

# List of Contents

<b>Title</b>	<b>Page</b>
<b>1. INTRODUCTION</b>	<b>1</b>
<b>2. REVIEW OF LITERATURE</b>	<b>5</b>
<i>2.1. Incidence of Listeria species in milk and dairy products.</i>	<b>5</b>
<i>2.2 Sources of contamination of milk and milk products with listeria.</i>	<b>23</b>
<i>2.3. Methods of isolation and bacteriological characters of Listeria species.</i>	<b>29</b>
<i>2.4. Antimicrobial susceptibility of Listeria.</i>	<b>38</b>
<i>2.5. Detection of listeria monocytogenes and their Virulence factors using PCR assay.</i>	<b>41</b>
<b>3. MATERIAL AND METHODS</b>	<b>58</b>
<b>3.1. MATERIAL</b>	<b>58</b>
<b>3.1.1. Samples</b>	<b>58</b>
<b>3.1.2. Bacteriological Media</b>	<b>58</b>
<b>3.1.3. Reagents and solutions</b>	<b>60</b>
<b>3.1.4. Stain</b>	<b>61</b>
<b>3.1.5. Antimicrobial Sensitivity discs</b>	<b>61</b>

<b>3.1.6. Test strains for CAMP test</b>	<b>62</b>
<b>3.1.7. Rabbits</b>	<b>62</b>
<b>3.1.8. Materials used for polymerase chain reaction</b>	<b>62</b>
<b>3.1.9. Material used for agarose gel electrophoresis</b>	<b>64</b>
<b>3.1.10. Equipment and apparatuses used in cPCR</b>	<b>65</b>
<b>3.2. Methods</b>	<b>66</b>
<b>3.2.1. Isolation and identification of Listeria strains</b>	<b>66</b>
<b>3.2.2. In-Vitro antimicrobial Sensitivity test for isolated <i>L. monocytogenes</i></b>	<b>72</b>
<b>3.2.3. Virulence tests of isolated Listeria strains</b>	<b>72</b>
<b>3.2.4. Methods of PCR technique</b>	<b>73</b>
<b>4. RESULTS -----</b>	<b>77</b>
<b>5. DISCUSSION -----</b>	<b>92</b>
<b>6. CONCLUSIONS-----</b>	<b>98</b>
<b>7. SUMMARY-----</b>	<b>99</b>
<b>8. REFERENCES -----</b>	<b>101</b>
<b>9.ARABIC SUMMARY -----</b>	<b>-</b>

# **LIST OF TABLES**

<b>Table No.</b>	<b>Table</b>	<b>Page</b>
<b>I</b>	<b>Antibiotic standardized discs used.</b>	<b>62</b>
<b>II</b>	<b>Oligonucleotide primers sequences Source.</b>	<b>63</b>
<b>III</b>	<b>Biochemical reaction of Listeria species.</b>	<b>71</b>
<b>IV</b>	<b>PCR components used in the PCR technique.</b>	<b>75</b>
<b>V</b>	<b>Cycling conditions of the different primers during cPCR.</b>	<b>75</b>
<b>1</b>	<b>Percentage of positive samples of Listeria spp. isolated from milk and milk products.</b>	<b>77</b>
<b>2</b>	<b>Incidence of Listeria species strains isolated from milk and milk products.</b>	<b>79</b>
<b>3</b>	<b><i>In-vitro</i> antimicrobial Sensitivity test for isolated <i>L. monocytogenes</i> strains.</b>	<b>85</b>
<b>4</b>	<b>The results of PCR amplifications of different used genes of <i>L. monocytogenes</i>.</b>	<b>87</b>

# LIST OF FIGURES

Figure No.	Figures	Page
1	Percentage of positive samples of <i>Listeria</i> spp. isolated from studied samples.	78
2	Percentage of <i>Listeria</i> species in examined samples.	80
3	Total number of <i>Listeria</i> species isolated from examined samples.	80
4	<i>Listeria</i> on ALOA agar	82
5	<i>Listeria</i> on PALCAM agar	82
6	Sugar fermentation for <i>L. monocytogenes</i>	84
7	Sugar fermentation for <i>L. grayi</i>	84
8	Motility of <i>Listeria</i> in semisolid media	84
9	<i>In-vitro</i> antimicrobial Sensitivity test for isolated <i>L. monocytogenes</i> strains.	86
10	<i>16S rRNA</i> genes	88
11	internalin A ( <i>inlA</i> ) genes and Positive regulatory factor ( <i>PrfA</i> ) genes.	89
12	internalin B ( <i>inlB</i> ) genes and Listeriolysin O, haemolysin ( <i>hlyA</i> ) genes	90

## LIST OF ABBREVIATIONS

<b>ALOA</b>	<b>Ottaviani and Agosti agar</b>
<b>CAMP test</b>	<b>Christie Atkins Munch Peterson test</b>
<b>CIP</b>	<b>Caseinolytic proteins</b>
<b>hlyA</b>	<b>haemolysin gene</b>
<b>Iap</b>	<b>Invasion associated protein, gene coding for Protein P60</b>
<b>ICMSF</b>	<b>International commission of Microbiological Specification for Foods</b>
<b>IDF</b>	<b>International Dairy Federation</b>
<hr/>	
<b>InIA</b>	<b>internalin A gene</b>
<b>InIB</b>	<b>internalin B gene</b>
<b>ISO</b>	<b>International Organization for Standardization</b>
<i>L. grayi</i>	<i>Listeria grayi</i>
<i>L. innocua</i>	<i>Listeria innocua</i>
<i>L. ivanovii</i>	<i>Listeria ivanovii</i>
<hr/>	
<i>L. monocytogenes</i>	<i>Listeria monocytogenes</i>
<i>L. murrayi</i>	<i>Listeria murrayi</i>
<i>L. seeligeri</i>	<i>Listeria seeligeri</i>
<i>L. welshimeri</i>	<i>Listeria welshimeri</i>
<b>LLO</b>	<b>Listeriolysin O</b>
<b>Mpl</b>	<b>Gene coding for Metalloprotease</b>
<b>NCCLS</b>	<b>National Committee for Clinical Laboratory Standarts</b>
<b>PALCAM</b>	<b>Polymyxin B-acriflavine-Lithium chloride Ceftazidime-Aesculin-Mannitol agar base</b>
<b>PC-PLC</b>	<b>phosphatidycholine-specific phospholipase C</b>
<b>PlcA</b>	<b>gene encodes a secreted phosphatidylinositol-specific phospholipase C (PI-PLC)</b>
<b>PlcB</b>	<b>Gene encodes a phosphatidylcholine phospholipase C</b>
<b>PI-PLC</b>	<b>phosphatidylinositol-specific phospholipase C</b>

<b>PrfA</b>	<b>Positive regulatory factor gene</b>
<b><i>R. equi</i></b>	<b><i>Rhodococcus equi</i></b>
<b><i>S. aureus</i></b>	<b><i>Staphylococcus aureus</i></b>
<b>USDA</b>	<b>United State Department of Agriculture</b>
<b>USFDA</b>	<b>United State Department of Health and Human Services Food and Drug Administration Center for Food Safety and Applied Nutrition</b>
<b>WHO</b>	<b>World Health Organization</b>

### **7. SUMMARY**

Milk and dairy products have high nutritional value and they are very suitable for development of microorganisms, including pathogenic bacteria as **Listeria** species resulting in Listeriosis. Therefore, this study was conducted to estimate the prevalence of Listeria species in milk, soft cheese, Kariesh cheese and ice cream at Kaliobia Governorate with special interest to *L. monocytogenes* and studying their cultural, biochemical and In-vitro antimicrobial Sensitivity for them with special reference to some virulence genes. So, the present study was performed on a total of 200 random samples of raw milk, soft cheese, Kariesh cheese and ice cream (50 samples each) were collected from small retails and different supermarkets at Kaliobia Governorate.

The results of **Listeria spp.** isolation revealed that, 11 out of 200 samples were positive for isolation (5.5%); represented as 3 positive samples (1.5%) from each type of samples of raw milk ; Kariesh cheese and ice cream samples followed by 2 (1.0%) from soft cheese samples. Mixed isolates were present in raw milk samples only.

The results of bacteriological examination of examined samples revealed that, a total of 13(6.5%) isolates of Listeria species were recovered from 200 samples, includes 10 *L. monocytogenes* (5.0%) and 3 *L. grayi* (1.5%). *L. monocytogenes* was isolated with an incidence of 76.9% (3 from each samples of raw milk; Kariesh cheese and ice cream (23.1%) and 1(7.7%) from soft cheese). Meanwhile, *L. grayi* was isolated with an incidence of 23.1% (2 from raw milk samples (15.4%) and 1(7.7%) from soft cheese only). Moreover, the other 4 species (*L. ivanovii* ; *L. innocua* ; *L. seeligeri* and *L. welshimeri* ) could not isolated from all samples .

## ***Summary***

---

The results of antibiotic sensitivity tests for the isolated *L. monocytogenes* cleared that, the isolated *L. monocytogenes* were sensitive to amoxicillin and gentamycin (80.0%) followed by enrofloxacin; kanamycin and ampicillin (70.0%; 70.0% and 60.0% respectively). While the isolated strains were resistant to Nalidixic acid, streptomycin and tetracycline.

The results of virulence tests for isolated *Listeria* strains appeared that, all of *L. monocytogenes* strains were virulent strains, as all of them were positive to CAMP test; showed narrow zone of  $\beta$ -haemolysis on sheep blood agar and were positive for Anton's test. Meanwhile, *L. grayi* strains were non-virulent, as none of them could produce haemolysin (CAMP test negative) and negative for Anton's test.

The PCR results for *L. monocytogenes* showed that, all genes (*16S rRNA*; *inlA* ;*inlB*; *hlyA* and *prfA*) were detected in five studied strains (100.0%) i.e., all studied strains were *L. monocytogenes* and all of them were virulent strains.