NEAR EAST UNIVERSITY
INSTITUTE OF GRADUATE STUDIES

DEPARTMENT OF MEDICAL MICROBIOLOGY AND CLINICAL MICROBIOLOGY

INVESTIGATIONOF TWO DIFFERENT METHODS OF METALLO-BETA-LACTAMASE ACTIVITIES IN *PSEUDOMONAS AERUGINOSA*STRAINS

M.Sc. THESIS

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NICOSIA

DECEMBER-2021

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I hereby declare that all information, documents, analysis and results in this thesis have been collected and presented according to the academic rules and ethical guidelines of Institute of Graduate Studies, Near East University. I also declare that as required by these rules and conduct, I have fully cited and referenced information and data that are not original to this study.

CHRISTOPHER IDU ITIUNG

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LIST OF ABBREVIATIONS

%:Percentage

µg: Micro gram

µl: Micro Liter

PCA: Phenazine-1-carboxylic acid

HCAIs: Healthcare-Associated Infection

CLSI: Clinical and Laboratory Standards Institute

DNA: Deoxy Ribonucleic Acid

ESBL: Extended Spectrum Beta Lactamase

EAS-NET: European Antimicrobial Surveillance Network

PICU: Pediatric Intensive Care Unit

VAP: Ventilator-Associated Pneumonia

PDR: Pandrug-Resistant

MDR: Multi Drug Resistant

MHA: Muller Hinton Agar

MIC: Minimum Inhibitory Concentration

eCDC: European Center for Disease Prevention and Control

PCR: Polymerase Chain Reaction

pH: Power of Hydrogen

HIV: Human Immunodeficiency Virus

AIDS: Acquired Immunodeficiency Syndrome

XDR: Extremely Drug Resistant

CF: Cystic Fibrosis

ICU: Intensive Care Unit

FQ: Fluoroquinolones

APH: Aminoglycoside Phosphoryl transferase

AAC: Aminoglycoside Acetyltransferase

ANT: Aminoglycoside Adenyl transferase

BLI: Bioluminescent Imaging

PBP: Penicillin-binding Protein

MHT: Modified Hodge Test

RND: Resistance-Nodulation-Division

:Beta

EDTA: Ethylenediamine Tetra Acetic Acid

IPM-EDTA: Imipenem + Ethylenediamine Tetra Acetic Acid

DDST: Double Disk Synergy Test

CDT: Combined Disk Test

NDM: New Delhi Metallo-Lactamase

NaOH: Sodium Hydroxide

MBL-PA: Metallo-Beta-Lactamase-*Pseudomonas aeruginosa*

ABSTRACT

Christopher Idu Itiung. Investigation Of Two Different Methods of Metallo-Beta-Lactamase Activities in *Pseudomonas Aeruginosa*Strains.Near East University, Institute of Graduate Studies, Medical Microbiology and Clinical Microbiology Program, Master Thesis, Nicosia, 2021

The first report of MBL producing *P.aeruginosa* came from Japan in 1988, and since then it has been reported from all over the world. This investigation was designed with the goal of detecting MBL-producing *P.aeruginosa* isolates from the Near East University Hospital. To accomplish this, we compared and evaluated two alternative phenotypic analysis approaches already in use. The combined disc diffusion test (CDT) and the double synergy disc test(DDST) are two of these procedures. The use of two microbiological media, Muller Hinton agar and EMB agar, made the two approaches easier to analyze. A total of 65 clinical samples were compiled and analyzed from the hospital's various departments. The intensive care unit had the highest percentage rate of cases (21.5%), while the dermatology department had the lowest percentage rate (1.5%). Samples were collected from various clinical patients in these departments, with urine having the highest percentage rate of 33.8% and blood having the lowest percentage rate of 1.5%. The resistance percentage of the antibiotics used in this study were as follows; amikacin (10.8%), cefepime (29.2%), gentamicin (23.1%), imipenem (35.4%), meropenem (23.1%), ceftazidime (27.7%), aztreonam (62.3%), piperacillin-tazobactam (27.7%), colistin (7.7%), ciprofloxacin (32.3%), and netilmicin (30.8%). MBL production was found in 25 of the 65 isolates, and the results revealed that CDT had the highest sensitivity and specificity for detecting MBL-producing *P.aeruginosa*, with a data of 52 percent versus 48 percent for DDST. In addition, CDT was found to be extremely sensitive in detecting MBL in *P. aeruginosa*.

***Key words*: *Pseudomonas aeruginosa*, metallo-beta-lactamase, antibiotics, carbapenemases.**

Christopher Idu Itiung. Çe itli Klinik Örneklerden zole Edilen *Pseudomonas aeruginosa* Su larında Metallo-betalaktamaz Aktivitesinin Ara tırılması. Yakın Do u Üniversitesi, Sa lık Bilimleri Enstitüsü, Tıbbi Mikrobiyoloji ve Klinik Mikrobiyoloji Programı, Yüksek Lisans Tezi, Lefko a, 2021

ÖZET

Metallo beta-laktamaz*P. aeruginosa*'nın ilk raporu 1988'de Japonya'dan geldi ve o zamandan beri dünyanın her yerinden rapor edildi. Bu ara tırma, Yakın Do u Üniversitesi Hastanesi'nden MBL üreten *P. aeruginosa* izolatlarının saptanması amacıyla tasarlanmı tır. Bunu ba armak için halihazırda kullanımda olan iki alternatif fenotipik analiz yakla ımını kar ıla tırdık ve de erlendirdik. Kombine disk difüzyon testi (CDT) ve çift disk sinerji testi (DSDT) bu prosedürlerden ikisidir. Hastanenin çe itli bölümlerinden toplam 65 klinik örnek derlendi ve analiz edildi. Yo un bakım ünitesi en yüksek vaka yüzdesine (%21,5) sahipken, dermatoloji bölümü en dü ük yüzdeye (%1.5) sahipti. Bu bölümlerdeki çe itli klinik hastalardan numuneler toplandı, en yüksek yüzde oranı yüzde 33,8 olan idrar ve yüzde en dü ük yüzde oranı yüzde 1,5 olan kan. amikasin (%10,8), sefepim (%29,2), gentamisin (%23,1), imipenem (%35,4), meropenem (%23,1), seftazidim (%27,7), aztreonam (%50,8), trimethoprim-sulfamethoksazol (%27,7), siprofloksasin (%32,3) ve netilmisin (%32,3) duyarlılık ve direnç (%30,8) açısından test edilen antibiyotiklerdi. Çalı ılan 65 izolatın 25'inde MBL üretimi bulundu ve sonuçlar, DSDT için yüzde 52'ye kar ı yüzde 48'lik bir veriyle CDT'nin MBL üreten *P.aeruginosa*'yı saptamak için en yüksek duyarlılı a ve özgüllü e sahip oldu unu ortaya koydu. Ayrıca CDT'nin *P.aeruginosa*'da MBL'yi saptamada son derece duyarlı oldu u bulunmu tur.

Anahtar kelimeler: *Pseudomonas aeruginosa*, metallo-beta-laktamaz, antibiyotik, karbapenemaz.

SECTION ONE: INTRODUCTION

1. Background of the Study

Pseudomonas aeruginosa (P. aeruginosa) is a bacillus bacterium, which does not form spores and it is known to be a Gram-negative trait. This bacteria presence is ubiquitous in nature from soil, water and to divergent vegetations amidst the globe (Ekrem and Rokan, 2014, Fazeli *et al*., 2012, Nadeem *et al*., 2019). The genus of *P. aeruginosa* comprises of more than 140 species in existence, as it were few of these pathogens that are infectious to man while others are basically saprophytic and occur widely in nature (Adedeji *et al*., 2010, Crackshank *et. al.,* 1975, Adedeji *et al*., 2007). It is an opportunistic pathogen seen to cause most nosocomial infections. This pathogen strains causes infections in hospitalized patients predominantly pneumonia, and urinary tract infections. Also, it is involved in skin and soft-tissue infections (Pawel *et al*., 2008,Giamarellou, 2002). *Pseudomonas aeruginosa* are non-fermentative organism found in diverse environmental settings and since it is an opportunistic pathogen its' prowess for causing serious infections amongst patients with immunocompromised systems is high in comparison with other opportunistic pathogens (Springer Science and Business Media LLC, 1993, Coggan and Wolfgang, 2012). These bacteria generally have the tendency to resist a variety of antimicrobial as well, because it has the capacity to create resistance by transformation or procurement of foreign resistance genes against diverse anti-microbial classes (Strateva and Yordanov, 2009, Mahesh *et al*., 2017).

P. aeruginosa causes community-acquired and nosocomial diseases, based on the pathogenesis it leads to pneumonia, urinary tract infections, and bacteremia. The diseases can be especially imperative in patients who are immunocompromised, such as neutropenia or cancer patients (Lister *et al*., 2009, Shaikh *et al*., 2015). These days, the rates of morbidity and mortality has been expanded because of multidrug-resistant *P. aeruginosa* strains (Gaouar-Borsali *et al*. 2012). The expanded association possessed by these omnipresent bacteria in diseases is based on the number of components which includes the rising factors such as; invasive procedures, and immunocompromised patients along with the continuous utilization of antimicrobials that have

advanced the selection of resistant bacteria (Cristino, 1999, Pawel *et al*., 2008). The intensive care (ICU), oncology division, surgical wards and burns unit always have patients whom habitually appear to have multi-resistant isolates which comprise of high morbidity and mortality (Giamarellos-Bourboulis *et al.,* 2006, Pawel *et al*., 2008). The spread of this bacteria is troublesome to control in healthcare establishments based on the present of different inherent and procured components in antimicrobial resistance (Boucher *et al*., 2007, Livermore, 2002, Lombardi *et al*., 2002, Pawel *et al*., 2008). These bacteria accounts for 10% of all health care associated infections (HCAIs) as an opportunistic pathogen it is known to be the fourth most commonly cause of nosocomial infections. They can extend from superficial skin diseases to incendiary blood poison and also subjugation of these traits in critical systems are fatal (Amani *et al*., 2017, Sivaraj *et al*., 2012).

Thesebacteria pathogenicity is determined by theirprowess to produce a variety of toxins and proteases. Their capacity to resist phagocytosis is also important (Baltimore, 2000). *Pseudomonas spp*. are found in the gastrointestinal tracts of between 5% and 30% of healthy persons, but they seldom take over (Adedeji *et al*., 2010, Baker and Breach, 1980, Adedeji *et al*. 2007). There are multiple intrinsic and acquired mechanisms of antimicrobial resistance which allows these bacteria often difficult to control. Genes encoded metallo-beta-lactamases (MBLs) is driven by regional consumption of extended-spectrum cephalosporins and carbapenems (Boucher *et al*., 2007, Livermore, 2002, Lombardi *et al*., 2002, Pawel *et al.,* 2008). The bacteria also produce redox dynamic phenazine compounds, which incorporate phenazine-1-carboxylic acid which is abbreviated as (PCA),pyocyanin,phenazine-1-carboamide, and 1-hydroxyphenazine (Mavrodi *et al*., 2001, Allen *et al*., 2005, Ling-Qing *etal*., 2017). In spite of the fact that they can cause disease, *P. aeruginosa* are against the virulence of other opportunistic pathogens, and are also the leading cause of different acute infections, ventilator-associated pneumonia (VAP) included (Sadikot *et al*., 2005, Planquette *et al*., 2013, Nicholas *etal*., 2017).

Metallo-beta-lactamases contains multiple beta-lactamases which are highly dynamic hydrolysers of carbapenems, but they have strong enzymatic activities against other beta-lactam antimicrobials, besides monobactams (Jonas *et al*., 2005, Walsh *et al*., 2005). Conventional beta-lactamases inhibitors are usually unable to inhibit the production of MBLs. Furthermore, MBLs that are gene encoded are easily seen in cassette related to multi-drug resistance integrons (Poirel

et al., 2000, Riccio *et al*., 2005). *P.aeruginosa* strains that are producers of MBLs are of specific significance, based on their prowess for horizontal spread and little amount of potential therapeutic agents (Rossolini and Mantengoli, 2005, Hirakata *et al*., 2003,Parkins, 2007).

Arrangement of made work had been carried out on *P. aeruginosa* and MBLs activity. Hence, the design of a suitable investigation is of basic significance in *P. aeruginosa* and two different methods of MBLs activity to abdicate an alluring result. Earlier information and encounters in creating a reasonable explanatory arrangement may play an imperative part in assist expository investigation work.

Various studies have shown *P. aeruginosa* and MBLs. The aim of this study is to detect MBLs-producing *P. aeruginosa* isolates from a hospital, compare and evaluate different phenotypic methods currently in use.

1.2.General Information

P. aeruginosa is the most common bacteria that is associated with nosocomial infections, especially in immunocompromised individuals (Kaye *et al*., 2015), and they are known for theirprowess to develop antibiotic resistance mechanisms (Zavascki *et al*., 2010). Antimicrobials that contain beta-lactams are most effective in treating these bacterialinfections. Regardless, the production of beta-lactamases, such as cephalosporinases and carbapenemases, has been extensively studied using clinical isolates of *P. aeruginosa* from Latin America, and represents the most powerful mechanism of beta-lactam resistance documented among gram-negative bacteria worldwide (Poster Session', Clinical Microbiology and Infection,2012, Labarca *et al*., 2016). Given the importance of carbapenems in treating*P. aeruginosa* infections, it is critical understanding the components that make up aberrant and inefficient phenotypes. Information regarding these systems signals a change in the particular pressure exerted by antimicrobials and the improvement of antimicrobial resistance, altering this bacterial treatment of infections, which are frequently restricted to only polymyxin (Morita *et al*., 2012, Queenan and Bush, 2007,Grosso-Becerra *et al*., 2014, Eloiza *et al*., 2017).

Because carbapenem molecules are more resistant to hydrolysis by a large variety of spread serine-lactamases, they are useful in treating infections caused by cephalosporinase producing strains (Hawkey *et al*., 2009), which are nevertheless susceptible to carbapenems. Combining carbapenems with natural components such as down-regulation or deletion of OprD porin, efflux pump hyper expression, chromosomal Ampc beta-lactamases generation, and target alterations modifies carbapenem resistance in these bacteria(Zavascki *et al*., 2010, Labarca *et al*., 2016).

Gram-negative bacteria containing carbapenemase producing gene, on the other hand, are resistant to practically all beta-lactams, since carbapenemases can hydrolyze penicillin, cephalosporins, and carbapenems (Queenan and Bush, 2007).

Community-acquired and nosocomial illnesses caused by *P. aeruginosa* include urinary tract infections and bacteremia. Infections are extremely harmful in patients whose immune system is compromised, like those with neutropenia or cancer (Lister *et al*., 2009, Shaikh *et al*., 2015, Abstracts cont.' Clinical Microbiology and Infection, 2004). Because of stains of*P.*

*aeruginosa*resistant to drugs, the prevalence of morbidity and mortality continues toincrease in recent years (Poster session', Clinical Microbiology and Infection, 2012, Gaouar-Borsali*et al*. 2012). These omnipresent bacteria increase association with disease is due to a number of factors, including an increase in the number of factors, invasive procedures, and immunocompromised patients, as well as an increase in the use of antimicrobials, which has aided in the selection of resistant organisms. The intensive care (ICU), oncology division, surgical wards and burns unit always have patients whom habitually appear to have multi-resistant isolates which comprise of high morbidity and mortality (Giamarellos-Bourboulis *et al.,* 2006, Pawel *et al*., 2008). Because of the many inherent and acquired components of antimicrobial resistance, controlling the spread of thesebacteria in healthcare settings is frequently difficult(Boucher *et al*., 2007, Livermore, 2002, Lombardi *et al*., 2002,Pawel *et al*. 2008). These bacteria accounts for 10% of all health care associated infections (HCAIs) as an opportunistic pathogen it is known to be the fourth most commonly cause of nosocomial infections. They can extend from superficial skin diseases to incendiary blood poison and also subjugation of these traits in critical systems are fatal (Amani *et al*. 2017, Sivaraj *et al*., 2012).

Thesebacteria pathogenicity is determined by theirprowess to produce a variety of toxins and proteases. Their capacity to resist phagocytosis is also important (Baltimore, 2000). *Pseudomonas spp*. are found in the gastrointestinal tracts of between 5% and 30% of healthy persons, but they seldom take over (Baker and Breach, 1980, Adedeji *et al*. 2007). *P. aeruginosa* also produces auxiliary metabolites, phytotoxins, slime, and antifungals, all of which provide obvious selective advantages for the bacteria to produce redox dynamic phenazine compounds, which incorporate phenazine-1-carboxylic acid which is abbreviated as (PCA),pyocyanin, phenazine-1-carboamide, and 1-hydroxyphenazine (Youenou *et al*., 2016, Mavrodi *et al*., 2001, Allen *et al*., 2005,Ling-Qing *et al*. 2017). *P. aeruginosa* is named among the virulent opportunistic bacteria, and are prominent cause of dreadfuldiseases, includingpneumonia caused by ventilators (VAP) (Sadikot *et al*., 2005, Planquette *et al*., 2013,Nicholas *et al*. 2017).

For infections caused by these bacteria, there are fewer, and in some cases no, effective antimicrobial treatments available.Gram-positive and Gram-negative bacteria are affected by the rising and spreading of antibiotic resistance, as this problem worsens, standardized definitions for portraying and classifying microorganisms that are aversion to various antibacterial drugs

should be readily available in order for epidemiological data to be collected and analyzed over preventive medicine settings and countries. Bacteria are classified as multidrug-resistance when they are resistant to more than one antibacterial agent in vitro. MDR diseases are linked to lower patient outcomes due to inadequate or delayed antibiotic treatment (Wiley, 2019, Onlinelibrary, Wiley, 2021, Magiorakos, 2012, Ibrahim *et al*., 2000). Highly resistant gram-negative bacteria may be resistant to all currently available antibacterial medicines or only responsive to older, potentially more toxic antibacterial agents such as polymyxins, limiting and suboptimal treatment options. (Magiorakos, 2012, McGowan, 2006, Bonomo and Szabo, 2006, Pitout and Laupland, 2008).When considering the small number of novel antibacterial drugs in development, the problem of growing antibacterial resistance becomes much more onerous (European Center for Disease Prevention and Control, 2009,Morita *et al*., 2012, Boucher *et al*., 2009). However, no consensus has been reached on the definition and application of terms such as "multidrug-resistant," "extremely drug resistant," "extensively or extremely drug resistant", and "pandrug-resistant" (Onlinelibrary, Wiley, 2021, Falagas *et al*., 2006, Goossens, 2003, Falagas and Karageorgopoulos, 2008, Apisarnthanarak *et al*., 2008, Doi *et al*, 2009, Park *et al*. 2009, Griffin *et al*., 2013), which describe MDR resistance. This inconsistency prevents reliable comparison of MDR surveillance data, preventing the community from fully comprehending the scope of the antimicrobial resistance problem. Furthermore, detailed information on the escalating threat of MDR to public health cannot be communicated to the public and policymakers (Cohen *et al*., 2008, Hidron *et al*., 2008, Paterson and Doi, 2007), Implementing uniform global verbiage to characterize bacteria aversion to a diverse variety of therapeutically dynamic drugs would be a critical step toward improving the comparability of these organisms' surveillance data and better assessing their global, territorial, and local epidemiological importance and public health impact (Onlinelibrary, Wiley, 2021, Carmeli *et al*., 2010, APA, 2005, Jones and Masterton, 2001). XDR bacteria are epidemiologically significant not only for their aversion to various antibacterial drugs, but also for their unfavorable plausibility of being resistant to all of the antibacterial agents that have been approved(Onlinelibrary, Wiley, 2021, Brink *et al*., 2008, Tseng *et al*., 2007, Magiorakos *et al*. 2011).

As per the European Antimicrobial Surveillance Network (EAS-Net) data from 2015, mean strain ratios for piperacillin/tazobactam carbapenems and quinolones between many *P. aeruginosa* intrusive isolated strains were similar to 20%, while ceftazidime and

aminoglycosides were just 13% (Spanish Journal of Chemotherapy, 2021, European Center for Disease Prevention and Control, 2015). Between 2011 and 2015, piperacillin/tazobactam conductivity increases in Europe, while carbapenem and ceftazidime impedance stayed constant. Generally speaking, there must have been different socio - economic in resistance rates within and between Eu countries, with south and east nations having higher resistance rates than Nordic regions(Spanish Journal of Chemotherapy, 2021, European Center for Disease Prevention and Control, 2015, Rahman *et al*., 2018). These bacteria isolates recovered from circulatory system infections in Spanish hospitals werehigh in resistance to piperacillin/tazobactam drugs, ceftazidime drugs, fluoroquinolones drugs, and aminoglycosides drugs than those reported by EARS-Net in a multicenter investigation (Spanish Journal of Chemotherapy, 2021, European Center for Disease Prevention and Control, 2015, Rahman *et al*., 2018). Carbapenem resistance, on the other hand, was equivalent to that described by EARS-Net (European Center for Disease Prevention and Control, 2015, Rahman *et al*., 2018,Ruiz-Garbajosa *et al*. 2017). Piperacillin/tazobactam, ceftazidime, and carbapenems all had moderate in vitro activity against *P. aeruginosa* respiratory isolates obtained from hospitalized patients with pneumonia in the United States and Europe, furthermore, resistance rates in European hospitals were greater than in American hospitals (Spanish Journal of Chemotherapy, 2021, Sader *et al*., 2014). The most effective antimicrobials against blood and respiratory *P. aeruginosa* isolates were amikacin and colistin (Cabot *et al*., 2011, Sader *et al*., 2014). The prevalence of MDR *P. aeruginosa*, on the other hand, has increased over the previous decade, reaching 30 percent in some locations, such as Eastern European countries (Oliver *et al*., 2015).XDR strains, which are non-susceptible to at least one drug in all but two or fewer antimicrobial categories, account for a large proportion of MDR strains (Spanish Journal of Chemotherapy, 2021, European Center for Disease Prevention and Control, 2015). In a multicenter study of *P. aeruginosa* bloodstream infection in Spain, 15% of the isolates were determined to have XDR (Oliver *et al*., 2015). Invasive isolates with antibacterial aversion to three or more classes (piperacillin-tazobactam, ceftazidime, fluroquinolones, aminoglycosides, and carbapenems)have increased significantly in Spain, according to the EARS-Net, with rates ranging from 4% in 2005 to 14% in 2015 (Spanish Journal of Chemotherapy, 2021, eCDC, 2015). Polymyxins and amikacin were the most active antibiotic against the XDR serotypes(Spanish Journal of Chemotherapy, 2021, European Center for Disease Prevention and Control, 2015, Oliver*et al*. 2015).

1.3. History

The scientific study on *P. aeruginosa*, was first seen "On the blue and green coloring of bandages," byCarle Gessard in 1882 as a result of dye screening (Gessard, 1882). *Bacillus pyocyaneus*, *Pseudomonas polycolor*, *Bacterium aeruginosa*, and *Pseudomonas pyocyaneus* reflect the unique coloration, which was eventually linked to a phenazine derivative, pyocyanin,althoughthis bacterial prowess to cause infections had been discovered by 1889 (Bouchad, 1889), its pathogenicity was questioned (Fraenkel, 1917), and these bacteria was viewed primarily as source for potent antibiotic chemicals (Schoenthal, 1941, Botzenhart andDöring)

1.4. General Microbiological Characteristics

Pseudomonas aeruginosa is aerobic gram-negative bacterium with the ability to achieve motility, they arerod-like shaped and does not form spore, they are positive to oxidase test and lactose non-fermenters, they bacteriabelong to the genus Pseudomonas sometimes called pseudomonads, these bacteria characteristic blue-green color on solid medium is due to the water-soluble pigments pyocyanin and pyoverdine (Fatma and Sameh, 2018). The bacteriaproduce indophenol oxidase, this enzyme separates them from other gram-negative bacteria by making them positive to "oxidase" test,movements exhibited by these bacteria is achieved by their polar flagella and pili, like many other environmental bacteria, resides in slime-encased biofilms that allow it to survive and replicate in human tissues and medical equipment (Fatma and Sameh, 2018, Engleberg and Dermondi, 2007). The bacteria are immune to antibodies and phagocytes produced by the host, which contribute to their prowess to resist drugs.

The bacterium *Pseudomonas aeruginosa* thrives in damp settings like soil and water. They are common on fresh fruits and vegetables in huge quantities. These bacteria colonize humans in the gastrointestinal tract, then spreads to moist skin locations like the perineum and axilla (Fatma and Sameh, 2018, Engleberg and Dermondi, 2007). At 42 degrees Celsius, these bacteria are smooth fluorescent green colonies in appearance with sweet grape-like odor, this making them easy to identify on solid media. As a group, Pseudomonads needs low nutrition to survive, many are capable of obtaining nourishment from a range of substances in the environment; however, these bacteria frequently require acetate and ammonia as their carbon and nitrogen sources, respectively.Furthermore, they may grow anaerobically and does not rely on fermentation for

energy, instead relying on sugar oxidation. It can grow in a variety of conditions due to its adaptable nutritional requirements;they are also tough to eliminate from polluted healthcare environments(Fatma and Sameh, 2018, Engleberg and Dermondi, 2007).

1.5. Epidemiology

Ninety-one cases of septicemia caused by *P. aeruginosa* were described in the literature prior to 1947 (Stanlet, 1947,Springer Science and Business Media LLC, 1993). Their significance being pathogenic to immunocompromise system, came up at the second half of the twentieth century (Finland, 1980,Springer Science and Business Media LLC, 1993), despite the organism's presence in both the inanimate and human environments prior to that time. It's unlikely that clinical microbiologists overlooked *P. aeruginosa* because it's so easy to culture and diagnose. As a result, the significant shift in the importance of *P. aeruginosa* as a nosocomial infection is likely due to developments in changes in patients' immune systems (Botzenhart & Döring, 1993,Springer Science and Business Media LLC, 1993).

P. aeruginosa'spervasive existence permits it to play a role in human infections on a regular basis. It's a versatile bacterium that prefers to live in the soil. *P. aeruginosa*, on the other hand, may also thrive in water. *P. aeruginosa* may resist toxic waste degradation due to its dietary variety. They are also known to be pathogens that affects vegetable plants. It is found in fresh water habitats such as streams, lakes, and rivers, as well as domestic sinks, showers, and hospital breathing device, and it has even been seen to contaminate distilled water (Favero *et al*., 1971). *P. aeruginosa* can be consumed by humans accidentally from these sources, but they do not attach well to normal, intact epithelium. As a result, they are found as part of normal gut flora, and *P. aeruginosa* does not cause infection in those who have a sound immune system (Engleberg and Dermondi 2007).

Pseudomonas reproduction is aided by the warmth in hot tubs, which can contain millions of organisms per milliliter. These bacteriaare versatile, and theythrive in healthcare settings, where reservoirs of the bacteria grow in water,and breathing device. *P. aeruginosa* can be from domestic cleaning solutions due to their inherent and acquired resistance to many common antimicrobial agents.These bacteria contamination is especially dangerous to respiratory therapy equipment and dialysis tubing, both of which require a wet, skin environment. Contamination has indeed been linked to the dispersion of the bacteria in multi-use vials of respiratory drugs.

Synthetic fingernails or extenders are not recommended for use by healthcare workers due to widespread discovery of *P. aeruginosa* colonization of the fingernails.Some antiseptic treatments used to sterilize endoscopes and surgical tools can even support *Pseudomonas spp*(Center for Disease Prevention and Control, 2000,Engleberg and Dermondi, 2007, Kennedy *et al*., 2004,Wilson *et al*., 1981).

P. aeruginosa accounted for 4% of cases in a study of 24,179 people and one of the leadingcauses of gram-negative infection with nosocomial sepsisdiseases in America from 1995 to 2002 (Wisplinghoff *et al*., 2004),the rate of nosocomial infection in pediatric intensive care unit was 1.5 per hundred patient a day, patients undergoing heart surgerywas the highest at 2.3 per hundred patient a day. The most commonly encountered diseases discovered were bacteremia with 51.7 percent rate, respiratory infection with 19.0 percent rate, and urinary tract infection with 17.2 percent rate, all of which were linked to the use of intrusive devices.The most prevalent species isolated were staphylococci with 39 percent rate that are negative to coagulasetest and *P. aeruginosa* with 24 percent rate.*Pseudomonas aeruginosa* is responsible for a number of human diseases, including bloodstream infectionsand lung infections(Urrea *et al*., 2003, Wright and Romano, 2006). They cancause infections in people with persistent neutropenia and neutrophil dysfunction, hematologic malignancies, HIV/AIDS, and diabetes mellitus. Furthermore, persistent pulmonary illness is widespread in cystic fibrosis patients (Fujitani *et al*., 2008).

1.6. Antimicrobial Resistance

Reduced permeability, development of efflux systems, creation of antimicrobial inactivating enzymes, and target alterations are all examples of antibiotic resistance mechanisms in bacteria.The significant proportion of these known mechanisms of resistance are exhibited by *P. aeruginosa* through the use of genes encoding or hereditarily exporting resistance factor that affect vital antimicrobial categories such like beta-lactams, aminoglycosides, quinolones, and polymyxin. Antibiotic used to diagnose *P. aeruginosa* diseases are classified into eight groups: aminoglycosides which includes; ’gentamicin, tobramycin, amikacin, and netilmicin’, cephalosporins which includes; ’ceftazidime and cefepime’, fluoroquinolones which includes; ’ciprofloxacin and levofloxacin’, penicillin with beta-lactamase inhibitors (BLI) such as ’ticarcillin and ’colistin, polymyxin B’.MDR traits of these bacteria are resistant to one of three

antimicrobial agents; widely drug-resistant (XDR) bacteria are hostile to all except one antibacterial agent; and pandrug-resistant strains are hostile to all antibacterial agents (Patel *et al*., 2021, Magiorakos, 2012, Sader *et al*., 2014, El Zowalaty *et al*., 2015). The drug resistant strains emerge in a timely manner due to changes in regulatory mechanisms that controls the expression of resistance determinants, the change of the structure of a gene, changes in membrane permeability, and acquisition of antibiotic-inactivating enzymes or enzymes that induce target modifications on a horizontal scale.The simultaneous creation of these mechanisms confers multi-resistance to many strains, which is noteworthy (Poole, 2009, Fujii *et al*., 2014).

According to an eCDC analysis published in 2016, *P. aeruginosa*(33.9 percent) strains were resistant to one at most of the antibiotic groups used in Europe under investigation,this antibiotic includes; piperacillin, tazobactam, fluoroquinolones, ceftazidime, aminoglycosides, and carbapenems (eCDC, 2016, Bassetti *et al*. 2018).This study found significant inter-country differences in antimicrobial resistance across antibacterial classes, with higher rate of resistance in the southern and eastern portions of Europe than in the northern parts. In Latvia, Poland, Slovakia, Hungary, Croatia, Serbia, Bulgaria, or Greece, for example, 25 percent to 50 percent of invasive isolates were carbapenem resistant, whereas more than 50 percent are resistantRomania.Due to the combination resistance to three or more of the antibacterial previously mentioned, 25 percent to 50 percent of those invasive strains isolated incountries like Slovakia, Romania, Croatia, Bulgaria, and Greece were safe (Bassetti *et al*. 2018).

Table1. Chromosomally Encoded or Important Resistance Mechanisms of *P. aeruginosa*

(Bassetti *etal*. 2018).

Site	Mechanisms	Antibiotics	Types of Resistance
Chromosomal intrinsic	AmpC-type cephalosporinase	-lactams	Antibiotic inactivation
	Class D oxacillinase OXA-50	-lactams	Antibiotic inactivation
	Aminoglycosides inactivating enzymes	Aminoglycosides	Antibiotic inactivation
	Over-expression	Multiple antibiotic	Efflux system

	efflux systems	classes	
	Decreased membrane permeability	Multiple antibiotic classes	Membrane impermeability and purines
	DNA gyrase and topoisomerase IV	Fluoroquinolones	Target modification
	LPS modification	Colistin	Target modification
Genetic element	Class A serine-beta-lactamases (PSE, CARB, TEM)	-lactams	Antibiotic inactivation
	Class A serine ESBL (TEM, SHV, CTX-M, PER, VEB, GES, IBC)	-lactams	Antibiotic inactivation
	Class D ESBL (OXA-types)	-lactams	Antibiotic inactivation
	Class B metallo-beta-lactamase (IMP, VIM, SPM, GIM)	Carbapenems	Antibiotic inactivation
	Class A serine carbapenemase (KPC)	Carbapenems	Antibiotic inactivation
	Class D carbapenemase (OXA-types: OXA-40)	Carbapenems	Antibiotic inactivation
	Aminoglycosides inactivating enzymes	Aminoglycosides	Antibiotic inactivation
	Ribosomal methyltransferase enzymes	Aminoglycosides	Target modification

1.6.1. Resistance of Aminoglycoside

Antipseudomonal chemotherapy, which includes aminoglycosides, treat diseases which includeslungdiseases in cystic fibrosis (CF) (Poole, 2005, Bartlett, 2003, Gilbert *et al*., 2003) patients (Cheer *et al*., 2003). The above agents are effective in inhibiting and work synergistically with other antibacterial drugs, most remarkably beta-lactams, with which they have been commonly used to treat P. aeruginosa diseases; toxic effects appear to be ameliorated by enhancing high dose durations(Turnidge, 2003),and at least in pulmonarydiseases, by using aerosolized agents (e.g., tobramycin) (Cheer *et al*., 2003). On other hand, papers dated back to 1960s has detailed these bacteria resistance to aminoglycoside, emphasizing the general insusceptibility of the bacterial clinical isolates to antibiotics such as kanamycindrug (Griffith, 1966, Griffith *et al*., 1960). Resistance to antipseudomonal aminoglycosides, such as gentamicin drug and tobramycin drug, and also amikacin drug, are too widespread nowadays and can be found almost anywhere in the world,especially in Europe and Latin America (Ruedas-Lopez *et al*., 2021, Antimicrob Agents Chemother, 2020, Antimicrob Agents Chemother, 2019, Soliman *et al*., 2015, Mushtaq *et al*., Access Microbiology 2020). Respiratory isolates (Mathai *et al*., 2001), notably those from CF patients (Henwood *et al*., 2001, Livermore, 2002, Pitt *et al*., 2003, Schulin, 2002), as well as bloodstream (Lyytikainen *et al*., 2001), urine (Bouza *et al*., 2001), wound (Jones *et al*. 2003), burn (Estahbanati *et al*., 2002, Walton *et al*., 1997), ocular (Alexandrakis *et al*., 2000, Chalita *et al*., 2004), and auditory (Dohar *et al*., 1996, Roland and Stroman, 2002) isolates, all show signs of resistance. Resistance is usually caused by drug inactivation by resistant bacteria's plasmid or chromosome-encoded enzymes, although it also causes impermeability resistance which is widespread, especially in patientswith cystic fibrosis (Ruedas-Lopez *et al*., 2021, Antimicrob Agents Chemother, 2020, Antimicrob Agents Chemother, 2019, Soliman *et al*., 2015, Mushtaq *et al*., Access Microbiology 2020, Plumb *et al*., 2009, Miller *et al*., 1994, Miller *et al*., 1995, Miller *et al*., 1995, Price *et al*., 1981, Saavedra *et al*., 1986) and intensive care units (ICUs) (Bert and Lambert-Zechovski, 1996, Goossens, 2003, Hanberger *et al*., 2001).Since the 1960s and 1970s (Brzezinska *et al*., 1972, Doi *et al*., 1968, Kobayashi *et al*., 1971, Tseng *et al*., 1973), resistant *P. aeruginosa* isolates have been reported to inactivate aminoglycosides. Aminoglycoside inactivation in hostile strains has traditionally been

accomplished by phosphorylated enzymes such like 'aminoglycoside phosphoryltransferase [APH]', acetylate such like 'aminoglycoside acetyltransferase [AAC]', or adenylate such like 'aminoglycoside nucleotidyltransferase [ANT]; also known as aminoglycoside adenyltransferase' (Ruedas-Lopez *et al*., 2021, Antimicrob Agents Chemother, 2020, Antimicrob Agents Chemother, 2019, Soliman *et al*., 2015, Mushtaq *et al*., Access Microbiology 2020, Morita *et al*., 2012, Azucena and Mobashery, 2001, Patricia, 2008). Individual aminoglycoside-resistant *P. aeruginosa* isolates are increasingly carrying several modifying enzymes, resulting in aminoglycoside with broad-spectrum resistance (Ruedas-Lopez *et al*., 2021, Antimicrob Agents Chemother, 2020, Antimicrob Agents Chemother, 2019, Soliman *et al*., 2015, Mushtaq *et al*., Access Microbiology 2020, Kettner*et al*., 1995, Miller *et al*., 1997, *Miller et al*., 1994, Miller *et al*., 1995, Miller *et al*., 1995, Rodriguez *et al*., 2000, Saavedra *et al*., 1986, Patricia, 2008).

1.6.2. Resistance Mechanism of Quinolone

In 1962, Quinolones were found to be an effective treatment for a variety of clinical symptoms (Liu and Mulholland, 2005). The first was nalidixic acid, which was synthesized at the Sterling-Winthrop Research Institute by George Lesher. Years ago, it was created by isolating chloro-1-ethyl-1, 4-dihydro-4-oxo-3-quinoline carboxylic acid as a byproduct of chloroquine production (Lesher *et al*., 1962). Quinolone resistance has evolved into a severe concern among many developing resistant pathogens over time (Hooper, 2001). Bacterial mutations against quinolones are typically found in the target enzyme binding sites of DNA gyrase and topoisomerase IV (Aldred *et al*., 2013). Furthermore, resistance to this class of antibiotics can be acquired through horizontal transfer of a resistant plasmid from other sources in the environment, resulting in fast resistance propagation (Hooper *et al*., 2015).

Quinolones are the main antibacterial available for oral treatment in most countriesin treating*P. aeruginosa*diseases. However, these bacteria quickly develop resistance to these antibiotics, significantly limiting their utility. The main approaches of resistance are genetic defects in target genes that encode DNA gyrase (gyrA) and topoisomerase IV (parC), along with genetic changes in transcription factor for distinct mechanisms.MexAB-OprM, MexCD-OprJ, and MexEF-OprN are three distinct multidrug efflux pump systems that are controlled by mexR (nalB), nfxB, and mexT (nfxC), respectively (Jones, 1999, Marchetti and Viale, 2013, Jalal *et al*., 2000).

1.6.3. Resistance Mechanism of Beta-Lactamase

The wild-type strains of these pathogens incorporate an overexpression molecular class C ampC cephalosporinase that is unphased by BLI like clavulanic acid, tazobactam, or sulbactam (Sligl et al., 2015). Low permeability of the cell membrane and numerous efflux systems cause resistance to aminopenicillins alone or in pairing with BLI, first and second generation cephalosporins (C1G, C2G), cephamycins, the two third generation cephalosporins (C3G), cefotaxime and ceftriaxone, as well as carbapenem and ertapenem (Oncul *et al*., 2014, Lund-Palau *et al*., 2016).This same carboxypenicillin, ureidopenicillin, C3G ceftazidime, C4G cefepime, aztreonam, and carbapenems remain active against these pathogens wild-type strain. Nevertheless, with the exception of carbapenems, induced or inherent AmpC upregulation and point mutation can decrease sensitivity to lactamin categories(Oncul *et al*., 2014; Lund-Palau *et al*., 2016). Unlike Enterobacteriaceae's AmpC, *P. aeruginosa*'s AmpC can also influence cefepime (Oncul *et al*., 2014; Lund-Palau *et al*., 2016). These enzymes' main substrates are carboxypenicillin and ureidopenicillin, and they can sometimes withstand BLI. Cefepime, cefpirome, and aztreonam are all vulnerable to these enzymes to variable degrees, although ceftazidime and carbapenem are still active against *P. aeruginosa* strains containing these beta-lactamase types (Strateva and Yordanov, 2009).

1.6.3.1. Resistance that develops with changes in penicillin-binding proteins (PBP)

B-Lactam antibiotics remain critical components of our antibacterial therapeutic options for the diagnosis of life-threatening hospital - acquired infections caused by*Pseudomonas aeruginosa*(Ruedas-Lopez *et al*., 2021, Antimicrob Agents Chemother, 2020, Antimicrob Agents Chemother, 2019, Soliman *et al*., 2015, Mushtaq *et al*., Access Microbiology 2020, Mesaros *et al.*, 2007). Despite this, resistance to these first-line antibacterial agents is on the upsurge, and it is usually associated with Multi - drug resistant phenotypic traits(Ruedas-Lopez *et al*., 2021, Antimicrob Agents Chemother, 2020, Antimicrob Agents Chemother, 2019, Soliman *et al*., 2015, Mushtaq *et al*., Access Microbiology 2020, Cabot *et al*., 2011, Lister *et al*., 2009),while genetic engineering is a serious challenge to the merger of influential extracellular B-lactamases like class B carbapenemases or ESBLs (Moya *et al*., 2012), B-lactam resistance is also much more commonly caused by the accumulation of a difficult and complicated back catalogue of genetic change(Ruedas-Lopez *et al*., 2021, Antimicrob Agents Chemother, 2020, Antimicrob

Agents Chemother, 2019, Soliman *et al*., 2015, Mushtaq *et al*., Access Microbiology 2020, Lister *et al*., 2009, Livermore, 2002, Poole, 2004, Poole, 2011).Those that result in the violence and oppression or inhibition of the gene encoding OprD, culminating in multidrug resistance(El Amin *et al*., 2005, Gutierrez *et al*., 2007, Pirnay *et al*., 2002,Quale *et al*., 2006), or those that result in an increase in the power generation of the chromosomal cephalosporinase AmpC(Cabot *et al*., 2011, Juan *et al*., 2006, Moya *et al*., 2009, Botelho *et al*., 2019), conferring resistance to penicillin, cephalosporins, and monobactams, are particularly interesting. Furthermore, mutated genes that up-regulate MexAB-OprM and MexXY-OprM, which are expressed inside these bacteria genetic material, may significantly contribute to B-lactam resistance phenotypic traits, as well as potentially reducing fluoroquinolone and aminoglycoside activity(Ruedas-Lopez *et al*., 2021, Antimicrob Agents Chemother, 2020, Antimicrob Agents Chemother, 2019, Soliman *et al*., 2015, Mushtaq *et al*., Access Microbiology 2020, Cabot *et al*., 2011,Cavallo et al., 2007, Masuda *et al*., 2000, Poole, 2004, Botelho *et al*., 2019).

While the combination of these mechanisms results in aversion to all available at the moment B-lactams, a few diagnostically advanced derivative products, like the novel cephalosporin ceftolozane (formerly CXA-101), have seemed to become less affected by them, and hence reflect a successful potential therapeutic strategies of Resistant bacteria(Ruedas-Lopez *et al*., 2021, Antimicrob Agents Chemother, 2020, Antimicrob Agents Chemother, 2019, Soliman *et al*., 2015, Mushtaq *et al*., Access Microbiology 2020, Bulik *et al*., 2010, Juan *et al*., 2010, Livermore *et al*., 2009, Moya *et al*., 2010, Sader *et al*., 2011). A further signficant inhibitory action is indeed the alteration of a target of B-lactam antibacterial drugs, the vital penicillin-binding proteins (PBPs), that include PBP1a, PBP1b, PBP2, and PBP3 (Ruedas-Lopez *et al*., 2021, Antimicrob Agents Chemother, 2020, Antimicrob Agents Chemother, 2019, Soliman *et al*., 2015, Mushtaq *et al*., Access Microbiology 2020, Zapun*et al*., 2008).

1.6.3.2. Impairment of efflux and other membrane permeability

The involvement of an efflux structure first from resistance-nodulation-division (RND in short) groups has awhile back been clearly stated by investigation (Ruedas-Lopez *et al*., 2021, Antimicrob Agents Chemother, 2020, Antimicrob Agents Chemother, 2019, Soliman *et al*., 2015, Mushtaq *et al*., Access Microbiology 2020,Poole, 2004) and MexXY (Mine *et al*., 1999) (also known as AmrAB [Westbrock-Wadman *et al*., 1999]) in the lower scale of aminoglycoside

buildup that typifies both barrier properties tolerance(Ruedas-Lopez *et al*., 2021, Antimicrob Agents Chemother, 2020, Antimicrob Agents Chemother, 2019, Soliman *et al*., 2015, Mushtaq *et al*., Access Microbiology 2020,Sobel *et al*., 2003; Vogne *et al*., 2004; Mima, 2009) and adaptive aminoglycoside resistance (Hocquet *et al*., 2003) in these bacteria.The RND pump group is among five drug signaling pathways detailed in bacteria thus far(Ruedas-Lopez *et al*., 2021, Antimicrob Agents Chemother, 2020, Antimicrob Agents Chemother, 2019, Soliman *et al*., 2015, Mushtaq *et al*., Access Microbiology 2020, Poole, 2004). It is usually composed of three main parts: an endomembrane drug-proton antiporter, an OM channel-forming protein, and a periplasmic link protein that integrates the other two components(Ruedas-Lopez *et al*., 2021, Antimicrob Agents Chemother, 2020, Antimicrob Agents Chemother, 2019, Soliman *et al*., 2015, Mushtaq *et al*., Access Microbiology 2020, Poole, 2004).The mexXY gene product encodes periplasmic and inner membrane proteins, respectively, whereas OprM is indeed the obvious OMF for this mechanism(Ruedas-Lopez *et al*., 2021, Antimicrob Agents Chemother, 2020, Antimicrob Agents Chemother, 2019, Soliman *et al*., 2015, Mushtaq *et al*., Access Microbiology 2020, Morita *et al*., 2012), which is really the result of MexAB-OprM operon's third gene, which encodes some other three-component RND type pump(Ruedas-Lopez *et al*., 2021, Antimicrob Agents Chemother, 2020, Antimicrob Agents Chemother, 2019, Soliman *et al*., 2015, Mushtaq *et al*., Access Microbiology 2020, Aires *et al*., 1999; Masuda *et al*., 2000). However, the discovery that mutants sorely missing one of OM proteins, OpmG, OpmH, or OpmI, are - lactam antibiotics hypersusceptible implies that either one of these proteins may also collaborate with MexXY, possibly as the intended OMF for this efflux mechanism(Ruedas-Lopez *et al*., 2021, Antimicrob Agents Chemother, 2020, Antimicrob Agents Chemother, 2019, Soliman *et al*., 2015, Mushtaq *et al*., Access Microbiology 2020, Jo *et al*., 2003).MexXY wild-type cells are involved in resistance to many antibacterial drugs (Morita *et al*., 2012, Masuda *et al*., 2000, Okamoto *et al*., 2002, Poole, 2005).

1.6.3.3. Resistance due to beta-lactamase enzyme release

Some mechanisms tend to inhibit medications from interacting with their biological targets, while others lead to drug inactivation (Lister *et al*., 2009). *P. aeruginosa*, like several other Gram-negative bacteria, has blaAmpCthat modulates a diverse beta-lactamase of class C(Daikos et al., 2021, Sabath *et al*., 1965). The above enzyme catalyzes the bacterial invulnerability to

malleable and triggering chemical compounds like aminopenicillins and first- and second-generation cephalosporins(Masuda *et al*., 1999). AmpC becomes a significant source of aggression to extensively used antipseudomonal penicillin, monobactams, and third and fourth generation cephalosporins when produced in excess as a result of variations modifying the peptidoglycan recyclability(Livermore *et al*., 1982, Fung-Tomc *et al*., 1989, Moya *et al*., 2009, Cabot *et al*., 2012, Berrazeg *et al*., 2015).

1.6.3.3.1 Metallo-Beta-Lactamases

Pseudomonas aeruginosa producing MBLs was originally described in Japan in 1991 (Watanabi *et al*., 1991), and has since been seen in Asia (Lee *et al*., 2004, Yan *et al*., 2001, Yatsuyanagi *et al*., 2004), Europe (Lagatolla *et al*., 2004, Libisch *et al*., 2004, Patzer *et al*., 2004, Poirel *et al*., 2000), Australia (Peleg *et al*., 2004), South America (Gales *et al*., 2003), and North America (Toleman *et al*., 2004). MBLs are Ambler type B bacteria that can hydrolyze a wide range of beta-lactam antibiotics like penicillin, cephalosporin, and carbapenems (Livermore and Woodford, 2000). Metal chelators like EDTA and thiol-based chemicals block the effects of these enzymatic reactions, which necessitate zinc for catalytic reactions(Pitout, 2014, Livermore and Woodford, 2000, Johann *et al*. 2005).

Except for monobactams (Jones *et al*., 2005, Walsh *et al*., 2005), MBLs are a class of beta-lactamases that are highly dynamic carbapenem hydrolyzers with strong but variable enzyme activity against other beta-lactam antimicrobials (Poirel *et al*., 2000, Riccio *et al*., 2005). Traditional beta-lactam inhibitors are largely ineffective against MBLs. MBL genes are also frequently discovered in cassettes linked to multidrug resistance integrons (Rossolini and Mantengoli, 2005, Hirakata *et al*., 2003).Provided their own potency for horizontal spread as well as the scarcity of promising therapeutic antibiotics, *P. aeruginosa* strains are particularly important. Regardless, there is still a scarcity of information about the best way to treat these bacteria producing MBL diseases (Parkins, 2007, Parkins *et al*. 2007).

1.7. Laboratory Diagnosis of Metallo-beta-lactamases

Molecular and phenotypic approaches can be used to detect MBL synthesis in P. aeruginosa. MBL-positive genes can be detected using molecular methods like PCR, DNA probes, cloning, and sequencing. These methods are extremely exact and dependable, but they're only available in

reference labs. EDTA and thiol-based have the ability to block MBL activity, which is why the rise in using phenotypic methods to detect MBL production is trending.MHT, DDST, CDT employ imipenem and EDTA when these tests are carried out, while E-test when us to identify MBLs-producing traits uses all common procedures (Sachdeva *et al*., 2020, Sachdeva *et al*., 2017).

1.7.1. Modified Hodge Test

MHT is among the few phenotypic diagnostic tests recommended by the Clinical and Laboratory Standards Institute for detecting pathogens with increased carbapenem MICs or reduced disk diffusion inhibition zones (Pasteran *et al*., 2015, CLSI, 2015). This test is based on carbapenemase-producing microorganisms inactivating carbapenems, allowing a sensitive marker trait to expand forward towards a disk comprising this dose of medication all along tests performed trait's streak of inoculum. MHT demonstrated high accuracy in identifying carbapenemase makers from classes A and D(Pasteran *et al*., 2015, Doyle *et al*., 2012, Girlich *et al*., 2012, Castanheira *et al*., 2011, Saito *et al*., 2015). Unfortunately, the MHT has a low sensitivity for detecting NDM-producing isolates, with a sensitivity of less than 50% (Doyle *et al*., 2012, Girlich *et al*., 2012, Castanheira *et al*., 2011, Saito *et al*., 2015, Bonnin *et al*., 2012, Pasteran *et al*., 2015).

1.7.2. Double Disc Synergy Test

Standard disk susceptibility test is conducted to assess zones of inhibition within disks containing other different chemicals. A dilution process of about 0.1 McFarland suspension is use to inoculate Mueller Hinton agar plates, and disks containing10 µg of an amoxicillin-clavulanate disk, 30 µg ceftazidime, 30 µg cefepime, and 10 µg imipenem are used for evaluation. The synergy between disks of substrates and inhibitors is always tested, triplication occurs the next day under identical conditions. The distances are measure 20 mm between the disks from the center diameter of the blanc disk to the antibiotic disk used, this is adjusted for each strain based on the widths of the inhibition zonesexamined(Poster Session', Clinical Microbiology and Infection, 2012, Cavallo *et al*., 2007, Moya *et al*., 2009, Hocquet *et al.,* 2011).

1.7.3. Combined Disc Diffusion Test

The CDT compares the zones of inhibition obtained with and without EDTA IPM disks (Yong *et al.,* 2002). Cephalosporin alone (cefotaxime, ceftazidime, cefepime) and in combination with clavulanic acid discs are used in each test. The inhibition zone surrounding the cephalosporin disc when paired with clavulanic acid is contrasted to the zone of inhibition within the disk when the cephalosporin is used alone. If the inhibition zone diameter with clavulanic acid is 5 mm greater than without, the test is positive. Because of their convenience and low cost, combined disk (CD) synergy tests are commonly employed. Changes in carbapenemase-producing bacterial populations, on the other hand, could jeopardize the assays' results(Heba *et al*., 2021, Giakkoupi *et al*., 2009, Zioga *et al*., 2010, Meletis *et al*., 2010, Zagorianou *et al*., 2012, Tsakris *et al*., 2011, Miriagou *et al*., 2013).

1.7.4. E-test

This is aminimum inhibitory concentration methodthat employs a thin strip with a consistent antibacterial agent gradient on one side and a quantifiable inferential magnitude on another. To calculate the minimum inhibitory concentrations in E-test, the antibacterial potency expressed on the testing kit at its intersection with the expansion in zone of inhibition is read. If the MIC results that is found is consistently correlating with those obtained using the less expedient agar dilution technique, the ease of use of the E-simplicity Test would be a significant benefit. (Other Helicobacter', Helicobacter, 2003, Saxena and Gomber, 2016, Marley, 1995).

1.7.5. Molecular Test

This method employs primer-mediated enzymatic expression of DNA to create a new strand that is complementary to the specific target sequence(De Vos *et al*., 1997; Qin *et al*., 2003; Anuj *et al*., 2009). To overcome the existing existing challenges, multiplex-PCR parallel to testing for more than one gene sequence may be used. Multiplex-PCR can provide internal control systems, reduce reagent costs, conserve important samples, and more effectively determine template quality and quantity(Edwards and Gibbs, 1994; Elnifro *et al*., 2000).

SECTION TWO: MATERIALS AND METHODS

2.1. Study Group

The investigation was done in Near East University, this was centered on the out and in patients of Near East University Hospital. The research was performed in the MICROBIOLOGY LABORATORY of Near East University Hospital. The study samples were taken from diverse diagnostic specimens of hospitalized patients from various departments of the hospital, including patients from the In and Outpatients' wards, the Intensive Care Unit wards, the Coronary Care Unit wards, the Emergency unit wards, and various general wards (Pulmonology, oncology, neurology, gastroenterology, cardiology, general wards of male and female etc.).

2.2. Tools and Equipment

- Petri-dish
- Automatic pipette (Gilson Pipetman.Dk60063, Biyomedikal 2179. Made in France)
- Wire loop
- Dispenser (Dispensette Brand. Made in Germany)
- Weighing balance (Shimadzo. ELB300. Biyomedikal 2205)
- test-tubes
- Vitek 2-Compact system (Biomerieux)
- Vortex-genie 2 (VELP Scientifica. Code F20220176. Made in Europe)
- Autoclave (model OT40L. Miive Steam Art. Biyomedikal 2189)
- Spatula
- Masking tape
- Test tube wrack
- Photoelectric calorimeter (Densichek Plus. Biomerieux)

- Cotton wool
- Biosafety cabinet (HERASAFE KS. Biyomedikal 2172)
- Incubator (Heraeus Thermo Scientific. Biyomedikal 2184)
- Dish washer (LANCER)
- Refrigerator (SANYO Medicool. Biyomedikal 2170)
- Freezer (SANYO Biomedical 2165)

2.2.1. Kits and Chemicals

- Imipenem disc(Bioanalyse limited Ankara/Turkey)
- Blank disc(Bioanalyse limited Ankara/Turkey)
- Saline solution (Biomerieux SA. REF. V1204. LOT. C0265. France)
- EDTA (Sigma, Germany)
- Mueller Hinton Agar (Merck KGaA 64271. VM779137714. 1.05437.0500. Germany)
- EMB Agar (Dickinson and Company Sparks. Becton MD 211221. France)

2.2.2. Microbiological Media

Media used in this work include; Muller Hinton agar, and EMB agar. 38g of Muller Hinton agar was weighed for 1000mL of distilled water and 15g of EMB agar was weighed for 500mL of distilled water, which were each placed in a sterile conical flask of 1000mL and 500mL respectively, after properly mixed, the solution was sterilized with an autoclave for 15 minutes at 15lbs pressure (121°C). It was allowed to cool for 40°C and was poured into a partitioned petri dish to gel in the case of the EMB agar and for the Muller Hinton agar the sterile media was poured in a non-partitioned petri dish and allowed to gel for isolation of the bacteria.

2.2.3. Solutions

2.2.3.1. EDTA (0.5M, pH: 8.0)

Distilled water of 100 mL was used to dissolve 18.61 g of disodium EDTA to make 0.5 M EDTA. The pH of the solution was adjusted to 8.0 using NaOH. Sterilization by autoclave and stored at 4°C while in use.

2.3. Method

2.3.1. Evaluation of Microbiological Properties

Pure cultures isolates were stocked and stored in a refrigerator (at -20°C). The isolates were characterized by cultural, morphological and biochemical tests, using a Vitek 2 system, which include Gram stain, motility, catalase test. The colony count was performed on the various culture media used. Discrete colonies appearing on the plate after appropriate incubation were counted and recorded. The total bacteria counts were obtained by counting discrete colonies on EMB agar and Muller Hinton agar.

2.3.1.1. Identification and susceptibility ofisolates antimicrobial test

ID-GNB cards were used to identify isolates using the VITEK 2 system, 64-well plastic ID-GNB cards were used which comprisesof forty-oneexaminations, likeeighteen sugar assimilation tests, eighteen sugar fermentation tests, two decarboxylase tests, and three miscellaneous tests for urease, utilization of malonate, and tryptophane deaminase.

Susceptibility testing using this system were conducted using AST cards, as directed by the manufacturer. The following antimicrobial agents (as dehydrated compounds) are present on the 64-well AST card at the concentrations indicated: gentamicin 32 µg per ml,' imipenem, 10 and 16 µg per ml,' meropenem16 µg per ml,' piperacillin-tazobactam128 µg per ml,'cefepime16 µg per ml,' ceftazidime32 µg per ml,' ciprofloxacin4 µg per ml,' Ticarcillin-Clavulanate, Cefoperazone-Sulbactam, Tigecycline, Netilmicin, Nitrofurantoin, Fosfomycin w/G6p, Trimethoprim-Sulfamethoxazole, Colistin; Aztreonam, Ceftriaxone, Ertapenem, Amikacin 30µg, Amoxicillin-Clavulanate (f).

2.3.1.2. Phenotypic Identification of Metallo-beta-lactamases

2.3.1.2.1. Combine Disc Test

After the inoculation of the isolate on the EMB agar it was incubated at 35°C for 24 hours. The pure isolate from the EMB media was inoculated onto the Muller Hinton media using a McFarlanddilution process. The dispenser was calibrated to dispense the saline water at 30mm into each sterile test-tube according to the serial number of the *P. aeruginosa*isolate. This process was done one at a time to prevent contamination. Each of the isolate was picked with a light touch using a sterile swab stick to reduce the density of the isolates in which 0.5-0.63 was the target density for each dilution process. The swab stick with the inoculum is deepened into the test tube with the saline water and was carefully steered and emulsified, the tube is then vortexed to ensure a better reading by the calorimeter, after inserting the tube in the calorimeter the reading was written down and the swab stick was disposed, a new swab stick was use to streak the isolate from the test tube on to the Muller Hinton media labelled according to the two methods of experiment carried out.

It is based on the expansion of the imipenem inhibition zone in the presence of EDTA. Two imipenemdiscs were placed in the plate. After adding EDTA (10 μL) to one of them, the inhibition zone diameter difference was evaluated. Imipenem/EDTA to which 0.1 M EDTA solution is added,the zone of inhibition of the imipenem disk alone was 4 mm from the diameter of hectare solution. The inhibition zone of the imipenem/EDTA diskto which hectare solution was added was 7 mm broader than the inhibition zone seen in the diameter of the imipenem disk per MBL positive bacterial isolate accepted.

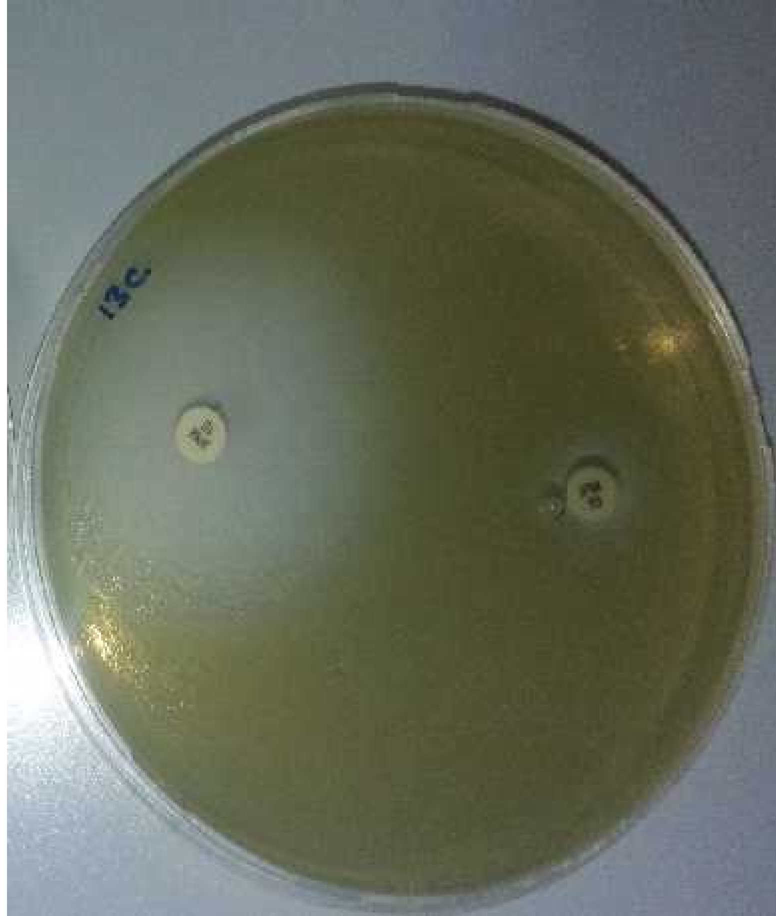

Figure 1. CDT inhibition zone of the phenotypic sensitivity test.

2.2.1.2.2. Double Disk Synergy Test

EMB agar was used to inoculate the isolate and incubated at 35°C for 24 hours after inoculation. Using a McFarland dilution technique, the pure isolate from the EMB media was inoculated onto Muller Hinton media. According to the serial number of the *Pseudomonas spp*., the dispenser was calibrated to administer saline water at 30mm into each sterile test-tube. To avoid contamination, each process was completed one at a time. Each isolate was picked lightly with sterile swab sticks to lower the density of the isolates, with a target density of 0.5-0.63 for each dilution operation. The inoculum swab stick was carefully steered and emulsified into the test tube with saline water, the tube was then vortexed to ensure a better reading by the calorimeter, the reading was written down and the swab stick was discarded, a new swab stick was used to

streak the isolate from the test tube on to the Muller Hinton media labelled according to the two methods of experiment carried out. A pair of sterile syringes were used to carefully remove the discs from the isolate's bottle after inoculation (Poster Session', Clinical Microbiology and Infection, 2012, Poster', Clinical Microbiology and Infection, 2011).

Imipenem disc is inserted 20 mm away from the center of the blank disc that has been previously prepared.10 µL, 0.5 M EDTA on after adding the imipenem disk inhibition zone. Its expansion towards the empty disc with added EDTA was evaluated as a zone of synergic inhibition.

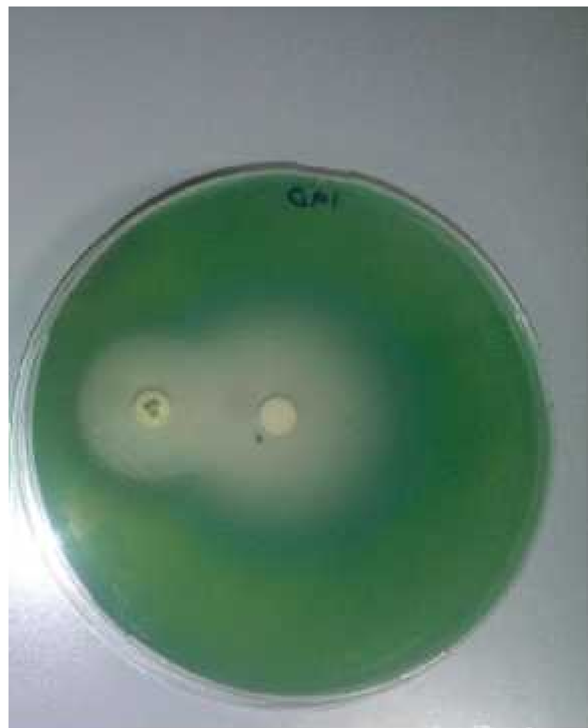

Figure 2. DDST inhibition zone of the phenotypic sensitivity test.

2.4 Statistical Analysis

Qualitative and quantitative data values along with the percentage and mean ±standard deviation (SD) is represented as frequency. The Chi-square test is tested as appropriate on the association between two or more variables. Pictorial explanations of the major results of the study were rendered using an appropriate statistical graph.SPSS version 25.00 statistical packages were used

for all statistical analysis (SPSS Inc. Chicago, IL, USA). Significance level was accepted to be 0.05.

2.5 Ethical Approval

The institutional review boards of near east university hospital gave their approval to the study protocol(2021/88). (Appendix-1)

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SECTION THREE: RESULTS

The result of this research work shows that 65 clinical sample were collected from the Near East University Hospital between the months April and August 2021. The study subjects were made up of patients of different age groups and gender attending the various departments of the hospital for *P. aeruginosa*. The carbapenemases cross-tabulation shows that there is no significant value between P-values of the clinical genders. The crosstabulation of In/Out patients carbapenemase has no significant difference between the P-values, this is also seen in the Carbapenemase Cross tabulation of the Ages of the patients. The CDT and the DDST had no significant difference in the P-values of both the negative and positive result derived from each phenotypical analysis.

Table 3.1. Distribution of the gender-based of *P. aeruginosa*samples

Gender	Number	%	Valid (%)	Cumulative (%)
Male	36	55.4	55.4	55.4
Female	29	44.6	44.6	100
Total	65	100	100	

The minimum age difference of patients from whom clinical samples were collected ranges from 2 – 95 years of age. Statistical Analysis of the collected samples reveals the following frequencies and percentages (%). Twenty-two(33.8%) samples of urine, 16(24.6%) samples were aspirate, 10(15.4%) samples were sputum, 11(16.9%) samples were wound/pus, 5(7.7%) samples were catheter and 1(1.5%)sample was blood.*P. aeruginosa* cases were predominant in urine samples.

Table 3.2. Distribution of the different clinical samples collected for the study.

Samples	Number	%	Valid (%)	Cumulative (%)
Urine	22	33.8	33.8	33.8
Aspirate	16	24.6	24.6	58.5
Sputum	10	15.4	15.4	73.8
Wound/Pus	11	16.9	16.9	90.8
Catheter	5	7.7	7.7	98.5
Blood	1	1.5	1.5	100
Total	65	100	100	

Based on the various department from which clinical samples were collected, Intensive Care Unit Department (ICU) shows the highest number of cases (21.5%) while Dermatology Department shows the lowest number (1.5%).

Table 3.3. Distribution of *P. aeruginosa* from the various hospital departments.

Department	Number	%	Valid (%)	Cumulative (%)
Infectious Diseases and Clinical	8	12.3	12.3	12.3
Laboratory	2	3.1	3.1	15.4
General Surgery	1	1.5	1.5	16.9
Dermatology	7	10.8	10.8	27.7
Cardiology	10	15.4	15.4	43.1
Chest Diseases	2	3.1	3.1	46.2
Ear Nose and Throat	3	4.6	4.6	50.8
Emergency Service	14	21.5	21.5	72.3
Intensive Care	8	12.3	12.3	84.6
Internal Medicine	5	7.7	7.7	92.3

Urology	5	7.7	7.7	100
Pediatrics	65	100	100	
Total				

Fourth-three (66.2%) samples were collected from in-patients whereas 22 samples (33.8%) were out-patients.The carbapenems inpatients and outpatients Cross-tabulation also shows no significant value

Table 3.4. Distribution of inpatient and outpatient samples

Patients	Number	%	Valid (%)	Cumulative (%)
Inpatients	43	66.2	66.2	66.2
Outpatients	22	33.8	33.8	100
Total	65	100	100	

The Sensitivity-Resistance Percentages (%) of *P. aeruginosa*list as tables above.

Table 3.5. The Sensitivity-Resistance Percentagesantimicrobial of *P.aeruginosa* strains

Amikacin	Number	%	Valid (%)	Cumulative(%)
Sensitive	56	88.9	88.9	88.9
Resistance	7	11.1	11.1	100
Total	63	100	100	
Gentamicin	**Number**	**%**	**Valid (%)**	**Cumulative (%)**
Sensitive	50	76.9	76.9	76.9
Resistance	15	23.1	23.1	100
Total	65	100	100	

Imipenem	Number	%	Valid (%)	Cumulative (%)
Sensitive Resistance Total	41 23 64	64.1 35.9 100	64.1 35.9 100	64.1 100

Meropenem	Number	%	Valid (%)	Cumulative (%)
Sensitive Resistance Total	50 15 65	76.9 23.1 100	76.9 23.1 100	76.9 100

Ceftazidime	Number	%	Valid (%)	Cumulative (%)
Sensitive Resistance Total	47 18 65	72.3 27.7 100	72.3 27.7 100	72.3 100

Cefepime	Number	%	Valid (%)	Cumulative (%)
Sensitive Resistance Total	43 19 62	69.4 30.6 100	69.4 30.6 100	69.4 100

Aztreonam	Number	%	Valid (%)	Cumulative (%)
Sensitive	20	37.7	37.7	37.7
Resistance	33	62.3	62.3	100
Total	53	100	100	

Piperacillin-Tazobactam	Number	%	Valid (%)	Cumulative (%)
Sensitive	47	72.3	72.3	72.3
Resistance	18	27.7	27.7	100
Total	65	100	100	

Ciprofloxacin	Number	%	Valid (%)	Cumulative (%)
Sensitive	43	67.2	67.2	67.2
Resistance	21	32.8	32.8	100
Total	64	100	100	

Netilmicin	Number	%	Valid (%)	Cumulative (%)
Sensitive	24	54.5	54.5	54.5
Resistance	20	45.5	45.5	100
Total	44	100	100	

Laboratory analysis reveals that 25 of the total samples collected exhibit carbapenemases activities, 52% were positive in Combined Disk Test (CDT), whereas 48% was analyzed in DDST.

Table 3.15. Results of combined disk test and double disk synergy test.

CDT	Number	%	Valid (%)	Cumulative (%)
Negative	12	48	48	48
Positive	13	52	52	100
Total	25	100	100	

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DDST	Number	%	Valid(%)	Cumulative (%)
Negative	13	52	52	52
Positive	12	48	48	100
Total	25	100	100	

Twenty-five carbapenemase positive isolates; It was isolated from 16 male (44.0%) and 9 female (31%) patients.The P-value for Carbapanemase Gender Crosstabulation of male and female is 0.198, with an odd ratio of 0.313. There were not any statistical differences between gender and carbapanemase. According to 25 carbapenamase isolates; 16 patients were inpatients and 9 patients were outpatients. The carbapenemase crosstabulations of inpatients and outpatients show P-values (Fisher's Exact Test) = 0.489 and odd ratio = 0.793, respectively. There were not any statistical differences between inpatient/outpatients and carbapanemase.

SECTION FOUR: DISCUSSION

Pseudomonas aeruginosa is an opportunistic bacterium that causes a variety nosocomial illness, including pneumonia, bacteremia and urinary infection. *P. aeruginosa's* status as a nosocomial pathogen is mostly influenced by a number of factors, including infectious delivery of broad-spectrum antibiotics, equipment, and the microorganism's natural resistance to a number of antimicrobial medications. Amongst the serious concerns of*P. aeruginosa* is its extraordinary potential to acquire drug resistance quickly. *P. aeruginosa* has a diverse variety of antimicrobial resistance mechanisms that rivals those found in other non-fermentative Gram-negative bacteria, demonstrating the organism's ability to adjust quickly to changes in selective environmental pressure. In the current study, the susceptibility patterns of *P. aeruginosa* isolates were investigated using various antibiotics in limited spectrum according to EUAST recommendations.

Several phenotypic methods for detecting metallo-beta-lactamase-producing bacteria are based on the capacity of metal chelators like EDTA and Thiol-based drugs to impede the MBLs' function. In the absence of crucial genes for carbapenem enzyme elaboration, phenotypic resistance to carbapenems most likely indicates that resistant mechanisms other than MBLs are at work in the isolates. Carbapenem resistance in *P. aeruginosa* may also be mediated through efflux pumps, outer membrane impermeability, target site alteration, and carbapenemases other than MBLs (Zubair and Iregbu, 2018). In this investigation, we looked at two phenotypic methods for detecting producers of MBLs in imipenem-resistant *P.aeruginosa* strains.

MBL E-test is the most commonly accepted standardized MBL screening test. Many clinical microbiology laboratories adopt alternative vetting process such as the double-disk synergy test (DDST) and the combination disk test due to the high cost and scarcity of E-test strips (CDT) (Amani *et al*., 2017). Despite the fact that the DDST and CDT assays are way more efficient and less pricey than the MBL E-test, findings have varied based on the methodology utilized, the B-lactam substrates used, the MBL inhibitors used, and the bacterial type examined (Amani *et al*., 2017, Pico *et al*., 2008 and Ranjan *et al*., 2015).CDT, DDST, and E-test are EDTA-based resistance reduction assays that use chelating chemicals to distinguish metal dependence of

carbapenemase enzymes. However, the test's validity is called into question because EDTA has been proven to have an inhibitory effect on bacterial growth, which could lead to false-positive results (Walsh *et al* 2005).The modified Hodge test, on the other hand, detects solely carbapenemase activity and does not require the addition of EDTA. It does not prove that carbapenemase is metal-dependent (Tan *et al*., 2008). Despite multiple writers' observations, none of these phenotypic tests were optimum due to low sensitivity or specificity (Samuelsen *et al*., 2008).

Despite its low specificity, some workers found CDT to be suitable for screening because it is a straightforward method and easy to interpret (Yong *et al*., 2002, Berges *et al*., 2007). Others, on the other hand, thought DDST was better and more dependable than CDT or the modified Hodge test (John *et al*., 2011). CDT had a greater association with the imipenem disk diffusion screening method, according to our findings.

Because CDT assesses the rise in inhibition zone above a cutoff value, there is less potential of subjective variation. DDST interpretation, on the other hand, is highly subjective. Temperature, aeration, pH, and media thickness are all parameters that limit CDT and DDST. Diffusion, on the other hand, affects the synergy between imipenem and imipenem+EDTA disks. To show a synergy, EDTA must diffuse near to the imipenem disk and reach a concentration with effective chelating activity (Kali *et al*., 2013). This could explain the discrepancy between CDT and DDST outcomes in our investigation.

In this investigation, 25 of 65 clinical isolates of *P. aeruginosa* were reported to be resistant to imipenem (Micron2018.com).A CDTand DDSTwere used to examine the MBL generation of imipenem-resistant isolates. For detecting MBL generation, the combination disk test has a sensitivity and specificity of around 52 percent, compared to 48 percent for the double disksynergy test. CDT with imipenem + EDTA was (Anwar *et al*., 2016) found more effective than the DDST with a threshold of >7 mm for imipenem and EDTA, CDT was also found to be highly sensitive for identifying MBL in *P. aeruginosa*, with positive and negative results being more easily distinguished.According to Sachdeva *et al*., 2020, the CDT for MBL generation is straightforward to perform, and the components employed are inexpensive, simple, and readily accessible, making it a useful diagnostic test for standard clinical laboratories.

P. aeruginosa was shown to be more prevalent in male clinical patients than female clinical patients, this is in line with the findings of Nedeem *et al*., 2019 and Amani *et al*., 2017. Furthermore, this research aligns with Nedeem *et al*., 2019's findings, which demonstrate that *P.aeruginosa* was predominantly isolated from urine samples. Both studies continued in accord about the antibiotics employed, concluding that *P.aeruginosa* was susceptible to all of the antibiotics utilized in this study.

The statistical analysis performed in comparison to the research works listed above has no significant value, but it does show a potential rise in MBL production in *P.aeruginosa*. One among the most serious issues regarding *P.aeruginosa* is its exceptional prowess tocause antimicrobial resistance quickly. *P.aeruginosa* has a diverse array of antimicrobial resistance mechanisms that rivals those of other non-fermentative Gram-negative pathogens, demonstrating the organism's ability to be able to react swiftly to changes in certain dimensions of environmental stresses (Amani *et al*., 2017, Zafer *et al.,* 2014).

The imipenem disk diffusion screening separated 65 study isolates into two groups: 25 imipenem resistant isolates (22.4 %) and 40 imipenem sensitive isolates (77.6%). This test was used as a screening test to identify possible MBL producing strains for further testing. This result was consistent with a study by Kali *et al*., 2013 that looked at the imipenem disk diffusion screening and found that the isolates were divided into two groups: 11 (22.4 %) imipenem resistant isolates and 38 (77.6%) imipenem sensitive isolates.As documented in earlier research, ceftazidime resistance is more prevalent in Enterobacteriaceae, where MBL generating strains can have low carbapenem MICs and look sensitive on disk diffusion (Walsh et al 2005, Lee et al 2003). Ceftazidime resistance was not evaluated for the first screening because this investigation only looked at *P. aeruginosa* isolates (Morita *et al*., 2012, Lee *et al* 2003).Tsakris *et al*. (2009) found 100% resistance to ceftazidime, cefepime, carbapenems, amikacin, netilmicin, and ciprofloxacin in VIM-2 type MBLPA, although gentamicin and piperacillin-tazobactam resistance was only 44% and 47%, respectively. In a recent Indian study, MBL-PA resistance to imipenem, gentamicin, ciprofloxacin, netilmicin, piperacillin, and amikacin was 77.5 percent, 72.1 percent, 67.3 percent, 57.7%, and 56.1 percent, respectively (John *et al*., 2011). De *et al*. (2010) found 100% resistance to all aminoglycosides, beta-lactams, and quinolones in a separate investigation.

These percentage resistances differ from those found in this study, in which all of the medicines tested were moderately resistant, with the exception of aztreonam (62.3%).

These regional variations in susceptibility patterns, according to De *et al*., 2010, reflect the antibiotic practices used in regional hospitals. During the study period, ICU had only four *Pseudomonas aeruginosa* isolates obtained, and all of them exhibited sensitivity to imipenem and most antipseudomonal medications, in contrast to the frequent observation of high prevalence *P. aeruginosa* with multidrug resistance in diverse investigations. In contrast to this study, ICU had 14 *Pseudomonas aeruginosa* isolates, which was the highest department among the other departments in which *P. aeruginosa* isolates were collected in Near East Hospital with 21.5 percent during the study period.This finding is consistent with the findings of Guvenir *et al*., 2021 and Ami *et al*., 2008, who discovered that 21% of *P. aeruginosa* isolates from ICU patients contain MBL enzyme, and Tanzinah *et al*., 2010, who found that 25% of isolates have MBL enzyme. These findings, on the other hand, contradict Johann *et al*., 2005and Shukriyah, 2013, who found that 66.66%and 69% of isolates contain MBL enzyme respectively. Our findings were similarly consistent with those of Hallem *et al*., 2011, who discovered that in Tehran, Iran 28% of *P. aeruginosa* isolates were producers of MBL. Using a combination Imipenem-EDTA disc technique, Johann *et al*., 2005 discovered that in a mostly centralized laboratory in Canada 30% of *P. aeruginosa* isolates were producers of MBL.

Several publications have regularly documented high mortality and multidrug resistance among ICU patients with *P. aeruginosa* infection. Excessive use of broad-spectrum antibiotics, invasive procedures, concomitant septicemia, and increased comorbidities among ICU patients could all be contributing factors (De *et al*., 2010, Wisplinghoff *et al*., 2004). In our case, however, the trial was only a few weeks long, and all *P. aeruginosa* isolates were susceptible to routinely used anti-pseudomonal medicines. As a result, these *P. aeruginosa* isolates were treatable. Furthermore, zero fatality rates in these patients could be explained by strong infection control methods and judicious antibiotic administration in therapy in the near east hospital.

Metallo-Beta-Lactamase producing *Pseudomonas aeruginosa*appeared to have the highest resistance in aztreonam (62.3%), followed by ciprofloxacin (32.3%). Furthermore, carbapenems appeared to be moderate in resistance such as imipenem (35.4%) and meropenem (23.1%). This contradicts Amani et al., 2017's study, reviewed that*P. aeruginosa* that produces MBL has the

highest rate of amikacin resistance (86.7%), Piperacillin and Ciprofloxacin come in second and third, respectively, with 80% of the market share., and imipenem and meropenem with 73.3 percent and Only 20 (37.7%) of the isolates were susceptible to aztreonam and conformed to this criterion in the current research, which found considerable hostility to monobactams such as aztreonam. Zafer *et al*.,2014 found 45.1% ofaztreonam reduction in resistance.

The study results on the phenotypic methods were discovered to be insignificant (the p-value for CDT = 0.000, odd ratio = 0.000, and the p-value for DDST = 0.000, odd ratio = 0.000), which is consistent with Kali et al., 2013 who found no significant value due to the length of the study period (the p value is 0.177360 and that of odd ratio is 0.337500) in determining the risk of MBL-PA infection.

SECTION FIVE: CONCLUSION

Conclusion

Finally, our findings support the use of phenotypic approaches for detecting MBL production in *P.aeruginosa* that are both simple and sensitive. Most notably, the combined disk test approach is mostresponsivetothe detection of carbapenem-susceptible MBL-producing *P.aeruginosa* isolates, which are becoming more common worrying Gram-negative trait.

Recommendation

Once *P.aeruginosa* has been isolated from the sample, I recommend that you work quickly to determine the cause of the infection and select an appropriate empirical antibiotic.

Appropriate *P.aeruginosa* management should be viewed as a substantial problem for doctors.

In terms of the level of aztreonam resistance found in our study, I recommend additional research be done using the aztreonam disk in phenotypic MBL detection to best explain the presence of different mechanisms of aztreonam resistance.

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Appendix-1

YAKIN DOĞU ÜNİVERSİTESİ
BİLİMSEL ARAŞTIRMALAR ETİK KURULU

ARAŞTIRMA PROJESİ DEĞERLENDİRME RAPORU

Toplantı Tarihi : 25.02.2021

Toplantı No **:** 2021/88

Proje No **:**1282

Yakın Doğu Üniversitesi SHMYO öğretim üyelerinden Doç. Dr. Meryem Güvenir'in sorumlu araştırmacısı olduğu, YDU/2021/88-1282 proje numaralı ve **"Çeşitli Klinik Örneklerden İzole Edilen Pseudomonas Aeruginosa Suşlarında Metallo beta laktamaz Aktivitesinin Araştırılması"** başlıklı proje önerisi kurulumuzca online toplantıda değerlendirilmiş olup, etik olarak uygun bulunmuştur.

Prof. Dr. Rüştü Onur

Yakın Doğu Üniversitesi

Bilimsel Araştırmalar Etik Kurulu Başkanı