# Bacterial Diversity In Goat Milk Produced In Suburbs Benghazi - Libya

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***Abstract***- The study's goal was to figure out the types of bacteria in goat milk produced on the outskirts of Benghazi. Goat milk samples were gathered from nineteen flocks in the suburbs of Benghazi city and the samples were coded from G1 to G19 respectively Milk samples were collected at random from private herds where the goats were milked by hand and two samples were taken at a rate of 200-250 ml from each herd. The milk samples were examined to determine the average total bacterial count (TBC), (total coliform count (TCC), and Staphylococcus aureus (TSC) and the likelihood of salmonella bacteria present as the suitability of goat milk for human consumption. The results showed that there were significant differences (P <0.05) between the averages of the total number of bacteria, coliform bacteria, and Staphylococcus aureus in the nineteen samples. The study reported that the average total bacterial count (TBC) ranged between (7.54 to 8.49 $\log_{10}$ CFU/ml). Statistical analysis of goat milk samples (total coliform count (TCC)) appeared that there were no significant differences at p< 0.05 between each of (G10, G11, G12, G14) (7.73, 7.75, 7.80, 7.73 log10 CFU/ml) respectively. Tests for detection of total staphylococcus counts (TSC) exhibited that G1, G8, and G2 (6.68, 6.68, and 6.67 log10 CFU/ml) were the greatest mean value (TSC), respectively. Tests have identified E. coli 41.7%, Bacillus spp. 16.7%, Enterobacter spp. 8.3%, Citrobacter spp. 8.3%, Klebsiella spp. 4.2%, Proteus spp. 4.2%, Shigella spp. 8.3%, Salmonella spp. 0.00%, and Brucella 8.3%, as a percentage of isolated bacteria produced in Benghazi cit. These results demonstrate that milking goats lack hygienic conditions.

***Index Terms***- Goat milk, E.coli, Bacterial pollution, Staphylococcus

## I. INTRODUCTION

In Libya, 2.6 million heads of goats have bred [1] Scientists pointed to Libya's goat (Mahali) populace, which represents over 90% of the goat in Libya and has scattered along the coast.[2],[3],[4]. The Common method used for goat production in Libya, where they are raised either individually or in the form of mixed flocks of sheep, is a comprehensive production[3]. In addition, in the same previous post, Akrim stated that in the mountainous area of Jabal Al Akhdar in the east or Nafusa in western Libya, herds usually consist only of goats[3]. Like every third world region, Libya produces very little milk from goats and sheep, which is found in conventional or small-scale farming systems with very low inputs. About 90% of the fresh goat milk consuming in Libya comes from informal markets.

Goat milk conformation varies from cow, buffalo, and human milk and varies by age, breed, male, season, feeding, management, environmental conditions, lactation location, and stage, and health status of the udder [5]). In comparison, goat milk has more health features and medicinal qualities than cow's milk[6]. Goat milk has shown greater digestibility, mineral bioavailability, and protein and fat profiles relative to cow's milk[7].In addition, while compared to cow and human milk, goat milk has superior digestibility, buffer capacity, alkalinity, and medicinal benefits[8].

Goat milk contains more Calcium, Magnesium, and phosphorus than cow and human milk, but the content of vitamin D, vitamin B12, and folate is smaller[9]. In contrast to cow milk, goat milk had lower quantities of C12:0, C14:0, C16:0, and the Na: K ratio, as well as higher concentrations of cis polyunsaturated fatty acids (PUFA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), isoflavones, B, Cu, Mg, Mn, P.[10].

The milk of goats has some unique characteristics that confer technical advantages. It has a smaller fat size relative to cow's milk, globules fat that give derived products a finer texture; lower levels of alpha1-casein resulting in softer gel products, higher ability to retain water, and lower viscosity [11],[12],[13],[14]. The presence of small fat granules and high levels of calcium salts distinguishes goat milk from ruminants milk others, so goat milk has considered one of the better foods for infants[11].

The European Food Safety Authority (EFSA) confirmed that cows, sheep and goats, horses and donkeys, and camels in the EU are microbiological risks that can be transmitted to humans through milk[15]. In addition, EFSA pointed out that the bacteria ( *Campylobacter spp. (thermophilic), Salmonella spp., shigatoxin-*

*producing Escherichia coli (STEC), Bacillus cereus, Brucella abortus, Brucella melitensis, Listeria monocytogenes, Mycobacterium bovis, Staphylococcus aureus, Yersinia enterocolitica, Yersinia pseudotuberculosis, Corynebacterium spp., Streptococcus suis subsp. zooepidemicus*, the parasites *Toxoplasma gondii* and *Cryptosporidium parvum*; as well as the virus tick-borne encephalitis virus (TBEV)) were as the microbiological hazards potentially transmissible via milk and present in the EU milk-producing animal population[15]. The study reported that isolated bacteria from 114 samples of milk in two districts in the Tanga region (Northern Tanzania) were *Escherichia coli, Staphylococcus aureus, Listeria spp., and Listeria monocytogenes*, as well as other microorganisms, included *Klebsiella spp., Proteus spp., Staphylococcus spp. Enterococcus faecalis, Bacillus cereus*, and *Pseudomonas spp*.[16].

Some studies stated that disease-causing and decay bacteria have been isolated from fresh goat milk in different parts of the world, such as *Listeria monocytogenes, Salmonella sp, Campylobacter, Staphylococcus aureus, Bacillus cereus, Escherichia coli, Streptococcus, Staphylococcus, and Micrococcus species* [17],[12]. From fresh goat milk, Saad et al. found that the mean values of total mesophilic count, total psychotrophic count, total lipolytic count, total staphylococci count, total coagulase positive staphylococci and coagulase negative staphylococci count, total yeast and mold count, and coliform count were (8.6 & 9.7), (4.5 & 5.0), (3.6 & 5.2), (7.7 & 8.9), (7.2 & 8.2), (7.5 & 7.4), (5.4 & 5.2) log CFU/ml. and (7.3 & 7.5) log MPN/ml respectively[18].

The purpose of the experiment is to study the bacterial content of raw goat milk sold in the Benghazi, as well as to understand the extent of contamination caused by hand milking of the goats.

## II. MATERIAL AND METHODS

### A. SAMPLE COLLECTION

Goat milk samples from nineteen herds around Benghazi city were collected. Milk samples have been obtained randomly where goats have been milked manually. From each flock, two samples of milk (200-250 mL each) were taken. The samples collected were immediately transported in an icebox to the laboratory, where they were begun to be numbered (G1 to G19), diagnosed, and bacteria were identified.

### B. PROCESSING

Once the samples arrived at the laboratory, each sample gave a unique number and analyzed as an independent analytical unit, as follows:

10 ml of each sample, after shaking well, were transferred to a bottle containing 90 ml of sterile peptone (0.1%) water, to preserve the vitality of the bacteria, and were mixed for two minutes to obtain a homogeneous mixture of 0.1 (1/10) concentration[19].

The homogenate mixture transferred to a sterile 500 ml bottle having the sample number, mixed well by swirling the bottle. Then the bottle cap was loosening, and incubated at 37°C for 24 hr., for the isolation of salmonella[20].

### C. MICROBIOLOGICAL ANALYSIS

In this study, numerous bacteria media were used (Plate count agar, MacConkey agar, S. S. agar, Mannitol salt agar, Xylose Lysine Deoxy cholate (XLD) Agar, Staphylococcus agar No. 110 (gelatin mannitol salt agar), and eosin methylene blue (EMB) agar for E. coli) (All selective media from OXOID limited, England). The bacteria media were used to detect total bacteria counts (TBC), total coliform counts (TCC), total Staphylococcus aureus counts (TSC), and the presence of Salmonella on goat milk samples. The typical colonies on these selective media were confirmed by Gram stain, morphological examination, and many standard biochemical tests (Motility test, Indole test and Sulfide production (SIM), Catalase test, Oxidase test, Citrate utilization, MRVP tests, Triple sugar iron (TSI) test, Brilliant green broth, and Lactose broth test)[21], [22]. Bacteria species were determined according to Bergy's manual of determinative bacteriology 9th edition[23]

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To prove the total number of bacteria, the number of poured plates containing 30 to 300 colonies was used to estimate the total number of bacteria in meat samples. Total number of bacteria was calculated by multiplying the inverse of dilution factor in the mean number of colonies in the plates (three replicates per dilution). For statistical analysis, colony forming units/ml (CFU/ml) was converted to log10 CFU/ml.

### D. STATISTICAL EXAMINATION

The data were analyzed with SPSS software (Statistical package for social science version 23, IBM/SPSS). Descriptive statistics were used to analyze the data. In addition, all bacterial counts were converted to log10CFU/g for analysis. ANOVA was performed. Duncan's test was used as a post hoc test. Mean differences were considered significant at $p < 0.05$. The comparison was between rows.

## III. RESULTS AND DISCUSSION

Microbial test of goat milk samples (chart 1) showed there were significant differences at $p< 0.05$ between goat milk samples from nineteen herds. Inasmuch as the results showed that the average total bacterial count (TBC) ranged between (7.54 to 8.49 $log_{10}$CFU/ml). In the same context, analyzes of samples have shown the highest mean of TBC were (G1 (8.46), G2 (8.49. G3 (8.49), G8( 8.47),G9(8.49) ($log_{10}$CFU/ml)). While, the lowest averages of TBC were (G15 (7.62), G16 (7.54), G17 (7.61), G18 (7.62), G19 (7.59) ($log_{10}$CFU/ml)).

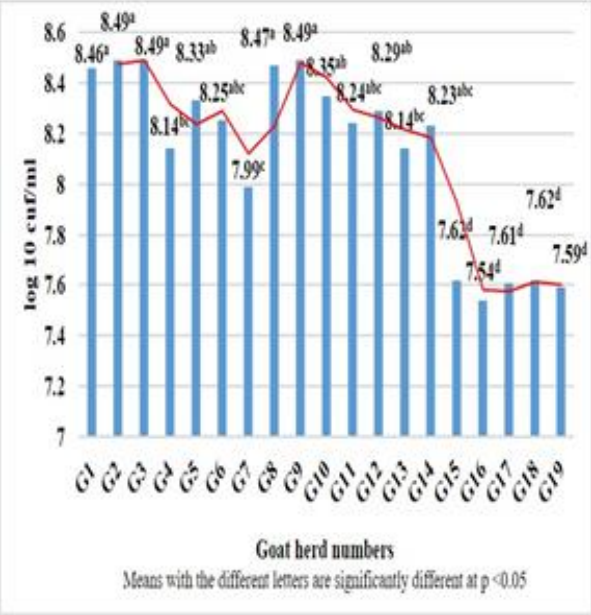


Chart ( 1 ) shows average total bacteria count (TBC) (log10CFU/ml) of goat milk produce in Benghazi

Statistical analysis of goat milk samples (total coliform count (TCC)) (chart 2) reported that there were significant differences at $p< 0.05$ between goat milk specimens. Minimal the mean of TCC was G16 (3.61 log 10 CFU/ml) and maximal the average of TCC was G4 and G6 (7.93 and 7.90 log 10 CFU/ml) respectively. Whilst, outcomes of the analysis of goat milk appeared that there were no significant differences at $p< 0.05$ between each of (G10, G11, G12, G14) (7.73, 7.75, 7.80, 7.73 log 10 CFU/ml) respectively.

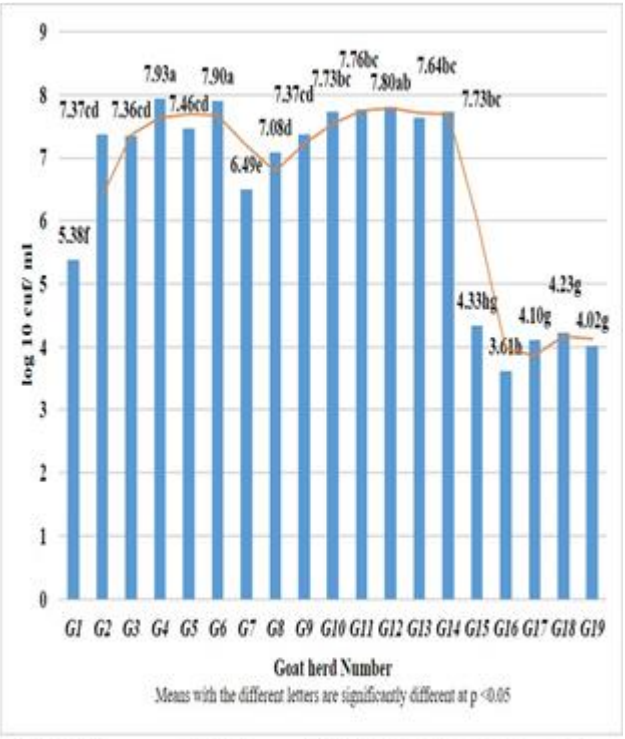


Chart ( 2 ) shows average total coliform count (TCC) (log10CFU/ml) of goat milk produce in Benghazi

Tests for detection of total staphylococcus counts (TSC) (chart 3) showed that there were significant differences at $p< 0.05$ between milk samples. The variances were very high, as the results show that G1, G8, and G2 (6.68, 6.68, and 6.67 log 10 CFU/ml) were the greatest mean value (TSC), respectively. On the contrary, the results reported that G5 and G16 (4.10 and 3.89 log 10 CFU/ml) were the smallest mean value (TSC) respectively.

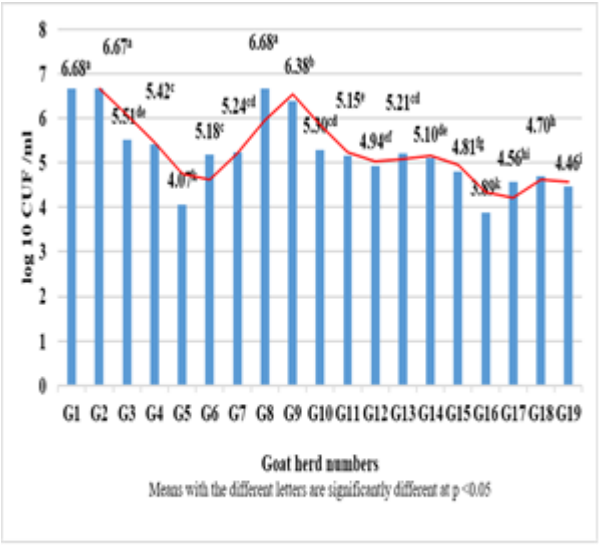


Chart ( 3 ) shows average total staphylococcus counts (TSC) (log10CFU/ml) of goat milk produce in Benghazi

The different biochemical reactions used to define various kinds of bacteria in goat milk are shown in table (1). The result of these tests (Table 2) was the identification of the following

bacterial species: *E. coli, Citrobacter spp., Enterobacter spp., Klebsiella spp., Proteus spp. Salmonella spp., Shigella spp. Bacillus spp., and Brucella spp.*( *Brucella* colonies appear as 1-2 mm diameter convex colonies with round entire edges, and may be identified by slide agglutination) (Bridson,2006). Besides, Shigella has been recognized for its distinct colony color using an XLD Agar setting. The number of isolates exhibiting Salmonella bacteria in the nineteen samples was zero% and in the two samples (G2 and G4) Shigella bacteria was 4% compared to other microbial groups.

Table 1: Biochemical reactions for identification of isolated bacteria from goat milk (Holt et al.)[23].

| Identification | TSI | | | | SIM | | | Simmon | VP | MR | Catalase | Gram Stain |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Slant | Bottom | $H_2S$ | Gas | Sulfide | Indole | Motility | | | | | |
| E. coli | A | A | - | d | - | + | + | - | - | + | + | - |
| Citrobacter | Ak/A | A | d | d | d | d | + | + | - | + | + | - |
| Enterobacter | A | A | - | - | - | + | + | - | - | + | + | - |
| Klebsiella | A | A | - | + | - | d | - | + | + | - | + | - |
| Proteus | Ak | A | + | + | + | - | + | - | - | + | + | - |
| Salmonella | Ak | Ak | - | V | V | - | + | V | - | + | + | - |
| Shigella | Ak | A | V | + | + | - | - | - | - | + | + | - |
| Bacillus | d | d | - | - | - | - | + | + | + | - | + | + |
| Brucella | A | A | - | + | - | - | - | - | - | - | + | - |

Alk: alkaline reaction, A: Acid reaction, butt: at bottom, MR: Methyl Red, VP: Vogas Proskauer,
(+): positive result, (-): Negative result, d: different results, V: Variable

Table (2) has identified *E. coli 41.7%, Bacillus spp. 16.7%, Enterobacter spp. 8.3%, Citrobacter spp. 8.3%, Klebsiella spp. 4.2%, Proteus spp. 4.2%, Shigella spp. 8.3%, Salmonella spp. 0.00%, and Brucella 8.3%* , as a percentage of isolated bacteria produced in Benghazi cit.

**Table 2 percentage of isolated bacteria of goat milk produce in Benghazi City**

| Bacteria | Number of sample | percentage |
|---|---|---|
| *E. coli* | 20 | 41.7% |
| *Bacillus spp.* | 8 | 16.7% |
| *Enterobacter spp.* | 4 | 8.3% |
| *Citrobacter spp.* | 4 | 8.3% |
| *Klebsiella spp.* | 2 | 4.2% |
| *Proteus spp.* | 2 | 4.2% |
| *#Shigella spp.* | 4 | 8.3% |
| *Salmonella spp.* | 0 | 0.0% |
| **Brucella spp.* | 4 | 8.3% |
| Total | 48 | 100% |

**Brucella* colonies appear as 1-2 mm diameter convex colonies with round entire edges and were identified by slide agglutination). *# Shigella* has been recognized for its distinct colony color using an XLD Agar setting.

In this analysis, the microbial content of raw goat milk from various pastures of the city of Benghazi was measured. The total number of bacteria permitted for direct human use of raw milk cannot be greater than 5 x $10^4$ colony-forming units/ml as opposed to European milk Requirements. As confirmed by European criteria the quality depends on the bacterial content, for industrial processes the total amount of raw milk bacteria shall not exceed 105 ml of colony-forming unit and must not exceed 100 to 500 cells / ml of staphylococcus bacteria (EEC 92/ 46)[23].

The total number of microbes (chart 1), as well as the numbers of coliforms (chart 2) and Staphylococcus aureus (chart 3), was above the permitted limitations for direct human use and the European standards for milk products were compared. The findings revealed that in the nineteen flocks, the overall gross number of bacteria surpassed 100 percent of samples, and the products under the conditions of good hygienic practice were not exceeded.

The method of total bacteria count bacteria reveals the amount of the microorganism in the food and the rise in the number of bacteria in dairy products suggests low processing, poor storage, or either of the processes of milk handling [24]. The average total amount of bacteria in raw goat milk samples was 2.4 x $10^9$ CFU/ml in this investigation. These findings are similar to those of Kateregga et al., who found that the overall bacterial count of milk samples obtained from three points of milk sale in Somalia was high, reaching 1.27 x $10^9$ CFU/ml [25].

Table 2 indicates that *E.coli and Bacillus spp*., which is accompanied by *Enterococcus spp*., and Citrobacter *spp*. were the most widespread occurrence of coliform bacteria in the raw goat milk samples, which has shown the health status of the milk samples with the microbiota distribution of the extracted items. The average coliform bacteria in the test samples were 3.1 x $10^6$, which was above the permitted number of standard FAD (no more than 10 CFU/mL throughout shelf life) [26]. It shows a lack of interest in health procedures, whether for livestock or for milk or the machinery used for manufacturing, storage, and delivery of milk and in general, there is a lack of attention to hygiene. Pantoja et al. observed that the coliform bacteria count quadrupled to 26-115 CFU per ml[27].

The average Staphylococcus aureus bacteria in the trial was 1.68 x $10^5$, while the average Staphylococcus aureus bacteria in the G5 sample was near to the upper limit and could not be accepted for marketing and consumption. These results are in agreement with many previous studies that demonstrated the

presence of coliform bacteria (*Staphylococcus aureus* and *E. coli* [28], as well as *Bacillus spp*., and Clostridium sp. [29], [30], and *Klebsiella pneumoniae* [31]. The prevalence of this amount of Staphylococcus aureus bacteria indicates that poor handling of the product during manual milking, handling, transport, and packaging operations.

The milking process, such as the cleanliness of the milking, the state of storage, the manner of transportation, and the health of the animal's udder, all influence the quality and cleanliness of the milk content[32]. Milk processing and multiple milk products can have both public health and economic pressures under unsafe conditions as well as bad production practices [30].

## IV. CONCLUSION

In the current study, some types of pathogenic bacteria in the goat milk collected from nineteen samples in the city of Benghazi was detected. These types were observed in various proportions in the collected milk samples and as follows E. coli 41.7%, Bacillus spp. 16.7%, Enterobacter spp. 8.3%, Citrobacter spp. 8.3%, Klebsiella spp. 4.2%, Proteus spp. 4.2%, Shigella spp. 8.3%, Salmonella spp. 0.00%, and Brucella 8.3%. These results demonstrate that milking goats lack hygienic conditions. Thus, from the current study, we conclude that the causes of bacterial goat milk contamination can come from plastic bottle contamination, goat's udder, milkman's hands, milking tools, or goat's infection with mastitis, which can lead to bacterial contamination of milk with pathogens.

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