Prevalence of Neutropenic Development in Gram-negative Bacterial Infections Treated with Piperacillin-Tazobactam in a Near East Hospital in Northern Cyprus

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Prevalence of Neutropenic Development in Gram-negative Bacterial Infections Treated with Piperacillin-Tazobactam in a Near East University Hospital in Northern Cyprus

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My Advisor

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APPROVAL

This study passed the thesis committee with a master thesis in medical and clinical microbiology.

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Declaration

I declare that I am the person who wrote this thesis study and that I have approached every aspect of its execution, from planning to writing, with honesty and adherence to ethical standards. Every piece of data in this thesis was gathered in compliance with ethical and academic guidelines. Everything I said and did that wasn't directly related to this thesis topic has been appropriately cited and added to the list of references. In addition, I have not done anything throughout the course of this research or the preparation of this thesis that would breach copyright or patent rights.

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ABSTRACT

Background: Piperacillin-tazobactam exhibits a wide range of antibacterial effects, specifically targeting enteric bacteria and other Gram-negative microbes. However, specific types of Enterobacter spp. bacteria exhibit resistance to various treatments. Pseudomonas aeruginosa is vulnerable to the piperacillin-tazobactam combination, which kills gram-negative bacteria. Thus, study assessed prevalence of neutropenic development in gram-negative bacterial infections treated with piperacillin-tazobactam in a Near East Hospital in Northern Cyprus.

Methods: This study design is intended to survey the population by selecting samples to be evaluated. A retrospective study was chosen for this study because it is effective. This study will investigate the prevalence of neutropenic development in gram-negative bacterial infections treated with piperacillin-tazobactam between January 2016 and December 2023 using samples of inpatients and outpatients from various hospital departments. The study investigated Gram-negative bacteria infections in patients treated with piperacillin-tazobactam, using 69 samples from 18+ individuals at Near East University Hospital. The investigation involved departments including anaesthesia, ICU, neurology, orthopaedics, urology, and emergency medicine.

Results: The observed neutropenia cases were 5 (7%) Two of these patients were women, while three of them were men. Three of these patients were in the age group of 70–79; one of them was 50–69, and the other was in 18-35. Previous publications have documented three cases of neutropenia in adults caused by piperacillin-tazobactam. The therapeutic duration in these instances was ≥17 days, as shown in a previous study.

Conclusion: The study indicated that the duration of neutropenia in patients treated with piperacillin-tazobactam was less than 10 days. However, one patient had neutropenia for more than 35 days after using the drug.

Additionally, neutropenia was observed in patients who had been using TZP for more than 18 days over the course of the research, which included 41 patients who had bone-related infections.

The cumulative treatment dosage for the patient was calculated at 4500 mg. According to the collected data, previous studies have consistently identified the total dose and duration of TZP therapy as the most commonly identified risk factors for the development of these side effects.

Keywords: Neutropenia. Piperacillin-tazobactam. Gram-negative Infection

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CHAPTER ONE

1.0 Introduction

Neutropenia is a medical disorder where there is a decrease in the number of neutrophils in the marginal pool to less than 1,500 cells per cubic millimeter. The absolute neutrophil count (ANC), which measures the quantity of neutrophil granulocytes in the blood, determines the condition's classification. The classification may vary from low to moderate to severe. An ANC (neutrophil count) ranging from 500 to 1000 cells/mm3 indicates moderate neutropenia, whereas a level between 1000 and 1500 cells/mm3 indicates mild neutropenia. Severe neutropenia is characterised by an absolute neutrophil count (ANC) below 500 cells/mm3. Neutrophils are most commonly seen in the bone marrow, where they are either actively dividing or have finished their maturation. A low neutrophil count, which is the defining feature of neutropenia, increases susceptibility to infections substantially. The duration and severity of neutropenia may have an impact on the likelihood of infection (1).

According to the specified criteria, the majority of laboratories would categorise neutropenia in newborns as an absolute neutrophil count (ANC) that is less than 2.5 109/L and in adults as an ANC that is less than 1.5 109/L. (2). Various strategies have been used to conceptualise the differential diagnosis of neutropenia. Neutropenia is categorised into three groups according to the absolute count of neutrophils: mild (ANC 1.0–1.5 109/L), moderate (ANC 0.5-0.90 109/L), and severe (ANC 0.5 109/L). The likelihood of infection is greatest in the severe classification (3). There are numerous conditions that can produce neutropenia, and these factors can be broadly classified as either neoplastic or nonneoplastic. Two classification systems are capable of recognising this. When neutropenia has a neoplastic etiology, the presence of abnormalities in other lineages can provide information about the precise etiology of the disease (4).

Neutropenia is linked to an increased likelihood of death; hence, it is crucial to promptly and efficiently administer empirical antibiotic treatment (5). The susceptibility patterns of numerous species in patients with febrile neutropenia have experienced a major global alteration in recent decades. *Staphylococcus aureus* became the dominant harmful bacteria infecting these people in

the 1950s and early 1960s. *Escherichia coli, Pseudomonas aeruginosa,* and *Klebsiella* species are all Gram-negative bacilli that subsequently ensued. (6) However, Gram-positive organisms have been more prevalent in this population since the 1980s. Non-fermenter Gram-negative rods, especially *Acinetobacter* species, have been identified as the pathogens in these people (7).

Moreover, the rise in the quantity of drug-resistant bacteria, which are resistant to many antibiotics, including both Gram-negative & Gram-positive bacteria, has been associated with the extensive use of broad-spectrum antibacterial. Hence, it is imperative to opt for an alternative empirical treatment, taking into account the current local strains and their patterns of resistance. Because other beta-lactamase drugs are becoming less effective, carbapenem is now generally seen as a good choice for empirical monotherapy in febrile neutropenic patients in most healthcare facilities. Vancomycin is the preferred initial treatment for suspected Gram-positive bacterial infections that are resistant to penicillin and methicillin (8).

Antibiotics of Beta-lactams are a group of very effective antibiotics that contain a beta-lactam ring. Key molecular structures have the potential to effectively target a diverse range of bacteria. These antimicrobial medications are commonly used to treat bacterial illnesses around the world. (9) They are the most often used antibacterial agents due to their safety, efficacy, and low cost (10). The molecular structure of beta-lactam antibiotics contains a beta-lactam ring. Ampicillin, amoxicillin, and piperacillin belong to the class of penicillin antibiotics. Cefazolin and cephalexin are examples of cephalosporin antibiotics. Aztreonam is a representative of monobactam antibiotics. Meropenem and imipenem are examples of carbapenem antibiotics. Cephalosporins are classified into five generations, namely first, second, third, fourth, and fifth. Examples of cephalosporins include cefotetan, cefoxitin, cefuroxime, ceftriaxone, cefotaxime, cefixime, and cefepime. Beta-lactam antibiotics are antimicrobial agents that have the ability to kill bacteria by blocking the synthesis of their cell walls (11).

The presence of a bicyclic ring structure in their constituent parts distinguishes a large class of chemical compounds known as penicillins. This structure consists of a β-lactam ring with four members, which is associated with a thiazolidine ring comprising five members (12).

Piperacillin and other semi-synthetic penicillins have been manufactured through the modification of the 6-aminopenicillanic acid 6-amino group (13). The specific substituent used determines penicillin properties such as lactamase resistance, acid stability, and antibacterial activity spectrum (14). Piperacillin, a ureidopenicillin, is synthesised by appending a hydrophilic heterocyclic group to the -amino group of ampicillins, which is a derivative of D (-)-amino benzyl-penicillin. This part makes piperacillin more effective against gram-negative bacteria by making it more likely to bind to penicillin-binding protein (PBP)-3. As a result, it demonstrates a wider range of effectiveness. Tazobactam possesses a chemical composition that closely resembles that of sulbactam, which is likewise an inhibitor of -lactamases. It is a compound derived from penicillanic acid, specifically a sulfone derivative (15).

Piperacillin resists B-lactamases more effectively when tazobactam is added to it. *Staphylococci*, *Enterobacteriaceae*, *Haemophilus influenzae*, and other bacteria produce toxins. Tazobactam has no inherent antibacterial activity (16).

The conventional therapy for serious infections now involves antibiotics that contain b-lactam. This is why they have such an exceptional safety record and are so effective. Adverse pharmacological responses are possible occasionally when using these medications, nevertheless. For the purpose of explaining these consequences, several theories have been put forth. The most prevalent is assumed to be the immune-mediated mechanism (17).

B-lactam antibiotics are widely regarded as being both safe and efficacious in the treatment of a wide spectrum of bacterial diseases in both paediatric and adult populations. Lactams rarely induce significant adverse effects. According to recent research, patients receiving prolonged piperacillin-tazobactam treatment at medical facilities have experienced skin rash, fever, and neutropenia, which is characterised by a decrease in white blood cells. The scientific literature extensively records the link between the use of lactam antibiotics and the incidence of neutropenia, notwithstanding its infrequency (18).

Beta-lactamases catalytically break down the beta-lactam ring structure seen in antibacterial medicines. These enzymes are present in gram-positive as well as gram-negative bacteria.

Beta-lactamase enzymes render beta-lactams inactive, so they serve as the primary catalyst for antimicrobial resistance. Lately, there has been significant focus on the advancement of beta-lactamase inhibitors. These are bioactive substances that have limited antibacterial effects but possess the capability to permanently attach to beta-lactamase enzymes (19). There are chemicals called clavulanate, sulbactam, and tazobactam that stop beta-lactamase enzymes from working. This makes some beta-lactam antibiotics more effective against bacteria. Piperacillin, a partially synthetic ureidopenicillin, demonstrates a wide range of effectiveness against bacteria that are gram-positive, gram-negative, and anaerobic. *Enterobacteriaceae*, which contain beta-lactamases and other plasmid-mediated bacteria, can degrade piperacillin. Tazobactam and piperacillin have been mixed to make them more effective at killing bacteria. Tazobactam stops beta-lactamases from working, which are enzymes that bacteria make (20).

Previous studies suggest that the mixture of a B-lactamase inhibitor with a B-lactam antibacterial can have a major effect. During the year 1991, Berlin hosted the 17th International Congress of Chemotherapy. Dr. Wise presented a portion of the findings that are currently on display. The results show that beta-lactam antibiotics work much better when tested against types of bacteria that can make beta-lactamase (21).

The widely used ureidopenicillin piperacillin has been linked to the newly created beta-lactamase inhibitor tazobactam in a way that makes them work better together. Besides that, tazobactam only slightly blocks Class 1 beta-lactamases, which is another benefit of its strong enzyme-blocking abilities (22). Utilising the Richmond-Sykes classification, tazobactam has demonstrated that various types of beta-lactamases, including Types I and VI, are present in *Klebsiella* pneumoniae, which is the causative agent of extended-spectrum beta-lactamase synthesis (23).

Over the years, academics have discussed the effectiveness of combining antimicrobial therapies for Gram-negative bacteria (GNB) infections, with studies showing diverse and occasionally contradicting results. People who support combination therapy say that it works better because it uses two antimicrobial drugs with different levels of activity to fight a wider range of pathogens, it stops resistance from developing, the two antibiotics work better together in the lab, and it lowers the death rate. Gram-negative bacteria (GNB)-related serious infections like sepsis, febrile

neutropenia, and severe pneumonia are best treated with combination therapy as a first step. There is a lot of evidence to support this (24).

However, it is widely agreed that, when it comes to infections caused by susceptible GNB, combining different therapies does not provide any further advantage in terms of reducing mortality. Therefore, it is common practice to reduce the use of antimicrobial drugs as soon as cultures reveal the presence of a sensitive bacterium. Regarding the avoidance of resistance, it is a challenging idea to substantiate, as most clinical trials primarily prioritise clinical efficacy and safety (25).

During the last three decades, B-lactam antibiotics have emerged as the primary option for treating a broad spectrum of infections, in particular those that are severe in nature. The primary determinants of this phenomenon were predominantly attributed to their extensive range of coverage and their exceptional safety record (26). Occasionally, there may be instances of unfavorable pharmacological reactions when these medications are used. The prevailing explanation for these negative effects is believed to be an immune-mediated mechanism, although various alternative hypotheses have been suggested. (27) Piperacillin-tazobactam, a type of B-Lactam antibiotic, has the capacity to induce reversible neutropenia in patients receiving prolonged therapy (28).

Piperacillin-tazobactam is a medication that consists of a fourth-generation extended-spectrum penicillin and a beta-lactamase inhibitor. This antibiotic is prescribed to treat infections that range in severity from moderate to severe and have broad-spectrum activity against bacteria that produce beta-lactamase enzymes. Moreover, it exhibits significant efficacy against *Pseudomonas aeruginosa*. Potential negative consequences encompass increased hypersensitivity reactions, neurotoxicity, hepatotoxicity, imbalances in electrolytes and acid-base levels, as well as issues related to bleeding. In addition, beta-lactam antibiotics have been linked to bone marrow suppression, a medical condition that can result in neutropenia, thrombocytopenia, and, in rare cases, hemolytic anaemia (1).

Piperacillin-tazobactam exhibits significant effectiveness against penicillin-resistant bacteria that generate beta-lactamase. This specific combination received authorization for use in the United

States of America in the year 1985. and is often restricted to the management of serious infections that necessitate intravenous therapy (29).

Both generic formulations of piperacillin-tazobactam and the branded product Zosyn are suitable for intravenous delivery. The suggested dosage of piperacillin is 3 to 4.5 grammes, together with 0.375 to 0.5 grammes of tazobactam, to be given every 6 to 8 hours for a duration of 7 to 14 days. Common side effects include cephalalgia, vertigo, emesis, diarrhoea, constipation, cutaneous eruptions, and hypersensitivity responses. Infrequent but possibly severe adverse effects encompass allergic Hypersensitivity responses, Stevens-Johnson syndrome, and chronic epidermal necrolysis are potential adverse effects (30).

Piperacillin-tazobactam works well and is well tolerated when used to treat infections in the lower respiratory tract, the abdomen, the skin, and soft tissues, as well as febrile neutropenia. According to the evidence, piperacillin-tazobactam has greater efficacy than other antibacterial medications. This is particularly accurate when managing individuals who have intra-abdominal infections and febrile neutropenia (31).

Piperacillin is a very effective penicillin that has broad-spectrum antibacterial action against a diverse array of bacterial species. Tazobactam, on the other hand, hinders the function of beta-lactamase enzymes. Both possess the ability to eliminate a wide range of Gram-negative pathogens and several Gram-positive bacteria, such as *P. aeruginosa*, as well as anaerobic pathogens (32).

The Food and Drug Administration granted approval in 1994 for the use of a specific combination of piperacillin mixed with tazobactam. Tazobactam is a beta-lactamase inhibitor that does not kill bacteria (33).

Piperacillin-tazobactam, commonly referred to as PIP/TAZO, has been extensively used in hospitalised patients with severe illnesses throughout the last decade. This pertains to the management of hospital-acquired illnesses caused by bacterial strains that exhibit resistance to primary antibiotics. Gin et al. have written an exceptional review that specifically examines the antibacterial properties of PIP/TAZO as well as the therapeutic applications for its administration in adult patients (34).

Piperacillin-tazobactam exhibits a wide range of antibacterial effects, specifically targeting enteric bacteria and other Gram-negative microbes. However, specific types of *Enterobacter* spp. bacteria exhibit resistance to various treatments. *Pseudomonas aeruginosa* is vulnerable to the piperacillin-tazobactam combination, which kills gram-negative bacteria. *E. faecalis, Listeria monocytogenes*, and *streptococci* are Gram-positive bacteria that are prone to infection. Piperacillin-tazobactam demonstrates efficacy against both Bacteroides fragilis and its cefoxitin-resistant variants. Furthermore, it demonstrates effectiveness against other bacteria that thrive in the absence of oxygen (35).

Piperacillin, a ureidopenicillin, inhibits the growth of *Enterobacteriaceae* and *Pseudomonas aeruginosa* in vitro (36). When taken together, tazobactam and piperacillin stop β-lactamases made by *Enterobacteriaceae* and other aerobic and anaerobic infections, whether they are Gram-negative or Gram-positive. Tazobactam expands the spectrum of bacteria that piperacillin may selectively target, enabling its use in the treatment of many clinical conditions. Piperacillin-tazobactam exhibits reduced effectiveness in laboratory tests against *Escherichia coli* and *Klebsiella* spp. (37).

Tazobactam has shown that it can make piperacillin more effective against a wider range of bacteria, including those that make both chromosomal and plasmid-mediated β-lactamases. Numerous studies have observed a synergistic effect between these two drugs. The compound exhibits reduced activity, ranging from 4 to 32 times, against strains that produce chromosomal enzymes compared to those that produce plasmid-mediated enzymes. It has the ability to eliminate specific strains of Gram-negative pathogens that have chromosomal class I β-lactamases that can be activated, though the strength of their effects varies between them. Several mechanisms of resistance reduce the susceptibility of microorganisms to piperacillin-tazobactam (38).

Piperacillin sodium has bactericidal effects on susceptible bacteria by inhibiting septum formation and cell wall synthesis. Tazobactam is a sulfone derivative of triazolyl methyl ethyl penicillanic acid. In addition, it makes piperacillin work better against more types of bacteria that make beta-lactamase enzymes and bacteria that are not sensitive to penicillins or cephalosporins because of plasmid-mediated mechanisms (39).

Piperacillin tazobactam is recognised for its capacity to counteract life-threatening neutropenia. One may easily disregard this potentially lethal side effect while utilising newer antibiotic formulations in clinical settings. Some reports say that giving piperacillin and the B-lactamase inhibitor tazobactam at the same time may have played a part in the development of acute neutropenia (40).

Since 1946, reports of penicillin-induced neutropenia have been available. The onset of neutropenia appears to be correlated with both the cumulative dosages and duration of penicillin therapy. According to one study, piperacillin-tazobactam has shown a higher occurrence of neutropenia compared to other penicillins (17).

Only 20% to 30% of people with neutropenia and fever have a confirmed infection based on clinical or microbiological evidence [2]. However, infections caused by gram-negative pathogens are becoming more common because of the rise of strains that are resistant to antibiotics. (41).

According to comparative clinical investigations, piperacillin-tazobactam exhibits superior efficacy in comparison to alternative antibacterial regimens. This is particularly true regarding the treatment of patients who have reduced neutrophil counts and intra-abdominal infections. Variability has been observed in the cost assessments of piperacillin-tazobactam, predominantly attributable to discrepancies in the inclusion of particular expenses (42).

1.1 Problem statement

There is a lack of adequate understanding of the prevalence and incidence of neutropenic development in gram-negative infections that are treated with piperacillin-tazobactam to this day. In 1946, not too much time had passed since the medicine was first made available to the public, when case reports of neutropenia connected with penicillin were discovered. Because of this, it is imperative to possess a comprehensive comprehension of the gravity of neutropenia within the context of this specific clinical setting. Despite its widespread application, piperacillin-tazobactam is commonly employed for the management of gram-negative bacterial infections. On the contrary, there is currently no knowledge regarding the incidence or progression of neutropenia in patients undergoing piperacillin-tazobactam treatment.

1.2 The objective of the study

Prevalence of Neutropenic Development in Gram negative Bacterial Infections Treated with Piperacillin-Tazobactam in a Near East University Hospital in North Cyprus

1.2.1 Specific purpose

1. To assess the risk of the development of neutropenia in adults with gram-negative bacterial infections treated with piperacillin-tazobactam
2. To determine the relationship between the duration of TZP treatment and the development of neutropenia.
3. To investigate Prevalence of neutropenic patients treated with piperacillin-tazobactam.

1.3 Research question

- What is the risk of the development of neutropenia in adults with gram-negative bacterial infections treated with piperacillin-tazobactam?
- How to determine the relationship between the duration of TZP treatment and the development of neutropenia?
- What is the prevalence of neutropenic patients treated with piperacillin-tazobactam?

1.4 Significance of the Study

This study investigates how often neutropenic complications happen in people in Cyprus who have gram-negative bacterial infections and are treated with piperacillin-tazobactam. This information will provide a fundamental reference point for any organisation that is ready to take on the issue. When taken together, piperacillin and tazobactam are a powerful combination of β-lactam and β-lactamase inhibitors that efficiently eliminate a wide range of bacteria. This chemical demonstrates effectiveness against most aerobic bacteria. Includes the two types of bacteria as well as anaerobic microorganisms. Furthermore, it showcases efficacy against a broad spectrum of infections that produce beta-lactamases. The efficacy of the piperacillin with tazobactam combination in an 8:1 ratio has been established for the treatment of infections in the lower respiratory tract, intra-abdominal region, urinary system, gynaecological area, and soft tissues of the skin. Moreover, it

is quite effective in treating fever in patients with neutropenia. This evidence was acquired from clinical trials where the participants were adults.

The mix of piperacillin and tazobactam worked much better than the mix of ticarcillin and clavulanic acid for people who got pneumonia in the community, both in terms of clinical and microbiological outcomes. People who were treated with piperacillin-tazobactam were much better at controlling intra-abdominal infections than people who were treated with imipenem/cilastatin, even though the latter was given at a lower dose than what is usually recommended outside of Scandinavia. In general, patients tolerate piperacillin-tazobactam well. Constipation and gastrointestinal disturbances, with diarrhea being the most prevalent adverse effect, are frequently encountered. The study's goal was to find out how often neutropenic progression happens in people with Gram-negative bacterial infections that have been treated with piperacillin-tazobactam.

1.5 Limitations

Sample size: Gram-negative bacteria are rarely observed; therefore, an analysis limited to a single hospital or a small sample may not provide an accurate representation of the broader population or the entire nation. As a result, research conducted during distinct time periods might lack direct comparability.

1.6 Abbreviation

(ANC), Absolute Neutrophil Count

(TZP) Piperacillin-Tazobactam

(ESBL) Extended-Spectrum Beta-Lactamase

(BLBLIs) β-Lactam Beta-Lactamase Inhibitors

(GNB) Gram Negative Bacteria

CHAPTER TWO
LITERATURE REVIEW

2.0 Introduction

Neutropenia is a medical illness defined by a decrease in the total number of neutrophils in the blood, decreasing below 1.5 x 109/l. microscopic cell counting is the established method for doing haematological analysis. Utilising this approach is crucial for verifying anomalies identified by automated cell counters and for examining cell shape. Neutrophil counts below 0.5 x 109/l are indicative of deep neutropenia, while chronic neutropenia is defined as continuing for more than three months, regardless of whether it is intermittent or persistent (43).

Neutropenia is often characterised by an absolute neutrophil count (ANC) that falls below 1500/L. Mild neutropenia is defined as having an absolute neutrophil count (ANC) between 1000/L and 1500/L. An absolute neutrophil count (ANC) between 500/L and 1000/L suggests the presence of mild neutropenia, while an ANC of less than 500/L indicates severe neutropenia. People who were receiving chemotherapy or other immunosuppressive treatments founded this group, which primarily originated in the Caucasian community. These people frequently have more extensive immunosuppression and skin or mucosal lesions that weaken their protective barriers (44, 45).

Additionally, neutropenia can be acquired or inherited. Variability in congenital neutropenia severity consequently influences the infection risk. Autosomal dominant mutations in the ELANE gene are primarily responsible for the most severe form of congenital neutropenia. However, it may also result from aberrations in an extensive array of additional genes, encompassing X-linked, autosomal dominant, and autosomal recessive variants (46, 47).

Viral infections, therapeutic radiation, and medications are the most frequent causes of acquired neutropenia. But acquired neutropenia can arise from a multitude of factors. Potential etiologies of acquired neutropenia include autoimmune disorders, malignant conditions, nutritional deficiencies, and various other factors (48)

2.1 Normal ranges of the laboratories

The absolute neutrophil count (ANC) of healthy individuals is usually within the range of 1.5 to 7.0 109/L in most laboratory settings. While certain laboratories may use established reference ranges, especially those catering to predominantly paediatric patients, it is more advantageous for the laboratory if the reference ranges of the hematologic criteria, including the absolute neutrophil count (ANC), are specific to this particular entity. The variation in the minimum value of the absolute neutrophil count (ANC) is ascribed to the patient's age and race (2).

2.2 The beta-lactam of antibiotics.

Antimicrobials known to cause agranulocytosis include B-lactam antibiotics. They are also prominently involved in the probe. Despite the fact that just a few case reports and literature studies on the subject have been published, the etiology of B-lactam-associated neutropenia is still unknown. A reduction in the quantity of neutrophils present in the bloodstream distinguishes antibiotic-associated neutropenia. This reduction can occur quickly or slowly. There is no commonly accepted definition of drug-induced neutropenia at the moment. There is a link between an increase in cumulative B-lactam antibiotic exposure and an increase in the prevalence of neutropenia, among other things (31).

2.2.1 The operational mechanism.

Piperacillin, like other β-lactam antibiotics, has bactericidal effects by irreversibly inhibiting PBP enzymes. Peptidoglycan transpeptidases (PBPs) are essential enzymes found only in bacteria. They aid in the ultimate step of connecting peptidoglycan strands through cross-linking. Peptidoglycan constitutes a vital constituent of the bacterial cell wall. It serves to safeguard the cell from osmotic rupture, uphold the cell's structure, and play a crucial part in cell development and division. The peptidoglycan strands are made up of N-acetyl glucosamine and N-acetyl muramic acid residues that are arranged in a linear polysaccharide chain (49).

It is made up of five peptide chains, and each one ends with a D-alanine residue. These chains are connected to another D-alanine residue all the way along their length. There is a specific link between the pentapeptide chains and a serine residue that it interacts with when it is added to the active site of PBPs. The coordinates are (49, 50).

These enzymes catalyse the hydrolysis of the last D-alanine residue in a peptide chain and then facilitate the formation of a new peptide bond between the remaining D-alanine residue and the peptide chain of a neighbouring peptidoglycan strand. Piperacillin has its inhibitory effect on these enzymes through the formation of a covalent link with the serine residue located at the active site of the enzymes. Facilitating this process is the similarity in stereochemistry between piperacillin and the D-alanine substrate of PBPs (49).

One effect of this penicilloyl-enzyme complex is that it messes up the normal transpeptidase process, which makes the cell wall lose its shape [13]. The release of autolysins results in bacterial cell wall lysis due to a sophisticated mechanism that occurs after the PBP attaches to the bacterial cell. (16).

2.2.2 Beta-lactam antibiotic history and activity against Gram-negative microorganisms

It has been found that the *Enterobacteriaceae* family, similar to other Gram-negative bacteria, possesses diverse forms of plasmid-mediated -lactamases. In the 1970s and 1980s, when cephalosporins, monobactams, and carbapenems were first being developed, it was seen that some strains of gram-negative bacteria were more likely to become resistant to lactamase. Both chromosomes and bacteria contributed to the development of this resistance (51).

In recent years, there have been discoveries of novel β-lactamases that are transmitted through plasmids and have the ability to degrade third-generation cephalosporins and monobactams. ESBL is the term used to refer to these enzymes. More and more people are exploring the use of β-lactamase inhibitors alongside β-lactam antibiotics to address this problem. These inhibitors operate by tightly binding to the active sites of β-lactamase enzymes in an irreversible manner. This mix keeps the β-lactam antibiotic's natural properties while making it more effective against β-lactamase-producing bacteria (52).

Tazobactam, a potent inhibitor of several lactamases, broadens the antibacterial range of piperacillin, the most potent penicillin against gram-negative bacteria, to encompass a wide variety of bacteria that produce B-lactamase. Studies conducted in a controlled laboratory environment have demonstrated that the combination of piperacillin and tazobactam is highly efficacious against a broad spectrum of Gram-negative bacteria that exhibit resistance to antibiotics (53).

2.3 Piperacillin-tazobactam can be explained

The combination of piperacillin and tazobactam effectively eradicates a broad spectrum of bacteria, encompassing both Gram-positive and Gram-negative strains that are capable of thriving in both aerobic and anaerobic environments. Piperacillin-tazobactam exhibits broad-spectrum antibacterial efficacy in laboratory-based testing. *Enterobacteriaceae* that produce beta-lactamase, as well as numerous extended-spectrum *Enterobacteriaceae*. *Enterobacteriaceae* that produce beta-lactamase, excluding Gram-negative bacilli that have AmpC. There are beta-lactamases. (16).

Tazobactam, a chemical that inhibits beta-lactamase enzymes, is frequently used intravenously in conjunction with piperacillin to treat bacterial infections. This medicine is frequently used to treat hospital-acquired diseases since it has the ability to kill both gram-positive and gram-negative bacteria, including *Pseudomonas aeruginosa*. Its ability to remain stable at room temperature makes it an excellent choice for infusion techniques that need continuous or long-term administration (54).

Piperacillin with tazobactam injection is used to treat bacterial infections that affect many body parts, such as the digestive system, respiratory system, integumentary system, and female reproductive systems. The coadministration of piperacillin with tazobactam leads to the synthesis of an antimicrobial agent. that is classified as a beta-lactamase inhibitor and penicillin. The microorganisms are eliminated through the inhibition of their proliferation. This medication is, however, ineffective against influenza, colds, and other viral infections (55).

Additionally, piperacillin-tazobactam (TZP) is provided as well. Frequent applications include the treatment of febrile neutropenia and other conditions requiring broad-spectrum antibiotics, including nosocomial infections. In addition to hypersensitivity reactions, TZP can cause complications of the gastrointestinal, renal, and hematologic systems. Although reversible neutropenia is a commonly Aside from the aforementioned hematologic adverse impact of TZP, there have also been documented cases of Coombs-positive hemolytic anaemia and thrombocytopenia (56).

Piperacillin-tazobactam (PPZ-TZB) is a medicine that has two parts: tazobactam (TZB), which stops beta-lactamase enzymes from working but doesn't kill many bacteria; and piperacillin (PPZ),

which is a semi-synthetic ureidopenicillin that does kill many bacteria. Antibiotics in this combination exhibit wide-ranging efficacy against diverse bacterial strains. The beta-lactam ring-containing penicillanic acid sulfone is the precursor to the compound TZB. By forming irreversible bonds with numerous bacterial beta-lactamases, it obstructs the degradation of PPZ. In addition, tazobactam functions as a B-lactamase enzyme inhibitor for bacteria, thereby reestablishing their susceptibility. Piperacillin sodium destroys susceptible bacteria by impeding their ability to produce septa and cell walls. It works better against bacteria that make beta-lactamase enzymes when piperacillin is mixed with tazobactam, which is a sulfone derivative of penicillanic acid. Plasmid-carried enzymes of this nature are frequently accountable for penicillin and cephalosporin resistance (57).

Giving PPZ-TZB through an IV is usually the best way to treat septicemia, severe respiratory infections (including *Pseudomonas aeruginosa*), and infections in the abdomen, pelvis, soft tissues, and bones. 1981 saw the initial issuance of sanctions for the PPZ-TZB. Piperacillin-tazobactam is administered intravenously due to its decreased oral bioavailability, specifically because of its half-life ranging from 0.8 to 1.1 hours (58, 59).

Piperacillin-tazobactam is a highly adaptable antibacterial medication that specifically targets Gram-positive, Gram-negative, and anaerobic species. Research has demonstrated the benefits of using it to treat serious and moderate infections, including pneumonia, difficult urinary tract infections, infections within the abdomen, infections in soft tissues, and infections in the female reproductive system (60).

The initial antibiotic has been on the market since 1993. It is particularly effective at treating serious illnesses and is safe for these patients. However, because it is so commonly used in the critical care unit (ICU), there are worries over the exorbitant costs associated with the provision of care for these individuals (61).

Generic medications must exhibit bioequivalence to the compounds that served as the basis for their creation. This means they must display the same level of efficacy and safety. Moreover, the utilisation of such pharmaceuticals can lead to substantial financial benefits linked to treatment. Health systems with limited resources typically invest between 20% and 60% of their budget in

drugs. As a result, the World Health Organisation has been promoting its careful and wise use since 1985. Their objective is to ensure that patients are provided with the appropriate treatment at the most effective dosage and schedule while minimizing expenses for both patients and the community. A highly effective approach to achieving cost-effective treatment is to use pharmaceuticals that are as effective but have a lesser cost. The new antibiotic has been commercially accessible since 1993. It has been proven to be efficacious and secure in the management of individuals with severe illness. However, the increasing use of this technology in the critical care unit (ICU) has raised concerns about the high costs of healthcare for these patients (60).

Piperacillin is a partially man-made ureidopenicillin with antibacterial properties, specifically designed to combat Gram-negative bacteria. Increasing numbers of lactamase-producing bacteria, which are resistant to the medicine piperacillin, have recently made it less effective as a medicine. Tazobactam is a derivative of penicillanic acid sulfone that functions as a lactamase inhibitor. It impedes the breakdown of piperacillin by beta-lactamase enzymes. When piperacillin and tazobactam are mixed, they improve the effectiveness of the antibiotic by including bacteria that make lactamase enzymes. The microorganisms included are *staphylococci*, some species of *Enterobacteriaceae, Haemophilus influenzae*, and *Bacteroides* spp. A previous medication assessment investigated the antibacterial effectiveness, pharmacokinetics, and therapeutic potential of piperacillin-tazobactam (40).

Piperacillin-tazobactam exemplifies such medications, and ensuring their appropriate administration is crucial in thwarting the rise of resistance. The mixture of piperacillin and tazobactam has undergone a thorough investigation. According to Raveh et al.'s research, 90% of patients in the study hospital considered piperacillin-tazobactam to be suitable. The number is 9. Appropriateness in this case means giving antibiotics according to the institution's rules, customising therapy based on sensitivity data, and following the medication recommended by an infectious disease physician (62).

2.3.1 Administration and dosing instructions

The recommended dosage of piperacillin-tazobactam for those aged 12 years or older is 4 grammes of piperacillin and 0.5 grammes of tazobactam. Administer this drug intravenously at intervals of either 6 or 8 hours. Piperacillin tazobactam 2/0.25g can be given via intramuscular route at intervals of six to twelve. It is recommended for the treatment of mild and moderate infections. To effectively treat acute infections, the combination should be given for a minimum of 5 days. Additionally, it is advisable to prolong the treatment for an extra 48 hours following the resolution of clinical symptoms and fever. The recommended dosing frequency for patients with a creatinine clearance of less than 1.2 L/h (< 20 ml/min) is twice daily (40). After hemolysis occurs, it is advisable to administer an extra dosage of piperacillin tazobactam. However, this is unnecessary for those who are receiving continuous ambulatory peritoneal dialysis therapy. No dosage modification is necessary for cirrhotic or elderly patients. Piperacillin tazobactam should not be used in patients who have previously experienced hypersensitivity responses to penicillins, cephalosporins, or β-lactamase inhibitors (63).

Piperacillin-tazobactam is well recognised as a secure and effective antibiotic. The most frequently documented adverse effects observed in clinical studies of piperacillin-tazobactam were gastrointestinal and dermatological responses. Piperacillin-tazobactam and ertapenem were both investigated lately, and the results revealed that there was not a significant distinction between the two treatment groups in terms of tolerability and security (64).

Adverse clinical effects were detected in 23.3% of patients receiving piperacillin-tazobactam therapy and in 23.2% of individuals in the ertapenem group. The therapy groups exhibited symptoms including diarrhoea, intravenous infusion responses, headaches, and nausea, with the majority of these adverse effects varying in intensity from mild to severe. (65). Diarrhoea was the predominant gastrointestinal symptom observed in clinical trials involving piperacillin-tazobactam (16).

Piperacillin-tazobactam has the potential to induce serious side effects, such as severe abdominal pain and the appearance of watery or bloody diarrhea that can persist for months after the last dose. confusion; involuntary muscle contractions or rigidity; impaired mobility; epileptic episode;

reduced number of white blood cells—fever, mouth ulcers, skin ulcers, sore throat, cough, breathing difficulties; decreased potassium levels—leg cramps, constipation, irregular heart rhythms, palpitations, excessive thirst or urination, numbness or tingling, muscle weakness, or loss of sensation (55).

Fever, pharyngitis, burning eyes, skin pain, a rash consisting of blistering and flaking, red or purple discoloration, respiratory distress, and facial or cervical edema are all indicative of a severe skin reaction. Hemorrhagic symptoms are indicative of an allergic reaction. A severe adverse drug reaction that has the capacity to impact various tissue systems. Muscle rash, extreme lethargy, unexpected bruising, fever, glandular swelling, and yellowing of the skin or eyes are all potential symptoms (22).

2.4 Neutropenia and fever are associated with piperacillin-tazobactam

Neutropenia is a condition that is not considered clinically serious until the absolute neutrophil count (ANC) drops below 500. Neutropenia is classified as such when the absolute neutrophil count (ANC) in newborns and adults is below 1500 cells/mL (45). An absolute neutrophil count (ANC) below 200 signifies an increased vulnerability to severe or opportunistic infections. Nevertheless, it is crucial to recognise that these concepts mostly relate to hypoproductive neutropenias that occur due to bone marrow suppression or dysfunction. Patients who experience neutropenia due to the peripheral elimination of mature neutrophils generally have a more favourable prognosis. This is likely because there are more neutrophils available at the infection sites. Therefore, it is feasible for people who are affected, especially infants and young children, to show no symptoms even when they have absolute neutrophil count (ANC) numbers that cause great concern among relatives and even medical professionals. Furthermore, the level of risk involved is strongly correlated with the length of time that neutropenia persists. Unlike temporary processes, chronic neutropenia is more likely to lead to serious infections (26).

Congenital neutropenia and maladies associated with bone marrow failure are the subjects of the educational evaluations included in this issue, which Klein and Shimamura present. Due to their similar favourable prognoses and diagnostic challenges, chronic benign (or idiopathic) neutropenia and chronic autoimmune neutropenia are frequently used synonymously in paediatric patients (66).

The diagnostic use of antineutrophil antibody testing is either highly limited or non-existent, as discussed in the section on diagnosis and management. This section provides a concise overview of typical attributes. Neutropenia is most prevalent in infants throughout their first year of life, namely between the ages of 2 and 54 months. Typically, the condition often disappears until the child reaches the age of two to four (67).

The prevalence of chronic neutropenia in children is extremely uncommon, manifesting in less than 1 in 100,000 children annually. Nevertheless, it is likely that numerous instances go undetected, as asymptomatic children do not exhibit any clinical signs that would prompt blood count monitoring. (68).

During the course of the illness, ANC levels are typically below 200 to 500 cells/mL, along with an increase in monocytes and a relative increase in eosinophils. During bacterial infections or stressful situations, the number of neutrophils normally increases, frequently reaching mildly neutropenic or even normal levels, but this may not occur with viral infections (69).

As well as being a key diagnostic sign that the baby has enough neutrophils stored in the bone marrow, this response rules out the possibility of hypoproductive disorders like severe congenital neutropenia, which is likely to have helped the baby's health improve (70).

The quantities of extra peripheral components align with the anticipated levels for an individual of the patient's age. Children exhibit a greater occurrence of selective IgA deficiency in comparison to the overall population, although it remains a rare disorder, affecting approximately 3% of persons. The presence of autoimmune cytopenias occurring simultaneously or in close succession is what differentiates Evans syndrome as a pathogenic condition. It is a commonly occurring and long-lasting condition that is closely linked to autoimmune lymphoproliferative syndrome, as well as other autoimmune disorders and diseases that decrease the immune system either from birth or as a result of other factors (71).

The presence of other nonimmune cytopenias may suggest an underlying bone marrow problem, including congenital bone marrow dysfunction disorder, acquired aplastic anaemia, myelodysplastic condition, or leukaemia. There aren't any reliable antineutrophil antibody tests for adults, so it's hard to tell the difference between chronic autoimmune and idiopathic neutropenias.

Unlike in newborns, the condition is more common in adult females (about 5–1) and has a higher likelihood of becoming chronic (45).

Individuals with profoundly low absolute neutrophil counts (ANCs) may experience recurring skin or respiratory infections, as well as stomatitis, although most of them may not suffer from severe infections. Acquired neutropenia is an intermittent, mild disease. It can happen alongside autoimmune and Chronic infections such as sarcoidosis, rheumatoid arthritis, and systemic lupus are characterised by long-lasting inflammation in the body and Sjogren syndrome. In the context of rheumatoid arthritis, "felty syndrome" denotes a more pronounced enlargement of the spleen and a decrease in the number of neutrophils (48).

2.5 Distinctive features of Gram-negative bacteria

The Hans Christian Gram stain, a technique developed in 1884, allows for the distinction between Gram-positive and Gram-negative bacteria. The process entailed the amalgamation of a crystal violet-iodine molecule with a safranin counterstain. Unlike Gram-positive bacteria, which display a violet or purple coloration, Gram-negative bacteria acquire a pink tint when counterstained with safranin instead of retaining the original stain. The variations in the content or structure of bacterial cell walls play a crucial role in determining the diversity of bacterial species (72).

Gram-negative bacteria contain trilaminar membranes. Gram-negative bacteria have an outer membrane (OM) that protects them from Gram-positive bacteria. Phospholipids connect the inner membrane layer and outer membrane (OM). In the outermost layer, lipopolysaccharide (LPS) causes endotoxic shock. Porins and other OMPs make up the cell's outer membrane (OM). To transport amino acids and small saccharides, OMPs are essential. The deepest layer's peptidoglycan cell wall provides a strong framework that shapes the cell. The disaccharide N-acetyl-glucosamine-N-acetylmuramic acid repeats (73).

The inner membrane (IM) of the third stratum is composed of a phospholipid bilayer. The intracellular matrix (IM) plays essential functions within the cell, such as maintaining structural integrity, facilitating cellular transport, and enabling metabolic processes. Additionally, it functions as the site for DNA binding and plays a vital role in the segregation of sister chromosomes (74).

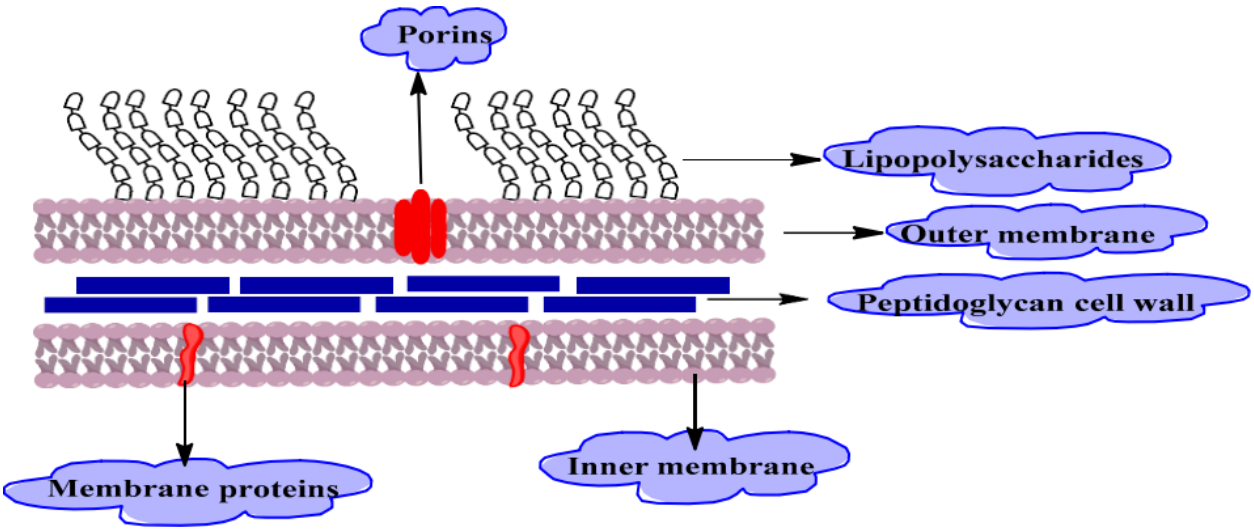


Figure: A diagram of the features and cellular structure of cell wall Gram-negative bacteria.

Unfortunately, some antibiotics, like colistins, B-lactams, and quinilons, don't work on Gram-negative bacteria because they still have an outer membrane. To achieve their intended functions, the majority of antibiotics must traverse the outer barrier. Hydrophobic medicines, which are resistant to water, can diffuse via a specific route, while hydrophilic antibiotics, such as B-lactams, which are attracted to water, can pass through porins. Vancomycin, on the other hand, lacks the ability to pass through the outer membrane, thereby preventing it from utilising any of these pathways (75).

Gram-negative bacteria can develop resistance by modifying their outer membrane through many mechanisms, including altering its hydrophobicity or modifying porins and other proteins. Gram-negative bacteria do not possess this essential layer, which renders them more resistant to antibiotics compared to gram-positive bacteria (76,77).

2.6 The study examines neutropenic bacterial infection epidemiology.

Gram-positive bacteria have been identified as the most prevalent causative agents of infections in neutropenic patients. This phenomenon emerged on a global scale during the 1990s. The

percentage of Gram-negative infections among patients with systemic infections and malignancies in the United States decreased from 22% to 15% between 1995 and 2000. On the contrary, an increase from 62% to 76% was observed in the proportion of Gram-positive bacteria among these individuals (78).

Individuals with neutrophil deficiencies are prone to developing Gram-positive sepsis as a result of various potential risk factors. Many individuals get mucositis as a result of chemotherapy; a significant number of individuals use central venous catheters; a considerable proportion of individuals receive quinolone therapy as a preventive precaution; and a substantial number of individuals consume proton pump inhibitors. Notably, in the early 2000s, there was a significant rise in the occurrence of infections caused by Gram-negative bacteria in patients with neutropenia (79,80).

2.7 Piperacillin-tazobactam's susceptibility to gram-negative bacteria

Beta-lactamase production is a vital mechanism that significantly contributes to bacterial drug resistance. Both gram-positive and gram-negative bacteria synthesise a diverse array of distinctive beta-lactamases. Plasmids or transposons can transfer beta-lactamases, facilitating their spread to other bacteria. In contrast, the DNA that the chromosomes contain controls them. Gram-negative bacteria, such as *staphylococci*, can possess a diverse range of plasmid-encoded beta-lactamases. Beta-lactamases are widely distributed (81).

Gram-negative bacteria have TEM-1, TEM-2, and SHV-1 enzymes. These enzymes break down ampicillin, first-generation cephalosporins, and penicillins. *Enterobacteria* and most *Escherichia coli* bacteria have TEM-1 and its enzymes. In clinical settings, ampicillin, first-generation cephalosporins, and monobactams are less common because of their widespread use and good efficacy. Enhanced enzyme production makes organisms resistant to extended-spectrum penicillins like ticarcillin and piperacillin. Recently, point mutations were discovered in the TEM-1, TEM-2, and SHV-1 enzymes. This enabled superior extended-spectrum beta-lactamases. These enzymes break down third- and fourth-generation cephalosporins. The phenomenon was first identified in *Klebsiella* species in Germany (82)

Presently, these enzymes are found in various species of bacteria, including *E. coli* and *Klebsiella*, on a global scale. Also, giving B-lactam antibiotics and B-lactam inhibitors at the same time makes it harder to get rid of bacteria because too many TEM-1 and SHV-1 enzymes are made (81).

Class 1 betalactamases, which are highly significant, are particularly vulnerable to chromosomal modifications. Enzymes of this nature are present in *enterobacteria* and *Pseudomonas aeruginosa*, and they possess the capability of hydrolyzing an extensive range of broad-spectrum penicillins and a medicine containing Despite the fact that the majority of gram-negative bacteria have the genetic material required to produce beta-lactamases, the amount produced is insufficient to cause resistance to antibiotics. The bacteria *Enterobacter, Citrobacter, Serratia, Proteus vulgaris, Morganella, Providencia species,* and *P. aeruginosa* are all capable of activating enzymes. Nevertheless, specific novel broad-spectrum penicillins and cephalosporins may continue to impact these microorganisms due to their inability to induce enzyme production in an efficient manner (83).

It is crucial to acknowledge that class 1 enzymes are consistently generated at elevated levels in mutant subpopulations of all three species. Therefore, when dealing with infections caused by organisms that manufacture inducible beta-lactamases, it is not advisable to employ beta-lactam antibiotics. This is due to the potential for identifying resistance mutants and converting them into resistant strains, thereby rendering the therapy ineffective. Some bacteria, like Gram-negative ones and *staphylococca*l beta-lactamases, have enzymes called TEM and SHV that can stop piperacillin from working. This ureidopenicillin possesses an extensive range of functions and is therefore versatile. Although piperacillin is still effective against organisms that can make inducible enzymes, it is no longer effective against class I enzymes because it can't activate beta-lactamases. (84).

Although organisms that consistently produce class 1 enzymes are not vulnerable to piperacillin. Furthermore, the beta-lactamases made by Bacteroides species neutralize the activity of piperacillin. Beta-lactamases are enzymes that can stop other enzymes from working. Tazobactam is a compound made from penicillanic acid sulphone. It is known that *staphylococci* bacteria have these enzymes. Other bacteria that have them include *E. coli, H. influenzae, Klebsiella* species, and several anaerobic bacteria, such as *Bacteroides* species (82,83).

Betalactamases of class 1 are present in *Enterobacter, Citrobacter, Serratia, Proteus vulgaris, Pseudomonas,* and other bacteria, it does not stop their activity. We expect that adding tazobactam to piperacillin will make it work again against bacteria that make beta-lactamases, since tazobactam stops these enzymes from working (81).

2.7.1 Piperacillin-tazobactam susceptibility in Gram-negative bacteria.

Piperacillin-tazobactam medications demonstrate effectiveness against a wide range of Gram-negative microorganisms. The bacteria in question possess the capacity to produce B-lactamase enzymes via plasmid mediation. Certain non-fermentative bacilli demonstrate resistance to the given mixture. Piperacillin-tazobactam has been shown to kill a wide range of *Enterobacteriaceae* bacteria. *E. coli, Serratia marcescens, Proteus mirabilis, Proteus vulgaris, Klebsiella* pneumoniae, *Klebsiella* oxytoca (along with additional *Klebsiella* species), *Enterobacter P aerogenes*, and *various Citrobacter* species were among the microorganisms discovered. Nevertheless, certain *Citrobacter freundii* and *E. cloacae* strains demonstrated signs of resistance (85).

The studies revealed that the isolates of *Morganella morganii* had a high degree of susceptibility to the mixture of piperacillin and tazobactam. This combination demonstrated efficacy against specific strains of *Providencia stuartii* but exhibited reduced effectiveness against specific strains of *Providencia rettgeri*. Piperacillin-tazobactam demonstrated efficacy against specific strains of *Providencia stuartii* (86).

Extra gram-negative bacteria from the combination of Piperacillin and tazobactam have demonstrated significant efficacy against isolates of *H. influenzae* that produce or do not produce β-lactamase. However, it exhibited ineffectiveness against bacteria that displayed resistance to β-lactamase and ampicillin, as well as *Moraxella catarrhalis*. A notable disparity existed between the susceptibility breakpoint and the average MIC90 values observed in all conducted experiments. Giving piperacillin and tazobactam together is an effective way to treat *Pseudomonas aeruginosa* strains that are sensitive to the antibiotic piperacillin (40). However, the susceptibility rates to piperacillin-tazobactam can differ among various types of gram-negative bacteria, depending on the institution or nation. The coordinates are (87, 88).

2.8 Risk of the development of neutropenia in adults with gram-negative bacterial infections treated with piperacillin-tazobactam.

This medication is a combination of two distinct classes of antibiotics: a b-lactamase inhibitor (BL-BLI) and a b-lactam, which is a broad-spectrum antibiotic. This medication is frequently employed to treat serious infections, including those acquired in healthcare environments. When it comes to treating serious bacterial infections with extended-spectrum antibiotics, TZP is widely regarded as a reliable option by many experts. To effectively control the spread and increase of carbapenemases, it is imperative to limit the use of carbapenems and tackle the problem of Gram-negative bacteria that produce extended-spectrum β-lactamase (ESBL) (89).

Piperacillin-tazobactam (TZP) is a potent synthetic antibiotic that has a wide spectrum of antibacterial activity against diverse infections. It exhibits better efficacy against gram-negative bacteria in comparison to other penicillins. It is frequently employed for the treatment of nosocomial infections and other conditions that necessitate the use of broad-spectrum antibiotics, such as febrile neutropenia. TZP is correlated with adverse consequences. such as hypersensitivity reactions as well as complications with the gastrointestinal, renal, and hematologic systems. While reversible neutropenia is commonly reported as a hematologic adverse reaction to TZP, there have also been documented cases of Coombs-positive hemolytic anaemia and thrombocytopenia. Following an extended course of treatment with TZP, certain people had symptoms of fever and neutropenia (90–92).

When given through an IV, the combination of piperacillin and tazobactam worked better than the other two beta-lactamase inhibitors. Published clinical research has primarily focused on adults. However, the use of this combination for the treatment of severe illnesses in children has demonstrated both safety and effectiveness. Piperacillin-tazobactam and ticarcillin-clavulanate both kill bacteria in the same range of situations when tested in a lab. Piperacillin-tazobactam exhibits superior efficacy compared to ticarcillin-clavulanate against specific bacteria, particularly *enterococci*. This could be attributed to the inherent potency of piperacillin (93).

Clinical investigations have demonstrated that piperacillin-tazobactam is equally or even more efficacious than other broad-spectrum antibiotics such as ticarcillin-clavulanate, carbapenems, and third-generation cephalosporins. This is particularly accurate when it is employed in conjunction

with an aminoglycoside to treat pneumonia, skin and soft tissue infections, intraabdominal infections, polymicrobial infections, and febrile neutropenia (16).

The recommended dosage for adults is 12 grammes of piperacillin and 1.5 grammes of tazobactam. The recommended dosage is 3.375 grammes (3 grammes of piperacillin and 0.375 grammes of tazobactam) administered every 6 hours, or 4.5 grammes every 8 hours for persons with a creatinine clearance exceeding 40 mL/min (94). The objective of this research endeavour is to evaluate the occurrence and prevalence of neutropenia among adult patients undergoing piperacillin-tazobactam therapy for gram-negative bacterial infections.

2.9 The relationship between TZP duration and neutropenia.

BLBLIs are antibiotics that are the subject of controversy due to their usage in treating infections caused by gram-negative bacteria that produce extended-spectrum beta-lactamase (ESBL-GNR). Piperacillin-tazobactam (PTZ) is an antibiotic that is specifically employed for the treatment of gram-negative bacterial infections. A multitude of ESBL-GNR strains are affected. Antimicrobial stewardship promotes treatment regimens for ESBL-GNR infections that do not involve the use of carbapenems. This matter continues to be relevant when clinical microbiology laboratories utilize rapid diagnostic methods to detect CTX-M-type or other ESBL resistance mechanisms. Multiple studies suggest that PTZ may have reduced efficacy against ESBLs. Minimum inhibitory concentration (MIC), site and severity of the illness, and the resistance mechanism may all contribute to the prescription of BLBLIs. (95).

Antibiotics at large doses over long periods of time are used to treat bone infections. Piperacillin-tazobactam is a b-lactamase inhibitor and synthetic ureidopenicillin. It treats many types of bacteria, including gram-negative bacteria that cause diabetic foot infections and osteomyelitis (96). In 110-day regimens, B-lactam antibiotics such as piperacillin and piperacillin-tazobactam can temporarily lower neutrophil numbers. Gram-negative bacterial infections have not been the subject of extensive research on the neutropenia that b-lactam medications produce in endocarditis, cystic fibrosis, or osteomyelitis. Piperacillin-tazobactam treats infections (97).

Most neutrophils are actively dividing or completely growing in the bone marrow. Low neutrophil counts make patients highly susceptible to infection, and neutropenia severity and duration affect

bacterial infection risk. Neutropenia appears to be linked to TZP therapy duration. Like piperacillin, ticarcillin causes neutropenia after 21 days of treatment. (18).

According to the majority of the trials evaluated, neutropenia after 10 days of treatment is extremely unusual. Agranulocytosis developed in a patient currently undergoing TZP therapy for a cranial infection, which has been ongoing for 17 days. One of the published case reports discussed the medical condition of this patient. The fact that different study designs and definitions of neutropenia are used in different ways contributes to the variability reported in studies. Piperacillin-induced neutropenia is noticed approximately 19 days following the start of piperacillin medication, based on the findings of one cohort study and many case reports. (1)

The patients developed neutropenia 20 days after starting piperacillin-tazobactam. Previous research has shown that neutropenia can arise 11–17 days following therapy. The combined dose of piperacillin and tazobactam was 4919 mg/kg, or 4372 mg/kg of body weight. These patients also had bone-marrow suppression. Piperacillin-tazobactam can inhibit bone marrow, especially neutropenia, which is a factor that physicians should take into account when treating patients. This particularly applies to patients who have been administered a substantial cumulative dosage of the medication. (31).

While the exact mechanism by which TZP induces neutropenia remains unclear, it is hypothesis that piperacillin is responsible for suppressing the growth of myeloid cells [11]. A case investigation revealed the presence of immunologically caused neutropenia when IgG antibodies for penicillins were discovered (48). Monitoring the patient's haematological levels is crucial when administering piperacillin, even for a brief duration.

Neutropenia seems to be associated with the total amount of penicillin administered, the duration of treatment, and the period of therapy. Based on an extensive research review investigating the association between piperacillin usage and the occurrence of neutropenia, the prevalence of neutropenia varied from 0.04% to 34% in individuals exposed to this medication (31).

In addition, it appears that the duration of TZP medicine treatment correlates with the start of neutropenia. Furthermore, prior to the conclusion of 10 days of treatment, neutropenia was found to be exceptionally rare in the majority of investigations (28).

CHAPTER THREE
MATERIAL AND METHODS

3.0 Study Design and Statistical Analysis

The researchers used Nucleus, the hospital data system, to retrieve the results of patients admitted to Near East University Hospital between January 2016 and December 2023 who received piperacillin-tazobactam treatment. Once the data was collected, the samples were analysed using SPSS version 25 to determine the results. This study design is intended to survey the population by selecting samples to be evaluated. A retrospective study was chosen for this study because it is effective, less expensive, and easier to collect information from the target population. This study will investigate the prevalence of neutropenic development in gram-negative bacterial infections treated with piperacillin-tazobactam.

For this study on *Gram-negative bacteria* infections in patients treated with piperacillin-tazobactam, a total of 69 samples were used, covering the period from January 2016 to December 2023. The samples used were obtained from individuals over the age of 18 who visited Near East University Hospital as both inpatients and outpatients. Our investigation took place within the microbiology laboratory department of Near East University Hospital. Involved patients from departments such as anaesthesia, ICU, neurology, orthopaedics, urology, and emergency medicine, among others.

3.1 Tools and Equipment

The following equipment was used in the investigation: an Antic medical petri dish, an automated pipette, a wire loop, test tubes, a dispenser, a measuring ruler, a sterile swab, an incubator, a selection of agars suitable for cultivating Gram-negative bacteria, an autoclave, a syringe, and a 1000-millilitre conical flask.

3.2 Specimens Collection

From January 2016 to December 2023, the microbiology laboratory will be collecting clinical specimens of gram-negative bacterial infections who are using piperacillin-tazobactam.

3.3 Study population

This study was selected gram negative the patients who takes piperacillin-tazobactam from January 2016 to December 2023.

3.3.1 Inclusion criteria

Patients with Gram-negative infections who use Piperacillin-tazobactam and are over the age of eighteen years.

3.3.2 Exclusion criteria

Patients under the age of eighteen with gram-negative infections who are using piperacillin-tazobactam, as well as patients taking other antibiotics,

3.4 Specimen Processing

Gram stains yield results more rapidly than bacterial cultures; they are generally incapable of distinguishing the specific species of bacteria, in contrast to bacterial cultures. Nevertheless, Gram stains may assist physicians in directing patients towards the most appropriate treatment. Gram stain testing consists of the following three general procedures: Assemble the prototype. Analysing the specimen. Investigating the sample. A healthcare provider will collect a sample from the location of the suspected infection in order to perform a Gram stain test. There are a few methods by which a provider may acquire Gram stain test samples.

Gram-positive cells possess cell walls with abundant layers of peptidoglycan, a carbohydrate, while Gram-negative bacteria have minimal amounts of it. Unlike Gram-negative bacteria, Gram-positive bacteria possess teichoic acids. The outer membrane of Gram-negative cells exhibits similarities to the phospholipid bilayer present in the cell membrane. A medical laboratory scientist categorises bacteria on a Gramme stain slide as either gram-positive or gram-negative based on the alteration in colour that takes place while the slide undergoes a sequence of staining procedures. In addition, the sample consists of gram-variable bacteria, which are distinguished by their uneven staining, as well as a combination of bacteria displaying pink and purple colours.

Gram staining procedure

3.4.1 Preparation of a slide smear:

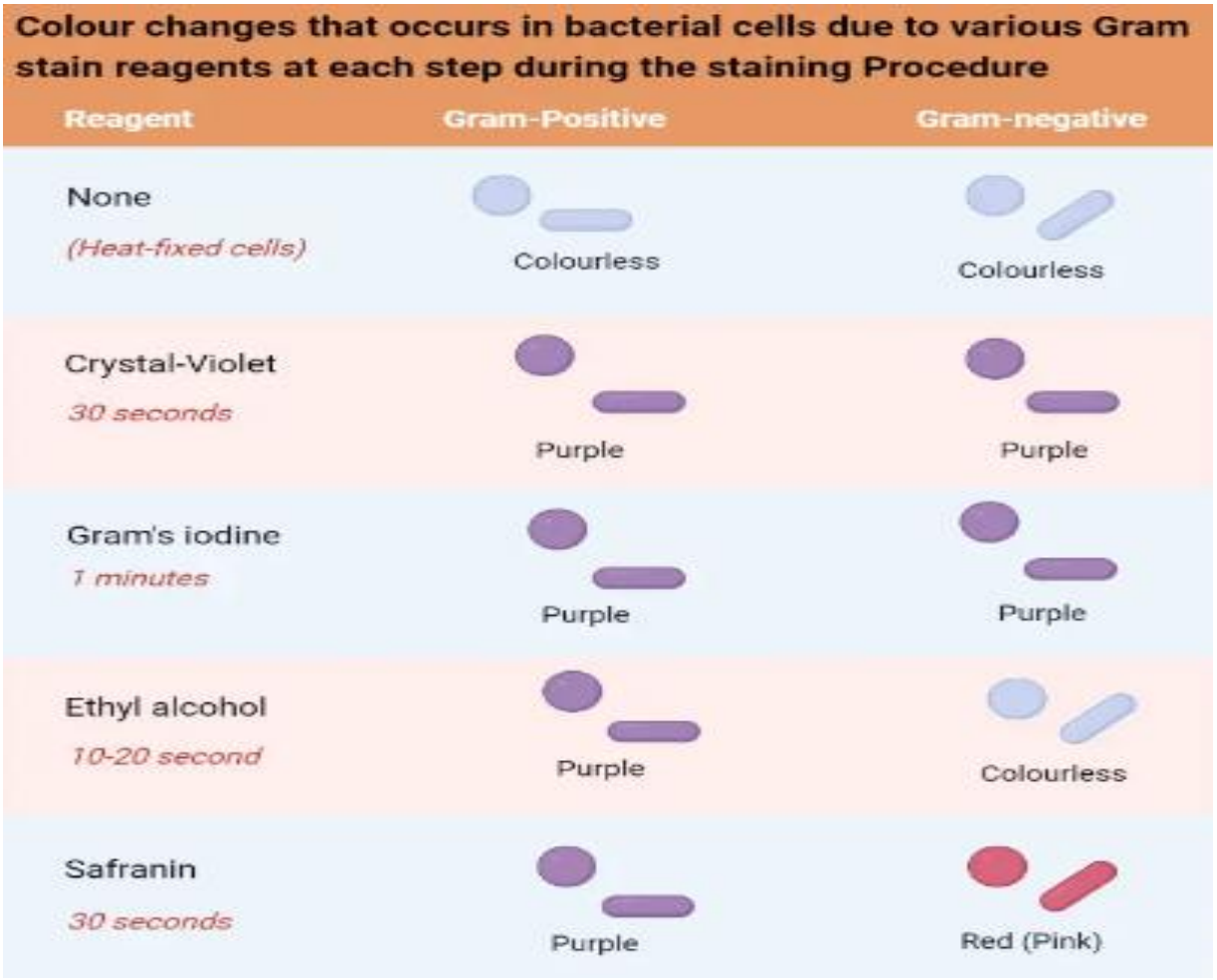
- A microscope slide is used in conjunction with an inoculation loop to get a small amount of suspension culture.
- To transfer a little amount of colony onto the inspection slide, water is injected into the Petri dish or slant culture tube containing the colony.
- Having a basic awareness of culture is vital. When a colony is detectable on an inoculation loop, it means that an excessive amount of culture has been collected.
- A sterilised loop is used to evenly distribute the culture on a circular surface of 15 mm in diameter. When examining numerous cultures, it is typical to observe up to four little dots on an average slide.
- The slide can be evaporated using either natural air drying or by applying mild heat using a moderate flame. In order to prevent excessive heat buildup or the development of a circular pattern, it is necessary to rotate the slide in a circular motion above the flame. Applying heat promotes the attachment of cells to the glass slide and minimises the substantial loss of culture during the washing process.

3.4.2 Staining processes:

- ❖ After applying the crystal violet dye to the fixed culture, it is allowed to settle for sixty seconds before being removed. Any remaining stain is then washed away with water. Stain removal is carried out with the aim of preserving the established culture.
- ❖ The smear is submerged in an iodine solution for a period of 60 seconds. The process is referred to as dye fixing. After the iodine solution has been drained, the slide is washed with running water. The surplus water on the surface is eliminated through agitation.
- ❖ Apply a small amount of decolorizer to the slide. Several decolorizers employ mixtures of ethanol and acetone. This process is referred to as "solvent treatment". The saturation of the slide reached its maximum value of 100% after a duration of 5

seconds. To prevent excessive decolourization of gram-positive cells, cease the addition of decolourizer after the solvent ceases to change colour as it traverses the plate.

- The smear is stained with a safranin solution for a duration of 60 seconds. Water is employed for rinsing off the fuchsine solution, while bibulous paper is used to eliminate any remaining water. Following the removal of excess water, the slide can be dried using air before conducting oil immersion germ screening.



CHAPTER FOUR

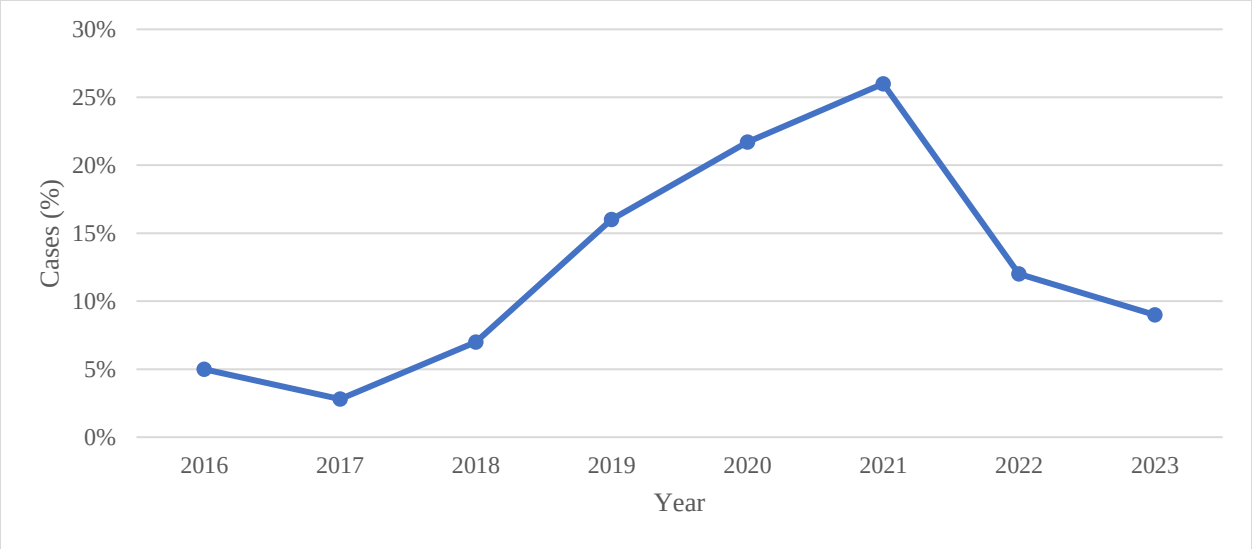
4.0 Results

This chapter provides an analysis and interpretation of the findings from the research on the development of neutropenia and its relationship with the intake of piperacillin-tazobactam.

Table:4.1 Distribution of Patients Taking Piperacillin-Tazobactam Treatment in Years

Years	**Patients number**	**Precentage %**
2016	4	5%
2017	2	3%
2018	5	6.5%
2019	11	16%
2020	15	23%
2021	18	26%
2022	8	11.5%
2023	6	9%
Total	69	100%

Figer 4.1 Distribution of patients taking Piperacillin-Tazobactam treatment in years

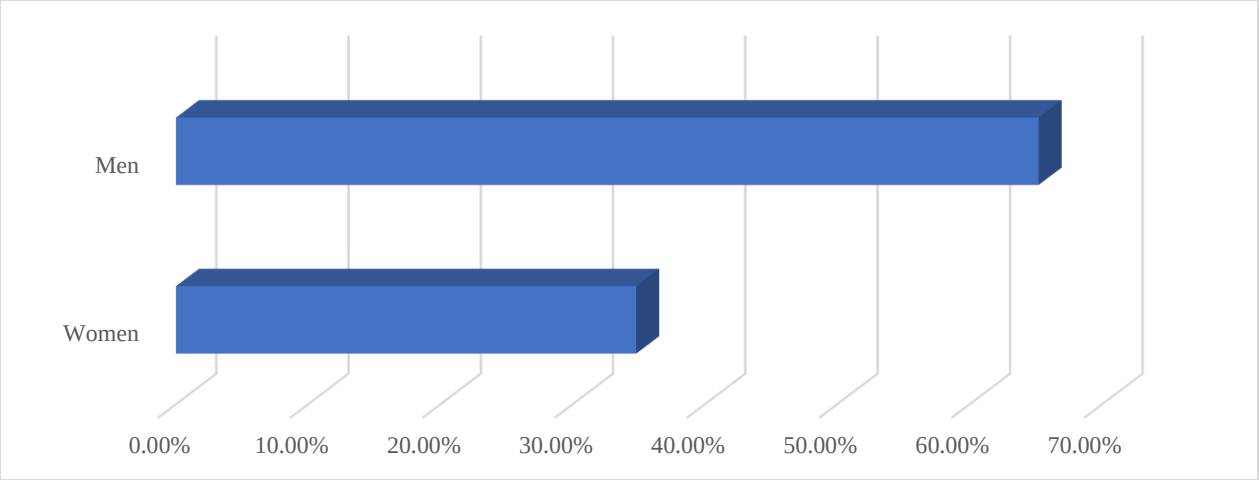


At NEU Hospital, the distribution of patients who got piperacillin-tazobactam from 2016 to 2023 is provided in Table 4.1, which can be seen above. This table contains detailed information regarding the distribution of recipients. Regarding the year 2017, for instance, we noticed a decrease in the proportion of years, which amounted to 3%. In the years 2021 (26%), 2020 (23%), 2019 (16%), 2022 (11.5%), and 2023 (9%), the percentage rates came in at their highest levels. This rate was also 6.5% and 5% for the years 2018, 2016.

Table:4.2 **Distribution of patients taking Piperacillin-Tazobactam treatment according to gender**

Gender	**Patients number**	**Precentage %**
Male	48	70%
Female	21	30%
Total	69	100%

Figer 4.2 Distribution of patients taking Piperacillin-Tazobactam treatment according to gender

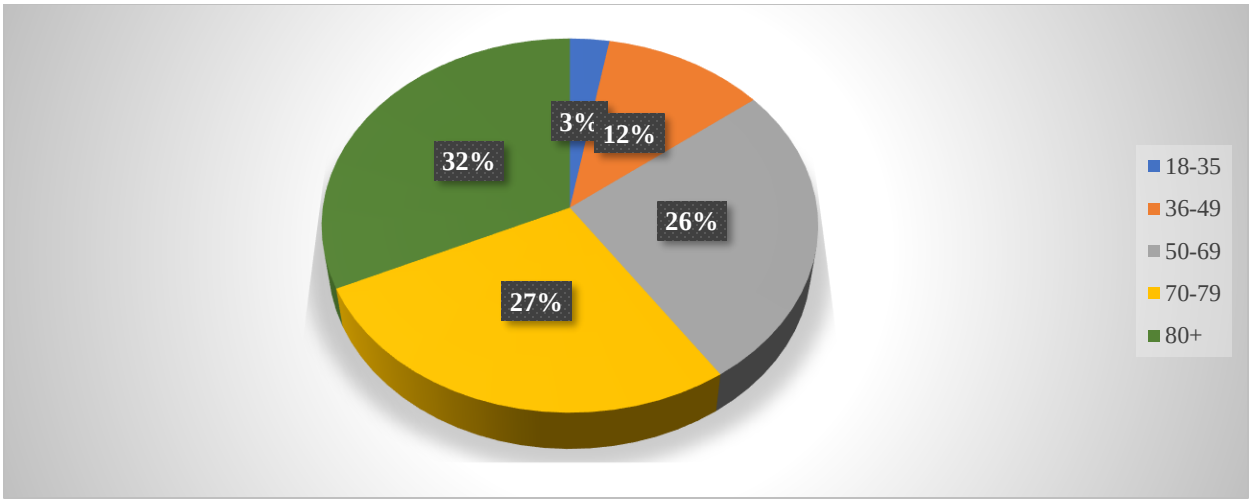


As shown in Table 4.2, the proportion of male patients who used piperacillin-tazobactam is significantly higher than that of female patients. 48 male patients, or 70%, and 21 female patients, or 30%, were included in the study.

Table 4.3 Distribution of patients taking Piperacillin-Tazobactam treatment according to the age group

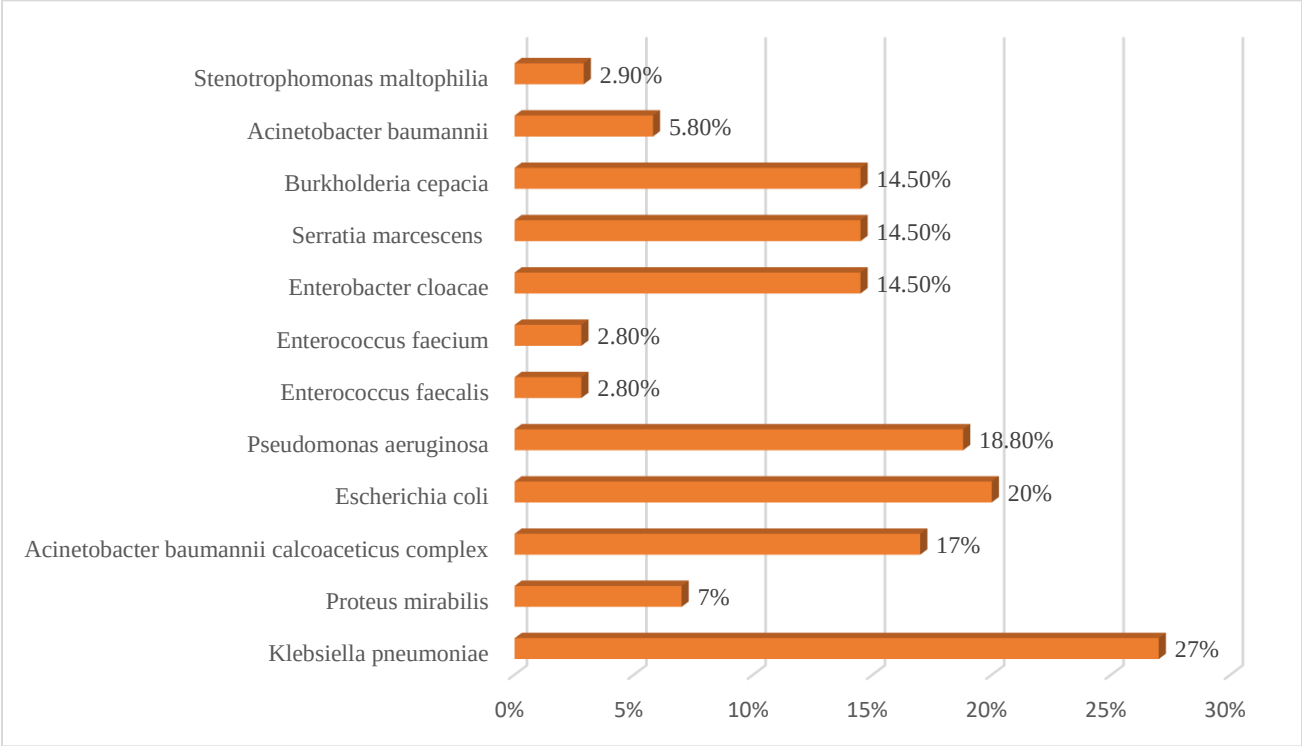
Accoding to the age group	Patients number	Precentage %
18-35	2	3%
36-49	8	12%
50-69	18	26%
70-79	19	27%
80+	22	32%
Total	69	100%

Figer 4.3 Distribution of patients taking Piperacillin-Tazobactam treatment according to the age group



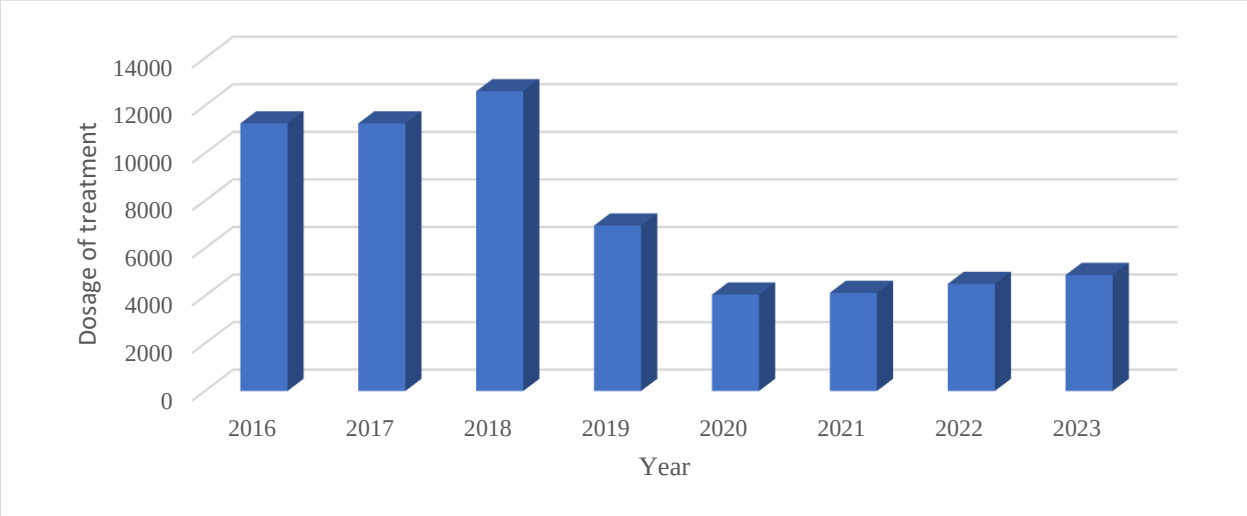
The patients' ages are classified into five basic groups. The first category comprises 3% of patients aged 18–35, 12% aged 36–49, 26% aged 50–69, 27% aged 70–79, and 32% aged 80 or above.

Figer 4.4 Distribution of bacteria detected in the patients' samples taking Piperacillin-Tazobactam treatment



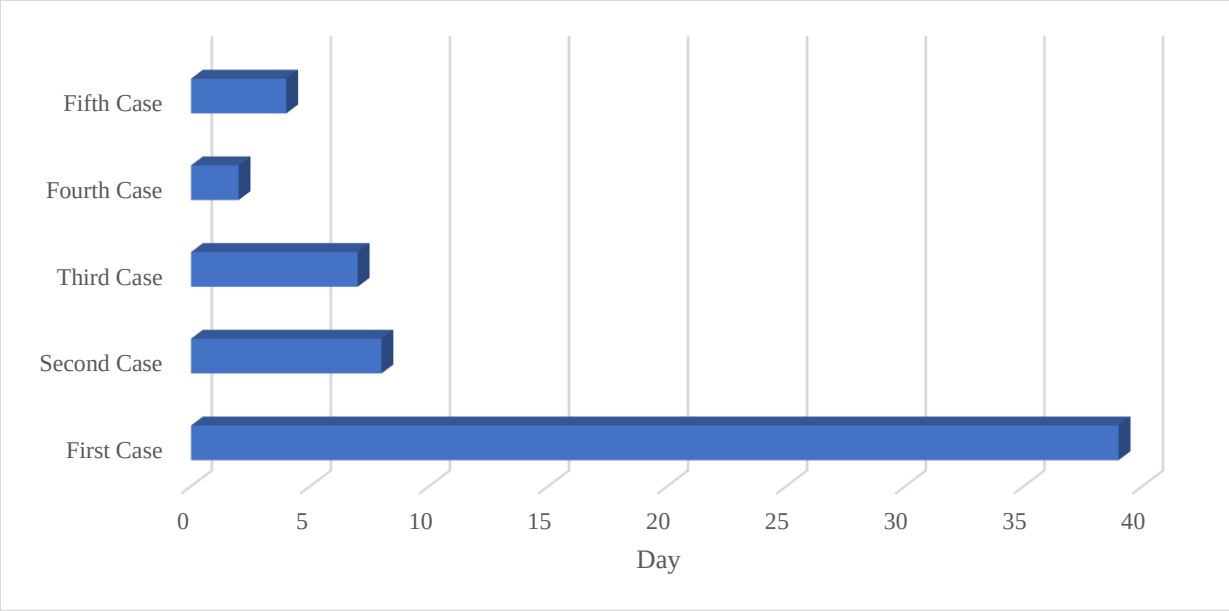
Patients who tested positive for *Stenotrophomonas maltophilia* were administered piperacillin-tazobactam due to the presence of other bacteria in their samples. Meanwhile, 5.80% of the sample patients tested positive for *Acinetobacter baumannii*, *Burkholderia cepacia*, *Serratia marcescens* (14.50%), and *Enterobacter cloacae*. The percentages *for Enterococcus faecium* and *Enterococcus facelis* were both approximately 2.80%, whereas *pseudomonas aeruginosa* accounted for 18.80% and *Escherichia coli* accounted for 20%. The prevalence of *Acinetobacter baumannii calcoaceticus* was 17%, while *Proteus mirabilis* accounted for 7%. *Klebsiella* pneumoniae was the most commonly found pathogen, representing 27% of the cases among patients treated with piperacillin-tazobactam.

Figer 4.5 Cumulative treatment dosages of patients whose taking Piperacillin-Tazobactam treatment



Observed neutropenia cases were 5 (7%). 2 of these patients were women while 3 of them were men. 3 of these patients were in the age group 70-79, one of them were in 50-69 and the other one was in 18-35. Cumulative treatment dosage of these patient were calculated as 4500 mg. According to the collected data, no increase were observed for neutropenia cases in North Cyprus.

Figer 4.6 Treatment period of neutropenia cases

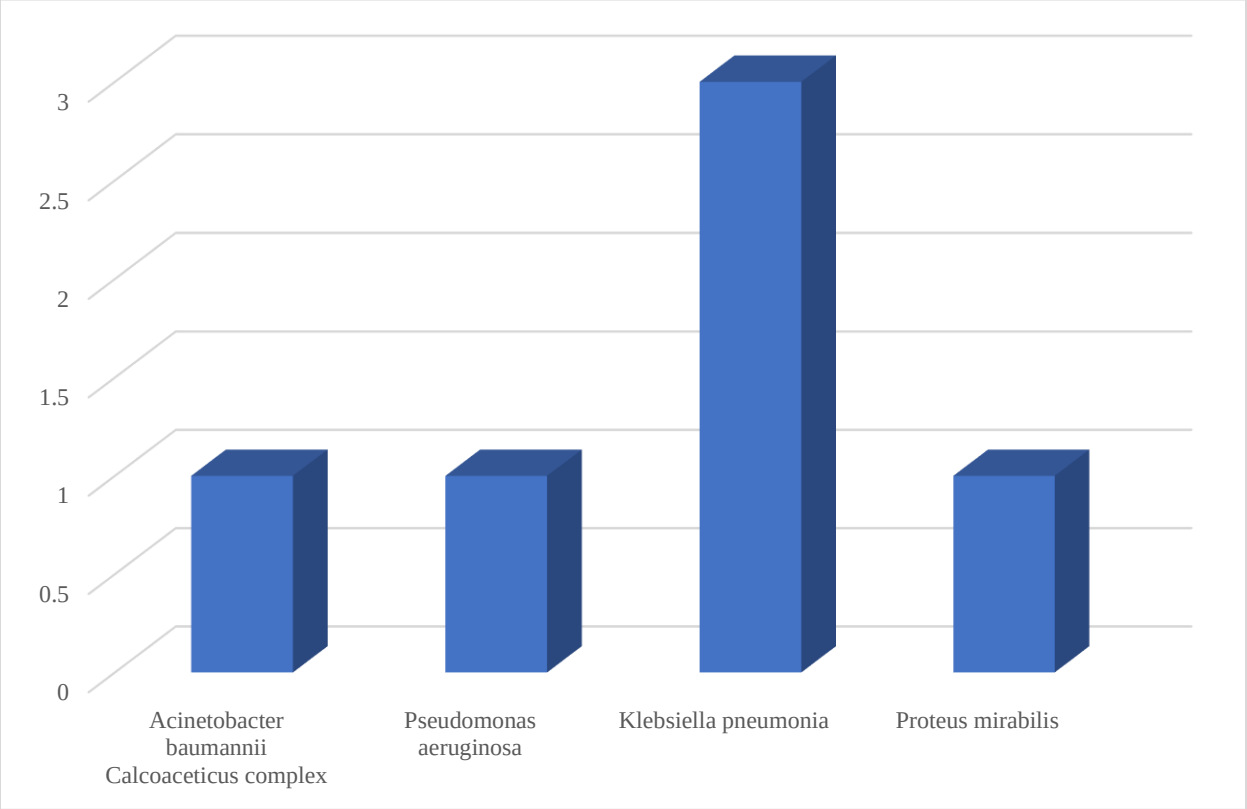


The figure indicates that out of the five patients, one experienced neutropenia after more than thirty-five days of starting the medication, while the remaining four patients got neutropenia within 10 days.

Table 4.7 Distribution of detected bacteria in neutropenia cases

Detected bacteria in neutropenia cases	**Number of cases**	**Presentage %**
Klebsiella pneumonia	2	3%
Acinetobacter baumannii Calcoaceticus complex	1	1.33%
protues mirabilis	1	1.33%
Pseudomonas aeruginosa	1	1.33%
Total	5	7%

Figer 4.7 Distribution of detected bacteria in neutropenia cases



Neutropenia was observed in two instances of *Klebsiella* pneumonia, indicating that 3% of patients exhibited this illness. *Klebsiella* pneumoniae is more strongly correlated with occurrences of neutropenia compared to other bacteria, although other bacteria demonstrate a similar rate of development in neutropenic situations. Other bacteria exhibit a similar rate of development in neutropenic conditions. which indicates the above table and chart.

CHAPTER FIVE

5.0 Conclusion And findings.

Piperacillin-tazobactam (TZP) is a beta-lactam antibiotic that is commonly prescribed to treat an extensive variety of infections. It is generally regarded as a safe medication. TZP can cause neutropenia, which is a rare but dangerous side effect. This paper presents a case of neutropenia generated by piperacillin-tazobactam in an older patient, from whose clinical data samples were obtained.

When observed in the context of a clinical trial, piperacillin-induced neutropenia is also typically reversible. Serious adverse events are more prevalent in non-clinical trial settings, according to MedWatch reports.

In our study, As shown in Table 4.2, the proportion of male patients who used piperacillin-tazobactam is significantly higher than that of female patients. 48 male patients, or 70%, and 21 female patients, or 30%, were included in the study.

The observed neutropenia cases were 5 (7%) Two of these patients were women, while three of them were men. Three of these patients were in the age group of 70–79; one of them was 50–69, and the other was in 18-35. Previous publications have documented three cases of neutropenia in adults caused by piperacillin-tazobactam. The therapeutic duration in these instances was ≥17 days, as shown in a previous study.

The study indicated that the duration of neutropenia in patients treated with piperacillin-tazobactam was less than 10 days. However, one patient had neutropenia for more than 35 days after using the drug.

The cumulative treatment dosage for the patient was calculated at 4500 mg. According to the collected data, previous studies have consistently identified the total dose and duration of TZP therapy as the most commonly identified risk factors for the development of these side effects.

In 2017 case report research conducted by Diana Darwichea, Katia Iskandara, et al., it was noted that a patient developed neutropenia after receiving therapy with TZP for pneumonia within 10 days. Regularly monitoring haematological markers is essential in patients receiving piperacillin-tazobactam, even for a short duration.

The findings of our analysis confirm the occurrence of neutropenia associated with piperacillin-tazobactam treatment. However, it is important to note that neutropenia developed after 10 days of treatment, as stated in our study. Consequently, it is important to do additional studies to investigate the connection between the duration of administered TZP medication and the development of neutropenia.

Additionally, neutropenia was observed in patients who had been using TZP for more than 18 days over the course of the research, which included 41 patients who had bone-related infections.

Based on our results; Patients who tested positive for *Stenotrophomonas maltophilia* were administered piperacillin-tazobactam due to the presence of other bacteria in their samples. Meanwhile, 5.80% of the sample patients tested positive for *Acinetobacter baumannii, Burkholderia cepacia, Serratia marcescens* (14.50%), and *Enterobacter cloacae*. The percentages for *Enterococcus faecium* and *Enterococcus facelis* were both approximately 2.80%, whereas *pseudomonas aeruginosa* accounted for 18.80% and Escherichia coli accounted for 20%. The prevalence of *Acinetobacter baumannii calcoaceticus* was 17%, while *Proteus mirabilis* accounted for 7%. *Klebsiella* pneumoniae was the most commonly found pathogen, representing 27% of the cases among patients treated with piperacillin-tazobactam.

However, neutropenia was observed in two instances of *Klebsiella* pneumonia, indicating that 3% of patients exhibited this illness. *Klebsiella* pneumoniae is more strongly correlated with occurrences of neutropenia compared to other bacteria, although other bacteria demonstrate a similar rate of development in neutropenic situations. Other bacteria exhibit a similar rate of development in neutropenic conditions. which indicates the above table and chart.

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