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Rapid *Salmonella* Detection in Different Food Samples by Direct-PCR

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The aim of the present work was to compare Direct-PCR on food samples or combined with pre-enrichment and / or selective enrichment media with the standard conventional microbiological methods and to investigate the most rapid & sensitive technique for the detection & identification of *Salmonella* species. Two hundred samples of retail chicken meat & byproducts in addition to 200 samples of beef meat & byproducts were tested by conventional isolation method & Direct-PCR techniques. Direct-PCR was performed on food samples, pre-enrichment and selective enrichment broth without DNA extraction. The less sensitive detection method was Direct-PCR on food samples 5% for both chicken & meat samples followed by conventional isolation method 7% for chicken & 5% for meat. The most optimized technique was Direct-PCR on selective 9.5 & 11.5 % for chicken & meat samples, respectively followed by the Direct-PCR on pre-enrichment.

Keywords: ***Salmonella*, Direct-PCR , Rapid, Detection, Food, Meat, Poultry, invA**

INTRODUCTION

Foodborne diseases are a serious threat to public health (Yang et al., 2010). *Salmonella* species are considered to be among the most important foodborne pathogens in the world and salmonellosis is still one of the most widespread foodborne bacterial illnesses in humans, with clinical manifestations ranging from asymptomatic state to severe disease (Galanis 2006). In recent years salmonellosis has increased considerably both in incidence and severity. (Plym & Wierup 2006).

It was stated that *Salmonella* outbreaks are linked to unhygienic food preparation, cooking, reheating and storage practices (Gorman et al. 2004). Meat and poultry products are contaminated with *Salmonella* during slaughtering and continuing processing methods. These methods provide many opportunities for contamination as well as cross contamination within a production batch (Borch and Arinder 2002 and Li et al. 2005).The traditional *Salmonella* detection includes pre-enrichment, selective enrichments and plating on selective agar media followed by biochemical and serological tests which requires 5 to 7 d for completion and is time consuming, labor intensive and costly to meet food safety control in routine food analytical laboratories (Okamura et al., 2009, Techathuvanan et al, 2010). PCR has become an important detection tool for pathogens in foods; PCR can also identify strain differences by targeting gene(s) or sequences exhibiting polymorphisms or variability in its distribution within the bacterial population (Hong et al. 2009). Several genes have been used to detect *Salmonella* in natural environmental samples as well as food and feces samples. Virulence chromosomal genes including; invA (Malorny et al, 2003a; Malorny et al, 2003b) is target gene for PCR amplification of *Salmonella* species. The

*inv*A gene of *Salmonella* contains sequences unique to this genus and has been proved as a suitable PCR target, with potential diagnostic applications (Rhan et al, 1992). Amplification of this gene now has been recognized as an international standard for detection of *Salmonella* genus (Malorny et al, 2003a). The present work is a comparative study for rapid detection methods of *Salmonella* in different food samples using Direct-PCR on food samples or combined with pre-enrichment and / or selective enrichment media with the standard conventional microbiological methods.

MATERIALS AND METHODS

Sampling

A total of 400 samples were collected as follows: 200 poultry byproducts (50 shawerma, 50 minced chicken meat, 50 chicken wings and legs and 50 chicken fillets) , while 200 meat byproducts were 50 oriental sausage, 50 minced meat, 50 livers and kidneys and 50 frozen meat. All samples were aseptically collected and sent to the laboratory

Isolation and identification:

The standard cultivation method for *Salmonella* isolation was carried out as recommended by ISO 6579 (ISO, 2002), From each sample 25 g were placed in a sterile stomacher bag containing 225 ml of Buffered Peptone Water. After stomacher homogenization for 2 min at 320 rpm and overnight incubation at 37°C, 0.1 ml aliquots were inoculated into tubes containing 10 ml Rappaport Vassiliadis (RV) broth and incubated for 48 h at 42 °C. Then, Xylose Lysine Deoxycholate (XLD) agar plates were inoculated from each of the RV broths and incubated for 18–24 h at 37°C. Suspect colonies with typical *Salmonella* morphology were confirmed biochemically.

Direct PCR detection:

The examined food samples & microbiological broth (preselective and/ or enrichment) were exposed for Direct-PCR for direct detection of *Salmonella* species. The tissue samples were processed according to Samir et al. 2015.The Direct-PCR was performed using (Phire Animal Tissue direct PCR Kit) according Hossam et al. 2016. The primer pairs *inv*A were used for detection of *Salmonella* spp. (Rhan et al. 1992)**.**

RESULTS

The results of the standard conventional microbiological method and Direct-PCR on food sample, buffered peptone water (PBW) and Rappaport Vassiliadis (RV) broth using the invA primers specific for *Salmonella* species are depicted in (Fig.1).

The detection of *Salmonella* was applied on the 400 different food samples and results of are shown in Tables 1 & 2. Not all samples, which were *Salmonella*-negative by standard conventional isolation method, had given the same result by the Direct-PCR approaches. There was variable positive percent of recovery between different types of examined sample either chicken or meat byproducts. The less sensitive detection method was Direct-PCR on food samples 5% for both chicken & meat samples, followed by conventional isolation method 7% for chicken & 5% for meat. The most optimized technique was Direct-PCR on selective & pre-enrichment together 9.5 & 11.5 % for chicken & meat samples, respectively.

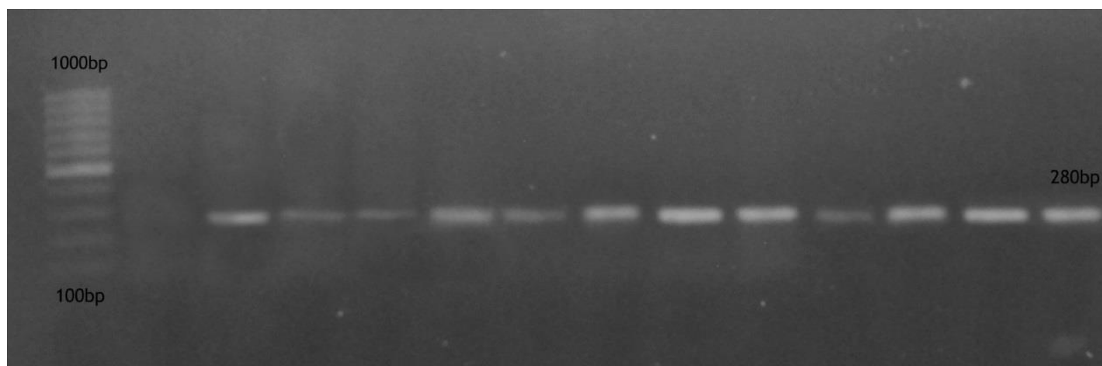

Figure 1: Electrophoretic profile of amplification of invA gene by Direct-PCR in different examined food samples, The size of amplicons 270 bp , Marker DNA ladder 100bp (Jena Bioscience-Germany)

Table (1): Identification of *Salmonella* species by standard conventional microbiological methods & Direct-PCR with invA primers in poultry meat & byproducts samples using pre-enrichment (BPW) and selective enrichment (RV)

Poultry Meat & byproducts	Samples No.	Conventional isolation method		Direct-PCR on					
				Food sample		Pre-enrichment		Selective enrichment	
		No.	%	No.	%	No.	%	No.	%
Shawarma	50	6	12	4	8	6	12	7	14
Minced chicken meat	50	4	8	4	8	5	10	6	12
Chicken wings and legs	50	3	6	2	4	3	6	4	8
Chicken fillets	50	1	2	-	-	2	4	2	4
Total	200	14	7	10	5	16	8	19	9.5

Table (2): Identification of *Salmonella* species by standard conventional microbiological methods & Direct-PCR with invA primers in beef meat & byproducts samples using pre-enrichment (BPW) and selective enrichment (RV)

Beef Meat & byproducts	Samples No.	Conventional isolation method		Direct-PCR on					
				Food sample		pre-enrichment		Selective enrichment	
		No.	%	No.	%	No.	%	No.	%
Oriental sausage	50	5	10	3	6	6	12	8	16
Minced beef	50	3	6	3	6	3	6	6	12
Livers and kidneys	50	1	2	2	4	2	4	2	4
Frozen meat	50	1	2	2	4	3	6	7	14
Total	200	10	5	10	5	14	7	23	11.5

DISCUSSION

The tracking of sentinel health events to detect and manage disease risks facing a human population is an important mission. However the maximum capacity of connecting animal and human health information to provide warning of such “shared risks” (Rabinowitz et al, 2009, Mansour et al. 2017). Animal, plants & birds or food of animal origin acting as a potential human health hazard (Abdel-Moein et al. 2012, Elhelw et al. 2014, Samir et al. 2015, Elhariri et al.2016, Abdel-Moein et al. 2017, Elhariri et al.2017a,b). *Salmonella* is ubiquitous in the environment originating from the gastrointestinal tracts of domesticated and wild animals and can be present without causing apparent illness. Most infections result from the ingestion of foods of animal origin contaminated with *Salmonella* species such as beef, chicken, turkey, pork, eggs, and milk (D’Aoust 1997; D’Aoust 2000; Olsen et al. 2000). Standard conventional methods for Diagnosis of *Salmonella* infections is still based primarily on cultivation and identification of the organism from veterinary clinical samples. However, cultivation by the traditional *Salmonella* detection includes pre-enrichment, selective enrichments and plating on selective agar media followed by biochemical and serological tests which requires 5 to 7 d for completion and is time consuming, laborious and costly to meet food safety control in routine food analytical

laboratories (Techathuvanan et al. 2010).

A few elements can meddle with the separation of *Salmonella* serovars from clinical examples: the condition of the specimen can allow contaminating organisms to hinder *Salmonella* isolation, anti-microbial in infected animals can impede the development of *Salmonella* life forms, or *Salmonella* organism might be shed just occasionally and in low numbers, especially in carrier individuals (WHO, 1988). In the present study, it was presented different approaches in comparison with standard conventional methods to overcome the previous mentioned factors in detection of *Salmonella* in food samples. The results show that greater numbers of samples positive for *Salmonella* species were detected by the Direct-PCR on selective broth (RV) by 9.5% & 11.5 for poultry & meat respectively , followed by Direct-PCR on enrichment broth (BPW) by 8 for poultry & 7 for meat and that the Direct-PCR on food samples was 5% for poultry lower that standard methods 7%, while in meat the detection percent was the same for Direct-PCR on food and standard method (5%). In recent years, diagnostic and food hygiene laboratories have been concerned with lessening those time needed for the detection of *Salmonella* species and a more rapid, applied and touchy approach for detection and identification of *Salmonella serovars* from clinical specimens is necessary to help in the prevention & distribution of contaminated food products. Diminishing the detection time to less than 24 hr with comparable sensitivity and specificity to traditional *Salmonella* culture methods is urgently needed. The Direct-PCR on RV broth will reduce the time required for the decision about the *Salmonella* positive samples. Amplification of DNA sequences unique to an organism utilizing the PCR enhances both the detection speed and the level of sensitivity at which organisms can be distinguished (Ramamurthy et al. 1993) and has been increasingly used to identify several bacterial species from food and clinical samples (Stone et al. 1994, Hossam et al. 2016, Elhariri et al 2017c). Direct-PCR is deals with genomic DNA of bacterial pathogen not dependent on the utilization of a substrate or the expression of antigens, in this way dodging phenotypic misdiagnosis (Hoorfar et al. 1999). Direct-PCR combined with broth has been applied to the detection of many bacterial pathogens (Candrian 1995; Schrank et al. 2001) to improve sensitivity and dilute substances which inhibit the PCR (Fluit et al. 1993)

CONCLUSION

It was concluded that the Direct-PCR on 18 hours selective proceeded by 24 hours buffered peptone water is more sensitive and time decreasing approach necessary to detect and identify *Salmonella*. The applied Direct-PCR on selective broth can be an intense tool for the rapid and accurate detection and identification of *Salmonella* and can be executed in diagnostic and food analysis laboratories

CONFLICT OF INTEREST

The present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

MKR planned the study and revised the manuscript, MDE supervised the practical work, YSA collected the samples and detected the *Salmonella*. All authors read and approved the final version.

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