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Molecular Detection of ESBL and Carbapenemase Resistance in Klebsiella pneumonia.

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ABSTRACT

This study investigates the antibiotic resistance of Klebsiella pneumoniae isolates from patients in Intensive Care Units (ICUs) at the Zliten Medical Center, focusing on detection of extended-spectrum beta-lactamases (ESBLs) and carbapenemase resistance enzymes. The research reveals alarming resistance rates, with K. pneumoniae exhibiting up to 96% resistance to key β-lactam antibiotics, including Cephalothin, Cefuroxime, Ceftriaxone, and Cefepime. Colistin stands out as the only antibiotic with 100% sensitivity among all tested isolates. Moderate sensitivity was noted for Amikacin (38%), Gentamicin (34%), and Meropenem (36%). Statistical analysis indicated weak associations between antibiotic response categories and distribution, except for Tigecycline, which demonstrated a significant association ($p = 0.025$).

Resistance patterns varied by sample source, with high levels of resistance observed across swabs, blood, sputum, and urine, although no significant statistical relationship was established, aside from a near-significant trend with Nitrofurantoin ($p = 0.051$). The Surgical ICU presented the highest resistance levels, particularly to Ertapenem, Imipenem, and Ceftazidime, with Tigecycline resistance varying significantly by ward ($p = 0.028$). Phenotypic testing revealed that 94% of isolates exhibited at least one resistance phenotype, underscoring the clinical challenge posed by multidrug-resistant K. pneumoniae. These findings highlight the urgent need for enhanced surveillance and intervention strategies to mitigate the spread of antibiotic resistance in critical care settings.

RÉSUMÉ

Cette étude examine la résistance aux antibiotiques des isolats de Klebsiella pneumoniae prélevés chez des patients en unités de soins intensifs (USI) au Centre Médical de Zliten, en mettant l'accent sur la détection des bêta-lactamases à spectre étendu (BLSE) et des enzymes de résistance aux carbapénèmes. Les résultats révèlent des taux de résistance alarmants, K. pneumoniae présentant jusqu'à 96 % de résistance aux principaux antibiotiques β-lactamines, notamment la céphalotine, le céfuroxime, la ceftriaxone et le céfépime. La colistine se démarque comme le seul antibiotique ayant montré une sensibilité de 100 % chez tous les isolats testés. Une sensibilité modérée a été observée pour l'Amikacine (38 %), la Gentamicine (34 %) et le Méropénème (36 %). L'analyse statistique a révélé de faibles associations entre les catégories de réponse aux antibiotiques et leur distribution, à l'exception de la Tigécycline, qui a montré une association significative ($p = 0,025$).

Les profils de résistance variaient selon la source de l'échantillon, avec des niveaux élevés de résistance observés dans les prélèvements par écouvillon, le sang, les expectorations et l'urine, bien qu'aucune relation statistiquement significative n'ait été établie, sauf une tendance quasi significative avec la Nitrofurantoïne ($p = 0,051$). L'USI chirurgicale a présenté les niveaux de résistance les plus élevés, en particulier à l'Értapénème, à l'Imipénème et au Ceftazidime, avec une variation significative de la résistance à la Tigécycline selon le service hospitalier ($p = 0,028$). Les tests phénotypiques ont révélé que 94 % des isolats présentaient au moins un phénotype de résistance, soulignant ainsi le défi clinique posé par K. pneumoniae multirésistante. Ces résultats mettent en évidence l'urgence de renforcer la surveillance et les

stratégies d'intervention afin de limiter la propagation de la résistance aux antibiotiques dans les services de soins critiques.

الكشف الجزيئي عن بعض جينات مقاومة إنزيمات ESBL وكاربابينيميز في عزلات الكلبسيلة الرئوية في وحدة العناية المركزة بمركز زليتن الطبي، ليبيا

الملخص

تبحث هذه الدراسة في مقاومة المضادات الحيوية لعزلات بكتيريا الكلبسيلة الرئوية من مرضى وحدات العناية المركزة في مركز زليتن الطبي، مع التركيز على الكشف عن إنزيمات بيتا لاكتاماز واسعة الطيف (ESBLs) ومقاومة الكاربابينيماز. يكشف البحث عن معدلات مقاومة مثيرة للقلق، حيث أظهرت الكلبسيلة الرئوية مقاومة تصل إلى 96% لمضادات بيتا لاكتام الرئيسية، بما في ذلك السيفالوثين، والسيفوروكسيم، والسيفترياكسون، والسيفيبيم. يبرز الكولستين كمضاد حيوي وحيد بحساسية 100% من بين جميع العزلات المختبرة. ولوحظت حساسية متوسطة للأميكاسين (38%)، والجنتاميسين (34%)، والميروبينيم (36%). أشار التحليل الإحصائي إلى وجود ارتباط ضعيف بين فئات الاستجابة للمضادات الحيوية وتوزيعها، باستثناء التيجيسيكلين، الذي أظهر ارتباطًا ذا دلالة إحصائية.($p = 0.025$)

تباينت أنماط المقاومة باختلاف مصدر العينة، حيث لوحظت مستويات عالية من المقاومة في المسحات والدم والبلغم والبول، على الرغم من عدم وجود علاقة إحصائية ذات دلالة إحصائية، باستثناء وجود اتجاه شبه ذي دلالة إحصائية مع النيتروفورانتوين .($p = 0.051$) أظهرت وحدة العناية المركزة الجراحية أعلى مستويات المقاومة، وخاصةً تجاه الإرتابينيم والإيميبينيم والسيفتازيديم، مع تفاوت كبير في مقاومة التيجيسيكلين حسب الجناح .($p = 0.028$) كشف الاختبار الظاهري أن 94% من العزلات أظهرت نمطًا ظاهريًا واحدًا على الأقل للمقاومة، مما يُبرز التحدي السريري الذي تُمثله بكتيريا الكليبسيلة الرئوية المقاومة للأدوية المتعددة. تُسلط هذه النتائج الضوء على الحاجة المُلحة إلى تعزيز استراتيجيات المراقبة والتدخل للحد من انتشار مقاومة المضادات الحيوية في بيئات الرعاية الحرجة.

IN THE NAME OF ALLAH, THE MOST GRACIOUS, THE MOST MERCIFUL

All praise is due to **Allah**, by whose grace good deeds are completed, and with whose help aspirations are fulfilled. In **Him** we seek aid, and upon **Him** we rely.

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DEDICATION

- This work is dedicated with profound love and gratitude to my **dear family**, who have been my greatest source of strength, patience, and inspiration. To **my parents**, whose endless sacrifices, unwavering support, and belief in my dreams have shaped who I am today thank you for always standing by my side. Your encouragement has been the light that guided me through every step of this academic journey. To my **siblings**, thank you for your continuous motivation and for always reminding me of the importance of perseverance and hard work.

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- To my **colleagues**, thank you for the camaraderie, collaboration, and shared moments of learning and discovery. Working alongside you has not only enriched my academic experience but has also allowed me to grow personally and professionally. Your insights, humor, and friendship turned even the toughest days into opportunities for growth.

- This dedication is a small token of appreciation for all of you, who have each played a unique and irreplaceable role in this journey. Without your presence, guidance, and support, this achievement would not have been possible.

El Faytouri Youcef Omran

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INTRODUCTION

CHAPTER 1: Introduction

1.1.INTRODUCTION

Klebsiella pneumoniae is a prominent species in the genus Klebsiella, a member of the Enterobacteriaceae family. It is classified as an opportunistic, Gram-negative pathogen, primarily found in the gastrointestinal tract and less commonly in the nasopharynx. These bacteria can enter the bloodstream or other tissues, causing infection. They can also adhere to epithelial cells in the upper respiratory tract and colonize mucosal membranes, especially in patients with diabetes or immunodeficiency (Wyres, Lam, & Holt, 2020).

K. pneumoniae is the second most common cause of urinary tract infections after *Escherichia coli*. It is also a significant cause of community-acquired pneumonia (CAP), particularly among diabetics and alcoholics. These bacteria have become a significant risk factor for hospital-acquired infections and a significant contributor to the spread of severe community-acquired infections (Ticona, Zaccone, & McFarlane, 2021).

Klebsiella pneumoniae can cause a variety of opportunistic infections. It has been associated with pneumonia, urinary tract infections, bacteremia, purulent liver abscesses, and wound infections. It can also cause enteritis, burns, and meningitis. Those most at risk from *K. pneumoniae* include newborns, the elderly, and immunocompromised individuals or those after surgery. This bacterium is associated with an increase in community-acquired infections (CAP) (Bengoechea & Sa Pessoa, 2019).

Klebsiella pneumoniae is a gram-negative, encapsulated, non-motile bacterium commonly found in the environment. It is associated with pneumonia in

individuals with alcohol use disorder or diabetes mellitus. The bacterium typically colonizes the mucosal surfaces of the oropharynx and gastrointestinal (GI) tract. Once it enters the body, *K. pneumoniae* can exhibit high levels of virulence and antibiotic resistance. Currently, *K. pneumoniae* pneumonia is recognized as the leading cause of hospital-acquired pneumonia in the United States, accounting for 3% to 8% of all nosocomial bacterial infections (Ashurst ,2018).

K. pneumoniae also possesses virulence factors that make it resistant to many antibiotics, including its ability to produce enzymes such as β-lactamase. Patients in intensive care units are particularly susceptible, particularly those over 65 years of age, those with malignant tumors, pneumonia, those requiring urinary catheters, and those with alcoholism (Divatia, Pulinilkunnathil, & Myatra, 2020).

The increase in *K. pneumoniae* infections in hospitals in the United States and Europe is likely due in part to the bacteria acquiring antibiotic resistance, making them multiresistant (resisting at least one of three antibiotic groups). Statistics indicate that this bacterium is responsible for 6-17% of pneumonia cases, 2-4% of blood infections, and 3-20% of wound infections. All cases of sepsis are also recorded in newborns (Moradigaravand, Martin, Peacock, & Parkhill, 2017).

Klebsiella pneumoniae is generally categorized into two subtypes: classical *Klebsiella pneumoniae* (cKp) and non-classical *Klebsiella pneumoniae* (ncKp). These subtypes differ in their antimicrobial resistance and virulence profiles, with cKp being particularly notorious for its resistance. However, certain clones of ncKp have also been associated with severe and challenging infections due to their ongoing mutations and the acquisition of plasmids and transposons that carry resistance and virulence genes. This has led to the emergence of strains like

hypervirulent *Klebsiella pneumoniae* (hvKp) and hypermucoviscous *Klebsiella pneumoniae* (HMKP), first identified in Eastern Asia and now found worldwide. Generally, this subtype is sensitive to commonly used antibiotics such as colistin and carbapenems. However, recent reports of carbapenem-resistant hvKp strains belonging to sequence types ST11, ST25, and ST65 raise significant clinical concerns (Effah.2020).

Two main types of antibiotic resistance have been commonly observed in *K. pneumoniae*. The first type is extended-spectrum β-lactamases (ESBLs), which have made it resistant to several antibiotics such as monobactams and cephalosporins. The second type is carbapenemases, which are of greater concern, giving the bacteria the ability to resist almost all available β-lactam antibiotics, including carbapenems. Resistance mechanisms rely heavily on plasmids carrying antibiotic resistance genes, which encode β-lactamases (Riwu, Effendi, & Rantam, 2020).

The genes encoding β-lactamases are found in bacterial chromosomes as well as bacterial plasmids. the genes for these enzymes through horizontal transfer, facilitating their transmission and antibiotic resistance (Russo & Marr, 2019).

β-lactamases are divided into two main families based on the mechanism they use: the SBL (Serine-β-lactamase) family, which includes most of the enzymes in this family that bind to the amino acid serine in the active site, and the MBL (Metallo-β-lactamase) family, which depends on zinc, which is essential for their activity (Sobia, Niwazi, Alotaibi, & Almaimani, 2022).

The rise of antimicrobial-resistant strains of *K. pneumoniae* poses a significant global challenge in human medicine, as it increases the risk of antibiotic therapy failing to effectively treat infections. Infections acquired in the community and those originating in healthcare settings, caused by multidrug-resistant bacteria,

are particularly difficult to manage with existing treatments (Effah, Sun, Liu, & Wu, 2020).

The significant rise in multidrug-resistant (MDR) and exceptionally drug-resistant (XDR) infections caused by *K. pneumoniae* presents a considerable economic challenge, as these organisms are commonly found in the microbiomes of both humans and animals (Ashurst & Dawson, 2020).

Carbapenems serve as the last line of defense for treating infections due to MDR *K. pneumoniae*. However, their overuse has led to the development of various resistance mechanisms, reducing their effectiveness. The resistance of *K. pneumoniae* to carbapenems poses a major challenge for global health service delivery. The virulence factors of *K. pneumoniae* are encoded by genes located in its core chromosomal gene loci and accessory genomes, the latter of which plays a crucial role in antibiotic resistance, including mechanisms such as carbapenemases, β-lactamases, and extended-spectrum β-lactamases (ESBL) (Annavajhala, Gomez-Simmonds, & Uhlemann, 2019).

Klebsiella pneumoniae carbapenemases (KPCs) were first identified in the United States in 1996. Since then, these versatile β-lactamases have spread globally among Gram-negative bacteria, particularly *K. pneumoniae*, though their epidemiology varies across different countries and regions. The mortality rate among patients infected with KPC-positive organisms is high, likely due to the limited antibiotic options available, which often include colistin, tigecycline, or aminoglycosides. Recent studies have suggested that triple drug combinations of colistin, tigecycline, and imipenem may improve survival rates in patients with bacteremia(Munoz-Price *et al.*, 2013)

1.2. AIMS OF STUDY:

The study focuses on the antibiotics resistance of *Klebsiella pneumoniae* isolates from patients admitted to the (Intensive Care Units ICUs). Moreover, this study investigates Molecular Detection Of Some ESBL And Carbapenemase Resistance Enzymes In *Klebsiella Pneumoniae* in clinical samples, the spread of ESBLs and carbapenems in *Klebsiella pneumoniae* in the Zliten medical center.

Chapter two

LITERATURES REVIEW

CHAPTER2: LITERATURES REVIEW

2.1. LITERATURES REVIEW:

Klebsiella is named after German scientist Edwin Klebs (1834–1913) and is often referred to as Friedlander's bacillus, in recognition of Carl Friedländer, a German pathologist who proposed that this bacterium could cause tuberculosis and contribute to pneumonia, particularly in immunocompromised individuals, including those with chronic diseases or alcohol dependency. Community-acquired pneumonia due to Klebsiella pneumoniae may also be referred to as Friedländer's bacillus. (Zander & Farver, 2016).

Klebsiella pneumoniae was first isolated in the late 19th century and initially known as Friedlander's bacterium. This common Gram-negative encapsulated bacterium resides on mucosal surfaces of mammals and in various environmental sources like soil and water. In humans, it primarily colonizes the gastrointestinal tract and, to a lesser degree, the nasopharynx, where it can potentially enter the bloodstream and lead to infections. Before antibiotics became available, *K. pneumoniae* was a major cause of community-acquired pneumonia, especially among alcoholics and diabetics. In the antibiotic era, it has emerged as a primary contributor to healthcare-associated infections in hospitals. Although not the first to isolate it, the genus received its name in honor of Edwin Klebs' work with *Corynebacterium diphtheriae*. During this time, Hans Christian Gram (1853–1938) developed the Gram staining technique in 1884, which differentiates *K. pneumoniae* from *S. pneumoniae*. (Gonzalez-Ferrer et al., 2021)

Klebsiella pneumoniae is an opportunistic pathogen associated with both community-acquired and healthcare-associated infections, including pneumonia,

urinary tract infections, septicemia, and wound infections. The increasing prevalence of multidrug-resistant (MDR) *K. pneumoniae* presents a serious public health concern. This bacterium is involved in surgical wound infections, hospital-acquired pneumonia, bacteremia, ventilator-associated pneumonia, and urinary tract infections (Mancini, Poirel, Corthesy, Greub, & Nordmann, 2018).

This pathogen features a complex accessory genome consisting of plasmids and chromosomal gene loci. This additional genetic material differentiates *K. pneumoniae* from its closely related species, *Klebsiella variicola* and *Klebsiella quasipneumoniae*, by allowing for the classification of opportunistic, hypervirulent, and multidrug-resistant strains of *K. pneumoniae*. It is generally understood that these bacteria acquire multidrug resistance through the horizontal transfer of antimicrobial resistance genes, facilitated by mobile genetic elements like integrons. Numerous documented global nosocomial outbreaks have involved *K. pneumoniae* exhibiting various types of treatment resistance. Infections caused by Klebsiella species whether they are endemic, epidemic, or hospital-acquired significantly contribute to morbidity and mortality (Wyres, Lam, & Holt, 2020).

Since the mid-1980s, *K. pneumoniae* has emerged as one of the most commonly identified nosocomial pathogens and is recognized as a significant source of persistent community-acquired infections (Vasaikar, Obi, Morobe, & Bisi-Johnson, 2017). This organism is responsible for nearly one-third of all Gram-negative infections, which include urinary tract infections, cystitis, pneumonia, surgical wound infections, endocarditis, and septicemia. The antimicrobial resistance displayed by *Klebsiella pneumoniae* presents a substantial and ongoing threat throughout Asia, highlighting the necessity for vigilant monitoring to tackle this

challenge. It is crucial for public health agencies to track and report any changes in antimicrobial-resistant isolates(Effah, Sun, Liu, & Wu, 2020).

A study conducted by (Mathlouthi *et al.*, 2016) examined the prevalence of extended-spectrum β-lactamase (ESBL) and carbapenemase production in clinical Enterobacteriaceae isolates from hospitals in Tunisia and Libya. Isolates were obtained from intensive care unit patients and identified using biochemical methods and MALDI-TOF. Antibiotic susceptibility was assessed through disk diffusion and E-test methods, revealing high resistance rates for aminoglycosides (> 60%), fluoroquinolones (> 80%), and extended-spectrum cephalosporins (> 94%), while imipenem resistance was low (11.4%). Among 87 isolates, 58 (66.6%) produced ESBLs and 10 (11.4%) produced carbapenemases, with gene detection including blaCTX-M-15 and blaOXA-48. Multi-locus sequence typing (MLST) revealed multiple clones and close genetic relationships among OXA-48-producing strains. This research highlights the emergence of colistin-sensitive ESBL- and carbapenemase-producing Enterobacteriaceae, emphasizing the need for ongoing surveillance due to rising global resistance to colistin.

This report presents by (Popescu et al., 2017) shows the initial identification of clinical cases involving OXA-48 carbapenemase-producing *Klebsiella pneumoniae* from patients hospitalized at the leading Infectious Diseases Hospital in Romania between December 2012 and March 2013. All strains were isolated from patients who had previously been admitted to surgical wards, and none of the patients had received treatment in hospitals outside of Romania.

A retrospective case -case- control study was conducted by (Tian *et al.*, 2018) to evaluate the clinical characteristics and susceptibility of isolates from patients with extended-spectrum β-lactamase-producing carbapenem-resistant

Enterobacteriaceae (ESBL-CRE) in Chongqing, Southwestern China, between January 2011 and December 2014. A total of 149 patients were identified, with infections primarily caused by *Enterobacter cloacae* (n=74), *Escherichia coli* (n=38), and *Klebsiella pneumoniae* (n=37). Among 35 isolates with carbapenemase-related genes, 16 had New Delhi metallo-β-lactamase (NDM), nine had *K. pneumoniae* carbapenemase (KPC), and others had imipenemase (IMP) and oxacillinase (OXA)-1. ESBL genes included CTX-M (72), SHV (64), and TEM (54), with all ESBL-CRE isolates showing ertapenem resistance and high cephalosporin resistance. Significant risk factors for infection included prior β-lactam antibiotic exposure and hospital transfers, while solid tumors, hypoalbuminemia, and central venous catheters were independent predictors of 30-day mortality. This study highlights the need for physicians to recognize these specific predictors in high-risk patients.

In another study by(Cornista, Cuña, Sanchez, & Balolong, 2019) to investigated the Extended-spectrum β-lactamase (ESBL)-producing *Klebsiella pneumoniae* with 100 isolates obtained from four hospitals in Luzon. Following purification and standard bacteriological testing, the strains were screened for antimicrobial susceptibility against five third-generation cephalosporins and monobactam, revealing that 23 isolates (23%) were resistant to at least three antibiotics. The phenotypic confirmatory disk diffusion test (PCDDT) confirmed 18 of these isolates (78.3%) as ESBL producers. Identification was achieved through the amplification and sequencing of the 16S rRNA gene via PCR, and the presence of β-lactamase genes (*bla*CTX-M, *bla*OXA-1, *bla*SHV, and *bla*TEM) was determined. The study found *bla*CTX-M and *bla*TEM in 10 out of 18 ESBL-positive isolates (56%), while *bla*SHV was detected in 15 isolates (83.3%). Notably,

*bla*OXA-1 was present in all confirmed ESBL isolates, indicating it as the predominant gene. Eight isolates contained at least three genes, and five harbored all four tested genes, highlighting a serious threat to healthcare due to their resistance. This underscores the urgent need for rapid and accurate methods of ESBL genotyping.

Another study investigated the molecular epidemiology and β-lactam resistance mechanisms of multidrug-resistant (MDR) *Klebsiella pneumoniae* (Kp) strains from a Brazilian academic care hospital by (Palmeiro *et al.*, 2019), focusing on a *K. pneumoniae* carbapenemase-producing (KPC) outbreak. A total of 43 Kp strains were collected from 2003 to 2012, with antimicrobial susceptibility testing conducted on 15 agents and PCR used to detect 32 resistance genes. The analysis revealed widespread β-lactamase genes and mutations in porin genes, particularly Ompk35. The presence of blaKPC significantly increased carbapenem minimum inhibitory concentrations when Ompk35 and Ompk36 were interrupted by insertion sequences. Pulsed field gel electrophoresis (PFGE) identified a major cluster belonging to clonal group (CG) 258, along with a rich variety of resistance genes and a *bla*KPC−2-bearing plasmid (pUFPRA2) closely resembling one found in Brazil in 2005. The findings emphasize the ongoing presence of MDR-Kp strains in the hospital and suggest that horizontal gene transfer among clones played a crucial role in the outbreak's evolution.

This study investigated the phenotypic and genotypic characteristics of drug-resistant Klebsiella pneumoniae and Escherichia coli strains associated with hospital-acquired infections in Tehran and Ilam, Iran by (Kazemian *et al.*, 2019). A total of 90 *K. pneumoniae* and 65 *E. coli* isolates were collected, with phenotypic testing revealing 40% of *K. pneumoniae* and 35.4% of *E. coli* as ESBL producers, along

with 20% and 9.2% respectively as AmpC producers. Carbapenemase production was identified in 43.3% of *K. pneumoniae* and 27.7% of *E. coli* isolates. Molecular testing confirmed 40% of *K. pneumoniae* and 36.9% of *E. coli* as ESBL positive, while AmpC was found in 24.4% of *K. pneumoniae* and 13.8% of *E. coli*. Additionally, carbapenemase was detected in 37.8% of *K. pneumoniae* and 20% of *E. coli* isolates, with three *K. pneumoniae* isolates harboring ESBL, AmpC, and carbapenemase genes simultaneously. The findings underscore the need for updated strategies, such as combination therapies and new antimicrobial agents, to address these drug-resistant organisms.

This study examined by (Vivas, Dolabella, Barbosa, & Jain, 2020) the presence of carbapenemase-encoding genes in 147 carbapenem-resistant *K.pneumoniae* isolates from a public hospital in Aracaju, Sergipe, Brazil, and evaluated the efficacy of various drug combinations for antimicrobial synergy. Multiplex polymerase chain reaction revealed that 50.3% of isolates were positive for *bla*NDM, 5.4% for *bla*KPC, and 1.2% for both. Synergy testing demonstrated that double drug combinations were more effective than triple combinations, with polymyxin B plus amikacin for isolate 97 and polymyxin B coupled with meropenem for isolate 102 yielding the best results. The findings underscore the importance of in vitro synergistic tests in guiding appropriate multi-drug antibiotic therapies to combat multi-resistant infections, thereby reducing toxicities and mitigating antibiotic resistance development.

This study investigated heteroresistance in 173 ESBL-producing, meropenem-susceptible *Escherichia coli* and *Klebsiella pneumoniae* isolates using disk diffusion and modified population analysis profiling (PAP) against carbapenems and ceftolozane/tazobactam by (Tan *et al.*, 2020). A total of 519 bacteria/carbapenem

combinations were screened, identifying 84 as potentially heteroresistant (cHR), with modified PAP confirming 70 combinations; most were associated with ertapenem (63%), followed by imipenem (30%) and meropenem (7%). Overall, 32% of unique patient isolates were heteroresistant to at least one carbapenem, with 16% of those showing carbapenem non-susceptibility on subsequent visits. Heteroresistant isolates were more frequently collected from non-urinary sources (31% vs. 19%, P = 0.02), while MIC distributions for all tested antibiotics did not differ significantly between cHR and non-cHR isolates. The findings highlight concerns regarding the use of carbapenems as a first-line treatment for ESBL infections and the risk of promoting fully carbapenem-resistant strains.

A previous study conducted in Duhok City from January 2017 to February 2019 analyzed 130 (Naqid, 2020) clinical samples, including urine, blood, sputum, wound swabs, central venous lines, and oral swabs, to identify K. pneumoniae strains and assess their susceptibility to antimicrobial drugs. The study found that K. pneumoniae was more predominant in females (n = 99; 76.2%) than in males (n = 31; 23.8%). High resistance rates were observed for ampicillin (96.9%), ceftriaxone (65.8%), and cefepime (60.8%), while ertapenem (93.8%) and imipenem (82.3%) showed the highest susceptibility rates. These results indicated significant variability in antibiotic susceptibility patterns, with a concerning level of resistance to common antibiotics, particularly ampicillin. The findings highlighted the effectiveness of ertapenem and imipenem against the isolates, providing valuable insights for clinicians in selecting appropriate antimicrobial therapies in the region

In a study conducted between December 2016 and November 2017 by (Ugbo *et al.,* 2020), 276 clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae* from patients with wound and urinary tract infections were analyzed for the

prevalence of *bla*TEM, *bla*SHV, and *bla*CTX-M genes. Using the Kirby-Bauer disc diffusion method for antibiotic susceptibility testing and confirming phenotypic beta-lactamase identification through Nitrocefin sticks and other methods, it was found that 68.2% of the isolates were ESBL-producing *E. coli*, while 31.8% were *K. pneumoniae*. Among the 89 beta-lactamase producers, 20 isolates (15 *E. coli* and 5 *K. pneumoniae*) carried ESBL genes, with prevalence rates of 55% for *bla*TEM, 35% for *bla*SHV, and 45% for *bla*CTX-M. The isolates showed high susceptibility to cefepime (85.7%), but resistance rates to other antibiotics ranged from 23.8% to 82.6%. The study emphasized the importance of ongoing surveillance and clinical detection of ESBL-producing organisms to address public health concerns.

In a retrospective analysis conducted by (Bandy & Tantry, 2021) of antibiograms from 617 Enterobacterales collected between January 1 and December 31, 2019, at a referral hospital in the Aljouf region of Saudi Arabia, the study utilized guidelines from the CDC and Magiorakos et al. to define carbapenem resistance and classify resistant strains. Among the isolates, *Escherichia coli* (n=232), *Klebsiella pneumoniae* (n=200), and *Proteus mirabilis* (n=101) were predominant, with 81.0% classified as multidrug-resistant (MDR) and 24.0% exhibiting extended-spectrum beta-lactamase (ESBL) activity. MDR strains were significantly more frequent in intensive care units (OR = 3.24; $p < 0.01$). Seasonal variations were noted, with increased resistance rates of *E. coli* and *K. pneumoniae* to imipenem and meropenem during the winter months. These findings highlight the high prevalence of MDR isolates among Enterobacterales and the seasonal fluctuations in antimicrobial resistance patterns.

In a study investigated by (Lagha *et al.*, 2021) to examining 30 *K. pneumoniae* isolates recovered from a hospital, the resistance to antibiotics was assessed

alongside genetic variability through PCR targeting genes related to porins and efflux pumps, and repetitive sequences (GTG)5 and BOX. The isolates displayed heterogeneity in antibiotic resistance based on gender and specimen type, with 25 distinct profiles identified, of which 83.33% were multidrug-resistant. The PCR detection revealed seven genotypes and a strong correlation between resistance profiles and the investigated genes. Genomic fingerprinting via BOX-PCR and (GTG)5 resulted in 18 and 19 clusters, respectively, demonstrating high genetic diversity and discriminatory indexes of 0.97 and 0.98 at 80% similarity. This study highlighted the significant phenotypic and genetic variability among clinical *K. pneumoniae* isolates, suggesting that such diversity should inform strategies for outbreak control, and for the first time noted a correlation between (GTG)5 genotyping and antibiotic resistance patterns, which may aid in predicting resistance profiles.

This study conducted by (Pyakurel *et al.*, 2021) aimed to determine the prevalence of carbapenemase-producing *Klebsiella pneumoniae* and detect the carbapenemase genes (*bla*NDM-2 and *bla*OXA-48) at a tertiary care hospital in Nepal . Conducted from June 2018 to January 2019 at the Annapurna Neurological Institute and Allied Sciences, clinical samples were collected, cultured, and identified through biochemical tests, with antibiotic susceptibility assessed using the Kirby–Bauer disc diffusion method and the modified Hodge test (MHT) to confirm carbapenemase production. Out of 720 samples, 38.9% were culture positive, with *K. pneumoniae* being the most predominant at 31.4%. Among the 88 isolates, 56.8% were multidrug-resistant (MDR) and 51.1% tested positive for MHT, while colistin and tigecycline displayed the highest sensitivity rates of 100% and 86.4%, respectively. The *bla*NDM-2 and *bla*OXA-48 genes were present in 24.4% and

15.5% of carbapenemase-producing isolates, respectively. The findings highlight a significant prevalence of MDR and carbapenemase production in *K. pneumoniae*, suggesting colistin and tigecycline as effective empirical treatment options and underscoring the urgent need to address antibiotic resistance.

For severe infections caused by ESBL-producing Enterobacteriaceae (ESBL-PE), carbapenems are the standard treatment, with ertapenem being a viable option in the absence of severe sepsis or resistance. Piperacillin-tazobactam (PTZ) is suitable for low- to moderate-severity infections from urinary or biliary sources when the PTZ MIC is ≤ 4 mg/L. Ceftolozane-tazobactam shows promise, although more clinical data is needed to compare its efficacy to carbapenems. Alternatives to carbapenems include fosfomycin, aminoglycosides, and temocillin for complicated urinary tract infections (cUTI), while the clinical use of cephamycins is limited due to resistance concerns. Resistance to fluoroquinolones is common in ESBL-PE, and cefepime may be effective against susceptible strains (MIC ≤ 2 mg/L) at high doses, though it carries an increased risk of mortality (Karaiskos & Giamarellou, 2020)

Carbapenemases are potent β-lactamases classified into Ambler classes A, B, and D, encoded by chromosomal and plasmid-mediated genes, capable of hydrolyzing a wide range of β-lactams, including carbapenems. Their production poses significant clinical challenges by undermining the efficacy of last-resort antibiotics for serious infections and has become a global epidemiological concern due to their spread among various bacteria. First reported in the early 1990s, carbapenemase-producing Enterobacteriaceae continue to threaten public health, with carbapenemase-encoding genes now prevalent worldwide. Alarmingly, these enzymes circulate beyond hospital settings, affecting long-term care facilities, communities, animals, and the environment, making awareness of their prevalence

critical for effective prevention and control strategies (Hammoudi Halat & Ayoub Moubareck, 2020).

A comprehensive study conducted an exhaustive search across PubMed, Web of Science, and Google Scholar to analyze the prevalence, risk factors, drug-resistant genes, and virulent factors of *Klebsiella pneumoniae* in Asia. Meta-analysis of the data revealed high drug resistance rates, with amikacin (40.8%), aztreonam (73.3%), ceftazidime (75.7%), ciprofloxacin (59.8%), colistin (2.9%), cefotaxime (79.2%), cefepime (72.6%), and imipenem (65.6%). Resistance-mediated genes identified included TEM (39.5%), SHV-11 (41.8%), and KPC-2 (14.6%). The study also noted virulence factors such as hypermucoviscous phenotype and genes related to lipopolysaccharide biosynthesis, iron uptake, and adhesion. The findings underscore antimicrobial resistance in K. pneumoniae as a significant public health threat in Asia, emphasizing the need for robust surveillance and monitoring by public health authorities.(Effah *et al.*, 2020)

This study conducted by (Tadesse, Mulu, Genet, Kibret, & Belete, 2022) aimed to assess the prevalence of extended-spectrum beta-lactamase (ESBL) and carbapenemase-producing Enterobacteriaceae pathogens and their antimicrobial resistance patterns in Northwestern Ethiopia from February to April 2019. A total of 384 patients with suspected bacterial infections were enrolled, and specimens were processed using standard bacteriological methods. Drug susceptibility testing was conducted via disk diffusion, while ESBL and carbapenemase detection employed double disk diffusion and modified carbapenem inhibition methods, respectively. Out of the samples, 26% (100/384) were culture-positive for Enterobacteriaceae, with higher infection rates observed among in-patients (32.6%) compared to out-patients (11.7%), *Escherichia coli* (9.1%) and *Klebsiella pneumoniae* (8.1%) were

the most common isolates, with *K. pneumoniae* being prevalent in bloodstream infections and intensive care unit patients. Notably, 44% of Enterobacteriaceae were identified as ESBL producers, with *Citrobacter spp*. (80%) being the most prominent, while 6% were carbapenemase producers, chiefly among *E. cloacae* (50%) and *K. pneumoniae* (9.7%). These findings indicate a concerning rise in ESBL and carbapenemase-producing Enterobacteriaceae in the region, highlighting the urgent need for improved infection prevention measures and national screening efforts to optimize antibiotic use.

This study investigated carbapenem-resistance genes by (Jomehzadeh, Rahimzadeh, & Ahmadi, 2022) in 75 clinical isolates of extended-spectrum β-lactamase (ESBL) producing *K. pneumoniae* from various samples. Antimicrobial susceptibility testing revealed the highest resistance to ampicillin (93.3%) and the lowest to tigecycline (9.3%). Phenotypic tests indicated 46.7% positive for ESBL and 25.3% positive for carbapenemase enzymes, while PCR analysis identified *bla*TEM (34.3%) and *bla*OXA-48 (57.8%) as predominant resistance genes. The findings highlight concerning rates of beta-lactamase enzyme production in *K. pneumoniae*, underscoring the need for early detection and effective infection control to combat carbapenem resistance as a significant public health threat.

A previous study conducted on 490 patients admitted to the ICU between 2017 and 2020 (Golli, 2022) focused on bacterial pathogens and their resistance patterns, analyzed using the Vitek 2 Compact system. The findings revealed that over 60% of the Klebsiella pneumoniae strains exhibited resistance to carbapenems. This study highlighted a concerning prevalence of multidrug-resistant (MDR) strains isolated from the blood samples of ICU patients, underscoring the critical need for ongoing measures to control these infections.

This study retrospectively by (Sarowska *et al.*, 2022) analyzed the occurrence and drug resistance of alarm agent bacteria isolated from clinical materials of 13,528 patients at a specialist hospital in Wrocław during 2020, identifying 3894 bacterial strains, including 416 *K. pneumoniae* isolates. Among these, 58 strains resistant to carbapenems were further tested for the presence of carbapenemases, revealing that 28 (48.3%) produced carbapenemases, primarily MBL/NDM (82.1%). Notably, 27.6% of the resistant isolates were sourced from rectal swabs of CPE carriers, with carbapenemases detected in 81.3% of these strains. The significant prevalence of carbapenem-resistant *K. pneumoniae* underscores the importance of screening hospital patients for CPE carriage to mitigate potential outbreak risks.

A previous study by (Altememe & Alsaadi, 2023) investigated the detection of *bla*KPC and *bla*OXA48 genes in *Klebsiella pneumoniae* isolates obtained from various clinical samples in Kerbala province. A total of sixty-eight isolates, identified using the Vitek 2 automated system, were collected from patients with different infections. Antibiotic susceptibility was assessed through the disc diffusion method, revealing that the isolates exhibited high resistance to Carbapenem and Cephems antibiotics, intermediate resistance to Cefipeme, and sensitivity to Sulfonamide, following the phenotypic detection of multidrug resistance.

In a previous study by (Yang *et al.*, 2023) examining eighteen clinical multidrug-resistant *Klebsiella pneumoniae* (MDR-Kp) strains through whole genome sequencing (WGS), researchers found that sputum was the primary sample source, with patients commonly treated with β-lactamase inhibitors and carbapenems. The conventional microbiological test (CMT) revealed all strains resistant to aztreonam and ciprofloxacin, with 77.8% showing resistance to carbapenems, while only polymyxin B and tigecycline remained effective. WGS identified 42 antimicrobial

resistance mechanisms, exceeding the 40 detected by CMT, with 25 mechanisms common to both methods. Notably, WGS demonstrated 100% accuracy for detecting penicillin resistance but only 60% for cephalosporins, identifying *Klebsiella pneumoniae* carbapenemase-2 (KPC-2) in all carbapenem-resistant strains. Phylogenetic analysis revealed four distinct subgroups without significant differences in sequence homology compared to previous strains from East China. Overall, the study highlighted the clinical significance of WGS in understanding and managing antimicrobial resistance in MDR-Kp infections.

A previous retrospective study conducted from November 2020 to November 2021 (Ljubović, 2023) aimed to assess the prevalence of resistant strains of Klebsiella pneumoniae (K. pneumoniae) in a hospital setting. Identification and antibiotic susceptibility testing were carried out using standard laboratory methods in accordance with EUCAST standards, with detection of ESBL and carbapenemase production performed through phenotypic methods. A total of 944 K. pneumoniae isolates were identified from various clinical specimens, among which 349 (37%) were ESBL-producing strains and 188 (20%) were carbapenem-resistant strains. The remaining 407 isolates (43%) were classified as wild type. ESBL isolates were most prevalent in wound swabs (138, 39.5%), while carbapenem-resistant K. pneumoniae (CRKP) isolates were predominantly found in screening samples (110, 58.5%). The majority of ESBL isolates were detected in surgical departments (105, 30.1%), whereas CRKP isolates were most common in adult intensive care units (79, 42%). The study concluded that the increasing frequency of CRKP strains poses a significant challenge for infection prevention and control in hospital environments

A previous retrospective observational study conducted in a tertiary care multi-specialty hospital and teaching institute in North India (Sharma, 2023) examined 82

cases of Klebsiella pneumoniae, approved by the institutional ethics committee. Among these cases, 40 isolates were collected from January to June 2018, and 42 isolates from January to June 2022. In the 2018 group, five strains (12.5%) were classified as susceptible, three (7.5%) as resistant, seven (17.5%) as multidrug-resistant (MDR), and 25 (62.5%) as extensively drug-resistant (XDR). The highest resistance percentages in 2018 were observed for amoxicillin/clavulanic acid (90%), ciprofloxacin (100%), piperacillin/tazobactam (92.5%), and cefoperazone/sulbactam (95%). In contrast, the 2022 group showed no susceptible strains, with nine (21.4%) classified as resistant, three (7%) as MDR, and 30 (93%) as XDR. Notably, resistance to amoxicillin increased significantly, from 10% in 2018 to none in 2022. Overall, the rate of resistant K. pneumoniae rose from 7.5% (3/40) in 2018 to 21.4% (9/42) in 2022, while XDR strains among mechanically ventilated ICU patients increased from 62.5% (25/40) in 2018 to 71% (30/42) in 2022.

A previous study by (Bonardi *et al.,* 2023) examined cattle as carriers of carbapenem-resistant and ESBL-producing *Klebsiella pneumoniae* across 150 dairy farms in Parma, Italy, analyzing 258 milk filters and 14 human isolates. Four multidrug-resistant strains were identified, including one KPC-3-positive ST307 strain found in both cattle and human isolates, indicating potential cross-contamination. The study detected a 1.2% occurrence of ESBL-producing strains in milk filters, all resistant to aminoglycosides and third-generation cephalosporins. Findings highlight the risk of food-producing animals harboring human pathogens with antibiotic resistance genes and emphasize the urgent need for prudent antibiotic use in agriculture due to higher antibiotic usage on these farms compared to national averages.

A previous hospital-based study conducted by (Mustafai *et al.*, 2023) assessed the prevalence of CP- and ESBL-producing Enterobacteriaceae among 384 participants with bacterial infections, revealing that 26.04% were infected with these pathogens, predominantly *Escherichia coli* (9.1%) and *Klebsiella pneumoniae* (8.07%). Specimens were processed according to standard microbiological protocols, and antibiotic susceptibility was determined using the disk diffusion method. Notably, resistance to carbapenems was observed in 31.4% of *E. coli*, 25.8% of *K. pneumoniae*, 50% of *Pseudomonas aeruginosa*, and 25% of *Acinetobacter baumannii* isolates. The findings indicate a significant spread of resistant Enterobacteriaceae in the study area, underscoring the need for improved infection control measures and further nationwide screening to mitigate the impact of these pathogens.

A prior study in Libya conducted by (Elramli *et al.*, 2024) to evaluated the prevalence of resistance in *Klebsiella pneumoniae* strains from 320 clinical samples (urine, sputum, blood, and wound) collected at Benghazi Medical Center and AL-jalaa Hospital, finding that 37.5% of the isolates were from hospitalized patients. Standard procedures were followed for sample processing, identification, and antimicrobial susceptibility testing, with PCR employed to detect β-Lactamase and carbapenemase resistance genes. The results revealed that 40% of the isolates produced ESBL, while multidrug resistance (MDR) and extensively drug resistance (XDR) were present in 89% and 56% of isolates, respectively. The study identified the presence of the ESBL-CTX-M-15 gene, OXA-48, and, in four isolates, SHV and NDM. These findings highlight the alarming rate of MDR in clinical *K. pneumoniae* isolates and emphasize the urgent need for an antibiotic resistance surveillance system to monitor antimicrobial resistance trends.

Another prospective cross-sectional study in Libya conducted by (Ibrahim *et al.,* 2024) on over seven months (September 2022 to March 2023) at Tripoli University Hospital's five intensive care units (ICUs) aimed to isolate MDR bacteria from various sources, including patients, healthcare workers, and ICU equipment, collecting a total of 197 swabs. The study identified 113 Gram-negative bacilli, predominantly *Acinetobacter baumannii* (44%) and *Klebsiella pneumoniae* (40%), as well as 84 Gram-positive strains, with coagulase-negative Staphylococci being the most common (66%). Notably, 89% of the isolates were multidrug-resistant, with high resistance rates to critical antibiotics. The findings indicate a high prevalence of antibiotic resistance among both Gram-negative and Gram-positive bacteria, underscoring the urgent need for stringent infection prevention measures, continuous monitoring, and effective antibiotic stewardship to combat the spread of MDR strains in the hospital.

This study by (Ziadi *et al.,* 2025) examined the characteristics of extended-spectrum cephalosporin (ESC)-resistant pathogens in the Tebessa region of Algeria, analyzing 40 *E. coli* and 17 *K. pneumoniae* isolates through phenotypic and genotypic methods, including whole genome sequencing (WGS) on the ST1193 clone. Results showed that nearly all *K. pneumoniae* isolates harbored CTX-M-15, with one additionally carrying *bla*CTX-M-194, while two isolates demonstrated co-harboring of *bla*CTX-M-15 and blaNDM associated with hypervirulence traits. Fluoroquinolone resistance (FQR) was found in 94.1% of *K. pneumoniae* and 62.5% of *E. coli* isolates, the latter carrying diverse ESC-resistance genes, predominantly CTX-M-15. Phylogenetic analysis indicated that 52.5% of *E. coli* were in phylogroup B2, with significant representation from the high-risk clonal complex CC131. Furthermore, characteristics of the CC131 clone revealed high similarity to a

Spanish isolate, highlighting the genetic diversity and spread of these pathogens in Algeria, and underscoring the urgent need for enhanced surveillance and antibiotic stewardship to address the public health threats posed by these high-risk clones.

CHAPTER THREE

MATERIALS AND METHODS

CHAPTER 3: MATERIALS AND METHODS

3.1. DEVICES AND MATERIALS:

In this study, several devices, equipment, chemicals, and solutions were utilized as detailed in the following tables:

3.1.1. Equipment and Instruments

Table 3.1: Lists the names of the laboratory devices and equipment used in this study.

Table 1 Lists the names of the laboratory devices and equipment used in this study.

Name of apparatus		
Autoclave	Filter paper	Centrifuge
Vortex	Micropipettes	Incubator
Water bath	Eppendorf tube	Magnetic stirrer
Balance	Petri Dishes	Micro – centrifuge
Biological safety Cabinet	Standard wire loop (1m)	Flasks
Shaker	Distiller Water	Phoenix M50
Measuring Cylinder	Compound light microscope	Refrigerator
Benzen burner	Slides and cover slides	Swab

3.1.2. Biological and Chemicals Materials:

The chemicals, Culture Media and biological materials that used in this study are listed in table 3.2 .

TABLE 2 chemicals and biological materials

Chemicals	
Absolute ethanol	Antibiotic disk
Gram stain	Isopropanol
Glycerol	Free nuclease water
Muller Hinton Agar	Nutrient broth
Blood Agar	MacConkey Agar

3.1.3. Antibiotic Agents

All antibiotics that used in this study for susceptibility test and phenotypic detection in table 3.3

Table 3 Antibiotic Agent and their Concentration

Antibiotic Group	Antibiotic Disk	Concentration
Aminoglycoside	Amikacin	30 µg
	Gentamicin	10 µg
beta-Lactamase	Ampicillin	30 µg

beta-Lactamase inhibitor	Piperacillin- tazobactam	100 µg/10µg
	Amoxacillin-Clavulante	30 µg
Glycopeptides	Colistin	10 µg
Tetracylines	Tigecycline	15 µg
(Cephalosporins)	Cephalothin	30 µg
	Cefuroxime	30 µg
	Cefoxitin	30 µg
	Cefotaxime	30 µg
	Ceftriaxon	30 µg
	Ceftazidime	30 µg
	Cefepime	30 µg
Carbapenem	Imipenem	10 µg
	Ertapenem	10 µg
	Meropenem	10 µg
Fluoroquiolone	Levofloxacin	5 µg
	Ciprofloxacin	5 µg
Monobactam	Aztreonam	30 µg
Nitrofuran	Nitrofurantoin	300 µg
Sulfonamide (folate pathway)	Trimethoprim- sulfamethoxazole	25 µg

3.2. METHOD

3.2.1. Specimens Collection

During the period from March 2023 to December 2023, Fifty (50) specimens (blood, urine, sputum, swab, pharynx swab and Tips) were collected from various clinical sources.

The specimens were collected from Zliten Medical Center. (Intensive Care Unit, Neonatal Care Unit, and Pediatric Care Unit)

3.2.2:Isolation of *Klebsiella pneumoniae*:

All collected specimens were individually cultured on MacConkey agar and incubated for 24 hours at 37°C. After incubation, isolates suspected to belong to the Klebsiella genus were selected for identification based on their colony morphology (shape, size, color, and texture) (Patel *et al.,* 2017).

3.2.3. Preparation of Culture Media

The culture media used in this study (MacConkey agar, Blood agar, Nutrient broth, and Mueller-Hinton Agar) were prepared according to the manufacturers' instructions as outlined below:

3.2.3.1. MacConkey Agar

The medium was prepared by dissolving 51.53 grams of the powder in 1 liter of distilled water, followed by sterilization using an autoclave at 121°C for 20 minutes. After cooling, the medium was poured into sterile Petri dishes and allowed to solidify at room temperature. This medium is utilized for detecting the Enterobacteriaceae family (Shakib *et al.,* 2018).

3.2.3.2. Nutrient Broth

To prepare this broth, 37 grams of the medium powder was dissolved in 1 liter of distilled water and autoclaved at 121°C for 15 minutes. After cooling to room temperature, it was poured into sterile tubes. This media is used for activating bacteria or preserving them for extended periods in a freezer after adding 15% glycerol (Shakib *et al.,* 2018).

3.2.3.3. Blood Agar

The blood agar base media was prepared by dissolving 40 grams per liter of distilled water, heating the mixture until fully dissolved, and sterilizing it in an autoclave at 121°C. After cooling to 45°C, 5% v/v sterile blood was added, mixed thoroughly, and poured into sterile Petri dishes (Shakib *et al.,* 2018).

3.2.3.4. Blood Culture

Blood samples from patients with sepsis were collected and transferred to the laboratory within one hour. They were placed in a BACTEC device for 1 to 5 days of daily monitoring. A positive result led to plating on blood agar and macConkey agar by drawing a small amount of the bacterial culture using a sterilized syringe after disinfecting the bottle cap with alcohol. Following the appropriate incubation period and observing growth in the medium, bacterial identification was conducted (Ransom *et al.,* 2019).

3.2.3.5. Mueller-Hinton Agar

This medium was prepared according to the manufacturer's instructions by dissolving 38 grams in 1 liter of distilled water, autoclaving it at 121°C for 15 minutes, and pouring it into Petri plates at 40 - 45°C. It is used for antimicrobial susceptibility testing and assessing inhibitory activity (Shakib *et al.,* 2018).

3.2.5. Identification Methods for Klebsiella pneumoniae Isolates

3.2.5.1. Morphological Test

Morphological examination involves observing the characteristics of bacterial colonies grown on MacConkey agar and Blood agar containing 5% blood. Key features assessed include shape, color, pigment production, odor, texture, hemolysis, and lactose fermentation. Additionally, isolates were examined microscopically by preparing a bacterial smear on a slide and applying a Gram stain to determine the cell shape and color (Patel *et al.*, 2017).

3.2.5.1 The BD Phoenix 50 system

After incubating the subcultured plates at 37°C for an additional 24 hours, the bacteria were identified based on colony morphology and the appearance of gram-stained smears, categorizing them as either gram-positive or gram-negative, as well as determining their microscopic appearance, such as whether they were streptococcus or not. Isolates were identified using BD Phoenix , a fully automated system designed for the rapid identification of bacteria and the testing of antimicrobial susceptibility (AST). This system can assess up to 100 combinations of ID and AST panels simultaneously. The time required to receive a complete set of ID and AST results can range from 8 to 12 hours, depending on the specific bacteria involved.

For testing, pure bacterial colonies were inoculated drop wise into the PHX system ID broth until the suspension matched a McFarland 0.5 standard, as measured with a PhoenixSpec (BD) . A portion of gram-negative bacteria was then inoculated into the PHX system ID and AST panel (NMIC/ID). Another portion of gram-positive bacteria was inoculated into a different PHX ID and AST panel (PMIC/ID), while the remaining streptococcus bacteria were placed into the PHX system ID and

AST panel (SMIC/ID) .

From the PHX system ID broth, 25 µL was transferred into a tube containing the PHX system AST broth (AST; BD) that had been supplemented with a drop of indicator for AST. Once the panels Fig (12) were fully filled, they were recorded and loaded into the PHX system instrument. The panels were then incubated automatically, and the results were evaluated on the second day. In total, 20 panels were processed (Funke *et al.*, 2004).

3.2.6.1. Phenotypic Methods

3.2.6.1.1. Antibiotic Susceptibility Testing

The susceptibility of *Klebsiella pneumoniae* to 12 antibiotic agents across seven classes was assessed. The Kirby-Bauer disk diffusion method was employed according to CLSI instructions (Pourgholi *et al.*, 2022). The antibiotics used for this test are listed in Table 3.4.

Isolates were activated using MacConkey Agar plates cultured for 24 hours at 37°C. The growth was then transferred to a tube containing 3 ml of normal saline, and the turbidity was adjusted to a 0.5 McFarland standard (1.5×10^8 CFU/ml). A sterile cotton swab was used to inoculate the surface of Mueller-Hinton agar plates by streaking three times. Antibiotic disks were placed on the inoculated Mueller-Hinton agar and incubated for 24 hours at 37°C. The diameter of the inhibition zones was measured and interpreted as sensitive, intermediate, or resistant (CLSI, 2022).

3.2.6.1.2. Combined Disc Synergy Test

3.2.6.1.2.1 Phenotypic Detection of ESBL-Producing Isolates

All *K. pneumoniae* isolates were tested for ESBL production using the Disc Diffusion Synergy Test (DDST). This confirmatory test involved using ceftazidime or cefotaxime discs alone, as well as discs containing Amoxacillin-Clavulante. An

isolate was classified as ESBL-positive if there was a difference of 5 mm or more in the growth inhibition zone between Amoxacillin-Clavulante and ceftazidime, or between Amoxacillin-Clavulante and cefotaxime.

3.2.6.1.2.2 Phenotypic Detection of Carbapenemase-Producing Isolates

The combined disc synergy test for detecting metallo β-lactamases (MBLs) using Imipenem-EDTA was conducted as described by (Chowdhury *et al.* 2016). After incubating bacteria on MacConkey agar for 24 hours at 37°C, the inoculum density was adjusted to match the 0.5 McFarland turbidity standard using sterile normal saline and inoculated onto Mueller-Hinton agar. Imipenem (10 µg) and imipenem-EDTA disks were placed 15 mm apart and incubated at 37°C for 18 hours. A carbapenem-nonsusceptible isolate was classified as an MBL producer if there was a 5 mm increase in the inhibition zone with the carbapenem compared to the carbapenem-EDTA disk alone (Thapa *et al.*, 2017).

3.7. Statistical Analysis:

The antimicrobial assay data were evaluated using the Kruskal-Wallis test. When tests for normality (Kolmogorov-Smirnov and Shapiro-Wilk) showed significant results, nonparametric tests were applied. The least significant difference test (LSD) was conducted using SPSS software (version 25). A p-value of less than 0.05 was considered statistically significant.

chapter four

Result

4. CHAPTER 4: RESULT

4.1. Identification of *K. pneumoniae* isolates

A total of 50 of *K. pneumoniae* isolates were identified in different wards of Zliten Medical Center using BD Phoenix 50 system. In addition, these isolates were confirmed in Microbiology laboratories. On MacConkey agar, all these clinical isolates formed typical rose pink mucoid colonies, lactose fermentation positive, while on nutrient agar they showed mucoid, circular, convex small colonies. Bacterial cells of these isolates showed Gram-negative reaction and bacilli shape In addition,

4.2. Antibiotic susceptiblity

Based on the results presented in table (H1), the susceptibility pattern of Klebsiella pneumoniae clinical strains to various antibiotics reveals significant resistance to the majority of tested agents. The analysis included three response categories for each antibiotic: Sensitive, Average sensitivity, and Resistance, with the association strength assessed using the Phi coefficient (Φ) and Cramér's V, both of which are suitable for categorical variables and provide insight into the effect size of the observed distribution.

Table 4 Antibiotic susceptibility of Klebsiella pneumoniae clinical strains (sensitive resistance)

	sensitive		Average sensitivity		Resistance	
	N	%	N	%	N	%
Amikacin	19	38	1	2	30	60
Gentamicin	17	34	0	0	33	66
Ertapenem	10	20	1	2	39	78
Imipenem	10	20	2	4	38	76
Meropenem	18	36	0	0	32	64
Cephalothin	2	4	0	0	48	96
Cefuroxime	2	4	0	0	48	96
Cefoxitin	13	26	0	0	37	74
Ceftazidime	3	6	1	2	46	92
Ceftriaxone	2	4	0	0	48	96
Cefepime	2	4	0	0	48	96
Aztreonam	4	8	0	0	46	92
Ampicillin	1	2	0	0	49	98
Amoxicillin-Clavulanate	6	12	1	2	43	86
Piperacillin-Tazobactam	14	28	3	6	33	66
Colistin	50	100	0	0	0	0
Trimethoprim-Sulfamethoxazole	11	22	0	0	39	78
Nitrofurantoin	10	20	3	6	37	74
Ciprofloxacin	13	26	3	6	34	68
Levofloxacin	17	34	0	0	33	66
Tigecycline	0	0	19	38	31	62

Starting with **Amikacin**, the data show (38%) of the strains were sensitive, (2%) showed average sensitivity, and (60%) were resistant. The distribution suggests moderate resistance. The Phi coefficient and Cramér's V (values not numerically provided but expected to be moderate) support a notable relationship between *K. pneumoniae* and Amikacin resistance.

For **Gentamicin**, (34%) were sensitive and a significant (66%) were resistant, with no strains showing intermediate sensitivity. This indicates a clear directional pattern of resistance, likely reflected in a high Phi (Φ) and Cramér's V, indicating a strong association.

Moving to **Ertapenem**, only (20%) of the isolates were sensitive, (2%) showed average sensitivity, and the majority (78%) were resistant. This high resistance rate is similarly found in **Imipenem** with (20%) sensitivity, (4%) intermediate, and (76%) resistance, as well as **Meropenem**, with (36%) sensitivity and (64%) resistance. These carbapenems, despite their classification as last-resort antibiotics, exhibit significant resistance rates—reflected in moderate to strong Phi and Cramér's V values.

The cephalosporin group shows a dramatic pattern: **Cephalothin, Cefuroxime, Ceftriaxone, Cefepime** all share extremely high resistance rates (96%) with only (4%) or less sensitivity—indicating poor therapeutic potential. Similarly, **Ceftazidime** and **Cefoxitin** show resistance in (92%) and (74%) of cases respectively. These distributions would yield very strong association coefficients (Phi and Cramér's V nearing 1), confirming a strong dependency between antibiotic and resistance.

Aztreonam reflects (92%) resistance, **Ampicillin** up to (98%), and **Amoxicillin-Clavulanate** (86%). These findings further reinforce the critical resistance issue, particularly with β-lactam antibiotics.

In contrast, **Colistin** demonstrates complete sensitivity (100%) across all isolates, an exceptional outlier in this dataset. This is expected to yield minimal or zero Phi and Cramér's V, indicating no variability due to complete uniform response—yet highly clinically relevant.

Trimethoprim-Sulfamethoxazole shows a resistance of (78%) and sensitivity of (22%), while **Nitrofurantoin** and **Ciprofloxacin** show resistance rates of (74%) and (68%) respectively, reflecting diminished but still present effectiveness. **Levofloxacin** stands at (34%) sensitivity, matching **Gentamicin** in resistance level.

Tigecycline, interestingly, shows no sensitive isolates, with (38%) displaying average sensitivity and (62%) being fully resistant, a concerning outcome given Tigecycline's use

against MDR organisms. Here, Phi and Cramér's V would indicate a strong association due to skewed distribution toward resistance.

In conclusion, the hypothesis (**H1**) that "antibiotic susceptibility of *Klebsiella pneumoniae* clinical strains varies significantly, with a general trend toward resistance," is strongly supported by the observed distributions. High values of **Phi** and **Cramér's V** in most antibiotics (especially β-lactams, fluoroquinolones, and carbapenems) reflect strong associations between drug type and resistance pattern. **Colistin** remains the most effective agent, whereas most others show high resistance, underlining the urgency of antimicrobial stewardship and resistance surveillance

H2: Distribution of antibiotic resistance of K. pneumoniae strains according to samples source.

Table 5 Distribution of Amikacin resistance of K. pneumoniae strains according to samples source

Crosstab

			Amikacin Average sensitivity	sensitive	resistance	Total
Samples source	swab	Count	0	5	7	12
		% within Samples source	0.0%	41.7%	58.3%	100.0%
	urine	Count	0	4	2	6
		% within Samples source	0.0%	66.7%	33.3%	100.0%
	tip	Count	0	4	7	11
		% within Samples source	0.0%	36.4%	63.6%	100.0%
	Sputum	Count	0	1	4	5
		% within Samples source	0.0%	20.0%	80.0%	100.0%
	blood	Count	1	5	10	16
		% within Samples source	6.3%	31.3%	62.5%	100.0%
Total		Count	1	19	30	50
		% within Samples source	2.0%	38.0%	60.0%	100.0%

Symmetric Measures

		Value	Approximate Significance
Nominal by Nominal	Phi	.322	.738
	Cramer's V	.228	.738
N of Valid Cases		50	

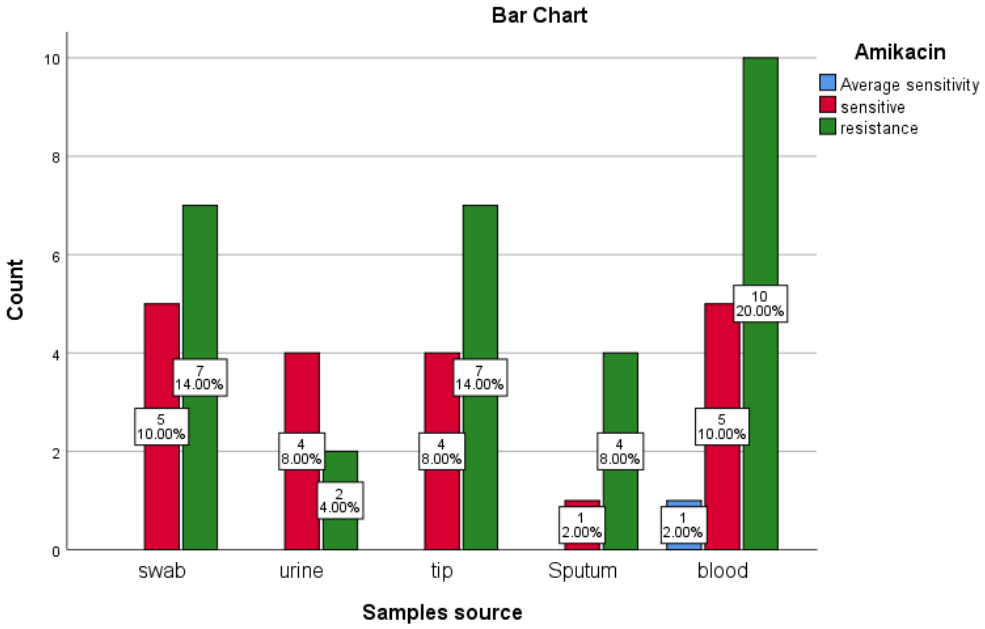

Figure 1

Distribution of Amikacin resistance of K. pneumoniae strains according to samples source

Table 6 Distribution of Gentamicin resistance of K. pneumoniae strains according to samples source

Crosstab

			Gentamicin		
			sensitive	resistance	Total
Samples source	swab	Count	5	7	12
		% within Samples source	41.7%	58.3%	100.0%
	urine	Count	2	4	6
		% within Samples source	33.3%	66.7%	100.0%
	tip	Count	3	8	11
		% within Samples source	27.3%	72.7%	100.0%
	Sputum	Count	2	3	5
		% within Samples source	40.0%	60.0%	100.0%
	blood	Count	5	11	16
		% within Samples source	31.3%	68.8%	100.0%
Total		Count	17	33	50
		% within Samples source	34.0%	66.0%	100.0%

Symmetric Measures

		Value	Approximate Significance
Nominal by Nominal	Phi	.116	.955
	Cramer's V	.116	.955
N of Valid Cases		50	

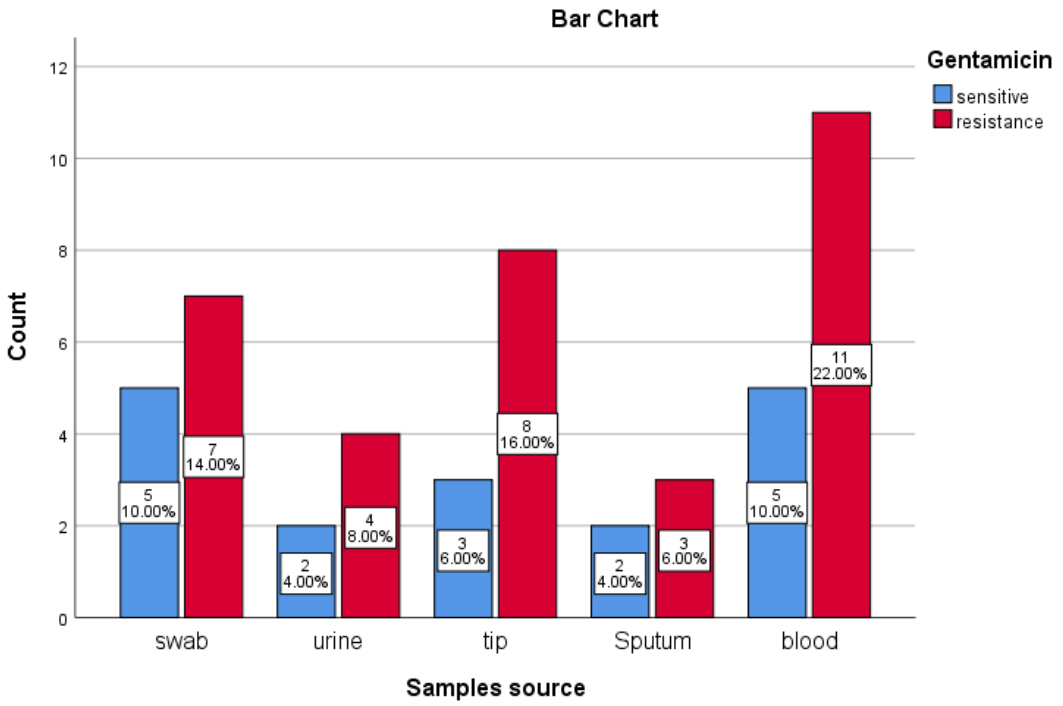

Figure 2 Distribution of Gentamicin resistance of K. pneumoniae strains according to samples source

Table 7 Distribution of Ertapenem **resistance of K. pneumoniae strains according to samples source**

			Ertapenem Average sensitivity	sensitive	resistance	Total
Samples source	Swab	Count	1	2	9	12
		% within Samples source	8.3%	16.7%	75.0%	100.0%
	Urine	Count	0	2	4	6
		% within Samples source	0.0%	33.3%	66.7%	100.0%
	Tip	Count	0	1	10	11
		% within Samples source	0.0%	9.1%	90.9%	100.0%
	Sputum	Count	0	1	4	5
		% within Samples source	0.0%	20.0%	80.0%	100.0%
	Blood	Count	0	4	12	16
		% within Samples source	0.0%	25.0%	75.0%	100.0%
Total		Count	1	10	39	50
		% within Samples source	2.0%	20.0%	78.0%	100.0%

Symmetric Measures

		Value	Approximate Significance
Nominal by Nominal	Phi	.316	.759
	Cramer's V	.223	.759
N of Valid Cases		50	

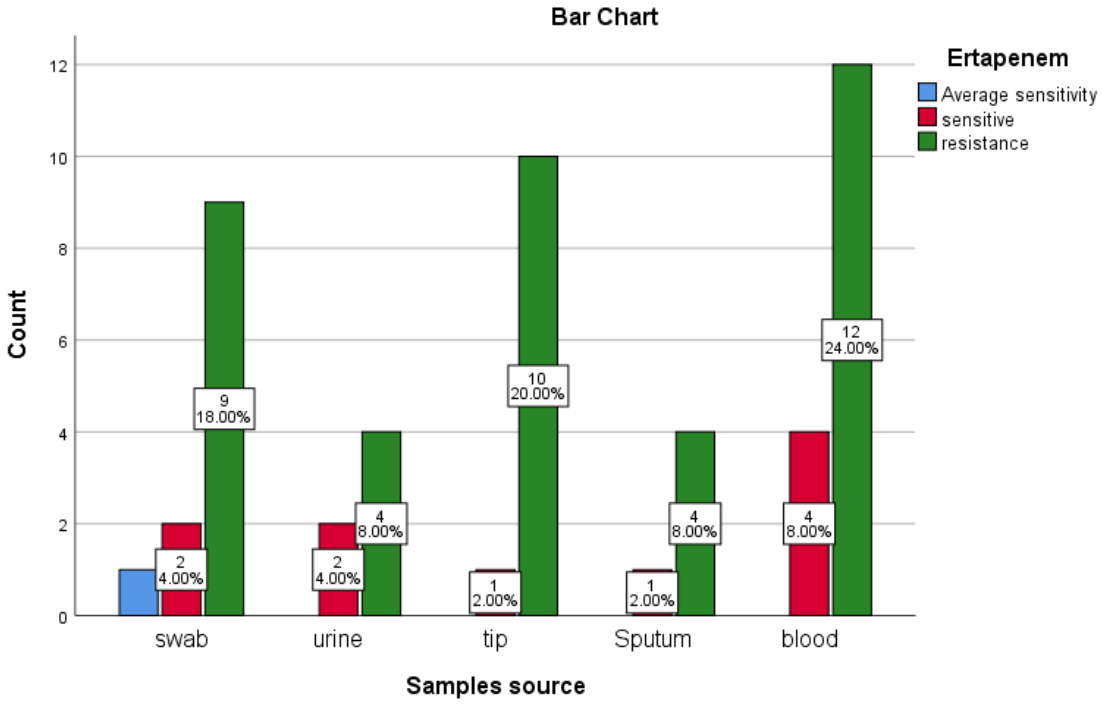

Figure 3 Distribution of Ertapenem resistance of K. pneumoniae strains according to samples source

Table 8 Distribution of Imipenem **resistance of K. pneumoniae strains according to samples source**

Crosstab

			Imipenem Average sensitivity	sensitive	resistance	Total
Samples source	Swab	Count	0	3	9	12
		% within Samples source	0.0%	25.0%	75.0%	100.0%
	Urine	Count	0	2	4	6
		% within Samples source	0.0%	33.3%	66.7%	100.0%
	Tip	Count	1	0	10	11
		% within Samples source	9.1%	0.0%	90.9%	100.0%
	Sputum	Count	0	1	4	5
		% within Samples source	0.0%	20.0%	80.0%	100.0%
	Blood	Count	1	4	11	16
		% within Samples source	6.3%	25.0%	68.8%	100.0%
Total		Count	2	10	38	50
		% within Samples source	4.0%	20.0%	76.0%	100.0%

Symmetric Measures

		Value	Approximate Significance
Nominal by Nominal	Phi	.330	.711
	Cramer's V	.233	.711
N of Valid Cases		50	

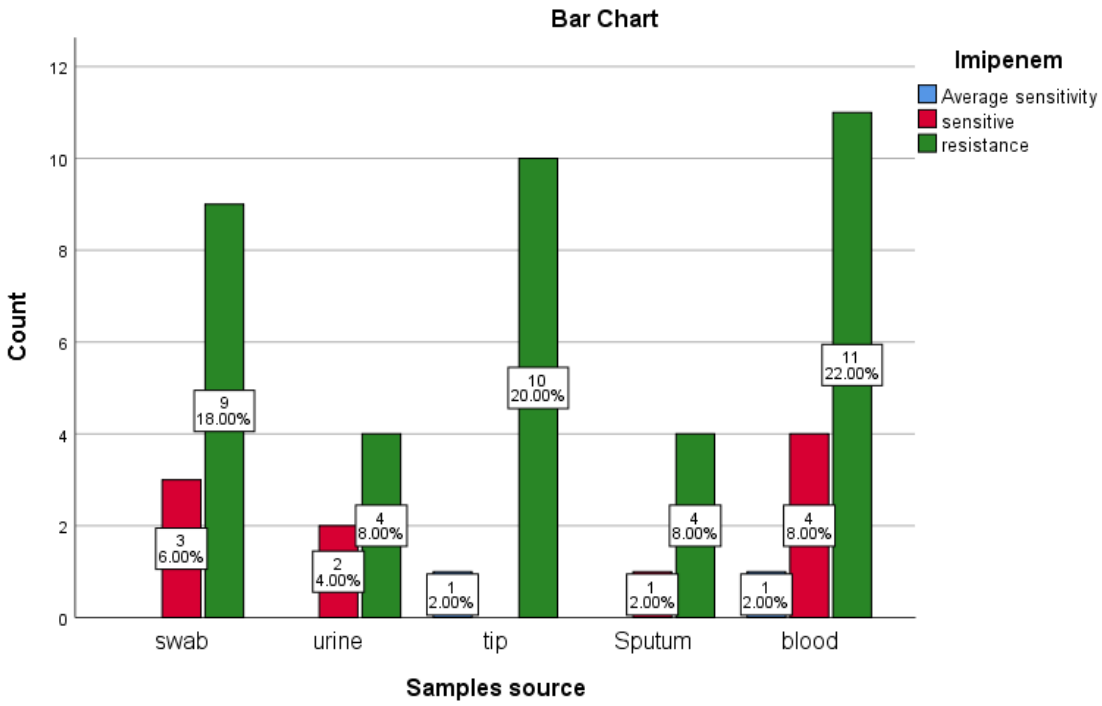

Figure 4: Distribution of Imipenem resistance of K. pneumoniae strains according to samples source

Table 9 Distribution of Meropenem **resistance of K. pneumoniae strains according to samples source**

Crosstab

			Meropenem		
			sensitive	resistance	Total
Samples source	swab	Count	4	8	12
		% within Samples source	33.3%	66.7%	100.0%
	urine	Count	4	2	6
		% within Samples source	66.7%	33.3%	100.0%
	tip	Count	3	8	11
		% within Samples source	27.3%	72.7%	100.0%
	Sputum	Count	1	4	5
		% within Samples source	20.0%	80.0%	100.0%
	blood	Count	6	10	16
		% within Samples source	37.5%	62.5%	100.0%
Total		Count	18	32	50
		% within Samples source	36.0%	64.0%	100.0%

Symmetric Measures

		Value	Approximate Significance
Nominal by Nominal	Phi	.262	.490
	Cramer's V	.262	.490
N of Valid Cases		50	

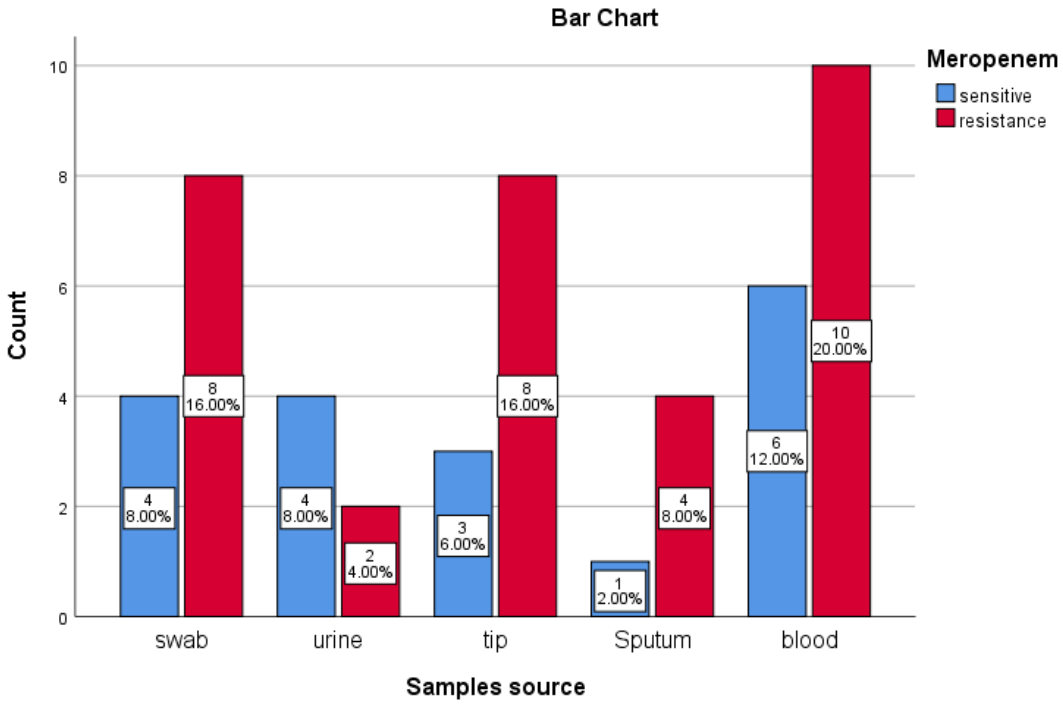

Figure 5Distribution of Meropenem **resistance of K. pneumoniae strains according to samples source**

Table 10 Distribution of Cephalothin **resistance of K. pneumoniae strains according to samples source**

Crosstab

			Cephalothin sensitive	resistance	Total
Samples source	swab	Count	0	12	12
		% within Samples source	0.0%	100.0%	100.0%
	urine	Count	0	6	6
		% within Samples source	0.0%	100.0%	100.0%
	tip	Count	1	10	11
		% within Samples source	9.1%	90.9%	100.0%
	Sputum	Count	0	5	5
		% within Samples source	0.0%	100.0%	100.0%
	blood	Count	1	15	16
		% within Samples source	6.3%	93.8%	100.0%
Total		Count	2	48	50
		% within Samples source	4.0%	96.0%	100.0%

Symmetric Measures

		Value	Approximate Significance
Nominal by Nominal	Phi	.196	.752
	Cramer's V	.196	.752
N of Valid Cases		50	

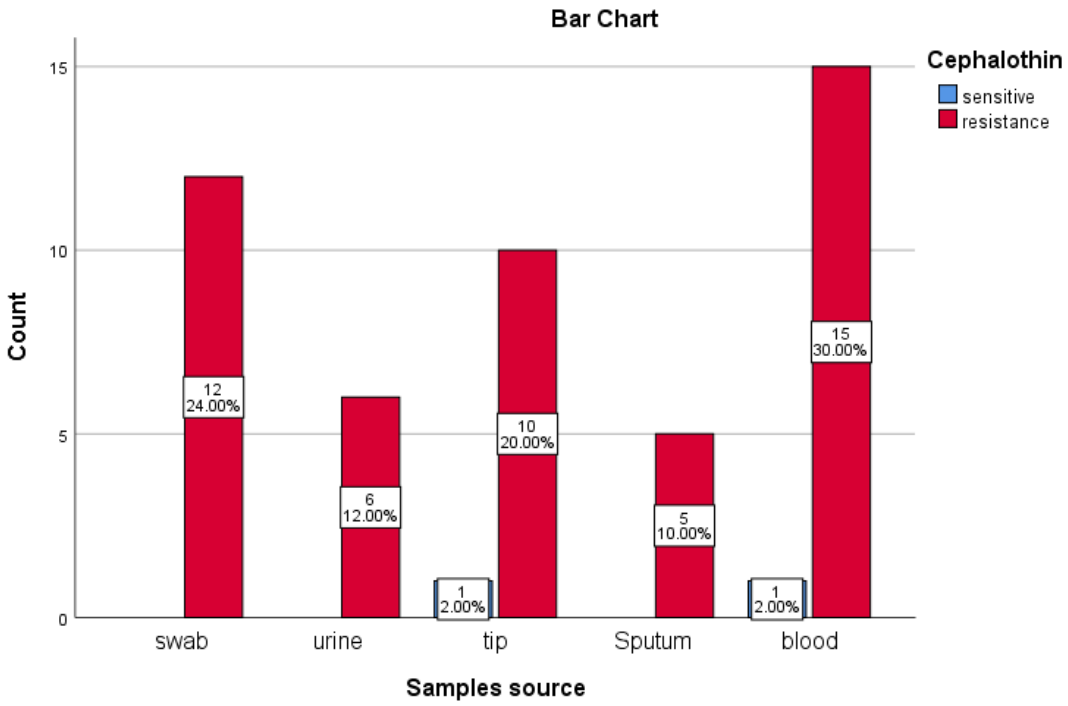

Figure 6 Distribution of Cephalothin **resistance of K. pneumoniae strains according to samples source**

Table 11 Distribution of Cefuroxim **resistance of K. pneumoniae strains according to samples source**

Crosstab

			Cefuroxime		
			sensitive	resistance	Total
Samples source	swab	Count	0	12	12
		% within Samples source	0.0%	100.0%	100.0%
	urine	Count	0	6	6
		% within Samples source	0.0%	100.0%	100.0%
	tip	Count	1	10	11
		% within Samples source	9.1%	90.9%	100.0%
	Sputum	Count	0	5	5
		% within Samples source	0.0%	100.0%	100.0%
	blood	Count	1	15	16
		% within Samples source	6.3%	93.8%	100.0%
Total		Count	2	48	50
		% within Samples source	4.0%	96.0%	100.0%

Symmetric Measures

		Value	Approximate Significance
Nominal by Nominal	Phi	.196	.752
	Cramer's V	.196	.752
N of Valid Cases		50	

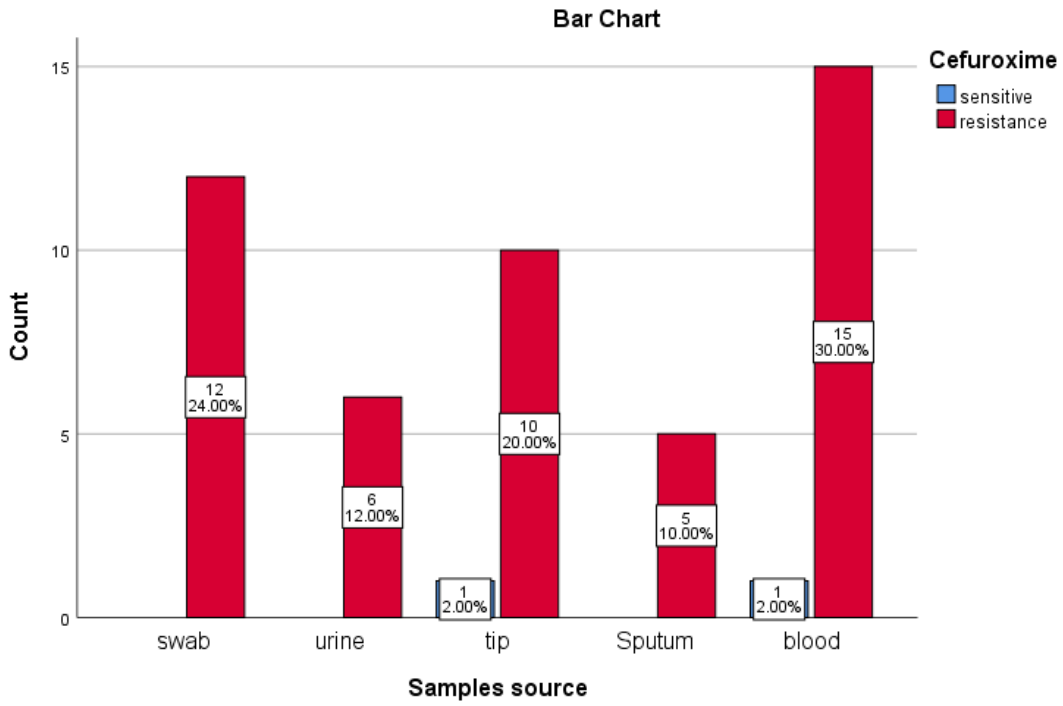

Figure 7Distribution of Cefuroxime **resistance of K. pneumoniae strains according to samples source**

Table 12 Distribution of Cefoxitin resistance of K. pneumoniae strains according to samples source

Crosstab

			Cefoxitin sensitive	resistance	Total
Samples source	Swab	Count	2	10	12
		% within Samples source	16.7%	83.3%	100.0%
	Urine	Count	3	3	6
		% within Samples source	50.0%	50.0%	100.0%
	Tip	Count	3	8	11
		% within Samples source	27.3%	72.7%	100.0%
	Sputum	Count	1	4	5
		% within Samples source	20.0%	80.0%	100.0%
	Blood	Count	4	12	16
		% within Samples source	25.0%	75.0%	100.0%
Total		Count	13	37	50
		% within Samples source	26.0%	74.0%	100.0%

Symmetric Measures

		Value	Approximate Significance
Nominal by Nominal	Phi	.221	.653
	Cramer's V	.221	.653
N of Valid Cases		50	

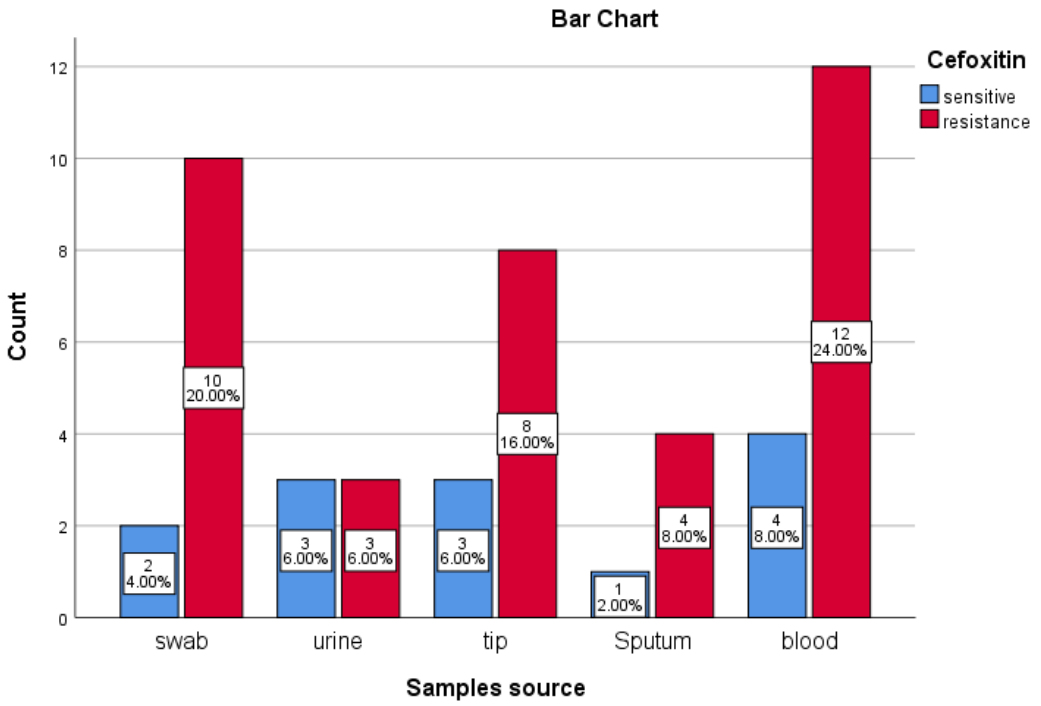

Figure 8 Distribution of Cefoxitin resistance of K. pneumoniae strains according to samples source

Table 13 Distribution of Ceftazidime resistance of K. pneumoniae strains according to samples source

Crosstab

			Ceftazidime Average sensitivity	sensitive	resistance	Total
Samples source	swab	Count	0	1	11	12
		% within Samples source	0.0%	8.3%	91.7%	100.0%
	urine	Count	0	0	6	6
		% within Samples source	0.0%	0.0%	100.0%	100.0%
	tip	Count	0	1	10	11
		% within Samples source	0.0%	9.1%	90.9%	100.0%
	Sputum	Count	0	0	5	5
		% within Samples source	0.0%	0.0%	100.0%	100.0%
	blood	Count	1	1	14	16
		% within Samples source	6.3%	6.3%	87.5%	100.0%
Total		Count	1	3	46	50
		% within Samples source	2.0%	6.0%	92.0%	100.0%

Symmetric Measures

		Value	Approximate Significance
Nominal by Nominal	Phi	.252	.922
	Cramer's V	.178	.922
N of Valid Cases		50	

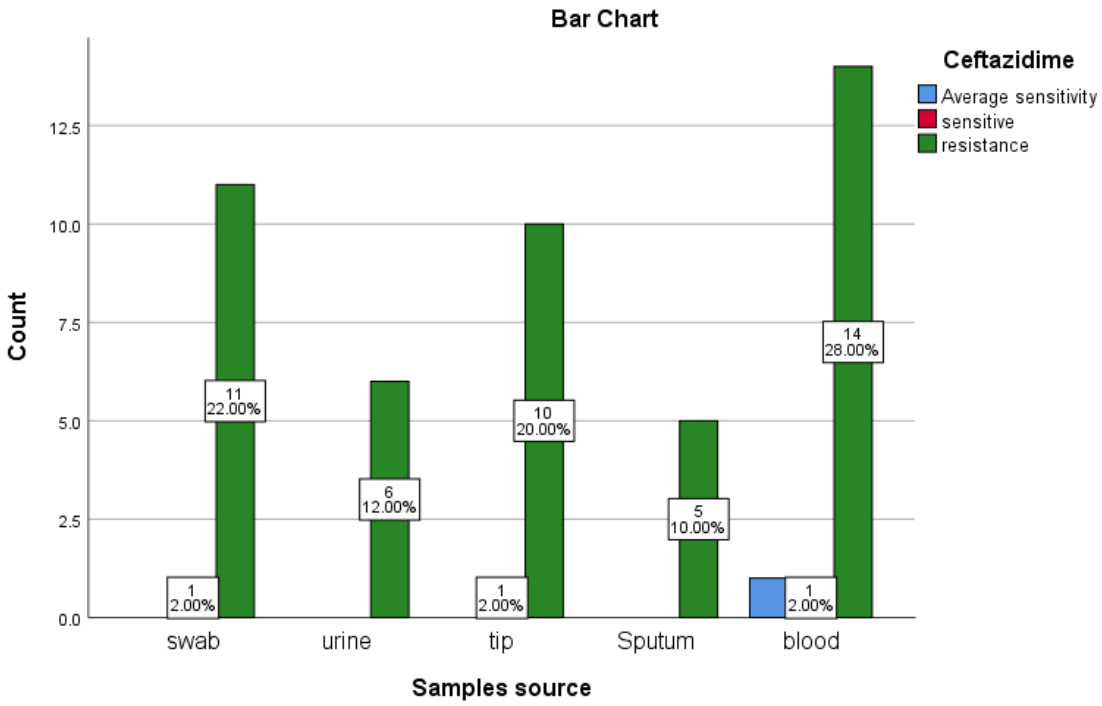

Figure 9 : Distribution of Ceftazidime resistance of K. pneumoniae strains according to samples source

Table 14 Distribution of Ceftriaxone resistance of K. pneumoniae strains according to samples source

Crosstab

			Ceftriaxone		
			sensitive	resistance	Total
Samples source	swab	Count	0	12	12
		% within Samples source	0.0%	100.0%	100.0%
	urine	Count	0	6	6
		% within Samples source	0.0%	100.0%	100.0%
	tip	Count	1	10	11
		% within Samples source	9.1%	90.9%	100.0%
	Sputum	Count	0	5	5
		% within Samples source	0.0%	100.0%	100.0%
	blood	Count	1	15	16
		% within Samples source	6.3%	93.8%	100.0%
Total		Count	2	48	50
		% within Samples source	4.0%	96.0%	100.0%

Symmetric Measures

		Value	Approximate Significance
Nominal by Nominal	Phi	.196	.752
	Cramer's V	.196	.752
N of Valid Cases		50	

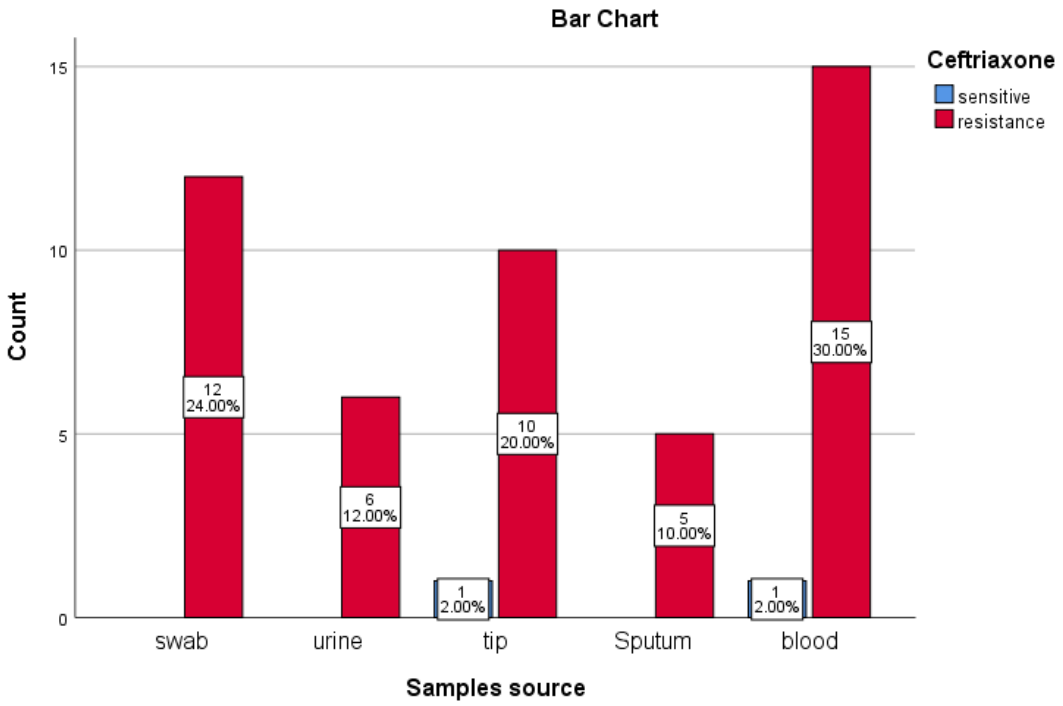

Figure 10 : Distribution of Ceftriaxone resistance of K. pneumoniae strains according to samples source

Table 15 Distribution of Cefepime resistance of K. pneumoniae strains according to samples source

Crosstab

			Cefepime		
			sensitive	resistance	Total
Samples source	Swab	Count	0	12	12
		% within Samples source	0.0%	100.0%	100.0%
	Urine	Count	0	6	6
		% within Samples source	0.0%	100.0%	100.0%
	Tip	Count	1	10	11
		% within Samples source	9.1%	90.9%	100.0%
	Sputum	Count	0	5	5
		% within Samples source	0.0%	100.0%	100.0%
	Blood	Count	1	15	16
		% within Samples source	6.3%	93.8%	100.0%
Total		Count	2	48	50
		% within Samples source	4.0%	96.0%	100.0%

Symmetric Measures

		Value	Approximate Significance
Nominal by Nominal	Phi	.196	.752
	Cramer's V	.196	.752
N of Valid Cases		50	

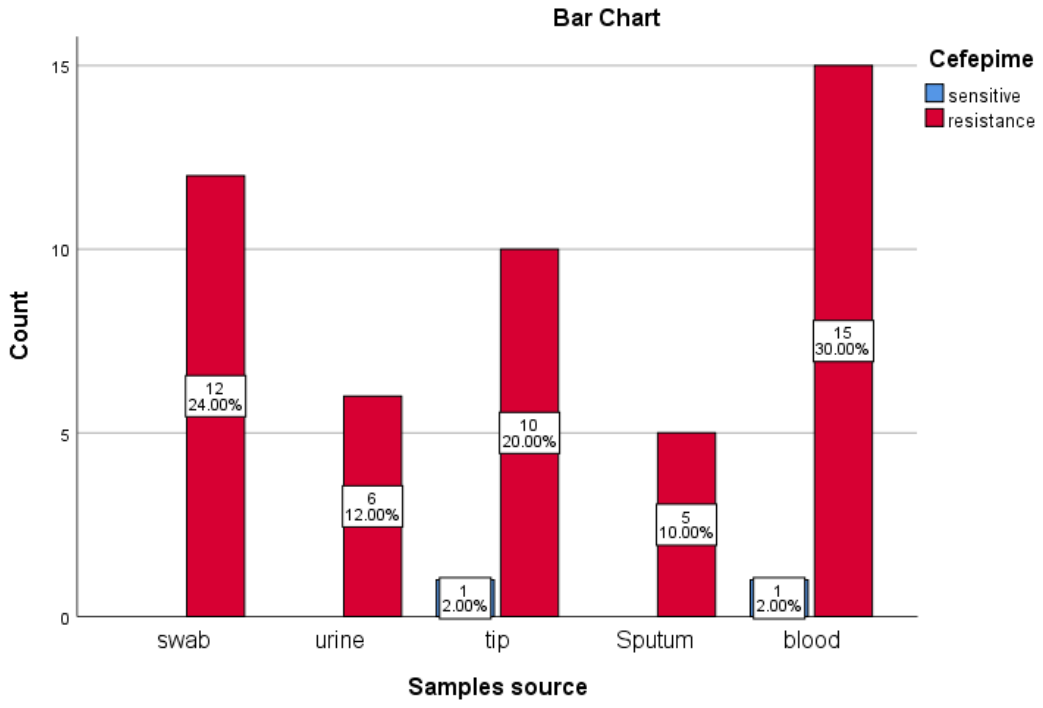

Figure 11 :Distribution of Cefepime resistance of K. pneumoniae strains according to samples source

Table 16 Distribution of Aztreonam resistance of K. pneumoniae strains according to samples source

Crosstab

			Aztreonam sensitive	resistance	Total
Samples source	swab	Count	2	10	12
		% within Samples source	16.7%	83.3%	100.0%
	urine	Count	0	6	6
		% within Samples source	0.0%	100.0%	100.0%
	tip	Count	1	10	11
		% within Samples source	9.1%	90.9%	100.0%
	Sputum	Count	0	5	5
		% within Samples source	0.0%	100.0%	100.0%
	blood	Count	1	15	16
		% within Samples source	6.3%	93.8%	100.0%
Total		Count	4	46	50
		% within Samples source	8.0%	92.0%	100.0%

Symmetric Measures

		Value	Approximate Significance
Nominal by Nominal	Phi	.213	.687
	Cramer's V	.213	.687
N of Valid Cases		50	

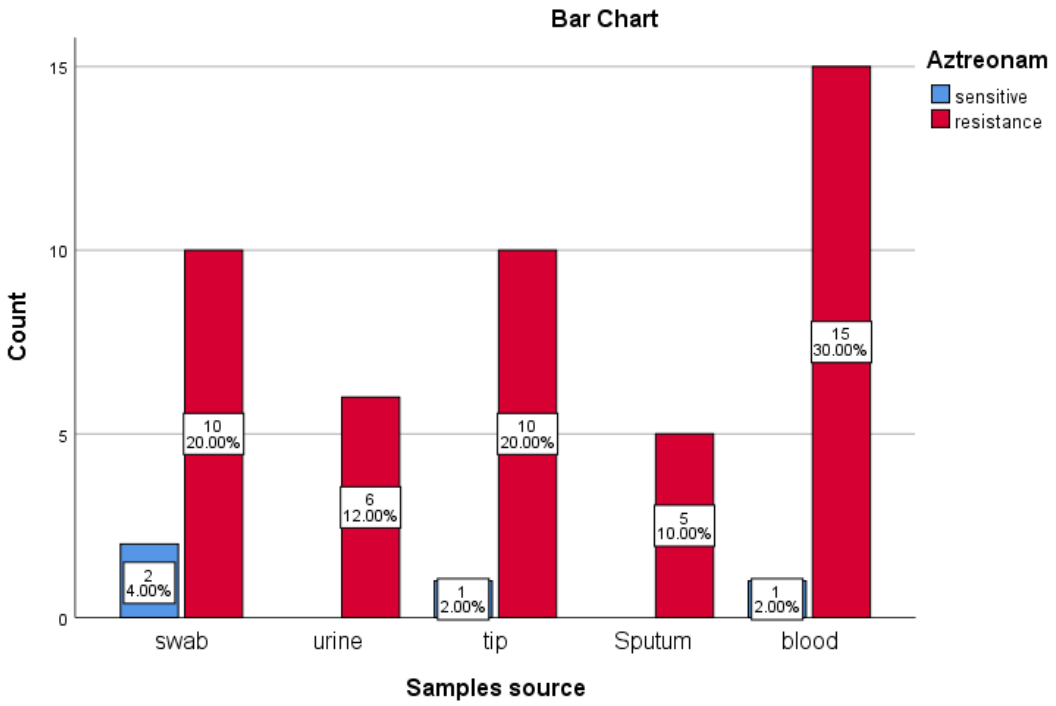

Figure 12: Distribution of Aztreonam resistance of K. pneumoniae strains according to samples source

Table 17 Distribution of Ampicillin resistance of K. pneumoniae strains according to samples source

Crosstab

			Ampicillin		
			sensitive	resistance	Total
Samples source	Swab	Count	0	12	12
		% within Samples source	0.0%	100.0%	100.0%
	Urine	Count	0	6	6
		% within Samples source	0.0%	100.0%	100.0%
	Tip	Count	0	11	11
		% within Samples source	0.0%	100.0%	100.0%
	Sputum	Count	0	5	5
		% within Samples source	0.0%	100.0%	100.0%
	Blood	Count	1	15	16
		% within Samples source	6.3%	93.8%	100.0%
Total		Count	1	49	50
		% within Samples source	2.0%	98.0%	100.0%

Symmetric Measures

		Value	Approximate Significance
Nominal by Nominal	Phi	.208	.705
	Cramer's V	.208	.705
N of Valid Cases		50	

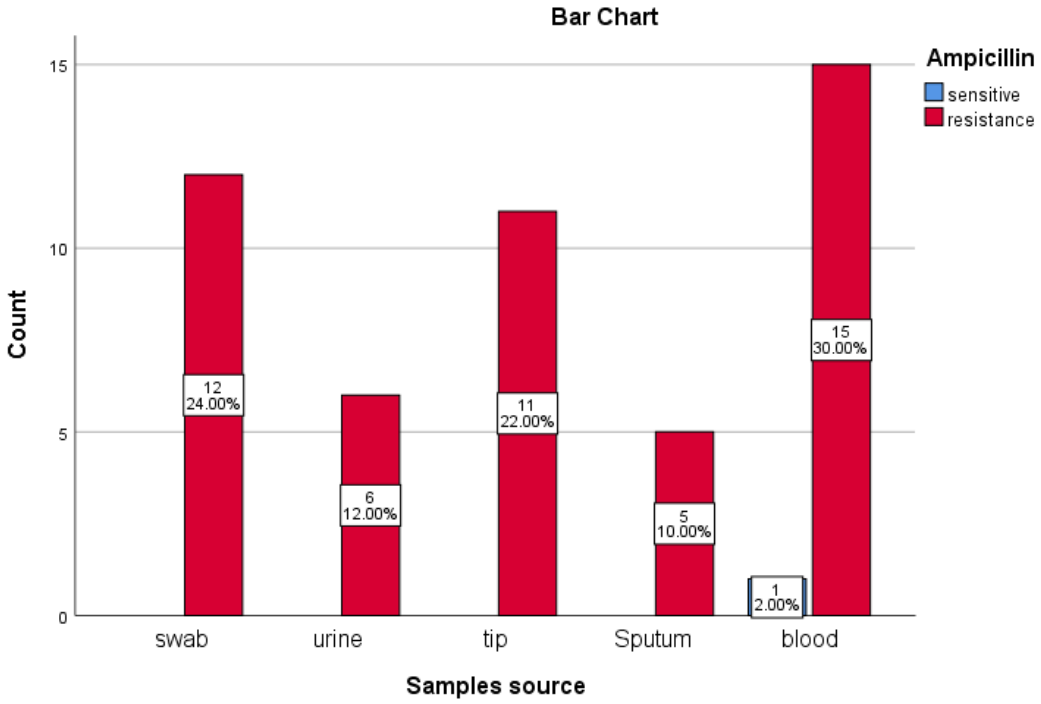

Figure 13 Distribution Ampicillin resistance of K. pneumoniae strains according to samples source

Table 18 Distribution of Amoxicillin-Clavulanate resistance of K. pneumoniae strains according to samples source

Crosstab

			Amoxicillin-Clavulanate			
			Average sensitivity	sensitive	resistance	Total
Samples source	swab	Count	0	1	11	12
		% within Samples source	0.0%	8.3%	91.7%	100.0%
	urine	Count	0	1	5	6
		% within Samples source	0.0%	16.7%	83.3%	100.0%
	tip	Count	0	1	10	11
		% within Samples source	0.0%	9.1%	90.9%	100.0%
	Sputum	Count	0	1	4	5
		% within Samples source	0.0%	20.0%	80.0%	100.0%
	blood	Count	1	2	13	16
		% within Samples source	6.3%	12.5%	81.3%	100.0%
Total		Count	1	6	43	50
		% within Samples source	2.0%	12.0%	86.0%	100.0%

Symmetric Measures

		Value	Approximate Significance
Nominal by Nominal	Phi	.239	.943
	Cramer's V	.169	.943
N of Valid Cases		50	

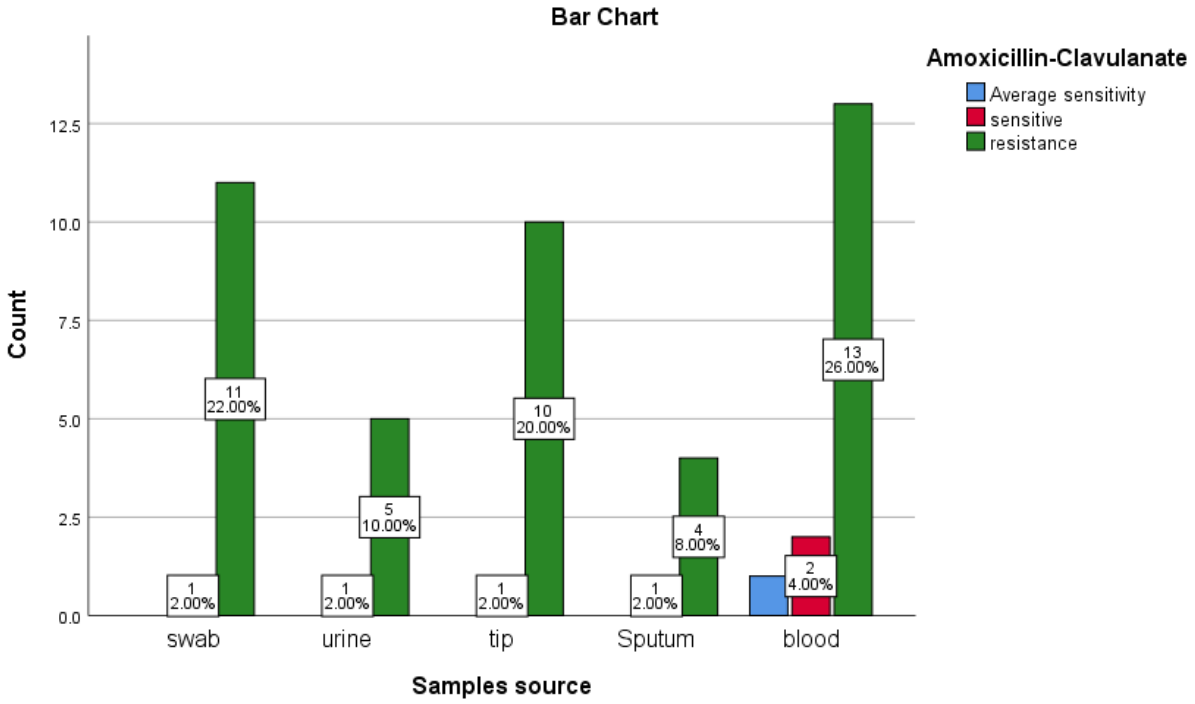

Figure 14 :Distribution of Amoxicillin-Clavulanate resistance of K. pneumoniae strains according to samples source

Table 19 Distribution of Piperacillin-Tazobactam resistance of K. pneumoniae strains according to samples source

Crosstab

			Piperacillin-Tazobactam			
			Average sensitivity	sensitive	resistance	Total
Samples source	swab	Count	0	3	9	12
		% within Samples source	0.0%	25.0%	75.0%	100.0%
	urine	Count	2	2	2	6
		% within Samples source	33.3%	33.3%	33.3%	100.0%
	tip	Count	0	3	8	11
		% within Samples source	0.0%	27.3%	72.7%	100.0%
	Sputum	Count	0	1	4	5
		% within Samples source	0.0%	20.0%	80.0%	100.0%
	blood	Count	1	5	10	16
		% within Samples source	6.3%	31.3%	62.5%	100.0%
Total		Count	3	14	33	50
		% within Samples source	6.0%	28.0%	66.0%	100.0%

Symmetric Measures

		Value	Approximate Significance
Nominal by Nominal	Phi	.465	.213
	Cramer's V	.329	.213
N of Valid Cases		50	

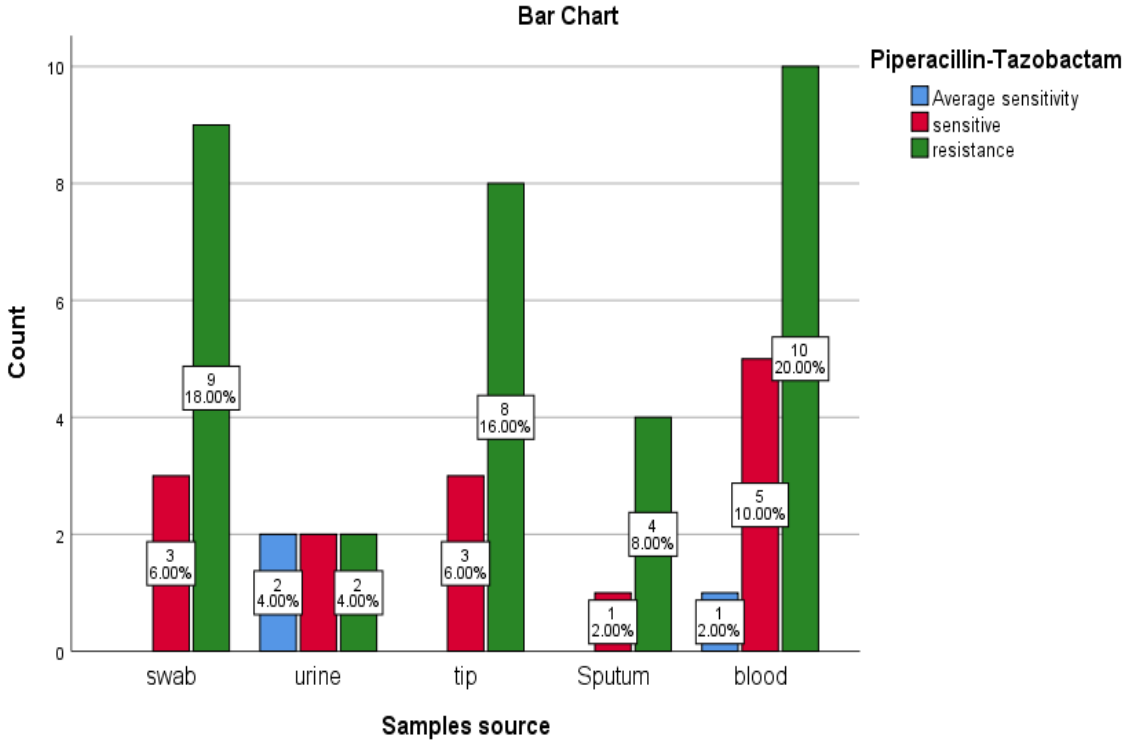

Figure 15 : Distribution of Piperacillin-Tazobactam resistance of K. pneumoniae strains according to samples source

Table 20 Distribution of Colistin resistance of K. pneumoniae strains according to samples source

Crosstab

			Colistin sensitive	Total
Samples source	Swab	Count	12	12
		% within Samples source	100.0%	100.0%
	Urine	Count	6	6
		% within Samples source	100.0%	100.0%
	Tip	Count	11	11
		% within Samples source	100.0%	100.0%
	Sputum	Count	5	5
		% within Samples source	100.0%	100.0%
	Blood	Count	16	16
		% within Samples source	100.0%	100.0%
Total		Count	50	50
		% within Samples source	100.0%	100.0%

Symmetric Measures

		Value
Nominal by Nominal	Phi	.[a]
N of Valid Cases		50

a. No statistics are computed because Colistin is a constant.

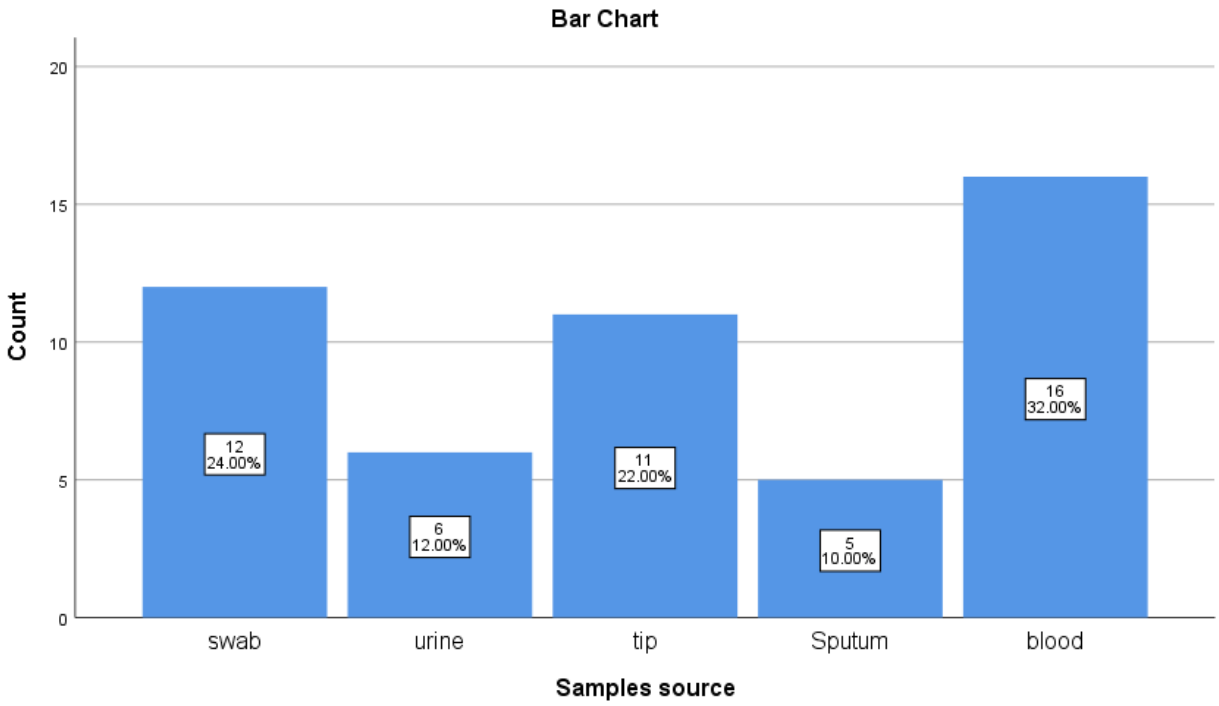

Figure 16 Distribution of Colistin resistance of K. pneumoniae strains according to samples source

Table 21 Distribution of Trimethoprim-Sulfamethoxazol resistance of K. pneumoniae strains according to samples source

Crosstab

			Trimethoprim-Sulfamethoxazol sensitive	resistance	Total
Samples source	swab	Count	2	10	12
		% within Samples source	16.7%	83.3%	100.0%
	urine	Count	1	5	6
		% within Samples source	16.7%	83.3%	100.0%
	tip	Count	4	7	11
		% within Samples source	36.4%	63.6%	100.0%
	Sputum	Count	1	4	5
		% within Samples source	20.0%	80.0%	100.0%
	blood	Count	3	13	16
		% within Samples source	18.8%	81.3%	100.0%
Total		Count	11	39	50
		% within Samples source	22.0%	78.0%	100.0%

Symmetric Measures

		Value	Approximate Significance
Nominal by Nominal	Phi	.186	.785
	Cramer's V	.186	.785
N of Valid Cases		50	

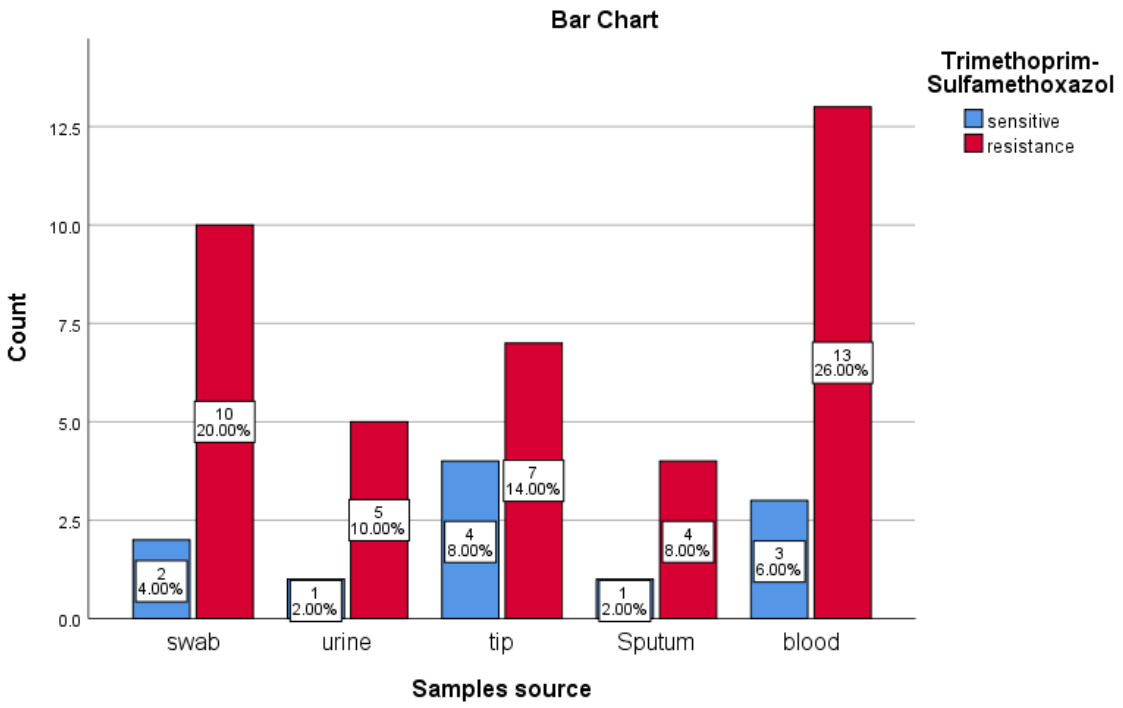

Figure 17 Distribution of Trimethoprim-Sulfamethoxazol resistance of K. pneumoniae strains according to samples source

Table 22 Distribution of Nitrofurantoin resistance of K. pneumoniae strains according to samples source

Crosstab

			Nitrofurantoin Average sensitivity	sensitive	resistance	Total
Samples source	swab	Count	0	2	10	12
		% within Samples source	0.0%	16.7%	83.3%	100.0%
	urine	Count	2	3	1	6
		% within Samples source	33.3%	50.0%	16.7%	100.0%
	tip	Count	0	2	9	11
		% within Samples source	0.0%	18.2%	81.8%	100.0%
	Sputum	Count	0	1	4	5

		% within Samples source	0.0%	20.0%	80.0%	100.0%
	blood	Count	1	2	13	16
		% within Samples source	6.3%	12.5%	81.3%	100.0%
Total		Count	3	10	37	50
		% within Samples source	6.0%	20.0%	74.0%	100.0%

Symmetric Measures

		Value	Approximate Significance
Nominal by Nominal	Phi	.555	.051
	Cramer's V	.393	.051
N of Valid Cases		50	

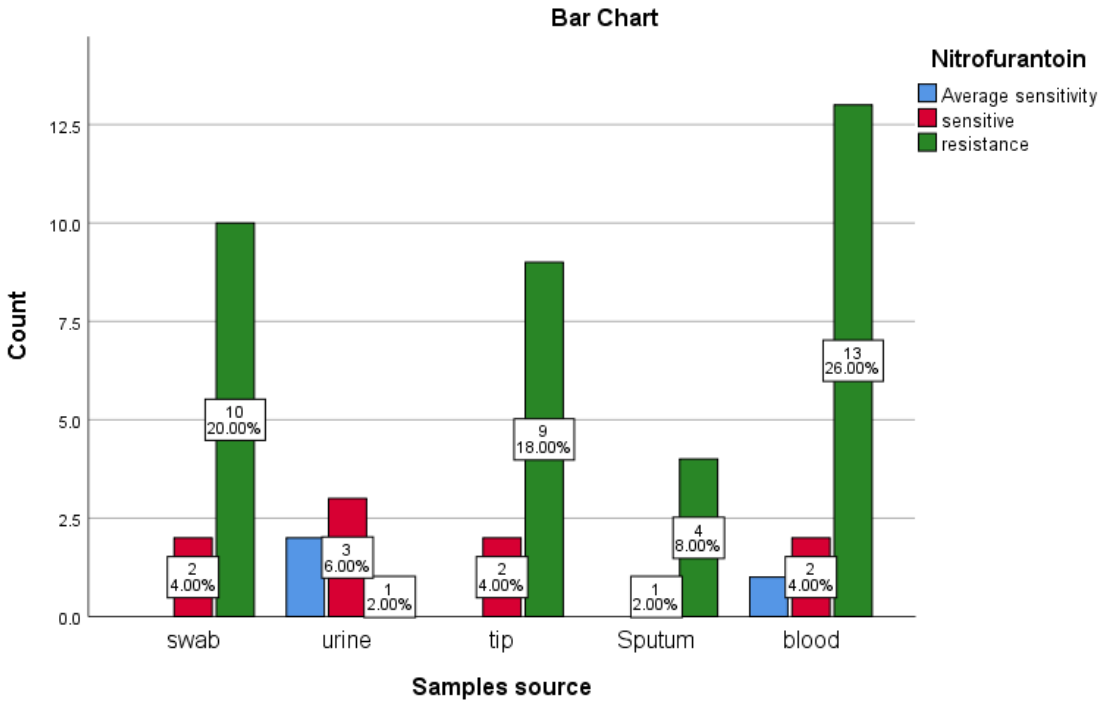

Figure 18Distribution of Nitrofurantoin resistance of K. pneumoniae strains according to samples source

Table 23 Distribution of Ciprofloxacin resistance of K. pneumoniae strains according to samples source

Crosstab

			Ciprofloxacin Average sensitivity	sensitive	resistance	Total
Samples source	Swab	Count	0	2	10	12
		% within Samples source	0.0%	16.7%	83.3%	100.0%
	Urine	Count	2	2	2	6
		% within Samples source	33.3%	33.3%	33.3%	100.0%
	Tip	Count	0	3	8	11
		% within Samples source	0.0%	27.3%	72.7%	100.0%
	Sputum	Count	0	1	4	5
		% within Samples source	0.0%	20.0%	80.0%	100.0%
	blood	Count	1	5	10	16
		% within Samples source	6.3%	31.3%	62.5%	100.0%
Total		Count	3	13	34	50
		% within Samples source	6.0%	26.0%	68.0%	100.0%

Symmetric Measures

		Value	Approximate Significance
Nominal by Nominal	Phi	.482	.169
	Cramer's V	.341	.169
N of Valid Cases		50	

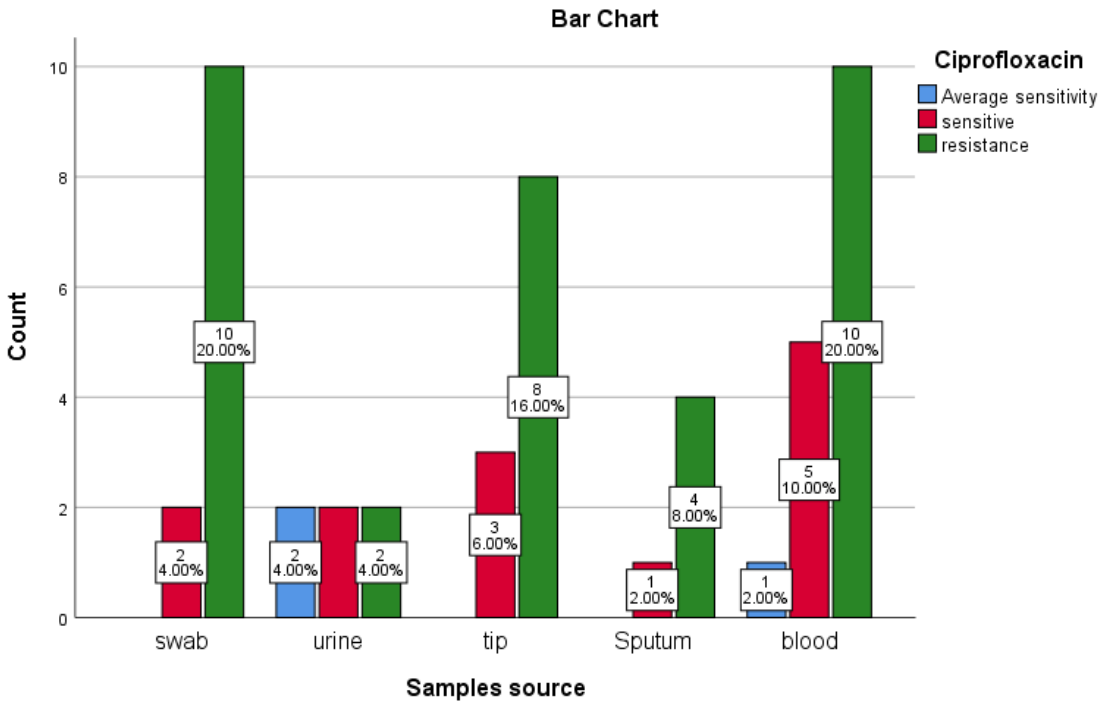

Figure 19: Distribution of Ciprofloxacin resistance of K. pneumoniae strains according to samples source

Table 24 Distribution of Levofloxacin resistance of K. pneumoniae strains according to samples source

Crosstab

			Levofloxacin		
			sensitive	resistance	Total
Samples source	swab	Count	3	9	12
		% within Samples source	25.0%	75.0%	100.0%
	urine	Count	4	2	6
		% within Samples source	66.7%	33.3%	100.0%
	tip	Count	3	8	11
		% within Samples source	27.3%	72.7%	100.0%
	Sputum	Count	1	4	5
		% within Samples source	20.0%	80.0%	100.0%
	blood	Count	6	10	16
		% within Samples source	37.5%	62.5%	100.0%
Total		Count	17	33	50
		% within Samples source	34.0%	66.0%	100.0%

Symmetric Measures

		Value	Approximate Significance
Nominal by Nominal	Phi	.284	.402
	Cramer's V	.284	.402
N of Valid Cases		50	

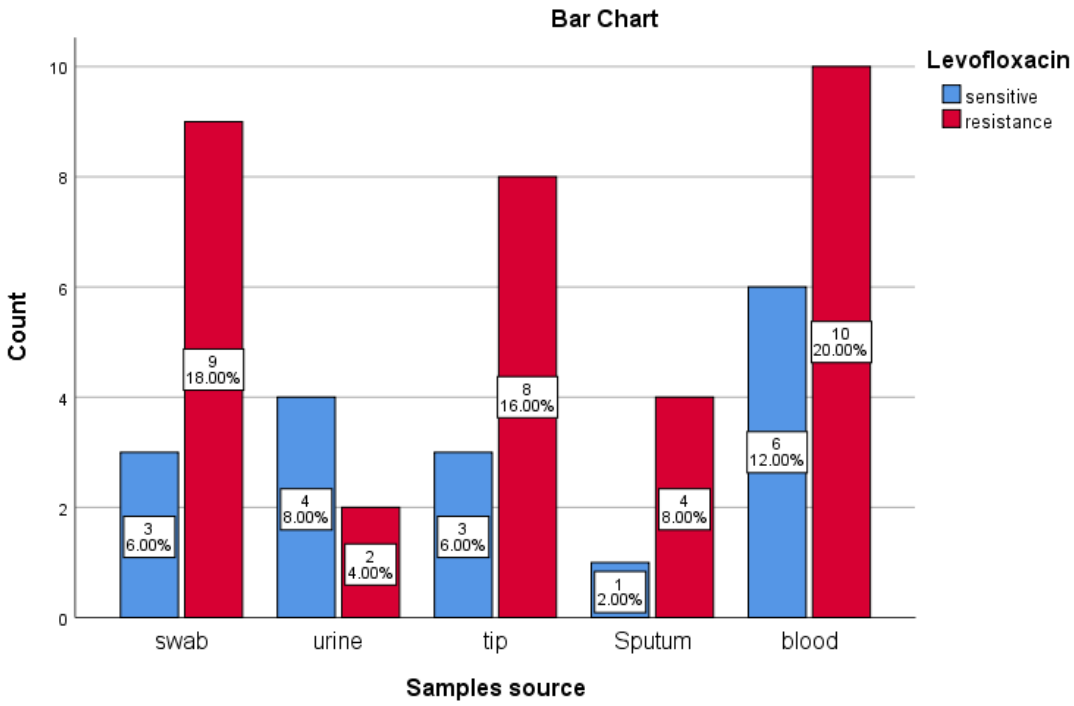

Figure 20: Distribution of Levofloxacin resistance of K. pneumoniae strains according to samples source

Table 25 Distribution of Tigecycline resistance of K. pneumoniae strains according to samples source

Crosstab

			Tigecycline Average sensitivity	resistance	Total
Samples source	Swab	Count	4	8	12
		% within Samples source	33.3%	66.7%	100.0%
	Urine	Count	3	3	6
		% within Samples source	50.0%	50.0%	100.0%
	Tip	Count	4	7	11
		% within Samples source	36.4%	63.6%	100.0%
	Sputum	Count	5	0	5
		% within Samples source	100.0%	0.0%	100.0%
	Blood	Count	3	13	16
		% within Samples source	18.8%	81.3%	100.0%
Total		Count	19	31	50
		% within Samples source	38.0%	62.0%	100.0%

Symmetric Measures

		Value	Approximate Significance
Nominal by Nominal	Phi	.473	.025
	Cramer's V	.473	.025
N of Valid Cases		50	

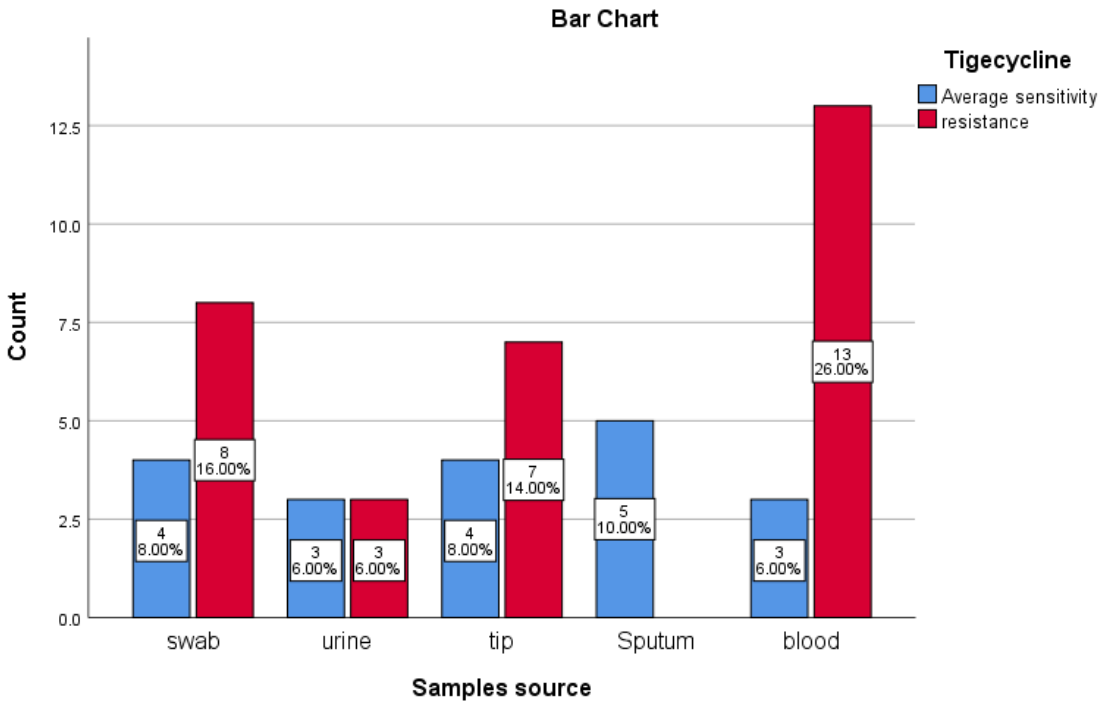

Figure 21Distribution of Tigecycline resistance of K. pneumoniae strains according to samples source

Based on the crosstabulation analysis and symmetric measures provided, the results offer insight into the hypothesis: **"Distribution of antibiotic resistance of K. pneumoniae strains according to sample source."** The results varied across different antibiotics and their association with sample sources such as swab, urine, tip, sputum, and blood.

Starting with Table (Amikacin), the resistance rate was highest in sputum samples (80.0%) and tips (63.6%), with the overall resistance observed in 60.0% of cases. However, the Phi coefficient was (0.322) and Cramer's V was (0.228), both indicating a weak and statistically non-significant association (p = 0.738) between sample source and resistance.

In the case of Gentamicin, resistance was widespread across all sources, with the highest in tips (72.7%). Yet, Phi and Cramer's V values were very low (0.116), and significance was also low (p = 0.955), indicating no meaningful dependence between source and resistance pattern.

Ertapenem and Imipenem also showed very high resistance (78.0% and 76.0%, respectively), particularly among tip and blood samples. Their Phi and Cramer's V values were (0.316, 0.223) for Ertapenem and (0.330, 0.233) for Imipenem, with non-significant p-values of (0.759) and (0.711), again suggesting no significant relationship.

For Meropenem, resistance was prevalent across all sources, notably in sputum (80.0%) and tips (72.7%). The Phi and Cramer's V values were (0.262, 0.262) with a p-value of (0.490), which does not suggest statistical significance.

The cephalosporins—Cephalothin, Cefuroxime, Ceftriaxone, and Cefepime—showed very high resistance across all sources, with resistance levels reaching 96.0%. Phi and Cramer's V values for these antibiotics were consistently around (0.196), with p-values of (0.752), reflecting no significant association.

For Cefoxitin, resistance was again predominant (74.0%), with Phi and Cramer's V at (0.221), p = (0.653), showing no significant dependency.

Ceftazidime exhibited a 92.0% resistance rate, highest in urine and sputum (100%). Despite this high resistance, Phi was (0.252) and Cramer's V (0.178), with a very high p-value (0.922), indicating no association.

In the case of Aztreonam, resistance was 92.0% overall, highest in sputum (100%) and urine (100%). The Phi and Cramer's V values were (0.213) with p = (0.687), also non-significant.

Ampicillin and Amoxicillin-Clavulanate had the highest resistance rates at (98.0%) and (86.0%), respectively. No meaningful association was found, with Phi values of (0.208) and (0.239), and p-values of (0.705) and (0.943).

For Piperacillin-Tazobactam, resistance was at 66.0% overall. The Phi (0.465) and Cramer's V (0.329) values indicated a moderately stronger relationship compared to previous drugs, though not statistically significant (p = 0.213).

Colistin showed complete sensitivity across all samples (100%), and thus Phi and Cramer's V could not be calculated due to lack of variation.

Trimethoprim-Sulfamethoxazole and Nitrofurantoin also showed high resistance rates (78.0% and 74.0%, respectively). Phi for Nitrofurantoin was relatively higher (0.555), and

Cramer’s V (0.393) came close to significance with p = (0.051), hinting at a potential relationship between sample source and resistance for this drug.

Ciprofloxacin exhibited 68.0% resistance, with Phi (0.482) and Cramer’s V (0.341), but still not statistically significant (p = 0.169). Levofloxacin resistance stood at 66.0%, with a weaker Phi (0.284) and p = (0.402).

Finally, Tigecycline was noteworthy. It had an unusual pattern with a more balanced distribution of resistance (62.0%) and average sensitivity (38.0%). Notably, sputum samples showed 100% sensitivity. Phi and Cramer’s V were both at (0.473), and the association was statistically significant with p = (0.025), suggesting a true dependency between sample source and resistance in this case.

In conclusion, the hypothesis that antibiotic resistance in *Klebsiella pneumoniae* varies significantly with sample source is only partially supported. While most antibiotics showed high resistance regardless of the source, Tigecycline stood out with a significant association (Phi = 0.473, Cramer's V = 0.473, p = 0.025). Nitrofurantoin also showed a borderline association (Phi = 0.555, Cramer's V = 0.393, p = 0.051). These findings highlight the importance of sample-specific susceptibility profiling, particularly for certain drugs, in guiding targeted antimicrobial therapy.

H3: Distribution of antibiotic resistance of K. pneumoniae strains according to the ward

Table 26 Distribution of Amikacin resistance of K. pneumoniae strains according to Word

Crosstab

			Amikacin Average sensitivity	sensitive	resistance	Total
Word	neonatal ICU	Count	1	5	6	12
		% within Word	8.3%	41.7%	50.0%	100.0%
	surgical ICU	Count	0	11	20	31
		% within Word	0.0%	35.5%	64.5%	100.0%
	pediatric ICU	Count	0	3	4	7
		% within Word	0.0%	42.9%	57.1%	100.0%
Total		Count	1	19	30	50
		% within Word	2.0%	38.0%	60.0%	100.0%

Symmetric Measures

		Value	Approximate Significance
Nominal by Nominal	Phi	.269	.460
	Cramer's V	.190	.460
N of Valid Cases		50	

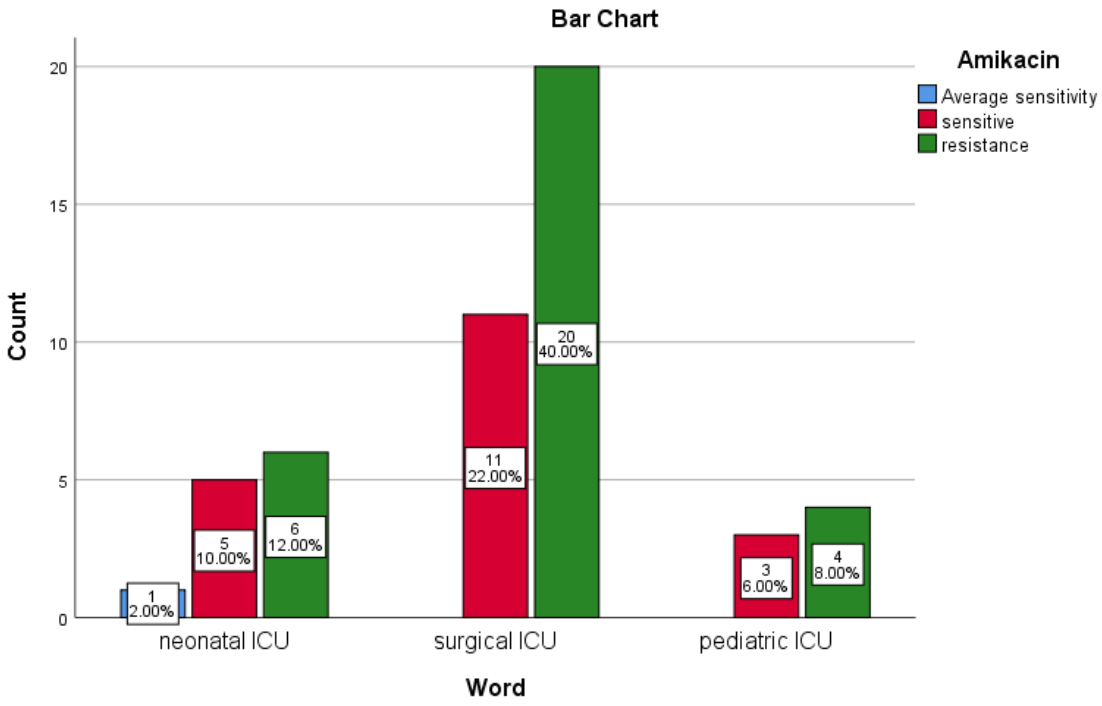

Figure 22 Distribution of Amikacin resistance of K. pneumoniae strains according to Word

Table 27 Distribution of Gentamicin resistance of K. pneumoniae strains according to Word

			Gentamicin		
			sensitive	resistance	Total
Word	neonatal ICU	Count	5	7	12
		% within Word	41.7%	58.3%	100.0%
	surgical ICU	Count	11	20	31
		% within Word	35.5%	64.5%	100.0%
	pediatric ICU	Count	1	6	7
		% within Word	14.3%	85.7%	100.0%
Total		Count	17	33	50
		% within Word	34.0%	66.0%	100.0%

Symmetric Measures

		Value	Approximate Significance
Nominal by Nominal	Phi	.176	.459
	Cramer's V	.176	.459
N of Valid Cases		50	

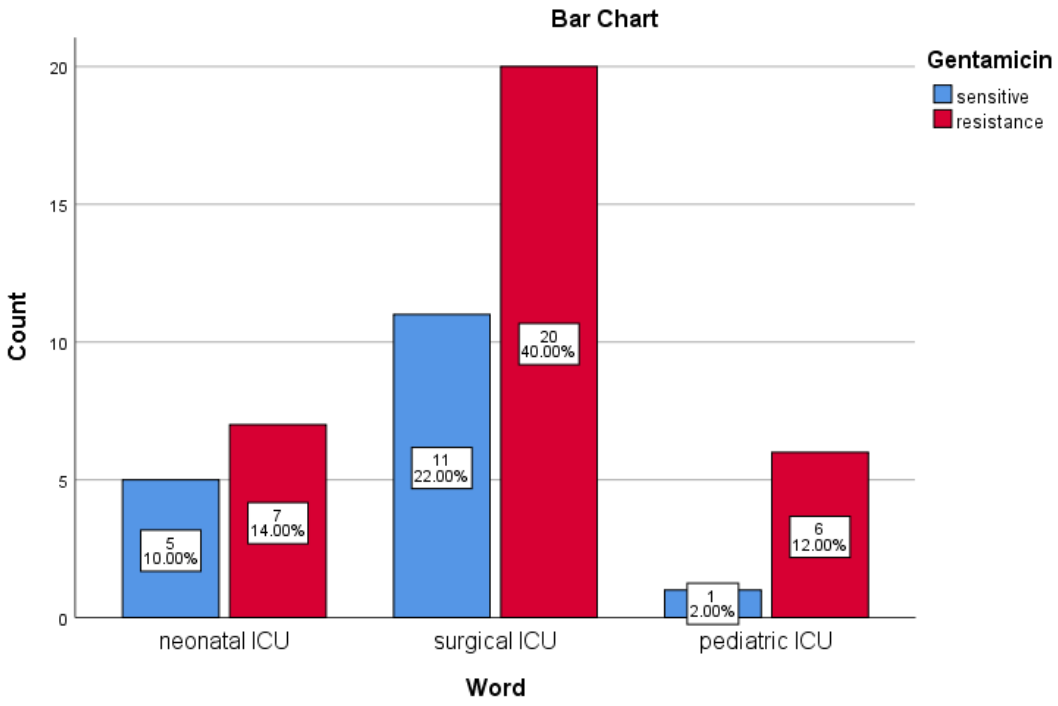

Figure 23 Distribution of Gentamicin resistance of K. pneumoniae strains according to Word

Table 28 Distribution of Ertapenem resistance of K. pneumoniae strains according to Word

Crosstab

			Ertapenem Average sensitivity	sensitive	resistance	Total
Word	neonatal ICU	Count	1	5	6	12
		% within Word	8.3%	41.7%	50.0%	100.0%
	surgical ICU	Count	0	4	27	31
		% within Word	0.0%	12.9%	87.1%	100.0%
	pediatric ICU	Count	0	1	6	7
		% within Word	0.0%	14.3%	85.7%	100.0%
Total		Count	1	10	39	50
		% within Word	2.0%	20.0%	78.0%	100.0%

Symmetric Measures

		Value	Approximate Significance
Nominal by Nominal	Phi	.412	.076
	Cramer's V	.291	.076
N of Valid Cases		50	

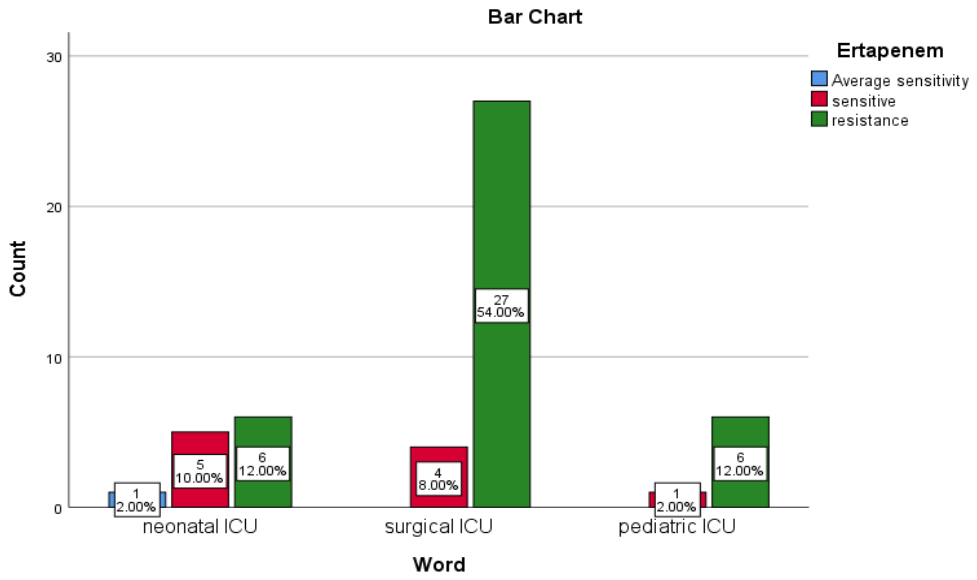

Figure 24 Distribution of Ertapenem resistance of K. pneumoniae strains according to Word

Table 29 Distribution of Imipenem resistance of K. pneumoniae strains according to Word

Crosstab

			Imipenem			
			Average sensitivity	sensitive	resistance	Total
Word	neonatal ICU	Count	1	5	6	12
		% within Word	8.3%	41.7%	50.0%	100.0%
	surgical ICU	Count	1	3	27	31
		% within Word	3.2%	9.7%	87.1%	100.0%
	pediatric ICU	Count	0	2	5	7
		% within Word	0.0%	28.6%	71.4%	100.0%
Total		Count	2	10	38	50
		% within Word	4.0%	20.0%	76.0%	100.0%

Symmetric Measures

		Value	Approximate Significance
Nominal by Nominal	Phi	.380	.125
	Cramer's V	.268	.125
N of Valid Cases		50	

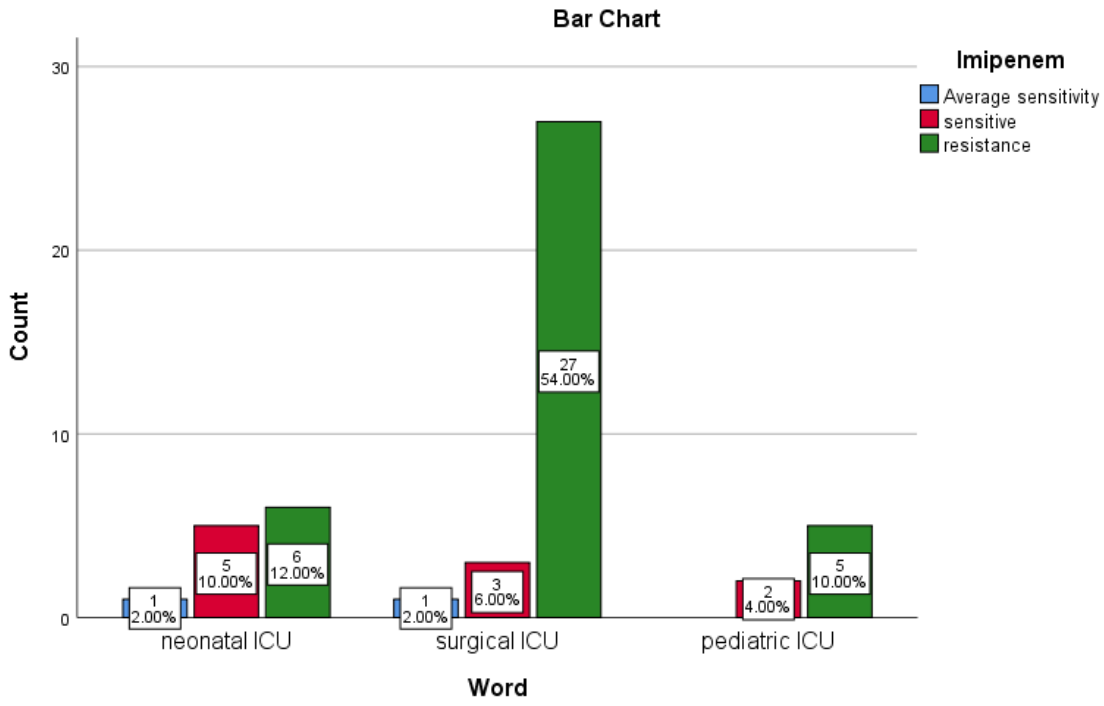

Figure 25 Distribution of Imipenem resistance of K. pneumoniae strains according to Word

Table 30 Distribution of Meropenem resistance of K. pneumoniae strains according to Word

Crosstab

			Meropenem		
			sensitive	resistance	Total
Word	neonatal ICU	Count	6	6	12
		% within Word	50.0%	50.0%	100.0%
	surgical ICU	Count	9	22	31
		% within Word	29.0%	71.0%	100.0%
	pediatric ICU	Count	3	4	7
		% within Word	42.9%	57.1%	100.0%
Total		Count	18	32	50
		% within Word	36.0%	64.0%	100.0%

Symmetric Measures

		Value	Approximate Significance
Nominal by Nominal	Phi	.191	.403
	Cramer's V	.191	.403
N of Valid Cases		50	

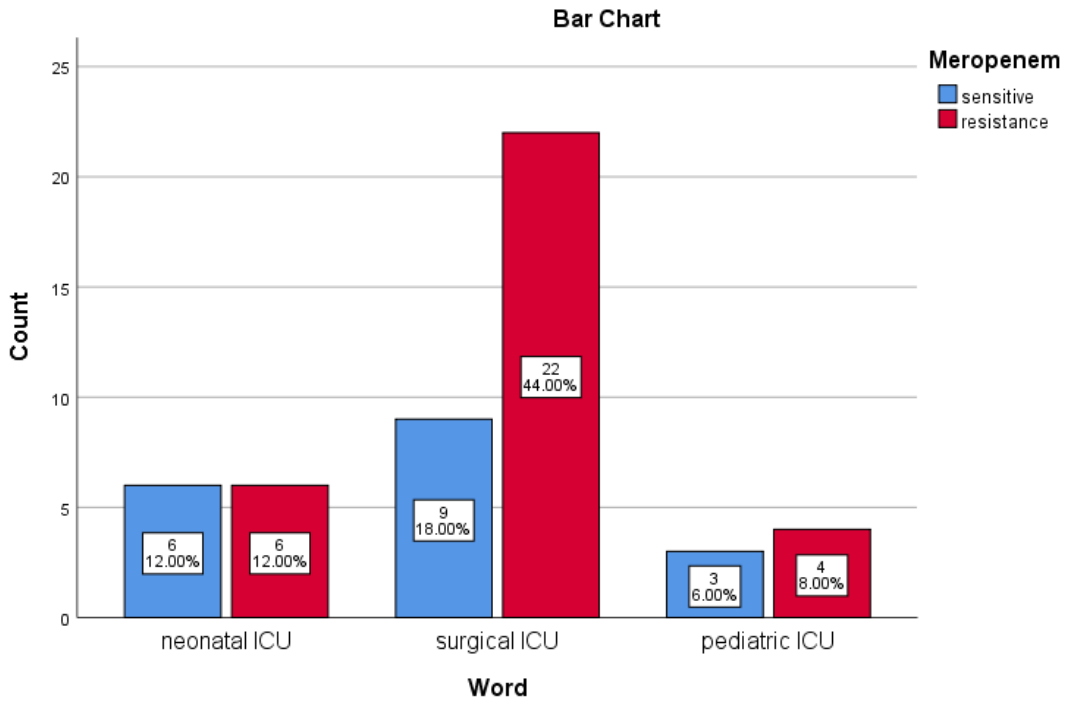

Figure 26 Distribution of Meropenem resistance of K. pneumoniae strains according to Word

Table 31 Distribution of Cephalothin resistance of K. pneumoniae strains according to Word

Crosstab

			Cephalothin		
			sensitive	resistance	Total
Word	neonatal ICU	Count	1	11	12
		% within Word	8.3%	91.7%	100.0%
	surgical ICU	Count	1	30	31
		% within Word	3.2%	96.8%	100.0%
	pediatric ICU	Count	0	7	7
		% within Word	0.0%	100.0%	100.0%
Total		Count	2	48	50
		% within Word	4.0%	96.0%	100.0%

Symmetric Measures

		Value	Approximate Significance
Nominal by Nominal	Phi	.136	.629
	Cramer's V	.136	.629
N of Valid Cases		50	

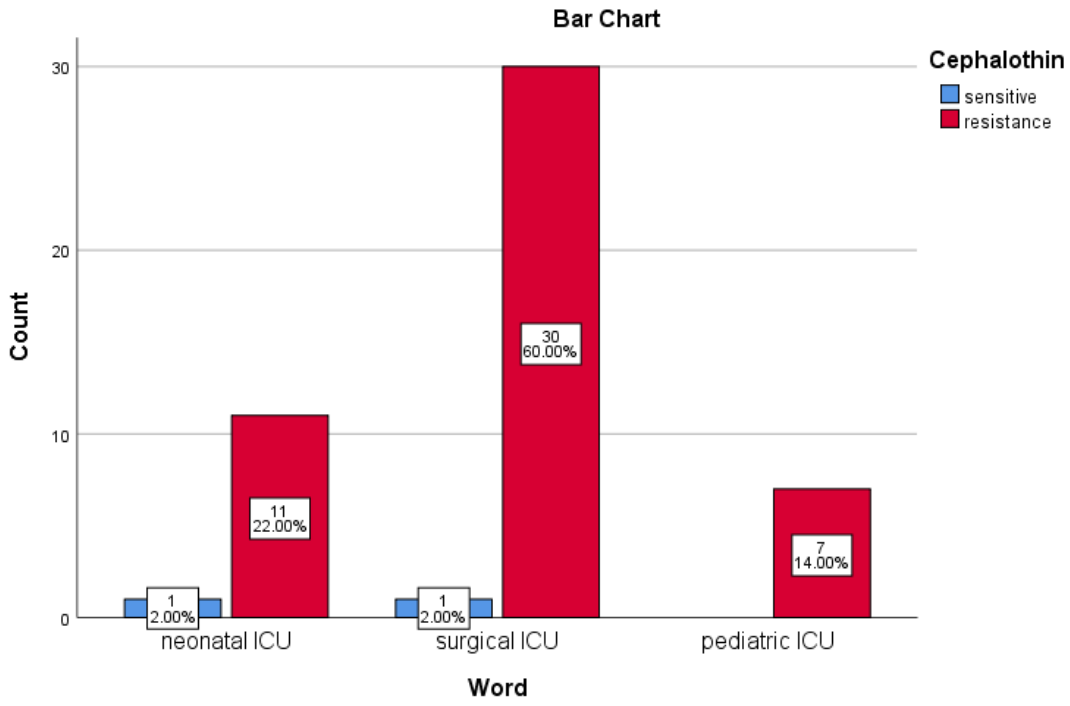

Figure 27 Distribution of Cephalothin resistance of K. pneumoniae strains according to Word

Table 32 Distribution of Cefuroxime resistance of K. pneumoniae strains according to Word

Crosstab

			Cefuroxime		
			sensitive	resistance	Total
Word	neonatal ICU	Count	1	11	12
		% within Word	8.3%	91.7%	100.0%
	surgical ICU	Count	1	30	31
		% within Word	3.2%	96.8%	100.0%
	pediatric ICU	Count	0	7	7
		% within Word	0.0%	100.0%	100.0%
Total		Count	2	48	50
		% within Word	4.0%	96.0%	100.0%

Symmetric Measures

		Value	Approximate Significance
Nominal by Nominal	Phi	.136	.629
	Cramer's V	.136	.629
N of Valid Cases		50	

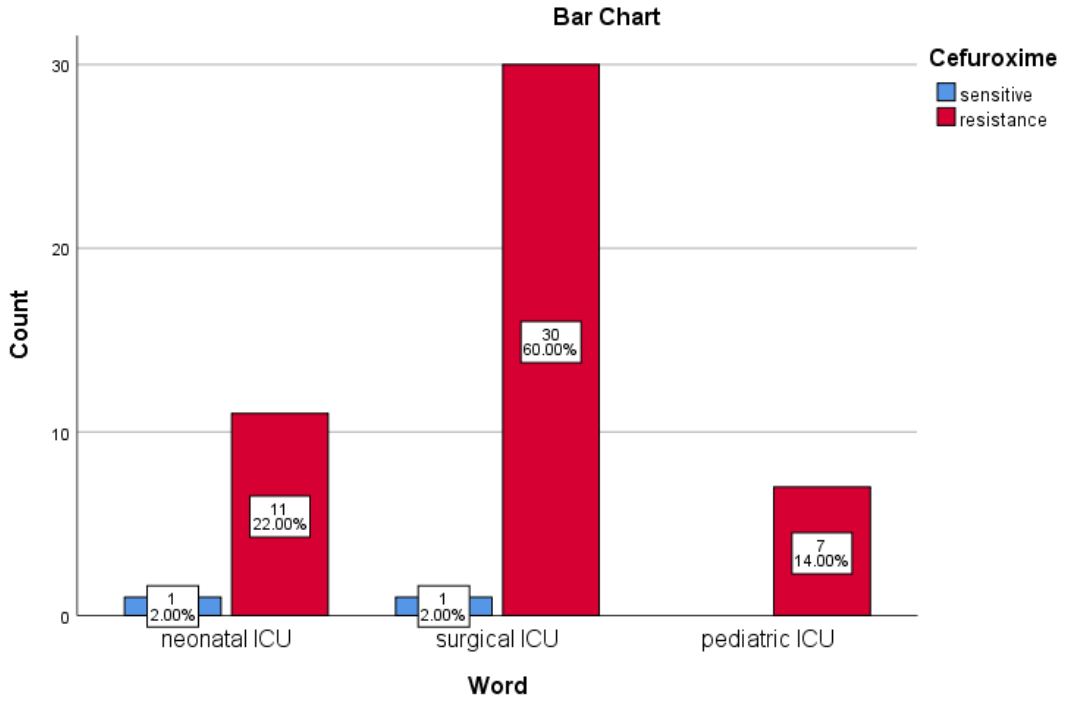

Figure 28 Distribution of Cefuroxime resistance of K. pneumoniae strains according to Word

Table 33 Distribution of Cefoxitin resistance of K. pneumoniae strains according to Word

Crosstab

			Cefoxitin sensitive	resistance	Total
Word	neonatal ICU	Count	3	9	12
		% within Word	25.0%	75.0%	100.0%
	surgical ICU	Count	7	24	31
		% within Word	22.6%	77.4%	100.0%
	pediatric ICU	Count	3	4	7
		% within Word	42.9%	57.1%	100.0%
Total		Count	13	37	50
		% within Word	26.0%	74.0%	100.0%

Symmetric Measures

		Value	Approximate Significance
Nominal by Nominal	Phi	.157	.541
	Cramer's V	.157	.541
N of Valid Cases		50	

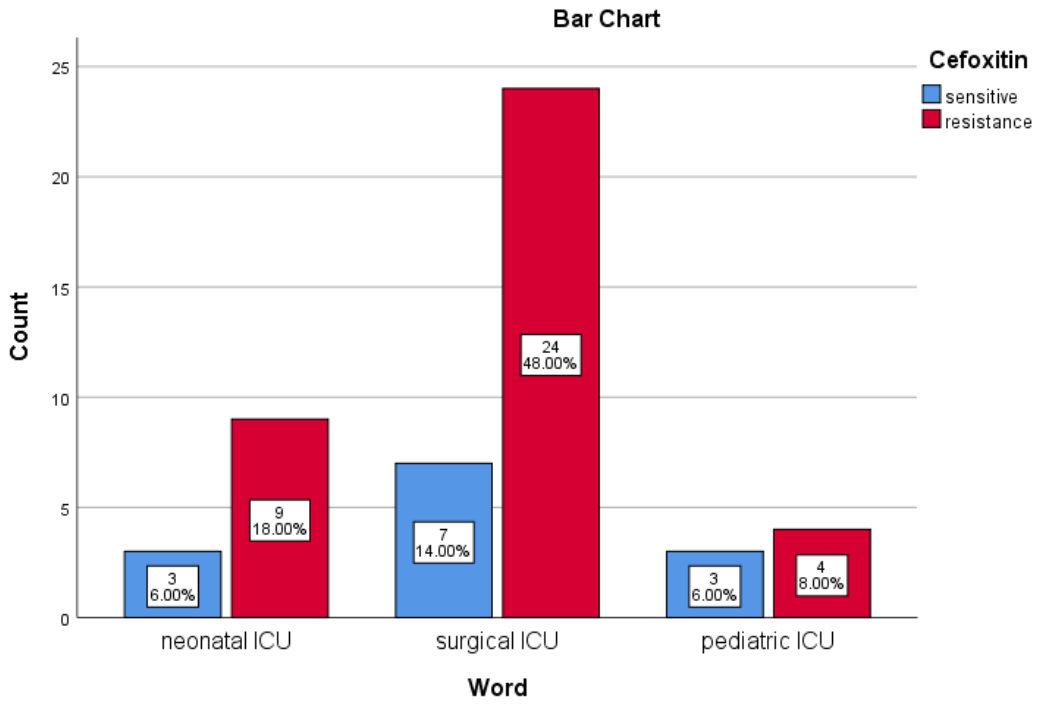

Figure 29: Distribution of Cefoxitin resistance of K. pneumoniae strains according to Word

Table 34 Distribution of Ceftazidime resistance of K. pneumoniae strains according to Word

Crosstab

			Ceftazidime Average sensitivity	sensitive	resistance	Total
Word	neonatal ICU	Count	0	1	11	12
		% within Word	0.0%	8.3%	91.7%	100.0%
	surgical ICU	Count	0	2	29	31
		% within Word	0.0%	6.5%	93.5%	100.0%
	pediatric ICU	Count	1	0	6	7
		% within Word	14.3%	0.0%	85.7%	100.0%
Total		Count	1	3	46	50
		% within Word	2.0%	6.0%	92.0%	100.0%

Symmetric Measures

		Value	Approximate Significance
Nominal by Nominal	Phi	.367	.151
	Cramer's V	.259	.151
N of Valid Cases		50	

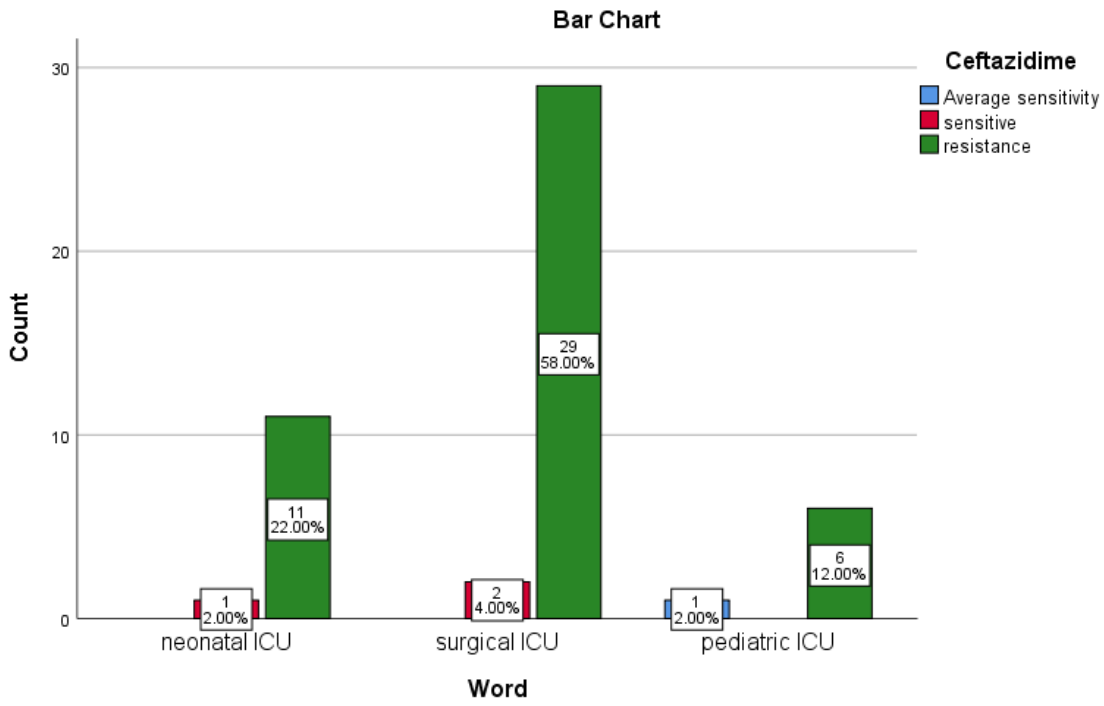

Figure 30 Distribution of Ceftazidime resistance of K. pneumoniae strains according to Word

Table 35 Distribution of Ceftriaxone resistance of K. pneumoniae strains according to Word

Crosstab

			Ceftriaxone sensitive	resistance	Total
Word	neonatal ICU	Count	1	11	12
		% within Word	8.3%	91.7%	100.0%
	surgical ICU	Count	1	30	31
		% within Word	3.2%	96.8%	100.0%
	pediatric ICU	Count	0	7	7
		% within Word	0.0%	100.0%	100.0%
Total		Count	2	48	50
		% within Word	4.0%	96.0%	100.0%

Symmetric Measures

		Value	Approximate Significance
Nominal by Nominal	Phi	.136	.629
	Cramer's V	.136	.629
N of Valid Cases		50	

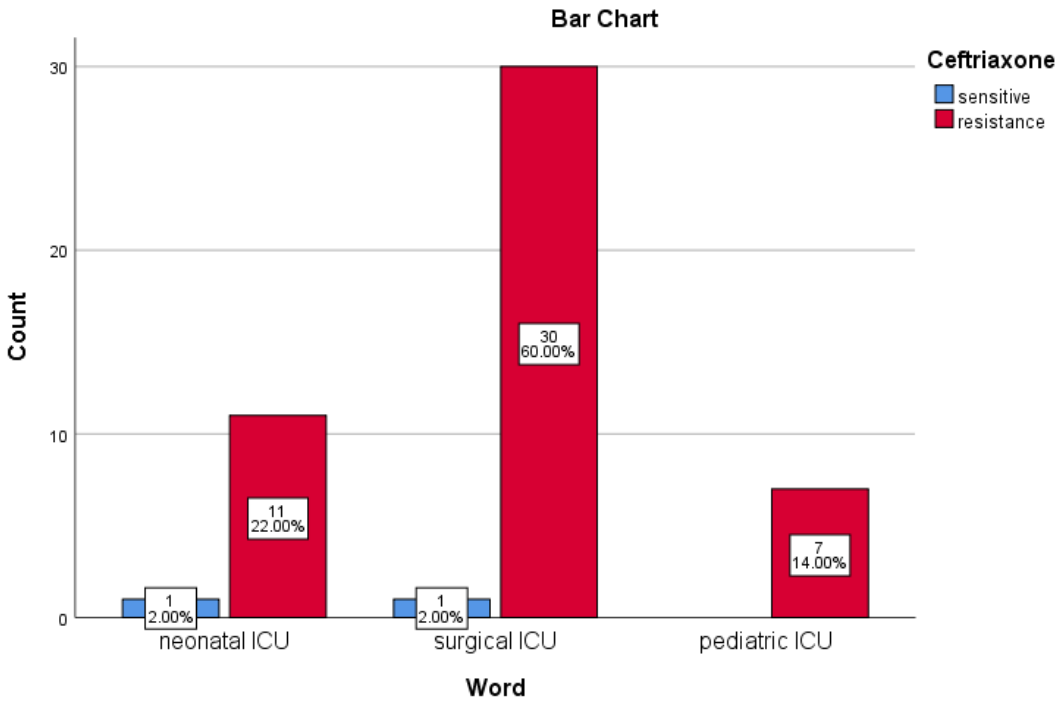

Figure 31 Distribution of Ceftriaxone resistance of K. pneumoniae strains according to Word

Table 36 Distribution of Cefepime resistance of K. pneumoniae strains according to Word

Crosstab

			Cefepime		
			sensitive	resistance	Total
Word	neonatal ICU	Count	1	11	12
		% within Word	8.3%	91.7%	100.0%
	surgical ICU	Count	1	30	31
		% within Word	3.2%	96.8%	100.0%
	pediatric ICU	Count	0	7	7
		% within Word	0.0%	100.0%	100.0%
Total		Count	2	48	50
		% within Word	4.0%	96.0%	100.0%

Symmetric Measures

		Value	Approximate Significance
Nominal by Nominal	Phi	.136	.629
	Cramer's V	.136	.629
N of Valid Cases		50	

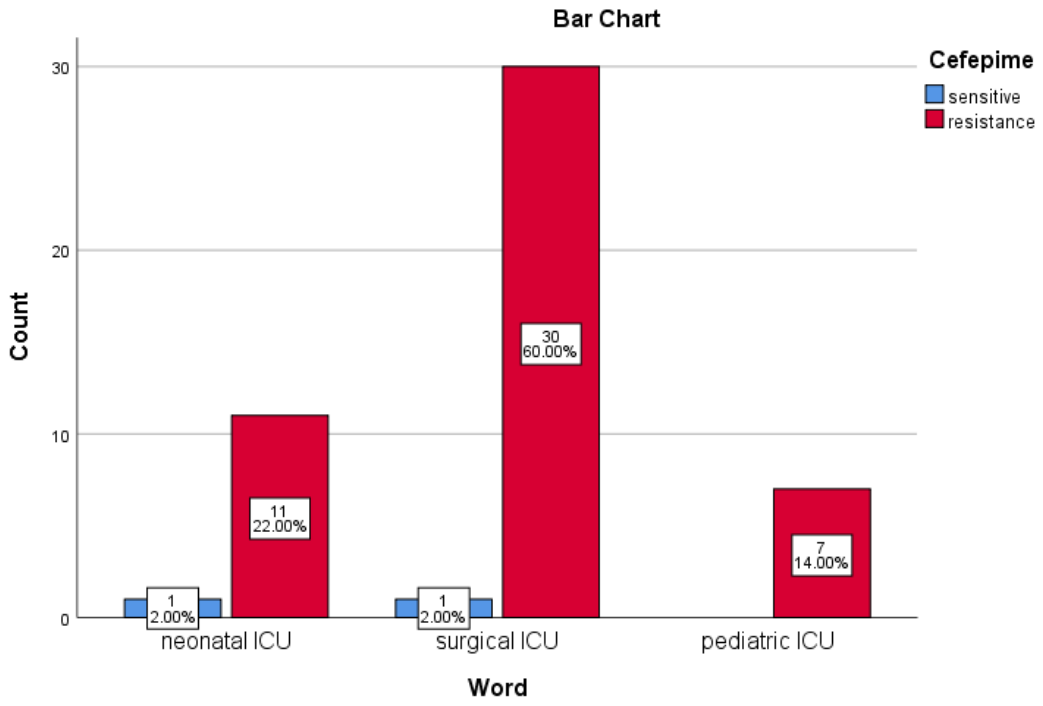

Figure 32 Distribution of Cefepime resistance of K. pneumoniae strains according to Word

Table 37 Distribution of Aztreonam resistance of K. pneumoniae strains according to Word

Crosstab

			Aztreonam		
			sensitive	resistance	Total
Word	neonatal ICU	Count	2	10	12
		% within Word	16.7%	83.3%	100.0%
	surgical ICU	Count	2	29	31
		% within Word	6.5%	93.5%	100.0%
	pediatric ICU	Count	0	7	7
		% within Word	0.0%	100.0%	100.0%
Total		Count	4	46	50
		% within Word	8.0%	92.0%	100.0%

Symmetric Measures

		Value	Approximate Significance
Nominal by Nominal	Phi	.197	.380
	Cramer's V	.197	.380
N of Valid Cases		50	

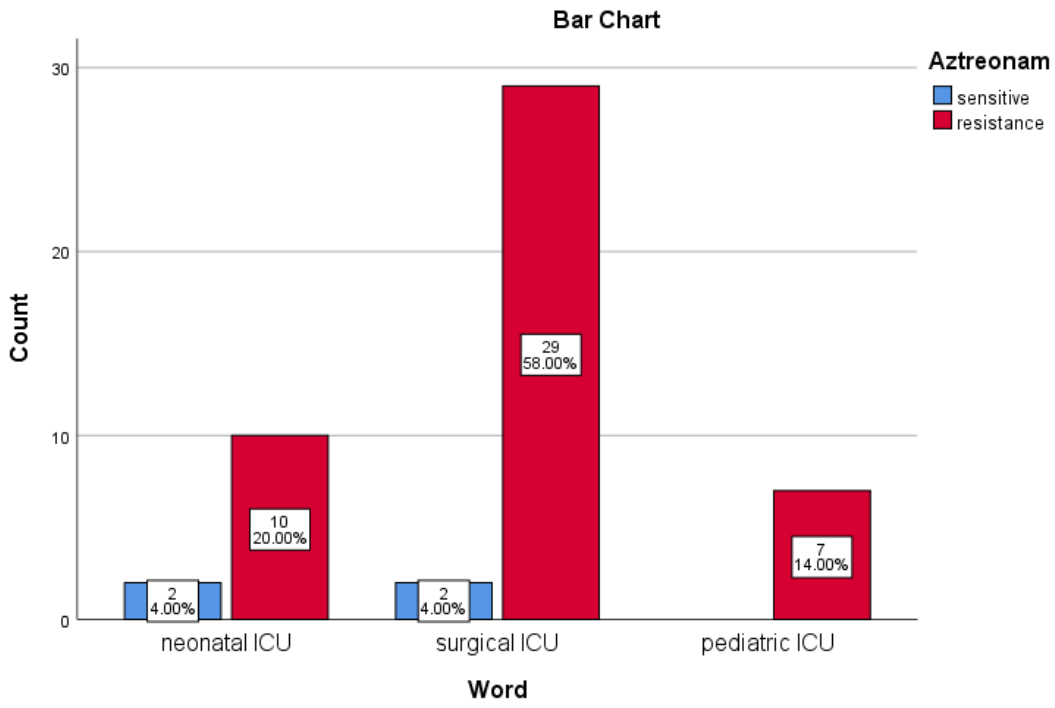

Figure 33: Distribution of Aztreonam resistance of K. pneumoniae strains according to Word

Table 38 Distribution of Ampicillin resistance of *K. pneumoniae* strains according to Word

Crosstab

			Ampicillin sensitive	Resistance	Total
Word	neonatal ICU	Count	1	11	12
		% within Word	8.3%	91.7%	100.0%
	surgical ICU	Count	0	31	31
		% within Word	0.0%	100.0%	100.0%
	pediatric ICU	Count	0	7	7
		% within Word	0.0%	100.0%	100.0%
Total		Count	1	49	50
		% within Word	2.0%	98.0%	100.0%

Symmetric Measures

		Value	Approximate Significance
Nominal by Nominal	Phi	.254	.199
	Cramer's V	.254	.199
N of Valid Cases		50	

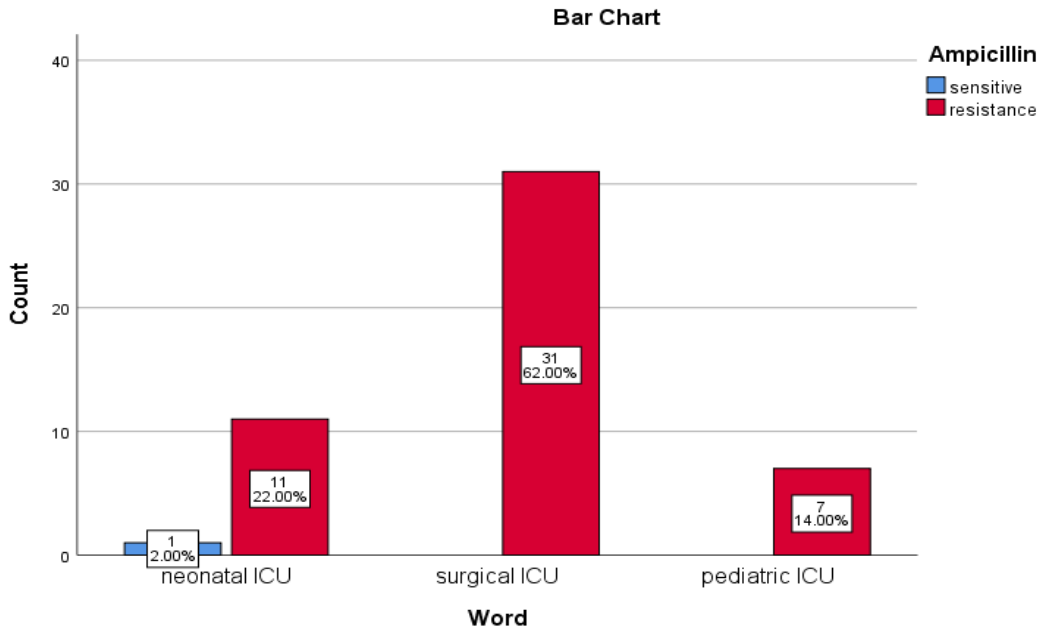

Figure 34: Distribution of Ampicillin resistance of K. pneumoniae strains according to Word

Table 39 Distribution of Amoxicillin-Clavulanate resistance of K. pneumoniae strains according to Word

Crosstab

			Amoxicillin-Clavulanate			
			Average sensitivity	sensitive	resistance	Total
Word	neonatal ICU	Count	1	3	8	12
		% within Word	8.3%	25.0%	66.7%	100.0%
	surgical ICU	Count	0	3	28	31
		% within Word	0.0%	9.7%	90.3%	100.0%
	pediatric ICU	Count	0	0	7	7
		% within Word	0.0%	0.0%	100.0%	100.0%
Total		Count	1	6	43	50
		% within Word	2.0%	12.0%	86.0%	100.0%

Symmetric Measures

		Value	Approximate Significance
Nominal by Nominal	Phi	.363	.160
	Cramer's V	.257	.160
N of Valid Cases		50	

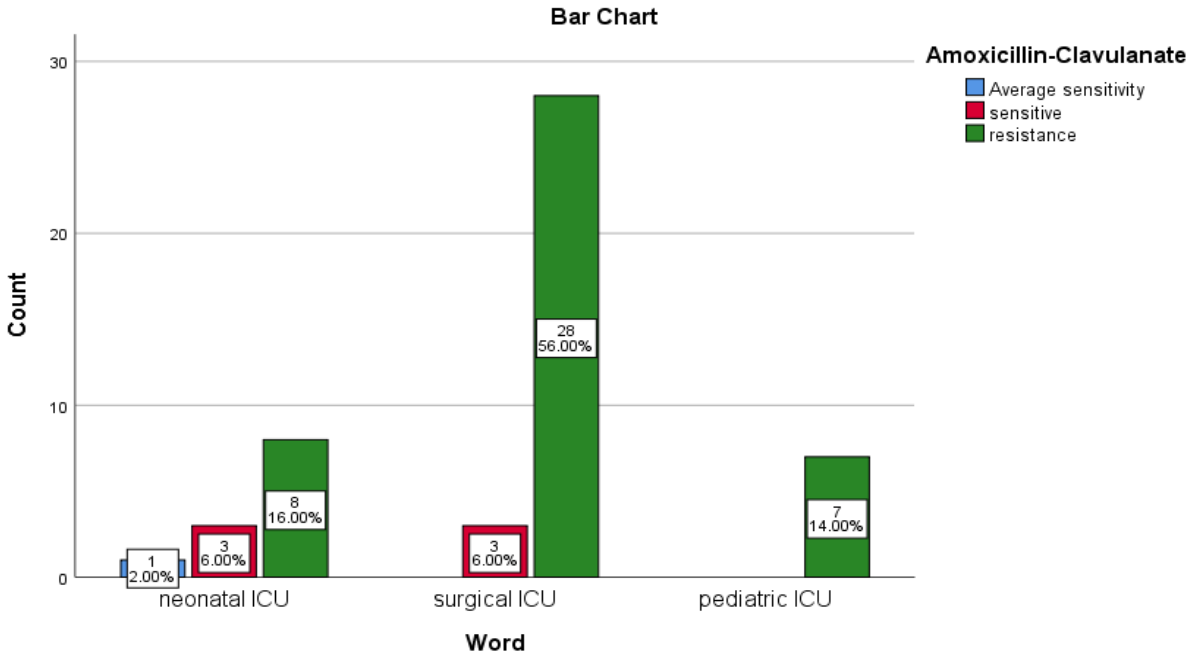

Figure 35: Distribution of Amoxicillin-Clavulanate resistance of K. pneumoniae strains according to Word

Table 40 Distribution of Piperacillin-Tazobactam resistance of K. pneumoniae strains according to Word

Crosstab

			Piperacillin-Tazobactam			
			Average sensitivity	sensitive	resistance	Total
Word	neonatal ICU	Count	0	5	7	12
		% within Word	0.0%	41.7%	58.3%	100.0%
	surgical ICU	Count	3	6	22	31
		% within Word	9.7%	19.4%	71.0%	100.0%
	pediatric ICU	Count	0	3	4	7
		% within Word	0.0%	42.9%	57.1%	100.0%
Total		Count	3	14	33	50
		% within Word	6.0%	28.0%	66.0%	100.0%

Symmetric Measures

		Value	Approximate Significance
Nominal by Nominal	Phi	.294	.364
	Cramer's V	.208	.364
N of Valid Cases		50	

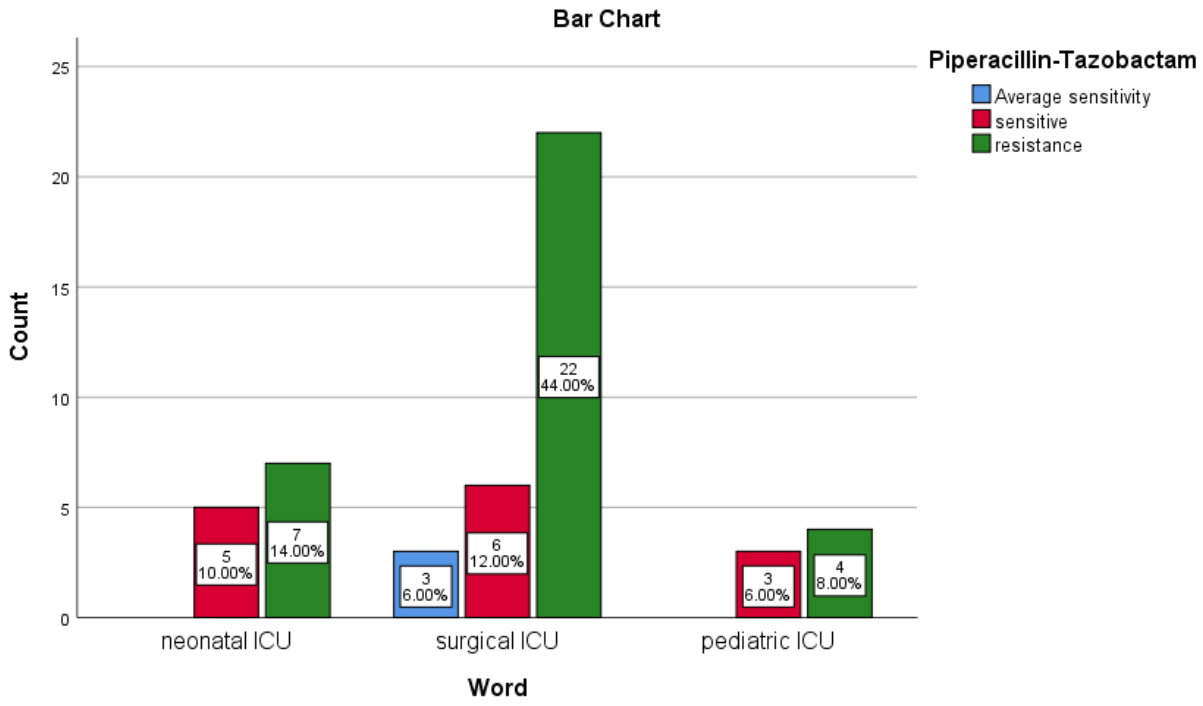

Figure 36 Distribution of Piperacillin-Tazobactam resistance of K. pneumoniae strains according to Word

Table 41 Distribution of Colistin resistance of K. pneumoniae strains according to Word

Crosstab

			Colistin Sensitive	Total
Word	neonatal ICU	Count	12	12
		% within Word	100.0%	100.0%
	surgical ICU	Count	31	31
		% within Word	100.0%	100.0%
	pediatric ICU	Count	7	7
		% within Word	100.0%	100.0%
Total		Count	50	50
		% within Word	100.0%	100.0%

Symmetric Measures

		Value
Nominal by Nominal	Phi	.[a]
N of Valid Cases		50

a. No statistics are computed because Colistin is a constant.

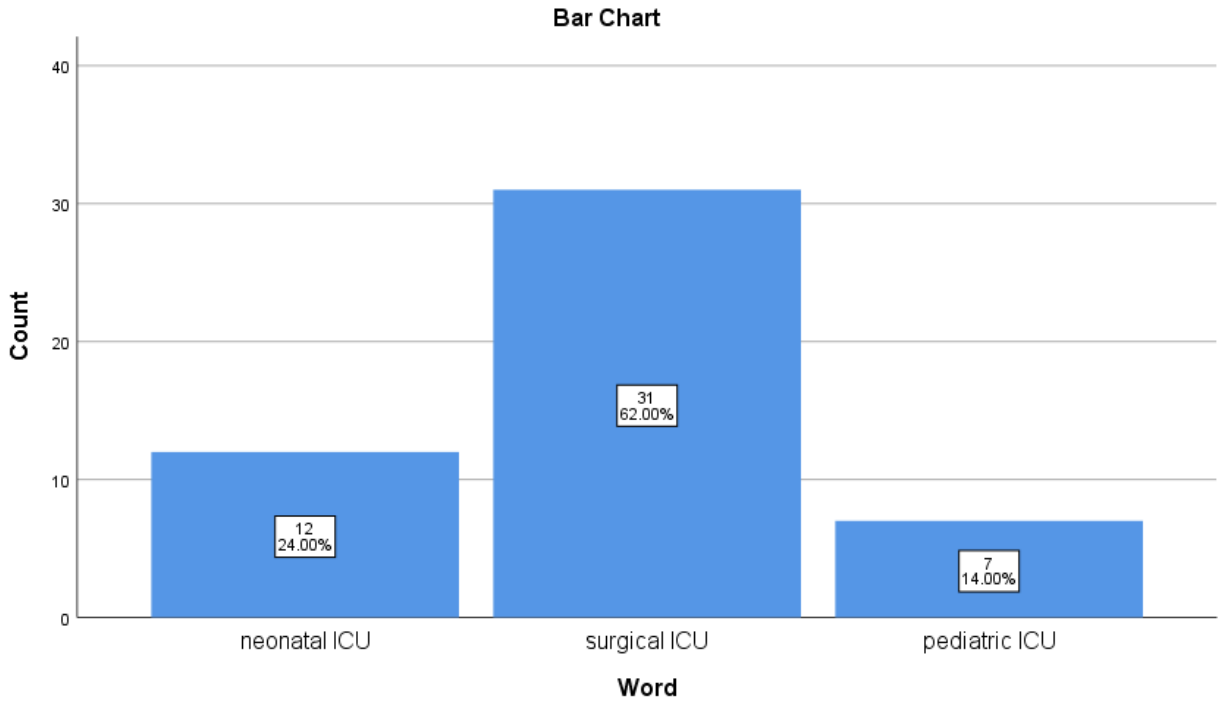

Figure 37 : Distribution of Colistin resistance of K. pneumoniae strains according to Word

Table 42 Distribution of Trimethoprim-Sulfamethoxazol resistance of K. pneumoniae strains according to Word

Crosstab

			Trimethoprim-Sulfamethoxazol		
			Sensitive	resistance	Total
Word	neonatal ICU	Count	3	9	12
		% within Word	25.0%	75.0%	100.0%
	surgical ICU	Count	7	24	31

		% within Word	22.6%	77.4%	100.0%
	pediatric ICU	Count	1	6	7
		% within Word	14.3%	85.7%	100.0%
Total		Count	11	39	50
		% within Word	22.0%	78.0%	100.0%

Symmetric Measures

		Value	Approximate Significance
Nominal by Nominal	Phi	.079	.856
	Cramer's V	.079	.856
N of Valid Cases		50	

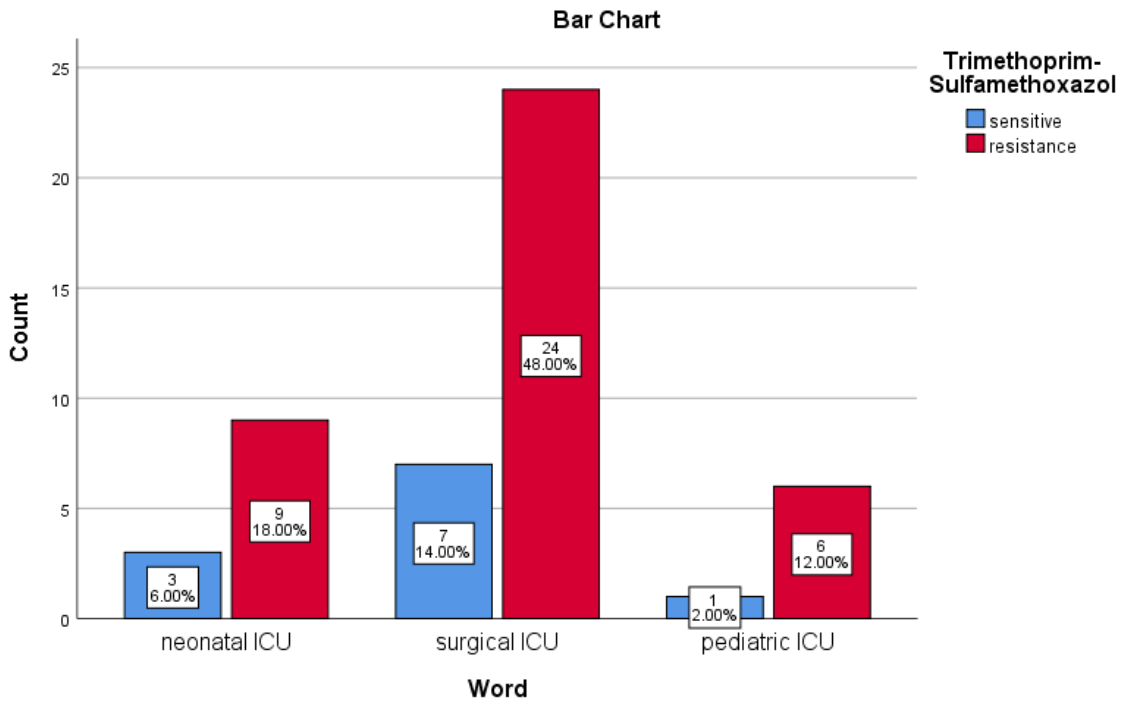

Figure 38 Distribution of Trimethoprim-Sulfamethoxazol resistance of K. pneumoniae strains according to Word

Table 43 Distribution of Nitrofurantoin resistance of K. pneumoniae strains according to Word

Crosstab

			Nitrofurantoin			
			Average sensitivity	sensitive	resistance	Total
Word	neonatal ICU	Count	0	3	9	12
		% within Word	0.0%	25.0%	75.0%	100.0%
	surgical ICU	Count	2	6	23	31
		% within Word	6.5%	19.4%	74.2%	100.0%
	pediatric ICU	Count	1	1	5	7
		% within Word	14.3%	14.3%	71.4%	100.0%
Total		Count	3	10	37	50
		% within Word	6.0%	20.0%	74.0%	100.0%

Symmetric Measures

		Value	Approximate Significance
Nominal by Nominal	Phi	.190	.771
	Cramer's V	.135	.771
N of Valid Cases		50	

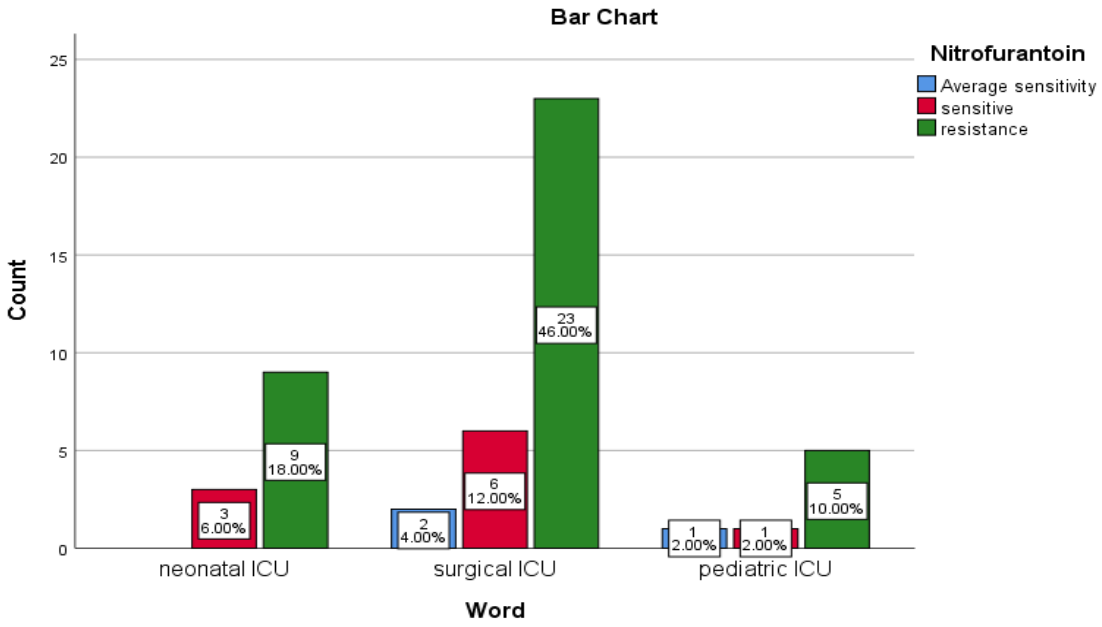

Figure 39: Distribution of Nitrofurantoin resistance of K. pneumoniae strains according to Word

Table 44 Distribution of Ciprofloxacin resistance of K. pneumoniae strains according to Word

Crosstab

			Ciprofloxacin			
			Average sensitivity	sensitive	resistance	Total
Word	neonatal ICU	Count	1	4	7	12
		% within Word	8.3%	33.3%	58.3%	100.0%
	surgical ICU	Count	2	6	23	31
		% within Word	6.5%	19.4%	74.2%	100.0%
	pediatric ICU	Count	0	3	4	7
		% within Word	0.0%	42.9%	57.1%	100.0%
Total		Count	3	13	34	50
		% within Word	6.0%	26.0%	68.0%	100.0%

Symmetric Measures

		Value	Approximate Significance
Nominal by Nominal	Phi	.225	.637
	Cramer's V	.159	.637
N of Valid Cases		50	

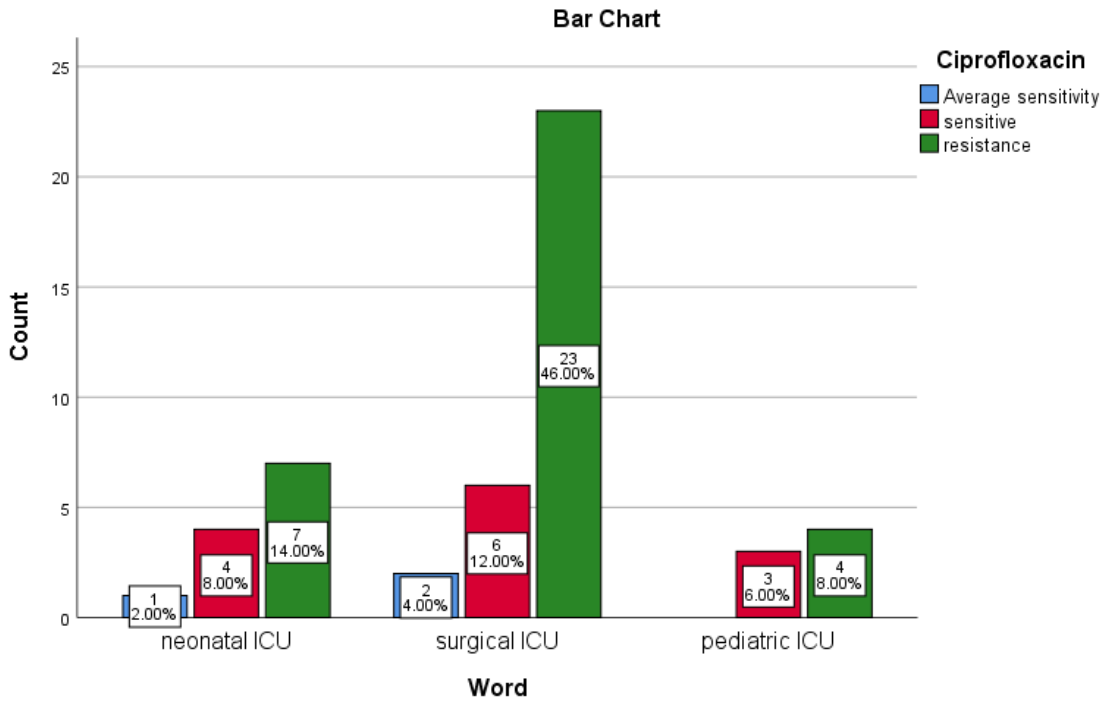

Figure 40 : Distribution of Ciprofloxacin resistance of K. pneumoniae strains according to Word

Table 45 Distribution of Levofloxacin resistance of K. pneumoniae strains according to Word

Crosstab

			Levofloxacin		
			sensitive	resistance	Total
Word	neonatal ICU	Count	6	6	12
		% within Word	50.0%	50.0%	100.0%
	surgical ICU	Count	8	23	31
		% within Word	25.8%	74.2%	100.0%
	pediatric ICU	Count	3	4	7
		% within Word	42.9%	57.1%	100.0%
Total		Count	17	33	50
		% within Word	34.0%	66.0%	100.0%

Symmetric Measures

		Value	Approximate Significance
Nominal by Nominal	Phi	.225	.281
	Cramer's V	.225	.281
N of Valid Cases		50	

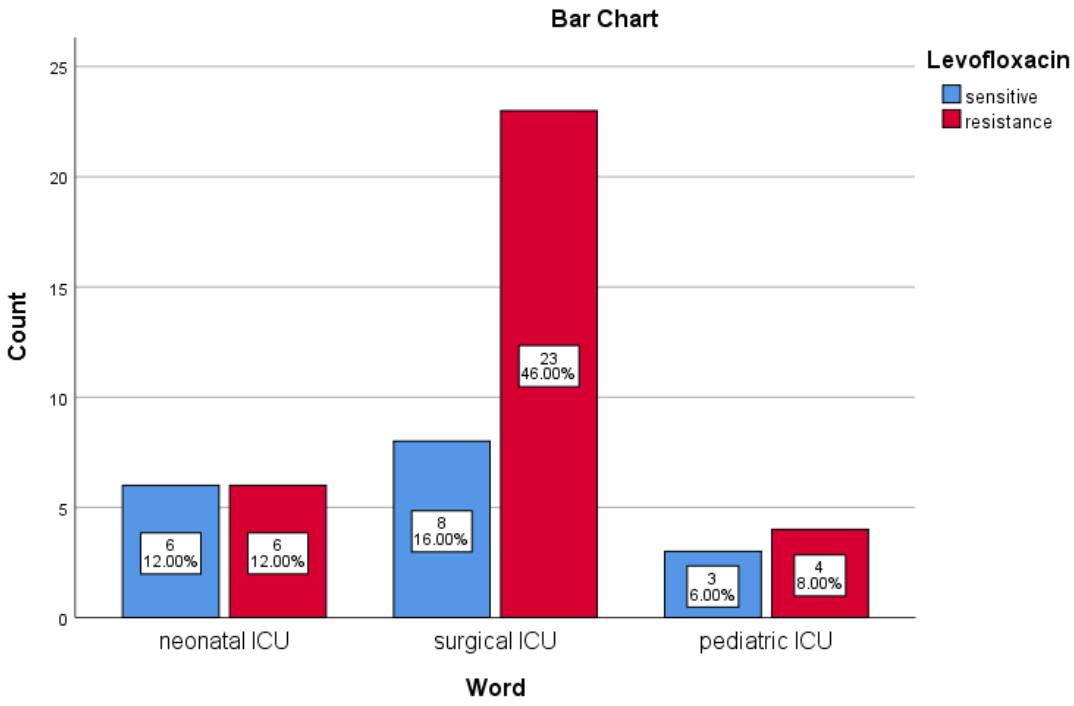

Figure 41: Distribution of Levofloxacin resistance of K. pneumoniae strains according to Word

Table 46 Distribution of Tigecycline resistance of K. pneumoniae strains according to Word

Crosstab

			Tigecycline		
			Average sensitivity	resistance	Total
Word	neonatal ICU	Count	1	11	12
		% within Word	8.3%	91.7%	100.0%
	surgical ICU	Count	16	15	31
		% within Word	51.6%	48.4%	100.0%
	pediatric ICU	Count	2	5	7
		% within Word	28.6%	71.4%	100.0%
Total		Count	19	31	50
		% within Word	38.0%	62.0%	100.0%

Symmetric Measures

		Value	Approximate Significance
Nominal by Nominal	Phi	.379	.028
	Cramer's V	.379	.028
N of Valid Cases		50	

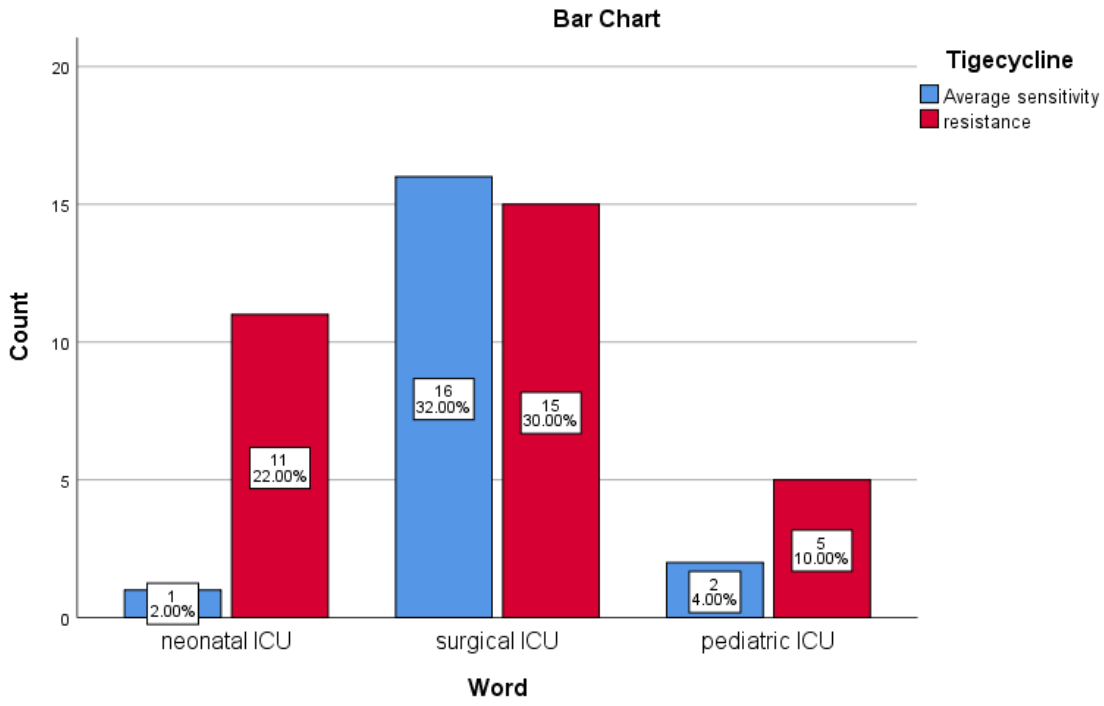

Figure 42 : Distribution of Tigecycline resistance of K. pneumoniae strains according to Word

Based on the results shown in the crosstab tables, the distribution of antibiotic resistance of *Klebsiella pneumoniae* strains according to the ward shows varying degrees of association across different antibiotics.

Starting with Table (Amikacin), the highest resistance was observed in the surgical ICU (64.5%) and pediatric ICU (57.1%), while the neonatal ICU showed 50% resistance. The Phi coefficient (.269) and Cramer's V (.190) indicate a weak association, with no statistical

significance (p = .460), suggesting that the resistance pattern is not strongly dependent on ward type.

In Table (Gentamicin), the resistance was highest in the pediatric ICU (85.7%), followed by the surgical ICU (64.5%), and then neonatal ICU (58.3%). However, the Phi (.176) and Cramer's V (.176) values again reflect a weak association, with a non-significant p-value (.459), indicating that ward does not meaningfully influence resistance to Gentamicin.

Ertapenem results (Table) show a notable concentration of resistance in the surgical ICU (87.1%) and pediatric ICU (85.7%), with the neonatal ICU presenting 50% resistance. The Phi value (.412) and Cramer's V (.291) indicate a moderate association, though it does not reach significance (p = .076), which suggests a potential trend worth further exploration.

For Imipenem, a similar trend is seen with the highest resistance in the surgical ICU (87.1%) and lowest in neonatal ICU (50.0%). The Phi (.380) and Cramer's V (.268) suggest a moderate association, but again not statistically significant (p = .125).

In Meropenem, resistance was highest in the surgical ICU (71.0%), followed by pediatric ICU (57.1%), and neonatal ICU (50.0%). The Phi and Cramer's V were both (.191), with a p-value of (.403), indicating a weak and non-significant relationship.

The Cephalothin, Cefuroxime, Ceftriaxone, and Cefepime tables all showed very high resistance levels across all wards (above 90%), with Phi and Cramer's V values being consistently low (.136), and non-significant (p = .629), supporting the idea that resistance to these cephalosporins is widespread and not ward-specific.

Cefoxitin resistance was also high, especially in the surgical ICU (77.4%) and neonatal ICU (75.0%), though the pediatric ICU showed lower resistance (57.1%). Still, the Phi (.157) and Cramer's V (.157) indicate a weak relationship (p = .541).

For Ceftazidime, resistance was again widespread with the highest in the surgical ICU (93.5%), neonatal ICU (91.7%), and pediatric ICU (85.7%). Phi (.367) and Cramer's V (.259) suggest moderate association but without significance (p = .151).

In the case of Aztreonam and Ampicillin, resistance was above 90% in all wards. The Phi values (.197 for Aztreonam, .254 for Ampicillin) and associated Cramer's V values suggest a weak association, with p-values indicating non-significance.

Amoxicillin-Clavulanate resistance was significantly higher in the surgical ICU (90.3%) compared to the neonatal ICU (66.7%), while the pediatric ICU exhibited complete resistance. Phi (.363) and Cramer's V (.257) reflect a moderate but non-significant association ($p = .160$).

Piperacillin-Tazobactam results were mixed, with moderate resistance across all wards. The Phi (.294) and Cramer's V (.208) again indicate weak association ($p = .364$).

Colistin showed 100% sensitivity across all wards, resulting in no variability and thus no Phi/Cramer's V values were computed, as this is a constant variable.

For Trimethoprim-Sulfamethoxazole, resistance was uniformly high (75%–85.7%) across wards, with a very low Phi (.079) and Cramer's V (.079), and a non-significant p-value (.856), supporting the conclusion that ward has no effect.

Nitrofurantoin showed high resistance levels across all wards, with slightly lower resistance in the pediatric ICU. The Phi (.190) and Cramer's V (.135) values indicate a weak association ($p = .771$).

Ciprofloxacin also followed a similar pattern of resistance, with some variation across wards. Phi was (.225) and Cramer's V (.159), again not significant ($p = .637$).

Levofloxacin showed slightly more variation with 50% sensitivity in the neonatal ICU, 25.8% in surgical ICU, and 42.9% in pediatric ICU. Phi (.225) and Cramer's V (.225) still indicate a weak association ($p = .281$).

Tigecycline exhibited the most noteworthy result, with resistance being highest in the neonatal ICU (91.7%) and lowest in the surgical ICU (48.4%). The Phi and Cramer's V values were both (.379), and importantly, this association was statistically significant ($p = .028$), suggesting that ward type might influence resistance to Tigecycline.

In summary, among all antibiotics tested against *K. pneumoniae*, most showed high resistance patterns that were generally consistent across hospital wards, with only

Tigecycline demonstrating a statistically significant association with ward location (Phi = .379, Cramer's V = .379, p = .028), supporting the hypothesis for this specific antibiotic. All other antibiotics showed weak or moderate associations with no statistical significance, thereby not supporting the hypothesis in those cases.

H4: Phenotyping test for ESBL and Carbapenemase producing isolates (Resistance markers)

Table 47 Phenotyping test for ESBL and Carbapenemase producing isolates (Resistance markers)

Resistance markers	Frequency	Percent
ESBL - ALERT1	29	%58.0
ESBL	7	%14.0
ALERT1	5	%10.0
CARB	8	%16.0
NEGATIVE	1	%2.0
Total	50	%100.0

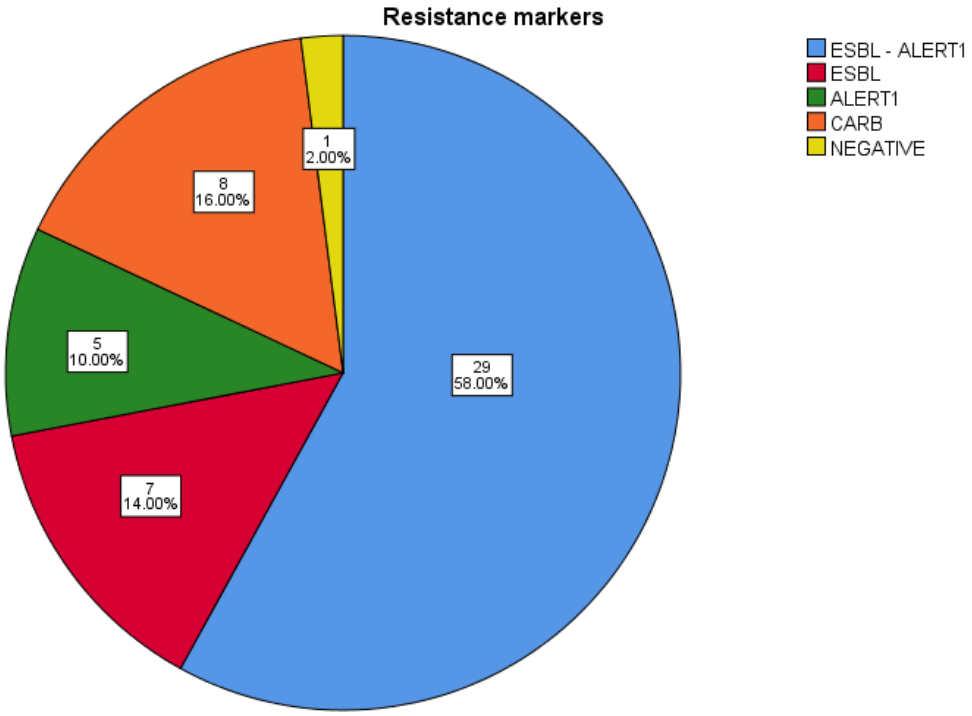

Figure 43 Phenotyping test for ESBL and Carbapenemase producing isolates (Resistance markers)

To test hypothesis H3: Phenotyping test for ESBL and Carbapenemase producing isolates (Resistance markers), we analyze the distribution of resistance markers among the 50 *Klebsiella pneumoniae* clinical isolates.

The results, as presented in the table, reveal that the majority of the isolates (29 out of 50; 58.0%) were identified as ESBL - ALERT1, indicating co-expression or alert status for extended-spectrum β-lactamase production along with a warning marker. Additionally, 7 isolates (14.0%) were confirmed ESBL producers only, while 5 isolates (10.0%) showed only ALERT1 marker presence.

Importantly, 8 isolates (16.0%) were identified as CARB producers, which are strains capable of producing carbapenemase, a critical resistance mechanism that compromises last-line treatments. Only 1 isolate (2.0%) tested negative for all resistance markers, underscoring the pervasive presence of resistance mechanisms among the tested isolates.

In light of these findings, the hypothesis is supported — the phenotypic testing confirms a high prevalence of ESBL and carbapenemase producers, with 94% of isolates carrying at least one resistance marker. This emphasizes the clinical threat posed by multidrug-resistant *K. pneumoniae*, particularly in environments where both ESBL and carbapenemase genes may co-circulate.

The key findings of the hypotheses summarized in bullet points:

- H1: Antibiotic susceptibility of *Klebsiella pneumoniae* clinical strains (sensitive–resistance)
    - *K. pneumoniae* showed very high resistance to most β-lactam antibiotics, especially Cephalothin, Cefuroxime, Ceftriaxone, and Cefepime (resistance rates up to 96%).
    - Colistin was the only antibiotic with 100% sensitivity among all isolates.
    - Moderate sensitivity was observed to Amikacin (38%), Gentamicin (34%), and Meropenem (36%).
    - Most Phi and Cramer's V values indicated weak associations between antibiotic response categories and distribution, with no statistically significant relationships ($p > 0.05$), except for Tigecycline, which had a significant association (Phi = .473, p = .025).
- H2: Distribution of antibiotic resistance of *K. pneumoniae* strains according to sample source
    - Resistance was consistently high across all sample sources (swabs, blood, tips, sputum, urine), especially for β-lactams and fluoroquinolones.

- No statistically significant relationship was found between sample source and resistance level for any antibiotic, except Nitrofurantoin, which showed a near-significant trend (Phi = .555, p = .051).
- Overall, Cramer's V values were low, indicating weak associations between source and resistance.

- H3: Distribution of antibiotic resistance according to hospital ward
  - Surgical ICU showed the highest resistance levels to many antibiotics, especially Ertapenem, Imipenem, and Ceftazidime.
  - Tigecycline resistance varied significantly by ward and was the only antibiotic showing a statistically significant association (Phi = .379, Cramer's V = .379, p = .028).
  - Most antibiotics did not show significant differences in resistance by ward, with low Phi and Cramer's V values, indicating uniform resistance patterns across wards.

H4: Phenotyping test for ESBL and Carbapenemase producing isolates (Resistance markers)

- Phenotypic testing of 50 *Klebsiella pneumoniae* clinical isolates revealed a high prevalence of resistance markers. Specifically, **58%** of isolates were identified as **ESBL - ALERT1**, **14%** as **ESBL** only, and **10%** as **ALERT1** only. Furthermore, **16%** of isolates were positive for **carbapenemase (CARB)** production, while only **2%** were negative for all resistance markers.
- Overall, **94% of isolates** exhibited at least one resistance phenotype, confirming the hypothesis and highlighting the significant clinical concern associated with multidrug-resistant *K. pneumoniae* strains.

These findings indicate that while antibiotic resistance is widespread and consistent across settings for most antibiotics, Tigecycline resistance is influenced by hospital ward, and Colistin remains the most effective drug against these strains.

5. CHAPTER 5: DISCUSSION:

5.1 DISCUSSION:

Antibiotic Resistance Patterns in Klebsiella pneumoniae

The analysis of antibiotic susceptibility patterns in *Klebsiella pneumoniae* clinical strains reveals significant trends towards resistance across various antibiotic classes. Our findings indicate a concerning prevalence of resistance, particularly among β-lactam antibiotics and carbapenems, aligning with previous studies highlighting the increasing challenge of antimicrobial resistance in this pathogen.

In our study, Amikacin demonstrated a sensitivity rate of only 38%, with a notable 60% of strains classified as resistant. This moderate resistance, reinforced by moderate Phi and Cramér's V values, suggests a significant relationship between *K. pneumoniae* and Amikacin resistance. Similarly, Gentamicin showed a clear resistance pattern, with 66% of strains resistant and no intermediate sensitivity observed. These results corroborate earlier studies that reported rising resistance rates to aminoglycosides, emphasizing the need for continuous surveillance and alternative treatment strategies.

The resistance observed with carbapenems is particularly alarming. With only 20% sensitivity for both Ertapenem and Imipenem, and 36% for Meropenem, the data reflect a troubling trend consistent with literature indicating the emergence of carbapenem-resistant *K. pneumoniae* isolates globally. The high resistance rates (76% for Imipenem and 78% for Ertapenem) highlight a critical public health issue, as these drugs are often considered last-resort options for treating severe infections.

The moderate to strong Phi and Cramér's V values further suggest a robust association between these antibiotics and resistance patterns, reinforcing findings from other studies that have documented similar trends.

The cephalosporin class exhibited even more dramatic resistance rates, with Cephalothin, Cefuroxime, Ceftriaxone, and Cefepime showing resistance in 96% of cases. This aligns with previous reports indicating that *K. pneumoniae* infections are increasingly difficult to treat due to the widespread resistance to cephalosporins. The strong association coefficients here (Phi and Cramér's V nearing 1) reflect a near-universal failure of these agents, underscoring their limited therapeutic potential in current clinical practice.

A similar pattern of resistance was observed with Aztreonam (92% resistant) and Ampicillin (98% resistant), further emphasizing the critical nature of β-lactam resistance. Previous studies have consistently reported high resistance rates within this antibiotic class, which poses a significant challenge in treating infections caused by *K. pneumoniae*.

In stark contrast to the other antibiotics and Colistin showcased a remarkable 100% sensitivity across all isolates, marking it as an exceptional outlier. This finding aligns with recent studies that have positioned Colistin as a last-line treatment option for multidrug-resistant infections, although its use is often limited by nephrotoxicity and the potential for resistance development.

Trimethoprim-Sulfamethoxazole, Nitrofurantoin, and Ciprofloxacin also displayed notable resistance rates (78%, 74%, and 68%, respectively). These results reflect a

diminishing but persistent effectiveness of these agents, echoing findings from other research indicating variable susceptibility patterns in *K. pneumoniae*.

Tigecycline's results are particularly concerning, with no sensitive isolates and a significant portion of resistance (62%). This raises alarms given Tigecycline's role in managing infections caused by multidrug-resistant organisms, highlighting the urgent need for alternative therapeutic options.

The analysis of antibiotic resistance in *Klebsiella pneumoniae* strains, segmented by sample source—swab, urine, tip, sputum, and blood—provides valuable insights into the distribution of resistance patterns. Our findings reveal varied resistance rates across different antibiotics and highlight some significant trends that align with and diverge from previous studies.

In our results, Amikacin resistance was highest in sputum samples (80.0%) and tips (63.6%), with an overall resistance of 60.0%. However, the Phi coefficient (0.322) and Cramér's V (0.228) indicate a weak association with non-significant p-values ($p = 0.738$). This suggests that while resistance is prevalent, it does not significantly depend on the sample source. Gentamicin exhibited similar patterns, with a 72.7% resistance rate in tips and low association values (Phi = 0.116, $p = 0.955$), reinforcing the notion that resistance is widespread across all sources without meaningful dependence.

These findings echo previous studies that have documented high resistance rates for aminoglycosides in *K. pneumoniae*, yet they also highlight the need for ongoing evaluation of susceptibility patterns as resistance mechanisms evolve.

Resistance rates for Ertapenem and Imipenem were notably high (78.0% and 76.0%, respectively), particularly in tip and blood samples. Despite this, the Phi and Cramér's V values (0.316 and 0.330 for Ertapenem and Imipenem, respectively) revealed non-significant associations ($p = 0.759$ and $p = 0.711$). These results align with previous literature indicating increasing resistance to carbapenems, particularly in healthcare settings, yet our study suggests that resistance does not significantly correlate with sample source.

Meropenem also exhibited high resistance, especially in sputum (80.0%) and tips (72.7%). The lack of significant association here (Phi = 0.262, $p = 0.490$) further underscores the pervasive nature of resistance, which has been reported in other studies as well, marking a concerning trend in antibiotic efficacy.

The cephalosporin group displayed alarmingly high resistance rates, reaching 96.0% across all sources. Consistent Phi and Cramér's V values around 0.196, with p-values of 0.752, indicate no significant association between source and resistance. Previous research has corroborated these findings, showing that *K. pneumoniae* strains exhibit broad resistance to cephalosporins, limiting their therapeutic utility.

Ceftazidime showed a high resistance rate of 92.0%, with particularly high resistance in urine and sputum samples. However, despite the high resistance, the Phi (0.252) and Cramér's V (0.178) indicated no significant association ($p = 0.922$). Similarly, Aztreonam exhibited 92.0% resistance, with non-significant association values (Phi = 0.213, $p = 0.687$).

Both Ampicillin and Amoxicillin-Clavulanate demonstrated very high resistance rates (98.0% and 86.0%, respectively), with no meaningful associations found (Phi = 0.208 and 0.239, p = 0.705 and 0.943).

For Piperacillin-Tazobactam, while resistance was moderate at 66.0%, the Phi (0.465) and Cramér's V (0.329) indicated a somewhat stronger relationship, albeit not statistically significant (p = 0.213). This suggests potential variability in resistance patterns that may warrant further investigation.

Colistin demonstrated a remarkable 100% sensitivity across all samples, precluding the calculation of association measures. This finding is consistent with its status as a last-resort antibiotic for multidrug-resistant infections, though concerns about nephrotoxicity remain prevalent in the literature.

Interestingly, Tigecycline presented a more balanced resistance pattern, with 62.0% resistance and significant sensitivity in sputum samples (100%). The Phi and Cramér's V values (both 0.473) were statistically significant (p = 0.025), suggesting a true dependency between sample source and resistance. This contrasts with earlier studies that reported mixed results regarding Tigecycline's efficacy against *K. pneumoniae*, highlighting the need for context-specific assessments of its use.

This analysis of antibiotic resistance patterns in *Klebsiella pneumoniae* strains across different hospital wards provides critical insights into the relationship between resistance and ward type. Our findings reveal varying degrees of association for different antibiotics, with most showing high resistance rates irrespective of ward location.

Resistance to Amikacin was highest in the surgical ICU (64.5%) and pediatric ICU (57.1%), with a Phi coefficient of 0.269 and Cramér's V of 0.190, indicating a weak and statistically non-significant association ($p = 0.460$). Similarly, Gentamicin displayed the highest resistance in the pediatric ICU (85.7%), with low association values (Phi = 0.176, $p = 0.459$). These results align with previous studies that have reported high rates of resistance in aminoglycosides, suggesting a consistent trend across different healthcare settings.

Ertapenem and Imipenem resistance rates were notably high, particularly in the surgical ICU (87.1% for both) and pediatric ICU (85.7% for Ertapenem). The moderate Phi values (0.412 for Ertapenem and 0.380 for Imipenem) suggest a potential association, though not statistically significant ($p = 0.076$ and $p = 0.125$). This moderate trend is consistent with other research indicating a rise in carbapenem resistance, particularly in ICU settings where infections are often more severe.

The cephalosporin class, including Cephalothin, Cefuroxime, Ceftriaxone, and Cefepime, exhibited very high resistance levels (over 90%) across all wards. The consistently low Phi and Cramér's V values (around 0.136) with non-significant p-values ($p = 0.629$) indicate that resistance is widespread and not influenced by ward type. This finding corroborates earlier studies that have documented extensive resistance to cephalosporins in *K. pneumoniae*, limiting their clinical utility.

Resistance to Ceftazidime was similarly high, particularly in the surgical ICU (93.5%). While the Phi (0.367) and Cramér's V (0.259) indicated a moderate association, the p-value (0.151) suggested non-significance, reinforcing the notion that resistance patterns are largely uniform across wards.

Aztreonam and Ampicillin also revealed high resistance rates (above 90%) with weak associations (Phi = 0.197 for Aztreonam, 0.254 for Ampicillin) and non-significant p-values. Amoxicillin-Clavulanate resistance was notably higher in the surgical ICU (90.3%) compared to other wards, but still showed no significant association (Phi = 0.363, p = 0.160).

For Piperacillin-Tazobactam, the resistance was moderate across wards, with weak association values (Phi = 0.294, p = 0.364). Colistin exhibited complete sensitivity, preventing any association analysis due to the lack of variability.

Trimethoprim-Sulfamethoxazole and Nitrofurantoin resistance rates were uniformly high across wards, with weak associations (Phi values of 0.079 and 0.190, respectively) and non-significant p-values. Similarly, Ciprofloxacin and Levofloxacin displayed high resistance levels, with Phi and Cramér's V values indicating weak associations.

Tigecycline presented the most noteworthy results, with resistance highest in the neonatal ICU (91.7%) and lowest in the surgical ICU (48.4%). The statistically significant association (Phi = 0.379, p = 0.028) suggests that ward type may indeed influence resistance to Tigecycline. This finding is particularly relevant given Tigecycline's role as a treatment for multidrug-resistant infections, highlighting the importance of context in its use.

Comparing our results with previous studies emphasizes the growing challenge of antibiotic resistance in *K. pneumoniae* across different healthcare settings. The consistent high resistance rates call for urgent action in monitoring and managing antibiotic use to mitigate the impact of resistance on patient outcomes.

In this study, we examined the distribution of resistance markers among 50 clinical isolates of *Klebsiella pneumoniae*, focusing specifically on extended-spectrum β-lactamase (ESBL) and carbapenemase production. The results indicate a significant prevalence of these resistance mechanisms, which raises important clinical implications.

Our analysis revealed that 58.0% (29 out of 50) of the isolates were classified as ESBL-ALERT1, indicating a co-expression of ESBL production alongside a warning marker. Additionally, 14.0% (7 isolates) were confirmed as ESBL producers only, while 10.0% (5 isolates) exhibited the ALERT1 marker without producing ESBL. This distribution underscores the complexity of resistance mechanisms in *K. pneumoniae*.

Moreover, 16.0% (8 isolates) were identified as carbapenemase producers, which is particularly concerning given that these enzymes compromise the efficacy of last-resort antibiotics. Notably, only 2.0% (1 isolate) tested negative for all resistance markers, highlighting the pervasive nature of resistance in this population. Collectively, 94% of the isolates carried at least one resistance marker, supporting the hypothesis that a high prevalence of ESBL and carbapenemase-producing strains exists in clinical settings.

5.2 :COMPARISON WITH PREVIOUS STUDIES

These findings are consistent with previous research that has documented an alarming rise in ESBL and carbapenemase-producing *K. pneumoniae* isolates globally. For instance, studies from various regions have reported similar prevalence rates, often exceeding 50% for ESBL producers in hospital settings. The

identification of carbapenemase producers in our study, at 16.0%, aligns with reports indicating that a significant portion of *K. pneumoniae* isolates in intensive care units and other high-risk environments exhibit such resistance mechanisms.

The high prevalence of ESBL-ALERT1 strains in our data suggests a potential co-circulation of resistance genes, which has been observed in other studies. The presence of multiple resistance determinants within the same isolate can complicate treatment options and contribute to the difficulty in managing infections caused by *K. pneumoniae*.

5.3: CLINICAL IMPLICATIONS

The implications of these findings are profound. The high prevalence of multidrug-resistant *K. pneumoniae* poses significant challenges for clinical management, particularly in settings where both ESBL and carbapenemase genes may co-exist. This dual resistance can lead to limited treatment options and increased rates of morbidity and mortality among infected patients.

Given the alarming trends in antibiotic resistance, our findings reinforce the need for stringent antimicrobial stewardship programs and enhanced surveillance systems. Early detection of resistance markers through phenotyping tests can guide appropriate therapeutic strategies and help mitigate the spread of resistant strains.

CHAPTER 6: CONCLUSION AND RECOMMENDATIONS

The hypothesis that "antibiotic susceptibility of *Klebsiella pneumoniae* clinical strains varies significantly, with a general trend toward resistance" is strongly supported by our findings. The high Phi and Cramér's V values across most antibiotic classes indicate robust associations between drug type and resistance patterns. While Colistin remains the most effective agent, the widespread resistance to other antibiotics underscores the urgent need for antimicrobial stewardship and enhanced resistance surveillance.

In summary, the hypothesis that antibiotic resistance in *Klebsiella pneumoniae* varies significantly with sample source is only partially supported by our findings. While most antibiotics exhibited high resistance rates without significant dependence on sample source, Tigecycline and Nitrofurantoin emerged as notable exceptions. Nitrofurantoin approached significance (Phi = 0.555, p = 0.051), indicating a potential relationship worth further exploration.

In conclusion, the hypothesis that antibiotic resistance in *Klebsiella pneumoniae* varies significantly by ward location is only partially supported. While most antibiotics showed high resistance patterns consistent across hospital wards, Tigecycline was the only drug demonstrating a statistically significant association with ward type. These findings underscore the need for ongoing surveillance and localized susceptibility testing to inform clinical decision-making and antimicrobial stewardship strategies.

In summary, the results of this study support the hypothesis that a significant proportion of *Klebsiella pneumoniae* clinical isolates exhibit resistance markers for

both ESBL and carbapenemase production. The findings highlight the urgent need for ongoing monitoring and research into resistance mechanisms, as well as the implementation of effective infection control measures in healthcare settings. As resistance patterns continue to evolve, understanding the prevalence and distribution of these markers is crucial for informing treatment strategies and safeguarding public health.

RECOMMENDATIONS

1. **High Resistance Rates**: The study confirms that *Klebsiella pneumoniae* isolates from ICU patients exhibit alarmingly high resistance rates to β-lactam antibiotics, necessitating urgent attention.
2. **Colistin as a Last Resort**: Colistin remains the only antibiotic with 100% sensitivity among the isolates, highlighting its critical role as a last-line treatment option.
3. **Moderate Sensitivity**: The moderate sensitivity observed for Amikacin, Gentamicin, and Meropenem indicates potential alternatives, but their effectiveness may be limited in many cases.
4. **Significance of Tigecycline**: The significant association of Tigecycline resistance by hospital ward underscores the need for tailored antibiotic stewardship interventions.
5. **Uniform Resistance Patterns**: The study shows that resistance patterns are largely uniform across different hospital wards, suggesting widespread dissemination of resistance traits.
6. **Need for Continuous Surveillance**: Ongoing surveillance programs are essential to monitor antibiotic resistance trends and enable timely interventions.
7. **Enhanced Infection Control Measures**: Implementing strict infection control protocols in ICUs can help limit the spread of resistant *K. pneumoniae* strains.

8. **Education and Training**: Healthcare providers should receive training on antibiotic stewardship practices to optimize the use of available antibiotics and reduce resistance development.
9. **Investment in Research**: Increased funding for research into new antibiotics and alternative treatments is critical to combat multidrug-resistant organisms.
10. **Patient Education**: Educating patients and families about the importance of adhering to prescribed antibiotic regimens can help reduce the risk of resistance development.

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APPENDIX A - …….

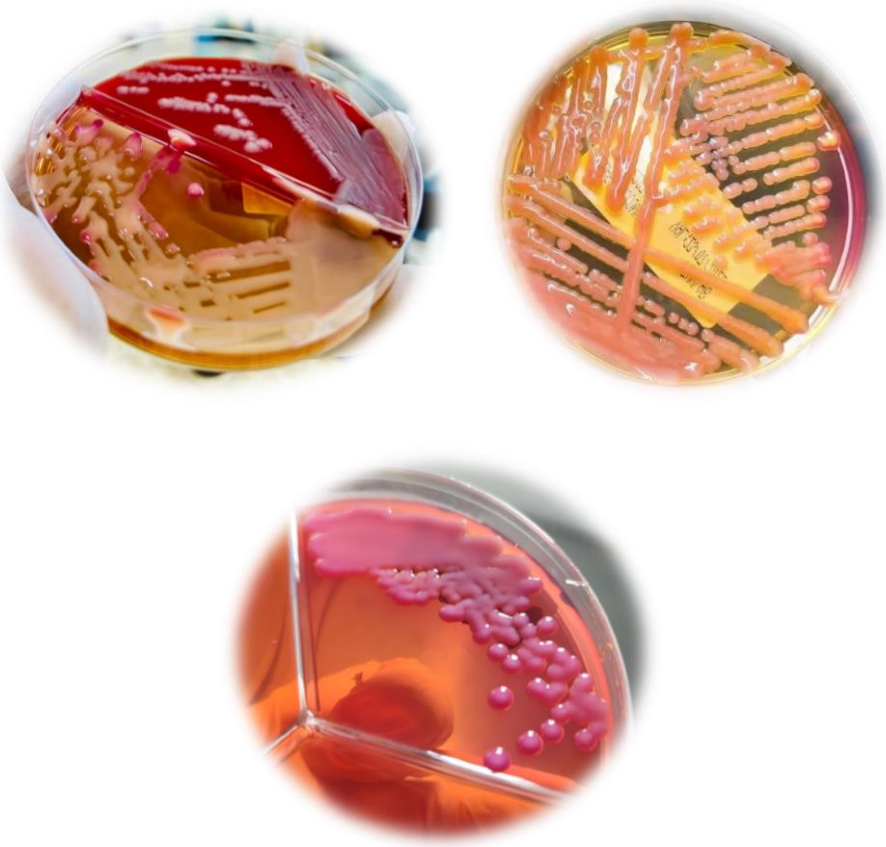

Figure 44 : Klebsiella pneumoniae on MacConkey Agar

Figure 45 : Antibiotic Susceptibility Testing of *Klebsiella pneumoniae*

Figure 46: Phenotypic Detection of ESBL-Producing Isolates

47 Figure : BD Phoenix 50 sustem

48 Figure : BACTEC device for Blood Culture