بسم الله الرحمن الرحيم

Sudan University of Science and Technology
College of Graduate Studies

Detection of Carbapenem-resistant *Klebsiella pneumoniae* in Tawam Hospital, United Arab Emerates

الكشف عن الكلبسيلة الرئوية المقاومة للكربابنم في مستشفى توام، الإمارات العربية المتحدة

A dissertation submitted in partial fulfillment for the requirements of MSc in Medical Laboratory Science (Microbiology)

By:
Osama Omer Ahmed Omer
BSc, Qualifying Certificate, in Medical Laboratory Science, Sudan university of Science & Technology, 2006

Supervisor:
Prof. Humodi Ahmed Saeed

2015

DEDICATION

To my father Mr. Omar

To mymother Mrs. Hanim

To my Daughter Asil and

To my son Basil

To my friend Wifaq

ACKNOWLEDGEMENT

First of all, thanks to ALMIGHTY ALLAH for giving me strength to complete this research.

I would like to thank my supervisor, **Prof. Humodi Ahmed Saeed** for his great help, starting from topic selection throughout the practical work till completion.

I am also grateful to staff of the Research Laboratory, Sudan University of Science and Technology for their help and support during laboratory work.

Thanks are extended to my friends and colleagues for their support and encouragement throughout this research. Especial thanks to Miss. Wifaq Mohamed for her unlimited help and support to conduct this research.

ABSTRACT

Carbapenems are one of the most powerful B-lactam antibiotics against bacteria especially multidrug resistant isolates. This is a laboratory-based study conducted to detect carbapenem-resistant *Klebsiella pneumoniae* (*K. pneumoniae*) isolated from patients with different diseases.

A total of sixty (n=60) *Klebsiella* isolates were obtained from Microbiology Laboratory, Tawam Hospital, UAE. The isolates were recovered from both males and females with different age and nationality. All of the isolates were checked for purity by streaking on nutrient agar, then examined microscopically. Gram stain and Vitek2 was used to confirm the identity of the isolates. Antibiotic susceptibility tests were carried out using Kirby Bauer technique and confirmed by Vitek2 machine. Carbapenem-resistant *Klebsiella pneumoniae* was detected by Modified Hodge Test.

Re-identification of the isolates confirmed that all isolates (n=60) were *K. pneumoniae*. Study on antibiotic susceptibility of the isolates revealed that the resistant was 100% to Ampicllin, Ceftaxidime, Cefruxime/Axetil, Cephalothin, Aztroenam, and Ertapenem. The resistant to the rest of the antibiotics range from 3.3-98.3 (Table 2)

The result revealed that all isolates of *K. pneumoniae* were carbapemen-resistat. The study concluded that *K. pneumoniae* were highly carbapemease produce. Deriving policies for treatment plan nationwide to control KPCs and strict hospital monitoring, sterilization and intervention plans are highly recommended. Further studies with large number of isolates are required to validate the results of this study.

المستخلص

هذه الدراسة المختبرية أجريت لكشف مقاومة للكاربينيمات الكلبسيلة الرئوية المعزولة من المرضى الذين يعانون من أمراض مختلفة. الكاربابينيمات هي واحدة من أقوى المضادات الحيوية(بيتا لاكتام) ضد بكتيرياالكلبسيلة الرئوية وخاصة المقاومة للأدوية. تم الحصول على ما مجموعه ستين كليبسيلا رئوية معزولة مسبقآ من مختبر الأحياء الدقيقة، مستشفى توام، الإمارات العربية المتحدة. تم عزل الكلبسيلة الرئوية من الذكور والإناث من مختلف الأعمار. وتم فحص جميع السلالات المعزولة بغرض تنقيتها وذلك بزرعها على الآجار المغذي المناسب لها، وتلى ذلك الفحص المجهري. استخدمت صبغة الغرام وجهازالفايتك-2 لتأكيد هوية المعزولات. أجريت اختبارات الحساسية للمضادات الحيوية باستخدام تقنية كيربي باور وأكده جهاز الفايتك-2. تم الكشف مقاومة الكلبسيلة الرئوية للكاربينيمات عن طريق اختبارالهودج المعدل.

أكدت عملية إعادة تحديد العزلات أن جميع العزلات (ن = 60) هي الكلبسيلة الرئوية. كشفت الدراسة مقاومة الكلبسيلة الرئوية بنسبة 100٪ للمضادات الحيوية الاتيه: للأمبسلين، سيفاتاكسايم، سيفروكسايم/أكستيل، سيفالوتين، أزترونام، وأرتابينم. أماالمقاومة لبقية المضادات الحيوية تتراوح من 3.3٪ حتى 98.3٪ (الجدول 2).

وكشفت النتائج أن جميع عزلات الكلبسيلة الرئوية الرئوية كانت مقاومة للكاربينيمات. وخلصت الدراسة إلى أن الكلبسيلة الرئوية لديها انتاج مرنفع لانزيم الكاربابينيمز. من توصيات هذه الدراسة للسيطرة على انتشار الكلبسيلة الرئويةالمقاومة للكاربينيمات هي سن السياسات الصارمة لوضع خطة علاج شاملة في البلاد ورقابة صارمة للمستشفيات ويوصى بشدة خطط التعقيم والتدخل. هناك حوجة لدراسات إضافية مع عدد أكبر من الكلبسيلة الرئوية المطلوبة للتحقق من صحة نتائج هذه الدراسة.

TABLE OF CONTENTS

CHAPTER THREE

MATERIALS AND METHODS

CHAPTER FOUR

RESULTS

CHAPTER FIVE

DISCUSSION

CHAPTER ONE

INTRODUCTION AND OBJECTIVES

CHAPTER ONE
INTRODUCTION AND OBJECTIVES

1.1- Introduction

Beta-lactam (β-lactam) antibiotics are wide range group of antibiotics that are commonly used to treat infectious diseases (CDC, 2009). They consist of the antibiotic agents that enclose in their molecular structure a β- lactam ring. Many examples may be encountered of such as carbapenems, monobactams, cephalosporins and penicillins (Lee *et al.,* 2009). Members of this group act by destructing the peptidoglycan of bacterial cell wall to stop their biosynthesis (Nordmann *et al.,* 2009). Many strains of bacteria developed resistance against β-lactam antibiotics via production of β-lactamase which is an enzyme that break down the β-lactam ring (Lee *et al.,* 2009). To overcome this resistance, scientists developed a new class of antibiotics. Which are β- lactamase inhibitors, for example clavamox which is combination of amoxicillin and clavulanic acid (Bratu *et al*., 2005a).

Members of the class carbapenems such as impenem, meropenem and atrapenem are antibacterial agents inactivate several types of Gram-negative bacteria (CDC, 2009). Carbapenems as sub-group of β-lactam are used successfully to treat the vast majority of ***Klebsiella*** infections especially that associated with hospitalization (Lee *et al.,* 2009). As opportunistic pathogens, ***Klebsiella*** spp. primarily attack immunocompromised individuals who are hospitalized and suffer from severe

underlying diseases such as diabetes mellitus or chronic pulmonary obstruction (**Falagas *et al*., 2007)**. Nosocomial ***Klebsiella*** infections are caused mainly by ***Klebsiella pneumoniae* (*K. pneumoniae*), which is considered** the most medically important species of the genus *Klebsiella*. **Many scientists stated that** *K. pneumoniae* is a repeated nosocomial pathogen that rank number four of the common cause of pneumonia and bacteremia in intensive care units (Patel *et al*., 2008).

Antibiotic resistance especially multi-drug resistance within the whole family of the genus *Klebsiella* (i.e. enterobacteriaceae) is not a new phenomenon. The rise of extended-spectrum β-lactamase (ESBL)-producing enterobacteriaceae (resistant to penicillins, cephalosporins, and monobactams) has reached high levels, and the number of unique ESBL protein sequences exceeded 1000 in 2011 (Weinstein and Logan, 2012). For serious infections, carbapenems have been the preferred, and at times only, treatment (Paterson and Bonomo, 2006). In 2003, resistance to third-generation cephalosporins among *K. pneumoniae* isolates recovered from patients in intensive care units was increased to 47%. Carbapenem-resistant enterobacteriaceae were first described in the early 1990s (Patel *et al*., 2008). The isolation of carbapenem-resistant *K. pneumoniae* strains occurred sporadically throughout that decade. The recovery of carbapenem-resistant *K. pneumoniae* strains from clinical specimens has increased at an alarming rate (Patel *et al*., 2008).

Recently, the emergence of carbapenemases carried on mobile genetic elements, such as transposons or plasmids that can harbor additional resistance genes affecting multiple

classes of antibiotics, has led to high level antibiotic-resistant bacteria, and the mobile resistance elements often have transferred into strains capable of efficient person-to-person spread (Weinstein and Logan, 2012). Researchers confirmed that carbapenem-resistant enterobacteriaceae especially *K. pneumoniae* has spread rapidly. It is now clear that carbapenem-resistant *K. pneumoniae* infections can be associated with significant morbidity and mortality.

1.2. Rationale

Worldwide the prevalence of carbapenem-resistant *K. pneumoniae* (CRKP) has been investigated in several countries such as India, Pakistan and United Kindom (Kumarasamy *et al*., 2010). Revising the literature, few researches were conducted in United Arab Emirates about CRKP among patients. Hence the importance of the current study increased to draw attention to carbapenem-resistant in Tawam Hospital, UAE.

1.2. Objectives

1.2.1. General objective

To detect carbapenem-resistant *K. pneumoniae* in patients attending Tawam Hospital UAE.

1.2.2. Specific objectives

A) To re-identify *K. pneumoniae* isolated in patients attending Tawam Hospital UAE.

B) To perform antibiotic susceptibility test for deferent types of antibiotics.

C) To detect carbapenem-resistant *K. pneumoniae.*

CHAPTER TWO

LITERATURE REVIEW

CHAPTER TWO

LITERATURE REVIEW

2.1. Carbapenems

Carbapenems possess the broadest spectrum of activity and greatest potency against Gram-positive and Gram-negative bacteria (Papp-Wallace *et al*., 2011). Members of carbapenems are considered first-line agents in treating infections caused by ESBL-producing organisms. Most of the evidence instead originates from case series and retrospective studies, which collect the responses and outcomes of patients with bacteremia receiving carbapenem therapy (Kitchel *et al.,* 2009). In a multinational study of 85 patients with ESBL-producing *K. pneumoniae* bacteremia, carbapenem was used as an independent predictor of lower mortality rate compared with the use of other antibiotic agents (CDC, 2009). The therapeutic advantage of carbapenems has been attributed to the high inoculums effect as well as high MICs of other agents that are close to the susceptibility breakpoints (Lee *et al.,* 2009).

2.2. Carbapenem-resistant enterobacteriaceae

Recognizing carbapenemase expression is the key to the appropriate management of infections caused by carbapenem-resistant enterobacteriaceae (Bratu *et al.,* 2005b). Unusually elevated MICs to carbapenems should arouse suspicion for a carbapenem-resistant isolate and preclude the use of carbapenems even if the MICs do not exceed the breakpoints for resistance (Kitchel *et al.,* 2009). As with ESBL-producing organisms, carbapenemase-producing strains are likely to exhibit simultaneous resistance to aminoglycosides and fluoroquinolones (Li *et al.,* 2014).

2.3. *Klebsiella pneumoniae* (*K. pneumoniae*)

K. pneumoniae is Gram-negative, non-motile, encapsulated, lactose-fermenter facultative anaerobic rod-shaped bacterium (Lee *et al.,* 2009). Although found as normal flora of the mouth, skin, and intestines, it can cause destructive changes to human and animal lungs if aspirated, specifically in lung alveoli resulting in bloody sputum in the clinical setting. The most other medically significant members of the genus *Klebsiella* are *K. lebsiella*, *K. oxytoca* and *K. rhinosmts.* All of them have also been demonstrated in human clinical specimens (CDC, 2009). In recent years, *klebsiellae* have become important pathogens in nosocomial infections. It naturally occurs in the soil, and about 30% of strains can fix nitrogen in anaerobic conditions (Li *et al.,* 2014). Members of the genus *Klebsiella* typically express two types of antigens on their cell surfaces (Kitchel *et al.,* 2009). The first is O antigen, a component of the lipopolysaccharide (LPS), of which 9 varieties exist (CDC, 2009). The second is K antigen, a capsular polysaccharide with

more than 80 varieties (Lee *et al.,* 2009). Both contribute to pathogenicity and form the basis for serogrouping.

2.3.1 Clinical significance

The vast majority of *Klebsiella* infections, however, are associated with hospitalization (Kitchel *et al.,* 2009). As opportunistic pathogens, *Klebsiella* spp. primarily attack immunocompromised individuals who are hospitalized and suffer from severe underlying diseases such as diabetes mellitus or chronic pulmonary obstruction (Falagas *et al*., 2007). Nosocomial *Klebsiella* infections are caused mainly by *K. pneumoniae*, the medically most important species of the genus (Lee *et al.,* 2009). The bacterium is a repeated nosocomial pathogen that is considered the fourth most common cause of pneumonia and bacteremia in intensive care patients (Patel *et al*., 2008). Since, 1990s *Klebsiella* species holding extended-spectrum β-lactamases (ESBLs) emerged, resistance is due to cephalosporins (Bratu *et al.,* 2005a). *K. pneumoniae* can cause destructive changes to human lungs via inflammation and hemorrhage with cell death and sometimes production of a thick, bloody, mucoid sputum (currant jelly sputum). These bacteria gain access typically after a person aspirates colonizing oropharyngeal microbes into the lower respiratory tract (CDC, 2009).

As a general rule, *Klebsiella* infections are seen mostly in people with a weakened immune system. Most often, illness affects middle-aged and older men with debilitating diseases (Kitchel *et al*., 2009). This patient population is believed to have impaired respiratory host defenses, including persons with diabetes, alcoholism, malignancy, liver disease, chronic obstructive pulmonary diseases, glucocorticoid therapy, renal failure, and certain occupational exposures (such as papermill workers). Many of these infections are obtained when a person is in the hospital for some other reason (a nosocomial infection). Feces are the most significant source of patient infection, followed by contact with contaminated instruments (Lee *et. al.,* 2009). The most common condition caused by *Klebsiella* bacteria outside the hospital is pneumonia, typically in the form of bronchopneumonia and also bronchitis. These patients have an increased tendency to develop lung abscess, cavitation, empyema, and pleural adhesions. It has a death rate of about 50%, even with antimicrobial therapy (CDC, 2009). The mortality rate can be nearly 100% for people with alcoholism and bacteremia (Kitchel *et al.,* 2009).

In addition to pneumonia, *Klebsiella* can also cause infections in the urinary tract, lower biliary tract, and surgical wound sites (Lee *et al.,* 2009). The range of clinical cases includes pneumonia, thrombophlebitis, urinary tract infection, cholecystitis, enteritis, upper respiratory tract infection, wound infection, osteomelitis, meningitis, bacteremia and septicemia. For patients with invasive devices in their bodies, contamination of these devices becomes a risk; for example, neonatal ward devices, respiratory support equipment, and urinary catheters put patients at increased risk (Kitchel *et al.,* 2009). Also,

the use of antibiotics can be a factor that increases the risk of nosocomial infection with *Klebsiella* bacteria. Sepsis and septic shock can follow entry of the bacteria into the blood. Two unusual infections of note from *Klebsiella* are rhinoscleroma and ozena. Rhinoscleroma is a chronic inflammatory process involving the naso-pharynx (Kitchel *et al.,* 2009). Ozena is of chronic atrophic inflammation that produces necrosis of nasal mucosa and mucopurulent nasal discharge (Lee *et.al.,* 2009). *Klebsiella* ranks second to *E. coli* for urinary tract infections in older people (CDC, 2009). It is also an opportunistic pathogen for patients with chronic pulmonary disease, enteric pathogenicity, nasal mucosa atrophy, and rhinoscleroma.

2.3.2 Resistant strains

New antibiotic-resistant strains of *K. pneumoniae* are appearing. These organisms are often resistant to multiple antibiotics (Kitchel *et al.,* 2009). Current evidence implicates plasmids as the primary source of the resistance genes. *Klebsiella* with the ability to produce (ESBL) is resistant to many classes of antibiotics. The most frequent are resistance to aminoglycosides, fluoroquinolones, tetracyclines, chloramphenicol, and trimethoprim/sulfamethoxazole (Lee *et al.,* 2009).

Infection with carbapenem-resistant enterobacteriaceae (CRE) or carbapenemase-producing enterobacteriaceae is emerging as an important challenge in health-care settings (Bratu *et al.,* 2005b). One of many CREs is carbapenem-resistant *Klebsiella* pneumoniae (CRKP) (Kitchel *et al.,* 2009). Over the past 10 years, a progressive increase in CRKP has been seen worldwide. This new emerging nosocomial pathogen is probably

best known for an outbreak that began around 2006 within the healthcare system in the USA. CRKP has been identified in 41 states; and is recovered routinely in certain hospitals in New York and New Jersey (CDC, 2009). It is now the most common CRE species encountered within the United States (Lee *et al.,* 2009).

The CRKP is resistant to almost all available antimicrobial agents, and infections with CRKP have caused high rates of morbidity and mortality, in particular among persons with prolonged hospitalization and those critically ill and exposed to invasive devices (e.g. ventilators or central venous catheters) (Kitchel *et al.,* 2009). The concern is that carbapenem is often used as a drug of last resort when battling resistant bacterial strains. Bratu *et al.,* (2005b) said that new slight mutations could result in infections for which healthcare professionals can do very little, if anything, to treat patients with resistant organisms.

A number of mechanisms cause carbapenem resistance in the enterobacteriaceae. These include hyperproduction of *ampC* β-lactamase with an outer membrane porin mutation, CTX-M extended-spectrum β-lactamase with a porin mutation or drug efflux, and carbapenemase production death (CDC, 2009). The most important mechanism of resistance by CRKP is the production of a carbapenemase enzyme*, blakpc* (Kitchel *et al.,* 2009).These strains are susceptible to carbapenems, they are not identified as potential clinical or infection control risks using standard susceptibility testing guidelines. Patients with unrecognized CRKP colonization have been reservoirs for transmission during nosocomial outbreaks (Lee *et al.,* 2009).

The extent and prevalence of CRKP within the environment in USA is currently unknown. The mortality rate is also unknown, but is suspected to be within a range of 12.5% to 44% and the probability of an epidemic or pandemic in the future remains uncertain (Kitchel *et al.,* 2009). The Centers for Disease Control and Prevention released guidance for aggressive infection control to combat CRKP: Place all patients colonized or infected with carbapenemase-producing enterobacteriaceae on contact precautions (Kitchel *et al.,* 2009). Acute-care facilities are to establish a protocol, in conjunction with the guidelines of the Clinical and Laboratory Standards Institute to detect non susceptibility and carbapenemase production in enterobacteriaceae, in particular *Klebsiella* spp. and *Escherichia coli*, and immediately alert epidemiology and infection-control staff members if identified death (CDC, 2009). All acute-care facilities are to review microbiology records for the preceding 6–12 months to ensure that there have not been previously unrecognized CRE cases (Bratu *et al.,* 2005a). If they do identify previously unrecognized cases, a point prevalence survey (a single round of active surveillance cultures) in units with patients at high risk (e.g., intensive-care units, units where previous cases have been identified, and units where many patients are exposed to broad-spectrum antimicrobials) is needed to identify any additional patients colonized with carbapenem-resistant or carbapenemase-producing *Klebsiella* spp. and *E. coli* (Bratu *et al.,* 2005b). When a case of hospital-associated CRE is identified, facilities should conduct a round of active surveillance testing of patients with epidemiologic links to the CRE case (Kitchel *et al.,* 2009) (e.g., those patients in the same unit or patients having been cared for by the same health-care personnel).

2.3.3 Significance *of K. pneumoniae* carbapenemases

In 1983, the first report of plasmid-mediated β-lactamases capable of hydrolyzing extended-spectrum cephalosporins was made (CDC, 2009). They were named extended-spectrum β-lactamases (ESBLs) and they have since been described worldwide (Lee *et al.,* 2009).The fact that carbapenems are the treatment of choice for serious infections caused by ESBLs, along with an increasing incidence of fluoroquinolone resistance among enterobacteriaceae, has led to an increased reliance on carbapenems in clinical practice (Bratu *et al.,* 2005b). In 2001, the first KPC-producing *K. pneumoniae* isolate was reported in North Carolina. The production of KPC enzymes has become the most prevalent mechanism of carbapenem resistance in the US today according to (CDC, 2009).

In 2009, (CDC) released a report on KPC-producing bacteria in which the term Carbapenem-Resistant Enterobacteriaceae (CRE) was proposed as more accurate, given the understanding that multiple species of Gram-negative bacteria can harbor the KPC-resistant element (Bratu *et al.,* 2005b). The use of the term 'KPC,' disseminated in the majority of the literature is in use to date (Kitchel *et al.,* 2009).

2.3.4. Clinical Features of KPCs

Infections caused by KPC-producing have been associated with increased cost and length of hospital stay as well as frequent treatment failures and death (CDC, 2009). Risk factors for infection include advanced age, being severely ill, previous treatment with antibiotics, organ or stem-cell transplantation, mechanical ventilation, and long hospital stays (Kitchel *et al.,* 2009). Reports are mixed as to whether previous carbapenem use is associated with the development of infections caused by KPC-producing bacteria. In at least one study, prior fluoroquinolone and extended-spectrum cephalosporin use were both independently associated with infection or colonization with KPCs.

Poor outcomes from infections with KPC-producing bacteria have been reported since the first reports of KPC outbreaks in New York City hospitals (Bratu *et al.,* 2005a). A small series of patients with bloodstream infections caused by KPC-producing bacteria from New York City hospitals in 2005 revealed mortality rates of 47% to 66% (Kitchel *et al.,* 2009). The experience outside the USA has been similar, as shown by a matched retrospective historical cohort study of 32 patients with bacteremia caused by carbapenem-resistant *K. pneumoniae* compared to patients with infections caused by susceptible *K. pneumoniae* that showed a crude morbidity of 72%, and mortality of 50% (Kitchel *et al.,* 2009). None of the patients in this study received appropriate empiric antibiotics (Bratu *et al.,* 2005b). In a cohort of 99 cases with KPC-producing *K pneumoniae* and 99 controls with susceptible *K. pneumoniae*, KPC-production was associated with greater than two-fold increased risk of death (Lee *et al.,* 2009).

The difficulty of detecting KPC production with routine testing appears to have attributed to the poor outcomes observed with infections caused by KPC-producing bacteria by causing a critical delay in treatment (Bratu *et al.,* 2005a). This applies to serious infections such as bacteremia, but also extends to other infections in patients undergoing organ transplants and cancer treatment, where the immunocompromised status of patients requires effective empiric antibiotics (CDC, 2009). Scientists reported two cases of orthotropic liver transplant recipients that died as a result of infections caused by KPC-producing (Lee *et al.,* 2009). Both patients were initially treated with meropenem based on the results of routine susceptibility testing (Kitchel *et al.,* 2009)

CHAPTER THREE

MATERIALS & METHODS

CHAPTER THREE
MATERIALS AND METHODS

3.1.1 Type of Study

This is a laboratory-based study.

3.1.2 Study Area

The study was carried out in Microbiology Laboratory, Tawam Hospital, UAE.

3.1.3 Study Duration

The study was conducted during the period January to April 2015.

3.2. Source of Isolates

The isolates of 60 *Klebsiella pneumoniae* were obtained from the Research Laboratory, Tawam Hospital, UAE. Which previously isolated clinical specimens urine, blood, wound swabs and sputum.

3.3. Re-identification of the isolates

3.3.1. Checking purity

The isolates were cultured on nutrient agar and incubated at 37°C for 18-24 hrs. Purity of each isolate was checked microscopically following simple stain as described by Cheesbrough, (2006).

3.3.2. Gram Stain

Gram stain was essential technique for initial identification of bacterial isolates. The procedure was carried out according to Cheesbrough (2006) as follows; smear was prepared from overnight culture on a clean and dry slide. The smear was left to air dry. Fixation was done by rapid pass of the slide three times through the flame of a Bunsen burner then allowed to cool before staining. Crystal violet stain was added to smear for 30–60 seconds, and then washed by tap water. Lugol's iodine was added for 30-60 minutes then washed by tap water and decolorized rapidly (few seconds) with acetone alcohol and washed immediately by tap water. Finally, the smear was covered with saffranin stain for 2 minutes and washed by tap water. The back of slide was wiped clean and placed in a draining rack for smear to air dry. Drop of oil was added to the dried smear and examined under the light microscope (Carl Zeiss, Germany) by oil lens 100X.

3.3.3. Identification and antibiotic susceptibility

The identification and antibiotic susceptibility test were done by VITEK Machine (BioMerieux, France) which is automated and semi-automated technology in microbiology.

3.3.3.1. Principle of the VITEK

The VITEK is an automated microbiology system utilizing growth-based technology. The system is available in three formats (VITEK 2 compact, VITEK 2, and VITEK 2 XL) that differ in increasing levels of capacity and automation. All three systems accommodate the same colorimetric reagent cards that are incubated and interpreted automatically.

3.3.3.2. Preparation of bacterial suspension

The suspension was prepared by emulsifying 2-3 colonies from an overnight culture in 5ml normal saline. The obtained suspension was adjusted to 0.5 McFarland using the Densichek. This suspension was used for both identification and antibiotic sensitivity tests for the VITEK 2 system (CLSI, 2009).

3.3.3.3. Inoculation of VITEK cards

The cards intended for identification and antimicrobial susceptibility testing were inserted in the VITEK tubes and then were put in VITEK machine. In the second day the identification and the susceptibility was read from the VITEK screen.

3.3.4. Confirmation of Susceptibility of bacterial isolates to antibiotics

Antibiotic susceptibility testing of *K. pneumoniae* was carried out by the disk diffusion technique. Seventeen (n=17) antibiotics commercially available discs (MAST Diagnostic Ltd, USA) were tested. The antibiotics used were amoxicillin (AX, 30 g), ampicillin (AM, 10 g), cefotaxime (CTX, 30 g), ceftazidime (CAZ, 30 g), ceftriaxone (CT,30 g), cephalexin (CL,30 g), ciprofloxacin (CIP, 5 g), nalidixic acid (NA,30 g), norfloxacin

(NX, 10 g), ofloxacin (OF, 5 g), amikacin (AK, 30 g), gentamicin (GEN, 10 g), tobramycin (TB, 10 g), imipenem (IPM, 10 g), nitrofurantoin (NIT, 300 g) and Cotrimoxazole (COT, 30 g).

The test was performed using Kirby-Bauer disc diffusion method according to CLSI (2009) as follows:

3.3.4.1. Culture medium

Sterilized molten Muller-Hinton agar (PH 7.4±2) was prepared, cooled to 45-50^{0}C and poured in sterile dry Petri plates on a level surface, to a depth of 4mm.

3.3.4.2. Quality control

Quality control was performed to measure the effectiveness of antimicrobial agents by using a control *E. coli* ATCC **25922** obtained from the Central Public Health Laboratory.

3.3.4.3. Preparation of inoculums

The inoculum was prepared by transfer of 3-5 well isolated colonies of same appearance with sterile wire loop to 2.0 ml of sterile physiological saline. The turbidity of this suspension was adjusted to a 0.5 McFarland standard. This suspension was used within 15 minutes of preparation.

3.3.4.4. Seeding of plates

A sterile non toxic cotton swab was dipped into the inoculums tube and then the swab was rotated against the side of the tube above the level of the suspension to remove excess fluid. The plate of Muller-Hinton agar was inoculated by streaking the swab

evenly over the surface of the medium in three directions. The surface of agar was allowed 3-5 minutes to dry.

3.3.4.5. Antibiotic disc application

The selected antibiotics were applied on the surface of agar by using sterile forceps which evenly distributed in the inculcated plate. Each disc was pressed down to ensure its contact with the agar.

3.3.4.6. Incubation

The inverted plates were incubated aerobically at 35^{o}C for 16-18 hours.

3.3.4.7. Reading of zones of inhibition

Following overnight incubation, by using a ruler on the underside of the plate, the diameter of each zone of the inhibition was measured in millimeters.

3.3.4.8. Interpretation of the results

The zone of each antibiotic was compared to their standard inhibition zone on the chart provided by manufacture. The results were interpreted as sensitive (S) or resistance (R).

3.3.5. Modified Hodge Test for carbapenemase detection

The Modified Hodge Test (MHT) detects carbapenemase production in isolates of enterobacteriaceae. The most common carbapenemase found enterobacteriaceae is *K. pneumoniae.*

3.3.5.1. Preparation of 0.5 McFarland dilution of the *E. coli* ATCC 25922

From fresh E. *coli* ATCC 25922 culture bacterial suspension was prepared of by transferring 2-3 colonies in 5.0 ml normal saline. The obtained suspension was adjusted to McFarland using the Densichek.

3.3.5.2. Procedure

1. Dilution 1:10 was done by adding 0.5 ml of the 0.5 McFarland to 4.5 ml of normal saline.
2. Streaking a lawn of the 1:10 dilution of *E. coli* ATCC 25922 to a Mueller-Hinton agar plate was done.
3. The plates were allowed to dry for 3–5 minutes.
4. A 10 µg meropenem disk was placed in the center of the test area.
5. In a straight line, the test organism (*K. pneumoniae*) was streaked from the edge of the disk to the edge of the plate.
6. The plate was incubated overnight at 35°C ± 2°C for 16–24 hours. Then the result was read and interpreted.

CHAPTER FOUR

RESULTS

CHAPTER FOUR

RESULTS

A total of sixty of *K. pneumoniae* isolates were obtained from Microbiology Laboratory, Tawam Hospital, UAE. Data registered in the log book of the laboratory indicated that the isolates were recovered from different clinical specimens: urine, blood, wound swabs, sputum and body fluids (Fig 1). The specimens were collected from both males 36 (60%) and females 24(40%). According to the World Health Organization (WHO) and Twama hospital the human age categories are children 1- 18 years old, adults from above 18- 60 and elderly above 60. So, in this study children are found to be (5%), adults (45%) and old age (50%). Re-identification of the isolates confirmed that all isolates were *K. pneumoniae*.

Study on antibiotic susceptibility of *K. pneumoniae* that includes seventeen antibiotics revealed that the resistance of six antibiotics was 100%; they are Ampicillin, ceftaxidime, Cefruxime/Axetil, Cephalothin, Aztroenam and Ertapenem. The resistance to the rest of the antibiotics range from 3.3%- 98.3 % (Table 1).

Table 1. Susceptibility of *K. pneumoniae* (n=60) to different antibiotics

Antibiotic	R	R (%)	S	S (%)	I	I (%)
Amoxicillin/ Clavulanate	60	100 %	-	-	-	-
Ampicillin	60	100 %	-	-	-	-
Ceftazidine	60	100 %	-	-	-	-
Cefuroxime/ Axetil	60	100 %	-	-	-	-
Cefuroxime	60	100 %	-	-	-	-
Cephalothin	60	100 %	-	-	-	-
Ciprofloxacin	54	90 %	6	10 %	-	-
Colistin	15	25 %	45	75 %	-	-
Gentamicin	35	58.3 %	21	35 %	4	6.7 %
Imipenem	50	83.3 %	-	-	10	60.7 %
Meropenem	53	88.3 %	-	-	7	11.7 %
Nitrofurantoin	52	86.7%	5	8.3 %	3	5.0 %
Piperacillin/Tazobactam	59	89.3 %	-	-	1	1.7 %
Trimethoprim/Sulfa	59	89.3 %	1	1.7 %	-	-
Tigecycline	2	3.3 %	52	86.7 %	6	10 %
Astroeonam	60	100 %	-	-	-	-

Key: S=Sensitive; R=Resistant; I=Intermediate

Adoption of Modified Hodge Test (MHT) for detection of carbapenemase in *K. pneumoniae* revealed that all isolates (n=60) were carbapenemase producers (Color plate 1). Distribution of positive MHT according to type of diseases and word is shown in fig (2 & 3).

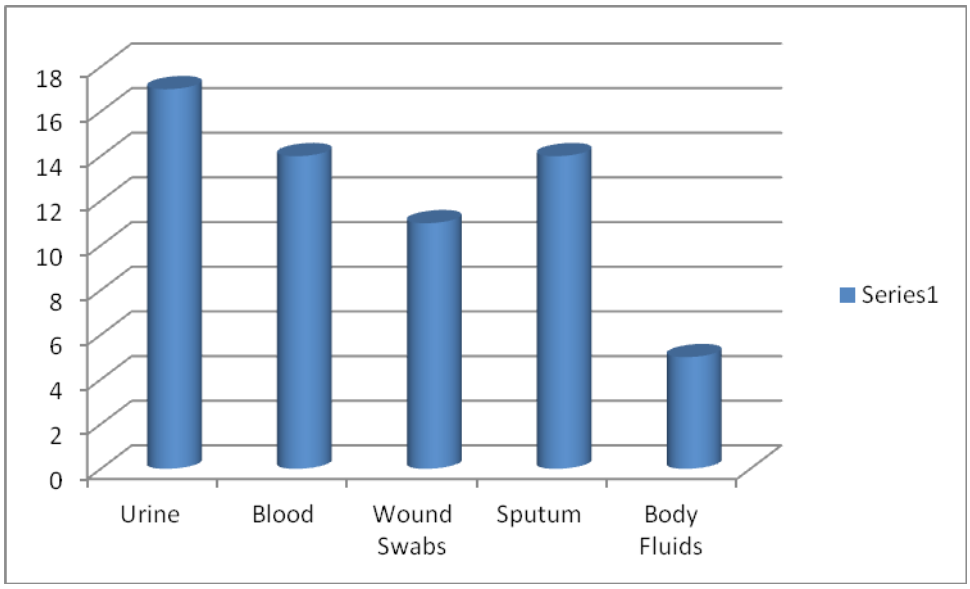

Fig 1. Types and frequency of specimens

Samples in general were obtained from various resources is found to be the highest from urine and equal amounts from blood and sputum as well.

Color plate 1. Shows carbapenemase positive test

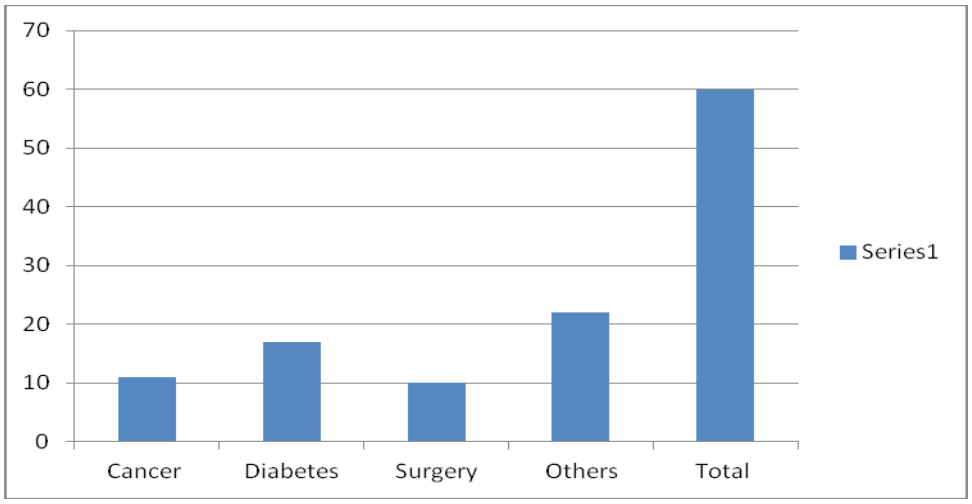

Fig. 2. Distribution of positive MHT according to type of disease

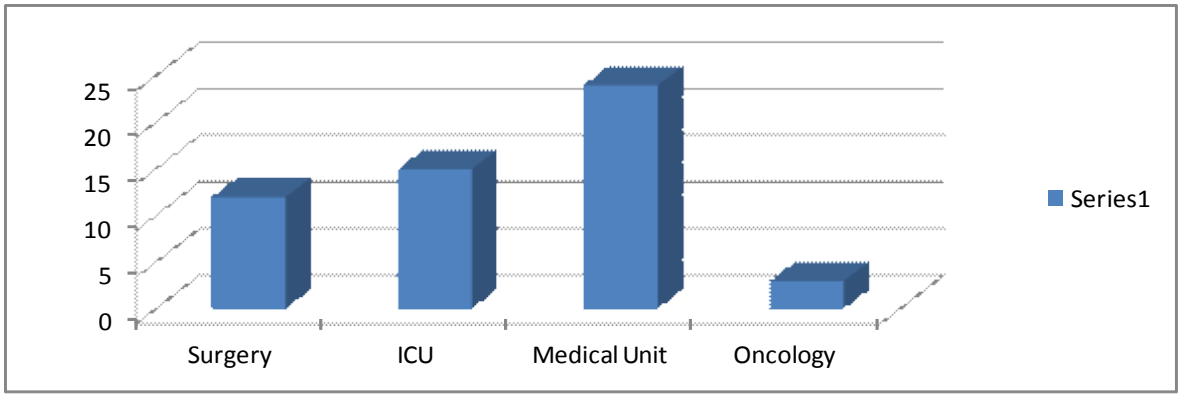

Fig. 3. Distribution of positive MHT according to word

CHAPTER FIVE

DISCUSSION

CHAPTER FIVE

DISCUSSION

5.1. DISCUSSION

Carbapenem antibiotics are used to treat serious infections caused by extended-spectrum β-lactamase-carrying pathogens. Carbapenem resistance has been unusual in isolates of *K. pneumoniae*, the opportunistic pathogen that commonly cause nosocomial infections. The bacterium, particularly in children is the cause of pneumonia, meningitis, sepsis, diarrhea and bacteremia. The increasing appearance of multidrug resistance among *K. pneumoniae* isolates has confined the suitable therapeutic choices for the treatment of these infections (Bina *et al*., 2015).

In this study Gram stain and VITEK system confirmed the identity of the sixty isolates as *K. pneumoniae* which indicated the accuracy of the primary recovery of these isolates. Study on susceptibility of the isolates showed high resistance (100%) to most antibiotics tested. The lowest rate of resistance was associated to Tigecycline (3.3%) and Colistin

(25%). These results are in agreement with that reported by Bratu *et al*., (2005a). The high resistance to the antibiotics detected during this study may be attributed to abuse of antibiotics.

Adoption of the Modified Hodge Test (MHT) showed that all *K. pneumoniae* were positive for carbapenemase. The test MHT is approved by the (CLSI) and is accepted as a specific and sensitive test for detection of carbapenemase. Similar studies conducted elsewhere reported carbapenemase positive *K. pneumoniae* is 98,9% (Kazi *et al*., 2015), 91.51% (Chauhan *et al*., 2015), 88.33% (Sood, 2014) and 75.0% (Li *et al*., 2014).

The number of *K. pneumoniae* carbapenemase (KPC)-producing where found to be higher in males than in females. This result is in agreement with several researches such as (Kazi *et al*., 2015), (Chauhan *et al*., 2015), (Sood, 2014) and (Li *et al*., 2014). Concerning, in the age factors numerous studies showed that KPC is much common in elderly patients followed by mid- age individuals. Elderly patients are the most susceptible individuals to KPC due to the age factor and the long exposure to different antibiotics (Bratu *et al*., 2005a). Also, researches focused on cancerous, immune-compromised and surgical patients, especially who are in medical devices, as the most common patients susceptible to be KPC.

5.2. CONCLUSION

The study concluded that carbapenem-resistant *K. pneumoniae* infection is highly prevalent among patients attending Tawam Hospital. Presence of multi-drug resistant *K. pneumoniae* is common which necessitate the need for detection of carbapenem-resistant *K. pneumoniae* routinely to avoid to further spread among patients and deeper complications.

5.3. RECOMMENDATIONS

1. Treatment of Carbapenem-resistant *K. pneumoniae* with Tigecycline, Tigecycline and glycylcycline is recommended.
2. Adoption of simple technique such as Modified Hodge Test for detection of carbapenemase production in isolates of enterobacteriaceae.
3. Further studies with molecular techniques are required to explore genes responsible for production of carbapenemase found in *K. pneumoniae.*

REFERENCES

REFERENCES

1. **Bina M, Pournajaf A, Mirkalantari S, Talebi M, Irajian G. (2015).** Detection of the Klebsiella pneumoniae carbapenemase (KPC) in K. pneumoniae Isolated from the Clinical Samples by the Phenotypic and Genotypic Methods. *Iran J Pathol*; 10(3):199-205.

2. **Bratu S., Landman D. and Haag R. (2005a)**. Rapid spread of carbapenem-resistant *Klebsiella pneumoniae* in New York City. *Arch Intern Med*; **165**:1430-1435.

3. **Bratu S**., **Tolaney P**. and **Karumudi U. (2005b).** Carbapenemase producing *Klebsiella pneumoniae* in Brooklyn- Molecular epidemiology and *in vitro* activity of polymyxin B and other agents. *J Antimicrobial Chemother*, **56**:128–132.

4. **CDC (2009).** Guidance for control of infections with carbapenem-resistant or carbapenemase-producing enterobacteriaceae in acute care facilities. *Morb Mortal Wkly Rep*; **58**:256–260.

5. **Chauhan K, Pandey A, Asthana AK, Madan M. (2015).** Evaluation of phenotypic tests for detection of Klebsiella pneumoniae carbapenemase and metallo-beta-lactamase in clinical isolates of Escherichia coli and Klebsiella species. *Indian J Pathol Microbiol*; 58(1):31-5.

6. **Cheesbrough M. (2006).** *District Laboratory Practice in Tropical Countries*, Part 2, Cambridge University Press, United Kingdom, PP. 60-64.

7. CLSI **(2009).** ***Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically*** (8).

8. **Falagas M., Rafailidis P., Kofteridis D., Virtzili S., Chelvatzoglou F., Papaioannou V., Sofia Maraki S., Samonis G., Michalopoulos A. (2007).** *Risk factors of carbapenem-resistant* ***Klebsiella pneumoniae*** *infections*: a matched case–control study', Journal of Antimicrobial Chemotherapy, Available at [http://jac.oxfordjournals.org/content/60/5/1124.long] Accessed on [Jan., 2015]

9. **Kazi M, Drego L, Nikam C, Ajbani K, Soman R, Shetty A, *et al*.** (2015). Molecular characterization of carbapenem-resistant Enterobacteriaceae at a tertiary care laboratory in Mumbai. *Eur J Clin Microbiol Infect Dis*; 34(3):467-72.

10. **Kitchel B., Rasheed K. and Patel B. (2009).** Molecular epidemiology of KPC-producing *Klebsiella pneumoniae* isolates in the United States- clonal expansion of multilocus sequence type 258. *Antimicrobial Agents and Chemother*, **53**:3365–3370.

11. **Kumarasamy K., Toleman M., Walsh T. and Bagaria J. (2010).** Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. *Lancet Infect Dis*, Available at [www.thelancet.com/infection] Accessed on Mar., 2015.

12. **Lee J., Patel G. and Huprikar S. (2009).** Decreased susceptibility to polymyxin B during treatment of carbapenem-resistant *Klebsiella pneumoniae* infection. *J Clin Microbiol*; 47:1611–1612.

13. **Li B, Xu XH, Zhao ZC, Wang MH, Cao YP. (2014)**. High prevalence of metallo-beta-lactamase among carbapenem-resistant Klebsiella pneumoniae in a teaching hospital in China. *Can J Microbiol*; 60(10):691-5.

14. **Nordmann P., Naas T. and Vedel G. (2009).** 'Plasmid-mediated carbapenem-hydrolyzing beta-lactamase KPC in a *Klebsiella pneumoniae* isolate in France *Antimicrobial Agents and Chemother*, **49**:4423–4424

15. **Papp-Wallace K. M., Endimiani A. Magdalena A. T. and Robert A. B. (2011)**. Carbapenems: Past, Present, and Future. *Antimicrobial Agent Chemother;* **55**(11): 4943-4960

16. **Patel G; Huprikar S., Stephanie H. Factor, M., Stephen G. J. and David P. C. (2008).** Outcomes of Carbapenem-Resistant *Klebsiella pneumoniae* Infection and the Impact of Antimicrobial and Adjunctive Therapies. *Infect Control Hosp Epidemiol*; **29(**12):

17. **Paterson L. and Bonomo A (2006).** 'Extended-spectrum beta-lactamases- a clinical update', *Clini Microbiol Rev*, **18**:657-686).

18. **Sood S. (2014).** Identification and differentiation of carbapenemases in Klebsiella pneumoniae: a phenotypic test evaluation study from jaipur, India. *J Clin Diagn Res*; 8(7):DC01-3.

19. **Weinstein R. and Logan L. (2012).** Carbapenem-Resistant Enterobacteriaceae: An Emerging Problem in Children. Clini Infect Dis 55(6): 852-859

APPENDICES

APPENDICES

Appendix 1: VITEK principle

OBJECTIVE This chapter describes the VITEK 2 automated microbiology system and its application in the identification of microorganisms.	PRINCIPLES The VITEK 2 is an automated microbiology system utilizing growth-based technology. The system is available in three formats (VITEK 2 compact, VITEK 2, and VITEK 2 XL) that differ in increasing levels of capacity and automation. Figure 1 shows the VITEK 2 compact system. All three systems accommodate the same colorimetric reagent cards that are incubated and interpreted automatically.

Appendix 2

1. Purpose

1.1 Define the criterion to classify the tested isolates of the *Enterobacteriaceae* family and *Pseudomonas* species for antimicrobial susceptibility, as MDR (Multi Drug Resistant)

2. Policy/Principle

2.1 Multi Drug Resistance isolates are considered critical results

2.2 The infection control team is informed for quick intervention.

2.3 Prevent nosocomial infections with MDR (Multi Drug Resistant).

2.4 For epidemiological and infection control purpose

3. Sample

3.1 Sample includes:

Organism Identification	**Source of culture**	**Clinical Significance of culture**	**Antibiotic Susceptibility Expert Analysis**	**Antibiotic Susceptibility Kirby Bauer method**
Enterobacteriaceae family	Any Body Site	Pathogen Requires antibiotic	The expert analysis is consistent with the	Proper confluent Growth of the
Pseudomonas species				

		susceptibility testing	organism Identification (Green circle seen)	Isolate (i.e. proper inoculum used)

3.2 Criteria for rejection:

3.2.1 Organism identification other than*Enterobacteriaceae family, and Pseudomonas species*

3.2.2 The expert analysis is not consistent with the organism Identification (Yellow or red square)

3.2.3 Antibiotic Susceptibility by Kirby Bauer method shows heavy or light Growth of the Isolate (i.e. improper inoculum used)

4. Reagents /Media and Supplies

N/A

5. Equipment Calibration and Maintenance

6. Special Safety Precautions

6.1 Refer to TOL: LAB-MIC-TOP-SAF-009

6.2 Refer to TOL: LAB-GEN-SOP-LAB-09

6.3 Refer to MSDS sheets

7. Quality Control

Refer to Microbiology LAB-MIC- TOP-QUA- 004

6.1 Refer to Microbiology LAB-MIC- TOP- ANT- 013

8. Quality Assurance

8.1

9. Procedure Instructions

9.1 Procedure

9.1.1 MDR (Multi Drug Resistant) Definition for :

Gram negative bacilli: from the *Enterobacteriaceae family*

(Not Pseudomonas species)

9.1.1.1 The isolate of Gram negative bacilli from the *Enterobacteriaceae* family is considered MDR if it is resistant or intermediate to **3 different Antibiotic Groups at the same time**

9.1.1.2 Each antibiotic group includes different antibiotics within the group.

9.1.1.3 **HOW TO COUNT**:

9.1.1.3.1 The isolate is considered resistant to an antibiotic group, if it is resistant or intermediate to any one antibiotic within a certain antibiotic group.

9.1.1.3.2 The isolate could be resistant or Intermediate to one, two or more antibiotics within the Antibiotic Group , but you count that specific group as one

9.1.1.3.3 So, regardless of how many resistant or Intermediate antibiotics are within the same Antibiotic Group, you count that Antibiotic Group as ONE.

9.1.1.3.4 Gram negative bacilli from the *Enterobacteriaceae* family are considered as MDR(Multi Drug Resistant) IF Resistant or Intermediate to 3 different antibiotic groups Listed in the following table

9.1.1.3.5 Table of the antibiotic groups to be considered for the

MDR Criteria for *Enterobacteriaceae* family(*NOT Pseudomonas species):*

Antibiotic Group	**Antibiotic Name**
Quinolones	Ciprofloxacin
Aminoglycosides	Amikacin
	Gentamicin
B-lactam /β-lactamase inhibitor combinations	Piperacillin/Tazobactam (Tazocin)
Carbapenems	Imipenem
	Meropenem
	Ertapenem
Cephems	Cefepime
	Cefatazidime
(3rd generation Cephalosporins)	Ceftriaxone or Cefotaxime

9.1.2 MDR (Multi Drug Resistant) Definition FOR :***Pseudomonas* species**

9.2.1 The isolate**OF *Pseudomonas* species** is considered MDR if it is resistant or intermediate to **3 different Antibiotic Groups at the same time.**

9.2.2 Each antibiotic group include different antibiotics within the group.

9.2.3 HOW TO COUNT:

9.2.3.1 The isolate is considered resistant to an antibiotic group, if it is resistant or intermediate to any one antibiotic within a certain antibiotic group.

9.2.3.2 The isolate could be resistant or Intermediate to one, two or more antibiotics within the Antibiotic Group , but you count that specific group as one

9.2.3.3 So, regardless of how many resistant or Intermediate antibiotics are within the same Antibiotic Group, you count that Antibiotic Group as ONE.

9.2.3.4 Gram negative bacilli of *Pseudomonas* species MDR(Multi Drug Resistant) IF Resistant or Intermediate to 3 different antibiotic groups Listed in the following table

9.2.3.5 Table of the antibiotic groups to be considered for ***Pseudomonas* species ONLY**

MDR Criteria for *Pseudomonas species:*

Antibiotic Group	**Antibiotic Name**
Aminoglycosides	Amikacin
	Gentamicin
B-lactam /β-lactamase inhibitor combinations	Piperacillin/Tazobactam (Tazocin)
	Piperacillin
Carbapenems	Imipenem
	Meropenem
Cephems (3rd generation Cephalosporins)	Cefepime
	Cefatazidime
Monobactams	Aztreonam
Quinolones	Ciprofloxacin

8.3 Reporting MDR results

8.3.1 On Cerner, verify the VITEK 2 MIC or Kirby-Bauer results .

8.3.2 On Cerner, add contact comment, phone the result to the ward and report and the time and name of the nurse who was notified. Finalize the result as MDR isolated.

8.3.3 Save the isolate and write the information in the Saving Isolates Log book.

10. Method Performance Specifications

N/A

11. Calculations

N/A

12. Results/Interpretation/Alert Values

12.1 The decision to adopt the above mentioned criteria for defining an isolate as MDR was taken by a committee of the Microbiology Consultant, Microbiology Section Chief and the TAWAM hospital infection Control Committee.

12.2 Defining MDR varies among different hospitals in different countries.

12.3 We define MDR to prevent the organism from establishing itself in the Patient and also to prevent its spread.

12.4 Always consult the Clinical Microbiologist and Senior medical technologists for technical or results interpretation advice, or when in trouble.

13. References

13.1 JCIA, Accreditation Standards for Hospitals, 4th edition, 2011, AOP.5

13.2 Manual of Clinical Microbiology, 9th Edition

13.3 TOP: LAB-MIC-TOP-QUA-004

13.4 ANTIBIOTIC SENSITIVITY TESTING LAB-MIC- TOP-ANT-013.5

14 APPENDICE- 3

Modified Hodge Test for Carbapenemase Detection inEnterobacteriaceae

Background

The Modified Hodge Test (MHT) detects carbapenemase production in isolates of Enterobacteriaceae. In the United States, the most common carbapenemase found inEnterobacteriaceae is the Klebsiella pneumoniae carbapenemase (KPC).

Purpose

Carbapenemase production is detected by the MHT when the test isolate produces the enzyme

and allows growth of a carbapenem susceptible strain (*E.coli* ATCC 25922) towards acarbapenem disk. The result is a characteristic cloverleaf-like indentation.

Reagents

1. 5 ml Mueller Hinton broth (MHB) or 0.85% physiological saline

2. Mueller Hinton agar (MHA)

3. 10 µg meropenem or ertapenem susceptibility disk

4. *E. coli* ATCC 25922: 18–24hr subculture

Equipment

1. Turbidity meter

2. 35OC ± 2OC ambient air incubator

Supplies

1. Sterile cotton-tipped swabs

2. 1 ml sterile pipette

3. Sterile loop

Specimen

Test organisms: 18–24 hr subculture

Special safety precautions

Biosaftey Level 2

Quality control

Perform quality control of the carbapenem disks according to CLSI guidelines.

Perform quality control with each run.

• MHT Positive *K. pneumoniae* ATCC BAA-1705

• MHT Negative *K. pneumoniae* ATCC BAA-170

Interpretation/Results

• After 16–24 hours of incubation, examine the plate for a clover leaf-type indentation at the intersection of the test organism and the *E. coli* 25922, within the zone of inhibition of the carbapenem susceptibility disk.

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

• MHT Negative test has no growth of the *E.coli* 25922 along the test organism growth streak within the disc diffusion. See the CLSI guidelines (M100) for recommendations on detection of carbapenemase production in Enterobacteriaceae that test susceptible to carbapenem.

Expected values

A positive MHT indicates that this isolate is producing a carbapenemase. A negative MHT indicates that this isolate is not producing a carbapenemase.

Method limitations

The class of carbapenemase cannot be determined by the results of the MHT. Some isolates show a slight indentation but do not produce carbapenmase. - 3 - Procedure notes Up to four organisms can be tested on the same MHA plate with

Procedure notes

Up to four organisms can be tested on the same MHA plate with one drug. Two drugs with up to 4 organisms can be tested on a 150 mm Mueller Hinton agar plate.