Journal of Current Veterinary Research

ISSN: 2636-4026

Journal homepage: http://www.jcvr.journals.ekb.eg

Meat Hygiene

Isolation and Molecular Characterization of *Salmonellae* Isolated from Some Meat Products

Zakaria Hassan Elbayoumi[1], Rasha Nabil Zahran[2] and Reyad R. Shawish[1]

(1) Department of Food Hygiene & Control, Faculty of Veterinary Medicine, University of Sadat City, Egypt.

(2) Department of Bacteriology, Mycology and Immunology, Faculty of Veterinary Medicine, University of Sadat City, Egypt.

**corresponding author: zico76us@yahoo.com* *Received: 7/12/2020* *Accepted: 16/1/2021*

ABSTRACT

A Total of 200 random samples of meat products (minced meat, beef burger, kofta, sausage and luncheon) collected from different shops and supermarkets at El Menofiya Governorate, Egypt. The collected samples were examined for the isolation of *Salmonella spp*, serological identifications and molecular characterization by using PCR. *Salmonella* spp was isolated from the examined samples with the percentage of 25% (10) ,35% (14) ,27.5% (11), 30% (12) and 7.5% (3) respectively. The isolated *Salmonella* serologically identified as *S. Enteritidis*, *S. Infantis*, *S. Paratyphi* A and *S. Typhimurium*, also multiplex PCR methods were used for detection of virulence genes (invA and hilA) genes of Salmonella. The PCR results revealed that invasion gene (inv A) and hyper-invasive locus gene (hilA) in S.Entertidis , *S. Paratyphi*a A and *S. Typhimurium* . While S. Infants only positive for Invasion gene (invA) could be detected.

Keyword: *Meat products, salmonella, PCR and virulence genes*

INTRODUCTION

Meat and meat products have not only been following convenience trends, they have been at the heart of them also meat products such as minced meat, beef burger, kofta, sausage and luncheon are highly demanded and considered more attractive for consumers than fresh meat due to their high nutritive value, reasonable price, good taste, quick easily prepared and also easily serving. In in spite of the importance of meat products to consumers, they can be contaminated with several types of food borne microorganisms from different sources during handling, preparation and storage practices .They are considered as an ideal culture medium for growth of many organisms because of the high moisture, the high percentage of nitrogenous compounds, plentiful supply of minerals , some fermentable carbohydrates (glycogen) and of a favorable pH for most microorganisms (Al-Mutairi ., 2011).

According to WHO estimations, 600 million cases of diseases caused by contaminated food were noted in 2010 Pathogenic bacteria also penetrate food production areas and may remain there in the form of a biofilm covering the surfaces of machines and equipment. A common occurrence of microbes in food products, as well as their improper or careless processing, leads to common poisonings. Symptoms of foodborne infections may be mild, sometimes flu-like, but they also may be accompanied by severe complications, some even fatal (Cetinkaya *et al.*, 2012).

Salmonella spp. is one of the most severe foodborne pathogens worldwide. The World Health Organization (WHO) estimated an annual global incidence of more than 60 million foodborne cases of nontyphoid *Salmonella* and 7 million cases in the Latin American and Caribbean region (Godínez-Oviedo *et al.*, 2019). Infections with *Salmonella* species represent a major health problem and a

significant burden on food industry (Cetinkaya *et al.,* 2012) Worldwide, foodborne diseases are a growing public health problem. Among the infectious bacteria, non-typhoidal *Salmonella* enterica serovars (NTS) are the major cause of hospitalization and death (Karmi *et al.,*2013)) *Salmonella,* a food-borne pathogen, has a recurrent incidence in meat and poultry products. Currently, cases of salmonellosis represent very important economic losses in many countries (Dallal *et al.,* 2019).

Polymerase Chain Reaction (PCR) based methods are powerful diagnostic tool for the detection of pathogenic microorganisms (Malorny *et al.,* 2003). Compared to other methods of detection, these methods are rapid, highly specific and sensitive in the identification of target organisms (Guillier *et al.,*2013).

Therefor the aim of the present study isolation and molecular characterization of *Salmonella* spp isolated from examined samples.

MATERIAL AND METHOD

Collection of samples:

A total of 200 random samples of raw meat products (minced meat, beef burger, kofta , sausage and luncheon) (40 of each) were collected from different shops and supermarkets with different sanitation levels at Menofiya Governorate .The collected samples were transferred directly to the laboratory in an ice box under complete aseptic conditions without undue delay, thawed at room-temperature to be examined bacteriologically for detection of Salmonella.

Preparation of samples:

according to the technique recommended by **APHA (1992)** as follows: 25 grams from each beef meat product samples were transferred to a sterile polyethylene bag, and 225 ml of 0.1 % sterile buffered peptone water were aseptically added to the content of the bag. Each sample was then homogenized in a blender at 2000 r.p.m for 1-2 minutes to provide a homogenate. The prepared samples were subjected to the following examination:

Isolation and identification of *Salmonella* spp according to (ISO, 2017)

Serological identification of Salmonellae:

Serological identification of Salmonellae was carried out according to Kauffman – White scheme (Kauffman, 1974) for the determination of Somatic (O) and flagellar (H) antigens using *Salmonella* antiserum (DENKA SEIKEN Co., Japan).

Polymerase Chain Reaction (PCR) of *Salmonella*

1.1. Genomic DNA extraction:

Using GeneJET Genomic DNA Purification Kit DNA amplified products "PCR master Mix "(Fermentis): Gel Elecrophoresis: Sambrook *et al.* (1989).

DNA ladder (molecular marker):

100 bp (Fermentas, lot No: 00052518).

Primer sequences of Salmonellae used for PCR system

The primers for detection of virulence factors including Enterotoxin (*stn), and hyper-invasive locus (hilA*) *genes of Salmonella species* were synthesized as follow:

Target gene	Oligonucleotide sequence (5′ → 3′)	Product size (bp)	References
invA (F)	**5′** GTGAAATTATCGCCACGTTCGGGCA **′3**	284	**Shanmugasamy *et al.*, (2011)**
invA (R)	**5′** TCATCGCACCGTCAAAGGAACC **′3**		
hilA (F)	**5′** CTGCCGCAGTGTTAAGGATA **′3**	497	**Guo *et al.*, (2000)**
hilA (R)	**5′** CTGTCGCCTTAATCGCATGT **′3**		

DNA amplification of virulence genes of Salmonella (Singh et al., 2013):

The reaction mix (25 µl) invariably consisted of 5 µl of the bacterial lysate, 5 µl of 10x assay buffer for Taq polymerase containing 1.5 mM MgCl2, 2 µl of 10mM dNTP mix 1 µl each of forward and reverse primer (10 pmol) and 1.25 U of Taq DNA polymerase made upto50 µl using sterile distilled water.

The PCR cycling protocol was applied as following: An initial denaturation at 94°C for 60 sec, followed by 35 cycles of denaturation at 94°C for 60 sec, annealing at 64°C for 30 sec and extension at 72°C for 30 sec, followed by a final extension at 72°C for 7 min. Finally, 5 µl of each amplicon was electrophoresed in 1.5 % agrose gel stained with ethidium bromide and

visualized and captured on UV transilluminator.

RESULTS

Table (1): Incidence of *Salmonella* spp in the examined samples of meat products. (n=40)

Meat products	No. of ex. samples	No.	%
Minced meat	40	10	25
Beef burger	40	14	35
Kofta	40	11	27.5
Sausage	40	12	30
Luncheon	40	3	7.5
Total (200)	200	50	25

Table (2): Incidence of *Salmonella* serovars isolated from the examined samples of meat products (n=40).

Products	Minced meat		Beef burger		Kofta		Sausage		Luncheon		Antigenic structure	
Salmonellae	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**O**	**H**
S. Enteritidis	3	7.5	2	5	5	12.5	2	5	1	2.5	1,9,12	g,m : -
S. Infantis	-	-	1	2.5	3	7.5	6	15	-	-	6,7	r : 1,5
S. Paratyphi A	2	5	4	10	3	7.5	3	7.5	-	-	1,2,12	i : 1,5
S. Typhimurium	5	12.5	7	17.5	-	-	1	2.5	2	5	1,4,5,12	i : 1,2
Total	10	25	14	35	11	27.5	12	30	3	7.5		

Table (3): Acceptability of the examined meat products samples dependin on their contamination with Salmonellae (n=40).

Meat products	Salmonellae /25 g*	Accepted samples		Unaccepted samples	
		No.	**%**	**No.**	**%**
Minced meat	Free	30	75	10	25
Beef burger	Free	26	55	14	35
Kofta	Free	29	62.5	11	27.5
Sausage	Free	28	70	12	30
Luncheon	Free	37	92.5	3	7.5
Total (200)		150	75	50	25

*Egyptian Organization for Standardization "EOS" No 1694-2005 for minced meat No 1688-2005 for beef burger No 1973-2005 for kofta No 1972-2005 for sausage No 1114-2005 for luncheon

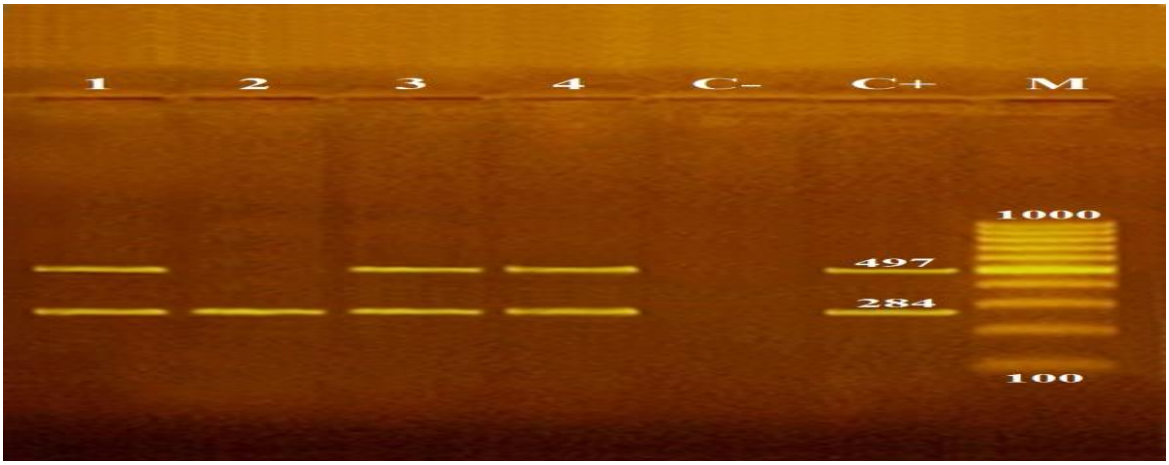

Fig. (1): Agarose gel electrophoresis of multiplex PCR of *invA* (284 bp) and *hilA* (497 bp) virulence genes for characterization of *Salmonella* species.

Lane M: 100 bp ladder as molecular size DNA marker.
Lane C+: Control positive strain for *invA* and *hilA* genes.
Lane C-: Control negative.
Lanes 1 (*S. Enteritidis*), 3 (*S. paratyphi*) & 4 (*S. Typhimurium*): Positive Salmonellae for *invA* and *hilA* genes.
Lane 2 (*S. Infantis*): Positive *Salmonella* strain for *invA* gene.

Table (4): Incidence of virulence genes of Salmonella strains isolated from the examined samples of meat products (n= 4 strains).

***Salmonella* strains**	***invA***	***hilA***
S. Enteritidis	+	+
S. Infantis	+	-
S. paratyphi	+	+
S. Typhimurium	+	+

***invA*:** Invasion gene. ***hilA*:** hyper-invasive locus gen

DISCUSSION

Meat products such as minced meat, beef burger, kofta, sausage and luncheon are highly demanded than fresh meat due to their high nutritive value, reasonable price, good taste, quick easily prepared and also easily serving but they can be contaminated by several types of food borne microorganisms from different sources during handling, preparation and storage practices (Mrema *et al.*, 2006).

The results obtained in table (1) revealed that the incidence of isolation of *Salmonella* in the examined samples of meat products (minced meat ,beef burger ,kofta ,sausage and luncheon)were 25% (10) ,35% (14) , 27,5% (11) ,30% (12) and 7,5% (3) respectively .

Incidence of isolation of *Salmonella* in the examined minced meat was (25% (10). Comparatively lower results were obtained by Karmi *et al.*, (2013) who found number of positive samples in minced meat 23/160 (14.4%). Also, lower incidence from minced meat reported by Mrema *et al.,* (2006) isolated *Salmonella* with prevalence rate (20%).On the other hand higher incidence in minced meat reported by Karmi *et al.,* (2013) isolated *Salmonella* from minced meat by the percentage of 40% (4/10) and by Abd El Tawab *et al.,* (2015) who isolated *Salmonella* from minced meat by the percentage of (7 = 35%).

Incidence of isolation of *Salmonella* in the examined samples of beef burger was 35% (14). lower incidence from beef burger reported by Hassanin *et al., (2014*) isolated *Salmonella* with the percentage of (13.13%). also, lower incidence from beef burger reported by Sallam *et al.,* (2014) and Abd El Tawab *et al.,* (2015) isolated *Salmonella* with the percentage of12.2% (11/90), 10% (2) respectively. While results nearly agree with Essa *et al.,* (2009) isolated *Salmonella* from beef burger by the percentage of 7 (23.3%) and Karmi *et al.,* (2013) who isolated *Salmonella* by the percentage of 20% while Ed-dra *et al.,* (2017) who isolated *Salmonella* by the percentage of 34 (21.79%).

Incidence of isolation of *Salmonella* in the examined samples of kofta was 27,5% (11) these results nearly agree with Hassanin *et al., (2014)* who isolated *Salmonella* with the percentage of 26.67%. lower incidence from kofta obtained by Cetinkaya *et al.,* (2012) isolated *Salmonella* by the percentage of (2%) and by Eldesouky, *et al.,* (2016) isolated *Salmonella* by the percentage of (8.5%).

The higher rate of microbial contamination of the examined samples of kofta may be attributed to Karmi *et al.,* (2013) isolated *Salmonella* by the percentage of 60% (6/10) and Hassanin *et al., (2014)* isolated *Salmonella* by the percentage of (33.3%).

Incidence of isolation of *Salmonella* in the examined samples of sausage was 30% (12). comparatively lower results were obtained by M Osman *et al.,* (2018) isolated *Salmonella* by the percentage of (4%). also, by Bingol *et al.,* (2013) and Samax *et al.,* (2012) isolated *Salmonella* by the percentage of 1.18%, 21.7% respectively. while Abd El Tawab *et al.,* (2015) isolated *Salmonella* from sausage by the percentage of (20%).

Incidence of isolation of *Salmonella* in the examined samples of luncheon was 7.5% (3). These results nearly agree with Eldesouky, *et al.,* (2016) isolated *Salmonella* with the percentage of 8.5% but El-Dosoky *et al.,* (2013) failed to isolate *Salmonella* from luncheon .On the other hand higher incidence of luncheon reported by Karmi *et al.,* (2013) who isolated *Salmonella* by the percentage of 10% (1/10) and Essa *et al.,* (2009) isolated *Salmonella* by the percentage of 16.7% (5) .

Lower incidence of *Salmonella* spp. in luncheon, could be due to heat treatment during manufacture and presence of chemical preservatives .Cutting boards, surfaces used for preparation of meat , equipments like meat

grinders, mincers and blinders are important sourses of *Salmonella* contamination , other studies stated that trucks, lairages, slaughter line, quartering, knives and surface of table are sources of *Salmonella* contamination of meat products ,also contaminated water used to clean equipment and cutting/slicing machines leading to cross-contamination especially if used with raw foods, handlers not practising proper sanitation and monitoring devices ,Survival of *Salmonella* in ready to-eat products has the potential to cause illness. (Karmi, 2013).

Results given in table (2) revealed that incidence of isolated serotypes of *Salmonella* in examined samples of minced meat were *S. Enteritidis* 7.5%(3) *S. Paratyphi A* 5%(2) and *S. Typhimurium* 12.5%(5).These results agree with Stock and Stolle (2001) who isolated *S. Typhimurium* and Mrema *et al.,* (2006) who isolated *S. Typhi, S. Enteritidis, S. Typhimurium* and *S. Paratyphi* .

Results given in table (2) revealed that incidence of isolated serotypes of *Salmonella* in examined samples of beef burger were *S. Enteritidis* 5% (2), *S. Infantis* 2.5% (1), *S. Paratyphia A* 10% (4*), S. Typhimurium* 17.5% (7). These results agree with Abd El Tawab *et al.,* (2015) who isolated *S. Typhimurium, S. Enteritidis and S. Typhi* and Essa *et al.,* (2009) who isolated 4 strains of *Salmonella typhimurium* and 3 strains of *Salmonella enteritidis* and dis agree with El-Dosoky *et al.,* (2013) who failed to isolate *Salmonella* from beef burger .

Morever the results given in table (2) revealed that incidence of isolated serotypes of *Salmonella* in the examined samples of kofta were *S. Enteritidis* 12.5% (5*), S. Infantis* 7.5% (3) *and S.Paratyphi A* 7.5%(3). These results agree with Eldesouky, *et al.,* (2016) THE isolated *S. enteritidis* was the predominant one followed by *S. infantis* and dis agree with Cetinkaya et al (2012) who isolated (*S. Typhimurium*).

Results given in table(2) revealed that incidence of isolated serotypes of *Salmonella* in the examined samples of sausage were *S. Enteritidis* 5% (2) ,*S. Infantis* 5% (6) , *S. Paratyphi* A 7.5% (3) and S.Typhimurium 2.5%(1) , these results disagree with Samax *et al.,*(2012) isolated *Salmonella enterica* subsp, *salamae* II . and agree with Mrema *et al.,* (2006) who isolated *S. Typhi, S. Enteritidis, S. Typhimurium, S. Paratyphi and S. Infantis*.

Finally the results given in table (2) revealed that incidence of isolated serotypes of *Salmonella* in the examined luncheon samples were S.Enteridis 2.5% (1) and *S. Typhimurium* 5% (2), these results agree with Eldesouky, *et al.,* (2016) who isolated *S. enteritidis* was the predominant one (47%) followed by *S. typhimurium* (23.52%).and dis agree with Essa *et al.,* (2009) who isolated 3 *Salmonella paratyphi-B* and 2 *Salmonella newport* strains.

The results showed that the examined beef burger were the more contaminated than other meat products and this may be due to destruction of the thermal system during freezing ,the very high initial contamination level in frozen beef burgers is the primary cause of this large outbreak and bad cooking practices(Guillier, *et al.,* 2013). there is no reason for concern in consuming ground beef burgers. In case of raw samples, microbes could originate from the vendors. Vendors have to be educated on hygienic practices which could help to reduce risks of food- borne infection. These data indicate that food handlers may contribute to contamination and that there are some handling practices that require more attention. (Soltan, *et al.,* 2009)

The results given in table (3) revealed that the accepted samples of *Salmonella* in minced meat (30) with the percentage of (75%) and the unaccepted samples (10) with the percentage (25%) , while in beef burger (26) with the percentage (55%) and the unaccepted samples (14) with the percentage (35%).kofta (29) with the percentage (62.5%) and the unaccepted samples (11) with the percentage (27.5%). sausage (28) with the percentage (70%) and the unaccepted samples (12) with the percentage (30%) and luncheon (37) with the percentage (92.5%) and the unaccepted samples (3) with the percentage (7.5%).

This study was directed to recognize some virulance genes that play an important role in virulence of Salmonella strains by using one of the resent development molecular biological techniques (PCR). The genes were invasion gene (*invA*) and hyper –invasive locus gene(*hilA*).

PCR result show that invasion gene (*invA*) and hyper –invasive locus gene (*hilA*) were detected in *S. Enteritis, S. Paratyphi and S.Typhimurium,* but *S. Infantis* only positive for invasion gene (*invA)*. These results agree with Ed-dra, *et al.,* (2017) who found that all *Salmonella* strains (34) were positive for

invasion gene *invA* and negative for the virulence gene *spvC*, and agree with Sallam, *et al.*, (2014) detected *gyrB* and *invA* genes in all isolated serotypes while Karmi. (2013) All *Salmonella* isolates were positive for the *invA* gene, also Eldesouky, *et al.,* (2016) detected *invA* gene and hyper-invasive locus (*hilA*) in isolated serotype of *Salmonella* and dis agree with Salehi *et al.,* (2010) who isolated *spvA*, *spvB* and *spvC* genes from *S. enteritidis*. (Table 4 and phase 1)

REFERENCES

Al-Mutairi, M. F. (2011): The incidence of Enterobacteriaceae causing food poisoning in some meat products. *Advance Journal of Food Science and Technology*, *3*(2), 116-121.

Abd El Tawab, A. A., El-Hofy, F. I., Maarouf, A. A., & El-Said, A. A. (2015): Bacteriological studies on some food borne bacteria isolated from Chicken meat and meat products in Kaliobia Governorate. *Benha Veterinary Medical Journal*, *29*(2), 47-59.

American Public Health Association "APHA'' (1992): Compendium methods the microbiological examination of food. 3rd Ed. APHA, microbiological methods for foods, Washington, D. C., USA.

Bingol, E. B., Dumen, E., Kahraman, T., Akhan, M., Issa, G., & Ergun, O. (2013): Prevalence of *Salmonella* spp., Listeria monocytogenes and Escherichia coli O157 in meat and meat products consumed in Istanbul. *Med weter*, *69*, 488-491.

Cetinkaya, F., Mus, T. E., Cibik, R., Levent, B., & Gulesen, R. (2012): Assessment of microbiological quality of cig kofte (raw consumed spiced meatball): Prevalence and antimicrobial susceptibility of Salmonella. *Food Control*, *26*(1), 15-18.

Ed-dra, A., Filali, F. R., Karraouan, B., El Allaoui, A., Aboulkacem, A., & Bouchrif, B. (2017). Prevalence, molecular and antimicrobial resistance of *Salmonella* isolated from sausages in Meknes, Morocco. *Microbial pathogenesis*, *105*, 340-345.

Eldesouky, I. E., Eissa, M. O., Nada, H. S., & Satar, A. A. (2016):Molecular characterization of *Salmonella* species isolated from some meat products. *J. Nat. Sci.*, *14*, 83-89.

El-Dosoky, H. F. A., Shafik, S., & Baher, M. (2013):Detection of spoilage and food poisoning bacteria in some ready to eat meat products in Dakahlia Governorate. Assiut Vet. Med. J, 59(138), 71-78.

Essa, H. H., Manaa, A. M., Makar, N. H., & Sayed, S. M. (2009): Studies on *Salmonella* and E. coli in some meat products (beef burgers and luncheon) sold in Assiut city. *Assiut Veterinary Medical Journal*, *55*(121), 126-135.

Guillier, L., Danan, C., Bergis, H., Delignette-Muller, M. L., Granier, S.,Rudelle, S., & Brisabois, A. (2013): Use of quantitative microbial risk assessment when investigating foodborne illness outbreaks: The example of a monophasic *Salmonella* Typhimurium 4, 5, 12: i:− outbreak implicating beef burgers. *International journal of food microbiology*, *166*(3), 471-478.

Guo X., Chen J., Beuchat, L. and Brackett, R. (2000): PCR detection of Harbor, NY, USA, 3rd edition, 2001.

Hassanin, F. S., Reham, A. A., Shawky, N. A., & Gomaa, W. M. (2014): Incidence of Escherichia coli and *Salmonella* in Ready to eat Foods. *Benha Vet Med J*, *27*(1), 84-91.

ISO, International Organization for Standardization. 2017. Microbiology of the food chain - Horizontal method for the detection, enumeration and serotyping of *Salmonella.*

Karmi, M. (2013): Detection of virulence gene (invA) in *Salmonella* isolated from meat and poultry products. *Int. J. Genet*, *3*(2), 07-12.

Kauffman, G. (1974): Kauffmann white scheme. J. Acta. Path. Microbiol. Sci., 61:385.

M Osman, N. A., E Suliman, S., Y Alian, Y., & A Abdalla, M. (2018): Prevalence of Salmonella, Escherichia coli in Meat Products in Khartoum State.

Mrema, N., Mpuchane, S., & Gashe, B. A. (2006): Prevalence of *Salmonella* in raw minced meat, raw fresh sausages and raw burger patties from retail outlets in Gaborone, Botswana. *Food Control*, *17*(3), 207-212.

Saleh, E. A., Ali, H. A., & Abu-Khadra, A. M. (2010): Detection of some food poisoning microorganisms in some meat products. *Alexandria Journal of Veterinary Sciences*, *31*(1), 27-33.

Sallam, K. I., Mohammed, M. A., Hassan, M. A., & Tamura, T. (2014): Prevalence, molecular identification and antimicrobial resistance profile of *Salmonella* serovars isolated from retail beef products in Mansoura, Egypt. *Food control*, *38*, 209-214.

Samaxa, R. G., Matsheka, M. I., Mpoloka, S. W., & Gashe, B. A. (2012): Prevalence and antimicrobial susceptibility of *Salmonella* isolated from a variety of raw meat sausages in Gaborone (Botswana) retail stores. *Journal of food protection*, *75*(4), 637-642.

Sambrook, J.; Fritsch, E. F. and Maniatis, T. (1989): Molecular cloning: Laboratory Manual. 2nd Edition, Cold spring, Harbor, New York, USA.

Shanmugasamy, M.; Velayutham, T. and Rajeswar, J. (2011): Inv A gene.

Singh, S., Singh, H., Tewari, S., Prejit, N. and Agarwal R. (2013): Characterization of virulence factors among diverse *Salmonella* serotypes and sources. Adv. Anim. Vet. Sci., 1(2): 69–74.

SOLTAN, D. M. M., Vahedi, S., ZERAATI, H., & Kalantar, E. (2009): Incidence of *Salmonella* serovars and its antimicrobial pattern in barbecued meat and ground beef burgers in Tehran.

Stock, K., & Stolle, A. (2001): Incidence of *Salmonella* in minced meat produced in a European Union–approved cutting plant. *Journal of food protection*, *64*(9), 1435-1438.