A Comparative Analysis of Expanded Quantitative Urine Culture and Standard Urine Culture Methods in Patients with Urinary Tract Infections

By

Tajri Hasan Srabony
20226012

Stella Bijoyee Kunder
22136053

K M Abdullah Hel Shafi
22126026

A thesis submitted to the Department of Microbiology in partial fulfillment of the requirements for the degree of
Bachelor of Science in Microbiology,
Bachelor of Science in Biotechnology.

Department of Microbiology and Department of Biotechnology
BRAC University
January 2026

Declaration

It is hereby declared that

1. The thesis submitted is our own original work while completing degree at BRAC University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. We have acknowledged all main sources of help.

Student's Full Name & Signature:

Tajri Hasan Srabony
20226012

Stella Bijoyee Kunder
22136053

K M Abdullah Hel Shafi
22126026

Approval

The thesis/project titled "A Comparative analysis of Expanded Quantitative Urine Culture and Standard Urine Culture methods in Bangladeshi Patients with Urinary Tract Infections" submitted by

Tajri Hasan Srabony (20226012)

Stella Bijoyee Kunder (22136053)

K M Abdullah Hel Shafi (22126026)

has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Science in Microbiology on 29th January, 2026.

Examining Committee:

Supervisor and
Chairperson
(Member)

_____

Nadia Sultana Deen, PhD
Chairperson,
Department of Microbiology,
BRAC University.

Ethics Statement

All experimental work was carried out according to the ethical guidelines of the Helsinki Declaration. The plagiarism level of this thesis is below 10%.

Abstract

Urinary tract infections (UTI) are one of the most common bacterial infections of humans. Accurate diagnosis of the cause of UTI is necessary for effective treatment. Even though clinically Standard Urine Culture (SUC) is common for detecting bacterial presence in urine samples, latest reports show that Expanded Quantitative Urine Culture (EQUC) provides better diagnostic sensitivity compared to SUC. To test this, SUC and EQUC methods were compared to analyse bacterial load and diversity using urine samples collected from 20 patients with UTI symptoms and 20 healthy controls in this study. Samples were diluted and inoculated on nutrient agar, HiChrome UTI agar, MacConkey agar and blood agar for SUC method, then incubated at 37°C for 24 hours under aerobic conditions. On the other hand, for EQUC, in addition to all the media, chocolate agar was used with different conditions like carbon-enriched, anaerobic environment with incubation at 37°C for 48 hours. Colony characteristics and polymerase chain reaction (PCR) were performed for bacterial detection. Antibiotic susceptibility test was conducted by the Kirby-Baur method. On nutrient agar medium, EQUC yielded 5-fold higher bacteria than SUC and portrayed higher bacterial diversity. While several bacterial species for example, *klebsiella* spp., *Staphylococcus aureus*, and *Pseudomonas aeruginosa* was detected by SUC and EQUC based on colony characteristics, EQUC exclusively detected the presence of *Staphylococcus haemolyticus*. Seventy-five percent of presumptive *Klebsiella* and 73.3% of presumptive *E. coli* were confirmed by PCR. AST was performed on *E. coli* and *Klebsiella*. Notably, bacteria grown via EQUC showed a diverse resistance pattern compared to SUC. Further molecular diagnoses will be performed to validate these findings.

Keywords: Urinary tract infection (UTI), standard urine culture (SUC), expanded quantitative urine culture (EQUC)

Acknowledgement

At first, we express our gratitude to the Almighty for granting us the resilience and clarity needed to complete this undergraduate thesis.

We sincerely acknowledge our supervisor, Dr. Nadia Sultana Deen, Chairperson of the Microbiology department, for her academic leadership, critical evaluation, and methodological guidance throughout this research. Her ability to challenge our thinking, refine our approach, and maintain academic rigor significantly contributed to the quality of this work.

We also acknowledge the contribution of our lab research assistant, Nazifa Tabassum Tasnim, whose technical expertise, hands-on supervision, and attention to laboratory protocols were essential for the successful execution of the experimental work. We appreciate the support of the lab officers, Shamima Akhter and Md. Mahmudul Hasan, for their cooperation, logistical assistance, and support in maintaining laboratory operations.

Lastly, we extend our heartfelt thanks to our families, especially our parents, for their enduring patience, emotional support, and encouragement, which sustained us throughout this demanding academic endeavor.

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List of Acronyms

AST	Antimicrobial Susceptibility Test
BA	Blood Agar
BAP	Blood Agar Plate
CA	Chocolate Agar
CDC	Centers for Disease Control
CFU	Colony-Forming Unit
CLSI	Clinical and Laboratory Standards Institute
cUTI	Complicated Urinary Tract Infection
dNTPs	Deoxynucleotide Triphosphates
EDTA	Ethylenediaminetetraacetic acid
EMA	European Medicines Agency
EQUC	Expanded Quantitative Urine Culture
ESBL	Extended Spectrum **β**-lactamase
EUCAST	European Committee on Antimicrobial Susceptibility Test
FDA	Food and Drug Administration
MAC	MacConkey
MHA	Mueller-Hinton Agar
MSCC	Midstream Clean Catch

NA	Nutrient Agar
OAB	Over Active Bladder
PBS	Phosphate-Buffered Saline
PCR	Polymerase Chain Reaction
SUC	Standard Urine Culture
TBE	Tris-Borate-EDTA
TE	Tris-EDTA
UPEC	Uropathogenic E. coli
UTIs	Urinary Tract Infections
UUI	Urgency Urinary Incontinence
uUTI	Uncomplicated Urinary Tract Infection

Chapter 1:

Introduction

1.1 Background

Urinary Tract Infections (UTIs) are among the most prevalent bacterial infections globally, affecting individuals across all age groups and representing a significant public health burden, creating crucial healthcare economic disruption, high recurrence rate and concerningly increasing antibiotic resistance (Werneberg, et. al., 2025). Around 50% people at least once suffered from this in their lifetime (Baimakhanova, et. al., 2025). US-based reports show up to 12.6% women suffering from UTI annually (Werneberg, et. al., 2025). Prevalence of UTI among infants aged from 0-24 months was 7% and for 0-19 years old children it was 7.8% (Lai, et. al., 2021). 50-60% of pregnant women get diagnosed with UTI, among them 2-8% of cases are asymptomatic UTI (Baimakhanova, et. al., 2025). Asymptomatic UTI remarkably increases during the peri and post menopause stage, affecting 4-19% women. Epileptic and diabetic women have higher prevalence of UTI occurrence and recurrence.

According to the European Medicines Agency (EMA) and US Food and Drug Administration (FDA), uncomplicated UTI (uUTI) and complicated (cUTI) can be explained based on the symptoms patients experience, host factors and presence of pyuria and bacteriuria (Bilsen, et. al., 2023) (**Table 1.1**).

Table 1.1: Definition of uUTI and cUTI by EMA and FDA

Category	Uncomplicated UTI (uUTI)		Complicated UTI (cUTI)		References
	FDA	EMA	FDA	EMA	
Symptoms	Painful urination, suprapubic pain, no systemic illness (fever, shaking etc.)	Minimal symptoms like urgency and painful urination.	Chills, fever, pelvic pain, frequent, urgent and painful urination.	Tenderness, pelvic pain, frequent, urgent and painful urination.	(Bilsen, et al., 2023).

Host conditions	Female patients due to their anatomy		Renal disease, urinary retention. Urinary catheter, neurogenic bladder, obstructive uropathy	Catheter, urinary retention, neurogenic bladder condition	(Bilsen, et al., 2023).
Pyuria	Analysis of leukocytes (nitrites/catalase test) to figure out pyuria	>10 leukocytes/μL	Dipstick test positive for leukocyte esterase and >10 leukocytes/μL	>10 leukocytes/μL	(Bilsen, et al., 2023).
Bacteriuria	$\geq 10^5$ CFU/mL of a particular species of bacteria	$>10^5$ CFU/mL of a uropathogen	$\geq 10^5$ CFU/mL of a particular species of bacteria	$>10^5$ CFU/mL of ≥2 relevant uropathogens	(Bilsen, et al., 2023).

*FDA (US Food and Drug Administration) *EMA (European Medicines Agency)

The possible reasons and necessary diagnostic approaches for both UTI are slightly different (Baimakhanova, et. al., 2025) (**Table 1.2**). Elevation of pH from 7.5 has been a key factor of identifying UTI. *Proteus* species and *Pseudomonas aeruginosa* reportedly increase the pH. *Proteus milabilis* can increase pH up to 8.3, whereas *Staphylococcus aureus* can elevate it to 7.4. On the other hand, *Klebsiella pneumoniae* can only take it to 6.4 (Lai, et. al., 2021).

Table 1.2: Microbial cause and diagnostic approach of uUTI, cUTI and asymptomatic bacteriuria

Aspect	**uUTI**	**cUTI**	**Asymptomatic Bacteriuria**	**References**
Microbial cause	80%- *E. coli* Others- *Proteus mirabilis, Klebsiella* species, *Staphylococcus,*	*E. coli, Enterococcus faecalis, Pseudomonas aeruginosa,* other	Any uropathogen with bacterial load of $\geq 10^5$ CFU/mL	(Baimakhanova, et. al., 2025)

	Enterococcus faecalis	*Enterobacterales*		
Diagnostic approach	No initial testing required. If recurrent UTI, then urinalysis and culture needed for pyelonephritis	Urinalysis and culture, kidney ultrasound needed for testing pyelonephritis	Not required unless urological procedure done prior did any damage to bladder mucosa	(Baimakhanova, et. al., 2025)

The primary etiological agent is *Escherichia coli* (*E. coli*), specifically uropathogenic *E. coli* (UPEC), which accounts for approximately 60-80% of both uncomplicated and complicated UTIs (Mancuso et al., 2023). UPEC possesses specialized virulence factors that enable it to colonize the urinary tract effectively, including Type 1 fimbriae that facilitate adhesion to the bladder epithelium by the binding to mannose receptors on the urothelial cells, thereby resisting mechanical clearance through urination. These adhesins allow UPEC to establish intracellular bacterial communities within the superficial umbrella cells of the bladder mucosa, contributing to persistence and recurrence.

In addition to UPEC **(Figure 1.1)**, other pathogens contribute significantly to the microbial spectrum of UTIs, particularly in complicated cases or specific populations. *Klebsiella pneumoniae*, a gram-negative bacterium, is frequently implicated in healthcare-associated UTIs and demonstrates increasing resistance to extended-spectrum beta-lactam antibiotics, posing therapeutic challenges (Mancuso et al., 2023). *Proteus mirabilis* is notable for its urease-producing capability, which hydrolyzes urea into ammonia and carbon dioxide, leading to alkalinization of urine and precipitate of struvite and calcium phosphate crystals– factors that promote stone formation and catheter encrustation, making it a common pathogen in patients with indwelling urinary devices (Mancuso et al., 2023). *Enterococcus* species, particularly *Enterococcus faecalis*, are gram-positive cocci often isolated in complicated UTIs, especially among hospitalized patients, those with diabetes, or individuals with prolonged antibiotic exposure, where disruption of normal flora facilitates their overgrowth (Mancuso et al., 2023). Furthermore, *Staphylococcus saprophyticus* is a well-recognized cause of uncomplicated UTIs in young, sexually active women, second only to UPEC in this demographic, due to its ability to adhere to uroepithelial cells and resist host defenses (Mancuso et al., 2023).

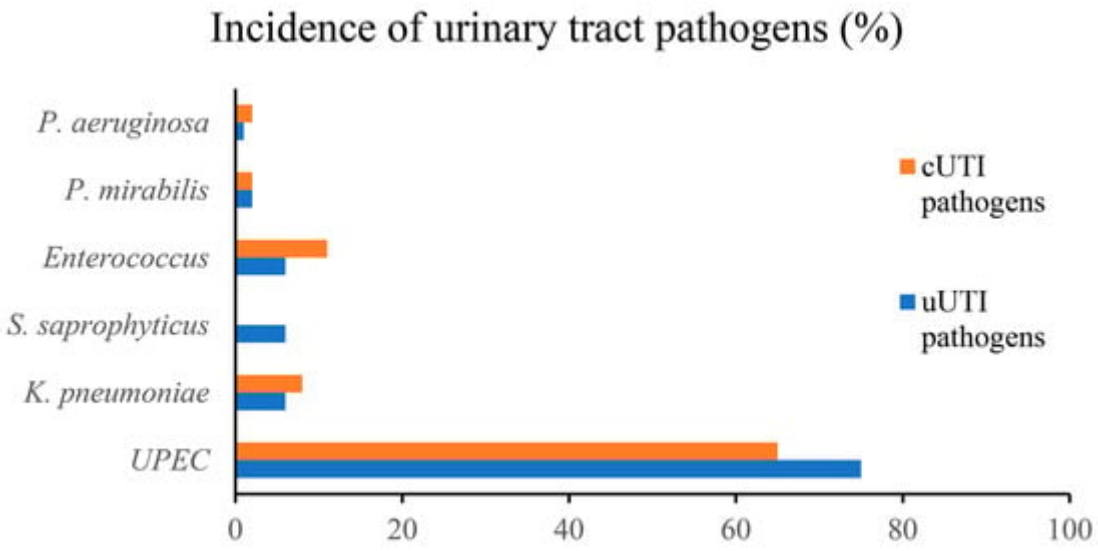

Figure 1.1: Epidemiology of uropathogens in uUTI and cUTI (Mancuso et al., 2023).

UTI might arise from gastrointestinal tract, urethra, poor hygiene and spread to bladder, ureters, and kidney (Moreland, et. al., 2024). The ascending route of infection remains the predominant mechanism, wherein bacteria from the perineal and gastrointestinal flora migrate through the urethra into the bladder and potentially ascend further to the kidneys via the ureters. This process is facilitated by anatomical and physiological factors: females are disproportionately affected due to their shorter urethral length (≅ 4 cm), proximity of the urethral meatus to the anus, and hormonal fluctuations that influence vaginal microbiota composition (Thamer Hadi & M. Jayashankar, 2022). Additional risk factors include sexual intercourse, spermicide use, menopause related estrogen deficiency leading to loss of protective *Lactobacillus*-dominant flora, and underlying conditions such as diabetes mellitus, neurogenic bladder, urinary obstruction, or vesicoureteral reflux (Mancuso et al., 2023). Structural abnormalities or functional impairments compromise natural defense mechanisms like continuous urine flow, acidic pH, antimicrobial peptides (e.g. defensins), and Tamm-Horsfall protein, which normally inhibit bacterial adherence and promote clearance.

Pediatric populations also experience a high incidence of UTI, with febrile UTI being the most common source of serious bacterial infection after respiratory illnesses in children under two years of age (Heshmat Hassan et al., 2022). In this group, *E. coli* remains the predominant pathogen, though delayed diagnosis can lead to acute pyelonephritis and long-term renal scarring, emphasizing the importance of early detection and appropriate management (Heshmat Hassan et al., 2022). Collectively, these findings underscore the multifactorial nature

of UTI causation, involving pathogen-specific virulence traits, host susceptibility, and environmental determinants that converge to initiate infection. The rising prevalence of antimicrobial resistance among uropathogens further complicates treatment paradigms, necessitating precise diagnostic stewardship and targeted therapeutic strategies.

UTI present with a range of clinical symptoms that vary depending on the anatomical site of infection, broadly categorized as lower or upper urinary tract involvement. Lower UTI primarily affect the bladder (cystitis) and urethra (urethritis), while upper UTI involve the kidneys (pyelonephritis). The distinction is critical for appropriate diagnosis and management. Lower urinary tract infections are the most commonly manifested by cystitis, which presents with dysuria, pain or burning during urination, which occurs in over 90% of symptomatic cases (Mancuso et al., 2023). Patients frequently report increased urinary frequency and urgency, often with only small volumes of urine voided due to inflammation and irritation of the bladder mucosa. Suprapubic discomfort or pressure is another hallmark symptom, localized in the pelvic region above the pubic bone. Hematuria, or the presence of blood in the urine, may be visible (gross hematuria) or detected microscopically, resulting from epithelial damage caused by bacterial invasion and host immune response. Additionally, urine may appear cloudy or have a foul odor due to the presence of white blood cells (WBCs), bacteria, and cellular debris. (Zhou et al., 2023). Urethritis, though less specifically delineated in routine clinical practice, contributes to dysuria and may be associated with the urethral discharge. In women, these symptoms can overlap significantly with other genitourinary conditions, necessitating careful differential diagnosis.

In contrast, upper urinary tract infections, particularly acute pyelonephritis, are more systemic and severe. They typically arise from the ascension of bacteria from the lower urinary tract but can also occur via hematogenous spread. Classic symptoms include high fever (often exceeding 38.5°C), chills, and flank pain: unilateral or bilateral pain in the costovertebral angle corresponding to the location of the kidneys, nausea and vomiting are common accompanying features, reflecting the systemic, inflammatory response. When evaluated, urinalysis of pyelonephritis may reveal leukocyte casts, which indicates tubular involvement and are diagnostic of renal parenchymal infection rather than isolated lower tract disease. (Mancuso et al., 2023).

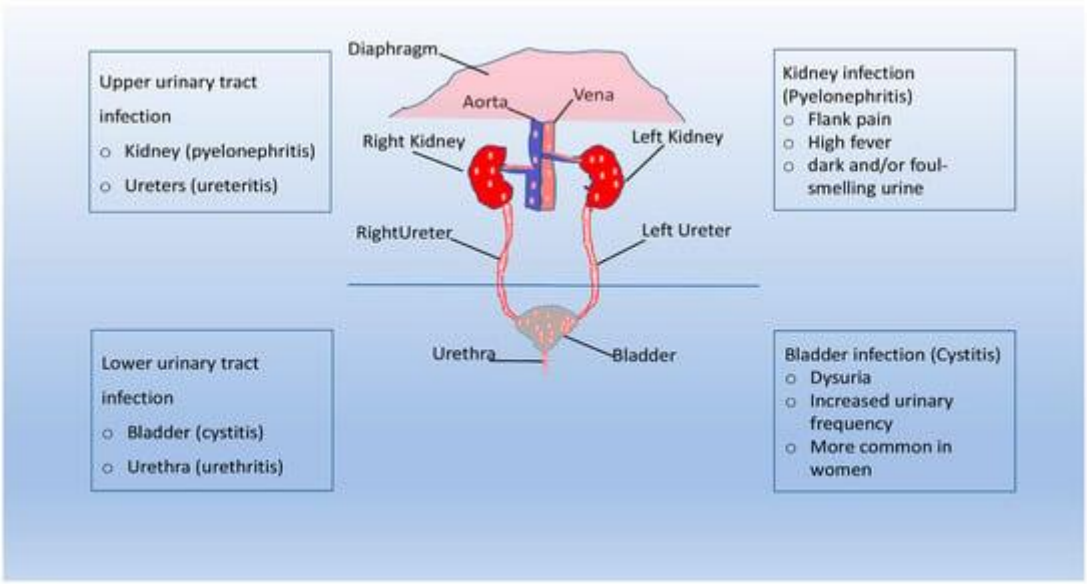

Figure 1.2: UTI based on the location.

UTI start when uropathogens colonize the urethra and subsequently the bladder through the action of specific adhesins. If the bacteria are able to evade the immune system, they begin to multiply and biofilms form. Bacteria can reach the kidney from the lower urinary tract, and UTI can evolve into bacteremia. In complicated UTI, uropathogens are usually able to bind to the catheter and multiply due to the protection of the biofilm. If left untreated, the infection can progress to pyelonephritis and bacteraemia (Mancuso et al., 2023) (**Figure 1.2**).

The illustration above provides a schematic overview of the urinary system and correlates anatomical sites with respective infection types and symptoms. On the right side, “Bladder infection (Cystitis)” is linked to dysuria, increased urinary frequency, and higher prevalence in women— consistent with epidemiological data showing that approximately half of all women will at least one UTI episode in their lifetime (Thamer Hadi & M. Jayashankar, 2022). Similarly, “Kidney infection (Pyelonephritis)” is associated with flank pain, high fever, and dark or foul-smelling urine, reinforcing the clinical differentiation between lower and upper UTI.

It is important to distinguish symptomatic bacteriuria from asymptomatic bacteriuria, the latter defined by the presence of ≥10^5 colony-forming units (CFU)/mL of urine without signs or symptoms of infection. Asymptomatic cases generally do not require treatment except in

specific populations such as pregnant women or those undergoing urological procedures, where intervention reduces the risk of complication (Mancuso et al., 2023).

Recurrent UTI affect up to 25-30% of women after an initial episode and may manifest with similar symptom profiles, though some individuals report milder or atypical presentations over time. Diagnostic accuracy relies on integrating patient reported symptoms with objective findings such as positive nitrite and leukocyte esterase tests on dipstick urinalysis, supported by culture when necessary. Misinterpretation of non-specific symptoms can lead to overdiagnosis and inappropriate antibiotic use, contributing to the growing challenge of antimicrobial resistance among uropathogens (Zhou et al., 2023).

1.2 Pathophysiology and Pathogenesis of UTI

The pathophysiology of UTI depends on the classification of UTI based on the site of infection and presence of leading conditions for infection (Mancuso, et. al., 2023) (**Figure 1.3**). Uncomplicated UTI or cystitis refers to the most common and recurring bacterial infection in the lower urinary tract resulting in urethritis, bladder inflammation after residing in the intestine (Bono & Leslie, 2025). Uncomplicated UTI occurs in healthy individuals, having no other comorbidities (such as- diabetes, renal failure, presence of calculi, obstructive uropathy, and history of urologic surgery, urinary retention, pregnancy, or immunocompromised state) or unusual structure (Mancuso, et. al., 2023). Enteric coliforms causing UTI populate in periurethral vaginal introitus. While ascending from urethra to bladder, the bacteria invade bladder mucosal wall that triggers the inflammatory reaction called cystitis (Bono & Leslie, 2025). Repeated injury and invasion to the mucosal wall is a major predisposing factor for recurring UTI. Complicated UTI poses higher risk of treatment failure and can occur to people suffering from other functional and structural irregularities in the urinary tract. The site of infection causes the symptoms to differ for patients suffering from UTI. Upper UTI, also known as Pyelonephritis, usually presents high fever and lower pain (Walsh & Collyns, 2017). Lower UTI brings dysuria, haematuria and tenderness in the suprapubic region.

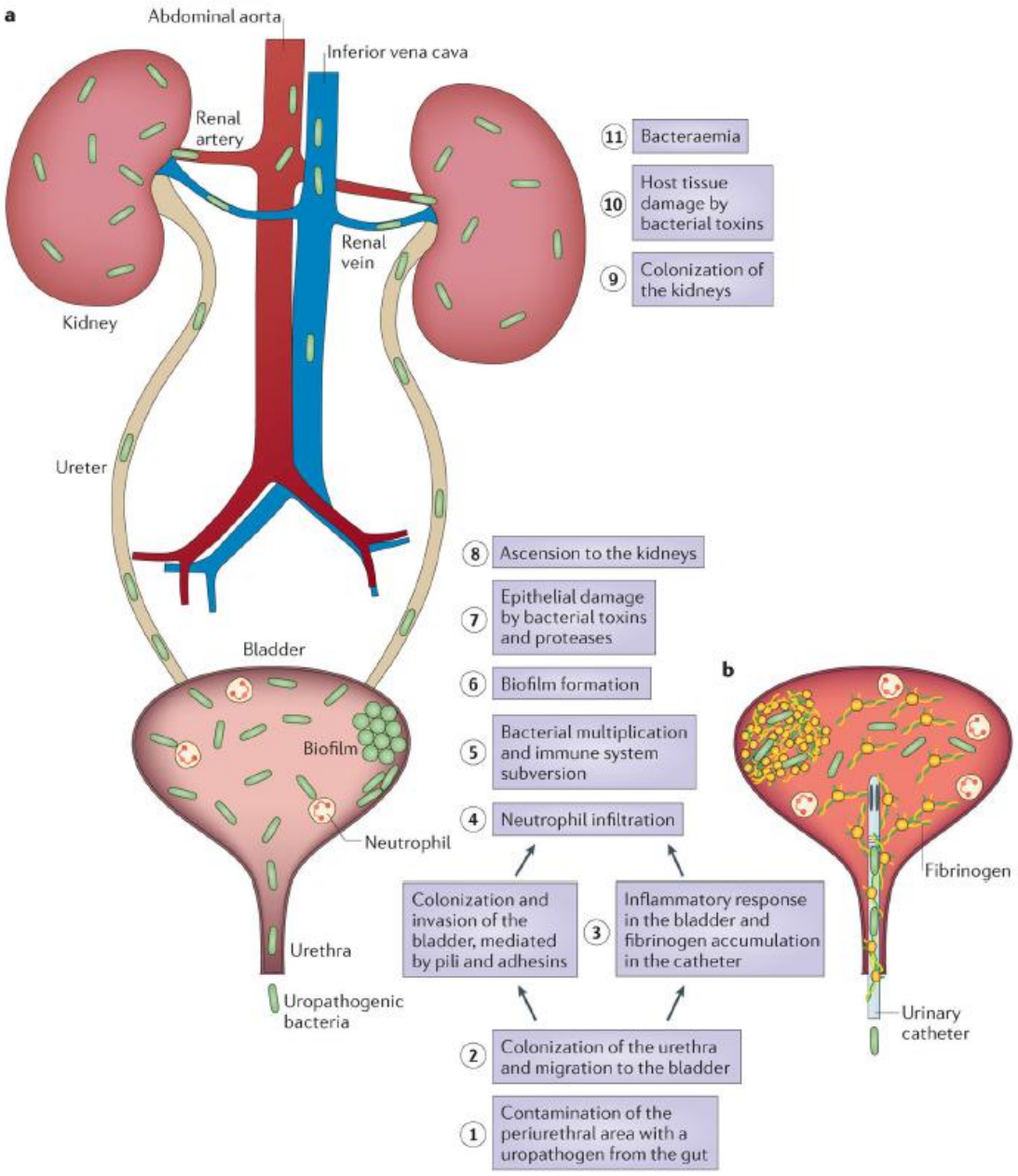

Figure 1.3: Pathogenesis of UTI*;* (a) uUTI, here, 1. Contamination in periurethral region and colonization, 2. Migration to bladder, 3. Expression of adhesins and pili and colonization of umbrella cells, 4. Neutrophil infiltration, 5. Bacterial multiplication, 6. Biofilm formation, 7. Toxins and proteases perform host cell damage, 8. Ascend to kidney, 9. Kidney colonization, 10. Host tissue damage, 11. Cross tubular epithelial barrier. (b) cUTI, compromised bladder caused by catheterization (Flores-Mireles et al., 2015).

1.3 Diagnosis of UTI

Diagnosis of UTI includes evaluating symptoms, conducting a urinalysis and confirming with urine culture. Molecular diagnosis can also be done for rapid results.

1.3.1 Standard Urine Culture (SUC)

Standard urine culture is known as the "Gold Standard" of diagnosis of UTI, which follows a 24hour incubation time to evaluate the presence of bacteria in urine samples based on the growth on selective media like MacConkey, Blood agar (Moreland, et. al., 2024). Though this method allows growth of fast-growing, facultative gram positive and negative anaerobes, it often can lead to misdiagnosis as SUC can rarely detect fastidious or slow-growing microbes because of the fast-growing gram (Moreland, et. al., 2024). Only 1µL urine is plated in the SUC method (Price et al.,2016).

1.3.2 Enhanced Quantitative Urine Culture (EQUC)

The extended version of SUC that utilizes different conditions and 100 times more urine on different media (BAP, MAC) with 48 hours incubation time is known as the Enhanced Quantitative Urine Culture method (Moreland, et. al., 2024). EQUC allows the growth of slow-growing microbes that are anaerobic, fastidious and microaerophilic that are undetected by SUC (Deen, et. al., 2023). This enables EQUC to detect a variety of clinically crucial microbes that are significant for diagnostic and therapeutic purposes.

1.3.3 Molecular Diagnosis

Molecular diagnosis of UTI includes nucleic acid amplification tests for directly detecting bacterial DNA/ RNA in urine (Szlachta-McGinn, et al., 2022). This provides a significant advantage as there is no prolonged culture time, highly sensitive against fastidious organisms and can successfully detect anaerobic pathogens (Baiken, et al., 2025). Multiplex PCR can allow detection of 26 different uropathogens including *Escherichia coli, Klebsiella pneumoniae, Enterococcus faecalis, Pseudomonas aeruginosa* (Leech et al., 2025). 49 antimicrobial resistance (AMR) markers like- *vanA, mecA* can be detected directly from unprocessed urine within an hour (Neopane, et al., 2022). Clinical studies show that multiplex PCR has 94.3% sensitivity and 98.7% specificity compared to SUC for resistance gene identification (Harris, et al., 2023).

Quantitative Multiplex PCR (q-MPCR) provides clinical validity by reporting pathogen load in genomic copies per milliliter that correlates with colony forming units (CFU/mL) from SUC (Festa, et al,., 2023). Next Generation Sequencing (NGS) provides extensive profiling of polymicrobial communities associated with recurrent UTI (Jaworska, et al., 2023).

1.4 Risk Factors of UTI

There are several factors that can contribute and influence the impact and recurrence of UTI. The factors are mentioned in the table below. Females are more prone to UTI due to their shorter urethra. Besides female anatomy, there are other risk factors that contribute to the progression and recurrence of UTI.

Table 1.3: Risk Factors of UTI

Risk Factor	**Possible impact**	**References**
Dehydration	Less urination increases bacterial load	Bono & Leslie, 2025
Diabetes	Impairment of immune system	Bono & Leslie, 2025
Pregnancy	Increased progesterone, increased volume of residual urine, uterine growth, slowed peristalsis	Storme et al., 2019
Menopause	Estrogen deficiency occurs, colonization of *Lactobacilli* decreases, increases pH	Storme et al., 2019
Bacterial Prostitis	Bacterial persistence in urinary tract	Walsh & Collyns, 2017
Hormonal status	Uropathogen binds to receptor in estrogen-dependant stage	Storme et al., 2019
Sexual intercourse, use of spermicide	Changes vaginal pH, bacterial flora	Storme et al., 2019
Family history of UTI, genetic disposition	Increased adherence of bacteria due to the receptors on vaginal epithelial cells	Storme et al., 2019
Blood Group	Blood group O have increased susceptibility as they are non-secretor due to absence of ABO antigens in body fluids.	Storme et al., 2019
Abnormal urinary tract	Reduce protection of unidirectional urine flow	Bono & Leslie, 2025

anatomy, neurologic bladder		
Urinary catheters, prosthetic devices, urethral manipulation	Reason behind formation of biofilm by bacteria like- *P. aeruginosa, P. mirabilis, E. faecalis.*	Walsh & Collyns, 2017
Kidney transplant	Excessive use of immunosuppressive drugs	Bono & Leslie, 2025
Surgery	Patients going through urological, gynecological face increased risk if they are above 60 years, diabetic, overweight	(Baimakhanova, et. al., 2025)

1.5 Antimicrobial Resistance

Carbapenems are known as broad spectrum antibiotics that are more effective against gram-negative organisms and slightly less effective against gram-positive organisms (Mishra, et. al., 2025). The beta-lactam antibiotic kills bacteria cells by binding to the penicillin binding proteins that disrupt the cell wall synthesis. Carbapenems and colistin are known as last resort antibiotics for treating infections caused by *Enterobacteriaceae*, which are able to produce extended-spectrum β-lactamase. A study revealed 6.87% of *Enterobacteriaceae* were carbapenem resistant out of 509 samples collected from a tertiary hospital of North India (Mishra, et. al., 2025).

Another study from Uganda revealed that 93.1% of gram-negative isolates were sensitive to carbapenem, which could be due to less availability of drugs in that area (Tesfa, et. al., 2025). 96.7% of Gram-negative bacteria were resistant to ampicillin. Ampicillin is an aminopenicillin antibiotic that inhibits bacterial cell wall synthesis and causes cell lysis of both gram positive and gram negative (Peechakara, et. al., 2023). 87.9% of Gram-negative isolates were resistant to co-trimoxazole, which is in the sulfonamide class that disrupts the folate synthesis system and inhibits the nucleic acids. The antibiotic is a combination of Sulfamethoxazole, that inhibits the first enzyme of production of folic acid, and Trimethoprim, that ensures the disruption of folic acid production and promotes stronger anti-folate effect (Kemnic & Coleman, 2022). Total 71.4% of the *E. coli* isolates were multidrug resistant (Tesfa, et. al., 2025). A study from Ghana also found sulfonamide-resistant Gram-negative bacteria (Wireko, et. al., 2025). 40% *K. pneumoniae* and 60% of *E. coli* were multidrug resistant. Concerningly high resistance of E. coli and K. pneumoniae against ampicillin, ciprofloxacin and cefixime in a study of a tertiary

center in Nepal (Karna, 2025). Out of the isolates, 27.9% were Multi Drug Resistant (MDR) and 67.5% were Extensively Drug Resistant (XDR).

Table 1.4: Differences of number of urotype isolated from SUC and EQUC

Objective	**Protocol**	**Level of bacteria**	**Percentage of culture positive sample**	**Urotype number**	**Reference**
Analysis of concordance of urinary microbiota observed by 16S rRNA sequencing vs EQUC	SUC : 0.001ml urine cultured in 5% sheep BAP and MAC, aerobically for 24 hours at 35^0C. EQUC: 0.1ml urine cultured in BAP, CA and CNA in 5% CO_2 at 35^0C for 48 hours for aerobic fastidious bacteria BAPs at 35^0C and 30^0C for 48 hours for aerobic bacteria CDC anaerobe 5% sheep BAP under anaerobic conditions 35^0C for 48 hours.	EQUC: 10 CFU/mL	Not specified	Not specified	(Vaughan et al., 2022)
Comparing urobiome changes based on OAB treatment in adult women.	SUC: not performed EQUC: As described by (Vaughan et al., 2022)	EQUC: 10 CFU/mL	Not specified	Not specified	(Halverson et al., 2022)
Assessment of the pediatric urinary microbiota	SUC: Not performed EQUC: As described by (Vaughan et al., 2022)	EQUC: 10 CFU/mL	EQUC: 60%	Not specified	(Storm et al., 2022)
Evaluation of collection and culture methods to identify recurring UTI urinary microbiota of	SUC: As described by (Vaughan et al., 2022) EQUC: 0.1ml urine cultured in BAP, CNA and MAC in 5% CO_2 at 35^0C for 48 hours	SUC and EQUC: varied result in different condition	SUC: Catheterized UTI-associated microbe (39.5%) and	SUC: Catheterized UTI-associated microbe (9) and voided	(Hochstedler et al., 2022)

women	0.1, 0.01, 0.001ml urine cultured in BAP, MAC at 5% CO_2 at 35^0C for 24 and 48 hours for aerobic bacteria 0.1, 0.01 and 0.001ml urine cultured in BAP, CA and CNA in 5% CO_2 at 35^0C for 24 and 48 hours for aerobic fastidious bacteria		voided UTI-associated microbes (57.1%) EQUC: Catheterized UTI-associated microbe (53.5%) and voided UTI-associated microbes (90.5%)	UTI-associated microbes (24) EQUC: Catheterized UTI-associated microbe (24) and voided UTI-associated microbes (56)	
Evaluation of correlation of symptom improvement and urobiome characteristics in adult women prescribed mirabegron for UUI treatment	SUC: Not performed EQUC: As described by (Vaughan et al., 2022)	EQUC: 10 CFU/mL	EQUC: 90%	EQUC: 34 genera	(Halverson et al., 2022)
Assessment of microbiome of women suffering from lower urinary tract symptoms	SUC: Not performed EQUC: As described by (Vaughan et al., 2022)	EQUC: 10 CFU/mL	EQUC: UTI (94.4%), UUI (92.1%), Stress UI (99.5%), IC/PBS (60.2%) and controls (57.5%)	EQUC: Not specified	(Joyce et al., 2022)

Identification of cultivable bacteria in urine of women with interstitial cystitis.	SUC: Not performed EQUC: As described by (Vaughan et al., 2022)	EQUC: 10 CFU/mL	EQUC: Control (40%) and interstitial cystitis (IC)/ painful bladder syndrome (PBS) (49%)	EQUC: Control : 14 species and IC/PBS: 19 species	(Jacobs et al., 2021)
Detection of UTI in women by Standard versus Expanded culture	SUC: As described by (Vaughan et al., 2022) EQUC: 0.1mL urine cultured in BAP, MAC, CNA in CO_2 at 37^0C for 48 hours.	SUC: 10^3 CFU/mL EQUC: 10 CFU/mL	SUC: 63% EQUC: 74%	EQUC: 28 genera	(Barnes et al., 2021)
Detection of urinary microbiome in postmenopausal women with recurrent UTI	SUC and EQUC: As described by (Vaughan et al., 2022)	SUC: 10^3 CFU/mL EQUC: 10 CFU/mL	SUC: No growth EQUC: 59.40%	SUC: No bacteria detected EQUC: Not specified	(Vaughan et al., 2021)
Determination of correlation of clinical profiles and urobiome composition in women	SUC and EQUC: As described by (Hochstedler et al., 2022)	SUC and EQUC: As described by (Hochstedler et al., 2022)	SUC, EQUC: not specified	SUC, EQUC: not specified	(Burnett et al., 2021)
Evaluation of the urine sampling to detect the male urinary microbiota	EQUC: 0.1mL urine cultured in Columbia BA at 37^0C for 48 hours, CBA at 30^0C for 48 hours, CBA at 37^0C for 48 hours in 5% CO_2, Schaedler BA at 37^0C for 5 days in campy gas mixture, then CBA at 37^0C for 48 hours,	EQUC: 10 CFU/mL	EQUC: 97% (first-catch voided urine), 87% (mid-stream voided), 13% (catheterized)	EQUC: 13 genera	(Hrbacek et al., 2021)
Assessment of male bladder	SUC: As described by (Hochstedler et	SUC: 10^3	SUC: Not	SUC:Not	(Bajic et al.,

microbiome related to lower urinary tract symptoms	al., 2022) EQUC: 0.1mL urine cultured in BAP, CNA, CA, BA in aerobic, anaerobic and CO_2 conditions at 35^0C for 48 hours.	CFU/mL EQUC: 10 CFU/mL	specified EQUC: 96% (voided urine specimen), 29% (catheterized urine specimen)	specified EQUC: 37 genera	2020)
Assessment of bladder bacterial diversity in continent and incontinent women	SUC and EQUC: As described by (Vaughan et al., 2022)	SUC: 10^3 CFU/mL EQUC: 10 CFU/mL	SUC: No growth EQUC: Control (57%), UUI (81%), stress UI (86%)	SUC: No bacteria detected EQUC: Not specified	(Price et al., 2020a)
Detection of underdiagnosed pediatric UTI	SUC: As described by (Hochstedler et al., 2022) EQUC: 0.1, 0.01, 0.001ml urine cultured in BAP, MAC at 5% CO_2 at 35^0C for 24 and 48 hours for aerobic bacteria	SUC, EQUC: Different varying result per condition	SUC: 12.8% EQUC: 16.15%	SUC: 8 species EQUC: 10 species	(Thapaliya et al., 2020)
Comparing between urethral microbiota and bladder urinary microbiota present in lower urinary tract symptom	SUC: Not performed EQUC: 0.1ml catheterized/ periurethral/ urethral urine cultured into diverse types of media in different conditions at 35^0C for 48 hours.	EQUC: 10 CFU/mL (catheterized), 100 CFU/mL for other samples	EQUC: not specified	EQUC: 35 genera	Chen et al., (2020)
Evaluation of temporal dynamics of adult female lower urinary tract	SUC: not performed EQUC: 0.1mL urine cultured in BAP, CNA, CDC in aerobic, anaerobic and	EQUC: Not specified	EQUC: Not specified	EQUC: Not specified	(Price et al., 2020b)

microbiota	CO_2 conditions at 35^0C for 48 hours.				
Comparison of Standard urine culture to Peezy midstream device in women	SUC: not performed EQUC: 0.1mL urine cultured in BAP, CA, CNA, CDC in aerobic, anaerobic and CO_2 conditions at 35^0C for 48 hours.	EQUC: 10 CFU/mL	EQUC: Not specified	EQUC: Not specified	(Southworth et al., 2019)
Culturing and genome sequencing of female bladder bacteria	SUC and EQUC: As described by (Hochstedler et al., 2022)	SUC: 10^3 CFU/mL EQUC: 10 CFU/mL	SUC: not specified EQUC: 66.4%	SUC: not specified EQUC: 36 genera and 78 species	(Thomas-White et al., 2018)
Detection of maternal bladder microbiota	SUC: As described by (Vaughan et al., 2022) EQUC: 0.1mL urine cultured in BAP, CA, CNA in aerobic, anaerobic and CO_2 conditions at 35^0C for 48 hours.	SUC: 10^3 CFU/mL EQUC: 10 CFU/mL	SUC: no growth EQUC: 66.7%	SUC: No bacteria EQUC: 34 genera	(Jacobs et al., 2017)
Assessment of urinary symptoms and their associations with UTI in urogynecologic patients	SUC: As described by (Vaughan et al., 2022) EQUC: 0.1, 0.01, 0.001ml urine cultured in BAP, CNA and MAC in 5% CO_2 at 35^0C for 24 hours 0.1, 0.01, 0.001ml urine cultured in BAP, MAC at 5% CO_2 at 35^0C for 24 and 48 hours for aerobic bacteria 0.1, 0.01 and 0.001ml urine cultured in BAP, CA and CNA in 5% CO_2 at 35^0C for 24 and 48 hours for aerobic fastidious bacteria	SUC: 10^3 CFU/mL EQUC: 10 CFU/mL	SUC: 38% EQUC: 93%	SUC: 11 species EQUC: 36 genera and 98 species	(Dune et al., 2017)
Detection of	SUC and EQUC: As described by	SUC: 10^3	SUC: 7.88%	SUC: Not	(Thomas-

association of the characteristics of microbiota with clinically relevant treatment response to oral UUI medication	(Vaughan et al., 2022)	CFU/mL EQUC: 10 CFU/mL	EQUC: 81.3%	specified EQUC: Control (36) and UUI (80)	White et al., 2016)
Assessment of enhanced techniques to improve identification of clinically relevant microorganisms from female with and without UTI	SUC: As described by (Vaughan et al., 2022) EQUC: 0.1, 0.01, 0.001ml urine cultured in BAP, CNA and MAC in 5% CO_2 at 35^0C for 24 hours 0.1, 0.01, 0.001ml urine cultured in BAP, MAC at 5% CO_2 at 35^0C for 24 and 48 hours for aerobic bacteria 0.1, 0.01 and 0.001ml urine cultured in BAP, CA and CNA in 5% CO_2 at 35^0C for 24 and 48 hours for aerobic fastidious bacteria For microaerophilic and anaerobic, 0.1, 0.01 and 0.001ml urine into two CDC anaerobe 5% sheep BAP in campy gas mixture.	SUC and EQUC: varied result per condition	SUC: 33% EQUC: 93%	SUC: not specified EQUC: Non UTI (75), UTI (66)	(Price et al., 2016)
Comparison of female urinary microbiome with and without urgency urinary incontinence (UUI)	SUC and EQUC: As described by (Vaughan et al., 2022)	SUC: 10^3 CFU/mL EQUC: 10 CFU/mL	SUC: 7.78% EQUC: 78.9%	SUC: not specified EQUC: 36 genera	(Pearce et al., 2014)
Detection of cultivable bacteria in adult females with or without overactive bladder	SUC: As described by (Vaughan et al., 2022) EQUC: 0.1, 0.01, 0.001ml urine cultured in BAP, CNA and MAC in 5%	SUC: 10^3 CFU/mL EQUC: 10 CFU/mL	SUC: 6.15% EQUC: 80%	SUC: Not specified EQUC: 35 genera and	(Hilt et al., 2014)

(OAB)	CO_2 at 35^0C for 24 hours 0.1, 0.01, 0.001ml urine cultured in BAP, MAC at 5% CO_2 at 35^0C for 24 and 48 hours for aerobic bacteria 0.1, 0.01 and 0.001ml urine cultured in BAP, CA and CNA in 5% CO_2 at 35^0C for 24 and 48 hours for aerobic fastidious bacteria For microaerophilic and anaerobic, 0.1, 0.01 and 0.001ml urine into two CDC anaerobe 5% sheep BAP in campy gas mixture.			85 species	

*BAP- Blood Agar Plate, MAC- MacConkey Agar, CFU- Colony Forming Unit, CNA- Columbia Nalidixic Acid Agar, CDC- Centers for Disease Control, OAB- Over Active Bladder, UUI- Urgency Urinary Incontinence.

1.6 Aims and Objectives

1. To determine the bacteria load in urine samples collected from UTI patients and healthy controls.
2. To isolate the bacteria load in urine samples collected from UTI patients and healthy controls.
3. To assess the efficiency of the EQUC method in isolating more accurate and diverse bacterial colonies than SUC method.
4. To determine the presence of *E. coli* and *K. pneumoniae* in specimens from both UTI patients and healthy controls.
5. To evaluate antibiotic resistance of isolated *E. coli* and *K. pneumoniae*.
6. To determine possible significant differences between antibiotic resistance of *E. coli* and *K. pneumoniae* from both UTI patients and healthy controls, isolated from SUC and EQUC method.

Chapter 2:

Methodology

2.1 Study Design

This study was conducted as a case-control design. Clinically suspected UTI patients were enrolled as the cases, while apparently healthy individuals without UTI symptoms served as controls. Midstream urine samples collected aseptically by the participants were processed for bacterial culture, and identification, and antibiotic susceptibility testing (**Figure 2.1**).

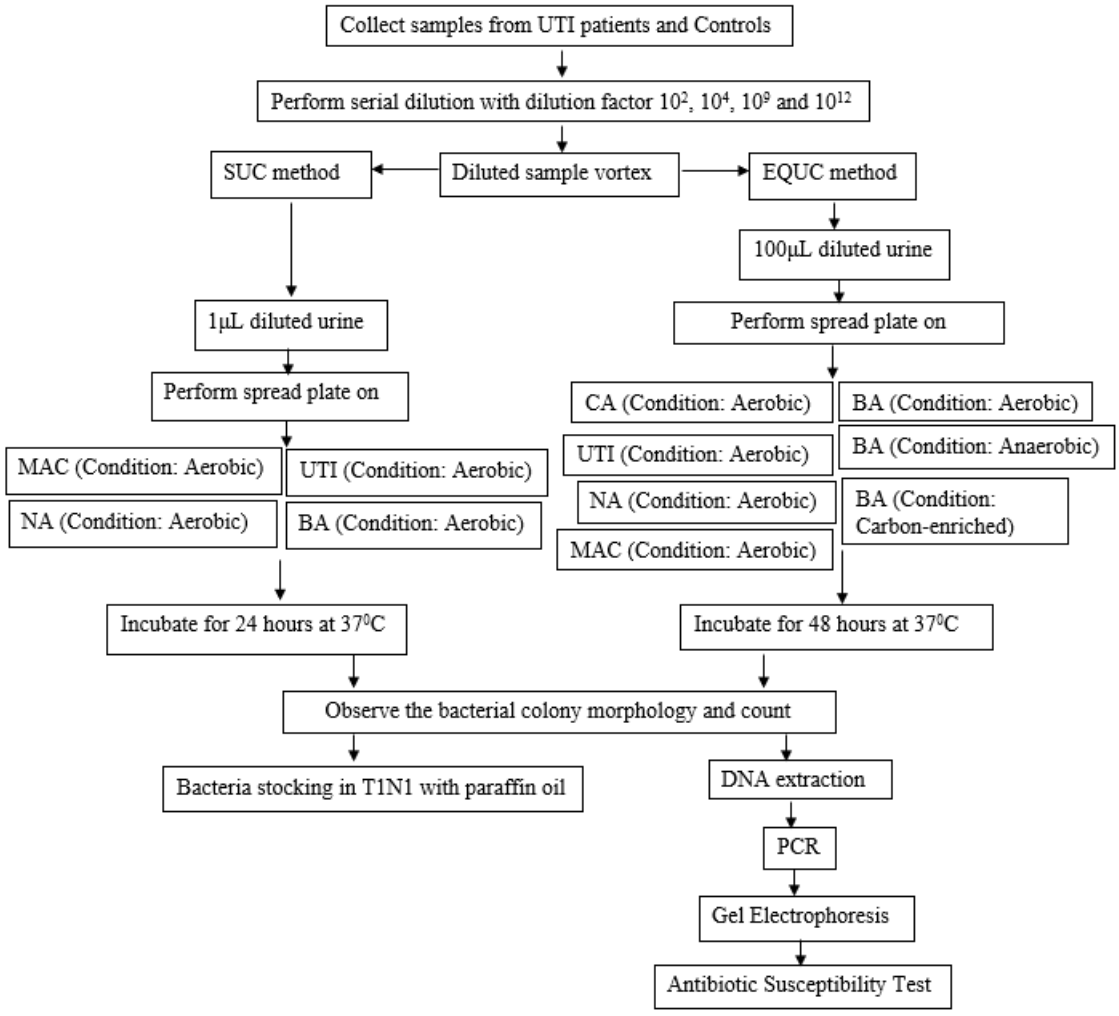

Figure 2.1: A flow diagram of the study design

2.2 Selection of Study Participants

For the case group, urine samples from UTI positive patients' were collected from Universal Medical College and Hospital. Controls were recruited from BRAC University. Selection criteria for the study participants are given in **Table 2.1**.

Table 2.1: Inclusion and Exclusion Criteria

Participant	**Inclusion criteria**	**Exclusion criteria**
Cases	Age- Above 18 Both male and female Individuals with the symptoms of UTI	Age- Below 18 Pregnancy Individuals on antibiotics
Controls	Age- Above 18 Both male and female	Age- Below 18 Individuals with the symptoms of UTI Pregnancy Individuals on antibiotics

2.3 Consent and Ethical issues

Written informed consent was taken from all participants prior to interview and sample collection for the control group. Consent was taken from the corresponding hospital for using samples from the cases.

2.4 Sample Size

A total of 40 samples were collected from Dhaka City for this study. Wherefrom, 20 samples were randomly collected from students and staff of BRAC University for Control. Meanwhile, 20 samples were collected from UTI patients of Universal Medical College & Hospital Ltd. for Case.

2.5 Sample Collection

A midstream clean catch (MSCC) urine sample is the recommended method to obtain a urine culture to confirm a diagnosis of UTI in symptomatic adults ((Lough et al., 2019). MSCC method is a safe and non-invasive urine sampling technique designed to obtain a more accurate urine specimen within the bladder by reducing contamination from urethral and external genital flora (Sinawe & Casadesus, 2023). This method includes discarding the initial portion of urine flow and collecting the middle portion of the stream in a sterile container, thereby minimizing the introduction of skin or vaginal bacteria into the urine sample (Llor et al., 2022).

Individual adults were instructed to provide around 10-30 mL of urine samples following the MSCC method in a sterile urine container maintaining cleanliness. The urine sample container was labelled with sample number, age, sex and date of collection. For sanitary reasons, it was recommended that the urine sample container be enclosed in a clear plastic bag with adhesive. Then, the urine samples were transported using a portable cooler box with ice or an insulated cooler box to keep the sample cool during transportation. Finally, the urine samples were delivered to the laboratory as soon as possible after completion of collection, preferably within 1-2 hours. Otherwise, the urine samples were refrigerated at 4-8 °C. Urine samples refrigerated for more than 24 hours cannot be used for ‘Culture’ and were rejected by the laboratory.

2.5.1 Urine Collection Pot

For urine collection, red lids, leak-proof urine collecting containers were used. There were no preservatives included in the pot. The pots were properly labelled with the patient's age, gender and serial number.

Figure 2.2: Urine collection pot

2.5.2 Protocols for Sample Processing

After the urine samples are transported to the laboratory for processing, each urine sample container with urine specimen is vortexed to homogenize the urine sample. From each urine sample, 1 mL of urine was transferred to 9 mL of saline and vortexed, then labelled as 10^-1. Similarly, 1 mL of diluted sample from 10^-1 is transferred to 9 mL of saline to be labelled as 10^-2, and further diluted to desired dilution factor by serial dilution for solution of EQUC. Serial dilution is a step-wise series of dilution which involves diluting the concentration of sample through a series of standard volumes of sterile diluent, e.g. distilled water or 0.9 % saline, to obtain an incubated plate with an easily countable number of colonies (*Basic Practical Microbiology: A Manual*, 2016). Meanwhile, 10 µL of urine is diluted in 990 µL of PBS and vortexed to prepare the solution for SUC.

Afterwards, 100 µL sample solution is individually inoculated from both SUC and EQUC solution, and spread evenly over different culture mediums at a time by spread plate method. Spread plate method is a plating technique where a sample is evenly distributed over the surface of solid culture medium by a glass rod spreader to enumerate viable bacterial colonies (Sanders, 2012). For SUC, diluted sample solution is cultured over NA, MAC, HiCrome UTI and BA medium. On the other hand, diluted sample solutions for EQUC are cultured over NA, MAC, HiCrome UTI, CA and BA medium. Last but not least, 100 µL of raw sample was inoculated on NA medium for both SUC and EQUC.

Finally, the cultured plates were incubated for optimal time and conditions as per protocol. For SUC, all NA, MAC, HiCrome UTI and BA plates were incubated under aerobic conditions at 37°C for 24 hours. Meanwhile, all NA, MAC, HiCrome UTI, CA and BA plates for EQUC were incubated under aerobic conditions at 37°C for 48 hours. However, two BA plates for EQUC were incubated under different conditions: one BA plate incubated under anaerobic condition at 37°C for 48 hours, and another incubated under carbon condition at 37°C for 48 hours.

Table 2.2: Protocol for Sample Processing

Method	Description of Protocol			
	Volume of Urine	**Media**	**Conditions**	**Incubation Time**
Standard Urine Culture	1 µL of urine sample + 99 µL of PBS = 100 µL/ 0.1 mL for spreading	Blood Agar	Aerobic 37°C	24 hours
		MacConkey Agar	Aerobic 37°C	
		Nutrient Agar	Aerobic 37°C	
		HiCrome UTI Agar	Aerobic 37°C	
Expanded Quantitative Urine Culture	100 µL/ 0.1 mL for spreading	Blood Agar	Aerobic 37°C	48 hours
		Blood Agar (carbon incubated)	5% CO2 37°C	
		Blood Agar (Anaerobic)	Anaerobic 37°C	
		Chocolate Agar	5% CO2 37°C	
		MacConkey Agar	Aerobic 37°C	
		NA	Aerobic 37°C	
		HiCrome UTI	Aerobic 37°C	

2.6 Bacterial Stocking by T1N1

Differential bacteria were subcultured on NA by streaking to obtain isolated colonies. The subcultured plates were incubated at 37°C for 24 hours. Meanwhile, T1N1 medium is prepared by dissolving tryptone, NaCl and agar in distilled water, followed by sterilization through autoclaving at 121°C for 15 minutes. After sterilization, 3 mL of T1N1 medium was transferred in each 4 mL vials. All T1N1 vials were kept vertically and cooled down until T1N1 medium solidifies. After T1N1 medium is prepared, a pure bacterial colony isolated from subculturing was aseptically inoculated by a needle and stabbed three times in a T1N1 vial. Afterwards, all the stabbed vials were kept vertically in a beaker and were incubated at 37°C for 24 hours. When significant bacterial growth was observed, 200-400 µL of sterile paraffin oil was transferred into the vials to cover the surface of T1N1 medium and bacterial growth. Finally, the vials were sealed with parafilm and stored vertically.

2.7 Suspected Bacteria Identification

After the isolation and stocking of bacterial colonies, these were identified by PCR and Gel Electrophoresis methods.

2.7.1 DNA Extraction: Boiling Method

DNA extraction by boiling method, specifically using TE buffer, is a refined thermal lysis technique designed to isolate nucleic acids while maintaining a stable pH and protecting the DNA from enzymatic degradation, where the DNA templates are later prepared for Polymerase Chain Reaction (PCR) (Boza et al., 2024).

A loopful of pure isolated bacterial colonies were resuspended in a sterile microcentrifuge tube with 200 µL of 1X TE buffer to remove exogenous inhibitors, such extracellular polysaccharide or culture media protein. Here, the TE buffer acts as both the lysis medium and DNA stabilization medium (Shin et al., 2021). Following a centrifugation for 10 minutes at 13000 RPM, after which the supernatant containing the impurities was discarded, while the cell pellet was resuspended and re-pipetted with 200 µL of 1X TE buffer. This resuspension was essential to ensure that the cells were uniformly distributed in the buffering medium, allowing for even heat distribution during the subsequent lysis step (Queipo-OrtuñO et al., 2008).

The core of the procedure involves submerging the microcentrifuge tubes in a dry heating block or water bath for 10 minutes at 100 ℃. This intense thermal energy causes the rapid expansion of intracellular fluids and denaturation lipid bilayer, leading to the physical rupture of cell walls and cell membranes, while intracellular components including genomic DNA were released into the TE buffer (Shwani et al., 2023). As there were no detergents in the buffer, some proteins and cell debris may remain intact, but the DNA becomes solubilized in the buffer (Shin et al., 2021).

After boiling, the DNA sample is immediately subjected to thermal shock by being placed on ice for approximately 5 minutes. This rapid cooling facilitates the precipitation of denatured proteins and cellular debris, while the DNA remains soluble in TE buffer (Yamagishi et al., 2016). The mixture is then centrifuged at 13000 RPM for 10 minutes to pellet large debris. The supernatant, which contains the extracted DNA dissolved in the TE buffer, was aseptically transferred to a new microcentrifuge tube and can be used directly for molecular analysis (Yamagishi et al., 2016). Depending on downstream requirements, the DNA extract can be used directly in PCR or or stored at -20 ℃ or lower for later analysis (Shin et al., 2021).

2.7.2. Polymerase Chain Reaction (PCR)

PCR is a highly sensitive molecular technique used for bacterial identification through amplification of specific DNA sequence, most commonly targets conserved genetic markers such as 16S ribosomal RNA (rRNA) gene, which contains both highly conserved regions allowing universal amplification across bacteria and hypervariable regions enabling discrimination at genus or species level (Yadav et al., 2024; Janda & Abbott, 2007).

Successful PCR amplification depends on the precise formulation of PCR reaction mix and each component of PCR mix has a specific and essential role in ensuring specificity, efficiency and reproducibility of bacterial DNA amplification. Commercial PCR master mix combines polymerase, buffer, dNTPs and magnesium ions into a single solution to improve reproducibility and reduce error.

A thermosable polymerase, typically Taq DNA polymerase, is responsible for synthesizing new DNA strands during PCR, while its thermostability allows it to remain active despite repeated exposure to high denaturation temperature (Khehra et al., 2025).

The PCR buffer maintains optimal pH and ionic strength, ensuring consistent enzyme performance throughout the thermal cycle, while Tris-HCl and monovalent ions enhance primer annealing and polymerase stability (Mohtar et al., 2024).

Deoxynucleotide Triphosphates (dNTPs) is a mix of dATPs, dCTPs, dGTPs and dTTPs which serve as the building blocks for new DNA strand synthesis, here balanced concentration of dNTPs are essential to maintain efficient polymerase activity and prevent incorporation errors that may compromise downstream sequence analysis (Yadav et al., 2024).
Magnesium ions, usually supplied as magnesium chloride, acts as essential cofactors for DNA polymerase activity by stabilizing the interaction between primer and template DNA as well as between polymerase and dNTPs. Suboptimal magnesium concentrations can either reduce amplification efficiency or increase non-specific binding, making magnesium concentration a critical parameter in PCR optimization (Khehra et al., 2025).

Table 2.3: Primer details for K. pneumoniae and E. coli

Name	**Sequence**	**Gene**	**Product size**	**Reference**
ECO-F	5'GACCTCGGTTTAGTTCACAGA3'	16SrDNA	585bp	(Punom, et al., 2019)
ECO-R	5'CACCACGCTGACGCTGACCA3'			
K.pneumoniae-F	5'-TGCAGATAATTCACGCCCAG-3'	Diguanylate -cyclase	130bp	(Mahmudunnabi, et al., 2017)
K.pneumoniae-R	5'-ACCCGCTGGACGCCAT-3'			

Primer are short single-stranded oligonucleotides which define the target DNA region to be amplified, often targeting conserved regions of the 16S rRNA gene, flanking variable regions that provide taxonomic information. Primer specificity and concentration are critical, as improper primer design can lead to non-specific amplification or false negative results (Janda & Abbott, 2007).

The template DNA consists of extracted bacterial genomic DNA containing the target sequence. DNA purity significantly affects PCR performance, as inhibitors commonly found in clinical or environmental samples can interfere with enzyme activity amplification efficiency (Yadav et al., 2024).

Nuclease-free water is primarily used to adjust the final reaction volume while ensuring that no contaminating enzymes interfere with DNA integrity or amplification efficiency. Unlike ordinary distilled or deionized water, nuclease-free water is specifically treated and quality controlled to be free from DNase, RNase, protease, microbial DNA, all of which could compromise PCR performance (Khehra et al., 2025).

Table 2.4: Amount of PCR Mix Components in 25 µL Volume for *K. pneumoniae and E. coli*

Amount of PCR Mix Components in 25 µL Volume		
Components	***Klebsiella pneumoniae* (in µL)**	***Escherichia coli* (in µL)**
Master Mix	12.5	12.5
Forward Primer	1.0	0.25
Reverse Primer	1.0	0.25
Primer Concentration	10 picomole/mL	2.5 picomole/mL
Template DNA	2.0	2.0
Nuclease-free Water	8.5	10

Five Steps of PCR Cycling Conditions

PCR amplification is achieved through thermal cycling, where controlled temperature changes drive sequential molecular events. A standard PCR protocol for bacterial identification involves five key steps:

The initial denaturation step is performed at a high temperature, typically 94-95 ℃, for several minutes. This step ensures complete separation of double-stranded DNA into single-strands and activates hot-start polymerases when used. Complete denaturation is especially important for complex bacterial genomic DNA, which may contain secondary structures (Khehra et al., 2025).

Each amplification cycle begins with a short denaturation phase approximately 94-98 ℃. During this step, newly synthesized DNA strands from the previous cycle are separated into single-strand, allowing primers to bind in the next step. Repeated denaturation is essential for maintaining exponential amplification throughout cycling (Mohtar et al., 2024).

In the annealing step, the temperature is lowered, usually 50-65 ℃, to allow the primer to hybridize to complementary sequences on the template DNA. The annealing temperature is determined by the primer melting temperature and directly affects amplification specificity. In bacterial identification PCR, precise annealing ensures selective amplification of conserved bacterial gene regions while minimizing non-specific products (Janda & Abbott, 2007).

During extension (elongation), the temperature is raised to approximately 72 ℃, the optimal working temperature for Taq polymerase. The enzyme extends the primer by incorporating dNTPs in the 5'→3' direction, synthesizing new DNA strands complementary to the template. The duration of this step depends on the length of the target sequence and determines the completeness of amplification (Khehra et al., 2025).

After completion of all cycles, a final extension step at 72 ℃ is included to allow full extension of any partially synthesized DNA strands. This step improves yield and ensures uniformity of PCR products, which is particularly important for downstream applications such as sequencing or cloning (Mohtar et al., 2024).

Table 2.5: Thermal Cycling conditions for *K. pneumoniae and E. coli*

Thermal Cycling Conditions						
Steps	***Klebsiella pneumoniae***			***Escherichia coli***		
	Temp. ℃	**Time**	**No. of cycle**	**Temp. ℃**	**Time**	**No. of cycle**
Initial Denaturation	94	4 mins	1	95	5 mins	1
Denaturation	94	1 min	35	94	45 secs	30
Annealing	55	1 min		52	45 secs	
Extension	72	1.5 mins		72	1 min	
Final Extension	72	10 mins	1	72	5 mins	1
Final Storage: 4 ℃ for ∞ holds						

2.8.3. Gel Electrophoresis

Gel electrophoresis is a standard molecular technique used to separate DNA fragments based on size as they migrate through a porous matrix under an applied electric field (Lee et al., 2012). As DNA has a uniform negative charge on its sugar-phosphate backbone, fragments move towards the positively charged electrode during the run, and smaller molecules migrate faster and further than larger molecules through the gel's matrix, enabling size-based separation into distinct bands that can be visualized under UV light (Lee et al., 2012).

Agarose was used to formulate the gel for this gel electrophoresis, since agarose gel forms a three-dimensional network of pores that DNA molecules must navigate during migration. Agarose gel concentration plays a key role in gel electrophoresis, as lower concentrations produce larger pores, facilitating the separation of larger DNA fragments, whereas higher concentrations produce smaller pores that improves resolution of smaller DNA fragments (Lee et al., 2012).

For gel preparation, 1.5% agarose for *K. pneumoniae* and 1.2% *E. coli*, was dissolved in the appropriate amount of 1X TBE buffer by heating. After the agarose gel mixture was cooled moderately, 4 µL of Ethidium Bromide (EtBr) was added to 100 mL of gel mixture. EtBr is the most common reagent used to stain DNA in agarose gel, so when exposed to UV light, electrons in the aromatic ring of the ethidium molecule are activated, which leads to the release of energy (light) as the electrons return to ground state (Lee et al., 2012). After adding EtBr, the gel mixture is poured into the gel apparatus and kept until solidification. After the gel solidifies, the comb is removed from the gel forming wells and gel tray in place in the gel box. An appropriate amount of running buffer (1X TBE) is poured into the gel box till the marked line. Afterwards, the wells in the gel were loaded with appropriate amounts of PCR product according to the size of well. A 50 base pair (bp) ladder was also loaded at the first and last wells, as a 50 bp ladder specifically contains bands spaced every 50 bp in the lower size range, making it particularly useful sizing for smaller PCR products. In gel electrophoresis, a ladder's band serves as a molecular size marker by comparing the migration distance of unknown bands to the ladder's band, thus can estimate the length of sample fragments based on the logarithmic relationship between distance migrated and fragment size (Lee et al., 2012).. Lastly, the lid of the gel box is closed, while the time, voltage and current is set according to the requirements and turned on.

Table 2.6: Gel Electrophoresis Conditions for *K. pneumoniae and E. coli*

Gel Electrophoresis Conditions		
Conditions	***Klebsiella pneumoniae***	***Escherichia coli***
Gel Concentration (%)	1.5	1.2

Ladder (bp)	50	50
Voltage (V)	100	100
Current (mA)	300	300
Time (minutes)	50	50
Band Size (bp)	130	585

2.9. Antimicrobial Susceptibility Test (AST)

An AST is a standardized laboratory procedure in clinical microbiology to determine whether a bacterial pathogen is sensitive or resistant to specific antibiotics, which is an essential information for guiding effective antimicrobial therapy and combating the global rise of antimicrobial resistance by avoiding the use of ineffective drugs and promoting targeted treatment strategies (Khan et al., 2019). AST results can prevent misuse of antibiotics and help tailor treatment to the causative pathogen, improving patient's outcome and slowing the spread of resistant strains (Khan et al., 2019).

One of the most widely used AST methods is the disk diffusion method, often called the Kirby-Bauer disk diffusion test. The procedure of AST typically begins after a bacterial isolate has been obtained and identified from a clinical specimen. A pure culture of the bacteria is grown on solid NA medium. This pure culture is inoculated in 0.9% saline solution and was vortexed to prepare a standardized bacterial suspension, usually adjusted to a turbidity equivalent to a 0.5 McFarland standard to ensure a uniform inoculum density. This standardized suspension is essential for reproducibility and accurate comparisons between tests. For disk diffusion methods, the inoculum is swabbed uniformly across the surface of a Mueller-Hinton agar plate, chosen for its ability to support the growth of non-fastidious bacteria while allowing consistent antibiotic diffusion. Antibiotic-impregnated paper discs are placed on the inoculated agar surface before incubation at a controlled temperature, usually 37 ℃, for about 18-24 hours.

After incubation, zones of inhibited growth around each disc were measured, and results were interpreted by comparing zone diameters to clinical breakpoint defined by standards such as those from the Clinical and Laboratory Standards Institute (CLSI) or the European Committee on Antimicrobial Susceptibility Test (EUCAST). The zone of inhibition is the cornerstone of disk diffusion interpretation. A large zone indicates that the bacteria is more susceptible to the antibiotic, meaning it was unable to grow where sufficient drug concentration exists in the agar, whereas small or absence of zone suggests resistance (Hombach et al., 2013).

Antibiotics used in disk diffusion testing come from various antibiotic classes, each with distinct mechanisms of action and clinical uses. Kanamycin is an aminoglycoside that binds irreversibly to the 30S ribosomal subunits in bacteria, causing mistranslation of mRNA and inhibition of protein synthesis (Dogan et al., 2021). Cefixime is an orally administered third-generation cephalosporin that inhibits cell wall synthesis by binding to penicillin-binding proteins (PBPs) (Qahtani et al., 2025). Cefepime is a fourth-generation cephalosporin with improved stability against many **β**-lactamases and broader gram-negative coverage compared to earlier cephalosporin (Shama et al., 2024). Ampicillin is broad spectrum aminopenicillin, which targets PBPs to disrupt cell wall synthesis, however it develops resistance through **β**-lactamase production (Rahman et al., 2025). Amoxicillin, similar to ampicillin, is susceptible to **β**-lactamase degradation (Rahman et al., 2025). Azithromycin is a macrolide antibiotic which interferes with protein synthesis by binding the 50S ribosomal subunit (Shama et al., 2024). Imipenem is a carbapenem with broad-spectrum activity against gram-negative bacteria, including many strains resistant to other **β**-lactams (Mouanga-Ndzime et al., 2024). Tetracycline is a broad-spectrum protein synthesis inhibitor that binds the 30S ribosomal subunit, preventing attachment of the tRNA to the ribosome (Gunduz & Altun, 2018). Ciprofloxacin is a fluoroquinolone that targets DNA gyrase and topoisomerase IV, blocking DNA replication and transcription (Mouanga-Ndzime et al., 2024) .

Aside from these antibiotics, vancomycin, aztreonam and linezolid were also incorporated, but were invalid against gram-negative bacteria. Vancomycin is a glycopeptide antibiotic that inhibits bacterial cell wall synthesis by binding to the D-Ala-D-Ala terminus of the peptidoglycan precursors, blocking cross-linking and weakening the cell wall, however gram-negative bacteria possess an additional outer membrane composed of lipopolysaccharide (LPS) which acts a protective barrier that large, hydrophilic molecules like vancomycin are unable to

penetrate (Li et al., 2025). Linezolid is a oxazolidinone antibiotic that bind to the 23S rRNA of the 50S ribosomal subunit, blocking the initiation of protein synthesis, but its spectrum does not extend to most gram-negative bacteria due to the outer membrane and efflux systems prevent efficient intracellular accumulation of linezolid (Kaya et al., 2020). Unlike linezolid and vancomycin, aztreonam is actually a **β**-lactam antibiotic that does target gram-negative bacteria by binding to PBPs, especially PBP-3, thereby inhibiting cell wall synthesis, however its spectrum is narrow and focused on aerobic gram-negative bacilli and its effectiveness can be compromised by resistance mechanisms such as extended spectrum **β**-lactamases (ESBLs) and other hydrolyzing enzymes that can inactivate aztreonam (Morroni et al., 2021).

Chapter 3:

Result

3.1 Baseline Characteristics of the Study Participants

A total of 40 samples were collected. 20 were from cases and 20 were from controls. Age criteria for collecting samples were above 18 years. From **Figure 3.1**, it is observed that among the control group, four individuals were aged 18–19 years, while within the age group of 20-29 the number of individuals were 14. Only two participants were from the range 30-39 years. In the case group, five individuals were aged 20–29 years, two individuals aged 40–49 years, five aged 50–59 years, three aged 60–69 years, four aged 70–79 years, and one individual aged 80–89 years.

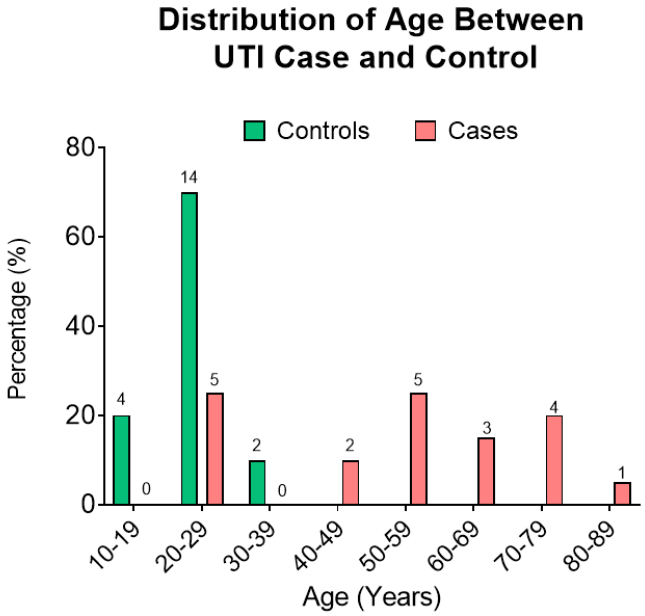

Figure 3.1: Age distribution of the controls and cases

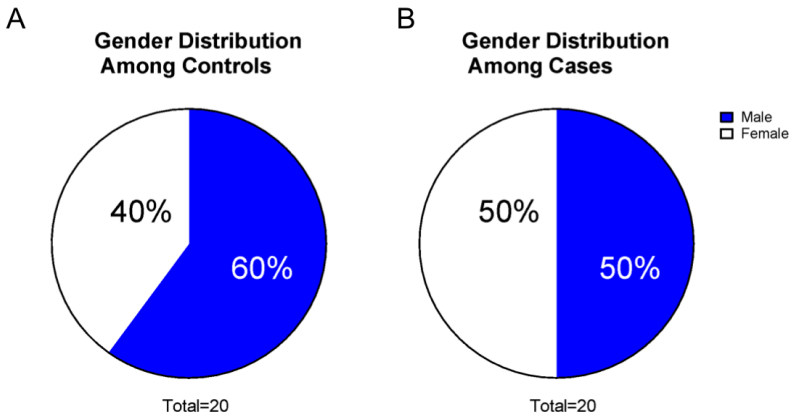

Figure 3.2: Gender distribution among (A) controls and (B) UTI patient

From **Figure 3.2** (A and B), it is observed that 8 (40%) of the controls were female and 12 (60%) of them were males. On the other hand, male vs. female ratio was 1:1.

3.2 Isolation of bacteria

Urine from both controls and cases was diluted and then inoculated onto the culture media. For SUC the desired dilution was further introduced into PBS and then inoculated onto the culture media. For SUC, the specimens were inoculated on Nutrient Agar (A), MacConkey Agar (B), HiChrome UTI agar (C) and BAP agar media (D) (**Figure 3.3**). For EQUC, Nutrient Agar (A), MacConkey Agar (B), HiChrome UTI agar (C) and BAP agar media (D) were used (**Figure 3.4**). Anaerobic incubation was additionally performed for BAP agar media. After incubation, each type of bacterial isolates based on their colony morphology were isolated and preserved.

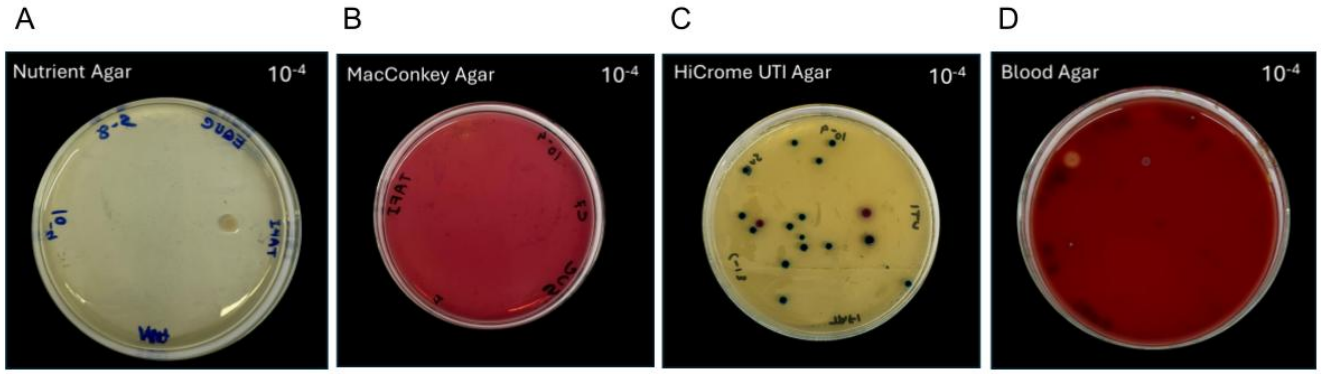

Figure 3.3: Cultured plates (A) Nutrient Agar, (B MacConkey Agar, (C) HiCrome UTI Agar and (D) Blood Agar media after incubation using SUC method.

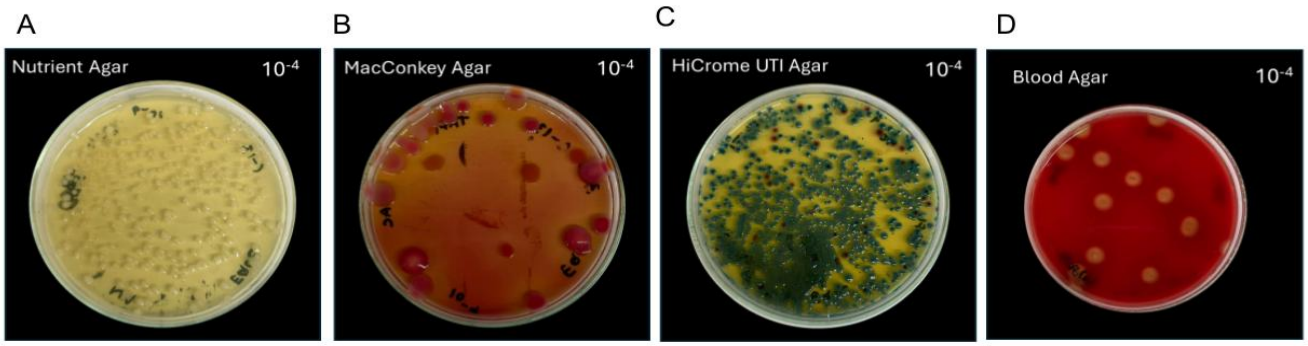

Figure 3.4: Culture plates (A) Nutrient Agar, (B) HiCrome UTI Agar, (C) MacConkey Agar and (D) Blood Agar media after incubation using EQUC method.

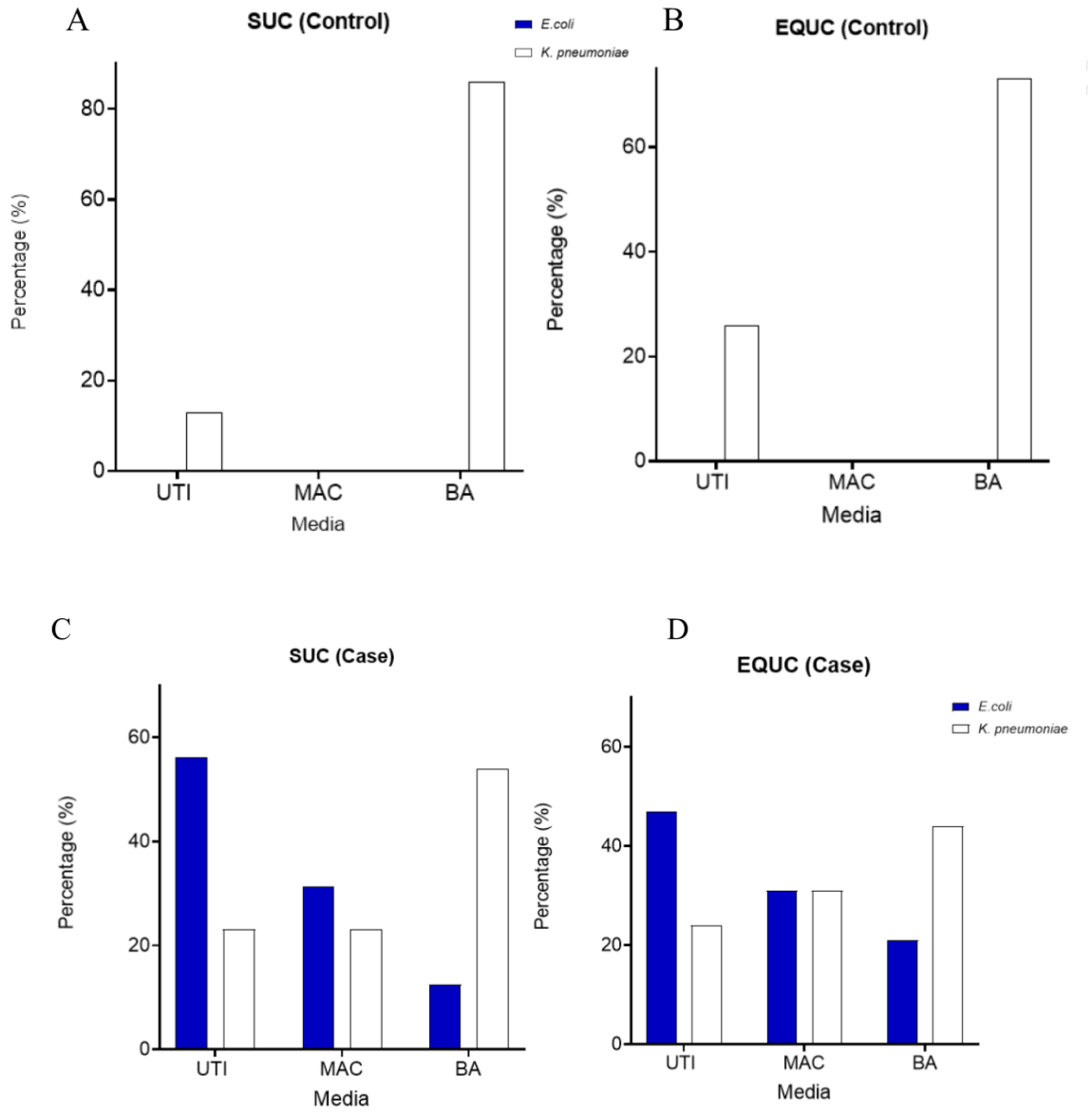

Figure 3.5: Percentage of *Klebsiella pneumoniae* and *Escherichia coli* isolated on different culture media

A media-based comparison (**Figure 3.5**) showed that for controls, 86.7% K. pneumoniae was isolated from BA, 13.3% was isolated from UTI of SUC method. There were no presence of E.coli in controls. Similar result was derived from EQUC, where 73.91% of K. pneumoniae was isolated from BA and 26.09% from UTI. From cases, equal amount of K. pneumoniae was isolated from MAC and UTI, which is 23.08% in SUC. The rest 53.85% was isolated from BA. In Cases, 24.14% K. pneumoniae was isolated from UTI, 31.03% from MAC and 44.83% from BA of EQUC. 56.25% of E. coli was isolated from UTI, 31.25% from MAC and 12.5% from

BA of SUC. And for EQUC, 47.37% was isolated from UTI, 31.58% from MAC and 21.05% from BA.

3.3 Total Aerobic Count

Each of the plates from both SUC and EQUC methods were observed and the number of colonies was counted and recorded. For the control group, from HiCrome UTI agar, the average colony count was 1.56×10^6 with the SUC method and an average count of 4.58×10^4 was detected using the EQUC method. In NA medium, the SUC method yielded an average colony count of 6.35×10^4 and the EQUC method showed an average of 6.71×10^4. No bacterial growth was present on MAC with either of the methods. On BA media, an average count of 1.13×10^6 was produced by SUC method and an average count of 2.21×10^6 were detected by EQUC method. In CA, EQUC produced an average colony count of 7.91×10^5.

For the case group, in HiCrome UTI agar the average number of colonies using SUC method was 1.92×10^5 while using the EQUC method the average count was 2.89×10^5. In NA, the average colony count was 4.26×10^4 using SUC, and in case of EQUC it is 2.37×10^5. On MAC, SUC yielded an average of 1.12×10^5 colonies, whereas EQUC detected 1.7785×10^5 colonies on average. The highest colony counts were observed on BAP. Using the SUC method, the average colony count was 9.03×10^{11}, while the EQUC method showed an average of 3.39×10^{14}. Similarly, for CA medium, which was used only in the EQUC method, yielded an average colony count of 1.42×10^{13}.

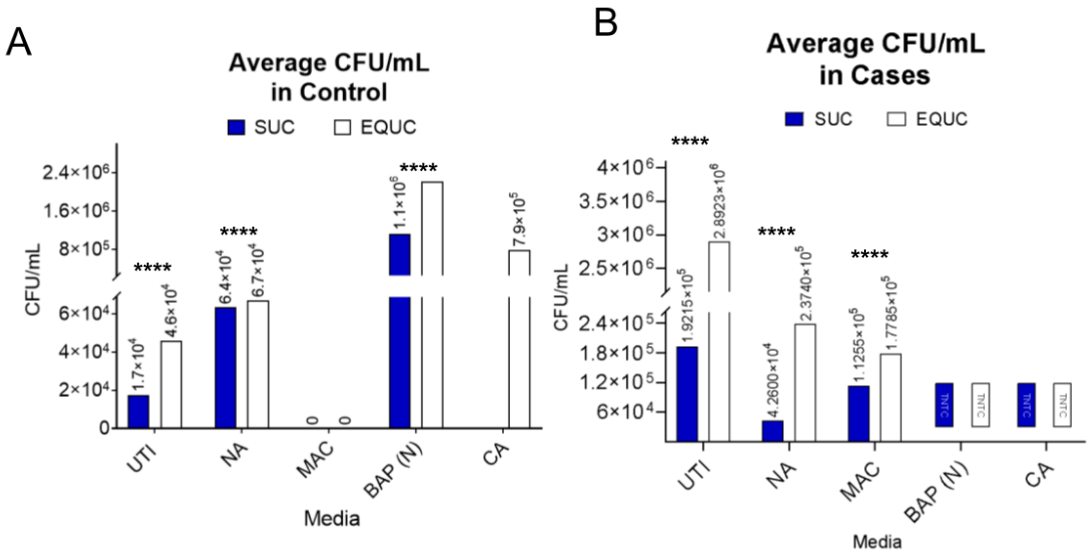

Figure3.6: Average CFU/mL count in (A) Controls (B) Cases (****p-value <0.0001)

From **Figure 3.6 (A)**, it is clear that the highest average CFU/mL is observed on BAP. In the case group **(Figure 3.6)** the highest average CFU/mL was observed UTI. Additionally, in both control and cases EQUC showed more growth than SUC.

3.4 PCR based Identification

Various types of bacteria were isolated from different media used in SUC and EQUC methods. Based on colony morphology and cultural characteristics, two major bacterial species found were *Escherichia coli* and *Klebsiella pneumoniae.* To confirm these bacteria, Polymerase Chain Reaction (PCR) method was used. Isolated *E. coli* and *K. pneumoniae* from both SUC and EQUC methods were identified and confirmed using the PCR method.

For PCR, a 130 bp product of the Diguanylate cyclase gene from *klebsiella pneumoniae* was targeted and amplified. A previously identified strain of *klebsiella pneumoniae* was used as a positive control. A negative control containing all reagents except DNA template was also used (**Figure 3.7**).

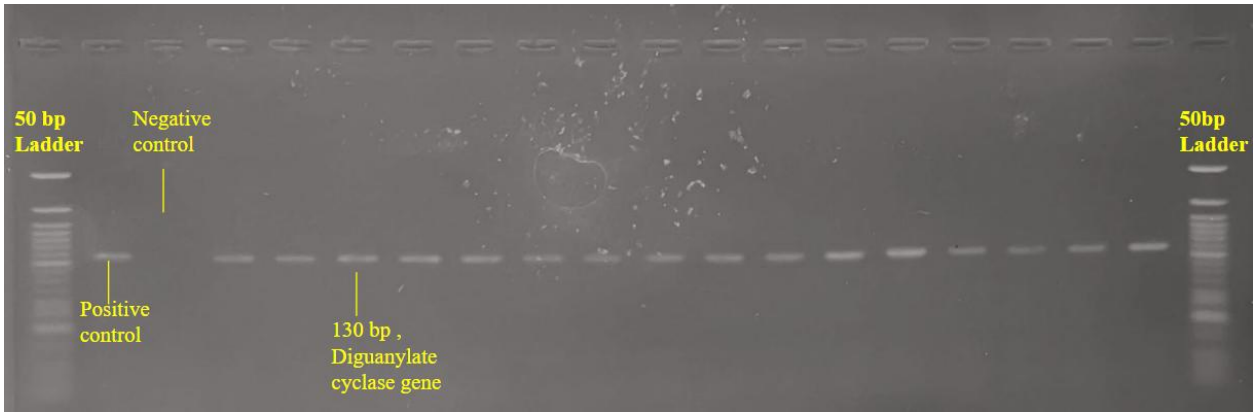

Figure 3.7: Gel electrophoresis result of *Klebsiella pneumoniae*

E. coli was identified by targeting its 16SrDNA (585 bp). A previously identified strain of *E. coli* was used as a positive control. A negative control containing all reagents except DNA template was also used **(Figure 3.8).**

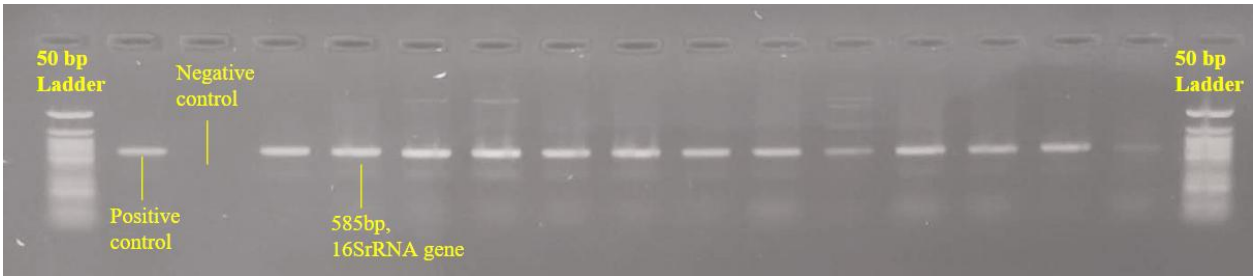

Figure 3.8: Gel electrophoresis result of *Escherichia coli*

Out of suspected 124 K. pneumoniae isolates from cases (59) and controls (65), 40 from cases and 53 from controls were confirmed after PCR. Out of 45 E. coli from cases, 33 were confirmed via PCR.

3.5 Antibiotic Susceptibility Test

To test the antibiotic susceptibility of the identified *E. coli* and *K. pneumoniae* isolates, 9 antibiotics were used: Kanamycin (30), Cefixime, Azithromycin, Cefepime (30), Ampicillin (10), Imipenem (10), Tetracycline (30), Amoxicillin and clavulanic acid and ciprofloxacin are the antibiotic used.

The graph A (**Figure 3.9**) represents the results from Antibiotic Susceptibility Test (AST) of *Klebsiella pneumoniae* of SUC method from controls. The highest value of resistance of *K. pneumoniae* is 100%, which is against Cefixime and Azithromycin. The highest sensitivity (100%) is against Kanamycin.

Graph B (**Figure 3.9**) shows the results from AST of *Klebsiella pneumoniae* of EQUC method from controls. Here the highest resistance is shown against Cefixime and Cefepime, which is

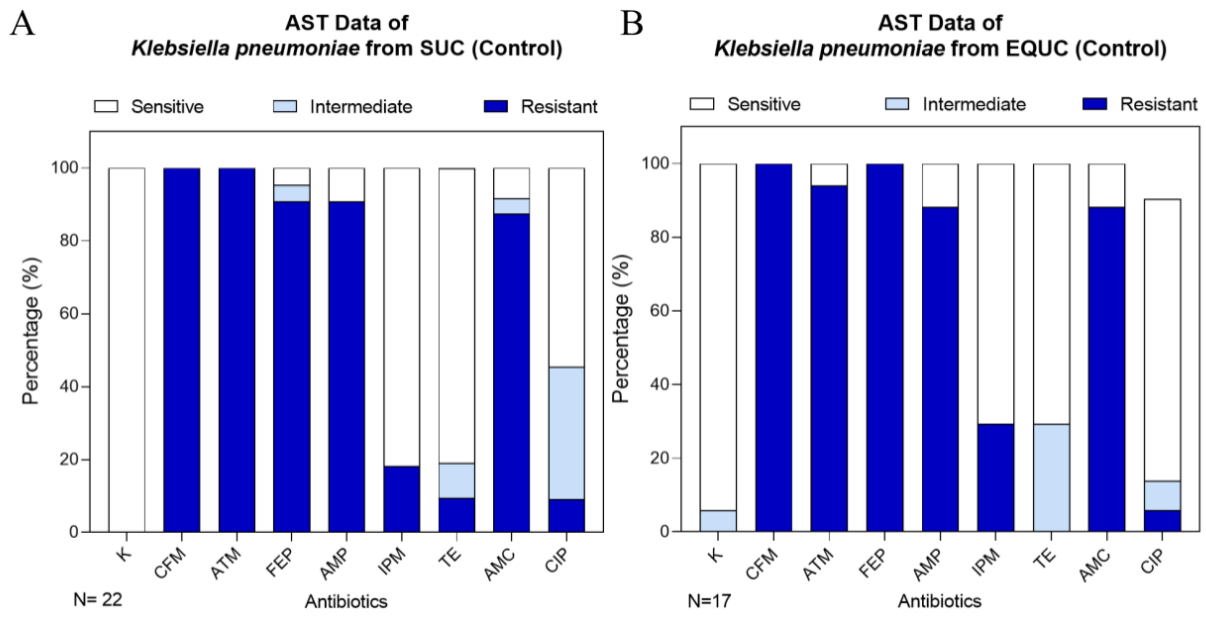

Figure 3.9: AST Data of *Klebsiella pneumoniae* (Control)

100%. Then against Azithromycin *K. pneumoniae* shows resistance of 94.12%. Around 94% of the *K. pneumoniae* shows sensitivity towards Kanamycin. .

Graph A (**Figure 3.10**) shows the results from AST of *K. pneumoniae* of SUC method from cases. Here the highest resistance is 75% against Amoxicillin. Against Azithromycin, Cefepime, Tetracyclin and Ciprofloxacin, it shows 100% sensitivity.

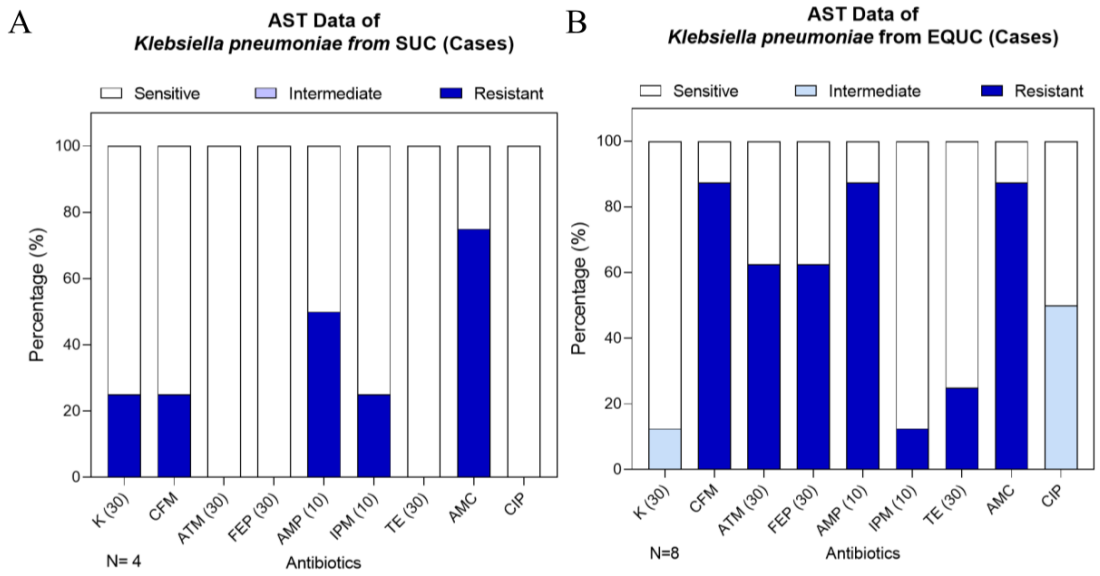

Figure 3.10: AST Data of *Klebsiella pneumoniae* (Case)

Graph B (**Figure 3.10**) shows the results from AST of *K. pneumoniae* of EQUC method from cases. It shows 75 % resistance against Cefixime, Ampicillin and Amoxicillin. Here the highest sensitivity is 75% which is towards Kanamycin.

Graph A (**Figure 3.11**) shows the AST result of *E. coli* from SUC method. Highest resistance is observed against Cefixime which is 86.96%. Then it shows 60% resistance against Azithromycin. Graph B (**Figure 3.11**) shows the results from EQUC method. Here the highest

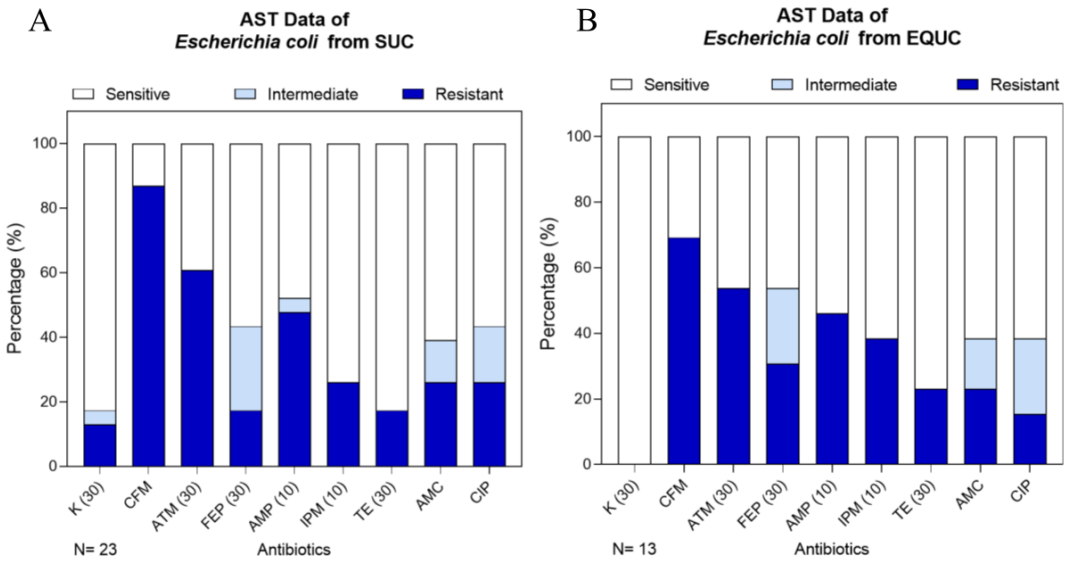

Figure 3.11: AST Data of *Escherichia coli* from SUC and EQUC (Case)

resistance is 69.23% which is against Cefixime. Around 54% *E. coli* is resistant against Azithromycin. *E. coli* in EQUC shows 100% sensitivity towards Kanamycin.

Chapter 4:

Discussion

4.1 Sample Collection

Various studies show that women are more prone to UTI than men across all age groups (Rowe & Juthani-Mehta, 2013). However, in this study, 50% men and 50% women were patients. This could be due to the small sample size. Higher incidence rate is visible in people aged above 60 years (Price et al., 2016). This study also shows 40% of the UTI patients belonging to the age group of above 60. Less incidence rate among younger population is shown in studies where the incidence rate is only 0.01 per person-year (Rowe & Juthani-Mehta, 2013). However, it can be observed from this study that 25% of UTI patients belong to the age group of 20-29, where the majority of patients are women. The risk factors, poor hygiene and unawareness could be prime reasons behind this. Control samples were collected mostly from students or the staff of BRAC University, as a result, 90% of the samples were from people aged below 29.

4.2 Culture Based Result Analysis

Both Gram-positive and Gram-negative bacteria were observed in the samples. The abundance of bacteria was higher in the EQUC method for both cases and control groups. In both cases and controls, the EQUC method showed greater bacterial diversity. Some bacteria did not grow in the SUC method but grew in the EQUC method. These included *Staphylococcus epidermidis*, *Enterococcus faecalis*, *Proteus mirabilis*, *Micrococcus luteus*, *Candida albicans*, *Roseomonas gilardii*, *Roseomonas mucosa*, and *Haemophilus influenzae*. In the EQUC method, culture media were incubated for 48 hours under different conditions. For some bacteria, 24 hours of incubation is not sufficient to produce visible growth (Lagier et al., 2015)**.** This result shows that the SUC method is not always enough to recognize the pathogenic agent causing UTI. More than 16 types were isolated from urine samples, different in number in each urine sample.

Escherichia coli was not detected in the UTI negative or control group. In the UTI positive group (cases), *E. coli* showed a 50:50 growth ratio between the EQUC and SUC methods. This indicates that both methods had similar efficacy in detecting this organism in this study. But in total bacterial abundance was much higher in the EQUC method than SUC. A study conducted by Price et al. (2016) reported that the EQUC method was more efficient in detecting *E. coli*. This difference may be due to the larger sample size used in their study. Despite this variation,

both studies identified many similar bacterial species. In addition, Price et al. (2016) showed that some bacteria did not grow using the SUC method but were detected using the EQUC method. In their study, two of the most abundant bacteria in UTI-positive patients were *E. coli* and *Klebsiella pneumoniae*. Similarly, in the present study, *E. coli* and *Klebsiella pneumoniae* were also among the most abundant bacteria.

EQUC identified a higher proportion of Klebsiella pneumoniae and Escherichia coli across blood agar (BA), MacConkey agar (MAC), and UTI-specific media, suggesting that expanded culture conditions improve bacterial detection. For example, controls had most K. pneumoniae isolated from BA by both SUC (86.7%) and EQUC (73.9%), and cases showed enhanced detection across all media with EQUC compared with SUC. E. coli in cases was recovered more evenly across media with EQUC (47.37% UTI, 31.58% MAC, 21.05% BA) than SUC (56.25% UTI, 31.25% MAC, 12.5% BA). These findings are consistent with published evidence that EQUC detects significantly more uropathogens than standard protocols because EQUC uses larger inocula, multiple media types, and extended incubation (Hilt et al., 2016; Southworth et al., 2019).

4.3 Total Aerobic Count

The average CFU/mL was higher in the EQUC method than in the SUC method on all media. This pattern was observed in both the control and case groups. In the control group, the average CFU/mL on HiCrome UTI agar and blood agar was two-fold higher with the EQUC method. In NA it was 1.09-fold higher in EQUC compared to SUC. In the case group, the difference was more observable. The average CFU/mL on HiCrome UTI agar was 15-fold higher in the EQUC method. On nutrient agar, it was 5-fold higher, while on MacConkey agar (MAC) it was 1.58-fold higher compared to the SUC method.The higher average CFU/mL obtained from the EQUC method is mainly because of the longer incubation period of 48 hours. Hochstedler et al. (2022) conducted a study comparing EQUC and SUC using both catheterized and non-catheterized urine samples. In all cases, the EQUC method showed higher average CFU/mL than the SUC method, which is consistent with findings reported by Hochstedler et al. (2022). Additionally, the case group showed a significantly higher average CFU/mL compared to the control group.

4.4 PCR Results Analysis

According to the study by Mohmadunnabi et al., (2017), primers *K. pneumoniae*-F and *K. pneumoniae*-R amplify the diguanylate cyclase gene of *K. pneumoniae* in PCR and in gel electrophoresis gives band at 130 bp region. In this study, using a 50 bp ladder, visible bands in 130 bp were observed for the *Klebsiella pneumoniae* derived from cases and controls. *K. pneumoniae* from all the cases and controls were confirmed and identified via PCR and gel electrophoresis.ECO-F and ECO-R primers amplify 16SrDNA gene of *E. coli* in PCR and in gel electrophoresis gives band at 585 bp region (Punom et al., 2019). In this study, ECO-F and ECO-R were used and visible bands were observed in 585 bp region, using 50 bp ladder. E. coli isolated from all the cases (UTI patients) were confirmed and identified via PCR and gel electrophoresis.

4.5 AST Result analysis

Klebsiella pneumoniae, isolated from UTI patients in SUC and EQUC method, was highly sensitive to kanamycin (75% and 87.5%, respectively). This was also observed in *K. pneumoniae* isolated from controls. As for SUC, the *K. Pneumoniae* were 100% sensitive to kanamycin and for EQUC, it was 94.12%. This indicates the potential use of kanamycin as an antibiotic to treat the people suffering from UTI. From non-patients, 100% resistance of *Klebsiella pneumoniae*, isolated from both SUC and EQUC, for cefixime was observed, which is highly concerning. A study also reported high resistance for cefixime in clinical *K. pneumoniae* samples, which was 95% (Iqbal, et al., 2021). For UTI patients, in SUC, the reported resistance is surprisingly lower (25%). However, in EQUC, the resistance increased to 75%. This could refer to the ability of the EQUC method to isolate more resistant bacteria efficiently. In case of controls, other than cefixime, high resistance was observed for aztreonam (in SUC- 100%, EQUC- 94.12%), cefepime (in SUC- 90.91%, EQUC- 100%) and ampicillin (in SUC- 90.91%, EQUC- 88.24%). A clinical study showed relatively lower resistance in *K. pneumoniae* for aztreonam (44.7%), cefepime (50%) and ampicillin (76.3%), compared to this study (Jalil & Atbee, 2022). However, resistance of *K. pneumoniae* isolated from cases show varying results in SUC and EQUC. As, resistance for aztreonam (62.5%), cefepime (62.5%) and ampicillin (87.5%) are relatively closer to the reported result of the study conducted by Jalil and Atbee (2022). But the results obtained from EQUC showed high sensitivity for aztreonam (100%), cefepime (100%), and ampicillin (50%).

E. coli, isolated from EQUC, was 100% sensitive to kanamycin, and 82.61% in case of SUC, indicating possible treatment for the patients suffering from UTI. For cefixime, highest resistance was observed in SUC and EQUC both, which is 86.96% and 69.23% respectively. This was also shown in a clinical study where the *E. coli* samples were 93% resistant to cefixime (Lopez-Sampedro, et al., 2023). Second highest resistance was observed for aztreonam, 60.87% and 53.85% for SUC and EQUC respectively. High resistance for aztreonam (74.4%) was also reported in *E. coli* isolated from clinical samples (Jalil & Atbee, 2022). More than 80% of *E. coli* isolates were reported to be resistant to ciprofloxacin (Iqbal, et al., 2021). However, in this study, the *E. coli* isolates from UTI patients were less resistant to ciprofloxacin in SUC (26.09%) and EQUC (15.38%). While this study shows relatively low resistance of *E. coli* to ampicillin (47.83% for SUC, and 46.15% for EQUC), other studies report high resistance up to 86.6% for ampicillin (Jalil & Atbee, 2022). High sensitivity of *E. coli* was observed for imipenem in both SUC and EQUC, 73.91% and 61.54% respectively. This was also reported in other studies where the resistance was 13.4% only (Golsha, et al., 2021).

Chapter 5:
Conclusion

In conclusion, this study shows that the EQUC method can detect more types of uropathogens and gives higher colony counts than the SUC. This means EQUC is more sensitive for detecting urinary tract infections. Although SUC is still useful for routine testing, EQUC may help improve the diagnosis and management of UTIs.

5.1 Strengths

This study has several notable strengths that enhance the reliability, depth and clinical relevance of its findings. The strengths are-

1. The study included samples from both male and female patients, which allowed a more comprehensive understanding of UTI across gender and improves the generalizability of the findings
2. Participants were selected from a wide range of adult age groups, enabling the analysis of age related variations in UTI prevalence and bacterial pattern among adults.
3. The EQUC method revealed a broader spectrum of bacteria compared to SUC providing deeper insights into the microbial diversity associated with UTI.
4. The direct comparison between SUC and EQUC strengthens the study by highlighting the limitations of routine diagnostics and the advantages of EQUC technique.
5. AST results enhanced understanding of the relationship between bacterial diversity and antimicrobial resistance.
6. Findings from this study may contribute to improved diagnostic accuracy and better targeted antimicrobial therapy essential for effective UTI management.

5.2 Limitations

Every study that has been conducted has some limitations. This study has also some limitations. They are,

1. The sample size was small, limiting the generalizability of the findings.
2. Anaerobic could not be adequately maintained due to lab limitations, leading to the underdetection of anaerobic uropathogens.
3. Maximum older adult samples were included in the case group, while the control group consisted exclusively of younger adult samples, which may introduce age-related bias.

4. Fewer female samples were included in the control group due to hesitation in participation, which may affect gender-based comparison.
5. The absence of specific primers in the lab restricted molecular identification of several bacteria.

5.3 Future Prospects

1. The sample will be increased to improve statistical power and enhance the generalizability of finding across different populations and healthcare settings.
2. Identification of suspected bacteria by PCR will be carried out from previous samples after obtaining specific primers.
3. Data from the EQUC method may contribute to updating diagnostic protocols of UTI, particularly in cases of recurrent or culture-negative infections.
4. Future research could incorporate detection of resistant genes using specific primers to correlate phenotypic AST results with genotypic resistance patterns.

Chapter 6:

Reference

Alkhafaji, R. T. H., & Jayashankar, M. (2022). Etiological agents of urinary tract infection (UTI). *International Journal of Scientific Research in Science and Technology*, 381–394. https://doi.org/10.32628/ijsrst22925

Baimakhanova, B., Sadanov, A., Trenozhnikova, L., Balgimbaeva, A., Baimakhanova, G., Orasymbet, S., Tleubekova, D., Amangeldi, A., Turlybaeva, Z., Nurgaliyeva, Z., Seisebayeva, R., Kozhekenova, Z., Sairankyzy, S., Shynykul, Z., Yerkenova, S., & Turgumbayeva, A. (2025). Understanding the burden and management of urinary tract infections in women. *Diseases*, *13*(2), 59. https://doi.org/10.3390/diseases13020059

Bajic, P., Van Kuiken, M. E., Burge, B. K., Kirshenbaum, E. J., Joyce, C. J., Wolfe, A. J., Branch, J. D., Bresler, L., & Farooq, A. V. (2018). Male bladder microbiome relates to lower urinary tract symptoms. *European Urology Focus*, *6*(2), 376–382. https://doi.org/10.1016/j.euf.2018.08.001

Barnes, H. C., Wolff, B., Abdul-Rahim, O., Harrington, A., Hilt, E. E., Price, T. K., Halverson, T., Hochstedler, B. R., Pham, T., Acevedo-Alvarez, M., Joyce, C., Fitzgerald, C. M., Schreckenberger, P. C., Brubaker, L., Wolfe, A. J., & Mueller, E. R. (2021). A Randomized Clinical Trial of Standard versus Expanded Cultures to Diagnose Urinary Tract Infections in Women. *The Journal of Urology*, *206*(5), 1212–1221. https://doi.org/10.1097/ju.0000000000001949

Basic Practical Microbiology: a manual. (2016, January 1). Microbiology Society. https://microbiologysociety.org/publication/education-outreach-resources/basic-practical-microbiology-a-manual.html

Bilsen, M. P., Jongeneel, R. M. H., Schneeberger, C., Platteel, T. N., Van Nieuwkoop, C., Mody, L., Caterino, J. M., Geerlings, S. E., Köves, B., Wagenlehner, F., Conroy, S. P., Visser, L. G., & Lambregts, M. M. C. (2023). Definitions of Urinary Tract Infection in Current Research: A Systematic review. *Open Forum Infectious Diseases*, *10*(7), ofad332. https://doi.org/10.1093/ofid/ofad332

Bono, M. J., & Leslie, S. W. (2025, February 21). *Uncomplicated urinary tract infections*. StatPearls - NCBI Bookshelf. https://www.ncbi.nlm.nih.gov/books/NBK470195/

Boza, J. M., Manning, J. C., & Erickson, D. C. (2024). Comparison and optimization of simple DNA extraction methods for LAMP-Based Point-of-Care applications employing submillimeter skin biopsies. *ACS Omega*, *9*(37), 38855–38863. https://doi.org/10.1021/acsomega.4c05025

Brown, T. A. (2016). *Gene cloning and DNA analysis: An Introduction*. John Wiley & Sons.

Cappuccino, J. G., & Welsh, C. T. (2017). *Microbiology: A Laboratory Manual, Global Edition*. Pearson Higher Ed.

Chen, Y. B., Hochstedler, B., Pham, T. T., Alvarez, M. A., Mueller, E. R., & Wolfe, A. J. (2020). The urethral microbiota: a missing link in the female urinary microbiota. *The Journal of Urology*, *204*(2), 303–309. https://doi.org/10.1097/ju.0000000000000910

CLSI publishes CLSI M100—Performance Standards for Antimicrobial Susceptibility Testing, 33rd Edition. (2025, June 16). https://clsi.org/about/news/clsi-publishes-m100-performance-standards-for-antimicrobial-susceptibility-testing-33rd-edition/

Deen, N. S., Ahmed, A., Tasnim, N. T., & Khan, N. (2023). Clinical relevance of expanded quantitative urine culture in health and disease. *Frontiers in Cellular and Infection Microbiology*, *13*, 1210161. https://doi.org/10.3389/fcimb.2023.1210161

Dogan, A., Kose, A., Gezer, Y., Ersoy, Y., Bayindir, Y., Ozden, M., Memisoglu, F., & Toplu, S. (2021). Antimicrobial susceptibility pattern of Escherichia coli and Klebsiella pneumoniae isolated from patients with urinary tract infections in a tertiary care hospital. *Annals of Medical Research*, *28*(6), 1406. https://doi.org/10.5455/annalsmedres.2021.02.172

Dune, T. J., Price, T. K., Hilt, E. E., Thomas-White, K. J., Kliethermes, S., Brincat, C., Brubaker, L., Schreckenberger, P., Wolfe, A. J., & Mueller, E. R. (2017). Urinary symptoms and their associations with urinary tract infections in urogynecologic patients. *Obstetrics and Gynecology*, *130*(4), 718–725. https://doi.org/10.1097/aog.0000000000002239

Flores-Mireles, A. L., Walker, J. N., Caparon, M., & Hultgren, S. J. (2015). Urinary tract infections: epidemiology, mechanisms of infection and treatment options. *Nature Reviews Microbiology*, *13*(5), 269–284. https://doi.org/10.1038/nrmicro3432

Gunduz, S., & Altun, H. U. (2018). Antibiotic resistance patterns of urinary tract pathogens in Turkish children. *Global Health Research and Policy*, *3*(1), 10. https://doi.org/10.1186/s41256-018-0063-1

Halverson, T., Mueller, E. R., Brubaker, L., & Wolfe, A. J. (2022a). Symptom improvement with mirabegron treatment is associated with urobiome changes in adult women. *International Urogynecology Journal*, *33*(5), 1319–1328. https://doi.org/10.1007/s00192-022-05190-w

Halverson, T., Mueller, E. R., Brubaker, L., & Wolfe, A. J. (2022b). Urobiome changes differ based on OAB treatment in adult females. *International Urogynecology Journal*, *34*(6), 1271–1277. https://doi.org/10.1007/s00192-022-05416-x

Heshmat Hassan, H., Ahmed Meheissen, M., Hamza Hassan, E., & Farid Ahmed, A. (2022). STUDY OF PREVALENCE AND BACTERIAL CAUSES OF UTI AMONG CHILDREN WITH FEVER AT ALEXANDRIA UNIVERSITY CHILDREN'S HOSPITAL. *ALEXMED ePosters*, *4*(2), 37–38. https://doi.org/10.21608/alexpo.2022.144919.1422

Hilt, E. E., McKinley, K., Pearce, M. M., Rosenfeld, A. B., Zilliox, M. J., Mueller, E. R., Brubaker, L., Gai, X., Wolfe, A. J., & Schreckenberger, P. C. (2013). Urine is not sterile: Use of enhanced urine culture techniques to detect resident bacterial flora in the adult female bladder. *Journal of Clinical Microbiology*, *52*(3), 871–876. https://doi.org/10.1128/jcm.02876-13

HiMedia Laboratories. (n.d.). https://www.himedialabs.com/us/

Hochstedler, B. R., Burnett, L., Price, T. K., Jung, C., Wolfe, A. J., & Brubaker, L. (2021). Urinary microbiota of women with recurrent urinary tract infection: collection and culture methods. *International Urogynecology Journal*, *33*(3), 563–570. https://doi.org/10.1007/s00192-021-04780-4

Hombach, M., Zbinden, R., & C Böttger, E. (2013). Standardisation of disk diffusion results for antibiotic susceptibility testing using the sirscan automated zone reader. *BMC Microbiol*, *13*, 225. https://doi.org/10.1186/1471-2180-13-225

Hrbacek, J., Morais, D., Cermak, P., Hanacek, V., & Zachoval, R. (2021). Alpha-diversity and microbial community structure of the male urinary microbiota depend on urine sampling method. *Scientific Reports*, *11*(1), 23758. https://doi.org/10.1038/s41598-021-03292-x

Inmaculada López-Sampedro, Itahisa Hernández-Chico, Gómez-Vicente, E., Expósito-Ruiz, M., José María Navarro-Marí, & José Gutiérrez-Fernández. (2023). Evolution of Antibiotic Resistance in Escherichia coli and Klebsiella pneumoniae from Urine Cultures. Archivos Españoles de Urología, 76(3), 203–203. https://doi.org/10.56434/j.arch.esp.urol.20237603.24

Iqbal, Z., Mumtaz, M. Z., & Malik, A. (2021). Extensive drug-resistance in strains of Escherichia coli and Klebsiella pneumoniae isolated from paediatric urinary tract infections. *Journal of Taibah University Medical Sciences.* 16(4), 565–574. https://doi.org/10.1016/j.jtumed.2021.03.004

Jacobs, K., Thomas-White, K., Hilt, E., Wolfe, A., & Waters, T. (2017). Microorganisms identified in the maternal bladder: Discovery of the maternal bladder microbiota. *American Journal of Perinatology Reports*, *07*(03), e188–e196. https://doi.org/10.1055/s-0037-1606860

Jalil, M. B., & Al Atbee, M. Y. N. (2022). The prevalence of multiple drug resistance Escherichia coli and Klebsiella pneumoniae isolated from patients with urinary tract infections. *Journal of Clinical Laboratory Analysis*, 36(9). https://doi.org/10.1002/jcla.24619

Janda, J. M., & Abbott, S. L. (2007). 16S RRNA gene sequencing for bacterial identification in the diagnostic laboratory: pluses, perils, and pitfalls. *Journal of Clinical Microbiology*, *45*(9), 2761–2764. https://doi.org/10.1128/jcm.01228-07

Joyce, C., Halverson, T., Gonzalez, C., Brubaker, L., & Wolfe, A. J. (2022). The urobiomes of adult women with various lower urinary tract symptoms status differ: A Re-Analysis. *Frontiers in Cellular and Infection Microbiology*, *12*, 860408. https://doi.org/10.3389/fcimb.2022.860408

Karna, S. (2025). Burden of antibiotic resistance in urinary isolates: a retrospective cross-sectional study from a tertiary center in Far-Western Nepal. *BMC Infectious Diseases*, *25*(1), 1634. https://doi.org/10.1186/s12879-025-12074-z

Kaya, G. G., Medaglia, S., Candela-Noguera, V., Tormo-Mas, M. Á., Marcos, M. D., Aznar, E., Deveci, H., & Martínez-Máñez, R. (2020). Antibacterial Activity of Linezolid against Gram-Negative Bacteria: Utilization of ε-Poly-l-Lysine Capped Silica Xerogel as an Activating Carrier. *Pharmaceutics*, *12*(11), 1126. https://doi.org/10.3390/pharmaceutics12111126

Khan, Z. A., Siddiqui, M. F., & Park, S. (2019). Current and emerging methods of antibiotic susceptibility testing. *Diagnostics*, *9*(2), 49. https://doi.org/10.3390/diagnostics9020049

Khehra, N., Padda, I. S., & Zubair, M. (2025, July 7). *Polymerase chain Reaction (PCR)*. StatPearls - NCBI Bookshelf. https://www.ncbi.nlm.nih.gov/books/NBK589663/

Lagier, J., Edouard, S., Pagnier, I., Mediannikov, O., Drancourt, M., & Raoult, D. (2015). Current and past strategies for bacterial culture in clinical microbiology. *Clinical Microbiology Reviews*, *28*(1), 208–236. https://doi.org/10.1128/cmr.00110-14

Lai, H., Chang, S., Lin, H., Hsu, Y., Wei, H., Kuo, C., Hwang, K., & Chiang, H. (2019). Association between urine pH and common uropathogens in children with urinary tract infections. *Journal of Microbiology Immunology and Infection*, *54*(2), 290–298. https://doi.org/10.1016/j.jmii.2019.08.002

Lee, P. Y., Costumbrado, J., Hsu, C., & Kim, Y. H. (2012). Agarose gel electrophoresis for the separation of DNA fragments. *Journal of Visualized Experiments*, *62*. https://doi.org/10.3791/3923

Li, T., Zhang, R., Gong, H., Tang, Z., Li, X., Gong, Z., Challa, M., Zou, C., Zhang, S., Guo, J., & He, Y. (2025). Synthesis and antibacterial evaluations of novel vancomycin analogues targeting bacteria membrane to combat Gram-negative infections. *European Journal of Medicinal Chemistry*, *289*, 117483. https://doi.org/10.1016/j.ejmech.2025.117483

Llor, C., Moragas, A., Aguilar-Sánchez, M., García-Sangenís, A., Monfà, R., & Morros, R. (2022). Best methods for urine sample collection for diagnostic accuracy in women

with urinary tract infection symptoms: a systematic review. *Family Practice*, *40*(1), 176–182. https://doi.org/10.1093/fampra/cmac058

Lough, M. E., Shradar, E., Hsieh, C., & Hedlin, H. (2019). Contamination in adult midstream Clean-Catch urine cultures in the emergency Department: a randomized controlled trial. *Journal of Emergency Nursing*, *45*(5), 488–501. https://doi.org/10.1016/j.jen.2019.06.001

Madigan, M. T., Aiyer, J., Buckley, D. H., Sattley, W. M., & Stahl, D. A. (2021). *Brock Biology of Microorganisms, Global Edition*. Pearson Higher Ed.

Mahmood, N., Siddiqui, M. R., Giasuddin, R. S., Islam, M., & Siddiqui, M. N. K. K. (2024). Recurrent urinary tract infection- Etiology, risk factors and outcome in a tertiary care hospital of Bangladesh. *Bangladesh Journal of Medicine*, *35*(3), 173–179. https://doi.org/10.3329/bjm.v53i3.75306

Mancuso, G., Midiri, A., Gerace, E., Marra, M., Zummo, S., & Biondo, C. (2023). Urinary tract infections: the current scenario and future prospects. *Pathogens*, *12*(4), 623. https://doi.org/10.3390/pathogens12040623

Mohtar, J., Mallah, H., Mardirossian, J. M., El-Bikai, R., Jisr, T. E., Soussi, S., Naoufal, R., Alam, G., & Chaar, M. E. (2024). Enhancing enteric pathogen detection: implementation and impact of multiplex PCR for improved diagnosis and surveillance. *BMC Infectious Diseases*, *24*(1), 171. https://doi.org/10.1186/s12879-024-09047-z

Moreland, R. B., Brubaker, L., Tinawi, L., & Wolfe, A. J. (2024). Rapid and accurate testing for urinary tract infection: new clothes for the emperor. *Clinical Microbiology Reviews*, *38*(1), e0012924. https://doi.org/10.1128/cmr.00129-24

Morroni, G., Bressan, R., Fioriti, S., D'Achille, G., Mingoia, M., Cirioni, O., Di Bella, S., Piazza, A., Comandatore, F., Mauri, C., Migliavacca, R., Luzzaro, F., Principe, L., & Lagatolla, C. (2021). Antimicrobial Activity of Aztreonam in Combination with Old and New β-Lactamase Inhibitors against MBL and ESBL Co-Producing Gram-Negative Clinical Isolates: Possible Options for the Treatment of Complicated Infections. *Antibiotics*, *10*(11), 1341. https://doi.org/10.3390/antibiotics10111341

Mouanga-Ndzime, Y., Bisseye, C., Longo-Pendy, N., Bignoumba, M., Dikoumba, A., & Onanga, R. (2024). Trends in Escherichia coli and Klebsiella pneumoniae Urinary Tract

Infections and Antibiotic Resistance over a 5-Year Period in Southeastern Gabon. *Antibiotics*, *14*(1), 14. https://doi.org/10.3390/antibiotics14010014

PBS (Phosphate Buffered Saline) (1X, pH 7.4) Preparation and recipe | AAT BioQuest. (n.d.). https://www.aatbio.com/resources/buffer-preparations-and-recipes/pbs-phosphate-buffered-saline

Pearce, M. M., Hilt, E. E., Rosenfeld, A. B., Zilliox, M. J., Thomas-White, K., Fok, C., Kliethermes, S., Schreckenberger, P. C., Brubaker, L., Gai, X., & Wolfe, A. J. (2014). The Female Urinary Microbiome: a Comparison of Women with and without Urgency Urinary Incontinence. *mBio*, *5*(4), e01283-14. https://doi.org/10.1128/mbio.01283-14

Peechakara, B. V., Basit, H., & Gupta, M. (2023, August 28). *Ampicillin*. StatPearls - NCBI Bookshelf. https://www.ncbi.nlm.nih.gov/books/NBK519569/

Price, T. K., Dune, T., Hilt, E. E., Thomas-White, K. J., Kliethermes, S., Brincat, C., Brubaker, L., Wolfe, A. J., Mueller, E. R., & Schreckenberger, P. C. (2016). The clinical urine culture: Enhanced techniques improve detection of clinically relevant microorganisms. *Journal of Clinical Microbiology*, *54*(5), 1216–1222. https://doi.org/10.1128/jcm.00044-16

Price, T. K., Lin, H., Gao, X., Thomas-White, K. J., Hilt, E. E., Mueller, E. R., Wolfe, A. J., Dong, Q., & Brubaker, L. (2020). Bladder bacterial diversity differs in continent and incontinent women: a cross-sectional study. *American Journal of Obstetrics and Gynecology*, *223*(5), 729.e1-729.e10. https://doi.org/10.1016/j.ajog.2020.04.033

Price, T. K., Wolff, B., Halverson, T., Limeira, R., Brubaker, L., Dong, Q., Mueller, E. R., & Wolfe, A. J. (2020). Temporal dynamics of the adult female lower urinary tract microbiota. *mBio*, *11*(2). https://doi.org/10.1128/mbio.00475-20

Program, H. F. (2017, October 16). *BAM Media M163: Tryptone salt agar (T1N1 agar and T1N2 agar)*. U.S. Food And Drug Administration. https://www.fda.gov/food/laboratory-methods-food/bam-media-m163-tryptone-salt-agar-t1n1-agar-and-t1n2-agar

Qahtani, W. a. A., Zaghlol, M. S., Qasem, Y. a. M., Mashi, M. M., Daghriri, A. A., Kubaysi, Y., Hakami, H. N., Khawaji, A. F., Hakami, N. T., Jeraiby, M. A., Moafa, H. N., Ezzi, M., & Moshi, J. M. (2025). Antimicrobial susceptibility patterns of Escherichia coli

from various clinical sources. *Antimicrobial Stewardship & Healthcare Epidemiology*, *5*(1), e184. https://doi.org/10.1017/ash.2025.177

Queipo-OrtuñO, M. I., De Dios Colmenero, J., Macias, M., Bravo, M. J., & Morata, P. (2008). Preparation of Bacterial DNA Template by Boiling and Effect of Immunoglobulin G as an Inhibitor in Real-Time PCR for Serum Samples from Patients with Brucellosis. *Clinical and Vaccine Immunology*, *15*(2), 293–296. https://doi.org/10.1128/cvi.00270-07

Rahman, A., Sujan, M. J., Rizvi, S. M. S., Barua, H. T., Habib, Z. H., Jannat, H., Deb, P. K., Hasnat, A., Kwon, S. Y., Aboushady, A. T., Clark, A., Stelling, J., Gautam, S., Shaw, A., Holm, M., Marks, F., & Poudyal, N. (2025). Patterns of Antimicrobial Resistance Among Major Bacterial Pathogens Isolated from Clinical Samples in Bangladesh (2017–2020): A Nationwide Cross-Sectional Study. *Microbiology Research*, *16*(6), 122. https://doi.org/10.3390/microbiolres16060122

Sanders, E. R. (2012). Aseptic Laboratory Techniques: plating methods. *Journal of Visualized Experiments*, *63*, e3064. https://doi.org/10.3791/3064

Senaratne, W. M. T. N., & Jayaweera, J. a. a. S. (2024). Comparison of microbial preservation methods: a narrative review. *GERMS*, *14*(4), 375–386. https://doi.org/10.18683/germs.2024.1447

Shama, A. I., Sultana, R., Ferdous, M., Alam, S. T., Momo, M. F., Mehjabin, T., Ferdous, K., Snikdho, M. S. N., Pranto, A. a. H., & Uddin, M. A. (2024). Recent Trends of Antibiotic Resistance Patterns of Gram-negative Bacteria Collected from Clinical Isolates in Dhaka City. *Journal of Preventive Diagnostic and Treatment Strategies in Medicine*, *3*(2), 122–130. https://doi.org/10.4103/jpdtsm.jpdtsm_39_24

Shin, S. K., Lee, Y., Kwon, H., Rhee, J., & Kim, J. K. (2021). Validation of direct boiling method for simple and efficient genomic DNA extraction and PCR-based macroalgal species determination. *Journal of Phycology*, *57*(4), 1368–1372. https://doi.org/10.1111/jpy.13175

Shwani, A., Zuo, B., Alrubaye, A., Zhao, J., & Rhoads, D., D. (2023). A Simple, Inexpensive Alkaline Method for Bacterial DNA Extraction from Environmental Samples for PCR Surveillance and Microbiome Analyses. *Applied Sciences*, *14*(1), 141. https://doi.org/10.3390/app14010141

Sinawe, H., & Casadesus, D. (2023, May 1). *Urine culture*. StatPearls - NCBI Bookshelf. https://www.ncbi.nlm.nih.gov/books/NBK557569/

Southworth, E., Hochstedler, B., Price, T. K., Joyce, C., Wolfe, A. J., & Mueller, E. R. (2019). A cross-sectional pilot cohort study comparing standard urine collection to the Peezy Midstream device for research studies involving women. *Female Pelvic Medicine & Reconstructive Surgery*, *25*(2), e28–e33. https://doi.org/10.1097/spv.0000000000000693

Storm, D. W., Copp, H. L., Halverson, T. M., Du, J., Juhr, D., & Wolfe, A. J. (2022). A Child's urine is not sterile: A pilot study evaluating the Pediatric Urinary Microbiome. *Journal of Pediatric Urology*, *18*(3), 383–392. https://doi.org/10.1016/j.jpurol.2022.02.025

Storme, O., Saucedo, J. T., Garcia-Mora, A., Dehesa-Dávila, M., & Naber, K. G. (2019). Risk factors and predisposing conditions for urinary tract infection. *Therapeutic Advances in Urology*, *11*, 1756287218814382. https://doi.org/10.1177/1756287218814382

TE Buffer 10X Preparation and recipe | AAT BioQuest. (n.d.). https://www.aatbio.com/resources/buffer-preparations-and-recipes/te-buffer-10x

Tenny, S., Kerndt, C. C., & Hoffman, M. R. (2023, March 27). *Case control studies*. StatPearls - NCBI Bookshelf. https://www.ncbi.nlm.nih.gov/books/NBK448143/#

Tesfa, K., Belete, D., Mulu, M., & Gelaw, A. (2025). Antimicrobial susceptibility patterns and associated risk factors of bacterial isolate among urinary tract infection suspected patients at University of Gondar Comprehensive Specialized Hospital North west Ethiopia. *BMC Infectious Diseases*, *25*(1), 1723. https://doi.org/10.1186/s12879-025-12125-5

Thapaliya, J., Khadka, P., Thapa, S., & Gongal, C. (2020). Enhanced quantitative urine culture technique, a slight modification, in detecting under-diagnosed pediatric urinary tract infection. *BMC Research Notes*, *13*(1), 5. https://doi.org/10.1186/s13104-019-4875-y

Thomas-White, K., Brady, M., Wolfe, A. J., & Mueller, E. R. (2016). The bladder is not sterile: History and current discoveries on the urinary microbiome. *Current Bladder Dysfunction Reports*, *11*(1), 18–24. https://doi.org/10.1007/s11884-016-0345-8

Thomas-White, K., Forster, S. C., Kumar, N., Van Kuiken, M., Putonti, C., Stares, M. D., Hilt, E. E., Price, T. K., Wolfe, A. J., & Lawley, T. D. (2018). Culturing of female bladder

bacteria reveals an interconnected urogenital microbiota. *Nature Communications*, *9*(1), 1557. https://doi.org/10.1038/s41467-018-03968-5

Thomas-White, K. J., Hilt, E. E., Fok, C., Pearce, M. M., Mueller, E. R., Kliethermes, S., Jacobs, K., Zilliox, M. J., Brincat, C., Price, T. K., Kuffel, G., Schreckenberger, P., Gai, X., Brubaker, L., & Wolfe, A. J. (2015). Incontinence medication response relates to the female urinary microbiota. *International Urogynecology Journal*, *27*(5), 723–733. https://doi.org/10.1007/s00192-015-2847-x

Thomas-White, K. J., Kliethermes, S., Rickey, L., Lukacz, E. S., Richter, H. E., Moalli, P., Zimmern, P., Norton, P., Kusek, J. W., Wolfe, A. J., & Brubaker, L. (2016). Evaluation of the urinary microbiota of women with uncomplicated stress urinary incontinence. *American Journal of Obstetrics and Gynecology*, *216*(1), 55.e1-55.e16. https://doi.org/10.1016/j.ajog.2016.07.049

Tripathi, K., Srivastava, Y., & Kumar, N. (2025). *Biotechnology lab techniques: culture media, microscopy, and microbial analysis*. https://doi.org/10.70593/978-93-49307-52-0

Vaughan, M. H., Mao, J., Karstens, L. A., Ma, L., Amundsen, C. L., Schmader, K. E., & Siddiqui, N. Y. (2021). The Urinary Microbiome in Postmenopausal Women with Recurrent Urinary Tract Infections. *The Journal of Urology*, *206*(5), 1222–1231. https://doi.org/10.1097/ju.0000000000001940

Vaughan, M. H., Zemtsov, G. E., Dahl, E. M., Karstens, L., Ma, L., & Siddiqui, N. Y. (2022). Concordance of urinary microbiota detected by 16S ribosomal RNA amplicon sequencing vs expanded quantitative urine culture. *American Journal of Obstetrics and Gynecology*, *227*(5), 773–775. https://doi.org/10.1016/j.ajog.2022.06.031

Walsh, C., & Collyns, T. (2017). The pathophysiology of urinary tract infections. *Surgery (Oxford)*, *35*(6), 293–298. https://doi.org/10.1016/j.mpsur.2017.03.007

Wireko, S., Ngmaakan, G., Dwomoh, D., Hansen, C., Nartey, S., & Senu, E. (2025). Regional Burden of Urinary Tract Infection, its aetiologies, and antibiotic Resistance Patterns, 2020–2023: A Retrospective study. *Health Science Reports*, *8*(11), e71503. https://doi.org/10.1002/hsr2.71503

Wolfe, A. J., & Brubaker, L. (2015). “Sterile urine” and the presence of bacteria. *European Urology*, *68*(2), 173–174. https://doi.org/10.1016/j.eururo.2015.02.041

Yadav, P. K., Singh, S., Paul, M., Kumar, S., Ponmariappan, S., & Thavaselvam, D. (2024). Development of a novel sequence based real-time PCR assay for specific and sensitive detection of Burkholderia pseudomallei in clinical and environmental matrices. *Annals of Clinical Microbiology and Antimicrobials*, *23*(1), 30. https://doi.org/10.1186/s12941-024-00693-4

Yamagishi, J., Sato, Y., Shinozaki, N., Ye, B., Tsuboi, A., Nagasaki, M., & Yamashita, R. (2016). Comparison of boiling and robotics automation method in DNA extraction for metagenomic sequencing of human oral microbes. *PLoS ONE*, *11*(4), e0154389. https://doi.org/10.1371/journal.pone.0154389

Zhou, Y., Zhou, Z., Zheng, L., Gong, Z., Li, Y., Jin, Y., Huang, Y., & Chi, M. (2023). Urinary Tract Infections Caused by Uropathogenic Escherichia coli: Mechanisms of Infection and Treatment Options. *International Journal of Molecular Sciences*, *24*(13), 10537. https://doi.org/10.3390/ijms241310537.

Appendix:

Media and Reagents

Nutrient Agar (NA)

According to HiMedia Laboratories (2024), NA is a general purpose medium utilized for the cultivation of non-fastidious microorganisms. Due to its basic formula and non-selective nature, it provides an optimal condition for a wide range of microorganisms to grow (HiMedia Laboratories, 2024).

Table A.1: Composition of Nutrient Agar (NA)

Composition of NA		
Ingredients	**Amount (g / L)**	**Function**
Peptone	5	Provides necessary nutrients for growth
Beef Extract	1.5	
Yeast Extract	1.5	
Sodium Chloride	5	Maintains osmotic equilibrium
Agar	15	Solidifying agent
Final pH (at 25°C): 7.4 ± 0.2		

This medium consists of peptone, beef extract and yeast extract which provides essential nitrogen and carbon compounds, vitamins, and some other essential trace ingredients that supports the growth of a wide range of bacteria; sodium chloride maintains the osmotic balance of the medium; while agar acts as an solidifying agent (HiMedia Laboratories, 2024). According to HiMedia Laboratories (2024), NA can be used for clinical samples such as urine and faeces; food and dairy samples; and water sample for routine culture of microorganism, enumeration of bacteria and subculturing to maintain bacterial strain.

HiCrome UTI Agar

As recommended by HiMedia Laboratories (2025), Hicrome UTI agar is a chromogenic, differential culture medium used in clinical microbiology to isolate, differentiate, and achieve presumptive identification of uropathogens directly from urine specimens in urinary tract infection (UTI) diagnostics. The medium contains two chromogenic substrates that react with specific bacterial enzymes, allowing distinct bacteria to produce differential colored colonies based on their metabolic properties (HiMedia Laboratories, 2025).

Table A2: Composition of HiCrome UTI Agar

Compositions of HiCrome UTI Agar		
Ingredients	**Amount (g / L)**	**Function**
Peptone (special)	15	Provides essential growth nutrients
Chromogenic mixture	2.45	Allows visible color change
Agar	15	Solidifying agent
Final pH (at 25°C): 6.8 ± 0.2		

As stated in HiMedia Laboratories (2025), the principle behind HiCrome UTI agar is presumptive identification of uropathogens on the basis of contrasted colony colors produced by the reaction of genus or species specific enzymes: β-glucosidase and β-D-galactosidase with two chromogenic substrates. For instance, *Escherichia coli* produces β-D-galactosidase, which cleaves the galactoside chromogen to form pink or magenta colonies; *Klebsiella* spp. or *Enterococcus* spp. express β-glucosidase cleaved by glucoside chromogen to produce blue colonies; meanwhile, presence of amino acids like phenylalanine and tryptophan from peptone detects tryptophan deaminase activities that appears as brown colonies, indicating the presence of *Proteus*, *Morganella*, and *Providencia* species (HiMedia Laboratories, 2025).

MacConkey Agar (MAC)

MAC is a selective and differential medium, primarily utilized for the isolation and differentiation of Gram-negative bacteria, particularly members of the family *Enterobacteriaceae.* However, some Gram-negative bacteria such as *Haemophilus influenzae*

and *Neisseria meningitidis* struggle to grow due to their sensitivity towards bile salts. This medium's effectiveness stems from its unique composition, which simultaneously inhibits the growth of most Gram-positive bacteria and promotes the differentiation of Gram-negative bacteria based on their ability to ferment lactose (Madigan et al., 2021).

Table A3: Composition of MAC

Compositions of MAC		
Ingredients	**Amount (g / L)**	**Function**
Peptone (pancreatic digest of gelatin)	17	Provides essential nutrients, vitamins, and nitrogenous factors for growth of microorganisms
Proteose peptone (meat and casein)	3	
Lactose monohydrate	10	Fermentable source of carbohydrate
Bile salts	1.5	Inhibits Gram-positive bacteria
Crystal violet	0.001	
Sodium chloride	5	Maintains osmotic balance
Neutral red	0.03	pH indicator
Agar	13.5	Solidifying agent
Final pH (at 25°C): 7.1 ± 0.2		

The selective properties of MAC are attributed to the inclusion of bile salts and crystal violet in its formation. Bile salts inhibit most Gram-positive bacteria, while crystal violet further enhances this inhibitory effect. This selectivity of MAC makes it invaluable for isolating Gram-negative enteric pathogens from various samples, such as clinical specimens, food, and environmental sources (Cappuccino & Welsh, 2017).

Beyond selection, MAC is a differential medium, distinguishing Gram-negative bacteria based on lactose fermentation. It contains lactose as the sole carbohydrate source and neutral red as

pH indicator. Lactose fermenting bacteria such as *Escherichia coli* and *Klebsiella spp.*, produces acid, which lowers the pH of the medium below 6.8 and causes the neutral red indicator to turn pink or red, often resulting in pink-red colonies. Meanwhile, non-lactose fermenting bacteria such as *Salmonella* and *Shigella* utilize peptone, forming ammonia, which increases the pH of the medium above 6.8, typically resulting in colorless or transparent colonies, reflecting the color of the medium (HiMedia Laboratories, 2025).

Blood Agar (BA)

BA is an enriched and differential medium widely used for the isolation and cultivation of fastidious microorganisms, particularly those with complex nutrient requirements. Its distinctive feature is the incorporation of 5% mammalian blood, typically sheep, in a blood agar base. This blood provides essential growth factors and nutrients required by many bacteria to thrive, making it an excellent enrichment medium (Cappuccino & Welsh, 2017).

Table A4: Composition of Blood Agar Base

Compositions of BA Base		
Ingredients	**Amount (g / L)**	**Function**
Peptone	10	Provides carbon, nitrogen, amino acids, vitamins and minerals
Tryptose	10	
Sodium chloride	5	Maintains osmotic equilibrium
Agar	15	Solidifying agent
Final pH (at 25°C): 7.3 ± 0.2		

The differential property of BA primarily revolves around its ability to detect hemolytic activity, which is the lysis of red blood cells (RBCs) by bacterial exotoxins called hemolysins. There are three main types of hemolysis observed on BA:

Alpha (**α**) hemolysis: This appears as partial lysis of RBCs around bacterial colonies, resulting in a greenish discoloration of the medium. This green color is caused by the reduction of

hemoglobin to methemoglobin due to the production of H2O2 by **α**-hemolytic bacteria. Example of **α**-hemolytic bacteria include *Streptococcus pneumoniae* and some strains of *Streptococcus viridans* (Cappuccino & Welsh, 2017).

Beta (**β**) hemolysis: This indicates the complete hemolysis of RBCs, leading to a clear, transparent zone around bacterial colonies. This complete clearing is due to denaturation of hemoglobin by potent hemolysins. Classic examples of **β**-hemolytic bacteria are *Streptococcus pyogenes* (Group A Streptococcus) and *Staphylococcus aureus*.

Gamma (**γ**) hemolysis or non-hemolytic: This signifies no hemolysis or discoloration of the BA around the bacterial colonies. The RBCs remain intact. Many non-pathogenic bacteria exhibit **γ** hemolysis, such as *Enterococcus faecalis* (Cappuccino & Welsh, 2017).
According to HiMedia Laboratories (2024), to prepare BA, create a sterile BA base by suspending 40 g of BA base powder in 1 L of distilled water and heated up to boiling to dissolve the medium completely. It is sterilized by autoclaving at 121 ℃ and 15 lbs pressure for about 15 minutes. The autoclaved medium is cooled down to about 45-50 ℃ to which 5% sterile, defibrinated sheep blood to room temperature is aseptically added and mixed well. The media is then poured in sterile plates under sterile conditions and stored in 2-8 ℃.

Chocolate Agar (CA)

CA is a non-selective, enriched growth medium used in the isolation and cultivation of fastidious microorganisms, particularly *Haemophilus* and *Neisseria* species (Madigan et al., 2021).

Table A5: Compositions of Chocolate Agar

Composition of CA		
Ingredients	**Amount (g / L)**	**Function**
Casein	15	Provides nitrogenous nutrients, amino acids, and essential elements for growth
Cornstarch	1	Neutralize possible toxic metabolites

Sodium Chloride	5	Maintains osmotic equilibrium
Dipotassium Phosphate	4	Maintains uniform pH during growth
Monopotassium Phosphate	1	
Hemoglobin solution	5%	Provides essential nutrients for fastidious bacteria
Koenzyme enrichment	10	Provides essential growth factors for fastidious bacteria
Agar	10	Solidifying agent
Final pH (at 25℃): 7.3 ± 0.2		

CA is prepared by adding 5% defibrinated sheep blood to a sterile molten CA base and heating it gently at 80℃ for 10 minutes. Unlike BA, this heating process causes RBCs to lyse, releasing essential intracellular nutrients for fastidious bacteria, so hemolysis cannot be observed . During this process, hemoglobin is converted to Factor X (hemin), which is an iron-containing porphyrin essential for bacterial respiration, and Factor V (Nicotinamide adenine dinucleotide, NAD), which is a coenzyme that supports metabolism (oxidation-reduction reactions). These two factors are essential for the growth of certain fastidious bacteria (HiMedia Laboratories 2024).

Unlike BA, CA is not differential as the RBCs are already lysed. However, various pathogenic bacteria lack the ability to synthesize these factors on their own; therefore, they are unable to grow on standard BA where these factors remain trapped inside intact RBCs. CA effectively pre-digest these RBCs, releasing a banquet of freely available growth factors (Cappuccino & Welsh, 2017).

This medium is designed for fastidious organisms–bacteria that are picky eaters and requires a specific environment to survive. It is the gold standard for culturing respiratory and sexually transmitted pathogens. For instance, *Haemophilus influenzae*, a major cause of respiratory infections and meningitis, requires both Factor X and Factor V and thrives on CA. Similarly, Neisseria species, such as Neisseria meningitidis which causes bacterial meningitis, and

Neisseria gonorrhoeae which causes gonorrhea, rely on this medium to provide the NAD they are unable to produce themselves (Madigan et al., 2021).

Tryptone Salt Agar (T1N1)

T1N1 is a low nutrient agar made with basic formulation, specifically tryptone solution with 1% NaCl, supporting the survival of many non-fastidious bacteria, which is used to maintain stock cultures over short to medium periods because the limited nutrient environment slows bacterial metabolism and reduces overgrowth while preserving viability (Program, 2017; Senaratne, 2024)

Table A11: Compositions of T1N1

Compositions of T1N1		
Ingredients	**Amount (g / L)**	**Function**
Tryptone	10	Source of amino acids for bacterial growth
Sodium Chloride	10	Maintains osmotic balance
Agar	20	Solidifying agent
Final pH (at 25℃): 7.1 ± 0.2		

Mueller Hinton Agar (MHA)

According to Clinical and Laboratory Standards Institute (CLSI), MHA is a non-selective, non-differential culture medium which is internationally recommended as the standard medium for antimicrobial susceptibility test (AST), particularly by the Kirby-Bauer disk diffusion method. The formulation of MHA supports the growth of a wide range of non-fastidious bacteria while allowing uniform diffusion of antibiotics, ensuring accurate and reproducible susceptibility results (CLSI, 2023).

Table A6: Composition of MHA

Composition of MHA		
Ingredients	**Amount (g / L)**	**Function**
Casein acid hydrolysate	17.5	Provides essential nutrients for growth
Beef extract	2	
Starch	1.5	Absorbs toxic metabolites
Agar	17	Solidifying agent
Final pH (at 25°C): 7.3 ± 0.2		

As mentioned in ™ Media (2022), MHA include beef extract and casein acid hydrolysate which supply nitrogen and carbon source, vitamins, amino acids and other essential growth factors; starch acts as a protective colloid which absorbs bacterial toxins which prevents interference with antibiotic activity and enhance the clarity of inhibition zone during AST, and starch hydrolyse dextrose, which serves as an energy source for bacterial growth; agar acts as a solidifying agent. Moreover, the low content of thymidine and thymine prevents false resistance results with sulfonamides and trimethoprim; while the level of calcium and magnesium is maintained for accurate zones of inhibition as per specified diameters in the CLSI standard.

Phosphate-Buffered Saline (PBS)

PBS is an isotonic, non-toxic buffer solution formulated to maintain a constant physiological pH of microorganisms, typically around pH 7.2-7.4, which closely resembles the pH of human body fluids (Madigan et al., 2021). The buffering capacity of PBS is provided by the phosphate system, which resists changes in pH when small amounts of acids or bases are added; the saline component maintains osmotic equilibrium, preventing cell lysis or shrinkage during laboratory procedure; due to its isotonic and non-toxic nature, PBS does not interfere with cellular integrity and biological reactions, making it highly suitable for washing microbial cells, diluting bacterial suspension, culture transfer, or molecular analysis (Cappuccino & Welsh, 2017).

Table A7: Composition of PBS (1x)

Composition of PBS (1X)		
Ingredients	**Amount (g / L)**	**Concentration (mM)**
Sodium Chloride (NaCl)	8	136.893
Potassium Chloride (KCl)	0.2	2.683
Sodium Phosphate Dibasic (Na_2HPO_4)	1.44	10.144
Potassium Phosphate Monobasic (KH_2PO_4)	0.245	1.8
Final pH (at 25°C): typically pH ≈ 7.4		

Tris-EDTA (TE) Buffer

TE buffer is primarily formulated to dissolve and preserve DNA and RNA extracted from microorganisms, where Tris (Tris(hydroxymethyl)aminomethane) maintains a stable alkaline pH to stabilize nucleic acids by preventing pH fluctuation, while ethylenediaminetetraacetic acid (EDTA) acts as a chelating agent which inhibits nuclease activity by removing metal ions such as magnesium ions and calcium ions for these enzyme activities (Brown, 2016)

Table A8: Composition of TE buffer (10x)

Composition of TE Buffer (10X)		
Ingredients	**Amount (g / L)**	**Concentration (mM)**
Tris-Cl	15.759	99.997
EDTA	2.92	9.992
Final pH (at 25°C): pH 7.5 for RNA and pH8.0 for DNA		

Tris-Borate-EDTA (TBE) Buffer

TBE buffer is commonly formulated as an electrophoresis buffer which is preferred for electrophoretic separation of small DNA fragments because it offers high buffering capacity and produces sharp, well-resolved bands (Madigan et al., 2021). TBE buffer consists of Tris

which maintains stable alkaline pH, while borate provides buffering capacity, and EDTA chelates divalent metal ions such as magnesium ions, thereby protecting DNA from nuclease activity (Brown, 2016).

Table A9: Composition of TBE Buffer (1X)

Composition of TBE Buffer (1X)		
Ingredients	**Amount (g / L)**	**Concentration (mM)**
Tris base	10.8	89
Boric acid	5.5	89
EDTA (disodium salt)	0.74	2
Final pH (at 25°C): typically pH ≈ 8.3		

Normal Saline Solution

According to HiMedia Laboratories (2024), 0.9% saline solution (normal saline) is an isotonic solution which maintains the osmotic balance in microbial cells and helps to maintain the cell integrity and viability. Normally used sterile after autoclaved, to prepare stock solutions and serial dilutions of antimicrobial agents, or microbial suspensions for detection of antimicrobial agents, or to growth media used for disk susceptibility testing (HiMedia Laboratories, 2024)

Table A10: Composition of Normal Saline Solution

Composition of Normal Saline Solution		
Ingredients	**Amount (g / L)**	**Function**
Sodium Chloride (NaCl)	9	Maintains osmotic balance, cell integrity and viability in microbial cells.

Agarose

Agarose is neutral, linear polysaccharide derived from agar and extracted from seaweeds genera *Gelidium* and *Gracilaria* consisting of repeated agarobiose (L- and D-galactose)

subunits, this uniform and low charge structure allows agarose to form a stable, porous gel matrix suitable for gel electrophoresis to separate DNA and RNA fragments based on molecular size (Lee et al., 2012). Moreover, due to its low sulfate content and minimal interaction with nucleic acids, agarose produces sharp, reproducible bands and is preferred over crude agar in molecular biology applications (Lee et al., 2012).

Paraffin Oil

Paraffin oil is a form of mineral oil used as a simple cost-effective method for stocking bacterial cultures on solid medium as covering the top layer of the medium and the microorganism forms a barrier between the microorganism and oxygen, which creates an oxygen-free environment that prevents the growth and multiplication of microorganisms, preserving their viability for an extended period (Senaratne, 2024).