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MICROBIOLOGICAL STUDIES ON *SALMONELLA* MICROORGANISM IN SOME MEAT PRODUCTS

(With 4 Tables and One Photo)

By

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دراسات ميكروبيولوجية علي ميكروب السالمونيلا في بعض منتجات اللحوم

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تم دراسة مدي تواجد ميكروب السالمونيلا في عينات اللانشون والسجق واللحم المفروم المجمد في ٥٠ عينة من كل نوع وتم عزل السالمونيلا بنسبة صفر و٨% و ٦% من اللانشون والسجق واللحم المفروم المجمد علي التوالي وتم تصنيف السالمونيلا إلي سالمونيلا تيفيوريم وانتريديدس وتم إجراء اختبار الحساسية لكل منها ووجد ان كل منهما حساس الي الاموكسيللين والسيفروكسيم صوديوم والسيبروفلوكساسين والدانوفلوكساسين والاوكسى تتراسيكلين وكل منهما مقاوم للاستربتومايسين وأيضا تم إجراء اختبار البلمرة المتسلسل وكان إيجابي بالنسبة للنوعين.

SUMMARY

Hundred and fifty random samples of various meat products represented by luncheon, sausage and frozen minced meat (50 of each) were collected from different markets for detection of *Salmonella* spp., Obtained results declared that *Salmonella* could be detected in zero, 8 and 6% of the examined samples of luncheon, sausage and frozen minced meat respectively. The recovered salmonellae were serotyped as *Salmonella typhimurium* and *Salmonella enteritidis*. The in vitro senstivity tests for the isolated bacteria were determined, *Salmonella typhimurium* and *Salmonella enteritidis* were sensitive to Amoxicillin, cefuroxime sod. Ciprofloxacin, Danofloxacin and oxytetracycline and were resistant to streptomycin. *Salmonella typhimurium* and *Salmonella enteritidis* can be detected by Real-Time PCR when used Quantitect probe RT-PCR kit cat. no. 20 4443 which based on fluoroqenic primers and probe (TAMRA dye).

Key words: *Salmonella microorganism, meat products, minced meat, sausage, luncheon, antibiogram, PCR.*

INTRODUCTION

Meat products such as luncheon, sausage and minced meat have a popularity because they represent quick, easy prepared meat meals and solve the problem of the storage in fresh meat of high price which is not within the reach of large number of families with limited income (Mohamed 2006). On the other side, meat products are liable to harbour different types of microorganisms through along chain of handling, processing, distribution and storage as well as preparation (Hassanien, 2004). Within this respect meat in general and poultry in particular are the commonest source of food borne diseases and have been frequently linked to outbreaks of food poisoning by *Salmonella* (Antunes *et al.,* 2003). *Salmonella* spp. typically cause an intestinal infection with or without fever, the spread to human is usually caused by consumption of contaminated food stuffs (Bhan *et al.,* 2005). *Salmonella* is one the most common cause of food borne diseases (Tirado *and Schmidt,* 2001) for this reason, the number of rapid test methods for *Salmonella* has grown rapidly in the last decade. PCR and real time PCR have become powerful tools for detection of pathogens in food. (Malorny *et al.,* 2003 and 2004). The real time PCR in food borne outbreak investigations provides an opportunity in food and clinical settings (Burkhard *et al.,* 2004). This study was undertaken for isolation of *Salmonella* from some meat products and identification of the isolates by real- time PCR.

MATERIALS and METHOD

I- Collection of samples:

A total of 150 random samples of luncheon, sausage and frozen raw minced meat(50 of each) were collected from different markets in Egypt an examined for the presence of *Salmonella* species.

II- Isolation and Identification. (FAO, 1979).

The method for isolation and identification of *Salmonella* recommended by Edwards and Ewing (1972), Cowan and Steel (1975) and FAO (1979) was followed.

Pre-enrichment

25gm of each sample were blended with 225ml of buffered peptone water and transfered aseptically to a sterile 500ml flask then incubated at 37°C for 10-20h.

Enrichment

10ml of each pre-enrichment medium were transfered to 100ml tetrathionat broth medium and another 10ml to 100ml selenite F. broth medium previously warmed to 42-43°C and incubated at 42-43°C for 48h.

Plating on selective media:

Brilliant green agar, MacConkey agar and Salmonella-shigella agar media were streaked from each enrichment flask and incubated at 37°C for 24h., then examined for typical colonies for *Salmonella*.

Typical or suspected colonies were selected from each selective medium and streaked on nutrient agar medium which incubated at 37°C for 24h.

Morphological, biochemical and serological confirmation were performed according to Edwards and Ewing (1972), and Cowan and Steel (1975).

III- Antibiogram:

Salmonella isolates were tested for sensitivity to 15 antibiotics by the disc and agar diffusion method. The interpretation of the results was carried out according to NCCLS (2002).

V- Real-time PCR (Petra *et al.*, 2005):

A real-time PCR assay was developed based on fluorogenic primers and probe, sal-F (5' -GCGTTCTGAACCTTTGGTAATAA-3'), sal-R (5'-CGTTCGGGCAATTGGTTA-3'),and probe (5'-FAM-TGGCGGTGGGTTTGTTGTCTTCT-TAMRA-3') used for amplification of a 102-pbregio on of the inVA gene of *Salmonella.* The PCR mixture consisted of 12.5µl 2X Quantitect probe RT-PCR master Mix, 0.2 µl primer F 50 pmol, 0.2V primer R 50 pmol, 0.25V probe 30 pmol 4-5 µl RNase free water 2-5 µl template RNA (Quantitect probe RT-PCR kit catalogue no-20 4443).

RESULTS

Table 1: Incidence of *Salmonella* in the examined samples of meat products

Samples	No of samples	Positive samples	%
Luncheon	50	-	-
Sausage	50	4	8
Frozen minced meat	50	3	6

Table 2: Serotyping of *Salmonella* isolated from the examined samples of meat products (N= 50)

Serotypes	Frequency of isolation					
	Luncheon		Sausage		Frozen minced meat	
	No	%	No	%	No	%
S.typhimurium	-	-	2	4	2	4
S. enteritidis	-	-	2	4	1	2

Table 3: Antigenic formula of *Salmonella* recovered from meat products samples.

Isolated *Salmonella*	Antigenic formula		
	O	Phase 1	Phase 2
S.typhimurium	1, 4, (5), 12	I	1, 2
S. enteritidis	1, 9, 12	g,m	[1, 7]

Table 4: Antibiogram patterns of isolated *Salmonella* serovars

Antibiotic and chemotherapeutic agent	Conc.	Symbol	*Salmonella typhimurium*	*Salmonella enteritidis*
Amoxicillin	10	AML	S	S
Ampicillin	10	AMP	M	S
Cefoperazone	75	CFP	M	M
Cefotaxime	30	CTX	M	M
Cefuroxime sod	30	C	S	S
Ciprofloxacin	5	CIP	S	S
Colistin sulphate	25	CT	S	S
Danofloxacin	5	DFX	S	S
Flumequine	30	UB	S	S
Enrofloxacin	5	ENR	S	S
Nalidixic acid	30	NA	S	S
Oxalinic acid	2ug	OA	M	M
Oxytetracycline	30u	OT	S	S
Streptomycin	10ug	S	R	R

S= sensitive
M= Moderate sensitivity R= Resistant

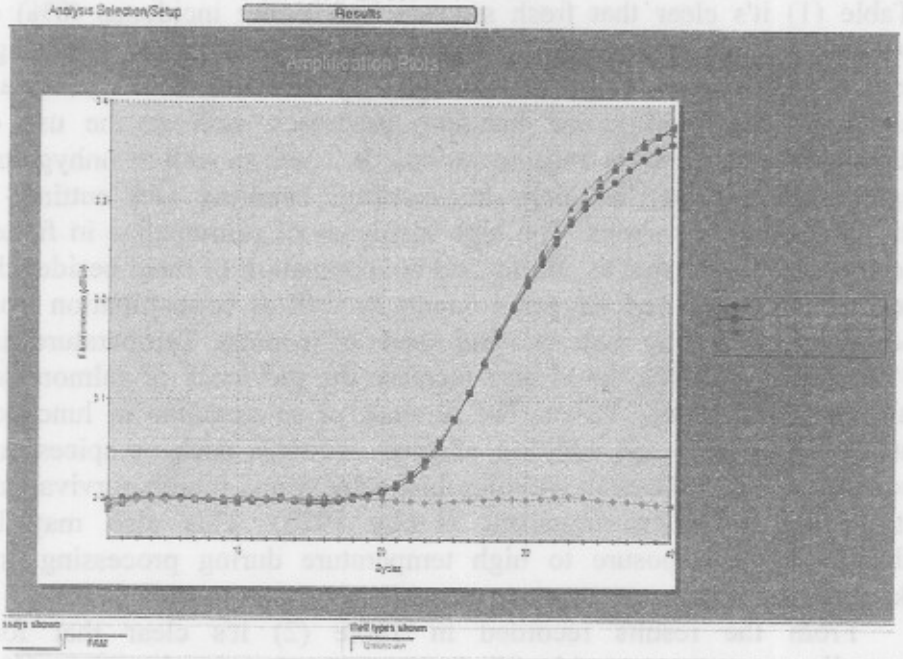

Photo 1: Analysis of Real-time PCR for *Salmonella typhimurium* and *Salmonella enteritidis.*

DISCUSSION

Meat and meat products are considered as a major vehicle of most reported outbreaks of foodborne disease. Epidemiology data have identified improperly handled meat products as important vehicles for infection (ICMSF, 1978), *Salmonella* remains as one of the major food borne health hazards and meat plays an important role, as a reservoir, in disseminating *Salmonella* (Mohamed, 2006).

In present study a total of one handred and fifty random samples of meat products (50 samples of luncheon, 50 samples of fresh sausage and 50 samples of frozen minced meat) were examined for salmonellae. Results obtained in Table (1) revealed zero, 8 and 6%that *Salmonella* were detected in the examined luncheon, sausage and frozen minced meat respectively. These results agree with that reported by Saleh (1991); Edris (1993); Moussa *et al.* (1993); Fathi *et al.* (1994); Aiedia (1995); Abd-El-Aziz *et al.* (1996); Ouf (2001) and Eleiwa (2003). The result disagrees with that reported by Mohamed (1988) who recorded that salmonellae could not be detected in the examined luncheon samples. From the results recorded

in Table (1) it's clear that fresh sausage had higher incidence (8%) of *Salmonella* contamination followed by frozen minced meat(6%). High incidence of salmonellae in fresh sausage may be due to faults in certain practices of slaughtering and handling processes such as the use of contaminated knives, tools, rags, saws, boards ....etc as well as unhygienic slaughtering, dressing, washing, transporting, handling and cutting in abattoirs and butcher shops. The high incidence of salmonellae in frozen minced meat may be due to cutting and contamination of meat besides the increase in its water and oxygen contents as well as contamination from grinders, dir, packaging materials and hands of workers. Temperature rise (2-4°C) during grinding could also increase the incidence of salmonellae organisms (Field *et al.,* 1977). The absence of salmonellae in luncheon meat may be due to the addition of food additives such as spices and preservatives, which have an antimicrobial activity and inhibit survival and multiplication of micro-organisms (Libby 1975). This also may be attributed to the exposure to high temperature during processing and cooking procedures.

From the results recorded in Table (2) it's clear that four *Salmonella* serovars were identified from sausage samples, two (4%) strains as *S. typhimurium* and two (4%) strains as *S.enteritidis.* Nearly similar results were obtained by Rao and Nandy (1977). It's evident that three *Salmonella* serovars were isolated from the examined frozen minced meat samples and identified as two (4%) strains as *S. typhimurium* and one (2%) as *S. enteritidis*. These results agree with that obtained by Gobran (1985).

Antibiogram patterns (Table 4) showed that *Salmonella typhimurium* isolates were resistant to streptomycin and sensitive to amoxicillin, cefuroxime sod., ciprofloxacin, Danofloxacin, flumequine, Enrofloxacin and oxytetracycline. *Salmonella enteritidis* was similar to *Salmonella typhimurium*. These results are similar to those recorded by Frech and Schwarz (1998); Guerra *et al.* (2000); Wiuff *et al.* (2000) and Gebreyes *et al.* (2004).

Photo (2) showed that *Salmonella typhimurium* and *Salmonella enteritidis* were detected by Real-time PCR when used fluorogenic primers and TAMARA-probe used for amplification of 102-bp region of the in VA gene of *Salmonella* (Quantitect probe RT-PCR kit cat.no.20 4443), according to Kura *et al.* (1999); Livak *et al.* (1995); Paszko *et al.* (1997); Hoorfar and Radstrom (2000). Detection probability of *Salmonella* real-time PCR assay at serially 10- fold- diluted cell concenterations of serotype *typhimurium* and *enteritidis* reference strains was determined in the presence of 150 copies of IAC DNA. Five microliters of each suspension

(10^0 to 10^6 CFU/ unit) was used as the template in the PCR. The graph shows a sigmoidal fit of data points generated by 30 repetitive PCRs. The real-time PCR in food borne outbreak investigations provides an opportunity for rapid detection of pathogens in food and clinical settings (Burkhard *et al.*, 2004).

Apart from saving time, real-time PCR is sensitive highly specific and offers the potential for quantification, the risk of cross-contamination is significantly reduced and high-through put performance and automation are possible, since no post-PCR manipulations are required (Harnai *et al.*, 1997; Lubeck and Hoorfar, 2003).

From the obtained results we can concluded that the possibility of contamination of meat products with such serious pathogens remains as a public health problem. Thus all precautions of proper sanitation during manufacture, handling and storage of such meat products should be adopted to control these serious pathogens and to obtain a maximum limit of safety to consumers. The RT- PCR may be considered as a rapid, sensitive highly specific and offers the potential for quantification of *Salmonella* isolates.

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