

TABLE OF CONTENTS

<u>CHAPTER</u>	<u>PAGE</u>
1 INTRODUCTION.....	1
- Features of <i>Escherichia coli</i>	3
- Antimicrobial Agents.....	5
- Mechanisms of Action	5
- β -lactam Antibiotics.....	7
- Cephalosporins and Cephamycins.....	9
- Bacterial Resistance.....	10
- Mechanisms of Action.....	10
- Resistance to β -lactam Antibiotics	11
- Classification of β -lactamases	12
- Extended Spectrum β -lactamases (ESBLs)	14
- Structure of β -lactamases.....	14
- The Origin and Genetic Determinants of ESBLs	14
- ESBL Types:	17
- SHV.....	17
- TEM.....	18
- CTX-M.....	19
- GES.....	20
- OXA.....	20
- Other ESBL Types	21
- Risk Factors for Colonization and Infection	21
- Epidemiology of ESBL-Producing Organisms	22
- Molecular Epidemiology of ESBL Types	25
- Detection Methods for ESBLs.....	26
- Recommended Methods by CLSI.....	27
- Double Disk Synergy Test	28
- Molecular Detection of ESBL.....	29
- Control of Infections Due to ESBL-Producing Organisms	30
- Treatment	31
- Statement of the Problem.....	33
- Objectives.....	33

TABLE OF CONTENTS (continued)

<u>CHAPTER</u>	<u>PAGE</u>
2 MATERIALS AND METHODS	34
- Specimens Collection	34
- Bacterial Identification.....	34
- Susceptibility Detection Methods of ESBL Producers	35
- Screening Method	35
- Confirmation Method.....	36
- Minimum inhibitory concentration	36
- Molecular Methods	38
- DNA Extraction	38
- Control Strains.....	38
- Single PCR Amplification for <i>bla</i> _{SHV} , <i>bla</i> _{TEM} and <i>bla</i> _{CTX-M}	39
- Amplification of <i>bla</i> _{SHV} , <i>bla</i> _{TEM} , and <i>bla</i> _{CTX-M} by Multiplex PCR.....	40
- Detection of CTX-M Groups by Multiplex PCR.....	40
- Agarose Gel Electrophoresis	41
3 RESULTS	43
- Sample Identification.....	43
- Antimicrobial Susceptibility Testing.....	44
- ESBL Screening.....	44
- Double Disc Synergy Test.....	44
- Combination Disk Assay	45
- Susceptibility Tests by Agar Dilution Method.....	46
- Molecular Detection of β -lactamases Genes.....	48
- Single PCR.....	48
- Multiplex PCR.....	50
- Detection of <i>bla</i> _{CTX-M} Groups by Multiplex PCR.....	52
- CTX-M Types and Susceptibility to Third Generation Cephalosporins.....	54
- Clavulanate Inhibitor Resistant Isolates.....	55
- UTI with ESBL-producing <i>E. coli</i> and Faecal Carriage.....	56
4 DISCUSSION.....	58
5 RECOMMENDATIONS.....	69
6 REFERENCES.....	70
7 APPENDIX.....	82