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Sudan University of Science and Technology

College of Graduate Studies

**Phenotypic Detection of Extended Spectrum β –
Lactamases in Gram Negative Bacilli among
Hospitalized Pregnant Women - Omdurman**

الكشف عن النمط المظهري لإنزيمات البيتا لاكتاماز واسعة الطيف في
العصويات سالبة الجرام وسط الحوامل المنومات للعلاج في المستشفيات -

أم درمان

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قال تعالى:

(تَبَارَكَ الَّذِي بِيَدِهِ الْمُلْكُ وَهُوَ عَلَى كُلِّ شَيْءٍ قَدِيرٌ)

صدق الله العظيم

سورة الملك الآية 1

Dedication

To souls of my parents

To my family

To my colleagues

Acknowledgment

All thanks and praise to **ALLAH** the worthy of all praise for all that I am with sincere thanks and gratitude, I would like to present my thanks to my supervisor **Dr.Elfadil Mustafa Abass** for his patience, advice and guidance. My thanks also extend to staff of Omdurman Maternity Hospital, Omdurman New Hospital (Al-Saudi Hospital) and Omdurman Military Hospital for their help .All thanks for everyone who helps me somehow in this work.

ABSTRACT

This study was conducted to detect Extended Spectrum β - Lactamase (ESBL) in Gram negative bacilli causing urinary tract infection in hospitalized pregnant women, who admitted to Omdurman Maternity Hospital, Omdurman New Hospital (Al- Saudi Hospital) and Omdurman Military Hospital (Obstetrics and Gynaecology department) between April and July 2015.

One hundred and fifty (n= 150) urine samples were collected and cultured on CLED media for primary isolation. Gram stain and conventional biochemical tests were used to identify the causative agents. Antimicrobial susceptibility test was performed using Modified Kirby-Bauer method and Double Disc Synergy Test (DDST) to determine ESBL production in Gram negative bacilli isolates.

Positive urine cultures were reported in 33(22%) of pregnant women, among which 7.3% were symptomatic and 14.7% were asymptomatic. Most common isolates were *E. coli* (36.4%) followed by *Staphylococcus spp*, accounting 27.3%. *E. faecalis*, *K. pneumoniae* and *K. oxytoca* were less common; represented 18.2%, 12.1% and 6.0%, respectively. Among Gram negative bacilli isolates, ESBL was detected in 8 isolates (44.4%). Of these; *E. coli* accounted 75% and *K. pneumoniae* accounted 25%.

Imepemen was the most effective antibiotic for Gram negative bacilli. That showed 100% sensitive followed by a little resistance pattern for Ciprofloxacin (22.2%); while other antibiotics showed moderate

antimicrobial effects. Ceftazidime, Cefotaxime, Ceftriaxone and Co-trimoxazole. Gram negative bacilli showed resistance of 55.6%, 61.1%, 61.1% and 66.7%, respectively.

Cefuroxime and Amoxicillin were the lowest effective antibiotics for Gram negative isolates. Resistances were 100% and 83.3%, respectively.

This study revealed that there is a need to apply urine culture and sensitivity test to assess urinary tract infection (UTI) among hospitalized pregnant women and detection of resistant bacteria like ESBL Gram negative bacilli. This will help these patients to get safe and effective treatment.

الخلاصة

أقيمت هذه الدراسة للكشف عن أنزيمات البيتا لاكتاماز واسعة الطيف في العصويات سالبة الجرام المسببة لالتهابات المجاري البولية لدى السيدات الحوامل الخاضعات للعلاج في مستشفى أم درمان للولادة ومستشفى أم درمان الجديد (المستشفى السعودي) ومستشفى السلاح الطبي أم درمان قسم النساء والتوليد في الفترة ما بين شهر ي ابريل ويوليو سنة 2015.

تم جمع 150 عينة بول وتزريعها في وسط اللاكتوز و السيستين المنقوص الشوارد لغرض العزل الأولي للبكتريا وتم استخدام صبغة جرام و الأختبارات الكيموحيوية في التعرف علي مسببات المرض تم إجراء إختبارات الحساسية للمضادات الحيوية بإستخدام طريقة كيربي باور المعدلة وإختبار الدبل ديسك سينرجي للكشف عن إنتاج العصويات سالبة الجرام لإنزيمات البيتا لاكتاماز واسعة الطيف 22% من العينات أظهرت نموا إيجابيا على الأوساط التزريبية وكانت البكتريا الأكثر عزلا هي الإشريكية القولونية بنسبة 36.4% تلتها المكورات العنقودية بنسبة 27.3%. المكورات المعوية البرازية و الكلبسيلا الرئوية و الكلبسيلا الأوكسيتوكية كانت الأقل عزلا حيث مثلت 18.2% و 12.1% و 6.0% على التوالي. العصويات سالبة الجرام المنتجة لإنزيمات البيتا لاكتاماز واسعة الطيف كانت في 8 من بين العصويات سالبة الجرام التي تم عزلها بنسبة (44.4%) حيث كانت الإشريكية القولونية 75% والكلبسيلا الرئوية 25%. الإيميبينم كان المضاد الحيوي الأكثر فعالية حيث لم تظهر له أي مقاومة تبعه السيبروفلوكساسين حيث كانت المقاومة له بنسبة

22.2%. بينما المضادات الحيوية السيفتازيديم و السيفوتاكسيم و السيفترايكزون والكوترايموكسازول أظهرت العصويات سالبة الجرام مقاومة بنسبة 55.5 % و 61.1 % و 61.1 % و 66.7 % تباعا. السيفروكسيم و الأموكسيسيلين كانا الأقل فعالية حيث أظهرت العصويات سالبة الجرام مقاومة بنسبة 100% و 83.3% علي التوالي.

كشفت هذه لدراسة الحاجة لتطبيق فحص تزرع البول و اختبار الحساسية لتقييم حالة إلتهاب البول لدى الحوامل الخاضعات للعلاج داخل المستشفى و الكشف عن البكتريا المقاومة للمضادات الحيوية مثل العصويات سالبة الجرام المنتجة لإنزيمات البيتا لاكتاماز واسعة الطيف و الذايسوف يساعد هؤلاء المرضى في تلقي علاجات فعالة وامنة.

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LIST OF ABBREVIATIONS

ATCC	American type culture collection
ASB	Asymptomatic bacteriuria
CLED	Cystine lactose electrolyte deficient
CLSI	Clinical and laboratory standards institute
DDCT	Double disc combination test
DDST	Double disc synergy test
DNAse	Deoxyribonuclease
ESBL	Extended spectrum beta lactamase
H₂O₂	Hydrogen peroxide
H₂S	Hydrogen sulphide
ICU	Intensive care unit
KIA	Kligler iron agar
MSA	Mannitol salt agar
MSU	Mid-stream urine
1N HCL	1 Normality hydrochloric acid
UTI	Urinary tract infection

CHAPTER ONE

INTRODUCTION AND

OBJECTIVES

CHAPTER ONE

INTRODUCTION AND OBJECTIVES

1.1. Background

β - Lactam agents such as Penicillins, Cephalosporins, Monobactams and Carbapenems, are among the most frequently prescribed antibiotics (Pitout *et al.*, 2005).

Cephalosporins antibiotics is known for its broad spectrum activity, proven efficacy and favorable safety profile, making it the most commonly prescribed class of antimicrobials (Laudano, 2011).

In Gram-negative pathogens, β -lactamases remain the most important contributing factor to β -lactam resistance. β - Lactamases are bacterial enzymes that inactivate β -lactam antibiotics by hydrolysis, which result in ineffective compounds (Pitout *et al.*, 2005).

The problem of antimicrobial-drug resistance is the immediate threat of a reduction in the discovery and development of new antibiotics. Several factors have contributed to this decline, including the increasing challenges of screening for new compounds, the high capital costs and long time required for drug development, the growing complexity of designing and performing definitive clinical trials and the concern about reduced drug longevity due to the emergence of resistance (Peleg and Hooper, 2010).

Resistance in Gram-negative bacteria is increasing; this is mainly due to the spread of strains producing Extended-Spectrum β -Lactamases (ESBLs).

Many of the isolates producing these enzymes are also resistant to Trimethoprim, Quinolones and Aminoglycosides, often plasmid has co-expression of other resistance mechanisms(Pallett and Hand., 2010).

Extended-Spectrum β -Lactamases (ESBLs) are a rapidly evolving groups of β -lactamases which share the ability to hydrolyze third-generation Cephalosporins and Aztreonam, yet are inhibited by Clavulanic acid (Paterson and Bonomo., 2005).

ESBL-producing bacteria are associated with severe infections such as bacteraemias, intra-abdominal infection, urinary tract infections, and respiratory tract infections(Dhillon and Clark., 2012).

Options in the treatment of ESBL-producing organism infections are extremely limited;Carbapenems are the treatment of choice for serious infections due to such organisms.The presence of ESBLs carries tremendous clinical significance(Paterson and Bonomo., 2005).

Urinary tract infections (UTIs) are the most common bacterial infections during pregnancy. It may be either an asymptomatic bacteriuria (ASB of pregnancy) or symptomatic acute cystitis and acute pyelonephritis. Asymptomatic bacteriuria (ASB), occurring in 2–11% of pregnancies, is a major predisposition to the development of pyelonephritis, which is associated with obstetrical complications, such as preterm labor and low birth weight infants.Untreated ASB is found to be associated with subsequent acute pyelonephritis;20-50% of thecases (Celenet *al.*, 2011).

1.2. Rationale

In Sudan, urine for culture and sensitivity is not requisite routinely for pregnant women suffering from UTIs. Hamdan *et al.*, (2011) had reported the prevalence of asymptomatic and symptomatic UTI in Sudanese pregnant women as 14.7% and 12%, respectively and Gram negative bacteria were the predominant causative agent. For most cases antibiotics are prescribed without evaluating the drug-resistance patterns of the causative agent.

ESBL producing Gram negative bacteria are a problem in health care field, because they are capable to inactivate Penicillins, third generation Cephalosporins and Azteronam. The most worrying about these bacteria, their increasingly spread especially in health care settings. Pregnant women, who admitted to antenatal wards at high risk to get infections, mainly UTIs. Laboratory detection of ESBL producing Gram negative bacteria will help patients to get effective treatment and reduce the subsequent complications of UTIs. Thus it was interesting to detect distribution of Gram negative bacilli producing ESBLs and causing urinary tract infections among pregnant women.

1.3. Objectives

1.3.1. General objective

To detect Extended Spectrum β -Lactamases (ESBLs) phenotype in Gramnegative bacilli causing urinary tract infection in pregnant women admitted to antenatal wards.

1.3.2. Specific objectives

- a) To determine resistance to third generation Cephalosporins in isolates of Gramnegative bacilli.
- b) To detect Extended Spectrum β -Lactamases (ESBLs) among third generation Cephalosporins resistant Gram negative bacilli isolates.

CHAPTER TWO

LITRETURE REVIEW

CHAPTER TWO

LITRETURE REVIEW

2.1. Resistance in Gram negative bacteria

Gram negative bacteria are organisms acquiring genes that code for mechanisms of antibiotic drug resistance, especially in the presence of antibiotic selection pressure. They often using multiple mechanisms against the same antibiotic or using a single mechanism to affect multiple antibiotics (Pelegand Hooper., 2010).

Seven mechanisms of resistance can be used by Gram negative bacteria, with some being mediated by a mobile plasmid. These mechanisms include the loss of porins, which reduces the movement of drug through the cell membrane; the presence of β -Lactamases in the periplasmic space, which degrades the β -lactam; increased expression of the transmembrane efflux pump, which expels the drug from the bacterium before it can have an effect; the presence of antibiotic-modifying enzymes, which make the antibiotic incapable of interacting with its target; target site mutations, which prevent the antibiotic from binding to its site of action; ribosomal mutations or modifications, which prevent the antibiotic from binding and inhibiting protein synthesis; metabolic bypass mechanisms, which use an alternative resistant enzyme to bypass the inhibitory effect of the antibiotic; and a mutation in the lipopolysaccharide, which renders the polymyxin class of antibiotics unable to bind this target(Peleg and Hooper., 2010).

β - Lactamases are the primary mechanism of conferring bacterial resistance to β -Lactam antibiotics, such as Penicillins and Cephalosporins (Dhillon and Clark., 2012).

2.2. β –Lactamases

β - Lactamases are hydrolytic enzymes with the ability to inactivate β -Lactam antibiotics before they reach the penicillin-binding proteins located at the cytoplasmic membrane (Falagas and Karageorgopoulos ., 2009; Tham, 2012).

Many of the Gram negative bacteria possess a naturally occurring chromosomally mediated β -lactamase, which probably assists the bacteria in finding a niche when faced with competition from other bacteria that naturally produce β -lactams (Turner, 2005; Tham, 2012).

The first plasmid-mediated β -lactamase in Gram negative bacteria, TEM-1, was described in 1965. This occurred in a strain of *Escherichia coli* isolated from blood culture of a patient in Greece (“TEM” came from the patient’s name, Temoniera). Because this β -lactamase was plasmid - borne, has been spread to other members of the *Enterobacteriaceae* family, *H. influenzae*, *Neisseria gonorrhoeae* and *Pseudomonas aeruginosa*. Another plasmid-mediated β -lactamase, known as “SHV-1” (sulfhydryl variable), was found in *Klebsiella pneumoniae* and *E. coli* (Turner, 2005).

The presence of these enzymes influenced the efforts of pharmaceutical companies’ to negate their effects. One such development was that of the Oxyimino-Cephalosporins (third generation of Cephalosporins), which showed good stability against the TEM-1 and SHV-1 β -lactamases. This class of antibiotics was widely used for the treatment of serious hospital infections due to Gram negative organisms (Turner, 2005).

2.3. Extended Spectrum β –Lactamases (ESBLs)

Extended-Spectrum β -Lactamases (ESBLs) are a rapidly evolving group of β -lactamases which share the ability to hydrolyze third-generation Cephalosporins and Aztreonam, yet are inhibited by Clavulanic acid. Typically, they derive from genes of TEM-1, TEM-2, or SHV-1 by mutations that alter the amino acid configuration around the active site of these β -lactamases. The first report of plasmid-encoded β -lactamases capable of hydrolyzing the Extended-Spectrum Cephalosporins (SHV-2) was published in Germany, 1983 (Paterson and Bonomo., 2005).

These enzymes can be carried on bacterial chromosomes, that is, inherent to the organism, or may be plasmid-mediated with the potential to move between bacterial populations. ESBLs are primarily produced by the *Enterobacteriaceae* family, in particular *Klebsiella pneumonia* and *Escherichia coli*. They are also produced by non -fermentative Gram negative organisms, such as *Acinetobacter baumannii* and *Pseudomonas aeruginosa* (Dhillon and Clark., 2012).

2.3.1. ESBLs classification

The total number of ESBLs now exceeds 200 enzymes. β -Lactamases are most commonly classified according to two general schemes: the Ambler molecular classification scheme and the Bush-Jacoby-Mederos classification scheme. The Ambler scheme divides β -lactamases into four major classes (A to D) according to protein homology (ESBLs are in class A) (Paterson and Bonomo., 2005; Dhillon and Clark., 2012).

The Bush-Jacoby-Medeiros classification scheme groups β -lactamases according to functional similarities (substrate and inhibitor profile). ESBLs classified in Bush-Jacoby-Mederos functional classification as 2be β –

lactamases. 2be designation shows that these enzymes are derived from group 2b β -lactamases (for example, TEM-1, TEM-2, and SHV-1); the “e” of 2be denotes that the β -lactamases have an extended spectrum (Paterson and Bonomo., 2005).

There are various genotypes of ESBLs; the most common are the SHV, TEM, and CTX-M types. Other clinically important types include VEB, PER, BEL-1, BES-1, SFO-1, TLA, and IBC(Dhillon and Clark., 2012).

These classificationsexclude allother β - lactamases such as plasmid-borne AmpC orOXA-type Cephalosporinases,metallo- β -lactamases (MBL),OXA-type Carbapenemases, the *Klebsiellapneumoniae* class Acarbapenemases (KPC) and certain GES-variant β -lactamases which have differentfunctional and/or structural classes, but they all certainly share an extended spectrum of β -lactam hydrolysis(Giskeet *al.*, 2009).

Giskeet *al.*, (2009)propose that the classical, functional class 2be β -lactamases could be designated as ‘class A ESBLs’ (ESBLA), whereas plasmid-mediated AmpC and OXA-ESBLs could be labeled ‘miscellaneous ESBLs’ (ESBLM) and ESBLs with hydrolytic activity against Carbapenems’ (ESBLCARBA).

2.3.2.Epidemiology

When ESBLs were first recognized in the early 1980s, they have become a major cause of hospital-acquiredinfection, particularly in the intensive care units (ICU).TEM and SHV-types have been recognized across theworld with over 100 mutations (Dhillon and Clark., 2012).

The CTX-M enzymes appear to have a greater ability tospread and cause outbreaks.There are over 50 variants of CTX-M to date, and theyhave been

associated with numerous outbreaks of infections both in hospitals and in the community (Dhillon and Clark., 2012).

Data from the last 10 years establishes CTX-M genotype as the predominant ESBL in Europe and East Asia. The prevalence of bacteria producing ESBLs varies worldwide, with reports from North America, South America, Europe, Africa, and Asia. Data from the Tigecycline Evaluation and Surveillance Trial (TEST) global surveillance database shows the rate of ESBL production was highest among the *K. pneumoniae* isolates collected in Latin America, followed by Asia/Pacific Rim, Europe, and North America (44.0%, 22.4%, 13.3%, and 7.5%, respectively) (Dhillon and Clark., 2012).

In comparison with the rest of the world, there is generally a lack of comprehensive data regarding ESBL-producing *Enterobacteriaceae* in African countries. However, there is sufficient evidence to highlight the prevalence of ESBLs in Africa. It is recognized that Egypt has an extremely high rate of ESBL producers, with up to 70% of isolates producing the enzyme. The CTX-M genotype appears to be the most common type in North Africa. There have also been reports of CTX-M *K. pneumoniae* in Kenya and SHV and TEM—types in South Africa (Dhillon and Clark., 2012).

2.3.3. ESBL infections and treatment

ESBL-producing organisms have an enormous clinical and microbiological significance. Such bacteria are associated with severe infections such as bacteraemias, intra-abdominal infection, urinary tract infections and respiratory tract infections. They inactivate Cephalosporins, which are often used in treating the septic patient in a variety of clinical settings. Therefore, this often renders empiric antibiotic treatment ineffective. Many ESBL

genes have the propensity to jump between organisms, thus leading to outbreaks of infection, if this occurs in an easily transmissible pathogen. It is also known that organisms producing ESBLs also have the ready capacity to acquire resistance to other antimicrobial classes such as the Quinolones, Tetracyclines, Cotrimoxazole, Trimethoprim, and Aminoglycosides, which further limits therapeutic options (Dhillon and Clark., 2012).

Nosocomial infections caused by these organisms complicate therapy and limit treatment options, in addition, patients infected with ESBL-producing bacteria may have a higher mortality rate and may require longer hospital stays because they are generally sicker and have received more antibiotics than patients who are not infected with ESBL-producing strains (Ramphal and Ambrose., 2006).

Effective strategies for the empirical and directed treatment of infections caused by ESBL-producing pathogens include the use of Carbapenems and, possibly, the fourth-generation Cephalosporin Cefepime. Studies indicate that the use of Cefepime to treat serious nosocomial infections (e.g., bacteremia, pneumonia, and urinary tract infections) is associated with high rates of microbiological and clinical success(Ramphal and Ambrose., 2006).

2.4. Urinary tract infections in pregnancy

2.4.1. Description and complications

Pregnancy is a unique state with anatomic and physiologic urinary tract changes, urinary tract infections represent the most common bacterial infection in pregnancy and classified as either asymptomatic or symptomatic. Asymptomatic bacteriuria occurs in 2–10% of all pregnancies (Schnarr and Smaill., 2008).

Hamdanet *al.*, (2010), in their study reported 14.7% prevalence of asymptomatic bacteriuria in Sudanese women during pregnancy and 12% symptomatic bacteriuria.

If asymptomatic bacteriuria is left untreated 30% of mothers develop acute pyelonephritis. Pyelonephritis in pregnancy has been associated with many perinatal complications including bacteraemia, respiratory insufficiency, anemia, renal disease, hypertension, preterm labor and low birth weight (Schnarr and Smaill., 2008; Banhidi *et al.*, 2007).

Beside the above consequences, Rizvi *et al.*, (2011) reported that untreated bacteriuria is associated with 50% increase the risk of pre-eclampsia and postpartum endometritis.

2.4.2. Bacterial causative agents

E. coli is the most common pathogen associated with both symptomatic and asymptomatic bacteriuria, representing 70–80% of isolates, but was found to be greater than 90% in one study (Schnarr and Smaill., 2008).

Other Gram negative rods such as *P. mirabilis* and *K. pneumoniae* can also be cultured. Gram positive Cocci such as *Staphylococcus saprophyticus*, other coagulase negative Staphylococci and group B Streptococci are less common (Perera, 2009).

2.4.3. Treatment and bacterial resistance

The choice of a Sulfonamide or Sulfonamide-containing combination, Penicillin, Cephalosporin or Nitrofurantoin, based on the results of susceptibility testing, are appropriate regimens for the management of asymptomatic bacteriuria. Increasing antibiotic resistance, however, complicates the choice of empiric regimens and is likely to become an

increasing problem (Smaill and Vazquez., 2007). *Enterobacteriaceae* expressing Extended Spectrum β -Lactamase (ESBL) are among the most multidrug-resistant pathogens in hospital and spreading worldwide. Transient carriage of bacteria on hands of health care workers may lead to transmission to patients (Tschudin-sutter *et al.*, 2010).

A report done by Tschudin-sutter *et al.*, (2010) revealed that an outbreak caused by transmission of ESBL *E.coli* from a mother to her new born twins and subsequently spread to other neonates and one health care worker. The mother was most colonized before hospitalization and UTI developed peripartum. Transmission by contact during vaginal delivery of twins and transmission by physical contact to health care worker and other neonates was the most likely mode of transmission.

2.5. Phenotypic detection of ESBL enzymes

2.5.1. Disk-Diffusion method

Screening test with an indicator Cephalosporin which looks for resistance or diminished susceptibility, thus identifying isolates likely to be harboring ESBLs. The Clinical and Laboratory Standards Institute (CLSI) has proposed disk-diffusion methods for screening for ESBL production by *Klebsiella pneumoniae*, *K. oxytoca*, *Escherichia coli* and *Proteus mirabilis*. Disk-diffusion methods were used for antibiotic susceptibility testing can screen for ESBL production by noting specific zone diameters which indicate a high level of suspicion for ESBL production. Cefpodoxime, Ceftazidime, Aztreonam, Cefotaxime or Ceftriaxone disks are used. Since the affinity of ESBLs for different substrates is variable, the use of more than one of these agents for screening improves the sensitivity of detection. However, it is adequate to use Cefotaxime, which is consistently

susceptible to CTX-M; and Ceftazidime, which is a consistently good substrate for TEM and SHV variants. If isolates show resistance or diminished susceptibility to any of these agents, it indicates suspicion for ESBL production, and phenotypic confirmatory tests should be used (Rawat and Nair., 2010).

2.5.2. Dilution antimicrobial susceptibility tests

The CLSI has proposed dilution methods for screening for ESBL production by *Klebsiella pneumoniae*, *K. oxytoca*, *Escherichia coli* and *Proteus mirabilis*. Ceftazidime, Aztreonam, Cefotaxime or Ceftriaxone can be used at a screening concentration of 1 µg/mL or Cefpodoxime at a concentration of 1 µg/mL for *Proteus mirabilis*; or 4 µg/mL, for the others. Growth at or above this screening antibiotic concentration is suspicious of ESBL production and is an indication for the organism to be tested by a phenotypic confirmatory test (Rawat and Nair., 2010).

2.5.3. Double Disk Combination Test (DDCT)

The British Society for Antimicrobial Chemotherapy has recommended the disk-diffusion method for phenotypic confirmation of ESBL presence using Ceftazidime/Clavulanate and Cefotaxime/Clavulanate combination disks. Using this method, the zone diameters of each combination compared with those of the Cephalosporin alone, and calculated a ratio of Cephalosporin/Clavulanate zone size divided by cephalosporin zone size. A ratio of 1.5 or greater was taken to signify the presence of ESBL activity (Rawat and Nair., 2010).

2.5.4. Double Disk Synergy Test (DDST)

In this, test disks of third-generation Cephalosporins and Amoxicillin/clavulanate (Augmentin) are kept 30 mm apart, center to center, on inoculated Mueller-Hinton agar. A clear extension of the edge of the inhibition zone of cephalosporin towards Augmentin disk is interpreted as positive for ESBL production. Evaluations of the double-disk diffusion test have revealed sensitivities of the method ranging from 79% to 97% and specificities ranging from 94% to 100%. In isolates which are suspicious for harboring ESBLs but are negative using the standard distance of 30 mm between disks, the test should be repeated using closer (for example, 20 mm) or more distant (for example, 40 mm) spacing (Rawat and Nair., 2010).

2.5.5. Etest for ESBLs

plastic drug-impregnated strips, one end of which contains a gradient of Ceftazidime (MIC test range 0.5 to 32 µg/ml) and the other with a gradient of Ceftazidime plus a constant concentration of Clavulanate (4 µg/ml). Similar strips containing Cefotaxime and Cefotaxime/Clavulanate. These strips are useful for both screening and phenotypic confirmation of ESBL production. The reported sensitivity of the method as a phenotypic confirmatory test for ESBLs is 87 to 100% and the specificity is 95 to 100%. The sensitivity and specificity of the method depend on the ratio of MICs of the Cephalosporin versus Cephalosporin/Clavulanate combination used (Rawat and Nair., 2010).

2.6. Previous studies

In study done by Mekki *et al.*, (2010) carried out in Khartoum state hospitalsto evaluate emergence of ESBL among multidrug- resistant *Escherichia coli* and *Klebsiella* species causing nosocomial UTI, β -Lactamase was produced by all isolates; high resistance level for third generation Cephalosporin was noticed. ESBLs were detected in high prevalence among all multidrug -resistant *E. coli* and *Klebsiella* species isolates 53%.

Ahmed *et al.*, (2013) in another Sudanese study under title - Increasing prevalence of ESBL-producing *Enterobacteriaceae* in Sudan community patients with UTIs, found ESBL producing bacteria was 59.6% mostly were in *K. pneumoniae* 68.8. % followed by *E. coli* 65.0 %. ESBL producing bacteria showed maximum resistance to Ceftazidime 95.4%, followed by Cefotaxime 94.6%, while minimum resistance was seen with Imepenem 0%.

Prevalence of Extended-Spectrum β -Lactamases-producing *E. coli* from Hospitals in Khartoum State, Sudan a study done by Ibrahim *et al.*, (2013) aimed to determine the prevalence and assess antimicrobial susceptibility of Extended- Spectrum β -Lactamases-producing *Escherichia coli* isolated from clinical specimens of patients at hospitalsin Khartoum State, Sudan; showed thatout of 232 *E. coli* isolates, 70 (30.2%) were found positive for ESBL by the applied phenotypic methods.

In Pakistan, Ejaz *et al.*, (2011) detect ESBLproduction in *E. coli* 57.4% and *K. pneumoniae* 71.7% out of total of 13638 urine samples were processed for culture and antimicrobial sensitivity testing.

In Nigeria ESBL was detected in 47.1% of the 85 isolates and *E. coli* was the major ESBL producer 52.5% followed by *K. pneumoniae* 47.5%. This study done by Azekhueme et al., (2015) aimed to investigate the prevalence of ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* in clinical samples and determine their antimicrobial susceptibility profile.

In (2006) a study done by Yu et al., in Taiwan showed the ESBLs producer *E. coli* were 1.5-16.7% and ESBLs producer *K. pneumoniae* were 8.5-29.8%.

Indian study done by Sharma et al., published in (2013) showed that ESBLs producer positive in 57.2% and *K. pneumoniae* producing ESBL were 67.04%. Kateregga et al., (2015) in Uganda revealed that frequency of ESBLs producers among Gram negative organisms were 62% and non ESBLs producers were 38%, *K. pneumoniae* represent 72.7% of ESBL producers. They reported that resistance to Ceftazidime and Cefotaxime were 73% and 57.5%, respectively.

CHAPTER THREE

MATERIALS AND METHODS

CHAPTER THREE

MATERIALS AND METHODS

3.1. Study design

This was a cross sectional descriptive hospital based study.

3.2. Study setting area

The sample was collected from three hospitals in Omdurman, Khartoum State; Omdurman Maternity Hospital, Omdurman New Hospital (Al- Saudi Hospital) and Omdurman Military Hospital (Obstetrics and Gynaecology department).

3.3. Study population and sample size

The study population was pregnant women who were admitted to antenatal wards, included one hundred and fifty (n= 150) pregnant women.

3.3.1. Inclusion criteria

Pregnant women admitted to antenatal wards with or without signs and symptoms of urinary tract infection.

3.3.2. Exclusion criteria

Non- pregnant women and pregnant women who were attended to hospital without admission to antenatal wards.

3.4. Type of sampling

The type of sampling was non –probability convenience sampling.

3.5. Data collection

3.5.1. Data collection tool

A questionnaire was used for collection of primary data from all participants in the study (appendix NO1).

3.5.2. Study variables

The study variables were qualitative variables and included participants age, pregnancy trimester, signs and symptoms of UTI, contraceptive use, other diseases with pregnancy, Gram- negative bacilli isolation, resistance to third generation Cephalosporins, susceptibility pattern to antibiotics, Extended Spectrum β - Lactamases(ESBLs) in Gram negative bacilli.

3.6. Ethical consideration

3.6.1. Ethical clearance

The study was ethically approved by Sudan University of Science and Technology, Research Board; Curative Medicine Department, Planning Unit of Ministry of Health and the respective hospitals.

3.6.2. Informed consent

All Participants were informed about objectives and aspects of the study and signed informed consent statements (appendix NO2).

3.7. Study duration

This study was conducted between April and July 2015.

3.8. Urine sample collection

Mid-stream urine (MSU) samples were collected in sterile wide mouth universal containers and transported with ice box to Research Laboratory of Sudan University of Science and Technology, as soon as possible.

3.9. Isolation technique

Under aseptic conditions, all urine samples were cultured on Cysteine Lactose Electrolytes Deficient media, CLED (HiMedia, India) and incubated aerobically for 18-24 hrs at 37°C.

3.10. Wet preparation

Samples were examined for presence of pus cells and epithelial cells in urine. Pus cells were counted and epithelial cells were reported as crosses by using 40x lens.

3.11. Identification techniques

3.11.1. Colonial features

Samples which showed growth on CLED media were examined for colonial morphology based on size, shape, colour and lactose fermentation.

3.11.2. Gram stain

Gram stain was used in identification of the causative agents by determining their Gram reactions, cell shape and arrangement. The method was performed as follow:

The smears of tested bacteria was prepared in clean slides by emulsifying a portion of a colony in a drop of normal saline, after drying fixed by rapidly passing the slides through benzene flame then smears were covered with

crystal violet and left for 1 minute and rinsed with tap water. Iodine was added to smears for 1 minute then rinsed gently with tap water. Alcohol was used to decolourize the colour of stain for seconds then rinsed with tap water. Safranin was added to smears for 2 minutes then the result assessed by light microscope using 100x lens (Washington *et al.*, 2006).

3.11.3. Biochemical tests

Sets of biochemical tests were used for identification of causative agents, including KIA medium, Citrate utilization test, Urease test, Indole test, Catalase test, DNase test, Mannitol fermentation test and Esculin hydrolysis test.

3.11.3.1. Kligler iron agar (KIA)

KIA media were used for identification of bacteria having the ability to ferment lactose with or without gas and hydrogen sulfide (H₂S) production. Tested bacteria were inoculated in KIA media (HiMedia, India) under aseptic conditions and incubated overnight at 37°C. At end of the incubation period; colour, gas and H₂S were observed. Fermenting Lactose is producing acid which convert the pH of media to acidic pH which in presence of phenol red (indicator) change colour of medium from red to yellow. Gas detected by air bubbles and cracking and Hydrogen sulfide (H₂S) by blackening the media (Cheesbrough, 2006).

3.11.3.2. Citrate utilization test

This test was used to identify bacteria which have ability to utilize sodium citrate as sole source of carbon. After inoculation the tested bacteria in Simmons citrate agar (HiMedia, India), incubated overnight at 37°C. The

colour of media was observed at end of incubation period and the results were reported. Bromothymole blue (indicator) is green in neutral pH and converted to blue colour due to presence of sodium carbonate which is alkaline compound (Cheesbrough, 2006).

3.11.3.3. Urease test

Urease test was used to detect bacteria which have ability to secrete urease enzyme. Under aseptic conditions Christensen media (HiMedia, India) were inoculated with tested bacteria and incubated for overnight at 37°C and at the end of incubation period the results were reported. This enzyme can breakdown urea into ammonia and carbon dioxide. Ammonia converts pH of media to alkaline which changes the colour of the Christensen medium from colourless to magenta or pink color due to presence of phenol red as indicator, which is considered as positive test (Cheesbrough, 2006).

3.11.3.4. Indole test

This test was used to detect bacteria which have ability to produce indole after breakdown of the amino acid tryptophan. Tested bacteria were inoculated in peptone water which contains tryptophan (HiMedia, India) and incubated for overnight at 37°C. Indole production was detected by adding drops of Kovac's reagent (HiMedia, India). When red ring appears in seconds, tested organism was reported as positive result (Cheesbrough, 2006).

3.11.3.5. Catalase test

Catalase test was used to differentiate the Staphylococci from the Streptococci. This enzyme detected by adding some colonies of tested bacteria to 2ml of 3% hydrogen peroxide (H_2O_2) in clean test tubes. Catalase enzyme breakdown hydrogen peroxide (H_2O_2) into water and oxygen. Test was reported as positive when in few seconds' air bubbles appear (Cheesbrough, 2006).

3.11.3.6. DNase test

This test was used to detect bacteria which can produce DNase enzyme. This enzyme secreted by some bacteria to break down DNA in DNA agar. The test organism was heavily streaked on DNase test agar base (HiMedia, India) and incubated for overnight at 37°C. After incubation, 1 N HCL solution was added to precipitate unhydrolyzed DNA in the media. When clear zone appears, test considered as positive (Cheesbrough, 2006).

3.11.3.7. Mannitol fermentation test

Mannitol salt agar (MSA) is a medium used to differentiate *S.aureus* from other *Staphylococcus* species, by its ability to grow and ferment Mannitol sugar in salty media. Tested organisms were streaked on MSA (HiMedia, India) and incubated at 37°C for overnight, at the end of incubation period the growth and color of media were observed. Mannitol fermentation leads to acid production, which converts the colour of Phenol red (indicator) from red to yellow, which consider as positive test. (Cheesbrough, 2006).

3.11.3.8. Esculin hydrolysis test

The purpose of this test is to examine ability of bacteria to hydrolyze the compound Esculin as carbon source. Some bacteria can metabolize Esculin into dark compound Escultin – ferric citrate which obviously discoloured the Bile Esculin agar slant. Bile Esculin agar slant (HiMedia, India) was inoculated with tested bacteria and incubated for 24 hrs at 37°C. After incubation time positive and negative results have been reported (Washington *et al.*, 2006).

3.12. Susceptibility test

A modified Kirby- Bauer susceptibility testing method was used to assess the sensitivity and resistance patterns of the isolates. On Mueller Hinton agar (HiMedia, India), a suspension of tested isolate which was compared with 0.5 % Macfarland standard was seeded. A set of antibiotics discs were applied include Imepenem 10µg, Ciprofloxacin 30µg, Co-trimoxazole 30µg, Amoxicillin 30µg, Cefruxime 30µg, Ceftazidime 30µg, Cefotaxime 30µg and Ceftriaxone 30µg (HiMedia, India). Plates were incubated aerobically for overnight at 37°C. Zones of inhibition were measured in mm and compared to a standard interpretation chart (Cheesbrough, 2006).

3.13. Double Disc Synergy Test (DDST)

This test was used to detect Extended Spectrum β -Lactamases (ESBLs). All Gram negative bacilli isolates which showed a diameter of or less than 17 mm for Ceftazidime and of or less than 22 mm for Cefotaxime were selected for checking the ESBLs production. The production of ESBL was tested by using a disc of Amoxicillin/Clavulanic acid (20/10µg HiMedia, India) along with two third generation Cephalosporins; Ceftazidime (30µg) and

Cefotaxime (30µg) discs. On Mueller Hinton agar plates lawn of tested strains and *E.coli* ATCC 22925(negative control) were made. Amoxicillin /Clavulanic acid (20/10µg) disc was placed in the center of the plate and Ceftazidime(30µg) and Cefotaxime (30µg) discs were placed 15 mm apart center to center to Amoxicillin /Clavulanic acid and incubated for 18-24 hrs at 37°C. Any increase in the zone towards the disc of Amoxicillin /Clavulanic acid was considered as positive result for the ESBL production (Kauretal., 2013).

3.14 purification and storage of isolates

All isolated Gram negative bacilli were purified in nutrient agar (HiMedia, India) and were used in identification and susceptibility tests. 16% v/v glycerol broth media were used for storing bacteria at -20°C.

CHAPTER FOUR

RESULTS

CHAPTER FOUR

RESULTS

This study was conducted to detect Extended Spectrum β - Lactamases in Gram negative bacilli causing urinary tract infections in hospitalized pregnant women. One hundred and fifty (n=150) pregnant women who admitted to antenatal wards were included from three different hospitals, Omdurman Maternity Hospital (n=138), Omdurman New Hospital (AL-Saudi) (n=7) and Omdurman Military Hospital; department of Obstetrics and Gynaecology (n=5).

Positive urine cultures were reported in symptomatic and asymptomatic pregnant women. As shown in Table 4.1, 33(22%) of the study population showed positive culture, among which, 7.3% were symptomatic and 14.7% were asymptomatic.

The frequency of isolated bacteria among pregnant women is shown in Table 4.2. The most common pathogen was *E. coli* followed by *Staphylococcus spp*, accounting 36.4% and 27.3%, respectively.

Cefuroxime, Ceftazidime, Cefotaxime and Ceftriaxone were used to estimate third generation Cephalosporins resistance in 18 Gram negative bacilli isolates. The highest resistance was reported for Cefuroxime, 100%. Ceftazidime, Cefotaxime and Ceftriaxone showed moderate resistance

pattern (Table 4.3).Resistance to bothCeftazidime and Cefotaxime were detected inisolates of *E. coli*,*K. pneumoniae*and*K. oxytoca* (Table 4.4).

Among the Gram negative bacilli isolates (n=18), ESBL was detected in 8 (44.4%) of these isolates (Figures 4.1 & 4.2).the most prevalent ESBL isolates were *E. coli*accounting,75%.Type of ESBL producer Gram negative bacilli isolates is shown in Table 4.5.

Antibiotics susceptibility patterns of Gram negative bacilli isolates appeared resistance to some antibiotics as follow, Ciprofloxacin (22.2%), Co-trimoxazole (66.7%) and Amoxicillin (83.3%) (Table 4.6).

In this study,ESBL producers were more frequent in age group 15-25 years(62.5%) and in third trimester(87.5%). Distribution of ESBL producers among pregnant women according to their age group and pregnancy trimester is shown in Tables 4.7 and 4.8, respectively.

Health status and some diseases were assessed; distribution of ESBL producers in pregnant women with recurrent UTI was thehighest among other characteristics, accounting 50% (Table 4.9).

Table4.1: Bacterial growth on CLED media of symptomatic and asymptomatic pregnant women (study group n =150).

Urine culture	Study group	Positive culture
Symptomatic	11 (7.3%)	
Growth (n = 33)		
	Asymptomatic	22 (14.7%)
Urine culture	Study group	Negative culture
Symptomatic	42 (28%)	
No growth (n =117)		
	Asymptomatic	75 (50%)
Total		150 (100%)

Values are numbers and percentages of positive and negative urine cultures in symptomatic and asymptomatic pregnant women.

Table 4.2: Identification and frequency of isolated bacteria among positive urine cultures.

Isolated bacteria Frequency (%)	
<i>E. coli</i> 12 (36.4%)	
<i>Staphylococcus spp</i> 9 (27.3%)	
<i>E. faecalis</i> 6 (18.2%)	
<i>K. pneumoniae</i> 4 (12.1%)	
<i>K. oxytoca</i> 2 (6.0%)	
Total	33 (100%)

Table 4.3: Resistance to third generation Cephalosporins among 18 Gram negative bacilli isolates.

Antibiotic	Resistant isolates	Susceptible isolates	Total
Cefuroxime	18 (100%)	0 (0%)	18 (100%)
Cefotaxime	11 (61.1%)	7 (38.9%)	18 (100%)
Ceftriaxone	11 (61.1%)	7 (38.9%)	18 (100%)
Ceftazidime	10 (55.6%)	8 (44.4%)	18 (100%)

Table 4.4: Resistance to both Ceftazidime and Cefotaxime in 18 Gram negative bacilli isolates.

Gram negative bacilli	Resistant isolates
<i>E.coli</i> (n=12) 7 (58.3%)	
<i>K.pneumoniae</i> (n=4) 2 (50%)	
<i>K. oxytoca</i> (n=2) 1 (50%)	

Table 4.5: Distribution of *E. coli*, *K. pneumoniae* and *K. oxytoca* in 8 ESBL producers.

Gram negative bacilli	ESBL producers
<i>E. coli</i> 6/8 (75%)	
<i>K. pneumoniae</i> 2/4 (25%)	
<i>K. oxytoca</i> 0 (0%)	

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Table 4.6: Antibiotics susceptibility patterns of 18 Gram negative bacilli isolates.

Susceptibility patterns					
Antibiotics	Sensitive	Intermediate	Resistant		
			ESBL	Non-ESBL	Total
IPM	18(100%)	0 (0%)	0(0%)	0(0%)	0(0%)
CIP	13(72.2%)	1(5.6%)	2(11.1%)	2(11.1%)	4(22.2%)
STX	6(33.3%)	0(0%)	5(27.8%)	7(38.9%)	12(66.7%)
AMC	2(11.1%)	1(5.6%)	8(44.4%)	7(38.9%)	15(83.3%)

Values are numbers and percentages of Gram negative isolates according to their susceptibility patterns to different antibiotics.

***Abbreviations:**

IPM=Imepenem.

CIP=Ciprofloxacin.

STX=Co-trimoxazole.

AMC=Amoxicillin.

ESBL= Extended Spectrum β - Lactamase producer.

Non-ESBL= non Extended Spectrum β - Lactamase producer.

Table4.7: Distribution of ESBL producers in pregnant women according to their age groups (study group n= 150).

Age group	Positive culture	ESBL producer
15-25years (n=41)	13/33(39.4%)	5/8(62.5%)
26-36 years (n=84)	14/33(42.4%)	2/8(25%)
37-47 years (n=25)	6/33(18.2%)	1/8(12.5%)

Table 4.8: Distribution of ESBL producers in pregnant women according to their pregnancy trimester (study group n= 150).

Pregnancy trimester	Positive culture	ESBL producer
Second trimester(n=8)	1/33 (3.0%)	1/8 (12.5%)
Third trimester (n=142)	32/33 (97.0%)	7/8 (87.5%)

Table 4.9: Distribution of ESBL producers in pregnant women according to their health status(study group n= 150).

Characteristic Positive cultureESBL producer		
(n=33)	(n=8)	
Contraceptive use(n=39)	7/33(21.2%)	3/8(37.5%)
Diabetes mellitus(n=14)	0/33(0%)	0/8(0%)
Recurrent UTI (n=82)	16/33(48.5%)	4/8(50%)
Antibiotic intake (n=78)	17/33(51.5%)	3/8(37.5%)
Hypertension(n=16)	3/33 (9%)	0/8 (0%)
Anemia (n=21)	1/33 (3%)	0/8 (0%)

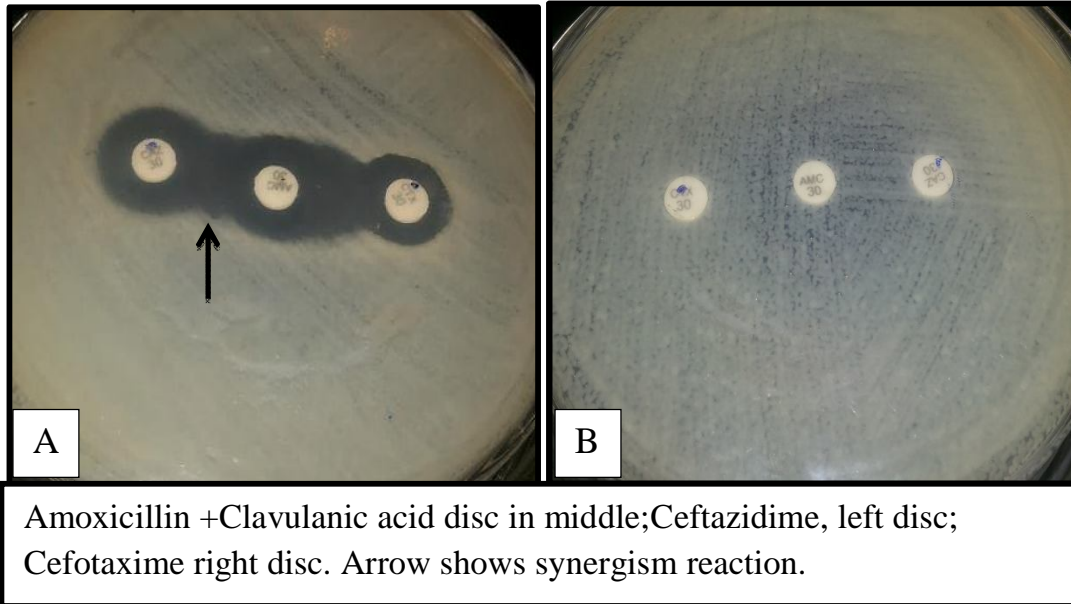


Figure 4.1: Double Disc Synergy Test (DDST).

Photo A: showing positive ESBL producer.

Photo B: showing negative ESBL producer.

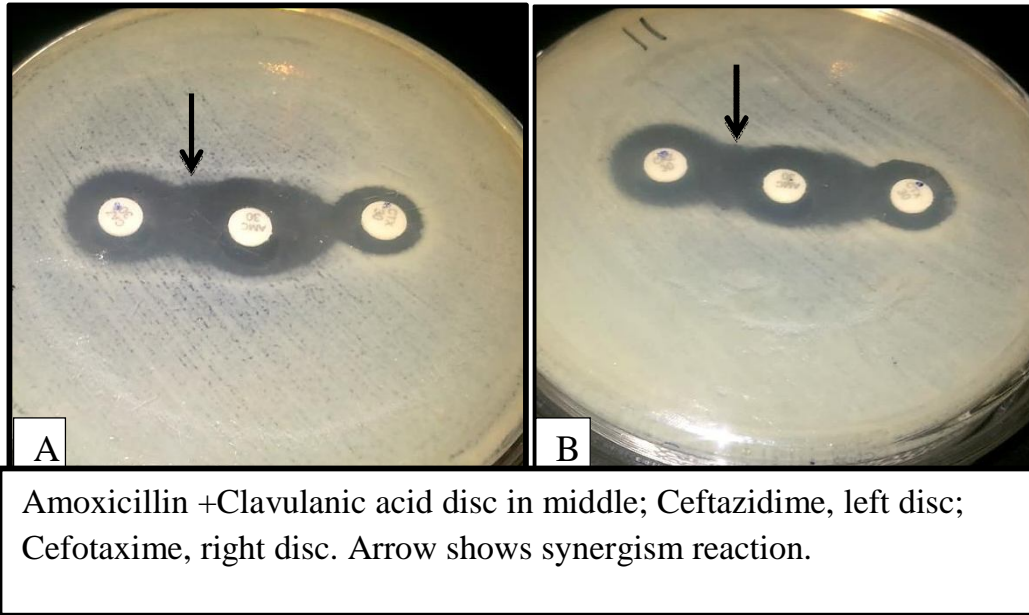


Figure 4.2: ESBL producing Gram negative bacilli.

Photo A: showing positive ESBL *E. coli*.

Photo B: showing positive ESBL *K. pneumoniae*.

CHAPTER FIVE
DISCUSSION, CONCLUSION &
RECOMMENDATIONS

CHAPTER FIVE

DISCUSSION, CONCLUSION & RECOMMENDATIONS

Discussion

Urinary tract infections (UTIs) is the common infection during pregnancy and can be symptomatic or asymptomatic, if untreated could lead to serious complications (Turpin *et al.*, 2007).

Prevalence of bacteriuria in symptomatic infection in this study was reported as 7.3%; which was lower than other studies conducted in Sudan and Tanzania. In these studies, prevalence of symptomatic bacteriuria was found as 12.1% and 17.9% in Sudan and Tanzania, respectively (Hamdan *et al.*, 2011; Masinde *et al.*, 2009).

In this study the prevalence of bacteriuria in asymptomatic infection was 14.7%, which was similar to what reported earlier (Hamdan *et al.*, 2011; Masinde *et al.*, 2009) who reported 14.7% and 13% prevalence of asymptomatic bacteriuria, respectively.

E. coli was the predominant causative agent found in studies done by several authors in many countries (Hamdan *et al.*, 2011; Masinde *et al.*, 2009; Obririkoraga *et al.*, 2012; Celen *et al.*, 2011). In present study the predominant causative agent was *E. coli* 36.4% followed by *Staphylococcus* spp 27.3%, this finding was in line with previous studies.

It was noted that *E. faecalis* in this study represent 18.2%, which is higher than that reported by Celen *et al.*, (2011). This variation may attribute to that all participants in this study under treatment inside antenatal wards.

E. faecalis consider among the most leading nosocomial pathogens, with intrinsic resistance to Cephalosporins (Vesic and Kristich., 2012).

Increase prevalence of ESBL producers among different bacterial strains and species is the most worrying issue in worldwide, especially in hospitals and health care settings (Dhillon and Clark., 2012).

Gram negative *Enterobacteriaceae* expressing ESBL are among the most multi-drug resistant pathogens in hospital and spreading worldwide. Infections caused by ESBL-producing organisms have resulted in poor outcomes, reduced rates of clinical and microbial response, longer hospital stays and greater hospital expenses (Tschdiu-sutter *et al.*, 2010).

In this study ESBL producers were found in 44.4% of Gram negative isolates. This result was similar to a previous study conducted in Nigeria (Azekhueme *et al.*, 2015), and less than those obtained by Mekki *et al.*, (2010) 53% and Ahmed *et al.*, (2013) 59.6% in Sudan. Another studies done by Sharma *et al.*, (2013) in India and Kateregga *et al.*, (2015) in Uganda showed that ESBL producers positive in 57.2% and 62%, respectively. Different wards patients were included in the study of Azekhueme *et al.*, (2015), that can explain the aspects of similarity.

This study revealed that *E. coli* was the most prevalent ESBL producing isolates 75%. This finding was different from previous studies; 65% (Ahmed *et al.*, 2013), 30.2% (Ibrahim *et al.*, 2013), 57% (Ejazet *et al.*, 2011),

52.5%(Azekhueme *et al.*, 2015). A study done by Yu *et al.*, (2006) in Taiwan showed the ESBLs producer *E. coli* were 1.5-16.7%.

ESBLs producing *K. pneumoniae* represent 25% in present study. Such result was less than other studies done by Ahmed *et al.*, (2013) 68.8%, Azekhueme *et al.*, (2015) 47.5%, Sharma *et al.*, (2013) 67.04%, Ejaz *et al.*, (2011) 71.7% and Kateregga *et al.*, (2015) 72.7%. Whereas it's agree with Yu *et al.*, (2006) 8.5-29.8%. This discrepancy in the frequency of ESBLs producers *E. coli* and *K. pneumoniae* in this study with other studies can be attributed to difference in sample size, number and type of Gram negative isolates, gender and even to complexity of ESBLs geographical distribution.

Resistance to Ceftazidime and Cefotaxime of Gram negative bacilli isolates were 55.6% and 61.1%, respectively. Ceftazidime and Cefotaxime resistance were lower than Ahmed *et al.*, report (2013) which were 95.4%, and 94.6%, respectively.

In comparison, Ceftazidime resistance result was less than Kateregga *et al.*, (2015) 73%, while met Kateregga *et al.*, (2015) result with Cefotaxime resistance 57.5%.

All ESBLs producers in present study showed no resistance to Imepenem, which match with results obtained by Ahmed *et al.*, (2013), Azekhueme *et al.*, (2015) and Mekki *et al.*, (2010).

ESBLs producing isolates showed little resistance pattern to Ciprofloxacin 22.2%, similar to study of Ahmed *et al.*, (2013) which was 23.4%. On the other side the result was much lower than Mekki *et al.*, (2010) 92-100%.

Resistance to Co-trimoxazole by ESBL producers was 66.7% in this study, which was higher than Ahmed *et al.*, (2013) result 48.2% and Azkhueme *et al.*, (2015) 14.6-24.3%.

Eighty three percent (83%) of ESBL producing isolates in present study were resistant to Amoxicillin that mismatch with Ahmed *et al.*, (2013) report 43.1%. Variation in the level of different antibiotics resistance pattern between present study and comparable studies could be due to difference in sample size and study setting.

In present study, in spite of high frequency of positive urine cultures among pregnant women in age group 26-36 years 42.4% which was in line with Girishbabu *et al.*, (2011) report 60% , the highest frequency of ESBL isolates was found in age group 15-25 years 62.5%.

Extremes of age, female gender, sexual activity, contraception, pregnancy, instrumentation, UT obstruction, neurologic dysfunction, previous antimicrobial use and other such factors act as predisposing factors for UTI development (Gururajan *et al.*, 2011)

In this study, recurrent UTIs, history of antibiotics intake and contraceptive use represented risk factors for acquiring ESBL producer's infection. In addition, diabetes mellitus, hypertension and anemia were not associated with ESBL producing isolates causing UTIs in hospitalized pregnant women.

Conclusion

The information from this study revealed that there was a high prevalence of asymptomatic bacteriuria among hospitalized pregnant women. *E. coli* was the most prevalent pathogen followed by *Staphylococcus spp.* ESBLs production showed high distribution in Gram negative isolates especially in *E. coli*.

Imepemen remain the most effective treatment for ESBL bacteria causing UTIs in pregnant women. The present study showed that some of ESBL isolates exhibited co-resistance to other antibiotics including Ciprofloxacin, Amoxicillin and Co-trimoxazole.

Recommendations

1. Using urine culture, sensitivity to antimicrobials and ESBL detection should be carried out for admitted pregnant women, in particular those with history of recurrent UTI and among symptomatic and asymptomatic individuals.
2. Genotype characterization of ESBL producing isolates.
3. Further studies in ESBL producing bacteria in pregnant women and other populations in larger sample size are needed to confirm these findings and assess other possible risk factors such as hospital stay duration.

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Appendices

Appendices

Appendix NO 1: Questionnaire.

Appendix NO 2: Informed consent.

Appendix NO 3: Colour plates.

Appendix NO 1

Questionnaire

Serial NO:

Date:

Name:Age:

Section A:

1. Did you have taken any kind of contraceptive?

Yes ☐

No ☐

2. Admission to hospital for:

3. Pregnancy trimester:

First ☐

Second ☐

Third ☐

4. Symptoms and signs of UTI?

Frequency to urinate ☐

Burning sensation ☐

Fever ☐

Lower abdominal pain ☐

5. Recurrence of UTI during present pregnancy?

Yes ☐

No ☐

6. Antibiotic intake?

Yes ☐

No ☐

7. Health status:

Diabetes ☐

Hyper tension ☐

Anemia ☐

Catheter ☐

Section B: Laboratory result form

1. Wet preparation:

Pus cells: HPF

2. Culture and sensitivity test:

Organism isolated:

Sensitive to:

.....

.....

Resistant to:

.....

.....

Susceptibility test to third generation Cephalosporin:

Sensitive ☐

Resistant ☐

3. Double Disc Synergy Test (DDST):

Positive ☐

Negative ☐

Appendix NO2

Informed consent

جامعة السودان للعلوم والتكنولوجيا

كلية الدراسات العليا

وثيقة موافقة للمشاركة في بحث علمي

الباحث :

عنوان البحث :

الجزء الاول :

أنت مدعوه من الباحث : لتشاركي في

دراسة بحثية بعنوان :

.....

الهدف منه : الكشف عن وجود إنزيم في بكتريا العصويات سالبة الجرام المسببة للإلتهابات المجارى

البولية لدى السيدات الحوامل اللواتى يخضعن للعلاج في المستشفى .

يتوقع من هذه الدراسة أنها ستساعد علي معرفة أسباب مقاومة بكتريا العصويات سالبة الجرام لبعض

المضادات الحيوية تتطلب مشاركتك في هذه الدراسة إجراء بعض الإختبارات لعينة البول التي لا

يزيد مقدارها عن 10 مل. ستفحص هذه العينات في معمل الأبحاث بجامعة السودان للعلوم

والتكنولوجيا وتحفظ بالمعمل حتى إكتمال مشروع البحث.

عند الإنتهاء من الفحص المعملّي سوف يتم إبلاغك عن النتائج .

المخاطر : لا يسبب اخذ العينة (البول) أى إزعاج أو ألام للمريضة .

الفوائد المحتملة : قد تجنى فوائد مباشرة لك من خلال معرفة المضادات المناسبة لك و يمكن للمعلومات المستخرجة من هذا البحث أن تساعد العلماء والباحثين علي معرفة المزيد عن إنتشار هذا الإنزيم في بكتريا العصويات سالبة الجرام.

البدائل : البديل للمشاركة في الدراسة هو عدم المشاركة ولك كل الحرية المطلقة لإختيار المشاركة أو عدم المشاركة في هذه الدراسة.

إنهاء المشاركة : سيتم إنهاء المشاركة فى الدراسة إذا قررت الإنسحاب من الدراسة او إذا قرر الباحث بأنك غير مستوفيه لشروط المشاركة فى البحث.

المشاركة التطوعية المشاركة في هذه الدراسة طوعية وا إذا قررت عدم المشاركة فإنك لن تتعرضى لأى مضايقات.

السرية : كمشاركة فى الدراسة ستكون هويتك ومحتويات الإختبارات المعملية سرية فى جميع المنشورات المتعلقة بنتائج الدراسة ويمكن الإطلاع عليها من قبل الباحثين ولجان الكلية فى حدود النظم والقوانين المطبقة بهذا الخصوص.

الأشخاص الذين يمكن الإتصال بهم للإستفسار عن نتائج البحث : يمكن الإتصال بالباحث على رقم الموبايل

الجزء الثاني

أنا أوقع

علي هذه الموافقة بعد ان شرح لي الباحث انني سأشارك في بحث علمي وأجاب علي كل تساؤلاتي
بخصوص هذا البحث.

وبتوقيعي هذا أقر بأنني موافقة علي اخذ العينة (البول) لغرض البحث.

المشاركة في البحث أو من يوقع عنها : الباحث:

الإسم : الإسم :

التوقيع :التوقيع :

التاريخ :التاريخ :

صلة القرابة :

(إذا كان الموقع غير المشاركة)

Appendix NO 3

Colour plates

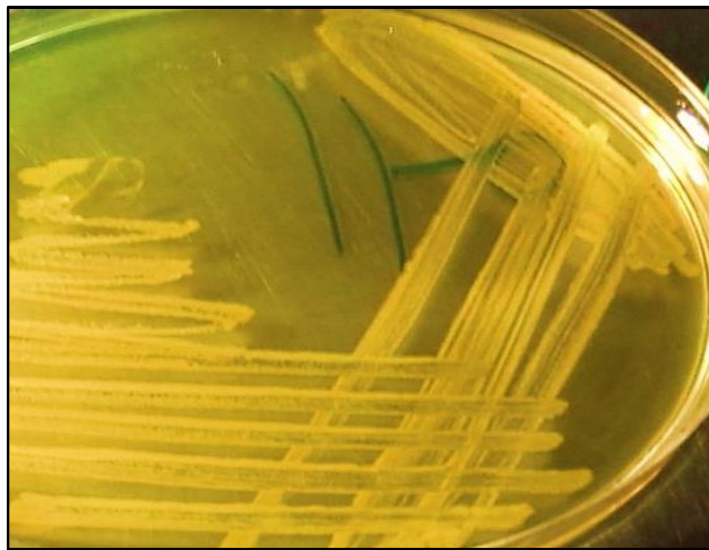


Plate 1: *E. coli* on CLED media showing yellow colour indicating lactose fermentation.

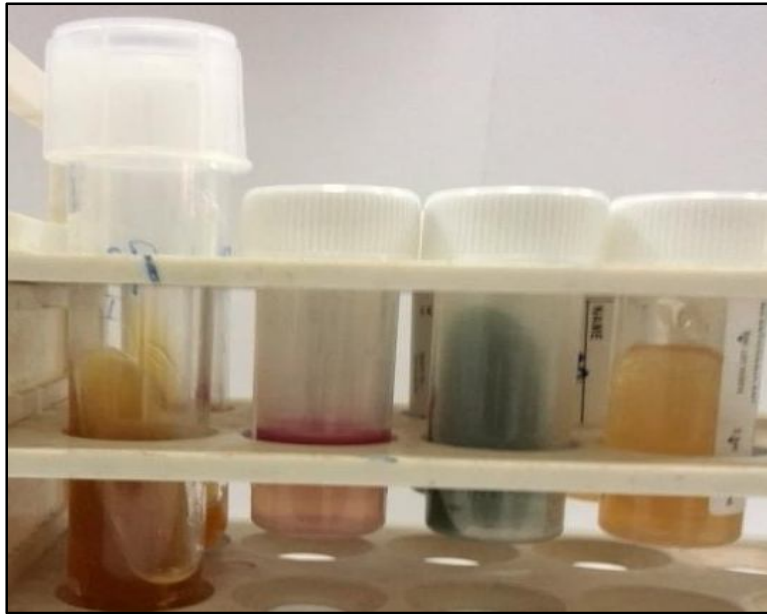


Plate 2: *E. coli* biochemical set/ from left to right, KIA slope yellow, butt yellow, Gas positive, H₂S negative; Indole test, positive; Citrate test, negative; Urease test, negative.



Plate 3: Pure urine culture of *K. pneumoniae* on Blood agar showing Non- hemolytic colonies.

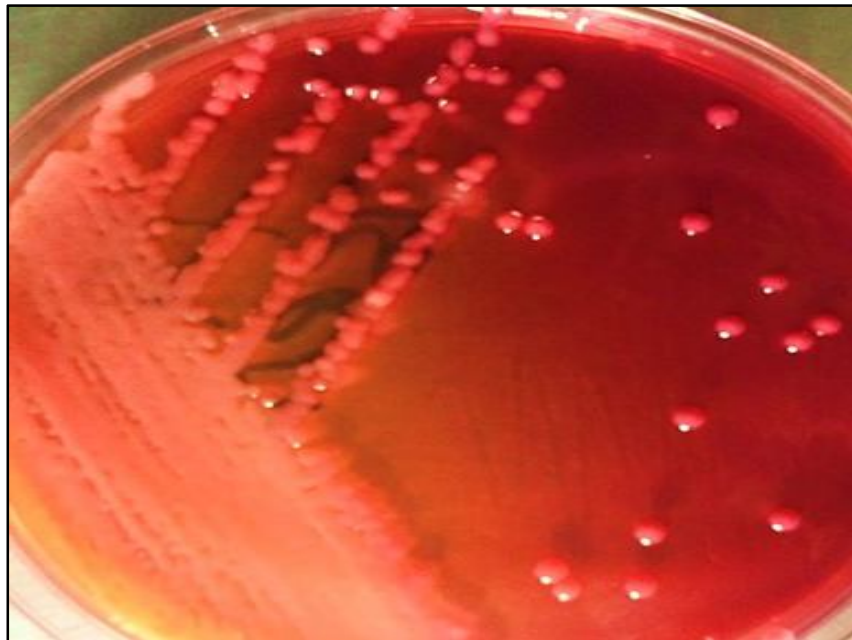


Plate 4: pure urine culture *K.pneumoniae* on MacConkey agar showing pink colour indicating lactose fermentation.

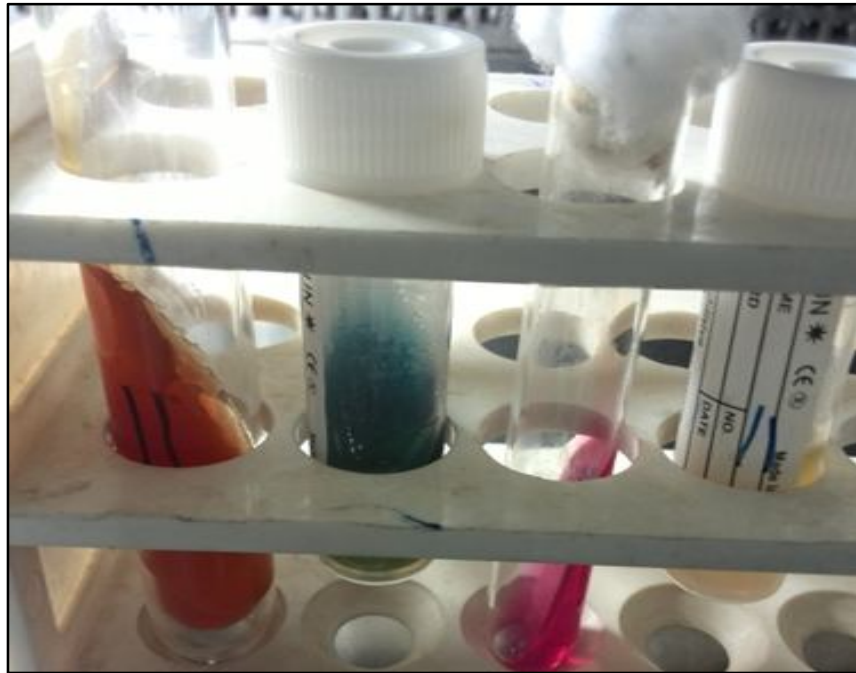


Plate 5: *K. pneumoniae* biochemical set /from left to right, KIA slope yellow, butt yellow, Gas positive, H₂S negative; Citrate test, positive; Urease test, positive; Indole test, negative.

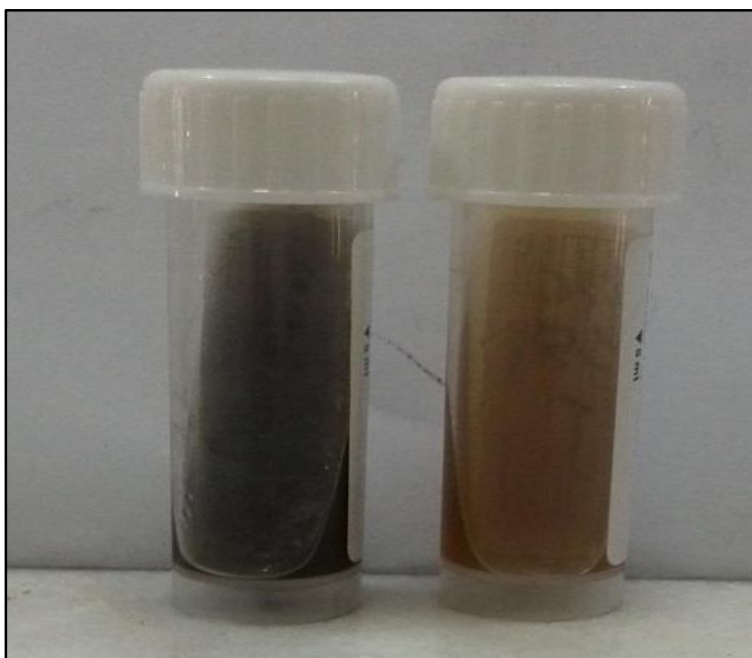


Plate 6:Esculin test of *E.faecalis*.

Left, positive; Right, negative.



Plate 7: *K. oxytoca* on CLED media showing yellow colour indicating lactose fermentation.



Plate 8: *K. oxytoca* biochemical set/ from left to right KIA slope yellow, butt yellow, Gas positive, H₂S negative; Citrate test, positive; Urease test, positive; Indole test, positive.

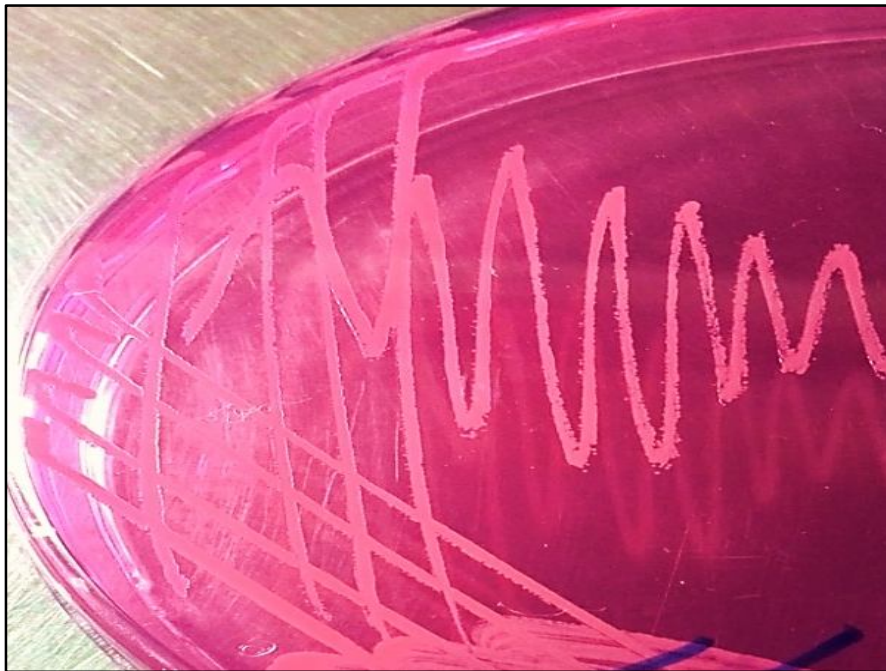


Plate 9: *Staphylococcus spp* on MSA showing pink colour indicating Non-mannitol fermentation.

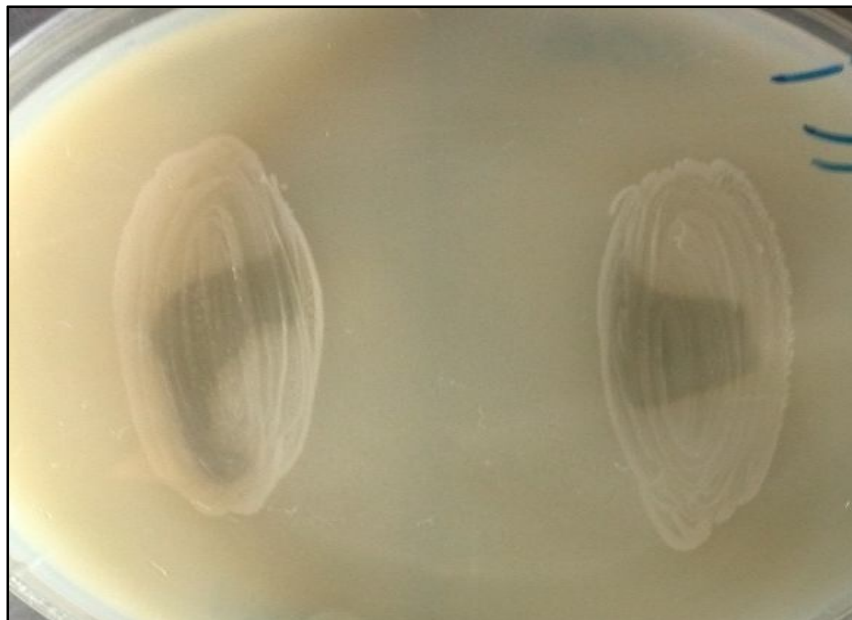


Plate 10: Negative DNase test showing no clear zone around bacterial colonies.



Plate 11: Resistance to antibiotics by Gram negative bacilli. The figure shows sensitivity to Imepenem and Ciprofloxacin and resistance to Amoxicillin and Co-trimoxazole.

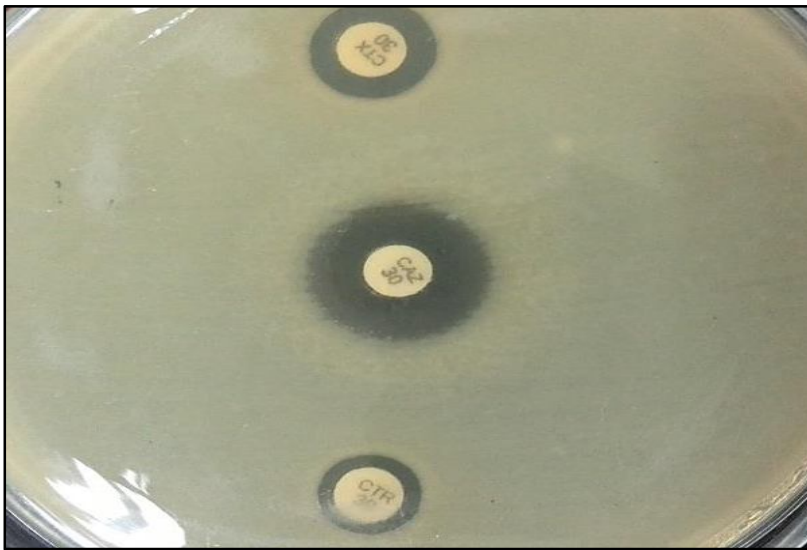


Plate 12: Resistance to third generation Cephalosporins(Ceftazidime; Cefotaxime and Ceftriaxone) by Gramnegative bacilli.