Peer reviewed **ORIGINAL ARTICLE**

INCIDENCE OF AEROBIC SPOILAGE- AND PSYCHROTROPHIC BACTERIA IN NON-PASTEURISED AND PASTEURISED BOVINE MILK FROM MASERU

SL Moshoeshoe[1] *(BTech Biomedical Technology)* | **D Olivier**[2] *(DTech Biomedical Technology)*

[1] *Maseru, Lesotho*
[2] *Central University of Technology, Free State, School of Health Technology, South Africa*

Corresponding author: Dr D Olivier | email: olivierd@cut.ac.za | tel: +27 51 507 3577

Abstract

The presence of pathogenic bacteria in milk is a possible source of food-borne diseases. One hundred and sixty non-pasteurised and forty pasteurised milk samples from the Maseru area were analysed for the presence of bacteria. It is recommended by legislation that milk be free from pathogenic bacteria, and microbial counts must be within the ranges as stipulated in Regulation 1555 of 21 November 1997. Eighty seven of the non-pasteurised milk samples had high total aerobic bacterial counts that were not within the stipulated range for microbial counts as outlined in Regulation 1555, whereas twenty seven pasteurised milk samples had unacceptably high aerobic bacterial counts. Unacceptably high counts of *E. coli* were detected in thirty five of the non-pasteurised samples. Bacterial phosphatase was detected in seven of the pasteurised samples, indicating possible post-processing contamination. *Listeria monocytogenes* was isolated from five non-pasteurised milk samples and *Listeria innocua* was isolated from one sample. The presence of psychrotrophic *Listeria* could contaminate the milk processing plant rendering pasteurised milk unsafe for human consumption. Community members should be informed regarding the dangers of consuming non-pasteurised milk. Small-scale farmers should be educated regarding the dangers of *Listeria* infection in the herds.

Keywords

Listeria monocytogenes, psychrotrophic, Maseru, pasteurised milk, non-pasteurised milk, *E. coli*.

INTRODUCTION

Food-borne diseases are a serious public health concern as they affect many people and contribute to the overall mortality rate[1, 2]. A broad spectrum of microbial pathogens can contaminate food and water supplies. This causes a range of illnesses, where the invasive organism can produce clinical syndromes and toxins that can affect almost any system in the body. Therefore, the term "foodborne disease" relates to many pathogens and many diseases[3]. The World Health Organization (WHO) defines food hazards as "biological, chemical, or physical agents in or property of food that may have an adverse health effect," and food-related risks are traditionally defined as "a function of the probability of an adverse effect and the magnitude of that effect, consequential to a hazard in food"[4].

Despite its nutritional qualities, milk also has the potential to cause food-borne diseases. The natural composition of milk makes it an excellent medium for the proliferation of spoilage and pathogenic microorganisms. Both pre-processing and post-processing aseptic and sterilisation measures are therefore crucial in maintaining low microbial numbers in milk or milk products[5, 6].

Non-pasteurised milk is susceptible to spoilage and must be kept refrigerated because if left standing without refrigeration, the lactic acid bacteria naturally ferment it to produce lactic acid, which gives non-pasteurised milk its sour taste[7]. Although pasteurisation of raw milk will initially destroy any potential pathogens and increase its shelf life[7], the presence of heat-resistant organisms, like streptococci, will eventually cause milk spoilage, rendering the milk unsuitable for consumption[7, 8].

Listeria monocytogenes is the causative agent of listeriosis and is a known pathogenic psychrotroph implicated in many cases of food-borne disease outbreaks[9, 10, 11]. Within the food and dairy industry, *Listeria* has been isolated from biofilms where these bacteria adhere to and colonise moist areas and equipment, thus posing a risk of recontamination of the finished product[12]. *Listeria* outbreaks were often associated with consumption of non-pasteurised milk and unfermented dairy products. However, in 1983, an outbreak of listeriosis due to the consumption of pasteurised milk was reported in Massachusetts[13, 14]. In France, soft cheese made from non-pasteurised milk was implicated in two outbreaks of listeriosis in 1995[15]. Several other outbreaks due to the consumption of pasteurised milk and dairy products have been reported on different occasions[16, 17, 18, 14]. However, milk and milk products are not the only vehicles of *Listeria* transmission in food products. Table 1 reflects some of the food products contaminated with *Listeria*.

Control of psychrotrophic *L. monocytogenes* is particularly difficult in terms of chill storage[25]. Storage temperatures of 4°C are not acceptable as even the presence of a small bacterial inoculum held at this temperature may grow and multiply to produce an infectious dose[14]. There are great differences in pathogenic potential among strains of *L. monocytogenes* and useful information can be obtained from serotyping[26]. This will help to identify and differentiate the organisms[6] so that in the event of epidemics, necessary measures can be taken to contain the infection and prevent further spread once the source has been identified.

Food safety failures usually receive much public attention. This

leads to demands for increased product testing in some countries and by large producers[27]. However, in some developing countries, such as Lesotho, not much attention is given to food safety failure due to the lack of food legislature. In addition, small-scale industries often cannot afford to implement quality assurance processes. In Maseru, there is only one large-scale dairy producer, and several small-scale milk producers sell the milk directly to the dairy. However, some of these farmers sell non-pasteurised milk directly to the community as well. Unlike large-scale production where the manufacturer implements quality and product control measures to ensure food safety, there is no quality and product control measures on the non-pasteurised milk sold directly to the community. This consequently poses a threat to the general health of consumers. The aim of this study was to determine the presence of aerobic spoilage bacteria and *L. monocytogenes* in 160 non-pasteurised bovine milk and 40 pasteurised bovine milk samples collected in the Maseru area in Lesotho.

METHODOLOGY

Sample Collection

Two hundred bovine milk samples were collected for this study. Hundred and sixty non-pasteurised milk samples were collected from local farmers as the milk was delivered at the dairy plant in Maseru, Lesotho. Approximately 15 samples per week were collected and analysed over a period of three months. Samples were collected in sterile 50 ml bottles and directly aliquoted into sterile labelled screw cap test tubes. Samples were transported on ice to the laboratory for microbial analysis on the day of collection.

Forty pasteurised milk samples, all within the stipulated expiry period, were bought from different local shops in Maseru. Ten samples per week were collected and transported on ice for microbial analysis on the day of purchase.

Geographic Study Area

The Maseru constituencies included in the study were Maseru, Stadium Area, Mabote, Motimposo, Tsosane, Lithabaneng, Lithoteng, Abia and Qoaling. Refer to Figure 1 for a geographical representation of the area.

Isolation And Identification Of Bacteria

All procedures were performed according to the standard operating procedures as stipulated by regulations related to milk and dairy products in South Africa incorporated in Act 54 of 1972, the Foodstuffs, Cosmetics and Disinfectants Act, in Regulation 1555 of 21 November 1997[28]. Bacterial counts were done on both the pasteurised and pasteurised milk samples.

Total Viable Count

Petrifilm™ (Merck, SA) aerobic count plates were used to determine the total number of viable aerobic bacteria present in each of the 200 samples. Tenfold serial dilutions of the samples (non-pasteurised and pasteurised) were made in Ringers solution (Oxoid, England). One millilitre of dilutions 10^{-3} and 10^{-4} respectively were dispensed onto the centre of the petrifilm and were evenly distributed over the surface using the provided spreader. Plates were left on a level surface for one minute to allow for gel formation and were then incubated aerobically at 37°C ± 1ºC for 24 h after which colonies were counted on each plate. Tests were performed in duplicate and the average of the two counts was taken as the total count and results were calculated as colony-forming units per millilitre.

Total Viable Coliform And Total Viable *E. Coli* Count

Total coliform and *E. coli* counts were determined for non-pasteurised and pasteurised milk using Chromocult agar (Merck, SA). The chromogenic substrates in the agar allows for the simultaneous detection of total coliforms and *E. coli*. The substrate X-glucuronide was used for the identification of ß-D-glucuronidase, which is characteristic for *E. coli*. *E. coli* cleaves both Salmon-GAL and X-glucuronide, and positive colonies take on a dark blue to violet colour. One millilitre milk was spread on the surface of the medium and incubated aerobically for 24 h at 37°C ± 1ºC. Total coliform count was recorded as the sum of red colonies (coliforms) and dark-blue colonies (*E. coli*). Tests were performed in duplicate and the average of the two counts was taken as the total count. In order to confirm *E. coli* colonies, KOVACS' indole reagent was used. A positive indole formation confirmed the presence of *E. coli*. The characteristic enzyme for coliforms, ß-D-galactosidase cleaves the Salmon-GAL substrate and causes a salmon to red colour of the coliform colonies.

Phosphatase Test

The Aschaffenburg and Mullen phosphatase test was used to determine the pasteurisation status of the commercial milk samples. The method was applied as stipulated in Regulation 1555 of 21 November 1997 (South Africa, 1997).

The temperature of the pasteurised milk was raised to 25 ºC ± 1ºC in a water bath (incubation time 30 minutes). In a sterile test tube, 5 ml of freshly prepared phosphate buffer was added to 1 ml of heated milk and vortexed. Samples were incubated in a water bath for 2 h at 37°C and colour changes observed every 10 minutes. Pasteurised milk, which turned bright yellow after 10-20 min, was recorded as having "bacterial phosphatase" (due to high bacterial counts). Samples that remained white or pale yellow after 30 min were recorded as pasteurised. Boiled milk was used as a negative control.

Isolation Of *Listeria*

Tests were performed in accordance with the International Organisation for Standardisation (ISO) 11290-1 (1996) and standard 143:1990 of the FDA/IDF-FIL for milk and milk products for the detection of *L. monocytogenes*.

Two-Stage Enrichment Method

Selective enrichment for *Listeria* was achieved by using a two-step enrichment method. One millilitre of the milk sample was inoculated into 10 ml *Listeria* enrichment broth (Merck, SA) and incubated aerobically up to 24 h at 30°C ± 1ºC. This was followed by the second enrichment step where 100 µl from the first cultures was inoculated into 10 ml of Fraser *Listeria* selective enrichment broth (Merck, SA) and incubated aerobically at 35°C ± 1ºC for 24 h. In the event where blackening of the broth was observed, Oxford *Listeria* selective agar (Merck, SA) was subcultured with 50 µl of the broth and incubated aerobically at 35°C ± 1ºC for up to 24-48 h. *Listeria monocytogenes* hydrolyses esculin to esculetin and forms a black complex with iron(III)ions, and produces brown-green coloured colonies with a black halo. All presumptive *Listeria* colonies were selected for further testing.

Identification Of *Listeria*

Culture purity on *Listeria* selective agar was confirmed by morphological examination of isolated colonies (observation of brown-green coloured colonies with a black halo). All presumptive *Listeria* isolates which were Gram-positive coccobacilli or bacilli, catalase positive, oxidase negative and motile at 25°C were selected for further biochemical analysis using the *Listeria* API (bioMérieux, SA). Sub-cultures were prepared on blood agar for confirmation and differentiation with the *Listeria* API according to the manufacturer's instructions.

RESULTS

Dairy products may serve as vehicles of transmission for pathogenic spoilage organisms. Since it is recommended by legislation that milk be free from pathogenic bacteria, non-pasteurised and pasteurised milk should therefore be tested for microbial content to ensure that microbial counts are within the ranges as stipulated in Regulation 1555 of 21 November 1997[7]. (See Table 2 for standard bacterial count values for milk.) Individual counts were therefore compared to the microbial values stated in Table 2.

Total Viable Counts

The total viable bacterial counts in 73 (n=160) non-pasteurised milk samples was within the acceptable range of < 200 000 cfu/ml. However, 87 samples had counts greater than 200 000 cfu/ml.

With regard to the pasteurised milk samples, only 13 (n=40) of these samples had total aerobic counts within the acceptable range of 50 000 cfu/ml in terms of the national standard, whereas 27 had counts above 50 000 cfu/ml.

Total Coliform Counts

Seventy one of the non-pasteurised milk samples (n=160) had coliform counts below 20 cfu/ml whereas 89 had coliforms counts higher than 20 cfu/ml.

Thirty three of the pasteurised milk samples (n=40) had a total coliform count of ≤ 20 cfu/ml, whereas 7 samples had higher counts.

Total *E. coli* Count

Hundred and twenty five (n=160) non-pasteurised milk samples complied with the standard count for *E. coli* whereas 35 samples had counts greater than 9 cfu/ml.

Table 1: Food products associated with *Listeria* contamination

Food	Organism	Reference
Meat salads, French salad, beef steak, chicken, cheese	*L. monocytogenes*	[19]
Mince, patty, trout, fish, salad, cheese salad	*L. monocytogenes*	[20]
Helix pomatia	*L. monocytogenes*	[21]
Cabbage, lettuce, squid	*L. monocytogenes*	[22]
Mussel, hake, mackerel	*L. innocua*	[22]
Non-pasteurised milk, cheese	*L. monocytogenes*	[23]
Sheep milk cheese	*L. monocytogenes*	[24]

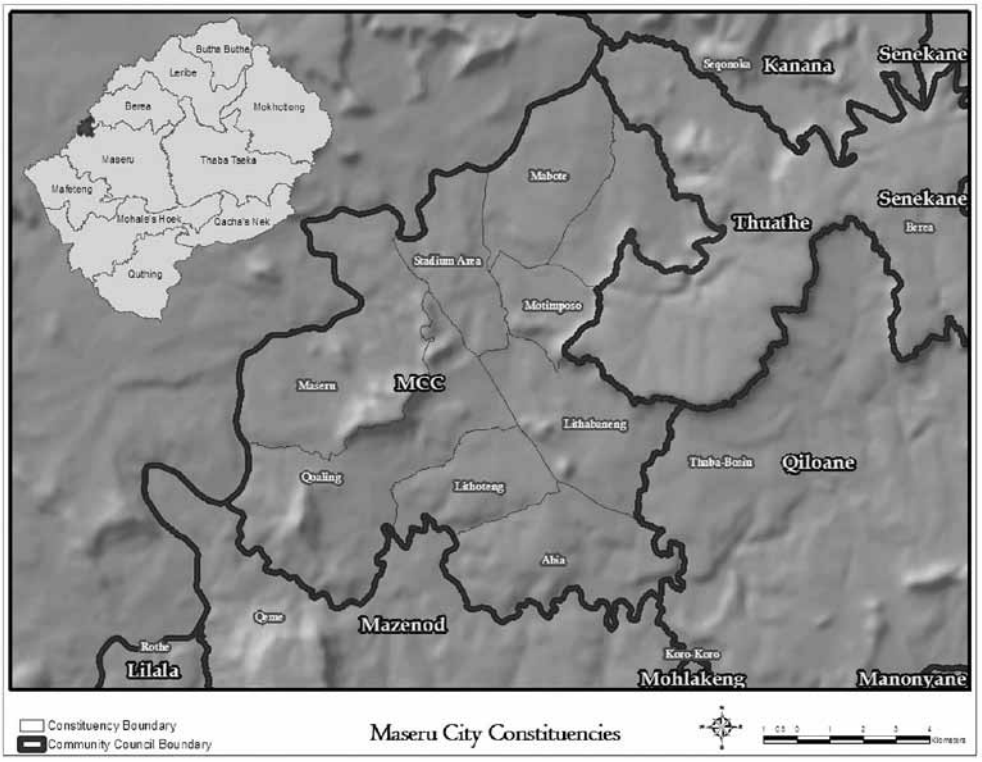

Figure 1: Maseru city constituencies (MCC) under study

Table 2: Standard bacterial count values for milk[28]

State of milk	Total viable count	Total coliforms (Dry film method)	Total *E. coli*
Non-pasteurised milk for processing	<200000 cfu/ml	<20 cfu/ml	<10 colonies/ml
Pasteurised milk	<50000 cfu/ml	<20 cfu/ml	Absent in 1ml

None of the 40 pasteurised milk samples showed the presence of *E. coli*. However, bacterial phosphatase was detected in 7 of the pasteurised milk samples.

Prevalence Of *Listeria*

Presumptive *Listeria* isolates were detected in 29 (n=200) of the milk samples tested. However, only 9 isolates were selected for API based on their biochemical reactions and Gram stain. The API confirmed six isolates to be *Listeria* species, five proved to be *L. monocytogenes* and one was *L. innocua*. The six isolates were identified only from the non-pasteurised milk samples.

DISCUSSION

Some non-pasteurised milk samples showed higher than expected ranges in all the parameters used to determine microbial quality (total aerobic count high in 54% samples; total coliform count high in 56% samples; *E. coli* high in 21% milk and *Listeria* present in 4% milk samples). This may be attributed to negligence of proper sanitation practices in the milking or transport environment. This emphasises the need for maintenance of hygienic conditions in the milking environment. This finding concurs with observations made by Van Kessel *et al.*[29] who found high levels of bacteria in bulk milk and emphasised the need for maintenance of hygienic conditions in both the milking and processing environment.

With regard to aerobic counts, a large percentage (68%) of the pasteurised milk samples showed unacceptably high counts. This shows that most of the pasteurised milk samples exceeded the national standard for total bacterial counts in pasteurised milk sold directly to the public. This could be attributed to the pasteurisation process not being effective in reducing aerobic count in the pasteurised milk or the result of post-processing contamination. Cagri-Mehmetoglu *et al.*[30] isolated both pathogenic *E. coli* and *L. monocytogenes* in two cheese processing environments. Although complete elimination of coliform bacteria entering the milk is considered very difficult[31], a very low total coliform count in pasteurised milk is essential for good quality milk. In the current study only 18% of the pasteurised samples showed non-conformance regarding total coliform counts compared to the 56% of the non-pasteurised milk samples. These results are similar to the findings by Van Kessel *et al.*[29] whereby a low percentage of the samples tested had unacceptably high total coliform counts.

Listeria species were detected in 6 of the 160 non-pasteurised milk samples, five of which were *Listeria monocytogenes* and one *Listeria innocua*. These results concur with those of other researchers. Waak *et al.*[32] reported that *L. monocytogenes* was prevalent in 1% of non-pasteurised milk samples, whilst Vardar-Unlu *et al.*[33] reported a 6% prevalence of *L. monocytogenes*. However, higher counts have been reported. According to Hayes *et al.*[34] *L. monocytogenes* was recovered from 12% of non-pasteurised milk samples and Holko *et al.*[35] found 13% non-pasteurised milk samples to be contaminated with *L. monocytogenes*. In a study by Rahimi *et al.*[36], the prevalence of *Listeria* in non-pasteurised milk in Iran was 22.6% followed by 19% prevalence in cheese. In the current study the presence of *Listeria* was not confirmed in any of the examined pasteurised milk samples. This concurs with the results from Kells & Gilmour[37] where no *Listeria* was found in pasteurised milk. The prevalence of *L. monocytogenes* and *L. innocua* was low in this study, nonetheless, these pathogens represent a potential risk to consumers of non-pasteurised milk and non-pasteurised milk products. *Listeria's* presence in food is an issue that raises much concern as this organism is responsible for cases of listeriosis. Prevention of listeriosis has to be of major importance as the mortality is very high.

Having confirmed the occurrence of *L. monocytogenes* in non-pasteurised milk samples, it is interesting that *L. innocua* was also identified although it was not within the scope of this study. However, this organism has been found on several occasions to co-exist together with *L. monocytogenes*[33, 22]. The *Listeria* contaminated samples also came from different producers in the Maseru City Constituent area, from the villages of Lithabaneng, Leqele (Lithabaneng), Tsosane, Semphetenyane (Qoaling) and Abia, indicating no specific regional incidence. Results regarding seasonality are inconclusive as the samples were only collected over a period of three months.

Identification of suspect *Listeria* colonies from the media was problematic, due to the occurrence of *Listeria*-like organisms. These organisms had the typical appearance and behaviour of *Listeria* in both the enrichment broth and selective media. As experienced by other authors, phenotypic properties by which bacteria are identified when using culture methods may not always be expressed and may be difficult to interpret[20, 14]. This was also found by Gebretsadik *et al.*[38] who observed cultures with similar growth and morphological characteristics as *Listeria* but were not confirmed as *Listeria*. Besse *et al.*[39] observed nutritional competition amongst *Listeria* species during the enrichment process. It is also important to note that some positive samples for *Listeria* may go undetected due to overgrowth by natural background flora during enrichment as some strains of *Listeria* may not be able to grow competitively. As bacterial adaptation to different environments causing similarities in phenotype, as well as resistance to ingredients in enrichment and selective media is often evident, conventional methods of detection cannot be exclusively relied upon, and genetic methods of detection should be included[20]. Such findings highlight the importance of using molecular methods as a confirmatory technique for isolating *L. monocytogenes* as well as for identification purposes. In a study conducted by Alessandria *et al.*[40] more positive samples were recovered when using molecular methods compared to traditional methods. As reported by other authors, molecular methods were not only developed to reduce analysis time but also because of their high specificity in identification and characterisation among species[41, 35, 24].

It is important to note the dangers of supplying contaminated milk to the dairy industry. Such milk increases the chances of post-pasteurisation contamination as most of the plant equipment may be in contact with the milk and serve as possible sites for contamination. If the prevalence of *L. monocytogenes* is successfully reduced in dairy products, the risk of acquiring listeriosis from the products will also be reduced. Numerous studies documented the presence of *Listeria* within the dairy processing plants where sample sites included floors, drains, freezers, silos, bottle washers, bottle fillers[37], milk filters[34], bulk milk tanks[29], environment of the cheese processing plant[24] and other equipment[40]. The formation of biofilms is another challenge that has to be considered when addressing post-processing contamination as bacteria usually adheres to and multiplies on such surfaces and become the source of contamination. When conducting molecular characterisation of L monocytogenes, Alessandria *et al.*[40] observed similarity in strains that were isolated from both the equipment and the final product in a dairy processing plant. It can thus be concluded that the supply of poor quality non-pasteurised milk to the plant will definitely impact the final product.

Bacterial phosphatase activity was detected in 17.5% the pasteurised milk samples, implicating ineffective pasteurisation or post pasteurisation contamination. Unacceptable levels of food-borne pathogens and spoilage organisms compromise the quality of the food product. These findings are a reflection of the poor quality of non-pasteurised milk in the study area and also indicate that some retail milk may not be suitable for public consumption.

CONCLUSION

Information about the risks involved in consuming non-pasteurised milk or milk products (e.g. maas or soft cheese) should be provided continuously to vulnerable groups of the population, such as pregnant women, the elderly and the immunocompromised[42]. Food safety issues should be discussed at health centres and health warnings should be issued in relation to high-risk foods. In cases where non-pasteurised milk sales are permitted directly from dairy farms, communities need to be informed that the milk may contain organisms that are harmful to health, and therefore has to be boiled prior to consumption. Special attention must be paid in educating pregnant women and immunocompromised individuals of the dangers of consuming *Listeria*-contaminated milk products.

In order to provide milk of acceptable quality, farmers also have to attend to the welfare or hygiene of the herd and the milking equipment. Environmental contaminants from bedding, manure and feeds are likely to affect the exterior of the udder. Since these contaminants can influence bacterial counts, measures such as proper teat sanitation before milking is crucial. Milk residue left on equipment contact surfaces supports the growth of a variety of microorganisms and thorough cleaning and sanitation of equipment should be emphasised. Farmers need to be informed of the presence of *Listeria* in the milk in order for them to take corrective measures to prevent spreading of the disease.

Food safety is an issue that cannot be avoided and to provide a safe product to the public, it is essential to improve hygiene standards in dairy industries. Frequent inspection of equipment and operations is necessary to produce safe products of acceptable quality[43]. At the same time testing for the microbial quality of milk can serve to help a dairy producer to identify inefficiencies in the production of milk and will also help monitoring the quality of pasteurised milk. This would ensure that the final product on the shelf meet the public's expectations for a safe and nutritious food. Food industries need access to rapid, reliable and sensitive methods of detecting bacteria. Even though rapid methods are valuable tools, they may not replace standard culture methods. However, the development and usefulness of culture techniques rely on continued studies to determine the on-going developing resistance of organisms to antimicrobial chemicals. The dairy management should take cognisance of the presence of *Listeria* in some of the milk they buy from the local farmers and measures should be implemented as a matter of urgency to avoid contamination of the pasteurisation plant. Emphasis should be placed on the role that biofilms can play in the contamination process and this should be addressed by specialised cleaning procedures.

It is worthwhile to expand this study and have it performed in a larger area to estimate geographical variation and seasonality. This will also help to monitor the prevalence of *Listeria* especially in those places where it was previously identified. Expansion of the study area will also allow analysis of a larger number of isolates which can be differentiated further through typing to establish an epidemiological profile. Further studies are also required to investigate the prevalence of diseases due to food-borne transmission. With such, estimates of incidence of listeriosis and other food-borne diseases can be established. In view of epidemiology, matching of implicated food and clinical isolates during an outbreak may help in recognising and containing the source of food-borne diseases.

REFERENCES

1. World Health Organization. Fact Sheet No. 237: Food safety and foodborne illness. [cited 2011 September 3]. Available from http://www.who.int/mediacentre/factsheets/fs237/en/index.html
2. Duff SB, Scott E, Malfios MM, Todd EC, Krilov LR, Geddes AM, Ackerman SJ. Cost-effectiveness of a targeted disinfection program in household kitchens to prevent foodborne illness in the United States, Canada and the United Kingdom. Journal of Food Protection. 2003;66(11):2103-2113.
3. Tauxe RV. Emerging foodborne pathogens. International Journal of Food Microbiology. 2002;78(1-2):31-41.
4. World Health Organization (WHO). Application of risk analysis to food standards issues. Report on the Joint FAO/WHO Expert Consultation, Geneva, Switzerland, 13-17 March. 1995, Geneva: WHO.
5. Goulet V, de Valk H, Pierre O, *et al.* Effect of prevention measures on incidences of human listeriosis, France, 1987-1997. Emerging Infectious Disease. 2001;7:983-989.
6. Lukinmaa S, Miettinen M, Nakari UM, Korkeala H & Siitonen A. *Listeria monocytogenes* isolates from invasive infections: variation of sero- and genotypes during an 11-year period in Finland. Journal of Clinical Microbiology. 2003;41:1694-1700.
7. Jay JM. Modern Food Microbiology, 4th edition. Van Nostrand Rainhold, New York; 1992.
8. Rowe MT, Dunstall G, Kilpatrick D & Wisdom GB. Effect of growth phase on the subsequent growth kinetics of psychrotrophic bacteria of raw milk origin. International Journal of Dairy Technology. 2003;56:35.
9. Hui H, Gorhan R, Murrell D, Cliver O. Food borne Disease Handbook, Diseases caused by bacteria. Marcel Dekker. New York; 1994.
10. Rodríguez-La´Zaro D, Herna´Ndez M, Mariela S, Esteve T, Va´Zquez-Boland JA, Pla M. Quantitative detection of *Listeria monocytogenes* and *Listeria innocua* by real-time PCR: Assessment of hly, Iap, and Lin 02483 targets and am

plifluor technology. Applied and Environmental Microbiology. 2004;70(3):1366-1377.
11. Jemmi T, Stephan R. *Listeria monocytogenes*: Food borne pathogen and hygiene indicator. Reviews in Science Technology, 2006, 25(2):571-80.
12. Holah JT, Taylor JH, Dawson DJ, Hall KE. Biocide use in the food industry and the disinfectant resistance of persistent strains of *Listeria monocytogenes* and Escherichia coli. Journal of Applied Microbiology. 2002;92:111-120.
13. Robinson R, Batt C, Patel P. Encyclopaedia of Food Microbiology; Volume 1. Academic Press, United Kingdom. 2000
14. Ryser ET, Marth EH. *Listeria*, Listeriosis and Food Safety, 3rd ed. CRC Press. Florida. 2007.
15. Lunden JN, Tolvanen R, Korkeala H. Human Listeriosis outbreaks linked to dairy products in Europe. Journal of Dairy Science. 2004;87:E6-E11.
16. McLauchlin J. Human listeriosis in Britain, 1967-85, a summary of 722 cases. Epidemiology and Infection. 1990;104:181-9.
17. Elcuaz R, Bordes A, Aladro Y, *et al.* Clinical characteristics and epidemiologic study of a listeriosis in Grand Canary. Enfermedades Infecciosas Microbilogia Clinica. 1996;14:416-422.
18. Robin LT, Churchill HL, Hall JC. Detection of *Listeria monocytogenes* and toxin listeriolysin O in food. Journal of Microbiological Methods. 2006;64(2):141-170.
19. Jersek B, Gilot P, Gubina M, *et al.* Typing of *Listeria monocytogenes* strains by repetitive element sequence-based PCR. Journal of Clinical Microbiology. 1999;37(1):103-109.
20. Gouws PA, Liedemann I. Detection of *L. monocytogenes* in food products, Food Technology and Biotechnology. 2005;43(2):201-205.
21. Kirkan S, Goksoy, EO, Kaya O. Detection of *Listeria monocytogenes* by using PCR in Helix Pomatia. Turkish Journal of Veterinary and Animal Sciences. 2006;30:375-380.
22. Laciar A, Vaca L, Lopresti R, Vega A, Mattana C, De Centorbi ONP. DNA fingerprinting by ERIC-PCR for comparing *Listeria* spp. strains isolated from different sources in San Luis, Argentina. Revista Argentina de Microbiologia. 2006;38:55-60.
23. Millet LA, Saubusse MA, Didienne RA, Tessier LB, Montel MC. Control of *Listeria monocytogenes* in raw-milk cheeses. International Journal of Food Microbiology. 2006;108:105-114.
24. De Santis EPL, Pilo AL, Cosseddu AM, Canu NA, Scarano C, Marongiu P. Multiplex PCR for the identification and serotyping of *L. monocytogenes* isolated from sheep cheese-processing plants. Veterinary Research Communications. 2007;31(1):359-363
25. Eley R. Microbial Food Poisoning. Chapman and Hall: UK. 1994.
26. Rocourt J, Jacquet C, Reilly A. Epidemiology of human listeriosis and seafoods. International Journal of Food Microbiology. 2000;62:197-209.
27. Hobbs B, Roberts D. Food Poisoning and Food Hygiene. 6th ed. Hodder & Stoughton: London. 1990.
28. SOUTH AFRICA. Regulations relating to milk and dairy products, foodstuffs, cosmetics and disinfectant (Act 54 of 1972). (Proclamation No. R 1555). Government Gazette, 18439. Pretoria: Government Printer. 1997.
29. Van Kessel JS, Karns JS, Gorski L, Mccluskey BJ, Perdue ML. Prevalence of Salmonellae, *Listeria monocytogenes*, and fecal coliforms in bulk tank milk on us dairies. Journal of Dairy Science. 2004;87:2822-2830.
30. Cagri-Mehmetoglu A, Yaldirak G, Bodur T, Simsek M, Bozkir HN, Eren M. Incidence of *Listeria monocytogenes* and Escherichia coli 0157:H7 in two Kasar cheese processing environments. Food Control. 2011;22:762-766.
31. Bell C, Kyriakides A. *Listeria*, A practical approach to the organism and its control in foods. Blackie Academic & Professional: London. 1998.
32. Waak E, Tham W, Dannielsson-Tham ML. Prevalence and fingerprinting of *Listeria monocytogenes* strains isolated from raw whole milk in farm bulk tanks and in dairy plant receiving tanks. Applied and Environmental Microbiology. 2008;68:3366-3370.
33. Vardar-Unlu G, Unlu MM, Bakici Z. Incidence of *Listeria* spp. from raw milk in Sivas. Turkish Journal. of Medical Sciences. 1998;28:389-392.
34. Hayes PS, Feeley JC, Graves LM, Ajello GW, Fleming DW. Isolation of *Listeria monocytogenes* from raw milk. Applied and Environmental Microbiology. 1986;51(2):438-440.
35. Holko U, Kantikova M, Pastorova K, Kmee V. PCR detection of *Listeria monocytogenes* in milk and milk products and differentiation of suspect isolates. Acta Veterinaria. 2002;71:125-131.
36. Rahimi E, Ameri M, Montaz H. Prevalence and antimicrobial resistance of *Listeria* species isolated from milk and dairy products in Iran. Food Control. 2010;21:1448-1452.
37. Kells J, Gilmour A. Incidence of *Listeria monocytogenes* in two milk processing environments, and assessment of *Listeria monocytogenes* blood agar for isolation. International Journal of Food Microbiology. 2004;91:167-174.
38. Gebretsadik S, Kassa T, Alemayehu H, Huruy K, Kebede N. Isolation and characterization of *Listeria monocytogenes* and other *Listeria* species in foods of animal origin in Addis Ababa, Ethiopia. Journal of Infection and Public Health. 2011;4:22-29.
39. Besse GN, Barre L, Buhariwalla C, *et al.* The overgrowth of *Listeria monocytogenes* by other *Listeria* spp. in food samples undergoing enrichment cultivation has a nutritional basis. International Journal of Food Microbiology. 2010;136:345-351.
40. Allessandria V, Rantsiou K, Dolci P, Cocolin L. Molecular methods to assess *Listeria monocytogenes* route of contamination in a dairy processing plant. International Journal of Food Microbiology. 2010;141:S156-S162
41. Bubert A, Hein I, Rauch M, *et al.* Detection and differentiation of *Listeria* spp. by a single reaction based on multiplex PCR. Applied and Environmental Microbiology. 1999;65(10):4688-4692.
42. Eley AR. Microbial Food Poisoning. Chapman and Hall; London. 1992.
43. Ryser ET, Marth EH. *Listeria*, Listeriosis and Food Safety. Marcer Dekker; New York. 1991.