TURKISH REPUBLIC
ERCIYES UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
DEPARTMENT OF BIOLOGY

EVALUATION OF MULTIPLEX PCR PLATFORM FOR SCREENING CARBAPENEM RESISTANT *Klebsiella pneumoniae* FROM RECTAL SWABS

Prepared by
Thowiba Yousif JAMEEL

Supervisors
Prof. Dr. Abdurrahman AYVAZ
Asistant Prof. Dr. Pınar SAĞIROĞLU

M.Sc. Thesis

September 2020
KAYSERİ

TURKISH REPUBLIC
ERCIYES UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
DEPARTMENT OF BIOLOGY

EVALUATION OF MULTIPLEX PCR PLATFORM FOR SCREENING CARBAPENEM RESISTANT *Klebsiella pneumoniae* FROM RECTAL SWABS

(M.Sc. Thesis)

Prepared by
Thowiba Yousif JAMEEL

Supervisor
Prof. Dr. Abdurrahman AYVAZ
Co-Advisor
Asistant Prof. Dr. Pınar SAĞIROĞLU

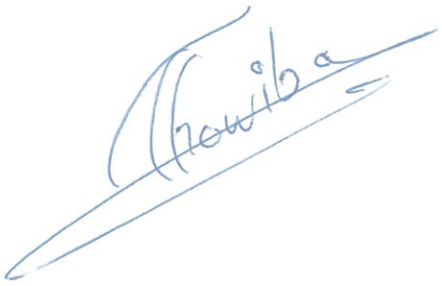
This study was supported by Erciyes University Scientific Research Projects Unit with project code FYL-2019-9331.

September 2020
KAYSERİ

COMPLIANCE WITH SCIENTIFIC ETHICS

I hereby declare that all information in this study has been obtained in accordance with academic rules and ethical conduct. At the same time, I also declare that, as required by these rules and conduct, I have fully cited and referenced all materials and results that are not original to this work.

Thowiba Yousif JAMEEL



SUITABILITY FOR GUIDE

This master's thesis writeup on the topic **"Evaluation of Multiplex PCR Platform for Screening Carbapenem Resistant *Klebsiella pneumoniae* From Rectal Swabs"** has been prepared in accordance with the Thesis Proposal and with the Guidelines for Writing Theses of Erciyes University.

Student

Thowiba Yousif JAMEEL

Supervisor

Prof. Dr. Abdurrahman AYVAZ

Head of Department

Prof. Dr. Abdurrahman AYVAZ

This study entitled **“Evaluation of Multiplex PCR Platform for Screening Carbapenem Resistant *Klebsiella pneumoniae* From Rectal Swabs"** submitted by **THOWIBA YOUSIF JAMEEL** under the supervision of **Prof. Dr. Abdurrahman AYVAZ and Asistant Prof. Dr. Pınar SAĞIROĞLU** has been accepted by the jury as a thesis for the degree of **M.Sc. Thesis of Biology** at Erciyes University, the Graduate School of Natural and Applied Sciences, the Department of Food Engineering.

…/06/2020

JURY:

Supervisor : Prof. Dr. Abdurrahman AYVAZ .....

Juror : .....

Juror : .....

APPROVAL:

That the acceptance of this thesis has been approved by the Institute Board with the decision number …… and the date of …….

..... /…… / ….

Director of the Institute

Prof. Dr. Mehmet AKKURT

ACKNOWLEDGEMENT

بِسْمِ اللَّـهِ الرَّحْمَـٰنِ الرَّحِيمِ

((الَّذِينَ قَالَ لَهُمُ النَّاسُ إِنَّ النَّاسَ قَدْ جَمَعُوا لَكُمْ فَاخْشَوْهُمْ فَزَادَهُمْ إِيمَانًا وَقَالُوا حَسْبُنَا اللَّهُ وَنِعْمَ الْوَكِيلُ فَانْقَلَبُوا بِنِعْمَةٍ مِنَ اللَّهِ وَفَضْلٍ لَمْ يَمْسَسْهُمْ سُوءٌ وَاتَّبَعُوا رِضْوَانَ اللَّهِ وَاللَّهُ ذُو فَضْلٍ عَظِيمٍ))

آل عمران (173-174) **صدق الله العظيم**

Alhamdulillah, all praises to Allah for the strengths and His blessing in completing this thesis.

Special appreciation goes to my supervisor, **Prof. Dr. Abdurrahman AYVAZ**, for his supervision and constant support. His invaluable help of constructive comments and suggestions throughout the experimental and thesis works have contributed to the success of this research. Allah blesses you.

Not forgotten, all my thanks, appreciation, and respect to Prof. **Dr. Mustafa ATALAY** and **Asistant Prof. Dr. Pınar SAĞIROĞLU** my co-supervisor, for all support, knowledge, many interesting, valuable, and sincere feedback throughout their supervision they have given me regarding this project.

Last but not least, my deepest gratitude goes to my beloved parents**; Mr. Yousif JAMEEL** and **Mrs. Ibtisam MAHMOOD**, and to my sister and my brothers for their endless love, prayers, and encouragement.

Omar JAMEEL, I am the luckiest girl in this world to have a brother like you, loving, supportive, and encouraging.

Thowiba Yousif JAMEEL

Kayseri, SEPTEMBER 2020

EVALUATION OF MULTIPLEX PCR PLATFORM FOR SCREENING CARBAPENEM RESISTANT *Klebsiella pneumoniae* FROM RECTAL SWABS

Thowiba Yousif JAMEEL

Erciyes University, Department of Biology
M.Sc. Thesis, September 2020
Supervisor: Prof. Dr. Abdurrahman AYVAZ
Co-Advisor: Assist. Prof. Dr. Pınar SAĞIROĞLU

ABSTRACT

The purpose of this research is to find the optimal, fastest, and accurate methods for diagnosing *Klebsiella pneumoniae*, and to investigate the presence of carbapenem resistance in these bacteria isolated from 48 rectal swabs that have taken from hospitalized patients. This study was conducted at Central Microbiology Laboratory of Erciyes University Hospital.

For the diagnosis and detection of *Klebsiella pneumoniae*, traditional routine cultural methods were compared with BD MAX real-time PCR system. Five carbapenem resistance genes (bla_{KPC}, bla_{OXA-48}, bla_{NDM}, bla_{IMP} and bla_{VIM}) were investigated using the BD MAX checkpoint CPO assay kit.

Chromagar KPC and MacConkey agar were used for bacterial cultures from rectal swap samples. Disk diffusion method with 10 µg Ertapenem, Imipenem and Meropenem discs were used for the antibiotic susceptibility test (AST). While Phoenix system was used for MIC determination, biochemical tests were performed for *Klebsiella pneumoniae* identification.

By using BD MAX system, we have determined that 29 out of 48 isolates gave positive results with above mentioned 5 resistance genes, 28 out of 29 were determined to be *Klebsiella pneumoniae* after the biochemical tests. When considering the distribution of these gene combinations, it was observed that 17 (58.62%) out of a total of 28 *Klebsiella pneumoniae* isolates gave bla_{OXA-48} positive results. The number of isolates carrying the bla_{OXA-48} and bla_{NDM} genes together (bla_{OXA-48} + bla_{NDM}) was found to be 4 (13.79%), while the number of (bla_{OXA-48} + bla_{VIM} / bla_{IMP}) was 3 (10.34%), as it's like in the number of isolates carrying the (bla_{OXA-48} + bla_{NDM} + bla_{VIM} / bla_{IMP}) gene

combination. Only one *bla*$_{NDM}$ gene was detected in 1 (3.45%) isolate. No *bla*$_{KPC}$ gene was observed in any of the isolates that were screened for resistance genes. It was also demonstrated with the Phoenix BD system that 10 of the 28 *Klebsiella pneumoniae* positive isolates were resistant to 19 different antibiotics and showed multi-drug resistance. Our findings have shown that the BD MAX CPO assay is suitable for use, providing rapid detection of carbapenemase genes with high accuracy and in a short time, which helps reduce the spread of antibiotic resistance through rapid diagnosis and detection. However, on the other hand, it has been observed to be insufficient in determining other antibiotic resistance mechanisms in routine laboratories.

Keywords: *Klebsiella pneumoniae*, BD MAX, PCR, *bla*$_{KPC}$, *bla*$_{OXA-48}$, *bla*$_{NDM}$, *bla*$_{IMP}$, *bla*$_{VIM}$, Ertapenem, Imipenem, Meropenem, Carbapenem Resistance

EVALUATION OF MULTIPLEX PCR PLATFORM FOR SCREENING CARBAPENEM RESISTANT *Klebsiella pneumoniae* FROM RECTAL SWABS

Thowiba Yousif JAMEEL
Erciyes Üniversitesi, Biyoloji Bölümü
Yüksek Lisans Tezi, Eylül 2020
Danışman: Prof. Dr. Abdurrahman AYVAZ
Eş Danışman: Dr. Ögr. Üyesi Pınar SAĞIROĞLU

ÖZET

Bu araştırmanın amacı, *Klebsiella* pneumoniae'yi teşhis etmek için en uygun, en hızlı ve doğru yöntemleri bulmak ve hastanede yatan hastalardan alınan 48 rektal sürüntüden izole edilen bu bakterilerde karbapenem direncinin varlığını araştırmaktır. Bu çalışma Erciyes Üniversitesi Hastanesi Merkez Mikrobiyoloji Laboratuvarında yapılmıştır.

Klebsiella pneumoniae tanısı ve tespiti için geleneksel rutin kültürel yöntemler BD MAX gerçek zamanlı PCR sistemi ile karşılaştırıldı. BD MAX kontrol noktası CPO test kiti kullanılarak beş karbapenem direnç geni (*bla*$_{KPC}$, *bla*$_{OXA-48}$, *bla*$_{NDM}$, *bla*$_{IMP}$ and *bla*$_{VIM}$) incelenmiştir.

Rektal swap örneklerinden bakteri kültürleri için Chromagar KPC ve MacConkey agar kullanıldı. Antibiyotik duyarlılık testi (AST) için 10 µg Ertapenem, Imipenem ve Meropenem disklerle disk difüzyon yöntemi kullanıldı. MİK tayini için Phoenix sistemi kullanılırken, *Klebsiella pneumoniae* tanımlaması için biyokimyasal testler yapıldı.

BD MAX sistemini kullanarak 48 izolattan 29'unun yukarıda belirtilen 5 direnç geni ile pozitif sonuç verdiğini, 29'unun 28'inin biyokimyasal testler sonucunda *Klebsiella pneumoniae* olduğunu belirledik. Bu gen kombinasyonlarının dağılımına bakıldığında, toplam 28 *Klebsiella pneumoniae* izolatından 17'sinin (% 58.62) *bla*$_{OXA-48}$ pozitif sonuç verdiği görüldü. *bla*$_{OXA-48}$ ve *bla*$_{NDM}$ genlerini bir arada taşıyan izolat sayısı (*bla*$_{OXA-48}$ + *bla*$_{NDM}$ 4 (% 13.79), (*bla*$_{OXA-48}$ + *bla*$_{VIM}$ / *bla*$_{IMP}$) sayısı 3 (% 10.34), (*bla*$_{OXA-48}$ + *bla*$_{NDM}$ + *bla*$_{VIM}$ / *bla*$_{IMP}$) gen kombinasyonunu taşıyan izolatların sayısındaki gibidir. 1 (% 3,45) izolatta sadece bir *bla*$_{NDM}$ geni tespit edilmiştir. Direnç genleri açısından taranan izolatların hiçbirinde *bla*$_{KPC}$ geni gözlenmedi. Phoenix BD sistemi ile 28 *Klebsiella pneumoniae* pozitif izolatından 10'unun 19 farklı antibiyotiğe dirençli olduğu ve çoklu

ilaç direnci gösterdiği de gösterilmiştir. Bulgularımız, BD MAX CPO testinin kullanıma uygun olduğunu, yüksek doğrulukta ve kısa sürede karbapenemaz genlerinin hızlı tespitini sağladığını ve hızlı tanı ve tespit yoluyla antibiyotik direncinin yayılmasını azaltmaya yardımcı olduğunu göstermiştir. Ancak rutin laboratuvarlarda diğer antibiyotik direnç mekanizmalarının belirlenmesinde yetersiz kaldığı görülmüştür.

Bu çalışma, Erciyes Üniversitesi Bilimsel Araştırma Projeleri Birimi tarafından FYL-2019-9331 proje kodu ile desteklenmiştir.

Anahtar Kelimeler: *Klebsiella pneumoniae*, BD MAX, PCR, bla_{KPC}, bla_{OXA-48}, bla_{NDM}, bla_{IMP}, bla_{VIM}, Ertapenem, Imipenem, Meropenem, Carbapenem Resistance

CONTENTS

EVALUATION OF MULTIPLEX PCR PLATFORM FOR SCREENING CARBAPENEM RESISTANT *Klebsiella pneumoniae* FROM RECTAL SWABS

LIST OF ABBREVIATIONS

AST	: Antibiotic Sensitivity Test
AUC	: Area Under the Curve
CP	: Carbapenemase-Producers
CP-CRE	: Carbapenemase Producing Carbapenem-Resistant Enterobacterales.
CPE	: Carbapenemase-Producing Enterobacterales
CPO	: Carbapenemase-producing organisms
CR-GNB	: Carbapenem-Resistant Gram-Negative Bacteria
CSF	: Cerebrospinal fluid
Ct	: Cycle Threshold
ERT	: Ertapenem
ESBL	: Extended-Spectrum β-Lactamases
EUCAST	: European Committee on Antimicrobial Susceptibility Testing
IPM	: Imipenem
MDR	: Multidrug Resistant
MEM	: Meropenem
MIC	: Minimal Inhibitory Concentration
MIO	: Motility Indole Ornithine Medium
NPV	: Negative Predictive Value
PBPs	: Penicillin-Binding Proteins
PCR	: Polymerase Chain Reaction
PPV	: Positive Predictive Value
ROC	: Receiver Operating Characteristic curve
TSI	: Triple Sugar Iron
UTI	: Urinary Tract Infection

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDIXS

CHAPTER 1

INTRODUCTION

Klebsiella pneumoniae is known as an opportunistic pathogen and is a serious cause of nosocomial infections (Effah et al., 2020). It is one of the most common pathogens in intensive care units causing severe respiratory (pneumonia) and urinary tract infections (UTI), bacteremia, sepsis, and liver abscesses (Paczosa and Mecsas, 2016).

It is a gram-negative, non-motile, encapsulated bacterium present in the normal intestinal flora. This capsule covers the entire cell membrane, signifies the organism's prominent appearance when tested in a Gram stain, and supplies resistance to several host's defensive mechanisms (Paczosa and Mecsas, 2016).

Klebsiella genus belongs to the Enterobacterales family, it was named after the scientist Edwin Klbs, a nineteenth-century German microbiologist (Sheff, 2000).

Customarily, hypervirulent *Klebsiella* strains considered one of the higher and biggest causes of life-threatening, community-acquired infections to hospitalized individuals, and more resistant to the antibiotics. *Klebsiella pneumoniae* is more medically significant than other *Klebsiella* species, where it represents a significant proportion of hospital-acquired UTIs, septicemia, pneumonia ,and soft tissue contagions (Pajand et al., 2020). *Klebsiella pneumoniae* causes pneumonia, a common lung infection where the air sacs in the lungs become inflamed. These vesicles may also be filled with affluent and pus, the inflammation can be mild or serious (Shahfiza et al., 2017).

Over the years, *Klebsiella pneumoniae* has induced serious infection mainly in immunocompromised persons but the recent appearance and dissemination of hypervirulent strains have broadened the number of people vulnerable to infection including other healthy individuals and immunodeficient. Additionally, *Klebsiella*

pneumoniae strains have been become highly resistant to antibiotics and have made infection with these strains extremely difficult to treat (Paczosa and Mecsas, 2016).

Hands, digestive system, patients, suction tubes, ventilator tubes, tables, beds, and hospital workers are the main causes of transmission of this bacterium. Since they are easily transmitted in the hospital environment, these bacteria have a very high outbreak potential and therefore they are among the most important nosocomial infectious agents. (Li et al., 2014; Abdallah et al., 2018).

The fact that *Klebsiella pneumoniae* is a risky pathogen is due to their potential to develop antibiotic-resistant strains and to transport these resistance mechanisms to other organisms via plasmids (Effah et al., 2020). The World Health Organization has defined this pathogen as resistant to antibiotics and emphasized the urgent requirement for new antibiotics in the treatment of diseases caused by this pathogen. (David et al., 2019).

Klebsiella pneumoniae was well known as a cause of bacterial pneumonia in the community, the nature and epidemiology of *Klebsiella pneumoniae* infections changed drastically in the early 1970's and this bacterium became a most important nosocomial infectious agent (Ciftci et al., 2019). Their tendency to spread in the hospital setting and the widespread use of antimicrobial therapy allowed *Klebsiella pneumoniae* to develop increased antibiotic resistance (Podschun and Ullmann, 1998). Resistance to aminoglycoside encoded by plasmids was widespread during the 1970s and 1980s. Thereafter, extended-spectrum β-lactamases (ESBL) has appeared. It was followed by fluoroquinolone resistance and eventually, carbapenem resistance (Jarvis et al., 1985). The primary reservoir of this bacterium represents human himself, where studies have shown that in the general population, 5 to 38% of these microorganisms transported in the feces and 1 to 6% in the nasopharynx. Especially, it was thought that the rate of transport in the stools of hospitalized patients reached 77% and this was due to the antibiotics used (Esposito et al., 2018; Walter et al., 2018).

Carbapenem resistance results from decreased permeability of the external membrane, overexpression of efflux pumps or carbapenemases genes (bla_{KPC}, $bla_{\text{OXA-48}}$, bla_{NDM}, bla_{IMP} and bla_{VIM}) (Nordmann et al., 2011a). Carbapenem-resistant *Klebsiella pneumoniae* poses the world's fastest-growing threat to antibiotic resistance in terms of human morbidity and mortality (Cassini et al., 2019). In *Klebsiella pneumoniae*, the

rapid expansion of carbapenem resistance has been due to the development of carbapenemase enzymes that hydrolyze carbapenems (a last-line class of antibiotics) and other β-lactam antibiotics at varying rates. Carbapenemase genes are associated with mobile elements that can spread horizontally in and above bacteria, promoting the spread of these genes to other bacteria. (Martin et al., 2017).

Klebsiella pneumoniae carbapenemase bla_{KPC} was first reported from the United States in 1996 and triggered epidemics within a couple of years (Nordmann et al., 2009). In 2003, bla_{OXA-48} was first recorded from Turkey and extensively determine in these country (Poirel et al., 2004). While, bla_{IMP} and bla_{VIM} are the more often determine Metallo β-lactamases worldwide. The foremost lately identified carbapenemases in Enterobacterales are bla_{NDM} enzymes (Nordmann et al., 2011b, 2009).

In other regions over the world, epidemiology is different, for example, in Israel, bla_{KPC} has been the most commonly carbapenemase; in Greece, bla_{VIM} is endemic; and in Japan, bla_{IMP} is endemic (Cantón et al., 2012). The bla_{NDM} and bla_{OXA-48} like carbapenemases started in India and Turkey, respectively, where they are endemic and have been increasingly disseminated globally (Nordmann et al., 2011). The frequent movement of people colonized or infected with CP-CRE through border crossings and exposed these colonized individuals to medical care treatment is a significant contributor to the transmission of CP-CRE (Lutgring and Limbago, 2016; Molton et al., 2013) .

Infections with strains that produce carbapenemase cause long-term hospitalization, high mortality rates, and morbidity. Moreover, carbapenemases are typically associated with several other resistance determinants, resulting in multidrug resistance. Therefore, if these strains are identified, steps to avoid the horizontal dissemination of resistance genes should be taken (Cohen et al., 2011).

Many methods for Carbapenem resistance gram-negative bacteria identification have been possible over the past 10 years, such methods include phenotypic-based methods that identify carbapenemase enzyme activity and molecular-based polymerase chain reaction (PCR) methods to identify carbapenemase genes (Al-Zahrani, 2018). However, there is no particular method that has been put forward as suitable for identifying all mechanisms of carbapenem resistance.

Compared between them on the basis of accuracy and speed in diagnosis. The faster and more accurate the diagnosis, the greater the control the outbreak of carbapenemase-producing *Klebsiella pneumoniae* in healthcare units particularly in the intensive care units (ICU), and for that, the aim of the research is to find the optimal, fastest, and accurate methods for diagnosing *Klebsiella pneumoniae*. In this study we aimed the determination of the outbreak rate of *Klebsiella pneumoniae* in Erciyes University Hospital by using the traditional conventional cultural techniques (chromagar KPC, MacConkey agar), biochemical tests (Citrate, TSI and MIO), antibiotic susceptibility of these isolates, percentage of resistance and sensitivity of these strains to carbapenem by the disk diffusion methods and to evaluate the efficiency of BD MAX check-points CPO Assay used for the screening of carbapenem resistance genes (bla_{KPC}, bla_{OXA-48}, bla_{NDM}, bla_{IMP} and bla_{VIM}). Finally, our main goal in this study is to compare traditional culture methods with the BD MAX system to determine the effectiveness of the system, which can complete diagnostic procedures in a very short time.

CHAPTER 2

LITERATURE REVIEW

Studies of environmental antibiotic resistance provide new insights into a challenge that has historically been limited to a subset of bacterial antibiotics resistant pathogens that are clinically applicable. It is obvious that the environmental microbiota, even in supposedly antibiotic-free environments, possesses a large amount and difference of antibiotic resistance genes, some of which are very related and linked to the genes circulating in pathogenic microbiota. (Aminov, 2009).

Drug-resistant bacteria are a considerable public health concern owing to the speed of their development and proliferation (Cirz et al., 2005). The resistance to antibiotics appears whenever microorganisms are modified once attacked by antimicrobial drugs, these drug-resistant microorganisms are also referred to as "superbugs." Therefore, the drugs become inefficient, and pathogens linger in the body, raising the risk of spread to others. Currently, the development of new mechanisms to resist the antimicrobials and spreading over the world consider as a serious problem, wherein it restricts our capacity to cure infectious diseases and leads to death in the worst case (WHO, 2020).

Resistance to carbapenem (last-resort antibiotic) in *Klebsiella pneumoniae* has developed and extended through all regions of the world. Due to this resistance, carbapenem antibiotics do not treat in over half of patients hospitalized for *Klebsiella pneumoniae* Infections (Codjoe and Donkor, 2018). The medical relevance of *Klebsiella pneumoniae* and its mechanisms of resistance to carbapenem and the methods for its diagnosis will be explained in this literature review.

2.1. Klebsiella Pneumoniae

Klebsiella pneumoniae was first defined through the scientist Carl Friedlander in 1882 as a bacterium secluded of the lungs of patients who became died from pneumonia (Wasekar et al., 2014). *Klebsiella pneumoniae* its environment is not bounded to humans, but it is observed everywhere in the environment. This includes surface water, sewage, and soil (Cabral, 2010). *Klebsiella pneumoniae* can colonize and live in the hospital, medical equipment, and healthcare places (Li et al., 2014). *Klebsiella pneumoniae* is a type of Gram-negative bacterium that is facultative anaerobic and relates to the Enterobacterales family. Contamination with bacteria is by an extensive variety of circumstances that can lead to infection and antibiotic resistance (Fair and Tor, 2014). *Klebsiella pneumoniae* is one of the bacteria that normally live in the intestines and human feces (Bengoechea and Sa Pessoa, 2019). *Klebsiella pneumoniae* are not dangerous when they are in the intestine, but if they spread to a different section of the body they may cause a hard infection that becomes more serious if the body has a disease where it can infect: liver, eyes, blood, wounds, lungs, Bladder, brain (Kus et al., 2017).

Klebsiella species are deemed opportunistic pathogens establishing mucosal surfaces externally causing pathology; however, from mucosae *Klebsiella* may propagate to other tissues producing life-threatening infections including pneumonia, UTIs, sepsis, and bloodstream infections (Mecsas, 2016).

It has defining features of these infections is the ability to metastatically spread and their significant morbidity and mortality (Shon et al., 2013).

The polysaccharide capsule in the organism is the most influential part of virulence and provide for *Klebsiella pneumoniae* to escape serum killing and phagocytosis (Ko, 2017). *Klebsiella pneumoniae* pathogenicity is caused by the thick capsule layer surrounding the bacteria. The soft fibre is 160 nm thick and projects from the external membrane at right angles (Schembri et al., 2005).

Klebsiella pneumoniae is one of the world's common serious superbugs and becoming resistant to practically every antibiotic available today. Increasing the clinical repetition of antibiotic-resistant bacteria is an important global health concentration issue.

Especially this is true when the infections by multidrug-resistant pathogens impose a significant and escalating burden on both patients and healthcare providers (Woldu, 2016). Klebsiella has become a significant pathogen in hospital environments producing nosocomial infections with disruptions of 20%, where the difficulty of antibiotic resistance is typically magnified (Ullmann, 1998).Therefore, immunocompetent people appear not to get Klebsiella infections unless they are critically suffering and they are on breathing machines or intravenous catheters, or unless they are getting long span courses of broad-spectrum antibiotics (Kallen, 2009; Paczosa and Mecsas, 2016).

2.2. *Klebsiella pneumoniae* resistance against antibiotics

The research, exploration and development of discovering the compounds that work and affect and inhibit the work of pathogens and these are called antimicrobials to heal infections have changed modern medicine and developed the form and treatment body. Indeed, antibiotics have become one from the best and most significant medical interferences needed to develop complex medical approaches like advanced surgical procedures, organ trans operations and cancer control, among other things. Unfortunately, the marked improvement in antimicrobial resistance between common bacterial pathogens now threatens this therapeutic achievement, threatening the successful outcomes of patients with serious diseases. In fact, the World Health Organization has classified antibiotic resistance as one of the three most important public health threats in the 21st century (Amann et al., 2019).

Klebsiella pneumoniae quickly became recognized for its resistance to the greatest antibiotics. It is particularly problematical in hospitals, where it produces a variety of acute infections (Effah et al., 2020). The mounting frequency of carbapenemase-producing *Klebsiella pneumoniae* has caused a much challenge to the communicable diseases (ID) physician due to lack of therapeutic selections. This became commenced to significant mortality (Patel et al., 2008). The increasing number of immunocompromised cases has extra led to a developed rate of infections with these highly resistant organisms. Receipt of Cephalosporin antibiotics including the oxyimino group (cefuroxime, ceftazidime, cefotaxime, ceftriaxone or aztreonam) is a well-known danger factor for getting Extended-spectrum beta-lactamases (Paterson et al., 2004).

Bacterial isolates contain carbapenemases are able to hydrolyze a broad spectrum of β-lactams including carbapenems, cephalosporins, monobactam and penicillins. Majority of these organisms belong to the ESCAPE (*Acinetobacter baumannii, Staphylococcus aureus, Pseudomonas aeruginosa, Clostridium difficille, Enterobacterales species, Enterococcus faecium*) collection of bacteria and are responsible for the main of the hospital- and common-acquired infections (Peterson, 2009).

The optimal treatment for carbapenem-producing *Klebsiella pneumoniae* has not yet been discovered. Although there are a few new antimicrobials in development, physicians tend to use older antimicrobials that were previously neglected, such as tigecycline and colistin to treat these infections (Urban et al., 2008). This statement has lately led to the appearance of organisms with resistance to antibiotics from all acknowledged groups, including the polymyxins with its attendant toxicities. tigecycline, which is bacteriostatic, is a glycylcycline with expanded activity facing many Enterobacterales, including *Klebsiella pneumoniae* carbapenemases and Extended-spectrum β-lactamases (Zarkotou et al., 2010).

The resistance to ampicillin by the production of β-lactamase SHV-1 to carbapenems impedance by different mechanisms, from the creation of extended-spectrum beta-lactamases, The Extended-spectrum β-lactamases producing organisms are among those responsible for antibiotic-resistant strains (Paterson and Bonomo, 2005). Extended-spectrum β-lactamases (ESBLs) are enzymes produced by specific bacteria, a rapidly evolving group of β-lactamases enzymes that participate the ability to hydrolyze third-generation Cephalosporins, Aztreonam, Ampicillin, Ceftazidime, Ceftriaxone, Oxyimino-monobactam, Cephamycins (Shah et al., 2004).

2.3. Carbapenems antibiotics

The antimicrobial resistance is a dangerous developing problem of public health. This improvement in bacterial resistance to antibiotics has resulted in the application of these antibiotics in patients medications (Mobarki et al., 2019).

Carbapenems are β-lactam antibiotics, it is very powerful antibiotic factors that are usually utilized to treat difficult or serious bacterial infections. targets bacterial cells by inhibiting transpeptidases (PBPs) which prevents the peptidoglycan synthesis (a

necessary structural component) that its absence leads to cell lysis (Hawkey and Livermore, 2012). This group of public or suspected multidrug-resistant bacterial infections is habitually classified. carbapenems are sections of the beta-lactam antibiotic class, which eliminate bacteria by bandaging them to penicillin-binding proteins, thereby inhibiting the synthesis of the bacterial cell wall. Moreover, carbapenems are not usually affected by resistance to emerging antibiotics, even with other beta-lactam kinds (Papp-Wallace et al., 2011).

Carbapenems antibiotics (ertapenem, meropenem, imipenem, biapenem, and doripenem) are used to treat serious Enterobacterales infections including *Klebsiella pneumoniae* and *E.coli* (Lee et al., 2016).

Ertapenem, meropenem and imipenem antibiotics have used in this research to investigate the resistance of *Klebsiella pneumoniae* isolates to them, and they will be discussed separately.

2.3.1. Ertapenem

Ertapenem is a 1-beta-methyl carbapenem and a broad-spectrum beta-lactam antibiotic that has a bactericidal property of the carbapenem family, and is a synthetic beta-supplement. It is used to treat infections caused by bacteria affected by the drug such as community-acquired pneumonia, diabetic foot inflammation, dermatitis, abdominal cavity inflammation, pelvic inflammation, urinary tract infections, and to prevent infections after colorectal surgery (Parakh and Krishnamurthy, 2009). Ertapenem bond to the penicillin-binding proteins present on the bacterial cell wall, especially PBPs 2 and 3, thereby inhibiting the concluding deposition step in peptidoglycan synthesis, which is an essential component of the bacterial cell wall. Inhibition commences to weakening of the cell wall and consequent deterioration, reaching to cell death from Gram-positive and Gram-negative anaerobic and anaerobic pathogens (Lecoq et al., 2013).

It’s in vitro activity against intestinal bacteria including beta-lactamases with plasmid or chromosomes, including AmpC and beta-lactamase of the extended-spectrum, is clinically important. It is very steady against almost all beta-lactamases, including AmpC and extended spectral beta-lactamases. Ertapenem has been shown to be

clinically and bacteriologically highly effective in randomized controlled trials for the treatment of community-acquired infections (Zhanel et al., 2014). Benefits related to the in-vitro activity are decreased collateral damage, and the development of special immunity during treatment, as reported in many clinical experiments, making ertapenem an excellent choice for treating complicated aerobic and anaerobic complicated infections caused by bacteria sensitive to carbapenems. On the other hand, due to its limited activity against *Acinetobacter spp*. *Pseudomonas aeruginosa* and *Enterococcus spp*., are less suitable for late hospital infections (Van Rijn et al., 2016).

2.3.2. Meropenem

Meropenem is an antibiotic from the carbapenem family, and is a synthetic beta-supplement. It is an anti-bacterial agent that interferes with the process of building the bacterial cell wall, making it unstable, which ultimately leads to the death of the bacterial cell. Meropenem is a broad-spectrum antibiotic, effective against most strains of Gram positive and negative, and beta-lactamase-resistant bacteria. It is used to treat infections caused by bacteria affected by the drug such as bacterial meningitis, dermatitis and subcutaneous tissue, inflammation of the abdominal cavity (Pournaras et al., 2010). Meropenem is a beta-lactam antibiotic relating to the carbapenem group. It varies structurally from imipenem, it was the first carbapenem has used and marketed, by containing an I-a-methyl collection on the carbapenem part and a replaced 2’ side sequence. Meropenem is almost stable to human dehydropeptidase-I (DHP-I), and therefore, unlike imipenem, it does not need to be administered with a DHP-I inhibitor such as cilastatin (Shah and Isaacs, 2003). Meropenem is stable against most β-lactamases produced by Gram-negative bacteria and has the biggest advantage in healing critical infections in hospitalized. It has immeasurable Cerebrospinal fluid (CSF) penetrability and beneficial in the treatment of childhood meningitis and infections in neutropenic children. Because of concern correlating to the development of resistance, this should be utilized as a reserve drug in severe-to-treat infections produced by resistant organisms or when conventional treatment fails (Hoffman and Weber, 2009).

Meropenem penetrates active and extensively into an area of body fluids and tissues. Meropenem also enters into CSF although, as with other β-lactam factors, permeability

is vaster in sufferers with meningeal inflammation. Meropenem is essentially secreted by the kidney with nearly half to three-fourths of the dose amount excreted unchanged in the urine and an extra one-fourth excreted as a microbiologically inactive open β-lactam metabolite. Unlike Imipenem, Meropenem is stable against hydrolysis by human renal dehydropeptidase (DHP-1) and concomitant administration of cilastatin (DHP inhibitor) is not required (Blassmann et al., 2016).

2.3.3. Imipenem

Imipenem is a carbapenem class antibacterial agent of β-lactams, with a very wide range of action which involves both Gram-negative and Gram-positive bacteria, aerobes and anaerobes, and identified action among β-lactamases-producing microbes. It is typically most effective among Enterobacter cloacae and Citrobacter freundii than 3-generation cephalosporins, as well as of comparable action to such antimicrobial agents against all other Enterobacteriaceae, and is typically less effective than ciprofloxacin against Escherichia coli, Klebsieila pneumoniae, E. cloacae and Serratia marcescens (Buckley et al., 1992). In order to an unfulfilled need for a highly active, broad-spectrum antimicrobial treatment including better safety features, Imipenem, the first carbapenem discovered, was produced over two centuries ago. Several 26 million patients have been treated with it since. Imipenem (N-formimidoyl-thienamycin) is a 5–10-fold highly stable than the original compound, amidine by-product of thienamycin (Rodloff et al., 2006). As all other beta lactams, imipenem prevent bacterial cell membrane synthesis by attaching to specific transpeptidases (PBP) and disabling them (Hashizume et al., 1984).

This contrasts with all other β-lactams, even carbapenems that attach selectively to PBP-1 and PBP-3. Correspondingly, imipenem promotes sphere formation with subsequent cell rupture but not the filamentous bacterial development noted with certain β-lactams. The imipenem treatment, therefore, decreases the number of lipopolysaccharides released throughout bacteriolysis (Jackson and Kropp, 1992). For more than two decades Imipenem had already maintained the clinical effectiveness, in vitro activity, and tolerability against significant bacterial pathogens (Buckley et al., 1992).

2.4. Carbapenemase Enzymes

Carbapenemase is β-lactamase with multiple-use hydrolyzes ability. It has the capacity to hydrolyzing penicillins, monobactams, cephalosporins, and carbapenems. Such β-lactamases producing bacteria can contribute to severe infections, with many β-lactams ineffectual by carbapenemase activity (Queenan and Bush, 2007).

Carbapenemases are the most versatile β-lactamases family, with an unrivaled spectrum width of other β-lactam- hydrolyzing enzymes. While several of these enzymes, known as "carbapenemases," recognize almost all hydrolyzable β-lactams, and most are resilient to inhibition by any commercially viable inhibitor of β-lactamase (Nordmann and Poirel, 2002; Walther-Rasmussen and Hoiby, 2006).

Several researchers preferred the nomenclature 'carbapenem-hydrolyzing enzymes' to the word 'carbapenemases,' indicating that carbapenems are just one segment of their substrates (Rasmussen and Bush, 1997). Nevertheless, the word carbapenemase has become rooted in the literature regarding β-lactamase and is used in this study.

Carbapenemases are classified into various groups, depending on the formation of the enzyme and the mechanism through which they hydrolyze the β-lactam ring. The two broad categories of carbapenemases are serine-carbapenemases, that contain serine at the active site, and metallocarbapenemases, which include zinc at the active site (Queenan and Bush, 2007).

Class A carbapenemases are serine carbapenemases and are encoded on either the chromosome of the bacteria or a plasmid. A serine at position 70 at the active site of this class of enzymes is required for hydrolysis of β-lactams to happen, this group includes bla_{KPC}, bla_{SME} bla_{IMI} bla_{NMC} bla_{GES} genes (Naas et al., 2016).

Class D carbapenemases also pointed to as the OXA β-lactamases, are serine β-lactamases. They are encoded on plasmids and carry a large variability in amino acid sequence. The resistance mechanism for class D carbapenemases is produced by the formation of an acyl intermediate when breaking the β-lactam ring, this group includes $bla_{\text{OXA-48}}$ gene (Antunes and Fisher, 2014).

Class B carbapenemases are metallolactamases and require zinc at the active site for hydrolysis, this group includes *bla*$_{IMP,}$ *bla*$_{VIM,}$ *bla*$_{NDM,}$ *bla*$_{GIM}$ and *bla*$_{SPM}$ genes (Queenan and Bush, 2007). Carbapenems antibiotics have obtained considered as a "last resort" antimicrobial to fight MDR *K.pneumoniae* infections. They are generally applied and are often the only therapeutic option for healing serious infections caused by *Klebsiella pneumoniae* that produce extended-spectrum β-lactamases (ESBLs). The modern emergence of carbapenem-resistant Klebsiella pneumonia in different countries is worrying as antimicrobial medication options are limited (Meletis, 2016).

Globally, carbapenemase-producing Enterobacterales (CPE) is estimated to be a severe issue, especially in hospitalized patients. The extraordinary-risk patients are those contaminated with CPE producer strains as it makes medicine to be ineffective in these patients. Thus, there an immediate needs to discover and determine CPE and formulate strategies to conquer their prevalence (Tzouvelekis et al., 2012).

2.5. Diagnosis of Klebsiella Pneumoniae

Diagnosis and detection of bacteria that are resistant to carbapenems is a real challenge for many clinical laboratories. Through this research, a variety of phenotypic or conventional methods and molecular methods used in the detection of bacteria resistant to anti-carbapenems were reviewed and discussed and no agreement was reached regarding the single, best, and optimal way to detect these bacteria.

Because of the differences in resistance activity between carbapenemase enzymes, the use of two or more conventional or outward detection methods can improve the accuracy of diagnosis compared to using only one method (Al-Zahrani, 2018). Molecular methods have become the best choice because of their rapid implementation and accurate results. DNA sequencing is one of the most sophisticated molecular methods, providing accurate and comprehensive results, and possibly used routinely in clinical laboratories. However, this technology is still very expensive and requires expertise to conduct it and special programs to analyze its results. Therefore, its routine use in clinical laboratories requires years, especially in developing countries (Jensen, 2018).

Detection systems in recent years, various technologies have been transformed to identify Carbapenem-resistant Gram-negative Bacteria (CR-GNB). These technologies involve phenotypic based methods which identify carbapenemase activity, such as growth-based assays, accelerated colorimetry techniques, immuno-color assays, molecular techniques, for example (PCR) and entire genome-based methods (Samanta and Bandyopadhyay, 2020).

The BD MAX system is an important way we applied, intended for use in vitro diagnosis (IVD) in a DNA test that has been scanned or approved by the FDA in clinical laboratories. It is capable of automatic extraction and purification of nucleic acids from multiple types of samples, as well as automatic amplification and detection of target DNA sequences by fluorescence-based PCR (Tsuyuki, 2017). It has the ability to extract and purify independent DNA, PCR amplification using thermal circulation across 24 lanes, real-time detection of amplification products, identification of each sample of buffer tubes with an external barcode reader, checking the working list with an internal barcode reader, fluorescence detection to Five wavelengths, which allow multiplex interactions by calling the threshold of the cycle (Ct), or multiplex reactions by dissolved analysis, qualitative and quantitative and dissolved analysis (Trushkin et al., 2013).

Rapid and accurate Carbapenemase identification is important for health safety, infection prevention, and surveillance. The BD MAX CPO analysis can be carried out at around 2.5 hours. This assay is faster compared to with growth-based methods for verifying carbapenemase production involving the modified Hodge analysis and the adapted carbapenem inactivation process that's taking 18–24-hour (Tamma and Simner, 2018).

Carba NP and lateral flow immunoassays are using for faster processes. Carba NP and variants take about 30 minutes and 2 hours. The immunochromatographic test gives findings in a time of 15 minutes (Chung and Lee, 2020).

Clinical microbiology laboratories use MALDI-TOF MS to meeting the need for rapid, cost-effective, and reliable microorganism identification since MALDI-TOF MS can efficiently distinguish carbapenem-resistant *Klebsiella pneumoniae* (CRKP) from

carbapenem-susceptible *Klebsiella pneumoniae* (CSKP) with high classification accuracy (Huang et al., 2020).

BD MAX system works to automatizes the test process and decreases the intrusion of the operator from the moment the specimen is placed inside the instrument till findings are obtained. Consequently, the test can be easily conducted by less professional staff and the possibility of contamination and human error can be significantly reduced. This assay can even test 24 specimens simultaneously and can test one specimen without losing the reagent. Also, assays that are run on the BD MAX device can be conducted simultaneously using various BD MAX assay kits, thereby allowing the clinical laboratory to meet the physicians' diverse needs. The BD MAX assay has various limitations, where this assay most of all has no ability to recognize bacteria. And identifying those gene sequences may not indicate that viable organisms are present. Due to the integration of bla_{IMP} and bla_{VIM} genes in the same optical channel of the BD MAX system, the BD MAX CPO Assay cannot differentiate between these two genes (Chung and Lee, 2020).

CHAPTER 3

MATERIALS AND METHODS

3.1. Materials

3.1.1. Instruments

Table 3.1. The instruments used in the research

Equipment	Company	Origin
BD MAX System	BD - Becton Dickinson	USA
Phoenix system	BD - Becton Dickinson	USA

3.1.2. Culture Media

The cultivation media and biochemical tests used in this research to diagnose Enterobacterales with their manufacturer and origin illustrated in Table 3.2.

Table 3.2. The culture media and biochemical tests used in this study

Materials	Company	Origin
KPC chromagar	RTA Laboratories	Istanbul Turkey
Mac Conkey agar	BD - Becton Dickinson	USA
Mueller-Hinton agar	BD - Becton Dickinson	USA
TSI agar	BD - Becton Dickinson	USA
CITRATE agar	BD - Becton Dickinson	USA
MIO test	BD - Becton Dickinson	USA

3.1.3. Antibiotics

Antibiotics used in the susceptibility test (AST) with their concentration, MIC value and manufacturer illustrated in Table 3.3.

Table 3.3. The carbapenem antibiotics used in this study

Antibiotics	Symbol	Conc. Of Antibiotic µg/ discs	MIC		Inhibition zone/mm		Company
			R	S	R	S	
Ertapenem	ERT	10	>1	<=0.25	≤15	29-35	Oxoid –Uk
Meropenem	MEM	10	>8	<=0.125	≤13	28-36	Oxoid –Uk
Imipenem	IPM	10	4-8	0.5	≤17	29	Oxoid –Uk

We used the carbapenem antibiotic discs Ertapenem, Imipenem and Meropenem in this study which considers the last resort for *Klebsiella pneumoniae* infection treatment. By cultivating the *Klebsiella pneumoniae* isolates on Mueller-Hinton agar, where antibiotic susceptibility tests were typically performed on it, antibiotic discs were put in media already inoculated with the *Klebsiella pneumoniae* strain where the colony grew intensively. The antibiotics in the discs began to disperse among the bacterial colonies, and inhibition zones around the antibiotic discs were observed after one day of incubation. The wider inhibition zone around the disc means the bacterium has a higher antibiotic sensitivity. The zones for inhibition are measured and contrasted with EUCAST guidelines.

MIC is minimum inhibition concentrations, meaning the low antibiotic concentration that inhibits the observable growth of a microorganism after overnight incubation, used to determine the antibiotic concentration that prevents bacterial development.

In Table 3.3. the MIC value of Ertapenem, Imipenem and Meropenem was given. The measuring of MIC is effectively and accurately selecting the antibiotics to ensure the success of the treatment in killing the pathogenic bacteria, hence the patient's recovery.

3.2. Method

This study was carried out in the clinical microbiology laboratory within the Erciyes University hospitals. All Clinical specimens have examined for Carbapenem resistance. Antibiotic susceptibility tests for these isolates were performed on Mueller-Hinton agar

using the Kirby-Bauer disk diffusion method, as per the EUCAST guidelines for 2020. Used as antimicrobial discs were Meropenem (10 μg), Imipenem (10 μg) and Ertapenem (10 μg). Before starting this study the approval of the institutional ethical committee was obtained.

While one group of 48 rectal swab samples taken randomly from different units of the hospital have been used for chromagar, conventional cultivation methods, and antibiotic sensitivity testing, the other 48 rectal swab group had been used to detect carbapenem resistance genes in the BD MAX system.

3.2.1. Samples collection

Forty-eight rectal swab duplicate specimens were obtained randomly from various ten hospital units (Intensive care unit, Neurosurgery unit, Pediatric intensive care unit, Anesthesia unit, Hematology unit, General surgery unit, Neonatal intensive care unit, Bone marrow unit, Nephrology unit, and Medical oncology unit) from patients with different age groups hospitalized at Erciyes University's Medical Faculty Hospital in Kayseri, Turkey, as indicated by Appendix (1). These specimens were kept at 4 ° C in the refrigerator.

3.2.2. Preparation of culture media, biochemical tests and bacterial broth.

The manufacturer has sterilized all media used for cultivation and biochemical testing and made them ready for use.

3.2.3. Cultivation media

3.2.3.1. KPC chromagar

KPC chromagar, is a selective and differential chromogenic agar, has a selective carbapenem compound used in obtaining a pure gram-negative bacteria colonies expresses decreased sensitivity to carbapenem antibiotics (Metian, 2017; Oliver, 2019).

It is enhanced by agents that are toxic to gram-positive /gram-negative carbapenem-sensitive bacteria, and thus inhibit their growth (Shahfiza et al., 2017).

KPC chromagar is used for the identification and distinguishing of Enterobacterales and after being cultured at 37 ° C and for 24 hours, different color formation is observed according to its unique enzymatic properties. (Samra et al., 2008).

3.2.3.2. MacConkey agar

MacConkey Agar Medium is a differential solid medium, selective and therefore indicator medium, which is used mainly for gram-negative and enteric bacilli strains It is mainly used for the distinction of lactose and non-lactose fermenters, for Enterobacterales in particular such as *E.coli* and *Klebsiella pneumoniae* (Fusion, 2005). It considered a selective medium for gram-negative bacteria because It is enhanced with crystal violet and bile salts which prohibit gram-positive bacteria from growing (Al-Zahrani, 2018). That the pH indicator is in the agar allows for the colonies to appear in two colors, whereas those fermenting the lactose appears as pink colonies by producing an acid that alters the pH from neutral to acidic, as with *E.coli* and *Klebsiella pneumoniae* do. Those not fermenting the lactose appear as colorless, translucent colonies. (Wilson, 2013).

3.2.3.3. Mueller-Hinton agar

It is a non-selective non-differential medium that encourages the growth of various organisms. It is the ideal medium for both antibiotic susceptibility test (AST) and microorganism cultivation. (Mueller and Ii, 2006).

3.2.4. Biochemical tests

3.2.4.1. TSI agar

It is a medium used for enteric bacteria detection, examines the fermentation of carbohydrates and H2S production. It consists of phenol red as a pH indicator, three sugars to be tested for fermentation of glucose, lactose, and sucrose, and iron and sulfur for H2S gas output. The fermentation of carbohydrates produces an acid that changes the acidity of the medium and changes its color from orange to yellow. If H2S gas is released along with the fermentation of carbohydrates, the medium will appear yellow with bubbles and cracks, as in the case of *Klebsiella pneumoniae* (Altmann et al., 1979).

A sterile inoculating needle was used to take Enterobacterales colonies from MacConkey agar and then inoculated into triple sugar iron agar tubes by gently stabbing and incubating in an aerobic environment at 37° C for one day (Bd, 2003).

3.2.4.2. Citrate agar

Citrate Agar is a type of biochemical test that focuses on citrate utilization to distinguish the gram-negative bacteria. In an aerobic environment at 37 ° C for one day, the citrate agar tubes were incubated after inoculated it with Enterobacterales colonies from MacConkey agar. After incubation, if the color changes from green to blue meaning the bacteria utilized citrate like *Klebsiella pneumoniae*, while if the appearance is green , that indicates bacteria can not utilize citrate like *E.coli* (Sulikowska, 2015). The medium was prepared by the manufacturer BD.

3.2.4.3. MIO test

It's a semi-solid medium used to identify Enterobacterales, is being used to demonstrate motility, indole production and the activity of ornithine decarboxylase, and therefore to distinguish Enterobacterales.

Enterobacterales colonies were inoculated into MIO agar tubes and incubated in an aerobic environment for one day at 37 ° C (Himedi, 2012). Motility indicated by the growth extending from the inoculation line. Some no motile species, including *Klebsiella pneumoniae*, grow only along the inoculation line. The medium was prepared by manufacturer BD.

3.2.4.4. Bacterial inoculum

In the Antibiotic Susceptibility Test (AST), the McFarland Criteria are used to standardize the approximate number of bacteria in the liquid suspension of a bacterial cell. In order to achieve more accurate results for the antibiotic susceptibility test, the bacterial suspension concentration must be between 0.5-0.65 McFarland. By collecting colonies from a bacteria grown overnight, using a sterile cotton swab and mixing with buffer saline (zero McFarland), the bacterial mixture had blended well using a vortex, then reading the density by McFarland densitometer, the suspension density for inoculum would be 0.5-0.65 McFarland.

3.2.5. Determination of the MIC value using the Phoenix system

The Phoenix system is a quick self-contained, automated, identification, and susceptibility test for clinically relevant bacteria, designed to identify and antibiotic sensitivity test results and to evaluate the minimum antibiotic inhibitory concentration (MIC). For AST, the Phoenix panel is capable of reporting up to 20 MIC values of antibiotics, and the device records turbidity changes following bacterial growth (Carroll et al., 2006).

The Phoenix system offers timely and accurate susceptibility results for clinicians to help them in their decisions about treatment and patient care.

Preparation procedure for Phoenix panel:

1. *Klebsiella pneumoniae* colonies that grown on MacConkey agar were picked and prepared 0.5 Mcfarland suspension of bacteria in the identification (ID) broth tube, and vortex the tube. Added one drop of AST indicator solution to AST broth and had inverted the tube.
2. Using a pipet, 25 µl had transferred from ID broth to AST broth and inverted the tube to mix.
3. Each tube was poured into the appropriate side of the panel and inserted it in the instrument and had incubated it at 37 °C in the Phoenix system.
4. MIC, AST and identification results were generated after 16 hours.

3.2.6. The Procedure

3.2.6.1. Conventional Method

1. On the KPC chromagar, which is used to diagnose Gram-negative bacteria expressing decreased exposure to antibiotics from the carbapenem family, 48 rectal swab samples were cultivated in a sterilized working area and incubated the KPC chromagar plates in the incubator at 37 ° C for 24 hours. Twenty-nine positive isolates of carbapenemase producers Enterobacterales colonies were grown in Metallic blue and pink colors after incubation and were classified morphologically as *Klebsiella pneumoniae* and *E. coli*, respectively.

2. After selecting a single colony of *Klebsiella pneumoniae* from KPC chromagar by sterilized inoculating loop, and streaked on MacConkey agar, which is an indicator and selective culture medium for bacteria designed to differentiate the Gram-negative bacteria and enteric (normally located in the intestinal tract). For 24 hours the McConkey agar plates were incubated at 37 ° C. On MacConkey agar were produced a very moist and sticky, large, brilliant, and dark pink mucoid colonies of *Klebsiella pneumoniae*.
3. According to EUCAST recommendations, AST was conducted using the disk diffusion method on Mueller Hinton agar by inoculuate the plates with previously formulated bacterial broth suspension by swabbing the inoculum in three directions and placing the Ertapenem, Imipenem and Meropenem antibiotic disks on. The susceptibility test findings revealed that 27 isolates of the overall positive samples were resistant after 24 hours of incubationwhereas only one sample was more susceptible to the Ertapenem, Imipenem and Meropenem antibiotic disks.
4. The biochemical test was conducted using Citrate agar, TSI agar, and motility tests for colonies taken from MacConkey agar, and after one-day incubation at 37 ° C, 28 isolates classified as *Klebsiella pneumoniae*, and one *as E. coli*, based on variations in the bacteria's biochemical activity.

The downsides of the conventional procedure have included a delay of the final positive findings up of 96 h.

3.2.6.2. Molecular Method

Real-time PCR has been an important tool for diagnosing infectious diseases for many years. Recently, the development of fully automated systems has created walk-away analyzes that provide rapid diagnosis and save resources for personnel (Ch, 2014).

The excessive and irresponsible antibiotic use has contributed to the proliferation of carbapenemase-producing pathogenic bacterial strains. These enzymes inhibit all carbapenem antibiotics considered a real threat to public health. Therefore, there was a growing need for rapid, sensitive methods in detecting these pathogens (Hara et al., 2013).

By using the BD Max assay, which was quick, easy to use, fully automated, and has excellent performance for CPE detection directly on rectal swabs, it was really easy to detect and screen carbapenemase-producers (CP) (Girlich et al., 2020).

It is a real-time PCR test, qualitative, automatically used to identify (*bla*$_{KPC}$, *bla*$_{OXA-48}$, *bla*$_{NDM}$, *bla*$_{IMP}$/*bla*$_{VIM}$) genes which are the resistance genes developed by bacteria insensitive to carbapenem, this bacteria colonizing patients in healthcare environments (Wang, 2017).

BD MAX offers shorter turn-around and hands-on-time. One technician can process 24 samples in 30 minutes. After approximately 3 hours the results are ready (Ch, 2014).

In our study, total of 48 rectal swab specimens, 24 samples per run, were tested. The samples were prepared by inserting the rectal swab into sample buffer tube that came with the BD MAX Check-Points CPO kit and mixing well manually, then removing the rectal swab and shaking the Buffer Tube for a few seconds by a vortex. The sample buffer tube closed with a septum cap that also came with the BD MAX Check-Points CPO kite, we vortexed it again, and inserted it into the BD MAX device. The run was started after the worklist was generated and the clinical samples were loaded onto the BD MAX system along with a BD MAX Check-Points CPO reagent strip and BD MAX PCR cartridge.

The BD MAX system prepares a sample automatically utilizing real-time PCR, this involves bacterial cell lysis, DNA extraction and concentration, reagent rehydration, amplification and sequence recognition. At high-temperature enzymatic lysis occurs for bacterial cells that lead to release the nucleic acids and cached on beads with magnetical affinity, with the bound nucleic acids, the beads are washed, and hence the nucleic acids are eluted. The eluted DNA is neutralized and moved to the master mixing tube for rehydration of PCR reagents, after rehydration, the BD MAX device distributes a constant amount of PCR ready solution into the BD MAX PCR cartridge. Microvalves are located in the BD MAX PCR cartridge to maintain the amplification mix until PCR is started so as to prevent evaporation and contamination. DNA amplified targets are identified using TaqMan probes hydrolysis, that labeled on one end with a fluorescent reporter dye and on the other with a quencher moiety. To detect the *bla*$_{KPC}$, *bla*$_{OXA-48}$, *bla*$_{NDM}$, *bla*$_{IMP}$ and *bla*$_{VIM}$ and sample processing control of the BD MAX system in

separate optical channels, these probes labeled with different fluorophores. One optical channel of the BD MAX system is shared with the *bla*$_{IMP}$ and *bla*$_{VIM}$ genes, and there are separate optical channels to the other genes. Owing to its proximity to the quencher, when the probes are at their native state, the fluorescence is quenched. However, the probes hybridize in the presence of target DNA with their complementary sequences and are hydrolyzed by the DNA polymerase 5'-3' exonuclease activity as it synthesizes the nascent strand along the DNA template. As a result, the fluorophores are isolated from the quencher molecules and fluorescence emissions.

In each cycle, the BD MAX system tracks these signals and interprets the data at the end of the program to report the final results after 2,5 hours, as shown in Figure 4.2.

CHAPTER 4

RESULTS

4.1. Isolation and Identification of *Klebsiella Pneumoniae*

In this study, *Klebsiella pneumoniae* isolates were screened at Erciyes University Hospital in February 2020. A total of 48 rectal swab isolates were collected randomly from patients most of them were hospitalized. Those patients, whose *Klebsiella pneumoniae* isolates were taken from them, were aged from 25 days to 83 years old as showed in Table 4.1.

Table 4.1. Distribution of the isolates according to age groups.

Age	Number	Frequency of positive isolates (%)
0-10 years	9	3 (10.7%)
11 - 30 years	12	9 (32.1%)
31 - 50 years	10	6 (21.4%)
51 - 83 years	17	10 (36%)
Total	**48**	**28**

The morphological diagnosis of bacterial isolates performed on the KPC chromagar and MacConkey agar, showed that 29 out of 48 isolates gave positive results, only one of them were E.coli and 28 were Klebsiella pneumoniae. The distribution of these 48 isolates according to age groups is given in the table 4.1. At a high rate of 57.4%, sixteen patients over 30 years were diagnosed with *Klebsiella pneumoniae,* of which 13 patients were found to be 81.25% extremely resistant to ERT, IPM and MEM, and thus the incidence rate of *Klebsiella pneumoniae* in the most advanced age group, was higher than in other age groups.

The highest rate of positive isolates was in males (70.4%) compared to females (29.9%) and in the older age group over 30 years (57.4%) compared to those under 30 years (42.8%) based on the results obtained from KPC chromagar and MacConkey agar.

Klebsiella pneumoniae colony morphology appeared as large metallic blue colonies with a mucous texture that distinguishes it from other Enterobacterales, after growing bacterial isolates on KPC chromagar and MacConkey agar, *Klebsiella pneumoniae* colonies were mucous, very moist and sticky, large, shiny and dark pink due to fermenting lactose.

It was confirmed that 28 of the 29 positive isolates were *Klebsiella pneumoniae* after performing biochemical identification tests used in the routine laboratory for the identification of *Klebsiella pneumoniae* and the findings were shown in Table 4.2.

In the citrate test, the results were positive due to the bacterium metabolizing the citrate used as the main source of energy and the ammonium ions as the main source of energy for nitrogen, which is essential for growth. When the bacteria use citrate, it produces carbonates and bicarbonates as by-products, and the degradation of ammonium salts produces ammonia, which leads to an increase in the medium pH resulting in a color change from green to blue.

The Triple and Iron Sugar test indicated the ability of *Klebsiella pneumoniae* to ferment these sugars (lactose, glucose, and sucrose) and release hydrogen gas, which appeared as bubbles, carbon dioxide is also released but does not appear as bubbles because it is more soluble.

When the bacteria begin to ferment the sugars, they produce an acid that changes the agar color from red to yellow in all mediums from top to bottom.

In the Motility test, *Klebsiella pneumoniae* did not show any growth around the stab line.

Table 4.2. Characteristics of *Klebsiella pneumoniae* in cultural media and as a result of biochemical tests.

Bacteria	**Citrate**	**TSI**	**MIO**	**KPC chromagar**	**MacConkey**
K.pneumoniae	+	+	-	Metallic blue colony	pink mucoid colony (Larger than *E. coli*)

Antibiotic susceptibility test (AST) was applied in Muller Hinton agar medium to isolates determined to be *Klebsiella pneumoniae* as a result of culture media and biochemical tests.

4.2. Determination of the antibiotic resistance of *Klebsiella pneumoniae*

AST performed on the Mueller Hinton agar medium by disk diffusion method, which is commonly used in routine laboratories for this purpose. The results showed that all *Klebsiella pneumoniae* isolates had similar resistance to Ertapenem, Imipenem and Meropenem antibiotic discs. It was determined that 27 (96.6%) of 28 *Klebsiella pneumoniae* strains were resistant to meropenem, Imipenem and ertapenem, Table 4.3.

Table 4.3. The resistance and sensitivity percentage of the *Klebsiella pneumoniae* strains for Ertapenem, Imipenem and Meropenem antibiotics.

Antibiotics	**Number of sensitive *K. pneumonia***	**Number of resistant *K. pneumonia***
ERT, IMP and MEM	1 (3.4%)	27 (96.6%)

Taking into consideration the medical history of patients who have been confirmed to infected with *Klebsiella pneumoniae*, it was found that 12 out of 28 had problems with the respiratory system, lung disease, and respiratory failure, which may be due to the development of *Klebsiella pneumoniae* infection. Of the 28 positive results, 10 patients who had a history of frequent infection with *Klebsiella pneumoniae*, where the AST test performed using Phoenix, exhibited that the *Klebsiella pneumoniae* strains that colonized them were resistant to 19 antibiotics, including three of the carbapenem antibiotics Ertapenem, Imipenem, Meropenem, and Imipenem as shown in Table 4.4.

This high resistance to antibiotics due to carbapenemase genes ($bla_{\text{OXA-48}}$ =6, $bla_{\text{OXA-48}}$+ bla_{NDM}=3, $bla_{\text{OXA-48}}$+ bla_{VIM} / bla_{IMP}=1) that have been detected in those 10 strains, or maybe due to ampC hyper-production or another enhanced-spectrum beta-lactamase. The results showed that *Klebsiella pneumoniae* was MDR at a very high rate.

4.3. MIC determination

The Minimum inhibition concentrations were determined using the Phoenix system for 28 *Klebsiella pneumoniae* isolates, where 27 were resistant to ERT, IPM and MEM antibiotics and only one strain was sensitive.

Table 4.4. The MIC value of ERT, IPM and MEM antibiotics of 28 *Klebsiella pneumoniae* strains.

Antibiotics	**Symbol**	**MIC**	
		R	**S**
Ertapenem	ERT	>1	<=0.25
Meropenem	MEM	>8	<=0.125
Imipenem	IPM	4-8	0.5

Of the 28 *Klebsiella pneumoniae* strains, 27 strains were found to be resistant, including10 of them found to be resistant to 19 antimicrobials including Ertapenem, Meropenem and Imipenem, which belong to carbapenems, these 10 isolates were from patients who had repeated infection with *Klebsiella pneumoniae*. These 19 antimicrobials shown in Table 4.5.

Table 4.5. MIC values and AST results of 10 *K. pneumonia* isolates determined by Phoenix system

No	ERT	MEM	IPM	AMK	AMC	AMP	SAM	CFZ	FEP	CAZ	C/T	CRO	CTX	CIP	CST	GEN	LVX	TZP	SXT
1.	>1, R	>8, R	8, R	>32, R	>16/2, R	>16, R	>8/8, R	>32, R	>8, R	>8, R	>4/4, R	>4, R	>16, R	>1, R	>4, R	>8, R	>2, R	>16/4, R	>8/152, R
2.	>1, R	>8, R	4, R	>32, R	>16/2, R	>16, R	>8/8, R	>32, R	>8, R	>8, R	>4/4, R	>4, R	>16, R	>1, R	>4, R	>8, R	>2, R	>16/4, R	>8/152, R
3.	>1, R	>8, R	8, R	>32, R	>16/2, R	>16, R	>8/8, R	>32, R	>8, R	>8, R	>4/4, R	>4, R	>16, R	>1, R	>4, R	>8, R	>2, R	>16/4, R	>8/152, R
4.	>1, R	>8, R	8, R	>32, R	>16/2, R	>16, R	>8/8, R	>32, R	>8, R	>8, R	>4/4, R	>4, R	>16, R	>1, R	>4, R	>8, R	>2, R	>16/4, R	>8/152, R
5.	>1, R	>8, R	>8, R	>32, R	>16/2, R	>16, R	>8/8, R	>32, R	>8, R	>8, R	>4/4, R	>4, R	>16, R	>1, R	<=1,X	>8, R	>2, R	>16/4, R	>8/152, R
6.	>1, R	>8, R	8, R	>32, R	>16/2, R	>16, R	>8/8, R	>32, R	>8, R	>8, R	>4/4, R	>4, R	>16, R	>1, R	2, X	>8, R	>2, R	>16/4, R	>8/152, R
7.	>1, R	>8, R	8, R	>32, R	>16/2, R	>16, R	>8/8, R	>32, R	>8, R	>8, R	>4/4, R	>4, R	>16, R	>1, R	<=1,X	>8, R	>2, R	>16/4, R	>8/152, R
8.	>1, R	>8, R	>8, R	>32, R	>16/2, R	>16, R	>8/8, R	>32, R	>8, R	>8, R	>4/4, R	>4, R	>16, R	>1, R	<=1,X	>8, R	>2, R	>16/4, R	>8/152, R
9.	>1, R	>8, R	8, R	>32, R	>16/2, R	>16, R	>8/8, R	>32, R	>8, R	>8, R	>4/4, R	>4, R	>16, R	>1, R	>4, R	>8, R	>2, R	>16/4, R	>8/152, R
10.	>1, R	>8, R	>8, R	>32, R	>16/2, R	>16, R	>8/8, R	>32, R	>8, R	>8, R	>4/4, R	>4, R	>16, R	>1, R	<=1,X	>8, R	>2, R	>16/4, R	>8/152, R

*Abbreviations, ERT: Ertapenem, MEM: Meropenem, IPM: Imipenem, AMK: Amikacin, AMC: Amoxicillin-Clavulanate (f), AMP: Ampicillin, SAM: Ampicillin-Sulbactam (f), CFZ: Cefazolin, FEP: Cefepime, CAZ: Ceftazidime, C/T: Ceftolozane-Tazobactam, CRO:ceftriaxone, CTX:cefotaxime, CIP: ciprofloxacin, CST: colistin, GEN: gentamicin, LVX: levofloxacin, TZP: Tazobactam , SXT: Trimethoprim-Sulfamethoxazole, I: intermediate, R: resistance.

4.4. BD MAX Assay Results

As mentioned before, one of the rectal isolates from 48 patients was used in culture media and biochemical tests, while the other was used in the BD MAX assay. Conventional culture methods and BD MAX results confirmed each other. According to these results, it was determined that a total of 29 isolates gave positive results and 28 of them were *Klebsiella pneumoniae*. The presence of carbapenem-resistant genes has been confirmed in *Klebsiella pneumoniae* isolates with more than one gene appearing in the same strain after the use of CPO checkpoint assay kite in BD MAX system, which is qualitative, fully automated in vitro diagnostic real-time PCR system (Becton Dickinson and Company, USA). The carbapenem-resistant genes of *Klebsiella pneumoniae* were screened by BD MAX CPO assay analysis and the results obtained are given in Table 4.6.

Table 4.6. Determined carbapenemase gene combination of *Klebsiella pneumoniae* isolates with the BD MAX checkpoint CPO assay.

BD MAX	**Positive Strain Number (%)**	
	Klebsiella pneumoniae	***E. coli***
bla_{OXA}	17 (58.62%)	1 (3.45%)
bla_{NDM}	1 (3.45%)	0
bla_{OXA}+bla_{NDM}	4 (13.79%)	0
bla_{OXA}+bla_{NDM}+bla_{VIM}/bla_{IMP}	3 (10.34%)	0
bla_{OXA}+bla_{VIM}/bla_{IMP}	3 (10.34%)	0
bla_{KPC}	0	0
Total Positive	29 (60.4%)	
Total Negative	19 (39.6%)	
Total isolates number	**48**	

As can be seen from the table, it was determined that 58.62% of the isolates (17) only carried the bla_{OXA-48} gene. This result confirms the findings given in other studies. Because the bla_{OXA-48} gene has been determined to be the most common carbapenem resistance gene in *Klebsiella pneumoniae* isolates obtained from Turkey, the Middle-East, North Africa and Europe (Ma et al., 2015). It was observed that none of the

isolates tested carries the *bla*$_{KPC}$ gene. The carbapenem resistance gene combinations carried by the isolates are given in Table 4.6.

As mentioned previously, *Klebsiella pneumoniae* is transmitted among hospitalized patients, which was reported in this study, it was diagnosed the combination genes in the same strain in 10 (34.47%) isolates of patients of different ages, distributed as follows: [***bla*$_{OXA}$+*bla*$_{NDM}$+*bla*$_{VIM}$/*bla*$_{IMP}$**] in three children patients (10.34%) were hospitalized in the pediatric intensive care unit, [***bla*$_{OXA}$+*bla*$_{NDM}$**] in four patients (13.79%) were hospitalized in the neurosurgery unit, and [***bla*$_{OXA}$+*bla*$_{VIM}$/*bla*$_{IMP}$**] in three patients (10.34%) were hospitalized in the intensive care unit.

Intensive care units have seen the highest incidence of *Klebsiella pneumoniae* infections among patients there with a relatively high ratio of 32.14%, followed by neurosurgery unit with rate reached to 28.57%, pediatric intensive care unit, anesthesia unit, and hematology unit with rate 10.71%, while general surgery unit with rate 7.14%, as the Table 4.7 showing.

Table 4.7. Indicates the prevalence of *Klebsiella pneumoniae* strains in the Erciyes University Hospital health care units.

Hospital units	**Number of isolates**	**Percentage %**
Intensive care unit	9	32.14%
Neurosurgery unit	8	28.57%
Pediatric intensive care unit	3	10.71%
Anesthesia unit	3	10.71%
Hematology	3	10.71%
General surgery unit	2	7.14%
Total	**28**	**100%**

Table 4.8. All positive results of AST (by disk diffusion method) and MIC values (by Phenix system) of the Carbapenems ERT, IPM and MEM, and Carbapenemase genes detected in *Klebsiella pneumoniae* strains after screened by the BD MAX assay.

NO.	Gender /Age	Genotype	AST / MIC (µ g / ml)		
			ERT	IPM	MEM
1.	M/24	bla_{OXA}	R, >1	R, 8	R, >8
2.	F/66	bla_{OXA}	R, >1	R, 8	R, >8
3.	M/11	bla_{OXA} + bla_{NDM} + bla_{VIM}/bla_{IMP}	R, >1	R, 8	R, >8
4.	M/21	bla_{OXA}	R, >1	R, 8	R, >8
5.	M/3	bla_{OXA}	R, >1	R, >8	R, >8
6.	F/75	bla_{OXA} + bla_{VIM}/bla_{IMP}	R, >1	R, 8	R, >8
7.	M/46	bla_{OXA}	R, >1	R, 8	R, >8
8.	M/53	bla_{OXA}	R, >1	R, 8	R, >8
9.	M/72	bla_{OXA}	R, >1	R, 8	R, >8
10.	M/68	bla_{OXA}	R, >1	R, 8	R, >8
11.	F/3	bla_{OXA} + bla_{NDM} + bla_{VIM}/bla_{IMP}	R, >1	R, 8	R, >8
12.	M/16	bla_{OXA}	R, >1	R, 8	R, >8
13.	M/83	bla_{OXA}	R, >1	R, 8	R, >8
14.	F/48	bla_{OXA}	R, >1	R, 8	R, >8
15.	M/18	bla_{OXA} + bla_{VIM}/bla_{IMP}	R, >1	R, 4	R, >8
16.	F/41	bla_{NDM}	R, >1	R, 8	R, >8
17.	M/46	bla_{OXA} + bla_{NDM}	R, >1	R, >8	R, >8
18.	F/15	bla_{OXA}	R, >1	R, >8	R, >8
19.	F/61	bla_{OXA}	R, >1	R, 8	R, >8
20.	M/25 day	bla_{OXA}	R, >1	R, 8	R, >8
21.	M/315 day	bla_{OXA} + bla_{NDM} + bla_{VIM}/bla_{IMP}	R, >1	R, 8	R, >8
22.	M/25	bla_{OXA}	R, >1	R, 8	R, >8
23.	M/75	bla_{OXA}	R, >1	R, 8	R, >8
24.	F/29	bla_{OXA} + bla_{NDM}	R, >1	R, 8	R, >8
25.	M/50	bla_{OXA} + bla_{VIM}/bla_{IMP}	S, <=0.25	S, 0.5	S, <=0.125
26.	M/58	bla_{OXA} + bla_{NDM}	R, >1	R, 8	R, >8
27.	F/25	bla_{OXA} + bla_{NDM}	R, >1	R, 8	R, >8
28.	M/81	bla_{OXA}	R, >1	R, 8	R, >8

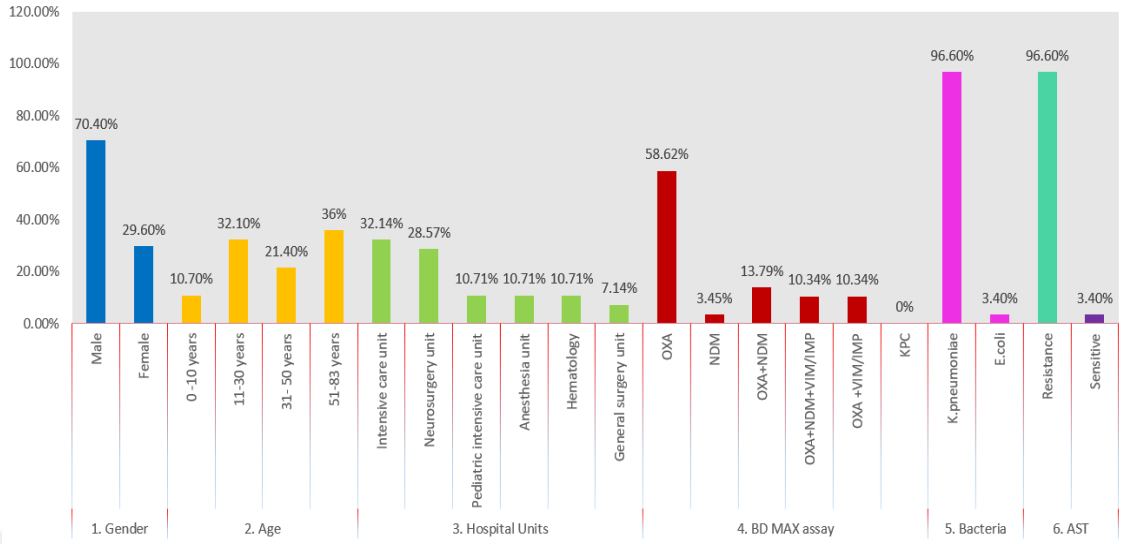
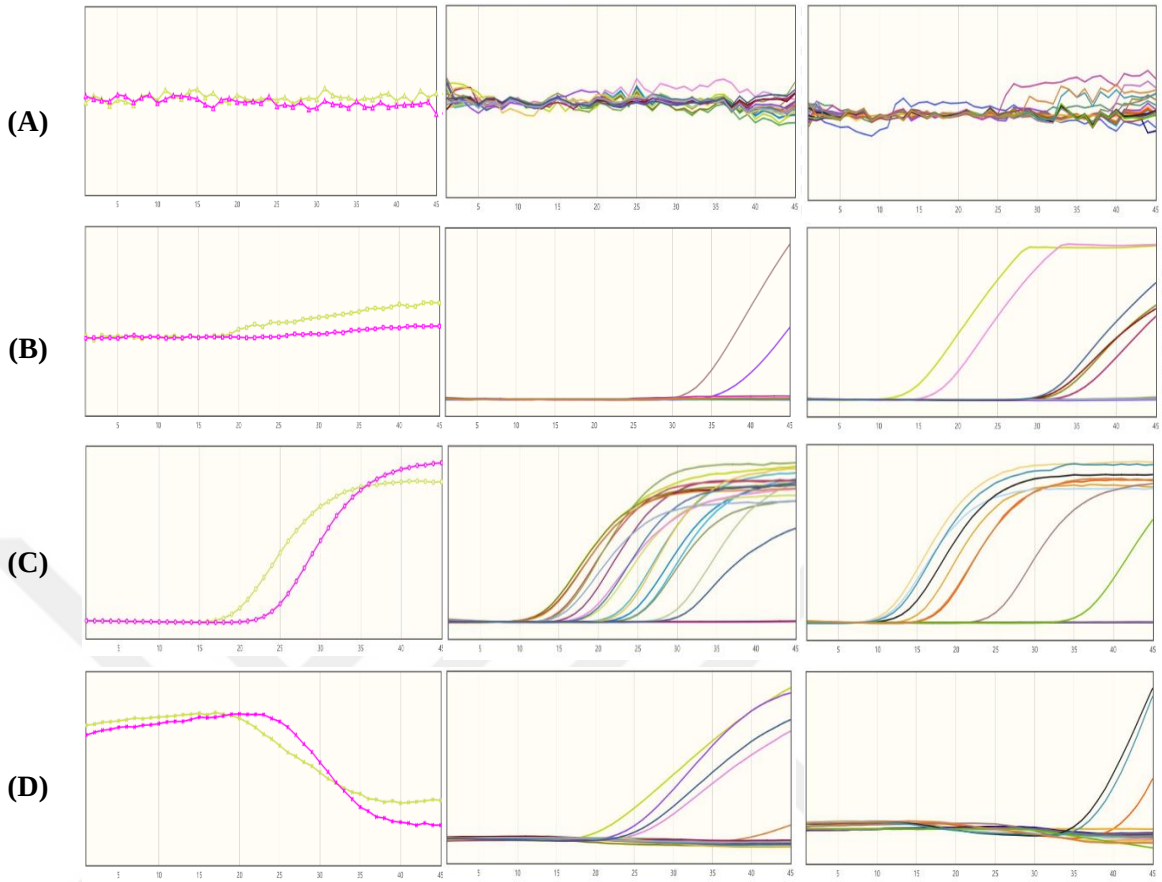


Figure 4.1. Summarizes all the findings of the current study

As can be seen from Figure 4.1, specimens taken from male patients were the majority of isolates which gave positive results. In addition, from male patients, 13 of the 17 isolates carried only *bla*$_{\text{OXA-48}}$ gene were obtained. Examining the age distribution of the patients from whom the isolates were taken, it was observed that a higher rate of positive isolates was obtained than the patients over 30 years of age compared to the younger ones.

As a result, the distribution of isolates with positive results during this study according to characteristics such as the patient's identity, age, hospital departments, carbapenem resistance gene profiles, types of bacteria and carbapenem resistance are summarized in the above Figure 4.1. Accordingly, the highest rate of positive isolates (70.40%) were obtained from male individuals. It was understood that most of the isolates giving positive results were obtained from patients over 30 years old (57.4%). It has been determined that 58.62% of the isolates only carry the *bla*$_{\text{OXA-48}}$ gene, and it has been found that this gene is very common in isolates. Again, it was observed that the highest rate of isolates was isolated from samples taken from the intensive care unit. The *bla*$_{\text{KPC}}$ gene was not observed in any of the isolates. *Klebsiella pneumoniae* constituted 96.6% of the bacteria isolates and it was determined that only one isolate was *E. coli*. More than 96% of the isolates obtained were resistant to carbapenems.



(A) Detection of *bla*$_{KPC}$ at 475/520 nm, (B) Detection of *bla*$_{VIM}$/ *bla*$_{IMP}$ at 530/565 nm, (C) Detection of *bla*$_{OXA}$ at 585/630 nm,
(D) Detection of *bla*$_{NDM}$ at 630/665 nm.

Figure 4.2. Detection findings of the five Carbapenemase genes in 48 rectal swab isolate obtained by the BD MAX Checkpoint CPO assay.

The BD MAX assay is a qualitative diagnostic test for the rapid detection of carbapenemase genes in Enterobacterales and other organisms as previously described. It gives no quantitative value, such as the DNA concentration or any information about the number of bacteria species present. BD MAX checkpoint CPO utilizes different fluorescent probes that present in each optical channel, it is possible to detect and to distinguish the five carbapenemase genes. At a certain wavelength, each carbapenemase gene is detected. Whereas, the *bla*$_{KPC}$ gene was detected at 475/520 nm as indicated by Figure 4.2. (A), as no amplification curves were shown due to the absence of the *bla*$_{KPC}$ gene in all 48 clinical isolates, (B) shows the detection of *bla*$_{VIM}$/ *bla*$_{IMP}$ at 530/565 nm in 6 clinical isolates, (C) Detection of *bla*$_{OXA-48}$ at 585/630 nm in 28 clinical isolates, (D) Detection of *bla*$_{NDM}$ at 630/665 nm in 8 clinical isolates. The threshold cycle (Ct) value of the genes for 29 positive isolates are given in Table 4.9 below.

Table 4.9. Ct value of Carbapenemase genes for 29 positive isolates obtained by BD MAX Checkpoint CPO assay.

NO.	Ct 475/520 nm *bla*$_{KPC}$	Ct 530/565 nm *bla*$_{VIM}$ *bla*$_{IMP}$	Ct 585/630 nm *bla*$_{OXA}$	Ct 630/665 nm *bla*$_{NDM}$
1	-1.0	-1.0	19.7	-1.0
2	-1.0	-1.0	24.2	-1.0
3	-1.0	14.6	13.3	19.4
4	-1.0	-1.0	25.6	-1.0
5	-1.0	-1.0	25.8	-1.0
6	-1.0	-1.0	24.5	-1.0
7	-1.0	33.9	13.2	-1.0
8	-1.0	-1.0	22.7	-1.0
9	-1.0	-1.0	17.6	-1.0
10	-1.0	-1.0	20.0	-1.0
11	-1.0	18.1	19.1	25.2
12	-1.0	-1.0	15.2	-1.0
13	-1.0	-1.0	15.5	-1.0
14	-1.0	-1.0	29.8	-1.0
15	-1.0	33.3	13.7	-1.0
16	-1.0	-1.0	13.8	41.3
17	-1.0	-1.0	23.2	-1.0
18	-1.0	-1.0	19.6	-1.0
19	-1.0	-1.0	16.0	-1.0
20	-1.0	32.5	31.5	24.2
21	-1.0	-1.0	15.8	-1.0
22	-1.0	-1.0	11.4	-1.0
23	-1.0	-1.0	18.0	41.9
24	-1.0	-1.0	12.0	-1.0
25	-1.0	33.9	25.4	-1.0
26	-1.0	-1.0	13.9	36.5
27	-1.0	-1.0	12.4	37.5
28	-1.0	-1.0	17.8	-1.0
29	-1.0	-1.0	-1.0	22.8

*__Ct:__ Cycle threshold, __-1.0__ : Negative

To investigate the accuracy of the conventional and molecular methods used in this study for the detection of carbapenems resistance, the BD MAX checkpoint assay was compared with AST (using disk diffusion method) to assess the accuracy, sensitivity, specificity, PPV, and NPV for each detection method, as indicated in Table 4.10, Figure 4.3.

Table 4.10. Criterion values and coordinates of the ROC curve of BD MAX checkpoint CPO assay and AST (disk diffusion method).

Test	Criterion	Sensitivity% (95%CI)	Specificity% (95%CI)	PPV	NPV	Accuracy	AUC	SE [a]
BD MAX assay	>0	90.62 (75.0 - 98)	100 (79.4 - 100)	100.0	84.2	0.9063	0.953	0.0313
AST	>0	84.37 (67.2 - 94.7)	100 (79.4 - 100)	100.0	76.2	0.8438	0.922	0.0401

***AUC**: Area Under the Curve, **PPV**: Positive Predictive Value, **NPV**: Negative Predictive Value, **SE** [a]: Standard Error

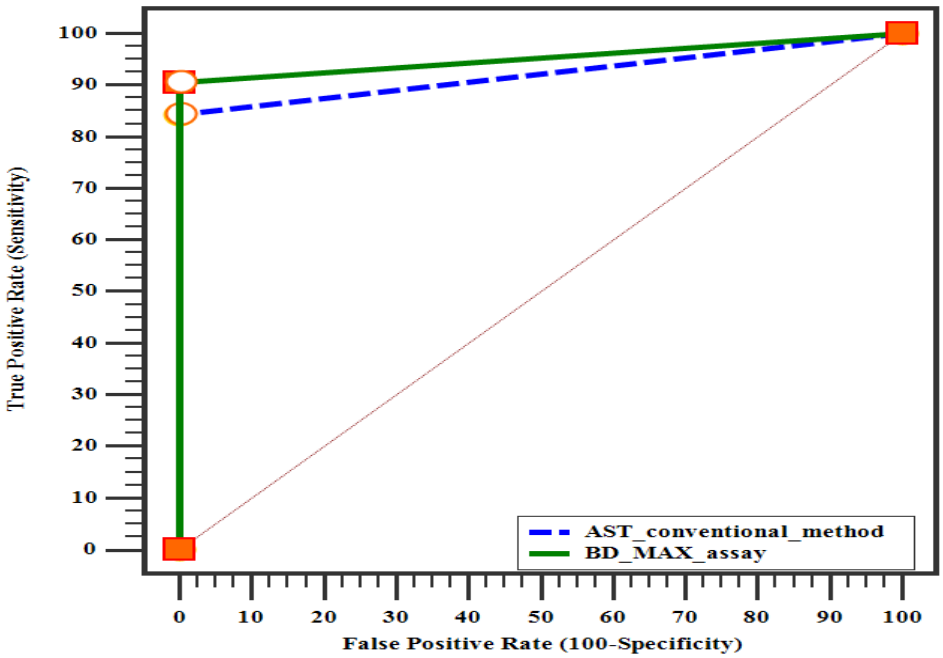


Figure 4.3. Comparison of BD MAX checkpoint CPO assay and AST (disk diffusion method) by ROC curve.

CHAPTER 5

DISCUSSION

The antimicrobial resistance is a dangerous developing problem of public health. This increase in bacterial immunity to antibiotics has evolved with the application of these antibiotics in patients treatments (Rahman et al., 2018).

Today, *Klebsiella pneumoniae* resistance to antibiotics is among the most serious threats to human health for its high resistance against most alternative antibiotics (Li et al., 2019). It represents a serious and critical problem worldwide due to the overuse and misuse of antibiotics (Effah et al., 2020).

The mingling of individuals colonized with carbapenemase-producing bacteria with other patients in the healthcare units and the rapid spread of the resistance strain among them shows the importance of using rapid tests in the detection of colonized patients in terms of infection control (Lin et al., 2013). The identification of carbapenem-resistant Enterobacterales is usually provided through diagnostic tests (genotype or phenotype tests) further to the sensitivity testing of isolates produced in culture, this method needs about 2-5 days for proven results. And because of the critical relationship between identifying carbapenemase-producing or carbapenem-resistant isolates and timely initiation of effective antibiotic therapy and preventing outbreaks, these isolates should be rapidly identified (EUCAST, 2015).

The BD MAX CPO assay, whose effectiveness was investigated in our study, is a real-time PCR kit that can be used with the BD MAX system. Molecular-based tests have significant advantages in detecting carbapenemase-producing bacteria over phenotypical tests. The specificity and sensitivity of these tests are quite high and, as with the BD MAX system, carbapenemase genes can be detected directly from the clinical specimens (Banerjee and Humphries, 2017).

The number of Enterobacterales isolates that come from the Erciyes University Hospital to the Central Microbiology Laboratory is about 500 rectal swabs per week, about 70 % of which are diagnosed as *Klebsiella pneumoniae*. This condition is primarily the result of pervasive, unreasonable antibiotic usage, bacterial strain virulence, and their potential to survive in the hospital setting (Skowska et al., 2014). In our research, we focused on the *Klebsiella pneumoniae* strains and their resistance to the Ertapenem, Imipenem and Meropenem antibiotics. Because this bacteria considered the main cause of nosocomial infections among hospitalized patients, such as UTI, respiratory tract infections, sepsis, and bloodstream infections (Li et al., 2014; Paczosa and Mecsas, 2016).

In the current study, 60.4 % (n = 29) of clinical specimens in the BD MAX assay were positive. The results of the biochemical tests showed that *Klebsiella pneumoniae* was 96.6% of all positive samples. In Erciyes University Hospital, *Klebsiella pneumoniae* strains showed the same high resistance to Ertapenem, Imipenem and Meropenem reached 96.6%. This was confirmed by (García-castillo et al., 2017) which reported similar resistance to Ertapenem, Imipenem and Meropenem.

A *Klebsiella pneumoniae* isolate which shows relatively high-level resistance to imipenem and meropenem as mentioned in (Yigit et al., 2001).A study in Israel in which *Klebsiella pneumoniae* demonstrated resistance to both ertapenem and meropenem (Raveh et al., 2007). In contrast, according to (Shah and Isaacs, 2003) and (García-Fernández et al., 2010) their study revealed *Klebsiella pneumoniae* resistance to ertapenem and its sensitivity to meropenem. But, on the other hand, (Jacoby et al., 2004; Landman et al., 2009; Leavitt et al., 2009; Orsi et al., 2011) revealed *Klebsiella pneumoniae* strains were resistant to ertapenem in their studies. While *Klebsiella pneumoniae* strains showed resistance to meropenem in other studies (Machuca et al., 2017; Shields et al., 2017; Tenover et al., 2006).

Twenty-eight of *Klebsiella pneumoniae* strains that analyzed via BD MAX checkpoint CPO assay, produced the carbapenemases genes. Where BD MAX CPO assay was applied on 48 rectal swab isolates to identify and detect the five carbapenemase genes $bla_{\text{OXA-48}}$, bla_{NDM}, bla_{KPC}, bla_{IMP} and bla_{VIM} and the outcomes were $bla_{\text{OXA-48}}$ alone in 58.62% (n=17) and in 3.45% (n=1) only the bla_{NDM} gene was identified. Gene combination appeared in 34.47% (n=10) distributed as follows: $bla_{\text{OXA-48}}$ + bla_{NDM} in

13.79% (n=4), *bla*$_{\text{OXA-48}}$ + *bla*$_{\text{IMP}}$ and *bla*$_{\text{VIM}}$ in 10.34% (n=3), *bla*$_{\text{OXA-48}}$ + *bla*$_{\text{NDM}}$ + *bla*$_{\text{IMP}}$ and *bla*$_{\text{VIM}}$ in 10.34% (n=3), and no *bla*$_{\text{KPC}}$ gene was observed in either of the isolates screened for resistance genes. Carbapenemase genes were reported in another study had distributed as *bla*$_{\text{KPC}}$ (72.58%), *bla*$_{\text{NDM}}$ (19.35%), *bla*$_{\text{OXA-48}}$, and *bla*$_{\text{OXA-48}}$ + *bla*$_{\text{NDM}}$ (3.2%), with non-identified *bla*$_{\text{IMP}}$ and *bla*$_{\text{VIM}}$ genes (Chung and Lee, 2020).

Also, a study in Antalya, Turkey, recorded that the most common carbapenemase gene was *bla*$_{\text{OXA-48}}$ (69.7 %), followed by *bla*$_{\text{VIM}}$,, *bla*$_{\text{NDM}}$ and *bla*$_{\text{IMP}}$, while no isolates examined were *bla*$_{\text{KPC}}$ positive (Su et al., 2020). Another research on Klebsiella spp isolates from 18 hospitals in 13 cities in Turkey verified that 104 harbored *bla*$_{\text{OXA-48}}$ isolates contained both *bla*$_{\text{OXA-48}}$ and *bla*$_{\text{NDM}}$; three had both *bla*$_{\text{OXA-48}}$ and *bla*$_{\text{VIM}}$ and nine isolates had *bla*$_{\text{NDM}}$ alone, four isolates contained only *bla*$_{\text{VIM}}$, and two isolates contained *bla*$_{\text{IMP}}$ alone. One isolated *bla*$_{\text{VIM}}$ and *bla*$_{\text{NDM}}$ co-harbored (Demirci-Duarte et al., 2020).

In Erciyes University Hospital, the high resistance of *Klebsiella pneumoniae* strains to ertapenem and meropenem is mainly due to the *bla*$_{\text{OXA-48}}$ gene, where appeared in 27 out of 28 positive *Klebsiella pneumoniae* isolates. Our outcome confirms the findings of other studies. Since the *bla*$_{\text{OXA-48}}$ gene is the most common carbapenem resistance gene in *Klebsiella pneumoniae* isolates obtained from Turkey, the Middle East, North Africa, and Europe (Ma et al., 2015). The *bla*$_{\text{KPC}}$ genes were not diagnosed in our study, also in (Ciftci et al., 2019) it has not been reported. Conversely, according to (Labarca et al., 2014) it was revealed in their studies in Turkey. The absence of the *bla*$_{\text{KPC}}$ gene may be attributed to the fact that the application of preventive measures in hospitals was successful, or a mutation occurs that led to the inactivation of this gene, and it was transmitted between *Klebsiella pneumoniae* strains via plasmids, or a change in the properties of bacteria during their spread and replication (Chen et al., 2014).

The appearance of the *bla*$_{\text{NDM}}$ gene alone (3.45%) in our study is attributed to the same reasons mentioned above. but it was appeared in (29.8%) in the study of (Ciftci et al., 2019) where was responsible for the high resistance to carbapenem along with *bla*$_{\text{OXA-48}}$ in Suleyman Demirel University Hospital in recent years. And because of its epidemic potential, the appearance of *bla*$_{\text{NDM}}$ at 3.45% in Erciyes University Hospital is proof of the effectiveness of preventive measures to reduce its spread among patients.

The CPO checkpoint assay reported excellent sensitivity and specificity as 90.62% and 100% respectively, for the detection of the carbapenemase genes, PPV (100%), and NPV (84.2%) for the predictive values and Accuracy (90.63%) in our results. Other studies reported sensitivity and specificity values close to ours, where in the (Girlich et al., 2020) study, the CPO checkpoint assay of BD MAX system showed high sensitivity (92.8%) and specificity (97.8%). Also in (García-castillo et al., 2017) study the sensitivity (96.3%) and specificity (96.9%) in the same assay. Compared to (Simner et al., 2016) study that used the BD MAX CPE assay for the detection of carbapenemase genes from Enterobacteriaceae, these values were: sensitivity (100%), specificity (97.6%), PPV (50%), and NPV (100%).

Whereas the overall sensitivity, specificity, PPV and NPV were (100%), (96.7%), (53.6%), and (100%) respectively, according to the Xpert Carba-R assay used in the (Ko et al., 2019) study. The BD Phoenix CPO test in (Simon et al., 2019) study showed an overall sensitivity of (89.7%) and specificity of (83.5%) for carbapenemase detection, the PPA, and NPA were (98.5%) and (97.2%) respectively. Due to the lack of references, there are limited data on the screening of BD MAX checkpoint CPO assay, especially in relation to the Turkish area, and that explains why we compared our findings with other studies that used different molecular methods to detect carbapenemase genes in carbapenemase-producing organisms CPO.

The most commonly isolated bacteria from patients in the intensive care unit are *Klebsiella pneumoniae* (Skowska et al., 2014). Where our findings suggest that patients in the intensive care unit are a major reservoir of Carbapenemase-producing *Klebsiella pneumoniae*. Where it formed the prevalence rate in intensive care unit 32.14% (n=9), followed by neurosurgery unit 28.57% (n=8), those patients have the potential to spread the multidrug-resistant organisms. Similar results were confirmed by (Abdallah et al., 2018; Lin et al., 2013; Ripabelli et al., 2018; Skowska et al., 2014) in their studies which indicated that the high prevalence of *Klebsiella pneumoniae* among patients in intensive care unit. Places responsible for the spread of *Klebsiella pneumoniae* carbapenem resistant involve suction tubing, respirator tubes, patient tables, and beds (Abdallah et al., 2018).

The purpose of the study was to evaluation of BD MAX real-time PCR assay for screening Carbapenem-Resistant *Klebsiella pneumoniae* from rectal swabs, where the detection and identification of the Carbapenemase genes (*bla*$_{\text{OXA-48}}$, *bla*$_{\text{NDM}}$, *bla*$_{\text{IMP}}$ and *bla*$_{\text{VIM}}$) were successful, super easy and the most important thing is the speed in diagnosis, the results were obtained after 2.5-3 hours. Where the faster the diagnosis, the greater the chance of controlling the spread of resistant strains to antibiotics, the application of accurate treatment as soon as possible and under medical supervision or at least the presence of full awareness of the patient regarding the use of antibiotics wisely, works to treat the infections and kill the bacteria and disrupting their ability to develop antibiotics resistance mechanisms, and prevent the spread of more virulent strains.

The most important advantage of the BD MAX CPO system is fast results and low workload, also, the BD MAX system was efficient, flexible, and scalable. In this study, the system also allows for culturing using the same swabs used in BD MAX assay (Sarı et al., 2016) confirmed this in their study. Thus, isolates can be obtained from samples that are positive in screening by conducting culture methods and hence allowing the identified carbapenem-resistant gram-negative bacteria to be stored for further studies.

Although there are positives for the BD MAX system, there were some negatives that could not be overlooked. The major disadvantage of BD MAX CPO is its cost, this disadvantage is found in most molecular-based rapid tests. Again, as in other PCR - based tests, determining only the targeted carbapenemase genes in the system requires caution in order not to overlook new carbapenemase genes. Not only that, but also, it could not be distinguished between *bla*$_{\text{IMP}}$ and *bla*$_{\text{VIM}}$ genes, because they were shared the same optical channel. Moreover, a false-negative result may occur due to the loss of nucleic acid from the insufficient collection, transport, or storage of specimens, or due to inadequate bacterial cell lysis.

This test is qualitative and does not has quantitative values, nor does it show the quantity of bacteria species present.

The BD MAX system demonstrated accuracy and effectiveness in diagnosing and detecting carbapenem resistance genes, and the most important thing is the speed at which results are obtained. Negative findings do not mean the BD MAX system's

absence of accuracy, but may indicate the existence of resistance mechanisms other than carbapenemase genes.

Through the results obtained in this study, we recommend using the BD MAX test as a diagnostic method in routine laboratories for the detection of carbapenem resistance, and if negative results occur, by using the AST (disk diffusion method that already used in routine labroutary) can be re-examined to determine that there are carbapenem resistance strains or not, this process reduces the time from 4-5 days to under 3 hours. Where AST is no less efficient than the BD MAX system, as the sensitivity, specificity and accuracy reached 84.37%, 100%, 84.38% respectively.

However, (García-castillo et al., 2017; Girlich et al., 2020; Hofmann-Thiel et al., 2020) confirmed, as we have done, the efficiency and accuracy of the BD MAX assay that have used to detect carbapenem genes.

In conclusion, the development of carbapenem resistance mechanisms and the lack of antimicrobials against *Klebsiella pneumoniae* is a significant treatment problem for clinicians and requires an accurate cure. Considering to our findings, While the routine cultivation method is easy to use, but it will take a long time almost 96 hours to obtain certain positive results, on the other hand, our findings indicate that BD MAX CPO assay is appropriate for use, it enables rapid detection of carbapenemase genes, with high accuracy and in a short time almost 2.5-3 hours, which helps to reduce the spread of antibiotic resistance through rapid diagnosis and detection. Hence, help the doctors treating the infection successfully with the correct antibiotics, but on the other hand, it cannot assist the routine laboratory to determine other mechanisms of antibiotic resistance. In addition, more studies are needed to evaluate BD MAX assay, involving a significant number of *Klebsiella pneumoniae* isolates to study carbapenem resistance in different areas.

Hope the findings of this study will be useful in applying an efficient approach to managing infectious diseases to prevent and reduce the prevalence of carbapenemase-producing by *Klebsiella pneumoniae*.

REFERENCES

Abdallah, S.A., Zaki, S.S., Hafez, S.S., Moustafa, E.E., 2018. Prevalence rate of Klebsiella pneumoniae in the intensive care unit: Epidemiology and molecular characteristics. **J. Biol. Res. 91**, 47–52. 10.4081/jbr.2018.7437

Al-Zahrani, I.A., 2018. Routine detection of carbapenem-resistant gram-negative bacilli in clinical laboratories.: A review of current challenges. **J. Saudi Med. J. 39**, 861–872. 10.15537/smj.2018.9.22840

Altmann, G., Eisenberg, E., Bogokowsky, B., 1979. Radiation sterilization of triple sugar iron agar. **Int. J. Appl. Radiat. Isot. 30**, 527–529. 10.1016/0020-708X(79)90165-0

Amann, S., Neef, K., Kohl, S., 2019. Antimicrobial resistance (AMR). **Eur. J. Hosp. Pharm. 26**, 175–177. 10.1136/ejhpharm-2018-001820

Aminov, R.I., 2009. The role of antibiotics and antibiotic resistance in nature. **Environ. Microbiol. 11**, 2970–2988. 10.1111/j.1462-2920.2009.01972.x

Antunes, N.T., Fisher, J.F., 2014. Acquired class D β-Lactamases. **J. Antibiot. (Tokyo). 3**, 398–434. 10.3390/antibiotics3030398

Banerjee, R., Humphries, R., 2017. Clinical and laboratory considerations for the rapid detection of carbapenem-resistant Enterobacteriaceae. **Virulence 8**, 427–439. 10.1080/21505594.2016.1185577

Bd, C., 2003. BD Triple Sugar Iron Agar (TSI Agar) Intended.

Bengoechea, J.A., Sa Pessoa, J., 2019. Klebsiella pneumoniae infection biology: Living to counteract host defences. **J. FEMS Microbiol. Rev. 43**, 123–144. 10.1093/femsre/fuy043

Blassmann, U., Roehr, A.C., Frey, O.R., Vetter-Kerkhoff, C., Thon, N., Hope, W., Briegel, J., Huge, V., 2016. Cerebrospinal fluid penetration of meropenem in neurocritical care patients with proven or suspected ventriculitis: A prospective observational study. **Crit. Care 20**, 1–9. 10.1186/s13054-016-1523-y

Buckley, M.M., Brogden, R.N., Barradell, L.B., Goa, K.L., 1992. Imipenem/cilastatin. A reappraisal of its antibacterial activity, pharmacokinetic properties and therapeutic efficacy. **Drugs 44**, 408—444. 10.2165/00003495-199244030-00008

Cabral, J.P.S., 2010. Water microbiology. Bacterial pathogens and water. **Int. J. Environ. Res. Public Health 7**, 3657–3703. 10.3390/ijerph7103657

Cantón, R., Akóva, M., Carmeli, Y., Giske, C.G., Glupczynski, Y., Gniadkowski, M., Livermore, D.M., Miriagou, V., Naas, T., Rossolini, G.M., Samuelsen, Ø., Seifert, H., Woodford, N., Nordmann, P., 2012. Rapid evolution and spread of carbapenemases among Enterobacteriaceae in Europe. **Clin. Microbiol. Infect. Off. Publ. Eur. Soc. Clin. Microbiol. Infect. Dis. 18**, 413–431. 10.1111/j. 1469-0691.2012.03821.x

Carroll, K.C., Glanz, B.D., Borek, A.P., Burger, C., Bhally, H.S., Henciak, S., Flayhart, D., 2006. Evaluation of the BD Phoenix automated microbiology system for identification and antimicrobial susceptibility testing of Enterobacteriaceae. **J. Clin. Microbiol. 44**, 3506–3509. 10.1128/JCM.00994-06

Cassini, A., Högberg, L.D., Plachouras, D., Quattrocchi, A., Hoxha, A., Simonsen, G.S., Colomb-Cotinat, M., 2019. Attributable deaths and disability-adjusted life-years caused by infections with antibiotic-resistant bacteria in the EU and the European Economic Area in 2015: a population-level modelling analysis. **Lancet Infect. Dis. 19**, 56–66. 10.1016/S1473-3099(18)30605-4

Ch, P., 2014. Clinical Microbiology & Microbial Genomics. **J. Clin. Mirobiology& Microb. Genomics 3**, 5073.

Chen, S., Feng, W., Chen, J., Liao, W., He, N., Wang, Q., Sun, F., Xia, P., 2014. Spread of carbapenemase-producing enterobacteria in a southwest hospital in China. **Ann. Clin. Microbiol. Antimicrob. 13**, 1–6. 10.1186/s12941-014-0042-4

Chung, H.S., Lee, M., 2020. Verification of the performance of the BD MAX Check-Points CPO Assay on clinical isolates. **J. Lab. Med. 44**, 165–168. 10.1515/labmed-2019-0145

Ciftci, E., Cetin, E.S., Us, E., Kutlu, H.H., Aridogan, B.C., 2019. Investigation of Carbapenem resistance mechanisms in Klebsiella pneumoniae by using phenotypic tests and a molecular assay. **J. Infect. Dev. Ctries. 13**, 992–1000. 10.3855/JIDC.10783

Cirz, R.T., Chin, J.K., Andes, D.R., De Crécy-Lagard, V., Craig, W.A., Romesberg, F.E., 2005. Inhibition of mutation and combating the evolution of antibiotic resistance. **PLoS Biol. 3**, 1024–1033. 10.1371/journal.pbio.0030176

Codjoe, F., Donkor, E., 2018. Carbapenem Resistance: A Review. **Med. Sci. 6**, 1. 10.3390/medsci6010001

Cohen, M.J., Block, C., Levin, P.D., Schwartz, C., Gross, I., Weiss, Y., Moses, A.E., Benenson, S., 2011. Institutional control measures to curtail the epidemic spread of carbapenem-resistant Klebsiella pneumoniae: a 4-year perspective. **Infect. Control Hosp. Epidemiol. 32**, 673—678. 10.1086/660358

David, S., Reuter, S., Harris, S.R., Glasner, C., Feltwell, T., Argimon, S., Abudahab, K., Goater, R., Giani, T., Errico, G., Aspbury, M., Sjunnebo, S., Feil, E.J., Rossolini, G.M., Aanensen, D.M., Grundmann, H., 2019. Epidemic of carbapenem-resistant Klebsiella pneumoniae in Europe is driven by nosocomial spread. **Nat. Microbiol. 4**, 1919–1929. 10.1038/s41564-019-0492-8

Demirci-Duarte, S., Unalan-Altintop, T., Gulay, Z., Kaygisiz, A.N.S., Cakar, A., Gur, D., 2020. In vitro susceptibility of OXA-48, NDM, VIM and IMP enzyme-producing Klebsiella spp. And Escherichia coli to fosfomycin. **J. Infect. Dev. Ctries. 14**, 394–397. 10.3855/JIDC.12456

Effah, C.Y., Sun, T., Liu, S., Wu, Y., 2020. Klebsiella pneumoniae: An increasing threat to public health. **Ann. Clin. Microbiol. Antimicrob. 19**, 1–9. 10.1186/s12941-019-0343-8

Esposito, E.P., Cervoni, M., Bernardo, M., Crivaro, V., Cuccurullo, S., Imperi, F., Zarrilli, R., 2018. Molecular Epidemiology and Virulence Profiles of Colistin-Resistant Klebsiella pneumoniae Blood Isolates From the Hospital Agency "Ospedale dei Colli," Naples, Italy. **Front. Microbiol. 9**, 1463. 10.3389/fmicb.2018.01463

EUCAST, 2015. The European Committee on Antimicrobial Susceptibility Testing. [WWW Document]. **Eur. Comm. Antimicrob. Susceptibility Test.** URL http://www.eucast.org.

Fair, R.J., Tor, Y., 2014. Perspectives in Medicinal Chemistry Antibiotics and Bacterial Resistance in the 21st Century. **J. Perspect. Med. Chem. 6**, 25–64. 10.4137/PMC.S14459.Received

Fusion, M., 2005. Technical Specification Sheet Triton(MacConkey Agar).

García-castillo, M., García-fernández, S., Isabel, M., Reilly, M., Ruiz-garbajosa, P., Moreno, R.C., 2017. P2321 Evaluation of the BD MAX Check-Points CPO assay for rapid detection of carbapenemase-producing organisms in rectal swab specimens from high-risk wards. **Jounal of ECCMID 7**, 1.

García-Fernández, A., Miriagou, V., Papagiannitsis, C.C., Giordano, A., Venditti, M., Mancini, C., Carattoli, A., 2010. An ertapenem-resistant extended-spectrum-β-lactamase-producing Klebsiella pneumoniae clone carries a novel OmpK36 porin variant. **Antimicrob. Agents Chemother. 54**, 4178–4184. 10.1128/AAC.01301-09

Girlich, D., Oueslati, S., Bernabeu, S., Langlois, I., Begasse, C., Arangia, N., Creton, E., Cotellon, G., Sauvadet, A., Dortet, L., Fortineau, N., Naas, T., 2020. Evaluation of the BD MAX Check-Points CPO Assay for the Detection of Carbapenemase Producers Directly from Rectal Swabs. **J. Mol. Diagnostics 22**, 294–300. 10.1016/j.jmoldx.2019.10.004

Hara, G.L., Gould, I., Endimiani, A., Pardo, P.R., Daikos, G., Hsueh, P.R., Mehtar, S., Petrikkos, G., Casellas, J.M., Daciuk, L., Paciel, D., Novelli, A., Saginur, R., Pryluka, D., Medina, J., Savio, E., 2013. Detection, treatment, and prevention of carbapenemase-producing Enterobacteriaceae: Recommendations from an international working group. **J. Chemother. 25**, 129–140. 10.1179/19739478 12Y.0000000062

Hashizume, T., Tamaki, S., Matsuhashi, M., Ishino, F., Nakagawa, J. ichi, 1984. Studies on the mechanism of action of imipenem (N-formimidoylthienamycin) in vitro: Binding to the penicillin-binding proteins (pbps) in escherichia coli and pseudomonas aeruginosa, and inhibition of enzyme activities due to the pbps in e. coli. **J. Antibiot. (Tokyo). 37**, 394–400. 10.7164/antibiotics.37.394

Hawkey, P.M., Livermore, D.M., 2012. Carbapenem antibiotics for serious infections. **BMJ 344**, 1–7. 10.1136/bmj.e3236

Himedi, A., 2012. MIO Medium Motility Indole Ornithine, Journal of BD company.

Hoffman, O., Weber, J.R., 2009. Pathophysiology and treatment of bacterial meningitis. **J. Ther. Adv. Neurol. Disord. 2**, 401–412. 10.1177/1756285609337975

Hofmann-Thiel, S., Plesnik, S., Mihalic, M., Heiß-Neumann, M., Avsar, K., Beutler, M., Hoffmann, H., 2020. Clinical evaluation of BD MAX MDR TB assay for direct detection of Mycobacterium tuberculosis complex and resistance markers. **J. Mol. Diagnostics 23**, 1–8. 10.1016/j.jmoldx.2020.06.013

Huang, T.S., Lee, S.S.J., Lee, C.C., Chang, F.C., 2020. Detection of carbapenem-resistant Klebsiella pneumoniae on the basis of matrix-assisted laser desorption ionization time-of-flight mass spectrometry by using supervised machine learning approach. **PLoS One 15**, 228–459. 10.1371/journal.pone.0228459

Jackson, J.J., Kropp, H., 1992. beta-Lactam antibiotic-induced release of free endotoxin: in vitro comparison of penicillin-binding protein (PBP) 2-specific imipenem and PBP 3-specific ceftazidime. **J. Infect. Dis. 165**, 1033–1041. 10.1093/ infdis/165.6.1033

Jacoby, G.A., Mills, D.M., Chow, N., 2004. Role of β-lactamases and porins in resistance to ertapenem and other β-lactams in Klebsiella pneumoniae. **Antimicrob. Agents Chemother. 48**, 3203–3206. 10.1128/AAC.48.8.3203-3206.2004

Jarvis, W.R., Munn, V.P., Highsmith, A.K., Culver, D.H., Hughes, J.M., 1985. The epidemiology of nosocomial infections caused by Klebsiella pneumoniae. **Infect. Control 6**, 68–74. 10.1017/s0195941700062639

Jensen, D.M., 2018. Molecular genetic testing and the future of clinical genomics Sara. **J. Physiol. Behav. 176**, 1570–1573. 10.1038/s41395-018-0061-4.

Kallen, A., 2009. Guidance for control of infections with carbapenem-resistant or carbapenemase-producing Enterobacteriaceae in acute care facilities. **MMWR. Morb. Mortal. Wkly. Rep. 58**, 256–260.

Ko, K.S., 2017. The contribution of capsule polysaccharide genes to virulence of Klebsiella pneumoniae. **J. Virulence 8**, 485–486. 10.1080/21505594. 2016.1240862

Ko, Y.J., Kim, J., Kim, H.N., Yoon, S.Y., Lim, C.S., Lee, C.K., 2019. Diagnostic performance of the Xpert Carba-R assay for active surveillance of rectal carbapenemase-producing organisms in intensive care unit patients. **Antimicrob. Resist. Infect. Control 8**, 2–9. 10.1186/s13756-019-0579-2

Kus, H., Arslan, U., Dagi, H.T., Findik, D., 2017. Hastane Enfeksiyonu Etkeni Klebsiella pneumoniae Izoiatlannda Cesitli Virulans Faktorlerinin Arastinlmasi. **J. Mikrobiyoloji Bul. 51**, 329–339. 10.5578/mb.59716

Labarca, J., Poirel, L., Özdamar, M., Turkoglü, S., Hakko, E., Nordmann, P., 2014. KPC-producing Klebsiella pneumoniae, finally targeting Turkey. **J. New Microbes New Infect. 2**, 50–51. 10.1002/nmi2.42

Landman, D., Bratu, S., Quale, J., 2009. Contribution of OmpK36 to carbapenem susceptibility in KPC-producing Klebsiella pneumoniae. **J. Med. Microbiol. 58**, 1303–1308. 10.1099/jmm.0.012575-0

Leavitt, A., Chmelnitsky, I., Colodner, R., Ofek, I., Carmeli, Y., Navon-Venezia, S., 2009. Ertapenem resistance among extended-spectrum-β-lactamase-producing Klebsiella pneumoniae isolates. **J. Clin. Microbiol. 47**, 969–974. 10.1128/JCM.00651-08

Lecoq, L., Dubée, V., Triboulet, S., Bougault, C., Hugonnet, J.E., Arthur, M., Simorre, J.P., 2013. Structure of enterococcus faecium l, d-transpeptidase acylated by ertapenem provides insight into the inactivation mechanism. **J. ACS Chem. Biol. 8**, 1–11. 10.1021/cb4001603

Lee, C.R., Lee, J.H., Park, K.S., Kim, Y.B., Jeong, B.C., Lee, S.H., 2016. Global dissemination of carbapenemase-producing Klebsiella pneumoniae: Epidemiology, genetic context, treatment options, and detection methods. **Front. Microbiol. 7**, 1–30. 10.3389/fmicb.2016.00895

Li, B., Zhao, Y., Liu, C., Chen, Z., Zhou, D., 2014. Molecular pathogenesis of Klebsiella pneumoniae. **J. Futur. Microbiol. 9**, 1071–1081. 10.2217/fmb.14.48

Li, Y., Shen, H., Zhu, C., Yu, Y., 2019. Carbapenem-resistant klebsiella pneumoniae infections among ICU admission patients in Central China: Prevalence and prediction model. **Biomed Res. Int. 9**, 1–10. 10.1155/2019/9767313

Lin, M.Y., Lyles-Banks, R.D., Lolans, K., Hines, D.W., Spear, J.B., Petrak, R., Trick, W.E., Weinstein, R.A., Hayden, M.K., 2013. The importance of long-term acute care hospitals in the regional epidemiology of Klebsiella pneumoniae carbapenemase-producing Enterobacteriaceae. **Clin. Infect. Dis. an Off. Publ. Infect. Dis. Soc. Am. 57**, 1246–1252. 10.1093/cid/cit500

Lutgring, J.D., Limbago, B.M., 2016. The Problem of Carbapenemase-Producing-Carbapenem-Resistant-Enterobacteriaceae Detection. **J. Clin. Microbiol. 54**, 529–534. 10.1128/JCM.02771-15

Ma, L., Wang, J.-T., Wu, T.-L., Siu, L.K., Chuang, Y.-C., Lin, J.-C., Lu, M.-C., Lu, P.-L., 2015. Emergence of OXA-48-Producing Klebsiella pneumoniae in Taiwan. **PLoS One 10**, 139_152. 10.1371/journal.pone.0139152

Machuca, I., Gutiérrez-Gutiérrez, B., Gracia-Ahufinger, I., Rivera Espinar, F., Cano, Á., Guzmán-Puche, J., Pérez-Nadales, E., Natera, C., Rodríguez, M., León, R., Castón, J.J., Rodríguez-López, F., Rodríguez-Baño, J., Torre-Cisneros, J., 2017. Mortality associated with bacteremia due to colistin-resistant Klebsiella pneumoniae with high-level meropenem resistance: Importance of combination therapy without colistin and carbapenems. **Antimicrob. Agents Chemother. 61**, 1–11. 10.1128/AAC.00406-17

Martin, J., Phan, H.T.T., Findlay, J.and et all, 2017. Covert dissemination of carbapenemase-producing Klebsiella pneumoniae (KPC) in a successfully controlled outbreak: long- and short-read whole-genome sequencing demonstrate multiple genetic modes of transmission. **J. Antimicrob. Chemother. 72**, 3025–3034. 10.1093/jac/dkx264

Meletis, G., 2016. Carbapenem resistance: overview of the problem and future perspectives. **J. Ther. Adv. Infect. Dis. 3**, 15–21. 10.1177/2049936115621709

Metian, M., 2017. Colorex ™ KPC Agar.

Mobarki, N., Almerabi, B., Hattan, A., 2019. Antibiotic Resistance Crisis. **Int. J. Med. Dev. Ctries. 40**, 561–564. 10.24911/ijmdc.51-1549060699

Molton, J.S., Tambyah, P.A., Ang, B.S.P., Ling, M.L., Fisher, D.A., 2013. The global spread of healthcare-associated multidrug-resistant bacteria: a perspective from Asia. **Clin. Infect. Dis. an Off. Publ. Infect. Dis. Soc. Am. 56**, 1310–1318. 10.1093/cid/cit020

Mueller, B.B.L., Ii, H., 2006. BBL ™ Mueller Hinton II Agar. **J. BD Syst. 16**, 5–9.

Naas, T., Dortet, L., I. Iorga, B., 2016. Structural and Functional Aspects of Class A Carbapenemases. **Curr. Drug Targets 17**, 1006–1028. 10.2174/1389450117666160310144501

Nordmann, P., Cuzon, G., Naas, T., 2009. The real threat of Klebsiella pneumoniae carbapenemase-producing bacteria. **Lancet. Infect. Dis. 9**, 228–236. 10.1016/S1473-3099(09)70054-4

Nordmann, P., Naas, T., Poirel, L., 2011a. Global spread of carbapenemase producing Enterobacteriaceae. **J. Emerg. Infect. Dis. 17**, 1791–1798. 10.3201/eid1710.110655

Nordmann, P., Poirel, L., 2002. Emerging carbapenemases in Gram-negative aerobes. **Clin. Microbiol. Infect. 8**, 321–331. 10.1046/j.1469-0691.2002.00401.x

Nordmann, P., Poirel, L., Walsh, T.R., Livermore, D.M., 2011b. The emerging NDM carbapenemases. **Trends Microbiol. 19**, 588–595. 10.1016/j.tim.2011.09.005

Oliver, J., 2019. For detection of Gram negative bacteria with a reduced susceptibility to most of the carbapenem agents Background. **J. Hilos Tensados 1**, 1–476. 10.1017/CBO9781107415324.004

Orsi, G.B., García-Fernández, A., Giordano, A., Venditti, C., Bencardino, A., Gianfreda, R., Falcone, M., Carattoli, A., Venditti, M., 2011. Risk factors and clinical significance of ertapenem-resistant Klebsiella pneumoniae in hospitalised patients. **J. Hosp. Infect. 78**, 54–58. 10.1016/j.jhin.2011.01.014

Paczosa, M.K., Mecsas, J., 2016. Klebsiella pneumoniae: Going on the Offense with a Strong Defense. **Microbiol. Mol. Biol. Rev. 80**, 629–661. 10.1128/mmbr.00078-15

Pajand, O., Darabi, N., Arab, M., Ghorbani, R., Bameri, Z., Ebrahimi, A., Hojabri, Z., 2020. The emergence of the hypervirulent Klebsiella pneumoniae (hvKp) strains among circulating clonal complex 147 (CC147) harbouring bla NDM/OXA-48 carbapenemases in a tertiary care center of Iran. **Ann. Clin. Microbiol. Antimicrob. 19**, 1–9. 10.1186/s12941-020-00349-z

Papp-Wallace, K.M., Endimiani, A., Taracila, M.A., Bonomo, R.A., 2011. Carbapenems: Past, present, and future. **Antimicrob. Agents Chemother. 55**, 4943–4960. 10.1128/AAC.00296-11

Parakh, A., Krishnamurthy, S., 2009. Ertapenem. **Kathmandu Univ. Med. J. 7**, 454–460. 10.3126/kumj.v7i4.2774

Patel, G., Huprikar, S., Factor, S.H., Jenkins, S.G., Calfee, D.P., 2008. Outcomes of carbapenem-resistant Klebsiella pneumoniae infection and the impact of antimicrobial and adjunctive therapies. **J. Infect. Control Hosp. Epidemiol. 29**, 1099–1106. 10.1086/592412

Paterson, D.L., Bonomo, R.A., 2005. Extended-Spectrum Lactamases: a Clinical Update. **J. Clin. Microbiol. Rev. 18**, 657–686. 10.1128/CMR.18.4.657

Paterson, D.L., Ko, W.-C., Von Gottberg, A., Mohapatra, S., Casellas, J.M., Goossens, H., Mulazimoglu, L., Trenholme, G., Klugman, K.P., Bonomo, R.A., Rice, L.B., Wagener, M.M., McCormack, J.G., Yu, V.L., 2004. International prospective study of Klebsiella pneumoniae bacteremia: implications of extended-spectrum beta-lactamase production in nosocomial Infections. **J. Ann. Intern. Med. 140**, 26–32. 10.7326/0003-4819-140-1-200401060-00008

Peterson, L.R., 2009. Bad bugs, no drugs: no ESCAPE revisited. **Clin. Infect. Dis. an Off. Publ. Infect. Dis. Soc. Am.** 10.1086/605539

Podschun, R., Ullmann, U., 1998. Klebsiella spp. as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. **Clin. Microbiol. Rev. 11**, 589–603.

Poirel, L., Héritier, C., Tolun, V., Nordmann, P., 2004. Emergence of Oxacillinanse-Mediated Resistance to Imipenem in Klebs. pneu. **Antimicrob. Agents Chemother. 48**, 15–22. 10.1128/AAC.48.1.15

Pournaras, S., Kristo, I., Vrioni, G., Ikonomidis, A., Poulou, A., Petropoulou, D., Tsakris, A., 2010. Characteristics of meropenem heteroresistance in Klebsiella pneumoniae carbapenemase (KPC)-producing clinical isolates of K. pneumoniae. **J. Clin. Microbiol. 48**, 2601–2604. 10.1128/JCM.02134-09

Queenan, A.M., Bush, K., 2007. Carbapenemases: The versatile β-lactamases. **J. Clin. Microbiol. Rev. 20**, 440–458. 10.1128/CMR.00001-07

Rahman, S., Ali, T., Ali, I., Khan, N.A., Han, B., Gao, J., 2018. The Growing Genetic and Functional Diversity of Extended Spectrum Beta-Lactamases. **J. BioMed Res. Int. 32**, 1–14. 10.1155/2018/9519718

Rasmussen, B.A., Bush, K., 1997. Carbapenem-hydrolyzing beta-lactamases. **Antimicrob. Agents Chemother. 41**, 223–232.

Raveh, D., Yinnon, A.M., Broide, E., Rudensky, B., 2007. Susceptibilities of ESBL-producing Enterobacteriaceae to ertapenem, meropenem and piperacillin-tazobactam with and without clavulanic acid. **Chemotherapy 53**, 185–189. 10.1159/000100516

Ripabelli, G., Tamburro, M., Guerrizio, G., Fanelli, I., Flocco, R., Scutellà, M., Sammarco, M.L., 2018. Tracking Multidrug-Resistant Klebsiella pneumoniae from an Italian Hospital: Molecular Epidemiology and Surveillance by PFGE, RAPD and PCR-Based Resistance Genes Prevalence. **Curr. Microbiol. 75**, 977–987. 10.1007/s00284-018-1475-3

Rodloff, A.C., Goldstein, E.J.C., Torres, A., 2006. Two decades of imipenem therapy. **J. Antimicrob. Chemother. 58**, 916–929. 10.1093/jac/dkl354

Samanta, I., Bandyopadhyay, S., 2020. Carbapenem Resistance. **Antimicrob. Resist. Agric. 8**, 59–69. 10.1016/b978-0-12-815770-1.00007-9

Samra, Z., Bahar, J., Madar-Shapiro, L., Aziz, N., Israel, S., Bishara, J., 2008. Evaluation of CHROMagar KPC for rapid detection of carbapenem-resistant Enterobacteriaceae. **J. Clin. Microbiol. 46**, 3110–3111. 10.1128/JCM.00249-08

Sarı, A.N., Alp Çavuş, S., Gülay, Z., 2016. Efficiency of BD MAXTM CRE Method on Detection of Carbapenemase-Producing Enterobacteriaceae spp. from Rectal Swab Samples. **Türk Mikrobiyoloji Cemiy. Derg. 46**, 112–121. 10.5222/tmcd.2016.112

Schembri, M.A., Blom, J., Krogfelt, K.A., Klemm, P., 2005. Capsule and Fimbria Interaction in. **Society 73**, 4626–4633. 10.1128/IAI.73.8.4626

Shah, A.A., Hasan, F., Ahmed, S., Hameed, A., 2004. Extended-spectrum β-lactamases (ESBLs): Characterization, epidemiology and detection. **J. Crit. Rev. Microbiol. 30**, 25–32. 10.1080/10408410490266429

Shah, P.M., Isaacs, R.D., 2003. Ertapenem, the first of a new group of carbapenems. **J. Antimicrob. Chemother. 52**, 538–542. 10.1093/jac/dkg404

Shahfiza, N., Osman, H., Hock, T.T., Abdel-Hamid, A.H.Z., 2017. Metabolomics approach for multibiomarkers determination to investigate dengue virus infection in human patients. **J. Acta Biochim. Pol. 64**, 1–9. 10.18388/abp.2015

Sheff, B., 2000. Klebsiella pneumoniae. **J. Nurs. 30**, 28. 10.1097/00152193-200030090-00008

Shields, R.K., Nguyen, M.H., Press, E.G., Chen, L., Kreiswirth, B.N., Clancy, C.J., 2017. In Vitro Selection of Meropenem Resistance among Ceftazidime-Avibactam-Resistant, Meropenem- Susceptible Klebsiella pneumoniae Isolates with Variant KPC-3 Carbapenemases. **Jounal Am. Soc. Microbiol. 61**, 1–5.

Shon, A.S., Bajwa, R.P.S., Russo, T.A., 2013. Hypervirulent (hypermucoviscous) Klebsiella Pneumoniae: A new and dangerous breed. **J. Virulence 4**, 107–118. 10.4161/viru.22718

Simner, P.J., Martin, I., Opene, B., Tamma, P.D., Carroll, K.C., Milstone, A.M., 2016. Evaluation of Multiple Methods for Detection of Gastrointestinal Colonization of Carbapenem-Resistant Organisms from Rectal Swabs. **J. Clin. Microbiol. 54**, 1664–1667. 10.1128/JCM.00548-16

Simon, M., Gatermann, S., Pfeifer, Y., Reischl, U., Gessner, A., Jantsch, J., 2019. Evaluation of the automated BD Phoenix CPO Detect panel in combination with the β-CARBA assay for detection and classification of carbapenemase-producing Enterobacterales. **J. Microbiol. Methods 156**, 29–33. 10.1016/j.mimet.2018.11.024

Skowska, A., Gospodarek, E., Kusza, K., 2014. The prevalence of infections and colonisation with Klebsiella pneumoniae strains isolated in ICU patients. **Anaesthesiol. Intensive Ther. 46**, 280–283. 10.5603/AIT.2014.045

Su, H.R., Turhan, Ö., Erman Daloğlu, C.A., Öğünç, M.D., Özhak, B., Öngüt, G., Kuşkucu, M.A., Midilli, K., Mamıkoğlu, L., 2020. Molecular Epidemiology of Carbapenem-Resistant Enterobacterales Strains Isolated from Blood Cultures in Antalya, Turkey. **Lab. Med. 17**, 1–20. 10.1093/labmed/lmaa017

Sulikowska, A., 2015. BBL ™ Simmons Citrate Agar Slants. **Jounal BD Syst. 31**, 1–2.

Tamma, P.D., Simner, P.J., 2018. Phenotypic detection of carbapenemase-producing organisms from clinical isolates. **J. Clin. Microbiol. 56**, 1–13. 10.1128/JCM.01140-18

Tenover, F.C., Kalsi, R.K., Williams, P.P., Carey, R.B., Stocker, S., Lonsway, D., Rasheed, J.K., Biddle, J.W., McGowan, J.E., Hanna, B., 2006. Carbapenem resistance in Klebsiella pneumoniae not detected by automated susceptibility testing. **Emerg. Infect. Dis. 12**, 1209–1213. 10.3201/eid1208.060291

Trushkin, E.V., Senyavina, N.V., Sakharov, D.A., Rusanov, A.L., Marx, W., Tonevitsky, A.G., 2013. Modern Technologies in Vitro Drug Testing inVitro: Using Micro Bioreactors. **J. Biotechnol. 0226**, 51–58.

Tsuyuki, Y., 2017. The BD MAX System.

Tzouvelekis, L.S., Markogiannakis, A., Psichogiou, M., Tassios, P.T., Daikos, G.L., 2012. Carbapenemases in Klebsiella pneumoniae and other Enterobacteriaceae: An evolving crisis of global dimensions. **J. Clin. Microbiol. Rev. 25**, 682–707. 10.1128/CMR.05035-11

Urban, C., Bradford, P.A., Tuckman, M., Segal-Maurer, S., Wehbeh, W., Grenner, L., Colon-Urban, R., Mariano, N., Rahal, J.J., 2008. Carbapenem-Resistant Escherichia coli Harboring Klebsiella pneumoniae Carbapenemase β-Lactamases Associated with Long-Term Care Facilities. **J. Clin. Infect. Dis. 46**, 1–4. 10.1086/588048

Van Rijn, S.P., Van Altena, R., Akkerman, O.W., Van Soolingen, D., Van Der Laan, T., De Lange, W.C.M., Kosterink, J.G.W., Van Der Werf, T.S., Alffenaar, J.W.C., 2016. Pharmacokinetics of ertapenem in patients with multidrug-resistant tuberculosis. **Eur. Respir. J. 47**, 1–6. 10.1183/13993003.01654-2015

Walter, J., Haller, S., Quinten, C., 2018. Healthcare-associated pneumonia in acute care hospitals in European union/European economic area countries: An analysis of data from a point prevalence survey, 2011 to 2012. **Eurosurveillance 23**. 10.2807/1560-7917.ES.2018.23.32.1700843

Walther-Rasmussen, J., Hoiby, N., 2006. OXA-type carbapenemases. **J. Antimicrob. Chemother. 57**, 373–383. 10.1093/jac/dki482

Wang, L., 2017. Gene sequence from colonies. C. Measurand: target dna sequence of the following genes:

Wasekar, M., Satpathy, P., Diggikar, P., Misra, R., 2014. Case of severe Friedlander′s pneumonia with review of the literature. **Med. J. Dr. D.Y. Patil Univ. 7**, 232. 10.4103/0975-2870.126365

WHO, 2020. Antimicrobial resistance [WWW Document]. **World Heal. Organ.** URL **https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance**

Wilson, C., 2013. BD MacConkey II Agar INTENDED.

Woldu, M., 2016. Klebsiella pneumoniae and Its Growing Concern in Healthcare Settings. **J. Clin. Exp. Pharmacol. 06**, 1–7. 10.4172/2161-1459.1000199

Yigit, H., Queenan, A.M., Anderson, G.J., Domenech-Sanchez, A., Biddle, J.W., Steward, C.D., Alberti, S., Bush, K., Tenover, F.C., 2001. Novel carbapenem-hydrolyzing β-lactamase, KPC-1, from a carbapenem-resistant strain of Klebsiella pneumoniae. **Antimicrob. Agents Chemother. 45**, 1151–1161. 10.1128/AAC.45.4.1151-1161.2001

Zarkotou, O., Pournaras, S., Voulgari, E., Chrysos, G., Prekates, A., Voutsinas, D., Themeli-Digalaki, K., Tsakris, A., 2010. Risk factors and outcomes associated with acquisition of colistin-resistant KPC-producing Klebsiella pneumoniae: A matched case-control study. **J. Clin. Microbiol. 48**, 2271–2274. 10.1128/JCM.02301-09

Zhanel, G.G., Johanson, C., Embil, J.M., Noreddin, A., Gin, A., Vercaigne, L., Hoban, D.J., 2014. Ertapenem: Review of a new carbapenem. **Expert Rev. Anti. Infect. Ther. 3**, 23–39. 10.1586/14787210.3.1.23

APPENDIX

Appendix (1) The distribution of forty-eight rectal swabs isolates that were collected from various age groups hospitalized from different hospital units.

Hospital units	**Age categories**				**Total**
	0-10 years	**11 – 30 years**	**31 – 50 years**	**51 – 83 years**	
Intensive care unit	0	4	2	6	**12**
Neurosurgery unit	1	3	2	3	**9**
Pediatric intensive care unit	3	1	0	0	**4**
Anesthesia unit	0	2	3	1	**6**
Hematology unit	1	2	2	4	**9**
General surgery unit	0	0	0	2	**2**
Neonatal intensive care unit	2	0	0	0	**2**
Bone marrow unit	1	0	0	0	**1**
Nephrology unit	1	0	0	0	**1**
Medical oncology unit	0	0	2	0	**2**
Total	**9**	**12**	**11**	**16**	**48**

Appendix (2) Comparison of conventional traditional method findings with BD MAX assay for all 48 rectal swab samples used in this study.

No.	Gender/Age	Conventional culturing method					BD MAX CPO assay
		Chromagar KPC	Biochemical tests	AST / MIC(µ g / ml)			
				ERT	MEM	IPM	
1	M/24	+	*K.pneumoniae*	R, >1	R, >8	R, 8	***bla*$_{OXA}$**
2	F/66	+	*K.pneumoniae*	R, >1	R, >8	R, 8	***bla*$_{OXA}$**
3	M/11	+	*K.pneumoniae*	R, >1	R, >8	R, 8	***bla*$_{OXA}$ + *bla*$_{NDM}$ + *bla*$_{VIM}$ /*bla*$_{IMP}$**
4	F/117D	-					-
5	M/3	+	*K.pneumoniae*	R, >1	R, >8	R, >8	-
6	M/21	-					***bla*$_{OXA}$**
7	M/210D	+	*K.pneumoniae*	R, >1	R, >8	R, 8	-
8	M/3	+	*K.pneumoniae*	R, >1	R, >8	R, 8	***bla*$_{OXA}$**
9	M/46	-					***bla*$_{OXA}$**
10	F/75	+	*K.pneumoniae*	R, >1	R, >8	R, 8	***bla*$_{OXA}$ + *bla*$_{VIM}$/ *bla*$_{IMP}$**
11	M/53	+	*K.pneumoniae*	R, >1	R, >8	R, 8	***bla*$_{OXA}$**
12	M/72	+	*K.pneumoniae*	R, >1	R, >8	R, 8	***bla*$_{OXA}$**
13	M/81	+	*K.pneumoniae*	R, >1	R, >8	R, 8	***bla*$_{OXA}$**
14	M/68	+	*K.pneumoniae*	R, >1	R, >8	R, 8	***bla*$_{OXA}$**
15	F/3	+	*K.pneumoniae*	R, >1	R, >8	R, 8	***bla*$_{OXA}$ + *bla*$_{NDM}$ + *bla*$_{VIM}$ /*bla*$_{IMP}$**
16	M/16	+	*K.pneumoniae*	R, >1	R, >8	R, 8	***bla*$_{OXA}$**
17	M/83	+	*K.pneumoniae*	R, >1	R, >8	R, 8	***bla*$_{OXA}$**
18	F/48	-					***bla*$_{OXA}$**
19	M/18	+	*K.pneumoniae*	R, >1	R, >8	R, 4	***bla*$_{OXA}$ + *bla*$_{VIM}$/ *bla*$_{IMP}$**
20	F/41	+	*K.pneumoniae*	R, >1	R, >8	R, 8	***bla*$_{NDM}$**
21	M/46	+	*K.pneumoniae*	R, >1	R, >8	R, >8	***bla*$_{OXA}$ + *bla*$_{NDM}$**
22	F/22	+	*K.pneumoniae*	R, >1	R, >8	R, 8	-
23	F/15	+	*K.pneumoniae*	R, >1	R, >8	R, >8	***bla*$_{OXA}$**
24	F/61	+	*K.pneumoniae*	R, >1	R, >8	R, 8	***bla*$_{OXA}$**
25	M/25D	+	*K.pneumoniae*	R, >1	R, >8	R, 8	***bla*$_{OXA}$**
26	M/315D	+	*K.pneumoniae*	R, >1	R, >8	R, 8	***bla*$_{OXA}$ + *bla*$_{NDM}$ + *bla*$_{VIM}$ /*bla*$_{IMP}$**
27	F/82	-					-
28	M/25	+	*K.pneumoniae*	R, >1	R, >8	R, 8	***bla*$_{OXA}$**
29	M/6	-					-
30	M/75	+	*K.pneumoniae*	R, >1	R, >8	R, 8	***bla*$_{OXA}$**
31	F/39	-					-
32	M/37	-					-
33	F/29	+	*K.pneumoniae*	R, >1	R, >8	R, 8	***bla*$_{OXA}$ + *bla*$_{NDM}$**
34	M/72	-					-
35	M/25	-					-
36	M/54	-					-
37	M/74	-					-
38	F/41	-					-
39	F/30	-					-
40	M/76	+	*E.coli*	S, <=0.25	S, <=0.125	S, <=0.25	***bla*$_{OXA}$**
41	F/44	-					-
42	M/50	+	*K.pneumoniae*	S, <=0.25	S, <=0.125	S, 0.5	***bla*$_{OXA}$ + *bla*$_{VIM}$/ *bla*$_{IMP}$**
43	F/80	-					-
44	F/10	-					-
45	F/82	-					-
46	M/58	+	*K.pneumoniae*	R, >1	R, >8	R, 8	***bla*$_{OXA}$ + *bla*$_{NDM}$**
47	M/50	-					-
48	M/25	+	*K.pneumoniae*	R, >1	R, >8	R, 8	***bla*$_{OXA}$ + *bla*$_{NDM}$**

*AST: Antibiotic Susceptibility Testing, MIC: Minimum Inhibitory Concentration ERT: Ertapenem, MEM: Meropenem, IPM: Imipenem, M: Male, F: Female, (+): Positive, (-): Negative, R: Resistant S: Sensitive.

CURRICULUM VITAE

Name: Thowiba Yousif JAMEEL

Nationality: Iraqi

Email: Thowiba112@gmai.com

Education information:

Bachelor's Degree, from the college of sciences / Biology / Tikrit University in 2012.

Master's Degree, from the graduate school of natural and applied sciences / Biology / Erciyes University in 2020.

Languages: Arabic, English, and Turkish.