

بسم الله الرحمن الرحيم

قال تعالى:

﴿ تَرْفَعُ دَرَجَاتٍ مَّنْ تَشَاءُ وَفَوْقَ كُلِّ ذِي عِلْمٍ
عَلِيمٌ ﴾

صدق الله العظيم

(سورة يوسف الآية 76)

Dedication

I dedicate this work

To the soul of my father

**To my great
mother**

To my kind brothers and sisters

Acknowledgment

All my praise and thanks to Allah who help me and give me confidence to complete this study.

With my great respect I want to thank my supervisor Dr. Humodi Ahmed Saeed for his kindness, great support and advices.

Also I want to thanks Ustaz. Mohammed Masaad and Ustaz. Asjad M. Mukhtar for their support.

Thanks are also to the staff of Microbiology Department staff and to the members of Research Laboratory for their efforts and patience during the practical part of this study.

I am very grateful to the members of Microbiology Departments in hospitals (Gaffar Ibn Auaf, Bashair and Alban Jadeed) for their helps, great support and permission to collect samples for this study, especially Alban Jadeed hospital.

My grateful thank to my family for their encouragement and support specially my kind mother.

Abstract

The study was conducted in Khartoum State during the period from November 2008 to April 2009, to isolate bacteria that cause acute diarrheal diseases in patients who have no previous history of diarrhea and to determine antimicrobial sensitivity of the isolated pathogens.

A total of two hundred diarrheal specimens were collected from Gaffar Ibn Auf Specialized hospital for Children (GIASH), Bashair Teaching Hospital and Alban Jadeed Teaching Hospital. The specimens were transported in transport medium and inoculated into a variety of selective media for primary isolation of pathogens. The bacteria identification was done by API 20 E and slide agglutination test. Modified Kirby-Bauer disc diffusion method was adopted to determine sensitivity of isolates to traditionally used antimicrobial agents. E test was adopted to determine the MIC of chloramphenicol, tetracycline, gentamicin, ciprofloxacin, ceftazidime and co-trimoxazole.

The results showed that *Escherichia coli* represent 57%, *Salmonella typhi* represent 2.5%, in which resistance rate was (100%) to tetracycline and ceftazidime, (60%) to co-trimoxazole, nalidixic acid and amoxicillin, (0%) to ciprofloxacin, gentamicin, ceftriaxone and chloramphenicol.

Shigella sonnei represent 2.0% and resistance rate was (100%) to co-trimoxazole, tetracycline and amoxicillin, (50%) to ceftazidime and (0%) to ciprofloxacin, chloramphenicol, gentamycin, ceftriaxone and nalidixic acid.

S. typhi MIC ranges to chloramphenicol were (0.1–0.5 µg/ml), tetracycline were (10-60 µg/ml), MIC range of gentamicin (0.1–0.25 µg/ml), MIC range of ciprofloxacin (0.004 -0.008 µg/ml), the MIC range of ceftazidime (1–7.5 µg/ml), MIC₅₀ and MIC₉₀ were 30µg/ml to tetracycline, 0.004µg/ml to ciprofloxacin, 0.1µg/ml to gentamicin respectively, 0.1µg/ml to chloramphenicol,

and the MIC₅₀, MIC₉₀ of ceftazidime were 1µg/ml and 3µg/ml respectively. *Shigella sonnei* MIC ranges of tetracycline were (120 – >240 µg/ml), were (5–7.5 µg/ml) to ceftazidime, (0.01–2 µg/ml) to ciprofloxacin and (4 - >240 µg/ml) to co-trimoxazole. The MIC₅₀ and MIC₉₀ were 120 µg/ml and >240 µg/ml to tetracycline, 5.0 µg/ml and 7.5 µg/ml to ceftazidime, 0.01 µg/ml and 0.1 µg/ml to ciprofloxacin, > 240 µg/ml to co-trimoxazole respectively.

The study concluded that responsibility of *Salmonella typhi* and *Shigella sonnei* in diarrheal disease was slightly high and resistance to antimicrobial agents also high.

النتائج

اجريت هذه الدراسة فى ولاية الخرطوم فى الفترة من نوفمبر للعام 2008 الى ابريل للعام 2009 لعزل المسببات البكتيرية للاسهالات وتحديد المضادات البكتيرية . و قد تم جمع مائتين عينة من مرضى الاسهالات من مستشفى جعفر بن عوف التخصصى للاطفال ،مستشفى بشائر التعليمى و مستشفى البان جديد التعليمى تم ذ قتل هذه العينات فى وسط نا قل ومن ثم تم ترريعها فى اوساط انتد قائية مختلفة للعزل الاولى للبكتيريا الممرضة. تم التعرف على البكتريا باستخدام دليل لمحة الحياة التحليلى و اختبار التراص باستخدام الشريحة . استخدمت طريقة انتشار قرص كيبرى – بير لتحديد الحساسية للمضادات الميكروبية المعتادة واختبار إى لتحديد ا قل تركيز يثبت البكتريا المعزولة. اظهرت الدراسة ان الاشريشية القولونية تمثل نسبة 57% ، السلمونيلة التيفية تمثل نسبة 2.5% و التى اظهرت فى اختبار الحساسية م مقاومة كلية لمضادات التتراسيكلين و السفتازيديم بينما اظهرت نسبة م مقاومة قدرها 60% لكل من الكوترايموكسازول ، النالدكسك اسد والاموكسيسيلين. بينما اظهرت حساسية لكل من السبروفلوكساسين، الجنتاميسين، السيفترياكسون والكلورامفينكول.

نجد ايضا ان الش قليلة سوناي تمثل نسبة 2.0% و قد اظهرت م مقاومة كلية لكل من مضادات الكوترايموكسازول ،التتراسيكلين و الاموكسيسيلين، اظهرت نسبة م مقاومة 50% لمضاد السفتازيديم بينما اظهرت حساسية لكل من السبروفلوكساسين، الكلورامفينكول، الجنتاميسين، السيفترياكسون والنالدكسيك اسد. كذلك تم تحديد مدى ا قل تركيز يثبت البكتريا للمضادات بالنسبة للسلمونيلة التيفية فوجد ان للكلورامفينكول هو (0.1- 0.5 ميكروغرام/مل) وايضا ا قل تركيز يثبط 50% و 90% هما 0.1 ميكروغرام/مل و 0.5 على التوالي ، التتراسيكلين هو (10 – 60 ميكروغرام/مل)؛ ا قل تركيز يثبط 50% و 90% هو 30 ميكروغرام/مل على حدالسواء، الجنتاميسين 0.1- 0.25 ميكروغرام/مل)؛ ا قل تركيز يثبط 50% و 90% هما 0.1 ميكروغرام/مل ، السبروفلوكساسين (0.004 – 0.008 ميكروغرام/مل)؛ ا قل تركيز يثبط 50% و 90% هما 0.004 ميكروغرام/مل كل على حدى و للسفتازيديم (1.0 - 57. ميكروغرام/مل)؛ ا قل تركيز يثبط 50% و 90% هما 1 µg/ml و 3 على التوالي.

اما بالنسبة للش قليلة سوناي فان ا قل تركيز يثبط هذه البكتريا قد حدد لكل مضاد علي حدى كالاتى ، التتراسيكلين (120 - 240ميكروغرام/مل)؛ ا قل تركيز يثبط 50% و 90% هما 120 و 240 < ميكروغرام/مل، السفتازيديم (5 – 7.5ميكروغرام/مل) هما 5.0 و 7.5 ميكروغرام/مل، السبروفلوكساسين (0.01 – 2.0 ميكروغرام/مل)؛ ا قل تركيز يثبط

50% و 90% تمثلا 0.01 و 0.1 ميكروغرام/مل على التوالي والكوترايموكسازول (4 - <240 ميكروغرام/مل) و اقل تركيز يثبط 50% و 90% هما <240 µg/ml كل على حدى .

خلصت هذه الدراسة الى ان السلمونية التيفية و الشيغلة سوناي تمثلا نسبة عالية كاحد مسببات الاسهالات و نسبة مقاومتها للمضادات الميكروبية اعلى ايضا.

Table of Contents

	الإهداء	I
	Dedication	II
	Acknowledgment	III
	Abstract	IV
	Abstract(Arabic)	VI
	Table of Contents	VIII
	List of Tables	XIII
	List of Figures	XIV
	List of Color Plates	XV
	Chapter One: Introduction and Objectives	
	Introduction	1
	Rationale	2
	Research Questions	2
	Objectives	2
	General Objective	2
	Specific objectives	2
	Chapter Two: Literature Review	
2.1	Diarrhea	4
2.1.1	Definition	4
2.1.2	Types	4
2.1.2.1	Watery diarrhea	4
2.1.2.2	Dysentery	4
2.1.2.3	Enteric fever	5
2.1.2.2	Other classification	5
2.1.2.2.1	Acute diarrhea	5
2.1.2.2.2	Chronic diarrhea	5
2.1.2.2.3	Spurious diarrhea	5
2.1.3	Bacterial causes	5
2.1.3.1	<i>Escherichia coli (E. coli)</i>	5
2.1.3.1.1	Antigenic structures	6
2.1.3.1.2	Extra- cellular products	6
2.1.3.1.3	Mode of transmission	7
2.1.3.1.4	Pathogenesis and Pathology	7
2.1.3.1.5	Laboratory diagnosis	8
2.1.3.2	<i>Salmonella</i> species	9
2.1.3.2.1	Antigenic structures	10
2.1.3.2.2	Extra-cellular products	10
2.1.3.2.3	Mode of transmission	10
2.1.3.2.4	Pathogenesis and Pathogenicity	10
2.1.3.2.5	Laboratory diagnosis	11

2.1.3.3	<i>Shigella</i> Species	12
2.1.3.3.1	Antigenic structures	12
2.1.3.3.2	Extra-cellular products	13
2.1.3.3.3	Mode of transmission	13
2.1.3.3.4	Pathogenesis and Pathogenicity	13
2.1.3.3.5	Laboratory diagnosis	14
2.1.3.4	<i>Vibrio</i> Species	14
2.1.3.4.1	Antigenic Structures	15
2.1.3.4.2	Extra-cellular Products	16
2.1.3.4.3	Mode of Transmission	16
2.1.3.4.4	Pathogenesis and Pathogenicity	16
2.1.3.4.5	Laboratory diagnosis	17
2.1.3.5	<i>Campylobacter</i> Species	17
2.1.3.5.1	Antigenic Structures	18
2.1.3.5.2	Extra-cellular Products	18
2.1.3.5.3	Mode of Transmission	18
2.1.3.5.4	Pathogenesis and Pathogenicity	19
2.1.3.5.5	Laboratory Diagnosis	19
2.1.3.6	<i>Yersinia enterocolitica</i>	20
2.1.3.6.1	Antigenic Structures	20
2.1.3.6.2	Extra-cellular Products	21
2.1.3.6.3	Pathogenesis and pathogenicity	21
2.1.3.6.4	Laboratory diagnosis	21
2.1.3.7	<i>Clostridium perfringens</i>	22
2.1.3.7.1	Extra-cellular Products	22
2.1.3.7.2	Mode of transmission	22
2.1.3.7.3	Pathogenesis and pathogenicity	22
2.1.3.7.4	Laboratory Diagnosis	23
2.1.3.8	<i>Clostridium difficile</i>	24
2.1.3.8.1	Extra-cellular Products	24
2.1.3.8.2	Mode of Transmission	25
2.1.3.8.3	Pathogenesis and pathogenicity	25
2.1.3.8.4	Laboratory Diagnosis	25
2.1.3.9	<i>Staphylococcus aureus</i> (<i>S. aureus</i>)	26
2.1.3.9.1	Antigenic Structures	26
2.1.3.9.2	Extra-cellular Products	26
2.1.3.9.3	Mode of Transmission	27
2.1.3.9.4	Pathogenesis and pathogenicity	27
2.1.3.9.5	Laboratory Diagnosis	28
2.1.3.10	<i>Bacillus cereus</i>	28
2.1.3.10.1	Extra-cellular products	29
2.1.3.10.2	Mode of Transmission	29

2.1.3.10.3	Pathogenesis and pathogenicity	30
2.1.3.10.4	Laboratory Diagnosis	30
2.1.4	Treatment of diarrheal disease	31
2.1.5	Prevention and control	31
	Chapter Three: Materials and Methods	
3.1	Study design	33
3.1.1	Types of study	33
3.1.2	Study area	33
3.1.3	Target population	33
3.1.4	Data Collection	33
3.2	Collection of specimens	34
3.3	Macroscopical examination	34
3.4	Direct microscope examination	34
3.5	Transportation of specimens	34
3.6	Carbol Fuchsin Stain	34
3.7	Cultivation of specimens	35
3.7.1	Culture Media	35
3.7.2	Inoculation of stool specimens	35
3.8	Examination of bacterial growth	35
3.9	Purification of bacterial growth	35
3.10	Identification of the isolated bacteria	36
3.10.1	Primary identification	36
3.10.1.1	Colonial morphology	36
3.10.1.2	Gram's stain	36
3.10.2	Confirmatory identification	36
3.10.2.1	Oxidase test	36
3.10.2.2	Analytical Profile Index (API 20 E)	37
3.10.2.2.1	Procedure	37
3.10.2.2.2	Interpretation	38
3.11	Identification of <i>verotoxinic E. coli</i>	38
3.12	Serotyping identification	39
3.12.1	Procedure	39
3.13	Antimicrobial Susceptibility test	39
3.13.1	Procedure	39
3.13.2	Interpretation of the zone size	41
3.13.3	Minimum Inhibitory Concentration	41
3.13.3.1	Procedure	41
3.13.3.2	Result and Interpretation	42
3.14	Statistical analysis	43
4.	Chapter Four: Results	
	Results	44
5.	Chapter Five: Discussion	
	Discussion	51
6.	Chapter Six: Conclusion and Recommendations	
6.1	Conclusion	57
6.2	Recommendations	58

References	59
Appendices	68

List of tables

Table 1. Distribution of specimens according to target aged group

Table 2. Frequency of isolated bacteria among enrolled patients

Table 3. Antimicrobial sensitivity test of *S. typhi* and *Sh. sonnei*

Table 4. MIC, MIC₅₀ and MIC₉₀ value of antimicrobial agents to *S. typhi*

Table 5. MIC, MIC₅₀ and MIC₉₀ value of antimicrobial agents of *S. sonnei*

List of Figures

Figure1. Distribution of specimens according to gender

**Figure2. Distribution of specimens according to residence of patients in Khartoum
State**

List of Color Plates

Plate1. Colonies of *Shigella sonnei* on Macconkey

Plate2. Gram negative coccobacilli of *Shigella sonnei*

Plate3. Biochemical result of *E.coli* using API 20 E test