

Full Length Research Paper

Differential Distribution of Pathogens from Raw Milk and Place of *Shigella* by Mode of Milking

Distribution Différentielle Des Germes Pathogènes Du Lait Cru et Place de *Shigella* Selon Le Mode de Traite

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Accepted 8 June, 2011

Milk is a biologic food that has clear nutritional benefits. Because of its composition, milk is a living environment composed of a diverse flora. Among the latter, we encounter pathogens that were dangerous to human health. Milk represents an important vector of germs in humans. Milk contamination by pathogenic bacteria such as *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus*, *Shigella*... is now a major concern of product chains in raw milk, because of their roles in food poisoning. In most cases, contamination of milk by these organisms occurs during milking by bacteria on the skin of the teats themselves soiled bedding contaminated by their droppings, the milking equipment or by water used for watering cows, either for operations hygiene at milking. In our study, selected pathogens were identified and counted from samples of raw milk from two types of milking and samples were collected from three different grocery stores. Varying proportions were recorded in relation to the mode of milking, *E. coli* occupies first place in hand milking (98.7%), followed by *Streptococcus* in 95%, *Staphylococcus* (79.3%) and *Shigella* with 16.5%, but these proportions diminish considerably when the milking is automatic or when the milk is collected from grocers. But in the automatic milking *Shigella* occupies the first place (80.7%) followed by *Staphylococcus* (19.9%), in contrast *E. coli* is almost absent and *Streptococcus* are present in small proportion (3.6%). The distribution of *Staphylococcus* and *Streptococcus* is homogeneous whatever the mode of milking but it is heterogeneous for *E. coli* and *Shigella*. Comparative analysis of milk from milking manually and automatically collected milk from 3 grocery stores shows that the *Streptococcus* which takes second place after *E. coli* in hand milking seems most representative and homogeneous. This differential distribution of germs is probably related to the environment of milking and its microbial ecological quality and probably the frequency and the relative volume of water used in each course operational. Further studies are needed to see the reasons for this unconventional redistribution to finding effective ways of prevention.

Keywords: raw milk, mode of milking, *Shigella*, dominance.

INTRODUCTION

Milk is a complex biologic fluid that plays an important role in human nutrition as it can be consumed in liquid or various derivatives developed aspects, taste and

nutritional values very diverse. This diversity is related to the original composition of milk which makes it, by its nature, a good growth medium for many microorganisms, some of which are hazardous to the health of consumers. A number of bacteria including *S. aureus*, *Escherichia Coli*, *Salmonella* have been recovered from

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raw milk and some of these have been determined to be pathogenic and toxicogenic, and implicated in milk borne gastroenteritis (Lingathurai 2010, De Buyser et al. 2001).

Milk contamination by pathogens, like *Staphylococcus*, may be intramammary when these germs infect the animal, reach the udder via the blood stream, and extramammary (Ben Hassen S. et al. 2003, Bergonier et al. 2003). Indeed, during milking, milk receiving a second injection of various species of microorganisms. The importance of this contribution varies considerably according to conditions of milking hygiene and environment. Bacterial contamination of raw milk can originate from different sources: air, milking equipment, feed, soil, faeces and grass (Coorevits *et al.*, 2008). The number and types of micro-organisms in milk immediately after milking are affected by factors such as animal health (including the skin of the udder) and equipment cleanliness, season, feed, and the milking equipment in a broad sense (from the machine to the tank when it comes to automatic milking), the milking machine, when improperly installed, improperly adjusted or poorly maintained, can help the emergence of new mammary infections by reducing the natural defenses of teats. It can also play a passive or active spread of germs pathogens from one neighborhood to another or from one cow to another (Bergonier et al. 2003). The environment (air-borne dust from the place of milking) and the bacteriological quality of water used for watering the animals and cleaning the milking equipment have a role in milk contamination (Michel V. 2005). These sources of contamination are fed by reservoirs of bacteria that are litter, faeces, soil, water ... Rinsing water for milking machine and milking equipment washing also involve some of the reasons for the presence of a higher number of micro-organisms including pathogens in raw milk (Bramley et al. 1990, Torkar K.G. et al.2008).

The hygienic quality of milk is a concern and the primary objective of heat treatment of raw milk to ensure destruction of pathogens may be present. The objective of this study is to determine the frequency of some pathogens may be present in raw milk, which is given special importance, because of the severity or frequency of their risks and are related environment and mode of trafficking and to identify sources of contamination in order to establish preventive measures to avoid these germs.

MATERIALS AND METHODS

Materials

Eight samples of cow's milk from two types of processes (manual and automatic) were conducted during the

months of February, March and April 2008, in two different regions; milks from hand milking of a few cows collected in a tank, milk from hand milking pasteurized, output milking machine, milk tank and milk tank pasteurized samples were collected in the region of Bizerte and finally 3 milk samples were collected from 3 different groceries in the region of Tunis.

Methods

The milk samples, collected in sterile bottles and sent the same day in the laboratory, have been selective enumeration of some selected pathogens whose characteristics are described in Tables 1 and 2.

Bacteriological analysis

For each sample of milk, 10 ml were analyzed, added in Erlenmeyer to 90 ml of sterile saline, we obtain a dilution mother 10^{-1} from which is produced decimal dilutions up to 6. One ml of each dilution was inoculated, in-depth selective agar for each gender.

The Fecal coliforms (*Escherichia coli*) were enumerated on Lactose Citrate Desoxicolate agar (DCL), incubated 24H at 44°C, *Staphylococcus* on Baird Parker agar supplemented with egg yolk and incubated 48H at 37°C, *Streptococcus* on agar D-Cocossel and incubated 24H at 37°C, and *Shigella* on SS (*Salmonella-Shigella*) agar, incubated 24H at 37°C.

Counting

Colony counts characteristics of each type was performed on boxes containing between 15 and 300 colonies at two boxes of successive dilutions. The total number of colonies expressed by CFU / ml is calculated using the formula: $N = \frac{\sum c}{v \cdot (n_1 + 0,1 \cdot n_2)} \cdot d$ with: $\sum c$: the sum of colonies, v = volume of inoculum, n_1 : number of selected box of the first dilution, n_2 : number of selected box of the second dilution and d : the dilution factor.

RESULTS AND DISCUSSION

In this study, pathogens were selected, identified and enumerated from raw milk and pasteurized milk samples from two types of milking and bulk samples collected from three different grocery stores. Varying proportions of germs were recorded in relation to the mode of milking, it appears that *E. coli* occupies first place in hand milking

Table 1. Characteristics of selected bacterial pathogens from milk

	Bacteria Gram +		Bacteria Gram -	
	<i>Staphylococcus</i>	<i>Streptococcus</i>	<i>E. coli</i>	<i>Shigella</i>
Family	Micrococcaceae	Streptococcaceae	Enterobacteriaceae	Enterobacteriaceae
Form and Gram	Cocci Gram+	Cocci Gram+	Bacille Gram -	Bacille Gram -
Respiratory Type	Aerobic strict	Aero-anaerobic optional	Aero-anaerobic optional	Aero-anaerobic optional
Mobility	-	-	+/- Ciliature peritrichous	- pendulum
Toxin production	+	+ /-	+	+
Invasion	+	+	+	-
Infection	chronic	chronic	acute	acute
Dissemination	In tissues and blood	In tissues (hemolysis) and blood	In blood	local infection
Reservoir	Human and animal	Human and animal	Human and animal	Human

Table 2. Distribution of bacteria in animals and human

	Animal			Human	
	Usual host	Infection	Reservoir	Usual host	Infection
<i>Staphylococcus</i>	Skin, teats (teatb lesions), tonsil, vagina	Clinical or subclinical mastitis	Mop, hands milking, milking equipement, tanks, pipes milk	Nasal cavity, throat, skin of face and hands, gut	Skin infections, pneumonia, meningitis, sepsis, poisoning and long-term inflammatory response in humans
<i>Streptococcus</i>	breast, oral cavity, vagina, respiratory tract and intestines	Acute or chronic mastitis	Litter, fecal matter	Mouth, nasopharynx, gastrointestinal tract	Skin infection, broncho-pulmonary infection, sore throat, pneumonia, vaginal infection
<i>E. coli</i>	Digestive tube	Diarrhea, mastitis	Environment, litter, fecal matter, water	Digestive tube	Diarrhea, gastroenteriti, u rinary infections, meningitis, septicemia
<i>Shigella</i>	Environment Germ	Mastitis	Water	Specific pathogen of the digestive tract	Shigellosis (intestinal infection specific rights), acute diarrhea, acute inflammatoryreaction of the intestine

(98.7%), followed by *Streptococcus* in 95%, *Staphylococcus* 79.3% and *Shigella* with 16.5% (Figure 1), but these proportions decreased significantly when it is automatic milking except for *Shigella*. In automatic milking, *Shigella* occupies the first place (80.7%) followed by *Staphylococcus* (19.9%) by cons *E. coli* was absent and *Streptococcus* were present with a little proportions

(3.6%).In the milk collected from 3 groceries (Figure 1), all the germs were presented in a little percentages.

Pasteurization of milk samples analyzed eliminated all the pathogens. So milk intended for consumption or processing to human nutrition must not contain any pathogenic microorganisms.

The number of all tested groups of micro-organisms was

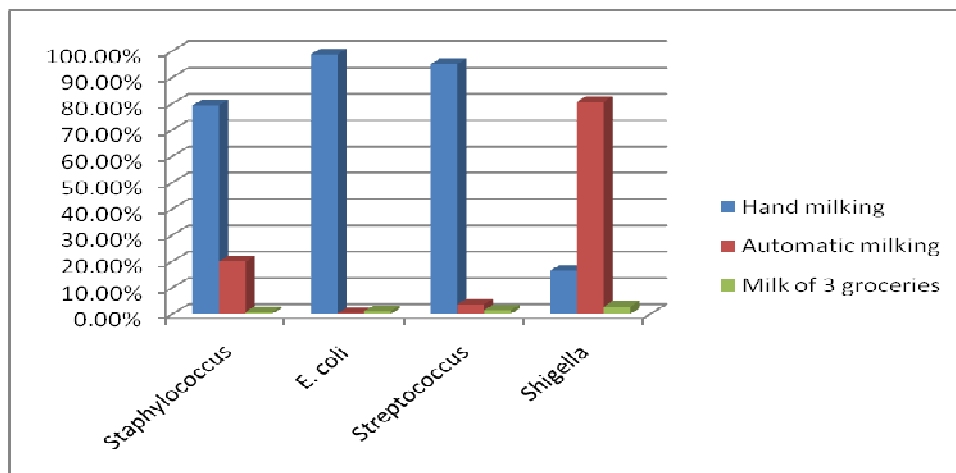


Figure 1. Variation in the percentage of pathogens by mode of milking

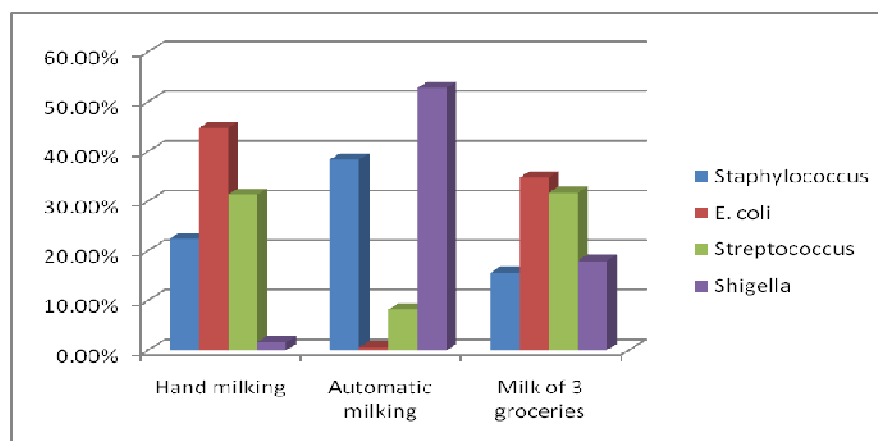


Figure 2. Proportion of each organism depending on mode of milking

higher in samples of milk from the three groceries, this result was found too by Torkar et al. 2008. The highest differences between samples taken from hand milking and from automatic milking, especially from tanks, were in number of fecal coliforms (98.7%), as it is represented in the Figure 2.

The search for microorganisms indicators of fecal contamination can judge the hygienic condition of a product. Even at low levels, they are evidence of hygienic conditions degraded during processing or during transportation. Contents-fecal-coliform are found below those listed by Hamama and El Mouktafi,

1990 (44.5×10^3 in all samples cons 1.8×10^5 cfu / ml fecal coliform) but higher than those reported by Labioui et al. 2009 (44.5×10^3 cons 5.2×10^3 cfu / ml fecal coliform).

The number of *Shigella* in milk from tanks and output milking machine was higher (80.7%) than in milk from hand milking (16.5%) and groceries (2.8%) Figure 1.

The high percentage of *Shigella* in the automatic milking is probably due to the excessive use of water for cleaning milking equipment, above all the water used for watering and cleaning of the animal and the milking equipment is the main source of contamination by this organism.

Comparative analysis of milk from hand and automatic milking and collected milk from 3 groceries shows that the *Staphylococcus* and *Streptococcus* seems most representative and homogeneous whatever the mode of milking but it is heterogeneous for *E. coli* and *Shigella* (Figures 1, 2).

Given its habitat and frequently implicated in mastitis, the presence of *Staphylococcus* in milk seems almost inevitable, but it is clear that these organisms are easily destroyed by pasteurization. This differential distribution of seeds is probably related to the environment of milking and its ecological microbial quality and/or frequency and the relative volume of water used in each course operational (Ben Hassen et al. 2003). The rate of *Streptococcus* is related to the health of cows and the hygienic conditions of milking.

The animal, the environment and humans may be the cause of the contamination of milk. The animal may indirectly contaminate milk by particles of feces, sputum and other discharges, or the neighborhood with sick animals of the same species or different species (goat, for example), it is the primary source of germs mainly *Staphylococcus* and *Streptococcus*.

The water, is the main source of *Shigella* and *E. coli* (Edberg et al, 2000, Chen et al. 2001), used for cleaning milking equipment and watering the cows, litter, dust, improperly cleaned equipment, etc, are important sources of secondary contamination of milk during manual milking and the various manipulations it is subjected. Through his hands, sputum, soiled clothes, etc., the sick or healthy carrier or infected may also be a cause of contamination of the animal or its environment and milk.

The machine-milking may increase the incidence of mammary infections either by a role as vectors of pathogens from infected areas to healthy neighborhoods, either by contamination of the teat force, its role is traumatic for the teat canal, while diminishing effect "barrier" (Boudry 2005).

Contamination of raw milk production by these pathogens is not always related to animal health problem. Taking the example of *E. coli* is a bacterium found in the digestive tract of the animal, whose natural host is transient or due to the ingestion of contaminated food themselves. This presence manifests itself most often a healthy carrier and a charge of fecal contamination of milk and its derivatives and the result of contamination of humans and trigger an inflammatory reaction.

E. coli is a germ of the environment, living in faeces (Edberg et al. 2000), polluted water and litter which explain its prevalence in raw milk from hand milking.

Shigella is a germ mainly found in water and faeces (Almanos et al. 2000, Chen et al. 2001, Koutsotoli et al.2006) presents a significant proportion in automatic milking and in the milk taken from three different

groceries. This germ can he represent a biomarker of water used by the groceries? These pathogens studied with several characters in common they may be a microbial community? How will the dynamics of this community?

Raw milk is a highly nutritious in terms of nutrition. Its production must be tightly controlled because of possible risk that he may pose to human health. Indeed, strains pathogenic for humans and animals (Table 2) may have acquired multiple resistances to antibiotics may proliferate. An Assessment of the hygienic quality of milk used to investigate the natural microflora and microbial contamination of witnesses extra-mammary possible (Labioui et al. 2009).

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