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Polymerase Chain Reaction (PCR): An Alternative Rapid Method for Detection of Some Microbial Contamination of Meat Products

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Key Words: PCR, Meat products, *E. coli*, *Staph. aureus*

ABSTRACT:

The use of advanced molecular techniques such as PCR has been introduced as a new rapid and reliable method for detection of microbial contamination of food stuff. In this study, a total of 160 meat products samples 40 of each luncheon, beef burger, minced meat, and fresh sausage) were examined for the presence of *E. coli* and pathogenic *Staph. aureus* using traditional methods of isolation and identification and RAPD-PCR. The data obtained from this study showed that *E. coli* was isolated from 9 (22.5%), 13 (32.5%), 19 (47.5%), and 24 (60%) by traditional method comparing with 8 (20%), 13 (32.5%), 17 (42.5%), 23 (57.5%) using RAPD-PCR method out of 40 samples of luncheon, beef burger, minced meat, and fresh sausage samples respectively. While, *Staph. aureus* was isolated from 17 (42.5%), 11 (27.5%), 28 (70%), and 18 (45%) using traditional methods in comparison with 13 (32.5%), 8 (20%) 23 (57.5%), and 14 (35%) using RAPD-PCR method out of 40 samples of luncheon, beef burger, minced meat, and fresh sausage samples correspondingly, with a sensitivity rate ranged from 87 to 96% in case of *E. coli* and 72 to 82% in case of *Staph. aureus.* Collectively, the obtained results in the current study clarified that that, PCR is considered as a rapid, reliable and sensitive tool for detection of microbial contamination of food stuff.

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1. INTRODUCTION

During the last few decades, a major development in food chain in the form of expansion of supermarkets that provided consumers with a wide range of retailing meat products, so, consumer's interest in meat as a food has been increased and is reflected by the increasing demand for high quality meat products (Scanga *et al.,* 2000; Stephan *et al.,* 2003). *Escherichia coli (E. coli)* and *Staphylococcus aureus* (*Staph. aureus*) are the most important foodborne pathogen causing food poisoning and many other disease conditions amongst food consumers. *E. coli* is a major component of the normal intestinal flora of humans and other mammals. Some *E. coli* strains represent primary pathogens with an enhanced potential to cause disease after acquiring specific virulence attributes. These virulence attributes are normally encoded on genetic elements that can be exchanged between different strains, the presence of theses virulence genes can magnitude the severity of infection caused by these strains (Li *et al.,* 2005). *Staph. aureus* is one of the most important amongst Staphylococci species. The species is found primarily on human skin, mucous membranes and can also be found in other areas of human contact including soil, water, and food products. *Staph. aureus* is considered the third worldwide cause amongst the food-borne illnesses reported cases (Tamarapu *et al.,* 2001). In Egypt, meat products such as sausage, beef burger and luncheon are gaining popularity as they represent quick easily prepared meat meals with low price which is within the reach of large numbers of families with limited income. Contamination of such products with some foodborne microorganisms including *E. coli* and *Staph. aureus* during processing makes the need for a rapid and accurate method for detection is urgent. In the last few years, the development of molecular typing methods such as Polymerase chain reactions (PCR) techniques in particular Multiplex PCR and RAPD-PCR has offered the possibility of accelerating a great deal of bacterial identification with limited troubles related to sampling preparations, use of specific media, excessive use of chemicals in the traditional methods (Aymerich *et al.,* 2003). Moreover, phenotypic identification of bacterial contamination of meat products is considered as time consuming and often problematic in many aspects.

Multiplex-PCR consists of multiple primer sets within a single PCR mixture to produce amplicons of various sizes that are specific to different DNA sequences, while RAPD PCR stands for random amplification of polymorphic DNA PCR. It is a type of PCR reaction, but the segments of DNA that are amplified are random. The scientist performing RAPD creates several arbitrary, short primers (8–12 nucleotides) then proceeds with the PCR using a large template of genomic DNA, hoping that fragments will amplify. By resolving the resulting patterns, a semi-unique profile can be gleaned from a RAPD reaction (Bartlett and Stirling, 2003). Herein, this study was aimed to validate the use of RAPD-PCR as an alternative rapid, reliable and accurate tool in the field of identification of microbial contamination of food stuff in comparison with traditional methods of isolation and identification (culture on specific media, biochemical and serological tests, …etc).

2. MATERIAL AND METHODS

I-Samples and samples preparation

The samples preparation was carried out according to **(**Petternel., *et al* 2014**).** Briefly, a total of 160 random meat product samples (40 each of luncheon, beef burger, minced beef, and fresh sausage) were collected from different super markets at Minufyia Governorate. The collected samples were then transferred immediately under full aseptic conditions for bacteriological isolation and identification of *E.coli* and *Staph. aureus* . 25 grams of the examined meat product samples were transferred to 225 ml of 0.1% sterile buffered peptone water, then stomached for 2 min to provide a homogenate. One ml from the original dilution was then transferred to another sterile tube containing 9 ml of sterile buffered peptone water and mixed will to make the next dilution from which further decimal dilutions were prepared **(Samaha *et al*., 2012)**.

II- Isolation and identification of *E. coli*

Isolation and identification of *E.coli* was carried on specfic media, then the isolates were confirmed to be *E.coli* by various biochemical assays, as per Bergey's manual of determinative bacteriology (Holt *et al.*,, 1994). The serological identification of isolates was carried out according to (Varnam and Evans, 1991). Isolated strains of *E. coli* were identified serologically using diagnostic Sera (Denka Seiken Co., LTD, Tokyo, Japan).

III- Isolation and identification of *Staph.aureus*

Isoaltion and identifcation of *Staphylococcus* was carried out according to per Bergey's manual of determinative bacteriology (Holt *et al.*, 1994). Screening for pathogenic *Staph. aureus*was done by performing various biochemical assays, including Coagulase test , DNase test (Baird, 1996), and Thermostable nuclease test (TNase) (Lachica *et al.*, 1971).

IV-Random Amplified Polymorphic DNA (RAPD)-Polymerase chain reaction (PCR) for *E. coli*

RAPD-PCR for *E. coli* was carried out according to (Maurer *et al.*, 1998). Briefly, 25 gm of sample were added to 225 of brain heart infusion broth and stomached (Seward stomacher 80 Biomaster, England) for 2 min then incubated over night. One ml of an overnight incubated broth was centrifuged at 13000 rpm for 2 min and the sediment was then resuspended in equal volume of Tris-EDTA buffer containing 100 µl of lysozyme solution (10 mg/L), 100 µl of protienase K enzyme (0.3 mg/L) and 1% dodecyl sulphate. The DNA lysate was extracted with chloroform/isoamyl alcohol (24:1, ratio by volume), then extracted with phenol/ chloroform/isoamyl alcohol (25:24:1, ratio by volume). The aqueous phase was mixed with isopropanol alcohol and incubated at -20◦C for 30 min. The precipitated DNA was spooled out, rinsed in 70 % ethanol and dissolved in 0.5 ml of Tris EDTA buffer. The RAPD-PCR reaction mix (50 µL) for each sample was made with (10 µL extracted DNA, 2.5 µL primers mix (0.5 µL from every primer table (1)), 1 µL deoxynucleoside triphosphate (dNTP-mix), 5 µL 10x buffer, 1µL Taq-DNA polymerase enzyme (5000 U/ml), and 30.5 µL ultra pure deionized water.

The reaction mixture was then overlaid with mineral oil and was incubated in the thermal cycler as follows, the first initial cycle 94◦C for one minute (denaturation), 35◦C for 2 min (annealing) and 72 ◦C for 2 min (extension). The consequent 35cycles of 94◦C for 30 seconds (denaturation), 35 ◦C for one minute (annealing) and 72 ◦C for 2 min (extension). The final extension step at 72 ◦C for 5 min then kept at 4◦C (hold temperature). The amplified products were analyzed on agarose gel (consisted of 2% agarose and 5 µL of ethidium bromide in 1x Tris – Acetate EDTA (TAE) buffer. The samples were then electrophoreses at 100 volts for one hour, shown under ultra violet transiluminator and photographed.

V-**Random Amplified Polymorphic DNA (RAPD)-Polymerase chain reaction (PCR) for *Staph. aureus***

The technique was carried out according to (Reinoso et al., 2004). Briefly, 25 gm of sample was added to 225 ml of brain heart infusion broth and stomached (Seward stomacher 80 Biomaster, England) for 2 min then incubated over night. Genomic DNA extraction was carried out using GeneJET Genomic DNA Purification Kit (Fermentas) following the instruction procedures. The collected DNA was then kept at -20 C untile used. 20 ng of chromosomal DNA was used per reaction . amplifications were performed in 25 µl of buffer solution containing 3 µM of oligonucleotides, 200µM of each deoxynucleoside triphosphate, 3.5 mM MgCL2 and 2.5U of DNA Taq polymerase. The oligonuleotides OLP6 (5\-GAGGGAAGAG-3\), OLP11 (5\-ACGATGAGCC-3\), OLP13 (5\-ACCGCCTGCT-3\). The mixtures were then overlaid with mineral oil and amplification was performed in PCR thermal cycler. The amplification consisted of a cycle of predenaturation at 94 C for 5 min. followed by 40 cycles of 1 min. at 93 C, 1.5 min. at 37C and 1 min at 72 C and final extension at 72 C for 8 mi. The amplified products was analyzed on agarose gel (consisted of 2% agarose and 5 µL of ethidium bromide in 1 x Tris – Acetate EDTA (TAE) buffer. The samples were then electrophoreses at 100 volts for one hour, the products were visulaised under ultra violet transiluminator and photographed.

3. Results and disscusion

3.1. Isolation and identification of E.coli with the traditional and RAPD-PCR method

E.coli is conisdered as one of the human and animal intestinal microflora, and most are found throughout the envrionment with little sanitary signficance (Geenberg and Hunt, 1985). Enteropathogenic *E. coli* been incrimintaed as apotential food poisoning ageny usually associated with infants diarrhoea and gastroenteritis in adults (Mayron, 1987). The data presented in table (2) showed that, *E .coli* was recovered from 9 (22.5%), 13 (32.5%), 19 (47.5%), and 24 (60%) using tradtional method (isolation and further identification) comparing with 8 (20%), 13 (32.5%), 17 (89.47), and 23 (95.83%) using RAPD-PCR. While that data presented in Fig (1) showed the specfifc band size of *E. Coli* starins using RAPD-PCR method. The sensitivity of RAPD-PCR in cmparion with tradtional method was ranged from 89 to 96%. The results obtained by the traditional method was agreed with obtained by (Nashed, 1993) and (Abou-Hussien, 2004). Conversely, a lower results were obtained by (Ouf, 2001) and (Eleiwa, 2003). The presence of *E.coli* in food is considered as an indicator of faults during preparation, handling, storage or service. Moreover, it is considered as index of fecal contamination. The contamination of meat products with *E.coli* especially the virulent strains will be associated with bad consequences, it may induce severe diarrhea in infants and young children, and food poisoning and gastroenteritis among the adults. The introduction of new method of bacterial identification was a time and chemicals saver. The use of RAPD-PCR offers an efficient microbiological tool for presumptive detection of *E. coli* in meat (Li *et al.*, 2005). Moreover, PCR has been introduced as an effcient and rapid tool for identification of enterotoxigenic and shigatoxin producing *E.coli* in fecal samples collected from human and animals (Zidan *et al.*, 2014; Zidan, 2010).

Table (1) Oligonucleotide primers sequences for RAPD-PCR amplification of *E.coli*.

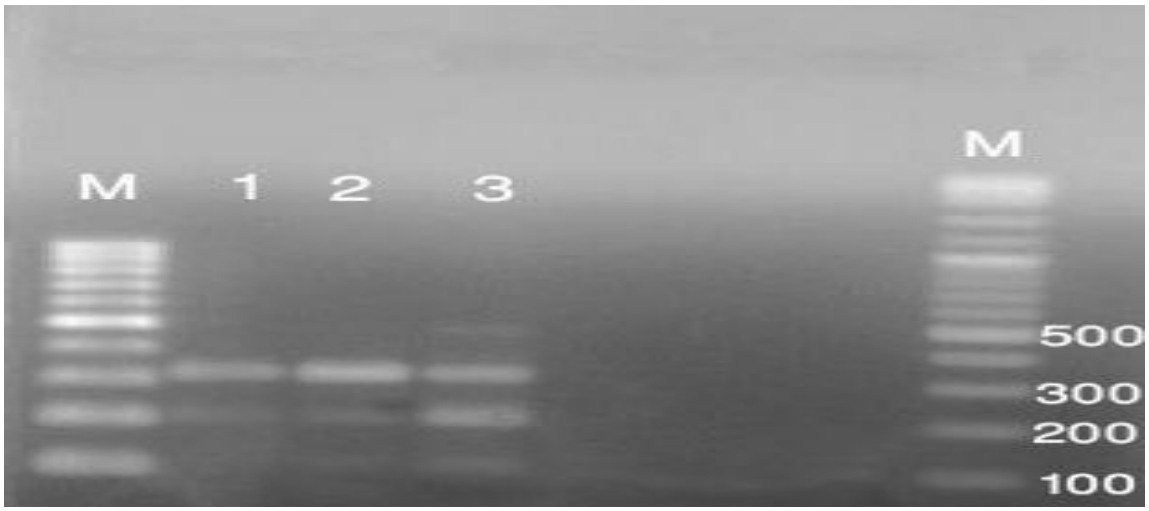
NO.	Primer sequence	Size	Melting point	Guanine cytosine%
1-	5' AAGAGCCCGT 3'	10-mer	32C	60
2-	5 ' AACGCGCAAC 3'	10-mer	32 C	60
3-	5' GCGATCCCCA 3'	10-mer	34 C	70
4-	5' GTGGATGCGA 3'	10-mer	32C	60
5-	5'AAACGGTTGGGTGAG3	15-mer	47.8C	53.3

Regarding the incidence of E.coli serovares isolated from meat products, the data presented in table (3) showed that many serovares are isolated with varied ratios. Pathogenic *E. coli* have been broadly classified into two major categories: the diarrheagenic *E. coli* and the extraintestinal pathogenic *E. coli*. Among the diarrheagenic *E. coli*, there are currently six categories:

enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC)/Shiga toxin-producing *E. coli* (STEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC), and diffusively adherent *E. coli* (DAEC) . The *E. coli* strains causing extraintestinal infections have been collectively called extraintestinal pathogenic *E. coli* (ExPEC), which includes two major pathotypes, uropathogenic *E. coli* (UPEC) and neonatal meningitis *E. coli* (NMEC) (Xiaodong, 2010). The data presented in table (3) showed that, *E.coli* serotypes were isolated from luncheon, beef burger, minced meat, and fresh sausage in a various ratios. The majority of isolated serotypes were EPEC, EHEC, ETEC,and EIEC. These data were nearly similar to that obtaine by (Abd-El-Aziz *et al.*, 1996; Aiedia, 1995) while lower ratios were obtained by Fantelli and Stephan and Saleh (Fantelli and Stephan, 2001; Saleh, 2001). EPEC (O126, O127, O114, and O142) strains are the major cause of infantile diarrhea (Varnam and Evans, 1991), while ETEC strains (O78 , O25 and O63) are considered the common cause of traveller's diarrhea and / or children diarrhea (Daved *et al.*,, 1990).

Table (2)Incidence of *E. coli* in the examined meat product using tarditional method and RAPD-PCR and sentivity of RAPD-PCR in comparison with traditional method.

Product	Traditional method		RAPD-PCR		
	No	%	No	%	
Luncheon	9	22.5	8	20	88.89
Beef burger	13	32.5	13	32.5	100
Minced meat	19	47.5	17	42.5	89.47
Fresh sausage	24	60	23	57.5	95.83



Figur (1); PCR Gel electerophersis pattern of *E.coli* starins using RAPD-PCR

Table (3). Incidence of *E. coli* serovars in various meat products (luncheon, beef burger, minced meat, and fresh sausage)

Serotypes	Meat products				
	Luncheon	Beef burger	Minced meat	Fresh sausage	Classification
O25		20%		20%	ETEC
O26			20%		EHEC
O63	20%				ETEC
O78			20%		ETEC
O86			20%		EAEC
O111	20%			20%	EHEC
O112		20%			EIEC
O114		20%			EPEC
O119		20%			EHEC
O124	20%				EIEC
O126	20%			20%	EPEC
O127		20%			EPEC
O128			20%	20%	EPEC
O136				20%	EIEC
O142	20%		20%		EPEC

Isolation and identification of *Staph. aureus* with the traditional and RAPD-PCR method

Staph. aureus is one of the most common causes of foodborne illness worldwide due to the widespread occurrence of *Staph. aureus*and to the ability of many strains to produce one or more Staphylococcus enterotoxins. The data presented in table (4) and Fig (2) showed that, *Staph. aureus* was recovered from 17 (42.5%), 11 (27.5%), 28 (70%), and 18 (45%) using traditional method of isolation and identification in comparsion with 13 (32.5%), 8 (20%), 23 (57.5%), and 14 (35%) using RAPD-PCR out 40 samples of luncheon, beef burger, minced meat and fesh sausage respectivelly, with a sensitivity rate ranged from 72 to 82%. Results of *Staph. aureus*counts in tested products werer varied between each other, undoubtedly, many factors are responsible for these variations, and mostly, the neglected hygienic practices were the foremost factors. The data presented in this study was lower comparing with that obtained by (Al-Kour, 2001; Ouf, 2001). On the contrary, a higher incidence was obtained by (Abou-Hussien, 2004; Hassanin, 2007). The data presented in Fig (2) showed the specific band size of examined meat products in comparsion with that of refernce strain. *Staph. aureus*is considered as an adaptable pathogen of humans and animals. It is present in the nares of about 20-30% of healthy

peoples; conversely about 60% of the population harbours the microorganism irregularly (Kluytsman *et al.*, 1997). The species is capable of causing a wide variety of diseases ranging in severity from slight skin infection to more severe diseases such as pneumonia and septicaemia (Franklin and Lowy, 1998). Although not all *Staph. aureus* food intoxication cases are recorded, yet *Staph. aureus* is still considered the third worldwide cause amongst the foodborne illnesses reported cases (Tamarapu *et al.*, 2001; Tirado and Schimdt, 2001; Zhang *et al.*, 1998). Staphylococcal food poisoning represents a considerable social burden in terms of hospital expenses, loss of patient's working days and productivity, together with the problems and the cost of disposing the contaminated food. Althought, culture methods are still conisered as standard methods for trdational confirmation of *Staph. Aureus* conatmination of food stuff. However, PCR has been introduced as alternative method that may decrease the time to 18 hrs or to less than 2 hrs (Anderson and Weese, 2007; Kilic *et al.*, 2010; Thomas *et al.*, 2007)

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CONCLUSION

The data presented in this study has focused on the use of advanced molecular techniques in particular RAPD-PCR in the field of identification of microbial contamination of meat products. RAPD-PCR is conisered as a rapid and senitive tool with limited mistakes related to phenotypic identification. On the other hand, it was not

possible to confirm these results by performing the cultural method as detection was carried out from DNA extracts only, and the cells had already been inactivated. Therefore, for accurate identification of microbial contamination in meat product we strongly recommend using PCR pararelle with cultural identification.

Table (4): Incidence of *Staph. aureus*in the examined meat product using tarditional method and RAPD-PCR and sentivity of RAPD-PCR in comparison with traditional method.

Product	Traditional method		RAPD-PCR		% of sensitivity
	No	%	No	%	
Luncheon	17	42.5	13	32.5	76.47
Beef burger	11	27.5	8	20	72.72
Minced meat	28	70	23	57.5	82.14
Fresh sausage	18	45	14	35	77.78

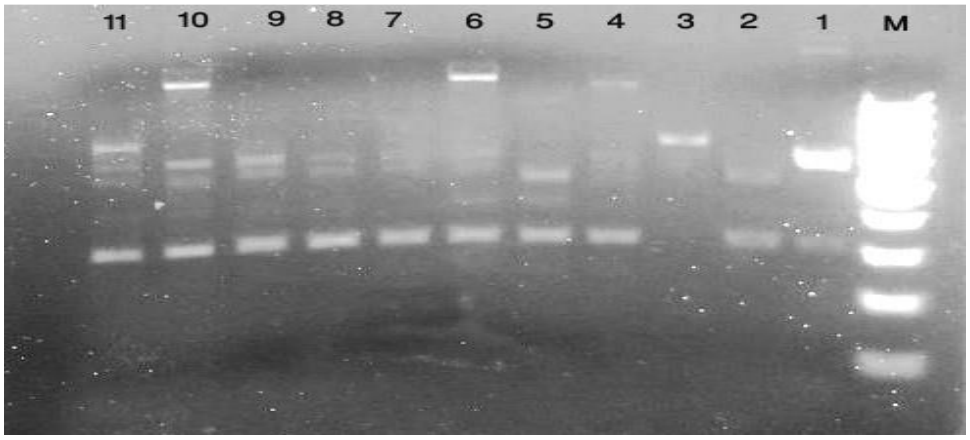

Fig (2); Agarose gel electrophoresis shows RAPD PCR products of *Staph. aureus*strains. Lane (1-10) several bands at different levels of the gel, lane (11) reference *Staph. aureus* strain.

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