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PREVALENCE OF *KLEBSIELLA SPECIES* AND THEIR SUSCEPTIBILITY PATTERN TO ANTIBIOTICS IN PATIENTS WITH URINARY TRACT INFECTION AT SUKKUR

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Abstract

Background: *Urinary Tract Infections (UTIs) present a significant global health challenge, particularly due to the escalating issue of antimicrobial resistance. This study specifically addresses the prevalence and antibiotic susceptibility patterns of Klebsiella species, a common uropathogen, within the Sukkur region of Pakistan.*

Objectives: *Our primary aims were to isolate UTI-causing bacteria from urine samples, identify Klebsiella species through biochemical testing, and determine their antibiotic susceptibility profiles.*

Methods: *A total of 150 urine samples were collected from suspected UTI patients in Sukkur. These samples underwent microbiological analysis, including isolation on CLED agar, Gram staining, and biochemical identification. Antibiotic susceptibility testing was performed using the Kirby-Bauer disk diffusion method.*

Results: *Out of 150 samples, 20 showed bacterial growth. Escherichia coli was the most frequent isolate (50%), followed by Klebsiella species (35%) and Streptococci (15%). Notably, all Klebsiella isolates exhibited 100% resistance to Cefoperazone/Sulbactam. High resistance was also observed against Amoxicillin (71%) and Sulfamethoxazole/Trimethoprim (71%). Conversely, Flucloxacillin demonstrated 72% sensitivity.*

Conclusion: *The findings highlight a concerning prevalence of antibiotic-resistant Klebsiella species in Sukkur. This underscores the urgent need for continuous surveillance of uropathogens and their resistance trends. Implementing judicious antibiotic stewardship and utilizing localized susceptibility data are crucial for effective UTI management and combating antimicrobial resistance in the region.*

INTRODUCTION

1.1 Background:

The kidneys, ureters, bladder, and urethra form the urinary tract (Figure 1). Sitting on each side of the spinal column in the abdomen, the kidneys and ureters make up the upper urinary system. According to Baransk (2023), the ureters are tiny tubes that connect the kidneys with the bladder. Everything else makes up the lower urinary tract. While males find their bladders

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above the prostate gland, women find theirs in the pelvis. The organ is formed like a balloon. The urethra is the passageway by which the pee leaves the urinary tract (Baranski, 2023).

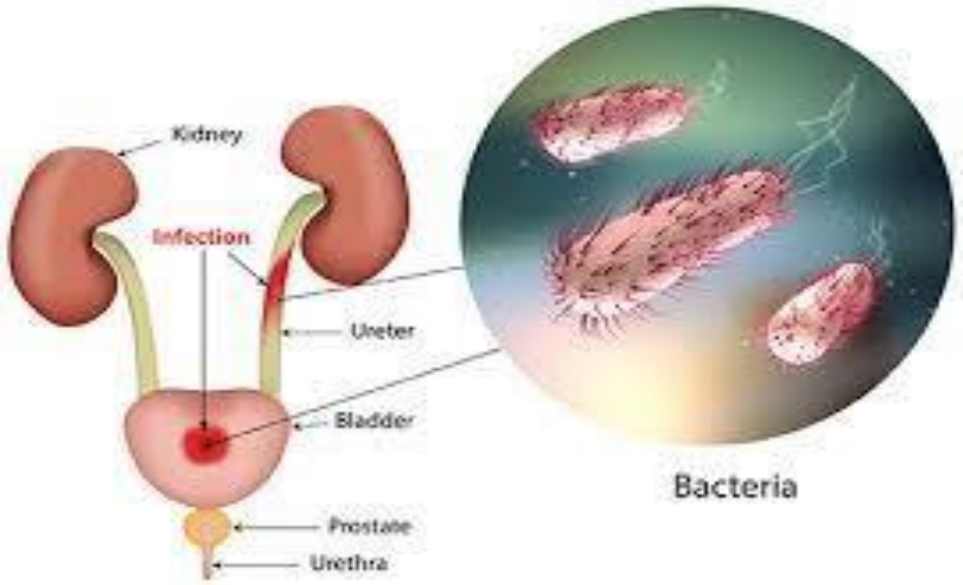

Figure1.1 Principal anatomical components of the urinary tract.

Urinary tract infection (UTI) is occurred due to multiplication of pathogen in urinary organs (Madwal and Borse, 2022), According to the criteria of the European Urological Association and the Infectious Diseases Society of America (IDSA), an infection caused by a bacterial pathogenic organism, exhibiting either symptoms or being asymptomatic, and involving acute pyelonephritis, is referred to as a UTI (Flores*et al.,* 2015).For overa century, urinary tract infections (UTIs) have been associated with diabetes, despite some earlier studies showing no variation in UTI frequency among individuals with diabetes. Diabetes, among numerous other causes, including immune changes such as granulocyte dysfunction, is considered one of the potential factors contributing to UTIs. (Geerlings, Hoepelman, 1999).

Urinary tract infection poses a significant challenge for individuals with diabetes, given the disease's manifold impacts on both the urinary tract and the host's immune system. In diabetic individuals, a considerable portion of urinary tract infections remains largely asymptomatic. Moreover, infections occur more frequently among diabetic patients compared to non-diabetic individuals due to diabetes heightening the susceptibility to severe illnesses, particularly when the condition is poorly managed. This asymptomatic infection can precipitate severe kidney damage, potentially culminating in kidney failure. The incidence of UTIs is on the rise among immune-compromised diabetic patients, with an estimated global prevalence reaching approximately 150 million people annually by 2022 (Oyewole, 2022).

Historically, complicated and non-complex UTIs have been categorized into two distinct groups. The symptoms of a urinary tract infection (UTI) might include burning or pain when you pee, the desire to urinate even when you don't have any urine in your bladder, and pain or pressure in your lower abdomen or groin. Consequently, these symptoms make it harder for afflicted persons to make a living, and treating UTIs is a top priority. Due to the bacterial nature of UTIs, antibiotics are often prescribed for their treatment. Never the less, UTIs may also be caused by viruses and fungus (Komala *et al.,* 2020). A urinary tract infection associated with

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structural, functional, or metabolic abnormalities of the genitor urinary tract is termed a complicated urinary tract infection (cUTI). (Neugent *et al.,* 2022). In addition to increasing the likelihood of recurrent infection, these underlying conditions compromise the host's defense mechanisms. (Flores *et al.,* 2019).

Underlying tuberculosis infections have significant monetary and health care costs. The general consensus is that acute uncomplicated UTIs in adult females who are not pregnant and who do not have any occluded ureters pose no serious health risks. Nevertheless, urinary tract infections (UTIs) increase the likelihood of pyelonephritis, preterm birth, and foetal death in pregnant women, and are linked to reduced renal function and end-stage renal disease in children. The monetary impact of community-acquired UTIs is substantial, amounting to almost $1.6 billion per year (Foxman, 2002). About 95% of UTI sare linked to uropathogens originating from the patient's own gut (GI) flora. UTIs are typically caused by bacterial infections,which include both Gram-2 positive and Gram-negative organisms. (Hyun *et al.,* 2019). For a UTI diagnosis, a positive urine culture indicating a known uropathogen at >1000 cfu/ml and the presence of urinary tract signs/symptoms are required. In this scenario, the identification of bacteria in the patient's collected urine was termed bacteriuria. (Cortes *et al.,* 2017).Depending on the location of the infection, symptoms vary ranging from mild irritation during voiding to sepsis (Table 1).

Clinical Symptoms and Signs of Upper and Lower Urinary Tract Infections

Urinary tract infections (UTIs) can be categorized based on whether they affect the lower urinary tract (such as the bladder and urethra) or the upper urinary tract (involving the kidneys and ureters). The clinical presentation differs between the two, helping clinicians distinguish between them.

Lower urinary tract infections (cystitis, urethritis) typically present with: Dysuria (painful urination), Burning sensation during urination, Increased urinary frequency, Malodorous urine, Suprapubic pain (discomfort above the pubic bone) or, in men, rectal pain, Normal body temperature (absence of fever), Possible haematuria (blood in urine),

In contrast, upper urinary tract infections (pyelonephritis) are more systemic and severe, often including: Generalized malaise (feeling systemically unwell), Nausea and vomiting, Rigors (uncontrollable shivering), Hypotension or even septic shock in severe cases, Loin pain and tenderness (discomfort in the upper back and flank areas), Fever (a key distinguishing feature from lower UTIs), Some patients may also exhibit symptoms of a lower UTI Understanding these differences is crucial for accurate diagnosis and appropriate treatment (Field, 2010).

The most important pathogen of urinary tract infections is *Escherichia coli*, followed by *Klebsiella pneumoniae, Staphylococcus saprophyticus, Enterococcus faecalis, group B streptococci, Pseudomonas aeruginosa, Candida spp, Staphylococcus aureusand, Proteus mirabilis* alr 2,701(including270). In hospitals, nursing homes, and communities around the world, *Klebsiella pneumoniae*—the most important human pathogen in the genus *Klebsiella*—causes a wide variety of infections, including those of the urinary tract, the abdomen, surgical sites, soft tissues, and bacteremia (Mo and Juthani, 2014). The typical flora of the stomach, skin, and mouth all include this encased Gram-negative bacterium (Li et al., 2014). The second most frequent microorganism causing UTIs in this patient population, according to research from Europe and Asia, was *Klebsiella pneumonia* after *Escherichia coli,* accounting for 5% to 55% (Krawczyk *et al.,* 2022) The production of extended-spectrum β-lactamases by multidrug-resistant (MDR) *Klebsiella pneumoniae* and other virulence factors determine the pathogenesis (Ahn *et al.,* 2017), which is growing issue on a worldwide scale is bacterial antimicrobial resistance. In both the general and hospital settings, urinary tract infections are among the most prevalent bacterial infections in people (Ullah *et al.,* 2009). Infectious microorganisms have different susceptibility to anti-microbial in different

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locations (Bashir *et al.*, 2017). Treatment of UTIs typically includes antibiotic therapy, urine culture, and antibiotic susceptibility testing (Ho *et al.*, 2019).

The most popular transmission mechanisms of bacterial resistance among gram-negative bacilli (chromosomal or plasmid mediated) are the development to β-lactamase, modification of penicillin binding proteins, outer membrane permeability, and mixture of unusual mechanisms (Lenchenko *et al.*, 2020).According to Yu et al. (2020), β-lactamase inhibitor substances such as clavulanate, sulbactam, or tazobactam can prevent pathogenic bacteria from producing β-lactamase enzymes, which is a common and important mechanism in antibiotic resistance. This includes all β-lactamases except cephamycins and carbapenems.

In addition, the severity of ESBL induced resistance *Klebsiella pneumoniae* isolates has been described as a major public health threat. The production of ESBL has increased the risk that antimicrobial medication failure has become unsuccessful, restricting the rapeutic choices, and triggering urinary tract infection (UTI), which remains the most prevalent bacterial infection in human populations,as well as other urinary tract infections (Walker, 2020) UTIs caused by *Klebsiella pneumoniae* happen when the pathogen pass in the urinary tract, or it can also happen by over growth of pathogenic bacteria resulted from the long time using of urinary catheter Likewise, the multidrug resisting bacteria frequently resulting from resistance plasmid that co-transferred by antibacterial agents such as amino glycosides, fluoroquinolones, tetracycline and chloramphenicol (Walker & Miller, 2020)) According to a study by Padmini et al., many β-lactam medicines, including third-generation cephalosporins, are unable to combat the *E. coli* and K. pneumoniae that cause ESBL (Zhang et al., 2020). In addition to β-lactamases, bacteria possess a range of additional enzymes that may break down antibiotics. These include aminoglycoside-modifying enzymes and chloramphenicol acetyl transferases, which render the antibiotics inactive prior to exerting their effects. One way antibiotics lose their effectiveness is when they alter the target site, making it impossible for them to attach (Livermor, 2007).

In Pakistan, UTIs are among the top ten most common infections encountered in outpatient and hospital settings. The situation is aggravated by the empirical use of antibiotics without laboratory diagnosis, lack of surveillance programs, and poor infection control measures. Regional studies from cities like Karachi, Lahore, and Islamabad have documented a concerning rise in antimicrobial resistance among uropathogens, particularly *Klebsiella species*. However, there is a noticeable lack of research specific to Sukkur, a prominent city in Sindh province, where climatic, socioeconomic, and healthcare-related factors may contribute uniquely to the epidemiology of UTIs. Given that resistance trends can vary significantly by region, there is a pressing need for localized data that can inform targeted treatment protocols.

The burden of UTIs in Sukkur is compounded by several risk factors, including poor hygiene practices, high rates of diabetes and other chronic illnesses, self-medication, and limited access to healthcare. The frequent and sometimes inappropriate use of antibiotics without prescriptions fosters an environment conducive to the emergence of resistant strains. Moreover, the use of antibiotics in agriculture and animal husbandry contributesto the overall antibiotic load in the community, indirectly promoting resistance in human pathogens. In such a setting, understanding the role of *Klebsiella species* in UTIs and their susceptibility patterns becomes critical for devising effective treatment and prevention strategies.

This study seeks to bridge that knowledge gap by providing detailed, context-specific data that can inform clinical and public health decision-making. The findings will not only benefit the local population by improving UTI management but also contribute to the national database on antimicrobial resistance. Given the urgency of the problem and the scarcity of localized data, this research is both relevant and necessary for effective healthcare delivery in Sukkur and beyond.

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1.2 Objectives

- To isolate UTI causing bacteria from urine sample of suspected patients of Sukkur
- To identify *Klebsiella species* through biochemical testing from isolated culture
- To determine antibiotic susceptibility assay.

1.3 Significance of Study

This suggested research has significant implications for our understanding of the prevalence and antibiotic susceptibility pattern of *Klebsiella* species in Sukkur's immune-compromised diabetic patients with UTIs. In short, this research is significant because it has the potential to improve patient care, assist antimicrobial stewardship initiatives, strengthen infection control procedures, and advance scientific understanding of infectious illnesses.

LITERATURE REVIEW

2.1 Urinary tract infections (UTIs)

Inflammation in the urinary tract may manifest in several parts of the body, including the urethra, bladder, kidneys, epididymis, and prostate gland. When an infection spreads to the bloodstream, it may cause complications such as sepsis, severe sepsis, or septic shock. Asymptomatic bacteriuria, simple UTI, complex UTI, and catheter-related UTI are the four clinical categories into which UTIs are divided for therapeutic purposes. The bacterial counts for clinically relevant diagnostic of UTI are below.

Asymptomatic Bacteriuria (ASB): No symptoms, but bacteria are present—≥100,000cfu/mL in two urine samples for women, one for men, or just 100cfu/mL if a catheter is in place.

Acute Cystitis/Pyelonephritis: For a simple bladder infection (>10 WBCs and ≥1,000 cfu/mL) or kidney infection (same WBCs but ≥10,000 cfu/mL).

Complicated UTI: More severe cases—≥100,000 cfu/mL for women, ≥10,000 for men or catheter samples.

Catheter-Related UTI: ≥1,000 cfu/mL with elevated WBCs (Grabe et al., 2015).

A symptomatic bacteriuria In the absence of any outward signs of infection, bacteriuria is defined as the presence of a single organism in urine at concentrations that are statistically significant (>105 cfu/mL for women and men or >102 cfu/mL for patients with an indwelling urethral catheter; see Table 1) According to Foster (2022), the overall incidence of ASB is 3.5% and tends to rise as people become older. Nicoll (2012) found that the ASB rate among independent older adults was 6% for males and 16% to 18% for women. According to Nicolle et al. (2006), the rates of ASB among senior women residing in nursing homes vary from 17% to 55%, whereas among elderly males the rates are 15% to 31%. According to Ipe et al. (2013), ASB is much more common among those residing in long-term institutions, with as many as 75% of women and 52% of men experiencing it. In addition to transplant and diabetic patients, who have an incidence rate of ASB that is two to four times greater than that of non-diabetic patients (%), pregnant women (1.9 to 15%) are another population that has considerable ASB (Ipe et al., 2013). (Foster, 2022)

2.1.1 Uncomplicated UTIs

In women in good health who do not have any systemic disorders that make them more susceptible to bacterial infections, a history of recent instrumentation, any underlying anatomical or neurological abnormalities of the urinary tract, or any other underlying condition, a simple UTI may develop in either the lower or upper urinary tract. Typically, a single bacterium, most often Escherichia coli, causes an infection. According to Czajkowsk et al. (2021), young women who are sexually active are the ones most likely to get simple UTIs, which often present as acute uncomplicated cystitis with colony counts of 103 cfu/mL or more. Infection may sometimes travel from the bladder to the kidneys or renal pelvis, causing simple pyelonephritis with a diagnostic cutoff of ≥104 cfu/mL (refer to Table 1) and accompanying symptoms. Upper urinary tract infections (UTIs) are characterized by a high temperature and discomfort in the upper back, rather than the more common symptoms seen in lower UTIs. Early research by Kass (Naboka et al.,

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2021) showed that mechanical compression of the growing uterus increases the incidence of pyelonephritis in pregnant women with asymptomatic bacteriuria (Denoble et al., 2022). Some of the consequences that may arise from pyelonephritis during pregnancy include bacteraemia, renal disease, hypertension, preterm labor, and low birth weight. The incidence of this condition varies from 0.5 to 9% during pregnancy (Sharma & Thapa, 2007). (Schnarr & Smaill, 2008).

Complicated UTIs (cUTIs)

In patients with a history of recurrent infection or instrumentation, or those who have anatomical, structural, or functional abnormalities caused by either internal or external sources, complicated UTIs may develop (Kim et l., 2021). In addition to or instead of the typical symptoms seen in lower and upper urinary tract infections, complicated UTIs may develop for unknown reasons. Although *E. coli* is the most common infection, other significant pathogens include non-fermenters (*Pseudomonas aeruginosa*) and Gram-positive *cocci* (e.g. *staphylococci* or *enterococci*). The incidence of virulence genes and the phenotypic expression of virulence factors are lower in *E. coli* isolates from patients with difficult urinary infections compared to those from simple illnesses (Josephs et al., 2021). Although both men and women may develop difficult UTIs at any age, men's UTIs are more likely to be accompanied with acute or chronic pyelonephritis, prostatitis, or perinephric and renal abscesses (Rando et al., 2022). With an average of 67 emergency admissions per 100,000 population every quarter, especially among senior patients, UTIs are a rising cause of hospitalization in the UK (NHS, 2014). With 35,676 cases reported in England in 2014–15, more than 60% of these infections originated in the urinary tract (PHE, 2016b), indicating a concerning trend in the rising frequency of *E. coli* bloodstream infections.

2.1.2 Catheter-related UTIs (CAUTIs)

People who have urinary catheters inserted into their urinary tracts are more likely to have catheter-associated infections (CA). In individuals who have an aurethral, suprapubic, or condom catheter—regardless of how recently it was removed—a microbiologically significant colony count of one bacterial species in a single urine specimen from the catheter is >103 cfu/mL (Isiaka, 2022). Hooton et al. (2010) found that 15% to 25% of hospitalized patients had urine catheterization, while Czwikla et al. (2024) found that a comparable percentage of nursing home residents have long-term indwelling catheters. The incidence rate of bacteriuria ranges from 3% to 10% every cathetered day, and it may happen in individuals with or without clinical symptoms and indications that are related to urine infections (Lo et al., 2014). Although patients' own intestinal flora is the most common source of catheter-associated UTIs, nosocomialpathogens (such as *Pseudomonas species*) pose a greater threat with long-term catheterization. The major risk factor of developing catheter related bacteriuria is the duration of catheterisation. Short-term episodes (less than 7 days) mostly are asymptomatic and often caused by a single organism, while along term catheterization (more than 30 days) increases the risk of polymicrobial infection (Tenke *et al.*, 2008). Several other factors can increase the likelihood of CA-bacteriuria, such as not taking systemic antimicrobial therapy, being female, having a catheter inserted outside of the operating room, not following catheter care instructions, being older, having diabetes, having a comorbidity, or having a fatal underlying illness (Chotiprasitsakul et al., 2021). Even though *Escherichia coli* is the most common pathogen, other possible infections may be caused by *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Staphylococcus epidermidis, Enterococcus species*., or *Candida* spp. (Palusiak, 2022) To adhere to a host cell or catheter, bacterial adhesins first need to find specific receptors on their surfaces. Bacteria adhere to the inside or outside of a catheter and proliferate by first creating micro colonies, and then a complete biofilm (Pugazhendhi et al., 2022) While cell dormancy protects bacteria against antibiotics, exopolysaccharides protect bacteria inside bio-

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films from mechanical flushing by urine flow and other host defenses (Tenke et al., 2008). It is not suggested to treat CA UTIs that do not cause any symptoms. Symptomatic infections are the only ones that need antibiotic therapy.

2.1.3 Urosepsis

Urosepsis, also known as septicaemia syndrome, is characterized by bloodstream overspill and usually develops after an infection in the male genital organs (prostate) or the upper urogenital tract (Guliciuc et al., 2021). Possible symptoms include hypotension, hypo-perfusion, or malfunction in many organs. A severe UTI obtained in the community or via a healthcare facility might progress to sepsis. On the other hand, Richards et al. found that UTIs accounted for 23% of all instances of hospital-acquired sepsis in the 1990s, with catheterized patients being the most often affected (Richards et al., 2000). While infections of the lungs and abdomen are the most common causes of severe sepsis, past infections of the urinary tract are responsible for around 5% of these patients (Hotchkiss & Karl, 2003). One research assessed the frequency of nosocomial urosepsis in urology patients at 12%, which is rather high. Around a quarter of all cases of sepsis are urosepsis (Wagenlehner et al., 2013).

Urosepsis has a significant death rate, with figures ranging from 20 to 42 percent in individuals at high risk. People who are already at a high risk due to factors such as old age, diabetes, immune-suppression (post-transplant), tumor treatment with chemotherapy or corticosteroids, or acquired immune deficiency syndrome might be considered high-risk. The commonest risk factor associated with developing urosepsis is structuralor functional genito-urinary abnormalities. Instrumentation (such as indwelling urethral catheters, nephrostomy tubes, or tumors), impaired voiding (such as neurogenic bladder or cystocele), metabolic abnormalities (such as diabetes), and immunodeficiency are among these (Christopeit et al., 2021).

The vast majority of urosepsis cases are caused by gram-negative bacilli. While Gram-positive bacteria are implicated less often, these bacteria and fungi include *E. coli* (50%), *Proteus* spp.(15%), *Enterobacter* spp.(15%), *Klebsiella* spp.(15%), and *P.aeruginosa* (5%). (Wagenlehner et al., 2007b). In sepsis in general, an 80% survival rate was linked to effective antimicrobial treatment during the first hour of recorded hypotension (Kumar et al., 2006). On average, survival rates dropped by 8% for every hour that elapsed in the six hours that followed.

2.2 Epidemiology and Burden of UTI

Over 150 million individuals are affected by UTIs each year, making it the second most frequent bacterial ailment (Flores-Mireles et al., 2015, Liu et al., 2021). We likely have a lower-than-real number of instances of UTIs since these illnesses are not reported in many nations. (Zeng et al., 2022; Foxman, 2003). In the course of their life times, between 60% and 50% of adult females will get UTIs (Medina and Castillo- Pino, 2019). Research shows that young women have almost half as many UTI episodes per person each year, with 27% of those cases being recurring, making it an even more common occurrence for this demographic. In a 1996 study, Rosen et al.The most prevalent kind of bacterium in humans, uropathogenic *Escherichia coli* (UPEC), is responsible for 80 to 90% of UTIs. In a review of the literature, it was shown that 81% of the UPEC strains found in patients belonged to the B2 and D phylogroups (Terlizzi et al., 2017; Flores-Mireles et al., 2015; Foxman, 2014). When Yun et al. Among these taxonomic groups, you're more likely to find the genes that encode the ferric Yersinia bactin uptake receptor (fyuA), the pyelonephritis-associated pili (pap), and the fimbrial-like protein (yfcV). These pathogenic factors influence cellular adhesion as well as iron uptake (Lara et al., 2017). According to Lara et al. (2017), additional virulence genes such sfa, CNF-1, and pic were found only in the B2 phylogroup. This lends credence to the findings of Dadi et al., who previously discovered a strong association between the B2 group and the afor ementioned virulence genes. Although it is somewhat lower than the data reported before, 57.5% of UPEC isolates belonged to the B2 and D phylo groups (Dadi et al., 2020).

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The increasing prevalence of germs that are resistant to many drugs poses a significant challenge to the treatment of recurrent UTIs. Severe instances of extraintestinal pathogenic *E. coli* (ExPEC) infections, such as sepsis and meningitis, and patients undergoing kidney transplants continue to pose a significant risk of mortality. Citations: (Halaji et al., 2020; Litwin et al., 2005).

Therefore, there are significant financial ramifications to effectively treating UTIs aswell. Annually, the United States spends $3.5 billion on medical consultations for UTIs, which account for 0.9-6% of all outpatient visits and lead to missed workdays. (Zeng *et al.*, 2022).

Etiologic Agents of UTI

A disproportionate amount of UTIs are caused by members of the *Enterobacteriaceae* family, which includes *Pseudomonas aeruginosa, Klebsiella, Proteus, E. coli* , and others. These bacteria are common in hospital settings(Manikandan et al., 2011; Onuoha and Fatokun, 2014).

Infections caused by *Klebsiella pneumoniae* may present in several ways. Some of these ways include the urinary system, the respiratory tract, wounds, and nosocomial infections. Nosocomial infections, such as UTIs, are common among members of the family *Enterobacteriaceae,* especially *Klebsiella spp*. and *E. coli* , which are known for their resistance to antibiotics(Alemu*etal.*,2012).The fact that *Klebsiella pneumoniae* may develop resistance to penicillins and cephalosporins if it gets plasmids that code for the production of extended-spectrum β-lactamase (ESBL) compounds the complexity of managing these infections (Alemu et al., 2012).However, persistent inflammation, bacteremia, acute pyelonephritis, and other serious UTI complications may be caused by *Proteus mirabilis* isolates. Hospital personnel are at risk of nosocomial transmission due to contaminated equipment, which increases the frequency of P. mirabilis infections in both inpatients and outpatients (Muder *et al.*,2005).

It is now critical to prevent the spread of *P. mirabilis* strains identified from community illnesses and hospital settings because to the increasing antibiotic resistance. Bacterial resistance to β-lactam antibiotics is prevalent and might become more severe if pharmaceutical consumption persists. Doing microbial culture, doing susceptibility testing, and ensuring that antibiotics are provided appropriately are important steps to decrease this risk [Maczynska and Kalemba, 2007]

It was previously believed that *Staphylococci* that do not generate coagulase were innocuous contaminants. However, they have only just been recognized as a major cause of urinary tract infections. Among sexually active young women, the Gram-positive bacteria *Staphylococcus saprophyticus* has been associated with UTIs (Sarathbaby et al., 2013). Patients hospitalized with UTIs have been associated with *Staphylococcus epidermidis* and *Enterococcus* species. These bacteria may cause endocarditis in hearts that have either natural or artificial valves, and they're resistant to a lot of medications (Sarathbaby et al., 2013).

Since coagulase-negative staphylococci have demonstrated resistance to numerous antibiotics, including penicillin, there is a dearth of treatment options for these bacteria. Because vancomycin is the last resort, the treatment options for resistant uropathogens are already limited (Nicolle et al., 2013; Sarathbaby et al., 2013). Staphylococcus aureus is the leading cause of urinary tract infections (UTIs) in hospitalized or catheterized individuals.

This increases the likelihood of colonization of the urinary tract by *S. aureus*, which may lead to *Staphylococcal* bacteremia (Muder et al., 2006; Ikeagwu et al., 2008).Even though the majority of *Staphylococcus aureus* UTIs don't show any symptoms, bacteriuria is a common side effect of long-term urinary catheterization. It is possible to isolate *Staphylococcus aureus* from the urine of individuals with or without symptoms of a urinary tract infection since it is a natural component of the skin flora (Ikeagwu *et al.*, 2008). S. aureus is a secondary urinary tract invader that may causes epticemiainaround 10% of cases (Sarathbabu*etal.*,2013).Despite accounting for less than 3% of positive urine

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cultures, bacteriuria produced by S. aureus is associated with a higher risk of developing bacteremia compared to bacteriuria caused by other pathogens. Asymptomatic bacteriuria and UTIs during pregnancy are also often caused by S. aureus in many developing countries. Because of the high likelihood of complications and the fact that methicillin resistance is as high as 86%, researchers have been driven to investigate the cause of S. aureus UTIs.

Urinary catheterization is the leading cause of severe MRSA UTIs. Comparatively, MRSA-induced CAUTIs are distinct from those caused by Enterococcus.Microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) aid bacterial adherence to fibrinogen-coated catheters.Critically, systemic infection requires clumping factor A (ClfA), whereas MRSA CAUTI development involves clumping factor B (ClfB). Klein and Hultgren (2020) found that MRSA exacerbates catheter-mediated inflammation in a mouse model of catheter-associated urinary tract infection (CAUTI) by raising the production of tumor necrosis factor (TNF), interleukin (IL)-1α, interleukin-1β, interleukin-6, and interleukin (IL)-1α. This leads to an increase in fibrinogen release and the persistence of germs in the bladder. One could have a UTI from a viral illness or a fungal infection. Nosocomial UTIs are caused by a variety of fungal infections, the second most common of which being Candida. A potentially life-threatening condition, it has the potential to metastasize (Samuel et al., 2012). Fungal infections are more common among patients with compromised immune systems, those who use invasive medical devices such as intravenous lines and catheters, and those who take long courses of antibiotics. Fungal infections, including Candida, are more common in children with UTIs, and these infections are often associated with UTI instruments (Mehta et al., 2013).

The incidence of Candida-related UTIs (Candiduria) rises steadily with increasing duration of hospital stay. In order to treat candiduria, one must discontinue the use of antibiotics, start taking antifungal medicine, and change or replace indwelling catheters. In addition to fungi, urinary tract infections may be caused by a variety of viruses, such as herpes simplex virus and adenovirus (Samuel et al., 2012).

2.3 *Enterobacteriaceae*

The *Enterobacteriaceae* family includes several Gram-negative bacteria that are prevalent in the gut flora of both humans and other animals. Many different types of bacteria belong to this heterologous family, including Escherichia coli, *Shigella*, *Salmonella*, *Enterobacter*, *Klebsiella*, *Proteus*, and countless more.The many illnesses that may be caused by members of this family include vaginal infections, UTIs, respiratory tract infections, nosocomial infections, and wound infections in both humans and animals.They might be aerobic or facultative anaerobe, have a rod form, and are Gram-negative. Optimal development temperature for the majority of members is 37 °C, and they are non-spore forming, flagellated, lactose fermenters, oxidase test negative, and indole test positive.A variety of virulence factors, including poisons, enzymes, capsules, flagella, and more, are present in these microbes (Riedel *et al.*, 2019; Oliveira *et al.*, 2017).

2.3.1 *Klebsiella pneumoniae*

K. pneumoniae is a rod-shaped gram-negative bacterium. As a pathogen that can be acquired both in the community and in a hospital, it is the most commonly encountered by doctors all over the world. In the *Klebsiella* genus, it belongs to the *Enterobacteriaceae* family. A German pathologist, Edwin Klebs, was honored with the honor of naming the genus after him when it was first described in 1885. The species' ability to cause life-threatening pneumonia gave rise to the name (ALATROSHI, 2022).

Community-acquired pneumocystis *pneumoniae*, or Friedländer's pneumonia in honor of Carl Friedländer, is another name for this pathogen. The lactose-fermenting, non-motile nature of other motile *Enterobacteriaceae* species and gram-negative rods, K.pneumoniaeis capable of distinguishing itself from both. MacConkeyagar

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displays large, round, mucoid pink colonies of this bacterium. (Kibuchi, 2021). Urea production distinguishes the *Klebsiella pneumoniae* strain from other *Klebsiella species*.

Another problem is to differentiate it from other counterparts. Decarboxylase assay (lysine + arginine - ornithine -), no indole production, lack of motility, capsule production, and lactose fermentation are additional important markers. *K. pneumoniae,* Raoultella planticola, and Raoultella terrigena can all be misidentified in a clinical microbiology laboratory using traditional biochemical assays (VENKATALAXMI *et al.*, 2023).

2.3.2 Pathogenesis and virulence factors

People with the disease with underlying medical issues are more likely to become infected by *K. pneumoniae*, a hospital-acquired type of bacteria. Virulence factors of *Klebsiella* can vary from one infection site to the next because the host's defense mechanisms are unique to each. For example, strains of *K.pneumoniae* that is responsible for UTIs have a different virulence profile than those that cause pneumonia in the lungs (Turton *et al.*, 2010).

It is the virulence characteristics of *K.pneumoniae*s trains that aid their readiness to infect and thrive in their hosts.Seven main microbial aspects were recorded by Podschunand

Ullmann (1998) as being part of the virulence factors of *K. pneumoniae*: capsule (to inhibit phagocytosis), lipopolysaccharide (host serum complement factors to be avoided), fimbriae (for adhesion), siderophores (to acquire iron), bacteriocin, serum resistance (extended spectrum cephalosporins to be protected from) (Pinsky *et al.*, 2009) Fig mentioned below 2.1

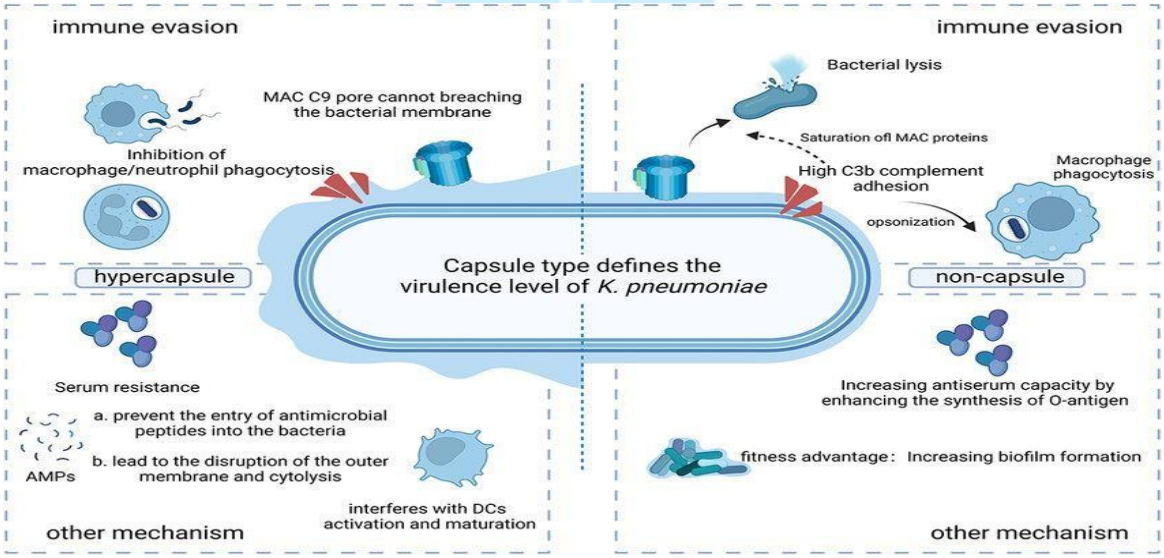

Fig2.1 Pathogenesis of *K.pneumonia*

Major virulence aspect of *K.pneumoniae* is the polysaccharide capsule, which is produced by the vast majority of these strains. It prevents polymorphonuclear cells from phagocytosing bacteria by inhibiting polymorphonuclear cell phagocytosis (Opoku *et al.*, 2019) . More than seventy-eight different capsular serotypes (known as the K-serotype) have been discovered so far. Variations in pathogenicity appear to exist among the various K-serotypes. When it comes to pathogenicity, isolates K1 and K2 posed a greater threat to human health than those that were not (ALATROSHI,2022).Gram-negative bacteria cannot function properly without lipopolysaccharides (LPSs). Inflammation canbe induced by binding to the Toll-like receptor 4 and increasing various pro-inflammatory mediators (cytokines, chemokines, and receptors

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of the major histocompatibility complex) (Du *et al.*, 2022)

If you find *K. pneumoniae*, it is likely that you will come across the adhesion factors type 1 and type 3. *Enterobacteraceae* species with Type 1 infundibulum are encrypted by the fim gene cluster, while enteric bacteria organisms with mrk gene cluster encoded type 3 fimbria. During an infection of the urinary tract, the expression of type 1 fimbria is activated, but it is inactive in the gastrointestinal tract and when the lungs are infected.Collagen type V and pulmonary epithel and endothel cell adhesion are all made easier by type 3 fimbria in biofilms (Riwu *et al.*, 2022)

Ferric ionis essential for bacteria because iron is scarce in the bloodstream and host tissue. A variety of iron acquisition systems have been linked to the pathophysiology of *K. pneumoniae* infections. *K. pneumoniae* isolates are most commonly found to contain enterobactin as a siderophore. Other siderophores found in *K. pneumoniae* include salmochelin (an enterobactin glycosylated derivative), yersiniabactin (which shows resistance binding by lipocalcin-2), and aerobactin (ALATROSHI, 2022).

2.4 Pathogenesis of UTI

When it comes to starting the different phases of UTI pathogenesis, adherence is very critical. It is common for uropathogenic microorganisms found in the digestive system to infect the periurethral region, marking the beginning of the UTI process. Afterwards, the infection spreads to the bladder via the urethra, where it uses structures like pili and flagella to move. Uropathogen colonization or elimination, once within the bladder, is decided by the result of complex interactions between the host and the pathogen (figure 2.2).

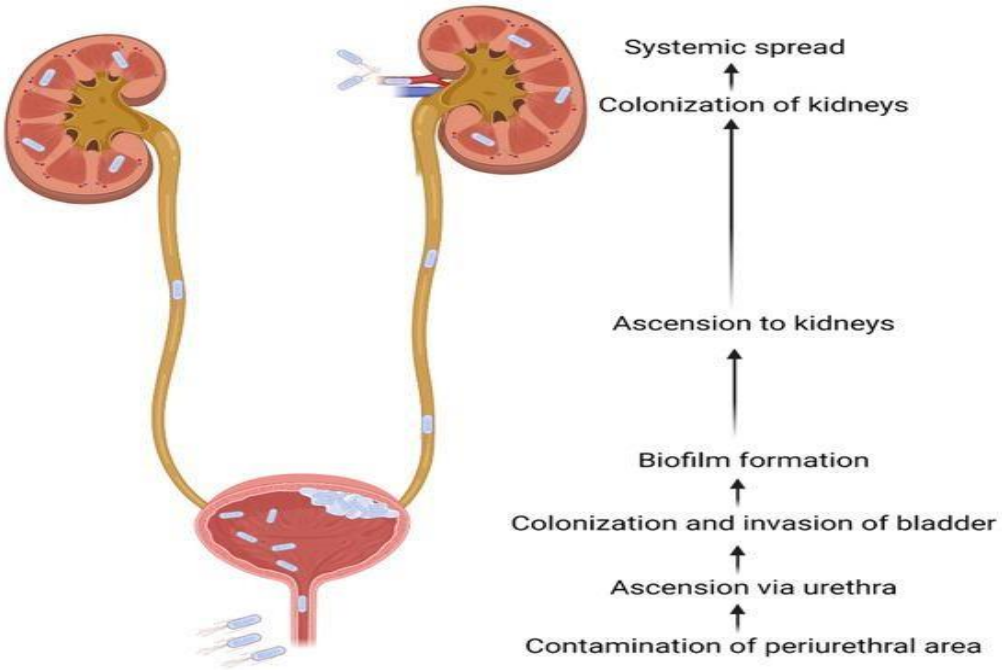

Figure2.2Pathogenesis of Urinary Tract Infection

Certain bacterial adhesins may connect to receptors on the uroepithelium (bladder epithelial cells), enabling the bacteria to infiltrate the cells. Bacteria that cause urinary tract infections (UTIs), such as Uropathogenic Escherichia coli (UPEC), have evolved to thrive in the bladder. To do this, it must first penetrate the bladder epithelium, produce siderophores to

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draw iron out of the host cells, and then release toxins and proteases to extract nutrients from the cells. Uropathogens may potentially replicate and elude the host's immune system prior to reaching the kidneys. They use adhesins or pili to cling to the kidney epithelium, and once inside, they unleash poisons that harm the tissues around them. When uropathogens penetrate the tubular epithelial barrier and reach the circulation, it may lead to bacteremia.

Uropathogens such as *Staphylococcus saprophyticus*, *Klebsiella pneumoniae*, and Uropathogenic *Escherichia coli* (UPEC) may cause uncomplicated UTIs by attaching directly to the bladder epithelium. Multiple cell types make up the bladder lining; they include basal cells, intermediate cells, superficial facet cells, and umbrella cells (Khandelwal et al., 2009). According to Khandelwal et al. (2009), UPEC and K. pneumoniae bind exclusively to uroplakins, which are the primary protein components of the apical membrane of umbrella cells.Uroplakins provide a protective barrier around the bladder tissue, keeping harmful substances in urine at bay (Lee, 2011). Eto et al. (2007) revealed that uroplakins and α3β1 integrins on uroepithelial cell surfaces might act as UPEC receptors.

Symptoms of a more complicated UTI could include bacterial adhesion to a urinary catheter, kidney stone, bladder stone, or blockage in the urinary system. One such bacterium is UPEC, which may induce a number of other issues associated with UTIs. However, other bacteria, including *Proteus mirabilis*, *Pseudomonas aeruginosa*, and *Enterococcus* spp., are the most common culprits behind complicated UTIs. The ability of uropathogens to colonize and survive over time is dependent on the biofilms they produce (Jacobsen and Shirtliff, 2011, Niveditha et al. 2012).

The pili of some uropathogens enable them to attach to surfaces both within and outside the host, penetrate host tissues, and promote bacterial interactions leading to biofilm development, which in turn causes UTIs(Wurpel *et al.*, 2013, Kline et al., 2010, Waksman and Hultgren, 2009, Vallet *et al.*, 2001). Many Gram-negative pathogenic bacteria, including *Escherichia coli*, *Klebsiella* spp., *Proteus* spp., *Pseudomonas* spp., *Haemophilus* spp., *Salmonella* spp., and *Yersinia* spp., express a family of adhesive fibers called chaperone-usher pathway (CUP) pili (Waksman and Hultgren, 2009, Wurpel et al., 2013). Kline et al. (2010) and Waksman and Hultgren (2009) describe a molecular process that assembles CUP pili: the chaperone-usher. The pili consist of pilin subunits that resemble immunoglobulins in their partial folds,but lack the customary seventh β-strand at the end (.Chorell et al. 2014, Piatek et al. 2013)

A specific periplasmic chaperone ensures the correct folding and stabilityof the immunoglobulin subunits bydonating a β-strand to finish their fold in a process known as donor-strand complementation. This complex forms with each subunit. The chaperone-subunit complex will next aim for the usher assembly protein, which is located in the outer membrane. Assisting in the sequential assembly of pili on the cell surface, the usher selectively distinguishes chaperone-subunit complexes via a mechanism called donor-strand exchange. The subunit completes its folding process, as stated by Geibel et al. (2013), when the incoming subunit's amino-terminal extension replaces the chaperone's provided β-strand during donor-strand exchange. Our expanding understanding of molecular biology is the key to creating antivirulence drugs. We know, for instance, that domains formed when proteins fold serve as the fundamental units for more complex supramolecular structures. Then, in order to put these structures together, a macromolecular machine with an outer membrane known as an usher coordinates the movement of individual subunits across a biological membrane. These compounds target the assembly or function of CUP pili in an effort to impair virulence factors. So, it's promising that they may be able to demonstrate activity against many Gram-negative bacteria.

2.5 Clinical management of *K.pneumoniae*

People with a compromised immune system are more likely to contract *K. pneumoniae*, the second

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most common cause of UTIs. For a variety of reasons, *K. pneumoniae* infection sare the most common in hospitalized patients. Nosocomial infections caused by

K. pneumonia include urinary tract infections (UTIs), pneumonia, surgical site infections, and bloodstream infections (Magill, 2014). A primary liver abscess or urinary tract infection in otherwise healthy members of the community are examples of both as part of an epidemic and as an isolated case of *K. pneumonia* infected infection (Hu *et al.,* 2021). To colonize, *K.pneumoniae* makes use of bio-films and anadhes in known as type1 fimbrial, which has aunique binding specificity to cause urinary tract infections (Timm *et al.,* 2025).

Carbapenem-resistant *K. pneumonia* is difficult to treat because it produces carbapenems that are resistant to ampicillin. Aminoglycoside and cephalosporin antibiotics are commonly used to treat it. Based on the patient's health, medical history, and the severity of the ailment, antibiotics can be prescribed (Totsika, 2012). Even though the beta- lactamases are inhibited to different degrees, the aminoglycosides and the cephalosporins are still effective against the bacteria. In spite of the fact that colistin can be used to treat infections caused by multidrug-resistant Gram-negative pathogens in intensive careunits,

*K.pneumonia*has been reported tobe resistant to colistin (Aslan, andAkova, 2022)

2.6 Typing of *K. pneumonia*

A range of measures can be used to distinguish *Klebsiella* spp. strains, including physiochemical and immunogenic tests, phages vulnerability and production typing, bacteriocin vulnerability and molecular and production typing methods (Anbazhagan *et al.,* 2010).

2.6.1 Phenotypic typing

HMV, bifido bacteria, and biotyping are all examples of phenotypictyping.It is well- known that the sticky polysaccharide net work of the capsuleisa phenotypic pattern of higher viscosity in *K. pneumonia* K1 and K2 sero-typing (Hyper muco-viscostiy-phenotype) (Lin *et al.,* 2011).

2.6.2 Molecular typing

DNA and protein-based molecular typing methods were discovered by Rebecca Lancefield after discovering Sero-typing. The differences in the genomic DNA sequences and structures were used to distinguish strains (Mirzaie and Ranjbar, 2021). *Klebsiella* strain outbreaks have been studied using a variety of molecular typing methods in recent years, and these methods have allowed researchers to evaluate strains among periods of different time and geographical locations (Kumar, S., Anwer and Azzi, 2023).

2.6.3 Using polymerase chain reaction (PCR) for diagnosis of *K. pneumonie*

Bacteria and other pathogens can be reliably identified using PCR ,which mimics the natural process of DNA replication (Songca, 2022) In order to provide objective evidence; this testing method is capable of amplifying a specific fragment million times. Because of its ability to amplify small amounts of pathogens, PCR can also improve detection in low quantity samples (SridapanandKrajaejun,2022).Essentially, it is a three-stepprocess that is repeated a predetermined number of times. Following the steps of denaturation, annealing and extension, each PCR cycle is completed (Sridapanand Krajaejun, 2022).

The ability to quickly and effectively identify *K. pneumoniae* is critical (Jasim,2012). A variety of virulence-associated genes and their biochemical characteristics were tested on the isolates by PCR. This gene has a defect in cps biosynthesis, which explains the importance of hyper muco-viscosity in this case (Chuang et al., 2006). Iron up take regulates the expression of cps synthesis genes (rcsA, rmpA, and rmpA2) via the fur gene (ferric uptake regulator), which Liu and colleagues (2008) found to be critical. This gene affects the expression of the fur region, which in turn affects the cps synthesis regulator genes.

2.7 Antibiotic resistance mechanisms

It is coming increasingly easy for bacteria to resistant antibiotics used to treat illnesses like

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urinary tract infections (UTI). In addition to reducing unnecessary antibiotic use, the discovery of these resistance mechanisms is essential for the development of more effective treatment methods. An increase in antibiotic use around the world is also due to an increase in bacteria becoming resistant to antibiotics. As resistance to multiple antibiotics increases, treating patients becomes more difficult. Instead of using narrow-spectrum antibiotics, we generally used broad-spectrum antibiotics. Inside the battle against bacteria, numerous procedures have been found, including inactivating the antibiotic, altering the antibiotic or its aim, and lowering the density of antibacterial meds. Most commonly, *K. pneumoniae's* resistance tolactam is due to the bacteria's ability to produce extended -lactam enzymes. Antimicrobial agents with broad spectrum – lactamases (Seifu, 2018).

2.7.1 β-lactamresistance

Bacteria, including *K. pneumoniae*, use the ability to produce—lactamases as the most common method of resistance to antibiotics. -lactam antimicrobials including such penicillins, cephalosporins, carbapenems, and monobactams are all resistant because of this property. Bacteriacan be treated with these antibiotics because they all have the same 4-atom structure: they are called beta lactamase (-lactam) ring antibiotics. Bacterial- actamases are encoded by plasmids, which can break down - lactam antibiotics. Enzymes differ significantly in protein structure and kinetics.Bacterial resistance has been reported to be gained via plasmid transfer or horizontal gene transfer using encoding by plasmids as one of the most common mechanisms (Ballén *et al.*, 2021)

Ambler (molecular) classification divides them into four classes (A through D), while Bush-Jacoby (functional) divides them into two classes (A and B). Ambler classification relies heavily on the amino-acid sequence as its primary criterion. In the active center of metallo-lactamases of class B, a divalent zinc ion is present, whereas in the active sites of classes A, C, and D, the serine group is present. In the Bush- Jacoby classification, - lactamases are grouped into three main groupings (groups 1, 2, and 3) based on their substrate specificity and sensitivity to inhibitors.

A member of the clavulanic acid-resistant first-generation carbapenems is hydrolyzed preferentially by class C-lactamases, which are difficult to impede. Cephalosporin- degrading Clactamases are found in group 1, which includes enzymes that are resistant to clavulanic acid inhibition. Class A and class D serine—lactamases are included in group 2, which exhibits a wide range of diversity. Members of group 3 include carbapenem- degrading metallo-lactamases (Veloo et al., 2022). Additionally, *K. pneumonia* has the ability to produce class C -lactamases (AmpC enzymes) and carbapenemases in addition to the ESBL-producing ability, which is the most important acquired -lactam resistance mechanism of *K. pneumonia*. All three mechanisms rely on enzymes to some degree or another. Multidrug resistance was caused by the action of these enzymes on lactam compounds. It is also common for multidrug-resistant bacteria to have additional resistance mechanisms to non-lactam antibiotics, further limiting treatment options. *K. pneumoniae* may be less susceptible to third-generation cephalosporins if it produces ESBL and/or AmpC simultaneously (Zeynali *et al.*, 2025)

2.7.2 Extended-spectrum-lactamases (ESBLs)

The first evidence of *K. pneumoniae* resistance to present broad-spectrum cephalosporins was in 1983. There is a steady rise in the amount of ESBL-producing isolates all over the world due to the proliferation of viable clones and mobile genetic elements (plasmids, transposons, etc.). While structurally diverse, ESBL enzymes are notable for spreading rapidly and conferring resistance to all three generations of cephalosporins as well as penicillins. They are also resistant to aztreonam, while beta-lactamase inhibitors like clavulanic acid primarily inhibit them.There are many different types of ESBL-producing illnesses, from simple urinary tract infections to life-threatening sepsis (Khater, 2014)The capacity to hydrolyze both narrow and elongated cephalosporins without affecting

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carbapenemsorcephamycins is widely documented,but not generally recognized. Other types of antibiotics, such as fluoroquinolones and glycopeptides, are also susceptible to resistance.Plasmodium sulbactam, tazobactam, and clavulanic acid have also been shown to reduce the activity of ESBLs, which are class A lactams (Elshafiee *et al.,* 2022).

Because of the proliferation of -lactamase point mutations, ESBL diversity has arisen. A total of over 200 ESBLs were identified until now in the literature. The progenitor enzyme is used to categorize them into several groups. CTX-M, TEM, and SHV are three of the more frequently encountered subgroups. Resistant strains of bacteria can develop resistance to the oxyimino cephalosporins that are hydrolyzed by the TEM and SHV enzymes. 40 members make up CTX-M, which can be divided into five subgroups. CTX- M -lactamases have been able to evolve due to their ability to migrate to mobile DNA. A point mutation's exact position and the group it belongs to determine how specific an ESBL substrate is (Ahmadi *et al.,* 2022).

Material and Methodology

Clean catch midstream urine samples were collected from patients with urinary infections in Sukkur. These samples were then transported to the Institute of Microbiology at Shah Abdul Latif University Khairpur. The first step was to isolate and preserve pure colonies of bacterial pathogens. In the second step, *Klebsiella species* were identified and isolates were recovered. Finally, antimicrobial sensitivity testing (AST) was conducted at the same facility.

Statistical studies and interpretations were also carried out utilizing several relevant programs, including Microsoft Excel 2016, the statistical application "Statistix" (Version 8.1), and others. Logistic regression and chi-square were among the statistical tests used to examine the relationships between the variables and the prevalence rates.

3.1 Participant's selection criteria

Patients at the Sindh Institute of Urology and Transplant (SIUT) in Sukkur as well as private clinics in the Sukkur region provided urine samples. The research comprised specimens from 150 patients, and all participants were required to fill out a written permission form in its entirety.

A total of 98 males and 52 females made up the 150 participants. While as per age wis: 26patients were 0-20years, 51were21-40years, 48 were 41-60 years, and 25 were 61-80 years age.

3.1.1 Inclusion criteria

- Patients with either age or eithers exhaving suspected to UTI.
- Exclusively for patients residing in the Sukkur Region.
- For the last three days, you have not taken any antibiotics.
- Obligated to participate in the study

3.1.2 Exclusion criteria

- Patients unsuspected to UTI.
- Kids hailing from places other than the Sukkur area
- Antibiotics used during the last three days.
- Not willing to be involved in the current study.

3.1.3 Sample calculation

If you want to know how often UTIs caused by Proteus spp. are, you may estimate the number of samples required using this equation:

Z1-a/22p(1-p)/d2

Where, P=11.33 %(11.33%of UTI with small changes (Akollo, 2024) Z1-a2= 1.96 (95% confidence level)

d=0.05(5%errorrate)

Therefore, Sample Size=1.962x11.33(1-11.33)/0.052

Sample size=150

For this study, we need 150 urine samples.

3.1.4 Materials

Following Supplies:

Tubes, Cotton swab, Wire loop, 0.5McFarland, Distilled water, Antibiotics, syringes

Equipment: Incubator, Autoclave

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Media:"I" is for indole test;"M" is for methyl red test;"V" is for Voges- Proskauer test, and "C" is for citrate test(IMVIC),Triple Sugar Iron (TSI), Mueller-Hinton Agar (MHA), Cystine Lactose Electrolyte Deficient (CLED), Urea. **Reagents:** Kovac's reagent

3.1.5 Media Preparation

PROCEDURE

Quantify the necessary amount of Mueller Hinton Agar (MHA), CLED, IMVIC, Triple Sugar Iron Agar (TSI), and Urea.

General Precautions:

1. Ensure all media contain ensure labeled properly.
2. Do not tighten caps completely to prevent explosion due to pressure buildup.
3. Use autoclave indicator tape to confirm successful sterilization.
4. Ensure media contain ersare not overfilled (no more than 2/3full) to avoid spillage.

Step-by-Step Autoclaving Procedure:

➢ **Preparation of Media:**

Prepare the following media as per manufacturer'sinstructions: CLED Agar, Mueller Hinton Agar (MHA) IMViC Media: Indole, broth MethylRed broth, Voges-Proskauer broth Simmon's Citrate, agar Triple Sugar Iron (TSI) Agar Urea Broth or Urease Slants

Dispense into appropriate containers (e.g.,screw-capped bottles for agar; culture tubes for biochemical media).

➢ **Loading the Autoclave:**

1. Place the prepared media in autoclavable baskets.
2. Loosen capsol bottles and tubes slightly to allow pressure equalization.
3. Do not stack containers too tightly; ensures team can circulatefreely.

➢ **Autoclave Settings:**

- Set autoclave to:
  - **Temperature:**121°C
  - **Pressure:**15psi (poundspersquare inch)
  - **Time:**15–20 minutes (time may vary slightly depending on volume)

Note: For high-volume media (e.g.,>500mL per bottle), extend the time to **25–30 minutes**.

➢ **Post-Sterilization Handling:**

1. Allow pressure to return to normal be for opening the autoclave door.
2. Wait an additional 10–15 minutes be for removing media to avoid burns.
3. Use heat-resistant gloves to remove containers.
4. Immediately tighten caps once containers have cooled slightly but are still warm.
5. Allow media to cool completely room temperature or our in to sterile Petri dishes if required (e.g., for CLED or MHA agar plates).

➢ **Quality Check:**

1. Check autoclave tape/indicators trip for color change to confirm successful sterilization.

Inspect media for contamination or abnormal appearance (e.g., color change, precipitation).

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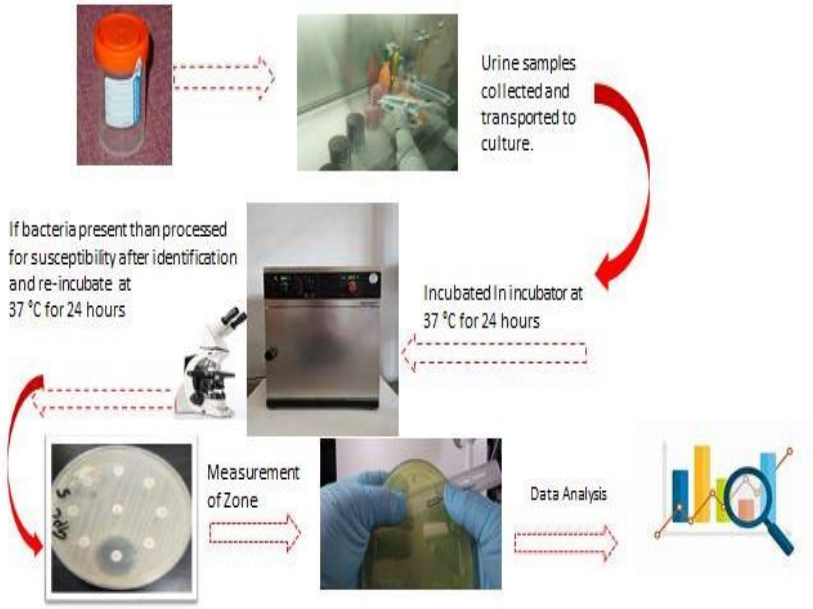
Methodology Overview:



Sample Collection and transportation

Urine samples were obtained from patients of both sexes who were suspected of having a UTI at the Sindh Institute of Urology and Transplant (SIUT) in Sukkur as well as from private clinics in the Sukkur Region. Midstream clean catch samples were collected in sterile containers to get the urine samples. Patients were told to empty their bladders into a container containing boric acid before the midstream urine sample was collected, and the volume should be more than 5 ml. Aseptic urine samples were collected from patients who underwent catheterization using the catheter port. The samples were immediately transferred for isolation and preservation to the Institute of Microbiology, Shah Abdul Latif University Khairpur following collection to ensure the accuracy of the results. Prior to shipment for further processing, the samples were kept at 2-8°C, as seen in figure 01.

Figure3.1 Collecting urine samples

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3.1.6 Isolation of *Klebsiella species*

1. Let the medium come to room temperature.
2. Petry plates were labeled with a distinct identification code.
3. As seen in figure 3.2, the samples were applied to the surface of CLED medium using sterilized urinary wire loops.
4. The plates were placed in an incubator set at 37°C for duration of 24 hours.

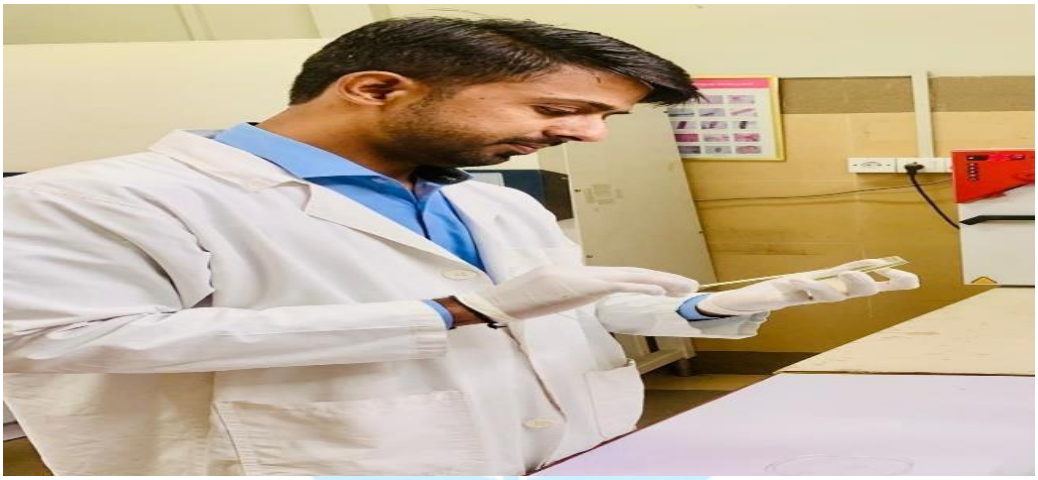

Figure3.2 Urine sample isolation on CLED medium

3.1.7 Cultures showing no growth:

1. The culture plates that did not show any development were re-incubated after the first 24-hour findings.
2. Confirmed as "No growth" after reexamining all 48-hour culture plates in the absence of development.

3.1.8 Cultures with growth:

Learn the colony count and morpho-type appearance in the culture.

Figure 4 shows the results of the microscopy analysis that used gram stain to identify colonies that may be harmful.

Figure3.3 Gram staining

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Figure 3.4 Gram staining under the microscope

3.2.1 Culture Interpretation:

The criteria of>=10^5CFU/ml for significance were considered with infection

A wide range of mucoid colors, from yellow to whitish-blue, characterize Klebsiella spp. colonies. .Figure 3.5 Colonial morphology and interpretation

3.2.3 Preservation of isolates in to 20% glycerol

- By mixing 30% glycerol with 70% water, an 80% glycerol (v/v) solution was created. The solution was autoclaved at 121°C for 15 minutes after being transferred to a glass container with a screw cover in order to sterilize it.
- Take off the top during autoclaving.
- Place 2 milliliters of sterile microfuge tubes containing 4 mm glass beads with 500 microliters of sterile 30% glycerol.
- Mix the glycerol in the tube with 500 µl of the bacterial culture using a vortex mixer.
- The tube should display the name, strain, and date of the organism.

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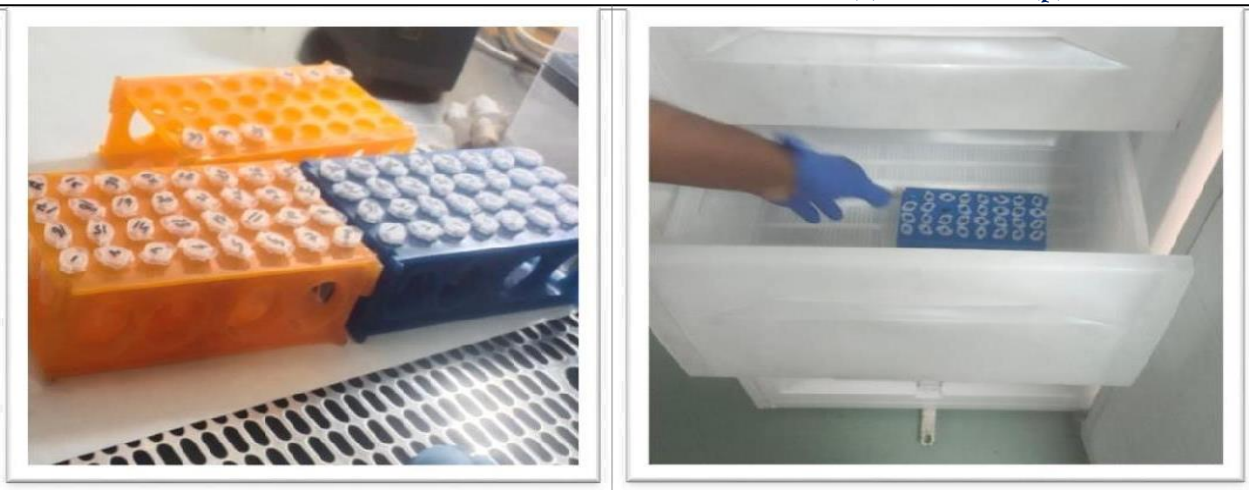

Figure3.6 Preservation of Isolates

3.3 Biochemical Identification of Isolates

3.3.1 Citrate Test

Procedure:

- Detailed coding was applied to a tube of Simmons Citrate Agar.
- The material was put to a tube of Simmons Citrate Agar, where a tiny colony grew.
- For 18 to 24 hours, the tube was incubated at a temperature of 35°C.
- The tube holding the Simmons Citrate Agar was checked for indications of growth after the incubation time. positive.

Interpretation:

Agar becomes blue as a result of a positive response.

A negative response would occur if citrate-negative bacteria were to colonize agar and cause its surface to become blue.

3.3.2 Urea Test Procedure:

- A Urea Agar tube was labeled with specific code
- A small colony of sample transferred to tube.
- The tube was incubated at 35°C for 18-24 hours.

Interpretation:

Optimal Reaction: A deep magenta to brilliant pink hue will develop between 15 minutes to 24 hours. Good Reaction: Bad Reaction Is Unchanged.

3.3.3 Triple Sugar Iron (TSI) Agar Test

Procedure:

- Each tube of Triple Sugar Iron Agar was assigned a unique code.
- Triple Sugar Iron Agar tube was used to transfer a tiny colony of material.
- 18–24 hours were spent incubating the tube at 35°C.

Interpretation:

- A response with a crimson slant and a yellow butt that shows signs of being acidic or alkaline. Everything it shows is the fermentation of glucose.
- An acid/acid reaction, which may alternatively be described as a yellow slant/yellow butt reaction, indicates that lactose, sucrose, or dextrose has fermented.
- Without carbohydrate fermentation, a red slant and red butt reaction, which is an alkaline/alkaline reaction, takes place.
- The medium becomes black in the presence of hydrogen gas.
- Gas production, including the formation of CO2 and H2, may be seen as the appearance of bubbles or cracks in the agar.

3.3.4 Sulfide Indole Motility agar test

Procedure:

- Each SIM Agar tube was assigned a

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unique code.

- A little sample colony was moved to a tube.
- For 18–24 hours, the tube was kept at a temperature of 35°C.

Interpretation

The presence of a black color in the medium suggests the production of hydrogen sulfide (H2S).

The presence of a red ring on the deep surface after the addition of Kovac's reagent signifies the completion of indole synthesis.

Motility is shown by the organism's capacity to move away from the streak in a fan-like manner.

Below in figure3.6 interpretation of biochemical test

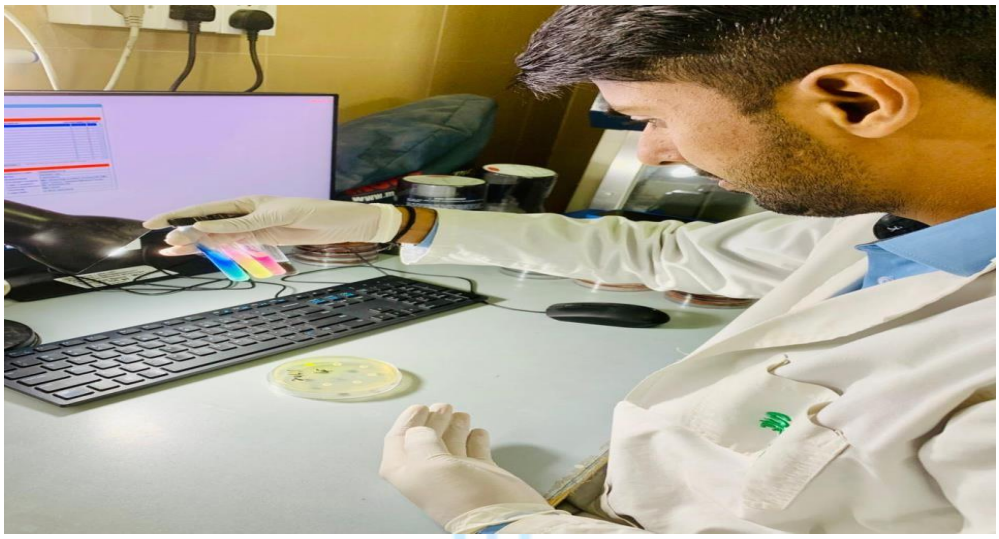

Figure3.7 Interpretation of biochemical tests

3.4 Antibiotic Susceptibility Testing

The effectiveness of antibiotics against uropathogenic bacteria was determined using the Kirby-Bauer disk diffusion susceptibility test, the results of which are shown in Table 3.

Procedure:

- Bring plates of mueller-Hinton agar to room temperature.
- The organism's name and the antibiotic(s) to be tested were written on the plates.
- A McFarland standard of 0.5 was used to create a bacterial suspension of the organism that was going to be examined.
- A sterile swab soaked in the bacterial solution was used to inoculate the plates by swabbing the agar surface.
- For a few minutes, the plates were left to dry naturally.
- With a minimum distance of 24mm, the antibiotic disks were positioned on top of the plates disjointed so as to avoid the consequences of both.
- Incubation of the plates at 35°C required 16 to 24 hours.

Following the incubation time, the diameter of the region around each antibiotic disk was measured to determine the extent to which bacterial growth was prevented. The zone diameters were compared with the antibiotic(s) under investigation's interpretation criteria to determine the organism's susceptibility.

List of Antibiotics and Their Susceptibility Breakpoints

This table outlines the antibiotics tested in this study, along with their disk concentrations and interpretive criteria for resistance, intermediate

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susceptibility, and sensitivity based on inhibition zone diameters (measured in millimeters).

Beta-Lactams and Beta-Lactamase Inhibitors

- **Amoxicillin/Clavulanic Acid (AMC, 20/10 µg)**: Resistance (<13 mm), Intermediate (14–16 mm), Sensitive (>17 mm).
- **Piperacillin/Tazobactam (TZP, 100/10 µg)**: Resistance (<17 mm), Intermediate (18–20 mm), Sensitive (>21 mm).
- **Cefoperazone/Sulbactam (SCF, 75/30 µg)**: Only sensitive breakpoint defined (>21 mm).
- **Ceftriaxone (CRO, 30 µg)**: Sensitive breakpoint (>24 mm); no resistance/intermediate criteria listed.
- **Ampicillin (AMP, 10 µg)**: Resistance (<12 mm), Intermediate (12–14 mm), Sensitive (>15 mm).

Carbapenems

- **Meropenem (MEM, 10 µg)**: Resistance (<15 mm), Intermediate (16–19 mm), Sensitive (>20 mm).

Aminoglycosides

- **Amikacin (AK, 30 µg)**: Resistance (<13 mm), Intermediate (14–17 mm), Sensitive (>18 mm).

Quinolones

- **Ciprofloxacin (CIP, 15 µg)**: Resistance (<13 mm), Intermediate (14–22 mm), Sensitive (>23 mm).

Other Antibiotics

- **Nitrofurantoin (F, 300 µg)**: Resistance (<14 mm), Intermediate (15–16 mm), Sensitive (>17 mm).
- **Co-trimoxazole (SXT, 1.25/23.75 µg)**: Resistance (<10 mm), Intermediate (11–15 mm), Sensitive (>16 mm).
- **Clindamycin (DA, 2 µg)**: Resistance (<15 mm), Intermediate (16–20 mm), Sensitive (>21 mm).
- **Erythromycin (E, 15 µg)**: Resistance (<13 mm), Intermediate (14–22 mm), Sensitive (>23 mm).
- **Vancomycin (VAN, 30 µg)**: Resistance (<14 mm), Intermediate (15–16 mm), Sensitive (>17 mm).

Notes on Testing

- For some antibiotics (e.g., SCF, CRO), only sensitive breakpoints were provided, suggesting these drugs may not have established resistance thresholds in this context.
- The term "Eucidisc acid" (FOT) appears to be a typographical error, possibly referring to **Fusidic Acid** (30 µg), with breakpoints: Resistance (<10 mm), Intermediate (11–15 mm), Sensitive (>16 mm).

3.5 Statistical analysis and report writing

We conducted all of our statistical analyses and comparisons using a statistical software set. "Statist ix" (8.1), 2011 Microsoft Excel editions. Statistical tests, such as logistic regression and chi-square, were used to examine the relationships between the variables and the prevalence rates.

Results

4.1 Frequency Distribution of Samples

We found that out of 150 total participants, 98 were male and 52 were female, according to the age- and gender-wise distribution study (see figure 4.1 below). Upon further examination of the age distribution, it was revealed, as seen in figure 4.2, that 26 patients belonged to the 0-20 age bracket, 51 to the 21-40 age bracket, 48 to the 41-60 age bracket, and 25 to the 61-80 age bracket. This detailed analysis lays the groundwork for future investigations into the correlation between the frequency of UTIs and the antibiotic susceptibility patterns of *Klebsiella* spp. isolated from patients in the Sukur area who are suspected of having a UTI, taking into account both gender and age.

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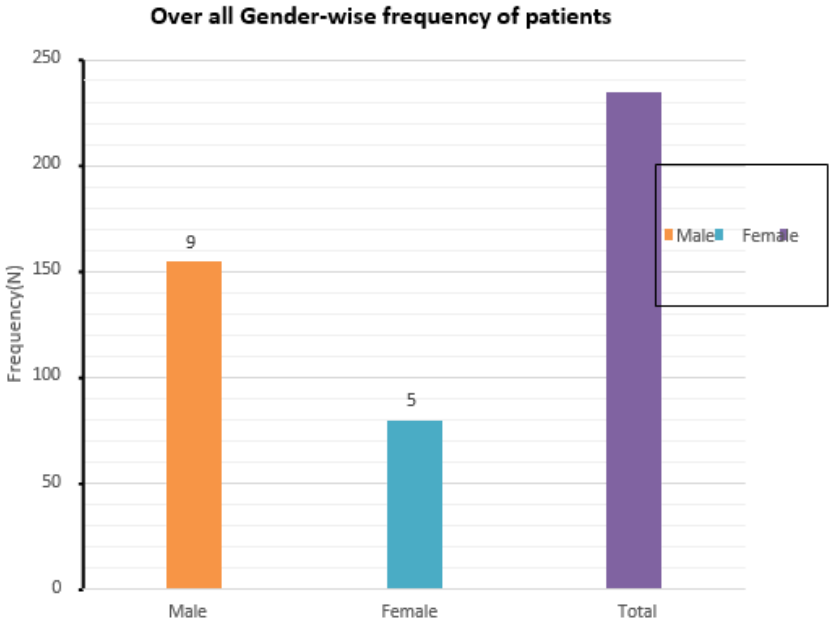

Figure4.1 A breakdown of the participants based on their gender

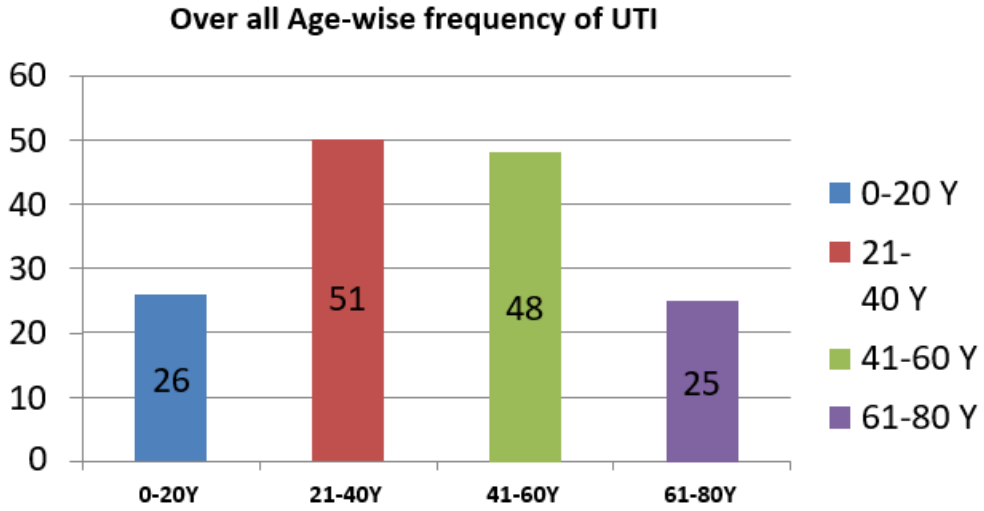

Figure4.2 Gender-wise Frequency distribution of participants

4.2 Distribution of Growth Pattern from Urine Specimen

Figure 4.3 (A) shows the results of inoculating 150 urine samples on Cystine-Lactose-Electrolyte-Deficient Agar (CLED) in order to isolate the pathogen responsible for the urinary tract infection. There was no evidence of bacterial growth in any of the 130 samples, hence the findings were negative. On the other hand, as shown in figure 4.3 (B), 20 samples tested positive for growth, including 10 *E. coli* , 7 *Klebsiella* species, and 3 *Streptococci* species. Important information on the microbiological make-up of the study's urine samples is uncovered by these results.

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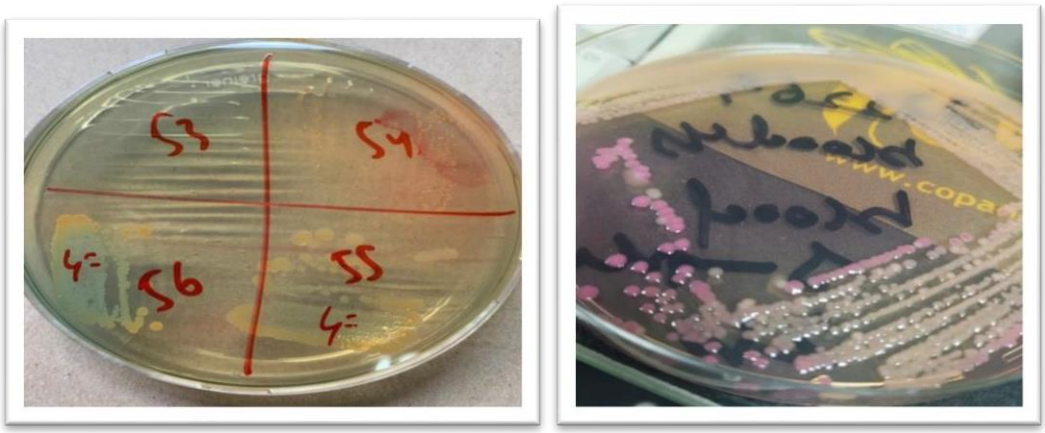

Figure4.3 A Isolates on culture media after incubation

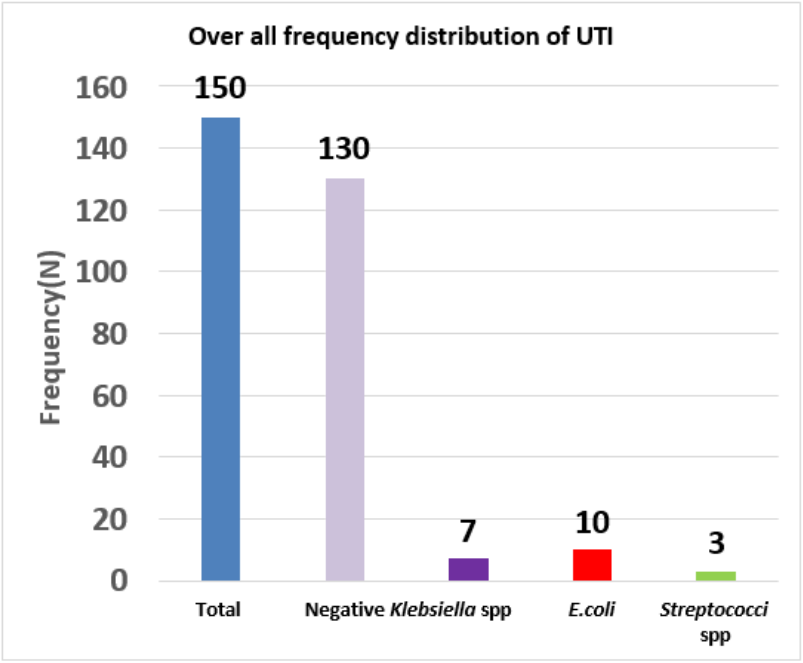

Figure4.3 B General distributions of frequencies including positive, negative, and mixed advances

Gram staining results

Twenty urine samples were found to have bacterial growth. The gram staining method was used to identify gram-negative bacteria among the 85% of positive samples. Figure 4.4 (A) shows a picture of the gram staining microscopy, while figure 4.4 (B) shows the percentage graph.

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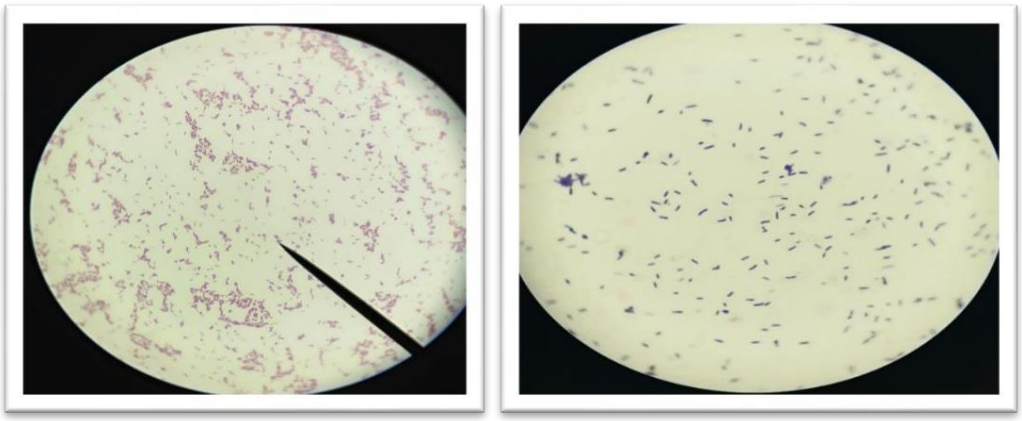

Figure4.4(A) Gram positive and Gram negative Bacteria in Microscopic Picture

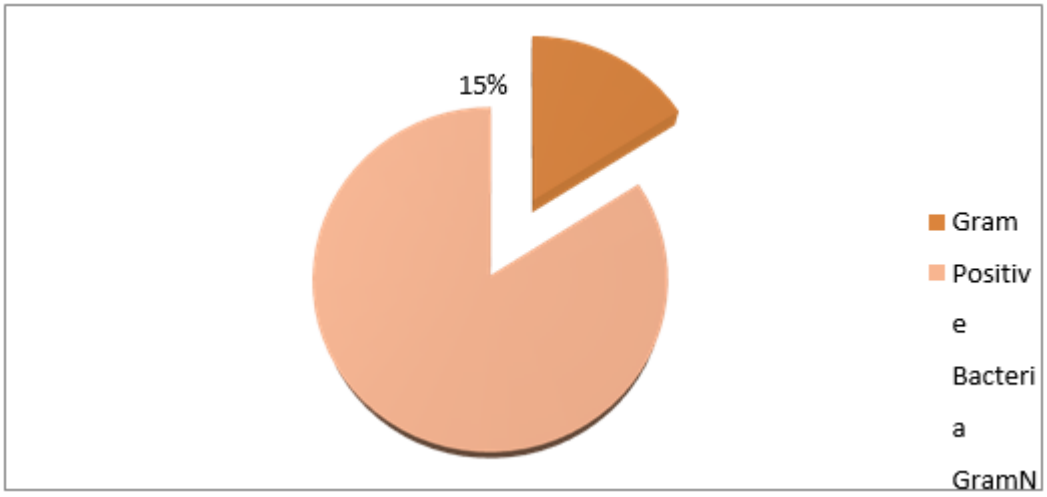

Figure4.4 (B) Gram positive and Gram negative percentage of isolates

4.3 Biochemical Identification of Bacteria

The presence of Streptococci spp. was confirmed by subjecting gram-positive isolates to catalase and coagulase assays after their gram response had been identified in bacteria. The most common bacteria were found to be *Escherichia coli*, *Klebsiella*, and *Streptococci*, as shown in figure 4.5 (A) and the percentage chart below figure 4.5 (B), respectively, based on the results of the biochemical tests conducted on the gram-negative isolates, as mentioned in table no. 4.1.

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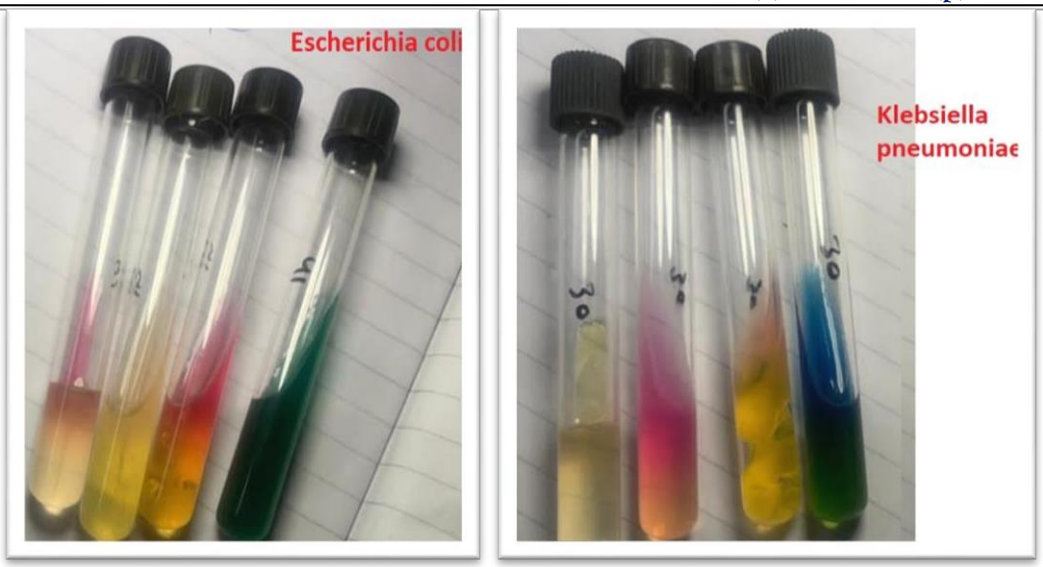

Figure4.5(A) Biochemical identification tests

Table4.1.Biochemical identification chart of bacteria

Bacteria	Gram reaction	Indole	TSI	Urease	Oxidase
E.coli	Negative	Positive	Positive	Negative	Negative
Klebsiella pneumoniae	Negative	Negative	Positive	Positive	Negative

Bacteria	Gram reaction	Catalase	Coagulase		
Streptococcispp	Positive	Negative	Negative		
Pseudomonasaeruginosa	Negative	Negative	Negative	Negative	Positive

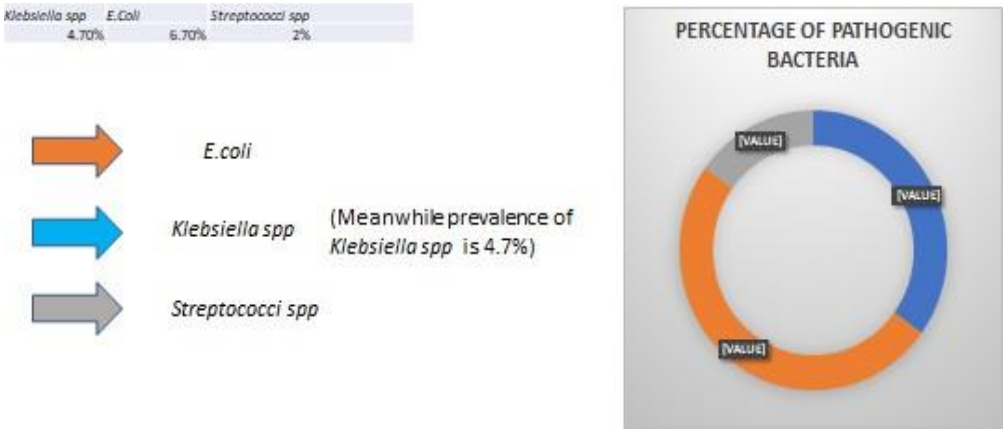

Figure4.5(B) Individual percentage of isolated

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4.4 Antimicrobial Susceptibility Testing

Gram-negative bacteria (*E.coli,K.pneumonia,Proteus*) were tested against eight Antimicrobial agents: Piperacillin/tazobactam, Trimethoprim/sulfamethoxazole, Nitrofurantoin, Fucidic acid, Meropenem, Sulbactam/Cefoperazone, Amoxicillin-clavulanic acid and Amikacin; some pictures are shown in figure 4.6 (A)one strain of *Klebsiella spp* weas 100% resitant to all drugs, described as PDR or XDR strain as shown in figure 4.6 (B).

Against gram-positive Streptococcus spp., seven antibiotics were tested: Clindamycin,Ciprofloxacin,Amoxicillin-clavulanic acid Amplicin,Vancomycin, Carbapenem and Erythromycin.

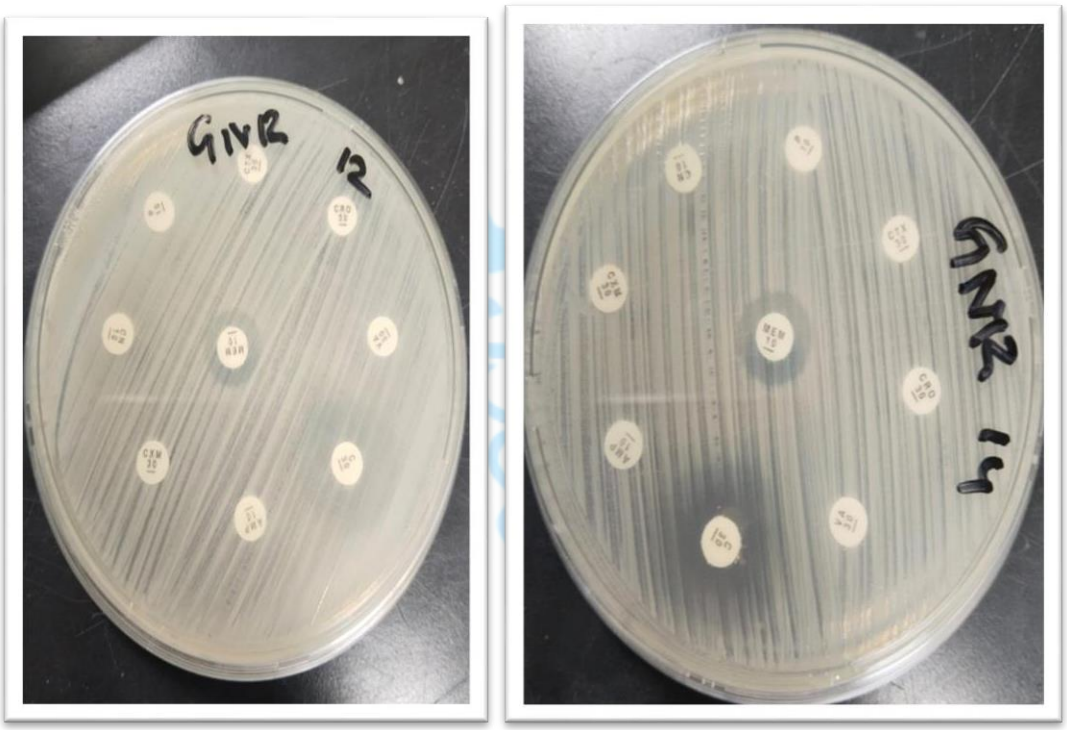

Fig4.6 (A) Antimicrobial susceptibility testing pattern of Bacterial isolates

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Fig4.6(B) Graph of Antimicrobial susceptibility testing pattern of Bacterial isolates

4.5 Percentage Resistance pattern of *Klebsiella species* against various Antibiotics

There are seven total strains were isolated from urine samples and 08 antibiotics were applied to know resistance pattern and as per percentage all *Klebsiella* stains were hundred percentage resistant to Cefoperazone/sulbactum (SCF) and followed by 71% to Amoxicillin and Salfamethoxazole/trimethoprim, 57% Meropenem (MEM) and Amikican (AK), 42% Fosfomycin/Trometamole (FOT), 29% Piperacillin/Tazobactam (TZP), and 28% Flucloxacillin (F) as shown in below figure 4.7 (A) and (B).

S.No	Isolates	Piperacillin/ Tazobactam	Salfamethoxazole/trim ethoprim	Flucloxacillin	Fosfomycin/tr ometamole	Meropenem	Cefoperazone/ sulbactum	Amoxicillin	Amikacin
1	Klebsiella spp	Resistant	Resistant	Sensitive	Sensitive	Sensitive	Resistant	Sensitive	Sensitive
2	Klebsiella spp	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Sensitive
3	Klebsiella spp	Sensitive	Resistant	Sensitive	Resistant	Sensitive	Resistant	Sensitive	Sensitive
4	Klebsiella spp	Sensitive	Sensitive	Sensitive	Sensitive	Resistant	Resistant	Resistant	Resistant
5	Klebsiella spp	Sensitive	Resistant	Resistant	Resistant	Resistant	Resistant	Intermedia te	Resistant
6	Klebsiella spp	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Resistant	Resistant	Resistant
7	Klebsiella spp	Sensitive	Resistant	Sensitive	Sensitive	Resistant	Resistant	Resistant	Resistant

Figure 4.7(A) Shows resistance pattern of *Klebsiella* against several antibiotics

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S.No	Antibiotics drugs	Drug abbreviation	Resistant percentage	Sensitive percentage
1	Piperacillin/Tazobactam	(TZP)	29%	71%
2	Salfamethoxazole/trimethoprim	(SXT)	71%	29%
3	Flucloxacillin	(F)	28%	72%
4	Fosfomycin/trometamole	(FOT)	42%	58%
5	Meropenem	(MEM)	57%	43%
6	Cefoperazone/sulbactum (Pandrug resistant PDR)	(SCF)	100%	00%
7	Amoxicillin	(AMC)	71%	29%
8	Amikacin	(AK)	57%	43%

(B)

5.1 Discussion:

Any infection that affects the urinary system is known as a urinary tract infection (UTI). The urethra, kidneys, ureters, and bladder make up the urinary system. The urethra and bladder are the most common sites of infection in the lower urinary system (Rao et al., 2022). Both symptomatic and silent UTIs are possible. The bacteriuria threshold in a clean-catch midstream urine sample is 100,000 CFU/mL pee, which is high for symptomatic UTIs (Renko et al., 2022). It is divided into two categories: complex and simple. An anomaly in the structure or function of the urinary system is linked with a complex UTI, while a healthy person without any underlying abnormalities in the urinary tract is associated with an uncomplicated UTI.

As per gender wise distribution, total 150 samples were collected among them 98 were male gender while 52 were participants were female as shown in Figure4.1 and similar study was done by Jahan, and Anwer (2025), total 7465 patients were included among them 4698 were females in gender and rest 2767 were males in gender. In above mentioned study female gender was indicated notable predominant but as per our study male gender shows predominance having 98 participants among 150 representing approximately, 65% while 35% male participants but above study showed 63% female and 37% male as mentioned in (Jahan, and Anwer, 2025). This gender disparity calls for more research into the unique health dynamics of each gender, since it raises the question of whether there are gender-specific variables that impact the frequency of UTIs and antibiotic susceptibility.

In age-wise study done by Zhan *et al* (2024), Age-related distribution of particiant was explored by categorizing patients into four groups: Age group 1, 0-49 years; age group 2, 50-69 years; age group 3, 70-79 years; and age group 4, ≥80 years, on other hand our results of age-wise distribution are categorized into four groups as well.Figure 4.2 shows that 51 participants were between the ages of 21 and 40, which is a significant proportion that highlights the participants' vulnerability within the context of the variables under investigation. Then there are 48 participants between the ages of 41 and 60, 26 patients

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between the ages of 0 and 20, and 25 participants between the ages of 61 and 80.

The research conducted by Bayaba *et al* (2025), research was conducted on 215 participants, among all 68 samples were positive for pathogen.There was a 31.62% prevalence of *Enterobacterales* (68/215 cases), 79.41% prevalence of *Escherichia coli* (54/68 cases), and 14.70% prevalence of *Klebsiella pneumoniae* (10/68 cases). Generalized ESBL-*Enterobacterales* prevalence was 64.70 percent (44 out of 68). In order to determine the incidence of *Klebsiella pneumoniae* in the Sukkur area and isolate urinary tract pathogens, we infected 150 samples on Cystine-Lactose-Electrolyte-Deficient Agar (CLED). As shown in Figure 4, 13% of the samples tested positive for culture. Of these, 50% were found to be *Escherichia coli*, 35% to be *Klebsiella Species*, and 15% to be *Streptococci* spp. Bayaba et al. (2025) demonstrated three comparable results.

NazirandKanth,(2024) Out of a total of 28, 252 urine samples, 804 (or 28.46% of the total) tested positive for bacterial infection when subjected to urine culture and sensitivity. A total of 33% (267/804) of the positive findings were caused by Gram-positive bacteria, whereas 58.5% (471/804) were caused by Gram-negative bacteria. Twenty urine samples were tested for bacterial growth using gram staining in our investigation. The fact that 85% of the samples were determined to be gram-negative and just 15% to be gram-positive is an intriguing discovery. The majority of studies have linked gram-negative bacteria, and *Escherichia coli* in particular, to UTIs (Nazirand Kanth, 2024).

One common way microbiologists classify bacteria is by looking at their cell walls; this process is called gram staining. Figure 4.4A, which shows gram staining microscopy, is helpful since it shows the bacterial shape visually. The percentage graph in Figure 4.4B provides further evidence that the positive samples were mostly composed of gram-negative bacteria. The microbiological profile within the examined population may be quickly grasped with the help of this visual depiction, which also improves the distribution's clarity.

The study's bacterial isolates, which were identified and characterized, give important details on the kinds and abundance of microbes in the Sukkur region's population.

The findings of the biochemical tests, which include indole, TSI (Triple Sugar Iron), urease, and citrate, provide further information about the metabolic and physiological features of each bacteria. Everything that is known about the metabolic profiles of these bacterial species is in line with these results.

In order to assess the antibiotic resistance and sensitivity of gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae*, and gram-positive *Streptococcus Species*), the Kirby-Bauer assay was used (Yin et al., 2021). This provided useful information about the antibiotic susceptibility patterns among the *Klebsiella* species found in the study, which looked at the prevalence of these infections in patients at Sukkur hospital.

Researched by Kebede et al. in 2025 It was discovered that Amikacin (83.3%), Gentamicin (81.5%), Imipenem (778.8%), and Nitrofurantoin (89.7%) were all effective against the detected microorganisms. Among the gram-negative isolates, *E. coli* showed susceptibility to 17 gram-negative antibiotics (71.4% of the time), 15 gram-negative antibiotics (71.4% of the time), 18 nitrofurantoin (85.7% of the time), and 11 gram-negative antibiotics (52.4% of the time) and 13 gram-negative antibiotics (61.9% of the time) respectively. Some *Klebsiella species* were also sensitive to imipenem (10/83.3%), trimethoprim-sulfamethoxazole (8/66.7%), and amikacin (10/83.3%). On the other hand, ciprofloxacin 5 (47.7%), tetracycline 4 (33.3%), and ampicillin 2 (16.7%) were all met with resistance by *Klebsiella species*. Piperacillin tazobactam, ciprofloxacin, and tetracycline resistance was also observed in three species of Citrobacter, accounting for 60.0% of the total. Nevertheless, according to (Kebede et al., 2025), three different isolates—P. vulgaris, P. mirabilis, and Serratia species—exhibited a resistance rate of 100% to different classes of antibiotics.

While in our study *Klebsiella species* are susceptible Piperacillin/Tazobactam (71%),

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Salfamethoxazole/trimethoprim (29%), Flucloxacillin (72%), Fosfomycin/trometamole (58%),Meropenem(43%), Amoxicillin(29%) and Amikacin(43%) and resistantto all above drugs respectively (29%, 71%, 28%, 42%, 57%, 69% and 57%). However 100% resistant rate of all strains of *kelbsiella* spps also detected to antibiotic Cefoperazone/sulbactum shown in 4.7 Band as my per opinion this is due to miss use and empirical use drug, therefore the situation is very alarming and it's a big issue.

Similarly, the other drugs will be ineffective in future if we are not taking it serious and sort out through proper recommendations of culture and susceptibility before use of antibiotics. The discussion of other pathogen isolated from clinical specimen is excluded from discussion because our title of research is "Prevalence of *Klebsiella species* and their Susceptibility Pattern of Antibiotics in Patients with Urinary Tract Infection at Sukkur." Due to the importance of comparing these findings in terms of antibiotic resistance patterns, this chapter primarily focuses on the antibiotic susceptibility of *Klebsiella species*. Sukkur patients with UTIs continue to face the problem of antibiotic resistance, which has recently emerged, notably in Klebsiella species. Prioritizing careful antibiotic usage and taking local resistance tendencies into account are crucial when developing treatment plans.

One of the primary causes of this serious issue is the rise in bacterial resistance to antibiotics. Antibiotics have a wide spectrum of action due to the overuse of these medications. Resistant to multiple classes of antibiotics, bacteria can cause urinary tract infections and pose a huge threat to public health. In our study, a big percentage of the *K. pneumonia* isolates conferred resistance to a wide range of antibiotics tested. The highest resistance of *K. pneumoniae* was observed against Cefoperazone/sulbactum with a ratio of 100%. The highest sensitivity of the isolates was against antibiotic Flucloxacillin 72% of the isolates revealed sensitivity, thus this antibiotic might be the best treatment for *K. pneumonia* infections. Not only did the bacteria isolated display high level of antibiotic resistance, but they also demonstrated sensitivity to the antibiotic Flucloxacillin.

In addition, a bacterium's virulence is greatly influenced by the formation of biofilms by the antibiotic-resistant bacteria when they are readily available and present in the environments. In transition from plank to growth of mode to biofilm producing bacteria, bacterial adaptation will change over time. The gene expression also changes with environmental condition in biofilms compared to planktonic counterparts (Cam and Brinkmeyer, 2020). The bacteria in the biofilm may undergo several transformations and changes to adapt to their new environment, which is why the biofilm forms on living or non-living surfaces. Exterior molecules, such as receptor sites on flagella and virulence factors, and metabolic waste, such as acids, carbohydrates, and toxins, allow the bacteriato better withstand adverse conditions.

5.2 Conclusion

Study "Prevalence of Prevalence of *Klebsiella species* and their Susceptibility Pattern to Antibiotics in Patients with Urinary Tract Infection at Sukkur" revealed several significant findings.

Among 20 positive UTI cases, *Klebsiella species.* prevalent 35%, while *E. coli* was the most prevalent organism at 50%, and *Streptococci* constituted 15%. The remaining samples tested negative for pathogenic growth.

Klebsiella species. demonstrated specific antibiotic resistance patterns Continuous surveillance of uropathogens and resistance trends is critical for effective management, especially in high-risk populations such as diabetics in Sukkur.

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