Egyptian Journal of Veterinary Sciences

https://ejvs.journals.ekb.eg/

Prevalence and Bacterial Isolation Causing Clinical and Subclinical Mastitis in Egyptian Dairy Cow in Kafer El-Sheikh, Egypt

Mohamed A. Abu El-Hamd[1*], Mohamed A. Egila[1], Nabeel B. Elgaml[1], Mohamed L. Salem[2], Saeed El-Ashram[3] Soliman H. Soliman[1] and Magdy E. Mahfouz[3]

[1]*Animal Production Research Institute, Agricultural Research Center, Ministry Of Agriculture, Giza, Egypt.*
[2]*Immunology and Biotechnology Unit, Zoology Department, Faculty of Science, Tanta University, Tanta, Egypt.*
[3]*Faculty of Science, Kafrelsheikh University, Kafr El-Sheikh 33516, Egypt.*

Abstract

THE prevalence of mastitis in Holstein Friesian cows was investigated. Culture-independent DNA-based techniques were used to analyze the isolated milk microbiota from mastitic cows. Among the 133 cows evaluated, subclinical (SCM) and clinical mastitis (CM) afflicted roughly 13.5% (18/133) and 20.3% (27/133), respectively. The isolated microbiota was dominated by gram-positive bacteria like *Escherichia coli* and *Staphylococcus aureus.* A high throughput sequencing platform identified *Firmicutes*, *Proteobacteria, Bacteroidetes*, and *Actinobacteria* as the most prevalent phyla of bacteria. The most often occurring genera were *Bifidobacterium* and *Lactobacillus*. In the same environment, *Lactococcus, Acinetobacter, Staphylococcus aureus, Streptococcus agalactiae,* and *Coagulase-negative staphylococci*-different bacterial species with higher potential roles in mastitis-were found. The scattering behaviour of several samples in PCoA plots-beta diversity and Alpha diversity indices demonstrated the mastitis microbiota's considerable diversity. Season, lactation, and infection all affected the alpha diversity of the milk microbiota in Egyptian cows; four dominant phyla were found, and despite the presence of distinct bacterial communities in infected samples, mastitis did not significantly change alpha diversity. In conclusions, the present study illuminates the prevalence of mastitis in Egyptian dairy cows, their microbiota and risk factors for mastitis. The findings can be reduce mastitis and improve dairy cow health and productivity.

Keywords: Mastitis, Dairy Cows, Microbiome, Risk Factors.

Introduction

Bovine mastitis is a condition typified by the persistent and inflammatory reaction of the udder tissue due to either physical trauma or infections caused by microorganisms [1]. Major forms of mastitis are subclinical (SCM) and clinical mastitis (CM) based on various factors such as season and nutritional conditions [2]. The CM is characterized by sudden onset of redness, swelling, heat, and pain in the diseased affected milk quarter, leading to a significant reduction in lactation, physiological changes thinning and yellowing of milk, symptoms of the flocculent material, and elevated body temperature [3,4]. Several factors strongly correlate with the condition, including season, fecundity, lactation, nutritional conditions, environmental health, and feeding management [5]. Usually, due to broken physical barriers in the mammary region, the disease develops when harmful bacteria enter the germ-free environment of the mammary gland. It takes appropriate host defences to stop colonization and the pathophysiology of ensuing diseases [6]. Various groups of microbes can colonize cows' mammary quarters and have evolved mechanisms that facilitate their proliferation, leading to clinical mastitis. Although bacteria are the main cause of mastitis, other microbes like archaea, viruses, and fungi might also be associated with the condition [7, 8]. Dysbiosis of the milk microbiota can arise during mastitis when opportunistic pathogenic bacteria proliferate and beneficial commensal bacteria decline [9]. Studies of the microbiota linked to bovine mastitis have, up to now, primarily focused on the isolation and characterization of specific pathogens [10]. Because of its variety of epidemiology, bovine mastitis occurs and spreads in different ways. Mastitis is classified as infectious and environmental

*Corresponding author: Mohamed A. Abu El-Hamd, E-mail: abuelhamd68@yahoo.com. Tel.:00201099033847
(Received 05 July 2024, accepted 10 October 2024)
DOI: 10.21608/EJVS.2024.301946.2231

[10]. Pathogens mostly present in the udder of sick cows are the source of infectious mastitis, which can spread from cow to cow during milking or other activities [11]. Most infectious agents include *Mycoplasma* species, *Corynebacterium bovis*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, and *Staphylococcus aureus*. Conversely, the bacteria *Escherichia coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Enterobacter aerogenes*, *Streptococcus dysgalactiae*, and *Streptococcus uberis* found in the cow's bedding, feed, and water are what induce environmental mastitis and by way of the teat canal, these germs might infect the udder [10]. A pervious study was conducted in Egypt collected 116-quarter milk samples from 29 cows to detect subclinical mastitis and found that 44.83% of the cows were subclinically mastitis, with *Staphylococcus aureus*, coagulase-negative Staphylococci, *Streptococcus* spp, *E. coli*, and *Aspergillus fumigatus* being the isolated microorganisms [12]. One thousand sixty-quarter milk samples were collected from 270 apparently healthy cows in three farms and examined in North Upper Egypt. The total prevalence of SCM was 46% and 44.8% based on the California Mastitis Test (CMT) and Somatic Cell Count (SCC), respectively. Bacteriological examination of CMT-positive quarters revealed that the prevalence of bacterial isolation in subclinically mastitic quarters was 90.4%. The most frequent bacterial isolates were *E. coli*, *Staphylococcus aureus*, Streptococci, and non-aureus staphylococci [9, 13].

Since its introduction ten years ago, culture-independent DNA-based mastitis diagnostic techniques have been commercially available in several nations [14]. These approaches frequently yield more species in the PCR test findings than can be found by traditional culture [14]. Critical evaluation of the results acquired by these techniques is necessary to guarantee their correctness and clinical applicability [15, 16]. Sequencing of the 16S rRNA gene is the most often used technique to investigate the milk microbiome; this technique has been applied in research on bovine mastitis [17-20]. The conventional wisdom regarding cow mastitis is that one or two bacteria species are responsible for the infection. Rather, a novel theory on the mammary gland's potential "dysbiosis" has been put out as a risk factor for mastitis and intramammary infection (IMI). Milk from quarters with mastitis contains a microbial diversity of a large range of taxa. Bacteria burden in Mastitic quarters is more than in healthy quarters [20]. Most microorganisms described in this research are entirely novel in terms of the phylogeny of microbial agents that cause mastitis. Even more varied than in quarters with clinical mastitis is the milk microbiota in bovine mammary quarters free from intramammary infection and inflammation, with a low milk somatic cell count [19-21]. The therapeutic relevance of these results in the milk microbiota is yet unknown, and more study is required to fully comprehend the consequences of these various microbial communities in bovine milk [21, 22]. The current work attempts to discover how common clinical and subclinical mastitis is in dairy cows in the province of Kafr El-Sheikh. The study aims to determine the cow and herd risk factors linked to mastitis and investigate the microbiota of clinical mastitis.

Material and Methods

Farm characterization

The microbiota and milk samples from a farm in the Egyptian governorate of Kafr El-Sheikh were analysed metagenomically. The cows were Local Holstein Friesian (HF) Egyptian local breed.

Samples of the study

Samples were taken from 133 Holstein Friesian dairy cows using standard operating protocols on these farm animals. Professional milkers prepared the udders as normal before taking milk samples. The first several milk strips were thrown away, the teat ends were cleansed with water and then with an alcohol swab, and a sample from each quarter was taken into a sterile cub per teat. The cows underwent clinical assessment.

The prevalence of mastitis

The samples were manually mixed with the commercial California mastitis test (CMT) reagent by a plastic rod. The condition of milk at each well was observed following the scale: negative (no change), +1 (thick slimy), +2 (thick lumpy) and +3 (thick gel) precipitation [23].

Heat maps, Venn diagrams, circle diagrams and bar graphs were used to evaluate gamma diversity. Using this knowledge, one can investigate the functional function of the bacteria in milk samples and create methods to control the bacterial population to raise milk quality and stop disease spread by milk.

Milk electrical conductivity determination

The udder abnormal condition of cows was recorded as the presence of any signs of inflammation such as pain, fever, redness and swelling. The electrical conductivity of milk was determined by a Hand-held EC meter (Draminski mastitis detector, Poland). The readings of the detector were interpreted based on readings below 250, 250 to 300 and above 300 units were SCM, CM and normal, respectively, provided by the manufacturer's manual [24].

Milk microbiological determination

Gathered milk in sterile containers, the samples were transported to the microbiological laboratory at 4 °C and then stored at -80 °C for further study. The samples were grown on a selection of media, such as

nutrition agar, Mackongy agar, blood agar, Ss agar, mannitol salt agar and Edward agar. Plates of culture were aerobically incubated at 37 °C for 48 h. After removing the tainted plates, every colony was stained and verified as positive or negative. Gram-positive bacteria were discovered on salt agar, and gram-negative bacteria on MacConkey agar. Catalase and coagulase tests were performed using blood sheep-blood agar as well as Bacillus spp. using Gram staining. The antibiotic sensitivity test of milk bacteria was examined using different antibiotics as Gentamycin (GN), Sulbactam/Ampicillin (SAM), Azithromycin (AZM), Norfloxacin (NOR), Rifampicin (RF), and chloramphenicol (C) [25]. The heatmap is a semi-quantitative method that provides a valuable snapshot of the relative abundance of bacteria in milk samples in a range between high and low abundance.

Genomic DNA extraction

We extracted sample DNA with SDS and CTAB. One per cent agarose gels were used to assess DNA purity and concentration. DNA was diluted with sterile water to 1 ng/μL after determining concentration [26].

Amplicon generation

Distinct regions of the 16S rRNA genes were amplified using specific primers (341F: CCTAYGGGRBGCASCAG; 806R: GGACTACNNGGGTATCTAAT) along with barcodes [27].

PCR reactions were performed in 30 μL volumes, with 15 μL of Phusion® High-Fidelity PCR Master Mix (New England Biolabs), 0.2 μM of forward and reverse primers, and approximately 10 ng of template DNA. The thermal cycling protocol included an initial denaturation step at 95 ℃ for 1 min, followed by 30 cycles of denaturation at 95 ℃ for 10 s, annealing at 50 ℃ for 30 s, and elongation at 72 ℃ for 30 s. A final extension step was performed at 72 ℃ for 5 min. [26].

Mixing and purifying PCR products

An equal 1 X loading buffer volume containing SYB Green was mixed with the PCR products. The resulting mixture was electrophoresed on a 2% agarose gel to detect and visualize DNA bands. Following the gel electrophoresis, the mixture of PCR products was purified using the GeneJETTM Gel Extraction Kit from Thermo Scientific [28].

Library preparation and sequencing

Following Thermo Scientific directions, the Ion Plus Fragment Library Kit 48 rxns created sequencing libraries. The Thermo Scientific Qubit@ 2.0 Fluorometer assessed library quality after preparation [29].

Data analysis

Single-end reads quality control

Data split

Samples were linked to single-end readings via unique barcodes. Barcode and primer sequences were truncated to get data ready for analysis. To leave genomic sequences of interest, barcode and primer sequences were deleted from reads [30].

Data filtration

Quality filtering was done to raw reads to provide clean reads. According to Cutadapt, this quality filtering process followed particular filtering parameters (V1.9.1,http://cutadapt.readthedocs.io/en/stable/)[31]

Chimera removal

We compared the measurements to the Silva database (https://www.arb-silva.de/) using the search tool (https://github.com/torognes/vsearch/) [32, 33]. Furthermore, chimeric sequences were eliminated from the sample [34]. Chimeric sequences were removed after the readings were compared to the reference database. We collected and got ready for analysis of the remaining clean readings.

Operational Taxonomic Units (OUT) cluster and species annotation

OTU production

The sequences were analyzed with UPARSE (v7.0.1001, http://drive5.com/uparse/) [35]. Sequences grouped into the same OTUs shared a minimum of 97% similarity. Sample sequencing from every OTU was annotated.

Species annotation

The Mothur algorithm was used to annotate each representative sequence's taxonomic information from the Silva Database (https://www.arb-silva.de/) [32].

Phylogenetic relationship construction

Multiple sequence alignment utilizing MUSCLE software was used to study evolutionary relationships between Operational Taxonomic Units (OTUs) and dominant species differences across samples or groupings (Version 3.8.31, http://www.drive5.com/muscle/)[36].

Data normalization

Operational Taxonomic Units (OTUs) abundance data was normalized using the sample with the fewest sequences. This normalized data was used for all alpha and beta diversity assessments.

Alpha diversity

Observed species Chao1, Shannon, Simpson, ACE, and Good-coverage alpha diversity indices were used to assess sample species diversity

complexity. The indices were generated using QIIME (1.7.0) and visualized using R (2.15.3). Shannon and Simpson's indices measured diversity, while Chao1 and ACE measured richness. Sequence depth was also measured using Good's coverage index, Coverage. The URLs have index documentation: Chao1, ACE, Shannon, Simpson, and Coverage are available at http://www.mothur.org/wiki/Chao, Ace, Shannon, Simpson, and Coverage.

Beta diversity

To compare species complexity, a beta diversity study was performed using QIIME (Version 1.7.0). Weighted and unweight UniFrac distances were determined. Cluster analysis reduced variable dimensionality after principal component analysis (PCA). The FactoMineR and ggplot2 R packages (2.15.3) were used. PCoA was used to visualize complex, multidimensional data and get primary coordinates. A distance matrix from weighted or unweight UniFrac sample distances was translated into orthogonal axes. The first principal coordinate indicates the largest variation factor, the second principal coordinate represents the second maximum variation, etc. The WGCNA, stat, and ggplot2 programs in R (Version 2.15.3) showed PCoA analysis findings. An Unweighted Pair-group Method with Arithmetic Means (UPGMA) hierarchical clustering was used to interpret the distance matrix using average linkage. The clustering analysis was done using QIIME (1.7.0).

Results

Reproductive performance

The prevalence of mastitis was ascertained by measuring the amount of milk precipitation after mixing with the CMT reagent using the Californian mastitis tests (CMT) and the NaOH. They demonstrated that the degree of precipitation that a solution of the reagent and milk produced matched the number of cells in the milk. Forty-five cows were confirmed to be either clinically or subclinically mastitis. Estimates of the prevalence of SCM were 20.3% (27/133) and CM of 13.5% (18/133). Because the diagnosis and treatment of mastitis depend on accurately identifying the bacterial species, milk samples were microbiologically examined. Table 1 illustrates the greater incidence of Gram-positive bacteria than of Gram-negative bacteria. The most often occurring Gram-positive bacteria were *Staphylococcus aureus*, *Streptococcus uberis*, *Streptococcus agalactiae*, and *Enterococcus fecalis*. Among the Gram-negative germs in the samples were *Klebsiella pneumoniae* and *E. Coli*. In addition, the Coagulase test separated coagulase-negative staphylococci from S. aureus. In this test, plasma fibrinogen clumps and forms a clot. Of all lactating dairy cow bacteria tested, 54.16% (26/50) were coagulase-positive (Table 2). The catalase test strongly suggests S. aureus. Conversely, a negative Catalase test may reveal additional streptococcal or staphylococcal species. The catalase test tests its ability to convert hydrogen peroxide into oxygen and water. While most *Streptococcus* species lack catalase, most *Staphylococcus* species do. Thus, 52.08% (25/48) of isolated bacteria were catalase-positive. Crucially, the sensitivity test determines how well antibiotics work against isolated bacteria. Most harmful to milk bacteria was chloramphenicol (C), followed by gentamycin (GN), rifampicin (RF), norfloxacin (NOR), and sulbactam/ampicillin (SAM). Chloramphenicol (C) was thus the antibiotic with the highest sensitivity (Table 2).

Table 3 shows composition and electrical Conductivity changes in milk of cows were examined in present study summarized in table 3 was showed–that the electrical conductivity of 27 cows (20.3%) was in the range of 250:300 units, which indicates the presence of SCM. While the CM appeared at 18 (13.53%) cows (< 250 units) and 88 (66.17%) cows were healthy (> 300 units). Milk yield, fat, protein, lactose and total solid percentage were significantly higher in normal cows than in subclinical and clinical mastitis.

Twelve bacterial isolates were selected for more investigation to find out what makes up their milk microbiota. Sample numbers (A6, A7, A8, and A9) are shown on the graph's x-axis, and sequences are listed on its y-axis from 0 to 125000 count. The line graph shows how widely the sequence counts differ among the samples; sample A6 has the highest sequence count (105800), and sample A7 has the lowest sequence count (93482) (Figure 1).

Alpha diversity dominance refers to a community or ecosystem where a small number of species strongly influence (Figure 2). This is very different from gamma diversity, which shows diversity across ecosystems or groups, and beta diversity, which measures variation in species composition. A few species dictate the number of species in an alpha diversity-dominating community or ecosystem. Biotic interactions, resource availability, and climatic circumstances can all play a role in triggering it. Chao1index measures the sample's total sequence count despite missing sequences or species richness. Observed features count sample unique sequences. The species richness and unique sequences were more similar in (A9 / A8) and (A7 / A6). Good coverage indicates how well the sample represents sequences overall. A7 has the highest goods coverage, indicating a good population representation. A higher dominance index means a few sequences are prevalent, and the others are rare. The graphs indicate the frequency of sequences vs their number. The first graph shows several sequences that exist exactly once in the sample; the second graph shows the number that appears exactly twice, etc. The last graphic shows sequences

in the sample five or more times. As sequence frequency increases, the graphs show fewer sequences.

Differently measured diversity is Simpson and Shannon's indices. Common sequences are favoured by the Simpson index and unique sequences by the Shannon index. Simpson: An indicator of diversity evaluating sample sequence distribution. The Simpson indices in some samples (more than ten) significantly increased from A7 to A8. Further, for sequences ≈ 92000, Simpson indices of A8, A6, A9, and A7 obtained maximum values of 0.95, 0.93, 0.91, and 0.16. Shannon is a diversity index derived from the samples' distribution evenness and unique sequences. About the Shannon index, A8 has the highest. The figure 2 was much more than A9 for samples ≥11000 and A7 for sequences ≥10. A6 and A8 differed not much from one another. Shannon indexes for A8, A6, A9, and A7 peaked at 5.7, 5.5, 5, and 2.7 for 90100 sequences (Figure 2).

Beta diversity was accomplished using a Principal Coordinate Analysis (PCoA) scatter plot to compare the compositional differences between two bacterial communities, PC1 and PC2. The percentages in parentheses next to each label indicate the amount of variance in the data explained by that principal component. Also, inside the rectangular coordinate system, the smaller the separation samples' distances, the higher the similarity. The results showed that it represents about 52.29% of the variance in the data, while PC2 represents about 31.54%. This means that the first two principal components explain over 80% of the variation in the data. Besides, the scatter plot shows that the two bacterial communities are well separated, with the red dots (community 1/sample 6) clustered on the left side of the plot and the blue dots (community 2/sample 7) clustered on the right side (Fig.3). This suggests that the two communities are very different in bacterial composition.

The eleven sectors that comprise the circular pattern in Figure 4 each stand for a phylum of bacteria. Section sizes show how many species are in the sample. Phylum abundance is shown by color-coding; pale pink indicates the most and medium blue the least. The genera are listed by phylum in the center text of the diagram. First in the sample are Firmicutes, followed by Proteobacteria, Bacteroidota, and Actinobacteriota. These four phyla account for more than 90% of the bacteria in samples, serving the most important roles in bacterial communities. Less numerous are the Campilobacterota, Crenarchaeota, Naroarchitects, WPS-2, Cyanobacteria, Desulfobacterota, and Patescibacteria.

The Venn diagram shows the dominant phyla of bacteria and the number of operational taxonomic units (OUTs) of bacteria that belong to multiple phyla (Fig. 5). It was observed that the most abundant OUTs found in all samples were 3. Also, the overlapping indicates the OUTs shared in multiple samples. The most observed overlapping OUTs was 24 between A7 and A9, 22between A9 and A8 as well as 21 between A8 and A7. Furthermore, the least overlapping was observed between A6 and A9 (4), A8 and A6 (7) as well as A6 and A7 (10).

The circles represent the different samples, and the numbers in each circle represent the number of OTUs in that sample. The areas where the circles overlap represent OTUs that belong to multiple samples.

Figure (6A) shows the heatmap of phyla and genera present in all samples. Samples showed high abundance different genera of bacteria include Fermicutes (*Lactobacillus*, *Metamycoplasma*, *Streptococcus* and *Veillona* sp.), Actinobacteria (*Gardnella* sp and *Rothia* sp.). Proteobacteria (*Acinobacter*, *Psychrobacter*, *Haemophilus* sp.) and Bacteroidata (*Sphingobacterium*, *Porphyromonas* and *Chryseobacterium* sp.) The second heatmap showed that the most abundant bacterial species present in milk samples are: *Lactococcuslactis, Streptococcus thermophiles, and Lactobacillus delbrueckii subsp. Bulgaricus, Lactobacillus helveticus, Pseudomonas fluorescens, Acinetobacter baumannii, Serratiamarcescens, Enterococcus faecalis, Escherichia coli, Staphylococcus aureus,* and *Klebsiella pneumoniae.* The heatmap also shows a great deal of variation in the abundance of different bacterial species in the milk samples. For example, *Lactococcus lactis* is highly abundant in all of the samples, while *Listeria monocytogenes* is very low in abundance in most of the samples (Figure 6B).

Furthermore, demonstrated by the bar plot is the variation in the relative abundance of several bacterial species according to the sample. *Lactobacillus* relative abundance, for instance, is greatest in sample A8 and lowest in sample A9. Samples A8 and A6 have the lowest relative abundance of *Clostridia_UCG-014* and sample A9 the highest. The gut microbiota of various samples varies greatly, as the graph likewise demonstrates. For instance, whereas it is hardly noticeable in the other samples, the species *Cellvibrio* is extremely abundant in the sample designated A9. Generally speaking, each dairy cow has a different gut flora (Fig. 7).

Discussion

The present study examined involved exam dairy cattle in Kafr El-Sheikh governorate Egypt. To investigate bovine mastitis and its internal and external causes. Multiple risk factors compound mastitis in dairy cows. Knowing these factors and taking targeted preventive measures can help dairy farmers ensure sustainable milk output, reduce

mastitis and improve cow health. The incidence of CM was found to be about 13.5% (18/133) and SCM to be 19.5% (26/133) in dairy cattle. It is important to remember that CM prevalence differs inside. El Oro Province, Ecuador, for example, has a prevalence of 12% [37]; China, 3.3%; Ethiopia, 12.5%; and India, 11.5% [38]. Moreover, this study revealed that 19.5% of the cattle examined in Kafr El-Sheikh, Egypt, had SCM. Furthermore, pertinent is the fact that the prevalence of SCM differs in African nations such as Tanzania (48.8%) [39] and Rwanda (50.4%) [40], as well as in Poland (36.7%) and Brazil (46.4%) [41]. These results imply that clinical and subclinical mastitis need ongoing study and attention as it is a serious worldwide problem. The prevalence of mastitis in Kafr El-Sheikh, Egypt, as found in present study is lower than recorded in several earlier studies, including those in Assosa town 39.32% [42, 43] and southern Ethiopia 40.4% [44], Hawassa and Wando Genet 63.11% [45], Adama 46.7% [46], Haramaya district 63.02% [47], Holeta town of central Ethiopia 71.05% [48], and around Addis Ababa 74.7% [49]. Still, our results are greater than the prevalence in southern Ethiopia (32.92%), Min Wolayita Sade (29.5%), and Bahir Dar (28.8%) [50]. Many reasons could be responsible for the variations in prevalence rates reported in different research, such as variations in management methods, surroundings, and diagnostic methods. More precisely, differences in cattle breeds, agroclimatic areas, and management practices could be responsible for the disparities in mastitis prevalence rates. Therefore, these elements must be considered when analyzing and contrasting prevalence rates from several research studies. In present study, the prevalence of CM is 13.5%, and SCM's is 19.5%, much higher than clinical instances. This is in line with other study [51] that found a clinical frequency of 3% and subclinical cases of 25.2% in Bahir Dar and its environs. CM is usually less common than SCM [52- 57]. This might be why SCM instances are frequently overlooked; infected animals may not exhibit overt signs and keep secreting milk that appears normal. Consequently, small-scale farmers could not be conscious of the unseen costs connected to SCM. Treatment of CM cases has always gotten more attention in Egypt than subclinical forms of mastitis. This emphasizes the significance of raising knowledge and instruction on the effects of SCM on dairy herds and the value of early identification and action.

Data of present study found that chloramphenicol, Sulbactam/Ampicillin, Gentamycin, Rifampicin, Norfloxacin, and Azithromycin worked on mastitis-causing bacterial isolates from milk samples. This result suggested that medicines can treat dairy cow mastitis. This investigation confirms previous findings that mastitis bacteria are sensitive to Ciprofloxacin, Norfloxacin, Gentamycin, and Chloramphenicol [58, 59]. However, shows that bacterial pathogens causing mastitis are resistant to chloramphenicol, gentamicin, cephalosporins, tetracyclines, vancomycin, penicillin, erythromycin, and ciprofloxacin across regions [60-63]. These results showed that suggests that geographic location, bacterial type, and antibiotic use may alter mastitis bacteria antibiotic susceptibility. Understanding the efficacy of antibiotics for mastitis in dairy cows affects its treatment and control. The present study examined the milk microbiota of twelve bacterial isolates using culture-independent DNA methods. This approach allows a deeper understanding of milk's microbial variety because bacterial cultivability does not limit it. Previous research has examined milk microbiota using culture-dependent and culture-independent methods [64]. A study studied microbes in raw milk from goats, sheep, cows, and people [65]. Milk may support a diversified microbiota due to its high nutritional content; thus, culture-dependent and culture-independent approaches were used. However, some studies have focused on culturally dependent methods. This approach may only partially represent the milk microbiota despite providing valuable information on cultivable bacteria. In previous bovine mastitis studies, milk samples from mastitis and healthy cows had different alpha diversity. Alpha diversity was substantially higher in healthy than mastitic quarters but not statistically different. *Firmicutes*, *Proteobacteria*, *Bacteroidota*, and *Actinobacteriota* dominate the milk microbiome [66, 67]. Contrary to certain findings [66, 68], symptomatic or subclinical mastitis does not affect milk diversity.

Principal coordinate analysis (PCoA) revealed significant changes in bacterial composition in samples of milk from cows with mastitis. Similar findings were obtained in earlier PCoA studies contrasting the milk microbiomes of healthy and mastitic cows [69]. Other studies that have discovered higher bacterial community overlap or clustering in milk samples suggest less bacterial composition variance. Variances in sample methods, sequencing platforms, data analysis, and environmental factors could be the reason for these differences [70- 73].

Patel *et al.* [74] observed that *Firmicutes* (57%) and *Proteobacteria* (16%) caused mastitis in India. *Firmicutes* and *Proteobacteria* are two of the principal pathogenic phyla. Several *Firmicutes* and *Proteobacteria* species can cause mastitis, affecting milk output, quality, and the dairy sector. Another study by Khasapane *et al.* [75] found that 97% of dairy mastitis cow bacteria in Free State Province, South Africa, belong to four phyla: *Actinobacteriota*,

Bacteroidota, *Firmicutes*, and *Proteobacteria*. Results supports present research bacterial profile and highlight the global importance of certain bacterial phyla in mastitis.

According to strong scientific data, *Lactobacillus* is a widespread and important genus of bacteria involved with SCM and CM in dairy cows. Depending on species, strain, and habitat, *Lactobacillus* can benefit or harm dairy cows. Probiotic *Lactobacillus* species and strains can improve host health by regulating gut and mammary microbiota, boosting the immune system, and blocking pathogenic bacteria [76,77]. *Lactobacillus plantarum* 17-5 reduces *E. coli*-induced inflammation in bovine mammary epithelial cells and nursing animals via suppressing NF-κB and MAPK signaling pathways [78]. *Lactobacillus sakei subsp. sakei* and hawthorn extract supplements increased common carp growth, digestive enzymes, immunity, and acetamiprid resistance [79].

These studies show that without medications, Lactobacillus may prevent and treat mastitis and other diseases in dairy cows and animals. Particular types and strains of Lactobacillus could be harmful to the host. Especially if their immune systems are compromised, or the bacterial burden is excessive, dairy cows can get mastitis or other illnesses from Lactobacillus [78]. Isolated from milk samples of cows suffering mastitis, *Lactobacillus fermentum, brevis, plantarum, paracasei, rhamnosus, pentosus, casei, raffinolactis,* and *mesenteroides* showed antibacterial activity against the main mastitis pathogen, *Staphylococcus aureus* [80]. Data from previous study imply that Lactobacillus may be a pathogen or an opportunistic pathogen in dairy cows and other hosts and that identifying and characterizing it is essential for mastitis diagnosis and treatment. Several bacterial species are involved in dairy fermentation procedures, such as the production of cheese and yoghurt and mastitis, an infection of the mammary gland in dairy cows. In dairy fermenting, gram-positive bacteria like *Lactococcus lactis, Streptococcus thermophilus, and Lactobacillus delbrueckii subsp. bulgaricus, Lactobacillus helveticus* and *Enterococcus fecalis* can result in mastitis depending on frequency and antibiotic resistance. Common in soil and water, gram-negative bacteria include *Pseudomonas fluorescens, Acinetobacter baumannii, Serratia marcescens, E. coli,* and *Klebsiella pneumoniae* can contaminate dairy products, induce mastitis, and resist many antibiotics. The facultative anaerobic bacteria *Staphylococcus aureus* on skin and mucous membranes is the cause of chronic mastitis resistant to antibiotics. The efficiency of dairy fermentation, the comfort of the animals, and milk quality depend on our understanding of these microbes [81- 83].

Conclusion

The SCM was 13.5% and CM 19.5% in Egypt's Kafr El-Sheikh Egypt. Gentamycin, Sulbactam/Ampicillin, Azithromycin, Norfloxacin, Rifampicin, and chloramphenicol all act on *Lactococcus, Acinetobacter, Pseudomonas, Enterococcus, Streptomyces, Mycoplasma, Staphylococcus aureus, Streptococcus agalactiae, Coagulase-negative staphylococci, Enterobacter, Ruminococcus, Flavobacterium, Escherichia-Shigella, Delftia, Ruminococcus torques* group, and *Actinomyces* spp. Present study on Holstein Friesian cow mastitis in Kafr El-Sheikh, Egypt, constrained by its small sample size and regional breadth. Researching risk, productivity, and environmental aspects may help prevent and treat mastitis. Through an analysis of the prevalence and microbiota of mastitis, this study will help to develop strategies to enhance milk quality and lower milk-borne infections in the Kafr El-Sheikh region of Egypt. Research should include risk factors, economic consequences, biomarkers, subclinical forms, and treatment/preventive methods to understand and treat mastitis better. The findings are specific to the farm studied and underscore the need for broader, multi-farm studies to accurately assess the prevalence, microbiome, and risk factors for mastitis in dairy cows nationwide.

Acknowledgements

We are grateful for the support provided by the laboratory personnel at the Sakha Animal Production Research Station while conducting the sample collection and parameter assessments.

Funding statement

No fund.

Conflict of Interest

No conflict of interest to declare.

Ethical statement

The Ethics Committee of Animal Production Research Institute, Agricultural Research Center approved all procedures involving animal subjects under protocol number ARC092017. The study adhered to the institutional and national guidelines, ensuring all efforts were made to minimize suffering and distress.

TABLE 1. Number of Gram-positive and Gram-negative bacteria in the milk samples collected from cows with mastitis.

Bacteria type	*Streptococcus agalactiae*	*Staphylococcus*	*Bacili sp.*	*Salmolella* and *shegilla sp.*	*Escherichia coli* and *Klebsiella*
Gram positive bacteria	30	36	58	8	8
Gram negative bacteria	27	29	6	0	57
Total	57	65	64	8	65

TABLE 2. Results of antibiotic sensitivity, Coagulase and Catalase test.

Bacteria	Antibiotic sensitivity						Coagulase test	Catalyse test	%
	GN	SAM	RF	AZM	NOR	C			
Coagulase-positive staphylococci	2	2	3.5	1	4	2	Positive	Positive	11.4
Coagulase-negative staphylococci	2	3	2	0	2	3	Negative	Positive	1.9
Streptococci	2.3	3.0	2.0	1.0	3.3	2.5	-----	Negative	12.4
Bacillus spp	2	3	2	175	3	3	-----	Negative	1.1
E-coli	1.7	2.6	1.8	2.8	3	2.5	-----	-----	25.5
Salmonella Shigella	175	1.5	1.3	00	3.0	3.0	-----	-----	0

Gentamycin (GN), Sulbactam/Ampicillin (SAM), Rifampicin (RF), Azithromycin (AZM), Norfloxacin (NOR) and chloramphenicol (C).

TABLE 3. Effect of Clinical or subclinical status on milk yield and composition and electrical Conductivity

Items	Milk yield	electrical Conductivity (units)	pH	Total solid	Fats	Proteins	Lactose
Sub clinical	15.60^{b}	269.14	6.64	10.15^{b}	3.03^{b}	2.81^{b}	4.33
Clinical	12.19^{b}	231.50	7.11	10.08^{b}	2.93^{b}	2.66^{b}	4.15
Normal	20.43^{a}	333.00	6.57	12.27^{a}	3.53^{a}	3.27^{a}	4.80

$^{a \text{ and } b}$: Means within the same row with different superscripts are significantly different (P<0.05).

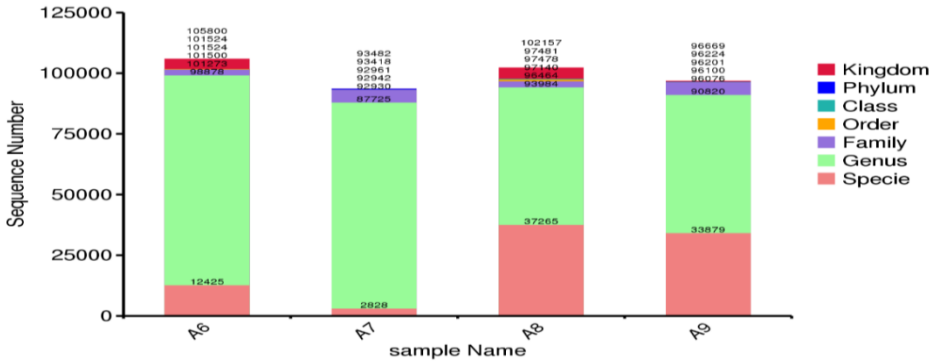


Fig. 1. Overview of the number of sequences in each sample. The x-axis shows the sample number (A6, A7, A8, and A9). The y-axis shows the number of sequences, from 0 to 125000.

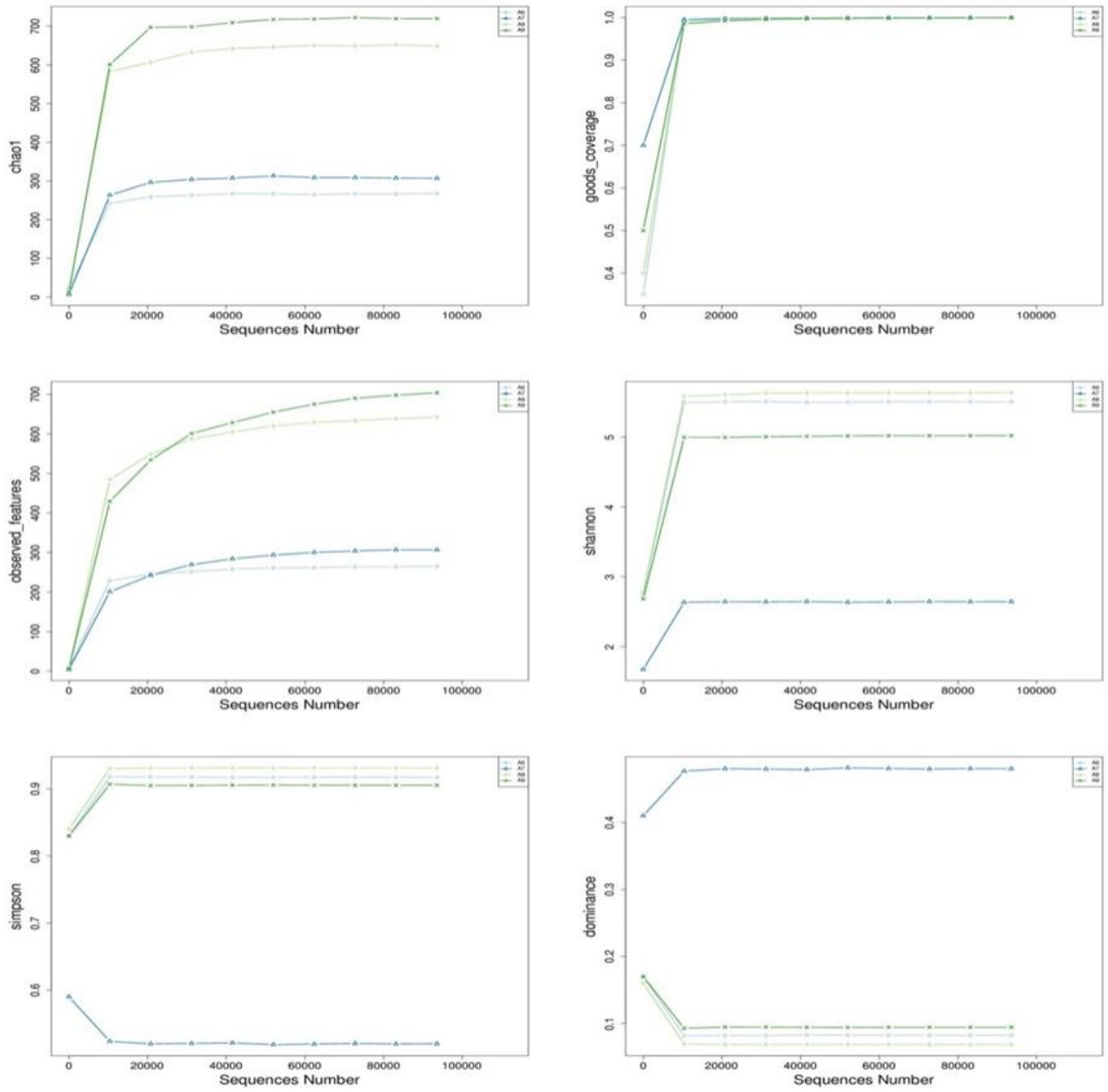


Fig. 2. Alpha Diversity Indices for comparing sample diversity. Plus (A8), Circles (A6), X (A9) and Triangles (A7).

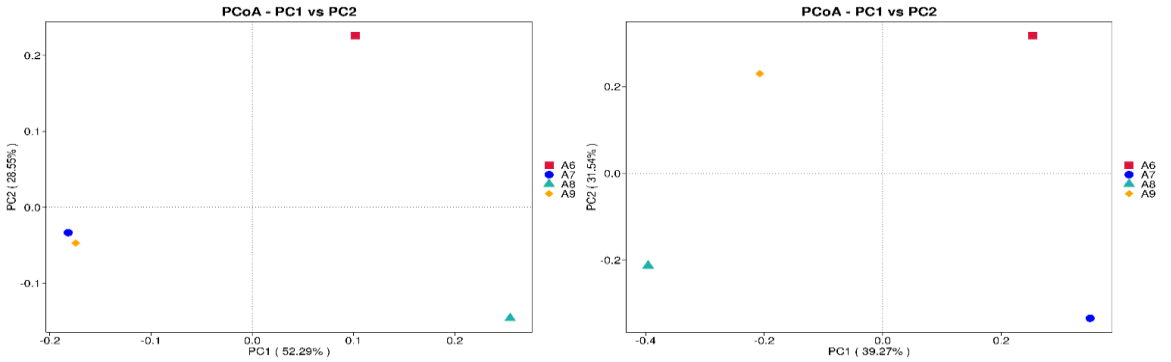


Fig. 3. Principal Coordinate Analysis (PCoA) reveals divergent bacterial communities

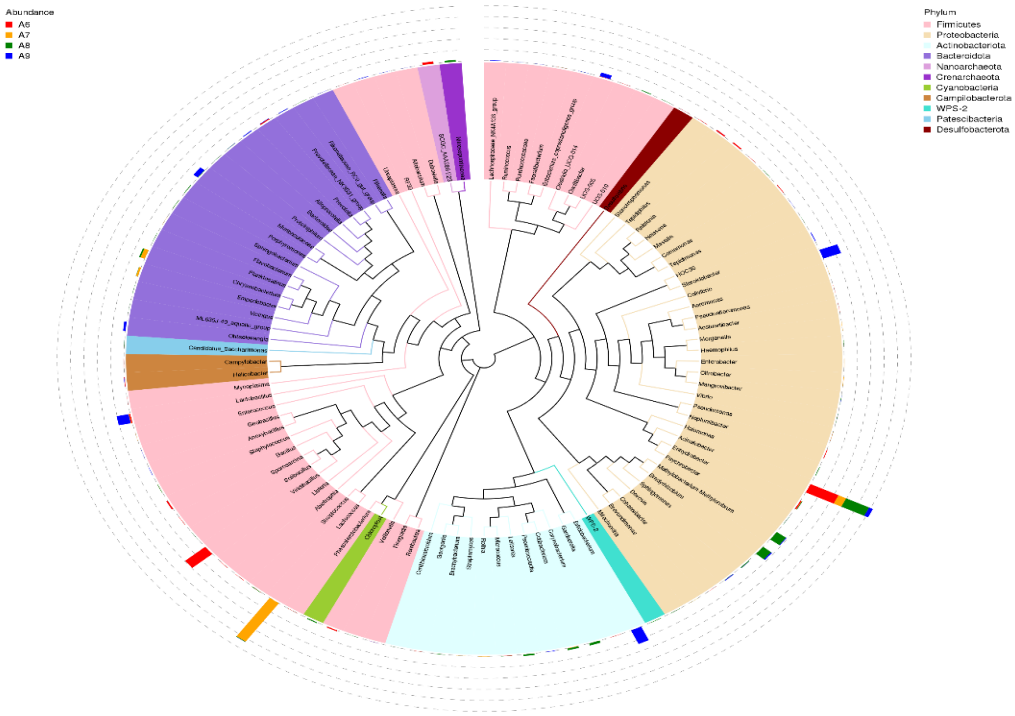


Fig. 4. Circular diagram of the distribution of different phyla of bacteria

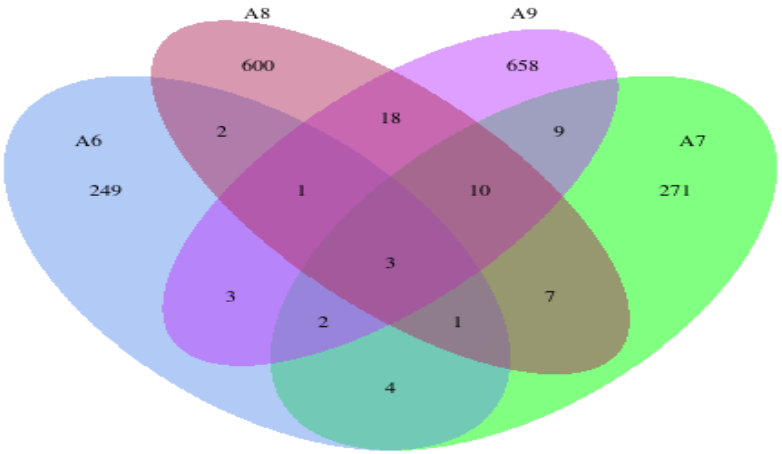
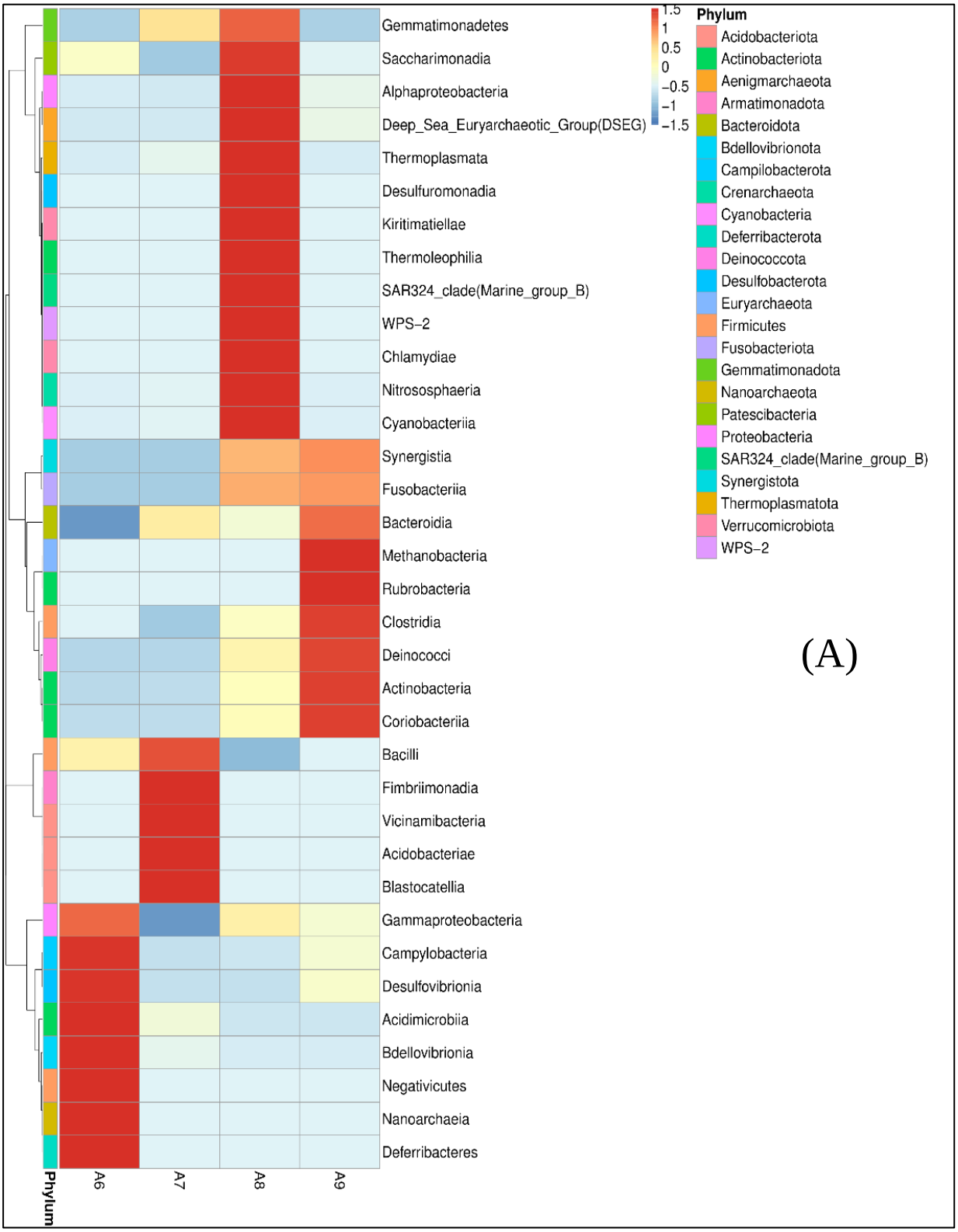


Fig. 5. Venn diagram showing the distribution of OTU (operational taxonomic unit) numbers of bacteria.


Gemmatimonadetes
Saccharimonadia
Alphaproteobacteria
Deep_Sea_Euryarchaeotic_Group(DSEG)
Thermoplasmata
Desulfuromonadia
Kiritimatiellae
Thermoleophilia
SAR324_clade(Marine_group_B)
WPS−2
Chlamydiae
Nitrososphaeria
Cyanobacteriia
Synergistia
Fusobacteriia
Bacteroidia
Methanobacteria
Rubrobacteria
Clostridia
Deinococci
Actinobacteria
Coriobacteriia
Bacilli
Fimbriimonadia
Vicinamibacteria
Acidobacteriae
Blastocatellia
Gammaproteobacteria
Campylobacteria
Desulfovibrionia
Acidimicrobiia
Bdellovibrionia
Negativicutes
Nanoarchaeia
Deferribacteres
1.5
1
0.5
0
−0.5
−1
−1.5
Phylum
Acidobacteriota
Actinobacteriota
Aenigmarchaeota
Armatimonadota
Bacteroidota
Bdellovibrionota
Campilobacterota
Crenarchaeota
Cyanobacteria
Deferribacterota
Deinococcota
Desulfobacterota
Euryarchaeota
Firmicutes
Fusobacteriota
Gemmatimonadota
Nanoarchaeota
Patescibacteria
Proteobacteria
SAR324_clade(Marine_group_B)
Synergistota
Thermoplasmatota
Verrucomicrobiota
WPS−2
A6
A7
A8
A9
Phylum
(A)

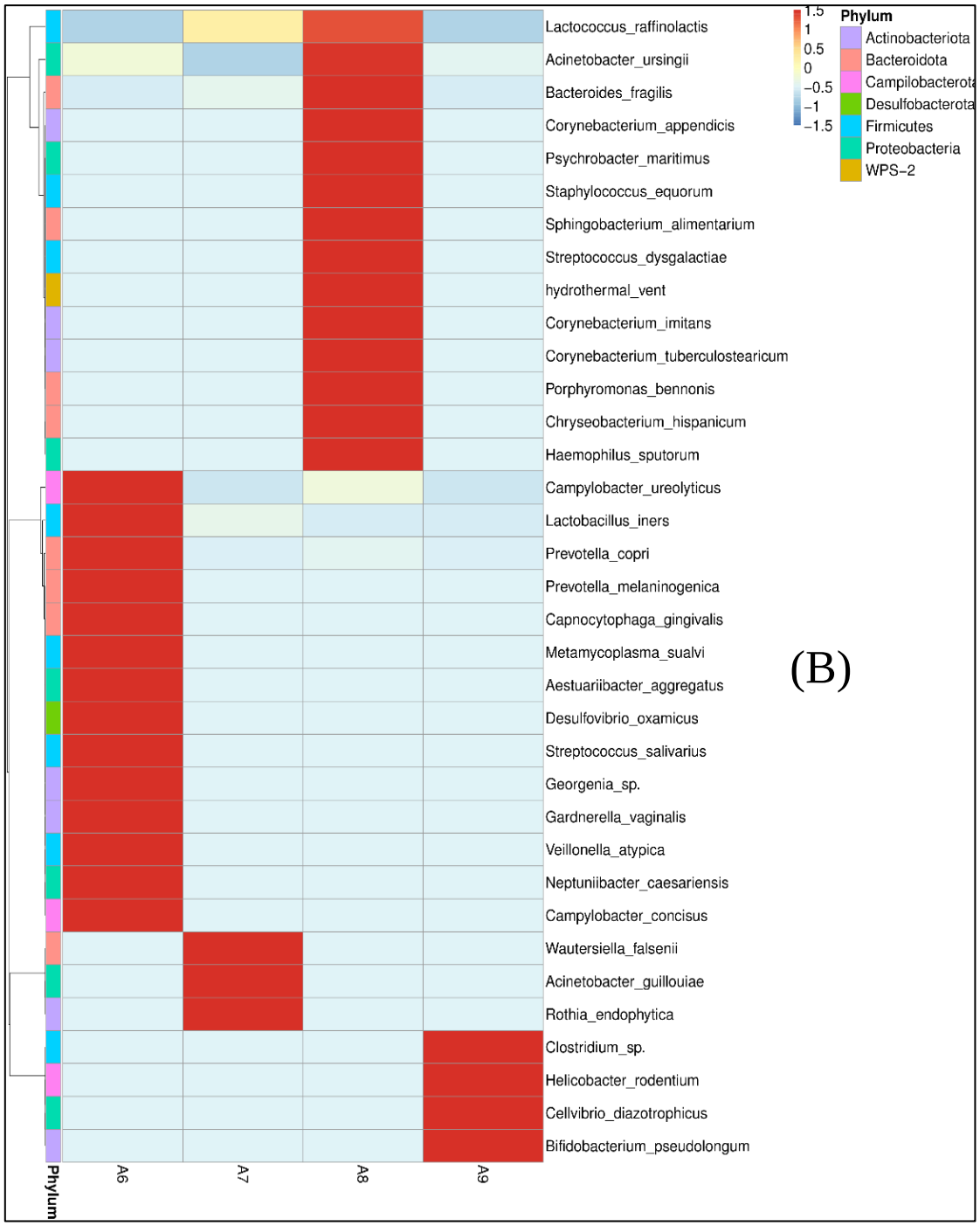


Fig. 6. The Heatmap shows the relative abundance of different bacterial phyla (A) and Species (B) in milk samples of dairy cows. The color of each square in the heatmap represents the abundance of that particular phylum, with red indicating high abundance and blue indicating low abundance. Red: High abundance; Orange: Medium abundance; Yellow: Low abundance; Blue: Very low abundance.

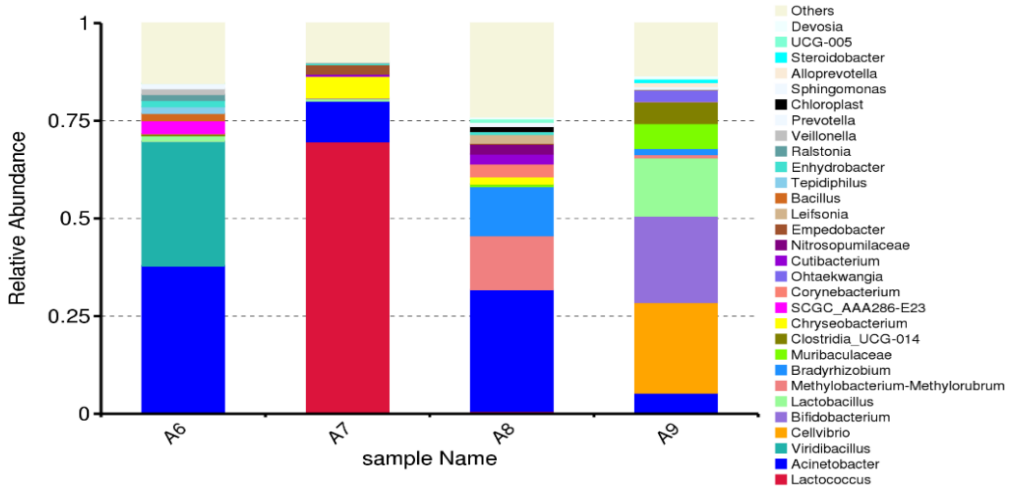


Fig. 7. A bar plot of the relative abundance of different bacterial dairy cow gut microbiota genera. The bar graph compares the relative abundance of other bacterial genera in the dairy cow's gut in different samples. The y-axis shows the relative abundance from 0 to 1. The x-axis shows the species of bacteria.

References

1. Falentin, H., Rault, L., Nicolas, A., Bouchard, D.S., Lassalas, J., Lamberton, P., Aubry, J.M., Marnet, P.-G., Le Loir, Y. and Even, S. Bovine teat microbiome analysis revealed reduced alpha diversity and significant changes in taxonomic profiles in quarters with a history of mastitis. *Frontiers in Microbiology*, **7**, 480 (2016).
2. Abu El-Hamd, M. A., Moussa, M. A. M. and Sayah, M. S. Effect of parity season and stage of lactation on somatic cell count milk yield and quality of friesian cows. *Journal of Food and Dairy Sciences, Mansoura Univ.*, **2**(8), 469 - 480 (2011).
3. Wilson, D. J., Gonzalez, R. N. and Das, H. H. Bovine mastitis pathogens in New York and Pennsylvania: prevalence and effects on somatic cell count and milk production. *J. Dairy Science*, **80**, 2592–2598 (1997).
4. Kaliwal, A. G. and Kulkarni, B. B. Bovine mastitis: a review. *Recent Sci Res.*, **4**, 543– 548 (2013).
5. Chen, S., Zhang, H., Zhai, J., Wang, H., Chen, X. and Qi, Y. Prevalence of clinical mastitis and its associated risk factors among dairy cattle in mainland China during 1982-2022: a systematic review and meta-analysis. *Frontiers in Veterinary Science*, **10**, 1185995 (2023).
6. Aitken, S. L., Corl, C. M. and Sordillo, L. M. Immunopathology of mastitis: insights into disease recognition and resolution. *Journal of mammary Gland Biology and Neoplasia*, **16**(4), 291-304 (2011).
7. Lin, S, Zhang, Y., Long, Y., Wan, H., Che, L., et al. Mammary inflammatory gene expression was associated with reproductive stage and regulated by docosahexenoic acid: in vitro and in vivo studies. *Lipids in Health and Disease*, **15**(1), 215 (2016).
8. Rinaldi, M., Li, R. W. and Capuco, A. V. Mastitis associated transcriptomic disruptions in cattle. *Veterinary Immunology and Immunopathology*, **138** (4), 267-279 (2010).
9. Patel, S. H., Vaidya, Y. H., Patel, R. J., Pandit, R. J., Joshi, C. G. and Kunjadiya, A. P. Culture independent assessment of human milk microbial community in lactational mastitis. *Scientific Reports*, **7**(1), 7804 (2017).
10. Kateete, D. P., Kabugo, U., Baluku, H., Nyakarahuka, L., Kyobe, S., Okee, M., Najjuka, C. F. and Joloba, M. L. Prevalence and antimicrobial susceptibility patterns of bacteria from milkmen and cows with clinical mastitis in and around Kampala, Uganda. *PLoS One*, **8**(5), e63413 (2013).
11. Cuo, L. Etiology, treatment and prevention of cow mastitis. *China Animal Health*, **23**, 21–22 (2021).
12. El-Kholy, A. M., Hassan, G. M., Zeinhom, M. M. A. and El-Klawy, M. A. A. M. Detection of subclinical mastitis in a dairy farm in Beni-Suef city, *Egyptian Assiut Veterinary Medical Journal*, **64**(157), 18-24 (2018)
13. Abed, A. H., Menshawy, A. M. S., Zeinhom, M. M. A., Hossain, D., Khalifa, E., Wareth, G., Awad, M.F. Subclinical Mastitis in Selected Bovine Dairy Herds in North Upper Egypt: Assessment of Prevalence, Causative Bacterial Pathogens, Antimicrobial Resistance and Virulence-Associated Genes. *Microorganisms*, **9**(6), 1175(2021).
14. Kajdanek, A., Kluska, M., Matusiak, R., Kazimierczak, J. and Dastych, J. A. Rapid and Inexpensive PCR Test for Mastitis Diagnosis Based on NGS Data. *Pathogens*, **13**, 423 (2024).
15. Vakkamäki, J., Taponen, S., Heikkilä, A. M. and Pyörälä, S. Bacteriological etiology and treatment of mastitis in Finnish dairy herds. *Acta Veterinaria Scandinavica*, **59**(1), 33 (2017).

16. Hiitiö, H., Simojoki, H., Kalmus, P., Holopainen, J., Pyörälä, S., & Taponen, S. The effect of sampling technique on PCR-based bacteriological results of bovine milk samples. *Journal of Dairy Sci*., **99**(8), 6532-6541 (2016).

17. Addis, M. F., Tanca, A., Uzzau, S., Oikonomou, G., Bicalho, R. C., and Moroni, P. The bovine milk microbiota: insights and perspectives from -omics studies. *Molecular Biosystems*, **12**(8), 2359-2372 (2016).

18. Oikonomou, G., Machado, V. S., Santisteban, C., Schukken, Y. H. and Bicalho, R. C. Microbial diversity of bovine mastitic milk as described by pyrosequencing of metagenomic 16s rDNA. *PLoS One*, **7**(10), e47671 (2012).

19. Kuehn, J. S., Gorden, P. J., Munro, D., Rong, R., Dong, Q., Plummer, P. J. Wang C, and Phillips, G. J. Bacterial community profiling of milk samples as a means to understand culture-negative bovine clinical mastitis. *PLoS One*, **8**(4), e61959 (2013).

20. Oikonomou, G., Bicalho M. L., Meira E., Rossi R. E., Foditsch C., Machado V. S., Teixeira A. G. V., Santisteban C., Schukken Y. H. and Bicalho R. C. Microbiota of cow's milk; distinguishing healthy, sub-clinically and clinically diseased quarters. *PLoS One*, **9**(1), e85904 (2014).

21. Ganda, E. K., Bisinotto R. S., Lima S. F., Kronauer K., Decter D. H., Oikonomou G., Schukken Y. H. and Bicalho R. C. Longitudinal metagenomic profiling of bovine milk to assess the impact of intramammary treatment using a third-generation cephalosporin. *Scientific Reports*, **6**, 37565 (2016).

22. Chen, S., Zhang, H., Zhai, J., Wang, H., Chen, X. and Qi, Y. Prevalence of clinical mastitis and its associated risk factors among dairy cattle in mainland China during 1982–2022: a systematic review and meta-analysis. *Frontiers in Veterinary Science*, **10**, 1185995 (2023).

23. Baloch, H., Rind, R., Kalhoro, D. H., Abro, S. H., Kolachi, H. A., Kakar, S., Muhammad Ahned F., Ali H. and Mahesar, M. Influence of Bacterial Species on Physical Characteristics and Somatic Cell Counts in Clinical and Subclinical Mastitis Milk of Kundhi Buffaloes *Pakistan Journal of Zoology*, 1-9. (2022).

24. Galfi, A., Radinović, M., Milanov, D., Boboš, S., Pajić, M., Savić, S. and Davidov, I. Electrical conductivity of milk and bacteriological findings in cows with subclinical mastitis. *Biotechnology in Animal Husbandry*, **31**(4), 533-541 (2015).

25. Smith, A. C. and Hussey, M. A. Gram stain protocols. *American Society for Microbiology*, **1**, 113–144 (2005).

26. Shome, B. R., Das Mitra, S., Bhuvana, M., Krithiga, N., Velu, D., Shome, R., Isloor, S., Barbuddhe, S. B. and Rahman, H. Multiplex PCR Assay for Species Identification of Bovine Mastitis Pathogens. *Journal of Applied Microbiology*, **111**, 1349–1356 (2011).

27. El-Ashram, S. and Suo, X. Exploring the microbial community (microflora) associated with ovine Haemonchus contortus (macroflora) field strains. *Scientific reports*, **7**(1), 70 (2017).

28. Lee, P. Y., Costumbrado, J., Hsu, C. Y. and Kim, Y. H. Agarose Gel Electrophoresis for the Separation of DNA Fragments. *Journal of Visualized Experiments*, **6**(2), e3923 (2012).

29. Head, S. R., Komori, H. K., LaMere, S. A., Whisenant, T., Van Nieuwerburgh, F., Salomon, D. R., & Ordoukhanian, P. Library construction for next-generation sequencing: overviews and challenges. *Biotechniques*, **56**(2), 61-passim (2014)

30. Piotrowski, J. S., Simpkins, S. W., Li, S. C., Deshpande, R., McIlwain, S., Ong, I., Myers, C. L., Boone, C. and Andersen, R. J. Chemical genomic profiling via barcode sequencing to predict compound mode of action. *Chemical Biology: Methods and Protocols*, **1263**, 299–318 (2015).

31. Martin, M. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet Journal*, **17,** 10-12 (2011).

32. Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P. Peplies J. and Glöckner, F. O. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Research*, **41**(Database issue), D590-596 (2013).

33. Rognes, T., Flouri, T., Nichols, B., Quince, C. and Mahé, F. VSearch: a versatile open-source tool for **metagenomics**. *Peer Journal for Life and Environment*, 4e2584, 1-22 (2016).

34. Haas, B. J., Gevers D,, Earl A, M., Feldgarden M,, Ward D, V., Giannoukos G., D. Ciulla, D. Tabbaa, Highlander S. K., Sodergren E., Methe B. and DeSantis T, Z., The Human Microbiome Consortium, Petrosino J. F., Knight R. and Birren B. W. Chimeric 16S rRNA sequence formation and detection in Sanger and 454-pyrosequenced PCR amplicons. *Genome Research*, **21**(3), 494-504 (2011).

35. Edgar, R. C. UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nature Method*s, **10**(10), 996-998 (2013).

36. Edgar, R. C. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*, **32**(5), 1792-1797 (2004).

37. Amer, S., Gálvez, F. L. A., Fukuda, Y., Tada, C., Jimenez, I. L., Valle, W. F. M. and Nakai, Y. Prevalence and etiology of mastitis in dairy cattle in El Oro Province, Ecuador. *Journal of Veterinary Medical Science*, **80**(6), 861-868 (2018).

38. Zeryehun, T. and Abera, G. Prevalence and bacterial isolates of mastitis in dairy farms in selected districts of Eastern Harrarghe Zone, EasternEthiopia. *Journal of Veterinary Medicine*, **6**, 498618 (2017).

39. Bhat, A. M., Soodan, J. S., Singh, R., Dhobi, I. A., Hussain, T., Dar, M. Y. and Mir, M. Incidence of bovine clinical mastitis in Jammu region and antibiogram of isolated pathogens. *Veterinary World* **10**, 984–989 (2017).

40. Mpatswenumugabo, J. P., Bebora, L. C., Gitao, G. C., Mobegi, V. A., Iraguha, B., Kamana, O. and Shumbusho, B. Prevalence of subclinicalmastitis and distribution of pathogens in dairy farms of Rubavu and Nyabihu Districts, Rwanda. *Journal of Veterinary Medicine*, **2017**, 8456713 (2017).

41. Busanello, M., Rossi, R. S., Cassoli, L. D., Pantoja, J. C. F. and Machado, P. F. Estimation of prevalence and incidence of subclinical mastitis in a large population of Brazilian dairy herds. *Journal of Dairy Science.* **100**, 6545–6553 (2017).

42. Asmelash, T., Asmamaw, A. and Kibeb, L. Isolation, Identification and Antimicrobial resistance profile of Staphylococcus aureus and occurrence of methicillin resistant S. aureus isolated from mastitic lactating cows in and around Assosa town, Benishangul-Gumuz Region, Ethiopia. *Journal of Dairy, Veterinary and Animal Research*, **6**(3), 00180 (2017).

43. Kerros, D. and Tareke, F. Bovine mastitis in selected areas of southern Ethiopia. *Tropical Animal Health and Production*, **35**(3), 197–205 (2003).

44. Dego, O. and Tareke, F. Bovine mastitis in selected areas of southern Ethiopia. *Tropical Animal Health and Production*, **35**, 197–205 (2003).

45. Kassa, F., Ayano, A., Abera, M. and Kiros, A. Longitudinal study of bovine mastitis in Hawassa and Wendo Genet Small Holder Dairy Farms. *Global Journal of Science Frontier Research*, **14**(2), 33–41 (2014).

46. Abera, M., Demie, B., Aragaw, K., Regassa, F. and Regassa, A. Isolation and identification of Staphylococcus aureus from bovine mastitic milk and their drug resistance patterns in Adama town, Ethiopia. *Journal of Veterinary Medicine and Animal Health*, **1**(2), 19–23 (2013).

47. Biniam, T., Taresa, F. and Yimer, M. Risk factors for bovine mastitis with the isolation and identification of Streptococcus agalactiae from farms in and around Haramaya district, eastern Ethiopia. *Tropical Animal Health and Production*, **51**(6), 1507–1513 (2019).

48. Mekibib, B., Fergasa, M., Abunna, F., Megersa, B., and Regassa, A. Bovine mastitis prevalence, risk factors and major pathogens in dairy farms of Holeta town, Central Ethiopia. *Veterinary World*, **3**, 397–403 (2010).

49. Zeryehun, T., Ayad, T. and Bayecha, R. Study on prevalence, bacterial pathogens and associated risk factors of bovine mastitis in small holder dairy farms in and around Addis Ababa, Ethiopia. *The Journal of Animal and Plant Sciences*, **23**(1), 50–55 (2013).

50. Bitew, M., Tafere, A. and Tolosa, T. Study on bovine mastitis in dairy farms of Bahir Dar and its environs. *Journal of Animal and Veterinary Advances*, **9**(23), 2912–2917 (2010).

51. Mulugeta, Y., and Wassie, M. Prevalence, risk factors and major bacterial causes of bovine mastitis in and around WolaitaSodo, Southern Ethiopia. *African Journal of Microbiology Research*, **7**, 5400–5405 (2013).

52. Endale, M., Eyob, E., Addisu, A., and Naod, T. A study on the prevalence of bovine mastitis and associated risk factors in and the surrounding areas of Sodo Town, Wolaita Zone, Ethiopia. *Global Journal of Science Frontier Research (GJSFR): (D) Agriculture & Veterinary*, **16**(2), 1–9 (2016).

53. Biffa, D., Debela, E. and Beyene, F. Prevalence and risk factors of mastitis in lactating dairy cows in Southern Ethiopia. *International Journal of Applied Research in Veterinary Medicine*, **3**(3), 189-198 (2005).

54. Haftu, R., Taddele, H., Gugsa, G. and Kelayou, S. Prevalence, bacterial causes, and antimicrobial susceptibility profile of mastitis isolates from cows in large-scale dairy farms of Northern Ethiopia. *Tropical Animal Health and Production*, **44**, 1765–1771 (2012).

55. Lakew, M., Tolosa, T. and Tigre, W. Prevalence and major bacterial causes of bovine mastitis in Asella, South Eastern Ethiopia. *Tropical Animal Health and Production*, **41**, 1525–1530. 10.1007/s11250-009-9343-6 (2009).

56. Mulugeta, Y. and Wassie, M. Prevalence, risk factors and major bacterial causes of bovine mastitis in and around WolaitaSodo, Southern Ethiopia. *African Journal of Microbiology Research*, **7**, 5400–5405 (2013).

57. Sori, H., Zerihun, A. and Abdicho, S. Dairy cattle mastitis in and around Sebeta Ethiopia. *The International Journal of Applied Research in Veterinary Medicine*, **3**(4), 332–338 (2005).

58. Singh, A., Chhabra, D., Sikrodia, R., Shukla, S., Sharda, R., and Audarya, S. Isolation of E. coli from bovine mastitis and their antibiotic sensitivity pattern. *International Journal of Current Microbiology and Applied Sciences*, **7**(10), 11-18 (2018).

59. Farooq, A. A., Inayat, S., Akhtar, M. S. and Mushtaq, M. Prevalence of mastitis and antibiotic sensitivity of bacterial isolates recovered from Nili-Ravi buffaloes. *Journal of Animal and Plant Sciences***, 18**, 2-3 (2008).

60. Hoque, M. N., Istiaq, A., Clement, R. A., Gibson, K. M., Saha, O., Islam, O. K., Abir R. A., Sultana M., Siddiki A. Z., Crandall K. A. and Hossain, M. A. Insights into the resistome of bovine clinical mastitis microbiome, a key factor in disease complication. *Frontiers in Microbiology*, **11**, 860 (2020).

61. Awandkar, S. P., Kulkarni, M. B. and Khode, N. V. Bacteria from bovine clinical mastitis showed multiple drug resistance. *Veterinary Research Communications*, **46**(1), 147-158 (2022).

62. Singh, K., Chandra, M., Kaur, G., Narang, D. and Gupta, D.K. Prevalence and antibiotic resistance pattern among the mastitis causing microorganisms. *Open Journal of Veterinary Medicine*, **8**(04), 54 (2018).

63. Salih, R. R. M. and Gibreel, H. H. Gentamicin is the best antibiotic in treatment all types of bovine mastitis caused by bacteria. *Agriculture and Forestry Journal*, **3**(1), 46-49 (2019).

64. Cheema, S. K., Li R. and. Cameron, Simon J. S. Culturomics as a tool to better understand the human milk microbiota and host–microbiota interactions. *Microbiota and Host*, **1**, e230001 (2023).

65. González, R., Mandomando, I., Fumadó, V., Sacoor, C., Macete, E., Alonso, P. L. and Menendez, C. Breast milk and gut microbiota in African mothers and infants from an area of high HIV prevalence. *PloS one*, **8**(11), e80299 (2013).

66. Falentin H, Rault L, Nicolas A, Bouchard DS, Lassalas J, Lamberton P, Aubry J-M, Marnet P-G, Le Loir Y and Even S. Bovine Teat Microbiome Analysis Revealed Reduced Alpha Diversity and Significant Changes in Taxonomic Profiles in Quarters with a History of Mastitis. *Frontiers in Microbiology*, **7,** 1-15 (2016).

67. Chen S, Zhang H, Zhai J, Wang H, Chen X. and Qi Y. Prevalence of clinical mastitis and its associated risk factors among dairy cattle in mainland China during 1982-2022: a systematic review and meta-analysis6. *Frontiers in Veterinary Science*, **10**,1-13 (2023).

68. Bouchard D. S., Seridan B., Saraoui T., Rault L., Germon P., Gonzalez-Moreno C., Nader-Macias F. M. E., Baud D., François P., Chuat V., Chain F., Langella P., Nicoli J., Le Loir Y. and Even S. Lactic acid bacteria isolated from bovine mammary microbiota: potential allies against bovine mastitis. *PLoS One*, **10**(12), e0144831 (2015).

69. Kuehn, J. S., Gorden P. J., Munro D., Rong R., Dong Q., Plummer P. J., Wang C. and Phillips G. J. Bacterial Community Profiling of Milk Samples as a Means to Understand Culture-Negative Bovine Clinical Mastitis. *PLoS one*, **8**(4), e61959 (2013).

70. Bhatt, V. D., Ahir, V. B., Koringa, P. G., Jakhesara, S. J., Rank, D. N., Nauriyal, D. S., Kunjadia A.P. and Joshi, C. G. Milk microbiome signatures of subclinical mastitis-affected cattle analysed by shotgun sequencing. *Journal of Applied Microbiology*, **112**(4), 639-650 (2012).

71. Oikonomou, G., Machado, V. S., Santisteban, C., Schukken, Y.H. and Bicalho, R.C. Microbial diversity of bovine mastitic milk as described by pyrosequencing of metagenomic 16s rDNA. *PloS one*, **7**(10), e47671 (2012).

72. Ganda, E. K., Gaeta, N., Sipka, A., Pomeroy, B., Oikonomou, G., Schukken, Y. H. and Bicalho, R. C. Longitudinal metagenomic profiling of bovine milk to assess the impact of intramammary treatment using a third-generation cephalosporin. *Scientific Reports*, **6**(1), 1-13 (2016).

73. Lima, S. F., Teixeira, A. G., Higgins, C. H., Lima, F. S. and Bicalho, R. C. The upper respiratory tract microbiome and its potential role in bovine respiratory disease and otitis media. *Scientific Reports*, **6**(1), 1-11 (2016).

74. Patel, R., Kunjadia, P., Koringa, P., Joshi, C. and Kunjadiya, A. Microbiological profiles in clinical and subclinical cases of mastitis in milking Jafarabadi buffalo. *Research in Veterinary Science*, **125**, 94-99 (2019).

75. Patel, R.J., Pandit R. J., Bhatt V, D., Kunjadia P. D., Nauriyal D. S., Koringa P. G., Joshi C. G. and Kunjadia A. P. Metagenomic approach to study the bacterial community in clinical and subclinical mastitis in buffalo. *Meta Gene*, **12**, 4-12 (2017).

76. Almallah, T. M., Khedr, S. I., El Nouby, K. A., Younis, S. S., Elazeem, M. A. and Elmehy, D. A. The synergetic potential of Lactobacillus delbrueckii and Lactobacillus fermentum probiotics in alleviating the outcome of acute toxoplasmosis in mice. *Parasitology Research*, **122**(4), 927-937 (2023).

77. Zhu, C., Yao, J., Zhu, M., Zhu, C., Yuan, L., Li, Z., Cai D., Chen S., Hu1 P. and Liu, H. Y. A meta-analysis of Lactobacillus-based probiotics for growth performance and intestinal morphology in piglets. *Frontiers in Veterinary Science*, **9**, 1045965 (2022).

78. Zommara, M., El-Ghaish, S., Haertle, T., Chobert, J. M. and Ghanimah, M. Probiotic and technological characterization of selected Lactobacillus strains isolated from different egyptian cheeses. *BMC Microbiology*, **23**(1), 160 (2023).

79. Salih, A. H. M., Patra, I., Sivaraman, R., Alhamzawi, R., Khalikov, K. M., Al-Qaim, Z. H., Golgouneh S., Abed Jawad M., Adhab A. H., Andrés Leonardo Vázquez-Cárdenas A. l. and Abarghouei, S. The Probiotic Lactobacillus sakei Subsp. Sakei and Hawthorn Extract Supplements Improved Growth Performance, Digestive Enzymes, Immunity, and Resistance to the Pesticide Acetamiprid in Common Carp (Cyprinus carpio). *Aquaculture Nutrition*, 1-14 (2023).

80. Wu, F., Xie, X., Du, T., Jiang, X., Miao, W. and Wang, T. Lactococcus lactis, a bacterium with probiotic functions and pathogenicity. *World Journal of Microbiology and Biotechnology*, **39**(12), 325 (2023).

81. Liu, P., Lu, Y., Li, R. and Chen, X. Use of probiotic lactobacilli in the treatment of vaginal infections: In vitro and in vivo investigations. *Frontiers in Cellular and Infection Microbiology*, **13**, 391(2023).

82. Moehring, R. and Mahlen, S. Infections due to Serratia species. *UpToDate. Waltham, MA.(accesat în Ianuarie* (2015).

83. Haxhiaj, K., Wishart, D. S. and Ametaj, B. N. Mastitis: What it is, current diagnostics, and the potential of metabolomics to identify new predictive biomarkers. *Dairy*, **3**(4), 722-746 (2022).

إنتشار وعزل البكتيريا المسببه لإتهاب الضرع السريري وتحت السريري في الأبقار المصرية الحلابة في كفر الشيخ، مصر

محمد عوض أبو الحمد[1] , محمد عمرو عجيلة[1] ، نبيل بسيوني الجمل[1] ، محمد لبيب سالم [2]، سعيد الاشرم[3] وسليمان حسونه سليمان[1] و مجدي محفوظ[3]

[1] معهد بحوث الإنتاج الحيواني ، مركز البحوث الزراعية، وزارة الزراعة ، جيزة ، مصر.

[2] وحدة المناعة والتكنولوجيا الحيوية، قسم علم الحيوان، كلية العلوم، جامعة طنطا، طنطا، مصر.

[3] كلية العلوم، جامعة كفر الشيخ، كفر الشيخ، مصر.

الملخص

تهدف هذه الدراسة لدراسة مدى انتشار التهاب الضرع في أبقار هولشتاين فريزيان الحلابة. تم استخدام التقنيات المعتمدة على الحمض النووي المستقلة عن تحليل ميكروبات اللبن المعزولة من الأبقار الحلابة. من بين 133 بقرة تم تقييمها، أصيب بالتهاب الضرع الحاد حوالي 13.5% (133/18) والتهاب الضرع تحت الحاد (SCM) و20.3% (133/27) على التوالي. سيطرت البكتيريا إيجابية الجرام مثل الإشريكية القولونية والمكورات العنقودية الذهبية على الكائنات الحية الدقيقة المعزولة. حددت تسلسل عالية الإنتاجية أن Firmicutes وProteobacteria وBacteroidetes وActinobacteria هي أكثر أنواع البكتيريا انتشارًا. وكانت الأجناس الأكثر شيوعًا هي Bifidobacterium و Lactobacillus. وفي نفس البيئة، تم العثور على المكورات اللبنية، والراكدة، والمكورات العنقودية الذهبية، والمكورات العقدية القاطعة للدر، والمكورات العنقودية السلبية المخثرة - وهي أنواع بكتيرية مختلفة ذات أدوار محتملة أعلى في التهاب الضرع. أظهر سلوك التشتت لعدة عينات في تنوع مخططات PCoA ومؤشرات تنوع ألفا التنوع الكبير في الكائنات الحية الدقيقة في التهاب الضرع. لقد أثر الموسم، والرضاعة، والعدوى على تنوع ألفا في ميكروبات الحليب في الأبقار المصرية؛ تم العثور على أربع شعب سائدة، وعلى الرغم من وجود مجتمعات بكتيرية متميزة في العينات المصابة، إلا أن التهاب الضرع لم يغير بشكل كبير تنوع ألفا. تسلط هذه الدراسة الضوء على مدى انتشار التهاب الضرع في أبقار الألبان المصرية، والميكروبات الحيوية الخاصة بها، وعوامل الخطر للإصابة بالتهاب الضرع. يمكن أن تقلل النتائج من التهاب الضرع وتحسين صحة وإنتاجية الابقار و الألبان

الكلمات الدالة: التهاب الضرع ، أبقار اللبن والميكروبات وعوامل الخطر.