



Journal of the Hellenic Veterinary Medical Society

Vol 73, No 4 (2022)



To cite this article:

Rahat, S., Ahmad, K., & Ali, A. (2023). Molecular identification of virulence features of Salmonella enterica isolated from fresh chicken meat sold at retail shops and markets in Peshawar, Pakistan. *Journal of the Hellenic Veterinary Medical Society*, *73*(4), 4773–4778. https://doi.org/10.12681/jhvms.27474

Molecular identification of virulence features of *Salmonella enterica* isolated from fresh chicken meat sold at retail shops and markets in Peshawar, Pakistan

R. Shaista¹, A. Kafeel¹, A. Amjad²

¹Center of Biotechnology and Microbiology, University of Peshawar, Peshawar, Pakistan

²Department of Biotechnology, Women University of Azad Kashmir, Bagh, Pakistan

ABSTRACT: Salmonella enterica is an important agent of food borne illnesses in humans. Poultry meat and products are easily contaminated with different strains of Salmonella enterica. Consumption of such food causes salmonellosis in human. The aim of present investigation was to estimate the prevalence of Salmonella enterica in fresh chicken meat available in retail shops and markets at District Peshawar, Pakistan and survey the prevalence of virulence associated genes in the isolated Salmonella enterica. Fresh chicken meat samples were collected from retail shops and markets at District Peshawar, Pakistan and survey the prevalence of virulence associated genes in the isolated Salmonella enterica. Fresh chicken meat samples were collected from retail shops and markets at District Peshawar, Pakistan. The samples were processed for isolation of Salmonella enterica using selective media. Presumptive isolates were confirmed as Salmonella enterica via different biochemical tests and by amplification of *invA* gene. Ninety samples were contaminated with Salmonella enterica out of 150 chicken meat samples. Isolates were tested for the presence of eight different virulence genes *invA*, *spiA*, *cdtB*, *prgH*, *orgA*, *lpfC*, *sopB*, and *pefA*. The prevalence of virulence genes was: *invA* (100%), *lfpC*(48.8%),*sopB* (44.4%),*orgA* (30%), 2*spiA* (26.6%),*pefA* (21.1%), *prgH* (20%) and *cdtB* (6.6%). Genetic profiles P1-P26 were identified based on the occurrence of virulence genes among the isolates. The findings of this study highlight an alarming public health risk and demand for strict bio-safety hygiene measures to be taken.

Key words: Salmonella enterica; Salmonellosis; pathogenesis; virulence genes; meat

Corresponding Author: Kafeel Ahmad, Center of Biotechnology and Microbiology, University of Peshawar, Peshawar, Pakistan, Post code: 25200 E-mail address: kafeelpbg@gmail.com

Date of initial submission: 10-07-2021 Date of acceptance: 05-05-2022

INTRODUCTION

Salmonella enterica subspecies enterica is the leading cause of foodborne illnesses worldwide. Mostly salmonellosis is caused by non-typhoidal Salmonellae (NTS) that leads to life threatening conditions (Agron et al., 2001; Piccini and Montomoli, 2020). Poultry meat and meat products easily get contaminated by *S. enteric* that are major sources of salmonellosis in human worldwide (Amavisit et al., 2001). Pathogenicity of *S. enterica* is due to the presence of different virulence genes. With the help of such genes, *Salmonella* can invade and destroy cells ((Latasa et al., 2012, Skyberg et al., 2006; Smith and Bayles, 2006; Zou et al., 2011).

Salmonella species possess many virulence genes that are located in Salmonella pathogenicity islands (SPIs). The most common pathogenicity islands are SPI1, SPI2, SPI3, SPI4 and SPI5. Collectively, these pathogenicity islands carry a total 60 virulence genes. These genes are responsible for adhesion, invasion, and multiplication of the pathogen inside the host cell. The invA gene participates in adhesion and invasion while mgtc5 mostly has role in survival inside the host cell (Oliveira et al., 2003). The invA genes is also associated with the structure of TTSS (Type Three Secretion System) and considered as a target gene for confirmation of S. enterica at genus level (Nikaido, 2003; Beceiro et al., 2013). Along with invA gene some other genes such as Salmonella outer proteins (sop) allow Salmonella to invade the host cell (Nayak et al., 2004; Huehn et al., 2010; Choudhury et al., 2016; Swamy et al., 1996). Pilus regulatory genes (Prg) operon is mainly composed of three genes i.e., PrgH, PrgI, and PrgK (Murugkar et al., 2003; Kubori et al., 1998). All these genes play crucial role in pilus assembly and its localization in membrane. The oxygen-regulated gene (OrgA) is involved in invasion of serovar S. typhimurium inside the host cells. The cytolethal distending toxin B (cdtB) is an important toxin found in many serovars of S. enteric that is involved in intestinal pathogenesis through cell cycle arrest, apoptosis, and cytoplasm distension in a range of mammalian host cells (Smith and Bayles., 2006).

Characterization of virulence genes is essential for better understanding the pathogenicity mechanisms of zoonotic *S. enterica*. Various studies from different parts of the world have focused on investigating the prevalence of virulence associated genes of *S. enterica* isolated from poultry and poultry products (Dione et al., 2011; Zou et al., 2011; Borges et al., 2013). Such data are not available in Pakistan. Hence, the current study was aimed at investigating the existence of virulence genes in *S. Enteric* recovered from chicken meat simples collected from different locations in Peshawar, Pakistan. To our knowledge, this is the first study from Pakistan on the occurrence of virulence genes in *S. enterica* isolates of chicken meat origin.

MATERIALS AND METHODS

This study was conducted at the Center of Biotechnology and Microbiology, University of Peshawar, Pakistan. A total of ninety isolates of *S. enterica* were isolated from 150 fresh chicken meat samples collected from different poultry markets and retail shops in Peshawar, Pakistan during 2014-2016.

Isolation and identification

For isolation of *S. enterica*, all samples were processed by enrichment and plating on selective media. Presumptive colonies of *S. enterica* were further confirmed by different biochemical tests according to standard procedures as previously described (Parija, 2006).

Molecular identification of S. enterica

DNA purification kit (GeneJET, K0721) was used for extraction of DNA. For genus level identification the *invA* gene was amplified via conventional PCR (Chiu and Ou 1996). A reaction mix(25 μ l) had 4 μ l master mix (BIORON, Cat. No. 1200126), 1 μ l of each primer (0.5 μ M), 2 μ l of template DNA and 18 μ l water (Sigma-Aldrich, US). The thermo cycler was set to initial denaturation for 1 min at 94°Cfollowed by35 cycles of denaturation at 94°C for 1 min, annealing at 53°C for 2 min and extension at 72°C for 3 min. Finally, reaction was kept for 7 min at 72°C. Amplified product of *invA* gene was analyzed using 1.5% agarose gel and 100 bp DNA ladder (BIORON,304105). Previously isolated *S. enteric* isolates were used as positive controls.

Detection of virulence genes

The occurrence of eight virulence genes (*invA*, *spiA*, *cdtB*, *prgH*, *orgA*, *lpfC*, *sopB*, and *pefA*) was analyzed by PCR (Skyberg et al., 2006). A PCR reaction mix of 25 μ l contained4 μ l master mix (so-lis-BioDyne,04-12-00126), 1 μ l DNA template, 1 μ l (0.5 μ M) of each primer and 18 μ l molecular grade water. Initial denaturation was set to 95°C for 5 min that was followed by 30 cycles of denaturation at 94C for 1 min, annealing at 66°C for 60 sec and extension

at 72°C for 90 sec. An additional final extension at 72°C for 10 min was also used. The products were analyzed using 1.5% agarose gel and a100 bp DNA ladder (Bioron, Cat no.304105) as size marker. The genetic profile of virulence genes i.e., co-occurrence of different virulence genes among the isolates was designated as P1, P2, P3 etc.

RESULTS

Out of 150 fresh meat chicken samples collected randomly at different locations in Peshawar, 90 samples (60%) were positive for *S. enterica*. Isolates were confirmed as *S. enterica* by different biochemical tests and amplification of *invA* gene via PCR. The target virulence genes (*invA*, *spiA*, *cdtB*, *prgH*, *orgA*, *lpfC*, *sopB*, and *pefA*) were detected in the isolates with variable frequencies. Virulence genes profiles were named as P1-P26 based on the occurrence of the different virulence genes among the isolates (Table-1). Among all the isolates, 90(100%) had *invA* gene, 44(48.8%) possessed *lfpC* gene,40(44.4%) possessed *sopB* gene, 27(30%) possessed *orgA* gene, 24(26.6%) possessed *spiA* gene, 19(21.1%) possessed *pefA* gene, 18(20%) possessed *prgH* gene and 6(6.6%) possessed *cdtB* gene. 15.5% had five genes and 21.1% had four genes simultaneously. 72.2% isolates harbored two virulence genes.

DISCUSSION

This study confirmed a high prevalence of *S. enterica* in meat samples collected from local markets at Peshawar, Pakistan. Similar results have been reported previously from Greece that showed a very high (97 %) contamination of chicken samples with

Table 1. Virulence genes profiles of S. enterica isolates				
No of resistance genes detected	Virulence gene	Genetic profile	No of isolates	Strain ID
One	invA	P1	25	B71, D63, L1, S2K, E2, E4, E6, E7, E9, E10,E12, E13, E14, E15, E17, E19,
Two	invA, sopB	P2	11	A1.2, A2, A2.3, 8, 10,11,12, 20, 14T FB, P2I, P4I, PIS, M61, E16, 9, 22, 8T, 9T, C5
	invA, lfpC	P3	12	F7, D61, C62, P3S, P2S, L2, E1, E3, E18, 13, 28T, 29T
	invA,orgA	P4	03	P5I, 25P, P3I
	invA, prgH	P5	02	E8, A3
	invA,pefA	P6	01	Al
Three	invA, lfpC, spiA	P7	01	2FP8
	invA,lfpC, sopB	P8	04	2M61, 3N62, L3, 14
	invA,orgA, sopB	P9	01	26P
	invA, lfpC, orgA	P10	01	15
	invA,orgA, pefA	P11	01	27
	invA,sopB, spiA	P12	01	28
Four	invA,orgA, prgH, sopB,	P13	01	S2G
	invA, lfpC, prgH, spiA	P14	02	INA, FD
	invA, lfpC, orgA, spiA	P15	01	PR7S
	invA, lfpC, pefA, spiA	P16	01	IS9
	invA,lfpC, sopB, spiA	P17	02	IS3, IS5
	invA,lfpC, orgA, sopB	P18	01	C63
	invA,lfpC, prgH, sopB	P19	01	P1I
	invA,lfpC, orgA, prgH	P20	01	S2M
Five	invA,lfpC, prgH, sopB,spiA	P21	03	F8S, S2A, S2I
	invA, lfpC, orgA, prgH, sopB	P22	01	IN3
	invA, lfpC, orgA, sopB, spiA	P23	04	211S, FIS, IS6, FA
	invA, orgA, prgH, sopB, spiA	P24	01	S2E
Six	invA,lfpC, orgA, prgH, sopB, spiA	P25	05	IN9, 201S, S2F, 1N14, IS4
Seven	invA, lfpc, OrgA, PefA, PrgH, SonB SniA	P26	03	S2C, INIS, S2B

J HELLENIC VET MED SOC 2022, 73 (4) ПЕКЕ 2022, 73 (4)

Salmonella (Karabaxoglou et al. 1996). The high contamination of chicken meat with Salmonella in the current study could be attributed to the unhygienic processing and storage of chicken meat in the local markets. These findings are alarming from health point of view as these bacteria are well equipped to cause Salmonella infections in human upon consumption of contaminated chicken meat or meat products as has been reported previously (Marin and Lainez, 2009; Papadopoulos et al., 2016, Zdragas et al., 2012). The isolated bacteria were also found to possess eight different virulence genes and many isolates had multiple types of virulence genes. Similar results have been reported previously involving clinical or other environmental and food samples (Uchiya and Nikai, 2008). This data confirmed that chicken meat isolates may cause human infections because of presence of similar virulence genes as reported in clinical isolates (Borges et al., 2013). All isolates were found positive for invA gene that play significant role in the host recognition and internalization of Salmonella inside the host epithelial cell during invasion process.

Fimbrial virulence genes such as *lpfA*, *lpfC*, and *pefA* have crucial importance in binding, attachment, and adhesion of the bacterium to enterocytes (Schechter and Lee, 2000; Chuanchuen et al., 2007; Miranda et al., 2008; Lekshmi et al., 2017). In current study *lpfC* and *pefA* showed high prevalence of 48.8% and 21.1% respectively as compared to past studies (Borges et al., 2013). The *cdtB* gene encodes an important toxin which plays a significant role in inducing apoptosis of the infected host cells. Only 6.6% isolates were found positive for *cdtB* gene as reported in previous studies (Tatavarthy and Cannons, 2010; El-Tawab, 2017). Some effectors proteins such as *orgA*,

prgH and sipB genes have a strong association with TTSS (Tatavarthy and Cannons, 2010; Borges et al., 2013; El-Tawab, 2017; Kim and Lee, 2017). In our findings orgA and prgH were found with prevalence of 26.6% and 20% respectively. Borges et al also reported similar genes in different Salmonella strains isolated from various food samples (El-Tawab, 2017). For better survival inside the host cells especially in presence of macrophages, genes such as msgA, spiA, pagC, and tolC are crucial. In current study spiA gene was detected in 26.6% isolates like previous findings (Tatavarthy and Cannons, 2010). The sopB gene that is mainly associated with prophages and helpful in construction of TTSS was found in 30% isolates. Current data is in close agreement with previous reports (Borges et al., 2019).

CONCLUSIONS

The presence of *Salmonella enterica* and its virulence genes in poultry meat is a great alarm for public health because these pathogens could be easily disseminated from poultry to human communities via food web. Hence, proper management and preventive measures need to be taken to prevent the spread of these pathogenic stains to human population. The study also had some limitations which were: This study covered a specific geographical location and the results could not be generalized. The study was conducted in 2014-16 and we do not have data for serovars. The result could, hence, be interpreted accordingly.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

REFERENCES

- Agron GP, Walker RL, Kind H, Sawyer SJ, Hayes DC, Wollard J, Andersen GL (2001) Identification by subtractive hybridization of sequences specific for *Salmonella* serovar *enteritidis*. Appl Environ Microbiol 67(11): 4984-4991.
- Amavisit P, Browing GF, Lightfood D, Anderson CS (2001) Rapid PCR detection of *Salmonella* in horse faecal samples. Vet Microbiol 79: 63-74.
- Beceiro A, Tomás M, Bou G (2013) Antimicrobial resistance and virulence: successful or deleterious association in the bacterial world. Clin Microbiol Rev 26:185-230.
- Borges KA, Furian TQ, Borsoi A, Moraes HL, Salle CT, Nascimento VP (2013) Detection of virulence-associated genes in *Salmonella enteritidis* isolates from chicken in South of Brazil Pesqui Vet Bras 33:1416-22.
- Borges KA, Furian TQ, Souza SN, Salle CT, Moraes HL Nascimento VP (2019) Antimicrobial resistance and molecular characterization of *Salmonella enterica* serotypes isolated from poultry sources in Brazil. Braz J Poult Sci 61:1-7.
- Chiu CH, Ou JT (1996) Rapid identification of *Salmonella* serovars in feces by specific detection of virulence genes, invA and spvC, by an enrichment broth culture-multiplex PCR combination assay. J Clin Microbiol 34:2619-2622.
- Choudhury M, Borah P, Sarma HK, Barkalita LM, Deka NK, Hussain I, Hussain MI (2016) Multiplex-PCR assay for detection of some major virulence genes of *Salmonella enterica* serovars from diverse sources. Curr Sci 111: 1252-1258.
- Chuanchuen R, Sirintip K, Pawin P (2007) Occurrence of qacE/qacE∆lgenes and their correlation with class 1 integrons in *Salmonella enterica* isolates from poultry and swine. Southeast Asian J trop med public health 38:855-62.
- Dione MM, Ikumapayi U, Saha D, Mohammed NI, Adegbola RA, Geerts S, Ieven M, Antonio M (2011) Antimicrobial resistance and virulence genes of non-typhoidal *Salmonella* isolates in The Gambia and Senegal. J Infect Dev Ctries, 5(11): 765-775.
- El-Tawab A, Ashraf A, Nabih AM, Agag MA, Ali A, Marwah H (2017) Molecular studies of virulence genes of *Salmonella typhimurium* causing clinical mastitis in dairy cattle. Benha Vet Med J 33(2): 27-37.
- Huehn S, Ragione RML, Anjum M, Saunders M, Woodward MJ, Bunge C, Helmuth R, Hauser E, Guerra B, Beutlich J, Brisabois A (2010) Virulotyping and antimicrobial resistance typing of *Salmonella enterica* serovars relevant to human health in Europe. Foodborne Pathog Dis 7: 523-535.
- Karabaxoglou D, Papa A, Kansouzidou A, Triantafyllou G, Mitka S, Amin A, Danielides B (1996) Incidence of *Salmonella* spp. in chickens consumed in hospitals of

Thessaloniki, Greece. Acta Microbiol Hellen 41: 230-233.

- Kim JE, Lee Y (2017) Molecular characterization of antimicrobial resistant non-typhoidal *Salmonella* from poultry industries in Korea. Irish Vet J 70(1):1-9.
- Kubori T, Matsushima Y, Nakamura D, Uralil J, Lara-Tejero M, Sukhan A, Galán JE, Aizawa SI (1998) Supramolecular structure of the Salmonella typhimurium type III protein secretion system. Science 280(5363): 602-605.
- Latasa C, García B, Echeverz M, Toledo-Arana A, Valle J, Campoy S, García-del Portillo F, Solano C, Lasa I (2012) Salmonella biofilm development depends on the phosphorylation status of RcsB. J Bacteriol 194(14): 3708-3722.
- Lekshmi M, Ammini P, Kumar S, Varela MF (2017) The food production environment and the development of antimicrobial resistance in human pathogens of animal origin. Microorganisms 5(1):11.
- Marin C,Lainez M (2009) Salmonella detection in faces during broiler rearing and after live transport to the slaughterhouse. Poult Sci 88: 1999-2005.
- Miranda JM, Vazquez BI, Fente CA, Calo-Mata P, Cepeda A, Franco CM (2008) Comparison of antimicrobial resistance in *Escherichia coli*, *Staphylococcus aureus*, and *Listeria monocytogenes* strains isolated from organic and conventional poultry meat. J food prot 71(12): 2537-2542.
- Murugkar HV, Rahman H, Dutta PK (2003) Distribution of virulence genes in *Salmonella* serovars isolated from man & animals. Indian J Med Res 117: 66–70.
- Nayak R, Stewart T, Wang RF, Lin J, Cerniglia CE, Kenney PB (2004) Genetic diversity and virulence gene determinants of antibiotic-resistance *Salmonella* isolated from preharvest turkey production sources. Int J Food Microbiol 91: 51-62.
- Nikaido H (2003) Molecular basis of bacterial outer membrane permeability revisited. Microbiol Mol Biol Rev 67:593-656.
- Oliveira SDD, Rodenbusch CR, Michael GB, Cardoso MI, Canal CW, Brandelli A (2003) Detection of virulence genes in *Salmonella Enteritidis* isolated from different sources. Braz J Microbiol 34: 123-124.
- Papadopoulos T, Petridou E, Zdragas A, Mandilara G, Nair S, Peters T, Chattaway M, de Pinna E, Passiotou M, Vatopoulos A (2016). Comparative study of all *Salmonella enterica* serovar Enteritidis strains isolated from food and food animals in Greece from 2008 to 2010 with clinical isolates. Eur J Clin Microbiol Infect Dis 35(5): 741-746.
- ParijaSC (2006). Textbook of practical microbiology. Ahuja Publishing, New Delhi, India.
- Piccini G, Montomoli E (2020) Pathogenic signature of invasive non-typhoidal *Salmonella* in Africa: implications for vaccine development. Hum Vaccin Immunother, 16(9): 2056-2071.

Schechter LM, Lee CA (2000) Salmonella invasion of non-phagocytic

J HELLENIC VET MED SOC 2022, 73 (4) ПЕКЕ 2022, 73 (4) cells. Subcell Biochem 33:289-320.

- Skyberg JA, Logue CM, Nolan LK (2006) Virulence genotyping of Salmonella spp with multiplex PCR. Avian Dis 50: 77-81.
- Smith JL Bayles DO (2006) The contribution of cytolethal distending toxin to bacterial pathogenesis Crit Rev Microbiol 32: 227-248.
- Swamy SC, Barnhart HM, Lee MD, Dreesen DW (1996) Virulence determinants invA and spvC in *Salmonellae* isolated from poultry products, wastewater, and human sources. Appl Environ Microbiol 62: 3768-3771.
- Tatavarthy A, Cannons A (2010) Real-time PCR detection of *Salmonella* species using a novel target: the outer membrane porin F gene (ompF). Lett Appl Microbiol 50:645-52.
- Uchiya K, Nikai T (2008) Salmonella virulence factor SpiC is involved in expression of flagellin protein and mediates activation of the signal transduction pathways in macrophages. Microbiology 154: 349-3502.
- Zdragas A, Mazaraki K, Vafeas G, Giantzi V, Papadopoulos T, Ekateriniadou L (2012) Prevalence, seasonal occurrence and antimicrobial resistance of *Salmonella* in poultry retail products in Greece. *Lett Appl Microbiol* 55(4): 308-313
- Zou W, Al-Khaldi SF, Branham WS, Han T, Fuscoe JC, Han J, Foley SL, Xu J, Fang H, Cerniglia CE, Nayak R (2011) Microarray analysis of virulence gene profiles in *Salmonella* serovars from food/food animal environment. J Infect Dev Ctries 5(2): 94-105.