Prevalence of Virulent Genes in Salmonella Isolated from Some Raw Meat Products

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Abstract

Two hundred random samples of beef burger, sausage, minced meat and hot dog were collected from the markets in Port- Said city under aseptic condition for isolation of *Salmonella* spp. The results revealed the isolation of 7 *Salmonella* isolates with a percentage of 3.5%. The isolated Salmonellae were *S.* Anatum, *S*. Enteritidis, *S*. Hato and *S*. Lamberhurst with a percentage of 28.6%, 14.3%, 28.6% and 28.6% respectively. The antibacterial resistance of the isolates was detected for 12 antibacterial agents by disk diffusion method. Isolated *Salmonellae* were resistant to Amoxicilline/clavulanic a., Doxycycline and Erythromycin. One hundred percent of the isolated serovars were sensitive to Ciprofloxacin, Chloramphenicol and Gentamycin. PCR assays using specific primers for the detection of different virulence genes of *Salmonella* spp. proved the presence of *inv*A, *csg*D, *stn*, *omp*A and *omp*F genes in all 7 *Salmonella* serovars. The public health hazard of *Salmonella* , as well as recommended measures to improve quality status of processed meet were discussed.

Key words: *Salmonella* spp., processed meat, antibacterial resistance genes, PCR, Public health.

Introduction

Salmonella considered one of the most preponderant bacterial causes of foodborne gastroenteritis all over the world after *Campylobacter*, *Salmonella* may spread through wildlife and domestic animals' fecal contamination, poor fertilization methods, contaminated water, and other activities **(*Meldruim and Wilson, 2005)***. *Salmonella* serotypes can grow and survive in many foods ***(kimura AC, et.al., 2005).*** *Salmonella* in foods is governed by a different type of ecological and environmental factors, as pH, water activity, chemical composition, the presence of synthetic or natural

antimicrobial agents, and also storage temperature. In addition, there are other factors such as the physical manipulation and heat treatment ***(Carraminana JJ., et.al., 2004).***

In developed countries in which active coordinated foodborne disease surveillance, serotypes such as Enteritidis and Typhimurium are frequently reported.

Nowadays, global meat markets, poultry, pets, fruits, vegetables, and farm animals, considered an important sources of *Salmonella* contamination which were complex, and sometimes its control is difficult. One of the main sources of *Salmonella* infections were found in meat products. As a result of this study, surveillance of *Salmonella* contamination in meat products is further most important for the control and prevention of severe diseases ***(Ye et al., 2011).***

Bacterial antibiotic resistance has been recognized as a major medical problem facing humankind and for preventing this problem, studying of their antibiotic susceptibilities, antibiotic resistance genes and their transmission is required ***(Adel and Sabiha, 2010).***

Food have been studied as important source of antibiotic resistant microorganisms, which may persist in processed food and subsequently transmitted to environment ***(Zhang, et al., 2009)*** and so, affecting bacterial virulence among bacterial population ***(Knezevic and Petrovic, 2008).***

An update technique based on molecular biology, such as PCR method, which is rapid, specific and sensitive method were used for the detection of food borne pathogens ***(Chiu et al., 1996).***

Material and methods

Two hundred random samples of beef burger, sausage, minced meat and hot dog samples were collected from different markets in Port- Said city, Egypt under aseptic condition. All collected samples were transported in ice box to be bacteriologically examined for ***Salmonella*** isolation and identification.

Isolation and Identification of *Salmonella* spp.:

Isolation and identification of *Salmonella* was done according to ***iso 6579 (2002).***

Serological identification of *Salmonella*.

Serological identification of Somatic (O) and flagellar (H) was carried out using *Salmonella* antisera (Denka Seiken Co., Japan), ***(Cruichkshank et al., 1975). (Kauffman, 1974).***

Antibiotic susceptibility testing:

Determination of *Salmonella* susceptibility to different antimicrobial agents made using disc diffusion technique according to ***(Finegold and Martin, 1982).***

Molecular examination of *Pseudomonas*:

DNA extraction: was performed using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with the modifications recommended by the manufacturer. Oligonucleotide Primers used were synthesized in reference lab. for veterinary quality control on poultry production **(Egypt)** and were listed in table (1).

PCR amplification: DNA samples. Primers were utilized in a 25- µl reaction containing 12.5 µl of Emerald Amp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmol concentrations, 4.5 µl of water, and 6 µl of DNA template. Extraction of DNA, preparation of PCR then cycling conditions of the primers during PCR.

Analysis of the PCR Products: Conventional PCR products. The products of PCR were separated by electrophoresis on 1.5% agarose gel stain with Athidium Promide (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 15 µl of the products was loaded in each gel slot. A 100 bp and 100 bp plus DNA Ladders (Qiagen, Germany, GmbH) were used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software.

Table (1): Oligonucleotide primers sequences. (Source: Metabion, Germany).

Primer	Sequence	Amplified product	References
Stn	TTG TGT CGC TAT CAC TGG CAA CC	617 bp	Murugkar *et al*., 2003
*inv*A	GTGAAATTATCGCCACGTTCGGGCAA	284 bp	Oliveira *et al*., 2003
	TCATCGCACCGTCAAAGGAACC		
*omp*A	AGT CGA GCT CAT GAA AAAGAC AGC TAT CGC	1052 bp	Kataria *et al*., 2013
	AGT CAA GCT TTT AAG CCT GCG GCT GAG TTA		
*omp*F	CCTGGCAGCGGTGATCC	519 bp	Tatavarthy and Cannons, 2010
	TGGTGTAACCTACGCCATC		
*csg*D	TTACCGCCTGAGATTATCGT	651 bp	Bhowmick *et al*., 2011
	ATGTTTAATGAAGTCCATAG		

Results

Results of Polymerase Chain Reaction technique for *inv*A gene from DNA of the isolated *Salmonella* species.

Seven *Salmonella* isolates from a total of 7 isolates were examined by PCR for *stn* gene. Seven isolates (7/7) (100%) were positive for *stn* genes giving amplification of 284 bp fragments. Also, the positive control showed 284 bp fragments whereas no amplification could be observed with the negative control.

Results of Polymerase Chain Reaction technique for *csg*D gene from DNA of the isolated *Salmonella* species.

Seven *Salmonella* isolates from a total of 7 isolates were examined by PCR for *csg*D gene. Seven isolates (7/7) (100%) were positive for this gene giving amplification of 651 bp fragments. Also the positive control showed 651 bp fragments whereas no amplification could be observed with the negative control.

Results of Polymerase Chain Reaction technique for *stn* gene from DNA of the isolated *Salmonella* species.

Seven *Salmonella* isolates from a total of 7 isolates were examined by PCR for *stn* gene. Seven isolates (7/7) (100%) were positive for this gene giving amplification of 617 bp fragments. Also, the positive control showed 617 bp fragments whereas no amplification could be observed with the negative control.

Results of Polymerase Chain Reaction technique for *omp*A gene from DNA of the isolated *Salmonella* species.

Seven *Salmonella* isolates from a total of 7 isolates were examined by PCR for *omp*A gene. Seven isolates (7/7) (100%) were positive for this gene giving amplification of 1052 bp fragments. Also, the positive control showed 1052 bp fragments whereas no amplification could be observed with the extracted DNA of the negative control.

Results of Polymerase Chain Reaction technique for *omp*F gene from DNA of the isolated *Salmonella* species.

Seven *Salmonella* isolates from a total of 7 isolates were examined by PCR for *omp*F gene. Seven isolates (7/7) (100%) were positive for this gene giving amplification of 519 bp fragments. Also, the positive control showed 519 bp fragments whereas no amplification could be observed with the extracted DNA of the negative control.

Table 2. Incidence, Numbers and percentage of different serotypes from the isolated *Salmonella* samples. (n=7)

Type of samples	Total sample processed	Total positive	serotypes	Positive cases	Percentage
Beef burger	50	2	*S.Anatum*	1	14.3
			S.Enteritidis	1	14.3
sausage	50	2	S.Hato	1	14.3
			S.Lamberhust	1	14.3
Minced meat	50	3	S.Anatum	1	14.3
			S.Hato	1	14.3
			S.Lamberhust	1	14.3
Hot dog	50	0	0	0	0
total	200	7	S.Anatum	1	14.3
			S.Hato	2	28.6
			S.Lamberhust	2	28.6
			S.Entritidis	2	28.6

Table 3. Antigenic structure of the isolated *Salmonella* samples.

No	*Salmonella* serotypes	O antigen	H antigen	
			Phase I	Phase II
1, 6	***S*. Anatum**	3,{10},{15},{15,34}	e,h	1,6
2	***S*. Enteritidis**	9, 12	g,m	-
3, 4	***S*. Hato**	1, 4, {5}, 12	g, m, s	{1, 2}
5, 7	***S*. Lamberhurst**	3,10	e,h	e,n, Z_{15}

Table 4. *Antibiogram of the obtained Salmonella isolates*.

Anti-bacterials	Code no. of samples						
	S. Anatum	S. Enteritidis	S. Hato	S. Hato	S. Lamberhut	S. Anatum	S. Lamberhurt
	1	2	3	4	5	6	7
AMC	R	R	R	R	R	R	R
CTX	R	I	I	I	S	R	S
C	S	S	S	S	S	S	S
CIP	S	S	S	S	S	S	S
CT	I	I	R	R	R	I	R
DO	R	R	R	R	R	R	R
E	R	R	R	R	R	R	R
CN	S	S	S	S	S	S	S
NA	I	I	S	S	S	I	S
NOR	I	S	S	S	I	I	I
RF	I	S	R	R	I	I	I
SXT	I	S	I	I	I	I	I

S: sensitive, R: Resistance, I: Intermediate
{AMC (amoxicillin / clavulinic acid), CN (gentamycin), CT (colistin - sulfate), CTX (cefotaxime), SXT (trimethoprim/sulphamethoxazole), DO (doxycycline), C (chloramphenicol), RA (rifampin), CIP (ciprofloxacine), NOR (norfloxacine), NA (nalidixic acid) E (erythromycin)}. So.these antibiotics are the most use at the field.

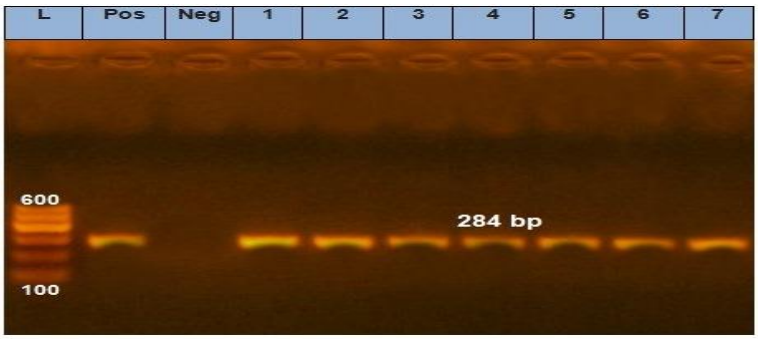

Figure 1. Agarose gel electrophoresis for *inv*A gene.
agarose gel electrophoresis showing specific PCR of Salmonella isolates using invA gene primer (284 bp).
Neg = negative control & Pos = positive control & L= ladder (100-1000 bp) & all lanes showed positive results confirmed that all Salmonella isolates were positive for invA gene.

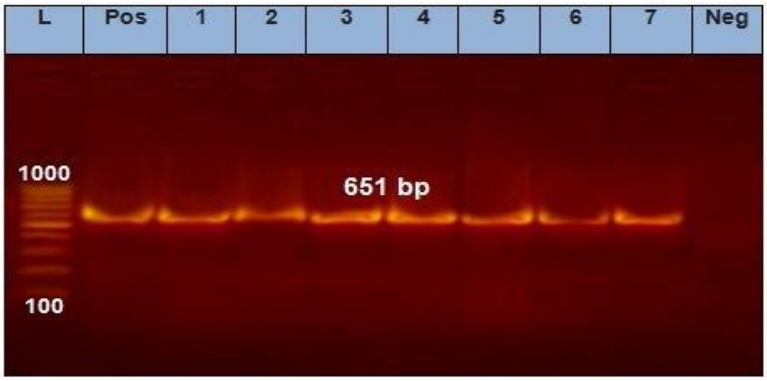

Figure 2. Agarose gel electrophoresis for *csg*D gene.
agarose gel electrophoresis showing Salmonella specific PCR using primer for csgD gene (651 bp).
Neg = negative control & Pos = positive control & L= ladder (100-1000 bp) & all lanes showed
positive results confirmed that Salmonella isolates were positive for csgD gene.

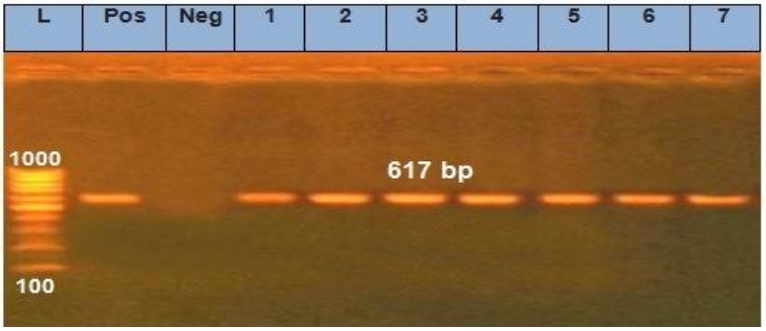

Figure 3. Agarose gel electrophoresis for *stn* gene.
agarose gel electrophoresis showing *Salmonella* specific PCR using primer for *stn* gene (617 bp).
Neg = negative control & Pos = positive control & L= ladder (100bp) & all lanes showed positive results confirmed that all *Salmonella* isolates were positive for *stn* gene.

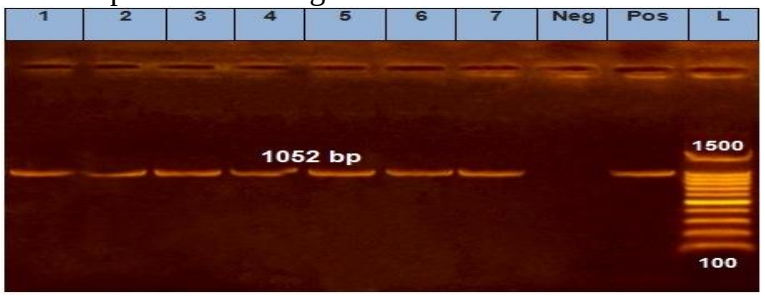

Figure 4. Agarose gel electrophoresis for *omp*A gene.
agarose gel electrophoresis showing Salmonella specific PCR using

primer for ompA gene (1052 bp).
Neg = negative control & Pos = positive control & L= ladder (100bp) & all lanes showed positive results confirmed that all Salmonella isolates were positive for ompA gene.

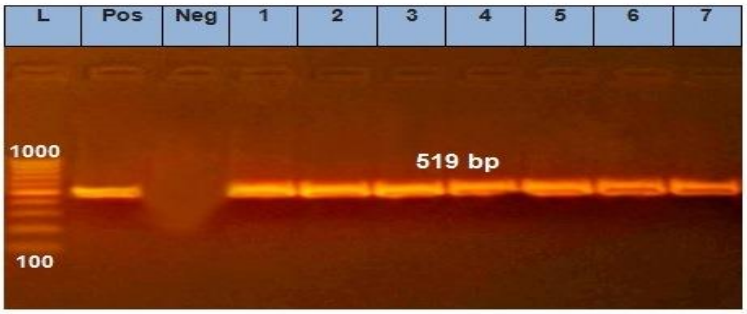

Figure 5. Agarose gel electrophoresis for *omp*F gene.
agarose gel electrophoresis showing Salmonella specific PCR using primer for ompF gene (519 bp).
Neg = negative control & Pos = positive control & L= ladder (100bp) & all lanes showed positive results confirmed that all Salmonella isolates were positive for ompF gene.

Discussion

Salmonella considered an important source for foodborne diseases, resulting in health problems worldwide. Differences in the prevalence of specific serotypes may be related to the movements of foods, animals and people. Nowadays, Typhimurium and Enteritidis considered the most frequent serotypes implicated in *Salmonella* outbreaks from foods all over the world ***(Zhou et al., 2019).***

In this study, the incidence of *Salmonella* in raw meat products (table2) was 3.5% (7 out of 200 raw meat products) and these results was nearly coincide with ***Bingol et al. (2013)*** who examined a total of 340 samples of meat and meat products (205 ground beef, 50 raw beef and 85 sausages) collected from producers and retailers in Istanbul. *Salmonella* spp. was detected in 1.18% of the tested meat and meat products. The isolated serovars were *S.* Anatum in ground beef and *S.* Reading and *S*. Meleagridis in sausage samples. Other studies stated the presence of *Salmonella* spp. in 1.4%, 2.0% and 2.08%, respectively ***(Scheelhaas et al., 1976; Kleinlein et al., 1989; Aabo et al., 1995).***

The incidence of *Salmonella* spp. in raw meat products in this study was lower in comparison to those detected by ***Pietzsch and Kawerau. (1981)***, (45.2%),

Al-Rajab et al. (1986) (18.0%), ***El-Leithy and Rashad. (1989)*** (15.0%), ***Baskaya et al. (2004)*** (11.1%) and ***Woldemariam et al. (2005)*** (12.1%). The reason for high contamination rates may be due to the use of contaminated raw materials, lack of proper heating and inadequate packaging as indicated in ***EFTA report journal (2012).***

In the present work, antibiogram was done by disk diffusion method to explore antibiogram result as shown in **table 5**.

Data in **table 5** illustrated all 7 serotypes (100%) were found to be resistant to Amoxycillin, doxycyclin and Erythromycin, 4 (57.14%) to Colistin sulphate, 2 (28.57%) to Ceftriaxone and Rifampicin.

While, all isolated serotypes (100%) were found to be sensitive to chloramphenicol, ciprofloxacin and gentamicin, 4 (57.14%) to Nalidixic acid, 3(42.86) to Norfloxacin, 2 (28.57%) to Ceftriaxone, 1(14.29%) to Sulfamethoxazole-trimethoprime.

In present study susceptibility of *Salmonella* isolates to Ciprofloxacin, Chloramphenicol and Gentamicin was 100%. These results go hand in hand with ***Fazlina et al. (2012)*** who found that, susceptibility of their *Salmonella* isolates to gentamicin, ciprofloxacin and chloramphenicol was 95%, 90% and 80%, respectively, high resistance was observed against amoxicillin-clavulanic acid (100%) and erythromycin (80%). Also, these results agreed with the results obtained by ***Miko et al. (2005)*** who detected a lesser number of isolates that were resistant to gentamycin and high percentage of resistance to amoxicillin and Sulfamethoxazole-trimethoprime.

On the other hand, these results differ from those obtained by ***Yang et al. (2001)*** who found that, resistance of their *Salmonella* isolates to Sulfamethoxazole-trimethoprime, nalidixic acid, Amoxycillin, chloramphincol and Gentamycin was 58%,35%,32%,26% and 26% respectively. The same results obtained by ***Harakeh et al. (2005)*** who found that, 86 and 57% of *Salmonella* isolates from fast meat-based food in Lebanon were resistant to trimethoprim-sulfamethoxazole and gentamicin, respectively.

Regarding antimicrobial susceptibility testing of *Salmonella* serovars to 12 different antibacterial, *Salmonella* isolates showed different degree of sensitivity to antimicrobial agents, higher degree of sensitivity were

observed to ciprofloxacin, gentamicin and Chloramphenicol with percentages matched to those found in many developing countries, especially Bangladesh, Nigeria, and Pakistan ***(Habrun et al., 2012; Putturu et al., 2013; Umeh et al., 2014).***

Resistance of *Salmonella* to Amoxicillin/clavulanic acid was (100%). These results agree with another report in South India, ***(Suresh et al., 2006)*** and higher than results discovered in Eastern China (80%) ***(Lu et al., 2014).*** resistance to doxycycline was 100%. These results agree with the discussed results in Eastern China ***(Lu et al., 2014).*** This result differences may be attributed to illegal use of antimicrobials at therapeutic doses as food additives to stimulate growth and as chemotherapeutic agent to control the spread of epizootic diseases in farms.

In the present investigation, it was noted an incidence of multidrug resistance among all 7 *Salmonella* isolates which was higher than that obtained previously by ***Shen et al.,(2008)*** (28.5%) and ***Ahmed et al., (2009)*** (14.4%). ***Schwarz et al. (2001)*** and ***Zouhairi et al. (2010)*** attributed the exacerbation of this MDR to the diminishing of new antibiotics and considered as a serious danger to public health.

Many authors have detected multi drug resistance of isolates from meat ***(Yang et al., 2001; Capita, 2003; Romani et al., 2008; Hur et al., 2011; Yildirim et al., 2011).***

From the above-mentioned results, it is important to note that, *Salmonella* can easily acquire multiple resistances to most antimicrobials and transfer them to human especially through food chain. Recently, the generation and transmission mechanisms of the drug resistance genes became an important research topic to control the spread of multidrug-resistant bacteria. The high occurrence of resistant bacteria reported in a lot of publications may be because of the worldwide overuse of antibacterial among different fields, inducing high pressure for the proper selection of antibiotic resistance among different bacterial pathogens.

Oliveira et al. (2003) revealed that, PCR is highly specific and sensitive and a fast procedure than traditional microbiological techniques for isolation and identification of *Salmonella*. PCR using primers of *inv*A specific for *Salmonella*, considerably decreases false-negative results which usually occur in laboratories. Amplification of *inv*A gene is

now considered as an international procedure for *Salmonella* identification.

The PCR targeting *Omp*A specific for *Salmonella* cosidered highly specific in detection of *Salmonella* serovar alone and its' sensitivity was up to 68.8 fg. ***(Kataria et al., 2013).*** In the present study, PCR assay made for the detection of *inv*A gene in seven isolated strains. It has been revealed that, it was present in all isolates (100%) that was detected by the presence of 284 bp PCR amplified fragment. The results in the present study were agreed with ***(Nagappa et al. (2007); Dione et al. (2011); Shanmugasamy et al. (2011).*** No amplified DNA fragments were obtained from non-*Salmonella* spp. The *inv*A gene contains sequences unique to *Salmonella* and proved to be a suitable PCR target, with an important diagnostic applications ***(Rahn et al., 1992).***

PCR was made for detection of *csg*D gene from isolated serotypes showed that, it was present in all of isolates (100%) which was proved by the presence of a 651 bp PCR product. The results obtained in this study was in corroboration with ***(Eckmann et al., 1997).***

In this study, PCR carried out for the detection of *stn* gene in *Salmonella* isolates showed that, the gene was present in all isolates (100%) and was detected by the presence of a 617 bp PCR product. These findings agreed with ***(Prager et al., 1995; Rahman, 1999; Murugkar et al., 2005)***. Observations from this study proved that, ***stn*** gene is widely distributed among *Salmonella* serotypes.

In addition to that, PCR carried out for the detection of *omp*A gene for *Salmonella* isolates showed that, the gene was present in all isolates (100%) that was detected by the presence of 1052 bp PCR product. We found an agreement with our results in previous studies ***(Nair et al., 2006; Dadmehr et al., 2011; Kataria et al., 2013; Fekry et al., 2018).***

Finally, the detection of *omp*F gene in the isolated *Salmonella* samples was carried out using PCR assay. The gene was present in all isolates (100%) that was detected by the presence of a 519 bp PCR product. These findings agreed with ***(Coldham et al., 2010; Al-Habsi et al., 2018).***

References

Aarestrup, F.M.; Hasman, H.; Jensen, L.B.; Moreno, M.; Herrero, I.A.; Domínguez, L.; Finn, M.; Franklin, A. (2002): antimicrobial resistance among

enterococci from pigs in three european countries. Appl. environ. microbiol. 68: 4127–4129.

Adel, K.K. and Sabiha, S.S. (2010): genetic site determination of antibiotic resistance genes in ***pseudomonas aeruginosa*** by genetic transformation. Br. J. Pharmacol. Toxicol. 1: 85–89

Adeyemi, K. and Oyetoran B.O (2014): presence of psychrophilic bacteria in meat and surface samples obtained from kara abattoir in ogun state (western region), nigeria. current research in microbiology and biotechnology, 2(4): 412-415.

Ahmed, A., Shimabukuro, H. and Shimamoto, T. (**2009**): isolation and molecular characterization of multidrug-resistant strains of *Escherichia coli* and *Salmonella* from retail chicken meat in japan', *journal of food science*, 74(7), pp. M405-10. doi: 10.1111/j.1750-3841.2009.01291.x.

Al-Rajab, W., Al-Chalabi, K. and Sulayman, S. (**1986**) 'incidence of *Salmonella* in poultry and meat products in iraq', *J. food microbiol*, 3, pp. 55–57.

''APHA'' American Public Health Association (2004): compendium of methods for the microbiological examination of foods, 5th edition. washington, dc 2005.

Arnaut-Rollier, I.; Vauterin, L.; De Vos, .;Massart, D.L.; Devriese, L.A.; De Zuler, L. and Van Hoof, J. (1999): a numerical taxonomic study of the *Pseudomonas* flora isolated from poultry meat. journal of applied micobiology, 87: 1528.

Baskaya, R., Karaca, T., Sevinc, I., Cakmak, O., Yýldýz, A. and Yoruk, M. (**2004**) 'the histological, microbiological and serological quality of ground beef marketed in ystanbul', *Y.Y.U. Vet. fak. derg*, 15, pp. 41–46.

Braden CR. Salmonella enterica serotype Enteritidis and eggs: a national epidemic in the united states. clin Infect dis. (2006); 43: 512-7.

Carraminana JJ, Rota C, Augutin I, Herrera Am. High prevalence of multiple resistance to antibiotics in Salmonella serovars isolated from poultry slaughterhouse in Spain. Veter Microbiol. (2004); 104: 133-9

Capita, R., Alonso-Calleja, C. and Prieto, M. (**2007**) 'Prevalence of *Salmonella* enterica serovars and genovars from chicken carcasses in \slaughterhouses in spain', *journal of applied microbiology*, 103(5), pp. 1366–1375. doi:

10.1111/j.1365-2672.2007.03368.x. Centers for disease control and prevention (CDC). Preliminary food net data on the incidence of infection with pathogen transmitted commonly through food 10 state/2005. MMWR Morb Mortal Wkly Rep. 2006; 55(14): 392-5. Centers for disease control and prevention (CDC). Preliminary food net data on the incidence of infection with pathogen transmitted commonly through food 10 state/2005. MMWR Morb Mortal Wkly Rep. 2006; 55(14): 392-5. Centers for disease control and prevention (CDC). Preliminary food net data on the incidence of infection with pathogen transmitted commonly through food 10 state/2005. MMWR Morb Mortal Wkly Rep. 2006; 55(14): 392-5.

Centers for disease control and prevention (CDC). Preliminary food net data on the incidence of infection with pathogen transmitted commonly through food 10 state/(2005). Mmwr morb mortal wkly rep. (2006); 55(14): 392-5.

Chiu, C. and Ou, J. (**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', *journal of clinical microbiology*, 34(10), pp. 2619–2622. doi: 10.1128/jcm. 34.10.2619-2622.

Cruickshank, R.; Duguid, J. P.; Marmion, B. P. and Swain, R. H. A. (1975): medical microbiology, 12th ed., vol. II. churchill livingstone, edinburg, london and new york.

Coldham, N., Webber, M., Woodward, M. and Piddock, L. J. (**2010**) 'A 96- \well plate fluorescence assay for assessment of cellular permeability and active efflux in *Salmonella enterica* serovar Typhimurium and *Escherichia coli*', *journal of antimicrobial chemotherapy*, 65(8), pp. 1655–1663. doi: 10.1093/jac/dkq169.

Cortez, A., Carvalho, A., Ikuno, A., Burger, K. and Vidal-Martins, A. (**2006**) 'identification of *Salmonella* spp. isolates from chicken abattoirs by multiplex-PCR.Res', *J. Vet. Sci*, 81(3), pp. 340–344.

Dalton CB, Gregory J, Kirk MD, et al. Foodborne disease outbreaks in Australia, (1995 to 2000). Commun Dis Intell. 2004; 28(2): 211-4.

Darwin, K. and Miller, V. (**1999**) 'molecular basis of the interaction of *Salmonella* with the intestinal mucosa', *clinical microbiology reviews*, 12(3), pp. 405–428. doi:

10.1128/cmr.12.3.405.

Eckmann, L. *et al.* (1997) 'd-myo-inositol 1,4,5,6-tetrakisphosphate produced in human intestinal epithelial cells in response to *Salmonella* invasion inhibits phosphoinositide 3-kinase signaling pathways', *proceedings of the national academy of sciences of the united states of america*, 94(26), pp. 14456–14460. doi: 10.1073/pnas.94.26.14456.

El-Leithy, M. and Rashad, M. (1989) 'bacteriological studies on ground meat and its products', *arch. lebensmittelhyg*, 4, pp. 49–72.

Fazlina, F., Al-Sultan, I. and Jasbir, S. (2012) 'antibiotic sensitivity of pathogenic bacteria isolated from beef samples obtained from kota bharu and Its surrounding provinces', *international journal*, 2, pp. 8–11.Gomez TM, Motarjemi Y, Miyagawa S, Kaferstein FK, Stohr K. Foodborne Salmonellosis. World Health Stat. Q 1997; 50: 81-9.

Habrun, B., Šimpraga, B., Kompes, G. and Krstulović, F. (2012) 'antimicrobial resistance and serotyping of *Salmonella enterica* subsp. *enterica* isolated from poultry in croatia', *veterinarski arhiv*, 82(4), pp. 371–381.

Harakeh, S. *et al.* (2005) 'isolation, molecular characterization and antimicrobial resistance patterns of *Salmonella* and *Escherichia coli* isolates from meat-based fast food in Lebanon.', *the science of the total environment*. netherlands, 341(1–3), pp. 33–44. doi: 10.1016/ j.scitotenv. 2004.09.025.

Kataria, J., Kumar, A., Rajagunalan, S., Jonathan, L. and Agarwal, R. (2013) 'detection of OmpA gene by PCR for specific detection of *Salmonella* serovars', *veterinary world*, 6(11), pp. 911–914. doi: 10.14202/vetworld.2013.911-914.

Kauffmann, F. (1950) 'international *Salmonella* Centre.', *bulletin of the world health organization*. world health organization, 3(1), pp. 171–5. available at: http://www.ncbi.nlm.nih.gov/pubmed/14792270

Kimura AC, Palumbo MS, Meyers H, Abbott S, Rodriguez R, Werner SB. amulti-state outbreak of *Salmonella* serotype thompson infection from commercially distributed bread contaminated by an ill food handler. Epidemiol Infect. 2005; 133: 823-8.

Knezevic, P. and Petrovic, O. (2008): antibiotic resistance of commensal *Escherichia coli* of food-producing animals from three vojvodinian farms, serbia. Int. J. antimicrob. agents, 31: 360–363.

Lampel, K., Orlandi, P. and Kornegay, L. (**2000**) 'improved template preparation for pcr-based assays for detection of food -borne bacterial pathogens', *appl. environ.microbiol*, 66, pp. 4539–4542.

Lu, Y. *et al.* (**2014**) 'characterization of multidrug-resistant *Salmonella enterica* serovars indiana and enteritidis from chickens in eastern china', *plos one* . edited by r. schuch. public library of science, 9(5), p. e96050. doi: 10.1371/journal.pone.0096050.

Meldrum RJ, Wilson IG.(2007) *Salmonella* and *Campylobacter* in United Kingdom retail raw chicken in(2005). J Food Protect. 2007; 70: 1937-9.

Miko, A., Pries, K., Schroeter, A. and Helmuth, R. (**2005**) 'molecular mechanisms of resistance in multidrug-resistant serovars of *Salmonella enterica* isolated from foods in germany', *journal of antimicrobial chemotherapy*, 56(6), pp. 1025–1033. doi: 10.1093/ jac/dki365.

Nagappa, K., Tamuly, S., Brajmadhuri, Saxena, M. and Singh, S. (**2007**) 'isolation of *Salmonella* Typhimurium from poultry eggs and meat of tarai region of uttaranchal', *indian journal of biotechnology*, 6(3), pp. 407–409.

Nair, M. and Venkitanarayanan, K. (**2006**) 'cloning and sequencing of the *omp*A gene of enterobacter sakazakii and development of an *omp*A-targeted pcr for rapid detection of enterobacter sakazakii in infant formula', *applied and environmental microbiology*, 72(4), pp. 2539–2546. doi: 10.1128/AEM.72.4.2539-2546.2006.

Oliveira, S., Rodenbusch, C., Cé, M., Rocha, S. and Canal, C. W. (**2003**) 'evaluation of selective and non-selective enrichment pcr procedures for *Salmonella* detection', *letters in applied microbiology*, 36(4), pp. 217–221. doi: 10.1046/j.1472-765X.2003.01294.x.

Prager, R., Fruth, A. and Tschäpe, H. (**1995**) 'Salmonella enterotoxin (*stn*) gene is prevalent among strains of *Salmonella enterica*, but not among *Salmonella bongori* and other enterobacteriaceae.', fems *immunology and medical microbiology*, 12(1), pp. 47–50. doi: 10.1111/j.1574-695X.1995.tb00173.x.

Rahn, K. *et al.* (1992) 'amplification of an *inv*A gene sequence of *Salmonella* Typhimurium by polymerase chain reaction as a specific method of detection of *Salmonella*', *molecular and cellular probes*, 6(4), pp. 271–279. doi: 10.1016/0890-8508(92)90002-F.

Schwarz, S. and Chaslus-Dancla, E. (2001) 'use of antimicrobials in veterinary medicine and mechanisms of resistance', in *veterinary research*, pp. 201–225. doi: 10.1051/vetres:2001120.

Shen, J. *et al.* (2008) 'detection and analysis of antibiotic resistance of *Salmonella* from retail meats in some districts of Shaanxi province.', *zhonghua yu fang yi xue za zhi [chinese journal of preventivemedicine]*,42(10),pp. 75861.Availableat:http://www.ncbi.nlm.nih.gov/ pubmed/ 19178840 (Accessed: 24 February (2020).

Suresh, T., Hatha, A., Sreenivasan, D., Sangeetha, N. and Lashmanaperumalsamy, P. (2006) 'prevalence and antimicrobial resistance of *Salmonella* Enteritidis and other Salmonellae in the eggs and egg-storing trays from retails markets of Coimbatore, South India', *food microbiology*. food microbiol, 23(3), pp. 294–299. doi: 10.1016/j.fm.2005.04.001.

Threlfall E J, Ward L R, Frost J A, Willshaw GA. The emergence and spread of antibiotic resistance in food-borne bacteria. Int J Food Microbial. 2000; 62: 1-5.

Woldemariam, E., Molla, B., Alemayehu, D. and Muckle, A. (2005)

'prevalence and distribution of *Salmonella* in apparently healthy slaughtered sheep and goats in debre Zeit, ethiopia', *small ruminant research*. elsevier, 58(1), pp. 19–24. doi: 10.1016/j.smallrumres. 2004. 08.008.

Yang, S. *et al.* (2001) 'multidrug-resistant *Salmonella* Typhimurium and

Salmonella Enteritidis identified by multiplex pcr from animals.', *journal*

of veterinary science, 2(3), pp. 181–8. Available at:

http://www.ncbi.nlm.nih.gov/pubmed/12441686 (Accessed: 24 February 2020).

Ye, Y., Wu, Q., Zhang, J., Lu, J. and Lin, L. (2011) 'isolation of *Salmonella* from meat samples and characterization by enterobacterial repetitive intergenic consensus-polymerase chain reaction and antibiotics test', *foodborne pathogens and disease*. mary

ann liebert, Inc. 140 huguenot Street, 3rd floor new rochelle, ny 10801 USA, 8(8), pp. 935–937. doi: 10.1089/fpd.2010.0799.

Zhou, G.H.; Xu, X.L. and Liu, Y. (2010): preservation technologies for fresh meat-a review. meat sci., 86: 119-128

Zhou, M., Li, X., Hou, W., Wang, H., Paoli, G. and Shi, X. (2019) 'incidence and characterization of *Salmonella* isolates from raw meat products sold at small markets in hubei province, china', *frontiers in microbiology*, 10, p. 2265.Availableat:

https://www.frontiersin.org/article/10.3389/fmicb.2019.02265.

الملخص العربى

في هذه الدراسة تم تجميع 200 عينة عشوائيا من مصنعات اللحوم المبردة المباعة في أسواق مدينة بورسعيد للكشف عن مدى تواجد ميكروبات ال *Salmonella*. و أظهرت نتائج الفحص البكتريولوجي أن الميكروب تم عزله بنسبة 3,5%من العينات. و كانت انواع العترات المعزولة من مصنفات اللحوم المبردة هي *S*.Anatum بنسبة (28.6%) و S.Enteritidesبنسبة (14.3 %)و S.Hato بنسبه (28.6%)و S. Lumberhurstبنسبه (28.6%) قد أجرى اختبار الحساسية لعدد 7 عترة من ميكروبات ال*Salmonella* واشارت النتائج الي انها حساسه للجنتاميسين -سيبروفلوكساسين الكلورمفينكول -سلفات الكوليستين والانروفلوكساسين بينما وجد الارثرومايسين والفلومكوين اقل تاثيرا علي انواع السالمونيلا .

, اظهرت النتائج تواجد جينات الضراوة (*inv*A,*csg*D,*stn*,*omp*A.*omp*F) وكانت النتائج ايجابيه بنسبه 100 % وذلك بظهور التتابع الجيني الموجب لتلك الجينات الذي يوجد في جنس السالمونيلا فقط وذلك بالتفريد الكهربي علي جهاز الفصل الكهربي لحمض الديوكسي ريبونيوكليز. . مما يدل ان تقنية PCR طريقة سريعة للتحري عن تواجد جينات المقاومة للمضادات الحيوية في ميكروبات ال *Salmonella* دون اللجوء للاختبار الحساسية التقليدية. وقد تم مناقشة النتائج وبيان أهمية الميكروب المعزول و خطورته علي الصحة العامة للمستهلك و عمل التوصيات للتقليل من مخاطرها.