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RESEARCH ARTICLE

Molecular Identification of Goat's Udder Microbes and Nutritional Value of Milk Using Dielectric Constants

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ABSTRACT

This study aims to determine which bacteria are present in goat's udder by using gram dye, nuclear nucleic acid technology, direct 16S rRNA gene sequencing, and dielectric constants. The 16S rRNA gene is frequently sequenced in modern approaches to identifying microorganisms and molecules. Bacteria like *Cohnella suwonensis, Cohnella yongneupensis, and Cohnella faecalis* were isolated and *Staphylococcus* species like *succinus, lentus, xylosus, equorum, stepanovicii, fleurettii, sciuri, kocurii,* and *faecalis* were recorded. The constants such as dielectric constant *(ε'),* spectra *(ε")* and dielectric loss factor *(ε'+ε")* spectra, and Total Bacterial Count (TBC) from raw goat milk samples were determined employing network analyzer and plate count methods, respectively. A poor relationship was found between TBC in logarithm and permittivities at single and multiple frequencies. Linear models like multiple linear regression, ridge regression, and absolute shrinking were used to determine TBC based on effective dielectric constant and spectra and their combination; and an excellent TBC performance was determined. In conclusion, various microbes found and isolated were, *Staphylococcus succinus, S. lentus, S. xylosus, S. equorum, S. stepanovicii, S. fleurettii, S. sciuri, S. kocurii, Cohnella suwonensis, C. yongneupensis,* and *C. faecalis***.** This study emphasizes the importance of improved hygienic procedures in ensuring food safety by emphasizing the importance of avoiding contamination and using the appropriate types of processing equipment.

Keywords: Dairy goat, DNA, Milk hygiene, Polymerase chain reaction, 16s rRNA gene

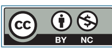
INTRODUCTION

The goat population of the country is very high and serves as an indispensable source of meat and milk producing 82.900 tons of meat and 96.900 tons of milk [1,2].

Mastitis, an infection of the udder, harms both the quantity and quality of milk produced in dairy farms [3,4]. Conventional treatment methods are factors that contribute to the disease's economic impact and therefore management becomes statistically non-significant [5,6]. Mastitis, cannot be completely cured and farmers and other dairy-related business owners are always in search of some alternate disease methods as it is critical to compare the costs of disease control with the costs and losses incurred directly by the disease [7,8]. The risk assessment's goal is to provide a fair and objective evaluation of various pieces of scientific research on the potential dangers of consuming unpasteurized goat's milk.

Molecular markers are used to determine whether phenotypic differences are hereditary or adapted. Many more variants in DNA sequences have been discovered using new DNA techniques, and one of the most important tools is molecular markers which are simply indicators of DNA changes in species. PCR has become extremely important in the field of molecular DNA research, as evidenced by genome mapping, genotyping of bonding strips with desired properties, and detection of polymorphisms in DNA (DNA fingerprint). The sequencing of the 16S rRNA gene is a necessary step in molecular diagnostics and other microbiological determinations [9-11]. The applications of genomic research rely heavily on the development of DNA markers and various molecular biology techniques. Furthermore, it opened up new avenues of possibility for genetic markers, genetic advancement, and animal selection markers.

In contrast to molecular markers, biosensors have also shown promising results in the estimation of various



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microbial populations in milk [12]. Therefore, due to high specificity and sensitivity, biosensors could detect a broad spectrum of analytes in complex sample matrices and give real-time results without pre-enrichment. The methods such as fluorescent microscopy [13], quantitative PCR [14], and flow cytometry [15] provide much information about the composition of milk and microbial count. Dielectric spectroscopy is one of the inexpensive methods employed for milk composition analysis [16,17]. Similar techniques have been used by several other workers for the determination of fat and protein from raw cow milk [17]. However, due to the high scale of farming goats in Saudi Arabia, raw goat milk quality determination is a must and a rapid method for detecting TBC of raw goat milk is very important for the local goat milk industry. The dielectric properties such as constant *(ε′)* and dielectric loss factor *(ε″)* are used to describe interactions of materials with electric fields. With the development of microbial populations and nutrient metabolization, the breakdown of macro-molecular occurs bringing change in energy and conductivity as well as resistance as recorded by electrodes. This energy change impacts the response of a material to the magnetic field [18].

It is critical to safeguard human health against illnesses caused by tainted milk or diseases that harm farm animals. Further, it is also very important to safeguard animal health against illnesses caused by contaminated milk. The FDA and the FDA have been implicated in milk contamination [19-21]. Traditional detection methods are time-consuming, costly, and involve several different steps. Molecular identification techniques, such as the 16S gene sequencing method, and dielectric spectroscopy are faster and more accurate [22]. Modern genomic techniques have enabled the discovery of the genetic underpinnings of a wide range of bacterial phenotypes by predicting the effects of variable mutations that occur during the progression of pathogenic populations in animal products. The Phylogenetic analysis was used to gain a better understanding of the diversity found in the expanded areas [23,24].

In the present study, cutting-edge methodologies such as molecular, and microbial identification, as well as 16S rRNA gene sequencing, and die-electric spectroscopy are used to identify and classify bacteria found in goat udder microbiomes.

Materials and Methods

Ethical Statement

This study was approved by the King Abdulaziz University Local Ethics Committee, and animal welfare is ensured through minimal handling and stress during the sampling step (Approval no: 9-15, 2021).

Bacterial Purification

The samples were collected from several goat farms, and all samples were serially diluted by pouring aliquots of 100 mL sterile distilled water (up to 104) with Nutritional Agar (NA) plates and incubating them at 30°C for 72 h. The scope of the investigation was expanded to include the maintenance of morphologically distinct bacterial colonies and bacterial isolates in a 20% glycerol solution. For the determination of bacterial counts and analysis of composition in raw milk, each milk sample was kept in a sterilized plastic bottle and delivered to the laboratory biological biology Dept., in a natural state within 10 min after milking. Samples were stirred for about 1 min using an electric to guarantee the uniform distribution of components. All samples taken were divided into 3 groups to perform chemical analyses, TBC determination, and dielectric spectra acquisition. Gram staining of samples was done exactly as per the procedure given by the Cerny method [25,26].

The Oxidase Test

As a testing reagent, 1% tetramethyl-phenylenediamine solution in water was used according to the method of Whatman [27]. In a glass petri dish, a piece of white paper was placed, followed by two drops of a freshly prepared 1% tetramethyl-phenylenediamine solution. We transferred a loop of bacteria from a culture that had been grown in NA media for 24 h to the impregnated portion of the strip using a sterile toothpick. If the color changed to purple within the first minute and a half, the results were considered positive.

Catalase is Used for Analysis

Bacterial cultures were grown for a full day in NA media to determine catalase activity [27]. A glass loop was mixed with a drop of hydrogen peroxide to see if gas bubbles formed, indicating a positive response to catalase. This process was repeated for each bacterial culture.

Isolation and Purification of DNA from Bacterial Genomes

Following the instructions given in QIAamp genomic DNA Purification Kit, the extraction process was followed and DNA was obtained using the method QIAGEN. Five milliliters of overnight-cultured Nutrient Broth (NB) were removed and centrifuged at 13.000 revolutions per minute to obtain 1.75 mL of each bacterial strain. After 24 h of incubation, the supernatant was removed from a test tube, 180 mL of enzyme lysis buffer was added, and the tube was shaken for the next 10-20 sec at 37°C (AL). After stirring, the liquid was heated to 56°C for 30 min. The centrifuged mixture was diluted with 200 microns of 100% ethanol. The filter was discarded and the column was again centrifuged for 1 min at 13.000 revolutions per minute after being washed with 500 L of AW1 wash buffer solution. The process was completed after incorporating

the mixture with 500 L of AW2 washing buffer and spinning it for 3 min at 13.000 revolutions per minute. In order to preserve the integrity of the DNA sample, it was chilled to -20°C before being frozen.

Polymerase Chain Reaction Amplification of the 16S Ribosomal RNA Gene

Primers with the sequences 5’-CAGCGGTACCAGTTT GCTGCTCAG-3’ and 5’-CTCTCTGCAGGCTACCTTGT ACGACTTT-3’ were used for 16S rDNA amplification. A denaturation polymerase chain reaction (PCR) was performed after 30 cycles of amplification at 94°C for 1 min, 58°C for 30 sec, 72°C for 1.5 min, 10°C for final extension, and 4°C for incubation. After being subjected to agarose gel electrophoresis, the amplified DNA was sequenced after being exposed to ultraviolet light (UV-radiations). A 1% agarose gel with a UV transilluminator was used to visualize the amplified PCR product. This was accomplished by shining a light through the gel.

Genealogical Tree Investigation

The 16S rDNA sequences were then BLAST searched against the GenBank database, which is kept up to date by the National Center for Biotechnology Information. MEGA 7.0.26 was used to create a phylogenetic tree and evolutionary distances were calculated using the Kimura 2-parameter model [28].

Milk Composition Determination

The selected milk samples from goat farms were taken and the nutrient composition including protein, fat, lactose, and SNF content, was determined using a Lactoscan milk analyzer. The moisture and PH were also determined following the method given by AOAC International [29] and PHSJ-3F China, respectively. Biochemical analysis was performed at room temperature (24±1°C).

Determination of Total Bacterial Count (TBC)- Palate Count Method

The palate count method was used to measure TBC following the Chinese National Standard GB4789.2-2010, as described by Zhu et al.[17]. TBC was logarithmically transformed (log_{10}) and the unit of the TBC in this study was log_{10} colony-forming units per milliliter.

Dielectric Spectra Acquisition

The dielectric measurement system is comprised of an Agilent E5071C vector network analyzer manufactured by Agilent Technologies, Penang, Malaysia. Other essential components include an Agilent 85070E open-ended coaxial line probe, and a few more accessories forming the bases of dielectric spectra acquisition. Frequency is important and the system network ranges from 1 to 10 MHz kept with sampling points in the logarithmic scale of 201. The coefficients or permittivities namely ε' and ε'' were calculated based on the reflection coefficient and material-probe interface, and the detailed information about the setup and calibration procedure of the system was followed as given by Zhu et al.[17]. The system was heated for 0.75-1 h to keep dielectric data steady. The warm-up of the system was immediately followed by calibrating the network analyzer and coaxial line probe. Once the temperature increases to 25°C within 2-5 min, 2 original dielectric spectra (i.e., ε' and ε'' spectra) were inserted and discrete frequencies were measured in periods of about 30-the 40s. Measurements were repeated thrice for each sample from each beaker, and a total mean value of 9 measurements for each milk sample was calculated. The data obtained were used for subsequent analyses.

Quantitative Determination of TBC Using Different Models

Various models used for the quantitative establishment of TBC are as:

a) Multiple Linear Regression (MLR)

b) Ridge Regression (RR)

c) Least Absolute Shrinkage and Selection Operator (LASSO)

MLR Model

This model is based on an output variable Y and an input variable X. The equation is given by:

$$Y = X\beta,$$

where β represents the regression coefficient matrix. It is determined by the Least Square Method to minimize the Residual Sum of Squares (RSS) as the points of data were represented as $(x_1, y_1), (x_2, y_2), (x_3, y_3), \ldots, (x_n, y_n)$. The x is an independent variable, while is the dependent one.

$$RSS_{MLR}=\| \check{Y}-X\beta \|^2$$

In the above formula, the yˆ is the predicted values vector of the output variables.

The estimated Regression Coefficient Matrix is expressed as:

$$\beta_{MLR}=\| (X^TX)^{-1} \| X^Ty$$

The value for the model limits are as $Model_1$<-^{l}m (y_1~x_1,data=df_1)

In the above equation, df represents a degree of freedom (n-1), y and x as dependent and independent variables, and T represents transposition.

RR Model

In order to limit overfitting and underfitting, the RR

model was used. It was done by introducing a penalizing term, $\alpha\|\beta\|^2$ in the RSS of the MLR model.

The regression Coefficient Matrix is as:

$$\beta_{RR} = (X^TX + \alpha I)^{-1}X^Ty$$

In the above equation, α is the ridge parameter. I represent the identity matrix, while α improves the regression condition and minimizes the variance of estimates. The α defines the mixing parameter between ridge (α = 0) and LASSO (α = 1), the choice of α is often conducted using a *k*-fold cross-validation approach.

LASSO Model

This LASSO model is a regression-based least squares algorithm (TS-LS-LR). The L_i constraint on the regression parameters ranges from 0 to 1. In the RSS model penalizing term, λ‖β‖1 is added and the regression of all variables is obtained by the formula:

$$\beta_j^{LASSO} = \beta\,(0{,}1\text{-}n\,\lambda/\|\,\beta^{MLR}\|)$$

Whereas, J = index variable; λ = parameter of LASSO; n = number of samples.

Using the k-fold cross-validation approach, optimal value λ is obtained. Contrary, to the RR model, the LASSO model is advantageous in estimating dependent and independent parameters and the simultaneous selection of discrete variables.

Based on LASSO regression the optimal value λ is as:

$\lambda = 0$, the same coefficient as in simple linear regression

$\lambda = \infty$, all coefficients are zero

$0 < \lambda < \infty$: The coefficient is between 0 and simple linear regression

Model Performance Evaluation

We performed the comparisons of simplified (usually linearized) versions of numerical models. The coefficients especially the calibration set (r_c), prediction set (r_p), RMSE (Root Mean Squared Error), RMSEP (Root Mean Square Error for prediction set), and RDO (Residual Predictive Deviation) were calculated to test model performances. We draw a fit Logistic Regression model with response variables as shown in *Fig. 4*.

Software Used

Matlab version 7.1 (The Mathworks Inc., Natick, MA) was used. Further, Spyder 3.2.6 *(https://www.spyder-ide.org)* was also used to establish the MLR, RR, and LSAAO models.

Results

The 345 samples of goat udder milk collected from ninety different locations in Saudi Arabia were streaked and cultured for 24 h at 30°C before being tested for total bacterial growth (TBG) *(Fig. 1)* and molecular determination of microbiota *(Fig. 2)*. After being placed in the Eppendorf tube, the pure culture was inoculated with blood chocolate and MacConkey media. The phenotypic, morphological, and molecular characteristics of genetically isolated populations were investigated, and a wide variety of useful microbes were found in samples that contributed to the generation of phenotypes *(Fig. 3)*. The microbes found were then used to identify pathogenic and nonpathogenic bacteria. Various morphological characteristics, such as gram-positive and gram-negative staining, were used to select the isolates. As shown in *Fig. 1*, each of the selected isolates has a unique set of morphological characteristics, one of which is the ability to be stained with both gram-positive and gram-negative pigments *(Fig. 1)*.

An Examination of 16S rRNA Gene Sequences-A Tool to Differentiate Bacterial Isolates

During the experiment, high-quality genomic DNA was extracted to investigate and identify bacterial isolates at

Table 1. *Representation of milk composition, total bacterial count, pH, and correlation analysis with total bacterial count*

Parameters	Observations	Power of H^+ Ion Concentration	Moisture Content %	SNF %	Sugar %	Protein %	Fatty Glycerides %	Log_{10} of Total Bacterial Count (TBC)
Static	Minimum	1.31	75	2.2	3.10	3.21	1.56	2.98
	Maximum	5.00	87	5.78	4.78	4.01	5.67	14.93
	Mean	3.21	81	3.99	3.94	3.23	3.61	8.955
	SD	0.54	1.23	0.25	0.15	0.31	0.85	4.43
Coefficient of Correlation	Pxy=Con (rx, ry)/σx σy Pearson's cofficent	-0.31	+0.003	+0.029	-0.01	-0.03	+0.011	-
	P-value	<0.001	0.76	0.29	0.451	0.101	0.09	-

Pxy=Con (rx, ry)/σx σy*; Where as;* ***ρxy*** *= Correlation between two variables;* ***Cov(rx, ry)*** *= Covariance of return X and Covariance of return of Y;* ***σx*** *= Standard deviation of X;* ***σy*** *= Standard deviation of Y*

Table 2. *Dimensional Constatnts from different models used for the determination of total bacterial count (TBC) based on dielectric spectra and dielectric constants and the log_{10} variations of dielectric spectra*

Model Type	Dielectric Constant (ε′)	Dielectric Spectra (ε″)	r_e	Log_{10} cfu /mL (RMSEC)	r_p	Log_{10} cfu /mL (RMSEP)	Residual Productive Derivations (RPD)
LASSO	ε′	ε″	0.87	0.81	0.85	0.80	2.5
	ε′	ε″	0.78	0.70	0.79	0.71	2.4
Dielectric Loss Factor	$\varepsilon'_{+}\varepsilon'$	ε″+ ε″	**1.76**	**1.51**	**1.64**	**1.51**	**4.9**
MLR	ε′	ε″	0.70	0.69	0.90	0.84	2.9
	ε′	ε″	0.88	0.80	0.98	0.90	2.8
Dielectric Loss Factor	$\varepsilon'_{+}\varepsilon'$	ε″+ ε″	**1.58**	**1.49**	**1.88**	**1.74**	**5.7**
RR	ε′	ε″	0.87	0.84	0.97	0.77	1.8
	ε′	ε″	0.60	0.63	0.80	0.88	1.9
Dielectric Loss Factor	$\varepsilon'_{+}\varepsilon'$	ε″+ ε″	**1.47**	**1.47**	**1.77**	**1.87**	**3.7**
P-value	-	-	-	<0.001	-	<0.001	-

ε′=dielectric constant; ε″= dielectric spectra; ε″+ ε′= dielectric loss factor; r_e= correlation cofficent of caliberation set, r_p= correlation cofficent of prediction set

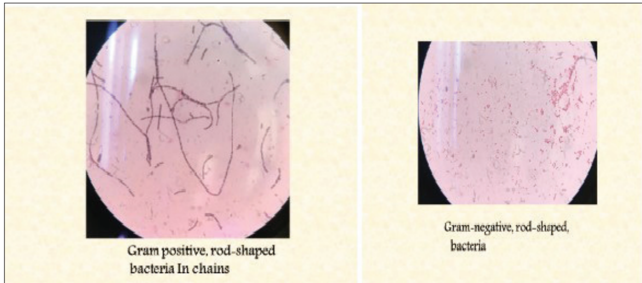

Fig 1. Showed goats udder colony gram staining. **A-** gram-positive *(spiral chain shaped),* and **B-** Gram-negative *(rod shaped)*

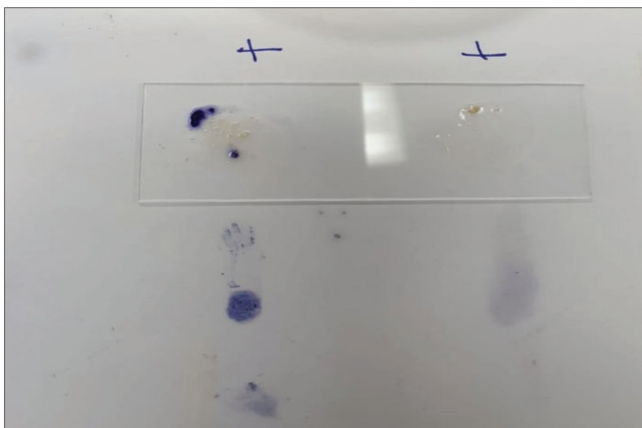

Fig 2. H200729-02 0A07 M A04 1492R *S. lentus* strain F4- 2-25 16S ribosomal RNA gene, partial sequence oxidase variable (catalase-positive). H200729-020 C07 M B04 1492R *S. lentus* strain DQ56 16S ribosomal RNA gene, catalase-positive and oxidase-positive

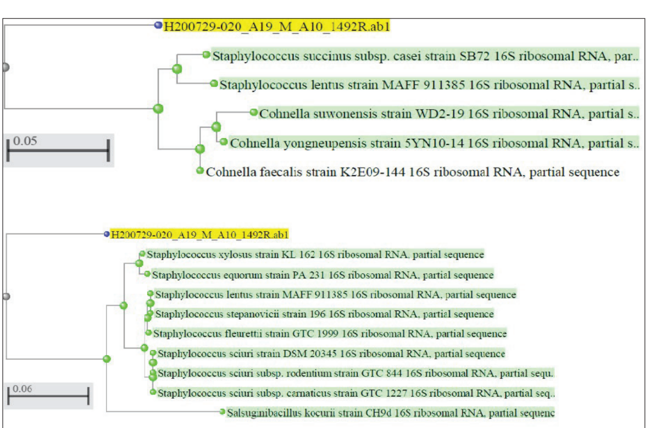

Fig 3. Phylogenetic analysis of bacteria isolates of *Staphylococcus succinus, S.lentus, S. xylosus, S. equorum, stepanovicii, S. fleurettii, S. sciuri, S. kocurii, Cohnella suwonensis, C. yongneupensis, C. faecalis* from goats udder samples based on partial nucleotide sequences of 16S rRNA gene. The tree was constructed using the neighbor-joining

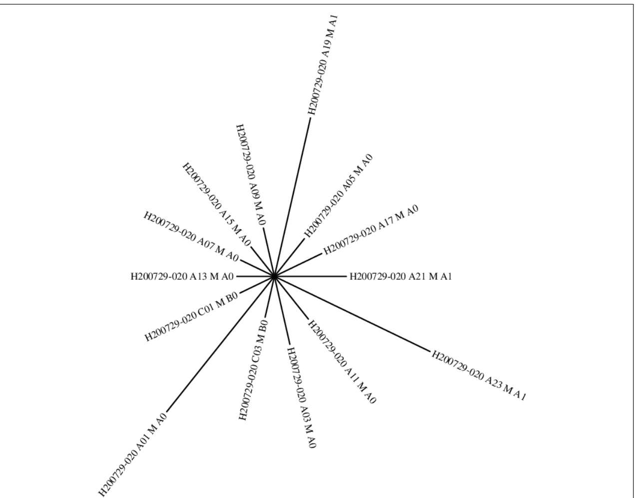

Fig 4. Based on partial nucleotide sequences of the 16S rRNA gene, a phylogenetic analysis of all bacterial isolates from goat udder samples was conducted. The LIAMM method was used to build the tree

the molecular level *(Fig. 4)*. The yield of genomic DNA was in the 60-140 ng/L range. Polymerase chain reaction (PCR) was used to amplify ribosomal DNA, with universal forward and reverse primers for 16S rDNA serving as the starting point *(Fig. 5)*. The dielectric constant (ε′), spectra (ε″), dielectric loss factor (ε′+ε″), and total Bacterial

Fig 5. Gblocks Results 0.91b, input. fasta was the processed file. Alignment is presumptively: DNA new total: 563 places (selected positions are underlined in blue)

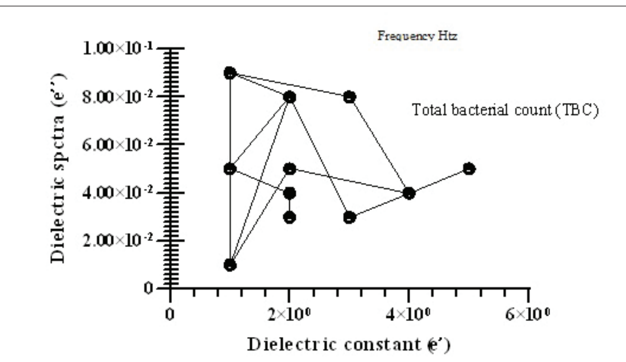

Fig 6. Representation of nonlinear relationship describing the variation of frequency (MHz) with Dielectric constant (ε′), Dielectric spectra (ε′′), and Total Bacterial Count (TBC)

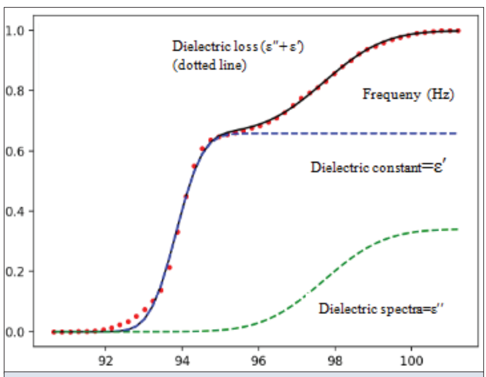

Fig 7. Dielectric constant (ε′) and dielectric spectra (ε′′) and frequency (Mhz) of variation of 345 raw milk samples of goat milk taken from goat forms

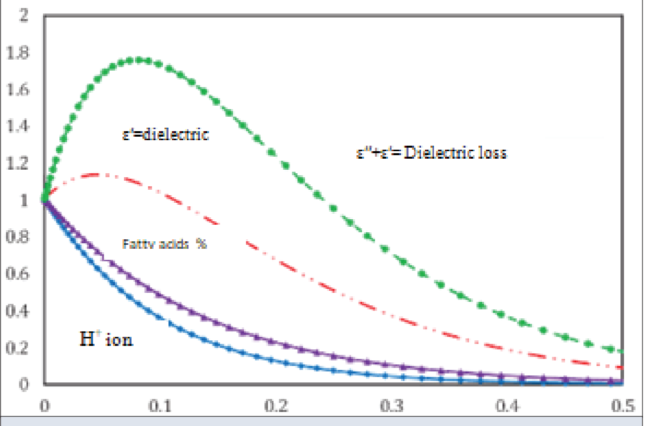

Fig 8. Linear relationship of correlation coefficient (r) between dielectric constant (ε′), dielectric loss (ε′′+ε′) and Fatty glycerides and pH of 345 raw goat samples and frequency (Mhz) of variation

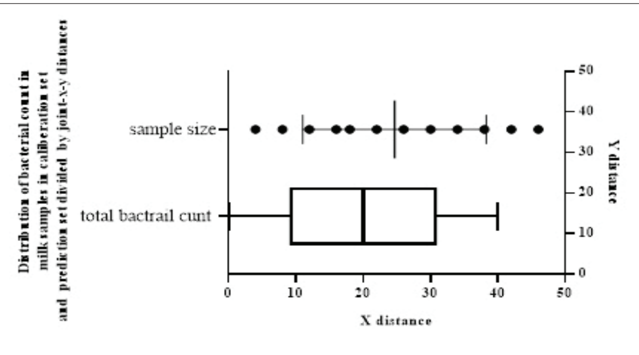

Fig 9. Graphical representation of the distribution of bacterial count in milk samples in calibration set and prediction set in -x-y distances

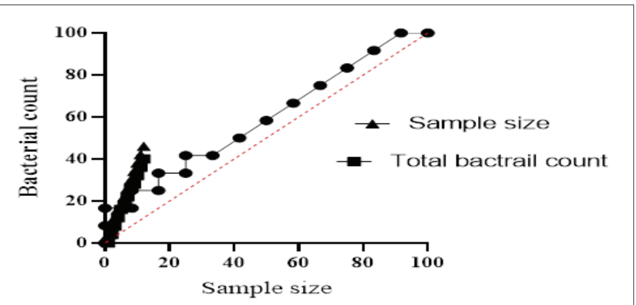

Fig 10. A Fit Logistic Regression Model with response variables Milk sample size and Total Bacterial Count (N=100). Note: in the Regression Fit Line we took only 100 milk samples

Count (TBC) were measured in raw goat milk samples using network analyzer and plate count procedures *(Fig. 6, Fig. 7)*. The association between logarithmic TBC and permittivities at single and multiple frequencies was unsatisfactory *(Fig. 7)*. Correlation studies *(Fig. 8)* and multiple linear regression and ridge regression *(Fig. 9, Fig. 10)*, and absolute shrinkage were utilized to predict TBC based on effective dielectric constant, spectra, and their combination *(Table 1, Table 2)*, resulting in outstanding TBC performance.

Discussion

According to current monitoring in Saudi Arabia, Jeddah. Mastitis disease in goats is significantly prevalent, and its severity can be reduced by improving hygienic practises. Early detection of the disease is critical. Various bacteria such as *Staphylococcus sucinus, S. Lentius, S. xylosus, S. equorum, stepanovicii, St. Flouretii, S. sciuri, S. kocurii, Cohnella suwonensis,* and *C. yongneupensis* were found in this study. Similarly, Alnefaie et al.[30] reported multiple bacteria in milk samples, such as *Klebsiella pneumonia, Serratia marcescens, Micrococcus, Coagulase-negative Staphylococcus, Diphtheroid,* and *Anthracoid.* Recent research has discovered a large number of harmful microorganisms which lead to disease especially Mastitis as it is becoming more common in animals, and this trend is expected to continue as animals become more susceptible at the early milking stage. This pattern has been observed in goats therefore routine monitoring is important. Mastitis was not previously recognized as a community-wide problem in goats (Arabic countries).

In the current study, the bacteria such as *Escherichia coli, Staphylococcus aureus, Citrobacter diversus,* and *Klebsiella* sp. were isolated from goat udder. Similar results were earlier found in Nigeria by Salihu et al.[31]. In Saudi Arabia, goat farms suffer huge economic losses to staphylococcal intramammary infections, *Staphylococcus aureus* is the major cause of clinical mastitis in goats and other ruminants [32].

To save the economic losses the Intramammary infections caused by *S. aureus* needs attention because of its huge prevalence and widespread forms of presentation of the disease. The *Coliform* bacteria cause feces infection contaminating the environment and causing poor hygiene; further, the unsanitary conditions in goat farms were caused by the presence of *E. coli, Klebsiella* spp., and *Citrobacter* species [16].

It has been reported that *coliform* bacteria were isolated from milk of unhygienic farms due to feces contamination and a lack of cleanliness. *Klebsiella* sp. is a gram-negative bacteria that is commonly found in poultry farms [25]. Because *Klebsiella* spp. are only found in unsanitary environments and it has significant public health implications as there is an increased risk of illness, particularly for young children and those with compromised immune systems. Further, when the pH of the urine changes, *Proteus mirabilis* can thrive, resulting in kidney stones [5]. *Citrobacter* can infect many different body systems, including the gastrointestinal tract, lungs, bones, peritoneum, endocardium, meninges, and circulation. The urinary system was the most frequently infected site, followed by the abdomen, skin, and soft tissue (including surgical infection), and caused pneumonia and other diseases in animals. The presence of *Klebsiella* and *Citrobacter* species increases the risk of eggs spoiling in poultry farms [30]. A variety of factors, including vertical and horizontal pollution, contribute to the spread of microbes on the outside and inside of goat farms in Saudi Arabia [10-14].

The goal of this study was to see the molecular identification of microbes and the composition of bacterial contamination in goat udders. There was a slight increase in the total number of microorganisms present in the goat udder in the afternoon samples taken from all farms (P>0.05). Because mesophile microorganisms thrive in temperatures ranging from 25 to 40°C, microbial loads are highest in the afternoon as opposed to the morning and evening. These results are consistent with those discovered by Theron et al.[21]. After being exposed to the high temperature, the number of different bacteria species was noticeably higher in the contents of the goats' udders [15,28].

Despite its high nutritional value, goat milk can cause serious health issues if contaminated with harmful germs, and generally goat farming appears to be poorly maintained, based on the high number of bacterial isolates discovered in goat udder and milk content and the unsanitary conditions under which goats were kept. Despite this, it is still advised that strict health regulations be followed in goat farms.

The current study will spend the majority of its time investigating the highly variable 16S rRNA region on chromosome 4. According to Zhang et al.[23]. Research, V4 is an excellent location for gathering information about the target's bacterial population. Furthermore, the ASV inference provides a clear definition of the bacterial diversity found in goat milk, allowing for the identification of variations found within the same species. Firmicutes and Actinobacteria are the two phyla with the highest frequency at the phylum level, according to our findings. Milk samples from various locations, as well as from the market, were found to share common characteristics. The similarities and differences between the core microbiota of goats were also investigated in this study and *Staphylococci, streptococcus*, and other bacteria were found in the core microbiota of all samples; the goats are the only exceptions to have *Corynebacterium* in milk samples. Lactobacillus appears to be the only organism that can be shared with the core microbiota when it comes to obtaining safe samples of cow's milk [14]. A previous study [13] found the same count of *Escherichia* and *Shaegen* in milk which is similar to the current study. Even though *Escherichia* and *Shigella* are not present in the core microbiota of humans, sheep, buffalo, and cow milk. In addition to a descriptive analysis of the microbiota found in goat milk, we presented the findings of a pilot study

designed to assess the potential relationship between SCS, a symptom of subclinical mastitis, and the milk microbiota. Even though the incidence of subclinical mastitis was frequently lower than 5%, it was estimated that the annual prevalence of clinical mastitis in small ruminants was anywhere from 5% to 30% higher than the incidence of subclinical mastitis. The small ruminants had significantly lower abundances of the bacteria species such as *Corynebacterium, Jeotgalicoccus,* and *Escherichia coli/Shigella* compared to the bigger ruminant (Cow) community. Six additional bacterial genera live in the SM community, each of which has the potential to spread illness and unsanitary settings

The most common pathogen that causes mastitis in goats is *Clostridium perfringens* which is consistent with what other researchers discovered in cattle. Further, several different studies have demonstrated this; however, we recognize that the validity of our findings is hampered by the small number of samples analyzed compared to the total population of goats and the conditions in which they are kept. The *Lactobacillus* levels were found to be higher and percentages increased from 12.2% to 19.3%. According to the findings of our study, no *Lactobacillus ASVs* were associated with the SCS trait, and the milk contains less *Lactobacillus* genus that may suppress some important mastitis pathogens. More research is needed to fully understand the role of the genus in subclinical cases of goat mastitis. Additional research on the composition of the microbiota in the goat's udder should be conducted with the assistance of a larger animal population to determine whether or not it can improve SCC quality. Based on previous knowledge [28], this is the completive study to characterize the microbiota found in goat udders using a culturally independent metagenomic method based on 16S V4 hypervariable region sequencing and dielectric properties and models.

If the TBC of the milk was greater than 8.955 log10 cfu/mL, the milk was regarded as hygienic. In the current studies, many of the milk samples analyzed were found to have good hygiene. As shown in *Table 1*, the Pearson correlation coefficients were -0.31, +0.003, +0.029, -0.01, -0.03, and +0.011 respectively for pH, Moisture, SNF, sugar, protein, fatty acids, and total bacterial count. Statistically, the P-value was high so significance was recorded *(Table1)*. *Table 1* shows the Pearson correlation coefficient and the P-value of each milk component and pH with TBC. Among the investigated compositions and pH, only the pH was highly correlated ($P<0.01$) with TBC, but The Pearson correlation coefficient was only -0.31. The poor correlation coefficients of the milk components and pH with TBC indicate that the milk composition and pH had difficulty in predicting the TBC of milk. So, any general conclusion from pH was difficult. Dimensional constants obtained are as shown in *Table 2* which showed the original dielectric constant (ε), dielectric spectra (ε′′), and dielectric loss (ε′′+ ε′) of 345 raw goat milk samples: dielectric constant (ε) decreased with the increase of frequency over the whole frequency range as shown in *Fig. 2*. While the dielectric spectra (ε′′) decreased with an increasing frequency and as shown in *Fig 2*. While the dielectric loss (ε′′+ ε′) showed an increase *(Fig 2)*. The correlation of calibration and prediction obtained are given in *Table 2*. Similar effects of frequency on ε′ and ε′′ of raw milk have been found in several studies [17]. Determination of milk quality and microbiota in milk is very important. In current studies, various microbes are found and isolated. The bacteria present in goats' udder were determined using gram dye, nuclear nucleic acid technology, and direct 16S rRNA gene sequencing. For quantitative estimation of bacteria count, dielectric spectra were used.

In conclusion, various microbes found in goat's udder and isolated microbes were *Staphylococcus succinus, S. lentus, S. xylosus, S. equorum, S. stepanovicii, S. fleurettii, S. sciuri, S. kocurii, Cohnella suwonensis, C. yongneupensis,* and *C. faecalis*. This study emphasizes the importance of improved hygienic procedures in ensuring food safety by emphasizing the importance of avoiding contamination and using the appropriate types of processing equipment.

Availability of Data and Materials

The authors declare that the data and materials are available on request from the corresponding author (M.M.M. Ahmed).

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Ethical Statement

This study was approved by the King Abdulaziz University Local Ethics Committee (Approval no: 9-15, 2021).

Competing Interest

The authors declared that there is no competing interest.

Author Contributions

F.A.A.: Desing of experiment, Mothdolgy, Lab. Collect samples; M.M.M.A.: Written MS and review; A.M.A.: Collect samples, Lab. and review; S.K.: Reviewer MS

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