

بسم الله الرحمن الرحيم

قال تعالى:

﴿هُوَ الَّذِي خَلَقَ لَكُمْ مَّا فِي الْأَرْضِ جَمِيعًا ثُمَّ اسْتَوَىٰ إِلَى السَّمَاءِ فَسَوَّاهُنَّ سَبْعَ سَمَاوَاتٍ وَهُوَ بِكُلِّ شَيْءٍ عَلِيمٌ﴾

صدق الله العظيم

(سورة البقرة، الآية (29)

# Dedication

To my parents, brothers and Sisters  
To my teachers  
To my colleagues

# **ACKNOWLEDGEMENTS**

I wish to express my thanks and gratitude to my advisor, Dr. Humodi Ahmed Saeed, The dean, College of Medical Laboratory Science Sudan University of Science and Technology for all of his support, guidance and encouragement to bring this thesis to light. Appreciation is also expressed to Dr. mogahid, Dr. Miskalyman, Ustaz Mansor for their support and guidance during my degree program. Special thanks are also extended to the staff of the research laboratory, especially the brothers Modather and Montaser for all of their help, and support. Finally, I am very much indebted to all my colleagues for their invaluable help.

## Abstract

This study was carried out in the period from May 2007- March 2008 to detect the presence of *Salmonella enteritidis* in patients suffering from gastroenteritis. Forty-seven stool specimens were collected from patients attending Albangadid teaching hospital and Alzahra medical centre.

Bacterial DNA was extracted from each stool specimen using phenol choloroform technique.

Real-time PCR technique was adopted to detect presence of *Salmonella enteritidis*.

The result revealed that only one (2.1%) specimen was positive.

The study concluded that the Real-time PCR technique facilitates detection of bacterial pathogens without bacteriological culture.

## الخلاصة

هذه دراسة تم اجراؤها في الفترة بين مايو 2007م إلى مارس 2008م لتحديد وجود بكتيريا (السلمونية الأمعائية) في مرضي التهاب المعدة والأمعاء.

تم تجميع سبع وأربعين عينة براز من هؤلاء المرضى من مستشفى البان جديد التعليمي ومركز صحي الزهراء.

تم استخلاص الحمض النووي للبكتيريا من عينات البراز باستخدام تقنية الفينول كلوروفورم.

وقد استخدم تفاعل البلمرة المتسلسلة الزمنية للكشف عن بكتيريا (السلمونية الأمعائية). وأظهرت النتائج وجود عينة ايجابية واحدة (2.1%).

خلصت الدراسة إلى أن تقنية تفاعل البلمرة المتسلسل الزمني تسهل كشف البكتيريا الممرضة بدون اللجوء لتقنيات الاستزراع المخبري.

## Table of Contents

No	Contents	Page
	□□□□□	I
	Dedication	II
	Acknowledgment	III
	Abstract (English)	IV
	Abstract (Arabic)	V
	Table of contents	VI
	List of Tables	VIII
	List of Figures	IX
	<b>Chapter One: Introduction and Literature Review</b>	
1.1	Introduction	1
1.1.1	Entrance	1
1.1.2	The Genus <i>Salmonella</i>	2
1.1.3	History	2
1.1.4	Classification	2
1.1.5	<i>Salmonella enteritidis</i>	3
1.1.5.1	Description	3
1.1.5.2	Pathogenesis	3
1.1.5.3	Habitat	4
1.1.5.4	Virulence factors	4
1.1.5.5	Diseases	4
1.1.5.6	Host defenses	5
1.1.5.7	Epidemiology	5
1.1.5.8	Control	6
1.1.5.9	Diagnosis	6
1.1.5.9.1	Traditional methods	6
1.1.5.9.2	The polymerase chain reaction (PCR)	7
1.1.5.9.2.1	Real-time polymerase chain reaction	7
1.1.5.9.2.1.1	The Evolution of PCR to Real-Time	8
1		
1.1.5.9.2.1.1.1	Real-Time Vs Traditional PCR	8
2		
1.1.5.9.2.1.1.1.1	Types of real time PCR	8
3		
1.1.5.9.2.1.1.1.1.1	Advantages of using Real-Time PCR	10
4		
1.1.5.9.2.1.1.1.1.1.1	Real-Time PCR Applications	10
5		
1.2	Literature Review	11
1.3	Objectives	11
1.3.1	General objective	11

1.3.2	Specific objectives	11
	<b>Chapter Two: Materials and Methods</b>	
2	Materials and Methods	12
1.1	Study Area	12
1.2	Subject	12
1.3	Age Group	12
1.4	Sample Size	12
2.5	Sterilization	12
2.6	Experimental work	12
2.6.1	Collection of specimens	12
2.6.2	Preparation of reagents	12
2.6.3	Extraction	12
2.6.4	DNA amplification and analysis	13
2.6.4.1	Equipments and reagents	13
2.6.4.2	Master Mix preparation	14
2.6.4.3	Plate preparation	15
2.6.4.4	Amplification	15
	<b>Chapter Three: Results</b>	
3	Results	16
3.1	Clinical specimens	16
3.2	DNA Extraction	16
3.3	Detection of <i>Salmonella enteritidis</i>	16
	<b>Chapter Four: Discussion</b>	
4	Discussion	20
	References	22
	Appendices	26

## **List of Tables**

	<b>Page No.</b>
Table 1: Distribution of specimens according to hospitals	16
Table 2: Distribution of specimens according to age	17
Table 3: Distribution of specimens according to gender	17



## **List of Figures**

	<b>Page No.</b>
Figure 1: Shows typical amplification of Real-time PCR	<b>18</b>
Figure 2: Shows a real sigmoid curve for all specimens	<b>18</b>
Figure 3: Shows the reading of non-template control (NTC)	<b>19</b>
Figure 4: Shows a real sigmoid curve for the positive specimen and NTC	<b>19</b>