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Prevalence of some food poisoning bacteria in semi cooked chicken meat products at Qaliubiya governorate by recent Vitek 2 compact and PCR techniques

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ABSTRACT

Bacterial foodborne pathogens are important food safety issue worldwide. Rapid and accurate identification of bacteriological pathogen isolated from food staff is important both for food quality assurance and for the tracing of outbreaks of bacterial pathogen. A total of ninety random samples of semi cooked chicken meat products represented by strips; pane and nuggets (30 of each) were collected from different supermarkets at Qaliubiya governorate for bacteriological examination using conventional culture method and rapid modern techniques as Vitek 2 compact system and molecular identification by PCR technique. The obtained results revealed that *Staph. aureus* and *E.coli* incidence were 20% and 10% in strips, 26.6% and 13.3% in chicken pane and 26.6% and 23.3% in nuggets for total examined samples. Also, *E.coli* was serologically typed as *O127, O128, O153, O157* and *O91. Staph. aureus and E. coli* were identified in all examined samples by confirmatory identification using Vitek 2 compact system in examined 10 random samples of the chicken products. Results recorded accuracy 100% for examined samples and showed that the Vitek 2 system is a suitable tool for rapid and direct identification of gram-positive cocci and gram-negative bacilli from chicken products. The PCR technique revealed that there were one or more virulence genes in *E. coli* (stx1&stx2) strains isolated from the examined samples of chicken products.

1. INTRODUCTION

Food processing is an important industry worldwide. One of the major problems threatening food industry is the contamination with foodborne microbes of human origin resulting from improper handling and processing. Microbial contamination reduces shelf life and food quality leading to food infection and poisoning outbreaks, some of which are life threatening. Continuous monitoring of food processing is essential to avoid potential health problems (Al-Bahry et al., 2014).

The coagulase positive Staphylococci, which include *Staph. aureus*, the most pathogenic species, that is considered the third important cause of food borne diseases in the world. This pathogen is considered an excellent indicator of thermal processing inefficiency, inadequate hygienic conditions during food production, preparation or inadequate cooling (Melheiros et al., 2010).

Enteropathogenic *E.coli* organisms constitute public health hazards as they may give rise to sever diarrhea in young children and adolescents as well as food poisoning and gastroenteritis among adult consumer (Bohaychuck et al., 2006). So, with the constant increase in semi cooked chicken meat products consumption worldwide and the variety of products and consumer demand.

Bacteriological criteria as are very important; they provide guidance in what concerns the acceptability of food and manufacturing processes, manipulation and distribution. The automated microbial identification system have become widely used in both clinical and food microbial laboratories .These systems offer some advantages over conventional methods including reduce labor ,reduce human error, increased samples throughput and rapid test result .Some example of automated microbial identification is Vitek systems and PCR technique (Darbandi, 2010).

2. MATERIAL AND METHODS

2.1. Collection of samples: Ninety of semi cooked chicken products of pane, strips and nuggets (30 of each) were collected from different supermarkets at Qaluobia governorate. Samples were transported directly and aseptically to the laboratory in an ice box.

2.2. Preparation of samples (APHA,2001): under aseptic condition twenty five grams of each sample were weighted and transferred into a sterile homogenizer flask containing 225 ml of 0.1% sterile buffered peptone water then homogenized at 2000 rpm for 1-2 min. to provide a homogenate of 1/10 dilution, one ml from the original dilution was transferred to another sterile tube containing 9 ml of 0.1% sterile buffered peptone water and mixed well to make the next dilution, from which further decimal serial dilutions were prepared. The prepared dilutions were subjected to the following examinations

2.3. Isolation and identification of staph. aureus: It was

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carried out according to ICMSF, 1996.

2.4. Isolation and identification of E.coli: It was carried out according to ISO, 2007.

2.5. Confirmatory biochemical identification of Staph. aureus and E.coli isolates by Vitek 2 compact system technique: It was carried out according to pincus (2006).

2.6. Molecular identification of coagulase gene of Staph. aureus and Shiga toxins virulence genes of E.coli: PCR technique was performed to 10 random samples of recorded results of traditional methods. Firstly, DNA extraction (Shah *et al*., 2009) then amplification of *E.coli* (Fagen *et al*., 1999) and amplification of *Staph. aureus* (Mehrorta *et al.,* 2000) was adopted.

3. RESULTS

As shown in table 1 results revealed that, a total of 22 isolates of coagulase positive *Staph. aureus* were isolated from examined samples those were 6 (22%) from strips, 8 (26.6%) from pane and 8 (26.6%) from nuggets. Moreover, the incidences of E.coli were 10%, 13.3% and 23.3% of examined chicken samples of strips, pane and nuggets respectively.

Table 1 Prevalence of some food-borne pathogens in examined chicken products samples (n=30)

microorganism	Examined chicken samples(n=30)					
	Chicken strips		Chicken pane		Chicken nuggets	
	No.	%	No.	%	No.	%
Staph. aureus	6	20	8	26.6	8	26.6
E. coli	3	10	4	13.3	7	23.3

Table 2 showed that the prevalence of serologically identified *E.coli* in strips samples were Enterotoxigenic *E.coli O127: H6* (66.6%) and Enteropathogenic *E.coli O128: H2* (33.33%) , identified *E. coli* in pane were Enterotoxigenic *E.coli O127: H6* (50%) and Enterohemorrhagic *E.coli O157:H7* (25%) and Enteropathogenic *E.coli O153:H2 (*25%) but the prevalence in nuggets samples were Enterohaemorrhagic *E.coli O157:H7* (28.5%), Enterotoxigenic *E.coli O127:H6* (57.1%) and Enteropathogenic *E.coli O91:H21 (*14.2%*).*

Table 2 Serotyping of *E.coli* isolated in examined chicken products samples.

E.coli serotypes	Examined chicken samples(n=30)					
	Chicken strips (n=3)		Chicken pane (n=4)		Chicken nuggets (n=7)	
	No.	%	No.	%	No.	%
O157:H7 (EHEC)	-	-	1	25	2	28.5
O127:H6 (ETEC)	2	66.6	2	50	4	57.1
O153:H2(EPEC)	-	-	1	25	-	-
O91:H21(EPEC)	-	-	-	-	1	14.2
O128:H2(ETEC)	1	33.3				

Table 3 and 4 showed confirmatory identification of 10 traditionally isolated *Staph. aureus* and *E.coli* (5 for each species) from examined products by Vitek 2 compact system. The results were identically recorded by conventional method. As, it reported that using of Vitek 2 compact system provides very good and trustable accuracy and reproducible results as shown in reported samples that mean 100% when compared with conventional method. Also, coagulase positive *Staph. aureus* in 5 random isolated *Staph. aureus* and virulence genes of *E.coli (*stx1 &stx2) were reexamined by using one of the most recent developments PCR techniques.

Table 3 Identification of *Staph. aureus* by using recent biochemical technique(Vitek 2 compact system).

Biochemical Details																
2	AMY	+	4	PIPLC	-	5	dXYL	-	8	ADH1	+	9	BGAL	+	11	AGLU	+
13	APPA	-	14	CDEX	-	15	AspA	-	16	BGAR	-	17	AMAN	-	19	PHOS	-
20	LeUA	+	23	ProA	-	24	BGURr	-	25	AGAL	-	26	pyrA	-	27	BGUR	-
28	AlaA	-	29	TYrA	-	30	dSOR	-	31	URE	-	32	POLYB	+	37	dGAL	-
38	dRIB	+	39	iLATk	+	42	LAC	-	44	NAG	+	45	dMAL	-	46	BACI	+
47	NOVO	-_	50	NC6.5	+	52	dMAN	+	53	dMNE	+	54	MBdG	+	56	PUL	-
57	dRAF	-	58	O129R	+	59	SAL	+	60	SAC	+	62	dTRE	+	63	ADH2s	-
64	OPT	++															

Table 4 Identification of *E.coli* by using recent biochemical technique (Vitek2 compact system).

Biochemical Details																
2	APPA	-	4	ADO	-	5	LeuA	+	7	IARL	+	10	ERYa	-	12	BGAL	-
10	BGAL	-	14	BANG	-	15	ARBa	-	18	AMYa	-	19	dGALa	+	20	GENa	-
17	APPA	+	23	LACa	-	24	MAdGa	+	26	dCELa	-	27	GGT	-	28	dMALa	+
25	ELLM	-	30	NAGA1	+	32	dMNEa	+	33	dMELa	-	34	dMLZa	-	38	ISBEa	-
32	IRHAa	-	40	XLTa	-	42	dSORa	+	44	SACa	+	45	URE	+	46	AGLU	(+)
43	dTURa	+	48	dTREa	-	49	NO3a	-	51	IARAa	-	54	DGATa	-	53	ESC	-
50	IGLTa	+	55	dXYLa	-	56	LATa	+	58	ACEa	+	59	CITa	+	60	GRTas	(-)
61	IPROa	+	62	2kGa		63	NAGa	+	64	dGNTa	+						

Fig. 1 showed that from 5 samples were confirmed for presence of coagulase gene in examined chicken products in 2 pane, 2 nuggets and one strip there were 4 (80%) coagulase positive *Staph. aureus.*

Fig. 2 indicated that agarose gel electrophoresis of multiplex PCR for detection of stx1 (614 bp) and stx2 (779 bp) virulence genes for characterization of Enteropathogenic *E.coli.* The results of molecular

identification showed the presence of 2 virulence genes in the chicken products in all *E.coli* isolates.

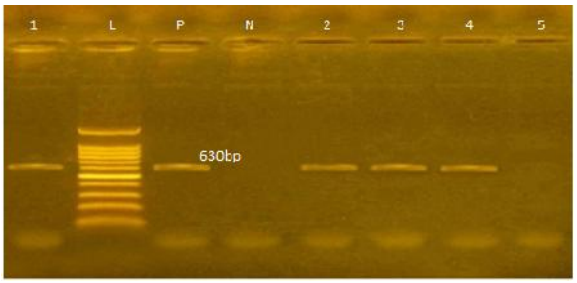

Fig 1 Agarose gel electrophoresis of positive amplification of *coa* gene fragment (630 bp)of *S.aureus* isolates. P: control positive, N: control negative, L : DNA ladder, Lane1, 2, 3&4: positive *coa* gene fragment (630 bp), Lane 5: negative *coa* gene fragment

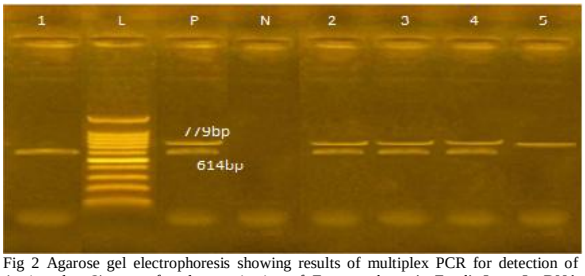

Fig 2 Agarose gel electrophoresis showing results of multiplex PCR for detection of *(stx1 and stx2)* genes for characterization of *Enteropathogenic E.coli*. Lane L: DNA ladder. Lane P: control positive .Lane N: control negative. Lane1(*E.coli* O128): Positive strain for stx1(614 bp). Lane2(*E.coli* O157), 3 (*E.coli* O127)and 4(*E.coli* O91): Positive of stx1(614 bp) and Positive of stx2(779 bp). Lane5 (*E.coli* O153): Positive of stx2 (779bp).

4. DISCUSSION

The current study was designed to isolate and identify *Staph. aureus* and *E.coli* from some semi cooked chicken meat products in Qaluobia governorate using conventional methods, automated biochemical method and molecular technique for identification of coagulase gene of *Staph. aureus* and virulence genes of *E.coli*.

Table [1] revealed that the occurrence of *Staph. aureus* was 22 (24.5%) of samples represented by 8 (26.6%) samples from pane, 8 (26.6%) samples for nuggets and 6 samples (22%) for strips .Moreover 68 out of 90 ones were accepted, as they were free from coagulase positive *Staph. aureus* isolates according to EOS (2005) by conventional culture method.

As collected samples were frozen or chilled raw products, the high incidence of *Staph. aureus* in chicken products especially in pane and nuggets could be attributed to that local manufacturers use of untreated and contaminated additives and spices and/or miss handling of these products.

These results came in accordance with those obtained by Atia (2017), El-Kholy (2018), and Arab (2010). furthermore, higher incidence reported by Abou-ElRoos (2010) include 44% in pane and 40% in nuggets; Amin (2015) reported 40% in nuggets and Shaltout *et al*.(2018) observed 56.6% in pane, 40% of nuggets and 43.3% in strips .While, lower incidence recorded by Shaltout et al., (2002) was 6% and Olimpia (2006) detected 15% in nuggets and Edris (2015) recorded 10% in nuggets. Otherwise, Shanab (2014) failed to detect *Staph. aureus* in examined samples. The presence of *Staph. aureus* in heat treated chicken products may be due to its contamination from food handlers inadequate cleaned equipment or post processing contamination (Duffy *et al*.,2000).

Also, table [1] showed that nuggets samples were contaminated by *E.coli* rather than other chicken products, the results is nearly similar to that obtained by Samaha *et al.*,(2012) where 12% in pane; by Edris (2015) where 25% in nuggets; and Elkohly (2018) where 16.6% of nuggets. Although lower results were recorded by Lee (2009) include 4.6%; while Arab (2010) reported 6.67% in pane and Khallaf (2019) observed 6.67% in pane but AbdEl-Sattar et al., (2016) recorded 36% in pane; Shaltout *et al.(*2018) detected 46.6% in pane, 30% in nuggets and 43% in strips as higher results. Although, Younes (2014) failed to isolate this microbe from pane and nuggets

Table [2] showed that the identified *E.coli* serotypes that is nearly similar to obtained by Karadal (2013) *O157*; Awadallah (2014) *O128*; Emara (2016) *O128, O91*; Atia (2017) *O91, O127,O153* and *O128*; Elkholy (2018) *O91,O153* and *O128*. The variation in the results between different authors may be due to the differences in manufacture practices, handling from producers to consumers and the effectiveness of hygienic measures applied during production. The presence of *E. coli* in food is considered as an indicator of faults during preparation, handling, storage or service. So, there are 3 main routes by which microorganisms enter the food through raw food used, food handlers and the surrounding environment. Fecal contamination can act as cross contamination of raw food which is never sterile and careful working practices are essential source of *E. coli* infection (ICMSF, 1996).

Table [3 and 4] showed identification of gram positive *Staphy. aureu*s and gram-negative *E.coli* of 10 traditionally isolated isolates by using Viteck2 compact system from examined chicken product samples. The correlations between the two methods were 100%. Nearly similar results were recorded by (Mellissa *et al.*, 2017) and (Spanu *et al*., 2003) was identified *Staph. aureus* with accuracy rate 99.6% when compared by PCR technique. But, Khalaf (2019) were identified isolated *Staph. aureus* and *E. coli* in a percentage 50% and 6.6% from chicken pane. This study showed that the PCR technique was very convenient to take DNA templates directly from the chicken meat products samples after DNA extraction and there is no need to take from the culture as it time-consuming, labor intensive and very costly as reported by Chen et al., (2012) and Kim et al., (2014) who examined directly from food samples without the use of bacterial cultures but with different primers used in this study unlike Latha *et al.*, (2014) who examined their PCR technique by the use of bacterial culture.

Five random *Staph. aureus* samples; 3 positive and 2 negative reexamined by PCR, there was great agreement between results of conventional method and PCR technique in four random samples; four positive and 1 negative while one sample was negative by conventional method for *Staph. aureus*, showed positive results with PCR (false negative) as in fig [1]. This result clarified the high sensitivity of the PCR technique in detection the false negative results of the traditional microbiological culture method. Similar results were obtained by Chen et al., (2012) and Moustafa (2016) who detected a false negative result negative by conventional method and positive by m-PCR. The false negative result may due to the low number of bacterial load which can't be detected by microbiological assay. 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.

Fig [2] revealed that there were one or more virulence genes in *E.coli* strains isolated from the examined samples of chicken products.*E.coliO127:H6,O157:H7&O91:H21* have the 2 virulence genes Stx1and Stx2), *WhileO128:H2* have Stx1 virulence gene and *O153:H2* have Stx2 virulence gene of *Escherichia coli*. Similar results were recorded by Emara (2016), Atia (2017) and Younes (2017) who recorded the different virulence genes in some heat-treated chicken meat products. *E.coli* strains possess that genes were more toxigenic and hazardous to consumer health.

The presence of Shiga toxins *E.coli* in all examined samples by PCR technique revealed that there were improper hygienic faults during preparation, distribution and storage. Shiga toxin genes are found in more than 200 serotypes of different strains of *E.coli* strains, of which O157 and non O157 serotypes are quite well known for their disease-causing ability (Page and Liles, 2013).

5. CONCULSION

Finally, the current study allows concluding that the possibility of contamination of semi cooked chicken 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 chicken products should be adopted to control these serious pathogens and to obtain a maximum limit of safety to consumers.Vitek 2 compact system and PCR technique showed more effective and rapid method for identification of food borne pathogen.

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