Escherichia Coli Isolated From Raw Milk at North Sinai Governorate

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Abstract:

The study was undertaken to detect the occurrence of *E.coli* in raw milk samples collected from different sources in and around El-Arish city, North Sinai Governorate.

A total of 450 raw milk samples were collected from different milk animals from farms, houses and market (supermarkets, dairy shops, groceries and vendors) situated in El-Arish city and surrounding areas. Various media were used for cultivation of the organism; biochemical identification was carried out by applying certain tests for *E.coli*, as well as serological identification by using specific antisera. Congo red test used as in vitro pathogenicity test; to detect the virulence markers of *E.coli* strains. *E.coli* was recovered from the raw milk samples; with a percentage of 8.9%. *E.coli* was detected in farm and market milk, with an incidence of 10% and 11.7% respectively. The serological identification revealed the detection of six serogroups (O1, O6, O55, O86, O128 and O158). *E.coli* strains showed 40% positivity for the Congo red dye uptake.

It is concluded that the contamination of raw milk with *E.coli* potentiates the alarm for the possibility of food poisoning occurrence for human being.

Keywords: Raw milk, *E.coli*, isolation, identification, pathogenicity.

Introduction:

Milk is a main source of protein and calcium for human being, No one can be healthy without taking a glass of milk daily or any milk product as a constitute; so the hygiene of milk is of great significance for the health of human being, especially the raw milk as some people still prefer consumption of raw milk than the pasteurized one.

Milk is considered also as an optimum growth media for cultivation of enormous types of microorganisms which can get entrance to the milk by various sources including the udder , the udders flanks, the water source ,the dairy man, the milking equipment and utensils, Vessels used for milk storage and transportation, the surrounding environment from air, litter, building ,insects, rodents and the atmosphere...etc. (Oliver et al., 2005 and Khan et al., 2008).

All of these circumstances can enhance the proliferation of pathogenic microorganisms in milk which has great human health interest (Mendelson, 2011), Furthermore, the occurrence of foodborne microbes in unprocessed milk elevate the danger of ingestion of such pathogenic bacteria and the transfer of highly dangerous toxins. Among the microorganisms which can enter to the milk and result in a potential effect is E. coli (Kaper et al.2004). In this study, a tensile focus was regarded to estimate the E. coli contamination in El-Arish city: as there is informally raw milk market; comes from the farm itself or from distributers of milk in dairy shops, supermarkets and groceries and also from street vendors.

Materials and Methods

Sample collection: A total of four hundred and fifty (450) milk samples gathered were and purchased from farms, houses and market (supermarket, dairy shops, groceries and vendors). The samples were taken from different milk animals (cows, goats and ewes), as shown in table (1).All samples were collected under aseptic condition, in sterilized sample bottles and were transported to the laboratory of animal health research institute, El-Arish; as soon as possible.

Microbiological analysis:

Isolation and identification:The samples were enriched in sterile buffered 1% pepton water and incubated at 37 °C for 24 hours. then a loopful from the enriched were media streaked onto MacConkey agar for 24-48 hours at 37°C, Pink colonies were further cultivated on EMB agar and blood investigated then the agar. characteristic colonies for E.coli (metallic sheen on EMB), according to Cruickshank et al. (1975) and APHA (2001).

Biochemical identification: was performed by applying various biochemical tests according to *Kreig and Holt (1984)*

identification: Serological The serogrouping of ten (n=10) E.coli isolates taken randomly from the samples was applied in the animal research institute in Dogi, Giza, by polyvalent using the and monovalent E.coli antisera. Serological grouping was performed according to methods described by Edwards and Ewing (1972). In Vitro pathogenicity testing for E. coli strains: This was performed by applying Congo red dye binding activity test according to Fodor et al. (2010). Appearance of red colonies was recorded as Congo red (CR+) positive and colonies that did not bind the dye and remained white or grey were considered as Congo red (CR -) negative

Kind of raw milk			Market			
samples Houses		Farms	Supermarkets, groceries and	Street		
			dairy shops(Dairy distributers)	vendors		
Bovine milk	15	50	200	100		
Caprine milk	50	-	-	-		
Ovine milk	35	-	-	-		
Total No.	100	50	200	100		

Table (1) Number and source of the examined raw milk samples:

Results:

The incidence of *E.coli* in raw milk samples observed in this study was summarized in table (2). According the present findings, E.coli to contamination was regarded high in the samples gathered from dairy distributers (supermarkets, groceries and dairy shops), with a percentage of 12%, and also from the street percentage vendors by а of contamination 11%.*E.coli* was recorded in the farm milk with a 10%.No of E.coli rate was estimated in the milk samples taken from houses, also caprine and ovine milk were devoid of any E.coli contamination.

The serogrouping of *E.coli* isolates revealed the detection of different

serogroups from Market(shops and street vendor) and farms, distributed following as the serogroups:O1(two isolates)from farm and street vendor,O6(two shops,O55(one isolates)from farm.O86(one isolates)from shops,O128(two isolates) from isolates)from shops and vendor and O158(two isolates)from shops and vendor; as shown in table (3). The Congo red dye binding activity

test for *E.coli* strains isolated from milk samples showed that four *E.coli* isolates;O158(2),O1(1) and O55 (1) bind actively with the Congo red dye (+ve), as shown in table(4).

Table (2) Inci	idence of E.co	li isolated fro	om the pooled	d examined	raw milk
samples:					
TEL 1 1 0				36.1	

The kind of	Houses		Farms		Market			
raw milk	(n=10	0)*	(n=	50)*	Sho	ops	S	treet
samples					(n=2	*(00	vendor	r(n=100)*
	+ve	%*	+ve	%*	+ve	%*	+ve	%*
Bovine milk	0	0	5	10%	24	12%	11	11%
Caprine milk	0	0	-	-	-	-	-	-
Ovine milk	0	0	-	-	-	-	-	-

* n =the total number of examined milk samples*% = the percentage of positive *E.coli* isolates

Type of samples							
Street vendor		Shops		Farm			
Polyvalent	monovalent	Polyvalent monovalent		Polyvalent	monovalent		
1	01	4	O6(2isolates)*	1	01		
1	(lisolate)*	1	O86(1isolate)*	1	(lisolate)*		
1	O128 (1isolate) *	1	O128(1isolate)*	2	O55		
3	O158 (1isolate)*	3	O158(1isolate)*	2	(1isolate)*		

Table (3)	Serogro	uping a	of the	E.coli	isolates:
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*the number of isolates

Table (4) In vitro pathogenicity of E.coli isolates by Congo red bindig test

Sanagnaun	CRB					
Serogroup	No.	No +ve*	%			
01	2	1	50%			
06	2	0	0%			
O55	1	1	100%			
O86	1	0	0%			
O128	2	0	0%			
O158	2	2	100%			
Total	10	4	40%			

* No +ve = the number of positive samples

Discussion:

In the present study; Among the 450 milk samples examined for the incidence of *E.coli* contamination. E.coli was isolated from 40 milk samples revealed a percentage of 8. 9%. Nearly similar results recorded by Samah(2006) who conducted study on milk samples from different sources (from market and from farm) with a total isolation of E.coli by 6.48% from the examined higher raw milk samples. Α percentage recorded from dairy farms (20%) and market (36.66%) samples Gwida and Elby Gohary(2013).

It was found that out of all milk samples; the highest contamination with *E.coli* was detected from market 11.7% (35\300), followed by dairy farms 10% (5\50) and none from house milk, the same results were estimated by *Kumar and Prasad* (2010), who isolated *E.coli* from raw milk samples collected from vendors, and dairy farm milk with percentages of 13% and 10% respectively; while no *E.coli* isolates were recovered from the house milk, with a total isolation of 8.15%.

Also; *Iqbal and Hussainy (2014)* found that the highest contamination with *E.coli* was recorded in the milk collected from milk sellers (33.3%) followed by dairy farms (26.6%) and finally the house milk (13.3%).

In spite of the difference between percentages the of E.coli contamination in market and farm milk recorded in this study is not so high, It is markedly noted by other studies which were performed through the milk value chain (Samah, 2006; Baloch et al., 2011 and Lubote et al., 2014); as a higher percentage of E.coli isolation was recorded from market milk than from the farm milk

This is may be due to; the increase handling of milk and the deficiency of good hygienic practice until reaching to the final consumers which were varying from locality to another.

Only 5 milk samples from cattle farms samples(no.50) were positive for *E.coli* by a percentage of 10%, these results go in accordance with the results of *Lira et al.* (2004), *Baloch et al.* (2011), *Zeinhom and Abdel-Latef* (2014) and *Al-Zogibi et al.* (2015) who conducted their studies on *E.coli* isolated from farm milk

On other hand, E.coli strains were observed in bovine raw milk only, no E.coli isolates were recovered from caprine and ovine milk. On contrary to the results of Ekici et al. (2004) and Bogdanovičová et al. (2016) who recovered E.coli isolates in ewes' milk samples and goats' milk samples by varying percentages, while E. coli was not detected in cows' milk samples, and also the results of Oprean et al. (2011) who detected E.coli strains from caprine and ovine milk and only one *E.coli* strain isolated from bovine milk.

Other studies showed a higher contamination of bovine raw milk samples with *E.coli* strains in comparison to caprine and ovine raw milk (*Rahimi et al.,2012 and Lye et al., 2013*).

There were many researchers who studied the food poisoning different microorganisms in governorates localities in Egypt; where E.coli isolation and identification in raw milk samples was estimated: either from farms or from markets.

A higher percentage of *E.coli* isolates was detected in raw milk samples obtained from Egyptian markets by; *Koraney (2016) and Ombarak et al.,(2016)* with a percentage of 21.5% and 76.4% respectively.

In respect to the different Markets in Egyptian Governorates, In Upper Egypt; from Assiut ; *El-Prince* (2010) recovered *E.coli* by a percentage of 3.3%. A much higher percentage was recorded by *Sadek et al.* (2014) in some Assiut city market; by 78.4%. From Qena governorate; an earlier study conducted by *Sabry and Laila* (2008) who recorded a percentage of 76%.

In the Delta Governorates; *Abd El-Latif (2012)* estimated the incidence of *E.coli* in examined raw cow and buffalo milk samples by a 20% in both types of raw milk which collected from different farmers in El-Behera Governorate. Also other

studies conducted in El-Menofia Governorates by *El-Nahas et al.*, (2015) and *El–Bagory et al.*, (2016) who isolated *E.coli* by percentages of 55% and 23.33% respectively.

In Cairo; also many studies were performed by many researcher in the last three decades; *Saudi* (1990), *Ahmed and Sallam* (1991) and *Ibrahim et al.* (2015); who recovered *E.coli* by different percentages as;9.33%,22% and 80%.

In concern of Suez Canal area, a reported study in El- Ismalia city was conducted by *Samah* (2006) who isolated *E.coli* from both market raw milk samples and farm milk samples by an incidence of 8.33% and 2.78% respectively.

In spite of the varied prevalence of *E.coli* recorded from the examined raw milk samples collected from Egyptian farms and markets; All the results contributed in the high prevalence of E.coli contamination of the milk available for the Egyptian who consumers unfortianly prefer the raw milk purchased from the informal sectors distributed in all Egyptian markets than the pasteurized and UHT milk. The serogrouping of isolated E.coli from examined raw milk samples taken randomly from the pooled samples as ten isolates (5 from the market, 3 from the vendors and 2 from the farm milk samples). revealed that among market milk were two O6 samples. there serotypes and one O86 serotype, O128 and O158 for the remaining

market milk samples while the milk samples from vendors were as; O1,O128 and O158 one isolate for each serotype, and that for farm milk samples were as O1 and O55,one for each. O158 is one of frequent serogroups which the isolated from raw milk samples, as by Carneiro et al. obtained (2006), Wenz, et al. (2006) and Koraney (2016) beside other serogroups were identified from E.coli isolates.

The O6 serogroup was also identified from *E.coli* isolated from food of animal origin by *Koraney* (2016).On the other hand, *E.coli* O128 poly1 was detected in raw milk samples by *Carneiro et al.* (2006), *Samah* (2006) and *Abike et al.* (2015).

It has been estimated that, Rashid et al. (2013) reported the presence of milk sample serologically one identified as O86 which comes in consistence with our results. Also; El-bagory et .al. (2016) recorded for the identification of one E.coli strain which belongs to the serogroup 055. the same as recorded for this study.

The serogroup O55 was also recovered by other researchers as; *Carneiro et al. (2006),Lamey et al. (2013) and Abike et al. (2015).*

Finally, O1 serogroup was found among the serogroups which determined by *Lamey et al. (2013)*, the same as the study findings.

In the current study, *E.coli* strains showed 40% positivity for the

Congo red dye activity test; (as shown in table 4).

The results go harmony with the results of *Lamey et al.*(2013), who detected the Congo red dye binding activity in *E.coli* isolates from milk samples by a percentage of 38.1%

Also similar results recorded by *El-Mahronki et al.*,(2006); *Sharma et al.*,(2006) ; *Parul et al.*,(2014); *Milanov et al.*,(2015) *and Ashraf* (2016) , with percentages of 46% ,47.42% ,44.28% ,44% and 43.5% respectively.

On the other hand, the results mismatched with the study of *Gupta et al.*, (2013), in which most of *E.coli* isolates failed to uptake the dye by a percentage of 88.89%.

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الملخص العريي

ايشيرشيا كولاى المعزولة من ألبان بمحافظة شمال سيناء

أحمد أحمد رفعت خفاجي ، حمزة محمد ابراهيم عيد، «فاطمة محمد أحمد يوسف، « » نور الهدى عبدالله عيد قسم البكتريولوجيا و المناعة و الفطريات ، كلية الطب البيطري جامعة قناة السويس معهد بحوث صحة الحيوان بالاسماعيلية * *مديرية الطب البيطري بشمال سيناء

تم اجراء هذه الدراسة لتعيين وجود ميكروب الايشيرشيا كولاى فى عينات اللبن الخام المأخوذة من مختلف المصادر في محافظة شمال سيناء؛ حيث تم تجميع عدد 450 عينة لبن خام من المزارع و الأسواق و البيوت الواقعة بمدينة العريش و المناطق المحيطة، و تم استخدام بيئات الزرع المناسبة لعزل الميكروب مع إجراء الاختبارات البيوكيميائية و السيرولوجية للتعرف على الميكروب، كما تم إجراء اختبار الضراوة للمعزولات.و لقد أظهرت النتائج عزل ميكروب الايشيرشيا كولامن اللبن الخام بمعدل عزل كلي 8.9%،كما أوضحت الاختبارات السيرولوجية تعيين عدد ستة مجموعات ميرولوجية، كذلك بينت المعزولات.والت البوبية بالنسبة لاختبار الضراوة. كل ذلك ساعد على استخلاص احتمال وجود تلوث للألبان الخام بميكروب الايشيرشيا كولاى ؛ مما ينبه على خطورة حدوث تسمم غذائي للانسان.