Hygienic Considerations of Pathogenic *Escherichia Coli* Contamination on Cattle Carcass Surfaces in Egypt

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A B S T R A C T

A total of 100 random samples were collected from surfaces of cattle carcasses in EL-Shouhada and Birket El –Sabaa Abattoirs in Menofyia governorate (50 of each).The results showed that the mean *E. coli* counts were 5.44×10^2 and 7.99×10^2 cfu/cm^2 in EL- Shouhada and Birket EL-Sabaa abattoirs, respectively. Also, the incidence of *E .coli* isolated from cattle carcass surfaces in EL-Shouhada and Birket El-Sabaa abattoirs were 26% and 28%, respectively. The isolated serotypes of *E. coli* were EHEC as O_{111} : H_2 (8%) and O_{26} : H_{11} (2%), EPEC as O_{15} : H_4 (2%), O_{55} : H_7 (2%), O_{114} : H_4 (4%) and O_{146} : H_{21}(2%), ETEC as O_{128} : H_2 (2%) and O_{125}: H_{21} (2%) and EIEC as O_{124} (2%) in EL-Shouhada abattoirs, while in Birket EL-Sabaa abattoir were EHEC as O_{111} : H_2 (6%) , O_{26} : H_{11} (8%) and O_{103} : H_4 (2%), EPEC as O_{55} : H_7 (2%) and O_{91} : H_{21}(2%) ETEC as O_{128}: H_2 (6%) and EIEC as O_{124} (2%). Findings of multiplex PCR showed that *eaeA* (intimin) gene was detected in (O_{91}:H_{21} and O_{114}:H_4), *hlyA* (haemolysin) gene was detected in (O_{125}:H_{21}), *stx*$_1$ gene was not detected in the all isolated *E. coli* serogroups, but *stx*$_2$ gene was detected in (O_{26}:H_{11} and O_{114}:H_4).

Keywords: *Escherichia Coli, Cattle Carcass, Menofyia governorate*.

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1. INTRODUCTION

The sanitary conditions of abattoirs and their surrounding environment are major factors contributing in bacterial contamination of meat (Gill *et al.*, 2000).

Escherichia coli infection is highly prevalent in abattoir environment, and can pose a major threat to human health in underdeveloped communities (Abu El naga-*et al*., 2014). Meanwhile, it is commonly non virulent but some strains have adapted pathogenic or toxigenic virulence factors that make them virulent for humans and animals (Malik and Memona, 2010). It is also commonly used as surrogate indicator; its presence in food generally indicates direct and indirect fecal contamination (Clarence *et al*., 2009).

Shiga-toxin producing *E. coli* (STEC) were found in a wide variety of animal species, including cattle, sheep, goats, pigs, water buffalos and wild ruminants (Caprioli *et al.*, 2005). Cattle form the main reservoir of (STEC) and fecal contamination of food represents the usual source of infection for humans but due to an apparently low infectious dose, human to human transmission

was also observed in outbreaks (Kuhnert *et al*., 2000). Pathogenic strains of *E. coli* were divided into intestinal pathogenic *E.coli* (INPEC) causing diarrhea and extraintestinal pathogenic *E.coli* (EXPEC) including urinary tract infection (UTI), meningitis and septicemia depend on virulence factors and clinical symptoms (Kaper *et al.*, 2004 and Eid and Erfan, 2013).

Polymerase Chain Reaction (PCR) based methods were identified as a powerful diagnostic tool for the detection of pathogenic microorganisms (Malorny *et al.*, 2003). Compared to other methods of detection, these methods were rapid, highly specific and sensitive in the identification of target organisms (Wang *et al.*, 2007).

2. Materials and methods

2.1. Collection of samples:

A total of 100 random samples were collected from surfaces of cattle carcasses slaughtered in EL-Shouhada and Birket El – Sabaa abattoirs (50 of each) in Menofyia governorate. The samples were taken using swab technique under aseptic conditions, just after washing and before stamping. The collected samples (Swabs) were preserved in an ice box then transferred to laboratory without undue delay and subjected to the microbiological examination.

٢*.2. Preparation of templates and Swabbing* (FSIS, USDA., 1996)

A templates made of metal having an exposed inner area 10 cm^2 (2x5cm) was used to delineate area of sampling.

Swabs from cattle carcasses surfaces were taken by using sterile cotton swabs and templates. The sterilized templates were placed firmly against the surface of examination to limit the examined area. The sterile cotton swab was rolled over the limited area inside the template, rolled in one direction and perpendicular to this direction to represent all the examined area. Finally, the cotton swabs were aseptically retained into the rinsing fluid screw capped tubes containing 10ml buffered peptone water (0.1%).

2.3. Preparation of swabs (APHA, 2001):

The collected swabs were mixed in 225ml of sterile buffered peptone water (0.1%) to give 1/10 dilution. One ml from the original dilution was transferred with sterile pipette to another sterile test tube containing 9ml of buffered peptone water and mixed well to make the next dilution, from which further decimal serial dilutions were prepared.

2.4. Detection of E. coli count was done according to McFadden (2000).

2.5. Isolation and identification of Escherichia coli was done according to McFadden (2000).

2.6. Serological identification of E. coli

The isolates were serologically identified according to *Kok et al. (1996)* by using rapid diagnostic *E.coli* antisera sets (DENKA SEIKEN Co., Japan).

2.7. Polymerase Chain Reaction (PCR):

2.7.1. Extraction of DNA

2.7.2. Preparation of PCR Master Mix

2.7.3. Cycling conditions of the primers during cPCR

2.7.4. DNA Molecular weight marker

2.7.5 Agarose gel electrophoreses

3. RESULTS

The results in table (1) revealed that the mean of *E. coli* counts were $5.44\times10^2\pm1.13^a$ and $7.99\times10^2\pm1.13^A$ in EL-Shouhada and Birket El-Sabaa abattoir, respectively. On the other hand, the results in Table (3) showed that the incidence of *E. coli* in the examined swab samples was (26%) and (28%) in EL-Shouhada and Birket EL-Sabaa abattoirs, respectively. Also, it is obvious from the results achieved in Table (5) that the serological identified *E. coli* serotypes were EHEC as O_{111} : H_2 (8%) and O_{26} : H_{11} (2%),

EPEC as O_{15} : H_4 (2%), O_{55} : H_7 (2%), O_{114} : H_4 (4%) and O_{146} : H_{21}(2%), ETEC as O_{128} : H_2 (2%) and O_{125}: H_{21} (2%) and EIEC as O_{124} (2%) in EL-Shouhada abattoir. Meanwhile in Birket El-Sabaa abattoir, EHEC as O_{111} : H_2 (6%) , O_{26} : H_{11} (8%) and O_{103} : H_4 (2%), EPEC as O_{55} : H_7 (2%) and O_{91} : H_{21}(2%) ETEC as O_{128}: H_2 (6%) and EIEC as O_{124} (2%). While, the results in Table (6) showed that the *eaeA* (intimin or *E. coli* attaching and effacing) gene was detected in (O_{91}:H_{21} and O_{114}:H_4), *hlyA* (haemolysin) gene was detected in (O_{125}:H_{21}), *stx*$_1$ gene was not detected in the all isolated *E. coli* serogroups, but *stx*$_2$ gene was detected in (O_{26}:H_{11} and O_{114}:H_4

Table (1): Mean values of *E. coli* count (cfu/cm^2) in swab of cattle carcass surfaces from abattoirs in Menofyia governorate (n=50).

Abattoirs	Min.	Max.	Mean± S.E*
EL- Shouhada	1.82×10^2	4.07×10^3	$5.44\times10^2\pm1.13^a$
Birket El-Sabaa	2.29×10^2	6.31×10^3	$7.99\times10^2\pm1.13^A$

S.E* = Standard error of mean

Table (2): Analysis of variance (ANOVA) of *E. coli* count in swab of cattle carcass surfaces from abattoirs in Menofyia governorate (n=50).

Source of Variance	S.S.	D.F.	M.S.	F. value	Sig.
Between Groups	.694	1	.694	4.729	.032
Within Groups	14.380	98	.147		
Total	15.074	99			

D.F = Degrees of freedom, S.S = Sum squares, M.S = Mean squares, = significant differences ($P<0.05$)

Table (3): Incidence of *E. coli* in the examined swab of cattle carcass surfaces from abattoirs in Menofyia governorate (n=50).

Abattoir	Positive samples	
	NO.	%
EL- Shouhada	13	26
Birket El-Saba	14	28
Total	27	54

Table (4): Serotypes of *E. coli* in the examined swab of cattle carcass surfaces from abattoirs in Menofyia governorate (n=50).

Swabs / Strains	EL- Shouhada abattoir		Birket-El-Sabaa abattoir		Strain Characteristics
	No.	%	No.	%	
O111 : H2	4	8	3	6	EHEC
O26 : H11	1	2	4	8	
O103 : H4	-	-	1	2	
O15 : H4	1	2	-	-	EPEC
O55 : H7	1	2	1	2	
O114 : H4	2	4	-	-	
O146 : H21	1	2	-	-	
O91 : H21	-	-	1	2	
O128 : H2	1	2	3	6	ETEC
O125 : H21	1	2	-	-	
O124	1	2	1	2	EIEC
Total	13	26	14	28	

Table (5): The results of PCR amplifications of different used genes of *E. coli* serogroups:

Sample	Results			
	EaeA	*HlyA*	*Stx1*	*Stx2*
1(O_{15}:H_4)	-	-	-	-
2(O_{26}:H_{11})	-	-	-	+
3(O_{55}:H_7)	-	-	-	-
4(O_{91}:H_{21})	+	-	-	-
5(O_{103}:H_4)	-	-	-	-
6(O_{111}:H_2)	-	-	-	-
7(O_{114}:H_4)	+	-	-	+
8(O_{124})	-	-	-	-
9(O_{125}:H_{21})	-	+	-	-
10(O_{128}:H_2)	-	-	-	-
11(O_{146}:H_{21})	-	-	-	-

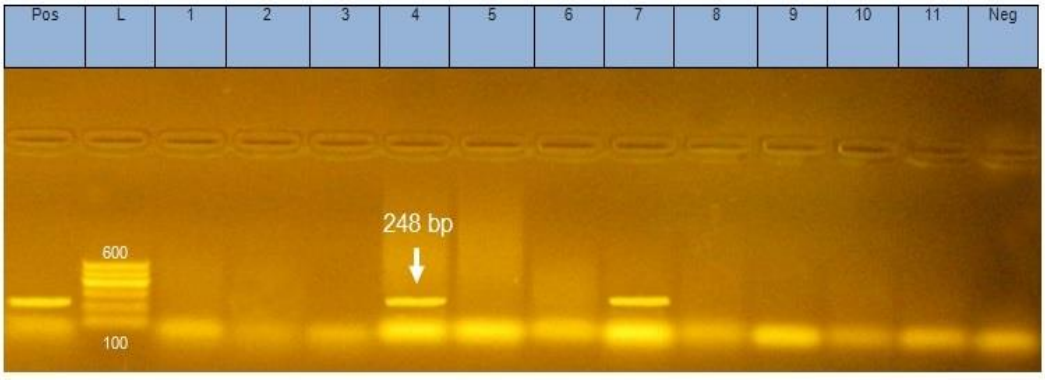

Figure (1): Agarose gel electrophoresis of PCR amplification products using specific primers of (*eaeA*) gene of *E. coli*. Lane L: 100-600bp DNA Ladder. Pos.: control Positive at (248bp). Neg.: control Negative. Lane1,2,3,5,6,8,9,10,11(Negative). Lane 4, 7 (Positive).

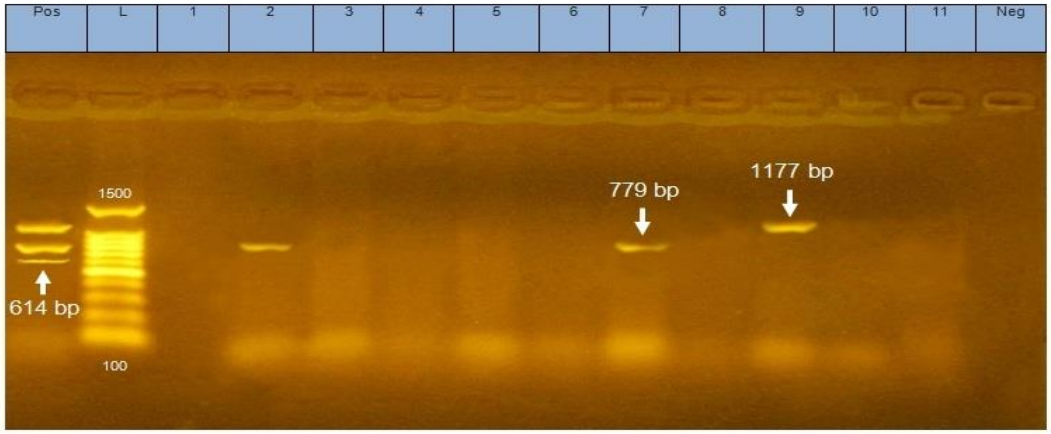

Figure (2): Agarose gel electrophoresis of PCR amplification products using specific primers of (*stx1*) gene, (*stx2*) gene and (*hlyA*) gene of *E.coli*.

**hlyA* : Haemolysin gene. Lane L: 100-1500bp DNA Ladder. Pos.: control Positive (at 1177bp). Neg.: control Negative. Lane 9 (Positive). Lane 1,2,3,4,5,6,7,8,10,11 (Negative). **Stx*$_1$: Shiga toxin1gene.Pos.: control Positive(at 614bp). Lane 1,2,3,4,5,6,7,8,9,10,11(Negative).

**stx*$_2$: Shiga toxin 2 gene. Pos.: control Positive (at 779bp).

Lane 1,3,4,5,6,8,9,10,11(Negative). Lane 2, 7 (positive).

4. DISCUSSION

Foodborne illnesses caused by *E. coli* represent a major public health problem worldwide. Every treatment done to meat from the point of slaughtering until it is ready for consumption could add to the bacterial load of this meat. Thus, meat products are considered as a major vehicle of most reported foodborne outbreaks, and may be contaminated with several types of organisms through long chain of preparation, handling of raw meat, processing, distribution, storage and retailing (Shawish, 2015).

The results recorded in Table (1) were similar with those reported by Bello *et al.*, (2011) (4.6 Log to 4.9 Log cfu/cm^2), Ahmad (2013) (2.81 log cfu/cm^2) and Eugène (2013) (0.8 to 3.0 log cfu/g). On the other hand, higher ones were recorded by EL-Morsi

(1994) (39×10^2cfu/cm^2) and Bogere, P. and Baluka, A. S. (2014) ($8.4x10^4$cfu/g). While, lower ones obtained by Fliss *et al.* (1991) (0.15×10^2 organisms/cm^2) and David *et al.* (2006) (0.8 log cfu/cm^2). The results obtained in Table (3) nearly agreed with Moustafa (1993) (34%), Barkocy-Gallagher *et al.* (2003) (26.7%) and Abdelrahman- Alromisaa (2015) (25%). On the other hand, higher incidence were obtained by EL-Bassiouny and Samaha (1991) (80%), Nashid-Heba (1993) (48%), Enabulele and Uraih (2009) (100%) and Bogere and Baluka (2014) (83.3%). While, lower incidence was obtained by Hassouba (2000) (17.2%), Abdallah *et al.* (2009b) (8.86%). The variation in the results between counts and incidences of *E. coli* in the examined swab samples of cattle carcass surfaces between both of EL-Shouhada and Birket EL-Sabaa abattoirs was attributed to the differences in slaughtering, preparation, handling and the effectiveness of hygienic measures applied in these abattoirs. It is obvious from the results recorded in Table (5) that they were nearly similar to those obtained by Moustafa (1993) (O_{26}, O_{55}, O_{111} and O_{124}), Abdelrahman- Alromisaa (2015) (O_{128}:H_2, O_{124} andO_{26}:H_{11}) and Ismail-Eman (2015) (O_{26}:H_{11} and O_{128}:H_2), while Barlow *et al.* (2006) could not isolated (O_{26} and O_{111}), while Abdallah (2009) isolate (O_{78} and O_{86}).The results in Table (7) and Fig (2) of PCR amplification of *Stx$_2$* gene substantiated what was reported by Adrienne and James (1998), Ram *et al.* (2007) and Fernández *et al.* (2010). Meanwhile, the results in Table (7) and Fig (1) of PCR amplification of *intimin* gene were nearly agreed with those obtained by Adrienne and James (1998), Ram *et al.* (2007). Finally, PCR amplification of *HlyA* gene in examined isolates in Table (7) and Fig (2) substantiated what was reported by Adrienne and James (1998) and Wang *et al.* (2013).

As conclusion, the examined swab samples recovered from surfaces of carcasses slaughtered at both abattoirs were contaminated by pathogenic *E. coli,* but there was a highly incidence in samples which were collected from Birket EL-Sabaa abattoir than those from EL-Shouhada one. This conclusion was attributed to the differences in effective hygienic measures adopted in both abattoirs.

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