IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB)
ISSN: 2455-264X, Volume 11, Issue 6 Series II (Nov. – Dec. 2025), PP 01-10
www.iosrjournals.org

Detection of multi drug resistant uropathogenic *E coli* through gene sequencing and assessment of cranberry based anti microbial potential: an emerging approach for UTI treatment.

Pranab Kumar Das[a*], Mayurakshi Basak[b], Debmalya Sarkar[b]
[a] *Assistant Professor,* [b] *Undergraduate Student, Dept. of Microbiology, Asutosh College, 92, S.P. Mukherjee Road, Kolkata-700026, India*

Abstract: *Urinary Tract Infections (UTIs) are infections that can impact any section of the urinary tract- the kidneys, bladder, ureters and urethra. They are among the most prevalent bacterial diseases, with over fifty percent of women experiencing at least one episode in their lifetime. Although urinary tract infections are usually managed with broad -spectrum antibiotics, the rising prevalence of multidrug-resistant uropathogens has underscored the pressing need for safe, effective, non- antibiotic alternatives for UTI prevention. This research seeks to examine the antimicrobial properties of plant extracts, particularly cranberry (Vaccinium macrocarpon), as a potential natural treatment for UTIs. In this investigation, human urine samples were cultured on enrichment media, followed by screening on selective media for the isolation and identification of pathogenic bacteria. The Gram-negative bacterium Escherichia coli was identified through gene sequencing and molecular biology techniques. It was then tested for antibiotic resistance against four standard antibiotics: streptomycin (25 µg), ampicillin (10 µg), chloramphenicol (30 µg) and tetracycline (30µg). Concurrently, bacteria were exposed to cranberry extract to assess its antibacterial activity. The results demonstrated that V. macrocarpon extract significantly inhibited the growth of the uropathogenic bacteria. Based on these findings, it can be concluded that cranberry extract, in vitro, may offer a positive alternative approach for the management and prophylaxis against UTIs.*

Keywords: *Urinary tract infections, plant extract, cranberry, antibiotic resistance, Vaccinium macrocarpon, gene sequencing, non-drug UTI prevention, alternative treatment, antimicrobial efficacy.*

Date of Submission: 08-12-2025 Date of Acceptance: 20-12-2025

I. INTRODUCTION

Historically, urine was considered a sterile fluid. However, extensive research over the years has demonstrated that urine may harbor urinary microbiota, potentially contributing to UTIs [1].

A Urinary Tract Infection is characterized by the detection of a substantial concentration of bacterial cells urine, typically exceeding 108 CFU/l (urinary white cell count [WCC] > 100/µl), often accompanied by symptoms affecting the bladder, ureters, or kidneys [2]. UTIs are highly prevalent, particularly among females, and rank as one of the most common bacterial infections, causing significant morbidity and incurring substantial healthcare costs globally [3]. It is estimated that 150 million individuals suffer from UTIs each year worldwide [4]. In US alone, UTIs lead to around 10.5 million outpatient consultations, and 3 million emergency visits annually. Furthermore, UTIs result in about 400,000 hospitalizations per year, with a total estimated cost exceeding $4.8 billion [5-7]. A notable 9.4% of patients have been diagnosed with healthcare-associated UTIs in various healthcare settings worldwide [8]. High incidence rates of UTIs have also been observed among pregnant women, particularly in the Asian and African subcontinents [9]. The prevalence of UTIs is influenced by numerous environmental factors and lifestyle choices. Older adults, particularly those with multiple comorbidities or undergoing certain medical treatments, face an elevated risk of UTIs. The use of urinary catheters, in particular, increases the likelihood of infections, especially those caused by Gram-negative bacteria [10]. Additionally, approximately 20% of men are also affected by UTI at least once in their lives [11].

UTIs exhibit considerable heterogeneity in both etiology and clinical manifestations. The severity of these infections can vary from moderate cases like "urethritis and cystitis, to more serious manifestations, including pyelonephritis, bacteremia, and septic shock" [12]. Among these, acute cystitis is most common. The primary etiology of such infections is the colonization of pathogenic bacteria on the epithelial urinary tract

lining. [13]. *Escherichia coli* is the predominant causative organism, a Gram-negative bacterium [14]. Other bacterial species, such as *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterococcus* spp., have also been implicated in UTIs [15].

Many of these bacterial species have developed multidrug resistance (MDR), rendering common antibiotics ineffective against these pathogens [16]. The rise of MDR bacteria represents a significant public health threat, as infections caused by these pathogens are challenging to manage and are frequently associated with elevated mortality levels [17]. This has sparked significant research into alternative therapeutic strategies that do not rely on traditional antibiotics for managing MDR pathogens.

The ongoing prevalence of UTIs has caused new prevention regimes. Notably, Uromune (MV140), a novel sublingual mucosal vaccine, has been formulated to prevent recurrent urinary tract infections (rUTIs) in women. rUTI refers to the occurrence of multiple UTIs within a specific timeframe, typically defined as three or more episodes within 12 months or two episodes within six months. Clinical trials, including observational, prospective, and randomized placebo -controlled studies, have shown that MV140 significantly reduces the incidence of UTIs, reduces the need for antibiotics, lowers overall management costs, and improves the life of women affected by rUTIs [18]. Additionally, in October 2024, Iterum Therapeutics' oral antibiotic, Orlynvah, containing sulopenem etzadroxil and probenecid, received FDA approval for treating uncomplicated UTIs in adult women, particularly those with limited antibiotic options [19]. These advancements illustrate a multifaceted approach to UTI management, integrating vaccines, alternative therapies, and novel antibiotics to address both common and resistant UTI pathogens.

Despite the use of antibiotics that appear to resolve the infection (as evidenced by negative urine cultures), a significant percentage of patients experience recurrent UTIs. Approximately 25% of patients who initially recover will develop a second UTI within six months, and this recurrence rate increases to 46% within 12 months [20].

Cranberry, especially in its extract form, has long been utilized in the control of UTIs [21]. Cranberry extract is believed to prevent bacterial adhesion to the bladder walls, thereby helping to limit the incidence of UTIs [22]. Cranberries are composed primarily of water (88%) and contain a variety of beneficial compounds, including organic acids (such as salicylate), fructose, vitamin C (around 200 mg/kg), flavonoids, anthocyanidins and triterpenoids [22]. Its unique flavour is attributed to iridoid glycosides, while the proanthocyanidins (PAC) and anthocyanidins, stable polyphenolic tannins present exclusively in vaccinium berries, act as antimicrobial agents [22,23].

II. MATERIALS AND METHODS

2.1 Source of samples, sample collection and transportation

Urine sample was collected from patient having symptoms and diagnosed with UTI. The urine samples were collected midstream during urination before antibiotic treatment for bacterial culture.
100 ml sample was taken and delivered to the lab by maintaining cold chain and following proper aseptic techniques. Sample with pus cells and epithelial cells > 5/hpf was considered as positive for UTI and proceeded for urine culture.

2.2 Isolation and identification of bacteria

The specimen was enriched in sterile nutrient broth (NB) and incubated at 37°C overnight, after which they were inoculated on solid media, Luria agar (LA) which was further incubated under the same conditions as before, to isolate and identify pathogenic bacteria. A single isolated colony was selected, and its characteristics were identified based on colony morphology, biochemical tests and gene sequencing.

2.3 Gene sequencing of isolated bacteria

Genomic DNA was extracted from the cultured isolate, and its integrity was evaluated through electrophoresis on a 1.0% agarose gel. The analysis revealed a single, distinct band corresponding to high-molecular-weight DNA. The 16S rRNA gene fragment was subsequently amplified using specific primers, 16SrRNA-F and 16SrRNA-R. Agarose gel electrophoresis confirmed the amplification by displaying a single, well-defined PCR product of approximately 1500 bp. The obtained amplicon was purified to eliminate any residual contaminants. Both forward and reverse sequencing reactions of the purified PCR product were performed using the 16SrRNA-F and 16SrRNA-R primers with the BigDye Terminator v3.1 Cycle Sequencing Kit on the ABI 3730xl Genetic Analyzer platform. The forward and reverse sequencing reads were assembled and aligned

through an aligner software to generate a consensus sequence of the 16S rRNA gene. The resulting consensus sequence was subjected to BLAST analysis against the NCBI GenBank 'nr' database. From the output, the top ten sequences with the highest similarity scores were retrieved and aligned using the ClustalW multiple sequence alignment program. A distance matrix was subsequently computed, and a phylogenetic tree was constructed using MEGA version 11 to determine the evolutionary relationship of the isolate with closely related taxa.

2.4 Antibiotic susceptibility testing with known antibiotics

For antibiotic susceptibility testing with known antibiotics, "The Clinical and Laboratory Standards Institute guidelines" [24] were referred to. Petri dishes having Mueller-Hinton Agar (MHA) weremade following instructions of the producer in order to test antibiotic sensitivity in accordance to the Kirby-Bauer disk diffusion method [25]. A micropipette was used to transfer 100µl of the isolated bacteria onto the MHA plates and then spread uniformly using a sterile glass spreader.

Subsequently, four antibiotic discs were employed : chloramphenicol (CHL, 30 µg), ampicillin (AMP, 10 µg), streptomycin (S, 25 µg), and tetracycline (TET, 30 µg). The discs were positioned into the media in the petri dishes using sterile forceps, such that each dish contained four discs out of which, two discs contained the antibiotics and the remaining two contained dimethyl sulfoxide (DMSO) which served as a negative control for the respective antibiotics. The petri dishes were transferred to the incubator at 37°C overnight to observe inhibitory zones for the standard antibiotics used.

2.5 Plant extract preparation

This study used dried cranberries (Family: *Ericaceae*, Genus: *Vaccinium*) purchased from local suppliers. Extracts of the berries were prepared at the laboratory, to test the susceptibility of UTI pathogenic bacteria. 10 gm cranberries were crushed with a mortar and pestle . While grinding, a few drops of dimethyl sulfoxide (DMSO), a widely used solvent that is miscible with water and a wide range of organic solvents, were added to make it into a fine paste. Filter paper was then used for filtration, and the cranberry paste was poured into the funnel to be filtered. The liquid was allowed to pass through the filter paper, and the filtrate collected was the plant extract.

2.6 Investigation of antimicrobial potential of plant extract

Antimicrobial potential investigation of plant extract means testing target extract's effectiveness against a specific bacterium. This was done using methods like agar well diffusion method which involved creating wells in Mueller-Hinton Agar plates seeded with bacteria, then inoculating the plant extract in the wells. They were then incubated for 24 hrs at a temperature of 37°C. The inhibitory zones were calculated to compare effectiveness of target extract.

2.7 Characterization of cranberry extract by phytochemical analysis

Phytochemical analysis was carried out for chemical identity of active component in cranberries. Stand ard phytochemical tests were carried by methods discussed by T r e a s e a n d E v a n s [2 6] .

2.7.1 To study the presence of steroid: To 1 m l of sample, 2 m l o f c o n c . H2SO4 w as carefully added. Reddish-brown coloured ring proved that steroids were present.

2.7.2 To study the presence of terpenoid: To 1 ml of sample, few drops of chlorof orm and conc. H2SO4 was add ed very slowly. Appearance of green-yellow ring within reddish- brown ring confirms the presence of terpenoid.

2.7.3 To study the presence of glycosides: Sodium-picrate solution was added to 1 ml of sample. Absence of brownish-orange colour confirms the absence of glycosides.

2.7.4 To study the presence of flavonoid: First, sample was dissolved in ethanol and few drops of it were d ropped on filter paper. Then 30% ammonia solution was add ed to the filter paper. Appearance of yellow colour on the filter paper proves the presence of flavonoid.

2.7.5 To study the presence of Tannins: 2-3 ml of $FeCl_3$ was ad ded to 1 ml of sample. Absence of dark green colour proves the absence of tannins.

2.7.6 To study the presence of carbohydrates: A small amount of Molish reagent was added to 1 ml of sample. Appearance of purple colour proves the presence of carbohydrates.

III. RESULTS

3.1 Identification of the pathogen by biochemical tests

The single isolated colony on Luria Agar was stained with Gram staining and observed under microscope 100X oil immersion. Gram negative rods were observed. It was further streaked onto Mac Conkey agar plates and incubated at 37°C for 24 hrs. Pink colonies characteristic of *Escherichia coli* were observed. Moreover, biochemical tests were performed viz. IMViC test (+ve+ve -ve -ve), catalase test (+ve), urease test (+ve), triple sugar iron test (characteristic reaction) were observed.

3.2 Gene sequencing results

The results for genomic DNA (g DNA) and 16S rRNA amplifications are shown in Fig 1. Primer used for 16S rRNA forward reaction is "AGAGTTTGATCCTGGCTCAG" and that for backward reaction is "CGGTTACCTTGTTACGACTT". A single separate PCR amplicon band was observed which was found to be 1500 bp when resolved on agarose gel. BLAST was carried out using 'nr'database of NCBI GeneBank data base. Only first ten sequences showing maximum identity score are selected. Sequences producing significant alignments are shown in Table 1, and the phylogenetic tree is shown in Fig 2.

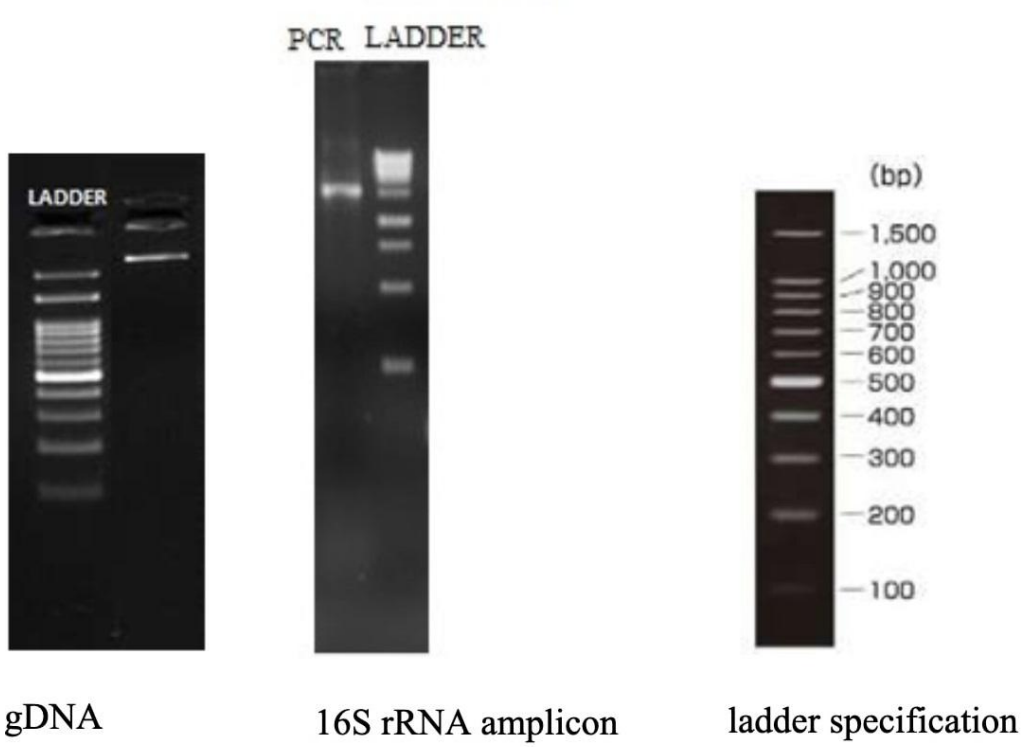

Fig 1: Results for genomic DNA (g DNA) and 16S rRNA amplicon

Table 1: Sequences producing significant alignments

Description	Max Score	Total Score	Query Cover	E value	% Identity	Accession
Escherichia coli strain SCDC-1	2761	2761	100%	0.0	99.80%	HM576813.1
Escherichia fergusonii ATCC 35469	2700	2700	100%	0.0	99.07%	NR_074902.1
Escherichia marmotae strain HT073016	2695	2695	100%	0.0	99.00%	NR_136472.1
Shigella flexneri strain ATCC 29903	2695	2695	98%	0.0	99.39%	NR_026331.1
Shigella sonnei strain CECT 4887	2687	2687	99%	0.0	99.20%	NR_104826.1
Shigella dysenteriae strain ATCC 13313	2671	2671	98%	0.0	99.13%	NR_026332.1
Escherichia fergusonii ATCC 35469	2658	2658	97%	0.0	99.25%	NR_027549.1

Shigella boydii strain P288	2652	2652	98%	0.0	98.92%	NR_104901.1
Escherichia fergusonii strain NBRC 102419	2643	2643	97%	0.0	99.05%	NR_114079.1
Escherichia coli strain NBRC 102203	2643	2643	97%	0.0	99.11%	NR_114042.1

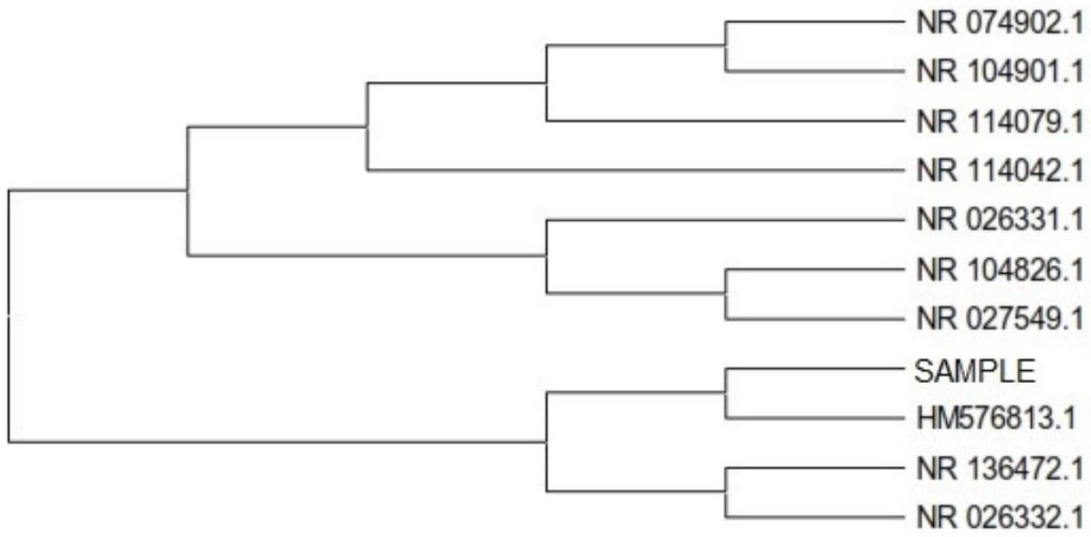

Fig 2: Phylogenertic tree (the isolate is represented by SAMPLE)

3.3 Evolutionary analysis by Maximum Likelihood method

The evolutionary history of the selected sequences was inferred using the Maximum Likelihood (ML) method based on the Tamura-Nei model [27]. The phylogenetic tree with the highest log likelihood value (-2426.22) is presented. For the heuristic search, the initial tree(s) were automatically generated by applying the Neighbor-Join and BioNJ algorithms to a distance matrix calculated using the Tamura-Nei model, followed by the selection of the topology that yielded the superior log likelihood score. A total of 11 nucleotide sequences were included in the analysis. Codon positions considered comprised 1st, 2nd, 3rd, and noncoding sites. The final dataset contained 1511 positions. All evolutionary analyses were performed using the MEGA11 software [28]. The estimated number of base substitutions per site between sequences is shown, with standard error values displayed above the diagonal. The Maximum Composite Likelihood model [27] was employed for these estimations. To ensure accuracy, all ambiguous positions were removed for each sequence pair through the pairwise deletion option. This analysis also involved 11 nucleotide sequences, with codon positions again including 1st, 2nd, 3rd, and noncoding regions. The final dataset retained a total of 1511 positions. Evolutionary analyses were conducted in MEGA11 [28] (Table 2).

Finally, Based on the microscopic examination, characteristic colony on Mac Conkey agar, biochemical tests, nucleotide homology and phylogenetic analysis, the isolated UTI pathogen was confirmed to be *Escherichia coli*.

Table 2: Estimates of evolutionary divergence between sequences (the isolate is represented by SAMPLE)

SAMPLE		0.001	0.003	0.002	0.002	0.003	0.003	0.003	0.003	0.002	0.002
HM576813.1	0.001		0.002	0.002	0.002	0.002	0.002	0.002	0.003	0.002	0.002
NR_074902.1	0.010	0.009		0.003	0.001	0.002	0.003	0.002	0.002	0.000	0.001
NR_136472.1	0.009	0.009	0.013		0.003	0.003	0.003	0.003	0.003	0.003	0.003
NR_026331.1	0.007	0.007	0.003	0.013		0.001	0.003	0.001	0.002	0.000	0.001
NR_104826.1	0.009	0.008	0.004	0.014	0.001		0.003	0.001	0.002	0.001	0.002
NR_026332.1	0.009	0.008	0.010	0.011	0.009	0.011		0.003	0.003	0.003	0.003
NR_027549.1	0.008	0.008	0.003	0.014	0.001	0.002	0.010		0.002	0.000	0.001
NR_104901.1	0.012	0.011	0.004	0.017	0.005	0.006	0.014	0.005		0.001	0.002
NR_114079.1	0.007	0.006	0.000	0.012	0.000	0.001	0.009	0.000	0.003		0.001
NR_114042.1	0.008	0.008	0.002	0.012	0.002	0.003	0.010	0.003	0.006	0.001	

3.4 Antibiotic sensitivity study of the pathogen *E coli*

This study shows that the isolated urinopathogenic *E coli* bacteria is resistant to several standard antibiotics due to development of multidrug resistance (MDR). MDR refers to the ability of microorganisms to resist multiple antimicrobial drugs of at least three or more antimicrobial categories, due to acquired resistance mechanisms. In this study, four antibiotics that has been used is already mentioned in Materials and Methods section. Resistance patterns of the isolate against the four standard antibiotics in terms of inhibition zone diameter (in mm) in plate 1 and plate 2include: Streptomycin (S, 25 µg) - 18 mm, 15mm; Chloramphenicol (CHL, 30 µg) - 0 mm, 0 mm; Ampicillin (AMP, 10 µg) - 0 mm, 0mm and Tetracycline (TET, 30 µg) - 0 mm, 0mm. The same hasbeen shown in Fig 3.

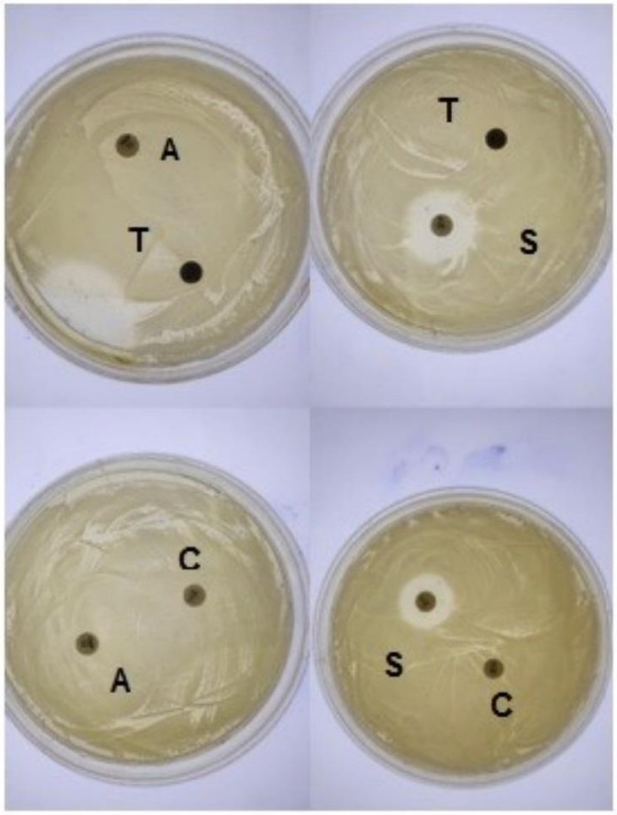

Fig 3: Antibiotic sensitivity tests showing Inhibition zone diameter against standard antibiotics.
A= ampicillin, T= tetracycline, C= chloramphenicol, S= streptomycin

3.5 Antimicrobial efficacy of the plant extract

This study illustrates the antimicrobial effectiveness of the cranberry extract against the isolated UTI pathogenic *E coli* strain. DMSO is used as negative control. The zone inhibition diameters are 12 mm and 14 mm respectively (Fig 4).

Fig 4: Antibacterial activity of cranberry against isolated *E coli* UTI pathogen in comparison to negative control DMSO

3.6 Characterization of cranberry extract by phytochemical analysis

Phytochemical analysis reveals that cranberry extract contains components as steroids terpinoides, flavnoides, carbohydrates but glycosoides and tanins are absent.

IV. DISCUSSION

UTIs are among the most prevalent infections that impact millions around the world , every year. They occur twice as often in females as in males [29]. Symptoms can consist of a constant need to urinate along with a burning feeling, foul-smelling urine, pelvic ache, and in more severe cases, fever and back ache. Most UTIs have *E. coli,* a normal inhabitant of our intestine, as their causative agent. The typical infection pathway involves bacteria invading the urethra or periurethral region followed by the bladder and then triggering an inflammatory reaction [30]. However, its pathogenic strain can cause an infection if it spreads to the urinary tract. Other factors that contribute to UTIs include poor hygiene, sexual activity, urinary catheter use, blockages in the urinary tract, and some health conditions like diabetes. In postmenopausal women, lower estrogen levels can also raise the risk of UTIs. Uropathogenic *Escherichia coli* is the primary cause of both uncomplicated and complicated UTIs [31]. Other causative agents include *Klebsiella pneumoniae*, *Staphylococcus saprophyticus*, *Enterococcus faecalis, Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida* spp [32].

This study aimed to isolate uropathogens present in the urine samples collected from patients having symptoms or diagnosed with UTI, identify them, study their resistance and sensitivity properties and draw a parallel between their treatments with specific plant extracts to that of standard treatment regimes. Microscopic observation, cultural characteristics, standard biochemical tests and gene sequencing results indicated the presence of gram-negative bacteria *Escherichia coli* in the urine sample. Such conclusions were also made by Johansen et al. [33] and Kaur et al. [34] with both describing the gram-negative bacteria (31% and 71.7% respectively), to be the most prevalent bacterial pathogen in case of UTIs. Odoki et al. [35] found the predominant agent to be *E. coli* (41.9%), followed by *S. aureus*, *K. pneumoniae*, *K. oxytoca.*

Antimicrobial susceptibility of isolate was tested against four different standard antibiotics: Streptomycin (S), Chloramphenicol (C), Ampicillin (AMP) and Tetracycline (TET), by the disc diffusion method already discussed in the Materials and Methods section of this manuscript. Streptomycin belongs to the aminoglycoside class that attach themselves to bacterial 30S ribosomal subunit, causing translational errors of mRNA, preventing the process of translation. Amphenicols constitute chloramphenicol, a broad -spectrum antibiotic that blocks translation by attaching to the 50S ribosomal subunit of prokaryotes [36,37]. Ampicillins are β-lactam antibiotics, specifically derived from penicillin that disrupts peptidoglycan biosynthesis [38]. Tetracyclines, on the other hand, show similar mode of action as that of Streptomycin, except that it prevents t-RNA attachment, blocking elongation of the peptide chain [39]. In the present study, our isolated uropathogen showed complete resistance to C, AMP and TET and partial resistance to S, exhibiting Multidrug Resistance (MDR). It refers to resistance against a minimum of three distinct antimicrobial classes [40]. While the study

lists several standard antibiotics used, including chloramphenicol (C), ampicillin (AMP), and tetracycline (TET), it directly states that the uropathogen was highly resistant to AMP and partially resistant to S. *E. coli* possess a virulence factor called beta-lactamase, that can break down ampicillin [41]. Such strains can also be MDR, with prevalence rates reported in studies. For example, *E. coli* isolates in one study showed resistance to ampicillin (100%), amoxicillin (100%), chloramphenicol (28.6%), and nalidixic acid (14.3%) [42]. Another study found that *E. coli* isolates showed resistance to AMP (90%) and CEP (83.33%). These isolates are genetically capable of inactivating antibiotics through reduced permeability and efflux pumps, contributing to MDR strains. Though UTIs are majorly caused by MRSA, extended -spectrum beta-lactamase-producing organisms, enterococci resistant to vancomycin, and carbapenem-resistant organisms increase morbidity and mortality [43]. However, the isolated MDR strains of *E. coli* were partially resistant to Streptomycin (S) with over 80% of patients with recurrent UTIs achieving cure [44].

The global statistical review studying correlation between biofilm development among *E. coli* variants responsible for UTI found that ~74.7% of uropathogenic *E. coli* (UPEC) isolates globally could form biofilm [45]. Whole genome analysis of MDR UPEC also identified biofilm-associated genes, various adhesins, and plasmids such as IncF, that contribute to antimicrobial resistance and biofilm-forming capabilities [46]. Hence, the isolated UPEC strain was capable of developing biofilm.

The global increase in bacterial resistance to different classes of antibiotics poses a significant community health hazard. Further, diminishing effectiveness of conventional treatments for common infections have resulted in a failure to adequately combat microbial threats. This, in turn, has led to prolonged illness, elevated healthcare expenditures, and increased mortality risks. Almost all infections showcase high MDR levels to increased toxicity and mortality [47]. Consequently, the pursuit of medicinal plants containing secondary metabolites capable of exhibiting antimicrobial properties is a pressing concern. Historically, medicinal plants have been recognized for their success in treating and preventing various diseases, leading to their widespread use by both the public and pharmaceutical industries [48]. Some of these plants possess compounds with antibacterial activity relevant to UTI. Thus, medicinal plants offer an alternative solution to the social, economic, and health consequences stemming from multidrug-resistant (MDR) bacteria like MRSA, *E. coli*, and *K. pneumoniae*, responsible for causing UTI [49]. This led us to study the antibacterial efficacy of plant extract, viz. cranberry (*Vaccinium macrocarpon*) against the tested uropathogenic bacteria. The plant extract displayed considerable antimicrobial activity depicted by their production of clear zone of inhibition against UTI -inducing microbes, thus affirming these findings.

Prior research has established that plant-obtained medicinal compounds could be a substitute to prevent and manage various diseases [50,51]. The fifth update of the Cochrane Review, which analysed 50 studies involving 8,857 participants, recently concluded that cranberry supplements can effectively tract infections among females experiencing periodic infections [51]. From the phytochemical analysis of cranberry, it is understood that cranberry is a notable source of polyphenols, specifically flavonoids and phenolic acids, which have been documented to offer beneficial effects in combating UTI [52]. Multiple in vitro and ex vivo studies highlight cranberry polyphenols and metabolites (e.g., myricetin, quercetin, A -type PACs) that inhibit *E. coli* adhesion to bladder cells, reduce biofilm formation by ≥50–75% and prevent P-type and Type-1 fimbriation [53,54]. Ethanol cranberry juice extract showed dose-dependent inhibition of *E. coli* (60%), *Proteus vulgaris* (75%), *Staph aureus* (90%) [55]. Thus, the antimicrobial activities of medicinal plants, such as *V. macrocarpon*, offer promising potential as adjunct or alternative therapies for UTIs, helping to mitigate the impact of drug-resistant bacteria. Future research should prioritize evaluating the synergistic effects of combining therapeutic plants along with conventional antibacterial drugs against infection causing pathogenic organism. A study stated that bactericidal efficacy against uropathogens like *E. coli* and *Proteus* spp. saw a substantial increase when antibiotics that were formerly ineffective like methicillin were combined with herbal compounds [56]. Hence, further research into these plant extracts may pave the way for novel therapeutic approaches to combat infectious diseases.

ACKNOWLEDGEMENT

The authors would like to express their sincere gratitude to Principal Sir and the Research & Development Cell of Asutosh College, Kolkata for their generous financial support in the form of Seed Grant Project, which has been instrumental in advancing this research work. Their encouragement and assistance are deeply appreciated.

REFERENCES

[1]. Morand, A., Cornu, F., Dufour, J.C., Tsimaratos, M., Lagier, J.C., Raoult, D., 2019. Human bacterial repertoire of the urinary tract: a potential paradigm shift. J. Clin. Microbiol. 57(3). doi: 10.1128/JCM.00675-18.

[2]. Williams, G., Stothart, C.I., Hahn, D., Stephens, J.H., Craig, J.C., Hodson, E.M., 2023. Cranberries for preventing urinary tract infections. Cochrane Database Syst. Rev. 2023(11):CD001321. doi: 10.1002/14651858.CD001321.pub7.

[3]. Sihra, N., Goodman, A., Zakri, R., Sahai, A., Malde, S., 2018. Nonantibiotic prevention and management of recurrent urinary tract infection. Nat. Rev. Urol. 15(9):1-8. doi: 10.1038/s41585-018-0106-x.

[4]. Qiao, L.D., Chen, S., Yang, Y., 2013. Characteristics of urinary tract infection pathogens and their in vitro susceptibility to antimicrobial agents in China: data from a multicenter study. BMJ Open 3(12):e004152. doi: 10.1136/bmjopen-2013-004152.

[5]. Schappert, S.M., Rechtsteiner, E.A., 2011. Ambulatory medical care utilization estimates for 2007. Vital Health Stat. 13:1-38.

[6]. Simmering, J.E., Tang, F., Cavanaugh, J.E., Polgreen, L.A., Polgreen, P.M., 2017. The increase in hospitalizations for urinary tract infections and the associated costs in the United States, 1998–2011. Open Forum Infect. Dis. 4:ofw281.

[7]. Foxman, B., 2010. The epidemiology of urinary tract infection. Nat. Rev. Urol. 7:653–60.

[8]. Wagenlehner, F., Tandogdu, Z., Bartoletti, R., Cai, T., Cek, M., Kulchavenya, E., et al., 2016. The global prevalence of infections in urology study: a long-term, worldwide surveillance study on urological infections. Pathogens 5(1):10. doi: 10.3390/pathogens5010010.

[9]. Belete, M.A., Saravanan, M., 2020. A systematic review on drug-resistant urinary tract infection among pregnant women in developing countries in Africa and Asia; 2005-2016. Infect. Drug Resist. 13:1465. doi: 10.2147/IDR.S250654.

[10]. Zhang, L., Huang, W., Zhang, S., et al., 2022. Rapid detection of bacterial pathogens and antimicrobial resistance genes in clinical urine samples with urinary tract infection by metagenomic nanopore sequencing. Front. Microbiol. 13:858777. doi: 10.3389/fmicb.2022.858777.

[11]. Farrell, K., Tandan, M., Hernandez Santiago, V., et al., 2021. Treatment of uncomplicated UTI in males: a systematic review of the literature. BJGP Open 5:bjgpopen20X101140.

[12]. Tandogdu, Z., Wagenlehner, F.M., 2016. Global epidemiology of urinary tract infections. Curr. Opin. Infect. Dis. 29:73–9. doi: 10.1097/QCO.0000000000000228.

[13]. Williams, G., Craig, J.C., 2019. Long-term antibiotics for preventing recurrent urinary tract infection in children. Cochrane Database Syst. Rev. 2019(4):CD001534. doi: 10.1002/14651858.CD001534.pub4.

[14]. Moges, A., Genetu, A., Mengistu, G., 2002. Antibiotic sensitivities of common bacterial pathogens in urinary tract infections at Gondar Hospital, Ethiopia. East Afr. Med. J. 79(3):140-142. doi: 10.4314/eamj.v79i3.8893.

[15]. Manges, A., Natarajan, P., Solberg, O.D., Dietrich, P.S., Riley, L.W., 2006. The changing prevalence of drug-resistant Escherichia coli clonal groups in a community: evidence for community outbreaks of urinary tract infections. Epidemiol. Infect. 134(2):425-431. doi: 10.1017/S0950268805005005.

[16]. Cox, G., Wright, G.D., 2013. Intrinsic antibiotic resistance: mechanisms, origins, challenges, and solutions. Int. J. Med. Microbiol. 303(6-7):287–292. doi: 10.1016/j.ijmm.2013.02.009.

[17]. Bharadwaj, A., Rastogi, A., Pandey, S., Gupta, S., Sohal, J.S., 2022. Multidrug-resistant bacteria: their mechanism of action and prophylaxis. Biomed. Res. Int. 2022:5419874. doi: 10.1155/2022/5419874.

[18]. Nickel, J.C., Doiron, R.C., 2023. An effective sublingual vaccine, MV140, safely reduces risk of recurrent urinary tract infection in women. Pathogens 12(3):359. doi: 10.3390/pathogens12030359.

[19]. Sneha, S.K., Unnamalai, L., 2023. US FDA approves Iterum's treatment for urinary infection. Pharm. News 2023.

[20]. Barber, A.E., Norton, J.P., Spivak, A.M., Mulvey, M.A., 2013. Urinary tract infections: current and emerging management strategies. Clin. Infect. Dis. 57(5):719–724. doi: 10.1093/cid/cit284.

[21]. Jepson, R.G., Mihaljevic, L., Craig, J.C., 1998. Cranberries for treating urinary tract infections. Cochrane Database Syst. Rev. 1998(4):CD001322. doi: 10.1002/14651858.CD001322.

[22]. Bruyere, F., 2006. [Use of cranberry in chronic urinary tract infections]. Med. Mal. Infect. 36(7):358–363. doi: 10.1016/j.medmal.2006.05.001.

[23]. Cimolai, N., Cimolai, T., 2007. The cranberry and the urinary tract. Eur. J. Clin. Microbiol. Infect. Dis. 26(11):767–776. doi: 10.1007/s10096-007-0379-0.

[24]. Abbey, T.C., Deak, E., 2019. What's new from the CLSI Subcommittee on Antimicrobial Susceptibility Testing M100, 29th Edition. Clin. Microbiol. Newsl. 41(23):203-209. doi: 10.1016/j.clinmicnews.2019.11.002.

[25]. Bauer, A., Kirby, W.M., Sherris, J.C., Turck, M., 1966. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol. 45(4):493. doi: 10.1093/ajcp/45.4_ts.493.

[26]. Trease, G., Evans, W., 2002 Phytochemicals. In: Pharmacognosy, fifteenth ed. Saunders Publishers, London, 42-393.

[27]. Kimura, M., 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. J Mol Evol. 16:111-120.

[28]. Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K., 2018. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Mol Biol Evol. 35:1547-1549.

[29]. Abou Heidar, N.F., Degheili, J.A., Yacoubian, A.A., Khauli, R.B., 2019. Management of urinary tract infection in women: A practical approach for everyday practice. Urol. Ann. 11(4):339–346. doi: 10.4103/UA.UA_104_19.

[30]. Al Lawati, H., Blair, B.M., Larnard, J., 2024. Urinary tract infections: Core curriculum 2024. Am. J. Kidney Dis. doi: 10.1053/j.ajkd.2023.08.009.

[31]. Parida, S., Mishra, S.K., 2013. Urinary tract infections in the critical care unit: a brief review. Indian J. Crit. Care Med. 17(6):370–374.

[32]. Choubey, S., Verma, M., 2022. Common cause of UTI and its regimen: a review paper. J. Pharm. Innov. Int. (submission and acceptance dates: 05-05-2022, 08-06-2022).

[33]. Johansen, T.E.B., Cek, M., Naber, K.G., et al., 2006. Hospital-acquired urinary tract infections in urology departments: pathogens, susceptibility, and use of antibiotics: data from the PEP and PEAP-studies. Int. J. Antimicrob. Agents 28(Suppl 1):91–107. doi: 10.1016/j.ijantimicag.2006.05.005.

[34]. Kaur, R., Walia, G., Mehta, M., 2012. Prevalence of urinary tract infections in children and their sensitivity to various antibiotics. J. Acad. Indus. Res. 1(4):161–163.

[35]. Odoki, M., Aliero, A.A., Tibyangye, J., et al., 2019. Prevalence of bacterial urinary tract infections and associated factors among patients attending hospitals in Bushenyi District, Uganda. Int. J. Microbiol. Article ID: 4246780. doi: 10.1155/2019/4246780.

[36]. Rohana, H., Hager-Cohen, A., Azrad, M., Peretz, A., 2023. Trend of changes in chloramphenicol resistance during the years 2017–2020: A retrospective report from Israel. Antibiotics 12(2):196. doi: 10.3390/antibiotics12020196.

[37]. Tevyashova, A.N., 2021. Recent trends in synthesis of chloramphenicol new derivatives. Antibiotics 10(4):370. doi: 10.3390/antibiotics10040370.

[38]. Kaushik, D., Mohan, M., Borade, D.M., Swami, O.C., 2014. Ampicillin: rise, fall and resurgence. J. Pharmacol. Pharmacother. PMCID: PMC4080027.

[39]. Sum, P.E., Sum, F.W., Projan, S.J., 2004. Recent developments in tetracycline antibiotics. Curr. Top. Med. Chem. doi: 10.2174/1381612804012210071132O2.

[40]. Al-Hilu, S.A., Al-Shujairi, W.H., 2021. Effectiveness of medicinal plant extract against pathogenic bacteria in the prevention and treatment of urinary tract infections. J. Ethnopharmacol. (Institutional affiliations provided, journal name inferred).

[41]. Soubeiga, A.P., Kpoda, D.S., Traoré, O., Zongo, C., Savadogo, A., 2020. Prevalence of β-lactamase genes and integrons among multidrug-resistant Escherichia coli and Salmonella spp recovered from food samples in Ouagadougou, Burkina Faso. J. Food Prot. (journal name inferred).
[42]. Beyene, G., Tsegaye, W., 2011. Bacterial uropathogens in urinary tract infection and antibiotic susceptibility pattern in Jimma University Specialized Hospital, Southwest Ethiopia. Ethiop. J. Health Sci. 21(2):141–146. doi: 10.4314/ejhs.v21i2.69055.
[43]. Gadisa, E., Tadesse, E., 2021. Antimicrobial activity of medicinal plants used for urinary tract infections in pastoralist community in Ethiopia. BMC Complement. Med. Ther. 21(1):74. doi: 10.1186/s12906-021-03249-7.
[44]. Ong, A., Pietropaolo, A., Brown, G., Somani, B.K., 2022. Are intravesical aminoglycosides the new gold standard in the management of refractory urinary tract infection: A systematic review of literature. Antibiotics PMCID: PMC9571321.
[45]. Mirzahosseini, H., K, Najmeddin, F, Najafi, A, Ahmadi, A, Sharifnia, H, Khaledi, A, Mojtahedzadeh, M. 2023. Correlation of biofilm formation, virulence factors, and phylogenetic groups among Escherichia coli strains causing urinary tract infection: A global systematic review and meta-analysis. J Res Med Sci. doi: 10.4103/jrms.jrms_637_22.
[46]. Roy, S, Jena, D, Swain, M, Gupta, B, Raghav, K, S, Mishra, N, S. 2025. Genetic landscape of biofilm forming uropathogenic E. coli from clinical samples. med Rxiv. doi: https://doi.org/10.1101/2025.04.14.25325849.
[47]. Tanwar, J., Das, S., Fatima, Z., Hameed, S., 2014. Multidrug resistance: an emerging crisis. Interdiscip. Perspect. Infect. Dis. 2014:541340. doi: 10.1155/2014/541340.
[48]. Elamary, R.B., Albarakaty, F.M., Salem, W.M., 2020. Efficacy of Acacia nilotica aqueous extract in treating biofilm-forming and multidrug-resistant uropathogens isolated from patients with UTI syndrome. Sci. Rep. 10(1):11125. doi: 10.1038/s41598-020-67732-w.
[49]. Gadisa, E., Weldearegay, G., Desta, K., et al., 2019. Combined antibacterial effect of essential oils from three most commonly used Ethiopian traditional medicinal plants on multidrug-resistant bacteria. BMC Complement. Altern. Med. 19(1):24. doi: 10.1186/s12906-019-2429-4.
[50]. World Health Organization, 2019. WHO global report on traditional and complementary medicine. World Health Organization Press.
[51]. Rossiter, S.E., Fletcher, M.H., Wuest, W.M., 2017. Natural products as platforms to overcome antibiotic resistance. Chem. Rev. 117(19):12415–12474. doi: 10.1021/acs.chemrev.7b00283.
[52]. González de Llano, D., Moreno-Arribas, M.V., Bartolomé, B., 2020. Cranberry polyphenols and prevention against urinary tract infections: relevant considerations. Molecules 25(15):3523. doi: 10.3390/molecules25153523.
[53]. Das, S., 2020. Natural therapeutics for urinary tract infections—a review. Futur. J. Pharm. Sci. 6(1):64. doi: 10.1186/s43094-020-00086-2.
[54]. Konesan, J., Wang, J., Moore, K.H., Mansfield, K.J., Liu, L., 2023. Urinary tract health and antibacterial benefits: in-vitro. Front. Microbiol. doi: 10.3389/fmicb.2023.1319785.
[55]. Al Kaabi, H.K.J., Hmood, B.A., 2025. Antimicrobial activity of cranberry juice (Vaccinium macrocarpon L.) ethanol extract against uropathogenic bacteria. Open Vet. J. 15(2):813–819. doi: 10.5455/OVJ.2025.v15.i2.30.
[56]. Acharjee, M., Zerin, N., Ishma, T., Mahmud, M.R., 2023. In-vitro anti-bacterial activity of medicinal plants against urinary tract infection (UTI) causing bacteria along with their synergistic effects with commercially available antibiotics. New Microbes New Infect. 51:101076. doi: 10.1016/j.nmni.2022.101076.