

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

فَالٰٓتِي عَالٰٓي

اَفَرَأَوْ يَا سَمِّ رَبِّكَ الَّذِي خَلَقَ (1) خَلَقَ الْإِنْسَانَ مِنْ عَلَقٍ (2) اَفَرَأَوْ
وَرَبُّكَ الْاَكْرَمُ (3) الَّذِي عَلَمَ بِالْقَلْمَنِ (4) عَلَمَ الْإِنْسَانَ مَا لَمْ يَعْلَمْ (5)

صدق الله العظيم
(سورة العلق الایات من ١-٥)

Dedication

I dedicate this research to my:
Parents,
Teachers,
Colleagues,
Friends and
All students of Sudan University.

Acknowledgement

I would like to express my thanks to my supervisor Dr. Humodi Ahmed Saeed for his keen supervision during the course of this study.

My thanks are extended to Dr. Mogahid Mohammed Elhassan for his efforts in the performance of Multiplex PCR.

Thanks are also to my colleagues for their help and comments.

Finally, my thanks to Miss. Suheir Ramadan and Miss. Igbal A. Ahmed for their technical assistance.

Abstract

Extended-spectrum β -lactamases (ESBLs) have become widespread throughout the world and are now found in a significant percentage of *Escherichia coli* and *Klebsiella pneumoniae* strains in certain countries. This study was conducted in

the Research Laboratory in Sudan University of Science and Technology. The study was carried out during the period from December 2009 to May 2010, to detect TEM, SHV and CTX-M genes in ESBLs-producing *E. coli*.

The *E. coli* strains were obtained from the Research Laboratory. All strains were checked for purity by sub-culturing on nutrient agar and examined microscopically. Bacterial DNA was extracted from each isolate using boiling method. Multiplex PCR was adopted to detect the different genes including (SHV, CTX-M). The result revealed presence of *TEM* gene only in six of the isolates. It is concluded that, *TEM* gene is the commonest gene in *E. coli* isolates. Thus, this gene may be the dominant one that responsible for ESBL phenomenon among *E. coli* infection in Sudanese patients. Further studies required for confirmation of presence of these genes in clinical Sudanese isolates.

المستخلص

تعتبر الإنزيمات واسعة الطيف منتشرة في كل العالم وتوجد بنسبة وافية في سلالات الإشريكية¹ القولونية و الكببسيلات الرئوية في دول معينة. هذه الدراسة نفذت في مختبر البحوث في جامعة السودان للعلوم والتكنولوجيا في الفترة من ديسمبر/ 2009 إلى مايو/ 2010، للكشف عن الجينات (*TEM* و *SHV* و *CTX-M*) في الأشرشيكية القولونية المنتجة لإنزيمات بيتا لاكتام واسعة الطيف.

تم الحصول على سلالات الإشريكية¹ القولونية من معمل الابحاث بالجامعة والتأكد من نقاوتها بإعادة تزرعها على الأغار المغذي ومن ثم فحصها مجهريا. تم استخلاص الحمض النووي منقوص الأكسجين للسلالات باستعمال طرقة الغليان. استخدمت طريقة تفاعل البلمرة المتسلسل المتعدد للإرسال للكشف هذه الجينات. اظهرت النتيجة وجود الجين *TEM* في سلالات الست. وخلصت الدراسة إلى أن الجين *TEM* هو الأكثر شيوعا في سلالات الإشريكية¹ القولونية، لذا يعتبر هذا الجين هو المسئول عن هذه الظاهرة في المرضى السودانيين بعدهوى الإشريكية¹ القولونية و ان مزيد من الدراسات مطلوبة للتأكد من وجود هذه الجينات في العزلات الاكلينيكية السودانية.

Table of Contents

| | | |
|---------------------------------------|---|-----|
| 1 | עמוד | I |
| 2 DEDIC | Dedication | II |
| 3 | Acknowledgements | III |
| 4 | Abstract | IV |
| 5 | Arabic Abstract | V |
| 6 | Table of Contents | VI |
| 7 | List of Abbreviations | VII |
| Chapter One: Introduction | | |
| 1.1 | Introduction | 1 |
| 1.2 | Rationale | 2 |
| 1.3 | Research questions | 3 |
| 1.4 | Objectives | 3 |
| Chapter Two: Literature Review | | |
| 2.1 | The species of <i>E. coli</i> | 4 |
| 2.1.1 | History | 4 |
| 2.1.2 | Classification | 4 |
| 2.1.3 | Natural habitat | 4 |
| 2.1.4 | Pathogenicity | 5 |
| 2.1.4.1 | Urinary tract infection | 5 |
| 2.1.4.2 | Gastrointestinal tract infection (GITI) | 5 |
| 2.1.4.3 | Neonatal meningitis | 5 |
| 2.1.5 | The pathogenesis | 6 |
| 2.1.5.1 | Enterotoxigenic <i>E. coli</i> (ETEC) | 6 |
| 2.1.5.2 | Enteroinvasive <i>E. coli</i> (EIEC) | 6 |
| 2.1.5.3 | Enteropathogenic <i>E. coli</i> (EPEC) | 6 |
| 2.1.5.4 | Enteroaggregative <i>E. coli</i> (EAEC) | 7 |
| 2.1.5.5 | Enterohemorrhagic <i>E. coli</i> (EHEC) | 7 |
| 2.2 | Extended-spectrum beta-lactamase (ESBL) | 8 |
| 2.2.1 | Types of beta-lactamases | 9 |
| 2.2.1.1 | TEM beta-lactamases (class A) | 9 |
| 2.2.1.2 | SHV beta-lactamases (class A) | 9 |
| 2.2.1.3 | CTX-M beta-lactamases (class A) | 10 |
| 2.2.1.4 | OXA beta-lactamases (class D) | 10 |
| 2.3 | Laboratory diagnosis | 11 |
| 2.3.1 | Isolation | 11 |

| | | |
|--|--|----|
| 2.3.2 | Identification | 11 |
| 2.3.3 | Molecular diagnostic assays | 12 |
| 2.3.3.1. | PCR, real-time PCR and RT-PCR | 12 |
| 2.3.3.2. | Nested PCR | 13 |
| 2.3.3.3. | Multiplex PCR | 13 |
| 2.3.4. | Double-disk test | 14 |
| 2.4 | Suitable Antibiotics | 14 |
| 2.5 | Prevention and control | 14 |
| Chapter Three: Materials and Methods | | |
| 3.1 | Study design | 15 |
| 3.1.1 | Type of study | 15 |
| 3.1.2 | Bacterial strains | 15 |
| 3.1.3 | Study area | 15 |
| 3.1.4 | Duration of study | 15 |
| 3.2 | Activation of bacterial strains | 15 |
| 3.2.1 | Purification of bacterial strains | 15 |
| 3.3 | Molecular Methods | 16 |
| 3.3.1 | Preparation of reagents | 16 |
| 3.3.1.1 | Primers | 16 |
| 3.3.1.2 | Preparation of 10x TBE buffer | 16 |
| 3.3.1.3 | Preparation of 1x TBE buffer | 17 |
| 3.3.1.4 | Preparation of Agarose gel | 17 |
| 3.3.1.5 | Preparation of Ethidium bromide | 17 |
| 3.3.1.6 | Preparation of loading dye | 17 |
| 3.4 | DNA extraction | 17 |
| 3.4.1 | Preparation of bacterial strains | 17 |
| 3.4.2 | Extraction procedure | 18 |
| 3.4.3 | Detection of DNA | 18 |
| 3.4.4 | Multiplex Polymerase Chain Reaction Techniques | 18 |
| 3.4.4.1 | Preparation of Master mix | 18 |
| 3.4.4.2 | PCR amplification | 19 |
| 3.4.4.3 | Visualization of PCR products | 19 |
| Chpater Four: Results | | |
| 4.1 | Source of <i>E. coli</i> clinical isolates | 20 |
| 4.2 | Reactivation of bacterial strains | 20 |
| 4.3 | Purification of bacterial strains | 20 |
| 4.4 | Multiplex PCR result | 20 |
| Chapter Five: Discussion, Concusion and Recommendations | | |
| 5.1 | Discussion | 23 |
| 5.2 | Concusion | 24 |
| 5.3 | Recommendations | 24 |
| | References | 25 |

List of Abbreviations

| | |
|-------------------|--|
| BP | Base pair |
| CTX-M | Cefotaxime |
| CLED | Cystine Lactose Electrolytes Deficient |
| DW | Deionized water |
| DNA | Deoxynucleic acid |
| dNTPs | Deoxynucleotide pyrimidines |
| DDD | Double Disc Diffusion |
| EAEC | Enteroaggregative <i>E. coli</i> |
| EHEC | Enterohaemorrhagic <i>E. coli</i> |
| EIEC | Enteroinvasive <i>E. coli</i> |
| EPEC | Enteropathogenic <i>E. coli</i> |
| ETEC | Enterotoxigenic <i>E. coli</i> |
| ELISA | Enzyme Linked Immune Sorbent Assay |
| EMB | Eosin Methylene Blue |
| ESBLs | Extended Spectrum Beta Lactamases |
| GIT | Gastrointestinal Tract |
| IMViC | Indol Motility Voges proskauer Citrate |
| KIA | Kligler Iron Agar |
| M MW | Marker Molecular Weight |
| MgCL ₂ | Magnesium Chloride |
| NA | Nutrient Agar |
| PCR | Polymerase Chain Reaction |
| SHV | Sulphydryl variable |

| | |
|------|------------------------------|
| TBE | Tris base Boric acid EDTA |
| TEM | Temoniera |
| TSI | Tri Sugar Iron |
| UTI | Urinary Tract Infection |
| UPEC | Uropathogenic <i>E. Coli</i> |
| UV | Ultraviolet Light |
| XLD | Xylose lactose deoxycholate |