56
11. Ahmer BMM (2004) Cell-to-cell signalling in Escherichia coli and Salmonella enterica. Molecular Microbiology, 52(4): 933-945. https://doi.org/10.1111/j.1365-2958.2004.04054.x
12. Kim W, Surette MG (2006) Coordinated regulation of two independent cell-cell signaling systems and swarmer differentiation in Salmonella enterica serovar typhimurium. Journal of Bacteriology, 188(2): 431-440. https://doi.org/10.1128/JB.188.2.431-440.2006
13. Rosa-Ferreira C, Munro S (2011) Arl8 and SKIP act together to link lysosomes to kinesin-1. Developmental cell, 21(6): 1171-1178.
14. Kaniuk NA, Canadien V, Bagshaw RD, Bakowski M, Braun V, Landekic M, Brumell JH (2011) Salmonella exploits Arl8B-directed kinesin activity to promote endosome tubulation and cell-to-cell transfer. Cellular Microbiology 13(11): 1812-1823.
15. Ohlson MB, Huang Z, Alto NM, Blanc MP, Dixon JE, Chai J, Miller SI (2008) Structure and Function of Salmonella SifA Indicate that Its Interactions with SKIP, SseJ, and RhoA Family GTPases Induce Endosomal Tubulation. Cell Host and Microbe., 4(5): 434-446. https://doi.org/10.1016/j.chom.2008.08.012
16. Diacovich L, Dumont A, Lafitte D, Soprano E, Guilhon AA, Bignon C, Méresse S (2009) Interaction between the SifA virulence factor and its host target SKIP is essential for Salmonella pathogenesis. Journal of Biological Chemistry, 284(48): 33151-33160 https://doi.org/10.1074/jbc.M109.034975
17. Arbeloa A, Garnett J, Lillington J, Bulgin

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RR, Berger CN, Lea SM, Frankel G (2010) EspM2 is a RhoA guanine nucleotide exchange factor. Cellular Microbiology. 12(5):654-664. https://doi.org/10.1111/j.1462-5822.2009.01423.x

18. Henry T, Couillault C, Rockenfeller P, Boucrot E, Dumont A, Schroeder N, Meresse S (2006) The Salmonella effector protein PipB2 is a linker for kinesin-1. Proceedings of the National Academy of Sciences, 9(18): 210-212. https://doi.org/10.1073/pnas.0605443103
19. Dumont A, Boucrot E, Drevensek S, Daire V, Gorvel JP, Poüs C, Méresse S (2010) SKIP, the host target of the salmonella virulence factor SifA, promotes kinesin-1-dependent vacuolar membrane exchanges. Traffic, 11(7): 899-911.
20. Cherneck CC, Berger BJ (2008) "Salmonella: Rectal Culture, Swab Diagnostic" In: Laboratory Test and Diagnostic Procedures. 5th Ed. Published by Saunders, an imprint of Elsevier Inc.
21. Ranjbar R, Mortazavi SM, Mehrabi Tavana A, Sarshar M, Najafi A, Soruri Zanjani R (2017) Simultaneous Molecular Detection of Salmonella enterica Serovars Typhi, Enteritidis, Infantis, and Typhimurium. Iranian Journal of Public Health, 46(1): 103
22. Halatsi K, Oikonomou I, Lambiri M, Mandilara G, Vatopoulos A, Kyriacou A (2006) PCR detection of Salmonella spp. using primers targeting the quorum sensing gene sdiA. FEMS Microbiology Letters, 259(2): 201-207. https://doi.org/10.1111/j.1574-6968.2006.00266.x
23. Bhatta DR, Bangtrakulnonth A, Tishyadhigama P, Saroj SD, Bandekar JR, Hendriksen R S, Kapadnis BP (2007) Serotyping, PCR, phage-typing and antibiotic sensitivity testing of Salmonella serovars isolated from urban drinking water supply systems of Nepal. Letters in Applied Microbiology, 44(6): 588-594 https://doi.org/10.1111/j.1472-765X.2007.02133.x
24. Mezal EH, Sabol A, Khan MA, Ali N, Stefanova R, Khan AA (2014) Isolation and molecular characterization of Salmonella enterica serovar Enteritidis from poultry house and clinical samples during 2010. Food Microbiology, 38: 67-74.
25. Nader MI, Rasheed MN, Hammed HH (2015) 'Molecular Identification of Salmonella typhimurium from Chicken, meat, and Human by PCR', in Intl Conf. on Medical Genetics, Cellular and Molecular Biology, Pharmaceutical and Food Science (GCMBPF-2015) 5-6: Istanbul, 1416-1422.
26. Akiba M, Kusumoto M, Iwata T (2011) Rapid identification of Salmonella enterica serovars, Typhimurium, Choleraesuis, Infantis, Hadar, Enteritidis, Dublin and Gallinarum, by multiplex PCR. Journal of Microbiological Methods, 85(1): 9-15.
27. Hanan ZK (2016) 'Isolation and Molecular Detection of Some Virulence Genes and Plasmids of Salmonella enterica from Diarrheal Children in Thi-Qar Province / Iraq'.
28. Proroga YTR, Capuano F, Capparelli R, Bilei S, Bernardo M, Cocco MP, Pasquale V (2018) Characterization of non-typhoidal Salmonella enterica strains of human origin in central and southern Italy. Italian Journal of Food Safety7 (32): 31-39. https://doi.org/10.4081/ijfs.2018.6888
29. Darwin KH, Miller VL (1999) Molecular basis of the interaction of Salmonella with the intestinal mucosa. Clinical Microbiology Reviews, 12(3): 405-428.
30. Bacci C, Paris A, Salsi A, Brindani F (2006) Genotypic and phenotypic virulence features in Salmonella enterica strains isolated from meat [Emilia-Romagna]. Annali della Facoltà di Medicina Veterinaria-Università di Parma (Italy) 26: 165-174.
31. Ginocchio CC, Rahn K, Clarke RC, Galán JE (1997) Naturally occurring deletions in the centisome 63 pathogenicity island of environmental isolates of Salmonella spp. Infection and Immunity, 65(4): 1267-1272.
32. Firouzi R, Derakhshandeh A, Khoshbakht R (2014) Distribution of sdiA quorum sensing gene and its two regulon among Salmonella serotypes isolated from different origins. Comparative Clinical Pathology, 23(5): 1435-1439. https://doi.org/10.1007/s00580-013-1801-x
33. Guo X, Chen J, Beuchat LR, Brackett RE (2000) PCR detection of Salmonella enterica serotype Montevideo in and on raw tomatoes using primers derived from hilA. Applied and Environmental

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Microbiology, 66(12): 5248-5252. https://doi.org/10.1128/AEM.66.12.5248-5252.2000

34. Soares JA, Ahmer BMM (2011) Detection of acyl-homoserine lactones by Escherichia and Salmonella. Current Opinion in Microbiology, 14(2): 188-193.

35. Tarabees R, Elsayed MS, Shawish R, Basiouni S, Shehata AA (2017) Isolation and characterization of Salmonella Enteritidis and Salmonella Typhimurium from chicken meat in Egypt. The Journal of Infection in Developing Countries, 11(04): 314-319.

36. Boucrot E, Beuzón CR, Holden DW, Gorvel JP, Méresse S (2003) Salmonella typhimurium SifA effector protein requires its membrane-anchoring C-terminal hexapeptide for its biological function. Journal of Biological Chemistry, 278(16): 14196-14202.

37. Stein MA, Leung KY, Zwick M, Garcia-del Portillo F, Finlay BB (1996) Identification of a Salmonella virulence gene required for formation of filamentous structures containing lysosomal membrane glycoproteins within epithelial cells. Molecular Microbiology, 64(12): 129-149 https://doi.org/10.1111/j.1365-2958.1996.tb02497.x

38. Beuzo Â N, CR Phane, SÂ Resse, MÂ Unsworth, KE Ruõ Âz-Albert, J Garvis S, Holden DW (2000) Salmonella maintains the integrity of its intracellular vacuole through the action of SifA. EMBO Journal, 19(13): 3235-3249.

39. Krieger V, Liebl D, Zhang Y, Rajashekar R, Chlanda P, Giesker K, Hensel M (2014) Reorganization of the Endosomal System in Salmonella-Infected Cells: The Ultrastructure of Salmonella-Induced Tubular Compartments 107(1): 23-37.

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