بسم الله الرحمن الرحيم

Shendi University

Faculty of Graduate Studies and Scientific Research

Title:

Detection of Carbapenem Resistant Enterobacteriacae Isolated from Urinary Tract Infection in Khartoum State

A dissertation submitted in fulfillment for the requirement of M.Sc.degree in Medical Laboratory Science (Microbiology)

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الآيـة

بسم الله الرحمن الرحيم

قال تعالى :

((وَقُل رَّبِّ زِدْنِي عِلْمًا))

سورة طه الأية (114)

Dedication

To

My life beats, for whom I work to make their dreams, my parents **Suad**, and

soul of my father **Mohammed Najeeb**…

To..

My life brightness, without whom I could not continue smile, my brothers

Amer, **Osama** & **Mazin..**

To..

My life flavor, my lovely sisters **Alaa** and **Duaa..**

To..

My life friend **Hala Ahmed**, she is filling all my days..

To..

The absolutely necessary person. The gentle reader..

Samar

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Allah enabled me to conduct this study by his blessing therefore thanks for my God **Allah** firstly and lastly.

Special regard and respect for my supervisor **Dr. Hadia** for his helping and skill full guides. Thanks for all **people**, they were very helpful. Thanks for all staff of **Microbiology Department** and **Research Laboratory** for their efforts and patience.

Great thanks for **my family** whom always encourage me for the better. Thanks for **my friends**, who helped me and made my work wonderful. Last thanks for **everyone** helped me in my research.

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List of abbreviation

AAP	American Academy of Pediatrics
APN	Acute pyelonephritis
CPE	Carbapenemase Producing Enterobacteriacae
DM	Diabetes mellitus
E.coli	Escherichia coli
ESPL	Extended-spectrum β lactamases
GBS	group B Streptococcus
MDRE	Multidrug resistant Enterobacteriacae
MHT	Modified hodge test
PN	Pyelonephritis
UPEC	Uropathogenic Escherichia coli
UTI	Urinary tract infections

Abstract

Background:

Urinary tract infection (UTI) is one of the most common infectious diseases and serious ailment in humans, spread of multidrug resistant Enterobacteriacae (MDRE) and recently Carbapenemase Producing Enterobacteriacae (CPE) have emerged as a major public health concern in patients with urinary tract infections (UTIs).The aim of the study to detect Carbapenem resistant Enterobacteriacae isolated from urinary tract infection.

Methods:

We conducted a cross sectional study in Alborge laboratory used 97 isolated organisms from urinary tract infected patients from March up to June using culture, Gram stain, biochemical test and sensitivity then Modified hodge test to detect Carbapenemase enzyme.

Results:

A total of 97 isolated organism from UTI patients, were collected from Alborg laboratories in Khartoum, the result show resistant to Carbapenem drugs 5(5.2%) for both Imipenem and Meropenem among the study group, *Escherichia coli* (highest resistant) 3 (5.2%) , 1(1.7%) respectively, then *Klebsiella spp* 1(3.4%),1 (3.4%) respectively, and *Proteus spp* 1 (12.5%) resist to Imipenem only.

Conclusion:

From 97 isolated organism of Enterobacteriacae , 5 organism resist to Carbapenem , most this organism *E.coli* , some of these resist by Carbapenemase producing and this detect by MHT , few resist by other mechanism.

ملخص الدراسة

خلفية:

يعد التهاب المجارى البولية من اخطر الامراض شيوعا بالنسبة للانسان بواسطة البكتريا المعوية مع انتشار الادوية متعددة المقاومة, وحديثا ظهور مقاومة بواسطة انزيم الكاربيبنيم الذى تفرزه البكتريا المعوية والذى يعد من اكبر المشاكل الصحية للمصابين باخماج المجارى البولية.

تهدف الدراسة لمعرفة البكتريا المعوية المقاومة للكاربيبنيم المعزولة من اخماج المجاري البولية.

الطريقة:

اجريت دراسة مقطعية على 97 عينة معزولة من مرضى بالتهاب المجارى البولية بمختبرات البرج الطبية فى الفترة من مارس الى يونيو 2018, تم استزراع العينات وعمل صبغة جرام , الاختبارات الكيميائية, واختبار الحساسية , واختبار هديج المعدل لمعرفة المقاومة للكاربيبنيم.

النتائج:

اظهرت الدراسة ل 97 عينة معزولة من مرضى التهاب المجارى البولية مقاومة للكاربينيم (اميبينم وميروبينيم) بنسبة بلغت 5 (5.2%), وقد اظهرت الاشريكية القولونية اعلى نسبة مقاومة 3 (5.2%), 1(1.7%) على التوالى, تليها الكلبسيلا, 1(3.4%), 1(3.4%) على التوالى بينما اظهرت المتقلبة الرائعة مقاومة للاميبينيم فقط 1(12.5%).

الخلاصة:

من 97 ممرض معزول من البكتريا المعوية, 5 ممرضات مقاومة للكاربيبنيم ومعظم هذه الممرضات الاشريكية القولونية , بعض هذه المقاومة بسبب انتاج انزيم الكاربيبنيم وهذا اكتشف باختبار هديج المعدل, قليل من هذه المقاومة بآليات أخرى.

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Chapter one

Introduction

Chapter one

Introduction

1.1. Introduction

Urinary tract infection (UTI) is one of the most common infectious diseases and serious ailment in humans. It is important because it may involve kidneys, ureters, bladder and urethra. It has been reported in all age groups and in both sexes. However, women are more susceptible than men, due to short urethra, absence of prostatic secretion; pregnancy and easy contamination of the urinary tract with fecal flora. Gram negative bacteria play an important role in urinary tract infection. *Escherichia coli* remained the most common causative agent of uncomplicated UTI for many years with (75-90%) causes of UTI infection. The other gram negative pathogens causing urinary tract infection are *Klebsiella spp., Proteus mirabilis* and *Pseudomonas aeruginosa* (Ameri *et al.*, 2014). Occur more often in women than in men, at a ratio of 8:1. Approximately (50-60%) of women report at least one UTI in their lifetime, and one in three will have at least one symptomatic UTI necessitating antibiotic treatment by age 24. (Badrand & Shaikh, 2013).The bacteria that cause urinary tract infections typically enter the bladder via the urethra. However, infection may also occur via the blood or lymph. It is believed that the bacteria are usually transmitted to the urethra from the bowel, with females at greater risk due to their anatomy (Salvatore *et al.*, 2011). From the late 1990s, multidrug-resistant *Enterobacteriaceae* (mostly *Escherichia coli)* that produce extended-spectrum β lactamases (ESBLs), have emerged within the community setting as an important cause of UTIs. The Carbapenems are widely regarded as the drugs of choice for the treatment of severe infections caused by ESBL-producing *Enterobacteriaceae* (Pitout & Laupland, 2008). Spread of multidrug

resistant *Enterobacteriaceae* (MDRE) and recently Carbapenemase Producing *Enterobacteriaceae* (CPE) have emerged as a major public health concern in patients with urinary tract infections (UTIs). The emergences of multidrug resistant among *Enterobacteriaceae* were mainly due to the production of enzymes, recently carbapenemase production is one of the main mechanisms in the occurrence of drug resistance in the family of *Enterobacteriaceae*. Carbapenemase Producing *Enterobacteriaceae* (CPE) are difficult to treat because they have high levels of resistance to antibiotics, which capable of break down all β-lactam agents including Carbapenems and make it ineffective. Carbapenem such as Imipenem, Meropenem, Ertapenem & Doripenem are considered as the last resort antibiotics to treat ESBL producing *Enterobacteriaceae* (Eshetie *et al*., 2015).

1.2. Rationale

Urinary tract infection are the common infection among Sudanese patients, the most of these infection cause by *Enterobacteriacae* which is become resist to wide range of antibiotic like Penicillin group, Carbapenem group and Fluoroquinolones group .

To assess the prevalence of Carbapenem resistant in Khartoum city and know resistance mechanism if by Carbapenemase producing or by other mechanism.

1.3. Objectives

1.3.1 General objective

To detect Carbapenem resistant *Enterobacteriacae* isolated from urinary tract infection.

1.3.2 Specific objectives

1. To identify *Enterobacteriacae* that resist to Carbapenem from isolates of urinary tract infection.
2. To determine the most common *Entrobacteriacae* produce Carbapenemase enzyme using Modified Hodge Test.

Chapter two

Literature Review

Chapter two

2. Literature Review

2.1. Anatomy of urinary tract:

Contiguous hollow-organ system, comprised from proximal to distal, of renal papillae, renal pelvis, ureters, bladder, and urethra, each component of the urinary tract has distinct anatomic features and performs critical functions (Hickling *et al*., 2015).

2.2. Background:

Urinary tract infection (UTI) is broadly defined as an infection of the urinary system, and may involve the lower urinary tract or both the lower and upper urinary tracts(Rowe & Juthani-Mehta, 2013), most often reflecting cystitis (infection of the bladder) or pyelonephritis (infection of the kidney) (McLellan & Hunstad, 2016). Symptom confined to lower urinary tract commonly dysuria, frequency and urgency. Pyelonephritis (inflammation of the renal parenchyma) is a clinical syndrome characterized by chills and fever, flank pain and constitutional symptoms caused by bacterial invasion of the kidney (Najar *et al*., 2009). Uropathogenic *Escherichia coli* (UPEC) is the most common cause of UTI (especially among community-onset infections) (McLellan & Hunstad, 2016).The risk factors associated with UTI include sex, genetic predisposition, sexual intercourse, use of spermicidal agents, diabetes, immune-suppression, pregnancy, hypertension, stone formation, nosocomial acquired infections and instrumentation like catheterization (BEHZADI *et al*., 2010). Diagnosis of a urinary tract infection is the detection of the pathogen in the presence of clinical symptoms. The pathogen is detected and identified by urine culture (using midstream urine) (Schmiemann *et*

al., 2010). Treatment for uncomplicated UTI uses Nitrofurantoin Monohydrate/Macrocrystals 100 mg twice daily for 5 days, or Trimethoprim–Sulfamethoxazole 160/800 mg twice daily for 3 days. Fluoroquinolones are among the most prescribed antibiotics for UTI, but resistance to these antimicrobials is high and they should only be used if sensitivity testing is performed (Rowe &Juthani-Mehta, 2013). Screening and treatment of asymptomatic bacteriuria is only necessary in exceptional cases for example, in pregnant women or before a urological operation (Schmiemann *et al.*, 2010) . Overtreatment with antibiotics for suspected urinary tract infection remains a significant problem, and leads to a variety of negative consequences including the development of multidrug-resistant organisms (Rowe & Juthani-Mehta, 2013). About (150) million people developed a urinary tract infection each year (Flores-Mireles *et al.*, 2015). UTIs are one of the most frequent clinical bacterial infections in women, accounting for nearly (25%) of all infections. Around (50–60%) of women will experience a UTI in their lifetime. Recurrences usually occur within three months of the original infection, and (80%) of recurrent UTIs are reinfections (Al-Badrand & Al-Shaikh, 2013).

2.3. Most common bacteria causing urinary tract infection:

Urinary tract infections are primarily caused by Gram-negative bacteria. The main pathogen responsible for uncomplicated cystitis and pyelonephritis is *Escherichia coli* followed by other species of *Enterobacteriaceae*, such as *Proteus mirabilis* and mostly *Klebsiella pneumoniae*, and by Gram-positive pathogens, such as *Enterococcus faecalis* and *Staphylococcus saprophyticus* (Mazzariol *et al*., 2017).

2.4. *Enterobacteriacae:*

The *Enterobacteriaceae* is a family of bacteria with many different genera and species. Members of this family are gram negative, non-spore forming, and facultative anaerobes, which include many opportunistic and pathogenic species.

Clinical isolates of *Enterobacteriaceae* that are commonly seen in acute and long term care centers are *Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis* (Mahon *et al*., 2010).

2.5. Signs and symptoms:

Cystitis, or infection of the lower urinary tract (i.e. bladder), traditionally presents with urinary urgency, frequency, dysuria or foul-smelling urine. In contrast, pyelonephritis, or infection of the upper urinary tract (i.e. kidney), is often associated with more severe or systemic symptoms, including fever, back pain, flank pain or vomiting (Lindsey *et al*., 2017).

2.5.1. Children

In infants and young children, these symptoms are often absent or difficult to identify. Fever may be the only symptom. Currently, the American Academy of Pediatrics (AAP) recommends that UTI be considered in any infant or child aged between 2 months and 2 years presenting with fever with no identifiable source of infection. In addition to fever, infants and young children with UTI can present with irritability, poor feeding or vomiting (Lindsey *et al*., 2017).

2.5.2. Elderly

When infected, are more likely to present with nonspecific symptoms, such as anorexia, confusion and a decline in functional status , fever may be absent or diminished (Rowe & Juthani-Mehta, 2013).

2.6. Causes and Risk factor of urinary tract infection:

UTIs are caused by both Gram-negative and Gram-positive bacteria, as well as by certain fungi. The most common causative agent for both uncomplicated and complicated UTIs is uropathogenic *Escherichia coli*. For the agents involved in uncomplicated UTIs, uropathogenic *Escherichia coli* is followed in prevalence by

Klebsiella pneumoniae, Staphylococcus saprophyticus, Enterococcus faecalis, group B Streptococcus (GBS), *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida spp*. For complicated UTIs, the order of prevalence for causative agents, following uropathogenic *Escherichia coli* as most common, is *Enterococcus spp, Klebsiella pneumoniae, Candida spp*., *Staphylococcus aureus, Proteus mirabilis, Pseudomonas aeruginosa* and *group B Streptococcus*. Several risk factors are associated with cystitis, including female gender, sexual activity, vaginal infection, diabetes, obesity and genetic susceptibility. Complicated UTIs are defined as UTIs associated with factors that compromise the urinary tract or host defense, including urinary obstruction, urinary retention caused by neurological disease, immunosuppression, renal failure, renal transplantation, pregnancy and the presence of foreign bodies such as calculi, indwelling catheters or other drainage devices (Flores-Mireles *et al*., 2015).

2.7. Pathogenesis:

Escherichia coli is the most common bacterial pathogen responsible for UTI and accounts for (85–90%) of cases. Uropathogenic *E.coli* are thought to originate from the faecal flora, spread across the perineum, and invade the bladder through the urethral opening. Bacterial attachment to the urothelium and internalization are essential in establishing UTI. If Uropathogenic *E.coli* attach to the urothelium and undergo internalization, they trigger a host inflammatory response which results in the production of distinct inflammatory mediators. This response is followed by the activation of innate immune cells and proteins which migrate to the infectious focus and facilitate eradication of the invading bacteria. Tissue damage following UTI is the result of this inflammatory response (Lindsey *et al*., 2017).

2.8. Antibiotic resistance:

Resistance occurs when a drug loses its ability to inhibit bacterial growth effectively. Bacteria become 'resistant' and continue to multiply in the presence of therapeutic levels of the antibiotics. The resistance process occurs via gene level mutations (Zaman *et al*., 2017).

2.9. Multidrug resistance gram negative organism:

Pathogens carrying resistance to one or more antimicrobials from at least three different classes. The most common mechanism of resistance is represented by intrinsic and acquired production of β-lactamases, which can be chromosomal or plasmid mediated. In the last decade, we have witnessed a dramatic increase worldwide in the number of multidrug resistant Gram-negative bacteria pathogens, with *Enterobacteriacae* (mostly *Klebsiella pneumoniae*), *Pseudomonas aeruginosa* and *Acinetobacter baumannii* being the major threats in clinical practice, multidrug resistant Gram negative bugs have been associated with delays in an adequate treatment, leading to significant increases in morbidity and mortality (Matteo *et al.*, 2016).

2.10. Carbapenem resistant:

Carbapenems are potent members of the β-lactam family of antimicrobials that are structurally related to the Penicillins. Mode of action of Carbapenems is initiated first by penetrating the bacterial cell wall and binding to enzymes known as Penicillin-binding proteins (PBPs). Development of resistance to Carbapenems may be due to intrinsic or acquired resistance mechanisms or both. Bacteria have acquired multiple mechanisms of resistance including enzymatic inactivation, target site mutation and efflux pumps (Codjoe & Donkor, 2018).

2.11. Diagnosis:

Accurate UTI diagnosis relies upon identification of the clinical symptoms in conjunction with positive laboratory testing. To establish the diagnosis of a UTI, positive laboratory testing includes (i) urinalysis indicating the presence of infection (pyuria or bacteriuria) and (ii) a positive urine culture growing at least 50,000 colony-forming units (CFU)/mL of auropathogen from an appropriately collected urine specimen. Obtaining an appropriately collected urine specimen before the administration of antibiotics is one of the fundamental barriers to the accurate diagnosis of UTI. Clinically, there are four methods of collecting urine, each with its own benefits and challenges. More appropriate methods include clean-catch midstream samples, transurethral bladder catheterization or suprapubic aspiration (Lindsey *et al*., 2017).

2.12. Classification:

UTIs are classified into 6 categories. The first category is an uncomplicated infection; this is when the urinary tract is normal, both structurally and physiologically, and there is no associated disorder that impairs the host defense mechanisms.The second category is a complicated infection; this is when infection occurs within an abnormal urinary tract, such as when there is ureteric obstruction, renal calculi, or vesicoureteric reflux. The third category, an isolated infection, is when it is the first episode of UTI, or the episodes are 6 months apart. Isolated infections affect (25–40%) of young females.The fourth category, an unresolved infection, is when therapy fails because of bacterial resistance or due to infection by two different bacteria with equally limited susceptibilities.The fifth category, reinfection, occurs where there has been no growth after a treated infection, but then the same organism regrows two weeks after therapy, or when a different microorganism grows during any period of time.The sixth category, relapse, is

when the same microorganism causes a UTI within two weeks of therapy; however, it is usually difficult to distinguish a reinfection from a relapse (Al-Badr & Al-Shaikh, 2013).

2.13. Differential diagnosis:

The most common cause of acute dysuria is infection, especially cystitis. Other infectious causes include urethritis, sexually transmitted infections, and vaginitis. Noninfectious inflammatory causes include a foreign body in the urinary tract and dermatologic conditions. Non inflammatory causes of dysuria include medication use, urethral anatomic abnormalities, local trauma, and interstitial cystitis/bladder pain syndrome (Michels & Sands, 2015).

2.14. Prevention:

The main strategy for preventing UTIs in men is to avoid the use of indwelling catheters (Beardsley, 2018). Prevention of catheter-associated UTI (CA-UTI) has recently become a top priority in many institutions, including aseptic insertion of urinary catheters, minimizing use of catheters and minimizing duration of catheter use, has led to a decrease in the incidence of catheter-associated UTI (CA-UTI). In adults who require catheterization, the use of antimicrobial-coated catheters may delay bacterial colonization and thus decreases the incidence of catheter-associated UTI. Prevention strategies for recurrent UTI in postmenopausal women have been studied and include use of antibiotic prophylaxis and non antimicrobial therapies, such as estrogen replacement therapy and cranberry formulations (Rowe & Juthani-Mehta, 2013). Factors that do not seem to increase the risk of UTI include diet, use of tampons, clothing and personal hygiene including methods of wiping after defecation and bathing practices (Najar *et al*., 2009).

2.15. Medications:

Phenazopyridine is a urinary tract analgesic for adjunctive treatment of pain associated with urinary tract infections in addition to antibiotics aimed at the underlying microbial infection. It is available as an over-the-counter medication for temporary relief of dysuria while the patient is awaiting medical evaluation and treatment (Shi *et al*., 2004).

2.15.1. Alternative medicine:

Although standard UTI therapy starts with antimicrobial therapy, alternative strategies are available to reduce exposure to antibiotics, such as the use of methenamine salts, probiotics, cranberry juice, immunoprophylaxis and vaginal oestrogens in post-menopausal women (Al-Badr & Al-Shaikh, 2013).

2.16. Treatment:

The goal of antimicrobial therapy is to eliminate the infecting organisms from the urinary tract and provide the resolution of symptoms. Clinicians should consider many factors when selecting an antibiotic for a urinary tract infection, such as the patient's allergy history, the cost and tolerability of the treatment, previous antibiotic therapy, and most important, the prevalence of resistance in the community.The antimicrobial agents most commonly used to treat uncomplicated urinary tract infections include the combination drug Trimethoprim and Sulfamethoxazole, Trimethoprim, β-lactams, Fluoroquinolones, Nitrofurantoin, and FosfomycinTromethamine. These agents are used primarily because of their tolerability, spectrum of activity against suspected uropathogens. Also, agents such as Trimethoprim-Sulfamethoxazole or the Fluoroquinolones that eradicate aerobic gram-negative flora but have little effect on the vaginal and fecal anaerobic flora

seem to provide the best long-term cures for uncomplicated urinary tract infections (Jancel & Dudas, 2002).

2.16.1. Asymptomatic bacteriuria

Isolation of a specified quantitative count of bacteria in an appropriately collected urine specimen obtained from a person without symptoms or signs referable to urinary infection. Its reported prevalence is higher in older persons, in women, and in the presence of genitourinary abnormalities ,asymptomatic bacteriuria a paradigm shift from treatment to non-treatment has occurred in recent years. Treating asymptomatic bacteruria does more harm than good and should be avoided (Sendi *et al*., 2017).

2.16.2. Uncomplicated

Uncomplicated UTI are represented by the acute uncomplicated cystitis and the uncomplicated pyelonephritis.They are mainly caused by *E.coli*. As an empirical therapy for uncomplicated cystitis Fosfomycin Trometamol, Nitrofurantoin or Pivmecillinam are recommended as first-line agents. As the oral first line therapy for uncomplicated pyelonephritis Fluroquinolonesin high dosages are recommended (Wagenlehner *et al*., 2011).

2.16.3. Complicated

A complicated urinary tract infection is a urinary infection occurring in a patient with a structural or functional abnormality of the genitourinary tract. Organisms isolated from patients with complicated urinary infection tend to be more resistant to antimicrobials. Repeated antimicrobial courses in patients with recurrent infection and nosocomial acquisition through urological interventions contribute to the increased prevalence of resistance (Nicolle, 2005).

2.16.4. Pyelonephritis

Pyelonephritis (PN) is a suppurative infection of the kidney, most commonly due to bacterial infection and may be either acute or chronic. Acute pyelonephritis (APN) subdivided into uncomplicated and complicated (Venkateshand & Hanumegowda, 2017). Diagnosis of acute pyelonephritis may be clinical. Severe cases should be treated with a Fluoroquinolone or extended-spectrum Cephalosporin associated or not with Aminoglycoside. Treatment should be continued for at least 10 days (Rollino, 2007).

2.17. Epidemiology:

UTIs are one of the most frequent clinical bacterial infections in women, accounting for nearly (25%) of all infections. Around (50–60%) of women will experience a UTI in their lifetime. The estimated number of UTIs per person per year is 0.5 in young females. Recurrences usually occur within three months of the original infection, and 80% of recurrent UTIs are reinfections. The incidence of UTI increases with age and sexual activity. Post-menopausal women have higher rates of UTIs because of pelvic prolapsed, lack of estrogen, loss of lactobacilli in the vaginal flora, increased per urethral colonization by *Escherichia coli (E.coli),* and a higher incidence of medical illnesses such as diabetes mellitus (DM). The microorganism that causes recurrent UTIs is similar, in most cases, to the sporadic infection. Most uropathogens from the rectal flora ascend to the bladder after colonizing the per urethral area and urethra (Al-Badr &Al-Shaikh, 2013).

2.18. Previous Studies:

Study done by Eshetie *et al.* Use urine sample in 2014 ,Multidrug resistant and carbapenemase producing Enterobacteriaceae among patients with urinary tract infection at referral Hospital, Northwest Ethiopia, the result showed that"160

(87.4%) were MDRE; the most common isolates were *Klebsiella pneumoniae* and *Escherichia coli*. Five (2.73%) of the isolates were found to be carbapenemase producers (Eshetie *et al.*, 2015).

Another study done in Asia during (2000-2012) by Xu, *et al*, Epidemiology of carbapenem resistant Enterobacteriaceae (CRE) during (2000-2012) in Asia, the result showed that ৳Resistance rates to Imipenem and Meropenem in *Enterobacteriaceae* are 0.8% (95% CI, 0.6-0.9%) and 1.0% (95% CI, 0.8-1.3%) respectively, resistant rates for Imipenem and Meropenem in *klebsiella* (highest resistant) 0.8(0.6-1.1),1.4 (0.8-2.1) respectively, for *Escherichia coli* (lowest resistant)0.2 (0.1-0.3),0.2 (0.1-0.4) respectively (Xu *et al.*, 2015).

Other study done by Leges *et al* in 2014 use urine sample in Addis Ababa ,extended-spectrum beta-lactamase and carbapenemase-producing Enterobacteriaceae among Ethiopian children,the result showed that ৳Among the 33 isolated Enterobacteriaceae, 18.2% (n=6/33) showed intermediate or high resistance to Imipenem and/or Meropenem, prevalence of Carbapenem-resistant Enterobacteriaceae (CRE) was 12.12% (n=4/33). Carbapenemase-producing organisms in this study were *Klebsiella pneumonia* (10.5%, n=2/19), *Klebsiella oxytoca* (100%,n=1/1), and *Morganella morganii* (50%, n=1/2) (Legese *et al*., 2017).

Also study done by Dahab *et al* in Khartoum state in the period from February to August 2016 use different clinical specimens, Phenotypic and genotypic detection of carbapenemase enzymes producing gram-negative bacilli isolated from patients in Khartoum State ,the result showed that "The most predominant Gram-negative bacilli isolates was *E.coli* (54.4%), followed by *Klebsiella* species (29.5%). More than fifty percent of the isolates were Carbapenem resistant. Fifty six percent of the resistant isolates were positive by Modified Hodge Test (Dahab *et al.*, 2017).

Last study done by Amjad *et al* use different sample from January 2010 to December 2010 in Pakistan, Modified Hodge test: A simple and effective test for detection of carbapenemase production, the result showed that "Out of 200 isolates, 138 (69%) were positive for carbapenemase production by Modified Hodge test. Out of 138 MHT positive organisms, the frequency of *Escherichia coli* was (38%), followed by *Pseudomonas aeruginosa* (30%), *Klebsiella pneumoniae* (17%), *Acinetobacter baumannii* (12%), *Citrobacter diversus* (2%) and *Enterobacter agglomerans* (1.4%) (Amjad *et al*., 2011).

Chapter three

Materials and methods

Chapter three

3. Materials and methods

3.1. Study Design:

A cross-sectional descriptive study.

3.2. Study Area:

Sample from Alborg laboratories in Khartoum, study done in Alnasim laboratory.

3.3. Study Population:

Patient with urinary tract infections in all age.

3.4. Duration of study:

The study done from March to June 2018

3.5. Sample size:

A total of (97) samples were be collected.

3.6.Ethical consideration:

Ethical approval was taken from the ethical committee of Shendi University to conduct this research. Also the patients was inform about the research and give the complete freedom to participate or not.

3.7. Methodology:

3.7.1. Culture and colonial morphology:

Culture of isolated organism was made on CLED agar (Appendix 10) after sterilization of the wire loop by flame , well area was made then lines were made and sterilization of the loop between the areas then zigzag was made. The plates were incubated at 37◦C for 24 hours (Collee *et al*., 2007)

Interpretation of Results:

Green colony …..non lactose fermenting.

Yellow colony…..lactose fermenting.

3.10.2.Gram's Stain:(Appendix 1)

Thin smear was made on clean ,dry microscope slide, then it left to dry and fixed by passing three times through Bunsen's flame, it covered by crystal violet for 1 minute then washed by running tap water , then it was covered by logol's iodine for 1 minute then washed by running tap water , decolorization done using acetone-alcohol for 10 seconds ,then it covered by safranin solution for 2 minutes then washed by running tap water (Collee *et al*., 2007).

Interpretation of Results

Violet color ….. Gram's positive.

Red color ….. Gram's negative.

3.10.3 Identification Test:

3.10.3.1 Oxidase Disks (Appendix 11)

The organism was taken by sterile wooden stick then it was put on the disk impregnated with the reagent (tetramethyl-p-phenylenediamine), changing the color of the disk within 5 seconds means that cytochrome c oxidase is present (Collee *et al*., 2007).

Interpretation of Results:

Purple color …..oxidase positive.

No purple color….. oxidase negative.

3.10.3.2.Kligler's Iron Agar:

The medium was stabbed after taking the colony by straight wire and then streaked on the surface of the slope of kligler iron agar medium (Appendix 5) (Collee *et al*., 2007).

Interpretation of Results:

Slant or Butt	**Color**	**Utilization**
Alkaline\acid	Red \yellow	Glucose fermentation
Acid\acid	Yellow\yellow	Glucose and lactose fermentation
Alkaline/alkaline	Red\red	No sugars fermentation
	Black	H2S production
Crack's		Gas production

3.10.3.3.Tryptophan Containing Medium:

The wire loop was sterilized by the flame then colony was taken and suspension was made on the tryptophan containing medium (Appendix 4) and incubated at 37◦C for 24 h, 0.5 ml of kovac's reagent (Appendix3)was added next day (Collee *et al*., 2007).

Interpretation of Results:

Red ring color…..Indole positive.

Yellow ring color…..Indole negative.

3.10.3.4 Semi Solid Medium

The colony was stabbed using sterile straight wire on the semi solid medium (Appendix 7) and incubated at 37 ◦C for 24 h (Collee *et al*., 2007).

Interpretation of Results:

Diffusible line …..positive.

No diffusible line …..negative.

3.10.3.5 Simmon's Citrate Medium

The colony was cultured on the citrate medium(Appendix 6) and incubated at 37 ◦C for 24 h (Collee *et al*., 2007).

Interpretation of Results:

Blue color…..positive.

Green color…..negative.

3.10.3.6 Urea Containing Medium

The colony was cultured on the urea medium (Appendix 8) and incubated at 37◦C for 24 h (Collee *et al*., 2007).

Interpretation of Results:

Pink color…..positive urease.

Yellow color…..negative urease.

3.10.4 Antimicrobial Susceptibility Test(Kirby-Bauer Method)

The colony was suspended on sterile normal saline (Appendix 2) and compared to the 0.5 McFarland turbidity standard (Appendix 9) ,then lawn streaking was made using sterile cotton swab on Mueller-hinton agar (Appendix 12),then antibiotic disks was put on the medium and incubated at 35◦C for 16-18h.

Table (3-1): Show the Antibiotics used and sensitivity zone (Appendix 13)

Antibiotics	**Abbreviation**	**Sensitive**	**Intermediate**	**Resistant**
Imipenem	IPM 10 units	**23**	**20-22**	**19**
Meropenem	MER10 units	**23**	**20-22**	**19**

Control organisms:

The control organisms were applied to all procedure by *Escherichia coli (ATCC 25922), Staphylococcus aureus (ATCC 25923), and Pseudomonas aeruginosa (ATCC 27853)* obtained from department of Microbiology in Central lab.

3.10.5. Modified Hodge Test:

The Modified hodge test was performed for all isolates Carbapenem resist. Ertapenem or Meropenem disks were placed on the agar plate seeded with *Escherichia coli ATCC 25922* (indicator strain). The isolates were inoculated in a straight line out from the edge of the disk to the edge of the plate. The plates were

incubated overnight at 35 ± 2◦C in ambient air for 16-24 hours (Amjad *et al*., 2011).

Interpretation of Result

-Positive test has a clover leaf-like indentation of the *Escherichia coli 25922* growing along the test organism growth streak within the disk diffusion zone.

- Negative test has no growth of the *Escherichia coli 25922* along the test organism growth streak within the disc diffusion.

Chapter four

Results

Chapter four

4.Results

Atotal of 97 isolated organisms of *Enterobacteriacae* were collected from Alborg laboratories in Khartoum. The aim of the study to detect *Enterobacteriacae* that resist to Carpabenem drugs 5(5.2%) these result show in table (4-1) and detect most Enterobacteriacae cause urinary tract infection show in table (4-2). For both Imipenem and Meropenem among urinary tract infection patient the result show *Escherichia coli* (highest resistant) 3(5.2%),1(1.7%) respectively.

Then *Klebsiella spp* 1(3.4%),1(3.4%) respectively, and *Proteus spp* 1(12.5%) resist to Imipenem only, *Serratia* and *Citrobacter* sensitive for both these show in below table (4-3).By using Modified Hodge Test to detect carbapenemase enzyme show this result in table (4-4).

Table (4-1) Resistance to Carbapenem:

Carpapenem	**Frequency**	**Percent**
Sensitive	92	94.8%
Resistance	5	5.2%
Total	**97**	**100%**

Table (4-2) Percentage of *Enterobacteriacae* in isolates from urinary tract infection:

organism	**Frequency**	**Percent**
E. coli	58	60
Klebsiella spp	29	30
Proteus spp	8	8
Serratia spp	1	1
Citrobacter spp	1	1
Total	97	100%

Table (4-3) Show Percentage of Carpabenem resistant (Imipenem and Meropenem)

		E. coli	*Klebsiella*	*Proteus*	*Serratia*	*Citrobacter*
IPM	Sensitive	54 (93.1%)	28(96.6%)	7(87.5%)	1(100%)	1(100%)
	Resistant	**3 (5.2%)**	**1(3.4%)**	**1(12.5%)**	0	0
	Intermediate	1(1.7%)	0	0	0	0
	Total	58 (100%)	29(100%)	8(100%)	1(100%)	1(100%)
MER	Sensitive	56(96.6)	28(96.6%)	8(100%)	1(100%)	1(100%)
	Resistant	**1(1.7%)**	**1(3.4%)**	0	0	0
	Intermediate	1(1.7%)	0	0	0	0
	Total	58 (100%)	29(100%)	8(100%)	1(100%)	1(100%)

IPM: Imipenem **MER: Meropenem**

Table (4-4) Result of Modified hodge test

organism name				
		E. coli	*Klebsiella*	*Proteus*
MOH	Positive	2	1	1
	Negative	1	0	0
	Total	3	1	1

Chapter five

Discussion

Conclusion

Recommendations

Chapter five

5.1. Discussion

This is cross sectional study was conducted in Khartoum city during the period from March to June 2018, to detect Carbapenem resistant among isolates of urinary tract infection.The main causative agents of urinary tract infection were *Esherichia coli* (58%) followed by *Klebsiella spp* (29%), most of the causative agents are sensitive to Meropenem and Imipenem disc, while few number of them were resist 5(5.2%). Study done by Eshetie *et al* (2014) in Ethiopia, found that the most common isolates were *Klebsiella pneumonia* and *Esherichia coli.* Five (2.73%) of the isolates were found to be carbapenemase producers, other study done by Legese *et al*, (2014) also in Ethiopia the most common isolates *Klebsiella pneumoniae*, 33 isolated *Enterobacteriaceae,* 18.2% (n=6/33) showed intermediate or high resistance to Imipenem and/or Meropenem, and study done by Xu *et al*, in Asia from (2000-2012), most causative agent were *Klebsiella* (highest resistant) and *Esherichia coli* (lowest resistant) that different from the current study, and these may be because of sample size ,sample type or the area of study.

Study done by Amjad *et al*, from January 2010 to December 2010 in Pakistan, the result showed that "Out of 200 isolates, 138 (69%) were positive for carbapenemase production by Modified Hodge test and this differ from current study, this may be to population habits and different in genetic background.

Also in the present study one of the *E.coli* show negative modified Hodge test and this can explained it is resistant may be by other mechanism. target site mutation and efflux pump.

Other study done by Dahab *et al,* (2016) in Khartoum state used different clinical specimens, the result showed that "The most predominant Gram-negative bacilli isolates was *E.coli* (54.4%), followed by *Klebsiella* species (29.5%). More than fifty percent of the isolates were Carbapenem resistant. Fifty six percent of the resistant isolates were positive by Modified Hodge Test, this study partially disagree to our study maybe because use combination isolates from different clinical specimens and at the same time agree with our study in the most isolates was *E.coli*, and this may be to use the same study area (Khartoum).

5.2. Conclusion

In the current study 97 isolated of Enterobacteriacae , 5 organism resist to Carbapenem, most these isolated *E.coli*, some of these organism resist by Carbapenemase producing and this detect by MHT, few resist by other mechanism.

5.3.Recommendations

- Covering largest number of areas in Sudan for detection of resistant.
- Future study with large samples size from different specimens must be done.
- Advance techniques such as PCR use to detect the resistant genes.
- In future use indicator strain *Klebsiella pneumonia (ATCC700603)* in modified Hodge test.

Chapter six

References

Appendix

Chapter six

6.1. Reference

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6.2.Appendix

Preparation of Reagents:

Appendix 1:

Gram's stain:

Is a routine bacterial stain to differentiate Gram positive bacteria (dark purple colour) from gram negative bacteria (red colour),using Crystal violet, Gram's iodine, Alcohol for decolonization and Safranine as a counter stain.

Crystal violet:

Standard formula:

To make 1 liter:

Ingredients	**Gram\liter**
Crystal violet	20
Ammonium oxalate	9
Ethanol or methanol absolute	95ml
Distilled water	To 1 liter

Method of preparation:

1-The crystal violet was weighted on a piece of a clean paper and transferred to clean brown bottle.

2-The absolute ethanol or methanol was added and mixed until the dye was completely dissolved.

3-The ammonium oxalate was weighted and dissolved in about 200 ml of distilled water and then added until 1 liter mark and mixed well.
4-The bottle was labeled and stored in the dark place in room temperature.

Lugal's iodine:

Standard formula:

Ingredients	**Gram\liter**
Potassium iodide	20
Iodine	10
Distilled water	To 1 liter

Method of preparation

1-The potassium iodide was weighted on a piece of clean paper and transferred to clean brown bottle.

2-About quarter of the volume of water was added and mixed until the potassium iodide was completely dissolved.

3-The iodine was weighted and added to potassium iodide solution, then mixed until the iodine was dissolved.

4-It was completed to the 1 liter mark with distilled water and mixed well.

5-The bottle was labeled and stored in the dark place in room temperature.

Safranine:

Safranine 0.5% (0.5 gram in 100 ml of distilled water).

Acetone alcohol:

Standard formula:

To make 1 liter:

Ingredients	**MI**
Acetone	500
Ethanol or methanol, absolute	475
Distilled water	25

Method of preparation

1-The distilled water was mixed with absolute ethanol or methanol .

2-The acetone was measured, and added immediately to the alcohol solution then mixed well.

3-The bottle was labeled and stored in the dark place in room temperature.

Appendix2

Normal saline:

Mainly used to emulsify for smear preparation, and can also act as a diluting solution whenever dilution procedure is required, and also used in antimicrobial sensitivity test

Ingredients	**Gram\liter**
Sodium chloride	8.5g
Distilled water	To 1 liter

Method of preparation:

1-The sodium chloride was weighted and transferred it to a leak-proof bottle pre-marked to hold 1 liter.

2-The distilled water was added to the 1 liter mark, and mixed until the salt was fully dissolved.

Appendix3:

Kovac's indole reagent

(HIMEDIA) Country of origin-INDIA

LOT: 0000298230

APR- 2020

Standard formula:

To make about 40 ml:

Ingredients	**Gram\liter**
4-dimethyamino benzaldehyde	2g
Isoamyl alcohol	30 ml
Hydrochloric acid concentrated	10 ml

Appendix 4:

Peptone water

(HIMEDIA) Country of origin – INDIA

LOT: 0000247467

OCT-2019

Standard formula :

Ingredients	**Gram\liter**
Peptic digest of animal tissue	10.00
Sodium chloride	5.00

Final pH 7.2 ± 2(at 25 C).

Method of preparation:

15 g of powder were dispensed in 1 liter of distilled water and mixed, and then it was distributed in tube and sterilized by autoclaving at 121◦C for 15 minutes.

Appendix5:

Kligler iron agar KIA:

(HIMEDIA) Country of origin – INDIA

LOT: 0000271235

JUL-2020

Standard formula:

Ingredients	**Gram\liter**
Beef extract	3.00
Peptone	15.00
Sodium thiosulphate	0.30
Phenol red	0.024
Ferrous sulphate	0.20

Yeast extract	3.00
Proteose peptone	5.00
Sodium chloride	5.00
Lactose	10.00
Dextrose	1.00
Agar	15.00

Final pH 7.4±0.2(at 25C).

Method of preparation:

1-Suspend 57.52 grams in 1000 ml distilled water.

2- Heat to boiling to dissolve the medium completely .cool to 45-50c.

3- Mix well and distribute into tube .sterilize by autoclaving at 15 ibs pressure (121◦C) for 15 minutes.

4- Allow the tubes to cool in slanted position to form slopes with about 1 inch butts.

Appendix 6:

Simmon's citrate media:

(HIMEDIA) Country of origin – INDIA

LOT: 0000220428

JAN – 2019

Standard formula:

Ingredients	Gram\liter
Magnesium sulphate	0.20
Ammonium di hydrogen phosphate	1.00
Dipotassium phosphate	1.00
Sodium citrate	2.00
Sodium chloride	5.00
Bromothymol blue	0.08
Agar	15.00

Final pH (at 25 C) 6.8±0.2

Method of preparation

1-Suspend 24.28 grams in 1 liter of distilled water and boiled to dissolve completely swirled to mix.

2- The solution was dispensed into tubes and sterilized by autoclaving at 121◦C for 15 minutes.

3-The tubes were put in sloped position and leaved to dry.

Appendix7:

Semi solid nutrient agar:

(HIMEDIA) Country of origin- INDIA

LOT : 0000149892

Standard formula:

Ingredients	Gram\liter
Tryptose	10.0
Sodium chloride	5.0
Agar	5.0

Final pH (at 25C) 7.2±0.2

Method of preparation:

1-28 grams were suspended in 1000 ml distilled water and boiled to dissolve the medium completely .

2-The solution was dispensed and sterilized by autoclaving at 15 pressure (121◦C) for 15 minutes.

3-The medium was allowed to cool in an upright position.

Appendix8

Urea agar base:

(HIMEDIA) Country of origin-INDIA

LOT:0000221147

OCT- 2020

Standard formula:

Ingredients	Gram\liter
Peptic digest of animal tissue	1.00
Dextrose	1.00

Sodium chloride	5.00
Disodium phosphate	1.20
Monopotassium phosphate	0.80
Phenol red	0.012
Agar	15.00

Final pH (at 25 C) 6.8±0.2

Method of preparation:

1-21 grams of powder were suspended in 950 ml distilled water and boiled to dissolve the medium completely.

2-The solution was sterilized in autoclave at 121◦C for 15 minutes and cooled down to 47c , then 50 ml of sterile 40% urea solution was aseptically added and mixed well.

3-The medium was distributed aseptically in to the tube and allowed to solidified standard in sloped position.

Appendix 9:

McFarland standard

McFarland standard was prepared by adding 1%solution (v\v) of sulphuric acid (H_2SO_4) 1ml of sulphuric acid was added to 100 ml distilled water and mixed well then 1% solution (w\v) of anhydrous barium chloride ($BaCL_2$)1ml of barium chloride was added to 100 ml of distilled water then 0.4 ml of barium chloride was added to 99.6 ml of sulphuric acid.

Appendix 10:

CLED agar media

(HIMEDIA) Country of origin – India

LOT: 0000268756

JUN-2020

Standard formula:

Ingredients	**Gram\liter**
Peptic digest of animal tissue	4.00
Casein enzymichydrolysate	400
Beef extract	3.00
lactose	10.00
L-cystine	0.128
Bromothymol blue	0.02
Agar	15.00

Final ph (at 25 C) 7.3±0.2

Method of preparation:

1-Suspend 36.15 grams in 1000 ml distilled water.

2-Heat to boiling to dissolve the medium completely.

3- Sterilize by autoclaving at 15 Ibs pressure (121c) for 15 minutes. Cool to 45-50.

4- Mix well and pour into sterile petri dish.

Appendix 11:

Oxidase disc:

(HIMEDIA) Country of origin -INDIA

LOT:0000300504

JUN-2018

Disc contain Tertramethyl-P-Phenylendiamin.

Appendix 12:

Muller – Hinton agar

(HIMEDIA) Country of origin -INDIA

LOT: 0000131020

JAN-2019

Standard formula:

Ingredients	**Gram\liter**
Beef, infusion from	300.00ml
Casein acid hydrolysate	17.50
Starch	1.50
Agar	17.00

Final pH (at 25C)7.3±0,1

Method of preparation:

1-Suspend 38 g of medium (or the components listed above) in 1 liter of distilled water.

2-Heat to boiling to dissolve the medium completely .

3-Sterilize by autoclaving at 15 ibs pressure (121◦C) for 15 minutes.

4- Mix well before pouring .

Appendix 13:

Antibiotic disks from(HIMEDIA **)**

-Meropenem: LOT NO.0000315994

Antibiotic disks from (Bioanalyse)

-Imipenem: LOT NO.171011D

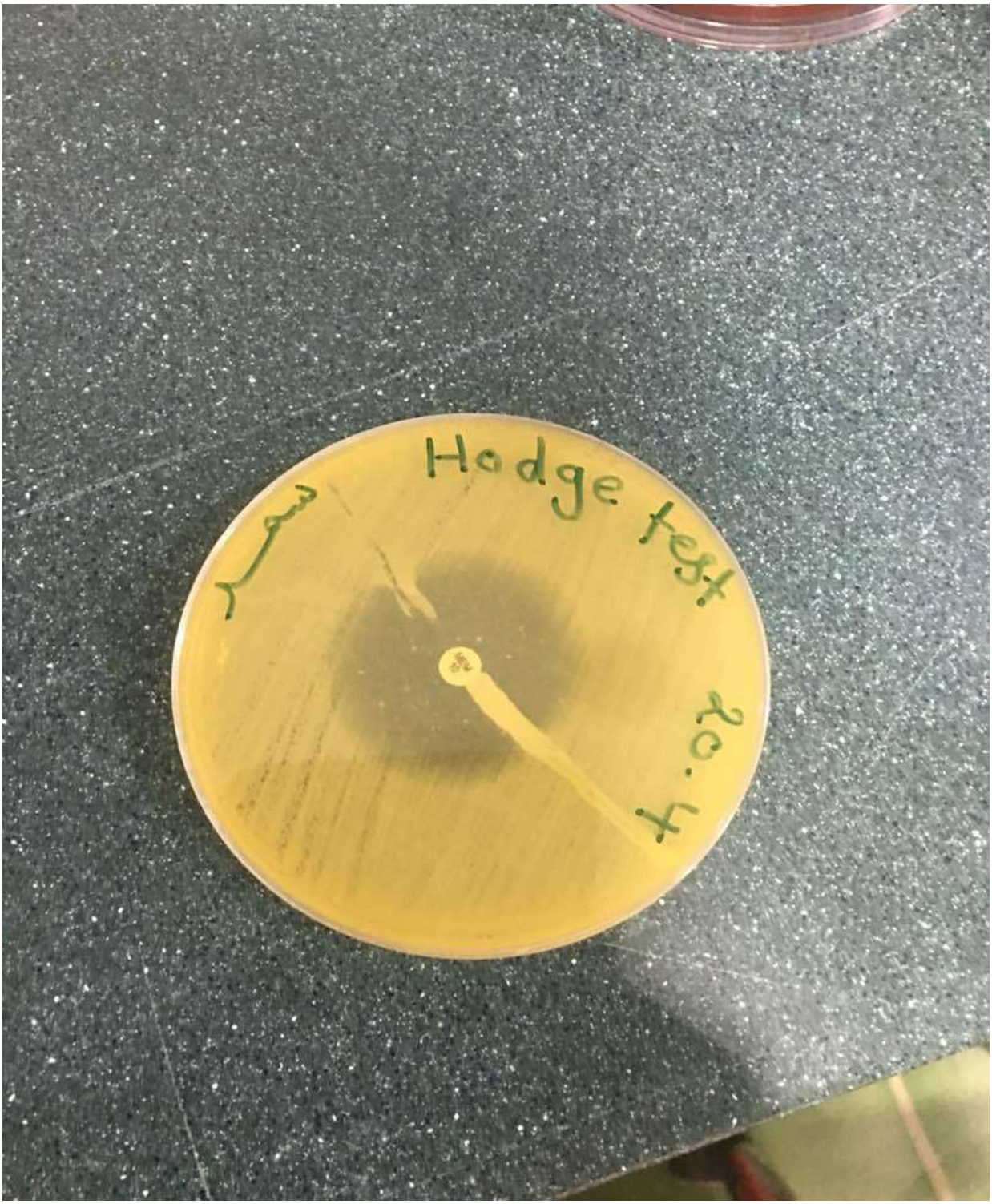

Figure (6.2.1) show Modified Hodge test