# Dedication

...To the soul of my father
Who worked hardly for us
..To my dear mother
..To my husband & lovely kids
..To my brothers & my sisters
..To whom I love

## Acknowledgment

First of all I would like to thank Allah who blessed me, and gave me the health, patience, and the will to achieve this project.

My greatest appreciation to the **German Academic Exchange Service (DAAD)** for their co-operation, and sponsoring this work by a complete scholarship.

A special appreciation and gratitude to my supervisor **Dr. Hummodi Ahmed Saeed**, Dean College of Medical
Laboratory Sciences, Sudan University of Science
&Technology, for his unlimited follow up, and who was in
constant contact with me and helped me greatly by providing
up-to-date information for this work,.

Professor Elfadhil Elobied Omer (Al Neelain University) who had suggested this topic which was to my interest and guided me throughout the thesis by his encouraging ideas. Thanks are also to Dr. Munsoor Mohammed Munsoor (Hematology Department, Sudan University) for his support and technical assistance, Dr. Abd Alla Osman (Khartoum University) and also Dr. Mogahid Mohammed El Hassan, and Dr. Misk Elyemen Abd Elatti Abdalla (Sudan University of Science & Technology). My thanks and gratitudes are also extended to the staff members of Microbiology Department, and Research Laboratory- Sudan University, and staff of Mandella and Wad Elbashir camps health clinics for their help.

I would like to thank every one who had a participating hand that caused this work to see the light. It was through the encouragement and support I received from friends and family that I was able to proceed. Therefore here I am not able to discriminate and name people.

Last, but not least, I would like to thank the dean and colleagues at the Faculty of Medical Laboratory Sciences, Al Neelain University for their un-lasting encouragement.

#### **ABSTRACT**

Over a period of 2 years (From January 2006 to January 2008), 710 faecal samples were collected from residents of two internally displaced persons camps, Khartoum State, with acute or chronic diarrhea, and investigated for bacterial, viral, and parasitic enteropathogens.

Specimens were collected from patients who were attending clinics (all age groups were included) using standard method, microbiological investigations were performed using conventional and molecular methods.

The results revealed that 77.5% of patients with diarrhea were infected with enteropathogens, among them 35.2% had a parasitic, 26.8% a bacterial, 13.9% a viral, and 1.5% had mixed infections.

*Shigella* spp. was the pathogen most frequently detected, accounting for 15.5% of all positive findings in the patients, followed by Rotavirus (14.8%), *Hymenolepsis nana* (13.4%), however, *Schistosoma mansoni* and *Cryptosporidium* spp. were the least detected pathogens (1.1% each). Most of patients affected with diarrhea were children under five years (46.5%), and Rotavirus was the most prevalent enteropathogen in this age group with 29.6% frequency rate, however it was *Shigella* spp. among adults, and children over 5years (18.3% both), and most of *Shigella* spp. were *S. dysenteriae* (42.5%).

Further characterization of identified Rotavirus isolates was done using real time polymerase chain reaction (RT-PCR), revealed that, they were type A (95.2%), and type C (4.8%).

Out of the total bacterial pathogens, high resistance was observed against amoxicillin (70.9%) then erythromycin (48.7%), and co-trimoxazole (45.2%). And the lowest resistance to cefuroxime (26.6%).

Most of the *Shigella* spp. isolates were sensitive to cefuroxime (65.5%), but high degree of resistance was observed against amoxicillin (73.6%). Most of the patients consumed untreated drinking water, depend on donkey carts as water source, live in very poor condition, they suffering from crowdness, and had no disposal latrines; all of them were risk factors for acquiring diarrhea.

In conclusion, these results revealed that most of diarrhea cases in displaced camps were of parasitic etiology. The most affected group was children under five years. The most frequently identified pathogen was *Shigella* spp., and most of it were multidrug resistant. Rotavirus had big role in diarrhea, mainly type A especially among children under five years. Further research is needed to adapt and simplify interventions, and to explore novel diagnostics, vaccines, and therapies.

#### 

على مدى سنتين (من يناير 2006 إلى يناير 2008)، تم جمع 710 عينة براز من الم قيمين بمخيمين من مخيمات النازحين داخليا، بولاية الخرطوم، و الذين كانوا يعانون من الإسهال الحاد أو المزمن، وذلك بهدف التد قق من الممرضات المعوية البكتيرية والطفيلية و الفيروسية.

جمعت العينات من المرضى الذين ترددوا على عيادات (و ضم ذلك جميع الفئات العمرية)، باستخدام الطرق المخبرية الاقياسية المعتادة، و تم التح قق باستخدام الطرق الت قليدية والجزيئية. أظهرت النتائج أن 77.5 ٪ من المرضى الذين يعانون من الاسهال كانوا مصابين بأمراض معوية، منهم 35.2 ٪ حالات مرضية بالطفيليات و 26.8 ٪ بالجراثيم و 1.5 ٪ حالات مرضية مختلطة.

وكانت 000000 هي الممرض المعوي الأكثر شيوعا، بما يمثل 15.5 ٪ من النتائج الإيجابية في جميع المرضى ، وتلاه فيروس الروتا (14.8 ٪) و 00000000 0000 (13.4 ٪) ، وكانت كلتا شستسوما مانسوناي و الكربتوسبورديم الله قل ترددا (1.1 % كل).

معظم المرضى المصابين بالإسهال هم من الأطفال دون سن خمس سنوات (46.5 ٪)، وفيروس الروتا كان الممرض المعوي الأكثر شيوعا في هذه الفئة العمرية (29.6 ٪)، ولكن كانت 000000 بين البالغين والأطفال فوق خمس سنوات ((18.3٪ على حد سواء، ومعظمها كانت 0000000 0000000 (42.5 ٪).

تم عمل توصيف لفيروس الروتا الذي تم التعرف عليه, وذلك باستخدام تفاعل البلمرة المتسلسل الزمني , و كشف ذلك ، عن نوع  $(4.8 \ \text{A})$  ، ونوع  $(4.8 \ \text{C})$  .

تم إجراء اختبار الحساسية تجاه المضادات الميكروبية لكل البكتيريا التي تم عزلها ، واظهرت النتائج وجود م قاومة عالية ضد أموكسيسيلين (70.9 ٪) ثم اريثروميسين (48.7 ٪) ، وأدنى م قاومة سيفروكسيم (26.6 ٪).

اظهرت معظم سلالات الالالال المساسية للسيفروكسيم ( 65.5 ٪)، ولكن درجة عالية من الم قاومة ضد أموكسيسيلين ( 73.6 ٪ ).

معظم المرضى يعتمدون على معدات تجرها الدواب لجلب المياه، غير المعالجة، ويعيشون في حالة بيئة متردية صحيا، حيث يعانون من الازدحام السكاني، و لا توجد دورات مياة، و هذه تعتبر من عوامل اكتساب الإسهال. خلصت نتائج الدراسة إلي أن اغلب حالات الإسهال بمخيمات النازحين تسببها الطفيليات, و معظم المرضى المصابين بالإسهال هم من الأطفال المصابين دون سن خمس سنوات, اللهامال هي الممرض المعوي الأكثر شيوعا و قد أظهرت اغلبها مقاومة متعددة لمضادات الجراثيم. بصورة عامة وجد أن السيفروكسيم هو المضاد الأكثر فاعلية لكل البكتيريا المعزولة. و كان لفيروس الروتا دور هام كمسبب للإسهال خاصة النوع A و خاصة في فئة الأطفال دون سن خمس سنوات و هذا يدفع الباحثين لإجراء المزيد من الدراسات، لاكتشاف طرق تشخيصية جديدة و ل قاحات و قائية، و أدوية علاحية.

### **Table of Contents**

Dedication		
Acknowledgment	II	
Abstract	III	
Abstract (Arabic)	V	
Table of Contents	VI	
List of Tables	X	
List of Figures	XI	
List of abbreviations	XIII	
Chapter One: Introduction		
1.1. Introduction	1	
1.2. Rationale	2	
1.3. Objectives	3	
1.3.1. General objectives	3	
1.3.2. Specific objectives	3	
Chapter Two: Literature Review		
2.1. Diarrhea	4	
2.1.1. Definition	4	
2.1.2. Signs & symptoms	4	
2.1.3. Types of Diarrhea	4	
2.1.4. Etiology	5	
2.1.4.1. Pathogenic mechanisms	7	
2.1.5. Epidemiology	8	

2.1.5.1. Transmission	8
2.1.5.2. Age	10
2.1.5.3. Seasonality	10
2.1.5.4. Diarrhea, environment and poverty	10
2.1.5.5. Morbidity	11
2.1.5.6. Mortality	12
2.1.6. Treatment	15
2.1.6.1. Antimicrobial therapy	15
2.1.6.1.1. The effect of antimicrobial resistance on the treatment of enteric infections	17
2.1.6.2. Non-antimicrobial therapy	18
2.1.8. Laboratory diagnosis	19
2.1.9. Syndromic diagnosis	20
2.2. Refugees and displaced communities	22
2.2.1 The displaced community in Sudan	23
2.3. Background studies	28
2.4. Prevention, and control of diarrhea	36
Chapter Three: Materials & Methods	
3.1. Study type	39
3.2. Study area	39
3.3. Target population	39
3.4. Inclusion and exclusion criteria	39
3.5. Study duration	39
3.6. Sample size	39

3.7. Data collection	39
3.8. Ethical consideration	39
3.9. Experimental work	40
3.9.1. Collection of specimens	40
3.9.2. Macroscopic examination	40
3.9.3. Microscopic examination	40
3.9.3.1. Wet mount preparation	40
3.9.3.2. Formal ether sedimentation technique	40
3.9.3.3. Modified Ziehl – Neelsen method	42
3.9.4. Culture for bacterial pathogens	42
3.9.5. Identification of bacterial pathogens	42
3.9.5.1. Colonial morphology	42
3.9.5.2. Gram stain	43
3.9.5.3. Biochemical tests	43
3.9.5.3.1. Identification of Gram negative oxidase negative isolates	43
positive isolates	45 46
3.9.5.5. Antimicrobial sensitivity tests	47
3.9.6. Rota and Adenovirus detection	48
3.9.6.1. Immuno- chromatography test (ICT)	48
3.9.6.2. Characterization of Rotavirus using reverse transcription real time PCR	50
3.9.6.2.1. RNA extraction	50
3.9.6.2.1. Real time- Reverse Transcription PCR Protocol	51
3.9.6.2.1.1. Preparation of real time- reverse	
transcription PCR mixture	51

3.9.6.2.1.2. Prin	ners design, and preparation		52
3.9.6.2.1.3. Prep	paration of a reaction master mix Frequency of different identified enteropathogens		53
	canations of planted for mean time PCR	62	53
3.9. <del>6</del> .2.1.5. PCF	Sondition of different enteropathogens according		54
	R Production and Spring Phosphology (Phosphology Phosphology Phosp	63	
me 3.1 <b>7</b> .ªÞæaanaly	cthods)	64	55 55
4. <b>Рредија</b> ѕ4	· Distribution of identified bacterial enteropathogens		56
	according to patients age group Discussion	65	
	· Distribution of Rota and Adenoviral enteropathogens according to patients age groups apter Six: Conclusion & Recommendations	66	85
Cn	apter Six: Conclusion & Recommendations		
6.1.Tablectusion	Type and frequency of mix pathogens among different age groups	75	96
	dations	75	97
Table 7. <b>References</b>	Antimicrobials resistance pattern among the total isolates (n=199)	77	98
Appendices	· Antimicrobials· resistance pattern among different		112
	Shigella spp. isolates (n= 87)	78	
Table 9.	Antimicrobials resistance pattern among different <i>Salmonella</i> spp. isolates (n= 51)	79	

## **List of Tables**

## **List of Figures**

Fig.1.	Age distribution of study group	60
Fig.2.	Frequency of different etiology of diarrhea amongdisplaced persons-Khartoum state	62
Fig3.	A real sigmoid curves for the specimens in plate No (1) (including positive and negative results)	67
Fig.4.	Plus/Minus scoring analysis showing positive samples of plate1, above the base line (threshold)	67
Fig.5. Fig.6.	The reading of non template control (NTC)(plate 1)  Plus/Minus scoring analysis of non template control (NTC) plate 1)	68 68
Fig.7.	A real sigmoid curves of specimens in plate No (2) (including positive and negative results)	69
Fig.8.	Plus/Minus scoring analysis showing positive samples (plate2), above base line (threshold)	69
Fig.9.	(The reading of non template control (NTC) (plate 2	70
Fig.10.	Plus/Minus scoring analysis of non template control (NTC) plate 2)	70
Fig.11.	A real sigmoid curve for specimens in plate No (3) (including positive and negative results)	71
Fig.12.	Plus/Minus scoring analysis showing positive samples of plate 3, above base line (threshold)	71
Fig.13	The reading of non template control (NTC)(plate 3	72
Fig.14	Plus/Minus scoring analysis of non template control (NTC) (plate 3)	72
Fig.15	A real sigmoid curves for specimens in plate No (4) including positive and negative results	73
Fig.16	Plus/Minus scoring analysis showing positive and negative samples of plate 4, above base line	73
Fig.17	The reading of non template control (NTC) (plate 4)	74
Fig.18	Plus/Minus scoring analysis of non template control (NTC) (plate 4)	74
Fig.19	Frequency of pathogens typenmong different patients age group	76

#### **List of Abbreviations**

WHO: World Health Organization.

IDPs: internally displaced persons.

RT-PCR: Reverse Transcription – Polymerase Chain Reaction.

ORT: Oral Rehydration Therapy.

UNICEF: United Nations Children's Fund.

CMR: Crude Mortality Rate.

CDC: Centre of Disease Control.

EPEC: enteropathogenic *E. coli*.

VTEC: vero-toxogenic *E. coli*.

EHEC: Enterohaemorrhagic *E. coli*.

OR: Odd Ratio.

API: Analytical Profile Index.

ICT: Immuno- chromatography test.

NCBI: National Center for Biotechnology Information.

NTC: Non Template Control.

ELISA: Enzyme Linked Immunosorbent Assay.