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Zoonotic Bacterial Pathogens Isolated from Raw Milk with Special Reference to *Escherichia coli* and *Staphylococcus aureus* in Dakahlia Governorate, Egypt

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Abstract

There are very few published data about the occurrence of *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) in raw milk (Market and Farm milk) in Dakahlia Governorate, Egypt. Therefore, the present study was conducted to clarify the role of raw milk in transmitting some zoonotic bacteria such as *S. aureus* and *E. coli* to man aiming to study the source of infection. Three hundred and twenty five random samples of market milk, bulk farm milk, milker hand swabs and human stool specimen were collected to be cultivated on the selective bacteriological media. Identification was performed using a series of different biochemical tests. The obtained results revealed that out of 150 examined market milk 36.66% (55 out 150) and 56.66% (85 out 150) harboring *E. coli* and *S. aureus* respectively. On the other hand, bacteriological examination of 100 raw milk samples collected from different dairy farms clarified that S.aureus and *E. coli* were isolated at a percent of (18 and 20%) respectively. Additionally seventy five samples were collected from man representing 50 stool samples (25 diarrheic cases and 25 apparently healthy dairy handlers) and 25 hand swabs from dairy handlers. *S. aureus* and E.coli were found to be positive in 7, 40 stool samples out of the total examined (50) with percentages of 14, 80 respectively. Out of 25 human diarrheic cases, *S. aureus* and *E. coli* were isolated with percentages of 8, 88 respectively. Meanwhile *S. aureus* and *E. coli* were isolated respectively from 5 (20%), 18 (72%) stool samples out of 25 apparently healthy dairy handlers. Out of 25 hand swabs from dairy handlers *S. aureus* was isolated with the percentage of 60. However, *E. coli* was isolated with percentage of 20. It could be concluded that the results of the present study clearly indicated that the quality of the raw milk sold in Dakahlia Governorate is considered unsatisfactory and strict hygienic measures are required to improve the quality of raw milk sold in Dakahlia Governorate.

Keywords: Raw milk; Zoonoses; *E. coli*; Staph aureus; Public health

Introduction

Milk is one of the greatest blessings that are given to Humans by Nature. Milk is considered a complete and nutritious food; not only for the new-born mammal and for the human beings, but it is considered as a good medium for many microorganisms. Raw untreated 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 [1]. For this reason, utilization of both raw untreated milk and raw milk cheeses has frequently been associated with food-borne illness. Zoonotic bacterial agent's presence in raw milk is of great public health and economic significance. As well as causing serious economic problems concerning the dairy industry, they constitute a major impediment to the trade of animals and animal products, and this can lead to obstruction of social and economic progress, especially in developing countries in Africa. Moreover, the level of cultural awareness among farmers about the importance of economic and public health from zoonotic diseases in most of these countries is low, and this increases the effort required to control these diseases [2]. One product that is commonly distributed in raw form is milk. Raw milk is usually colonized by a variety of many zoonotic pathogens such as *Campylobacter jejuni, enterohaemorragic Escherichia coli, Salmonella typhimurium, Listeria monocytogenes, Staphylococcus aureus* and *Yersinia enterocolitica* therefore; they represent an important source of foodborne pathogens. These pathogens in milk have been linked to the environment in the farm, mixing clean milk with mastitis milk and from livestock [3]. The natural raw milk obtained from the mammary gland of healthy animal is usually with low microbial load and the application of all hygienic measures during milking prevents milk from contaminating as well. The bacteria can access to the milk through colonization of the teat canal or an infected udder (clinical and subclinical mastitis) or gets contaminated from milk utensils or water supply used [4,5]. The presence of bacteria in milk has many undesirable effects on the quality and safety of milk and its products [6]. Milk contaminated by high levels of bacteria usually becomes unsuitable for further processing [7].

E. 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 enteropathogenic and/or toxigenic strains which lead to sever gastrointestinal disturbance [8]. However the other toxigenic strains like *E. coli* O_{157}:H_7 cause life threating syndromes [8,9]. *E. coli* is among many pathogenic microorganisms which can access to milk and some of dairy products [10] which considered a reliable indicator of contamination by manure, soil and contaminated water [11].

Milk is an excellent media for the growth and multiplication of *Staphylococcus aureus*. The organism is responsible for approximately

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30% to 40% of all mastitis cases [12]. Unhygienic measures, contaminated equipments, mammary gland infected with *S. aureus* and hands of milkers during handling and processing of raw milk are considered the main cause of milk contamination with *S. aureus* [13]. *S. aureus* is considered the most important cause of food borne illnesses all over the world [12].

On account of zoonotic importance of some opportunistic pathogens causing food poisoning. The present study was conducted to throw light on the role of raw milk in transmitting zoonotic bacteria such as *Staphylococcus aureus* and *E. coli* to man aiming to study the source of infection. This was done in the manner of studying the following points:

I- Occurrence of *S. aureus* and *E. coli* in human cases.

II- Occurrence of *S. aureus* and *E. coli* in raw milk samples.

Material and Methods

Collection of samples

A total of 250 raw milk samples were randomly collected (150 market milk, 100 bulk farm milk) (Table 1). Samples were collected from different dairy shops, groceries, supermarkets and dairy farms, in Dakahlia Governorate, Egypt. Milk was transferred directly to the Hygiene and Zoonoses Laboratory, Faculty of Veterinary Medicine, Mansoura University in clean and dry bottles. In addition to seventy five samples from man representing 50 stool samples were collected from 25 diarrheic patients attending the outpatient clinic of gastrointestinal tract Hospitals, Dakahlia Governorate and 25 apparently healthy dairy handlers. 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 [14]. Moreover, 25 hand swab samples were also collected from milk handlers from different dairy farms and shops in Dakahlia Governorate [14]. All swabs were transferred into sterile buffered peptone water (BPW) tubes under aseptic conditions. The tubes were labeled with respect to age and localities then ice packed and transferred immediately to the lab. The culture media used were according to Cruickshank et al. [15,16].

Samples	Number of samples
Raw market milk	150
Raw farm milk	100
Human samples:	
Milk handlers swabs	25
Stool samples:	
Diarrheic	25
Apparently healthy	25
Total	325

Table 1: Total numbers of collected samples.

Biochemical test	Reaction
Lactose fermentation	+
Catalase	+
Simmon's citrate	-
Indole Production	+
Nitrate Reduction	+
Methyl Red	+
Voges- Proskauer	-
Urease	-
Acid from sugar	
Glucose	+
Mannitole	+
Lactose	+
Salicin	+
Sucrose	+

Table 2: Biochemical characterization of *E. coli* isolated from raw milk.

Microbiological Methods

Isolation and identification of *Staphylococcus aureus* and *E. coli* from raw milk

Culturing was carried out according to standard protocols [15,17]. 25 ml from the collected samples were added to sterilized tubes containing 225 ml buffered peptone water and incubated aerobically at 37˚C for 18-24 hrs. A loopfull from the incubated broth was streaked onto Baird Parker agar base (Oxoid, CM 275) and incubated at 37˚C for 24-48 hrs. Black shinny colonies from each plate were picked up, streaked on nutrient agar plates and incubated at 37˚C for 18-24 hrs. The purified colonies were then streaked onto nutrient agar slants and incubated at 37˚C for 18-24 hrs for further identification. The isolated strains were subjected to series of different biochemical tests. For the isolation and identification of *E. coli*, one ml from incubated BPW was transferred to 5 ml MaCconkey broth (Oxoid, CM 5a) and incubated at 37˚C for 24 hr. A loopful from the incubated broth was streaked on Eosin methylene blue (EMB) (Oxoid, CM 69) agar and incubated at 37˚C for 24 hrs. Morphologically typical colonies (at least 5 per plate) producing metallic sheen were taken into nutrient broth for further identification.

Isolation and identification of *Staphylococcus aureus* and *E. coli* from human cases

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

Biochemical Characteristics

The isolated strains were subjected to a series of different biochemical tests (Tables 2 and 3) using the procedure of Barrow and Feltham [18] to confirm *E. coli* and *S. aureus*.

Results and Discussion

The results of the present study are summarized in the Tables 4 and 5.

The results presented in Table 4 show that out of 150 examined

Biochemical test	Reaction
Catalase	+
oxidase	-
Indole Production	-
Nitrate Reduction	+
Methyl Red	+
Voges- Proskauer	+
Hemolysis	+
Coagulase	+
Acid from sugar	
Glucose	+
Mannitole	+
Maltose	+
Lactose	+
Sucrose	+

Table 3: Biochemical characterization of *S. aureus* isolated from raw milk.

Isolated organisms	Raw market milk (150)		Raw farm milk (100)		Total (250)	
	No of positive	%	No of positive	%	No of positive	%
S. aureus	85	56.66	18	18	103	41.2
E. coli	55	36.66	20	20	75	30

Table 4: Percentage of *E. coli* and *S. aureus* in examined raw milk samples.

Samples		No of samples	*Staphylococcus aureus*		*E. coli*	
		No of positive	%	*No of positive*	%	%
Stool samples	Diarrheic cases	25	2	8	22	88
	Apparently healthy	25	5	20	18	72
Hand swabs		25	15	60	5	20
Total		75	22	29.33	45	60

Table 5: Percentage of *E. coli* and *S. aureus* in examined human samples.

raw market milk samples 55 were contaminated with *E. coli*. On the other hand, bacteriological examination of 100 bulk farm milk samples collected from different farms revealed that *E. coli* were isolated at a percent of 20 as 20 isolates from 100 examined samples. Other researchers reported high incidence of *E. coli* from different types of milk [19-23]. Recovery of *E. coli* from raw milk is not only regarded as an indicator of fecal contamination but more likely as an evidence of poor hygiene and sanitary practices during milking and further handling. The presence of *E. coli* itself in milk and milk products as a possible cause of food borne disease is insignificant because *E. coli* is normally a ubiquitous organism [24]. However, the occurrence of pathogenic strains of *E. coli* in milk products could be hazardous for consumers.

Staphylococcus aureus is one of the leading causes of food borne illnesses in humans worldwide and is associated with contaminated foods of animal origin. 85 isolates of *S. aureus* out of 150 examined market milk samples and 18 isolates out of 100 bulk farm milk samples were identified as *S. aureus* by culturing using selective culture media (Baired parker media) for isolation with a percentage of 56.66% and 18% respectively. Higher incidence of *S. aureus* mastitis reached (75.3%) in India were reported [25]. Wide variation in the prevalence of *S. aureus* has also been reported [26]. This variation is largely attributed to the changing management conditions and using of different diagnostic tests.

Concerning the type of examined milk samples, the high incidence of *S. aureus* and *E. coli* in street milk may be attributed to in Egypt bulk farm milk is mainly transported directly to the dairy plant for processing meanwhile 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 mastitis milk, unclean hands of workers, unclean utensils and unhygienic water supply for washing the utensils could be the source for accelerating the bacterial contamination [27].

Table 5 illustrates the percentage of *S. aureus* and *E. coli* in 50 stool specimen of man (25 diarrheic and 25 apparently healthy persons). The overall percentage of *S. aureus* in the total examined stool samples was 14% (7 out of 50). Higher and lower results were previously reported by Gebreselassie [28] whose result was 40.5%. However, lower percentage of 4.2% was reported by Bhalla et al. [29]. On the other hand the percentage of *E. coli* in the total examined stool samples was 80% (40 out of 50). As regards the frequency distribution of *S. aureus* and *E. coli* in stool specimen of man with respect to diarrheic or non-diarrheic status, Table 5 clarify that, *S. aureus* was isolated from diarrheic stool samples with a percentage of 8 (2 out of 25). Nearly similar results were previously reported by Flemming and Ackermann [30] who isolated *S. aureus* from patients with nosocomial diarrhea with a percentage of 7.3. Higher prevalence of *E. coli* than *S. aureus* in diarrheic samples. The percentage of isolated *E. coli* was 88 (22 out of 25).

Regarding the occurrence of *E. coli* in stool samples of apparently healthy persons *E. coli* was found to be positive in 18 out of 25 samples examined (72%). This percentage is logic as the organism is normally a ubiquitous. Nearly similar results were obtained by Haggag et al. [31].

Results illustrated in Table 5 show higher prevalence of *Staphylococcus aureus* in hand swabs of milk handlers, 15 out of 25 examined (60%). High prevalence rate were also reported by Deandrade and Zelante and Tondo et al. [32,33] whose results were 35.7% and 35.2%, respectively. In contrary, lower percentages of 11.67 and 4.2 were previously recorded by Samaha et al. and El- Khawas and Amani [34,35]. This may be attributed to staphylococci are ubiquitous organisms and at least 50% of individuals carry the organism in their nasal passages, throat and through coughing or sneezing they contaminate their hands.

It is obvious from the results recorded in Table 5 that the percentage of isolated *E. coli* from hand swabs of milk handlers was 20 (5 out of 25). Nearly similar results were recorded by Mohamed et al. [36] who isolated *E. coli* from hand swabs with percentages of 18.8. Meanwhile low percentages were mentioned by Samaha et al. [34] who isolated *E. coli* from hand swabs with percentages 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.

In conclusion, the results of the present study clearly indicated that microbial quality and safety of raw milk was unsatisfactory. The presences of fecal indicator organisms not only indicate poor hygiene but also itself may be pathogenic. The pathogenic bacteria such as *E. coli* and *S. aureus* may pass to the milk; this suggests that raw milk should be considered as a vehicle for the transmission of potentially pathogenic bacteria. Since a lot of people still drink raw milk, especially in rural areas, this emphasis's the need for educational efforts to improve dairy farmers' awareness of milk borne zoonoses, how these pathogens transmitted to milk, risk factors associated with milk borne pathogens and how to obtain fresh clean milk. It is of utmost importance to examine the stool specimens of apparently healthy dairy handlers (non diarrhoeic stool samples) to clarify their role in shedding bacterial pathogenic agents.

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