Milk-borne infections. An analysis of their potential effect on the milk industry

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Abstract

In developed countries such as the United States of America, foodborne illnesses account for 48 million infections per year. Developing countries such as India face greater simultaneous challenges particularly since incorrect processing or storage of dairy products can represent a transmission hazard for a large number of pathogens and can be responsible for outbreaks of brucellosis, listeriosis, tuberculosis, etc.

It is important to recognize the types of germs which can be transmitted through insufficient thermal preparation of milk or milk products or through post-pasteurization contamination, in order to successfully avoid transmission of milk-borne infections.

Keywords **Contamination, pathogenicity, milk processing, foodborne infection.**

Introduction

Foodborne illnesses account for 48 million infections per year in the United States of America, with Norovirus, *Salmonella spp* (nontyphoidal), *Clostridium perfringens*, *Campylobacter spp* and *Staphylococcus aureus* ranking as the top five pathogens contributing to domestically-acquired foodborne illnesses.[1] Incorrect processing or storage of dairy products can represent a transmission hazard for a large number of pathogens and can be responsible for outbreaks of brucellosis, listeriosis,[2] tuberculosis,[3] etc, posing a greater threat in developing countries, such as India.

Being rich in proteins, lipids and sugars, milk is an example of ideal culture medium for various microorganisms. Thus we may as well say that milk is a readymade vehicle for the omnipresent germs. Some of the bacteria contained in milk (such as *Lactobacillus spp* or *Bifidobacterium spp*) are also present in the healthy human gastrointestinal tract, aiding in digestion and protection from other infections,[4] while other bacteria can be extremely harmful to human health.

Milk-borne infections

In developing countries, the industrialization brought a series of problems along with the much appreciated progress, with the mass collection and distribution of milk from various sources playing the role of potential vehicle for disease transmission. In olden days, milk was collected from small groups of animals in farms and it was supplied to a small number of people living nearby. But with the advent of industrialization, population growth and urbanization, the demand increased drastically. Milk supply through the small farms no longer met the increasing demand. Hence commercialization of the milk industry ultimately took place.

Based on the available clinical records, some of the earliest documented outbreaks caused by

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the contamination of milk and other dairy products were probably due to infections with *Campylobacter spp*,[5] *Salmonella typhi*,[6] *Corynebacterium diphtheriae*,[7] or *Streptococcus pyogenes*,[8] although most germs had not yet been isolated at that time. With the current industrially-available tools for thermal processing of milk and given the international norms and regulations, the risk of infection has dramatically decreased but we need to be aware of the relatively long list of pathogens which can still cause sporadic cases or occasional outbreaks and of the much shorter list of pathogens which can still evade the current norms applied to the processing of dairy products.

Bacterial infections

The list of bacteria which can be responsible for milk-borne diseases is long and it includes *Brucella spp, Campylobacter jejuni*,[9] *Bacillus cereus*, Shiga toxin-producing *E. coli (E. coli O157:H7)*, *Coxiella burnetii, Listeria monocytogenes, Mycobacterium tuberculosis, Mycobacterium bovis, Mycobacterium avium subspecies paratuberculosis, Salmonella spp, Yersinia enterocolitica*, and certain strains of *Staphylococcus aureus* which are capable of producing highly heat-stable toxins.

Brucellosis is one classical example of milk-borne infection, *Brucella spp* being transmitted from goats to humans either through direct contact or through the milk of the infected animal, particularly since the appearance and taste of the milk are rarely affected by the presence of the bacteria. Once transmitted to humans, *Brucella* is responsible for a type of granulomatous hepatitis or an acute febrile illness which can, at times, persist and progress to a chronically incapacitating disease with serious complications.[10]

Coliform contamination ranks high among the most common types of contamination in the dairy industry. Microorganisms such as *Escherichia coli, Pseudomonas aeruginosa, Citrobacter spp, Klebsiella spp* and *Proteus mirabilis* can multiply in the normal summer temperatures and hence unpasteurized milk has every chance of containing *E coli*. Therefore, even nowadays, basic microbiology tests performed on milk or any dairy product are aimed at detecting coliforms.

The mechanism behind staphylococcal enterotoxin gastroenteritis is the production of a heat-stable enterotoxin by certain strains of *Staphylococcus aureus*.[11] Humans and dairy cows are the main carriers of this microbe, presenting mucosal or cutaneous lesions such as impetigo or cattle mastitis. Therefore, either the udder of cattle or the hands of milkers can be responsible for passing on the bacteria to milk, and staphylococcal mastitis is known to be prevalent in India even nowadays,[12] with an older study showing that staphylococci were isolated from 61.97% of the bacteriologically-positive samples, appearing to be the main etiological agents of bovine mastitis in India.[13] The enterotoxin is very resistant to heating and pasteurization, boiling of the milk for one hour leading to a decrease in the quantity of toxin but only autoclaving at 15 psi for 20 minutes being able to completely destroy the toxin. The sterilized milk needs to be refrigerated at 0°C to 4°C until further processing. Since staphylococci are known to grow well on saline media, the risk for contamination is higher with home-made salted cheeses.

Contamination of milk with group A streptococci may occur through humans or animals which act as carriers and the infection can sometimes be passed on to dairy cows, causing udder lesions. Group B streptococci represent another known cause of bovine mastitis[14] and a recent study has shown that group B streptococci of human or bovine origins seem to have similar virulence, being connected with possible but limited dissemination.[15]

Tuberculosis is yet another disease which can be transmitted through raw milk.[16] Infected cattle seem to be the most frequent source of infection, although buffalos, goats, sheep and camels can also pass on the bacteria.[17]

An interesting history where milk plays the role of vehicle for spreading diseases looks at a school in South Africa where several adults and 64 out of 125 children presented shigellosis seven hours after eating sour cream contaminated with *Shigella flexneri* from employees who had

shigellosis. The same strain was isolated from all the patients connected to this outbreak.[18]

Typhoid and paratyphoid fever are generally recognized as food-borne and water-borne illnesses but milk-borne infections have also been reported. The source of infection is generally a human carrier among dairy industry workers. Pasteurization is the best way of destroying *Salmonella typhi* and *paratyphi*.

Botulism caused by *Clostridium botulinum* and cholera caused by *Vibrio cholerae* are rarely transmitted through milk but the possibility cannot be completely ruled out.[19,20]

Another relatively rare milk-borne pathogen is *Bacillus anthracis*, a Gram-positive, spore-forming rod which has been shown to pass into the milk when it is present in cattle in large amounts. The contaminated milk often has an altered appearance and is secreted in smaller amounts therefore yielding a relatively low chance of transmission to humans through consumption of milk from sick cattle. The real risk is that of environmental contamination of milk or other food products from the discharges of infected animals. The vegetative form of the organism is destroyed with low-temperature-long-time (LTLT) pasteurization but the spores are resistant and can be destroyed by boiling the milk for 10 to 40 minutes plus autoclaving at 15 pounds per square inch (psi) for 10 minutes. For this reason, a first report of the Joint Food and Agriculture Organization and World Health Organization (FAO-WHO) Expert Committee on Milk Hygiene, back in 1957, recommended that in the event of disease outbreak in dairy herds, utmost care be taken to prevent environmental contamination of milk.[21]

Another well-known disease, less frequent since the advent of vaccination, is diphtheria. *Corynebacterium diphtheriae* can contaminate milk during the handling process if infected dairy workers sneeze or cough the bacilli into milk. Fortunately, the bacteria can be destroyed through high-temperature-short-time (HTST) pasteurization but milk can also be contaminated post-pasteurization.[22]

A different type of microorganism is the etiologic agent of Q fever, formerly categorized as rickettsia.[23] Most human *Coxiella burnetii* infections are caused by inhalation of contaminated dust or aerosols,[24] but consumption of contaminated milk has also been mentioned as transmission route.[25]

When referring to thermal death points, it is apparent that *Corynebacterium diphtheriae* can be destructed at the lowest temperature (58°C), while *Salmonella typhimurium*, *Brucella melitensis*, *Mycobacterium tuberculosis* and group A streptococci can all be destroyed at 62°C, if exposed to this temperature for a certain time span (ranging from 20-21 seconds for *Corynebacterium diphtheriae* to up to 135-144 seconds for group A streptococci). If the temperature increases, the duration of exposure decreases, with 2-4 seconds being enough for most of the abovementioned microorganisms at 80°C.[26]

Viral infections

A series of viruses can also be involved in milk-borne infections, particularly in developing countries with low sanitary conditions. Certain viruses may require heat inactivation temperatures slightly higher than those maintained during pasteurization (for example LTLT applies 61.5°C for 30 minutes while HTST applies 71°C to 72°C for 15 seconds) but generally speaking, the contamination appears to take place post-pasteurization in most developed countries.

In the pre-vaccination era, poliomyelitis outbreaks had debilitating consequences, infections with polioviruses being correlated with milk contamination. HTST for 30 seconds is required for completely inactivating polioviruses in water, milk and yoghurt.[27] Coxsackie viruses were found to be resistant to HTST, with increased thermal stability of viral strains suspended in cream. Therefore, alternate treatments such as ultra-high temperature (UHT) are recommended for contaminated milk.

Some other agents which can potentially contaminate milk are tick-borne encephalitis viruses, found more often in the milk of sheep, goats and less often in cow milk.[28] This virus also

resists LTLT procedures but it can be inactivated through HTST pasteurization.[29]

Hepatitis viruses, particularly hepatitis A virus (HAV)[30] and hepatitis E virus (HEV)[31] can also contaminate milk and a relatively recent study has demonstrated that increased fat content of dairy products appears to contribute to the heat stability of HAV.[30] Hepatitis B virus (HBV)[32,33] or hepatitis C virus (HCV) pose less of a threat since they recognize parenteral transmission, without a fecal-oral route.

Fungal infections

A series of pathogenic fungi can infect the udder of the cow and hence be excreted in large amounts in the milk. *Nocardia asteroides* has been found to cause bovine mastitis,[34] being excreted in milk for a period of several months. This fungus survives even if the milk is treated at a temperature of 74°C for 15 seconds or at 64°C for 30 minutes, but complete destruction of the organism is possible when the milk is heated at 66°C for 30 minutes. Other fungal species such as *Nocardia brasiliensis, Candida tropicalis,*[35] *Candida albicans*[36] or *Candida krusei*[37] have also been shown to cause bovine mastitis and therefore can be transmitted to humans through incorrectly processed milk, posing a threat of fungal infection[38] particularly in immunodepressed patients (for example in case of diabetes,[39] HIV-positive patients with decreased CD4 count,[40-44] patients with cirrhosis[45] or with chronic alcohol consumption).[46]

Parasitic infections

Certain parasites such as *Taenia* spp[47] or *Toxoplasma gondii*[48,49] can contaminate milk and be transmitted to humans. Other sources of infection include the environment of milk procurement, which is heavily controlled in industrialized farms. Soil contamination may also lead to the presence of soil-borne parasites in milk (e.g., *Ascaris lumbricoides, Trichuris trichiura*). Hence sanitary conditions, proper pasteurization and hygienic conditions should be maintained to avoid such contaminations.[50]

Preventing infection

Apart from individual measures for preventing milk-borne infections, such as: only consuming milk which comes from trustful sources and has undergone the standard pasteurization techniques; avoiding home-made cheeses, creams, yoghurts; respecting the cold-chain for milk-based products, etc, in order to avoid bacterial, viral, fungal or parasitic contamination of milk, there are a series of measures enforced in the dairy industry. For example, testing for any clinical infections or open wounds is required in milkers, workers who come in direct or indirect contact with the milk. The personnel is also required to wear face masks and hair covers and to use hand sanitizers every half hour or at regular intervals.

The facilities, such as milking sheds, silos, ice bank tanks where milk is stored, tankers used for transportation, milk processing plants, collecting tanks, pasteurizers, homogenizers, packing machines, packing materials, crates in which the milk sachets are transported should all be clean and periodically evaluated according to microbial counts per area, as prescribed by the governing bodies. All tanks, crates, silos, etc are regularly sanitized using hot water, caustic hot water, detergents or nitric acid solution. The silos and collection tanks are specifically cleaned according to Cleaning in Place (CIP) procedures, through a succession of hot water (80°C), caustic water and nitric acid solution at 65°C.

The large insulated storage rooms are sterilized by fumigation using potassium permanganate and formaldehyde. Linear low-density polyethylene (LLDPE), high-density polyethylene (HDPE) and other types of food grade plastic used for packing undergo ultraviolet sterilization.

Milk also undergoes microbial testing, organoleptic tests and a series of other biochemical tests (clot on boiling, phosphatase test, methylene blue reduction time test, milk adulteration test, etc.).

Testing of milk and milk products

Different samples undergo testing for different types of microbes (table I).

Sample type	Tests performed
Milk Byproducts	Coliforms, yeast and mould
Raw milk	Coliforms and standard plate count
Packed milk	Coliforms and standard plate count
Raw water	Coliforms and standard plate count
Processing water	Coliforms and standard plate count

Table I. Specific microbes in milk and milk based products

For culturing and identifying bacteria, different types of media have been used. Milk itself is full of all the nutrients needed for the microbes to grow. But when testing for different microbial species, in order to be able to differentiate and distinguish between different microbes, selective media are used (for example violet red bile agar for coliforms or potato dextrose agar for yeast and mould). For the standard plate count (SPC), the MacConkey medium is used, at room temperature. The media is essentially used in two dilutions based on the sample availability. According to the International Dairy Federation standards, SPC is performed with non-selective media such as PCA (Plate Count Agar), while MacConkey agar is a selective media used to detect and isolate Gram-negative bacteria. The recommended incubation conditions are 30°C for 3 days or 32°C for 2 days. The following options are available for detecting gas producing coliforms: SSLB (single-strength lactose broth – 4 g of media powder in 100 mL of distilled water with a sample addition of 1 or 0.1 mL) or DSLB (double-strength lactose broth – 8 g of media powder in 100 mL of distilled water with a sample addition of 10 mL).

When aiming to detect yeast and mould, the growth of bacteria needs to be inhibited (for example, that of coliforms and certain spore forming bacteria) and this can be done through the addition of 10% tartaric acid to the medium. Tartaric acid is used instead of other antibiotics because of its high effect at small concentrations and also because of its long shelf life. One important aspect is that media containing tartaric acid are single-use only and cannot be reused after storing because of the formation of gas and froth in the medium if stored along with the medium for a longer duration.

The samples are first collected from the following sampling points: milk tanker wash; sampling port of storage silos; random milk packs; swabs from the workers at the packing section; swabs from the crates used for storage; swabs from the milk packing rolls; butter sample from the output of Continuous Butter Making machines (CBM); swabs from butter handlers; swabs from the plastic sheets used for packing butter; sample of butter wash from CBM; random sample of ice cream from the manufacturing process; swabs from the handlers of the ice cream products; random paneer (Indian cheese) and doodh peda (sweets) samples; swabs from the handlers of paneer and doodh peda; random butter milk, curd and flavored milk samples; swabs from the respective handlers; water samples from the Effluent Treatment Plant, wash water and processing water.

The swabs are taken in small vials with trisodium nitrate or sodium chloride solution as medium. The Petri plates, pipettes and test tubes are first sterilized in the oven at 170°C for about 2 hours. The flasks with medium are also sterilized at 121°C for about 15 to 20 minutes at 15 lb pressure. The Petri plates are labeled with the date, batch number, organism to be tested for and product name for easy traceability. Then the samples are inoculated in the plates in accordance with the dilutions and procedure. The medium is then cooled and about 14 to 15 mL are added in each plate and stirred gently for uniformity. Then the plates are allowed time for hardening and are then incubated in a Biochemical Oxygen Demand (BOD) incubator. After inoculation, the plates should be incubated at 35°C for 24 hours. The coliforms take about 24 hours to grow, the yeasts, about three days and the moulds, about five days. After the stipulated time, the plates are taken out and examined for microbes.

Fumigation is one other method, used for reducing airborne pathogens. Potassium permanganate and formaldehyde are used for this purpose. The airborne pathogens are detected by exposing a sterile MacConkey agar plate to air in

the location for about 5 minutes and incubating it in the BOD incubator.

The exact specifications of the Prevention of Food Adulteration (PFA) Act regarding the microbial counts in milk and milk products are presented in Table II.

Type of milk product	Maximal bacterial load accepted
Butter	Coliform count < 5/g
	Yeast and mould < 20/g
Ice cream	Coliform count < 10/g
	Yeast and mould = nil
	SPC < 250,000/gm
Doodh peda and paneer	Coliform count < 90/g
	Yeast and mould < 250/g
Raw milk	Coliform count < 2,000/mL
	SPC < 2 million/mL
Packed milk	Coliform count = nil
	SPC < 30,000/mL
Water	Coliform count = nil for 100 mL MPN (coliform count based on the MPN index)
	SPC < 50/mL
Machinery swabs	Coliform count = nil
	SPC < 25,000/900 cm^2
Hand swabs	Coliform count = nil
	SPC < 2,000/mL of swab liquid
Flavored milk	SPC = nil
Air microbial count	< 60
Can rinse	Coliform count = nil
	SPC < 40,000/40 L
Skim Milk Powder	Coliform count = nil
	SPC < 50,000/0.1 g
Cream	Coliform count = nil
	SPC < 60,000/0.1 mL

MPN most-probable-number; **SPC** standard plate count.

Table II. Specifications for the microbial count according to the Prevention of Food Adulteration (PFA) standards[51]

Indian legislation

A large number of legal acts govern the safety of milk and milk-based products, with compulsory legislation that includes the PFA dating back to 1954, the Export (Quality Control and Inspection) Act from 1963, the Standards of Weights and Measures (Packaged Commodities) Rules from 1977, the Milk and Milk Products Order (MMPO) from 1992 and the more recent Food Safety and Standards Bill from 2005.[52]

Voluntary standards have also been set in place by the Bureau of Indian Standards (BIS), the Directorate of Marketing and Inspection (DMI) and the International Standards Organization (ISO), while other regulations include: Industrial License, Foreign Investment, Foreign Technology Agreements, Import of Capital Goods, Import of Second Hand Capital Goods, Dividend Balancing.[53]

Disadvantages of the present testing conditions

The current microbial testing undertaken by the large scale milk processing plants presents some disadvantages in the ever growing scenario where new virulent strains keep arising. Although testing is generally carried out for most universally found pathogens, the fact that there are millions of species and subspecies of microbes whose virulence may differ greatly both in prevalence and in strength cannot be neglected. The testing for the most virulent strains is time consuming and not feasible when performed on a large scale, nevertheless the need for testing is undeniable. According to our observations, in a leading milk processing plant at Hyderabad, Andhra Pradesh, India, testing for some virulent microbes such as *Coxiella burnetii* takes place periodically but not on daily basis *(unpublished data)*.

The avoidance of any contamination of milk has been possible in the plant by regular and stringent monitoring of the sanitary conditions in every step of milk processing. What seems to be encouraging is that the processing plant follows its own set of specifications and standards along with the set of standards laid down by the government such as BIS, ISO and DMI. These self-set standards have been found to be very stringent and fool-proof. The milk procurement centers and the ice bank tanks which are located elsewhere are regularly monitored for sanitation and hygiene. The workers also undergo regular health-related checkups and the food and water

in the plant which are provided to the workers have been found to be clean and hygienic. The waste water from the plant is not drained into the environment; rather there is a provision for the treatment of the effluents from the production. "Effluent Treatment Plant" and "Reverse Osmosis" facilities are present which enable recycling of water and efficient functioning of the production even during the harsh summers which are common in this region.

Conclusion

The advent of vaccination for a series of historically common milk-borne infections such as tuberculosis or diphtheria has significantly shifted the balance towards some other pathogens, for which vaccination is not available and for which good thermal preparation of milk and milk products remains essential.

The risks of milk-borne infections seem to be generally well understood in the dairy industry, with good testing strategies set in place for the most frequent pathogens that are known to contaminate milk. However, the risk of milk-borne infections in human remains a threat in small communities which grow their own cattle and apply their own set of hygiene rules in the milking process and in food preparation.

Conflicts of interest All authors-none to declare.

Author contributions SA provided the opportunity of working as In plant trainee in the leading milk processing plant in Andhra Pradesh. SA, AN and RD have contributed equally in the study on the production process for milk products and on milk-processing. The testing and analysis of milk and milk products for adulterants have been thoroughly performed on randomly picked products by SA, AN and RD. The microbiologic testing of the products and raw materials for some of the most commonly found pathogenic microbes have been performed under the guidance of RD, SA and AN.

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