ARTICLE / INVESTIGACIÓN

The direct microscopic examination of somatic cell count to detect sub-clinical mastitis in cows of Diyala province

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DOI. 10.21931/RB/2023.08.03.29

Abstract: Background Udder Inflammation is still one of the most significant economic issues that may affect milk production. Methods: Several tests were used to detect the pH of milk, such as the aerial mastitis test, Whiteside test, chloride test, Bovi-mastitis test (Card test), Somatic cell count, and bacterial culturing. Results showed that 32% of cows suffered abnormalities in the consistency of milk, whereas 68% of cows passed through subclinical mastitis. Furthermore, the results also showed that 80 % of cows were affected by Staph. Aureus, compared to other bacterial species. Conclusions: According to our findings, subclinical and clinical mastitis is significant in Diyala Province, and infectious and environmental pathogens are involved. Moreover, locally manufactured Arial mastitis test, Whiteside test, and the imported Bovi card test are appropriate tests for the early diagnosis of subclinical mastitis in cattle. *Staph. Aureus* was more prevalent than other inflammatory infections and ambient pathogens in cases of bovine mastitis.

Key words: Detection, mastitis clinical and sub-clinical, Dairy cows, Diyala Province.

Introduction

A parenchymal disease of the udder tissue is identified via various pathological alterations in the mammary cells and alterations in milk's physicochemical and biological composition[1]. Mastitis falls into two main categories. The first is clinical mastitis, which presents as swelling, a rise in temperature, and pain in the udder or milk that indicates milk clotting being present, milk color change, and excessive counts of white blood cells in affected milk in animals. The other is sub-clinical mastitis, which manifests no apparent symptoms in the udder unless diagnostic tools are used. Mastitis is regarded as 1 of the more essential causes of loss of finances in milk production in animals globally because it affects both the quality and quantity of milk[3]. Lymphocytes, macrophages, polymorphonuclear cells, and some epithelial cells are examples of somatic cells, a component of the body's natural defense system[4]. The California mastitis test) (CMT) allows for the quantitative measurement of (somatic cell count (SCC). A quick, easy, and Low-cost examination procedure is available for sub-clinical mastitis at dairy farms[5]. old, milking stage, variance, time, fatigue, organization, daily difference, and primarily intramammary infection (IMI) are just a few factors that may influence SCC. Understanding the factors that may affect the number of somatic cells is necessary to accurately interpret somatic cell counts[6-8]. Bacterial pathogens that can be divided into two groups typically cause inflammation of the udder[9]. Infectious microorganisms like *Staph. Aureus, Mycoplasma bovis, Streptococcus agalactiae*, and environmental pathogens like *Streptococcus species* (*Streptococcus uberis* and *Streptococcus dysgalactiae*). Additionally, there are environment-specific coliforms, which include gram-ve bacteria like *Serratia, Pseudomonas*, and *Proteus*, as well as *Escherichia coli, Klebsiella species*, *Citrobacter* species, *Enterobacter* species, Enterobacter faecalis & *Enterobacter faecium*[1].

Materials and methods

Area of Study

This research was done in Al-Khalis and Baquba district regions extending to the northeast of Baghdad. About 100 milk samples from 25 different crossbred dairy cows were collected for the study. These samples were divided into groups according to age (2.5 to 7 years), parity (one to four), and lactate phase (late and early).

Collection of samples

After properly cleaning the teat surface with 70 percent ethanol, 100 milk samples were taken. 10 mL of milk were collected aseptically from each of the four quarters in separate, sterile polyethylene screw-capped containers[10]. Specimen of milk was stored at four to ten C^0 in a refrigerator for additional lab testing after being placed in an icebox and transported to the diagnostic lab of the veterinary hospital in Diyala Governorate.

Diagnostic tests

The following diagnostic tests were performed on milk samples from cows that appeared to be in good health:

Indirect Examination:

For the detection of milk pH, various tests, including the Arial mastitis test (AMT), Whiteside test (WST), chloride test (CT), and the Bovi-mastitis test (Card test), are used: It was

Citation: Kamil Awad A, The direct microscopic examination of somatic cell count to detect sub-clinical mastitis in cows of Diyala province. Revis Bionatura 2023;8 (3) 29. http://dx.doi.org/10.21931/RB/2023.08.03.29
Received: 28 May 2023 / **Accepted:** 15 July 2023 / **Published:** 15 September 2023

prepared like the Surf field mastitis test (SFMT) according to (one) with a little alteration. Bromocresol purple was added to the three percent solution at a ratio of 1:10,000 after a basic washing ingredient weighing 3g (Arial) was added to a hundred ml of filtered regular water. One well is used to test each quarter of the cow on a four-well plastic paddle. Following that, the reaction was visually graded according to how much gel had formed:

Negative = no response trace = When the plate is rotating, streaks appear and can be seen as a trace.

1+ = remarkable thickening at rotating but no gel.

2+ = slight formation of gel to follow the rotating plate very slowly.

3+ = The solid gel that sticks to the plate base has formed.

The WST was carried out according to (11) by combining two drops of sodium hydroxide (4%) and five drops of milk on a dark.

Procedure

a- Mix the freshly collected milk samples thoroughly. Distribute ten µL of milk in a region (1 square cm^2) on clear slides. Distribute milk equally. Do not heat the slide; dry it on a flat, horizontal surface. Using Newman- Lambert stain (Methylene-blue twelve g, ethyl alcohol ninety-five percent fifty-four ml, tetrachloroethane forty ml, and glacial acetic acid six ml). The benefit of the glass plate is to stir for 20 and display the degree of coagulation. The Chloride test was conducted according to 11.The yellow color was demonstrated in milk with an abnormally high chloride level (> 0.14 percent), showing a positive sample. As a result of the formation of silver chromate, the color was continuously red, meaning a negative sample. The Bovie Vet. Indicator paper was used to determine the pH of the milk sample (Figure 1).

Figure 1. Card-test[12].

(VMED SUPPLY, INC). A Little drop of milk on the test sheet for the recording of spots' coloration. The company's recommendations are used to determine how pH and color change are related as follows: pH (6.6-6.7) pale green, (pH 6.8) moderates green, (pH 7.1) green, and (7.4) dark-blue green.

-Direct examination of Somatic cell count (SCC).

This Staining will be discarding fat. Fix. Staining bacteria and WBCs.

b- Based on the stain's goodness, soak an air-dried smear for fifteen seconds to a minute.

c- Drying within the air.

d- Use the liquid to wash.

e- Dry within the air.

f- Check the field of immersion in oil for the appearance of bacteria and WBCs.

g- In 20–30 microscopic fields, count the WBCs (Figure 2).

No of WBCs/ ml milks=

$$\frac{\text{A cell's numbers counted x microscopic agent } (4 \times 10^5)}{\text{No. of fields examined}}$$ [10].

H-Bacteriology

Bacterial culture was performed according to (13). After being collected, milk specimens from both clinical and sub-clinical status streaked for 24 hours on (blood) and (Mac-Conkey) agars plates. They were examined after being cultured for twenty-four and forty-four hours at thirty-seven °C under anaerobic conditions. If there was no growth after 48 hours of incubation, the culture was regarded as negative. based on those sizes, form, pigment, and hemolytic properties, G-stain, and colony creation of catalase, bacteria on culture +ve plates were) recognized. Various biochemical tests were used for verification after subculturing and isolating different cultures[13].

Results

All dairy cows had a clinical examination to determine whether or not they had reddish, suffering, swelling, a symmetrical or non-symmetrical udder, and abnormal in their (milk samples). Eight (32%) of 25 cows suffered abnormalities and inconsistency of milk in which clot, straw color, redness, and a non-symmetrical quarter of udder. When 68% of cows had sub-clinical mastitis, which doesn't manifest any symptoms in the udder unless diagnostic equipment is used. The test results revealed that indirect and direct examination shows a significant variation at ($p < 0.05$), as in Table 1.

The present study shows that the age, breed, parties, and stage of lactation of cows affected SCC values, as in Table 2.

Bacteriology

Milk samples from the clinical and sub-clinical areas were gathered for bacteriological culture. Table 3 shows 40 isolates were gram +ve and 19 gram-ve isolates.

Discussion

Clinically, clinical mastitis cases recorded in the present study agree with another result[14]. Sub-clinical mastitis is the most common in cattle raised in various Diyala Governorate regions. AMT and WST are recognized to be utilized as indirect processes for numeration or (count) WBCs into milk specimens on the farm and in the lab. Table 1 shows a strong relationship between the total affirmative responses to these two tests. This result was compatible with (15); they found that the Surf-mastitis tests (SMT), WST, and chloride -tests had the same sensitivity. The chloride test revealed that more chloride ions were present in all samples. There is variation in the standard level of chloride ions found in common milk specimens. Elango, Doraisamy, and Rajarajan Kumaresan[16] showed that healthy animal milk typically had a chloride concentration between 0.08 and 0.14 percent. While Sharma, SinghBhadwal[17] believed the usual milk sample's chloride concentration was 0.91 percent. The results of the chloride examination were in agreement with those obtained by (18), and they regarded them as an invaluable tool for identifying sub-clinical mastitis in buffaloes. Comparing multiple diagnostic tests for identifying subclinical mastitis revealed a 97 percent sensitivity for SCC, an 88 percent sensitivity for AMT, and an 85 percent compatibility with the result for WST reaction[19,20]. The current research demonstrated that the SCC was the most accurate and

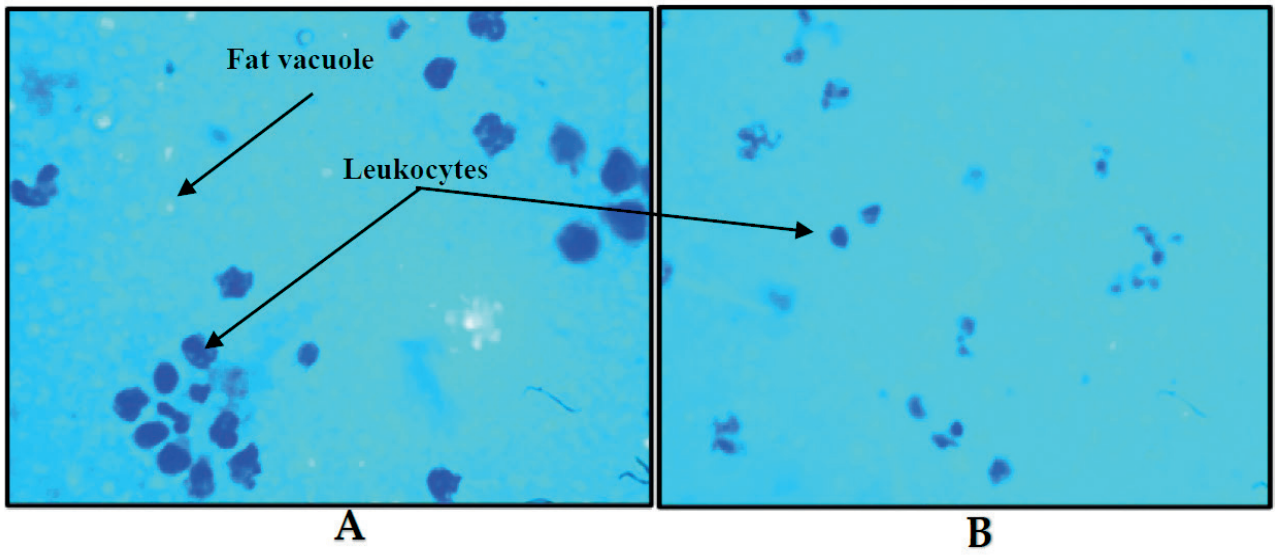

Figure 2. (A-B) Milk staining high somatic cells (WBCs) and vacuoles of fat (X 100).

Total number of milk samples	+ AMT	+ WST"	+ Chloride test	+ Increase pH	+ SCC
100	88*	85*	100*	80	97*
	(88%)	(85%)	(100%)	(80%)	(97%)
X^2=30.889 p value = 0.00000323 P< 0.05 X^2= Chi-square, S.D = Significant difference*				SD*	

AMT: Arial mastitis test. WST: Whiteside test.

Table 1. Number and percentage of milk samples giving +ve reaction using the 4 tests.

Case No	age	Breed	Clinical signs	Direct somatic cell count (DSCC)	(Mean ± SD)
1	3.5 years	Friesian	Pregnant in the eighth month	10680000 8460000 4580000 3540000	6815000.0 ± 3334921
2	3.5 years	local	birthing for months or longer and regular feeding	1100000 2620000 1520000 2940000	735000.0 ± 875652.1
3	2.5 years	local	first calving, and all signs are normal	1340000 1300000 1100000 2020000	1440000.0 ± 400666.1
4	3 years	cross	parturiated more than a month, no skin lesion	1640000 1040000 2680000 1140000	535000.0 ± 750710.8
5	2.5 years	Friesian	Normal signs	1600000 2640000 940000 2540000	660000.0 ± 809361.9
6	2.5 years	Friesian	Normal signs 2 parity	1140000 1220000 940000 1200	412050.0 ± 561878.1

Table 2. Case history, ages, breed of cattle, and (DSCC) of milk specimens.

7	3 years	local	It has a new parturient and normal lactation	760000 1360000 940000 900000	185000.0 ± 258457
8	2 years	locals	Mastitis symptoms are absent, and the udder is symmetrical.	1120000 980000 960000 840000	75000.0 ± 114746.1
9	3.5 years	locals	Mastitis symptoms are absent, and the udder is symmetrical.	1140000 1420000 1400000 1500000	112500.0 ± 156098.3
10	2 years	locals	"The udder, milking, and milk consistency are all normal."	740000 620000 860000 820000	80000.0 ± 105830.1
11	2.5 years	local	"Early in pregnancy and the asymmetrical quarter of the udder."	5240000 4960000 1340000 1400000	1865000.0 ± 2156687
12	2 years	locals	There are no signs and uniform shape of the quarter udder	720000 Fat presence 1120000 1000000	151111.1 ± 205264.1
13	2.5 years	Friesians	"No signs of 3parity hind quarter non-symmetrical"	2520000 2820000 3980000 3600000	560000.0 ± 676165.7
14	2.5 years	Friesian	Normal signs and 2 parties	3800000 900000 960000 1220000	1040000.0 ± 1393604
15	2.5 years	Friesian	Normal signs and symmetrical quarters of the udder	760000 1220000 1020000 920000	980000.0 ± 192527.1
16	4 years	cross	It has two cases of parturient and a non-symmetrical quarter of the udder	5440000 1960000 1300000 7320000	4005000.0 ± 2860507
17	3 years	cross	Normal signs and the milk consistency are normal	1860000 1140000 1000000 940000	312500.0 ± 425009.8
18	2.5 years	local	Abnormal milk consistency with blood, non-symmetrical quarter	3980000 4560000 4860000 6080000	4870000.0 ± 4560000
19	2.5 years	local	The front quarter is non-symmetrical, and the presence of blood in the milk	9860000 2480000 1660000 3060000	2797500.0 ± 3773959
20	4 years	local	Normal signs and normal milk	1760000 1820000 3380000 1420000	2095000.0 ± 874585.6

Table 2. Case history, ages, breed of cattle, and (DSCC) of milk specimens.

closely matched the bacteriological studies. Direct microscopic testing of the somatic cell count was a more reliable technique to diagnose subclinical inflammation of the udder in milk production. The modifying California-mastitis tests (MCMT), the modifying White-side tests (MWST), and SCC were the three tests most effective at identifying sub-clinical mastitis[21]. According to (22), CMT had more excellent dependability (85.69%) than MWST (79.74 percent). Dependent on the analysis data of SCC values in Table (2), the study shows SCC was affected by age and breed and increased as parity increased. These results are compatible with earlier studies (23-25) and revealed increased SCC for subsequent equivalencies. At younger and older ages, Haas[23] suggested that there was a different defense me-

Bacteria species	No. isolated bacteria	(%)
Staphylococcus aureus	32	80.0%
Staphylococcus coagulase-negative (CNS)	8	20.0%
Pseudomonas spp.	5	26.31%
Pasteurella spp.	3	15.79%
E. coli	3	15.79%
Klebsiella spp.	30	15.79%
Salmonella spp.	30	15.79%
Proteus spp.	20	10.52%

Table 3. Show the number and percentage of isolated bacteria.

chanism against udder infection. It does not matter if the cattle have an illness or if there is no SCC increases as lactation progresses and delayed feeding[8]. The neutrophil ratio appears to rise in earlier and delayed breastfeeding, whereas the ratio of lymphocytes appears to be low[17]. SCC often exceeds 1 million cells per ml before parturition and falls to 100,000 cells/ ml 7 to 10 days after delivery. In cases of disease or mammary infection, the SCC increased over the usual range. The complexity of sub-clinical mastitis and variations in farm management practices, lactation stage, parity, and breed may contribute to the variable results[26]. Differences in farm management practices, phase of lactating, valence, generation, and old and intramammary diseases may all contribute to the elevation of SCC values shown in the current research of diseased cattle with sub-clinical mastitis; this result is compatible with other authors[25-28]. The most common bacterium was S. aureus, as evidenced by the fact that it was isolated from 32 (57.14 percent) of the mammary glands with sub-clinical inflammation of the udder, whereas *Staphylococcus*. In comparison, coagulase-negative (CNS) were identified in eight (14,28 percent) specimens. The finding was in line with the majority of projects in cow inflammation of the udder, in which numerous researchers showed *S. aureus* was a more prevalent pathogen that separated a cow from the epidermis of the teat and teat-end, milk and the typical pathogens were separated[4,29,30]. The result of (Koivula, Pitkälä, PyöräläMäntysaari[31] showed that 10.5 percent of cow milk specimens were contaminated with CNS and that *Staph. Aureus* was present in 36.8 percent of them, supporting the predominance of *Staphylococcus spp.* it indicated that (36.8%) of bovine milk samples had been infected with *S.aureus* and 10.5% with CNS, supporting the notion that *Staphylococcus spp.* Predominate. Predominate. In Baghdad, *Staphylococcus* epidermidis was the more prevalent pathogen in subclinical inflammation of the udder (53.84%), while *Staph. Aureus* was the more common pathogen in clinical inflammation of the udder (44.44%) (27). This research also showed Enterobacteriaceae in many cases of bovine mastitis, including *E. coli* 3 (5.20%), and *Klebsiella spp.* 3 (5.3%), *Salmonella spp.* 3 (5.3%) & *Proteus spp.* 1 (1.78%). These findings were consistent with those of other studies conducted in Iraq and other regions of the world (31-33).

Conclusions

According to our findings, subclinical and clinical mastitis is significant in Diyala Province, and both communicable and environmental pathogens are involved. Following cultural isolation, Somatic cell count was the most accurate test, followed by the Arial mastitis and Whiteside tests. Despite minor variations in the recorded scores, we concluded that using the locally manufactured Arial mastitis test, Whiteside test, and the imported Bovi card test is appropriate for the early diagnosis of subclinical mastitis in cattle. *Staph. Aureus* was more prevalent than other intramammary infections and ambient pathogens in cases of bovine mastitis.

Author Contributions

AKA developed the research concepts, design, methodology, and data analysis and wrote the manuscript.

Funding

This research received no external funding.

Acknowledgments

The author sincerely thanked Ibrahim Kadhim Ibrahim for their insightful comments and suggestions that helped significantly improve this research work.

Conflicts of Interest

The authors declare no conflict of interest.

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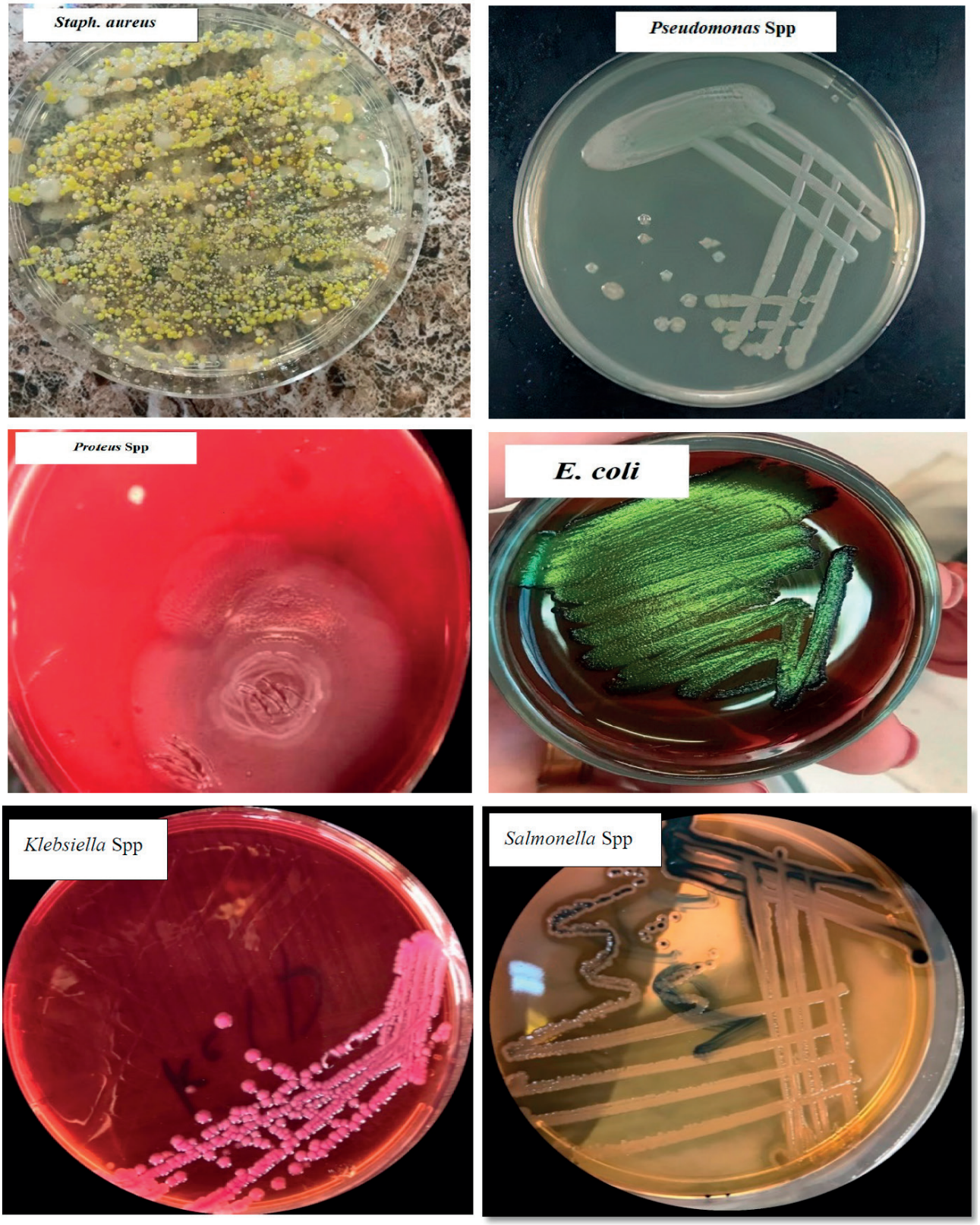

Figure 3. Morphological Characters of Isolated Colonies.

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