International Immunopharmacology 126 (2024) 111213

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

Comparative transcriptome analysis of *E. coli & Staphylococcus aureus* infected goat mammary epithelial cells reveals genes associated with infection

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ARTICLE INFO

Keywords:
RNA-Seq
Goat
E. coli
S. aureus
Transcriptomics
PPI
Mammary epithelial cells

ABSTRACT

Mastitis, an inflammatory disease of the mammary gland, imposes a significant financial burden on the dairy sector. However, the specific molecular mechanisms underlying their interactions with goat mammary epithelial cells (GMECs) remain poorly understood. This study aimed to investigate the transcriptomic response of GMECs during infection with *E. coli* and *S. aureus*, providing insights into the host-pathogen interactions. Differential expression of gene (DEGs) analysis was done to find genes and pathways dysregulated in the wake of infection. *E. coli* infection triggered a robust upregulation of immune response genes, including pro-inflammatory chemokines and cytokines as well as genes involved in tissue repair and remodeling. Conversely, *S. aureus* infection showed a more complex pattern, involving the activation of immune-related gene as well as those involved in autophagy, apoptosis and tissue remodeling. Furthermore, several key pathways, such as Toll-like receptor signaling and cytokine-cytokine receptor interaction, were differentially modulated in response to each pathogen. Understanding the specific responses of GMECs to these pathogens will provide a foundation for understanding the complex dynamics of infection and host response, offering potential avenues for the development of novel strategies to prevent and treat bacterial infections in both animals and humans.

1. Introduction

One of the most prevalent harmful conditions affecting dairy ruminants globally is mastitis. It causes inflammation of the mammary gland, low milk quantity and quality and thus, generates economic losses. A variety of bacteria, including Gram-negative *Escherichia coli (E. coli*) and Gram-positive *Staphylococcus aureus (S. aureus*), can cause mastitis [1]. Gram-negative bacteria including *E. coli* causes acute clinical mastitis which is associated with severe inflammation. The pathogens will be eventually cleared by the immune system within days or with antibiotic treatment [2]. *S. aureus* causes chronic mastitis and the pathogen clearance by antibiotics is often ineffective [1].

Bacterial infections commonly cause infection in the teat canal inducing the production of chemokines like IL-8 and pro-inflammatory cytokines including interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α) [3]. Activated cytokines will induce an acute-phase inflammation response which will recruit leukocytes to the infection spot [4]. Proteomic analysis of mammary epithelial cells isolated from bovine (bMECs) when stimulated with *E. coli* and *S. aureus* revealed differential expression of immune response proteins, chemokines and cytokines [5].

Different pathways in host immune responses to pathogens are revealed in mammary gland tissues that have been obtained after intramammary infection and/or after bovine mammary epithelial cells have been stimulated with mastitis pathogens [6–7]. Mastitis control strategies will involve understanding the host response mechanisms as well as developing appropriate control measures. Goat is considered best model species to study the mammary gland infections (**Mihevc and Peter 2013**). Current mastitis prevention in goats involves use of antimicrobials or culling to eliminate the pathogen infection. However, this raises the risk of developing antibiotic resistance which poses risk to human as well as animal health. Despite the tremendous progress in

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https://doi.org/10.1016/j.intimp.2023.111213
Received 4 September 2023; Received in revised form 21 October 2023; Accepted 9 November 2023

understanding bovine mastitis pathogenesis, it still remains a global burden in the dairy industry worldwide.

Advancements in RNA-sequencing (RNA-seq) and bioinformatics offers multiple opportunities to annotate and characterize mRNAs enabling us to comprehensively identify and quantify differentially expressed genes (DEGs) playing a role in mammary gland infections. Our previous study reported comparative transcriptome analysis to reveal striking variations between jersey and Kashmiri cattle in gene expression pathways which regulate synthesis of milk in MECs at various lactational phases [8]. Milk-derived gMECs culture has been used as a model of infection and stimulated with whole heat-killed *E. coli* [9]. In this study, mammary epithelial cells isolated from goat (gMECs) were stimulated with bacteria. The research of pathogen-host interactions relies significantly on RNA-seq, which also offers a potent method for delving deeply into the immunological response of eukaryotic cells and the response of gene expression. Using high-throughput transcriptome sequencing, potential candidate mRNAs, lncRNAs, and miRNAs implicated in particular biological processes have been investigated [10–11]. Several investigations on the mammary transcriptional response to *S. aureus* infection have been conducted [11–12]. The goal of the study was to investigate how *E. coli* and *S. aureus* infection affected the transcriptional regulation of gMECs. Cells were subjected to RNA sequencing to identify and characterize differentially expressed genes. We report the MEC transcriptome of goats and present valuable regarding the host-pathogen interactions which will offer prospective therapy targets for mastitis.

2. Materials and methods

2.1. Cell culture and pathogen stimulation

Goat mammary epithelial cell cultures were established as per our protocol described previously Mumtaz et al., 2022. Centrifugation was used to defat 30 ml of milk samples for 15 min at 800 g. The cell pellet was resuspended in Dulbecco's phosphate-buffered saline (DPBS) (Thermo Fisher Scientific) after the fatty layer and supernatant were removed. The cell pellet was then resuspended in the culture media after repeating the washing step twice. Cells were grown in a humidified incubator with 5% CO_2 at 37 °C. When cells are 80% confluent, they are seeded into a 6-well plate and grown in complete media for several days at 37 °C and 5% CO_2. As reported in previous study by Taban et al 2023, The gMECs cells were fully characterized for growth, proliferation, morphological characteristics, surface marker validation cytokeratin 18 (CKT 18) and karyotype analysis. The cells were also checked for their differentiation potential as well as transfection efficiency.

E. coli (ATCC 25922) and *Staphylococcus aureus* subsp. *aureus Rosenbach* (ATCC 31890) were used as the infection agents for pathogen stimulation against mammary epithelial cells. Luria Bertani (LB) agar and tryptic soy agar (TSA) was used to culture *E. coli* and *S. aureus* respectively aerobically in an incubator overnight for 37 °C. A single colony of *E. coli* and *S. aureus* was transferred to LB broth & tryptic soy broth (TSB) (20 ml) and incubated at 220 rpm in an open-air shaker at 37 °C.

Each bacterium was grown at 37 °C in their respective medium until OD600 reached 0.6. To accurately determine cell counts based on OD readings, serial dilutions were performed and the diluted samples were plated on plate count agar (PCA). Heat inactivation of bacteria was done at 63 °C for 30 min. Control plating was also done to verify the heat inactivation of bacteria. Bacteria were centrifuged at 3000 rpm for 15 min and allowed to resuspend at a density of 1×10^8 /mL.

In a 6-well plate, isolated gMECs (three biological replicates) with complete medium were seeded. 48 h after seeding, at 80% confluency, cells were stimulated with $1x10^7$ /mL heat-inactivated *E. coli* and *S. aureus*, respectively for 24 h. Following the incubation period, the cells were washed thrice with DPBS to eliminate any remaining bacteria. Subsequently, the cells were processed for total RNA isolation.

For verifying the adherence of bacteria on infected gMECs, a bacterial adhesion assay was carried. 1.5 × 105 gMECs were seeded in 12 well BD Falcon culture plates. The growth medium was replaced by an infection medium (a medium containing bacteria) at 80% confuency. At 37 °C and 5% CO2, monolayers/wells were infected with HK bacteria (106 cfu/ml) at a MOI of 1:100. Additionally, included were uninfected control wells with bacterial-free infection medium. After 30 min of staining the wells with 20% Giemsa dye (Sigma Aldrich), they were examined at 100× using an inverted microscope (IX73, Olympus, Japan).

2.2. RNA extraction, library preparation and sequencing

Using the Trizol method (Ambion-USA), RNA was extracted from gMECs in accordance with the manufacturer's recommendations. The quantity and quality of the extracted RNA were evaluated using a bioanalyzer, and the RNA was quantified using a spectrophotometer (ThermoFisher, USA). In accordance with with the manufacturer's recommendations, cDNA libraries were generated using the Illumina-TruSeq Stranded mRNA Sample Prep kit. Using the TruSeq RNA Sample Prep kits from Illumina, the RNA seq library was created using approximately 4 μg of total RNA. mRNA molecules carrying poly-A were isolated using magnetic beads with poly-T oligo attached. Following purification, mRNA was fragmented into small pieces using divalent cations at high temperatures. First-strand cDNA was synthesized from cleaved RNA fragments using reverse transcriptase and random primers, followed by second-strand cDNA synthesis using DNA polymerase I and RNAase H. The 3′ ends of DNA fragments are adenylated followed by hybridization with an Illumina PE adaptor and index. Using the Illumina PCR Primer Cocktail, cDNA fragments (200 bp) were generated and carefully selected to construct the final sequencing library. For library sequencing, the IlluminaHiSeq 2500 platform was employed.

2.3. Quality control, mapping and quantification

Raw reads from samples were cleaned. Adapter sequences were removed. Low-quality reads and undermined bases containing reads were removed using Trimmomatic software v0.32 (22). The clean reads undertook quality control analysis using FastQC program version 0.11.9 [13].

2.4. Differential expression analysis and pathway enrichment

The initial step in the data analysis pipeline involved the pre-processing of raw reads, encompassing the removal of adapter sequences, as well as the filtration of reads with low-quality bases, predominantly targeting the 3′-end of sequences.

Subsequent to the pre-processing phase, the refined reads were mapped to the *Capra hircus* reference assembly ARS1 using HISAT program, version 2.21 [14]. To identify differentially expressed genes (DEGs) between different contrast groups, the edgeR software, version 3.17 [15], was used, data normalization was performed using Trimmed Means of M−values (TMM) method [16]. DEGs across various contrast groups were identified by applying a filter of a false discovery rate (FDR) adjusted P-value < 0.05 and an absolute log2 (fold change) > 1. Subsequently, genes exhibiting significant dysregulation underwent functional annotation and pathway enrichment analysis utilizing KOBAS server v3.0 [17–18].

2.5. Reverse transcription-quantitative qPCR validation of DEGs

RNA-Seq results were validated by randomly selecting genes from top hits for reverse transcription-quantitative PCR (RT-qPCR) validation. The top hit is designated using two specific criteria larger log2(Fold Change) and smaller P-Value. Upregulated genes included (IL-21R, MST1R) for *E. coli* and for *S. aureus* (IRF5, MyD88). Downregulated

genes included (CISH, HAS3) for *E. coli* and (VMO1, SRXN1) for *S. aureus*. GAPDH and β-actin were used as internal controls to normalize the DEGs expression level. Primer information of the genes was provided in the Supplementary Table 1. For RT-PCR the cDNA was generated from 0.5 μg of the same RNA used in RNA-sequencing using the Thermo Scientific Revert Aid First Strand cDNA Synthesis Kit. Reactions were conducted in a 20 μl volume, comprising 0.5 μl of cDNA template, 10 μl of 2 × SYBR Green Master Mix, 0.3 μl of each 10 μmol/μl primer, and 8.9 μl nuclease-free water. The thermal cycling program consisted of an initial denaturation at 95 °C for 10 s, followed by 40 cycles of denaturation at 95 °C for 15 s and annealing/extension at 55 °C for 34 s. Each gene's qPCR analysis included three biological replicates. Relative gene expression was normalized to goat GAPDH using the $2^{-\Delta\Delta CT}$ method, and a Pearson correlation coefficient was used to compare gene expression levels obtained from both RNA-seq and qPCR analyses.

2.6. Network analysis

In order to identify the potential protein–protein interactions (PPI) among the DEGs, we utilized STRINGDB [27]. Two key topological metrics, Maximal Clique Centrality (MCC) and Degree, were calculated to pinpoint pivotal nodes. A gene's significance within the PPI network increases with higher values of these two quantitative measures and the network was generated using Cytoscape software, version 2.1 [28].

2.7. Drug-gene interaction networks

The drug-gene interaction network, which is also known as the drug-target interaction network or drug-gene association network, serves as a comprehensive framework for illustrating the intricate connections that exist between drugs and the specific genes that play pivotal roles in mediating their pharmacological effects. This network is a powerful tool for elucidating the complex web of interactions that underpin the effectiveness and mechanisms of action of pharmaceutical compounds.

Drug-gene interaction network was constructed using DrugBank database, version 5.0 [29]. The DrugBank database is a valuable resource that houses a wealth of information about the properties and interactions of drugs, including their targets and molecular mechanisms. Leveraging this database, the network was constructed to map out the relationships between various drugs and the genes they interact with target.

Furthermore, the development of the disease gene interaction network was also a crucial step in comprehending the genetic underpinnings of various diseases and their potential connections to drug treatments. This network was meticulously crafted by harnessing data from the DisGeNET database V7.0 [30]. The DisGeNET database is a reputable source for information on disease-gene associations, encompassing a wide range of genetic factors implicated in various diseases. By utilizing this database, researchers were able to construct a network that outlines the intricate connections between disease-related genes, shedding light on potential therapeutic targets and avenues for drug development.

3. Results

3.1. Transcriptome sequencing, aligning and mapping reads to the reference genome

Sequencing of gMECs transcriptome libraries generated a total of 779.9 million reads from all samples. Out of these 779.9 million reads of the gMECs transcriptome, 728.1 million reads passed QC and were aligned to the *Capra hircus* genome ARS1 downloaded from NCBI. All samples were mapped to 3-D Euclidean space for principal component analysis (PCA) (Supplementary Fig. 1). In the figure compact clustering of samples within the same group on the PCA plot underscores the consistency and similarity among samples sharing a common characteristic or trait. This reduced intra-group variability reinforces the notion that the differences observed in gene expression between groups are indeed meaningful and not simply the result of chance or random fluctuations. As a result, the downstream analysis can be considered reliable indicators of the genuine difference between the contrast groups.

3.2. Differentially expressed mRNAs in pathogen stimulated gMECs

In the group challenged with *E. coli*, we observed differential expression (DE) in a total of 942 genes, with 545 genes being upregulated and 397 genes downregulated. In contrast, the *S. aureus* treated group exhibited DE in 322 genes, consisting of 100 upregulated genes and 222 downregulated genes (Supplementary File 1 and 2). 2-D hierarchical clustering and heatmap of top DEGs were provided in Fig. 1. In Fig. 1, we have highlighted the most DEGs, specifically those ranked highest in terms of their p-value significance. This two-dimensional hierarchical clustering plot enables us to observe how genes cluster together based on their expression patterns, revealing potential relationships and similarities among them. In Fig. 2, we the DEGs were presented in the form of a volcano plot. Here, we accentuate the top DEGs in a manner that underscores both their statistical significance (p-value) and the magnitude of their fold changes (log2(FC)).

3.3. Reverse transcription-quantitative qPCR validation of DEGs

The DE status of the eight selected genes was confirmed through RT-PCR. The expression profiles of these genes, as determined by RT-PCR, exhibited a consistent pattern (Pearson's correlation coefficient = 0.93) when compared to the RNA-seq results. This high degree of correlation strongly affirms the accuracy and reliability of the RNA-seq findings, as illustrated in Fig. 3.

3.4. Protein-protein interaction network

It was discovered that several hub nodes play a crucial role in bacterial infections and other disorders. In the context of *E. coli* infection, noteworthy hub nodes identified encompassed *NOD1, TLR3, MX1, HERC5, IFIT2, IFIH1, EIF2AK2, WNT9A*, and *WNT2*, as depicted in Fig. 4a. This assembly of hub nodes reveals a network of interconnected genes and proteins that collectively wield substantial influence in response to E. coli infection.

In the case of *S. aureus* infection, a distinct set of hub nodes emerged, signifying their significance in the context of this specific bacterial challenge. These hub nodes encompassed *UBA52, UCHL1, USP28, TP53, MYC, KAT7, AKT1, MYD88*, and *TRAF6*, as depicted in Fig. 4b. Their presence within this network highlights their role in mediating cellular responses and immune reactions when faced with *S. aureus* infection. These findings provide valuable insights into the underlying mechanisms of bacterial infections and associated disorders, shedding light on potential targets for further investigation and therapeutic intervention.

3.5. Gene drug molecule interactions

Gene-drug interaction network involves integrating various types of biological and pharmacological data to identify and quantify the interactions between drugs and genes. Genes and drugs are shown as nodes, while their interactions are shown as edges. These networks can be further visualized and analyzed using network analysis tools to identify and quantify the interactions between drugs and genes.

Under *E. coli* infection, multiple genes were identified which can be targeted by multiple drugs. These genes may represent potential drug targets for the development of new therapies. *SIGMAR1, GABRB2, GRIK2, FAAH*, and *GRIA1* (Fig. 5) were identified with multiple drug targets. *GABRB2* and *GRIA1* had multiple drug targets such as ethanol, methoxyflurane, desflurane, enflurane, sevoflurane and isoflurane.

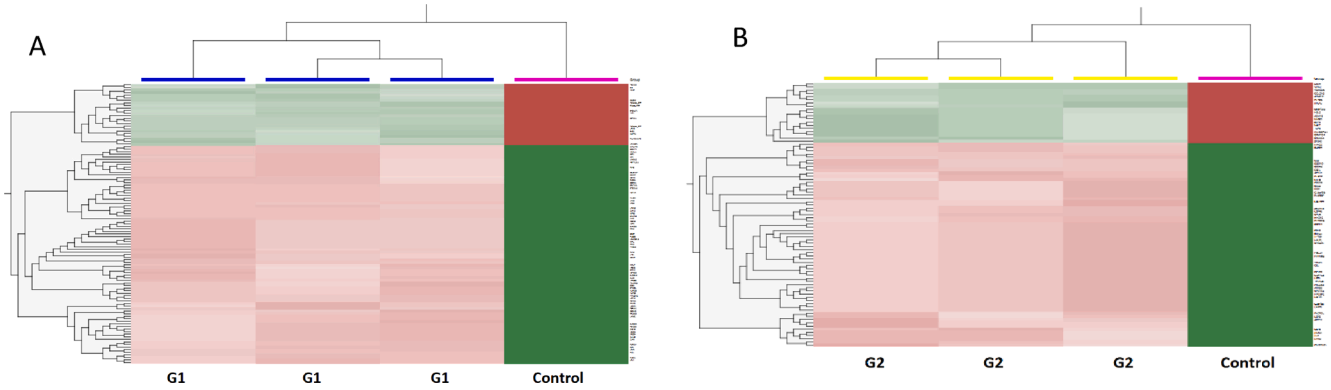

Fig. 1. 2D-hierarchical clustering was performed, and a heatmap was generated to visualize the top DEGs between the two compared groups. In the heatmap, columns represent individual samples, rows correspond to each DEG, and the color scale illustrates the relative expression levels of these DEGs. A) Top Differentially expressed genes in gMECs under E. coli (G1) infection, B) Top Differentially expressed genes in gMECs under S. aureus (G2) infection.

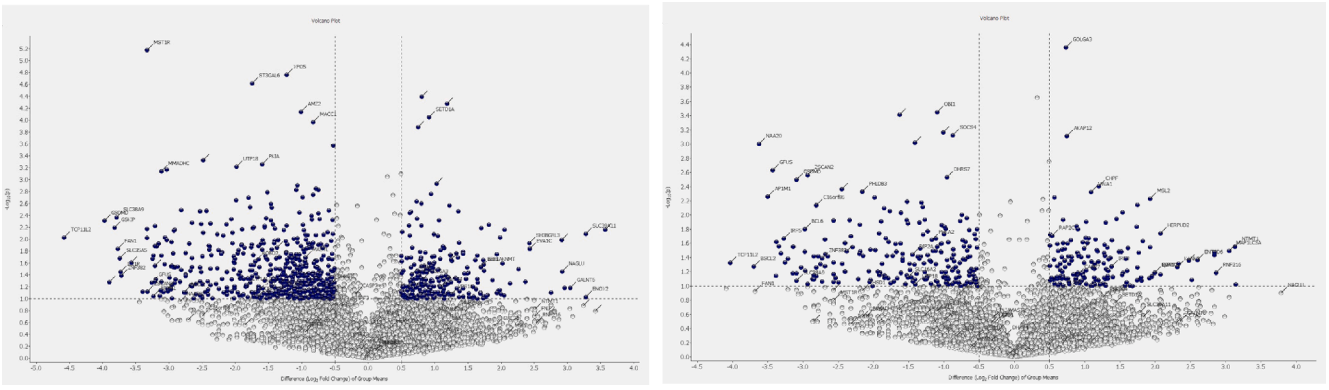

Fig. 2. Volcano plots between different contrast groups represent genes that are both highly significant and have meaningful changes in expression. a) Volcano plot in E. coli infection and b Volcano plot in S. aureus infection. Here x-axis represents the log2-fold change in gene expression between the two conditions and y- axis represents the statistical significance of the change in gene expression.

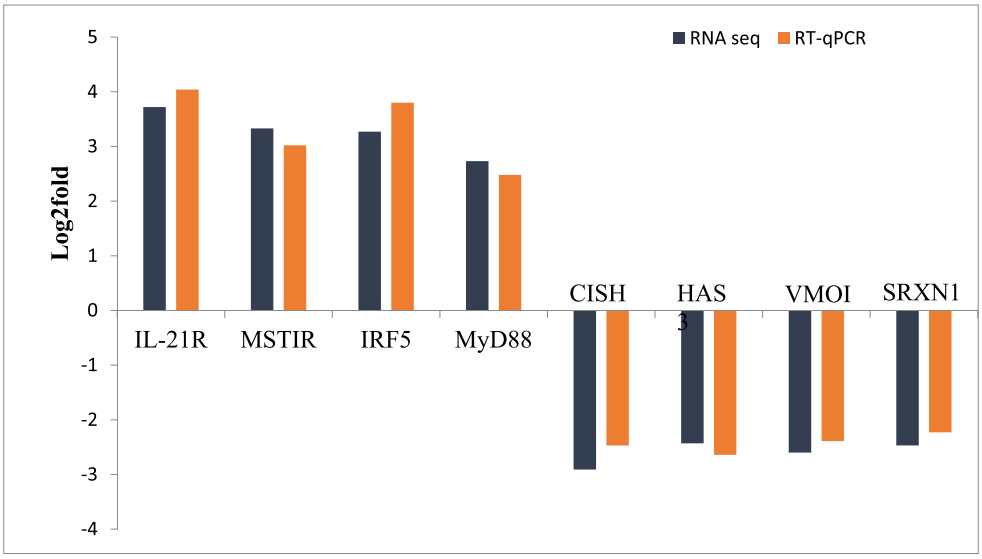

Fig. 3. Validation of RNA-seq results using RT-qPCR. To confirm the accuracy of the RNA-Seq results, eight representative genes were selected for validation using RT-qPCR. The mean -CT values derived from three biological replicates of RT-qPCR are depicted by the black bars, while the RNA-Seq data is represented by the red bars. This validation process ensures the reliability and consistency of the gene expression profiles obtained from both techniques. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

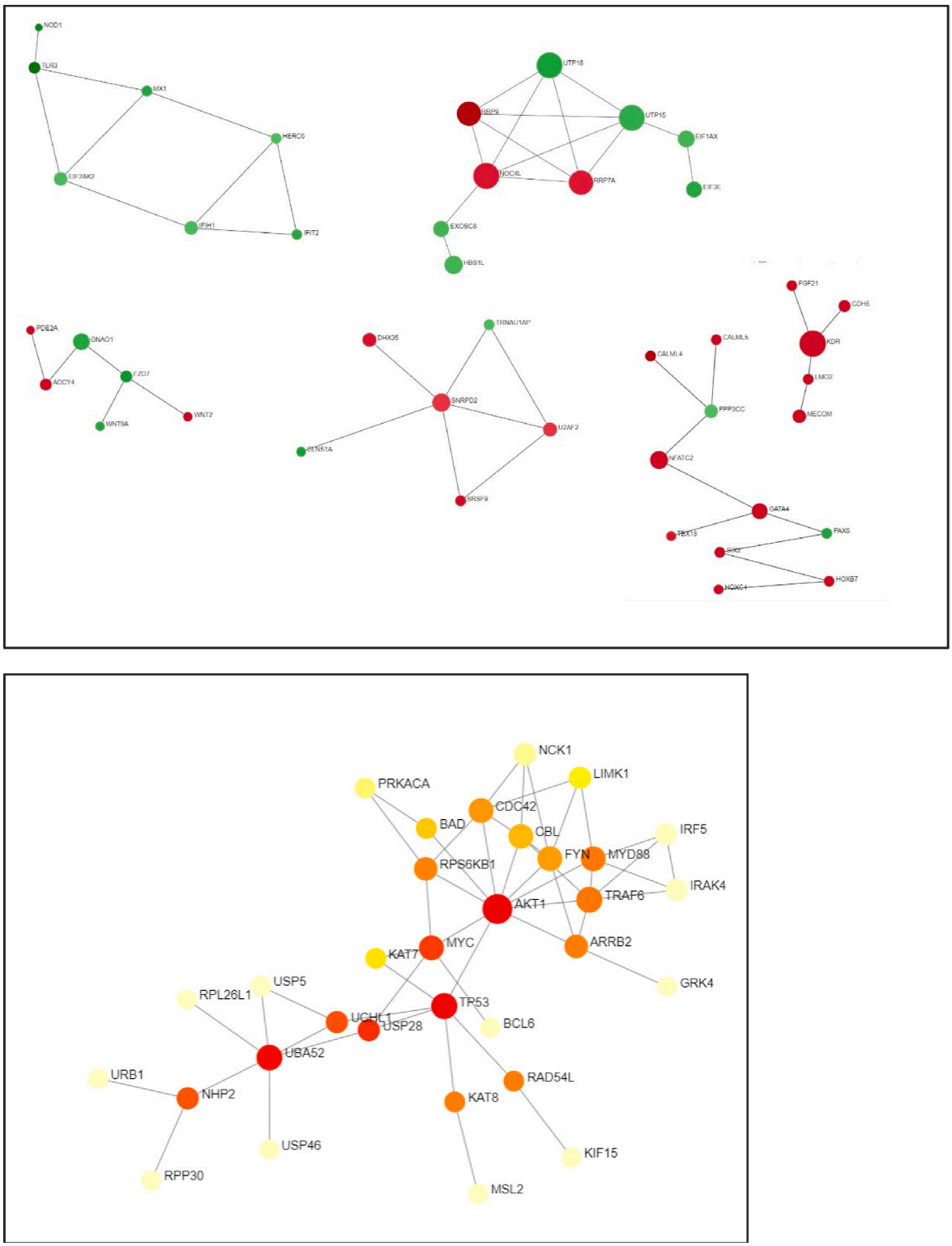

Fig. 4. PPI network and hub clustering modules. Size of node represents the importance of gene, larger the size of node more important is the gene. a) PPI network in E. coli infection green color genes aree up-regulated in infection and red are downregulated. b) PPI network in S. aureus infection. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Gene-drug interaction network can aid in finding drugs which targets genes, even if the drugs were initially developed for a different purpose.

4. Discussion

Mastitis in dairy cows is economically significant due to the damage it does to the dairy sector, its quality and composition, as well as dairy products. Understanding goat mammary cells and manipulating them is useful for studying mammary development and lactation biology, as well as disease, including mastitis. It is of the highest importance to develop an understanding of the molecular responses associated with mastitis. To address this, we evaluated the complete

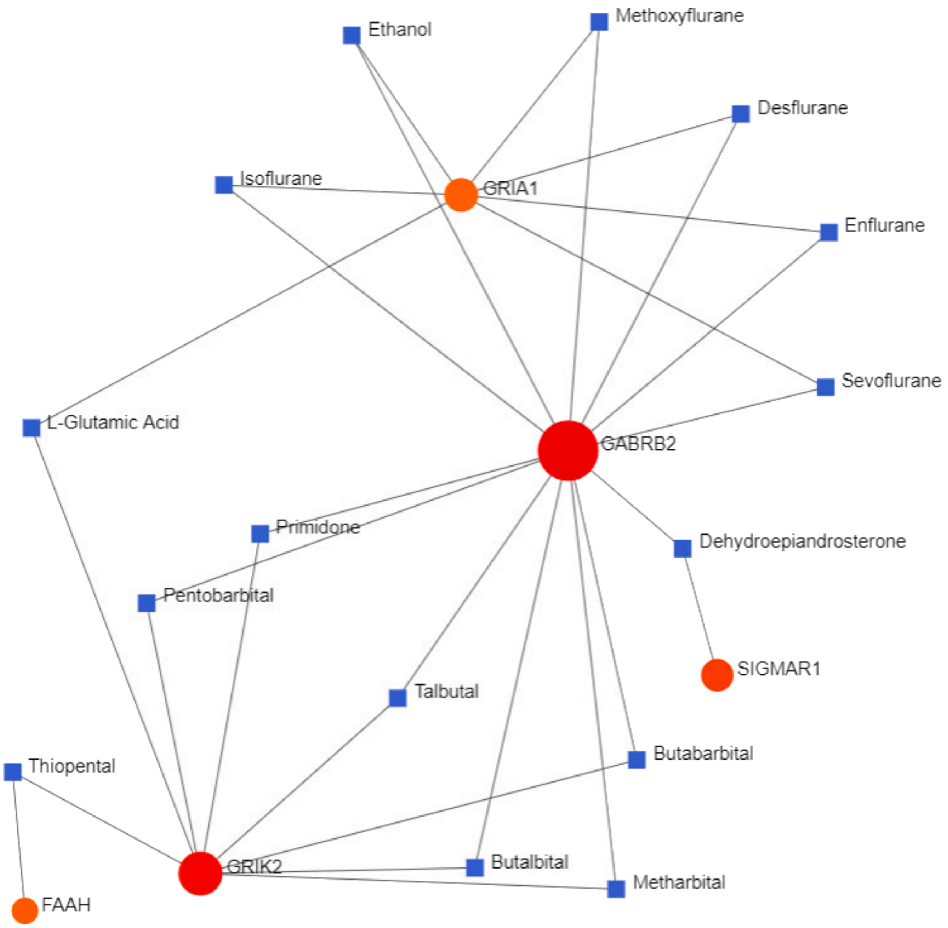

Fig. 5. Gene drug molecules interaction in E. coli infection. Nodes represent genes (round shape) and drug molecules (in square shape) connected by edges represents direct connections.

transcriptomic profile of gMECs following intracellular infection with *S. aureus* and *E. coli*. The goal of this study was to acquire a better knowledge of the intricate modulation of the transcriptome in gMECs during the bacterial stimulation, allowing us to better grasp the molecular mechanisms that underpin it.

In this study, we carried out a thorough examination of the genes and pathways in the infected goat MECs with *E. coli* and *S. aureus*. Infection with *E. coli* and *S. aureus* yielded 942 (397 upregulated and 545 downregulated) and 322 (222 upregulated and 100 downregulated) (p-value < 0.05, log2 (fold change) > 1) differentially expressed genes in gMECs respectively. The relevance of these gene families and genes in mounting a successful immune response against bacterial infections has been demonstrated in earlier investigations [33–35]. *E. coli* infection triggered a robust upregulation of genes associated with immune response & apoptosis, including pro-inflammatory cytokines and chemokines like GSDMD, GSK3β, IL21R. Gasdermin D (GSDMD) is a caspase substrate and is a member of the gasdermin family. It is cleaved during pyroptosis and is vital for IL-1β secretion. GSDMD has been shown to protect against cutaneous infection caused by *S. aureus* by decreasing Cxcl1-Cxcr2 signaling. It facilitates pathogen control and protects tissues during cutaneous *S. aureus* infection [36]. GSK3β has been shown to act as a positive regulator of inflammation [37]. It downregulates the anti-inflammatory cytokine IL-1Ra levels while simultaneously upregulating the IL1β levels in LPS-stimulated human monocytes [38]. IL-21 signaling is also important in Mycobacterium TB infection, as it increases CD8 + T cell priming, T cell accumulation in the lungs, and T cell cytokine output [42]. Another study discovered a link between IL and 21 and type I IFN in the innate immune response to methicillin-resistant *Staphylococcus aureus* [43].

Zinc finger protein 382 (ZNF382), which limits cell proliferation, promotes apoptosis, and suppresses several oncogenes, was shown to be significantly upregulated in our study [44]. Macrophage stimulating 1 receptor (MST1R) which is a tyrosine kinase receptor present on epithelial cells, macrophages and T-cell subsets. Its involvement in innate immunity was discovered to be critical, and it also plays a key role in host defense against viral infection [45]. These studies indicate that MST1R overexpression in gMECs following *E. coli* infection may be in response to host defense against bacterial infection.

Toll-like receptors (TLRs) set off a series of signaling events that stimulate the synthesis of chemokines, type I interferon, and pro-inflammatory cytokines. TLRs are essential for controlling the host immunological response [46]. Our results showed that infection with *E. coli* could increase the expression of TLR3 which will eventually resist the bacterial infection. DNAJC15 (MCJ) gene expression has been shown to be a transcriptional target of the cytokine IFN-γ, contributing to the regulation of inflammation.

Following infection, some genes participate in the apoptotic process. This include COMMD1 & Rad9a. A protein called COMMD1 is involved in sodium transport, the NF-kB signaling pathway, and copper homeostasis. Upregulation of COMMD1 in cell lines has been found to contribute to apoptosis-mediated cell death [49]. Rad9a is a checkpoint protein complex that acts as an early detector of DNA damage after genotoxic stress. It stimulates cellular checkpoints, which prevent or postpone cell division until either damaged DNA is repaired or the cell enters an apoptotic state [50].

Our findings also demonstrated that CNOT4, which is important in regulating interferon and interleukin-4 generated STAT-mediated gene responses in human HeLa cells, was significantly upregulated [53].

Chemokine and chemokine receptors in the host regulate tissue-specific migration as well as immune cells function and maintenance. Among these, CCR10 plays a special role in epithelial immunity. During an immune response, CCR10 is expressed for their migration and maintenance at mucosal sites [54]. Upregulation of CCR10 upon *E. coli* infection might be responsible for regulating immune cells in epithelial immunity. ZC2HC1A has been identified as a factor in the inflammatory response and cytokine production related with the macrophage response to acute ZIKV infection [55].

NOD1 becomes activated by γ-D-glutamyl-*meso*-diaminopimelic acid (iE-DAP) which is present in various Gram-negative bacteria (*E. coli*) and certain Gram-positive bacteria (*S. aureus*). The findings of our investigation are consistent with reports that NOD1 contributes to host defence by producing anti-microbial compounds and pro-inflammatory cytokines [56–57].

Another gene which was found to be significantly upregulated was Trex1. TLR4 ligand LPS has been shown to boost early Trex1 expression via an IFN-independent NF-κβ dependent signaling pathway, whereas DC activation via TLR3, −4, −7, and −9 augments Trex1 expression via autocrine IFN- synthesis and activates the IFN signaling pathway [60]. Fas-activated caspase 8 contributes to the cytokines production and processing of pro-IL-1β/ IL-18 [61]. Fas has been demonstrated to have a major impact on the innate immune system's response to infection with pathogenic microbes [62]. Selenoprotein T (SELENOT) was significantly upregulated upon *E. coli* infection which is in accordance with previous reports wherein overexpression of SELENOT has been shown to protect endothelial cells from LPS-induced activation and death [63]. These differentially expressed genes are implicated in various aspects of immune response, inflammation, and oxidative stress responses.

Conversely, there were many genes which were found to be downregulated including CISH, SUMO3, TIMM8B. CISH regulates T-cell function and cytokine signaling, modulating infectious disease susceptibility [64]. Immune system cell communications signals are slowed down by CISH and thus CISH downregulation will have a positive influence the host immunity to *E. coli* infection. SUMO regulatory pathway has been associated in response to infection including SUMO1, SUMO2, and SUMO3 as the three SUMO proteins. According to reports, *S. aureus* infection causes a dramatic drop in SUMOylation in infected macrophages, which encourages their intracellular survival and multiplication [65]. This is consistent with our results where expression of SUMO3 was significantly downregulated. Another gene that was significantly downregulated was the inner mitochondrial membrane protein TIMM8B. Downregulation of TIMM8B was also reported in SARS-CoV-2 infection [66].

Hyaluronic acid synthase 3 gene (HAS3) is an epithelial glycocalyx-associated gene which was found to be significantly downregulated in our study. This is consistent with previous studies wherein downregulation of HAS3 due to *E. coli* lead to decreased production of hyaluronic acid and weaker glycocalyx barrier formation, which may increase the likelihood of pathogen infection [67].

S. aureus infection exhibits a multifaceted impact characterized by the upregulation of various immune-related genes, as well as the activation of genes involved in autophagy, apoptosis, and tissue remodeling like expression of interferon regulatory factor 5 (IRF5), a gene implicated in multiple cellular processes. It is crucial for the expression of IFN-dependent genes, the expression of inflammatory cytokines, and the stimulation of apoptotic genes [68]. Another noteworthy finding was the significant up-regulation of CCR10. Chemokine receptor CCR10 has been reported to be uniquely involved in the epithelial immunity by regulation of immune cells [54]. Further support for the consistency of our findings was provided by [70] wherein they reported expression of CCR10 during infection by gut and BAL neutrophils. Our study reported significant upregulation of Zinc finger protein 382 (ZNF382) upon *S. aureus* infection which inhibits cell proliferation, induces apoptosis and suppresses multiple oncogenes [44].

The increase of type 2 deiodinase in our research was another interesting discovery (Dio 2). In cultures of rat astrocytes, bacterial LPS has been shown to stimulate type 2 deiodinase [72].

Another interesting discovery was the marked overexpression of the mitochondrial protein ERAL1, which controls innate immunity caused by RNA viruses and is released through the BAX/ BAK pore, activating RIG-I and MDA5 [81]. According to reports, Cbl ubiquitination facilitates the clathrin-dependent endocytosis machinery's recruitment and activity and is also necessary for bacterial entrance and internalization [82]. In addition to TRAF6, ARBB2 inhibits NF-κB activation and the production of pro-inflammatory genes, reducing inflammation brought on by *S. aureus* [83].

Upon *S. aureus* infection of gMECs, a number of genes displayed downregulation. Notably, VMO1 expression was markedly downregulated, which is consistent with other results showing that VMO1 was downregulated in HIV patients following respiratory infection [84]. Antioxidant protein SRXN1 guards against oxidative stress-related apoptosis [85]. However, viral replication is significantly aided by downregulation of the host's antioxidant system [86]. As stated above, CISH regulates T-cell function and cytokine signaling, influencing susceptibility to infectious diseases [64]. CISH downregulation will have a positive influence the host immune response to *E. coli* infection. Targeted downregulation of inner mitochondrial membrane protein TIMM8B was seen following SARS-CoV-2 infection [66]. Our findings support the findings of a prior study in which TRIM36 was discovered to be a negative regulator of IFN response and silencing of TRIM36 resulted in a considerable increase in IFN response [87].

The protein–protein interaction network was built, and various hub nodes were discovered. Nod1 is a signal transducer in epithelial cells that senses the microbial peptidoglycans components and signals the activation of NF-κB. NOD1 signaling in the intestinal epithelium rapidly activates the innate immunity during infection with gram-negative pathogenic bacteria [88]. According to reports, bacterial LPS improves the host's antiviral response by increasing TLR3 expression through a signaling pathway that is dependent on TLR4, MyD88, IRAK, TRAF6, and NF-κB [47]. Upon *E. coli* infections in lung cells, it leads to an increase in the the expression levels of interferon-stimulated genes (ISGs) such as MX1 which remarkably promotes the interferon secretion [89]. Other than the action of pro-inflammatory cytokines tumour necrosis factor α and interleukin 1β, HERC5 is a HECT E3 ubiquitin ligase that is selectively increased in LPS activated endothelial cells [90]. A type I interferon response gene called interferon induced tetratricopeptide repeat protein 2 (IFIT-2, P54) is strongly activated after stimulation with LPS [91].

Under *S. aureus* infection, hub nodes which were identified included UBA52. DUBs play a vital role in the ubiquitination system and are able to remove Ub from the target substrates. UBA52 is a gene implicated in apoptosis, cell cycle, DNA repair, and cytokine signaling [96]. By changing the dynamics of the actin cytoskeleton, the ubiquitin C-terminal hydrolase UCH-L1 facilitates bacterial invasion and raises the possibility that the ubiquitin cycle plays a previously undetected role in bacterial entry [79]. USP28 is crucial for the Chk2-p53-PUMA pathway in the DNA damage response, and it does it in part by controlling how p53 induces proapoptotic genes like PUMA [97]. Autophagy and apoptosis manipulation promote *S. aureus* intracellular survival in human neutrophils. In the defense against *S. aureus*, MyD88 mediates neutrophil recruitment that is started by IL-1R. TLR8 detects *S. aureus* in infected macrophages and monocytes, resulting in the generation of IFN-β through MYD88 signaling [101]. *S. aureus* triggers a TLR2-dependent endosomal signalling cascade including Mal, MyD88, TRAF6, and IKK-related kinases. These factors help to increase intracellular survival and IFN-1 production. This pathway manipulates the host to enhance intracellular survival while also triggering type I IFN responses [102].

The transcriptomic responses of gMECs to E. coli and Staphylococcus aureus infections in this study revealed intriguing differences and similarities in gene expression patterns and activated pathways. In terms of gene expression patterns, both infections led to the differential

expression of numerous genes associated with immune response, apoptosis, and inflammatory signaling. However, the magnitude and specific genes involved varied between the two pathogens. For example, E. coli infection triggered the robust upregulation of genes like GSDMD, GSK3β, and IL21R, while S. aureus infection resulted in the upregulation of IRF5 and CCR10, indicating unique immune responses. Conversely, certain genes, such as HAS3 and VMO1, were downregulated specifically in response to E. coli and S. aureus, respectively.

Regarding pathways activated, both infections shared the activation of innate immune response pathways involving Toll-like receptors (TLRs) and the complement system. However, the degree and nature of activation differed. Notably, S. aureus infection activated genes related to autophagy, apoptosis, and tissue remodeling, such as IRF5 and CCR10, more prominently, while E. coli infection exhibited a strong upregulation of TLR3 and genes like GSK3β and GADD45G.

5. Conclusion

The transcriptomic insights into goat mammary cell responses to E. coli and S. aureus infections offer valuable information for the pharmaceutical industry. The identified genes associated with *immune response, apoptosis,* and *inflammation* provide potential therapeutic avenues. Genes like *GSDMD, GSK3β,* and *IL21R*, which are upregulated during infections, present potential drug targets for enhancing the immune response against bacterial pathogens. Understanding how these genes function at the molecular level can inform the design of novel drugs that boost host defenses. Additionally, genes such as *CISH, SUMO3,* and *TIMM8B*, which are downregulated in response to infections, can be explored for drug development to modulate immune responses or mitigate the pathogen's impact. This research opens new avenues for pharmacological intervention and underscores the pharmaceutical industry's role in improving animal health, dairy production, and public health. Further research will be crucial to translate these findings into tangible drug therapies.

Funding

Not applicable.

Availability of data and materials

All data generated and analyzed during this study, including the supplementary information files, have been incorporated into this article.

Ethics statement

Not applicable.

Author contribution

AS and PTM conceived the study. SS, TM, QT designed the experiments. AS and PTM performed the experiments. MAD, BB*, and SMA* analyzed the data. AS wrote the original manuscript draft. SMA supervised the work. All the authors read and approved the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

Acknowledgement

None declared.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.intimp.2023.111213.

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