



Demonstration of some foodborne pathogens in different meat products: a comparison between conventional and innovative methods

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ABSTRACT

This study was conducted on 100 random samples of meat products viz: (minced meat, kofta, burger and sausage 25 of each). They were collected from different shops at El-Kaliobia Governorate, to be examined for bacteriological evaluation and detection of *Escherichia coli* and salmonella. These samples were examined for such pathogens by conventional bacteriological methods and confirmed by polymerase chain reaction (PCR). *E. coli* bacteriological results revealed the prevalence in minced meat, kofta, burger and sausage was (16%, 12%, 24%, 12%) respectively, While the prevalence of salmonella was 1% in minced meat. In contrast, kofta, burger and sausage were free from salmonella. Accordingly the traditional method is laborious, time consuming and less accurate as it could not detect some positive Salmonella and *E.coli* samples. Whilst PCR method was found to be accurate, sensitive, and remarkably low cost and shortening the time needed for the pathogenic agent identification. On other hand multiplex Polymerase Chain Reaction (m-PCR) was applied for detection of genes responsible for enterotoxins production from identified salmonella and *E.coli*.

Keywords: Meat products, *phoA*, *invA*, enterotoxin, mPCR.

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1. INTRODUCTION

In Egypt, Meat products are gaining popularity as they represent quick easily prepared meat meals and solve the problem of the shortage in fresh meat of high price, Although meat products may be derived as raw materials from a source less in microbial contamination, it could be contaminated in the course of manufacture, transport or from food handlers, utensils, air and soil, In addition to incomplete hygienic condition during manufacturing steps like packaging, storage and marketing of such products promoting the growth and

multiplication of various bacteria such as *E. coli* and salmonella.

The bacterial contamination and hygienic measures during meat production can be measured using the aerobic plate count and total coliforms (Hamed et al., 2015). Moreover, salmonella is an important pathogen in the food industry and has been frequently identified as the etiological agent of food borne outbreaks (Siqueira et al., 2003). *In the same context*, *E.coli* is considered as a good indicator of possible fecal contamination (Synge, 2000). It is commonly non-virulent but

some strains have adopted pathogenic or toxigenic virulence factors that make them pathogenic to human and animals. It has been associated with numerous out breaks of disease resulting from contaminated beef and meat products, including bacteremia, urinary tract infections, neonatal meningitis, pneumonia, deep surgical wound infections, endovascular infections, vertebral osteomyelitis, and septicemia (Datta et al., 2012). There was a need for development of more innovative methods for rapid identification of food borne pathogen including Polymerase Chain Reaction (PCR) that minimize manipulation, provide results in less time, and reduce costs (Naravaneni and Jamil, 2005).

Multiplex PCR has an advantage against the culturing methods as numerous amount of selective DNA can be used in one PCR reaction. Recent reports have been shown that m-PCR greatly improves specificity and sensitivity for the detection of pathogen many different target genes in a single PCR reaction tube simultaneously (Huang et al., 2009)

The present study was conducted to evaluate the safety and quality of some meat products at Kaliobia Governorate. These meat product samples were examined by conventional bacteriological methods for *E.coli* and salmonella and confirmed by detection of the *phoA* gene for *E. coli* and for *invA* gene for salmonella by polymerase chain reaction (PCR). Moreover m-PCR used for detection of enterotoxine *Stx1*, *Stx2* of *E.coli* and *stn* enterotoxin of salmonella for positive samples.

Materials and methods

2.1. Collection of samples:

A total of 100 random samples of fresh beef and meat products viz: Minced meat, kofta, burger and sausage (25 for each), were collected from different shops at Kaliobia Governorate to evaluate the bacterial status and detection of some food borne pathogens containing them.

2.2. Preparation of samples (APHA, 2001).

2.3. Determination of Aerobic Plate Count (APC)/ g, using the standard plate count following (FDA, 2001).

2.4. Total coliform count by the surface plating method of ICMSF (1996) using Violet Red Bile agar medium.

2.5. Isolation and identification of *E.coli* following (ISO, 2001): Typical *E.coli* colonies (Metallic green colonies) on E.M.B (Eosin methylene blue) were picked up for identification morphologically by Gram's stain and biochemically according to Quinn et al., (2002).

2.6. Isolation and identification of *Salmonella* following ISO (2002). Suspected *Salmonella* colonies that appeared as red with black centers on XLD agar were identified morphologically by Gram's stain and biochemically according to Quinn et al. (2002).

2.7. Polymerase Chain Reaction (PCR):

Using polymerase chain reaction (PCR) in 4 random sample for *E.coli* (two positive & two negative) and 2 random sample for salmonella (one positive& one negative).

2.8. Multiplex polymerase chain reaction (m-PCR):

Using multiplex PCR for detection of *stx1* and *stx2* gene in 4 positive *E.coli* samples (one from each products) and for detection of *stn* gene in 2 positive samples of salmonella.

DNA extraction and purification direct from the meat products samples, following QIAamp DNA Mini Kit (Catalogue no.51304) used to obtain purified. Emerald Amp GT PCR mastermix (Takara) with Code No. RR310A, 1.5% agarose gel electrophoreses (Sambrook et al., 1989) using the Primers sequence, target genes amplicons sizes and cycling conditions showed in Tables (1) and (2).

2.9. Statistical analysis:

Data obtained were analyzed according to Snedecor and Cochran (1969) using the computer software program (SPSS, 2001).

3. RESULTS

The results of bacteriological examination of meat products (minced meat, beef kofta,

sausage and beef burger) are presented in Tables (3-6) and Figures (1-4).

Table1: Cycling conditions of different primers during cPCR

Target	Gene	Primary denaturation	Secondary denaturation	Annealing	Extension	No. of cycles	Final extension
<i>E. coli</i>	<i>phoA</i>	94°C	94°C	55°C	72°C	35	72°C
		5 min.	30 sec.	40 sec.	45 sec.		10 min.
	<i>Stx1,2</i>	94°C	94°C	58°C	72°C	35	72°C
		5 min.	30 sec.	40 sec.	45 sec.		10 min.
<i>Salmonella</i>	<i>invA</i>	94°C	94°C	55°C	72°C	35	72°C
		5 min.	30 sec.	30 sec.	30 sec.		7 min.
	<i>stn</i>	94°C	94°C	59°C	72°C	35	72°C
		5 min.	30 sec.	40 sec.	45 sec.		10 min.

Table 2: A detailed descriptions of the designed oligonucleotide primers

Target	Primer	Gene	Sequence (5' -3')	Amplified product	Reference
<i>Salmonella</i>	F	<i>invA</i>	GTGAAATTATCGCCACGTTTCGGGCAA	284 bp	Oliveira <i>et al.</i> , 2003
	R		TCATCGCACCGTCAAAGGAACC		
	F	<i>stn</i>	TTG TGT CGC TAT CAC TGG CAA CC	617 bp	Murugkar <i>et al.</i> , 2003
	R		ATT CGT AAC CCG CTC TCG TCC		
<i>E. coli</i>	F	<i>phoA</i>	CGATTCTGGAAATGGCAAAAAG	720 bp	Hu <i>et al.</i> , 2011
	R		CGTGATCAGCGGTGACTATGAC		
	F	<i>Stx1</i>	ACACTGGATGATCTCAGTGG	614 bp	Dipineto <i>et al.</i> , 2006
	R		CTGAATCCCCCTCCATTATG		
F	<i>Stx2</i>	CCATGACAACGGACAGCAGTT	779 bp		
R		CCTGTCAACTGAGCAGCACTTTG			

Table 3: Total aerobic plate count (cfu/g) in the examined samples of meat products (n=25)

Samples	Min.	Max.	Mean ±SEM*
Minced Meat	0.06×10 ⁵	2.3×10 ⁵	.95×10 ⁵ ±0.16×10 ⁵ bc
Kofta	0.12×10 ⁵	7.8×10 ⁵	1.7×10 ⁵ ± 0.39×10 ⁵ a
Burger	1.1×10 ⁴	9.5×10 ⁴	3.3×10 ⁴ ±0.45×10 ⁴ c
Sausage	0.12×10 ⁵	2.8×10 ⁵	1.1×10 ⁵ ±0.18×10 ⁵ ab

Different abc in superscript letter within the same column indicate sig. difference (p<0.05). *Standard error of mean

Table 4: Total coliform counts/gm in the examined samples of meat products (n=25)

Samples	Positive		Min.	Max.	Mean ±SEM*
	No.	%*			
Minced Meat	16	64	0.01×10 ²	3.0×10 ²	0.58×10 ² ±0.21×10 ^{2a}
Kofta	15	60	0.07×10 ²	1.4×10 ²	0.39×10 ² ±0.10×10 ^{2a}
Burger	15	60	0.01×10 ²	1.2×10 ²	0.34×10 ² ±0.09×10 ^{2a}
Sausage	14	56	0.01×10 ²	1.3×10 ²	0.32×10 ² ±0.11×10 ^{2a}

*Standard error of mean

Table 5: Incidence of *E.coli* in the examined meat products samples (n=25)

Samples	Positive <i>E.coli</i> samples	
	No.	%
Minced Meat	4	16
Kofta	3	12
Burger	3	12
Sausage	6	24
Total	16	16

Table 6: Incidence of Salmonella in the examined meat products samples (n=25)

Samples	Positive Salmonella samples	
	No.	%
Minced Meat	1	4
Kofta	0	0
Burger	0	0
Sausage	0	0
Total	1	1

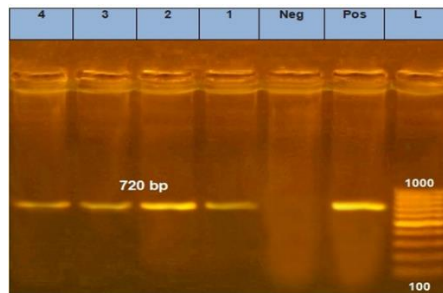


Fig.1. Agrose gel electrophoresis showing PCR products of *phoA* gene (720bp) specific for characterization of *E.coli* species. Lane L: 100-1000 bp DNA Ladder. Neg.: Negative control (*S. aureus* reference: ATCC25923 at 638 bp). Pos.: Positive control (*E.coli*: ATCC14028 at 720 bp). Lane 1., 2., 3 &4: *E.coli* (Positive).

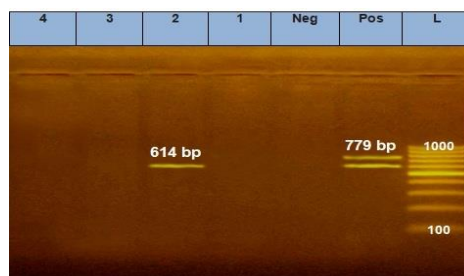


Fig.2. Agrose gel electrophoresis of multiplex PCR of *Stx1* (614bp), and *Stx2* (779bp) for characterization of positive *E.coli* samples Minced meat, kofta, burger and sausage (one from each products) (n=4). Lane L: 100-600 bp DNA Ladder. Neg.: Negative control. Pos.: Positive control for *stx1*, and *stx2*. Lane 2: Positive *E.coli* strain for *Stx1* gene (minced meat). Lane 1,3,4: (Negative) *E.coli* strain for *stx1&stx2*.

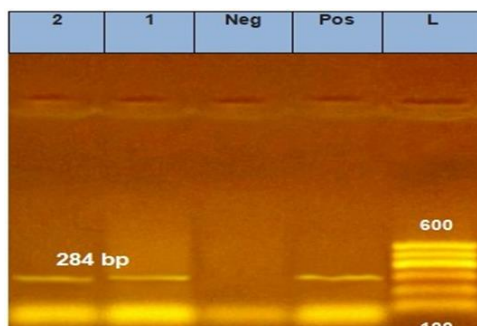


Fig.3. Agrose gel electrophoresis showing PCR products of *invA* gene (284bp) specific for characterization of all Salmonella species. Lane L: 100-600 bp DNA Ladder. Neg.: Negative control (*S. aureus* reference: ATCC25923 at 638 bp). Pos.: Positive control (Salmonella reference: ATCC14028 at 284 bp). Lane 1& 2: Salmonella (Positive).

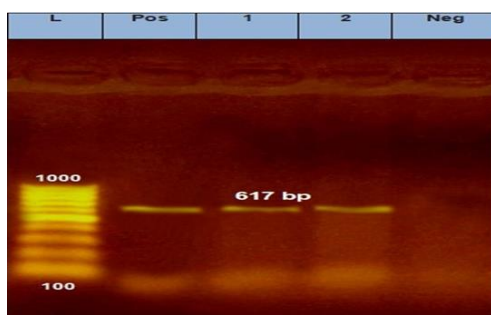


Fig.4. Agrose gel electrophoresis of PCR of *stn* enterotoxin genes (617bp) for characterization of positive salmonella samples (Minced meat and sausage one from each products). Lane L: 100-600 bp DNA Ladder. Neg.: Negative control. Pos.: Positive control for *stn*. Lane 1&2: Positive salmonella strain for *stn* gene.

4. DISCUSSION

4.1. Total Aerobic Plate Count:

The total aerobic reflect the bacterial contamination and declared the hygienic quality of both raw meat and meat products. Meanwhile, Coliform counts may indicate faecal contamination either from human or animal sources and its presence indicate poor sanitation and handling (Paulsen *et al.*, 2006). Food borne diseases caused by *E. coli* and Salmonella species that transmitted mainly through consumption of contaminated food

and the presence of them in meat and raw meat products has relevant public health implications (Normanno *et al.*, 2007).

The data shown in Table (3) revealed that the mean of aerobic plate counts (APC) in the examined of meat products (minced meat, kofta, burger and sausage) was $.95 \times 10^5 \pm 0.16 \times 10^5$., $1.7 \times 10^5 \pm 0.39 \times 10^5$., $3.3 \times 10^4 \pm 0.45 \times 10^4$ and $1.1 \times 10^5 \pm 0.18 \times 10^5$, respectively. Although 100% of samples showed bacterial contamination, the counts were considered lower than those suggested by Abu EL Hassan-Asmaa (2012) and Abd El-

Aziz- Wafaa (2015) and higher than those counts were recorded by Mousa *et al.* (2014) Abd El-Fatah-Rabab (2015) and Hamed *et al.* (2015).

Moreover, the statistical results revealed that, kofta samples showed a significant ($P \leq 0.05$) increase of APC when compared with burger and minced meat samples. In addition, sausage samples showed a significant ($P \leq 0.05$) increase of APC when compared with burger. Moreover, there were no significant difference ($P > 0.05$) of APC counts between kofta and sausage samples as well as between burger and minced meat samples in addition to between sausage minced meat.

4.2. Total Coliform Count:

Data presented in Table (4) showed that, the value of coliform count of meat products (minced meat, kofta, burger and sausage) was $0.58 \times 10^2 \pm 0.21 \times 10^2$, $0.39 \times 10^2 \pm 0.10 \times 10^2$, $0.34 \times 10^2 \pm 0.09 \times 10^2$ and $0.32 \times 10^2 \pm 0.11 \times 10^2$, respectively. These results came in parallel with those of Paulsen *et al.* (2006), Stagnitta *et al.* (2006), Al-Mutairi (2011), and Heweidly (2017). Moreover, the statistical results revealed that, there were no significant difference ($P > 0.05$) of *total Coliform count* between minced meat, kofta, burger and sausage samples.

4.3. Isolation and identification of *E.coli*.

The results in Tables (5) revealed that, 16 isolates of *E.coli* were isolated from examined meat samples represented as 4 (16%) from minced meat, 3 (12%) from kofta, 3 (12%) from burger and 6(24%) from sausage samples. Nearly similar results were obtained by Ahmed, M. N. (1992) and Fathi et al., (1992). while, higher than those obtained by (Blanco and Blanco, 1996) and lower than those of Shawki, (1990). The increased incidence of *E.coli* in the examined samples may be due to mishandling during production,

processing and distribution or to the use of contaminated water during evisceration and slaughtering (Gwida *et al.*, 2014).

4.4. Isolation and identification of *Salmonella*.

The results obtained in Table (6) revealed that, only one salmonella was isolated from minced meat represented as 1(4%) and failed to be detected from other products. These results were agreed with those recorded by Datta *et al.* (2012) and Abdel-Raouf et al., (2014). Meanwhile, disagreed with those of Maarouf and Nassif-Marionette (2008), Abdaslam *et al.* (2014).

The incidence of salmonella in frozen minced meat may due to cutting and contamination of meat besides the increase in its water and oxygen contents as well as contamination from grinders, air, and hands of the workers. Temperature rise ($2-4^{\circ}\text{C}$) during grinding could also increase the incidence of *Salmonella* organisms (Field et al. ,1977).

The results of the bacteriological examination of meat products revealed that APC were highest in kofta and sausage then minced meat then burger. While, coliform count was highest in minced meat then kofta and burger then sausage. As well as the incidence of *E. coli* was highest in sausage then minced meat then burger and kofta while only one sample salmonella serovars was detected bacteriologically in minced meat

Finally, the present study proved that meat products are considered public health hazard due to the presence of coliforms., *E.coli* salmonella may be due to mishandling and the negligence of hygienic aspects either at production levels where most workers did not have medical certificates or selling of meat with expired dates. Therefore, it was concluded that *E.coli* and salmonella are meat-borne pathogens of public health important.

4.5. Uniplex Polymerase Chain Reaction (PCR).

The PCR not only replace the more labour-intensive conventional culturing techniques, but also allow the detection of species that are present at low level that can remain undetected by plating so the m-PCR might be used for detection the minimal microbial concentration Settanni and Corsetti (2007). The current study showed that the PCR technique was very convenient to take DNA templates directly from the meat products samples after DNA extraction and there is no need to take from the culture as it time consuming, labour intensive and very costly. Guan et al., (2012) and Kim et al., (2014) subjected food samples directly to PCR without the use of bacterial cultures and also with the same primer for salmonella but with different primers used in this study for *E. coli*. This result was unlike to latha et al., (2014) who examined their multiplex PCR technique by the use of bacterial culture.

Regarding to *E. coli*, Four random samples (two positive and two negative) by conventional method, were reexamined by PCR, there were great agreement between results of conventional method for and PCR technique in two positive random samples (minced meat and sausage). While two samples were negative by conventional method (burger and kofta) showed positive results with PCR (false negative) as in fig. (1).

For Salmonella, two random samples (one positive and one negative) by conventional method, were reexamined by PCR, there were great agreement between results of conventional method for and PCR technique in one positive random samples (minced meat). While the other sample (sausage) was negative by conventional method showed positive results with PCR (false negative) as in fig. (3). These results were nearly similar to Gihan, M. et al., (2016) who confirmed the presence of *E.*

coli and *Salmonella* spp. using *phoA* and *invA* gene respectively by conventional PCR at 720 bp and 284 bp, respectively.

The false negative result may due to low number of bacterial load which can't be detected by microbiological assay and effect of freezing. Also, inhibition of some microbes to selective microbe appearance on the media. So the m-PCR assay has the potential to be used in routine diagnostic laboratories and also as a rapid screening tool in food testing laboratories to identify food samples quickly especially in case of outbreaks.

4.6. Multiplex PCR for detection of classic enterotoxin.

The m-PCR results showed that *stx1* was detected in one sample recovered from minced meat sample, while *stx2* was not detected in all samples, these results go parallel with Blanco and Blanco (1996) and Abd El Tawab et al. (2015). While *stn* gene was detected in both samples of minced meat and sausage Fig. (2-4). These results nearly similar to Amin and Abd El-Rahman (2015) who able to detected enterotoxin gene (*stn*) of salmonella in all examined sample. Meanwhile, disagreed with Gihan, M. et al (2016) who failed to detected *stn* gene.

5. CONCLUSION

In conclusion Meat products are considered as a good medium for the growth of different food borne pathogen and production of toxin. The presence of this bacterial species in the final products is a result of contamination during the manufacturing, distribution storage, mincing, packaging and retail sale of the products. This subsequently contributes to health risks to the consumer. Therefore, proper handling, adequate cleaning of hands, surfaces, disinfection of the market, good personal hygiene and good hygienic practices not only in the manufacture, but also in the retail sale can reduce the spreading of these serious

pathogens and to obtain final products with a maximum limit of safety.

PCR is rapid and specific method for detection of different food borne pathogen in meat products samples. It gives the ability to detect bacteria cells within a little time and PCR was demonstrated to be accurate methods for identification.

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