Comparative Analysis of Urinary Microbiome Diversity in Diabetic Patients With and Without Suspected UTI

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A thesis submitted to the Department of Mathematics and Natural Sciences in partial fulfillment of the requirements for the degree of Bachelor of science in Biotechnology

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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.

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Ethics Statement

This study was reviewed and approved by the Institutional Review Board (IRB) of BRAC University and BIRDEM General Hospital.

Prior to enrollment, informed written consent was obtained from all individual participants included in the study.

The non-invasive sample collection procedure was performed with standard hygienic procedures. All collected data from patients were handled with strict confidentiality. Personal identifiers were removed during data collection. Each participant was assigned an unique identifier (e.g. Sample 1, Sample 2, etc.) and all analyses were performed using these unique identifiers so that privacy remained uncompromised.

All procedures for urine collection, biochemical culture, and metagenomic sequencing were conducted in accordance with relevant institutional guidelines.

Abstract

Urinary tract infections (UTIs) represent one of the most common bacterial infections worldwide, imposing a considerable global health and economic burden. According to Hu et al. (2025), the incidence of UTIs among older women increased by 132% between 1990 and 2021, rising from 15.87 million to 36.77 million cases. In 2019 alone, UTIs accounted for approximately 404.61 million cases and 236,790 deaths globally. In light of these alarming trends, this study aims to address key gaps in microbial characterization of UTIs among diabetic patients. A cross-sectional survey was conducted involving both inpatient and outpatient participants, categorized into two groups: Group 1 (No UTI; patients with no clinical evidence of infection), and Group 2 (Suspected UTI; patients presenting clinical signs or symptoms suggestive of a UTI but not yet confirmed). The findings of this study provide compelling evidence that the clinical state of a suspected UTI in diabetic patients is defined by a profound ecological shift in the urinary microbiome, underscoring the complex interplay between host metabolic state and microbial community dynamics.

Keywords:
Urinary tract infection (UTI); Diabetes mellitus; Urinary microbiome; Microbial characterization; Dysbiosis; Hospital-based survey; Metagenomic analysis

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Chapter 1: Introduction

1.1 Background

The most common bacterial infections worldwide are urinary tract infections (UTIs), imposing significant costs in healthcare and affecting millions annually, with 236790 deaths globally and 404.61 million cases in 2019[1]. They occur when the urinary tract is colonized by pathogens, primarily by uropathogenic *Escherichia coli* (UPEC), resulting in conditions like pyelonephritis (kidney infection) and cystitis (bladder inflammation)[2]. UTIs are classified into complicated (associated with immunosuppression, catheters or anatomical abnormalities) and uncomplicated (among healthy individuals) with the former being often linked to biofilm formation which is one of the key virulence factors detected in 60% of the isolates[3]. Women suffer disproportionately from UTIs due to differences in physiological and anatomical factors making women 30% more likely than men to be affected[4]. Clinical biology enables a crucial role in management and diagnosis through accurate pathogen identification, differentiating true infections from contamination and antimicrobial susceptibility testing (AST). The rise in antibiotic-resistant uropathogens such as carbapenemase and ESBL producers emphasizes the necessity of improved diagnostics, alternative therapies and stewardship.[4] In high-income countries, the elderly and residents are more susceptible to frequent recurrent UTIs and severe complications due to antibiotic resistance and demographics compared to poorer nations where inadequate medical resources presents the main challenge.[1]

1.2 Routes of Infection and Pathogenesis

UTIs are broadly classified into complicated (cUTIs) and uncomplicated (uUTIs) through their distinction by clinical severity and underlying host factors. cUTIs occur in patients with comorbidities (e.g. diabetes, immunosuppression) or functional or anatomical abnormalities (e.g. neurogenic bladder, obstructions, catheters). These infections are caused by a broad-spectrum of pathogens, including resistant bacteria such as ESBL (Extended-Spectrum Beta-Lactamase) producing *E.coli*, *Klebsiella*, *Pseudomonas* and fungi, often requiring broad-spectrum antibiotics, hospitalization and sometimes surgical assistance. These infections impose a higher risk of severe complications, including prolonged hospital stays and sepsis, mainly in immunocompromised individuals or elderly.[5] In contrast, uUTIs arise in otherwise healthy

non-pregnant women without functional or structural urinary tract abnormalities, usually appearing as cystitis with dysuria (painful urination), urinary urgency and frequency. *E.coli* usually result in these infections and are empirically managed with first-line antibiotics such as fosfomycin or nitrofurantoin with urine cultures obtained for recurrent infections.[7] The main route of UTIs is ascending infection where the periurethral area are colonized by uropathogens from gut microbiota such as *E.coli*, *Klebsiella pneumoniae*, *Proteus mirabilis* due to fecal contamination and ascend into the bladder through the urethra. This method is enhanced by virulence factors such as P fimbriae and Type 1 fimbriae, enabling bacterial attachment to urothelial receptors. Upon entering the bladder, immune cells are invaded by pathogens like UPEC, leading to quiescent intracellular reservoirs (QIRs) or intracellular bacterial communities (IBCs) which evade immune responses and contribute to recurrent infections.[2,8] In catheter-associated UTIs (CAUTIs), urease activity and fibrinogen deposition are exploited by bacteria such as *P. mirabilis* to develop biofilm on abiotic surfaces, exacerbating treatment difficulties.[2] The less common route is the hematogenous spread where bacteria like *Staphylococcus aureus* infect the kidneys through the bloodstream, accounting for less than 5% of UTI cases, often in immunocompromised individuals. The lymphatic route is a rare mechanism that involves bacterial dissemination from adjacent infected organs through lymphatic vessels.[9] The pathogenesis of UTI depends on the combination of bacterial virulence factors which include toxin production, biofilm formation and siderophore-mediated iron acquisition. For instance, UPEC secretes α-hemolysin (HlyA), resulting in formation of pores in host cells, facilitating nutrient release and tissue damage. Additionally, IBCs by UPECs or biofilm formation by *P. mirabilis* further provide protection from antibiotics and immune defenses. Moreover, siderophores like yersiniabactin and aerobactin facilitate proliferation and survival by enabling bacteria to scavenge iron, a scarce nutrient in the urinary tract. On the contrary, host susceptibility factors such as conditions like diabetes, anatomical abnormalities or immunosuppression increases vulnerability. Therefore, these two factors combinedly dictate the persistence, severity and recurrence of UTIs.[8]

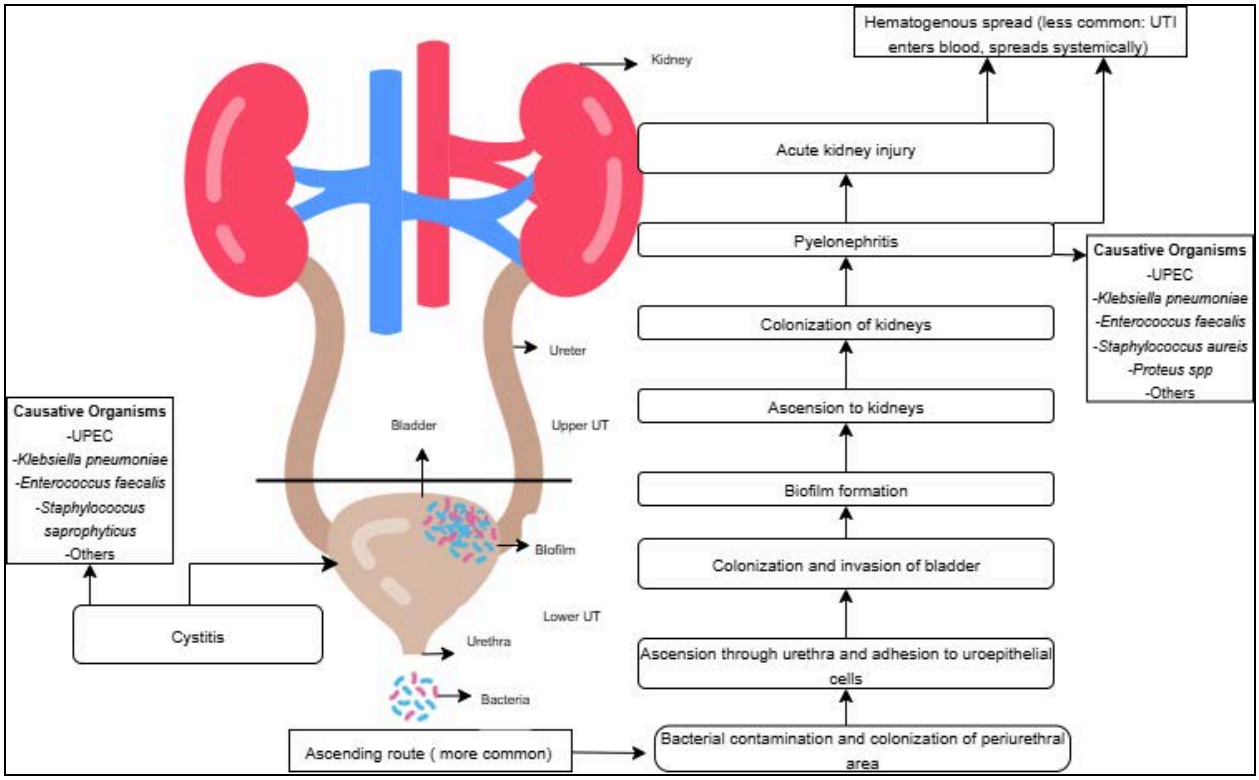


Figure 1. Illustration of the pathogenesis of urinary tract infections (UTIs) through ascending route (common) and hematogenous spread (less common). Diagram created using draw.io, icons sourced from FlatIcon.

1.3 Chronic and Recurrent UTI

Recurrent urinary tract infections (rUTIs) and chronic urinary tract infections (cUTIs) have distinct yet overlapping clinical definitions. rUTIs are defined as two or more confirmed infections within a 6-months period or three or more infections over a year span with patients recovering fully between distinct infection episodes.[10,11,12]cUTIs are inconsistently defined, usually referring to persistent symptoms between acute flares, possibly due to intracellular bacterial reservoirs with limited evidence in humans.[13,14] The primary risk groups include- sexually active young women where 25% develop rUTI with 27% recurrence within 6 months[10,14]; post-menopausal women displaying 50% higher risk due to estrogen deficiency[10,11,14]; older adults with atypical symptom patterns; diabetic patients with 23-37% recurrence rate or those with anatomical abnormalities.[11,14] The diagnostic challenges arise from limitations in standard urine cultures (24-72 hrs delay, repeated false negatives for internalized uropathogens), dipstick inaccuracy and challenges associated with distinguishing between true

recurrences and distinct infection episodes.[12,14]The treatment approaches are complicated by emerging antibiotic resistance, poor drug penetration into infected tissues and lack of standardised protocols for chronic UTI cases.[12,13,14] The efficacy of non-antibiotic strategies vary significantly where intravaginal estrogen is beneficial for post-menopausal women whereas the clinical benefits of immunostimulants (OM-89) and methenamine hippurate lack evidence.[11,12] Moreover, sexually active women are disproportionately affected by UTIs due to limitations in diagnosis and treatment. One of the most significant modifiable risk factors for UTIs is represented by sexual activity, particularly in women aged 18-39 years. In this age group, sexual frequency directly correlates to UTI averaging 0.5 episodes per person per year. UTI risk is significantly elevated with recent sexual intercourse (within 48 hours). Even though sexual activity elevates UTI risk in younger women, post-menopausal women are affected significantly due to estrogen deficiency. Therefore, addressing these interconnected challenges is essential for improving UTIs[15,16] . The multifactorial risk factors associated with UTIs are illustrated in the schematic diagram below;

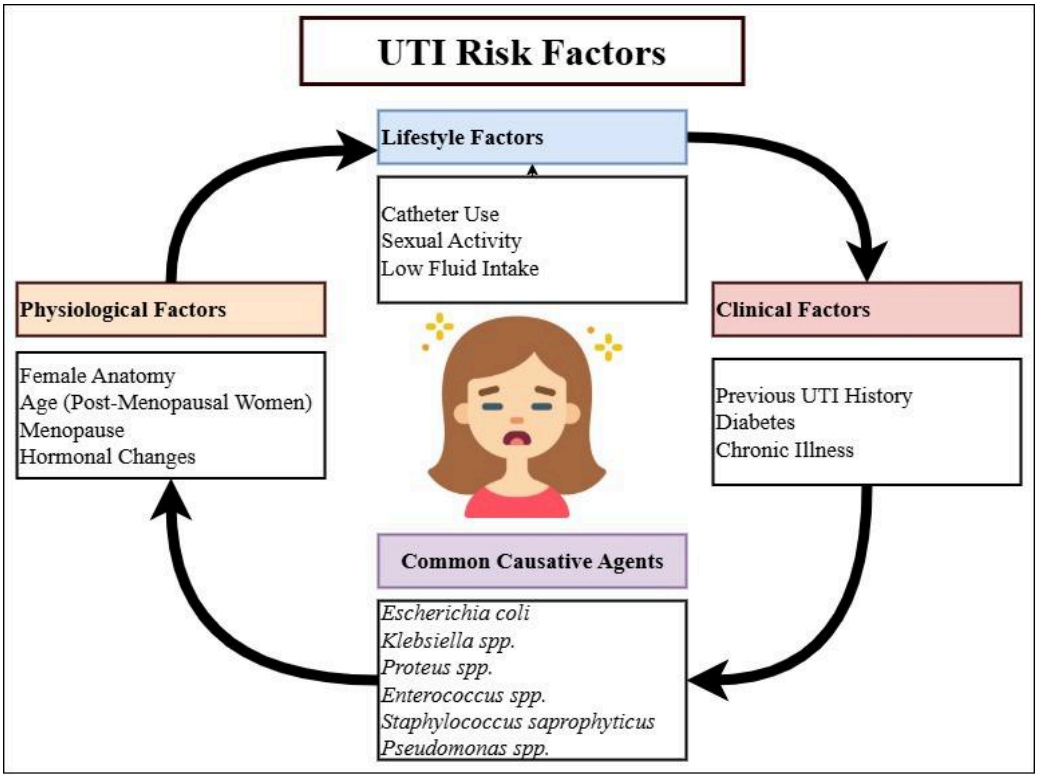


Figure 2. Schematic representation of risk factors associated with UTIs. Diagram created using draw.io, icons sourced from FlatIcon.

1.4 Microbiota Imbalance and UTI Susceptibility

The susceptibility and recurrence of UTI is critically influenced by an interconnected ecosystem of gut, vaginal and urinary microbiome. The gut microbiome serves as the main reservoir of uropathogens such UPEC which originate in the gut microbiome but can migrate to the urinary tract through fecal-perineal-urethral contamination. This process is exacerbated by dysbiosis due to an imbalance due to overabundance of *Enterobacteriaceae* and depletion of protective commensals such as *Faecalibacterium* and *Romboutsia*, disrupting microbial homeostasis.[17] Meanwhile, the vaginal microbiome serves as the biochemical and physical barrier against ascending UTIs with *Lactobacillus* species maintaining the crucial role in maintaining acidic pH, producing antimicrobial compounds and competitively inhibiting pathogenic bacteria. However, this protective mechanism is vulnerable and can be disrupted by estrogen deficiency, antimicrobial usage and bacterial vaginosis (BV), where the proliferation of anaerobic bacteria and decrease in *Lactobacillus* dominance provide a favorable environment for uropathogen colonization.[18] Particularly, BV with a global prevalence of 23-29% among reproductive-aged women with highest prevalence in South Asia (29%), is bidirectionally connected to UTI. Women with BV often exhibit microbiomes similar to those with rUTIs and BV treatments may further worsen this dysbiosis, further increasing UTI risk. [18,19] The urinary microbiome is now recognized as a dynamic community whose dysbiosis may trigger UTIs. Moreover, this microbiome is dominated by *Acinetobacteria*, *Proteobacteria* and *Firmicutes*, playing a crucial role in urinary health with imbalances associated with conditions like bladder cancer and overactive bladder besides UTIs.[17] Therefore, these findings highlights the necessity for microbiome-targeted therapies to restore balances among all three microbiomes which reduces dependence on antibiotics and addresses root causes of rUTIs.[18,19]

1.5 Global Burden and Trends

UTIs are a significant public health concern affecting individuals across all age groups. However they disproportionately impact women due to anatomical and hormonal factors [20]. The risk of infection is notably increased during menstruation, pregnancy, and menopause. According to a study by Hu et al. (2025) the incident cases of UTIs in older women increased by 132% between 1990 and 2021, increasing from 15. 87 million to 36,77 million cases [21]. In 2021, South Asia experienced the highest burden of UTIs among older women, a trend which can be attributed to

the region's large population [21]. In line with highest incidence rates the region also reported nearly twice the number of UTI related deaths compared to Western Europe [22]. The highest number of incident cases, deaths due to UTIs were predominantly concentrated in South Asia and Western Europe. However high income countries in North America exhibited the highest age-standardized incidence rate with Tropical America exhibiting the highest age-standardized mortality rate. These regional variations highlight the complex association between population demographics, healthcare infrastructure, and disease outcomes. These disparities, although region specific, reflect broader systematic challenges that collectively contribute to the rising global burden of UTIs. The clinical and economical burden of UTIs are on the rise with increasing antimicrobial resistance, and inadequate access to timely diagnosis contributing to healthcare costs. This burden is not limited to low income countries as studies have shown that the economic burden of UTI-related hospitalizations is substantial even in high income settings which is estimated to be approximately $2.8 billion annually in the United States alone [23]. In contrast, in low and middle income countries where healthcare is often under-resourced the cost of managing recurrent or complicated UTIs places a significant financial strain on patients and the healthcare system. For example, in Bangladesh, the average cost of treating a UTI including consultation and medication is approximately BDT 670, with societal costs ranging from BDT 642 to BDT 1,384 per episode [15]. Hospital-acquired urinary tract infections (HAUTIs) account for about 18% of UTIs globally, more than double the 8% prevalence of community-acquired cases. HAUTI rates are highest in Asia (22%) and South America (26%), while community UTIs are more common in Africa (17%)[24]. These regional differences may reflect disparities in healthcare infrastructure. The burden of UTIs on patients and society is multifactorial and is likely to increase due to the growing antimicrobial resistance trends observed globally [15].

1.6 UTI Burden and Epidemiological Significance in Bangladesh

UTIs pose a substantial public health burden in Bangladesh affecting individuals of all age groups with a higher incidence among women. National and regional studies indicate that the overall UTI prevalence ranges vary depending on the population subgroup and study setting. In Bangladesh, UTIs contribute significantly to outpatient visits, antimicrobial prescriptions, and hospitalization risks, particularly among vulnerable groups such as pregnant women, the elderly. The overall burden is exacerbated by limited diagnostic access in rural areas, misuse and overuse

of antimicrobials. Addressing these challenges requires a clear understanding of local epidemiological patterns, particularly those emerging from hospital-based surveillance and clinical studies. In a study by Rustom et al. (2021), out of 300 urine samples analyzed, the overall UTI prevalence was 42%, with 126 samples testing culture-positive [25]. Among the demographic groups studied, adult females exhibited the highest prevalence at 64%, followed by adult males and children, both at 31%[25]. Similar findings were reported in a study by Rahman et al. (2014) where adult women (aged above19) exhibited a higher UTI incidence rate (16.8%).[26] In a similar study, 400 urine samples were collected from young female students, of which 106 (26.5%) showed significant bacterial growth. Among these positive cases, 57% (60 samples) were symptomatic, while 43% (46 samples) were asymptomatic. The highest prevalence of uropathogens was observed in the 24–25-year age group, accounting for 51% of infections [27]. In one study involving 247 pregnant women, 31.5% were symptomatic and 68.4% were asymptomatic [28]. This high proportion of asymptomatic cases underscores the potential risks of untreated UTIs during pregnancy as they can lead to serious complications such as pyelonephritis. While pregnant women represent a high risk group for UTI in Bangladesh, UTIs also disproportionately affect other vulnerable populations such as newly married women. For this group, social and biological factors contribute to increased susceptibility. According to a cross-sectional study where the total sample size included 1662 newly married women (aged 18 to 25) the overall UTI prevalence was 23.4% with a notably higher rate during the first year of marriage (7%)[29]. This pattern suggests an association between marital status and UTI risk which is likely influenced by factors such as increased sexual activity, changes in personal hygiene practices, and limited awareness about UTI prevention practices. These findings from Bangladesh are consistent with global epidemiological studies, which also identify adult women as the demographic most commonly affected by UTIs. The burden of UTIs in Bangladesh encompasses significant clinical, social, and economic impacts. Clinically, UTIs contribute to frequent outpatient visits and hospitalizations. Economically, the high prevalence of multidrug-resistant pathogens has increased healthcare costs, prolonged treatment durations. The emergence of multidrug resistant pathogens causing UTIs have been reported in several studies in the country. For example, in a study conducted in Dhaka, Bangladesh, among 110 suspected UTI patients, *Escherichia coli* isolates showed high resistance rates to commonly used antibiotics, including 100% resistance to azithromycin, 80% to nalidixic acid, 54% to

cotrimoxazole, and 44% each to ciprofloxacin and cefixime[30]. This study also noted a predominance of female patients (76.4%) across all age groups consistent with earlier studies.

1.7 Socioeconomic and Cultural Factors Affecting UTI Risk and Management in Bangladesh

The risk, diagnosis, and treatment of UTIs are influenced by underlying socioeconomic and cultural factors, particularly in low- and middle-income countries like Bangladesh. Across multiple regions marginalized populations face disproportionate barriers in diagnosis, treatment, and prevention of UTIs. Studies have shown that women from lower socioeconomic backgrounds are disproportionately affected, as limited financial resources, restricted healthcare access, and poor sanitation conditions increase their vulnerability to infection. A cross-sectional study from Pakistan revealed that individuals from deprived and severely deprived backgrounds were more likely to seek informal treatment rather than accessing formal healthcare services [31]. These groups were also less likely to complete prescribed antibiotic courses which highlights how poverty directly impacts treatment adherence and further contributes to antibiotic misuse [31]. According to a study by Deng et al. (2024) globally, the age-standardized incidence of UTIs among adolescents and young adults (AYAs) increased between 1990 and 2019, with low- and middle-income countries (LMICs) bearing a disproportionate burden [32]. In the United States, a study of young adults revealed that socioeconomic and cultural barriers such as cost concerns, time constraints, lack of insurance, language barriers, and fear of stigma from parents or peers significantly hinder access to UTI care [33]. Such challenges are magnified in the Bangladeshi context. In Bangladesh low socioeconomic status has been recognized as a significant risk factor of UTIs [34]. According to different studies women from disadvantaged backgrounds from the country are more vulnerable to susceptibility due to occupational stress, long working hours, poor hygiene conditions in the workplace, and limited access to proper sanitation and healthcare [34]. A study comparing 310 garment workers and 297 other female workers found higher rates of UTI symptoms among garment workers [34]. Interestingly, financial strain serves both as a risk factor for and a consequence of UTIs. A study reported that self-identified financial distress is often a direct result of recurrent infections [35]. Limited healthcare access also contributes to socioeconomic disparities, including unequal diagnosis and treatment of UTIs in Bangladesh. Additionally the risk of uUTIs is significantly influenced by factors such as socioeconomic

status, place of birth, and urban residence [36]. In Bangladesh, economic barriers and out-of-pocket expenses pose a major challenge to UTI treatment. Public healthcare facilities, hospitals are often overcrowded or lack essential resources, leading many to seek care from private clinics. Managing multidrug-resistant UTIs and recurrent UTIs further increases financial strain, as it involves costlier antibiotics and frequent follow-up visits. Additionally, social and infrastructural challenges such as lack of privacy and safety often force women and girls to delay urination, a known risk factor for UTIs.

1.8 Hypothesis and Objectives of the Study

Given the high burden of urinary tract infections (UTIs) and the diagnostic challenges faced in resource-limited settings, this study focuses on addressing key gaps in microbial characterization. The first objective is to determine the prevalence and distribution of bacterial species associated with UTIs, emphasizing both commonly reported and underrecognized infectious agents. To achieve this, metagenomic analysis was employed to explore the diversity of the urinary microbiota, allowing simultaneous detection of both known and previously overlooked pathogens.

Emerging evidence suggests that diabetic patients may exhibit altered urinary tract microbial communities, with potential differences between those who develop urinary tract infections (UTIs) and those who do not. In the present study, it is hypothesized that microbial diversity and composition differ significantly between diabetic patients without UTI (Group 1) and those with suspected UTI (Group 2). Specifically, Group 2 is expected to harbor a higher relative abundance of classical uropathogens such as *Escherichia coli, Klebsiella, Proteus, Shigella, Providencia,* and *Enterobacter,* alongside increased monomicrobial interactions. Based on this hypothesis, the study sets out the following objectives:

1. To compare microbial diversity between Group 1 (without UTI) and Group 2 (with suspected UTI).
2. To determine the prevalence of monomicrobial versus polymicrobial communities in both groups
3. To identify the relative abundance of classical uropathogens in both groups.
4. To analyze shifts in microbial community composition associated with suspected UTI in diabetic patients.

Chapter 2: Literature review

2.1 Culture-Independent Microbial Analysis: Advancements in Sequencing Technology

Metagenomic sequencing is a powerful tool for diagnosing UTIs as it enables the characterization of urobiome, identification of uropathogens and detection of culture-negative infections. Besides shotgun metagenomic sequencing, 16s rRNA sequencing have also been employed by many studies to assess microbial diversity. These approaches are valuable in women as postmenopausal and recurrent UTIs are influenced by multiple factors such as age, hormonal status, antibiotic exposure, BMI and other comorbidities. Below are 10 related papers that applied sequence-based methods for UTI detection which sorted patients (mostly women) according to demographic characteristics, laboratory findings, and clinical presentation with some having healthy controls and others focusing on recurrent or symptomatic UTIs.

Table 1: Current advancements in sequencing technology for UTI detection

Year	Focus	Participants	Grouping Parameters
2022	Shotgun metagenomics to identify microbial and functional differences among postmenopausal women with varying UTI history, hormonal therapy influence and microbial resistance gene patterns	3 groups of postmenopausal women (n=75): no UTI history (control) , past rUTI but infection free, current rUTI	UTI history, bmi, race, smoking status, estradiol therapy, urinary creatinine concentration [42]
2022	Used metagenomic nanopore sequencing for rapid pathogen and antibiotic resistance gene detection as an alternative to traditional culture-based methods	76 suspected cases of acute or chronic UTI infection (40 female, 36 male)	Demographic and baseline characteristics, symptoms and laboratory findings for clinical diagnosis[43]
2024	Proof of concept rapid detection of UTI pathogens through nanopore sequencing	Not specified (pilot scale)	Healthy urine spiked with clinically relevant uropathogens concentration to imitate UTI[44]

2021	Used 16S rRNA to compare urinary microbiome diversity and composition among acute and recurrent cystitis in women	42 female patients categorized into acute uncomplicated cystitis and recurrent cystitis, no controls	Sorted into acute and current cystitis on the basis of age (>=20 years), urinalysis, urine culture and urine NCS; patients with structural abnormalities excluded[45]
2022	Used 16S rRNA to investigate urinary microbiota composition of women with rUTI (culture-negative) and healthy controls	90 rUTI women and and 62-age matched controls	rUTI clinical diagnosis (culture negative) and healthy asymptomatic controls with age (>=18 years); patients with structural abnormalities, antibiotic treatment, pregnant or lactating women and sexually transmitted infections were excluded [46]
2023	Used MinION nanopore sequencing for pathogen detection	3 groups (gender not specified): culture-positive (148), culture-negative (65) and healthy control (39)	On the basis of culture status and presence of symptoms [47]
2021	Used 16s rRna sequencing to evaluate the urobiome in postmenopausal women with rUTIs and age-matched controls without rUTIs	64 postmenopausal women divided into 3 groups: rUTI+ antibiotics (17), rUTI+ no antibiotics (23), control (23)	On the basis of age (>55), bmi, use of estrogen (>6 months prior sampling), and additional covariates considered (sexual activity, history of diabetes, prior surgeries)[48]
2025	Used 16s rRna sequencing to evaluate the urobiome in	50 postmenopausal women divided into 2	On the basis of age (>50), and additional

	postmenopausal women with rUTIs and without rUTIs (age-matched control)	groups: rUTI (30), no rUTI as control (20)	covariates considered: behavioral (sexual activity, hygiene), clinical (history of diabetes, antibiotics usage, UTI history), and sampling timing[49]
2018	Used 16s rRNA sequencing and shotgun sequencing to characterize microbial metagenome of UTI and to compare sequencing methods	Both men (29) and women (92) into 3 clusters: likely non-infected (9), infectious (63) and unclear (49)	Sorting based on clinical metadata, gender, and infection status[50]
2014	Used several sequencing methods to determine whether single clinical isolates from acute uncomplicated UTIs are representative of the dominant microbial populations and compared microbial communities obtained through culture-based methods	50 individuals: female (38), male (12)	Sorting based on age, gender, clinical parameters[51]

2.2 Discrepancies in Microbial Detection Between Culture and Sequencing

The accurate characterization of FUM (Female Urinary Microbiota) comes with a number of challenges, with contamination being a major factor that leads to misleading results in most cases. Urine is a low biomass sample meaning the amount of microbial DNA is relatively low, which increases the risk of contamination presenting a significant challenge in microbiome study. Standard culture conditions favor fast-growing, non-fastidious organisms, often resulting in the overrepresentation of taxa like *Enterobacteriaceae*. Slow-growing, anaerobic, or fastidious microbes may fail to grow or be outcompeted, leading to underestimation of true microbial diversity. Additionally contamination is a major problem with standard culture techniques. Potential sources of contamination include method of urine collection, laboratory settings, environmental exposure, reagents used, and human handling. The table below summarizes the common contamination sources and their impact on urine microbiome analysis.

Table 2: Challenges associated with standard culture techniques.

Contamination Source	Impact
Voided urine samples	May introduce vaginal bacteria increasing the diversity of the samples observed.
Catheterization and clinical equipment	Might lead to false positives for bladder microbiota. Additionally contamination could arise from handling
Laboratory cross contamination	Mislabeling and contamination from laboratory environments may lead to increased diversity
Environmental exposure	Contact with air, surfaces, or equipment may introduce taxa that were not originally present in the urine sample.
DNA extraction kits and reagents	Contaminating DNA is ubiquitous and varies between kits affecting low biomass samples in both 16S rRNA and shotgun metagenomics studies. [58, 59]

This highlights the importance of sequencing for bacterial identification and why culture-independent sequencing often reveals taxa that are undetected by conventional cultivation.

2.3 Contamination as a Confounding Factor in Microbial Community Analysis

Urine culture contamination introduces significant challenges in both culture-based and sequencing-based approaches. In culture-based approaches, contamination is introduced through errors during sample collection from patients or sample handling, while for sequencing of low-biomass samples, the vulnerability is presented by trace DNA from environment and reagents which can dominate or overwhelm true signals. The contamination profiles of both methods are contrasted in the table below;

Table 3: Contamination in both techniques.

Method	Contamination Profile	Key Findings
Culture-based	Commensal bacteria in the distal	Cleansing procedures may not

	urethra, skin and vaginal flora such as *Coagulase-negative Staphylococci*, diphtheroids	significantly reduce contamination but are challenging for elderly and impaired. Despite contamination, colony counts remained clinically reliable.[37]
Culture-based	Low for all methods (MSU and CSU). No significant difference in contamination was found in MSU and catheter	MSU spun sediment was the most productive method whereas high no-growth rate (53%) was observed in CSU sampling. The most comprehensive urinary sediment profile was presented by analysis of naturally voided samples, indicating that polymicrobial growth and epithelial cells' presence do not necessarily indicate contamination.[38]
Sequencing-based (low-biomass samples)	High contamination frequency and dominant (>50%) sequences with low biomass, contamination from reagent DNA and environment. The kitome included genera like *Acinetobacter*, *Ralstonia*, *Pseudomonas*, *Bradyrhizobium*, *Burkholderia*, and *Propionibacterium.*	Low-biomass microbiome profiles can be distorted and dominated by contaminants, leading to false conclusions. The contamination profile is batch and kit specific.[39]
Sequencing-based	Variable contamination; dominant and significant in samples with low-biomass without controls. The sources include: cross-well contamination, aerosolized DNA from handling, index hopping, laboratory surfaces, sampling equipment and most importantly from reagents and kits.	Ensuring data reliability necessitates extensive controls and proper lab setups as contamination is a major concern in low-biomass studies.[40]

2.4 Healthy Urinary Microbiome

The female urinary microbiota (FUM) shows variability among individuals leading to the use of distinct urotypes based on the dominant genus. There are six urotypes which include: (1) *Gardnerella*, (2) *Sneathia*, (3) *Staphylococcus*, (4) *Enterobacteriaceae*, (5) *Lactobacillus*, and (6) a "diverse". The diverse urotype is characterized by an even distribution of multiple genus [41]. At the phylum level, the FUM is composed primarily of *Firmicutes* (65%), with smaller contributions from *Bacteroidetes* (18%), *Actinobacteria* (12%), *Fusobacteria* (3%), and *Proteobacteria* (2%) [42]. Additionally, age-related differences have also been reported with older women exhibiting reduced bacterial diversity compared to younger women. While the FUM in healthy individuals is generally characterized by *Lactobacillus* dominant urotypes, this is significantly altered in diseased individuals. A comparison of findings from multiple studies is summarized in the table below in order to highlight the alterations in microbial composition.

Table 4: Microbial composition in different conditions.

Study	Condition	Dominant Phyla	Study Findings
Siddiqui et al. (2011) [43]	Healthy women	*Lactobacillus, Prevotella, Gardnerella, Peptoniphilus, Dialister, Finegoldia, Anaerococcus, Allisonella, Streptococcus, Staphylococcus*	Study revealed that the bacterial composition in human female urine specimens is polymicrobial with considerable variation.
Pearce et al. (2014) [44]	Urgency Urinary Incontinence (UUI)	*Actinobaculum, Actinomyces, Aerococcus, Arthrobacter, Corynebacterium, Gardnerella, Oligella, Staphylococcus,* and *Streptococcus*	Study revealed that the UUI and non-UUI urinary microbiomes differ based on both sequence and culture evidence. Additionally the findings revealed that the UUI microbiome was composed of increased *Gardnerella* and decreased *Lactobacillus.*

Curtiss et al. (2017)[45]	OAB patients vs Healthy women	*Staphylococcus* (59%), *Streptococcus* (51%), *Corynebacterium* (37%), *Lactobacillus* (28%)	Both groups had a mean of 5 genera. *Proteus* was more common in OAB patients. *Lactobacillus* was significantly more common in controls
Yoo et al. (2021)[46]	Acute uncomplicated cystitis (AUC)	*Pseudomonas, Acinetobacter, Enterobacteriaceae*	Lower microbial diversity with only typical uropathogens.
Yoo et al. (2021)[46]	Recurrent cystitis (RC)	*Sphingomonas, Staphylococcus, Streptococcus, Rothia*	RC patients had significantly higher diversity and different microbiome composition than AUC. NGS detected more taxa than urine culture

2.5 Microbiome Dysbiosis in Diabetic Women

In women with Type 2 diabetes mellitus (T2DM), significant alterations are observed in the urinary microbiome that increase the susceptibility to UTIs and related complications.[47] A systematic review highlighted consistent patterns of decrease in microbial diversity, with T2DM patients exhibiting decrease in beneficial taxa such as *Lachnospiraceae* (butyrate-producing bacteria associated with mucosal integrity), *Anaerococcus, Peptoniphilus* and *Finegoldia* while showing increase in inflammation-associated microbes, primarily in the Proteobacteria phyla (e.g. *Pseudomanadata*, *Lactobacillus* and *Enterococcus*), signalling a shift toward being more favourable for UTI-causing bacteria to thrive.[48] In elderly women with T2DM, *Lactobacillus* levels were significantly lower compared to non-diabetic individuals, whereas *Dialister* and *Peptoniphilus* were lower in those with asymptomatic bacteriuria, indicating that even minor imbalances in the urinary microbiome in diabetes can increase UTI risk.[49] Furthermore, T2DM patients with comorbid hypertension showed increased abundance of Proteobacteria with inclusion of *Escherichia*, whereas those with hyperlipidemia did not show such increase, a distinction matching trends with asymptomatic bacteriuria and nitrate-positive results, suggesting

host metabolic comorbidities can affect the urinary microbiome and UTI risk.[50] One mechanistic study revealed that increased levels of *Lactobacillus iners*, *Corynebacterium*, *Enterococcus* and *Akkermansia* correlated with elevated urinary interleukin-8 (IL-8) which is a proinflammatory chemokine. It highlighted that local inflammation can be triggered by an imbalanced microbiome and increase UTI risks.[51] Collectively, these findings present that in diabetes, the urinary microbiome shift away from protective commensals and shifts towards potentially pathogenic and inflammation promoting bacteria, creating favorable environments that increase risk of rUTIS, impaired bladder immunity, and asymptomatic infections.

Chapter 3: Methodology

3.1 Patient Selection and Eligibility

A cross-sectional survey was conducted among diabetic patients in this study. Samples were collected from both inpatient and outpatient departments. The two groups defined in this study are:

- Group 1 (No UTI): Patients with no clinical evidence of urinary tract infection.
- Group 2 (Suspected UTI): Patients with clinical signs or symptoms suggestive of a urinary tract infection, but not yet confirmed.

The inclusion criteria were as follows;

1. Adult female patients with a confirmed diagnosis of diabetes mellitus.
2. Patients clinically suspected of having UTI for Group 2, without clinical suspicion of UTI for Group 1.
3. Patients providing informed consent for survey data collection and sample collection.

The exclusion criteria for this study include;

1. Non-diabetic individuals.
2. Patients under the age of 18.
3. Patients with urinary catheters.

From the inpatient samples, a total of 30 samples were initially collected from patients admitted to the hospital wards for conditions unrelated to UTIs. After applying the inclusion criteria 24 samples from confirmed diabetic patients were retained. Six samples were excluded in order to focus the analysis on patients meeting the study's specific clinical characteristics and to maintain comparability across samples. In case of outpatient samples a total of 38 samples were collected from the general/outpatient department of which 36 met the inclusion criteria and were included in the study. A total of 27 samples were sent to ICDDR,B for metagenomic sequencing comprising 17 samples from Group 1 and 10 samples from Group 2.

3.2 Standard Microbiological Testing

3.2.1 Sample Collection

A total of 15 ml of midstream urine was collected for each sample in sterile falcon tubes. The samples were transported to the laboratory within 2 hours of collection (on ice). From each

sample 5 ml was stored for sequencing while the remaining volume was used for microbiological analysis.

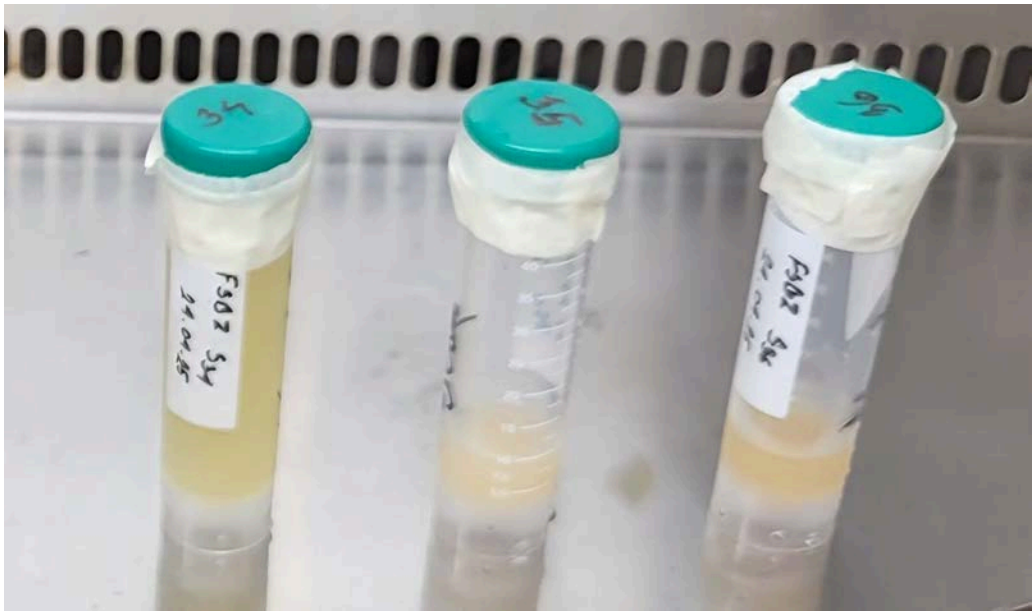


Figure 3. Falcon tubes used for urine collection.

The workflow for microbiological testing is illustrated in the flowchart below;

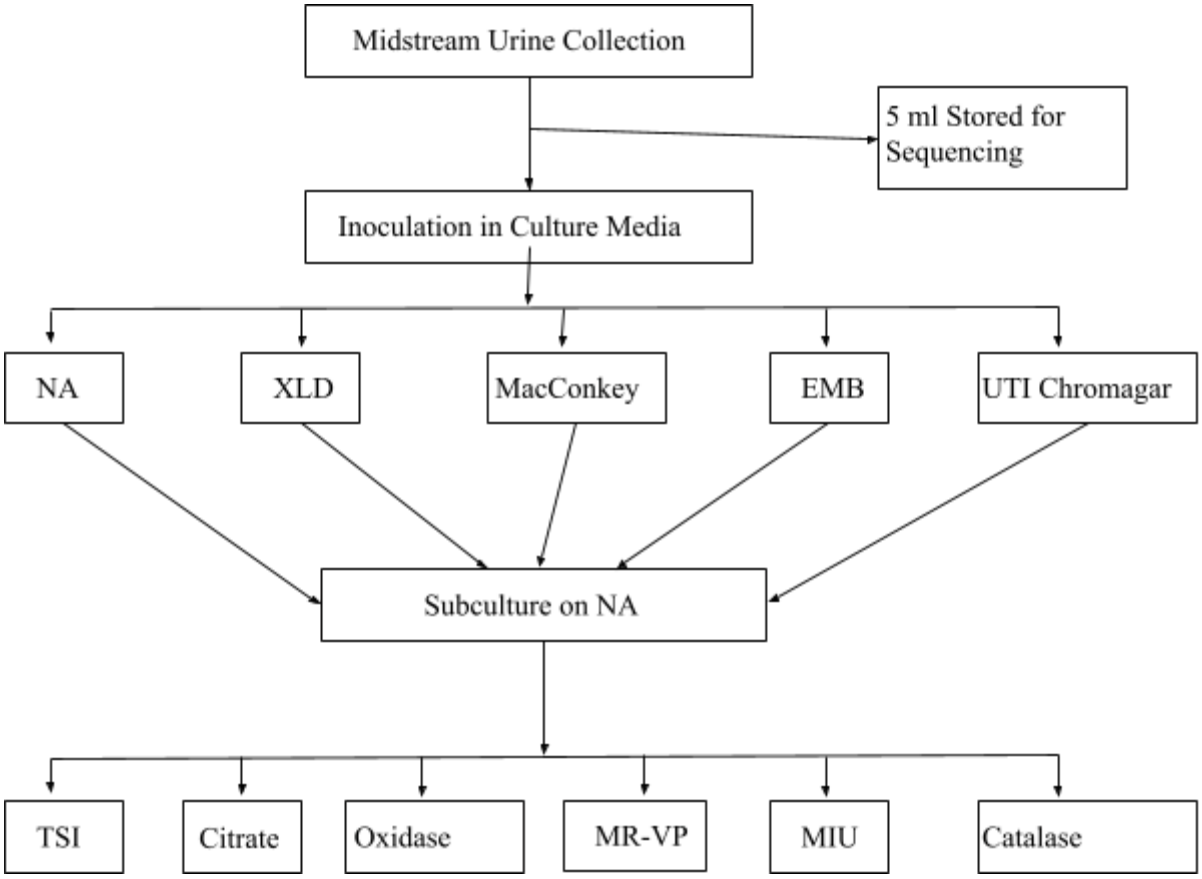


Figure 4. Workflow of urine sample collection, culture, and biochemical characterization.

3.2.2 Culture and Isolation

Urine samples were inoculated onto five different culture media which include Nutrient Agar (NA), MacConkey Agar, UTI Chromagar, Xylose Lysine Deoxycholate (XLD) Agar, Eosin Methylene Blue Agar. Inoculation was done under sterile conditions following standard laboratory protocols. Plates were incubated at 37° C for 24-48 hours. Microbial growth was observed and representative colonies were selected based on colony morphology and were subcultured on NA for subsequent biochemical tests. Each media aid in the selective growth and differentiation of specific microorganisms as described below;

1. Nutrient Agar: NA is a basic culture medium generally used for the culture of non-fastidious organisms. In this study NA was used for colony count and subsequent subculturing.
2. MacConkey Agar: MacConkey agar is both selective and differential culture medium. It is selective due to the presence of crystal violet which inhibits the growth of

gram-positive bacteria. The differential property of this media is due to the presence of pH indicator neutral red. Gram-negative bacteria can be differentiated based on their ability to ferment lactose.

3. XLD: XLD agar is selective and differential media commonly used for the isolation and differentiation of *Salmonella spp.* and *Shigella spp.* The growth of gram positive bacteria is inhibited by sodium deoxycholate and the differentiation is possible due to lysine decarboxylation and hydrogen sulfide production.
4. EMB: EMB agar is a selective and differential media which is used to isolate gram negative coliforms. The presence of Eosin Y and Methylene Blue inhibits the growth of gram positive bacteria. Differentiation is based on the microorganisms ability to ferment lactose.
5. UTI Chromagar: UTI chromogenic agar is a differential media generally used for the presumptive identification of bacterial pathogens present in urine samples. The differentiation is based on the chromogenic substrates that are metabolized by bacterial enzymes which produce colonies with distinct colors for different species of bacteria.

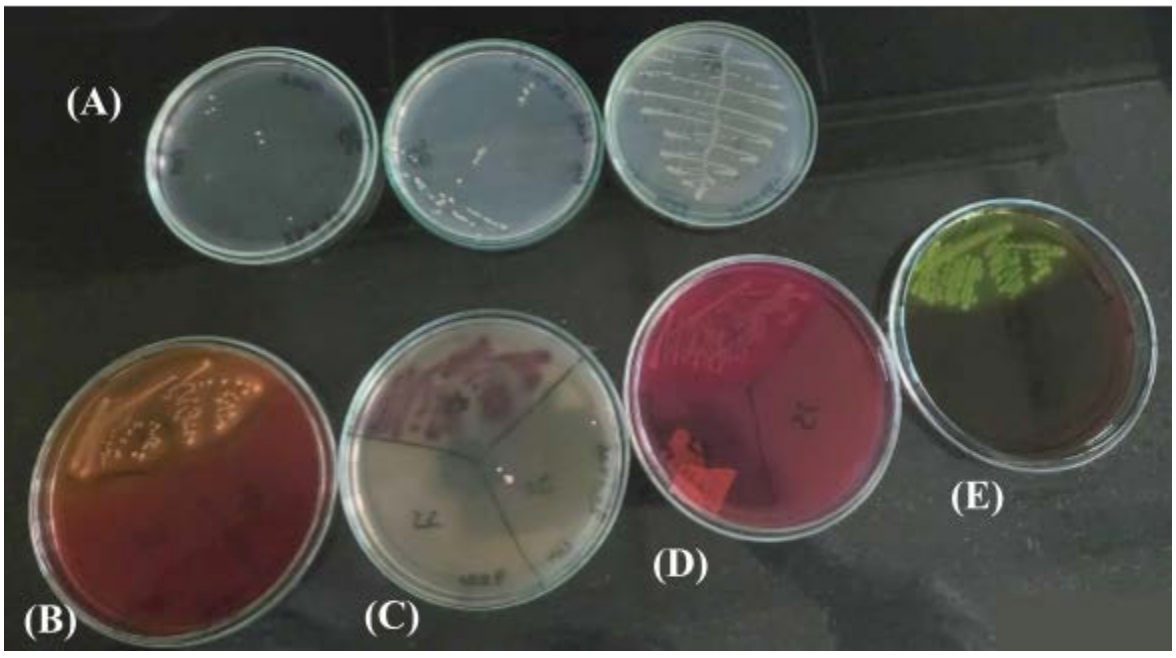


Figure 5. Representative growth of urine samples on different culture media; (A) Nutrient Agar, (B) XLD Agar, (C) UTI Chromagar, (D) MacConkey Agar, and (E) EMB Agar.

3.2.3 Biochemical Characterization

Representative isolates were identified and characterized using a series of standard biochemical tests. The following tests were performed;

- Triple Sugar Iron (TSI) Agar: TSI characterizes bacterial species in accordance with their carbohydrate fermentation patterns. A yellow slant and yellow butt (A/A) indicate fermentation of all three sugars, a red slant and yellow butt (K/A) indicate the fermentation of only glucose, a red slant and red butt (K/K) indicate non-fermentors. The production of hydrogen sulfide is indicated by blackening of the agar, the production of gas is indicated by cracking or lifting of the media.

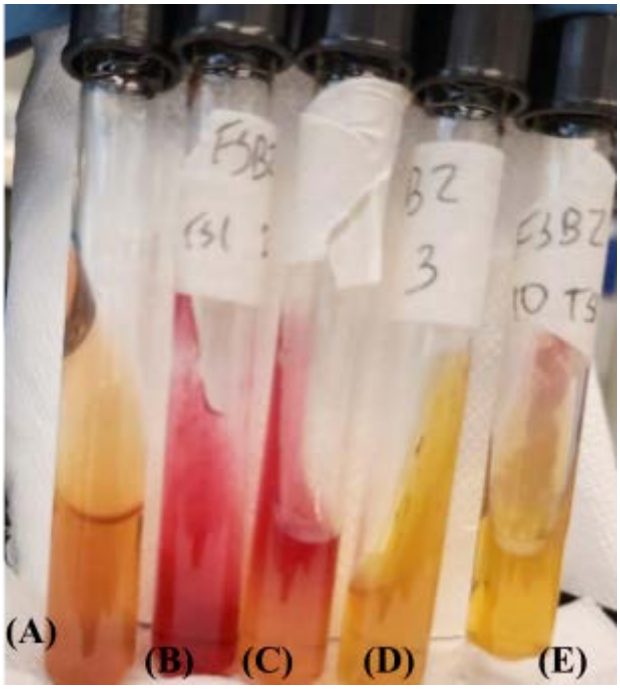


Figure 6. TSI test results. (A) Uninoculated control, (B) Red slant/Red butt, (C) Red slant/Yellow butt, (D) Yellow slant/Yellow butt, (E) Lifting of media due to gas production.

- Simmon's Citrate Agar: This test assesses an organism's ability to utilize citrate as its sole carbon source. A positive result is indicated by a colour change of the media from green to bright blue signifying an increase of pH in the media (alkalization).

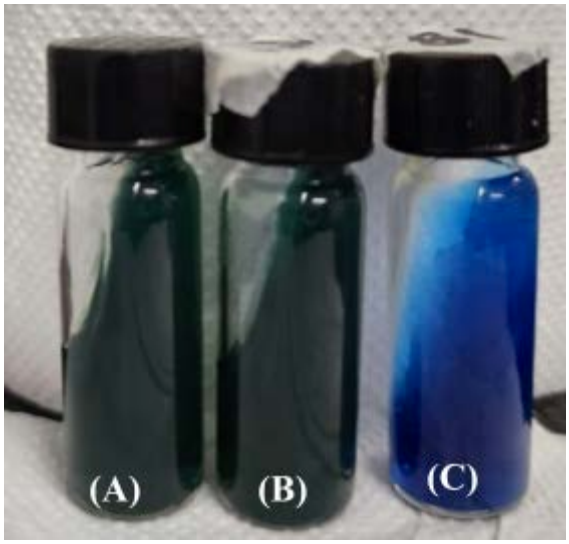


Figure 7. Citrate test results. (A) Uninoculated control, (B) Negative result, (C)Positive result.

- Methyl Red (MR) and Voges-Proskauer (VP) Tests: These two tests distinguish bacteria based on major glucose metabolism pathways. The MR test detects acid end products and detection is based on red colour formation due to the addition of methyl red. The VP test detects the presence of precursor acetoin in the butanediol fermentation pathway. The detection is based on the formation of red-brown colour after the addition of reagents.

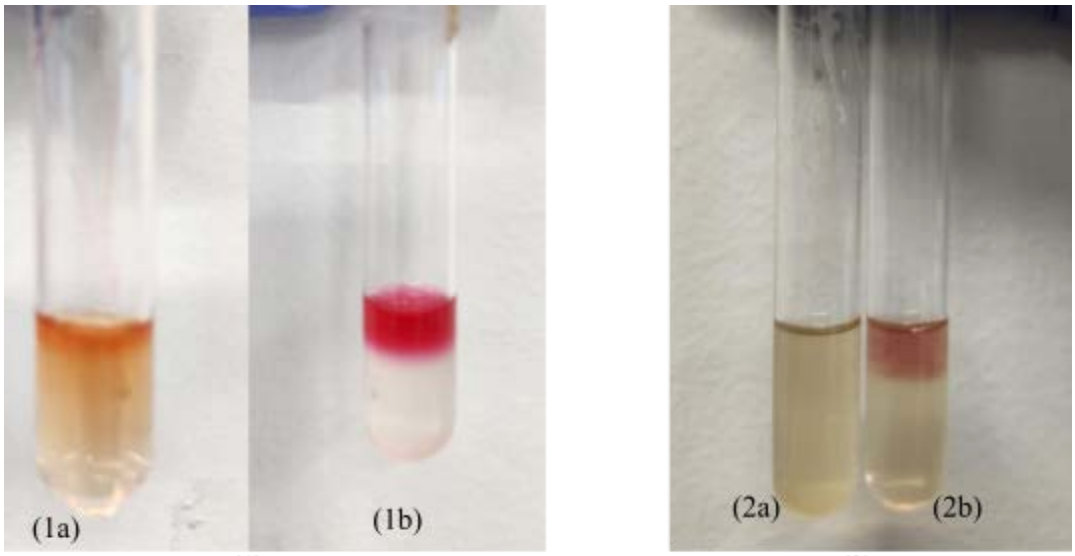


Figure 8. MR-VP test results. (1) represents the MR test where (1a) negative result and (1b) positive result. (2) represents the VP test where (2a) negative result and (2b) positive result.

- Motility Indole Urease Test: This is a combination of three different tests. Motility is

observed as diffuse growth radiating from the stab line. Indole production from tryptophan is detected by adding Kovac's reagent, forming a red ring for a positive result. Urease activity is indicated by a color change to pink-red, showing hydrolysis of urea to ammonia.

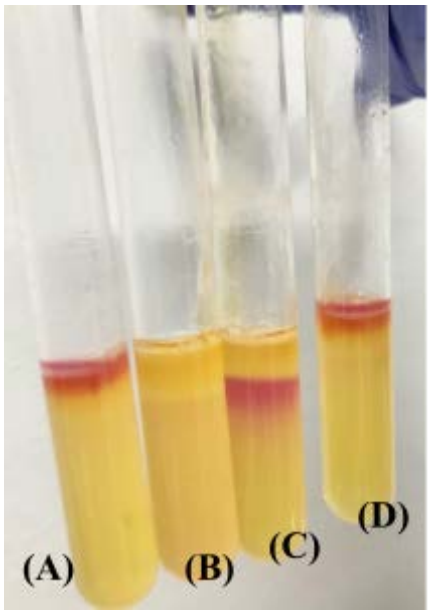


Figure 9. MIU test results: (A) Indole positive, (B) Indole negative, (C) Urease positive, (D) Motility positive.

- Oxidase Test: Oxidase test identifies the presence of cytochrome c oxidase, a key enzyme in the electron transport chain of aerobic bacteria. The formation of a dark blue-purple color within seconds after inoculation of a bacterial colony on a filter paper soaked in tetramethyl-p-phenylenediamine dihydrochloride is indicative of a positive result.

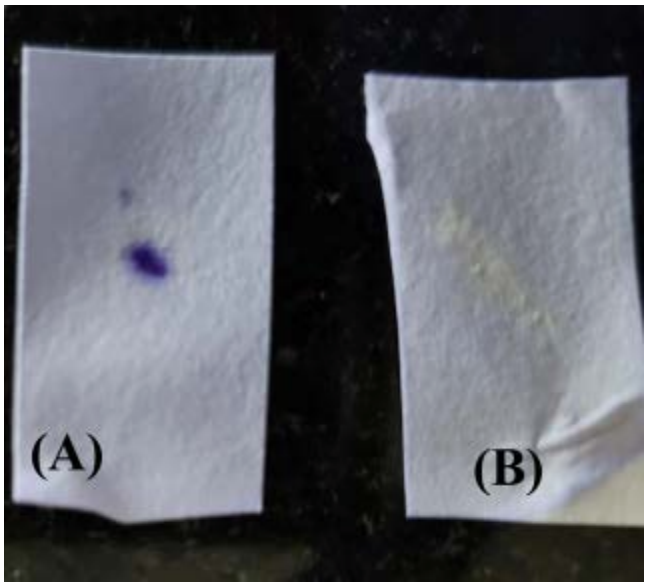


Figure 10. Oxidase test. (A) positive result, (B) negative result.

- Catalase Test: This test detects the production of the catalase enzyme, which breaks down hydrogen peroxide into water and oxygen. The addition of H_2O_2 to a bacterial colony and the immediate formation of oxygen bubbles indicates a positive result.

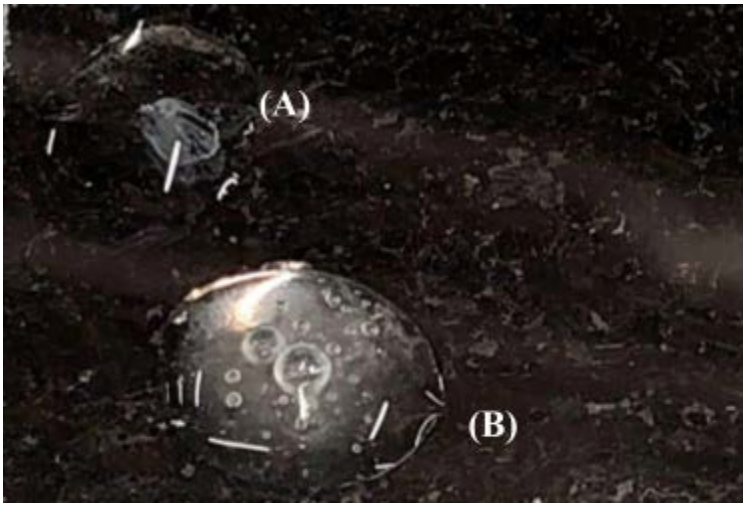


Figure 11. Catalase test. (A) negative result indicated by no bubble formation, (B) positive result indicated by bubble formation.

3.2.4 Data visualization of Biochemical Test Results

The data derived from biochemical characterization were compiled into a structured dataset for subsequent analysis. Statistical summaries were generated, and results were visualized using Python (3.10), using different libraries such as Pandas for data handling, Matplotlib and Seaborn

libraries for data visualization. A dissimilarity matrix was computed from the binary-coded biochemical test results which was generated using the Jaccard distance metric with Python using the scipy.spatial.distance library. Hierarchical clustering was subsequently performed with the average linkage method from scipy.cluster.hierarchy to cluster isolates. The resulting relationships were visualized as a dendrogram using the scikit-bio or matplotlib library.

3.3 Metagenomic Analysis

Microbial community analysis was based on genus-level abundance data. The raw abundance table was first transformed into a binary (presence-absence) matrix to focus on community membership rather than relative abundance. This distance matrix was generated and used as input for hierarchical clustering with the average linkage method, and the resultant dendrogram was visualized. This entire workflow was implemented in Python using libraries including Pandas, Scipy, and Scikit-learn. Additionally, microbiome analysis was carried out using Python with the scikit-bio library for calculating diversity. Alpha diversity was analyzed using Shannon, Simpson, and observed OTU metrics. Beta diversity was assessed using Bray-Curtis dissimilarity and principle co-ordinate analysis (PCoA). Statistical comparisons utilised Mann-Whitney U tests for alpha diversity and PERMANOVA for community structure differences. Differential abundance of uropathogens was tested through non-parametric statistics with false discovery detection rate correction. Taxonomic composition was visualized at both genus and family levels using, with the integration of results with biochemical culture data and sequencing data using Spearman correlation analysis. All visualizations were generated using matplotlib and seaborn.

Chapter 4: Results

4.1 Biochemical Analysis

The study population was divided into two groups for comparative analysis. Group 1 consisted of 24 diabetic patients who were hospitalized for conditions unrelated to urinary tract infections (UTIs). Group 2 included 36 diabetic patients from the outpatient department who were clinically suspected to have a UTI.

In Group 1 (diabetic patients without suspected UTI), out of the 24 urine samples, bacterial growth was observed in 13 samples. The isolated organisms from each culture-positive sample are presented in Table 5.

Table 5: Microorganisms isolated from Group 1

Sample No.	Microorganisms
1	*Escherichia coli, Proteus, Klebsiella*
8	*Klebsiella, Enterobacter, Escherichia coli*
12	*Shigella*
13	*Salmonella*
18	*Shigella, Klebsiella, Escherichia coli, Proteus*
19	*Salmonella, Escherichia coli, Klebsiella, Enterobacter*
21	*Klebsiella, Shigella Escherichia coli*
22	*Proteus, Pseudomonas*
23	*Shigella, Enterobacter (possible)*
24	*Pseudomonas*
27	*Shigella, Escherichia coli*
28	*Escherichia coli, Providencia*
29	*Escherichia coli*

In Group 2 (diabetic patients with suspected UTI), out of the 36 urine samples, bacterial growth was observed in 28 samples. The isolated organisms from each culture-positive sample are presented in Table 6.

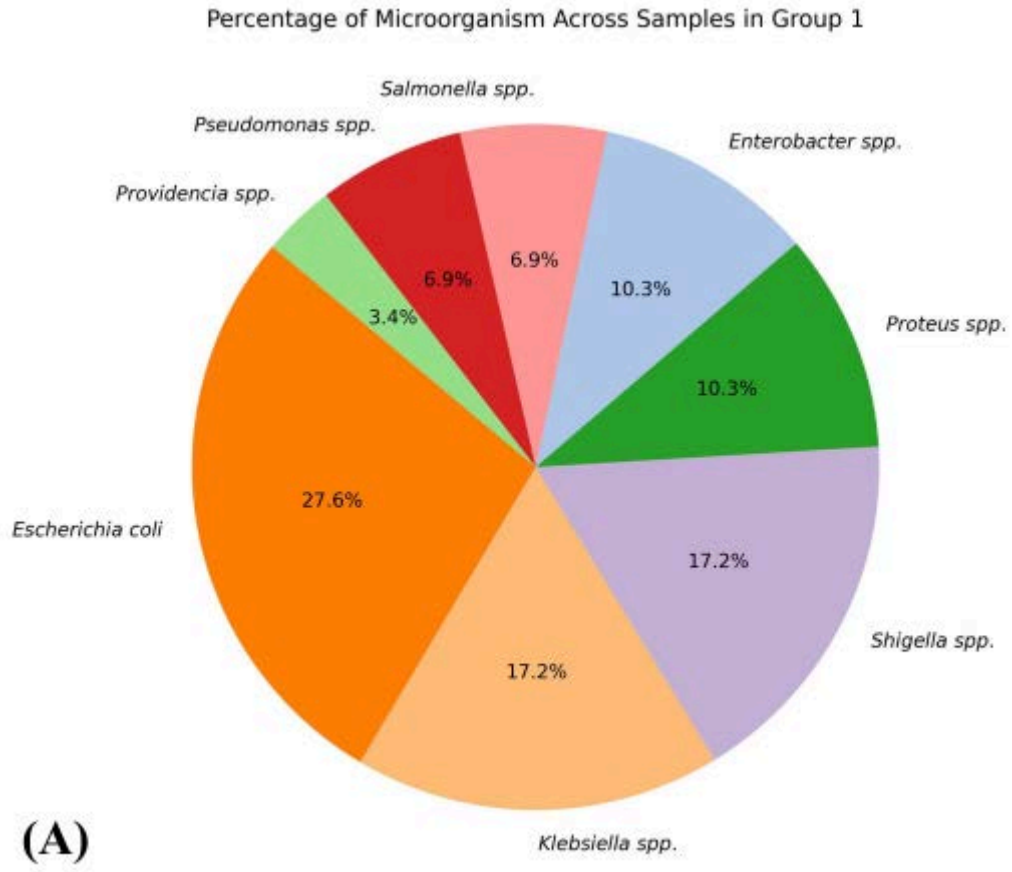
Table 6: Microorganisms isolated from Group 2.

Sample No.	Microorganisms
31	*Escherichia coli*

32	*Providencia, Enterobacter*
34	*Staphylococcus aureus*
35	*Staphylococcus aureus*
36	*Escherichia coli*
37	*Citrobacter freundii, Pseudomonas*
38	*Escherichia coli, Enterobacter*
39	*Pseudomonas*
40	*Enterobacter*
41	*Shigella, Proteus,*
42	*Escherichia coli, Klebsiella, Shigella*
43	*Enterobacter*
44	*Escherichia coli, Enterobacter*
45	*Enterobacter*
46	*Providencia*
47	*Enterobacter*
49	*Shigella, Enterobacter*
53	*Enterobacter*
54	*Enterobacter*
56	*Escherichia coli*
58	*Pseudomonas*
59	*Klebsiella, Enterobacter, Shigella*
67	*Pseudomonas, Enterobacter, Shigella*
68	*Escherichia coli, Klebsiella*
62	*Enterobacter*
63	*Serratia marcescens*
65	*Escherichia coli, Pseudomonas, Shigella*
57	*Escherichia coli*

Detailed biochemical test profiles of all isolates are provided in Supplementary Table S1. Among the culture-positive samples in Group 1, the predominant organism isolated was *Escherichia coli* (27.6%), followed by *Klebsiella spp.* (17.2%) and *Shigella spp.* (17.2%). Other isolates included *Proteus spp.* (10.3%), *Enterobacter spp.* (10.3%), *Salmonella spp.* (6.9%), *Pseudomonas spp*. (6.9%), and *Providencia spp.* (3.4%). In Group 2, 36 urine samples were processed, among

which 28 showed bacterial growth. The most frequent isolate was *Enterobacter spp.* (30.2%), followed by *Escherichia coli* (20.9%) and *Shigella spp.* (14.0%). Other bacteria included *Pseudomonas spp.* (11.6%), *Klebsiella spp.* (7.0%), *Providencia spp.* (4.7%), *Staphylococcus aureus* (4.7%), *Citrobacter freundii* (2.3%), *Proteus spp.* (2.3%), and *Serratia marcescens* (2.3%). The overall distribution of bacteria across samples in both groups is illustrated in Figure 11.



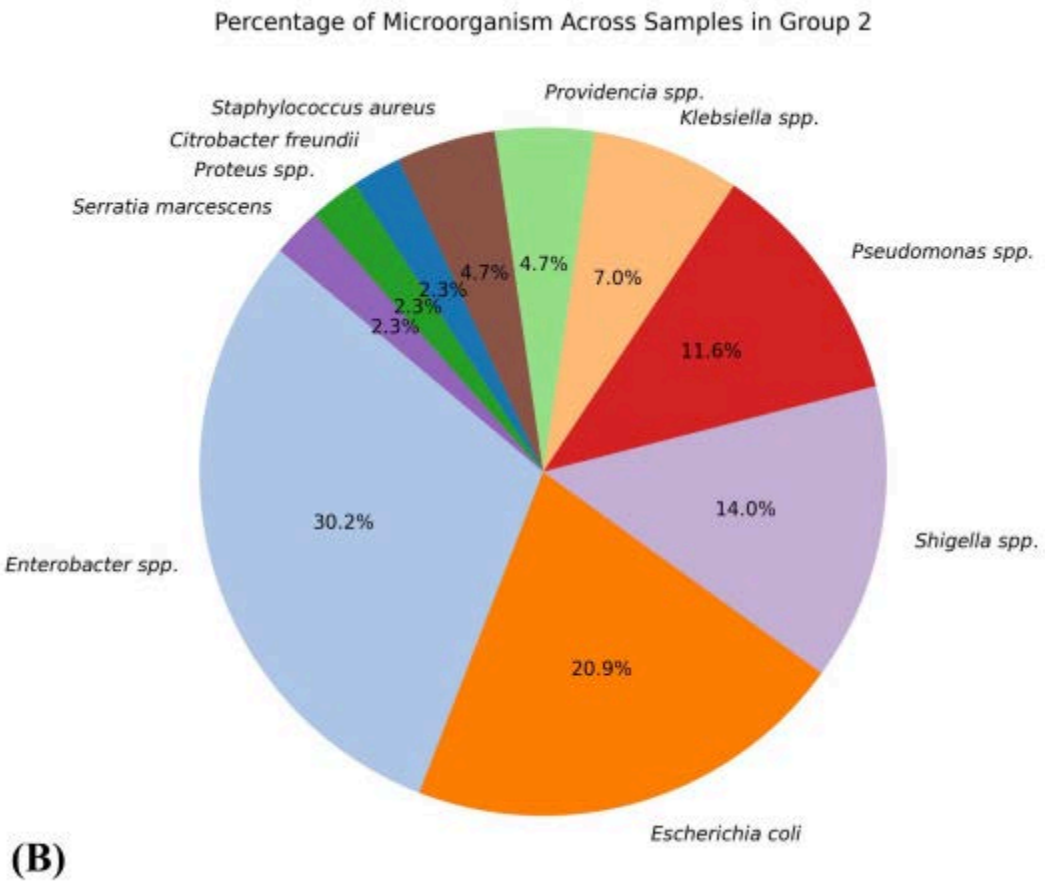
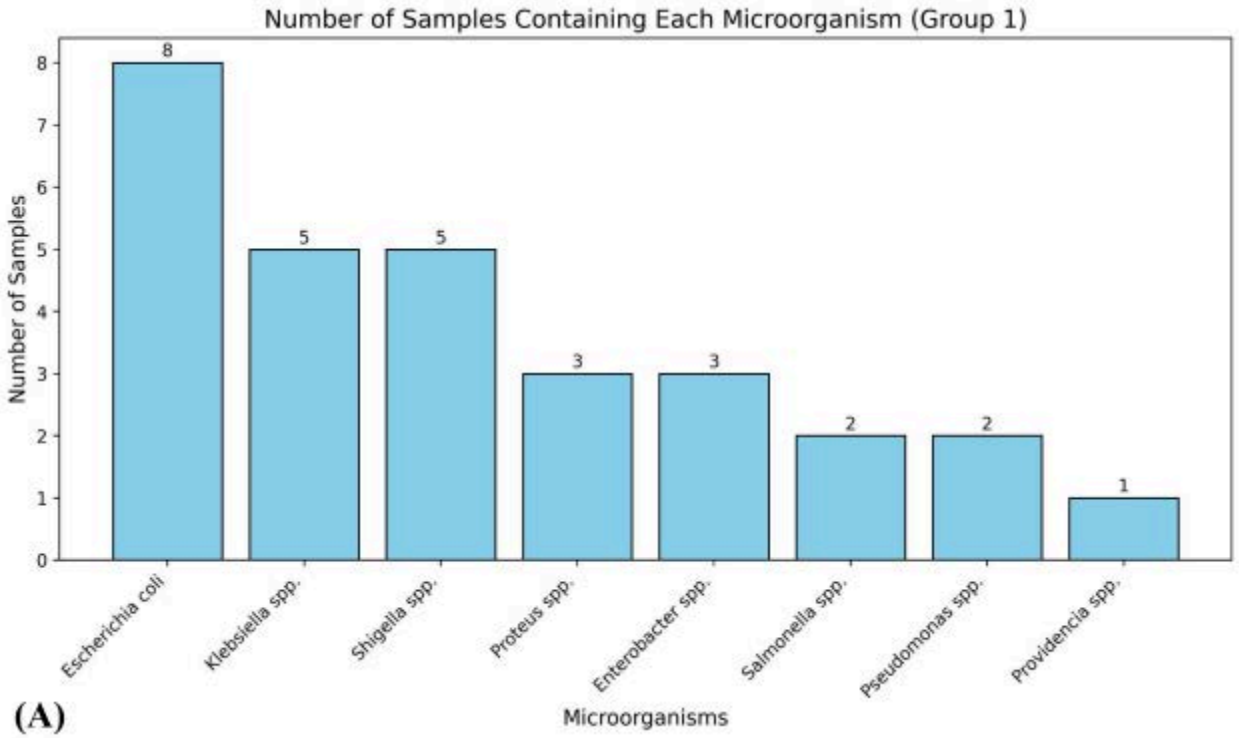


Figure 12. Pie chart demonstrating the distribution of bacterial isolates in urine cultures from diabetic patients with and without suspected UTI. (A) In group 1 (non-UTI), the isolate with the highest percentage were *E. coli* (27.6%), followed by *Klebsiella spp.* and *Shigella spp. (*17.2%). The isolate with the lowest percentage was *Providencia spp.* (3.4%). (B) In group 2 (suspected UTI), the isolate with the highest percentage were *Enterobacter spp.* (30.2%), followed by *E. coli* (20.9%). and *Shigella spp.* (14%). The isolate with the lowest percentage were *Proteus spp*., *Citrobacter freundii*, and *Serratia marcescens* (2.3%).

The following bar charts show the number of samples in which each microorganism was detected.


Number of Samples Containing Each Microorganism (Group 1)
Number of Samples
Microorganisms
Escherichia coli
Klebsiella spp.
Shigella spp.
Proteus spp.
Enterobacter spp.
Salmonella spp.
Pseudomonas spp.
Providencia spp.
8
5
5
3
3
2
2
1
0
1
2
3
4
5
6
7
8

(A)

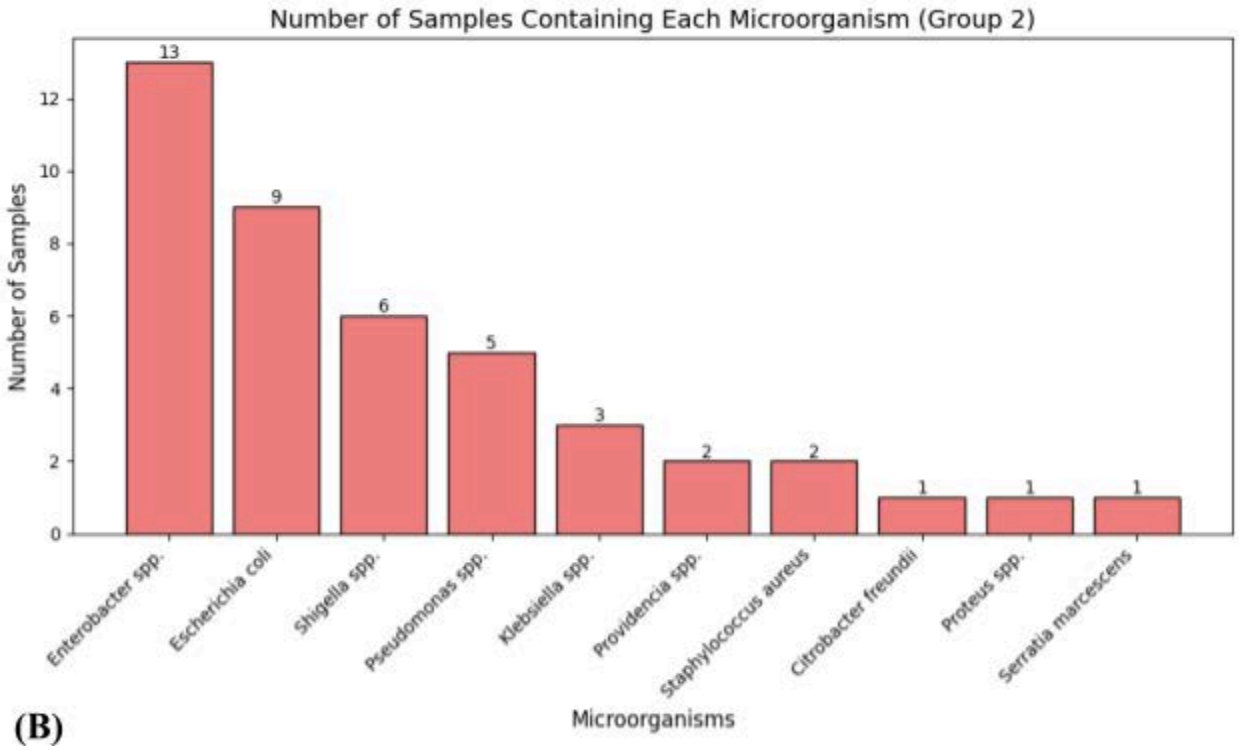
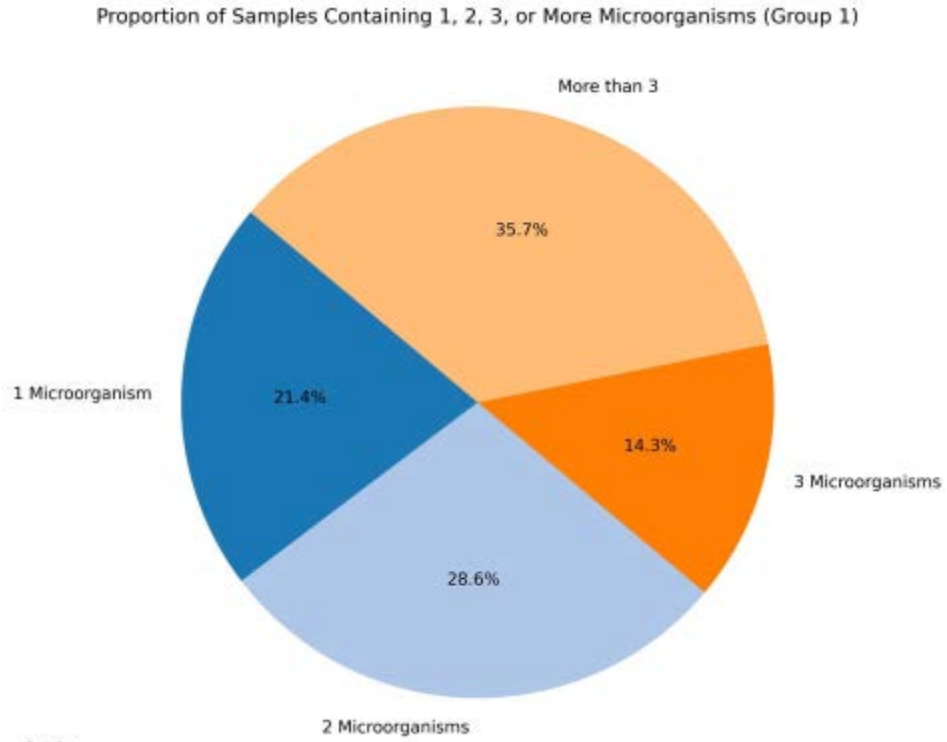


Figure 13. Bar chart presenting the distribution of microorganisms isolated from patient samples across two groups. (A) In group 1, *E. coli* was present in most of the samples (8), followed by *Klebsiella spp.* and *Shigella spp.* (5), and *Providencia spp.* was present in only 1 sample. (B) In group 2, *Enterobacter spp.* was present in most of the samples (13), followed by *E. coli* (9) and *Shigella spp.* (6) and *Proteus spp., Citrobacter freundii,* and *Serratia marcescens* were found in only 1 sample.

The frequency of samples containing one, two, three, or more microorganisms was analyzed for both groups. Among the positive samples from Group 1, 21.4% contained a single microorganism, 28.6% contained two, 14.3% contained three, and 35.7% contained more than three microorganisms. In Group 2 growth was observed in 28 samples, with 60.7% of positive samples containing one microorganism, 25.0% containing two, and 14.3% containing three microorganisms. The following Pie charts illustrate the proportion of samples with one, two, three, or more microorganisms in each group.


Proportion of Samples Containing 1, 2, 3, or More Microorganisms (Group 1)
More than 3
35.7%
1 Microorganism
21.4%
14.3%
3 Microorganisms
28.6%
2 Microorganisms

(A)

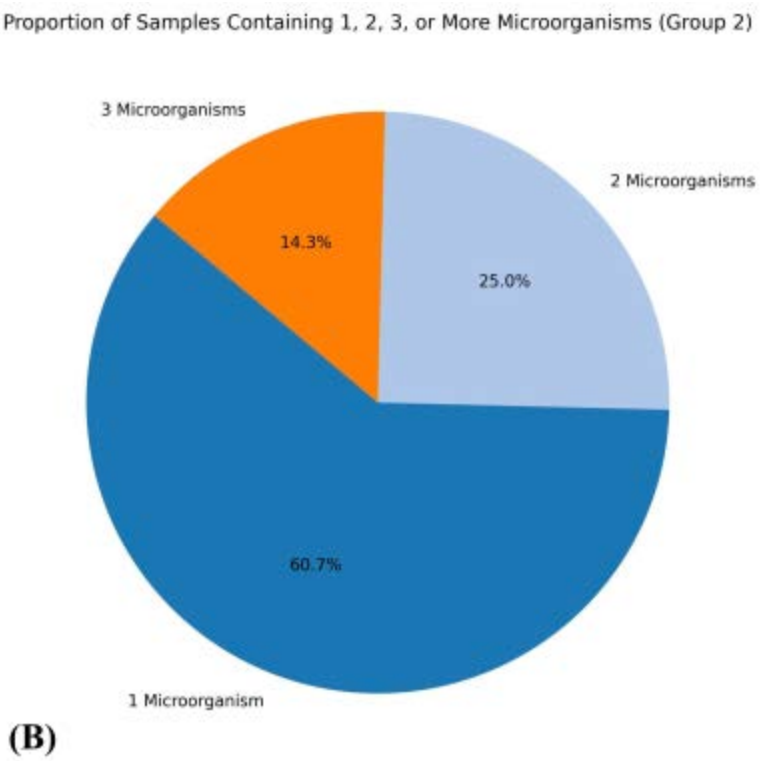


Figure 14. Pie chart showing the proportion of samples containing one, two, three, or more microorganisms in group 1 and group 2. (A) In group 1, the majority of the samples (35.7%) had more than 3 microorganisms and only 21.4% had 1 microorganism. (B) In group 2, the majority of the samples (60.7%) had 1 microorganism and only 14.3% had 3 microorganisms.

The microbial composition within individual patient samples was analyzed to assess co-occurrence patterns. Stacked bar charts were generated where each bar represents a single patient sample, and the colored segments within each bar indicate the different microorganisms present in that sample.

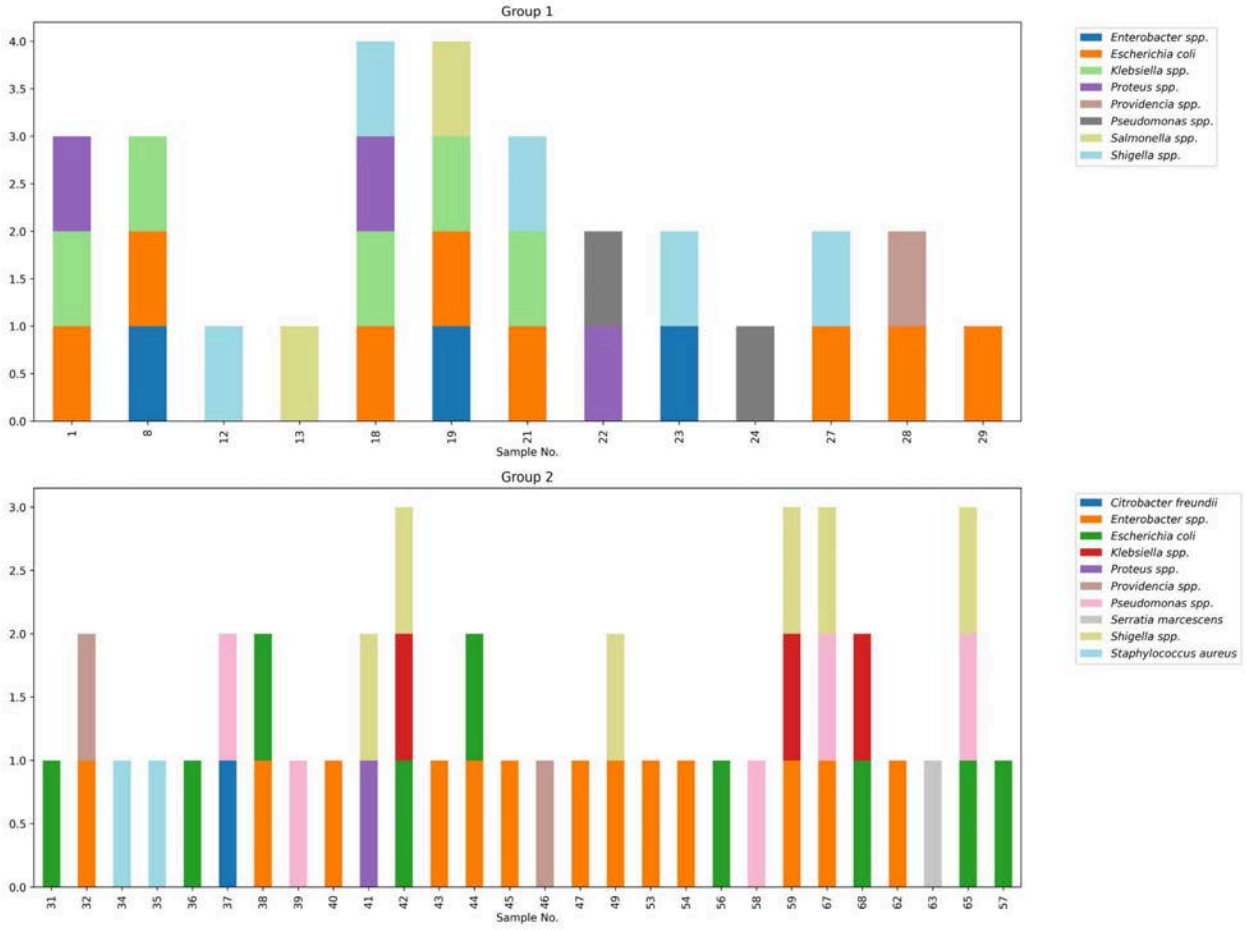
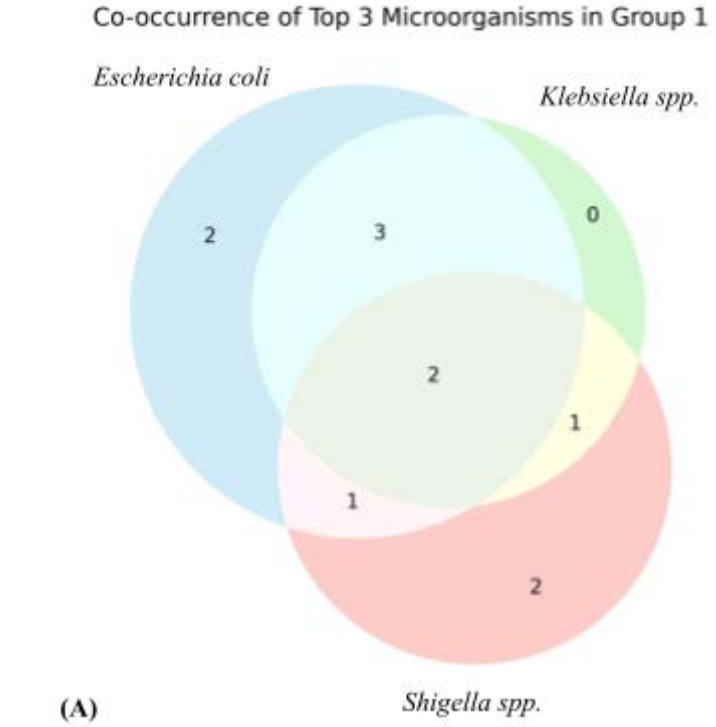


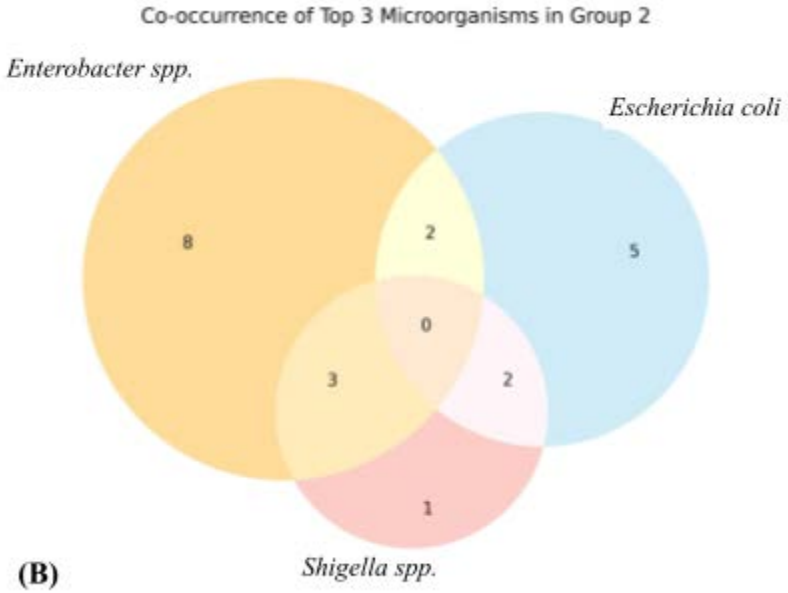
Figure 15. Stacked bar chart illustrating the microbial composition and co-occurrence in individual patient samples. In group 1, the most frequently detected microorganism, *E. coli* co-occurred mostly with *Klebsiella spp.* (35.7%) and *Shigella spp.* (21.4%). In group 2, the most frequently detected microorganism, *Enterobacter spp*. co-occurred mostly with *Shigella spp*. (10.7%) and *E. coli* (7.1%).

The co-occurrence patterns of the most prevalent microorganisms in each group were analyzed. In Group 1, *Escherichia coli* was the most frequently detected microorganism. The percentage of samples in which *Escherichia coli* co-occurred with other bacteria was, *Klebsiella spp.* 35.7%, *Shigella spp.* 21.4%, *Enterobacter spp*. 14.3%, *Proteus spp*. 14.3%, *Providencia spp.* 7.1%, *Salmonella spp*. 7.1%, and *Pseudomonas spp*. 0%. In Group 2, *Enterobacter spp*. was the most prevalent microorganism. The percentage of samples in which *Enterobacter spp*. co-occurred with other bacteria was: *Shigella spp*. 10.7%, *Escherichia coli* 7.1%, *Pseudomonas spp*. 3.6%, *Providencia spp*. 3.6%, *Klebsiella spp*. 3.6%, *Citrobacter freundii, Proteus spp.*, *Serratia marcescens,* and *Staphylococcus aureus* 0%. The Venn diagrams below illustrate the

co-occurrence patterns of microorganisms in the two patient groups visually representing the overlapping presence of the top bacteria in each group.



(A)



(B)

Figure 16. Venn diagrams showing the co-occurrence of the top microorganisms in patient samples for group 1 and group 2. (A) In group 1 (no-UTI), shows substantial overlap, with *E. coli* co-occurring with *Klebsiella spp.* (35.7%) and *Shigella spp.* (21.4%), indicating a polymicrobial community. (B) In group 2 (suspected UTI), shows minimal overlap, with *Enterobacter spp.* co-occurring mostly with *Shigella spp.* (10.7%) and *E. coli* (7.1%), indicating a state of single pathogen dominance.

4.2 Hierarchical Clustering of Microbial Composition

A hierarchical clustering analysis was performed to analyze the relationship between samples based on their microbial composition. The analysis included Jaccard distance metrics measuring the dissimilarity based on the presence or absence of microorganism species. The clusters were formed using the average linkage method with the distance threshold set at 0.75. The analysis led to eight clusters of the 41 samples (both Group1 and Group 2).

Table 7: Characteristics of clusters defined by a Jaccard distance threshold of 0.75.

Cluster	No. of Samples	Defining Microorganisms	Representative Sample Numbers
1	13	*Escherichia coli, Klebsiella spp., Shigella spp.*	1, 18, 21, 27, 28, 29, 31, 36, 42, 56, 57, 65, 68
2	12	*Enterobacter spp., Escherichia coli*	8, 19, 32, 38, 40, 43, 44, 45, 47, 53, 54, 62
3	7	*Shigella spp., Enterobacter spp., Klebsiella spp.*	12, 23, 30, 41, 49, 59, 67
4	1	*Providencia spp.*	46
5	5	*Pseudomonas spp.*	22, 24, 37, 39, 58
6	1	*Salmonella spp.*	13
7	2	*Staphylococcus aureus*	34, 35
8	1	*Serratia marcescens*	63

The dendrogram generated is as follows with sample numbers are shown on the x-axis and the y-axis represents the Jaccard distance at which the clusters merge. The threshold was chosen because it falls just below the longest vertical lines in the dendrogram, capturing coherent groups

before they merge with highly dissimilar samples. Additionally it balances cluster resolution and avoids overly small, fragmented clusters. It prevents the lumping of dissimilar groups and results in a manageable eight clusters that clearly separate the major groups from outliers such as *S. aureus* and *Serratia.*

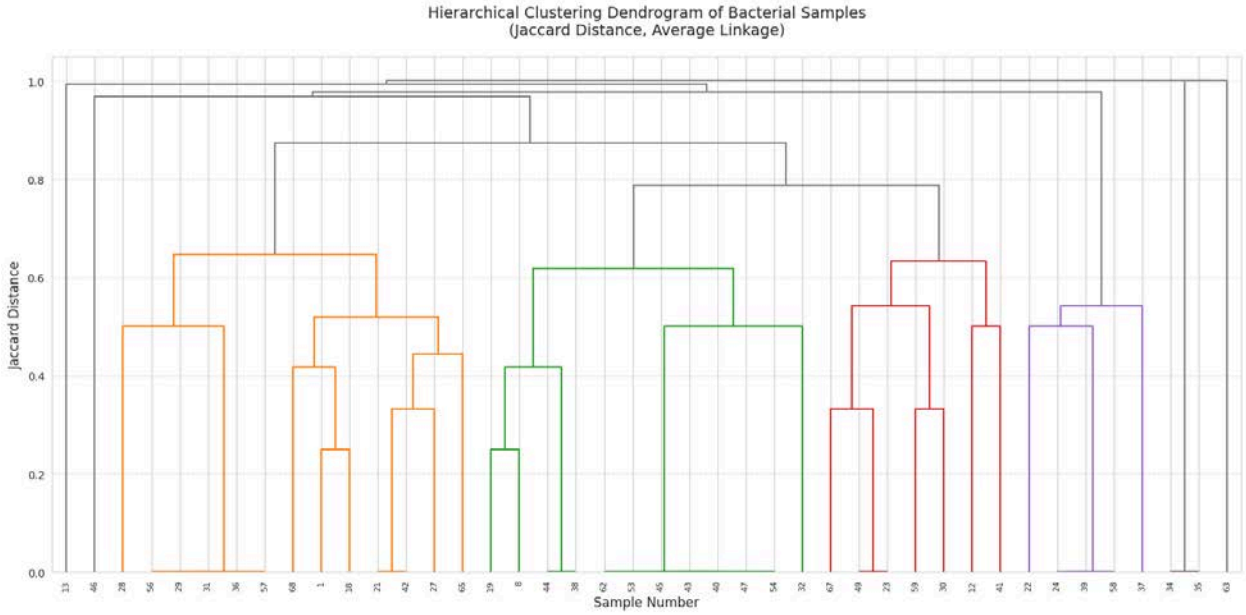


Figure 17. Schematic representation of the dendrogram based on Jaccard distance and average linkage clustering. Analysis revealed eight clusters, cluster 1 (n=13), defined by *E. coli*, *Klebsiella spp*., *Shigella spp.* (no-UTI group); cluster 2 (n=12), defined by *Enterobacter spp.*, *E. coli* (suspected UTI group). Smaller clusters include, cluster 3 (n=7*; Shigella spp., Enterobacter spp., Klebsiella spp.*), cluster 5 (n=5; *Pseudomonas spp*.) and singleton clusters 4, 6, 7, 8 (n=1-2; *Staphylococcus aureus, Providencia spp., Salmonella spp., Serratia marcescens*).

The two largest clusters are Cluster 1 (n = 13) and Cluster 2 (n = 12) comprising eight samples. These clusters accounted for 59.5% of all the samples (25 of 41 samples) representing the most common microbial communities. Cluster 1 was characterized by *Escherichia coli, Klebsiella spp., Shigella spp.* Cluster 2 on the other hand was defined by a strong association between *Escherichia coli* and *Enterobacter spp.*. Cluster 3 (n=7) represented a distinct profile dominated by the presence of *Shigella spp.* alongside *Enterobacter* and *Klebsiella* species. Several samples formed small, well-separated clusters; Cluster 5 (n=5) grouped all samples where Pseudomonas spp. was the major microorganism. Clusters 4, 6, and 8 contained single microorganisms Providencia spp., Salmonella spp., and Serratia marcescens, respectively. Most distantly related

were the two samples in Cluster 7, which contained only *Staphylococcus aureus*, a Gram-positive coccus sharply differentiated from all Gram-negative microorganisms.

4.3 Metagenome Sequencing Analysis

4.3.1 Hierarchical Clustering of Microbial Composition based on Sequencing Data

In order to further validate the patterns of microbial similarity, hierarchical clustering was performed using metagenomic sequencing data. Similar to the dendrogram generated from the biochemical data, hierarchical clustering of the metagenomic sequencing data was performed using Jaccard distance and average linkage with a threshold value of 0.75. The analysis resulted in six distinct clusters for the 27 samples that were sent for sequencing (17 samples from Group 1 and 10 samples from Group 2). Cluster 1 predominantly comprised samples from Group 1, reflecting a shared microbial profile characteristic of this patient group. In contrast, Cluster 3 mainly included samples from Group 2 indicating that these patients harbored a microbiome profile distinct from Group 1. The defining genus for Cluster 3 were *Escherichia-Shigella* and *Acinetobacter*. The smaller clusters (Clusters 2, 4, 5, and 6) primarily represented singleton or outlier samples. The cluster assignments are summarized in the table below:

Table 8: Characteristics of clusters defined by a Jaccard distance threshold of 0.75.

Cluster	No. of Samples	Defining Microorganisms	Representative Sample Numbers
1	14	*Acinetobacter, Aenigmarchaeales, Allobacillus, Alloprevotella, Arenimonas, Bacillus, Bacteroides, Bathyarchaeia, Bifidobacterium, Blautia, Candidatus_Nitrosotenuis, Candidatus_Omnitrophus, Candidatus_Peribacteria, Catenibacterium, Clostridium_sensu_stricto_1, Collinsella, Deinococcus, Dorea, Enterococcus, Escherichia-Shigella, Faecalibacterium, Ferriphaselus, Flavobacterium, Fusobacterium, Gallionella, Holdemanella, Imtechella, Intestinibacter,*	Brac-S13, Brac-S15, Brac-S18, Brac-S19, Brac-S21, Brac-S24, Brac-S25, Brac-S26, Brac-S27, Brac-S28, Brac-S29, Brac-S7, Brac-S8, Brac-S9

		Lactobacillus, Limnobacter, Listeria, Lysobacter, Megasphaera, Methanobacterium, Methanosphaera, Methylomonas, Methyloparacoccus, Nitrosomonas, Nitrospira, Pirellula, Prevotella, Pseudomonas, Psychrobacter, Rheinheimera, Romboutsia, Sarcina, Sideroxydans, Staphylococcus, Streptococcus, Subdoligranulum, Turicibacter, Veillonella, Weissella, Woesearchaeales, [Ruminococcus]_torques_group, uncultured.1	
2	1	*Acinetobacter, Agathobacter, Akkermansia, Allorhizobium-Neorhizobium-Para rhizobium-Rhizobium, Bdellovibrio, Bifidobacterium, Blautia, Brevundimonas, Candidatus_Obscuribacter, Candidatus_Omnitrophus, Candidatus_Peribacteria, Caulobacter, Cellvibrio, Chryseobacterium, Clostridium_sensu_stricto_1, Devosia, Dorea, Edaphobaculum, Enhydrobacter, Exiguobacterium, Flavobacterium, Fluviicola, Gracilibacteria, Holdemanella, Ignavibacterium, Lacunisphaera, Massilia, Megamonas, Megasphaera, Methylobacterium-Methylorubrum, Methylophilus, Methylotenera, Nitrosomonas, Nitrospira, Noviherbaspirillum, Paracoccus, Pedosphaeraceae, Peredibacter, Prevotella, Pseudarcicella, Pseudomonas, Pseudorhodobacter, Rheinheimera, Romboutsia,*	Brac-S6

		Sediminibacterium, Sphingobacterium, Spirosoma, Staphylococcus, Streptococcus, Terrimicrobium, Terrimonas, Woesearchaeales	
3	9	*Acinetobacter, Aeromonas, Bacillus, Bifidobacterium, Blautia, Clostridium_sensu_stricto_1, Corynebacterium, Deinococcus, Dialister, Enterococcus, Escherichia-Shigella, Kurthia, Lactobacillus, Lactococcus, Megasphaera, Meiothermus, Prevotella, Pseudomonas, Romboutsia, Streptococcus, Veillonella, Weissella.*	Brac-S-32, Brac-S-34, Brac-S-36, Brac-S-37, Brac-S-38, Brac-S-41, Brac-S-42, Brac-S-45, Brac-S-65
4	1	*Achromobacter, Akkermansia, Bacteroides, Blautia, Brevibacterium, Campylobacter, Collinsella, Corynebacterium, Dialister, Escherichia-Shigella, Fusobacterium, Gardnerella, Lactobacillus, Methylobacterium-Methylorubrum, Mycoplasma, Peptostreptococcus, Prevotella, Pseudomonas, Qipengyuania, Rhodococcus, Romboutsia, Streptococcus, Succinivibrio, Ureaplasma, Weissella*	Brac-S4
5	1	*Acinetobacter, Akkermansia, Anaerococcus, Bifidobacterium, Chryseobacterium, Comamonas, Empedobacter, Escherichia-Shigella, Lactococcus, Meiothermus, Prevotella, Pseudomonas, Sphingobacterium, Stenotrophomonas, Streptococcus, Weissella*	Brac-S1
6	1	*Bifidobacterium, Blautia, Deinococcus, Enterococcus, Escherichia-Shigella,*	Brac-S-57

		Lactobacillus, Paraclostridium, Pediococcus, Streptococcus, Subdoligranulum	

The dendrogram generated is as follows with sample numbers are shown on the x-axis and the y-axis represents the Jaccard distance at which the clusters merge. The red dashed line represents the threshold used to define the clusters.

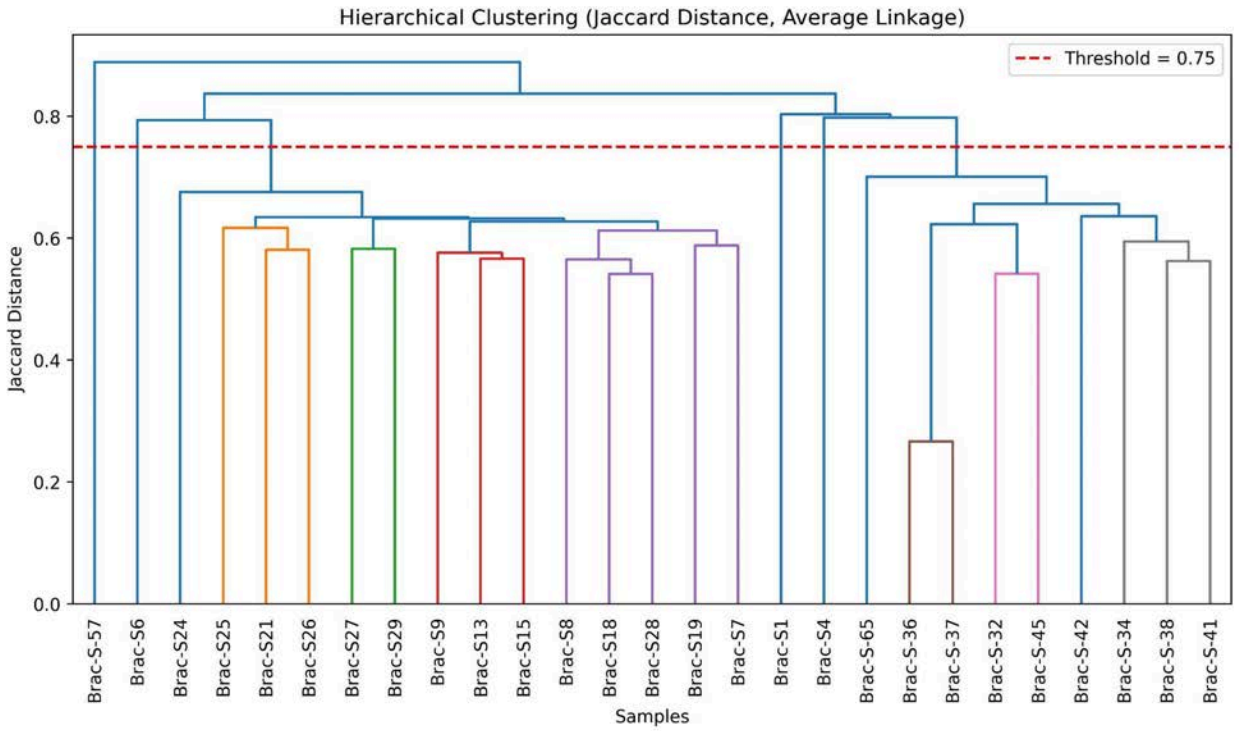


Figure 18. Schematic representation of the dendrogram showing the hierarchical clustering of the 27 sequenced samples. Cluster 1 predominantly composed of group 1 (no-UTI samples) and cluster 3 was primarily formed from group 2 (suspected UTI samples) and was defined by co-dominance of the genera *Escherichia-Shigella* and *Acinetobacter*. Cluster 2, 4, 5, 6 were singleton clusters formed from single samples (1, 4, 6, 57).

4.3.2 Alpha Diversity

Alpha diversity measures the microbial diversity within individual samples. To assess the overall microbial complexity in each group, we calculated three key within-sample metrics: Observed OTUs (species richness), the Shannon index (richness and evenness), and the Simpson index (community dominance). Across all metrics, alpha diversity was significantly reduced in the

suspected UTI group compared to the non-UTI group (Independent t-test, $p < 0.05$ for all comparisons).

- Species Richness (Observed OTUs): The non-UTI group exhibited 188% greater species richness than the UTI group (121.9 ± 34.7 vs. 42.3 ± 12.6; t=6.940, p<0.001), reflecting a substantial loss of microbial taxa in the infected state.
- Overall Diversity (Shannon Index): The Shannon index was 81% higher in the non-UTI group (3.17 ± 1.17) than in the UTI group (1.75 ± 0.99; t=3.216, p=0.004), indicating a pronounced reduction in both the number and equitable distribution of species.
- Community Dominance (Simpson Index): The Simpson index was 50% higher in the non-UTI group (0.82 ± 0.26 vs. 0.55 ± 0.28; t=2.567, p=0.017), confirming that the UTI microbiome is dominated by one or a few species rather than a balanced community.

The effect sizes were large across all metrics (Cohen's d: Observed OTUs=2.77, Shannon=1.28, Simpson=1.02), highlighting the magnitude of these differences. These findings are visually supported in Figure 8, where boxplots show consistently lower median values and narrower distributions for all three indices in the suspected UTI group compared to the non-UTI group. The most pronounced separation is observed for Observed OTUs, emphasizing the dramatic collapse in species richness associated with UTI.

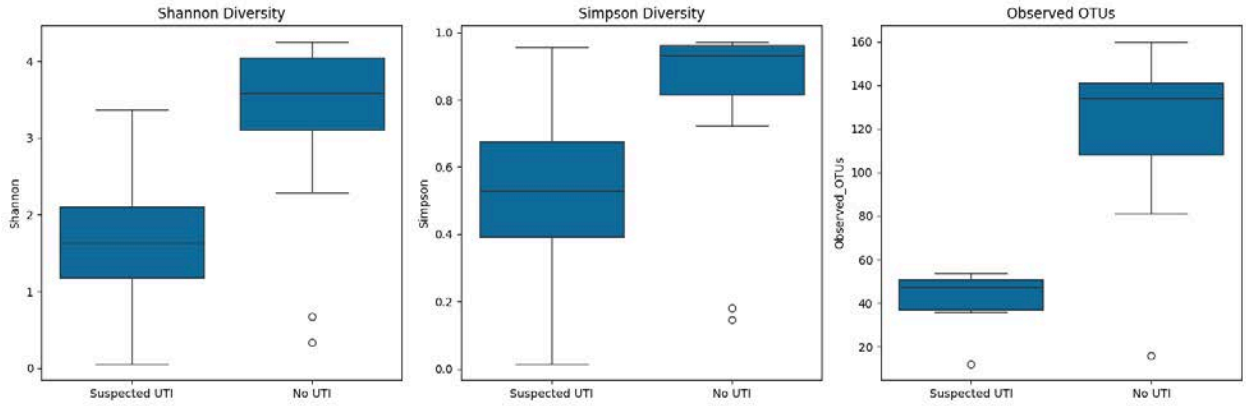


Figure 19. Comparison of alpha diversity metrics across two groups. Box plots show Shannon diversity (richness and evenness), Simpson diversity (community dominance) and Observed OTUs (species richness). The suspected UTI group (n=10) showed a significant reduction in all metrics ($p < 0.05$) compared to the no-UTI group (n=17), indicating a significant loss of microbial diversity and stability in the infected state.

4.3.3 Beta Diversity

To examine differences in overall microbial community composition between groups, a Principal Coordinates Analysis (PCoA) was performed using Bray-Curtis dissimilarity metrics. The PCoA plot demonstrated a clear separation between the 'Suspected UTI' and 'No UTI' groups along the primary axis of variation (PC1, 19.0%). This separation was statistically supported by a PERMANOVA test (Pseudo-F = 3.90, p = 0.001). This further confirms that the microbial communities in the suspected UTI group are fundamentally distinct from those in the non-UTI group. The PCoA plot is illustrated below;

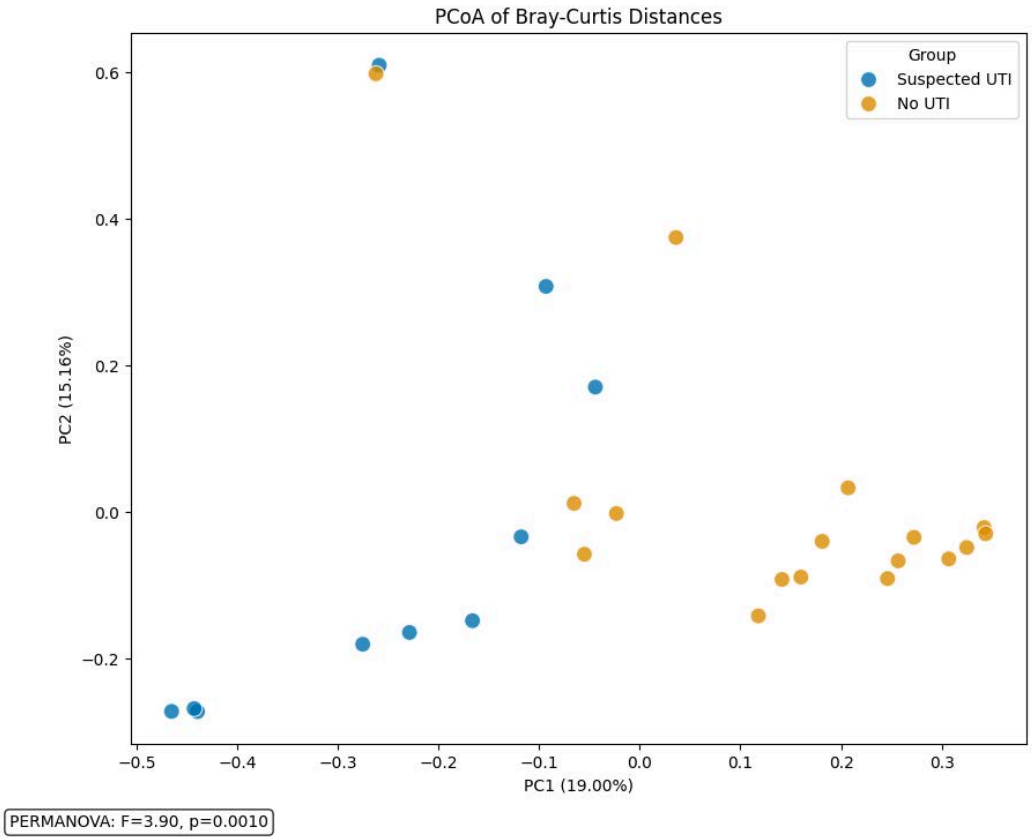


Figure 20. Beta-diversity PCoA plot: Bray-Curtis distances between suspected UTI and no-UTI groups. A clear separation was observed in the first principal co-ordinate (PC1), which was statistically significant, supported by the PERMANOVA test (Pseudo-F = 3.90, p = 0.001). The tight clustering of the suspected UTI samples indicates a uniform, dysbiotic state, while the broader dispersion of the non-UTI samples reflect the higher diversity and a much healthy urinary microbiome.

4.3.3 Differential Abundance

The analysis of the taxonomic composition at the genus level revealed profound differences in microbial community structure between groups. The most significant change was a near-doubling in the relative abundance of the *Escherichia-Shigella* genus (primarily containing uropathogenic *E. coli*) in the suspected UTI group (47.0%) compared to non-UTI (24.1%), identifying it as a primary driver of dysbiosis. Conversely, several genera were substantially depleted in the UTI state. *Acinetobacter* and *Enterococcus*, which were major constituents of the Group 1 microbiome (10.7% and 10.9%, respectively), were reduced to negligible levels among the top taxa in the UTI group. The UTI group was also characterized by a significant increase in

the relative abundance of *Lactobacillus* (17.5% vs. 4.3%) and *Prevotella* (16.3% vs. 4.1%), suggesting a complex dysbiotic shift beyond a simple pathogen bloom. The relative abundance of the top genera (by Group) is illustrated in the figure below;

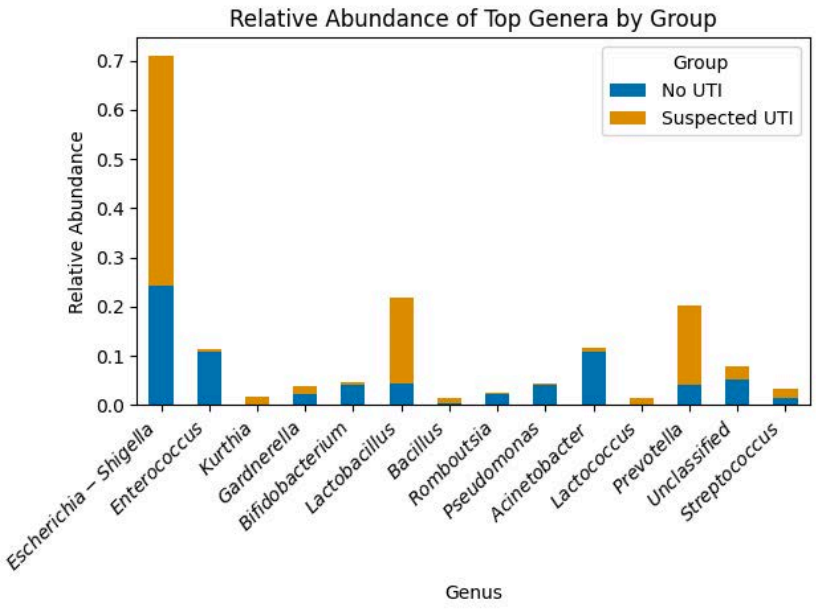


Figure 21. Relative abundance of top bacterial genera in suspected UTI vs. no UTI groups. The suspected UTI group is characterized by near-doubling of *Escherichia-Shigella* and a significant increase in *Lactobacillus* and *Prevotella*, with substantial reduction of genera such as *Acinetobacter* and *Enterococcus,* indicating the restructuring of the microbial community.

4.3.4 Enterobacteriaceae Abundance Across Groups

Given the near-doubling of *Escherichia-Shigella* in the UTI cohort the Enterobacteriaceae family was analyzed to determine if the increased abundance of *Escherichia-Shigella* in the suspected UTI group was indicative of broader shifts within the family. Summary statistics for each group are shown in the table below;

Table 9: Abundance of *Enterobacteriaceae* in both groups.

Group	Mean ± SD	Range (min–max)
Group 1	6,342 ± 15,548	28 – 64,501
Group 2	8,887 ±	18 – 67,357

	20,834	

Enterobacteriaceae were detected in all urine samples from both groups. In Group 1, counts ranged from 28 to 64,501 reads per sample (mean ± SD: 6,342 ± 15,548), whereas in Group 2, counts ranged from 18 to 67,357 reads per sample. These sequencing results were consistent with biochemical analysis. In Group 1, *Escherichia coli* was the predominant organism isolated, aligning with the high Enterobacteriaceae counts observed. In Group 2, *Enterobacter spp*. predominated, which is reflected in the elevated *Enterobacteriaceae* counts in the sequencing data. *Enterobacter* was detected across samples; however, the large standard deviation relative to the mean in both groups indicates that a subset of samples harbored disproportionately high *Enterobacter* levels, whereas some exhibited much lower abundances.

4.3.5 Prevalence of Commensal Flora and Uropathogens in Suspected UTI Samples

Following the analysis of Enterobacteriaceae abundance the prevalence of both commensal flora and uropathogenic taxa within the suspected UTI samples were evaluated to better characterize the microbial taxa associated with infection. Analysis of Group 2 (suspected UTI) samples revealed a high prevalence of commensal bacteria, notably *Lactococcus lactis* and *Lactococcus garvieae* (80.0% each). Among specific uropathogens, *Prevotella bivia* and *Streptococcus anginosus* were the most common, each identified in 40.0% of samples.

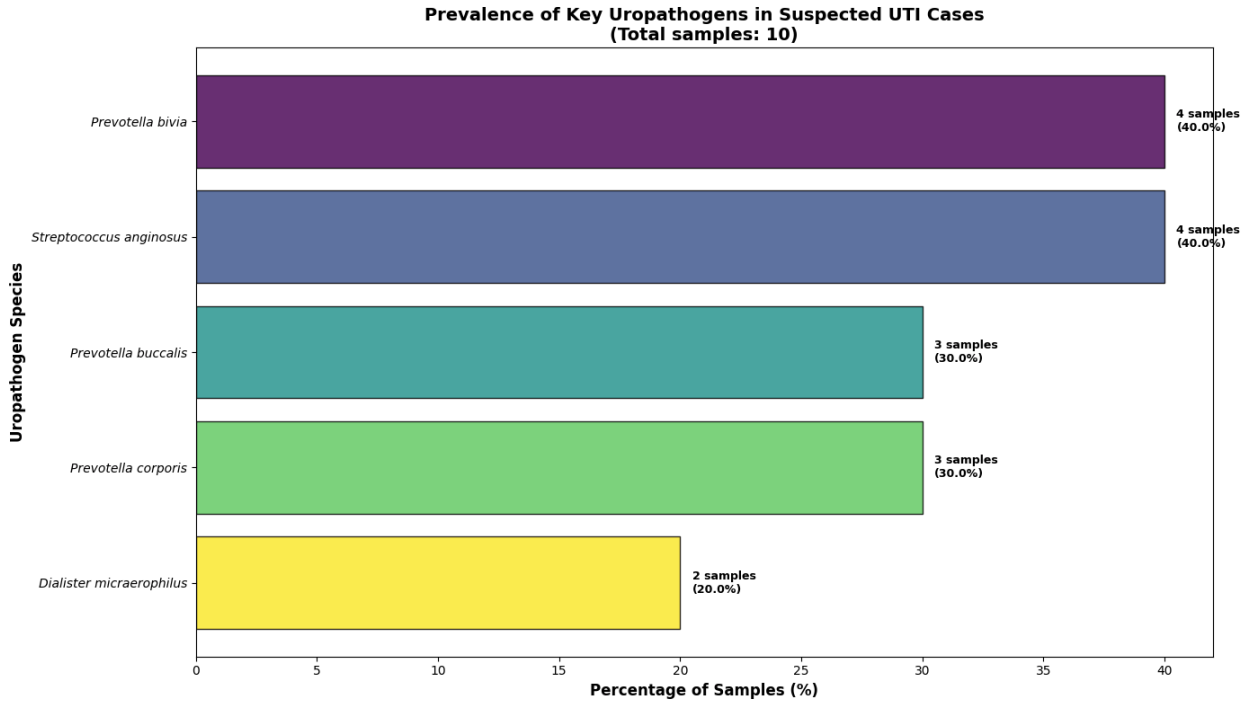


Figure 22. Prevalence of key uropathogens in suspected UTI cases (n=10). Sequencing analysis identified *Prevotella bivia* and *Streptococcus anginosus* as the most common uropathogens, each identified in 40% of samples, highlighting their role in UTI pathogenesis.

4.4 Comparison of Biochemical Culture and Sequencing Results in UTI and Non-UTI Groups

4.4.1 Genus-level Comparison Between Biochemical and Sequencing Analysis

To assess the consistency of results obtained between traditional microbiological methods and metagenomic sequencing, the biochemical culture results were compared with sequencing data across the UTI and non-UTI groups. In Group 1 (non-UTI patients) biochemical culture indicated a dominance of *Escherichia* (25.8%), *Klebsiella* (19.4%), and *Shigella* (19.4%). In contrast, sequencing revealed a more diverse microbiota, identifying genera such as *Acinetobacter* (13.9%), *Lactobacillus* (13.5%), *Enterococcus* (11.9%), and *Prevotella* (11.8%), which were not detected by culture. Traditional pathogens like *Escherichia* (9.9%), *Klebsiella* (10.3%), and *Shigella* (9.7%) were present in sequencing results but at lower relative abundances compared to culture. In Group 2 (suspected UTI), biochemical culture identified *Enterobacter* (30.2%), *Escherichia* (20.9%), and *Shigella* (14.0%) as dominant genera. Sequencing confirmed the prominence of *Escherichia* (31.8%) and *Enterobacter* (20.6%) but additionally revealed

Lactobacillus (11.6%) and *Prevotella* (10.9%). Minor genera such as *Pseudomonas, Providencia*, and *Serratia* were detected by both methods, though their relative abundances varied. The bar charts below compare the relative abundances of dominant genera identified by biochemical culture and sequencing across both clinical groups. A correlation analysis to assess how closely the microbial profiles obtained from biochemical culture aligned with those identified through sequencing. In the case of Group 1 the correlation between biochemical and sequencing results was moderate ($\rho = 0.606$, $p = 0.1112$), but not statistically significant. This indicates divergence between methods, as sequencing detected many uncultured taxa absent from culture. On the contrary, the correlation was strong and significant ($\rho = 0.899$, $p = 0.0004$) for Group 2 reflecting high agreement between sequencing and culture in detecting UTI-associated pathogens.

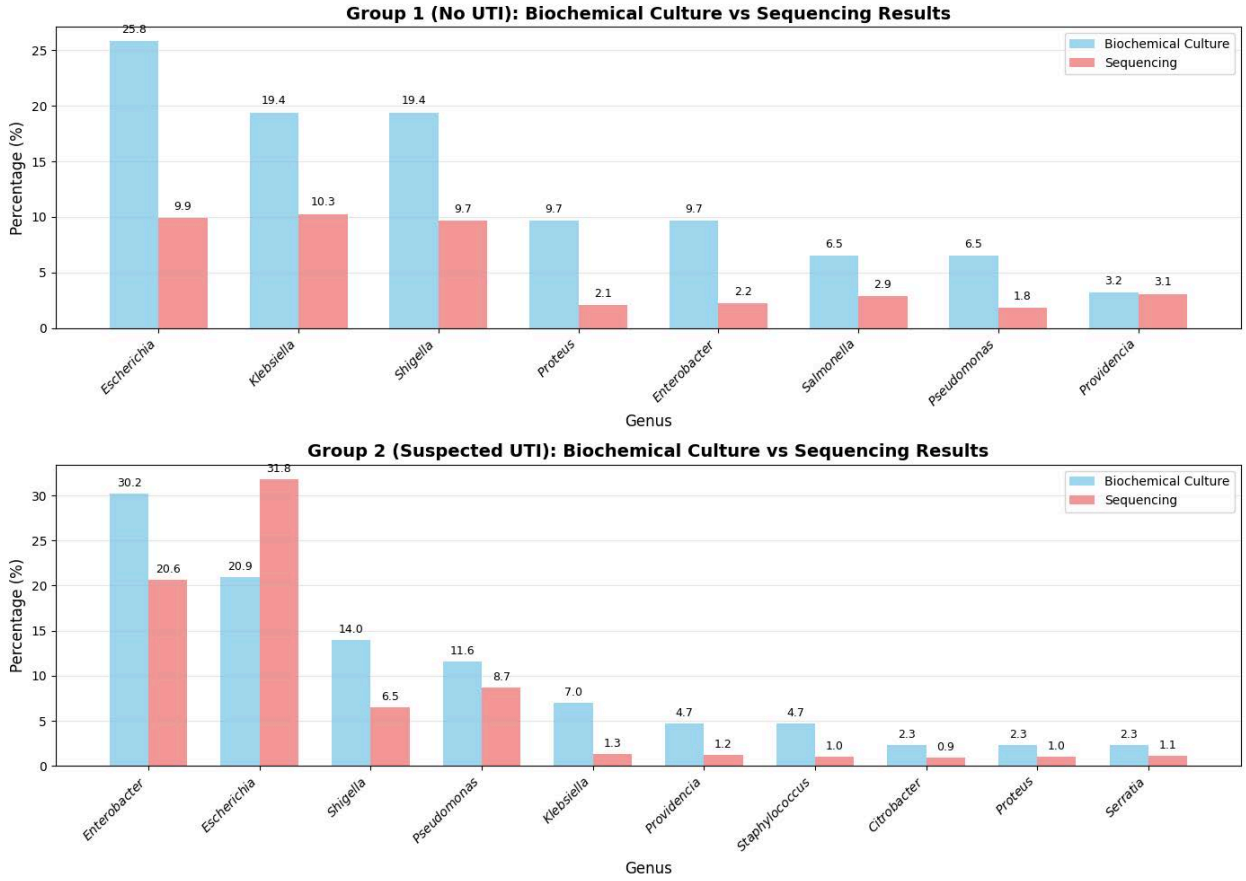


Figure 23. Genus-level comparison between biochemical and sequencing methods. A diverse microbiota was revealed by sequencing, including the commensal genera (*Acinetobacter*, *Lactobacillus*, *Enterococcus*, and *Prevotella*), which were not detected by culture. Correlation was strong and significant in group 2 ($\rho = 0.899$, $p = 0.0004$) whereas for group 1, it was moderate and non-significant ($\rho = 0.606$, $p = 0.1112$).

4.4.2 Family-level Comparison Between Biochemical and Sequencing Analysis

A comparative analysis was performed at the family level which revealed significant differences in microbial community profiles among biochemical and sequencing methods. Stronger taxonomic agreement was found at the family level than at the genus level. For group 1 (no-UTI), a perfect correlation was found between the methods ($r=1.000$, $p<0.001$) in comparison to a moderate level comparison at the genus level ($\rho = 0.606$, $p = 0.1112$). Similarly, a stronger family-level correlation ($r=0.949$, $p=0.051$) was shown by group 2 (suspected UTI) than the genus-level ($\rho = 0.899$, $p = 0.0004$). Pathogenic families, including Enterobacteriaceae (90.5% in group 1 and 79% in group 2), Pseudomonadaceae, Morganellaceae, and

Staphylococcaceae were exclusively detected by biochemical culture. Contrarily, commensal families such as Lactobacillaceae, Prevotellaceae, Enterococcaceae, and Moraxellaceae were identified additionally by sequencing, providing a more comparative profile of urinary microbiome.

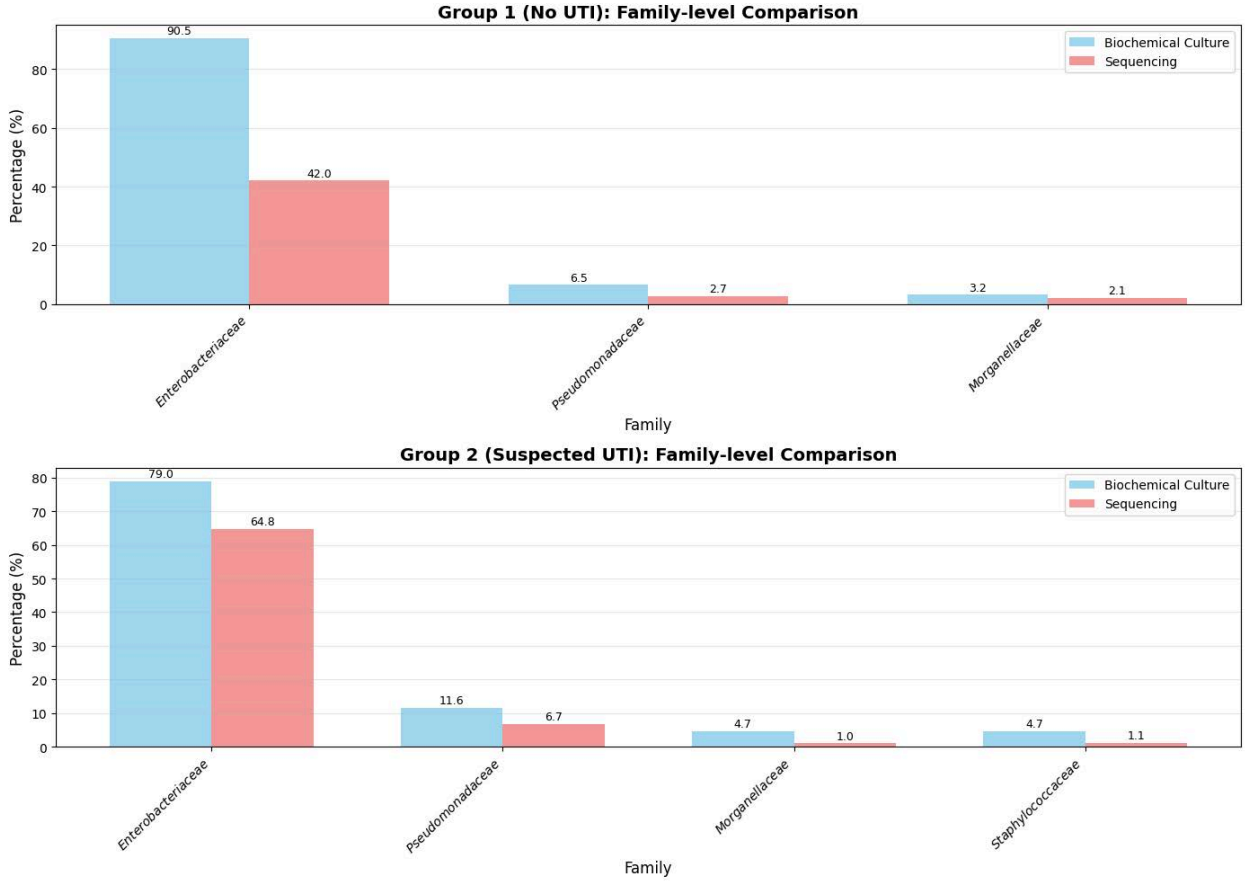


Figure 24. Family-level comparison between biochemical and sequencing methods. A significantly stronger correlation was observed between two methods at the family level (group 1: r=1.000, p<0.001; group 2: r=0.949, p=0.051) compared to the genus level. Biochemical culture exclusively detected pathogenic families such as *Enterobacteriaceae* (90.5% in group 1 and 79% in group 2), *Pseudomonadaceae*, *Morganellaceae*, and *Staphylococcaceae* but sequencing detected commensal families such as *Lactobacillaceae, Prevotellaceae*, *Enterococcaceae*, and *Moraxellaceae.*

Chapter 5: Discussion

5.1 Microbial Composition Across Groups

The analysis provided a fundamental shift of the urinary microbiome among the non-UTI and suspected UTI groups. The non-UTI group revealed a complex, polymicrobial environment where a variety of organisms were identified by the biochemical culture, including *Escherichia coli* (25.8%), *Klebsiella* (19.4%), and *Shigella* (19.4%). On the other hand, greater diversity was revealed through sequencing which provided a substantial presence of commensal and opportunistic genera such as *Acinetobacter* (13.9%), *Lactobacillus* (13.5%), *Enterococcus* (11.9%), and *Prevotella* (11.8%) that were absent from the culture results. This finding is consistent with multiple studies on 16S/NGS approaches for detection of additional commensals which cannot be identified by standard culture [52]. This pattern shows the existence of ecological balance in these diabetic patients without suspected UTI where a diverse community besides *Lactobacilli* and other commensals which may contribute to the stability of the urinary tract and provide resistance to pathogen growth as observed in other cohorts [53]. On the contrary, the suspected UTI group exhibits a state of dysbiosis characterized by a collapse in microbial diversity which was quantified through a significant reduction in all alpha diversity metrics as well as a shift in community structure. Known uropathogens dominated the microbiome as sequencing showed almost doubling in the relative abundance of *Escherichia-Shigella* genus (47%) compared to the non-UTI group (24.1%), and culture identified *Enterobacter spp.* as the most frequent isolate (30.2%). These observations are supported by other studies reporting reduced alpha diversity in UTI cases and increase in uropathogens, particularly *Escherichia*/*Shigella* and related *Enterobacterales* during active infection [54]. The increased prevalence of mono-microbial samples (60.7%) in the suspected UTI group with comparison to the high rate of complex co-occurring organisms in the non-UTI group (35.7% samples having more than 3 organisms) as found in biochemical culture demonstrates a shift from diverse, competitive environment to an unstable, pathogen-saturated environment. This transition is coherent with existing urobiome literature which states that symptomatic UTIs are not due to an increase in the abundance of a single pathogenic species but rather due to a decrease of community complexity and protective taxa, allowing that specific pathogenic species to dominate [54]. Moreover, several studies examining urobiome in diabetic patients report altered

community structure and decreased diversity compared to non-diabetic controls supporting the idea that changes in the host environment (immune modulation, glycosuria) due to diabetes may decrease resistance to pathogen takeover and increase dysbiosis during UTI episodes [55].

5.2 Microorganism Diversity and Polymicrobial Community

According to our findings, an inverse relationship between microbial diversity and likelihood of infection was observed, highlighting the importance of a balanced microbiome for maintaining urinary health. The non-UTI group (group 1) showed significantly higher alpha diversity with greater species richness (188%), 81% higher Shannon index and 50% higher Simpson index compared to group 2 (suspected UTI). A complex and stable ecosystem is indicated by these metrics which is not dominated by a single pathogen. Fouts et al. (2012) and Hilt et al. (2014) reported similar findings where culture-negative or asymptomatic individuals had significantly enriched urinary microbiota than symptomatic UTI patients, particularly when assessed through enhanced culture or sequence-based approaches [56,57]. This high diversity was observed with a notable increase in polymicrobial communities with 35.7% of positive samples in group-1 comprising more than 3 distinct organisms compared to 0% in group 2 where 60.7% of samples only contained one organism as revealed by culture. This finding is supported by other studies. Thomas-White et al. (2018) both found polymicrobial communities in urine of healthy or asymptomatic individuals compared to pathogen-dominated profiles of UTI cases [58]. As aforementioned, this diversity and comparative interactions may provide protection through prevention of unchecked proliferation of any single uropathogen which is supported by ecological reviews on function of urinary microbiota [53]. Contrarily, a profound decrease in diversity shown by significantly reduced alpha diversity metrics and community structure dominated by few or one pathogens such as *Escherichia coli* (31.8% relative abundance by sequencing) or *Enterobacter spp.* (20.6%). This finding is consistent with comparative cohort studies such as those by Brubaker and Wolfe (2017) and Adu-Oppong et al. (2022) which demonstrated increased dominance of *E.coli* and related *Enterobacterales* and decreased alpha diversity in symptomatic UTI cases [59,60]. The loss of diversity was associated with an alteration from complex polymicrobial interactions towards mono microbial interactions. Sequence-based frameworks provided by studies above show that samples dominated by a single pathogen with

high relative abundance are consistently correlated with likely UTI whereas diverse communities correspond to unlikely UTI categories.

5.3 Co-occurance Patterns and Hierarchical Clustering

On the basis of the observed shifts in diversity and composition, a deeper understanding was obtained through co-occurance patterns and hierarchical clustering, revealing the specific structural reorganization and highlighting the dysbiotic state with the integration of both biochemical and sequencing analysis data. The high diversity of the non-UTI group was not random but organized into distinct, reproducible communities of different microorganisms. These patterns were identified by hierarchical clustering of metagenomic data, most notably in cluster 1 (non-UTI group), defined by the co-association between *Shigella spp., E. coli* and *Klebsiella spp.* and cluster 3 (suspected UTI group) composed of *Enterobacter spp.,* and *Shigella spp.*. Predominantly, the clusters that are composed of non-UTI samples validate the culture finding of polymicrobial growth (greater than 3 or more samples in 50% positive samples), suggesting a structured network of bacterial interactions. The complexity of this group is further highlighted by the presence of non-culturable commensal organisms revealed by sequencing such as *Lactobacillus* and *Acinetobacter.* These were completely missed by culture-based methods which explains the moderate correlation (r=0.606, p=0.111) between the two methods at the genus level. However, the community structure of the suspected UTI group was more uniform and less complex. This was dominated by cluster 2 found through hierarchical clustering of biochemical data through strong, binary association of *E. coli* and *Enterobacter spp.* which is consistent with the biochemical culture result which identified *Enterobacter* as the most frequent isolate (30.2%) and is strongly supported by the almost doubling of *Enterobacteraceae* abundance in sequencing. The strong, significant correlation (r=0.899, p=0.0004) in this group demonstrates that the dysbiosis is due to increased dominance of readily culturable uropathogens. This is further shown by co-occurance rates where in the non-UTI group *E. coli* co-occured with *Klebsiella* in 35.7% of the samples but the most prevalent organism in the UTI group (*Enterobacter spp.*) only co-occured with *E. coli* in 7.1% of samples only. Moreover, several singleton clusters such as *Pseudomonas spp.* in cluster 5 and *Staphylococcus aureus* in cluster 7 act as ecological outliers in both groups, further suggesting the core dysbiosis in UTI results in the collapse of central, complex network of common uropathogens to a state where a

single pathogen dominates overwhelmingly, resulting effective loss of protective communities, leading to UTIs. These observations are reinforced by other global and regional evidence. The sequence-based studies globally reveal polymicrobial communities with other frequent co-occurring commensal taxa like *Cornebacterium, Gardenella* and others as mentioned above and shift toward a simpler, mono-microbial community is associated with symptomatic UTIs or recurrent infections [53]. For instance, a recent study identified distinct clusters of bacterial communities in women with rUTI as opposed to asymptomatic women with these clusters having less diversity and dominated by Enterobacteraceae. Regionally, culture-based studies from Bangladesh also report *Klebsiella, E. coli* and *Enterobacter* among the main uropathogens and mostly as single dominant species in UTI cases [61]. Another study in Dhaka also revealed the dominance of a single pathogen in UTI cases with decreased detection of co-occurring pathogens associated with infections [62]. These regional findings align with observations in both methods and highlight that much of the dominant taxa responsible for the dysbiotic state in the suspected UTI cases can be identified through culture-based approaches.

5.4 Enterobacteraceae Abundance

The collapse of the diverse, complex microbial communities into the dysbiotic state was further demonstrated by significant increase in members of the Enterobacteriaceae family (key group of common uropathogens). Sequencing analysis showed that despite Enterobacteriaceae being widespread (detected in all urine samples and common in both groups), their mean abundance was 40% higher in suspected UTI individuals (group 2) compared to group 1. This molecular finding aligned with the biochemical culture results which provided the shift in the detailed cultured genus within this family. In group 1 (non-UTI), *E. coli* was the predominant isolate (25.8%) whereas in group 2 (suspected UTI), *Enterobacter spp.* was the predominant isolate (30.2%). The concordance among these two methods highlighted that the dysbiotic state is characterized not through alteration of the community but also due to significant increase in the overall burden of Enterobacteriaceae pathogens. These observations are also documented in other studies. A meta-analysis of the urinary microbiome studies found that members of the Enterobacteriaceae family (mainly *Escherichia-Shigella*) were significantly more samples compared to healthy controls [63]. Despite limitations of data, culture-based studies from Bangladesh consistently document *Klebsiella* and *E. coli* as the most cultured pathogens in UTI

with high burden in most cases, highlighting the pattern of Enterobacteriaceae dominance in symptomatic individuals [64].

5.5 Alpha Diversity

The ecological collapse in group 2 was more clearly captured and quantified through alpha diversity analysis from sequencing data which quantifies the microbial diversity within each sample. Our results provided a statistically significant reduction of alpha diversity across all three metrics in the suspected UTI group (n=10) compared to the non-UTI group (n=17). Most importantly, species richness (Observed OTUs) demonstrated a profound 188% loss. The Shannon index reflecting both evenness and richness was 81% lower in the UTI group, and the Simpson index, showing community dominance was 50% lower. The magnitude of these reductions was underscored by large effect sizes. This reduction in alpha diversity measured in sequencing was perfectly aligned with the biochemical culture profile since the culture data showed a high rate of complex polymicrobial community in group 1 (35.7% of samples had more than 3 organisms) whereas group 2 showed mono-microbial growth (60.7% of samples having one organism). Together, these findings again suggest that UTI pathogenesis is due to reduction of within-sample ecological complexity and resilience, resulting in dominance of a single pathogen. This was supported by multiple studies as various cohorts, including women and pediatric patients confirmed the association of UTI or rUTI with significant reduction in evenness, richness and Shannon diversity in the sequencing data [65].

5.5 Beta Diversity

Along with alpha diversity assessing the within-sample differences, between-sample differences in overall microbial community structure was also assessed using beta diversity analysis of sequencing data on the basis of Bray-Curtis dissimilarity. The Principle Coordinates Analysis (PCoA) provided a clear, statistically significant separation among the suspected UTI and no-UTI groups along the primary axis of variation (PC1, 19%) which was confirmed by highly significant PERMANOVA test (Pseudo-F=3.90, p=0.001). This visual and statistical separation demonstrates the fundamentally distinct and more uniform microbial composition of the UTI group compared to the non-UTI group. The tight clustering of the UTI samples are reflective of their dysbiotic, pathogen-saturated state which is strongly supported by biochemical culture

results which showed a consistent pattern of dominant uropathogens such as *E. coli* and *Enterobacter*. On the other hand, the broader dispersion of the non-UTI samples in the PCoA plot show the higher, more variable healthy urinary microbiome, which was also evidenced by the wider variety of organisms (both pathogens and commensals) obtained from this group. Hence, beta diversity analysis confirms the association of UTI with a consistent shift towards a dysbiotic state alongside loss of internal diversity, as aforementioned. Numerous studies on the urinary microbiome globally support these findings and reveal that the UTI and healthy urinary microbiome significantly differ in beta diversity with samples from UTI clustering more tighter together, showing less inter-sample variation [66].

5.6 Differential Abundance

Differential abundance analysis identified *Escherichia–Shigella* as the primary driver of dysbiosis, nearly doubling in relative abundance in UTI cases. This aligns with its well-established role as a dominant uropathogen in urinary microbiome studies[67]. Interestingly, commensals such as *Acinetobacter* and *Enterococcus* were significantly depleted in UTI patients based on both biochemical characterization and sequencing. The loss of such taxa may be attributable to pathogen overgrowth and disruption of the balance [68]. Moreover, because commensals are thought to contribute to colonization resistance and maintenance of microbial stability, their depletion may further facilitate pathogen overgrowth [69]. Conversely, the observed increase in *Lactobacillus* and *Prevotella* suggests more complex, non-linear community restructuring rather than a simple bloom of pathogens. The increase of *Lactobacillus* is particularly intriguing, as it contrasts with its traditionally protective role in the urogenital tract [70]. This shift suggests that certain strains may act opportunistically under altered host biochemical conditions such as in the diabetic urinary tract. This finding is consistent with previous reports; for instance, Penckofer et al. (2020) reported that the genus *Lactobacillus* was detected more frequently in women with type 2 diabetes mellitus compared to the control group [71]. These results emphasize the critical need to interpret microbial roles contextually, as taxa can exhibit niche-specific functions, acting protectively in health but opportunistically in dysbiosis.

5.7 Comparison of Biochemical Culture and Sequencing

The comparative analysis between traditional culture and sequencing reveals a complex and complementary relationship, underscoring a paradigm shift in how we define and diagnose urinary tract infections. The observed discrepancies reflect the fundamental differences in what each method measures; culture detects only what is viable and readily cultivable on standard media under aerobic conditions, while sequencing identifies the presence of bacterial taxa based on genetic material, including organisms that are non-culturable, fastidious, anaerobic, or present in low abundance [72]. In this study, culture-based methods demonstrated a clear selection bias for members of the Enterobacteriaceae family, such as *Escherichia, Klebsiella*, and *Enterobacter*. This is a well-documented limitation where the culture conditions (aerobic incubation at 37°C) inherently favor fast-growing, non-fastidious organisms, causing them to dominate the microbial profile while limiting the understanding of the underlying diversity [73]. In contrast, sequencing provided a culture-independent, unbiased view of the urinary microbiota. It was instrumental in uncovering a "hidden microbiome" in Group 1 (non-UTI) patients, revealing significant proportions of commensal and opportunistic genera such as *Acinetobacter* (13.9%), *Lactobacillus* (13.5%), *Enterococcus* (11.9%), and *Prevotella* (11.8%) that were completely missed by culture. This finding is critical, as a growing body of evidence suggests these taxa may contribute to a protective microbial ecosystem that resists colonization by pathogens. The stronger correlation between the two methods in the suspected UTI group (Group 2) indicates that during acute infection the community becomes dominated by high-biomass, readily culturable pathogens causing the results of both techniques to converge. This convergence validates culture's utility in diagnosing infections but also highlights its limitation to uncover the broader ecological collapse that precedes or accompanies the infections in vulnerable populations. However culture remains clinically indispensable, as it provides live isolates essential for performing antibiotic susceptibility testing (AST) to guide targeted therapy. Culture remains the gold standard for confirming the viability of dominant pathogens and guiding antimicrobial stewardship. Meanwhile sequencing serves as an essential discovery tool, revealing the full ecological context of UTIs. It identifies patients with subclinical dysbiosis who might be at risk for future infection, detects polymicrobial contributions, and uncovers pathogens that culture misses.

5.8 Correlation Analysis (Culture vs Sequencing)

The correlation analysis between biochemical culture and sequencing data provides a nuanced, quantitative measure of their agreement. Further revealing that the relationship between these methods is heavily dependent on the clinical context and the taxonomic resolution examined. The moderate and statistically non-significant correlation observed in Group 1 (non-UTI patients, $\rho = 0.606$, $p = 0.1112$) at genus level is perhaps the most insightful finding. It quantifies the extent of culture's blind spots, confirming that a significant portion of the microbial community in asymptomatic states specifically, the diverse array of anaerobic, microaerophilic, and slow-growing commensals like *Lactobacillus*, *Prevotella*, *Acinetobacter*, *and Enterococcus*. The culture of these organisms is simply not possible using standard clinical culture protocols. The lack of statistical significance underscores that in the absence of a dominant, culturable pathogen, the two methods are capturing fundamentally different aspects of the microbial landscape. Conversely, the strong and highly significant correlation in Group 2 (suspected UTI, $\rho = 0.899$, $p = 0.0004$) at genus level demonstrates a convergence of evidence during active infection. This high degree of similarity indicates that the dysbiotic state of a UTI is characterized by a massive bloom of culturable Enterobacteriaceae (e.g., *Escherichia*, *Enterobacter*), whose biomass is so high that they are effortlessly detected by both techniques. This convergence validates culture's primary clinical role; to reliably identify the dominant microbial agents in acute infections to guide initial antibiotic therapy. The analysis at different taxonomic levels adds another critical layer of understanding. It demonstrates that while the methods frequently lead to differences of interpretation on the specific genus or species present, they consistently provide similar results on the broader taxonomic groups that dominate the community. For instance, both methods might conclude that Enterobacteriaceae is the dominant family, but culture may identify it as Klebsiella while sequencing identifies it as Escherichia. This suggests that although family-level identification from sequencing could be highly reliable, genus and species-level identification require careful interpretation based on clinical context. These correlation results have profound practical implications. For suspected UTI (Group 2) culture remains the gold standard, as it efficiently identifies the primary pathogens and provides essential data for AST. The strong correlation here means culture alone often suffices for acute diagnosis. However for complex, recurrent, or culture-negative cases (Group 1), sequencing becomes indispensable. The moderate correlation in non-UTI states is a strength, not a weakness,

as it reveals the dysbiosis that may be underlying the patient's symptoms. In these cases, sequencing provides the microbial diagnosis that culture misses.

Chapter 6: Future Directions

This study establishes a foundational understanding of urinary microbiome alterations in diabetic patients with suspected UTIs. Based on the findings of this study several important directions for future research are suggested. This study establishes a foundational understanding of urinary microbiome dysbiosis in diabetic patients with suspected UTI. Based on these findings, several important directions for future research are suggested. Firstly, future work should involve larger cohorts. This would enhance the detection of subtler microbial associations and improve the generalizability of findings across diverse diabetic populations with varying demographics, diabetic complications, and comorbidities. A longitudinal study design is also suggested as it is essential for tracking microbial dynamics through UTI onset, antibiotic treatment, and recovery. Future studies should focus on integrating microbiome profiles with antimicrobial susceptibility testing (AST) data from dominant cultured pathogens to better elucidate the clinical relevance. For deeper analysis the requirements include the integration of detailed patient metadata with microbiome profiles.

Chapter 7: Study Limitations

While the combined culture-sequencing approach is a key strength, this study has several limitations that should be acknowledged:

- Sample Size: The modest sample size for the sequencing analysis (n=27) limits the statistical power for more complex multivariate analysis and may reduce the generalizability of the conclusions. A larger cohort would strengthen the findings.
- Unmeasured Confounding Variables: The lack of detailed antibiotic exposure history for participants is a significant limitation, as recent antibiotic use is a major known disruptor of the microbiome.
- Inherent Culture Bias: The discrepancies between methods are partly attributable to the inherent cultivation bias of biochemical methods, which favor fast-growing, aerobic bacteria like Enterobacteriaceae, and fail to detect anaerobic, slow-growing, or unculturable bacteria.

Chapter 8: Conclusion

In conclusion, this study provides compelling evidence that the clinical state of a suspected UTI in diabetic patients is defined by a profound ecological shift in the urinary microbiome. The combined use of traditional culture and modern sequencing revealed complementary narratives; culture effectively identified the predominant, cultivable pathogens driving acute infection, while sequencing uncovered the hidden ecological collapse and the overlooked diversity of the broader microbial community. The findings of this study have direct clinical and public health implications, challenging the adequacy of culture-alone diagnostics.

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Appendices

Appendix A. Biochemical Test Results of Bacterial Isolates

Table A1. Biochemical profiles of bacterial isolates obtained from diabetic patients (Group 1).

Sample no	Colony Appearance	TSI			CITRATE	Catalase	Oxidase	MIU			Result Interpretation
		Slant/Butt	Gas production	H_2S production				Indole	Urea	Motility	
1EP	emb purple	yellow/ yellow	(+) ve	-	(+) ve	(+) ve	(-) ve	(-) ve	(+) ve	(-) ve	Klebsiella
1XY	xld yellow	yellow/ yellow	(+) ve	-	(-) ve	(-) ve	(-) ve	(+) ve	(-) ve	(+) ve	E. coli
1XBC	xld black center	red/black	(+) ve (weak)	(+) ve	(-) ve	(+) ve	(-) ve	(-) ve	(+) ve	(+) ve	Proteus
1XY	xld yellow	yellow/ yellow	(+) ve	-	(-) ve	(-) ve	(-) ve	(+) ve	(-) ve	(+) ve	E. coli
1UT	UTI chrome turquise	yellow/ yellow	(+) ve	-	(+) ve	(+) ve	(-) ve	(-) ve	(+) ve	(-) ve	Klebsiella
8XY	xld yellow	yellow/ yellow	(+) ve	-	(-) ve	(-) ve	(-) ve	(+) ve	(-) ve	(+) ve	E. coli
8XY	xld yellow	yellow/ yellow	(+) ve	-	(+) ve	(-) ve	(-) ve	(-) ve	(-) ve	(+) ve	Enterobacter
8EC	emb colourless	yellow/ yellow	(+) ve	-	(+) ve	(-) ve	(-) ve	(-) ve	(-) ve	(+) ve	Enterobacter
8UT	UTI chrome turquise	yellow/ yellow	(+) ve	-	(+) ve	(+) ve	(-) ve	(-) ve	(+) ve	(-) ve	Klebsiella
8UP	UTI chrome purple	yellow/ yellow	(+) ve	-	(-) ve	(-) ve	(-) ve	(+) ve	(-) ve	(+) ve	E. coli
8EP	emb purple	yellow/ yellow	(+) ve	-	(+) ve	(+) ve	(-) ve	(-) ve	(+) ve	(-) ve	Klebsiella

12UW	uti chrome white/colourless	red/yellow	-	-	(-) ve	(+) ve	(-) ve	(+) ve	(-) ve	(-) ve	Shigella
13XBC	xld black center	black/black	(+) ve	(+) ve	(+) ve	(+) ve	(-) ve	(-) ve	(-) ve	(+) ve	Salmonella
13UW	uti chrome white/colourless	red/yellow (black)	(+) ve	(+) ve	(+) ve	(+) ve	(-) ve	(-) ve	(-) ve	(+) ve	Salmonella
18UW	uti chrome white/colourless	red/yellow	-	-	(-) ve	(+) ve	(-) ve	(+) ve	(-) ve	(-) ve	Shigella
18UP	UTI chrome purple	yellow/yellow	(+) ve	-	(-) ve	(-) ve	(-) ve	(+) ve	(-) ve	(+) ve	E. coli
18UT	uti chrome turquoise	yellow/yellow	(+) ve	-	(+) ve	(+) ve	(-) ve	(-) ve	(+) ve	(-) ve	Klebsiella
18MP	maccon key pink	yellow/yellow	(+) ve	-	(-) ve	(-) ve	(-) ve	(+) ve	(-) ve	(+) ve	E. coli
18MP	maccon key pink (mucoid)	yellow/yellow	(+) ve	-	(+) ve	(+) ve	(-) ve	(-) ve	(+) ve	(-) ve	Klebsiella
18XBC	xld black center	red/black	(+) ve (weak)	(+) ve	(-) ve	(+) ve	(-) ve	(-) ve	(+) ve	(+) ve	Proteus
19XY	xld yellow	yellow/yellow	(+) ve	-	(+) ve	(-) ve	(-) ve	(-) ve	(-) ve	(+) ve	Enterobacter

19UP	UTI chrome purple	yellow/ yellow	(+) ve	-	(-) ve	(-) ve	(-) ve	(+) ve	(-) ve	(+) ve	E. coli
19UT	uti chrome turquoise	yellow/ yellow	(+) ve	-	(+) ve	(+) ve	(-) ve	(-) ve	(+) ve	(-) ve	Klebsiella
19UW	uti chrome white/colourless	red/black	(+) ve	(+) ve	(+) ve	(+) ve	(-) ve	(-) ve	(-) ve	(+) ve	Salmonella
21UT	uti chrome turquoise	yellow/ yellow	(+) ve	-	(+) ve	(+) ve	(-) ve	(-) ve	(+) ve	(-) ve	Klebsiella
21UW	uti chrome white	red/yellow	-	-	(-) ve	(+) ve	(-) ve	(+) ve	(-) ve	(-) ve	Shigella
21MP	maccon key pink	yellow/ yellow	(+) ve	-	(-) ve	(-) ve	(-) ve	(+) ve	(-) ve	(+) ve	E. coli
22XBC	xld black center	red/black	(+) ve (weak)	(+) ve	(-) ve	(+) ve	(-) ve	(-) ve	(+) ve	(+) ve	Proteus
22UC	uti chrome colourless	red/red	-	-	(+) ve	(+) ve	(+)ve	(-) ve	(-) ve	(+) ve	Pseudomonas
23UW	uti chrome white	red/yellow	-	-	(-) ve	(+) ve	(-) ve	(+) ve	(-) ve	(-) ve	Shigella
23EP	emb pink	yellow/ yellow	(+) ve	-	(+) ve	(-) ve	(-) ve	(-) ve	(-) ve	(+) ve	Enterobacter
24UC	uti chrome colourless	red/red	-	-	(+) ve	(+) ve	(+)ve	(-) ve	(-) ve	(+) ve	Pseudomonas

27UP	uti chrome purple	yellow/ yellow	(+) ve	-	(-) ve	(-) ve	(-) ve	(+) ve	(-) ve	(+) ve	E. coli
27MC	maccon key colourl ess	red/yell ow	-	-	(-) ve	(+) ve	(-) ve	(+) ve	(-) ve	(-) ve	Shigell a
28MC	maccon key colourl ess	yellow/ yellow	(+) ve	-	(+) ve	(+)	(-)	(+)	(+)	(+)	Provide ncia
28UP	UTI chrome purple	yellow/ yellow	(+) ve	-	(-) ve	(-) ve	(-) ve	(+) ve	(-) ve	(+) ve	E. coli
29UP	UTI chrome purple	yellow/ yellow	(+) ve	-	(-) ve	(-) ve	(-) ve	(+) ve	(-) ve	(+) ve	E. coli

Table A2. Biochemical profiles of bacterial isolates obtained from diabetic patients (Group 2).

Colony appearance	**Isolate Name**	**Number**	**Citrate**	**MIU**			**TSI**				**Catalase**	**Oxidase**	**Result Interpretation**
				Indole	**Urea**	**Motility**	**Slant**	**Butt**	**Gas**	**H2S**			
Green colonies in EMB	31EG	31	(-)	(+)	(-)	(+)	Y	Y	(+)	-	(+)	(-)	*Escherichia coli*
colourless colonies in Mackonkey	32MC	32	(+)	(+)	(+)	(+)	Y	Y	(+)	-	(+)	(-)	*Providencia*
blue colonies in UTI agar	32UB	32	(+)	(-)	(+)	(+)	Y	Y	(+)	-	(+)	(-)	*Enterobacter*

creamy white colonies in EMB	34EW	34	(-)	(-)	(+)	(-)	R	R	-	-	(+)	(-)	*Staphylococcus aureus*
creamy white colonies in EMB	35EW	35	(-)	(-)	(+)	(-)	R	R	-	-	(+)	(-)	*Staphylococcus aureus*
Green colonies in EMB	36EG	36	(-)	(+)	(-)	(+)	Y	Y	(+)	-	(+)	(-)	*Escherichia coli*
Flat brown colonies in EMB	37EB	37	(+)	(-)	(-)	(+)	Y	Y	(+)	(+)	(+)	(-)	*Citrobacter freundii*
uti chrome colourless	37UW	37	(+)	(-)	(-)	(+)	R	R	-	-	(+)	(+)	*Pseudomonas*
Green colonies in EMB	38EG	38	(-)	(+)	(-)	(+)	Y	Y	(+)	-	(+)	(-)	*Escherichia coli*
blue colonies in UTI agar	38UB	38	(+)	(-)	(+)	(+)	Y	Y	(+)	-	(+)	(-)	*Enterobacter*
uti chrome colourless	39UW	39	(+)	(-)	(-)	(+)	R	R	-	-	(+)	(+)	*Pseudomonas*

blue colonies in UTI agar	40UB	40	(+)	(-)	(+)	(+)	Y	Y	(+)	-	(+)	(-)	*Enterobacter*
uti chrome white/ colourless	41UC	41	(-)	(-)	(-)	(-)	R	Y	-	-	(+)	(-)	*Shigella*
xld black center	41XBC	41	(+)	(-)	(+)	(+)	R	Y	(+)	(+)	(+)	(-)	*Proteus*
Green colonies in EMB	42EG	42	(-)	(+)	(-)	(+)	Y	Y	(+)	-	(+)	(-)	*Escherichia coli*
uti chrome turquoise	42UT	42	(+)	(-)	(+)	(-)	Y	Y	(+)	-	(+)	(-)	*Klebsiella*
uti chrome white/ colourless	42UC	42	(-)	(-)	(-)	(-)	R	Y	-	-	(+)	(-)	*Shigella*
blue colonies in UTI agar	43UB	43	(+)	(-)	(+)	(+)	Y	Y	(+)	-	(+)	(-)	*Enterobacter*
blue colonies in UTI agar	44UB	44	(+)	(-)	(+)	(+)	Y	Y	(+)	-	(+)	(-)	*Enterobacter*
Green coloni	44EG	44	(-)	(+)	(-)	(+)	Y	Y	(+)	-	(+)	(-)	*Escherichia coli*

es in EMB													
blue colonies in UTI agar	45UB	45	(+)	(-)	(+)	(+)	Y	Y	(+)	-	(+)	(-)	*Enterobacter*
colourless colonies in Mackonkey	46MC	46	(+)	(+)	(+)	(+)	Y	Y	(+)	-	(+)	(-)	*Providencia*
blue colonies in UTI agar	47UB	47	(+)	(-)	(+)	(+)	Y	Y	(+)	-	(+)	(-)	*Enterobacter*
blue colonies in UTI agar	49UB	49	(+)	(-)	(+)	(+)	Y	Y	(+)	-	(+)	(-)	*Enterobacter*
uti chrome white/ colourless	49UC	49	(-)	(-)	(-)	(-)	R	Y	-	-	(+)	(-)	*Shigella*
blue colonies in UTI agar	53UB	53	(+)	(-)	(+)	(+)	Y	Y	(+)	-	(+)	(-)	*Enterobacter*
blue colonies in UTI agar	54UB	54	(+)	(-)	(+)	(+)	Y	Y	(+)	-	(+)	(-)	*Enterobacter*
Green coloni	56EG	56	(-)	(+)	(-)	(+)	Y	Y	(+)	-	(+)	(-)	*Escherichia coli*

es in EMB													
uti chro me colou rless	58U W	58	(+)	(-)	(-)	(+)	R	R	-	-	(+)	(+)	*Pseudom onas*
uti chro me turqu oise	59UT	59	(+)	(-)	(+)	(-)	Y	Y	(+)	-	(+)	(-)	*Klebsiella*
blue coloni es in UTI agar	59UB	59	(+)	(-)	(+)	(+)	Y	Y	(+)	-	(+)	(-)	*Enterobac ter*
uti chro me white/ colou rless	59UC	59	(-)	(-)	(-)	(-)	R	Y	-	-	(+)	(-)	*Shigella*
uti chro me white/ colou rless	67UC	67	(-)	(-)	(-)	(-)	R	Y	-	-	(+)	(-)	*Shigella*
blue coloni es in UTI agar	67UB	67	(+)	(-)	(+)	(+)	Y	Y	(+)	-	(+)	(-)	*Enterobac ter*
uti chro me colou rless	67U W	67	(+)	(-)	(-)	(+)	R	R	-	-	(+)	(+)	*Pseudom onas*
Green coloni	68EG	68	(-)	(+)	(-)	(+)	Y	Y	(+)	-	(+)	(-)	*Escherich ia coli*

es in EMB													
uti chro me turqu oise	68UT	68	(+)	(-)	(+)	(-)	Y	Y	(+)	-	(+)	(-)	*Klebsiella*
blue coloni es in UTI agar	62UB	62	(+)	(-)	(+)	(+)	Y	Y	(+)	-	(+)	(-)	*Enterobac ter*
	63	63	(+)	(-)	(-)	(+)	Y	Y	-	-	(+)	(-)	*Serratia marcesce ns*
uti chro me white/ colou rless	65U W	65	(-)	(-)	(-)	(-)	R	Y	-	-	(+)	(-)	*Shigella*
uti chro me colou rless	65U W	65	(+)	(-)	(-)	(+)	R	R	-	-	(+)	(+)	*Pseudom onas*
Green coloni es in EMB	65EG	65	(-)	(+)	(-)	(+)	Y	Y	(+)	-	(+)	(-)	*Escherich ia coli*
Green coloni es in EMB	57EG	57	(-)	(+)	(-)	(+)	Y	Y	(+)	-	(+)	(-)	*Escherich ia coli*