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Prevalence of *Escherichia coli and Citrobacter freundii* in Raw Beef from major Abattoirs located in Damascus and countryside, Syria

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Abstract: The aim of the study was to determine the prevalence of *E. coli* and *Citrobacter freundii* contamination in beef from major abattoirs. A total of 34 meat samples were examined for the prevalence of *Escherichia coli and Citrobacter freundii* . They were randomly collected from different areas in Damascus and its countryside, from Slaughterhouses (from the thigh area of beef) They were performed using macConkey agar and EMB (Eosin Methylene Blue) agar. The API 20E system BioMérieux company – France was used to distinguish, coliform bacteria and *E.coli* that isolated on the selective culture. Out of 117 total isolates, 35 isolates (29.9%) were *Escherichia coli* and 2 isolates (1.7%) were *Citrobacter freundii*. It must be used good healthy measures like, periodic cleaning for tools and surfaces contact to the meat during skinning and slicing of the meat in order to reduce the potential contamination.
Key words: *Escherichia coli, Citrobacter Freundii,* beef, abattoirs, API 20E.

Introduction :

Meat is every edible part of any slaughtered animal, whether the same is in its natural state or has been subjected to freezing, chilling, salting, canning or other preservative processes[1]. Raw meat is an ideal medium for bacteria growth, this is because of its high moisture contents, it is rich in protein, has fermentable carbohydrate, favourable pH and other growth factors[2].

Beef is meat that is produced from cattle. It is produced and consumed worldwide, with South America, Africa, Asia and Australia being the highest consumers of the product[3].

Microbial food safety is an increasing public health concern worldwide. It is estimated that each year in the United States there are approximately 76 million food-borne illnesses[4].

E. coli is a normal inhabitant of the intestinal tract of humans and warm-blooded animals. Its presence in raw food is considered an indication of direct or indirect fecal contamination. Thus, it is used as an indicator organism for possible presence of enteric pathogens in food and water[5,6]. Furthermore, some strains of E. coli are pathogens for humans and animals. Pathogenic *E. coli* strains are responsible for enteric and diarrheal diseases, urinary tract infections, and sepsis and meningitis. They are capable of causing disease under certain conditions when the immune system is compromised or disease may result from an environmental exposure[7]. Food-borne *E. coli* may constitute– indirectly via host fecal flora an important cause of urinary tract infections[8]. They have been increasingly recognized as the most important causes of foodborne diseases and outbreaks all over the world[9].

Citrobacter Freundii is present as intestinal commensal of man and animals. It is well known now that it has been associated with various nosocomial and community acquired infections in humans. The importance of this species lies in their association with serious nosocomial infections and high degree resistance to common antimicrobial agents used for the treatment of various infections[10].

Materials and methods:

A total of 34 meat samples (beef carcass) were collected from abattoirs in damascus and countryside, Syria The samples were placed into cool boxes (at 4°C) and transported to the laboratory[11]. Determination of the level of contamination of E. coli and *Citrobacter freundii* in meat samples was performed using macConkey agar[12,13]. 25 g sample was aseptically added to 225 ml of buffered peptone water (Merck), and homogenized for 2 min. 1 ml was inoculated into macConkey agar. After incubation at 37 and 44.5 °C for 48 h , The pink colonies were considered the typical ones and transport to EMB agar and incubated at 37°C and 44.5 °C for 24-48 h , then several biochemical tests were carried out for the colonies immediately. The system API 20E BioMérieux company – France was used to distinguish, coliform bacteria and, *E.coli* that isolated on the selective culture.

Results and discussion:

34 samples were collected randomly from different areas of Damascus and its countryside, from slaughterhouses (abattoirs), the samples were taking of the thigh part of the animal (beef carcass). The table (1) shows the distribution of samples and its number depending on the region.

After the incubation at 37 and 45.5 ° C for 48 hours on MacConkey agar culture,117 isolates were sellected from MacConkey agar culture, 91 isolates were found Gram-negative, rod-shaped bacteria and catalase positive, that is the first characteristics of the coliform bacteria[14].

Table (2) shows the the presence of coliform bacteria and the percentage of their presence.

And then the selected isolates (coliform isolates) transferred to the EMB culture, incubated at 37 and 44.5 degree C for 48 hours. And different forms of isolates were on EMB culture from shiny pink, mucous pink , light pink to green bluish. Table (3) shows the distribution of the isolates according to their growth on the EMB culture and the persentage of their presence and some of their characteristics by applying TSI (Triple Sugar Iron) test , cetrate test, VP(Voges Proskauer) test, Indole test, and Urease test. Table (3) illustrate that 38.46% were green bluish, 36.26% were shiny pink, 23.08% were mucous pink , and 2.2% light pink.

Depending on colonies's morphology (on EMB), figure (1) shows the distribution of isolated colonies.

API 20E system has been applied on the isolates that have grown on the EMB culture. And Table (4) shows the results of biochemical tests (contained in the API 20e system) for those isolates.

Table (5) and figure (2) show the coliform bacteria strains that have been isolated from EMB culture and its percentage depending on the results of the API 20E system. Table (5) shows that *Escherichia coli* is prevailing among the coliform bacteria contaminating Syrian raw beef (38.46%) . However *Citrobacter freundii* had a very low percentage compared to other coliform bacteria (2.2%).

Prevalence of *Escherichia coli and Citrobacter freundii* compared to total isolates (compared to gram negative bacteria):

Table (6) and figure (3) show that *Escherichia coli* had the highest percentage compared to the gram negative bacteria. Table (6) shows that 35 isolates out of 117 isolates (29.9%) were *Escherichia coli* ,and 2 isolates out of 117 (1.7%) were *Citrobacter freundi*.

Escherichia coli and fecal coliforms are considered to be the most important and compulsory measure of microbiological quality of food and food related products in terms of hygiene. Their presence is used as indicators of fecal pollution. Among these, *E. coli* is often preferred as a more specific indicator of fecal contamination because it is specific and most reliably reflects fecal origin[15,16].

our results shows that *Escherichia.coli* is the most common bacteria in raw meat among the coliform bacteria 38.46% . However the proportion of *Citrobacter freundii* is very low 2.2%. Also *Escherichia.coli* is the most common bacteria in raw meat among the total gram negative bacteria 29.9%, by contrast the percentage of *Citrobacter freundii* is only 1.7%.

There have been a number of studies on meat hygiene in different countries:

In Australia, *E. coli* was detected on 10.3% of carcasses and 5.1% of boneless beef samples[17]. In a study reported by Sumner and others[18], *E. coli* was isolated from 18.8% of beef carcasses. In Croatia, E. coli was found in 6% of the beef samples tested reported by[19]. In a study reported by Iroha and others[20] about bacterial contamination in fresh meat that is the most common bacteria were Respectively *E. coli* and *Klebsiella pneumonia.* Other study reported by[1] about Assessment of bacteriological quality of fresh meats sold in calabar metropolis, Nigeria, showed that *K. pneumoniae* (16.7%) was the most predominant pathogens. This was followed by *Enterobacter spp* (13.9%) and *C. freundii* (13.9%).

Compared to the results mentioned above, our results, which were 29.9% positive for *E. coli*, show the high level of contamination of *E. coli,* consistent with a risk to human health due to bacterial hazards in raw beef.

It must be used good healthy measures and periodic cleaning for tools and surfaces contact to the meat during skinning and slicing of the meat in order to reduce the potential contamination.

Comparison between the presence of *E.coli* in raw beef in Damascus and its presence in raw beef in countryside:

The table (7) shows high proportion of *E.coli* bacteria in the countryside of Damascus, it was about 39.1% (18 isolates out of 46) compared to total coliform. However it was in Damascus 37.7% (17 isolates out of 45) isolated.

The table (8) also shows high proportion of *E.coli* bacteria in the countryside of Damascus, it was about 35.29% (18 isolates out of 51) compared to total isolates. However it was in Damascus 25.76% (17 isolates out of 66) isolated.

The high proportion of *E.coli* in the countryside of Damascus is Associated with the bad health conditions with regard to contaminated wash water and the contaminated surface and tools used during slaughtering in slaughterhouses .

Table (1) The number of samples depending on region

Region	Number of samples
Bab al-jabia	3
Al-qassa'a	3
barzah	5
Reken al-dien	2
Al-mazah	4
almidan	4
katana	4
sehnaiya	2
jaramana	3
Qudsaiya suburb	2
Al-saboura	2
Total	**34**

Table (2) The proportion of the presence of isolated coliform bacteria

Total isolates	Coliform isolates	persentage %
117	91	77.8

Table (3) The distribution of isolates , according to thier growth on EMB culture and some of their characteristics

		Colonies color			
	Bluish green	**shiny pink**	**shiny pink**	**mucous pink**	**light pink**
Number of isolates	35	31	2	21	2
Percentage %	38.46	34.06	2.2	23.08	2.2
Catalase test	+	+	+	+	+
Oxidase test	-	-	-	-	-
test [1]TSI	+ **g+**	+ **g+**	+ **g+**	+ **g+**	+ **H2S**
Citrate test	-	+	+	+	+
VP[2]	-	+	+	+	+
Indol test	+	-	-	-	-
Urease test	-	-	+	+	-

1 Triple Sugar Iron Agar Test

2 Voges Proskauer Test

Table (4) The results of API 20e system on the isolates

isolates	API 20e results																					
	ONPG	ADH	LDC	ODC	CIT	H2S	URE	TDA	IND	VP	GEL	GLU	MAN	INO	SOR	RHA	SAC	MEL	AMY	ARA	NO2	N2
35	+	-	+	+	-	-	-	-	+	-	-	+	+	-	+	+	-	+	-	+	+	-
22	+	+	-	+	+	-	-	-	-	+	-	+	+	-	+	+	+	+	+	+	+	-
9	+	+	+	+	+	-	-	-	-	+	-	+	+	-	+	+	+	+	+	+	+	-
2	+	+	+	+	+	-	+	-	-	+	-	+	+	-	+	+	+	+	+	+	+	-
21	+	-	+	-	+	-	+	-	-	+	-	+	+	+	+	+	+	+	+	+	+	-
2	+	-	-	-	+	+	-	-		+	+	+	+	+	+	+	+	+	+	+	+	-

Table (5) Strains of coliform bacteria isolated from the EMB culture and percentage According to the API 20E system :

strains	Isolates Number	Percentage
Escherichia coli	35	38.46
Enterobacter cloacae	22	24.18
Enterobacter aerogenes	9	9.89
Enterobacter gergoviae	2	2.2
Klebsiella pneumoniae	21	23.08
Citrobacter freundii	2	2.2
Total coliform	91	100

Table (6) Strains of coliform and gram negative bacteria and percentage:

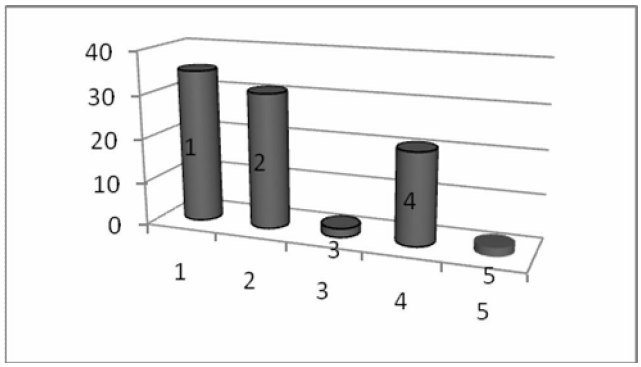
strains	Isolates Number	Percentage
Escherichia coli	35	29.9
Enterobacter cloacae	22	18.8
Enterobacter aerogenes	9	7.7
Enterobacter gergoviae	2	1.7
Klebsiella pneumoniae	21	18
Citrobacter freundii	2	1.7
The other gram negative isolates	26	22.2
Total isolates	117	77.8

Table (7): The proportion of *Escherichia coli* and *Citrobacter freundii* isolates out of coliform bacteria by region.

	Total coliform	*E.coli* isolates	%percentage	*Citrobacter freundii* isolates	%percentage
Damascus	45	17	37.7	0	0
Countryside	46	18	39.1	1	2.2

Table (8): The proportion of *Escherichia coli* and *Citrobacter freundii* isolates out of total isolates by region.

	Total isolates	*E.coli* isolates	% percentage	*Citrobacter freundii* isolates	%percentage
Damascus	66	17	25.76	0	0
Countryside	51	18	35.29	1	1.96



green bluish - 1 shiny pink - 2 shiny pink-3 mucous pink- 4 light pink- 5

Figure (1): Distribution of colonies depending on their morphology

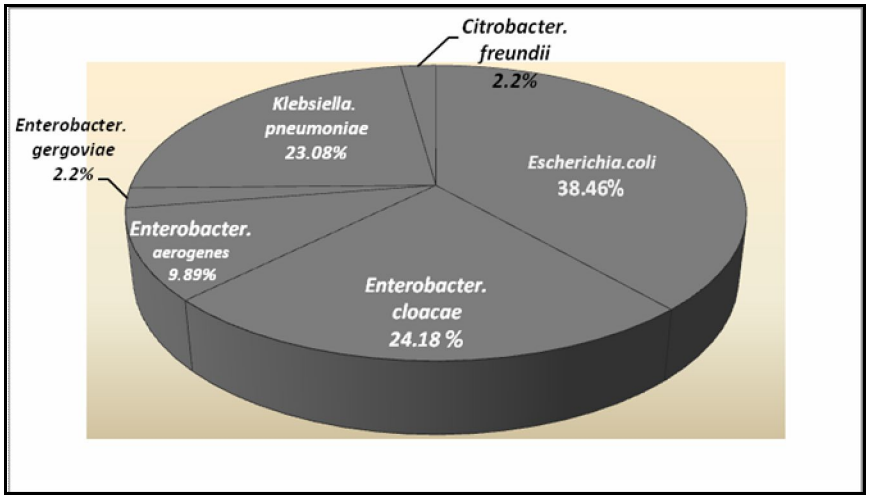


Figure (2): strains of coliform bacteria isolated from the EMB culture and percentage:

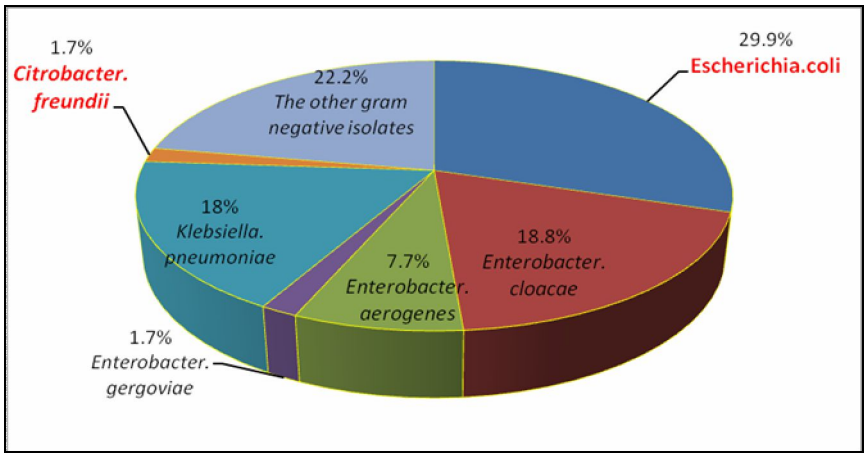


Figure (3): strains of coliform and gram negative bacteria. and their percentage:

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