IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)
e-ISSN: 2319-2380, p-ISSN: 2319-2372. Volume 7, Issue 12 Ver. IV (Dec. 2014), PP 08-11
www.iosrjournals.org

Prevalence of Listeria Monocytogenese in Raw Milk in Faisalabad, Pakistan

Muhammad Usman[1] Nabeela Mukhtar[2]
1Veterinary officer health, livestock and dairy development department, Sheikhupura, Punjab, Pakistan.
2Department of Wild life Fisheries, Government college university Faisalabad, 38000

Abstract: *Raw milk consumption is the main reason of many diseases caused by Listeria monocytogenese, E.coli and Samonella spp. Sometimes severe infections occur and death may happen by complications caused by these microbes. Listeriosis is one of the important dangerous bacterial zoonitic diseases that occur in humans. It occurs mainly by using the milk and products which are contaminated by different microbes including L. monocytogenese. The present study was undertaken to determine the prevalence of L. monocytogenes in raw milk samples obtained from different marketes in Faisalabad, one of the biggest city of Pakistan. A total of 400 samples of milk were randomly selected and purchased from market and food shops in Faisalabad from May 2012 to September 2012. . The results showed that, 30 (7.5%) samples were positive for Listeria spp. and 9 (2.25%) for Listeria monocytogenese. But presence of a single bacterium of L. monocytogenese is not acceptable in 25ml of milk. In conclusion, because the mortality rate of listeriosis is very high, due to this reason, so low prevalence of Listeria monocytogenese cannot be ignored.*

Key words: *Raw milk, Prevalence, Listeria monocytogenese, Listeriosis,*

I. Introduction

Milk is the secretion obtained from the mammals for the sustenance of their infants. Milk is composed of many components. Principle components are fat, water, lactose and protein. pH of milk is between 6.4-6.6 and a number of nutrients are present in milk. Due to presence of these nutrients and ample supply of water, milk is an excellent medium for growth of microbes [**1**].

Pathogenic bacteria in milk are responsible for many types of diseases and have been a major factor for public health concern. Contamination of milk is due to unhygienic conditions during handling and processing of milk. Many authors have reported that contamination may result from contact with the equipment. They also reported that contamination may also be due to mishandling of milk and other products [2]. The major factors of milk contamination are dairy cattle, food handler and dairy equipments. Raw milk consumption is the main reason of many diseases caused by Listeria monocytogenese, E.coli and Samonella spp. Sometimes severe infections occur and death may happen by complications caused by these microbes [3].L.monocytogenese can grow at low temperature and at low pH. Due to this uniqueness L. monocytogenes is very difficult to control in milk and milk byproducts. Due to this reason this bacteria is a major factor for public health concern. Contamination due to this bacterium can lead to high risk factor for public health [4].Listeriosis is one of the important dangerous bacterial zoonotic diseases that occur in humans. It occurs mainly by using the milk and products which are contaminated by different microbes including L. monocytogenese [5].Milk is known to be major source of Listeria spp. and thus appear to be major mean of Listeriosis. **[6]** stated that Listeria are secreted in milk so it constitutes to a potential public health hazard.Listeria monocytogenese has been remained a major reason of infection in all animals including man. Many listeriosis outbreaks were occurred in past years due to Listeria monocytogenese [7, 8]. Various reports show that Listeria spp. can be found in dairy products [9]. Furthermore, many listeriosis outbreaks were occurred due to the use of milk and are causing great concern in the dairy industry due to the number of cases and 30% mortality rate were noted due to these outbreaks [10]. Elder people, immunocompromised patients, newborn babies and pregnant women are at special risk [11]. Listeria monocytogenes were found to be survived in cheddar cheese for one year [12] Listeria monocytogenese is psychrophilic because it can grow temperature under zero. It also has tolerance against many preservatives.

It is the main cause of disease associated with the contamination and consumption of various types of foods such as milk, cheese and other dairy products [13].The present study was undertaken to determine the prevalence of L. monocytogenes in raw milk samples obtained from different market in Faisalabad, one of the biggest city of Pakistan.

II. Materials and Methods

The study was undertaken to determine the prevalence and distribution of Listeria monoctogenes and other Listeria species from milk samples. A total of 400 samples of milk were randomly selected and purchased

from market and food shops in Faisalabad from May 2012 to September 2012. The samples were kept in ice box and transported to microbiology laboratory of Institute of Microbiology at University of Agriculture Faisalabad. They were stored at freezing temperature until analyzed. Then frozen samples were thawed at room temperature before processing.

III. Isolation And Identification

First of all primary selective enrichment step were done. This step included a choosy liquid median in which there was a cheap concentration of selective agents and the name of this medium is half Fraser broth. This broth is composed of one volume of lithium chloride (3g/10 mo of distilled water) and half a volume of acriflavine hydrochloride (0.25 g/100 ml distilled water) and also half a volume sodium salt of nalidixic acid (0.1 g/10 ml sodium hydroxide solution) (half Fraser broth). After this, 25 ml of each sample was transferred to a stomacher bag containing 225 ml of half Fraser broth. Homogenization was done in laboratory blender (Stomacher 400, Seward, England) at high speed for 2 minutes. Then the material was incubated at 30oC for 24 hours. After primary selective enrichment, the second step was secondary selective enrichment. In this step the secondary selective enrichment medium called Fraser broth (Fraser broth: AES Lab., Combourg, France) containing full concentration of selective agents was used. 0.1 ml was taken from pre enrichment culture (half Fraser broth) and then mixed into 10 ml of Fraser broth. After this, incubation were done at 37oC for 48 hours.

In order to isolate listeria spp. colonies, a loopful from secondary enrichment was plated on PALCAM media and incubated at 30 ^{0}C for 24 to 48 hours.The plates were examined for the presence of characteristic colonies presumed to be Listeria. The base of identification of Listeria species on PALCAM agar plates is on the fact that all Listeria spp. hydrolyse aesculin and this was evidenced and illustrated by black colour of medium. Mannitol fermentation is all a base for isolation and identification of Listeria spp. This was evidenced because the colour of colonies was changed from grey to yellow and that was due to the acidic end products that were produced by fermentation of mannitol by bacteria. The surrounding medium of colony was also changed from grey to yellow. The selectivity of the PALCAM medium is achieved through the presence of lithium chloride, polymixin B sulphate and acriflavine hydrochloride present in the medium base and ceftazidime provided by PALCAM antimicrobial supplement. The compounds present in PALCAM (polymixin B sulphate, acriflavin hydrochloride, lithium chloride, ceftazidime and aesculin) do not allow the growth of most bacteria (non Listeria species) that occur in food samples.Colonies with black colour were selected from the plates and cultured onto pre-dried plates of trytic soya years extract agar (TSYEA) (Difco, Bacton, USA). They were incubated at 37 ^{0}C for 18 to 24 hours. After this, characterization of Listeria was done by using different tests. These tests includes Gram staining, oxidase, motility at 28oC and 37oC and catalase test, methyl red tests, voges proskauer tests (MR-VP), sheep blood haemolysis, nitrate reduction carbohydrate utilization and Christe Atkins Munch Peterson (CAMP). These tests were applied using standard methods as recommended by [14]. Xylose, rhamose and mannitole sugars were used for carbohydrate utilization tests. Tubes containing these sugars were cultured with colonies selected from TSYEA. Then incubation were done at 370C for up to 5 days. There was a change in colour (yellow) and this was due to acid formation. Acid formation was seen after 36 to 48 hours. There was no gas production [15].

IV. Results

Table 1. Prevalence of Listeria monocytogenese in raw milk and milk byproducts.

No. of samples	Listeria spp	Listeria monocytogenese
400	30 (7.5%)	9 (2.25%)

In this study, a total of 400 samples were studied. The results showed that, 30 (7.5%) samples were positive for Listeria spp. and 9 (2.25%) for Listeria monocytogenese. But presence of a single bacteria of L. monocytogenese is not acceptable in 25ml of milk.

V. Discussion

Pathogenic bacteria in milk are responsible for many types of diseases and have been a major factor for public health concern. Listeriosis is one of the dangerous diseases which is caused by L. monocytogenese. It is one of the emerging zoonotic diseases and mainly caused by the consumption of milk and byproducts and other types of food. Raw milk samples have been collected and studied in many countries for isolation of Listera species.

Table 2. Incidence of L. monocytogenese in raw milk in different countries

Country	N0. of samples	Incidence	References
Canada	315	17 (5.4%)	**[16]**
USA	300	9 (3%)	[17]
Spain	67	30 (44.7%)	[18]
Malaysia	930	18 (1.9%)	[19]

Turkey	100	3 (3.00%)	[20]
France	337	14 (4.2%)	[21]
USA	200	14 (7.00%)	[22]
Nigeria	150	1 (0.7%)	[23]
N. Ireland	176	27 (15.3%)	[24]

Lund et al. (1991) collected 300 samples in USA and when study was carried out, 9 (3%) was positive for L. monocytogenese. [20] carried out a study in Turkey for determination of prevalence of L.monocytogenese. they collected 100 samples and reported 3% occurrence of L.monocytogenese. our results are almost similar with their findings. But Garayzabal et al. (1987) carried out a study in Spain and they collected only 67 samples but their findings showed higher prevalence (44.7%) of L.monocytogenese. Another study which showed high prevalence of L.monocytogenese was carried out in USA by Lovett et al. (1987). They studied 200 samples and reported 14 positive for L.monocytogenese. The occurrence rate was 7%. A study by [24] also show a high incidence of L.monocytogenese. They studied 176 samples and reported 27 (15.3%) positive for L.monocytogenese. There are many sources of contamination. Listeria species are found in the environment. Contamination of milk is due to unhygienic conditions during handling and processing of milk. Many authors have reported that contamination may result from contact with the equipment. They also reported that contamination may also be due to mishandling of milk and other products [2]. L.monocytogenese is an intracellular parasite, so it can grow in white blood cells which are present in the milk. This intracellular state may give resistance to Listeria species against pasteurization [25]. So low concentration of L.monocytogenese has a major health hazard for public.

VI. Conclusion

Although the results obtained by this study show that the incidence rate of L.monocytogenese was low. However the listeriosis is one of the dangerous diseases in the world, so this low prevalence of L.monocytogenese cannot be ignored. Milk may be one one of the sources for listeriosis in the world. By keeping in mind all these conditions, we recommend the improvement and implementation of appropriate hygienic measure in milk handling and processing. There should be improvement in milk production technology. Consumers should take proper care for prevention this organism by storing at cold temperature. Public awareness programmes should be started.

Acknowledgment

The authors would like to thank Professor Dr. Muhammad Arshad (Institute of Microbiology, University of Agriculture, Faisalabad) for providing us financial support.

References

[1]. MR Adams and MO. Moss, Food Microbiology, 3rd Edition. RSC Publishing, UK, 2008.
[2]. SVS Malik, SB. Barbuddhe and SP. Chaudhari, Listeric infections in humans and animals in Indian subcontinent, A review. Tropical Animal Health Production, 34, 2002, 359-381.
[3]. RK Robinson, CA. Batt and PD. Patel, Encyclopedia of Food Microbiology, Part II. Academic Press, San Diego 2000.
[4]. BC Hobbs and D. Roberts, Food Poisoning and Food Hygiene. 6th edition, Edward Arnold, London1993.
[5]. PN Acha and B. Szyfres, Zoonoses and communicable diseases common to man and animals, 3rd ed. Pan American Health Organization Washington D.C. U.S.A, 168-176, 2001.
[6]. JM Farber, GW. Sanders and JI. Speias,. Growth of Listeria monocytogenes in naturally contaminated raw milk, Lebensm Wiss Technol, 23(252), 1990.
[7]. J Bille, Epidemiology of human listeriosis in Europe, with special reference to the Swiss outbreak. In: AL Miller, JL. Smith and GA. Somkuti, (Eds.), Foodborne Listeriosis, Elsevier Science Publishers B.V. (Biomedical Division), Amsterdam, 1990, 71–74.
[8]. CV Broome, B. Gellin and B. Schwartz, Epidemiology of listeriosis in the United States. In: AL Miller, JL. Smith and GA. Somkuti, (Eds.), Foodborne Listeriosis. Elsevier Science Publishers B.V. (Biomedical Division), Amsterdam, 1990, 61–65.
[9]. EH Marth and ET. Ryser,. Occurrence of Listeria in foods: milk and dairy foods. In: AL Miller, JL. Smith and GA. Somkuti, (Eds.), Foodborne Listeriosis. Elsevier Science Publishers B.V. (Biomedical Division), Amsterdam, 1990, 151–164.
[10]. MW Griffiths, Listeria monocytogenes, its importance in the dairy industry, Journal of Science and Food Agriculture, 47, 1989, 133–158.
[11]. A Schuchat, B. Swaminathan and C. Broome, Epidemiology of human listeriosis, Clinical and Microbiology Review, 4, 1991, 169–183.
[12]. ET Ryser and EH Marth, Behavior of L. monocytogenes during the manufacture and ripening of cheddar cheese, Journal of Food Protection, 50, 1987, 7-13.
[13]. A Sharif and N. Tunail, Investigation on Listeria monocytogenes contamination of raw milk obtained from different regions of Anatolia and pasteurized milk sold in Ankara, Mikrobiyoloji Bülteni, 25, 1991, 15-20.
[14]. French Association for Standardization (AFNOR). Food microbiology–detection of Listeria monocytogenes- routine method, 8(55), AFNOR, Paris, 1993.
[15]. AD Hitchins,. Food and drug administrations, bacteriological analytical manual. 8th Edition. Gaithersburg, USA: AOAC International, 1995 10.01-10.13.
[16]. PJ Slade, DL. Collins-Thompson, Two enrichment procedures for isolating Listeria monocytogenes from raw milk, Journal of Food Protection, 50, 1987, 904-908

[17]. AM Lund, EA. Zottola and DJ. Pusch, Comparison of methods for isolation of Listeria from raw milk, Journal of Food Protection, 54 (8), 1991, 602-606.
[18]. JFF Garayzabal, L. Dominquez, JA. Vazquez, E. Gomez-Lucia, ERR. Ferr and G. Suarez, Occurrence of Listeria monocytogenes in raw milk, Veterinary Record, 120, 1987, 258-259.
[19]. FY Chye, A. Abdullah and MK. Ayob, Bacteriological quality and safety of raw milk in Malaysia, Food Microbiology, 21, 2004, 535-541.
[20]. E Soyutemiz, F. Cetinkaya, C. Ozakın and S. Gedikoglu, Presence of Listeria monocytogenes in raw milk samples from West Anatolia, Turkish Journal of Infection, 15 (1), 2001, 5-9.
[21]. J Gledel, Listeria and the dairy industry in France. Foodborne Listeriosis, Proceedings of a Symposium, Wiesbaden, Germany, 1988, 72-82.
[22]. J Lovett, DW. Francis and JM. Hunt, Listeria monocytogenes in raw milk: detection, incidence, and pathogenicity, Journal of Food Protection, 50, 1987, 188-192.
[23]. OO Oni, AA. Adesiyun, JO. Adekeye and SNA. Saidu, Prevalence and some characteristics of Listeria monocytogenes isolated from cattle and milk in Kaduna State, Nigeria, Israel Journal of Veterinary Medicine, 45, 1989, 12-17.
[24]. J Harvey and A. Gilmour, Occurrence of Listeria species in raw milk and dairy products, Northern Journal of applied Bacteriology, 72, 1992, 119-125.
[25]. MP Doyle, KA. Glass, JT. Beery, GA. Garcia, DJ. Pollard and RD. Schultz, Survival of Listeria monocytogenes in milk during high temperature, short time pasteurization, Applied Environment Microbiology, 53(7), 1987, 1433-1438.