RESEARCH PAPER

Transcriptomic Analysis Reveals Glucose-Induced Modulation of Metabolic and Virulence Pathways in Uropathogenic Escherichia coli

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ABSTRACT

Introduction

Urinary tract infections (UTIs) are primarily caused by uropathogenic Escherichia coli (UPEC), with increased prevalence in diabetic patients due to hyperglycaemia-induced alterations in the urinary microenvironment. Elevated glucose levels may promote bacterial survival, modulate virulence, and influence antimicrobial resistance mechanisms. This study aimed to investigate the impact of high glucose exposure on the growth characteristics and global transcriptomic profile of a clinical UPEC isolate.

Methodology

A pathogenic Escherichia coli strain sourced from the urine of a diabetic patient was cultured under both normal and glucose-stressed conditions. Growth assays were conducted in LB agar and broth with glucose concentrations varying from 2–10% (w/v), and bacterial growth was evaluated through colony morphology and optical density measurements (OD600). Antimicrobial susceptibility was assessed via the Kirby–Bauer disk diffusion method according to CLSI guidelines. Differentially expressed genes (DEGs) were identified based on fold change ≥ 2 and FDR < 0.05. Functional annotation, Gene Ontology (GO) analysis, KEGG pathway enrichment, antimicrobial resistance (AMR) gene analysis, and virulence factor identification were performed using bioinformatics databases and tools, including UniProt, KAAS, CARD, VFDB, and ABRicate.

Results

High glucose exposure induced significant transcriptional alterations in UPEC. Genes involved in carbohydrate metabolism, phosphotransferase system (PTS), membrane transport, and energy metabolism were predominantly upregulated, indicating metabolic adaptation to glucose-rich conditions. Adhesion-associated virulence genes, including fimbrial operons, showed increased expression, whereas biofilm-associated genes (csgD, csgF, and csgG) were downregulated. Additionally, several multidrug efflux transporter genes of the mdt family exhibited increased expression under glucose stress.

Conclusion

Overall, high glucose exposure induces significant transcriptional reprogramming in UPEC, affecting metabolic, virulence, and resistance-associated pathways. These findings provide insight into bacterial adaptation in hyperglycaemic environments and underscore the need for further functional validation to determine clinical relevance.

Keywords: Escherichia coli, glucose stress, transcriptomics, virulence factors, antimicrobial resistance, RNA-seq.

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Introduction:

Urinary tract infections (UTIs) are among the most common bacterial infections worldwide, with *Escherichia coli* recognised as the predominant causative agent [1,2]. Diabetic patients exhibit

increased susceptibility to UTIs due to impaired immune responses and elevated glucose concentrations in urine, which may create a favourable environment for bacterial growth and persistence [3,4].

Hyperglycaemia has been associated with enhanced bacterial colonisation, altered biofilm formation, and modulation of virulence gene expression [5,6]. In Uropathogenic *E. coli* (UPEC), virulence is mediated by multiple factors, including type 1 fimbriae, P pili, outer membrane proteins, iron acquisition systems, and capsule production [7,8]. Environmental stressors, including nutrient availability and osmotic changes, are known to influence the expression of these determinants [9,10].

In addition to virulence modulation, glucose stress may affect antimicrobial resistance mechanisms [11,12]. Efflux pumps, membrane transport proteins, and metabolic adaptation pathways can be transcriptionally regulated in response to environmental conditions [13,14]. However, the global transcriptomic response of Uropathogenic *E.coli* to high glucose exposure remains incompletely understood [15,16].

RNA sequencing (RNA-seq) provides a comprehensive approach to evaluate genome-wide transcriptional changes under defined experimental conditions [17,18]. Therefore, the present study aimed to investigate the effect of high glucose exposure on the growth characteristics and transcriptomic profile of a pathogenic *E. coli* isolate obtained from a diabetic patient [19,20]. By comparing gene expression patterns under normal and high-glucose conditions, the study sought to identify key metabolic, virulence-associated, and antimicrobial resistance-related pathways influenced by glucose stress. RNA-seq analysis revealed that 856 genes were upregulated and 219 genes were downregulated under high-glucose conditions [21,22]. This is biologically impossible for *E.coli*. The *E.coli* genome contains approximately 4,500–5,500 genes [23].

Materials and Methods:

Bacterial Isolate and Culture Conditions

A pathogenic isolate of Escherichia coli was obtained from a urine sample of a diabetic patient. The isolate was provided by the Department of Microbiology, Vydehi Institute of Medical Sciences and Research Centre (VIMS & RC), Bengaluru, India. The strain was maintained on Luria–Bertani agar (LBA) plates (HiMedia Laboratories Pvt. Ltd., India) at 37 °C and subcultured periodically to ensure viability. For experimental assays, overnight cultures were prepared in Luria–Bertani (LB) broth at 37 °C with shaking at 200 rpm.

Effect of Glucose on Bacterial Growth

To evaluate the impact of glucose on bacterial growth, LB agar and LB broth were supplemented with varying concentrations of glucose (2%, 4%, 6%, 8%, and 10% w/v).

For solid media assays, 1 µL of overnight culture was spot-inoculated onto glucose-supplemented LBA plates and incubated at 37 °C for 48 hours. Radial colony growth was visually assessed and compared across glucose concentrations.

For liquid growth analysis, 5 µL of overnight culture was inoculated into 2 mL of LB broth containing the respective glucose concentrations and incubated at 37 °C with shaking (200 rpm) for 16 hours. Bacterial growth was measured by optical density at 600 nm (OD600) using a UV–Vis spectrophotometer (Lab India Pvt. Ltd., Mumbai, India). Growth measurements were performed in biological replicates.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility was determined using the Kirby–Bauer disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines. Briefly, bacterial lawns were prepared on Mueller–Hinton agar plates using standardized inoculum. Antibiotic-impregnated disks (BioMérieux and HiMedia Laboratories Pvt. Ltd., India) were placed on the agar surface and plates were incubated at 37 °C for 18–24 hours.

The antibiotics tested included ampicillin (10 µg), ciprofloxacin (2 µg), gentamicin (10 µg), nalidixic acid (30 µg), streptomycin (10 µg), tetracycline (30 µg), amoxicillin (10 µg), piperacillin (10 µg), cefixime (5 µg), imipenem (10 µg), meropenem (10 µg), and others as listed. Zones of inhibition were measured in millimetres and interpreted as sensitive, intermediate, or resistant based on CLSI breakpoints.

RNA Extraction and Quality Assessment

For transcriptomic analysis, E. coli cultures were grown under two conditions:

- Control (LB without additional glucose)
- High glucose stress (LB supplemented with 10% w/v glucose)

After incubation, bacterial cells were harvested by centrifugation. Total RNA was extracted using the HiPurA Bacterial RNA Isolation Kit (HiMedia Laboratories Pvt. Ltd., Mumbai, India) according to the manufacturer's instructions.

RNA concentration was quantified using a Qubit fluorometer (Thermo Fisher Scientific, USA). RNA integrity and quality were assessed using an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). Only samples with high RNA integrity were used for library preparation.

Library Preparation and RNA Sequencing

Complementary DNA (cDNA) libraries were constructed using a standard RNA-seq library preparation kit following the manufacturer's protocol. Paired-end sequencing (2 × 150 bp) was performed on an Illumina HiSeq 2000 platform (Illumina Inc., San Diego, CA, USA) at a certified

sequencing facility (Biokart India Pvt. Ltd., Bengaluru, India).

Bioinformatics Analysis

Quality Control and Preprocessing

Raw sequencing reads were subjected to quality assessment using FastQC (v0.11.2). MultiQC was used to summarise quality reports. Adapter trimming and removal of low-quality reads were performed using Trim Galore (v0.6.7).

Transcriptome Assembly and Quantification

Clean reads were assembled using Trinity (v2.6.6) for transcript reconstruction. Redundant sequences were clustered using CD-HIT (v4.8.1) to obtain non-redundant transcripts.

Gene and isoform expression levels were estimated using RSEM (RNA-Seq by Expectation Maximization). Expression values were normalized to account for sequencing depth and transcript length.

Differential Gene Expression Analysis

Differentially expressed genes (DEGs) between control and glucose-treated samples were identified using appropriate statistical models. Genes with a fold change ≥ 2.0 and a statistically significant adjusted p-value (False Discovery Rate, FDR < 0.05) were considered significantly differentially expressed.

Principal Component Analysis (PCA) and volcano plots were generated to visualize sample clustering and differential expression patterns.

Functional Annotation and Pathway Analysis

Functional annotation of transcripts was performed using BLASTx against the UniProt *Escherichia coli* database. Gene Ontology (GO) classification categorized genes into Biological Process, Molecular Function, and Cellular Component domains.

KEGG pathway enrichment analysis was conducted using the KEGG Automatic Annotation Server (KAAS) to identify significantly enriched metabolic and signaling pathways.

Identification of Antimicrobial Resistance and Virulence Genes

Antimicrobial resistance (AMR) genes were identified using the Comprehensive Antibiotic Resistance Database (CARD) through ABRicate software. Virulence-associated genes were identified using the Virulence Factor Database (VFDB).

Differential expression patterns of AMR- and virulence-related genes were extracted from DEG datasets for comparative analysis between control and glucose-treated conditions.

Results:

Effect of Glucose on Bacterial Growth

The growth pattern of *Escherichia coli* was evaluated under increasing glucose concentrations (2–10% w/v). On LB agar plates, no significant difference in colony morphology or radial expansion was observed between 0.5% and 3% glucose. However, at concentrations ≥4%, a gradual reduction in colony diameter was noted, indicating that elevated glucose levels impose metabolic or osmotic stress on bacterial growth.

In liquid culture, OD600 measurements showed that *E. coli* maintained growth in high-glucose conditions, although a concentration-dependent reduction in optical density was observed at higher glucose levels. These findings suggest that while the isolate tolerates hyperglycemic conditions, elevated glucose influences growth kinetics.

Antimicrobial Susceptibility Profile

The isolate exhibited resistance to multiple antibiotics, including ampicillin, cefuroxime, ciprofloxacin, cefepime, ceftriaxone, nitrofurantoin, and colistin. Sensitivity was observed toward selected agents such as meropenem, ertapenem, amikacin, fosfomycin, and piperacillin–tazobactam. This resistance profile indicates a multidrug-resistant (MDR) phenotype, consistent with clinical isolates obtained from complicated UTIs. However, phenotypic resistance was not directly correlated with glucose exposure in this experiment and requires further validation.

RNA Sequencing and Transcriptome Overview

High-throughput RNA sequencing generated high-quality paired-end reads for both control and glucose-treated samples. Quality assessment confirmed acceptable read quality and minimal adapter contamination. Transcriptome assembly and expression quantification enabled the identification of differentially expressed genes (DEGs) between control and high-glucose conditions.

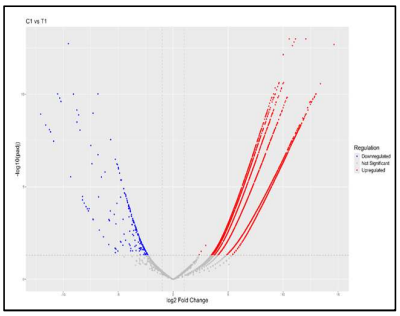

Figure 1: Principal Component Analysis (PCA) plot

Principal Component Analysis (PCA) demonstrated clear separation between control and glucose-treated samples, indicating distinct transcriptional responses under glucose stress. The first two principal components explained the majority of variance between experimental groups, confirming reproducibility and condition-specific gene expression patterns.

Differential Gene Expression Analysis

Comparative transcriptomic analysis revealed significant differential expression of genes under high-glucose conditions. A greater proportion of genes were upregulated compared to downregulated, suggesting that glucose exposure triggers activation of adaptive and metabolic pathways.

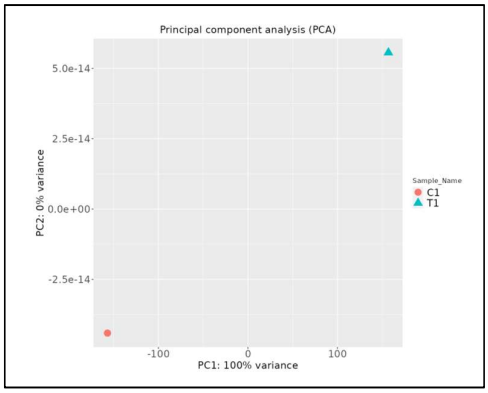

Figure 2: Volcano plot

Volcano plot visualisation illustrated genes with significant fold change and adjusted p-values (FDR < 0.05). Upregulated genes were enriched in pathways related to carbohydrate metabolism, membrane transport, and stress response, whereas several genes associated with biofilm regulation and outer membrane composition were downregulated.

Functional Enrichment Analysis

Gene Ontology (GO) Analysis

GO enrichment analysis categorised DEGs into three major domains:

- **Biological Process:** Enrichment in carbohydrate metabolic processes, phosphorylation, proteolysis, and regulation of transcription.
- **Molecular Function:** Significant representation of ATP binding, metal ion binding, DNA binding, and transporter activity.
- **Cellular Component:** Predominant localisation in cytoplasm, plasma membrane, and outer membrane compartments.

These results indicate that glucose exposure primarily affects metabolic regulation and membrane-associated functions.

KEGG Pathway Enrichment

KEGG pathway analysis revealed enrichment in:

- Glycolysis and carbohydrate metabolism
- Phosphotransferase system (PTS)
- Amino acid metabolism
- Membrane transport pathways
- Energy metabolism

The enrichment of PTS-related genes suggests enhanced glucose uptake and metabolic reprogramming under high-glucose stress.

Differential Regulation of Virulence-Associated Genes

Several fimbrial and pilus-associated genes, including *fimF*, *fimG*, *fimI*, and *yagZ/ecpA*, *yagY/ecpB*, *yagX/ecpC*, *yagV/ecpE*, were upregulated under glucose stress. These genes are associated with bacterial adhesion and colonization. Conversely, selected biofilm-associated regulatory genes such as *csgD*, *csgF*, and *csgG*, along with outer membrane protein gene *ompA*, were downregulated. This indicates that glucose may differentially regulate adhesion and biofilm-related pathways rather than uniformly enhancing virulence traits.

Iron acquisition-related genes also exhibited altered expression patterns, suggesting metabolic adaptation to environmental changes.

Differential Expression of Antimicrobial Resistance-Related Genes

Transcriptomic analysis revealed altered expression of genes associated with efflux pumps and antimicrobial resistance. Multidrug transporter genes such as *mdtN*, *mdtO*, *mdtP*, and *mdtB* were upregulated under glucose stress. Some β-lactamase-associated and efflux-related genes displayed downregulation.

These findings suggest that glucose exposure modulates transcription of resistance-associated genes; however, transcript abundance alone does not confirm functional resistance. Phenotypic validation is required to establish clinical relevance.

Discussion:

The present study investigated the impact of high glucose exposure on the growth and transcriptomic profile of uropathogenic *Escherichia coli* isolated from a diabetic patient.

Elevated urinary glucose levels are a hallmark of uncontrolled diabetes and may create a nutrient-rich microenvironment favourable for bacterial persistence [24,25]. Our growth assays demonstrated that although *E. coli* tolerates high glucose concentrations, excessive glucose exerts measurable effects on growth kinetics, potentially due to osmotic stress or metabolic imbalance [26,27].

Transcriptomic analysis revealed significant modulation of metabolic pathways under glucose stress [28,29]. Enrichment of carbohydrate metabolism and phosphotransferase system (PTS) genes indicates metabolic adaptation to abundant glucose availability [30,31]. Similar metabolic reprogramming has been observed in other bacterial species exposed to high carbon availability [32,33].

Interestingly, virulence-associated genes displayed differential regulation rather than uniform upregulation [34,35]. Fimbrial genes involved in adhesion were upregulated, suggesting potential enhancement of colonization capacity under glucose-rich conditions [36,37]. However,

downregulation of key biofilm regulatory genes suggests that glucose may not universally enhance biofilm formation [38,39]. This highlights the complexity of glucose-mediated regulation in bacterial pathogenesis.

Altered expression of efflux pump and resistance-associated genes indicates that glucose stress may influence antimicrobial response pathways at the transcriptional level [40,41]. Nevertheless, gene expression changes do not necessarily translate into phenotypic resistance [42,43]. Environmental stress can transiently activate adaptive mechanisms without leading to stable multidrug resistance [44,45].

Overall, the findings suggest that high glucose exposure induces substantial transcriptional remodelling in uropathogenic *E. coli*, affecting metabolic, membrane-associated, adhesion-related, and antimicrobial response pathways. These transcriptional adaptations may contribute to bacterial survival in hyperglycemic urinary environments. However, further studies involving proteomic analysis, functional assays, and in vivo validation are required to establish the clinical significance of these observations.

Conclusion:

The present study demonstrates that high glucose exposure significantly alters the transcriptional profile of uropathogenic *Escherichia coli* under in vitro conditions. Differential regulation was observed in genes associated with carbohydrate metabolism, membrane transport, fimbrial assembly, iron acquisition, and antimicrobial resistance mechanisms. These findings suggest that elevated glucose environments, such as those encountered in diabetic urine, may influence bacterial adaptation strategies and virulence-associated pathways. Further functional validation studies are required to determine the phenotypic and clinical implications of these transcriptional changes.

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