Detection of *salmonella enteritidis* in some meat products by using PCR

[1]Ashraf A. Abd El-Tawab, [1]Fatma I. El-Hofy, [2]Khalid I. Alekhnawy, Doaa M. Sharaf
[1]Bacteriology, Immunology and Mycology Dep., Fac .Vet .Med. Medicine ,Benha Uni., [2]Animal Health Research Institute, Tanta branch

A B S T R A C T

A total of 150 random samples were collected from different supermarkets and retailers of different sanitation levels at Gharbeia governorate, Egypt as follows 70 samples of minced meat, 40 samples of sausage and 40 samples of beef burger. The aforementioned samples were subjected to bacteriological and serological applications to assess the prevalence of *S. Enteritidis*. The obtained results revealed that the incidence of *S. Enteritidis* in the examined samples of minced meat, sausage and beef burger were 1/70 (1.4%), 1/40(2.5%) and 0/40(0%) respectively. The isolates were submitted for serological analysis and revealed that *Salmonella Enteritidis* O 1,9,12 ad monophasic H:g, m. The antibiogram sensitivity test was applied upon the two isolates of *S. Enteritidis* and revealed that they are sensitive to chloramphinicol, amoxicillin, levofloxacin, ciprofloxacin, enrofloxacin and gentamycin but they were resistant to oxytetracycline. The virulence genes of *S. Enteritidis* isolates were determined by using multiple PCR technique for the two serologically detected *Salmonella Enteritidis* by using the following genes *inv* A, *sef* A, *sop* B and *bcf* C at 284bp, 310bp, 517bp, 467bp, respectively.

Keywords: *S. Entritidis*, Meat products, simplex PCR and virulence genes.

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1. INTRODUCTION

Salmonellosis is a serious zoonotic food-borne pathogen, which causes outbreaks and sporadic cases of gastroenteritis in human worldwide. The clinical illness characterized by fever, nausea and diarrhea, vomition and abdominal pain after an incubation period of only 12 to 72 hrs. Salmonella spp. can pass through the food chain from feed to poultry and finally human. (Rabie *et al.,* 2012). In Egypt *S.* Enteritides was isolated from broiler chickens, chicken meat and food poisoning patients (Ammar *et al*., 2010). Salmonellae are Gram negative, non-spore-forming, usually motile, facultative anaerobic bacilli belong to the family Enterobacteriaceae. Infection with Salmonella may or may not lead to fatal Salmonellosis. (Abd El-Tawwab *et al*., 2013). Meat products are gaining popularity because they represent quick easily prepared meals of low price from one side and render the processors to convert the various types of meat into unified products. On the other side, meat products are liable to harbor different types of microorganisms through a long chain of handling, processing, distribution and storage as well as preparation. Within this respect, they are considered as serious sources of food borne diseases and have been frequently linked to major outbreaks of food poisoning all over the world (Ahmed, 1999). Detection of Salmonella by using PCR method is highly specific sensitive and more important for requiring a less time procedure (Malkawi, 2003). Therefore, the present study was performed for bacteriological and serological characterization of *S.* Entritidis isolated from meat products and detection of its virulent genes by using multiplex PCR technique.

2. MATERIAL AND METHODS

2.1. Samples collection

A total of 150 samples of meat products represented by 70 minced meat, 40 sausage and 40 beef burger were randomly collected from different supermarkets and retailers of different sanitation levels at Gharbeia Governorate, Egypt. Each sample was separately packed, identified and immediately transferred in icebox under sanitary precaution to the laboratory where they were subjected to the bacteriological examination within limited time.

2.2. Preparation of samples:

At the laboratory, frozen samples were thawed by overnight refrigeration. Each sample was aseptically and carefully freed from its casings and mixed thoroughly in sterile mixer. Twenty five grams of the examined samples were weighed aseptically into sterile blender container and thoroughly homogenized with 225 ml of sterile peptone broth (Oxoid) as pre-enrichment. The homogenate was incubated at 37°C for 24 hrs. One ml of the incubated pre-enrichment homogenate were transferred to Selenite cystine broth (SC) (Difco) as selective enrichment and incubated at 37°C for 24 h. At the end of the incubation period, a loopful from the selective enrichment broth was streaked onto XLD agar , MacConky's agar and Salmonella –Shigella agar (SS) (Oxoid) and incubated at 37°C for 24 h. The plates were examined for the presence of typical colonies of Salmonellae. Smears of suspected colonies were stained with Gram's stain and examined morphologically for staining characters. Presumptive Salmonella colonies were then subjected to initial screening tests using triple sugar iron agar (TSI), lysine iron agar (LIA) , urea broth(Merck) and lysine decarboxylase . All biochemical tests were performed at 37°C for 18–24 hours including citrate utilization, indol production test, methyl red,urea hydrolysis, and Voges- Proskauer (Andrews and Hammack, 1998) .

2.3. Antimicrobial susceptibility testing :

By the Kirby –Bauer disk diffusion method (Finegold and Martin 1982), Muller Hinton broth , Muller Hinton agar and antibiotic disks are used (levofloxacin ,gentamycin, , oxytetracyclinn, Amoxycillin ,chloramphinicole, enrofloxacin and ciprofloxacin) are used. The results were interpreted according to NCCLS (2002).

2.4. DNA extraction:

DNA was extracted from the isolated *S. Enteritidis* microorganism by using QIAamp DNA Mini Kit . The oligonucleotide primers that were used for *S. Enteritidis* genes are mentioned in the following table (1). Material used for agarose gel electrophoresis were used according to (Sambrook *et al*., 1989). Aliquots of amplified PCR products were electrophoresed in 1.5 % A multi-purpose, high gel strength agarose. PCR Master Mix prepared according to (Emerald Amp GT PCR mastermix (Takara) as follows: 12.5 *µl* Of Emerald AmpGT PCR Master MIX , 1 *µl* of each primer of 20 pmol concentrations, 4.5 *µl* of PCR grade water and 6 *µl* of template DNA with total of 25 *µl* .

3. RESULTS

3.1. Prevelance of S. Enteritidis:

After culturing onto XLD (Xylose Lysine Deoxycholate) medium ,Salmonella appeared as smooth colonies with black center while onto Salmonella –Shigella agar , it appeared pale colored colonies indicated non lactose fermenting with or without black centers and onto MacConkey's agar appeared as pale colorless smooth ,transparent and raised colonies. All isolates showed similar pattern of reaction despite of the source of isolation .Urea hydrolysis, Indole reaction and Voges –Proskauer reaction showed negative results ,while TSI, Lysine Iron ,Simmon's Citrate and Methyl Red reactions showed positive results. Further, *S. Entritidis* was isolated from minced meat ,sausage and

burger with a percentage of 1.4 % ,2.5 % and 0 % respectively(table 2).

3.2. Results of antibacterial sensitivity test for S. Entritidis

The two isolates show sensitivity to the following antibiotics (chloramphinicole, levofloxacin, gentamycin, amoxicillin, enrofloxacin and ciprofloxacin) while they were resistant to oxytetracycline.

3.3. PCR results :

The two isolates of *S. Enteritidis* were tested for *inv*A, *bcf*C, *sop*B and *sef*A genes at (284bp, 467bp, 517bp, and 310bp) respectively, and the results showed that the two isolates contained *inv*A at 284 bp and the *sef*A at 310 bp, one sample only was contained *bcf*C at 467 bp and the two samples weren't containing *sop*B .As shown in table (3), and figure(1).

Table (1) The oligonucleotide primers that were used for *S*.E.genes are mentioned in the following

Primer	Sequence	Amplified product	Reference
InvA	GTGAAATTATCGCCACGTTCGGGCAA TCATCGCACCGTCAAAGGAACC	284bp	Oliveira *et al*., 2003
SopB	TCA GAA GAC GTC TAA CCACTC TACCGTCCT CATGCA CAC TC	517bp	Huehn *et al*. 2010
BcfC	ACC AGA GAC ATT GCCTTC C TTC TGC TCGCCG CTA TTCG	467bp	Huehn *et al*. 2010
sefA	GCAGCGGTTACTATTGCAGC TGTGACAGGGACATTTAGCG	310bp	Akbarmehr *et al*., 2010

Table: (2) Prevalence of S. Entritidis in meat products are shown in the following:

Samples	No. of samples	Positive	%
Beef burger	40	0	0
sausage	40	1	2.5
Frozen packed minced meat	70	1	1.4
Total	150	2	1.33

Table (3) Result of PCR Detection of S.E.

sefA				L *(A)*	*bcfC*				*invA*				L (A)	*sopB*			
1	2	3	4		5	6	7	8	9	10	11	12		13	14	15	16

4. DISCUSSION

Salmonellosis is considered one of the anthropozoonotic disease of a serious medical problem and raises great concern in the food industry. (Ashton, 1990). The worldwide distribution of salmonellosis often parallels to the patterns of trade of animal products and food, and the migration patterns of humans and animals (Penfold *et al.,* 1979; Callaghan and Simmons, 2001, Wong *et al*., 2007and Gilbert *et al*., 2010). Meat products such as minced meat, sausage and burger have popularity because they represent quick and easy prepared meat meals and solve the problem of the shortage in fresh meat of high price, which is not within the reach of large

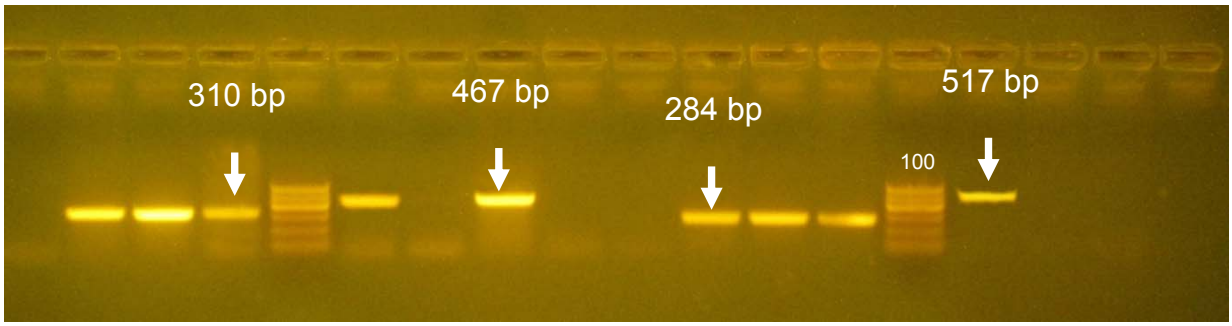

Figure (1) simplex PCR Detection for Virulence Genes of S.E. Neg: Negative Control, POS: Positive Control, Lane1 (A): marker 100-600bp DNA ladder. 1,2,3,4 show negative control for *sef*A gene, positive of sample (1), positive of sample (2) and positive control respectively. 5, 6, 7, 8 show positive control of *bcf*C gene, negative for sample (2), positive for sample (1) and negative control of the gene. 9,10,11,12 show negative control for invA gene, positive of sample (1), positive of sample (2), positive control of invA gene. 13,14,15,16 show positive control of *sop*B gene, negative of sample (2), negative of sample (1) and negative control of *sop*B gene .

number of societies with limited income. Despite of the traditional food hygiene efforts for eliminating of agents responsible for food borne illness, Salmonella remains as one of the major food borne health hazards, and meat and meat products plays an important role, as a reservoir, in disseminating Salmonellae. (Mohammed, 2000). In the present study a total of one hundred and fifty random samples of meat products, (70 samples of frozen minced meat 40 samples of frozen sausage and 40 samples of beef burger were examined for presence of *S.* Enteritidis

Salmonellae were detected in 1.33 % of the examined meat product samples. The percentage of Salmonellae in minced meat, frozen sausage and frozen beef burger, was 1.4 % , 2.5% and 0 % ,respectively. In the present study salmonellae failed to be detected in the examined beef burger samples as shown in table (2) .This result is similar to(Ismail,2006) .On the other hand, this result is not similar to (Torky,2004) who found that the incidence of salmonella was 5% in the examined samples and also (Mrema *et al*., 2006) who found that the prevalence rate of salmonella was 20%. Actually ,1.4% of the examined samples of frozen packed minced meat were positive for Salmonellae and such result is not agreed with that found by Ahmed,1992 who couldn't detect salmonella from his examined minced meat samples while (White *et al*., 2001 and Malkawi, 2003) who detected salmonella from the examined minced meat with a percentage of 20% and 87%, respectively. This variation in results might be due to difference in sampling procedure, locality and in methodology in use . In Egypt, the predominant serotype differs from one geographic area to another. This may be due to contamination during its production, handling, packing and storage (Rabei et al., 2012). The incidence of Salmonella in tested sausage was 2.5% which is nearly similar to Abdel-Aziz (1988) who found the incidence of Salmonella was 2% in the examined minced meat samples but Barrel (1982) found that the incidence of salmonella in sausage was 17.6%.While the result was not agreeds with(Elkhateib ,1982and Amal,1983) who not found salmonella in all examined. The meat product samples were collected and examined bacteriologically and the strain was typed as *S.* Entritidis which was one from minced meat and the other from sausage this agreed with (Turnbull and Phyllis, 1982). The isolated Salmonella species were (O 1,9,12 ,H1 g,m H2 -) for the two isolates which agreed with (Rabei *et al*.,2012). The antibiotic sensitivity test for the isolated strains they revealed that the two strains are resistant to oxytetracycline this disagree with(Boris *et al*., 2012), by using gentamycin the two strains also susceptible,enorfloxaqcin the

two samples are sensetiveto it ,and this agree with (Abd -El-Rahman et al., 2000) finally, by using amoxicillin they are highly sensitive this agree with (Taddele *et al.,* 2012). Multiplex-PCR assay may be available tool in epidemiological investigation and surveillance by relating isolates from different sources to a common origin(Rabie *et al*., 2012). By application of PCR the two isolates contained *inv*A (Salmonella invasion gene) gene which is a unique gene for Salmonella species amplified at 284bp . (Jamshidi *et al*., 2009). Also *sef*A (fimbrial antigen of *S.* Enteritidis) which is specific for the detection of *salmonella Entritidis* serovar was identified in all of the strains isolated at 310bp which agreed with Chagas *et al.,* (2013). When testing *bcf*C (bacterial colonization factor) gene presence in the tested samples, it was present in one sample at 467bp but not present in the second sample .Both samples show lacking of *sop*B (Salmonella outer protein B) gene that amplified at (517bp). Conventional diagnostic methods are laborious and time consuming so biotechnology detection can improve the time for reporting of the final result from several days to the next day.

5. REFERENCES

Abd El -Tawwab, A.A., Ammar, A.A., Aisha, R. Ali, Fatma, I. El-Hofy, Sayed Ahmed, M.E.E. 2013. Detection of common (*inv*A gene in Salmonellae isolated from poultry using polymerase chain reaction technique. B.V.M.J., m-25(2):70-77.

Abd El-Aziz, A.S. 1988. Salmonella in locally manufactured meat products. M .V .Sc., Thesis, Fac. Vet. Med., Cairo University.

Abd-El-Rahman, M.A., Moussa, H.M. 2000. Bacteriological and histopathological studies on *Salmonella* isolates from ducks in North Sinai. Egyptian Journal of Agricultural Research. 78(1):15-24.

Ahmed, N.M. 1992. Incidence and occurrence of salmonella and *E.Coli* organisms in packed meat products .M.V.Sc. Fac. Vet. Med. Assuit University.

Ahmed, A.F. 1999. Sanitary status of private and governmental meat cool stores. M. V. Sc. Thesis, Fac. Vet. Med., Moshtohor, Zagazig University, Benha branch.

Akbarmehr, J., Salehi, T.Z., Brujeni, G.H.N. 2010. Identification of Salmonella isolated from poultry by MPCR technique and evaluation of their hsp gro EL gene diversity based on the PCR-RFLP analysis . African Journal of Microbiology Research 4(15):1594-1598,

Amal, A.M. 1983. Sanitary Status of a meat factory. M.V.Sc. Thesis, (Meat Hygiene) Fac. Vet. Med., Cairo University.

Ammar, A.A., Ahmed, A., Asawy, Ibrahim, A. 2010. Bacteriological studies on salmonella entertidis isolated from different sources in Dakhlia governorat Assiut Vet. Med. J. 56(124):125-135.

Andrews, W.H., Hammack, T.S. 1998. Salmonella. In Bacteriological Analytical Manual Revision A, Chapter 5. Gaithersburg, MD: AOAC International.

Ashton, W.L.G. 1990. Enterobacteriaceace in Poultry Disease. 3 th. El. Baunere, pp: 11-41.

Barrel, R.A.F. 1982. Isolation of Salmonellae from human, food and environmental sources in the Manchester area: 1976 – 1980. J. Hyg. 88 (3):403 - 411.

Boris, H., Borka, S., Gordan, K., Fani, K. 2012. Antimicrobial resistance and serotyping of *Salmonella enterica* subsp. *enterica* isolated from poultry in Croatia. Vet. archive 82: 371-381.

Callaghan,M., Simmons, G.2001. Outbreak of *Salmonella* Typhimurium type 160 in Auckland linked to an umu function. NZ Public Health Report, 8:44-45.

Chagas, F.D.C., Bruno, D.A.C., Kelly, M.P.O., Alexeia, B.G. 2013. Identification and detection of Salmonella strains from chicken carcasses and environmental sources in Dourados, MS, Brazil .Afr.J. Microbiol. 7(25):3222-3228.

El-Khateb, T. 1982 .Sanitary Condition of Sausage in Assuit. M.V.Sc. Thesis (Meat hygiene), Fac. Vet. Med., Assuit. Univ.

Essam, M.I. 2006. Detection of Salmonella in some meat products using recent techniques .Fac Vet Med. Moshtohor, Benha branch, Zagazig University .

Finegold, S., Martin, W. 1982. Diagnostic Microbiology 6th Ed., C.V. Mosby Co. St. Louis Toranto, London.

Gilbert, S., Lake, R., Cressey, P., Hudson, A., King, N. 2010. Risk Profile: *Salmonella* (Non-Typoidal) in Pork and Pork Products. Institute of Environmental Science and Research Limited. India. Asian, Pac. J. 3(6):496-500.

Huen, S., La Ragione, R. M., Anjum Saunders, M., Woodward, M.J., Bunge, C., Helmuth, R., Hauser, E., Guerra, B., Beutlich, J., Brisabios, A. Peters, T. Sevnsson, L. Madajczak, G. Litrup, E. Imre A, Herrera –Leon, S., Mevius, D., Newell, D.G. ,Malorny, B. 2010. Virolotyping and antimicrobial resistance typing of salmonella enterica serovars relevant to human health in Europe. Food borne pathogens Dis 7:523-35.

Ismail, S.A. 2008. Microbiological quality of Hawawshy consumed in Ismailia, Egypt .J. Food Safety, 47:13.

Jamshidi, A., Bassami, M., Afshari-Nic, S. 2009. Identification of salmonella species and Salmonella Typhimurium by a multiplex PCR based assay from poultry carcasses in Mashhad-Iran. Int. J. Vet. Res. 3:43- 48.

Macfaddin, J. E. 1980. Biochemical test for identification of medical bacteria, 2nd Ed. The Williams and Wikins Company, Baltimore, USA.

Malkawi, H. 2003. Molecular identification of Salmonella isolates from poultry and meat products in Ibrid City, Jordan .World Journal of Microbiol & Biotechnol. 19: 455-459.

Mrema, N.M., puchane, S., Gashe, B. A. 2006. Prevalence of Salmonella in raw meat, raw fresh sausage and raw burger patties from retail outlets in Gaborone .Botswana .Trends in Plant Science, 17(3):207-212.

National Committee for clinical laboratory standards (NCCLS) 2002. Performance standards for antimicrobial disc susceptibility test. 7th Ed., approved standards M2-A8, National Committee for Clinical Laboratory Standards.

Oliveira, S.D., Rodenbusch, C.R., Cé, M.C., Rocha, S.L. 616-.S. Canal, C.W. 2003. Evaluation of selective and non-selective enrichment PCR procedures for *Salmonella* detection. Lett. Appl. Microbiol., 36(4):217-221.

Penfold, J.B., Amery, H.C., Peet, P.M. 1979. Gastroenteritis associated with wild birds in a hospital kitchen. British Med. J. 2: 802-803.

Rabie, N.S., Khalifa, N.O., Radwan, M.E.I., Afify, J.S.A. 2012. Epidemiological and Molecular Studies of Salmonella Isolates from Chicken, Chicken Meat and Human in Toukh, Egypt. Global Veterinaria 8(2):128-132.

Sambrook , J., Fritscgh , E.F. , Mentiates . 1989. Molecular cloning. A laboratory manual. Cold spring Harbor Laboratory press, New York.

Taddele, M.H., Rathore, R., Dhama, K. 2012. Antibiogram Assay of *Salmonella* Gallinarum and Other *Salmonella enterica* Serovars of Poultry Origin in India. Asi. J. Ani. and Vet. Advances, 7: 309-317.

Torky, A.A.S. 2004. Trials for inhibition of some food poisoning microorganisms in meat products PhD Thesis, Fac. Vet. Med, Cairo University.

Turnbull, M., Phyllis, R. 1982. Campylobacter jejune and Salmonella in raw red meat .J. Hyg. (Camp), 88:29-37.

White, D.G., ShaohuaZhao, D.V.M., Robert Sudler, M.S., Ayers, S., Sharton Friedman, B.A.,ShengChen,D.V.M., Mc Dermott, P.F., Shawn McDermott, B.S., Wagner, D.D., Jianghong Meng, D.V.M. 2001. Tge isolation of antibiotic resistant Salmonella from retail ground meat. The New England Journal of Medicine 345(16):1147.

Wong, T.L., Nicol, C., Cook, R., MacDiarmid, S. 2007. *Salmonella* in Uncooked Retail Meats in New Zealand. J Food Prot 70:1360-1365.