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Antibacterial activity of Pomegranate Peel Extract Against Listeria Monocytogenes Isolated From Refrigerated Meat

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Abstract:
The food samples such as ice cream, Raw meat, and Refrigerated meat were randomly collected from the shops in Chennai and were analyzed for the presence of *Listeria species*. Totally 15 food samples were analyzed. The samples were introduced in Primary enrichment medium Fraser broth and incubated at 30℃ for 24 hours. Then, 0.1 ml of the culture was inoculated into 10 ml of Fraser broth (Secondary enrichment) at 37℃ for 48 hours and then plated on selective media- Oxford and PALCAM agar. The colonies from the selective media are streaked on TSYEA (Tryptone Soya Yeast Extract Agar). The presence of bacterial colonies on TSYEA (Tryptone Soya Yeast Extract Agar) confirms the presence of *Listeria species*. Out of 15 samples, *Listeria species* were detected in 9 samples. *Listeria monocytogenes* was isolated, identified, and characterized from Refrigerated meat samples by Biochemical test, Haemolysis test, Carbohydrate utilization test, and CAMP test. The antibacterial activity of the Ethanolic extract of Pomegranate peel was tested against *Listeria monocytogenes*. 500 mg/ ml of the stock was prepared and the zone of inhibition was measured with a diameter of 16 mm, 18 mm, and 20 mm for 25, 50 and 100 μl.

Keywords: *Listeria monocytogenes*, Oxford agar, PALCAM agar, TSYEA agar, CAMP test

1. INTRODUCTION

Listeriosis is a food-borne infection mainly caused by *Listeria monocytogenes.*It causes high mortality rates (20-30%)worldwide when compared with other food-borne microbial pathogens.There are six species currently recognized: *Listeria monocytogenes, Listeria innocua, Listeria ivanovii, Listeria seeligeri, Listeria welshimeri and Listeria grayi.* Only two species of the genus are generally considered to be pathogenic, *Listeria monocytogenes*in humans and *Listeria ivanovii in other mammals* (UtaGasanov *et al*., 2004).

In mammals*, Listeria monocytogenes* can cause spontaneous abortions and the symptoms of Listeriosis range from a flu-like illness to severe complications including meningitis, septicemia, and spontaneous abortion. Various foods have been implicated in the spread of *Listeria monocytogenes*, namely meat and meat products, raw milk, soft cheese, pasteurized dairy products including ice cream, fish and fish products, and ready-to-eat foods (Nayak*et al*.,2015). The important characteristics of *L.monocytogenes*like the ability to grow as low as $4°C$, resist heat, salt, nitrite, and acidity, withstand osmotic stress, and survive mild preservation treatment measures that cause the bacteria to cause infection in Humans. (Alsheikh*et al.,*2012).

Listeria species are Gram-positive, facultatively anaerobic, non-spore-forming, rod-shaped bacteria with a low G+C content and capable of growing at 4℃. In cattle, it can result in abortion and mastitis, and the infected animals will shed the organism in the milk. Other infected animals, including sheep and chickens, can serve as a source of the organism in the food supply.The fatality ranges from 30-75,% especially in high-risk groups like pregnant women, unborn or newly delivered infants, and elderly people as well as persons with severe underlying disease conditions like immune suppression, AIDS, and

chronic conditions like cirrhosis. Though not phenomenal, the number of human Listeriosis cases in India has been on the rise with reports on sporadic cases and incidence in clinical samples and has been quoted as an emerging food-borne disease in India (Liu, 2006).

L.monocytogenes may cross-contaminateready-to-eat (RTE) meat and poultry products during post-processing steps such as slicing, peeling, and packaging.A highprevalence of*L.monocytogenes* in RTE food is commonly reported at high rates in different parts of the World. *L. monocytogenes* was detected in Spanish-style sausage (3.7%), Blood sausage (11.1%), Cooked meat samples (8.8%), different RTE foods (7.3%), and in in-store-packed deli meat products (8.5%) (Alsheikh *et al.,* 2013).Analysis and characterization of *Listeria monocytogenes* in food samples can be detected by using the method approved by the International Standards Organization (ISO 11290). In this method, *Listeria monocytogenes* has a selective enrichment process which includes primary and secondary enrichment. In Primary Enrichment, Fraser Broth containing half strength of selective supplements was added, and in Secondary Enrichment Fraser Broth containing full strength of selective supplement. The Selective media, Oxford and PALCAM agar are used for the growth of *Listeria spp*. The growth of *Listeria* on TSYEA (Tryptone soya yeast extract agar) confirms the presence of *Listeria spp.* The Biochemicaltests, CAMP test (Christie-Atkins-Munch-Peterson), and Haemolysis test are used to identify Listeria*monocytogenes.*

Listeria cells are slow-growing and can be rapidly out-grown by competitors, and hence bacteriostatic agents, such as acriflavin and nalidixic acid that specifically act to suppress competing microflora, have been introduced into enrichment media or selective agar (Welshimer, H.J., 1981). Human Listeriosis is rare with an incidence of 2 to 15 cases per 1 million people per year reported in developed countries. (Chan and Wiedmann, 2009).

Punica granatum commonly called Pomegranate, recently described as nature's power fruit, is a plant used in folkloric medicine for the treatment of various diseases. Pomegranate has strong antioxidant and anti-inflammatory properties and recent studies have demonstrated its anti-cancer activity in several human cancers. In addition, pomegranate peel extract with an abundance of flavonoids and tannins has been shown to have a high antioxidant activity (Yehia *et al*., 2011).The extract of pomegranate peel possesses antimicrobial activity against the microorganisms, such as *Bacillus subtilis, Staphylococcus aureus, Yersinia enterocolitica, Listeria monocytogenes, Candida utilis, Saccharomyces cerevisiae and Aspergillus niger* (Nuamsetti *et al.*, 2012).

In the present study, refrigerated samples like ice cream and Meat samples are analyzed for the presence of *Listeria monocytogenes,* and the antibacterial activity of Ethanolic extract of Pomegranate peel was tested against *Listeria monocytogenes*.

2. MATERIALS AND METHODS

2.1 Sample Collection

A total of 15 food samples, comprising 5 Ice-cream samples, 5 Fresh Meat samples, and 5 Refrigerated Meat samples were collected and analyzed for the presence of *Listeria species*.5 Ice-cream samples of different brands were collected randomly from Ice-cream shops in Kolathur, Chennai. Fresh Meat samples were collected from the chicken stall from different areas of Chennai. Fresh Meat samples are collected and stored in the refrigerator for 1 week (Refrigerated Meat sample).The meat samples are collected from Perambur, Kolathur, Kilpauk, T.BChathram, Ayanpuram, and Chennai. The samples are collected in sterile polythene bags and taken to the laboratory for the analysis of *Listeria monocytogenes* in the food samples.

2.2 Isolation of *Listeria Species* from the Food Samples

Food samples, such as ice cream, Fresh Meat, and Refrigerated Meat samples were tested for the presence of *Listeria species by*following the procedure recommended by the International Standards Organization (ISO 11290-1: 1996).

2.2.1 Primary Enrichment

10g of the representative portion from each sample was introduced aseptically into a sterile container containing 90 ml of Half Fraser Broth (Primary enrichment). The samples are homogenized for 1 minute and are incubated at 30℃ for 24 hours.

2.2.2 Secondary Enrichment

0.1 ml from each Half Fraser Broth culture (Primary enrichment) was added into the 10 ml of Fraser Broth (Secondary enrichment) and incubated at 37℃ for 48 hours.

2.3 Plating on Selective media

A loopful of the Fraser Broth secondary enrichment culture was streaked on the surface of Listeria selective agar such as Oxford and PALCAM agar and incubated for 48 hours at 37℃.

2.4 Confirmation of *Listeria Species*

For confirmation of *Listeria species*, the suspected colonies from Oxford and PALCAM agar were picked up and streaked on Tryptone Soya Yeast Extract Agar (TSYEA) and incubated at 37℃ for 24 hours. Growth on this medium confirms the presence of *Listeria species* (ISO 11290).

2.5 Identification of *Listeria monocytogenes*

2.5.1 Haemolysis Test

The isolated colonies on Tryptone Soya yeast extract agar were streaked on the blood agar plates and the plates were incubated at 37℃ for 24 hours.

2.5.2 Carbohydrate Utilization Test

The colonies are tested for carbohydrate utilization. Rhamnose and xylose broth were prepared, sterilized, and inoculated with culture from TSYEB and incubated at 37℃ for up to 5 days. Positive reactions are indicated by yellow color and occur mostly within 24 to 48 hours.

2.5.3 CAMP Test

Staphylococcus aureus and *Rhodococcus equi*were streaked in a single line across the sheep blood agar plate so that the two cultures were parallel and diametrically opposite. The test strain was streaked at a right angle to these cultures so that the test culture, *S. aureus,* and *R. equi* culture did not touch but at their closest about 1 mm to 2 mm apart, and incubated at 37℃ for 24 hours. A positive reaction with occur with *S. aureus* appears as a small zone of enhanced hemolysis extending only about 2 mm from the test strain.

2.6 Antibacterial Activity of Ethanolic Extract of Pomegranate Peel against*Listeriamonocytogenes*

2.6.1 Sample Collection

The pomegranate fruits were purchased from the local market in Perambur, Chennai. The collected pomegranate peels were washed with tap water or distilled water. After washing, pomegranate peels were dried in shade for 10 to 15 days. Then the pomegranate peels were fined into powder with a mechanical grinder to detect the antibacterial activity of pomegranate peelsagainst *Listeriamonocytogenes.*

2.6.2 Preparation of Ethanolic Extract of Pomegranate Peel

5 g of powdered pomegranate peel was mixed with 100 ml of 90% ethanol and leftat room temperature for 24 hours. After 24 hours, the mixture was filtered through the Whatman No. 2 filter paper. After filtration, the crude extract is evaporated and stored in the refrigerator.

2.6.3 Preparation of Bacterial Culture

Listeria monocytogenes isolated from food samples was inoculated in Tryptone Soya Yeast Extract Broth and incubated for 24 hours at 37°C.

2.6.4 Determination of Antibacterial Activity of Ethanolic Extract of Pomegranate Peel by Agar Well Diffusion Method

The agar well diffusion method is widely used to evaluate the antimicrobial activity of plants or microbial extracts. The antibacterial activity of pomegranate peel extract was determined by the agar well diffusion method. Mueller-Hinton agar was prepared, autoclaved and cooled medium was added to sterile petri dishes to give a uniform depth of 4mm and allowed to set. Optimally within 15 min after adjusting the turbidity of the inoculum suspension, a sterile cotton swab was dipped into the adjusted suspension and pressed firmly on the inside wall of the tube to remove excess inoculum from the swab. The swab was streaked over the entire sterile agar surface. Then, a hole with a diameter of 6 to 8 mm was punched aseptically with a sterile cork borer or a tip. 500mg/ml of the extract was prepared as the stock solution and a volume (25, 50, and 100 µL) of the antimicrobial agent or extract solution at the desired concentration was introduced into the well. Ampicillin was used as a positive control. The plates were incubated at 37˚C for 24 hrs and the diameter of the zone of inhibition was measured around the discs. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain tested.

3. RESULTS

3.1. Sample Enrichment

3.1.1 Primary Enrichment

Fig 1: Primary Enrichment of Ice cream, Fresh meat and refrigerated meat in Half Fraser Broth

Black coloration may develop during the incubation period of 24 hours

3.1.2 Secondary Enrichment

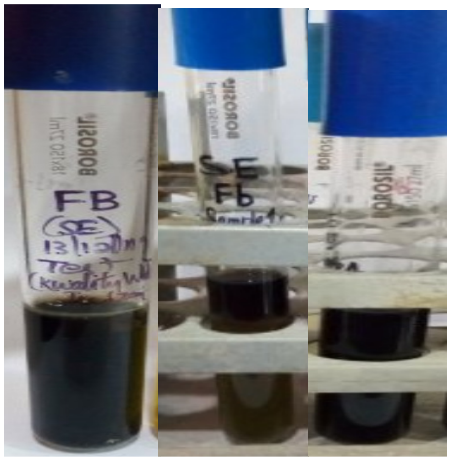

Fig2:Secondary Enrichment of Ice cream, Fresh meat, and refrigerated meat in Fraser Broth

3.3 Identification of *Listeria Species*

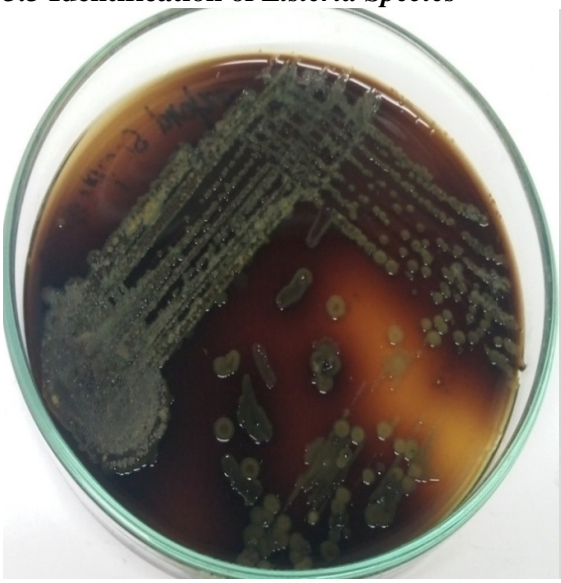

Fig3: Listeria colonies on Oxford agar

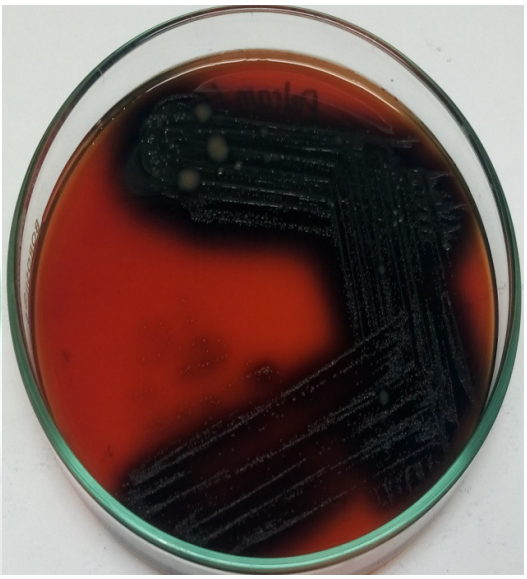

Fig 4: Listeria colonies on PALCAM agar

A loop full of culture from the Fraser culture medium was streaked on the surface of the two selective mediums Oxford agar and PALCAM agar and incubated at 37°C for 48 hours. On Oxford agar, colonies of *Listeria species* are small (1mm), greyish colonies surrounded by black halos, after 24 hours of incubation. After 48 hours, colonies become darker, with a possible greenish sheen, and are about 2 mm in diameter, with black halos and sunken centers.

On PALCAM agar, after 24 hours of incubation, *Listeria*grows as small or very small greyish-green or olive-green colonies 1.5 mm to 2 mm in diameter, sometimes with black halos observed. After 48 hours, *Listeria species* appear in the form of green colonies about 1.5 mm to 2 mm in diameter with a central depression and surrounded by black halos.

3.4 Confirmation of *Listeria Species*

3.4.1 Selective Agar (TSYEA)

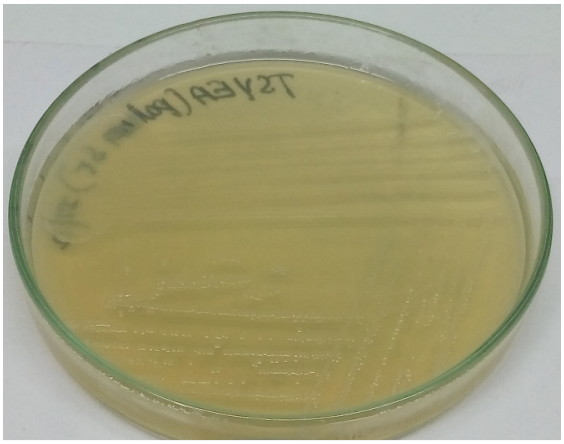

Fig5: TSYEA Plate after 24 hours

On TSYEA (Tryptone Soya Yeast Extract Agar) after 24 hours of incubation, *Listeria species* grow as 1 mm to 2 mm in diameter colonies, convex, colorless, and opaque colonies were observed.

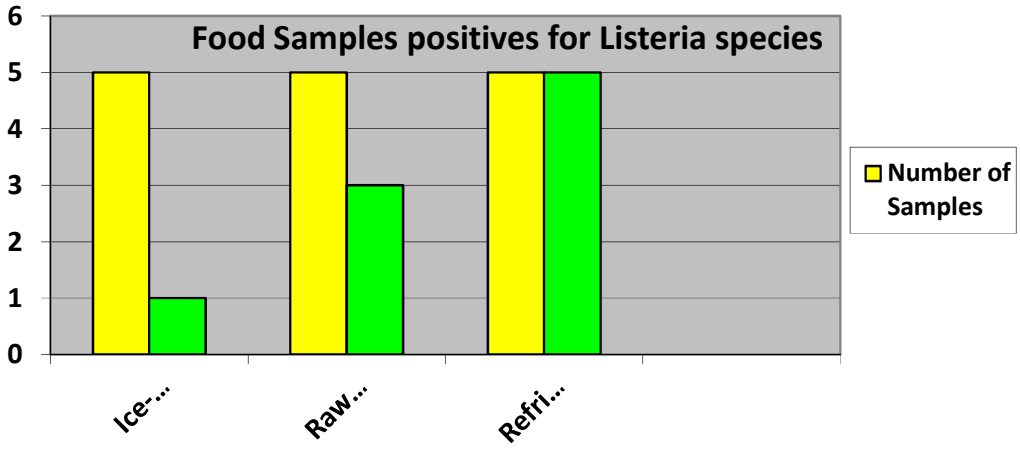

Chart 1: Occurrence of *Listeria species* in Food Samples

Out of 15 food samples, *Listeria species* was detected in 9 food samples. *Listeriamonocytogenes* was isolated, identified, and characterized from the Refrigerated Meat sample.Fresh raw meat and Refrigerated meat showed contamination with *Listeria species* than the Ice-cream sample.*Listeria species* was detected in 1/5 ice cream, 3/5 raw meat, and 5/5 refrigerated meat samples out of 15 samples. The presence of *Listeria species* was confirmed in 60% of the food samples analyzed.

3.5 Identification of *Listeria monocytogenes*

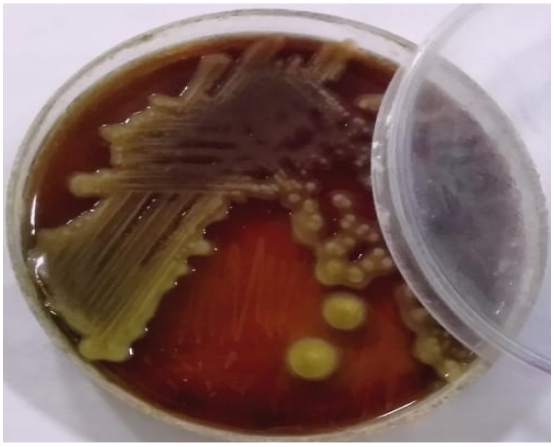

Fig 6: βHaemolysis on Blood agar

A narrow zone of hemolysis was observed surrounding the streak line.

CAMP Test

Fig7: CAMP Test (Christie, Atkins, Munch-Petersen) - Small Zone of Enhanced Haemolysis on *S.aureus* with *L. monocytogenes*

A positive reaction with *S. aureus* appears as a small zone of enhanced hemolysis extending only about 2 mm from the test strain large zones of hemolysis do not occur in*S. aureus* and *L. monocytogenes*.

S.No	Test	Result
1.	Gram's Staining	Gram-Positive rod
2.	Motility	Motile
3.	Catalase	Positive
4.	Oxidase	Negative
5.	Hemolysis	Positive
6.	CAMP Test	*S.aureus*(+), *R.equi*(-)
7.	Rhamnose	Positive
8.	Xylose	Negative

Table 1: Characteristics of *Listeria monocytogenes*

Based on the Morphological and physiological characteristics and catalase reaction, the presence of *Listeria species* is confirmed in the refrigerated meat. *Listeria monocytogenes* is confirmed based on the hemolysis test, carbohydrate utilization test, and CAMP test.

3.6Antibacterial activity against *Listeria monocytogenes* using Pomegranate peel extract

Fig8: Powdered Pomegranate Peel

Fig 9: Pomegranate peel Extract

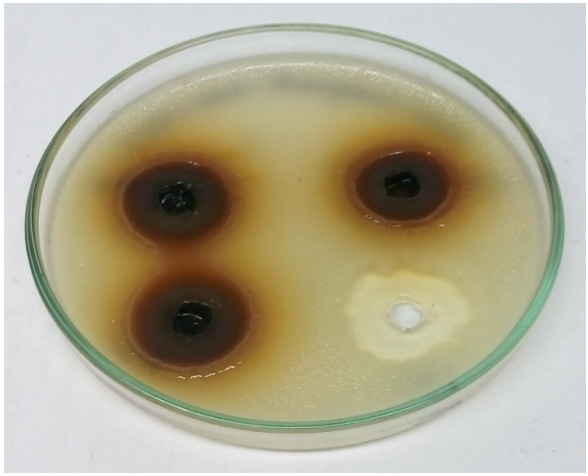

Fig 10: Zone of inhibition of Pomegranate peel extract against *Listeria monocytogenes*

Sample	**Volume of Crude Extract**	**Concentration of Crude Extract**	**Zone of Inhibition**
Ethanolic Extract	25 μl	12.5 mg	16 mm
	50 μl	25 mg	18 mm
	100 μl	50 mg	20 mm
Ampicillin	50 μl	10 mg	14 mm

Table 2: Antibacterial Activity of Pomegranate Peel Extract against Listeria monocytogenes

The antibacterial activity of the Ethanolic extract of Pomegranate peel was tested against *Listeriamonocytogenes*. The zone of inhibition was observed with a diameter of 16 mm, 18 mm, and 20 mm for 25, 50 and 100μl (500mg/ml of stock).

4. DISCUSSION

Listeriosis has been recognized to be one of the emerging zoonotic diseases during the last two decades and is contracted mainly from the consumption of contaminated foods and food products. In the present study, *Listeria species* were isolated from ready-to-eat food (RTE) like ice cream, fresh meat, and Refrigerated meat samples using ISO 11290 standards.

Fresh Raw meat and Refrigerated meat showed contamination with *Listeria species* than the Ice-cream sample. Molla et al., 2004 reported that the raw meat products showed a high level of contamination with *Listeria species* (50.6%). It is generally assumed that such products cannot be free from *Listeria* because of slaughter methods evisceration and food processing that allowa greater chance for contamination.

Listeria species are ubiquitous in nature and is widely present in plant, soil, silage and processing environments. Meat might be colonized by *Listeria species* due to consumption of contaminated feed and water (Beresford et al., 2001).

Listeria species was detected in 1 ice cream sample out of the 5 samples tested. *Listeria* is not killed by freezing. Growth is arrested altogether, but normal growth will be resumed after thawing.Comparatively low degrees ofincidence have been recorded in ice-cream samples which is indicative that marginally safe food hygiene practices might have been followed. This study correlates with the study of Nayak et al. One specific characteristic of *Listeria monocytogenes* that appears to be its ability to cause human food-borne illness is to capacity to grow at low temperatures. The presence of L. monocytogenes is confirmed in the refrigerated meat. *Listeria monocytogenes* has been shown togrow at temperatures ranging from -0.4 to 45℃.Of particular importance, many studies have shown that *Listeria monocytogenes* can proliferate in many refrigerated ready-to-eat (RTE) foods such as deli meats, smoked fish, and milk (U.S Department of Health and Human Services/ U. S Department of Agriculture, 2003).

Al-Zorkey (2009) reported that only methanol extract of pomegranate peel has marked inhibition against *Listeria monocytogenes, Pseudomonas aeruginosa*, *S. aureus, E. coli,* and *B. subtilis*. In the present study, ethanol extract of Pomegranate peel inhibited the growth of *Listeria monocytogenes* which was against the study of Al-Zorkey.Pomegranate peel extract with an abundance of flavonoids and tannins has been shown to have high antioxidant activity (Abdel Moneim *etal*., 2011).As Pomegranate peels have been used in traditional medicine for treating diarrhea and dysentery (Reddy *et al.,* 2007), In the present study Ethanolic extract of Pomegranate peel also inhibits Listeria which causes intestinal infections.

5.0 CONCLUSION

The presence of Listeria species was confirmed in 60% of the food samples analyzed. Listeria monocytogenes was isolated and identified from Refrigerated meat which causes food-borne Listeriosis. Listeria is killed by cooking. Thoroughly cooking the meat to a high temperature will kill the bacteria. Listeria is not killed by freezing but it survives and the growth is arrested and resumes normal growth after thawing. Both safe food handling and maintaining proper refrigeration temperature are critical to minimize the risk of Listeriosis. Ethanolic extract of Pomegranate peel showed antibacterial activity against Listeria monocytogenes. The extract can be further analyzed for the compound by GC-MS analysis. The compound can be detected, and purified by HPLC and could be used as an antibiotic against Listeria infection. The control of Listeria monocytogenes is required at all stages in the food chain. All sectors of the food chain should implement Good Manufacturing Practices (GMP) as well as implement a food safety management system based on the principles of Hazard Analysis Critical Control Points (HACCP).

6. BIBLIOGRAPHY

1. Alsheikh A D I, Mohammed G E, and Abodalla M A. First isolation and identification of *Listeria monocytogenes* from fresh raw dressed broiler chicken in sudan. Res J Microbiol 2012; 7(6): 319-326.
2. Al-Zoreky N S. Antimicrobial activity of pomegranate (Punica granatum L.0 fruit peels. Inn J Food Microbiol 2009; 134(1): 244-248.
3. Angela Di Pinto, Lucia Novello, Filomena Montemurro, Elisabetta Bonerba, Giuseppina Tantillo. Occurrence of *Listeria monocytogenes* in ready-to-eat foods from supermarkets in southern italy. New Microbiol 2010; 33: 249-252.
4. Abdollah Jamshidi and Tayebeh Zeinali. Significance and characteristics of *Listeria monocytogenes* in poultry products. Inn J Food Sci 2019: 1-7.
5. Ahmed SS, Tayeb BA, Ameen AM, Merza SM and Sharif YHM. Isolation and detection of *Listeria monocytogenes*and cheese in Duhokprovince Kurdistan region by using RT-PCR. J Food Indus Microbiol 2016; 2(1): 1-4.
6. Adzitey F and Huda N. *Listeria monocytogenes* in foods: Incidences and possible control measures. Afr J Microbiol Res 2010; 4(25): 2848-2855.
7. Alsheikh A D I, Mohammed G E, and Abdalla M A. Isolation and identification of *Listeria monocytogenes* from retail broiler chicken ready to eat meat products in Sudan. IntJ Anim Veter Adv2013; 5(1): 9-14.
8. Ahmed E. Abdel Moneim. Antioxidant activities of Punica granatum (pomegranate) peel extract on brain of rats. J Med Plants Res2012; 6(2): 195-199.
9. Bhilegaonkar K N, Kulshrestka S B, Kaapoor K N, Ashok kumar, Agarwal R K and Singh B R. Isolation of *Listeria monocytogenes* from Milk. J Food Sci Technol 1997; 34: 248-250.
10. Bayleyegn Molla, Roman Yilma, Daniel Alemayehu.*Listeria monocytogenes* and other *Listeria species* in retail meat and milk products in Addis Ababa, Ethiopia. Ethiop J Health Dev 2004; 18(3): 209-211.
11. Beyza H Ulusoy and Kefyalew Chirkena. Two perspectives of *Listeria monocytogenes* hazards in dairy products: the prevalence and antibiotic resistance. Food Quality Safety 2019; XX: 1-9.

12. Dongyou Liu. Identification,subtyping and virulence determination of *Listeriamonocytogenes* an important food borne pathogen. J Med Microbiol 2006; 55: 645-659.
13. Deepti N Nayak, Savalia C V, Kalyani H, Rajeev kumar and Kshrisagar D P. Isolation, identification, and characterization of *Listeria spp*. from various animal origin foods. Veterinary World 2015; 8(6): 695-701.
14. Hossein Jamali, Lay Ching Chai, Kwai Lin Thong. Detection and isolation of *Listeriaspp*. and *Listeria monocytogenes* in ready to eat foods with various selective culture media. Food Control 2013;32: 19-23.
15. Hany M. Yehia, Manal, F Elkhadragy and Ahmed E. Abdel Moneim. Antimicrobial activity of pomegranate rind peel extracts. Afr J Microbiol Res 2011; 4(22): 3664-3668.
16. Jami S, Jamshidi A and Khanzadi S. The presence of *Listeria monocytogenes* in raw milk samples in Mashhad, Iran. Iran J Vet Res Shiraz Univ 2010; 11(4): 363-367.
17. Katarzyna Kosek-Paszkowska, Jacek Bania, Jaroslaw Bystron, Jerzy Molenda and Malgorzata Czerw. Occurrence of *Listeria species* in raw poultry meat and poultry meat products. Bull Vet Inst Pulawy 2005; 49: 219-222.
18. Luca Cocolin, Kalliopi Rantsiou, Lucilla Iacumin, Carlo Cantoni, and Giuseppe Comi. Direct identification in food samples of *Listeria spp*. and *Listeriamonocytogenes* by molecular methods. Appl Environ Microbiol 2002; 68(12): 6273-6282.
19. Mir-Hassan Moosavy, Saber Esmaeli, Ehsan Mostafavi, Fahimeh Bagheri Amiri. Isolation of *Listeria monocytogenes* from milks used Iranian traditional cheese in Lighvan cheese factories. Ann Agric Environ Med 2014; 21(4): 728-729.
20. Nayak D N, Savalia C V, Kshirsagar D P, and Kumar R. Isolation, identification and Molecular characterization of *Listeria species* from Milk and Milk products in Navsari City of South Gujarat. J Vet pub Hlth 2015; 13(1): 19-23.
21. Nurcay Kocaman, Belgin Sarimehmetoglu. Isolation of *Listeria monocytogenes* in lamb meat and determination of the antibiotic resistance. Ankara UnivVet Fak Derg 2017; 64: 273-279.
22. Nirmaladevi D Shirinithivihahshini, Mariyaselvam Sheelamary, Duraisamy Mahamuni, Rengaraj Chithiradevi. Occurrence of *Listeria monocytogenes* in Food and Ready to Eat Food products available in Tiruchirappalli, Tamil Nadu, India. World J Life Sci and Medical Research 2011; 1(4):70-5.
23. Prem Saran Tirumalai. Listeriosis and *Listeria monocytogenes* in India. Wudpecker J Food Tech 2013; 1(6): 098-103.
24. Reddy MK, Gupta SK, Jacob MR, Khan SI, Ferreira D. Antioxidant, antimalarial and antimicrobial activities of tannin-rich fractions, ellagitannins, and phenolic acids from punica granatum L. Planta Med. 2007;73(5):461-467.
25. Saha M, Debnath C and Pramanik A K. *Listeria monocytogenes* An Emerging food borne Pathogen. Int J Curr Microbiol App Sci 2015; 4(11): 52-72.
26. Sindhuja P and Gomathi N. Antimicrobial, Antioxidant and Anticancer Activity of Flax seed. World Journal of Pharmaceutical Research 2018; 7(19): 644 - 659
27. Steve Harakeh, Imane Saleh, Omar Zouhairi, Elias Baaydoun, Elie Barbour, Nisreen Alwan. Antimicrobial resistance of *Listeria monocytogenes* from dairy-based food products. Science of the Total Environment 2009; 407: 4022-4027.
28. Tianchai Nuamsetti, Petlada Dechayuenyoung, Sukon Tantipalibulvut. Atibacterial activity of pomegranate fruit peels and arils. ScienceAsia 2012; 38: 319-322.
29. Uta Gasanov, Denise Hughes, Philip M. Hansbro. Methods for the isolation and identification of *Listeria spp*. and *Listeria monocytogenes*. FEMS Microbiology 2005; 29: 851-875.
30. Yvonne C Chan and Martin Wiedmann. Physiology and Genetics of *Listeriamonocytogenes* Survival and growth at cold temperatures. Critical Reviews in Food Science and Nutrition 2009; 49: 237-253.

31. Zeinali T, Jamshidi A, Bassami B and Rad M. Isolation and identification of *Listeriaspp*. in chicken carcasses marketed in the northeast of Iran. Int Food Res J 2017; 24(2): 881-887.

