سورة الفاتحة

قال تعالى:

سِنسِ إِلَّهُ الْحَالِمُ الْحَالُمُ الْحَالِمُ الْحَالُمُ الْحَال

صدق الله العظيم

Declaration

I hereby declare that this work is original research work; undertaken under supervision of prof/Dr. Humodi A. Saeed and has not been presented elsewhere for the award of a degree or a certificate. All sources have been cited and appropriately acknowledged.

Nnme: Ali Fadhil Kadhum

Signature: Date: 18/03/2012

Dedication

I dedicate this work:

To my father and mother mercy of Allah, brothers and sisters.

To whom I exceptionally love my wife Dr. Aliaa Fouad Baqir.

To my daughter Noor and my son Yaseen.

To my relative, friends and colleagues.

Acknowledgment

First of all thanks to Almighty Allah who gives me the power to complete this study. With a great deal of respect I would like to express my thanks to my supervisor Prof. Humodi Ahmed Saeed for his advice and patience. This work would not be done without his non-stop guidance. Thanks are extended to my co-supervisor Dr. Mogahid M. EL-Hassan. I am very grateful to the staff of the Microbiology Laboratory of Rashid hospital in UAE for their support. Thanks and appriatiation to Miss. Suheir Ramadan and to all staff of the Research Laboratory in Sudan University of Science and Technology for their utmost support.

Abstract

The presence of extended spectrum β-lactamase (ESBL)- producing bacteria significantly affects the infection management worldwide. The objective of this study was to detect and characterize ESBL-producing enterobacteria in Khartoum hospitals.

A total of 350 clinical specimens were collected from different hospitals in Khartoum State. The specimens cultivated on selective agar media. Identification was done by Gram's stain and API 20E. The presence of ESBLs was determined by double disk synergy test, combination test and E-test. The genotypic characterization of ESBLs was done by polymerase chain reaction technique.

The results revealed that 125 enterobacterial species were identified. The identified bacteria include *Escherichia coli* 47 (37.6%), *Klebsiella pneumoniae* 27 (21.6%) *Proteus mirabilis* 10 (8%), *Enterobacter cloacae* 2 (1.6%), *Pantoea spp.* 15(12.0%), *Klyvera spp.* 5 (4.0%), *Serratia odorifera* 3 (2.4%), *Serratia marcescens* 2 (11.6%), *Proteus vulgaris* 2 (1.6%), *Citrobacter spp.* 4 (3.2%), *Klebsiella ozania* 1 (0.8%), *Klebsiella oxytoca* 1 (0.8%), *Enterobacter sakazakii* 5 (4.0%), *Proteus peneri* 1 (0.8%). 50(40%) species were found ESBLs-producers by double disk synergy test. 36(28.8%) of the isolates gave ESBLs positive by combination test and E-test. 40 genes (CTX-M 26(65%), TEM 8(20%) and SHV 6(15%) were detected. 3 (*Proteus mirabilis*, *Enterobacter sakazaki* and *E. coli*) out of 36 isolates gave ESBLs positive when tested by multiplex PCR technique.Among three species only 4 genes were detected. These genes were CTX-M(2) and TEM (2).

It is concluded that the most prevalent enterobacterial species is *E. coli* followed by *Klebsiella pneumoniae*. The two genera wer ESBL- producers. The CTX-M gene was the most common, followed by TEM and SHV. Further studies about ESBLs-producing bacteria in community setting are highly recommended.

المستخلص

وجود البكتريا المنتجة لانزيمات البيتا لاكتام الممتدة الطيف تؤثر تاثيرًا واضحاً في ادارة العدوى في جميع أنحاء العالم. الهدف من هذه الدراسة لكشف و تمييز البكترية المعوية المنتجة لانزيم البيتا لاكتام ممتدة الطيف في مستشفيات الخرطوم.

جمعت 350 عينة سريرية من مختلف مستشفيات ولاية الخرطوم. أستزرعت هذه العينات على اوساط الأجار الأنتخابية تم التعرف على البكتيرية المعوية بواسطة صبغة الجرام واإلآختبارات الكيموحيوية. تم تحديد وجود إنزيمات البيتا لاكتام ممتدة الطيف بواسطة إختبار مزدوج التآزر؛ وفحص القرص المزدوج وفحص الاختبار ال E. تم التوصيف الوراثي لانزيمات البيتا لاكتام ممتدة الطيف بواسطة تقنية تفاعل البلمرة المتسلسل.

أظهرت النتائج التعرف على 125 نوع باكتيري معوي. شملت الباكتيريا التي تم التعرف عليها (%7(37) الأسكريشية القولونية و (%10(21) الكليبسيلة الرئوية و (%8) 10 المتقلبة الشائعة و (%10(2) الأمعائية المذرفية و (%1) الكليبسيلة الرئوية و (%8) 10 السراتية الذابلة و (%1) المتقلبة البونتية و (%4) انواع الكلافيرة و (%3.2) السراتية الورويفيرا و (%1) السراتية الذابلة و (%1.0) المتقلبة الاعتيادية و (%4) 10 الكليبسيلة الورونية و (%1) الكليبسيلة الأكسوتوكية و (%1) الأمعائية ساكاز اكي و (%1.0) الكليبسيلة الأكسوتوكية و (%1) المتقلبة بنيري. أظهرت (%10) الكليبسيلة الورودي وجود انزيمات البيتا لاكتام ممتدة الطيف بواسطة إختبار مزدوج التأزر. (%28.8) 36 من الأنواع الباكتيرية اعطت نتيجة موجبة لأنزيمات البيتا لاكتام ممتدة الطيف بواسطة إختبار القرص المردوج وفحص اختبار ال. ك. تم الكشف عن 40 جين (%65) CTX-M26/40(65%) و (%10) 3. SHV15/40(15%) و (%10) 10 كانت النتيجة موجبة لأنزيمات البيتا لاكتام ممتدة الطيف بواسطة اختبار تفاعل البلمرة المتسلسل المتعدد. كانت النتيجة معزولة اعطت نتيجة موجبة لأنزيمات البيتا لاكتام ممتدة الطيف بواسطة اختبار تفاعل البلمرة المتسلسل المتعدد. كانت النتيجة 2 CTX-M . TEM

خلصت الدراسة الى ان اكثر انواع البكتيرية المعوية شيوعاً هي الأشريشية القولونية تليها الكليبسيلة الرئوية وهما جنسان منتجان لأنزيمات البيتا لاكتام ممتدة الطيف وكان جين CTX-M الأكثر شيوعاً ويليه TEM ثم SHV ويوصى بشدة أجراء الدراسات الواسعة حول البكتيرية المنتجة لأنزيمات البيتا لاكتام ممتدة الطيف.

Table of Contents

Topic	Page
الآيات	I
Declaration	II
Dedication	III
Acknowledgment	IV
Abstract	V
ملخص البحث	VI
Table of Contents	VII
List of Figures	XII
List of Tables	XIII
CHAPTER ONE: INTRODUCTION AND OBJECTIVE	
1.1. Introduction	1
1.2. Rationale	2
1.3. Objectives	3
CHAPTER TWO: LITERATURE REVIEW	
2.1. Enterobacteraceae	4
2.2. Antimicrobial resistance	4
2.2.1. Mechanism of antimicrobial resistance	4
2.2.1.1. Inherent (Natural) resistance	4
2.2.1.2. Acquired resistance	5
2.2.1.3. Producing of β -lactamases	5
2.3 β-Lactam antibiotics	7
2.4. Beta-lactamase	8
2.5. Extended spectrum β lactamase ESBL	8

2.5.1. TEM β-lactamase	9
2.5.2. SHV β-lactamase	10
2.5.3. CTX -M β-Lactamase	10
2.5.4. OXA β -lactamase	12
2.6. Epidemiology of ESBL	12
2.7. Detection of β -lactamase in the laboratory	17
2.7.1. Phenotypic test	18
2.7.1.1. Doubl-disc potentiation method	18
2.7.1.2. Combination disc method	19
2.7.1.3 E test	19
2.7.1.4 Automated tests (e.g. Vitek)	20
2.7.2 Genotypic detection	21
2.7.2.1 Polymerase Chain Reaction (PCR)	21
2.7.2.2 Multiplex PCR	22
CHAPTER THREE: MATERIALS AND METHODS	
3.1 Type of study	23
3.2. Target population	23
3.3. Inclusion criteria	23
3.4. Exclusion criteria	23
3.5. Study period	24
3.6. Samples and sampling technique	24
3.8. Ethical considerations	24

3.9. Bacteriological culture	24
3.9.3. Cultivation of specimens	25
3.9.4. Colonial morphology	25
3.9.5. Microscopic examination	25
3.10. Identification of Enterobacteraceae by API 20E system	26
3.10.1. Loading the cupules	26
3.10.2. Incubating the API 20E	26
3.10.3. Reading API 20E Results	27
3.10.4. The API 20E interpretation	27
3.11. Sensitivity tests	27
3.11.1Methods	27
3.12. Beta Lactamase test	28
3.13. Detection ESBL production	29
3.13.1. Phenotyping detection	29
3.13.1.1. ESBL screening tests	29
3.13.1.2. Double disc synergy test	29
3.13.1.3. Cephalosporin/clavulanate combination disks	29
3.13.1.3.1. Procedure	30
3.13.1.4. Determination of MIC of ESBL- producing enterobacteria	30
3.13.1.4.1 Method	31
3.13.2. Preservation of culture organism	32
3.13.3.1. DNA extraction	31
3.13.3.2. Measurement of DNA concentration by spectrometry	31

3.13.3.3. Polymerase chain reaction (PCR)	32
3.13.3.3.1.Primers	32
3.13.3.2. Preparation of reaction mixture	33
3.13.3.3. Protocol used for amplification of CTX-M gene	33
3.13.3.3.4. Protocol used for amplification of SHV gene	33
3.13.3.3.5. Protocol used for amplification of TEM gene	34
3.13.4. Multiplex PCR	34
3.13.4.1. Preparation of reaction mixture	34
3.13.4.2. Protocol used for amplification of multiplex genes	34
3.14. Preparation reagents	34
3.14.1. Preparation of 10 X TBE buffer	34
3.14.2. Preparation of 1X TBE buffer .	35
3.14.3. Preparation of Ethidium bromide	35
3.14.4. Preparation of loading dye	35
3.11.5. Preparation of agarose gel	35
3.14.6. Visualization of PCR products	36
2.15. Data analysis	36
CHAPTER FOUR: RESULTS	
4.1. Collection of specimens	37
4.2. Epidemiology finding	38
4.2.1.Gender	38
4.2.2. Age group	38
4.2.3. Identification	39
4.2.3.1. Isolation	39

4.2.4. Morphology	40
4.2.5. API 20	40
4.3. Sensitivity tests	42
4.4. Beta Lactamase test	42
4.5. Detection ESBL	42
4.5.1. Screening tests	42
4.5.2. Double disc synergy test :standardized disc diffusion method	42
4.5.3. Cephalosporin/clavulanate combination disks	44
4.5.4. E test	45
4.6. Genotyping detection	46
4.6.1. ESBL genotyping	46
4.6.1.1 Polymerase chain Reaction	46
4.6.1.1.1 Amplification of the <i>CTX-M</i> (550bp) Target Sequences	47
4.6.1.1.2. Amplification of the <i>TEM</i> (752bp) Target Sequences	49
4.6.1.1.3. Amplification of the SHV (753bp) Target Sequences	50
4.6.1.2. multiplex PCR	51
CHAPTER FIVE: DISCUSSIONS, CONCLUSION and RECOMMENDATIONS	
5.1 Discussions	52
5.2.Conclusion	55
5.3 Recommendations	56
References	57
Appendices	76

List of Figure

Topic	Page
Figure 1. Distribution of specimens according to sex	38
Figure 2 .Distribution of specimens according to age group	39
Figure 3. Shows result of primary culture on different bacteriological culture media	40
Figure 4. Culture plates with ESBL -producing bacterial strains.	44
Figure 5. The phenotypic confirmatory test	45
Figure 6. ESBL genes positive and negative	46
Figure 6. ESBL positive for CTX-M gene.	47
Figure 7. ESBL positive for CTX-M gene.	48
Figure 8 . ESBL positive for TEM gene.	49
Figure 9 . ESBL positive for SHV gene	50
Figure 11. ESBL positive genes CTX-M,SHV by multiplex PCR	51

List of Table

Торіс	page
Table 1. primers for detection of SHV, TEM, CTX-M genes	32
Table 2. Distribution of specimens according to their types	37
Table 3. Distribution and intensity of isolates enterobacterial species	41
Table 4 .Number and percentage of ESBLs producers	43
Table 5: Result of combination test (confirmatory test) doing for ESBL producing Enterobacteraceae	76
Table 6: MIC for ESBL positive by synergy test	77
Table 7:ESBL positive by screening test	78
Table 8 : Total Results of ESBL	79