

TABLE OF CONTENTS

1. INTRODUCTION.....	1
1.1. <i>Escherichia coli</i>	2
1.1.1. Historical antecedents.....	2
1.1.2. Current taxonomic situation	2
1.1.3. Characteristics	3
1.1.4. Pathogenicity	5
1.1.5. <i>Escherichia coli</i> in food.....	8
1.1.6. <i>Escherichia coli</i> detection in food.....	9
1.2. <i>Salmonella</i> sp.	10
1.2.1. Historical antecedents.....	10
1.2.2. Current taxonomic situation	10
1.2.3. Characteristics	11
1.2.4. Pathogenicity	12
1.2.5. <i>Salmonella</i> sp. in food	13
1.2.6. <i>Salmonella</i> sp. detection in food	14
1.3. <i>Listeria monocytogenes</i>	15
1.3.1. Historical antecedents.....	15
1.3.2. Current taxonomic situation	16
1.3.3. Characteristics	16
1.3.4. Pathogenicity	17
1.3.5. <i>Listeria monocytogenes</i> in food.....	18
1.3.6. <i>Listeria monocytogenes</i> detection in food.....	19
1.4. <i>Staphylococcus aureus</i>	19
1.4.1. Historical antecedents.....	19
1.4.2. Current taxonomic situation	20

1.4.3.	Characteristics	22
1.4.4.	Pathogenicity	23
1.4.5.	<i>Staphylococcus aureus</i> in food	24
1.4.6.	<i>Staphylococcus aureus</i> detection in food	25
2.	OBJECTIVES	27
3.	MATERIAL AND METHODS	28
3.1.	Analysis of samples by cultural method, for the detection of <i>Escherichia coli</i> , <i>Salmonella</i> spp., <i>Staphylococcus aureus</i> and <i>Listeria monocytogenes</i>	28
3.1.1.	Samples studied	28
3.1.2.	Analysis of samples by cultural methods	28
3.1.2.1.	Sample preparation	28
3.1.2.2.	Enumeration of <i>Enterobacteriaceae</i>	29
3.1.2.3.	Isolation and identification of <i>Escherichia coli</i>	29
3.1.2.4.	Isolation and identification of <i>Salmonella</i> spp.	30
3.1.2.5.	Isolation and identification of <i>Staphylococcus aureus</i>	31
3.1.2.6.	Isolation and identification of <i>Listeria monocytogenes</i>	32
3.2.	Analysis of samples by molecular method, for the detection of <i>Escherichia coli</i> , <i>Salmonella</i> spp., <i>Staphylococcus aureus</i> and <i>Listeria monocytogenes</i>	33
3.2.1.	Reference strains	33
3.2.2.	DNA extraction	34
3.2.3.	Selection of primers.....	34
3.2.4.	Primers validation by simplex PCR and temperature gradient.....	35
3.2.5.	Evaluation of primers sensitivity in simplex PCR.....	36
3.2.6.	Analysis of samples by simplex PCR.....	36
3.3.	Simultaneous co-culture and detection of <i>Escherichia coli</i> , <i>Salmonella</i> spp., <i>Staphylococcus aureus</i> and <i>Listeria monocytogenes</i> by multiplex PCR	36
3.3.1.	Selection of a co-culture medium.....	36

3.3.1.1.	Effect of various culture broths on individual growth.....	37
3.3.1.2.	Buffered Peptone Water as co-culture broth.....	37
3.3.1.2.1.	Inoculum preparation	37
3.3.1.2.2.	Effect of BPW on individual and co-culture growth.....	37
3.3.1.2.3.	Effect of BPW on co-culture growth, from artificially inoculated food matrix.....	38
3.3.2.	Development and Optimization of multiplex PCR	39
3.3.2.1.	In silico validation of primers	39
3.3.2.2.	Development of multiplex PCR.....	39
3.3.2.3.	Optimization of multiplex PCR	40
3.3.2.4.	Evaluation of multiplex PCR specificity	41
3.3.2.5.	Evaluation of multiplex PCR sensitivity.....	41
3.3.3.	Detection limits by multiplex PCR from the co-culture.....	41
4.	RESULTS AND DISCUSSION	43
4.1.	Analysis of samples by cultural method, for the detection of <i>Escherichia coli</i> , <i>Salmonella</i> spp., <i>Staphylococcus aureus</i> and <i>Listeria monocytogenes</i>	43
4.1.1.	Enumeration of <i>Enterobacteriaceae</i>	43
4.1.2.	Isolation and identification of <i>Escherichia coli</i>	46
4.1.3.	Isolation and identification of <i>Salmonella</i> spp.	50
4.1.4.	Isolation and identification of <i>Staphylococcus aureus</i>	52
4.1.5.	Isolation and identification of <i>Listeria monocytogenes</i>	56
4.2.	Analysis of samples by molecular method, for the detection of <i>Escherichia coli</i> , <i>Salmonella</i> spp., <i>Staphylococcus aureus</i> and <i>Listeria monocytogenes</i>	57
4.2.1.	Selection of primers.....	57
4.2.2.	Primers validation by simplex PCR and temperature gradient.....	57
4.2.3.	Evaluation of primers sensitivity in simplex PCR.....	59
4.2.4.	Analysis of samples by simplex PCR.....	60

4.3. Simultaneous co-culture and detection of <i>Escherichia coli</i> , <i>Salmonella</i> spp., <i>Staphylococcus aureus</i> and <i>Listeria monocytogenes</i> by multiplex PCR	62
4.3.1. Selection of a co-culture medium.....	62
4.3.1.1. Effect of various culture broths on individual growth.....	62
4.3.1.2. Buffered Peptone Water as co-culture broth.....	64
4.3.1.2.1. Effect of BPW on individual and co-culture growth.....	64
4.3.1.2.2. Effect of BPW on co-culture growth, from artificially inoculated food matrix.....	65
4.3.2. Development and Optimization of multiplex PCR	67
4.3.2.1. In silico validation of primers	67
4.3.2.2. Development of multiplex PCR.....	67
4.3.2.3. Optimization of multiplex PCR	69
4.3.2.4. Evaluation of multiplex PCR specificity	72
4.3.2.5. Evaluation of multiplex PCR sensitivity.....	73
4.3.3. Detection limits by multiplex PCR from the co-culture.....	74
4.3.3.1. Detection limits from BPW co-culture recovery	74
4.3.3.2. Detection limits from BPW co-culture recovery, with artificially inoculated food matrix.....	75
4.4. Methodology proposed to analyze a food sample.....	78
CONCLUSIONS	80
REFERENCES	83