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Development of a real-time multiplex PCR assay for detection of *Salmonellae* in chicken samples

PHD

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Abstract:

Salmonella is the pathogenic agent of salmonellosis, a major cause of enteric illness and typhoid fever. *Salmonella* Typhimurium infects poultry causing significant losses, serovar 1, 4, [5], 12: i: - is considered a monophasic variant of *S. Typhimurium* that increasingly important as public health risk. A total of 58 *Salmonella* isolates were reidentified by slide agglutination test according to White-Kauffmann- Le Minor scheme. A duplex syber green real time PCR was applied for detection of genus *Salmonella* and *S. Typhimurium* using *16S rRNA* and *fliC* genes. All strains harbor *16S rRNA*. Twenty one strains harbor *fliC* gene that including *S. Typhimurium* (12), *S. Kentucky* (6) , *S.1,4,[5],12:i:-* (1) , *S. Lagos* (1) and *S. Kedougou* (1). A duplex Tagman real time -PCR was performed for differentiation between biphasic *S. Typhimurium* and monophasic variant 1,4,[5],12:i:- using *fljB1,2* and *fliB / IS200* in the *Fli A. B* intergenic region. Ten strains out of 12 *S. Typhimurium* harbor *flj B1,2* however strain *S 1,4,[5],12:i:-* don't possess this gene. While 13 strains were positive to *fliB / IS200* (12 *S. Typhimurium* and *S 1,4,[5],12:i:-*). The *fliB / IS200* is specific gene for *S. Typhimurium* (biphasic and monophasic). These results indicate that two strains serologically confirmed to be *S. Typhimurium* and *S.1,4,[5],12:i:-* don't possess *fljB1,2* and have *fliB/ IS200* genes are monophasic variants by a duplex Tagman real time -PCR. It was noticed that prolonged subculture and or repeat phase inversion method leads to formation of flakes that in turn cause wrongly serotyping identification. Real time –PCR is rapid and can be used to identifying and differentiation between biphasic and monophasic *S.1, 4, [5], 12: i: - S. Typhimurium*.

Keywords: Biphasic and monophasic *S. Typhimurium*, *fljB1,2* gene, Real - time PCR, *Salmonel*

LIST OF CONTENTS

Title	Page
Chapter 1:	
1. Introduction	1
Chapter 2:	
2. Review Of Literature	5
2.1. History of <i>Salmonella</i> isolation	5
2.2. Taxonomy and serotyping of <i>Salmonella</i>	5
2.3. <i>Identification of Salmonella and its major antigens</i>	7
2.4. <i>Salmonella</i> as important pathogen	11
2.5. Real time PCR for identification of <i>Salmonella</i>	15
2.6. <i>Salmonella</i> Typhimurium and monophasic variant	18
2.7. Gene sequence for <i>Salmonella</i> Typhimurium	23
Chapter 3:	
3. Published papers	27
Chapter 4:	
4. Discussion	24
Chapter 5:	
5. Conclusion and recommendations	50
Chapter 6:	
6. Summary	51
Chapter 7:	
7. References	53
الملخص العربي	
المستخلص العربي	

LIST OF TABLES

No.	Title	Page
1	Oligonucleotide primers used in this study for detection of genus <i>Salmonella</i> using 16S rRNA and <i>fliC</i> genes.	28
2	Oligonucleotide primers and probes used for differentiating between biphasic <i>Salmonella</i> Typhimurium and monophasic serovar <u>1</u> , 4, [5], 12:i:- using <i>fljB</i> 1,2 and <i>fliB</i> /IS200 in the <i>fliA</i> - <i>fliB</i> intergenic region using Tagmann real time PCR.	29
3	Antigenic structure of all <i>Salmonella</i> strains recovered using slide agglutination test.	30
4	Detection of 16S rRNA and <i>fliC</i> genes in <i>Salmonella</i> serovars using duplex Syber green real-time PCR	31
5	Detection of <i>fljB</i> 1,2 and <i>fliB</i> /IS200 in <i>Salmonella</i> serovars using duplex TaqMan real-time PCR	32
6	Comparison between results of conventional serotyping and real-time PCR for <i>Salmonella</i> Typhimurium (biphasic and monophasic strains)	35

LIST OF FIGURES

No .	Title	Page
1	Syber green real-time PCR targeting <i>16S rRNA</i> gene for 58 <i>Salmonella</i> strains isolated from chickens (fluorescence chart and melting curve).	33
2	Syber green real-time PCR targeting <i>fliC</i> gene for 58 <i>Salmonella</i> strains isolated from chicken (fluorescence chart and melting curve).	34
3	TaqMan real-time PCR amplification chart for <i>fliB</i> 1,2 gene among 58 <i>Salmonella</i> strains isolated from chickens.	35
4	TaqMan real-time PCR amplification chart for <i>fliB/IS200</i> gene among 13 <i>Salmonella</i> isolates (12 <i>S. Typhimurium</i> and the variant strain serotype 1, 4 [5], 12:i:-)	35
5	Amino acid sequence alignment report for <i>fliC</i> gene of two Egyptian <i>Salmonella</i> strains recorded in GenBank with accession number Mk103394 and Mk103395 for <i>S. Typhimurium</i> Egy 1(biphasic) and <i>S. Typhimurium</i> Egy 2 (monophasic).	36
6	Amino acid sequence distance performed using the CLUSTAL W multiple sequence alignment program and version 1.83 of MegAlign module of Lasergene DNASTar software Pairwise for <i>fliC</i> gene among two Egyptian <i>Salmonella</i> strains (<i>S. Typhimurium</i> Egy 1(biphasic) and <i>S. Typhimurium</i> Egy 2 (monophasic)).	37
7	Phylogenetic analysis of <i>Salmonella</i> Typhimurium using <i>fliC</i> gene sequence performed by maximum likelihood, neighbor-joining and maximum parsimony implemented in MEGA6.	38