

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

قال الله تعالى

اَفْرَأَ يَا سَمِّ رَبِّكَ الَّذِي خَلَقَ (1) خَلَقَ الْإِنْسَانَ
مِنْ عَلَقٍ (2) اَفْرَأَ وَرَبِّكَ الْاَكْرَمُ (3) الَّذِي
عَلَمَ بِالْقَلْمَ (4) عَلَمَ الْإِنْسَانَ مَا لَمْ يَعْلَمْ (5)

صدق الله العظيم

سورة العلق الآيات 5-1

Dedication

To my parents
To my brothers and my sisters
To my wife and my lovely sons

To my teachers and
my friends

Who give me the strength
And support

ACKNOWLEDGEMENT

First of all, I praise Allah Almighty for His infinite support and guidance. Secondly I would like to convey my deep gratitude to my supervisor Dr.Humodi Ahmed Saeed for his invaluable advice and directions I would also like to thank all my friends for their encouragement and support during the preparation of this dissertation. Last, but not least my special thanks go to Dr. Mogahid Mohammed Elhassan, Dr. Misk Elyaman and, Ustaz Mansour Mohammed Mansur for their technical support.

Abstract

This study was carried out during the period from May 2007 to March 2008 to detect the presence of *Enterobacter cloacae* in stool specimens from patients suffering from gastrointestinal disturbance. Forty six samples were collected from patients attended to Khartoum Teaching Hospital, Omdurman Teaching Hospital, Khartoum pediatrics Hospital and Omdurman pediatrics Hospital.

DNA was extracted directly from stool specimens and used for the subsequent experiment.

The forty six specimens were examined using Real time PCR Technique. The study revealed only seven (15%) specimens were positive and the remained specimens thirty nine (85%) were negative.

The study concluded that the real-time PCR technique facilitates rapid detection of bacterial pathogens directly from clinical specimen without culturing of bacteria

الخلاصة

هذه دراسة تم اجراؤها في الفترة بين مايو 2007م إلى مارس 2008م لتحديد وجود بكتيريا القولونية العصوية في عينات البراز من مرضى يعانون من اضطراب الجهاز الهضمي.

تم تجميع ست واربعون عينة من المرضى الذين يعانون من اضطرابات في الجهاز الهضمي حضروا إلى مستشفى الخرطوم التعليمي، مستشفى امدرمان التعليمي، مستشفى الخرطوم للأطفال، مستشفى امدرمان للأطفال. استخلص الحمض النووي مباشرة من جميع العينات واستخدمت لإجراء التجربة. اسفرت النتائج عن سبعة عينة ايجابية تمثل (15%) من مجموع العينات وما تبقى تسع وثلاثون (85%) نتائجها سلبية. خلصت الدراسة ان تقنية البلمرة المتسلسل الزمني يسهل الاكتشاف السريع للبكتيريا الممرضة مباشرة في العينة السريرية دون استخدام طريقة التزريع.

Table of Contents

No	Contents	page
	اللّا	I
	Dedication	II
	Acknowledgement	III
	(Abstract (English	IV
	(Abstract (Arabic	V
	Table of contents	VI
	List of Tables	VIII
	List of Figures	IX
	Chapter One: Introduction and literature review	
1.1.1	Entrance	1
1.1.2	The genus <i>Enterobacter</i>	2
1.1.2.1	Definition of genus	2
2.2 .1.1	Habitat	2
2.3 .1.1	Clinical significant	2
1.1.2.3.1	Transmission	2
2.3.2 .1.1	Antigenic structure	3
2.3.3 .1.1	Virulence Factors	3
1.1.2.3.3.1	Toxins	3
1.1.2.3.3.2	Antibiotic resistance	3
2.4 .1.1	Pathogenesis	4
2.5 .1.1	Diseases caused by <i>E.cloacae</i>	4
2.5.1 .1.1	Bacteremia	4
2.5.2 .1.1	Skin and soft tissues infections	4
3 .1.1.2.5	Urinary tract infections	4
1.1.2.5.4	Intra-abdominal infection	4
.1.1.2.6	Laboratory diagnosis	5
1.1.2.6.1	Microscopy	5
6.2 .1.1.2	Culture methods	5
6.3 .1.1.2	Molecular diagnosis	5
a.1.1.2.6.3	(Polymerase Chain Reaction (PCR	6
b.1.1.2.6.3	(Quantitative PCR (Real-time PCR	6
c.1.1.2.6.3	Real-time PCR Versus Traditional PCR	7
1.2	Literature Review	8
1.3	The Objectives of the study	9
	Chapter Two :Materials and Methods	
2.1	Study Area	10
2.2	Subjects	10
2.3	Age group	10
2.4	Sample size	10
2.5	Sterilization	10
2.6.1	Glass wares and other requirements	10

.2.7	Experimental work	10
2.7.1	Collection of specimens	10
2.7.2	Pre-examination treatment	10
2.7.3	Preparation of reagent for DNA extraction	10
2.7.4	Method of DNA extraction	11
2.7.3	DNA amplification and analysis	11
2.7.3.1	Equipments and reagents	11
2.7.3	PCR amplification	12
2.7.3.1	PCR Protocol	12
	Chapter Three: Results	13
3	Result	13
3.1	Clinical specimens	13
3.2	DNA Extraction	13
3.3	Detection of <i>E.cloacae</i>	13
	Chapter four: Discussion	
4	Discussion	20
	References	22
	Appendices	25

List of Tables

Table 1: Distribution of specimens according to hospitals 13

Table 2: Distribution of specimens according to age 13

List of Figures

Figure 1: sigmoid curve of the positive specimen	14
Figure 2: sigmoid curve of the positive specimen	14
Figure 3: sigmoid curve of the positive specimen	15
Figure 4: sigmoid curve of the positive specimens and control	
15	
Figure 5: sigmoid curve of the positive specimen	16
Figure 6: sigmoid curve of the positive specimen	16
Figure 7: sigmoid curve of the positive specimen	17
Figure 8: sigmoid curve of the positive specimen	17
Figure 9: positive results with sigmoid curve of specimens and control	18
Figure 10: Shows plate layout	19