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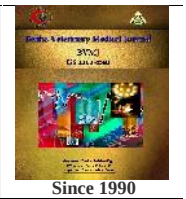

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Original Paper

E. coli and *Salmonella* serotyping in cattle carcasses at abattoir level

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ABSTRACT

Ninety random samples (100 gm) of fresh beef were collected from the cattle carcasses slaughtered at 3 different abattoirs in Menoufia Governorate namely A, B and C (30 of each), represented by neck region (chuck) during summer and winter seasons in 2018 (45 samples of each season). The obtained results of serotyping of *E. coli* revealed that EHEC O26: H11 was the most prevalent among other serogroups 6.67%, 6.67% &13.33% and 6.67%, 13.33% & 6.67% . The prevalence was 46.67% and 40%, 26.67% and 26.67%, and 26.67% and 20.00% for A, B & C abattoirs during the summer and the winter seasons, respectively. Serotyping of salmonella revealed *Salmonella Typhimurium* was the most prevalent during the summer; 13.33% for each abattoir. The total isolation rate was 33.33%, 26.67% and 20.00% for A, B and C abattoirs, respectively. The most prevalent serogroup during the winter was *Salmonella Enteritidis* 13.33%, 6.67% and 6.67%, while the total isolation rate was 26.67%, 20.00% and 6.67% for A, B and C abattoirs, respectively. It was concluded that the contamination rate increased in abattoir A than B than C, and the prevalence increased during summer than in winter.

1. INTRODUCTION

The bacterial contamination during slaughtering process is a safety problem in meat production with a direct relationship between microbial load of carcass meat and good processing practices occurred at abattoir during processing. Abattoirs are considered major sources of information for animal disease outbreaks and the environment for transmission of meat borne diseases which considered a major threat to public health and food safety. At slaughterhouses, different slaughtering steps could contaminate the meat of healthy animals which are naturally sterile and does not hold microbes (Ercolini et al., 2006).

The survival of E. coli in these environments is associated with certain raw materials attached with it not only the survival properties of the bacteria (Holah et al., 2004). The diarrheagenic E.coli are categorized into Enteropathogenic (EPEC), Enterotoxigenic (ETEC), Enterohaemorrhagic (EHEC), Enteroinvasive (EIEC) and Enteroaggregative (EAEC) and diffusively adherent E.coli (Xiaodong, 2010).

In the developed countries, salmonella species are the main cause of food poisoning and results in many diseases and even deaths annually (CDC, 2006).

The most important food poisoning pathogens were S. Typhimurium and S. Enteritidis which were isolated from human salmonellosis due to consumption of contaminated meat or offals (Freitas et al., 2010). The highest isolation rate of E.coli infection was during the summer (Chapman et al., 2001). The aim of this study was to serotype the identified and isolated E.coli and salmonella species during summer and winter seasons from the examined abattoirs.

2. MATERIAL AND METHODS

2.1. Collection of samples:

Approximately 100 g of ninety random fresh samples were collected from cattle carcasses (mainly neck region or chuck) slaughtered at three different abattoirs in Menoufia Governorate namely A, B and C (30 of each) from of each carcass. The samples were collected equally during summer and winter seasons in 2018 (45 samples of each season) and kept in a separate sterile plastic bags inside an ice box and transferred to the laboratory under complete aseptic conditions without undue delay.

2.2. Bacteriological examination:

Preparation of samples (ICMSF, 1996):

2.2.1. Total Enterobacteriaceae count (Gork, 1976)

2.2.2. Identification of family Enterobacteriaceae

2.2.3. Screening for Enteropathogenic *Escherichia coli*:

2.2.3.1. Isolation of *E .coli*:

Pre-enrichment (ICMSF, 1996)

Enrichment broth:

Plating media

2.2.3.2.Identification of *E .coli:*

2.2.3.3. Morphological examination:

2.2.3.4. Biochemical identification (Kreig and Holt, 1984):

2.2.3. 5.Serodiagnosis 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).

2.2.4. Screening for Salmonellae:
Pre-enrichment broth:
Enrichment broth (Harvey and Price, 1981):
Selective Plating:
2.2.4.1. Morphological examination:
2.2.4.2. Identification of Salmonellae
2.2.4.3. Biochemical tests:
2.2.2.4. Serological identification of Salmonellae:
The identification of Salmonellae was carried out according to Kauffman White scheme (Kauffman, 1974) for the determination of Somatic (O) and flagella (H) antigens using Salmonella antiserum (Denka Seiken Co., Japan).
Identification of Somatic (O) antigen was done by using agglutination test. Salmonella group Identification and the other somatic components of the group were also identified using by using separate "O" antiserum factors.
Identification of Flagellar (H) antigen was done by using agglutination test. Determination of Flagellar (H) antigens was carried out by using Polyvalent H antiserum for both phase 1 and phase 2 in order to determine the complete antigenic formula of the isolates.

3. RESULTS

Concerning to results recorded in table (1) the incidence of isolated pathogenic *E.coli* during the summer season were 46.67%, 40% and 26.67% at abattoir (A), (B) and (C), respectively. At abattoir (A) EHEC *as O111: H2* and *ETEC as O125: H21* were 13.33% for each strain and *EHEC as O26: H11, O91: H21* & *O113: H4* were 6.67% of each serotype. Concerning to *E.coli* isolated from abattoir (B) were ETEC *as O128: H2* which was isolated at the highest rate 13.33%, *EHEC O26: H11*&O111: H2 and *EPEC as O55: H7* & *O163: H2* were isolated at 6.67% for each serotype. While *E.coli* isolated from abattoir (C) resembled by EHEC *as O26: H11*which *was* 13.33% & *O111: H2* was 6.67% and *ETEC as O128: H2* was 6.67%.
The results in table (2) showed that the prevalence of isolated pathogenic *E.coli* during the winter season at abattoir (A) and abattoir (B) were 26.67%, while abattoir C had lower incidence 26.67%. *E.coli* isolated from abattoir (A) EHEC as *O26: H11* & *O111: H2*, EPEC *as O86* and *EIEC as O124* were 6.67% for each serotype. *E.coli* isolated from abattoir (B) was *EHEC as O26: H11* was 13.33%, *EPEC as O119: H6* and *ETEC O128: H2* were 6.67% for each serotype.

Table 1 Serotyping of Enteropathogenic *E. coli* isolated from the examined beef samples of Menoufia different abattoirs (n=15) at summer season.

E.coli strains	Abattoir						Strain Characteristics
	A		B		C		
	No.	%	No.	%	NO	%	
O26 : H11	1	6.67	1	6.67	2	13.33	EHEC
O91 : H21	1	6.67					
O111 : H2	2	1.333	1	6.67	1	6.67	
O113 : H4	1	6.67					
O124	-	-					
O86	-	-					
O125 : H21	2	1.333					ETEC
O128 : H2			2	13.33	1	6.67	
O55: H7			1	6.67%			EPEC
O119 : H6			-	-			
O163 : H2			1	6.67			
O121 : H6					-	-	
Total	7	46.67	6	40	4	26.67	

Table 2 Serotyping of Enteropathogenic *E. coli* isolated from the examined beef samples of Menoufia different abattoirs (n=15) at winter season.

E.coli strains	Abattoir						Strain Characteristics
	A		B		C		
	No.	%	No.	%	NO	%	
O26 : H11	1	6.67	2	13.33	1	6.67	EHEC
O91 : H21	-	-					
O111 : H2	1	6.67	-	-	-	-	
O113 : H4	-	-					
O124	1	6.67					EIEC
O128 : H2			1	6.67	1	6.67	ETEC
O125 : H21	-	-					
O55: H7			-	-			EPEC
O119 : H6			1	6.67			
O86	1	6.67					
O163 : H2			-	-			
O121 : H6					1	6.67	
Total	4	26.67	4	26.67	3	20%	

The prevalence of isolated *E.coli* at abattoir (C) was 6.67% for each strain of *EHEC as O26: H11*, *ETEC as O128: H2* and *EPEC as O121: H6*.
Results revealed in table (3) declared that the prevalence of isolated *Salmonellae* spp during the summer season was 33.33% 26.67% and 20% at abattoir (A), (B) and (C), respectively. Serotyping of *Salmonellae* revealed that *S. Typhimurium* was the most prevalent isolate 13.33% for abattoir (A), (B) and (C) abattoirs, while *S. Enteritidis*, *S. Essen and S. Tsevie* was at abattoir (A), *S. Enteritidis* and *S. Anatum* was at abattoir (B), and *S. Inganda* was at abattoir (C) were isolated at the rate of 6.67% for each serotype.
The results in table (4) stated that the prevalence of isolated *Salmonellae* spp during the winter were 26.67%, 20% and 6.67% at abattoir (A), (B) and (C) respectively, while serotyping of *Salmonellae* revealed *S. Typhimurium* was (6.67%) at (A) and (C) abattoirs, while *S. Enteritidis* was 13.33%, 6.67% and 6. 67% at abattoir (A), (B) and (C), respectively. While 6.67% *S. Apeyeme* only isolated from abattoir (A) and 6.67%. *S. Takoradi* only isolated from abattoir (B).

4. DISCUSSION

Enterotoxigenic E. coli (ETEC), Enteropathogenic E. coli (EPEC), Entero- hemorrhagic E. coli (EHEC), Enteroinvasive E. coli (EIEC) are serogroups of intestinal pathogenic *E. coli* (Croxen et al., 2013; Gaafar (2016).
The results in table (1) showed that EHEC (O26: H11) was the most prevalent isolate from the three abattoirs A, B and C. This agreed with Abdelrahman-Alromissa (2015) 5%, (Gaafar 2016) 12% and Abd Elfatah (2017) 8%. EHEC (O111: H2) was isolated from A, B and C abattoirs. This agreed with Abd Elfatah (2017) 8% and Gaafar (2016) 8%, while Barlow et al. (2006) failed to isolate O_{157}, O_{26} and O_{111} from the examined samples.
ETEC (O128: H2) were isolated from B and C abattoirs that agree with Abdelrahman-Alromissa (2015) 5% and Abd Elfatah (2017) 6%. Although lower results were recorded by Abd Elfatah (2017) for EHEC (O91: H2) 2% & ETEC (O125: H21) 2% at abattoir A and EPEC O55: H7 2% at abattoir B. The results recorded for O55: H7 agreed with Gaafar (2016) 8%.

Table 3 Serotyping of Salmonellae isolated from the examined beef samples of Menoufia different abattoirs (n=15) at summer season.

Salmonella strains	Abattoir						Group	Antigenic structure	
	A		B		C				
	No.	%	No.	%	NO	%		O	H
S. Apeyeme	-	-					C3	8,20	Z38 : -
S. Enteritidis	1	6.67	1	6.67	-	-	D1	1,9,12	g,m : -
S. Essen	1	6.67					B	4,12	g,m : -
S.Tsevie	1	6.67					B	4,5	i : e,n, z15
S. Typhimurium	2	13.33	2	13.33	2	13.33	B	1,4,5,12	i : 1,2
S. Anatum			1	6.67			E1	3,10	e,h : 1,6
S. Takoradi			-	-			C3	8,20	i : 1,5
S. Inganda					1	6.67	6,7	Z10 : 1,5	
Total	5	33.33	4	26.67	3	20			

Table 4 Serotyping of Salmonellae isolated from the examined beef samples of Menoufia different abattoirs (n=15) at winter season.

Salmonella strains	Abattoir						Group	Antigenic structure	
	A		B		C				
	1	6.67	No.	%	NO	%		O	H
S. Apeyeme	2	13.33	-	-			C3	8,20	Z38 : -
S. Enteritidis	-	-	1	6.67	1	6.67	D1	1,9,12	g,m : -
S. Essen	-	-					B	4,12	g,m : -
S.Tsevie	1	6.67					B	4,5	i : e,n, z15
S. Typhimurium			1	6.67	-	-	B	1,4,5,12	i : 1,2
S. Anatum							E1	3,10	e,h : 1,6
S. Takoradi			1	6.67			C3	8,20	i : 1,5
S. Inganda	4	26.67			-	-	6,7	Z10 : 1,5	
Total	1	6.67	3	20	1	6.67			

It was noticed that EHEC O26: H11 and O111: H2 were more prevalent than other serogroups isolated from the three examined abattoirs. This agreed with Abd Elfatah (2017), However, EPEC O86 was failed, and this disagreed with Gaafar (2016) 4%.

The results recorded in table (2) revelad E. coli serotyping of during winter season *EHEC (O26: H11*) was more prevalent than other serogroups for A, B and C abattoirs, respectively. Although Barlow *et al*. (2006) failed to isolate it from the examined samples. Lower results were revealed by Abd Elfatah (2017) 2% for EIEC (O124) at abattoir (A) and ETEC O128: H2 2%. The incidence of *E.coli* was the highest in abattoir A, while abattoir C was the lowest isolation rate. The variation between the examined abattoirs agreed with (EOS) (2008) and Gaafar (2016). The obtained results of the incidence *E. coli* agreed with Abd al slam et al. (2014), though lower results were recorded by Ahmed-Fatms (2003) and Barhoma (2016). Nevertheless, high results were reported by Edris et al. (2013). It was noticed that the isolation rate during summer season was higher than which during winter, This agreed with Chapman et al. (2001) and Mohammed et al. (2014) but disagreed with Ogden et al. (2004), who recorded lower incidence during the warmer months than cooler months.

Serotyping and identification of *E. coli* strains in the food or environmental samples considered a critical matter to be able to understand their epidemiology (Monaghan et al., 2012). The results of *S. Typhimurium* agreed with Edris et al. (2013) 10%, while higher results were recorded by Hejazi (2013) 46% and Abd al slam et al. (2014) 57%, and lower results were recorded by Gaafar (2016). S. *Enteritidis* at abattoir A and B abattoirs agreed to those reported by Edris et al. (2013) 5% but was lower than Hejazi (2013) 35.7% and Abd al slam et al. (2014) 19% and higher than which recorded by Gaafar (2016) 4%, although lower results of *S. Anatum* were obtained by *Ahmed-Fatms (2003)2%.*

The results recorded revealed that the predominant isolate in table (1) was *S. Typhimurium* and *S. Enteritidis* in table (2) that differ from Sumner et al. (2003), who reported that *S. Anatum* was the predominant serotype.

Concerning, the incidence *salmonella spp* results agreed with those reported by Abd al slam et al. (2014), while lower results were obtained Beyene et al. (2016) and higher results were reported by Hejazi (2013). The incidence of *salmonella* was the highest in abattoir (A), although abattoir (C) recorded the lowest isolation rate agreed with Gaafar (2016). The most common types of salmonellae were S. *typhimurium* and *S. enteritidis* which causing illness for more than 100 years ago, while a new resistant strain of *S. typhimurium* appeared causing more limited options for treatment (Zhao et al., 2002).

5. CONCULSION

In conclusion, good hygienic measures should be applied to decrease the microbial load at abattoir level for production of safe meat for consumption

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