BENI-SUEF UNIVERSITY JOURNAL OF BASIC AND APPLIED SCIENCES 3 (2014) 209–215

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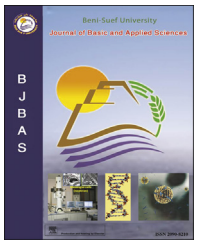

Full Length Article

# Public health risk of some milk borne pathogens

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ARTICLE INFO

*Article history:*
Received 21 May 2014
Received in revised form
23 August 2014
Accepted 26 August 2014
Available online 1 November 2014

*Keywords:*
Raw milk
Zoonoses
Aeromonas
*Escherichia coli*
Salmonella
Faecal samples

ABSTRACT

A total of 150 samples of raw milk, 75 each of farm and market milk were collected from different farms and supermarkets in Beni-Suef Governorate, in addition to 30 stool samples from milk handlers and 25 milker's hand swabs were examined for the presence of *Escherichia coli*, *E. coli* O157:H7, *Salmonella*, *Aeromonas* and *Yersinia*. Isolates were identified biochemically and serologically. The obtained results revealed that *E. coli* was detected in a percentage of 26.7% and 16% in the examined raw market and bulk farm milk respectively, while in stool and hand swabs samples were 16.6% and 16%, respectively. *E. coli* O157:H7 and *Salmonella* spp. failed to be detected in any of the examined samples. Additionally, 45% and 16.7% of the recovered *E. coli* strains from the examined raw market and farm milk samples were enteropathogenic O166, while 55% and 83.3 were untypable, respectively. On the other hand 60% of human stool samples isolates were O 148 and 40% of the isolates were untypable, while 100% of the hand swab isolates were untypable. The results also exhibits isolation rate of *Aeromonas hydrophila* in a percentage of 24%, 13.3%, 10% and 16% from market milk, farm milk samples, stool and hand swabs respectively. While *Yersinea enterocolitica* represent 3.3% in the stool samples only. The public health significance of isolated strains as well as suggested control measures were discussed.



## 1. Introduction

Milk ranks high among other foods and is considered as the most perfect food for human from birth to senility as it is not only has good sensory properties and all nutrients required for the body for rapid growth but also could prevent or reduce risks of many nutritional deficiency diseases (Kalkwarf et al., 2003; Marshall et al., 2003).

Raw milk is still used by large number of farm families and workers and by a growing segment of the general population who believe that the milk is not only safe but also imparts beneficial health effects that are destroyed by pasteurization (LeJeune and Rajala-Schultz, 2009). Milk and products derived from milk can harbor a variety of microorganisms and can be important sources of food borne pathogens. The presence of food borne pathogens in milk may be due to direct contact with contaminated sources in the dairy farm environment and to excretion from the udder of an infected animal.

*Escherichia coli* is a normal inhabitant of the intestines of animals and humans but its recovery from food may be of public health concern due to the possible presence of

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Peer review under the responsibility of Beni-Suef University.
http://dx.doi.org/10.1016/j.bjbas.2014.10.006

enteropathogenic and/or toxigenic strains which lead to sever gastrointestinal disturbance (Soomro et al., 2002). It is considered as the major indicator of fecal pollution in food production. Its presence in processed foods results from recontamination, because this bacterium usually does not survive food preservation processes. The main reasons for the presence of *E. coli* in food products are nonobservance of relevant technological regimes, incompliance with recommended process standards, and the lack of personal hygiene (Law, 2000).

The majority of *E. coli* rods do not constitute a serious health hazard, but some serotypes can cause food poisoning and alimentary intoxications. The most dangerous among them are enterohemorrhagic *E. coli* strains, especially serotype O157:H7. *E. coli* O157:H7 has become a pathogen of major concern in both food and dairy industries, and to the public, because of its ability to cause severe illness, in particular, haemorrhagic colitis, hemolytic uremic syndrome and thrombotic thrombocytopenic purpura (Picozzi et al., 2005; Reuben et al., 2013). The sources of infections with enterohemorrhagic *E. coli* strains are mostly meat products, especially underdone steaks and hamburgers (Chinen et al., 2001), but also other foodstuffs as unpasteurized milk and dairy products manufactured from raw milk, have been implicated in many outbreaks, (Maher et al., 2001).

Salmonellosis is the most common food-borne bacterial disease worldwide (Forshell and Wierup, 2006). *Salmonella* is the second leading cause of food borne illness in most developed countries causing diarrhea, cramps, vomiting, and often fever. Food-borne salmonellosis has remained a neglected zoonosis in Egypt and other developing countries of the world. Food borne Salmonellosis has been recognized due to consumption of raw or improperly pasteurized milk and milk products (Karshima et al., 2013).

The genus *Yersinia* comprises an important group of bacterial pathogens, with *Yersinia enterocolitica*, *Y. pseudotuberculosis*, and *Y. pestis* representing the species of interest. *Y. enterocolitica* is the most common agent of this genus that are associated with a spectrum of clinical syndromes in man, with gastroenteritis as the most frequently encountered manifestation. Most cases are sporadic or occur in small clusters, but large outbreaks have been reported worldwide in families, schools, hospitals, and in association with community gatherings (Bottone, 1997; Leclercq et al., 2005) although *Y. enterocolitica* has been isolated from a number of environmental, food, and water sources, there have been relatively few documented outbreaks of human illness where food was proved by culture to be the source of infection. According to (Ackers et al., 2000) the three well-documented outbreaks in which contaminated chocolate milk, raw milk, and tofu were the vehicles of transmission.

The genus *Aeromonas* includes at least 13 species, among which is the motile, mesophilic *Aeromonas hydrophila* (Abbott et al., 2003). The mesophilic species have been associated with a wide range of infections in humans that have been isolated frequently from various food products, and from patients with diarrhea. Drinking water and food are reservoirs of *A. hydrophila* and therefore may be important sources of human infections, leading to intestinal and non-intestinal diseases. Epidemiological studies implicated *Aeromonas* species in causing water and food-borne outbreaks and traveler's diarrhea (Vila et al., 2003) that are increasingly recognized by researchers as a cause of various clinical syndromes (Doyle and Hugdahl, 1983; Tsai et al., 2006). The presence of *Y. enterocolitica* and *A. hydrophila* in food products is of a special concern since those organisms are capable of growth at refrigerator temperatures.

Therefore, this study was carried out to determine the prevalence of some pathogenic bacteria spread by contamination of raw milk and among people who may be carriers as well as discussing the public health significance of the isolated microorganisms and suggestive control and preventive measures.

## 2. Materials and methods

### 2.1. Collection of samples

A total of 150 raw milk samples were collected randomly (75bulk farm milk and 75 raw market milk from different dairy shops, groceries and supermarkets) in Beni-Suef Governorate, Egypt. Milk samples were identified and rapidly delivered to the Food hygiene and control laboratory, Faculty of Veterinary Medicine, Beni-Suef University in an insulated ice-box to be examined. In addition to 25 hand swab samples and 30 stool samples were collected from milk handlers from the same examined dairy farm and shops in Beni-Suef Governorate (APHA, 1992). A swab was taken from each stool samples using a sterile swab and then inserted into sterile buffered peptone water (BPW) tubes under aseptic conditions (Sadoma, 1997). The tubes were labeled then ice packed and transferred immediately to the lab.

### 2.2. Isolation and identification of E. coli from raw milk (APHA, 1992)

25 ml from the collected raw milk samples were added to sterilized tubes containing 225 ml of BPW and incubated aerobically at 37 °C for 24 h. One ml from incubated BPW was transferred to 5 ml MaCconkey broth and incubated at 37 °C for 24 h. A loopful from the incubated broth was streaked on Eosin methylene blue (EMB) agar and incubated at 37 °C for 24 h. Morphologically typical colonies (at least 5 per plate) producing metallic sheen were taken into nutrient broth for further identification.

### 2.3. Isolation of E. coli O157 from raw milk (De-Boer and Heuvelink, 2000)

25 ml of each milk sample was directly added to modified Tryptone soy broth supplemented with novobiocin (20 mg/litter). The inoculated broth was incubated at 37 °C for 24 h. A loopful from the incubated broth was streaked onto Tellurite-Cefixime Sorbitol MacConkey agar plate and incubated at 37 °C for 24 h. Sorbitol negative colonies (colorless) were picked up and purified then examined Biochemically (tests were performed to confirm *E. coli* using Gram staining, Catalase test, Indole, Methyl red, Voges–Proskauer test, Nitrate

**Table 1 – Incidence of isolated pathogens from the examined raw milk samples.**

| Isolated organisms | Raw market milk (75) | | Raw farm milk(75) | | Total (150) | |
|---|---|---|---|---|---|---|
| | No of positive | % | No of positive | % | No of positive | % |
| *E. coli* | 20 | 26.7 | 12 | 16 | 32 | 21.3 |
| *E. coli* O157:H7 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Salmonella* | 0 | 0 | 0 | 0 | 0 | 0 |
| *Aeromonas hydrophila* | 18 | 24 | 10 | 13.3 | 28 | 18.6 |
| *Yersinia enterocolitica* | 0 | 0 | 0 | 0 | 0 | 0 |

reduction, Urease production, Simon citrate agar, and various sugar fermentation tests) and serologically.

### 2.4. *Isolation of* Salmonella *from raw milk* (Quinn et al., 2002)

25 ml of each well mixed raw milk sample were thoroughly mixed with 225 ml of sterile buffered peptone water. All samples were incubated at 35 °C for 24 ± 2 h. One hundred microliters from the pre-enriched sample was transferred to 10 ml of Rappaport Vassiliadis (RV) enrichment broth and incubated at 43 °C for 24 h. Loopfuls from enriched RV broth were separately streaked onto each of xylose lysine desoxycholate (XLD) agar and Salmonella- Shigella (SS) agar plates and incubated at 37 °C for 24 h. Two or three of typical or a typical colonies (colorless with black center on SS standard colonies with black center on XLD) were selected from each selective medium and streaked onto nutrient agar slope which incubated at 37 °C for 24 h for further biochemical and serological identification.

### 2.5. *Isolation and identification of* Y. enterocolitica *and* A. hydrophila *from raw milk samples* (Doyle and Hugdahl, 1983)

25 ml from the collected raw milk samples were added to sterilized tubes containing 225 ml of BPW and incubated aerobically at 37 °C for 24 h. All enrichments were streaked onto XLD, incubated at 32 °C for about 18 h, SS agar, was incubated at 30 °C for about 24 h, while MCA agar was incubated at 25 °C for 24 h. Each colonial type present was selected with neither fewer than two nor more than four colonies per plate used to individually inoculate Simmon's citrate agar, Christensen's urea agar and were incubated overnight at 28 °C. Isolates exhibiting typical reactions "Citrate –ve, urease + ve were subjected to additional biochemical API 20E system (BioMerieux) to confirm identification as *Y. enterocolitica* and *A. hydrophila*. (MacFaddin, 1981; Koneman et al., 1994).

### 2.6. *Human stool and hand swabs samples examination*

The collected swabs in BPW were incubated at 37 °C for 24 h then all samples were subjected to the same laboratory diagnostic techniques as done in milk samples as mentioned before.

### 2.7. *Serological identification of* E. coli *isolates*

Serological identification of the strains was carried out in the Clinical Microbiology Unite, Central Health Laboratories of Ministry of Health, Egypt.

## 3. Results

The results presented in Table 1, showed that 20 isolates of *E. coli* out of 75 examined raw market milk samples and 12 isolates out of 75 bulk farm milk samples were identified as *E. coli* with a percentages of 26.7% and 16% respectively. Both *E. coli* O157:H7, *Salmonella* and *Y. enterocolitica* failed to be detected in either raw market milk or bulk tank farm milk, while *A. hydrophila* was detected in 18 out of 75 raw market milk and 10 out of 75 farm milk samples with a percentage of 24% and 10%, respectively.

Results presented in Table 2, showed that *E. coli* were recovered from 5 (16.6%) out of 30 stool samples while it was isolated from 4 (16%) out of 25 of the examined hand swap samples. It was evident that *E. coli* O157:H7 and Salmonella couldn't be isolated from any of the examined stool or hand swap samples. *A. hydrophila were isolated in a rate of* 10% and 16% from stool and hand swabs respectively, while *Y. enterocolitica* represent 3.3% in the human stool samples, but failed to be isolated from the other samples.

Regarding to *E. coli* isolated from the examined raw market milk samples and as recorded in Table 3, 11(55%) out of 20 isolates were untypable, and the remaining isolates 9(45%) were serologically typed as O166. On the other hand 10 (83.3%)

**Table 2 – Incidence of isolated pathogens from the examined human samples.**

| Isolated organisms | Stool samples (30) | | Hand swaps(25) | | Total (55) | |
|---|---|---|---|---|---|---|
| | No of positive | % | No of positive | % | No of positive | % |
| *E. coli* | 5 | 16.6 | 4 | 16 | 9 | 16.4 |
| *E. coli* O157:H7 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Salmonella* | 0 | 0 | 0 | 0 | 0 | 0 |
| *Aeromonas hydrophila* | 3 | 10 | 4 | 16 | 7 | 12.7 |
| *Yersinia enterocolitica* | 1 | 3.3 | 0 | 0 | 1 | 1.8 |

**Table 3 – Serological identification of *Escherichia coli* isolated from the examined raw milk samples.**

| *Escherichia coli* Serogroups | Type of the sample | | | |
|---|---|---|---|---|
| | Raw market milk | | Raw farm milk | |
| | NO | % | NO | % |
| O:148 | 0 | 0.0 | 0 | 0.0 |
| O:166 | 9 | 45 | 2 | 16.7 |
| Untypable (poly1-9) | 11 | 55 | 10 | 83.3 |
| Total | 20 | 100 | 12 | 100 |

out of 12 isolates from the examined raw farm milk samples were untypable and the remaining isolates 2(16.7%) were serologically typed as O166.

Table 4, revealed that 2 (40%) out of 5 isolates from the examined stool samples were untypable and the remaining isolates 3(60%) were serologically typed as O148, while 100% of the isolated *E. coli* from the hand swab samples were untypable.

## 4. Discussion

*E. coli* is the most common species of facultative anaerobe found in the gastrointestinal tract of both man and animals and the most commonly encountered pathogen in the Enterobacteriaceae family, therefore the presence of such organism in foods is indicative of fecal pollution (Soomro et al., 2002; Benkerroum et al., 2004).

The results presented in Table 1, showed that *E. coli* was isolated within a percentages of 26.7% and 16% from raw market and bulk farm milk respectively. Nearly similar results was reported by (Rajeev and Amit, 2010) who found that out of all milk samples examined the highest contamination was recorded from the milk collected from vendors 26% flowed by dairy farms 20%, while (Ali and Abdelgadir, 2011; Gwida and EL-Gohary, 2013) could isolate *E. coli* from raw milk in a percentage of 63% and 41.2% respectively. Lower results were recorded by (Kivaria et al., 2006) who detected *E. coli* in 6.3% of the examined raw milk samples.

Although global importance of *E. coli* as a causative agent for diarrheal illness has decreased markedly over the past 50 years following the implementation of improved sanitary practices, it is still the major cause of illness in under developed nations (Ryser, 1998).

The high incidence of *E. coli* in market milk than bulk farm milk may be due to that bulk farm milk is mainly transported directly to the dairy plant for processing while market milk is usually collected from small farms or farmers therefore it will be liable to cross contamination by different ways as mixed fresh clean milk with unclean milk by hands of workers, containers of transportation or contaminated water used for cleaning utensils could be source of contamination.(Murphy and Boor, 2000).

Regarding the occurrence of *E. coli* in stool samples as shown in Table 2, *E. coli* was found to be positive in 16.6% of examined samples. This percentage is logic as the organism is normally a ubiquitous. Higher rate was recorded by (Gwida and EL-Gohary, 2013). Also it is obvious from the results that the percentage of isolated *E. coli* from hand swabs of milk handlers was 16%. Nearly similar results were recorded by (Mohamed et al., 2004) who isolated *E. coli* from hand swabs with a percentage of 18.8. Meanwhile low percentages were mentioned by (Samaha et al., 2004) who isolated *E. coli* from hand swabs with a percentage of 7.5. The presence of *E. coli* in milk handlers attributed to the handlers contaminates their hands with their stool due to lack of hygienic awareness.

*E. coli* O157:H7 is associated with life threatening diseases such as hemorrhagic colitis (HC), hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP). Raw milk is considered a high risk food as it is highly nutritious and serves as an ideal medium for bacterial growth. Several factors contribute to milk contamination such as poor hygienic milking conditions, contaminated equipments, milking utensils and milk handlers' poor personal hygiene.

The present study failed to isolate *E. coli* O157:H7 from any of the examined samples and these findings are not surprising, since also (Coia et al., 2001; Abd El-Atty and Meshref, 2007) did not detect *E. coli* O157 in raw milk samples. On contrary, (Picozzi et al., 2005; Rey et al., 2006; Lye et al., 2013) could isolate *E. coli* O157 from milk at various percentages.

The failure in detection of *E. coli* O157 in milk is mainly returned to isolation of *E. coli* O157 is often difficult as it is present sporadically at very low levels among very high levels of competitor organisms (Siriken et al., 2006).

Table 3, revealed that 9 (45%) and 2 (16.7%) from the examined market and farm milk samples for *E. coli* were belonged to the serovar O166, respectively. Similar *E. coli* serovars were isolated from milk products were previously recorded by (Madic et al., 2001), the rest of the isolated strains were untypable. The public health importance of isolated Enteropathogenic serovars had been attributed to its enterotoxin, which is implicated in causing gastroenteritis, epidemic children diarrhea, and sporadic diarrhea in children as well food poisoning (Hassan and Afify, 2007).

On the other hand, 2 (40%) out of 5 isolates from the examined stool samples were untypable and the remaining isolates 3(60%) were serologically typed as *E. coli* O148 (Table 4), nearly similar result was recorded by Neelam et al. (2006) who isolated *E. coli* from human stool samples which serotype was O148, O158, O 63, O15 and the other isolates were untypable. While 100% of the isolated *E. coli* from the hand swab samples were untypable.

*E. coli* O148 is isolated from one case of HUS in an outbreak occurred among wedding attendees in France in June 2002. (Espie et al., 2006) The presence of *E. coli* in milk handler's stool

**Table 4 – Serological identification of *Escherichia coli* isolated from the examined human samples.**

| *Escherichia coli* Serogroups | Type of the sample | | | |
|---|---|---|---|---|
| | Stool samples | | Hand swabs | |
| | NO | % | NO | % |
| O:148 | 3 | 60 | 0 | 0.0 |
| O:166 | 0 | 0.0 | 0 | 0.0 |
| Untypable (poly1-9) | 2 | 40 | 4 | 100 |
| Total | 5 | 100 | 4 | 100 |

samples and hand swabs due to lack of hygienic awareness act as a source of contamination of milk.

*Salmonellosis* is one of the most important zoonotic bacterial pathogen of food-borne infection all over the world. The most important serotypes of *Salmonella* are *Salmonella typhimurium* and *Salmonella enteritidis* (Fashae et al., 2010; Hendriksen et al., 2011). Salmonella spp can cause gastrointestinal disease. The main sources of transmission are water, eggs and raw foods (Karns et al., 2005).

In the present study *Salmonella* could not be detected in any of the examined raw milk samples and these results are in agreement with (Khan and Malik, 2002; Ekici et al., 2004; Zeinhom, 2011), while (Abd Elall et al., 2005; Karshima et al., 2013) could isolate *Salmonella* from raw milk with different values. Also failure of isolation of *salmonella* from human stool samples is in accordance with (Ibrahim et al., 2013) who found that all stool samples he examined reacted negatively to all *Salmonella* spp. This may be attributed to the fact that all the examined humans were apparently healthy (absence of fever and diarrhea). A high percentage of human salmonellosis occurs through consumption of raw milk or dairy products manufactured with raw milk (CDC, 2003).

Table (1&2) exhibits isolation rate of *A. hydrophila in a percentage of* 24%, 13.3%, 10% and 16% from market milk, farm milk, stool and hand swabs samples respectively, Abdelraouf and Naima (2011) isolated *A. hydrophila in a percentage of* 36% from the milk. Yucel et al. (2005) in Turkey found aeromonads in 49.2% out of 132 bulk raw milk samples and in 40% out of 25 raw milk samples sold in the street, These findings indicate that motile *Aeromonas* sp. are common species in raw milk (Neyts et al., 2000). On the contrary, studies from developed countries reported isolation rates of aeromonads of less than 5% from certain dairy products (Hunter and Burge, 1987). The presence of *A. hydrophila* in raw milk is of a special concern since this organism is capable of growth at refrigerator temperatures.

Abdelraouf and Naima (2011) found that 34.3% of the stool samples they examined were positive for *A. hydrophila*. which is lower than some parts of the world especially northern European countries with a frequency up to 13% (Fredriksson and Korkeala, 2003), and higher than other parts, 2.8% in Montreal, Canada, 2.1% from the Oneida County outbreak (Shayegani et al., 1981), and 1.04% were isolated from 7290 black Atlanta children during the Thanksgiving-Christmas holidays in 1988 (Metchock et al., 1991). Also Rahman et al. (2007) found that the total fecal carriage rate of normal humans is < 1–7% for most studies. Ghenghesh et al. (1999) isolated *Aeromons* sp. from 15% of diarrheic and from 18% of non-diarrheic children in Libya.

*Y. enterocolitica* failed to be detected in any of the examined milk samples (Table 1); these are similar to the results obtained by (Quaglio et al., 1988; Desmasures et al., 1997; Ramesh et al., 2002), on the other hand, higher results were reported by (Ozbas et al., 2000; Zeinhom, 2007).

*Y. enterocolitica* represent 3.3% in the stool samples which nearly in accordance to (Abdelraouf and Naima, 2011) who found that (4.7%) of the stool samples they examined were positive for *Y. enterocolitica*. Higher rate was found in some parts of the world especially northern European countries with a frequency up to 13% (Fredriksson and Korkeala, 2003). This might be partly due to the warmer climate in our country, while low rate was recorded by (Shayegani et al., 1981) (2.8%). Presence of *Y. enterocolitica* and *A. hydrophila* in the human samples act as a potential source for contamination of the milk.

Since the microbiological limits of raw milk are not established in Egypt: it is very likely that milk should often be tested, if found positive for pathogens then withheld from human consumption. The production of high-quality milk and safe milk should be of great importance to the economy of the farmer and the sustainability of the dairy industry in Egypt.

## 5. Conclusion

The results of the present study clearly indicated that microbial quality and safety of raw milk was unsatisfactory. The presences of faecal indicator organisms not only indicate poor hygiene but also itself may be pathogenic. The pathogenic bacteria such as *E. coli and A. hydrophila may* pass to the milk; also the presence of *A. hydrophila* in raw milk is of a special concern since this organism is capable of growth at refrigerator temperatures; this suggests that raw milk should be considered as a vehicle for the transmission of potentially pathogenic bacteria. Because of the fact that raw unpasteurized milk is consumed directly by a large number of people in rural areas and indirectly by a much larger segment of the population via consumption of several types of cheeses, this emphasises the need for effective and continuous training accompanied with emphasize on the safety and health issues related to raw milk hazards, educational efforts to improve dairy farmers' awareness of milk borne zoonoses,, risk factors associated with milk borne pathogens, efficient cleaning of all utensils and equipment and the consumers should take in consideration the cleanliness of sales persons. The final retail containers used are preferred to be dispensable and efficiently closed or covered. It is of outmost importance to examine the stool specimens of apparently healthy dairy handlers to clarify their role in shedding bacterial pathogenic agents. To protect public health, more stringent regulations and strategies are in demand.

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