Available online freely at www.isisn.org

Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2019 16(2): 1549-1555. **OPEN ACCESS**

Detection of some foodborne pathogens in meat by Duplex PCR

Abdelhalim A. Elshawadfy[1], Ashraf A. Abd El-Tawab[2], Hanan A. Fahmy[3], Sherin Mahmoud Noser[4], Fatma I. El-Hofy[2]and Ahmed Hikal[2]

[1]Animal Health Research Institute, Mansoura branch, Agricultural Research Center, Giza, **Egypt**.
[2]Bacteriology, Immunology and Mycology Department, Faculty of Veterinary Medicine, Benha University, **Egypt**.
[3]Biotechnology Department, Animal Health Research Institute, Agricultural Research Center, Giza, **Egypt**.
[4]Food Hygiene Department, Animal Health Research Institute, Agricultural Research Center, Giza, **Egypt**.

***Correspondence:** halim@drhalim.com **Accepted: 0**4 May 2019 **Published online:** 20 May. 2019

The study was aiming for detection of two foodborne pathogens in meat by duplex PCR assay. A total of 210 samples of frozen, minced meat and burger were collected from different shops at Dakahleyah Governorate, Egypt for bacteriological examination and duplex PCR assay. Results of Culture based method revealed that, 29 (13.8%) isolates were recovered from 210 samples represented as 10 positive samples (14.3%) from frozen meat, where 7 were single and 3 were mixed contaminations, meanwhile, 6 (8.6%) from minced meat samples where 2 were single and 4 were mixed contaminations and 13 positive samples (18.6%) from burger, where 10 were single and 3 were mixed contaminations. *E. coli* were the most isolated (18= 8.6%) followed by *S. aureus* (11=5.2%). Duplex PCR assay results revealed that six positive samples by culture method of mixed infection for *S. aureus* and *E. coli* were positive for *phoA* and *clfA* genes respectively in all six samples while results of four positive samples for *S. aureus* by culture method, were positive for *clfA* gene by PCR. On the other hand, results of four positive samples for *E. coli* by culture method were positive for *phoA* gene when reexamined by duplex PCR, which showed negative results for both genes in another four samples that were negative by culture method. The recorded results revealed that duplex PCR approach allows the accurate, rapid, sensitive and automated detection of some foodborne pathogens for routine analysis at a reasonable cost and short period.

Keywords: Foodborne, *E. coli, S. aureus*, PCR, *phoA, clfA*.

INTRODUCTION

Foodborne bacterial pathogens such as *Salmonella enterica*, *Escherichia coli* O157:H7, *Staphylococcus aureus*, *Listeria monocytogenes, Campylobacter jejuni, Bacillus cereus* and Vibrio spp. are major causes of foodborne illness. In latest years, diseases caused by foodborne pathogens have become an important public health issue causing a prominent morbidity and mortality rate in the globe (Oliver et al., 2005).

Escherichia coli causes health problems varying from gastrointestinal tract-related complications, urinary tract infection, pneumonia and even meningitis (Johnson et al., 2006).

One of the most known foodborne illness is staphylococcal food poisoning that is caused by ingestion of staphylococcal enterotoxins (SEs). There are over 20 type of SEs to date, they all have antigenic effect but only half of them have emetic effect, all this representing a potential hazard for human consumers (Hennekinne et al., 2012).

Specific detection and quantification of pathogens in food by quantitative PCR has been

assessed for a wide range of micro-organisms, with attention to the main foodborne pathogens responsible for critical medical and economical outbreaks: Salmonella spp., L. monocytogenes, *E. coli* O157:H7, and *S. aureus* (Rodríguez-Lázaro et al., 2013).

Rapid detection of pathogens in food is becoming significantly vital for assuring the consumers safety, because most of food-borne diseases and mortalities are caused by pathogenic bacteria. Thus, rapid, sensitive, affordable and convenient techniques to detect food-borne pathogenic bacteria is critical in managing food safety. The multiplex PCR assay for the simultaneous and rapid detection of, Listeria monocytogenes, Salmonella spp. and E. coli O157:H7 was developed (Nguyen et al*.,* 2016).

The objectives of this work were detection of *E. coli* and *S. aureus* from frozen, minced meat and burger directly by molecular technique**.**

MATERIALS AND METHODS

Collection of samples:

A total of 210 random samples of frozen meat, minced meat and burger (70 for each), were collected from different supermarkets and groceries at Dakahleyah Governorate. The samples were obtained as sold to the public and transferred as soon as possible in an icebox to the laboratory with a minimum of delay for bacteriological examination and duplex PCR assay.

Isolation and Identification of *E. coli* was carried out according to (APHA, 2001 and Quinn et al., 2002).

Isolation and identification of *S. aureus* was carried out according to (APHA, 2001 and Quinn et al., 2002).

Extraction of DNA directly from meat samples was done according to QIAamp DNA mini kit instructions:

DNA was directly extracted from samples.

PCR amplification of the target genes:

The *pho*A gene of *E. coli* was amplified using the primers R (5-CGATTCTGGAAATGGCAAAAG-3) and F (5-CGTGATCAGCGGTGACTATGAC-3) (Hu et al., 2011) while *clf*A gene of *S. aureus* was amplified using the primers R (5-CTTGATCTCCAGCCATAATTGGTGG-3) and F (5-CGTGATCAGCGGTGACTATGAC-3) (Mason et al., 2001). PCR tubes consisted of 25 µl Emerald Amp GT PCR Master Mix 2x premix (Takara Bio Inc.), 1 µl of each forward and reverse primer, 5 µl DNA template and the volume me was completed to 50 µl with sterile distilled water. The amplification protocol was done as follows: primary denaturation at 94˚C for five min. then 35 cycles each consists of the following: denaturation at 94˚C for 30 sec; primer annealing at 55˚C for 40 sec. and extension at 72˚C for 45 sec then incubation at 72˚C for 10 min. for final extension.

Detection of the amplified PCR product:

The PCR products were inoculated in agarose gel placed in the electrophoresis chamber and covered with electrolyte solution in 1X TBE running at 95 V for 30 minutes. The base pairs of the expected PCR product could be visualized by the presence of 100 bp marker (Qiagen) and using Gel documentation system. (Biometra).

RESULTS

Results of bacteriological examination

Prevalence of positive samples for foodborne pathogens isolated from examined samples: Results of duplex PCR:

Table (1): Total number and percentage of positive samples for pathogens isolation from studied samples

Samples	Number Of samples	Positive samples		Negative samples		Prevalence of single and mixed contaminant	
						single	mixed
		No.	%*	No.	%*	No.	No.
Frozen Meat	70	10	14.3	60	85.7	7	3
Minced Meat	70	6	8.6	64	91.4	2	4
Burger	70	13	18.6	57	81.4	10	3
Total	210	29	13.8	181	86.2	19	10

%* Percentage in relation to total number of each sample in each row (70 for each sample).

Percentage of foodborne pathogens isolated from examined samples:

Table (2): Percentage of foodborne pathogens in examined samples

samples	Frozen Meat		Minced Meat		Burger		Total	
isolates	No.	%*	No.	%*	No.	%*	No.	%**
E. coli	7	10	2	2.9	9	12.9	18	8.6
S. aureus	3	4.3	4	5.7	4	5.7	11	5.2
Total	10	14.3	6	8.6	13	18.6	29	13.8

%* Percentage in relation to total number of each sample (70).

%** Percentage in relation to total number of samples (210).

Results of duplex PCR:

The recorded results of duplex PCR in table (3&4) & fig. (1) revealed that out of 6 positive samples by culture method (2 minced meat, 2 frozen meat, 2 burger) of mixed infection for *S. aureus* and *E. coli*, PCR results were positive for *phoA* and *clfA* genes in all samples. Results of 4 positive samples (2 minced meat, 1 frozen meat, 1 burger) for *S. aureus* by culture method, were positive for *clfA* gene by PCR. The tested random 4 positive samples (1 minced meat, 2 frozen meat, 1 burger) for *E. coli* by culture method were positive for *phoA* gene by duplex PCR which showed negative results for both genes in 4 samples (1 minced meat, 1 frozen meat, 2 burger) that were negative by culture method.

Table (3): Duplex PCR results of previously examined meat products by conventional bacteriological method

Type of Samples Tested by conventional bacteriological method	No. Of Samples	Sources of Samples			PCR Results		Agreement %
		Frozen Meat	Minced Meat	Burger	No. of Positive	No. of Negative	
Mixed positive Samples	6	2	2	2	6	0	100
E. coli Positive Samples	4	1	2	1	4	0	100
S. aureus Positive Samples	4	2	1	1	4	0	100
Totally Negative Samples	4	1	1	2	0	4	100
Total	18	6	6	6	14	4	100

Mixed: contain both *E. coli* and *S. aureus*

Table (4): Results of duplex PCR for *detecting S. aureus clfA* & *E. coli phoA* genes.

Sample No.	Sample ID	clfA	phoA	Type of Infection
1	M1	+	+	Mixed
2	A5	-	-	-
3	A20	+	-	Single
4	B13	-	+	Single
5	F31	+	-	Single
6	A80	-	+	Single
7	M199	-	-	-
8	M116	+	+	Mixed
9	A164	-	-	-
10	A95	-	+	Single
11	F201	-	+	Single
12	A39	+	-	Single
13	A165	-	-	-
14	F77	+	+	Mixed
15	M27	+	+	Mixed
16	B71	+	+	Mixed
17	M65	+	-	Single
18	B208	+	+	Mixed

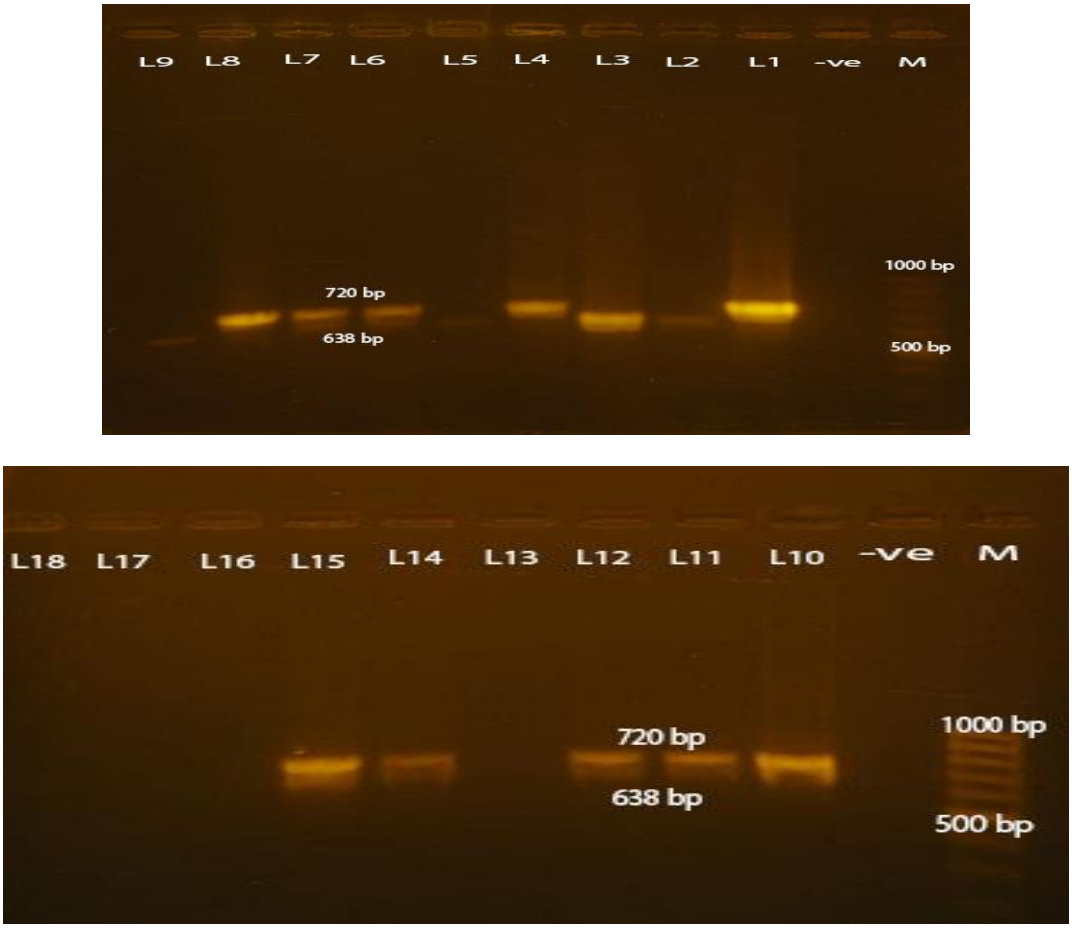

Figure. (1): Agarose gel electrophoresis of duplex PCR for *S. aureus clfA* & *E. coli phoA* genes amplified at 638 bp and 720 bp respectively.

M: Marker (100 bp)

Lane 1, 6, 7, 11, 12, 15: 6 positive samples (2 minced meat, 2 frozen meat, 2 burger) of mixed infection for *S. aureus and E. coli.*

Lane 2, 3, 5, 9: 4 positive samples (2 minced meat, 1 frozen meat, 1 burger) for *S. aureus.*

Lane 4, 8, 10, 14: 4 positive samples (1 minced meat, 2 frozen meat, 1 burger) for *E. coli.*

Lane 13, 16, 17, 18: 4 negative samples (1 minced meat, 1 frozen meat, 2 burger)

-ve: Negative Control.

DISCUSSION

The results of bacteriological examination of tested samples (Table 1&2) revealed that, a total of 29 (13.8%) isolates of foodborne pathogens were recovered from 210 samples (10 from frozen meat, 6 from minced meat and 13 from burger) where *E. coli* were the most isolated (18= 8.6%) followed by *S. aureus* (11=5.2%). For frozen meat samples, 10 (14.3%) isolates were isolated includes *E. coli* (7=10%) were the most isolated followed by *S. aureus* (3=4.3%). Meanwhile minced meat samples, 6 (8.6%) isolates were isolated includes *S. aureus* were the most isolated (4=5.7%) followed by *E. coli* (2=2.9%) while for burger samples, 13 (18.6%) isolates were isolated includes *E. coli* (9=12.9%) were the most isolated followed by *S. aureus* (4=5.7%). Suleiman et al., (2016), recorded nearly similar results. Moreover, these bacterial pathogens in meat are of public health importance for consumers (Son et al., 2014).

The results of *E. coli* isolation (Table 2)

showed that 18 strains were isolated. 7 (10%) from frozen meat, 2 (2.9%) from minced meat and 9 (12.9%) from burger, results come in accordance with that obtained by Geoff et al., (2008) from examined samples. In general, presence of *E. coli* in meat indicates general lack of cleanness which maybe during slaughtering, evisceration, dressing, transportation and handling of meat. As well as, *E. coli* may be taken as an indicator microorganism for the majority of common foodborne pathogens since it offers an estimation of faecal contamination and bad sanitation throughout processing or handling of meat by infected food handlers. Escherichia coli organism is damaged by heat treatment of meat, hence, the existence of *E. coli* in heat processed food means either failure or even more frequently, post processing contamination from equipment, employee or from cross contamination (Jindal et al., 1995).

The results of *S. aureus* isolation (Table 2) cleared that, 11 strains were isolated, three (4.3%) from frozen meat, 4 (5.7%) from minced meat and four (5.7%) from burger, this result were more than that obtained by Omar et al., (2009) and Heredia et al., (2001).

Polymerase chain reaction (PCR), particularly duplex PCR, is a widely used molecular technique, presenting rapid, highly sensitive and accurate results *(*Vidal et al., 2004*).* The experimental results also fortified the truth that using the DNA extraction kit to extract genomic DNA effectively separated DNA from PCR inhibitory materials. Duplex PCR assay established in this particular study was efficient for the detection of the target pathogens. The outcomes even illustrated that amplification of the two sets of primer was effective for yielding two obvious bands.

The current study showed that the duplex PCR technique was very convenient to take DNA templates directly from the meat product samples after DNA extraction and there is no need to take from the culture as it is time consuming, labor intensive and very costly as reported by Kim et al., (2014) and Al Jobori et al., (2015) who examined directly from the food samples without the use of bacterial cultures unlike Latha et al., (2014) and Adhikari et al., (2015) who examined their multiplex PCR technique by the use of bacterial culture.

Consequently, this study was instituted to help a rapid and proficient duplex PCR assay for simultaneous detection of two pathogens as shown in (Table, 3&4 and Fig., 1). For establishment of the duplex PCR assay, choosing of specific target gene for each one of the two target pathogens is critical. Alkaline Phosphatase *(phoA)* gene in *E. coli* strains is utilized in a lot of laboratories for the identification of *E. coli* isolates *(*Hu et al., 2011*)*. The Clumping factor A *(clfA)* present in all *S. aureus (*Mason et al., 2001*)*.

Random positive samples of the examined meat products by conventional bacteriological method, were reexamined with duplex PCR directly from the food samples not from the positive isolates. For *E. coli* and *S. aureus* 8 positive meat products samples by culture method 4 for each microbe were reexamined by duplex PCR, there were total agreement between results of culture method and PCR technique (Table, 3&4 and Fig., 1). For mixed infection, when 4 samples (2 minced meat, 2 frozen meat, 2 burger) that showed positive results by conventional bacteriological method reexamined by duplex PCR assay, the result was agreeing in 100% with that obtained by conventional bacteriological method (Table, 3&4 and Fig., 1). On the other hand, when duplex PCR technique was applied on 4 negative samples (1 minced meat, 1 frozen meat, 2 burger) by culture method, there were no difference in results obtained by both techniques (Table, 3&4 and Fig., 1).

The current results are very similar to those of Thapa et al., (2013) and Kim et al., (2014*)* where they used multiplex polymerase chain reaction on detection of *S. aureus, L. monocytogenes* and *E. coli*, but with different primers, while *Guan* et al., (2013*)* developed a rapid m-PCR method that would definitely enable the simultaneous detection of up to five serious foodborne bacterial pathogens where five pairs of primers were used for identification of *ail gene, nuc gene, rfbE gene, hlyA gene and invA gene for Y. enterocolitica, S. aureus, E. coli* O_{157}*:*$H_{7,}$ *L. monocytogenes and Salmonella,* respectively and Latha et al., *(*2014*)* who established m-PCR assay for the simultaneous detection of four popular food bacterial pathogens in which four pairs of primers were utilized to identify *stm gene, nuc gene, ail gene and hlyA gene for detection of S. enterica typhimurium, S. aureus, Y. enterocolitica and L. monocytogenes*, respectively.

CONCLUSION

Finally, the recorded results showed that duplex PCR approach allows the accurate, rapid, sensitive and automated detection of some foodborne pathogens directly from food sample for routine analysis at a reasonable cost and short

period.

CONFLICT OF INTEREST

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

ACKNOWLEGEMENT

The author would thank all participants

AUTHOR CONTRIBUTIONS

All authors contributed equally in all parts of this study.

REFERENCES

APHA "American Public Health Association", 2001. Compendium of Methods for Microbiological examination of Foods. 4th Ed. F.P. Downes and K.Ito (editors), APHA. Washington D.C., USA.

Bhandare SG, Sherikarv AT, Paturkar AM, Waskar VS, Zende RJ, 2007**.** A comparison of microbial contamination of sheep/goat carcasses in a modern Indian abattoir and traditional meat shops. Food Contr. 18: 854-868.

Geoff H, Andrew P, Michelle I, Andreas K, Geoff R, John S, 2008. Microbial profiles of carcasses and minced meat from kangaroos processed in South Australia. Inter. J. Food Microbiol., 123; (1-2): 88-92.

Guan P, Jiang Y, Gao F, Zhang L, Zhou H, Guan J, 2013. Rapid and simultaneous analysis of five foodborne pathogenic bacteria using multiplex PCR. European Food Research and Technology, 237 (4): 627- 637.

Hennekinne JA, De Buyser ML, Dragacci A, 2012. Staphylococcus aureus and its food poisoning toxins: characterization and outbreak investigation. FEMS Microbiology Reviews, 36: 815-836.

Heredia N, Garcia S, Rojas G, Salazar L, 2001. Microbiological condition of ground meat retailed in Monterrey, Mexico. J Food Prot., 64 (8): 49-51.

Hu Q, Tu J, Han X, Zhu Y, Ding C, Yu, S, 2011. Development of multiplex PCR assay for rapid detection of Riemerella anatipestifer, Escherichia coli, and Salmonella enterica simultaneously from ducks. J Microbiol Methods; 87:64–9.

Jindal N, Arora R, Bhushan B, Arora S, 1995. A study of infective aetiology of chronic diarrhea in children in Amritsar. J. Indian Med. Ass., 93 (5): 169-170.

Johnson J, Kuskowki M, Menard M, Gajewski A, Xercavins M, Garau J, 2006. Similarity between human and chicken Escherichia coli isolated in related to ciprofloxins resistance status. Infect. Dis., 194(1): 71-78.

Kim J, Rhim S, Kim K, Paik H, Lee J, 2014. Simultaneous Detection of *Listeria monocytogens, Escherichia coli* O_{157}:H_7, *Bacillus cereus, Salmonella spp.* and *Staphylococcus aureus* in Low- fatted Milk by Multiplex PCR. Korean J. Food Sci. An., 34(5): 715-716.

Latha A, Sunil A, Jolly D, 2014. Multiplex PCR assay for the simultaneous detection of four common food pathogens in meat. Journal of Foodborne and Zoonotic Diseases, 2(3): 45-49.

Mason W J, Blevins, JS, Beenken K, Wibowo N, Ojha N Smeltzer MS, 2001. Multiplex PCR protocol for the diagnosis of Staphylococcal Infection. J. Clinical Microbiology, 39 (9): 3332-3338.

Oliver SP, Jayarao BM, Almeida RA, 2005. Foodborne pathogens in milk and the dairy farm environment: food safety and public health implications. Foodborne Pathog. Dis. 2: 115-129.

Omar, HS, Ikbal AA, Entesar RA, 2009. Extraction of *Staphylococcus aureus* toxin from minced meat in Mosul City. The Iraq J. for Vet Med, 23(1): 27-32.

Quinn P, Markey B, Carter M, Donelly W, Leonard F, 2002. Veterinary microbiology and microbial disease. Black Well Science; chapters 26-36.

Rodríguez-Lázaro D, Cook N, Hernández M, 2013, Realtime PCR in food science: PCR diagnostics. Current Issues in Molecular Biology, 15, 39–44.

Son I, Binet R, Maounounen-Laasri A, Lin A, Hammack TS, Julie A, Kase JA, 2014. "Detection of five Shiga toxin-producing *Escherichia coli* genes with multiplex PCR".

J. Food Microbiol., 40:31-40.

Suleiman MA, Wasa AA, Na'ilatu MI, Titus I, Adamu S, 2016. Bacteriological Analysis of Chicken Parts of Fresh and Frozen Poultry Chicken Sold in Gombe Metropolis, Nigeria., International Journal of Adulteration, 1 (3): 58-73.

Vidal R, Vidal M, Lagos R, Levine M, Prado V, 2004. Multiplex PCR for diagnosis of enteric infections associated with diarrheagenic *Escherichia coli*. J Clin Microbiol, 42 (1): 1787-1789.