Molecular characterization of listeria monocytogenes isolated from raw milk and some dairy products at local markets in Damanhour city, Egypt

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ABSTRACT

Several outbreaks of L. monocytogenes have been involved with milk and dairy products consumption. The present study was conducted to investigate the prevalence listeria monocytogenes in raw milk and some dairy products. A total of 225 samples of raw milk (75), pasteurized milk (50), ice cream (50) and Ras cheese (50) were collected randomly from different supermarkets, retail outlets and other markets outlets in Damanhour city, El-Beihra governorate, Egypt. Out of 225 samples, 29 (12.88%) were positive for Listeria species. The occurrence of Listeria spp. in raw milk, pasteurized milk, ice cream and Ras cheese samples were 10 (13.33%), 6 (12%), 7 (14%) and 6 (12%), respectively. The most prevalent listeria species isolated from raw milk and dairy products in this study was L. innocua and L. monocytogenes. The biochemically identified isolates of L. monocytogenes (16) were molecularly characterized by multiplex PCR for detection of three virulence genes (iap, hlyA and actA); the results showed that iap gene was demonstrated in all isolates (100%); hlyA and actA were detected in 83.3 and 66.7% of isolates from raw milk; 66.7 and 66.7% of isolates from pasteurized milk; 80 and 80% of isolates from ice cream; 100 and 50% of isolated from Ras cheese samples. Concerning to antibiotic resistance, 16 isolates of L. monocytogenes were tested against 14 antibiotics disc and showed that all the isolates were resistant to Kanamycin (100%) and Nalidixic acid (93.75%), meanwhile, most of the isolates showed sensitivity against Ciprofloxacin (87.50%) and Ampicillin (68.75%). In conclusion, the results of this study emphasize the need for applying more strict hygienic control measures especially during processing, storage and marketing of dairy products.

Keywords: Listeria spp., Listeria monocytogenes, Dairy products, multiplex PCR

1. Introduction

Milk and dairy products, because of their high nutritional value, are very suitable for multiplication of microorganisms, including pathogenic bacteria (Kasalica et al, 2011). Listeria spp. are Gram positive and facultative anaerobic organisms. They are also non-spore forming, and rod-shaped bacteria (Odetokun and Adetunji, 2017). The genus Listeria has been divided into 17 species and 4 subtypes based on 16S rRNA sequences (Anonymous 2017). Classic Listeria species (L. monocytogenes, L. innocua, L. seeligeri, L. grayi, L. ivanovii, L. welshimeri) can be isolated from food. Recently, 11 new species of the Listeria were identified (Barre et al., 2016). L. monocytogenes and L. ivanovii are the main known pathogenic species within this genus. Although L. monocytogenes may lead to illness and death in humans and other mammals, L. ivanovii is primarily associated with ruminant animals (Hellberg et al., 2013). Listeria species are widely distributed in soil, surface water, sewage, animal feed, farm, food processing equipment, urban and suburban settlements (Korsak and Szuplewska, 2016). Listeria spp. are the most frequently prevalent in the milk processing environment.

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Received 5 April 2021, revised 20 April 2021, Published 26 April 2021

Also, dairy products may become contaminated with L. monocytogenes during subsequent stages of production (Seyoum et al., 2015). Eldaly et al., (2013) confirmed that insufficient heat treatment of food enhances the growth of L. monocytogenes in food. Several outbreaks of L. monocytogenes infection were mainly associated with the consumption of milk and dairy products because the Listeria organism capable of slow multiplication in refrigerated food which subjected to minimal further processing and post processing contamination (CDC, 2011; Gaulin et al., 2012).

Contamination of milk after pasteurization or due to defects in technology during pasteurization (inadequate temperature, technical errors) is responsible for the presence of L. monocytogenes in pasteurized milk. Therefore, the occurrence of L. monocytogenes in milk and dairy products could be due to failure in the pasteurization process or post-pasteurization contamination (Lee et al., 2019). Seyoum et al., (2015) reported high incidence of listeria species in pasteurized milk (60%).

Identification of Listeria species using biochemical tests is time-consuming, laborious and an inaccurate procedure. So, for better accuracy, PCR was used. PCR relying on nucleic acid composition rather than the phenotypic expression of the bacterium (Coocolin et al., 2002). The genes encoding for virulence (iap, hlyA and actA) are common to all Listeria species (Gitot and Content, 2002).

Antimicrobial resistance of L. monocytogenes may be associated with the presence of a plasmid, conjugated genes and chromosomal gene mutation (Poros-Gluchowska and Markiewicz, 2003). Also, resistance of listeria monocytogenes to antibiotics associated with misuse of antibiotics (Rahimi et al., 2012).

Contamination of milk and dairy products with Listeria species constitutes serious health problems for consumers, so, the aim of the present work is molecular characterization and antibiotic resistance profile of listeria monocytogenes isolated from raw milk and some dairy products produced in Damanhour city, El-Beihra governorate, Egypt.

2. Materials and Method

2.1. Collection of samples:
A total of 225 random samples represented by raw milk (75), pasteurized milk (50), ice cream (50) and Ras cheese (50) were collected from different markets and dairy shops located in Damanhour city, El-Beihra governorate, Egypt. All collected samples were separately collected in clean polyethylene bag and transferred without undue delay in an icebox to the Food analysis central Lab, Benha University for further examination.

2.2. Isolation and identification of the Listeria species according to ISO 11290-1 (2017):
For milk, ice cream and Ras cheese; 25 ml or 25 g of each sample was aseptically taken and homogenized in 225 ml of Listeria half Fraser broth (Oxoid) supplemented with Listeria selective enrichment supplement (Oxoid) using a stomacher for 2-4 minutes and then incubated for 48 hours at 30 °C. Accurately, 1 ml of the primary enrichment was transferred to 10 ml of Fraser broth and incubated for 48 hours at 30 °C. A loopful of the incubated Fraser broth was streaked onto Oxford agar and incubated at 30 °C for 48 hours. Characteristic colonies (2 mm darker greenish sheen with black halo and sunken centers) were subcultured onto tryptone soy agar supplemented with a 0.6% yeast extract (TSAYE) and incubated at 37o C for 24 hours.

All separated colonies were subjected to Conventional biochemical characterization according to (Aygun and Pehlivanlar , 2006).
2.3. Detection of Listeria monocytogenes virulence genes using multiplex PCR technique: -
Listeria monocytogenes were screened for the presence of invasive associated protein gene (iap), Listeriolysin O (hlyA) and Actin polymerization protein gene (actA).

2.3.1. DNA Extraction of L. monocytogenes using QIA amp kit according to Shah et al. (2009) :
All detected L. monocytogenes strains were grown in Brain Heart Infusion (BHI) broth overnight at 37°C, the suspension was then heated for 20 minutes at 100°C. Accurately, 50-200 μl of the culture were kept at -40°C till use. The obtained lysate (5 μl) was used in PCR reaction mixture as DNA template.

2.3.2. Amplification reaction of L. monocytogenes (Kaur et al., 2007):
The amplification was performed on a Thermal Cycler (Master cycler, Eppendorf, Hamburg, Germany). A multiplex PCR was attempted with comprising three virulence associated genes (iap, hlyA and actA). The multiplex PCR was set up in 50 μl reaction volume. The optimized reaction mixture was : 10 μl PCR buffer (consisting of Tris-HCl, pH 8.3(100 mM l); 500 mM KCl; 0.01%MgCl2, 7.5 mM MgCl2, 1 mM dNTP mix and 10 μM forward and reverse primer of each gene, 5 μl of cell lysate, 5 U of Taq DNA polymerase and sterilized milliQ water.

The cycling conditions for PCR included an initial denaturation of DNA at 95°C for 2 min followed by 35 cycles each of 15 sec denaturation at 95°C, 30 sec annealing at 60°C and 1 min extension at 72°C, followed by a final extension of 10 min at 72°C and held at 4°C. The same PCR amplification cycles was used for all the virulence gene primers.

Aagarose gel 1.5% electrophoresis (Applichem, Germany, GmbH) in 1x TBE buffer with ethidium bromide stain were used for visualization of Amplified DNA fragments on UV transilluminator. A DNA Ladder (100 bp plus, Qiagen, Germany, GmbH) was used to determine the fragment sizes.

2.4. Antibiotic Resistance of Isolated Listeria monocytogenes:
Antimicrobial susceptibility was assessed via 16S rDNA-biochemically identified L. monocytogenes was examined by the single diffusion method according to Jamali et al. (2013). Sensitivity antibiotic discs (Oxoid Limited, Basingstoke, Hampshire, UK) with different concentrations were used.

Agar plate method was applied by using of nutrient agar as a substrate for growth of the tested bacterium for its antibiotic sensitivity. The bacterial culture was uniformly spread on the surface of nutrient agar. The antibiotic discs were distributed on the surface of plate inoculated with L. monocytogenes. The plates were incubated at 25°C for 2-7 days and checked for the growth of the L. monocytogenes around the discs. Complete inhibition zones were measured and interpreted.

Therefore, the antimicrobial susceptibility testing was applied according to the guidelines stipulated Clinical and Laboratory Standards Institute (CLSI), (2018). Accordingly, the antimicrobial discs and their concentrations as well as the diameters of the inhibition zones for the tested strains are demonstrated in the table (2)

3.5. Statistical analysis:
The results expressed as the mean ±SE. Data analysis was preformed SPSS program (2008) (Statistical Package for Social Science, version 16).

3. Results and Discussion
The high prevalence of Listeria spp. in milk and some dairy products has a great hazard on public health and dairy industry (Scallan et al., 2011). Milk and dairy products are implicated in most outbreaks of listeriosis all over the world. listeriosis was firstly reported in USA was resulted from consumption of pasteurized milk in 1983 (Cartwright et al., 2013).

The obtained results in Table (3) illustrated that the highest incidence rate of listeria species was observed in ice cream samples 14% followed by raw milk 13.33%, and 12 % for both pasteurized milk and Ras cheese samples. Higher incidence of Listeria spp. in ice cream samples could be attributed to the contamination of raw milk, low quality of ingredients used, use of polluted water supplies and lack of hygienic measures during processing and handling besides the absence of pasteurization, especially in case of small scale produced ice cream.

Nearly similar prevalence of listeria species in raw milk was obtained by Saha et al., (2015) who reported that incidence was 13.46%. Higher incidence 45% and 54% in raw milk was reported by Hesham et al., (2017) and Hossein et al., (2013), respectively. Lower incidence of listeria species 7.5%, 7.33% and 5.49 % in raw milk was reported by El Hag et al., (2020); Haggag et al., (2019) and Shamloo et al., (2015), respectively. Higher incidence rate of listeria species in pasteurized milk was reported by Seyoum et al., (2015) who found that incidence rate was 60%. Lower prevalence was reported by Waghmare et al., (2012) who found that incidence of listeria species was 4% in pasteurized milk. Contrary to the postulated results, Listeria spp. couldn’t be isolated from examined pasteurized milk samples in studies performed by Şanlıbaba and Tezel (2018); Owsu-Kwarteng et al., (2018); Muthalakshimi et al., (2018) Higher incidence of listeria (45%) in ice cream was reported by Garedew et al., (2015) while, Lower incidence (3%) was reported by Abd El-Tawab et al., (2015). Contrary to the recorded results, Listeria spp. couldn’t be isolated from examined ice cream samples in studies performed by Kevenk and Gulei (2016); Akrami-Mohajeri et al., (2018); Mohamed et al., (2020). In addition, Mohamed et al., (2020) couldn’t isolated listeria species from Ras cheese samples.

The abovementioned result in Table (3) illustrated that the most prevalent listeria species isolated from raw milk was L. innocua 41.7% followed by L. monocytogenes 35.29%. In the examined pasteurized milk samples.

The incidence of listeria species was, L. monocytogenes 37.5% followed by L. innocua 25%, L. seeligeri 25%; from examined ice cream samples, L. monocytogenes incidence rate was 41.67% followed by L. innocua 33.33% finally from examined Ras cheese samples, L. innocua incidence rate was 42.86% followed by L. monocytogenes 28.57% and L. ivanovii 20.77%.

Listeria innocua was the most prevalent listeria species isolated from examined raw milk followed by L. monocytogenes, this result was agreed with Mshref et al., (2015) who reported that L. innocua was the main listeria species isolated from raw milk (35.71%) in Beni-suef, Egypt.

The most prevalent listeria species isolated from pasteurized milk samples was L. monocytogenes. This result agreed with Seyoum et al., (2015) who found that the most prevalent listeria species isolated from pasteurized milk was L. monocytogenes (20%), L. innocua (15.4%) and L. ivanovii (9.2%). The most prevalent listeria species isolated from ice cream samples was L. monocytogenes. This result disagreed with El-Shinawy et al., (2017) who found that the most prevalent listeria species isolated from ice cream was L. grayii.

The most prevalent listeria species isolated from examined Ras cheese samples was L. innocua followed by L. ivanovii and L. monocytogenes. Contrary to the postulated results, L. monocytogenes couldn’t be isolated from Ras cheese samples by Mohamed et al., (2020). Rahimi et al. (2010) reported higher incidence rate of L. monocytogenes in the examined milk samples (72.4%). Lower incidence rate (2.1%) of L. monocytogenes in raw milk was reported by Durmaz et al., (2015) (2.1%) and Seyoum et al., (2015) (2.04%). On the contrary, Listeria monocytogenes couldn’t be isolated from examined raw milk samples in study performed by Aygun and Pehlivanlar (2006).

Lower incidence rate of L. monocytogenes in examined ice cream was reported by Garedew et al., (2015) 15%. On the other hand, L. monocytogenes was failed to isolate from the examined ice cream samples in studies performed by Akya et al., (2013); Metwally and Ali (2014) and Akrami-Mohajeri et al., (2018).

According to Egyptian Standards, (2005), which stipulated that milk and dairy products should be free from L. monocytogenes, there are 13.33, 12, 14, and 12% of examined raw milk, pasteurized milk, ice cream and Ras cheese samples exceeding that permissible limit (Table 4). Three main virulence genes were screened in 16 biochemically identified L. monocytogenes using multiplex PCR (Table 5). The occurrence of iap gene was demonstrated in 100% of isolates isolated from raw milk, pasteurized milk, ice cream and Ras cheese samples; hlyA and actA were detected in 5 /6(83.3%) and 4 /6 (66.7%), respectively in raw milk; 2 /6 (66.7%) and 2/3 (66.7%), respectively in pasteurized milk; 4/5 (80%) and 4/5 (80%), respectively in ice cream; 2 /2 (100%) and 1/2 (50%), respectively in Ras cheese sample.

In the current study hlyA was detected in 83.3 and 80 of examined raw milk and ice cream samples. This results nearly agreed with Abd El Tawab et al. (2015) detected that the (hlyA) gene was amplified in 5 (100%) L. monocytogenes strains isolated from raw milk and ice cream samples. Also, Nayak et al. (2015) detected the gene analysis O (hlyA) gene in L. monocytogenes isolated from raw milk samples.
All the biochemically identified strains (16) of L. monocytogenes were examined for three virulence associated genes iap, hlyA and actA using multiplex PCR technique (Photo 1). hlyA and actA was found in 7 isolates of L. monocytogenes at 131, 465 and 839 bp, respectively; iap and hlyA was detected in 5 isolates; Finally, iap and actA was detected in 3 isolates. The examined virulence genes in the of current study were the most remarkable in determining the virulence L. monocytogenes (Osaili et al., 2011).

hlyA, inIA, inIB, prfA, plcA, plcB, mpl, and actA are the main virulence genes of L. monocytogenes (Alineida et al., 2017). Listeriolysin O (LOO) which encoded by hlyA gene and present only in virulent strains of the species and required for virulence Suriyapriya et al. (2016). Different studies employed the hlyA gene in order to detect L. monocytogenes and differentiate it from other Listeria spp. and other microorganisms (Norton and Butt, 1999). The intracellular mobility and cell-to- cell spread is facilitated by the surface protein act A (ActA). ActA is responsible for intracellular movement through actin polymerization and also has a role in cell adhesion and invasion (Travier et al., 2013).

Results from antimicrobial susceptibility testing are shown in Table (6). High resistance rates to kanamycin 100%, Nadilidic acid (93.75%), Streptomycin (81.25%), Neomycin (81.25%), Amikacin (62.50%) and oxytetracycline (56.25%) were observed. On the other hand, 87.50, 68.75, 62.50, 56.25, 56.25 and 50% of strains were susceptible to Ciprofloxacin, Ampicillin, Cefotaxime, enrofloxacin, Sulphamethoxazole and gentamycin, respectively. Our study showed that most of the L. monocytogenes isolates were sensitive to gentamicin and trimethoprim-sulfamethoxazole because these agents are not used frequently in veterinary practice (Harakeh et al., 2009).

The abovementioned results agreed with Sahnihba et al., (2018) reported that that L. monocytogenes strains from food products exhibit resistance to kanamycin, levofloxacin, amoxicillin. Also, Girma and Abebe (2018) reported resistance of L. monocytogenes isolated from raw milk to nalidixic acid, followed by tetracycline, chloramphenicol and streptomycin. On the other hand, Aksoy et al., (2018) reported high resistance of L. monocytogenes to trimethoprim-sulfamethoxazole.

Our results indicated that L. monocytogenes was sensitive to Ciprofloxacin and gentamycin. This result agrees with Sreeja et al., (2016) reported that ciprofloxacin was highly effective in inhibiting the growth of L. monocytogenes. Gohar et al., (2017) reported that all L. monocytogenes isolated from raw were sensitive to ciprofloxacin and gentamicin. On contrary to the postulated results, Saha et al., (2015) reported that highest resistant of L. monocytogenes recorded against Ciprofloxacin (100%).

Wang et al., (2013) suggest that a combination of gentamicin and ampicillin, or amoxicillin could be used for the remediation of listeriosis in human.

It is concluded that raw milk and some dairy products sold in Damanhour market may be considered a threat to consumers. They are significant vehicles of L. monocytogenes which regularly causing listeriosis outbreaks. Therefore, people with clear risk factors to be infected with listeriosis should not consume such products. This indicates importance and need for permanent control and detection of potential sources of contamination with L. monocytogenes. Introduction of HACCP (Hazard Analysis and Critical Control Points), as a way of control during production and processing of dairy products, could decrease the risk of contamination of these products with Listeria species. The presence of antimicrobial-resistant strains is alarming and constitutes a serious danger to the public health. It is obvious that the increase of the awareness to the importance of controlled use of antibiotics is crucial to limit the emergence of drug-resistant bacteria.

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Table 1 Primer used in this study in the following table.

| Target gene | Oligonucleotide sequence (5′ → 3′) | Product size (bp) | Reference |
|-------------|-----------------------------------|------------------|-----------|
| iap (F)     | 5′ ACAAGCTGCACCTGTGTCGAG 3′       | 131              | Sneath et al. (2013) |
| iap (R)     | 5′ TGACAGGGTGTGATAGGCA 3′         | 456              |           |
| hlyA (F)    | 5′ GCAGTGGAACGCTTGGAGGTGA A 3’    | 839              | Suarez and Boland (2001) |
| hlyA (R)    | 5′ GCAACGTATCCCTCAGATGTC 3’       | 621              |           |

Table 2 Antimicrobial discs and interpretation of their action on the isolated pathogens.

| Antimicrobial agent | Content (μg) | Resistant (mm) | Intermediate (mm) | Susceptible (mm) |
|---------------------|-------------|---------------|------------------|------------------|
| Neomycin (N)        | 30          | 12 or less    | 13-16            | 17 or more       |
| Ampicillin (AM)     | 10          | 13 or less    | 14-17            | 18 or more       |
| Cefotaxim (CF)      | 30          | 17 or less    | 18-22            | 23 or more       |
| Ciprofloxacin (CP)  | 5           | 15 or less    | 15-19            | 20 or more       |
| Erythromycin (E)    | 15          | 13 or less    | 14-22            | 23 or more       |
| Cephalothin (CN)    | 30          | 14 or less    | 15-17            | m18 or more      |
| Gentamicin (G)      | 10          | 12 or less    | 13-14            | 15 or more       |
| Enrofloxacin (EN)   | 5           | 11 or less    | 12               | 13 or more       |
| Kanamycin (K)       | 30          | 13 or less    | 14-17            | 18 or more       |
| Amikacin (AK)       | 30          | 12 or less    | 13-15            | 16 or more       |
| Streptomycin (S)    | 10          | 11 or less    | 12-14            | 15 or more       |
| Oxytetracycline (T) | 30          | 14 or less    | 15-18            | 19 or more       |
| Nalidixic acid (NA) | 30          | 13 or less    | 14-18            | 19 or more       |
| Sulfamethoxazole (SXT) | 25       | 10 or less    | 11-15            | 16 or more       |

Table (3): The frequency of Listeria species in raw milk and examined dairy products.

| Examined samples | No. of samples | Listeria species | Listeria monocytogenes | Listeria innocua | Listeria ivanovii | Listeria seeligeri | Listeria welshimeri |
|------------------|----------------|------------------|------------------------|------------------|-------------------|-------------------|---------------------|
| Raw milk         | 75             | 10               | (13.33%)               | 6 (35.29%)       | 7 (41.17%)        | 1 (5.89%)         | 1 (5.89%)           |
| Pasteurized milk | 50             | 6               | (12%)                  | 3 (37.5%)        | 2 (25%)           | 0 (0%)            | 0 (0%)              |
| Ice cream        | 50             | 7               | (14%)                  | 5 (41.67%)       | 4 (33.33%)        | 1 (8.33%)         | 0 (0%)              |
| Ras cheese       | 50             | 6               | (12%)                  | 2 (28.57%)       | 3 (42.86%)        | 0 (0%)            | 0 (0%)              |
| Total            | 225            | 29 (12.88%)     | 16 (7.11%)             | 16 (7.11%)       | 14 (6.25%)        | 5 (2.22%)         |                     |

Table (4): Prevalence of Listeria monocytogenes isolated from examined milk and dairy products samples in Comparison with Egyptian Standards.

| Products               | No. of examined samples | Egyptian Standards | Samples do not conform with Egyptian Standards | No. % |
|------------------------|-------------------------|---------------------|-----------------------------------------------|-------|
| Raw milk               | 75                      | Nil (ES:154-1/2005) | 10                                            | 13.33 |
| Pasteurized milk       | 50                      | Nil (ES:1616/2005)  | 6                                             | 12    |
| Ice cream              | 50                      | Nil (ES:1185-1/2005)| 7                                             | 14    |
| Ras cheese             | 50                      | Nil (ES:1007/5/2005)| 6                                             | 12    |

Table (5): Prevalence of virulence genes of Listeria monocytogenes isolated from examined milk and dairy products samples.

| Products               | No. of examined isolates | Virulence genes |
|------------------------|--------------------------|-----------------|
|                        | iap                      | hlyA             | actA             |
| Raw milk               | 6                        | 60               | 106              | 83.3 | 4  | 66.7 |
| Pasteurized milk       | 3                        | 10               | 2                | 66.7 | 2  | 66.7 |
| Ice cream              | 5                        | 5                | 10               | 4    | 80 | 4  |
| Ras cheese             | 2                        | 2                | 10               | 1    | 50 |    |
| Total                  | 16                       | 16               | 100              | 13   | 81.3| 11 | 68.8|

Table (6): Antimicrobial susceptibility profile of Listeria monocytogenes isolated from examined milk and dairy products samples (n=16).

| Antimicrobial agents   | Sensitivit y disc content (μg) | Susceptibility | Intermediate | Resistant |
|------------------------|--------------------------------|----------------|--------------|-----------|
| Kanamycin (K)          | 30                             | -              | -            | 16        |
| Nalidixic acid (NA)    | 30                             | -              | -            | 15        |
| Streptomycin (S)       | 10                             | 1              | 6.2          | 13        |
| Neomycin (N)           | 30                             | 3              | 18.75        | 9         |
| Amikacin (AK)          | 30                             | 4              | 25           | 0         |
| Oxytetracycline (T)    | 30                             | 2              | 12.5         | 5         |
| Cephalothin (CN)       | 30                             | 6              | 37.5         | 4         |
| Erythromycin (E)       | 15                             | 7              | 43.75        | 6         |
| Sulfamethoxazole (SXT) | 25                             | 9              | 56.25        | 1         |
| Enrofloxacin (EN)      | 5                              | 9              | 56.25        | 2         |
| Gentamicin (G)         | 10                             | 8              | 50           | 4         |
| Cefotaxime (CF)        | 30                             | 10             | 62.50        | 2         |
| Ampicillin (AM)        | 10                             | 11             | 68.75        | 3         |
| Ciprofloxacin (CP)     | 5                              | 14             | 87.50        | 1         |

5
Photograph (1): Agarose gel electrophoresis of multiplex PCR of iap (131 bp), hylA (456 bp) and actA (839 bp) virulence genes for characterization of L. monocytogenes.
Lane M: 100 bp ladder as molecular size DNA marker.
Lane C+: Control positive L. monocytogenes for iap, hylA and actA genes.
Lane C-: Control negative.
Lanes 1, 2, 4, 7, 12, 14 & 16: Positive L. monocytogenes strains for iap, hylA and actA genes.
Lanes 5, 6, 9, 11 & 15: Positive L. monocytogenes strains for iap and hylA genes.
Lanes 3, 8 & 10: Positive L. monocytogenes strains for iap and actA genes.