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## Research Article

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# Estimation and Detection of Microbial Contamination in Milk – South Khartoum State

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**Abstract:** The aim of this study was to assess the bacterial load in milk samples sold in selected cafeterias and grocery shops, focusing on the total count of aerobic bacteria and coliforms, as well as to identify the biochemical characteristics of the isolated bacteria. Ten samples were collected from various locations, including cafeterias at the International University of Africa and the Al-Azhari and Al-Inqaz neighbourhoods. The results showed a marked variation in the mean counts of aerobic bacteria among the samples, ranging from ($5.2 \times 10^5$) to ($1.03 \times 10^8$) cells/ml, reflecting differences in contamination levels and handling conditions. The presence of coliform bacteria was also detected in all samples, with averages ranging from ($1.5 \times 10^4$) to ($>1.1 \times 10^6$) cells/ml, a clear indication of faecal contamination and poor hygiene practices during production, transport or storage. The results of the biochemical tests revealed variations in the characteristics of the bacterial isolates between the different samples; however, the predominant bacterium in most samples was Klebsiella pneumoniae, whilst Bacillus subtilis was found in lower proportions in some samples. These results indicate the poor quality of the milk circulating in the studied areas and its non-compliance with health standards, posing a potential risk to public health. The study recommends the need to improve hygiene and sterilisation practices during the production and distribution stages, alongside strengthening health monitoring and raising awareness of the risks of bacterial contamination in food.

**Keywords:** *Milk, Aerobic Bacteria, Coliform Bacteria, Klebsiella Pneumoniae, Food Contamination, Food Safety.*



# INTRODUCTION

The dairy sector plays a significant role in society and in the country's economy, according to the Food and Agriculture Organisation (FAO, 2018). Food is one of the most essential human needs for survival; a person cannot maintain good health on a single type of food,and their diet must be balanced so that it provides them with the energy required for their activities (carbohydrates and fats) and also supplies them with the proteins necessary to build their bodies and repair damaged cells, in addition to containing the vitamins and minerals essential for metabolic processes within the body (Khalil, 2005) Food safety is one of the vital pillars of public health and economic development, as contaminated food is a direct cause of numerous diseases and places a significant burden on the health and economic systems of developing countries (WHO 2022) With the significant advances in microbiology associated with food contamination, numerous disease-causing microbes have been identified; such microbes have the ability to grow in food. As scientific progress continues, consumers have come to realise that the presence of these microbes is not always accompanied by the production of an unpleasant taste, odour or appearance. Consequently, some foods naturally appear to be safe and fit for consumption (Al-Aib *et al.*, 2024). Milk is considered a complete food due to its nutritional content, which includes proteins, carbohydrates, fats, and the salts necessary for growth, as well as certain vitamins (Khalif, 2014). and a small amount of enzymes. Milk also contains organic acids and their salts, as well as antibodies, particularly in colostrum (Abdullah, 2002). On the other hand, milk contains a large amount of water, which is the basis of biological activity in milk; it is a very important factor for this activity. If we add to this the fact that the temperature of milk at milking is usually 37°C—a temperature suitable for the growth of many microorganisms—microbial activity in milk is intense, leading to numerous undesirable changes in milk quality and potentially resulting in spoilage. Hence, a sufficient understanding of dairy microbiology enables us to grasp the key principles of safe handling of milk and its products, thereby ensuring the safety and quality of the milk. It also ensures the utilisation of the beneficial applications of dairy microbiology in the manufacture of diverse products that meet consumers' tastes and needs (Khalif, 2014). The presence of microorganisms in general, and bacteria in particular, in small numbers does not necessarily mean that the milk is of good quality, as

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it is possible that the milk was produced by an infected cow and that the low bacterial count may include pathogenic or toxin-producing bacteria (Qasim and Mazahra, 2003). Milk and dairy products are frequently exposed to contamination during the production stages, after heat treatment, and during processing, packaging, storage and consumption; furthermore, they can transmit many diseases to humans, whether of animal or human origin. and for this reason, milk and dairy products must be thoroughly inspected to ensure the elimination of pathogenic and spoilage microorganisms present in them, or to dispose of them if they are found to be unfit for consumption, as most microbes grow and multiply rapidly within it, causing many undesirable changes that have a significant impact on the quality of the milk and its suitability for processing into dairy products. Under operational conditions, large numbers of microbes from animals, humans and the containers used reach the milk unless these are clean and sterilised, and some of these microbes may be pathogenic, leading to the spread of disease among consumers (Morshedi, 1998). With the renaissance targeted by the third millennium, amidst the tremendous development in various sciences—particularly food science and nutrition—this renaissance has also been accompanied by an increase in consumer awareness of food, especially regarding healthier choices in general. As milk and dairy products are among the most important foods used in nutrition to improve health and as an adjunct even during periods of treatment and disease prevention (Abu Zeid, 2007). It is essential to educate consumers about the microbes present in milk; this is considered a vital issue that leads to increased health awareness. Given researchers' interest in this field, studies have identified the pathogenic microbes transmitted by humans, as well as those transmitted by dairy animals such as cows, and methods for eliminating these microbes (Khalif, 2014).

This study aims to assess the level of microbial contamination in milk in southern Khartoum State by determining the total aerobic bacterial count, as well as estimating the number of coliform bacteria. The study also seeks to identify and characterise other types of bacteria present in milk. **Materials and Methods.**

**Materials and Tools Used**
**First: Materials**
Culture media

1. Nutrient Agar (NA) → for the growth of general bacteria
2. Plate Count Agar (PCA) → for counting total aerobic bacteria
3. MacConkey Agar → for isolating coliform bacteria
4. Eosin Methylene Blue Agar (EMB) → for confirming Escherichia coli
5. Blood Agar → for the detection of pathogenic bacteria and blood decomposition
6. Mannitol Salt Agar (MSA) → for isolating staphylococci

**Reagents and Chemicals**

1. Sterile saline solution (0.85% NaCl) → for dilutions
2. Sterile distilled water
3. 70% ethyl alcohol → for sterilisation
4. Gram stains (Crystal violet, Iodine, Safranin, Alcohol) □ Biochemical test reagents such as:
   - Catalase test o Oxidase test o Indole test o Citrate test
   - Urease test

**Sample Collection Materials**

- Sterile containers/tubes for milk collection
- Medical gloves
- Cool boxes for storing samples during transport

**Second : Equipment**
**Basic tools used**

- Petri dishes
- Test tubes
- Pipettes and micropipettes
- Sterile pipette tips
- Test tube rack

**Preparation and Sterilisation Equipment**

- Autoclave
- Hot air oven
- Bunsen burner or alcohol lamp
- Refrigerator (4°C) for sample storage

**Culture and Analysis Equipment**

- Bacterial incubator at 37°C
- Analytical balance
- Vortex mixer
- Water bath

**Testing Equipment**

- Microscope
- Colony counter
- Slides

**Methods Used**
**Preparations**
The culture media, Petri dishes and test tubes were prepared and sterilised prior to collecting milk samples at the laboratory of the Faculty of Pure and Applied Sciences at the International University of Africa.

**Sampling**
Ten milk samples, each comprising 50 ml, were collected in pre-sterilised glass containers from milk dispensers in canteens and transported directly to the laboratory of the Faculty of Pure and Applied Sciences. All ten milk samples were collected from different areas in the south of Khartoum State. Two samples were from the International University of Africa; two from Al-Azhari District, Block 10; two from Al-Azhari District, Block 11; two from AlInqaz District, Block 2; and two from Al-Inqaz District, Block 3. All were taken from milk distribution outlets (grocery shops and cafeterias).

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**Microbiological analyses**

**Serial dilutions**

For the cultivation of various microorganisms in food media, the serial dilution method was used, where 1 ml of the sample was added to 9 ml of peptone water in a test tube and shaken well to achieve complete homogeneity of the solution and thus a homogeneous distribution of the micro-organism within the tube, resulting in a dilution of $10^{-1}$ . Then, 1 ml was taken from the first tube and added to the second test tube containing 9 ml of peptone water, and it was shaken to obtain a dilution of $10^{-2}$ . Then, 1 ml was taken from the second tube and added to the third test tube containing 9 ml of peptone water, and it was shaken to obtain a dilution of $10^{-3}$ . Then, 1 ml was taken from the third tube and added to the fourth test tube containing 9 ml of peptone water, and it was shaken to obtain a dilution of $10^{-4}$ . Then, 1 ml was taken from the fourth tube and added to the fifth test tube containing 9 ml of peptone water, and it was shaken to obtain the dilution $10^{-5}$ . Then, 1 ml was taken from the fifth tube and added to the sixth test tube containing 9 ml of peptone water, and it was shaken to obtain the dilution $10^{-5}$ . Then, 1 ml was taken from (Darrar, 2010).

**Estimation of the total number of aerobic bacteria (Total plate count)**:

For this purpose, the agar plate count method was used, preparing a Petri dish for each dilution (Al-Tamimi *et al.*, 2014) . One millilitre of each dilution was taken and placed in a sterile Petri dish; approximately 15–20 millilitres of sterile nutrient medium was added to this sample after it had been cooled to 45°C. This added volume was mixed thoroughly with the diluted sample and the mixture was left to solidify, All plates were then incubated in an inverted position at 37°C for 48 hours. The number of colony-forming units was counted using a colony counter. The number of colony-forming units was then calculated according to the following formula:

Number of cells in the original sample = number of colonies × inverse dilution factor. The calculation of colony counts focused on plates containing between 30 and 300 colonies (Darar, 2010). **Estimation of total coliform bacteria** count **Probabilistic testing for coliform bacteria:**

As stated in ISO/CD (1997) and Andreus (1997), the most probable number method was used in MacConkey broth to count these bacteria. Peptone water was used as the diluent, with the following dilutions: ($10^{-1}$ ,$10^{-2}$ ,$10^{-3}$ ,$10^{-4}$ ,$10^{-5}$). A Durham tube was placed in each tube containing MacConkey broth. One of each dilution was placed in three tubes containing MacConkey broth. All tubes were incubated at 37°C for 48 hours, after which the Durham tubes were examined for gas formation.

**Confirmatory test for E. coli:**

A tube containing positive MacConkey broth (in which gas had formed) was inoculated. A loopful was transferred to a tube containing brilliant green bile broth at 37°C. Any gas production and the appearance of slight turbidity in the Durham tubes were considered a positive confirmatory test. Results were calculated using MPN tables based on the positive MacConkey broth tubes from three consecutive dilutions (ISO/CD, 1997).

**Calculation of coliform counts using the most probable number method**:

*The most probable number in 1 ml = (value extracted from the table × reciprocal of the median dilution) ÷ 10.

* To extract the value from the table, consider the three positive results, i.e. the result before zero (Khalif, 2014).

**Supplementary test for coliform bacteria:**

According to ISO/CD (1997), Eosin Methylene Blue (EMB) was used. Plates were inoculated by filling a loopful of the confirmatory and positive brilliant green broth culture. The plates were incubated at 37°C for 24 hours. Single colonies with a dark centre, with or without a metallic sheen, were considered positive. Two or more colonies were taken from each EMB agar plate and transferred to slant nutrient agar for morphological and biochemical testing.

**Biochemical Tests**

**Gram stain**

A clean slide was taken and a drop of water was placed on it; then, using an inoculation loop, a small sample of the colony was taken and placed on the drop on the slide. This was then mixed, smeared, flamed, and left to fix and dry. Crystal violet stain was added to the bacteria after they had been fixed on the slide.After several minutes, this stain was washed off with water, then a quantity of iodine was added to cover the surface of the slide; this acts as a fixative for the stain. After two minutes, the iodine was poured off the slide; at this stage, it can be seen that both Gram-negative and Gram-positive bacterial groups appear dark violet. The slide was then washed with ethyl alcohol, which acts as a stain remover ( ). It was observed that washing with this substance removed the stain from some of the bacterial cells on the slide but not from others. Finally, the alcohol was washed off the slide with water, and it was stained with safranin (a red dye) for several minutes, washed with water, left to dry, and then examined under an oil immersion lens.It is observed that the violet dye (violet + iodine) binds to the dye-receptive part of the bacterial cell and colours it violet. Bacteria that retain this violet colour after washing with ethyl alcohol are called Gram-positive, whilst bacteria that lose the violet colour when washed with ethyl alcohol are called Gram-negative. Since Gram-negative bacteria become colourless when washed with alcohol, they are difficult to observe under a microscope; therefore, we stain them with safranin, which gives them a red colour http://www.paldf.net/forum/showthread.php?: 1117650.

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**Indole test**

It is used to detect the presence of indole, which is one of the metabolic products of the amino acid tryptophan, as a result of the bacterium possessing the enzyme tryptophanase. The was inoculated into liquid peptone broth containing the isolate under study; the liquid peptone broth, which had been sterilised, and the inoculum was pipetted into test tubes that had been previously prepared and incubated at 37°C for 24 hours. then 5 drops of Kovac's reagent were added to the inner surface of the test tube; the result is considered positive if a red ring ( ) appears within seconds of adding the reagent (Koneman *et al.*, 1992).

**Catalase test**

This test is used to detect the ability of bacteria to produce the enzyme catalase, which breaks down hydrogen peroxide into water and releases oxygen gas in the form of air bubbles. A portion of each bacterial isolate was transferred individually to a clean glass slide, and a few drops of 3% hydrogen peroxide ($H_2O_2$) were added using a pipette; the appearance of oxygen gas bubbles is evidence of a positive test result (Koneman *et al.*, 1992).

**Kligler Iron Agar Test**

This medium is primarily used to diagnose the Enterobacteriaceae family to determine the organism's ability to ferment specific carbohydrates with or without the production of sulphuric acid. In a positive result, a yellow colour is produced; in a negative result, the colour is red (http://www.bd.co//ds.clincalcenter.

**Methyl Red**

This test is used to determine the ability of certain microbes to produce a large amount of acid when fermenting glucose, which in turn will lower the pH of the culture medium to a level below 4, at which point the colour of the indicator will change. The liquid peptone broth is inoculated with the isolate under study, The sterile liquid peptone broth was inoculated into test tubes, each containing a separate culture, and the medium was incubated at 37°C for 24 hours. Drops of methyl red were then added to the surface inside the test tube; the result is considered positive if a red ring appears within seconds of adding the methyl red (Koneman *et al.*, 1992).

**Urease test**

The tubes containing the urea agar medium were inoculated by the stab-and-streak method on the slant and then incubated at 37°C for 24 hours. A pink colour change in the medium indicated a positive test (Koneman *et al.*, 1992).

**Citrate utilisation test**

The isolates were inoculated onto slanted Simon-Strat medium; the tubes were incubated at 37 °C for 24 hours. A positive result was indicated by a colour change in the medium from green to blue due to the consumption of streptomycin (Koneman *et al.*, 1992).

The results of the microbiological analysis of the milk samples showed a clear variation in the total viable count and coliforms between the different samples. In samples (1) and (2) taken from cafeterias (A) and (B) at the International University of Africa, the average number of aerobic bacteria was $1.308 \times 10^6$ cells/ml and $3.383 \times 10^6$ cells/ml, respectively (Tables 1 and 5).

Coliforms were also detected at an average of $2.8 \times 10^4$ and $1.5 \times 10^4$ cells/ml respectively (Tables 2 and 6). The results of the biochemical tests revealed some differences in the characteristics of the bacterial isolates, with Klebsiella pneumoniae being the predominant bacterium (Tables 4 and 8). As for samples (3) and (4) taken from grocery shops in the Al-Azhari neighbourhood (Block 11), the average number of aerobic bacteria was $4.923 \times 10^7$ cells/ml and $2.56 \times 10^7$ cells/ml, respectively (Tables 9 and 12). E. coli was also detected at an average of $3.5 \times 10^4$ and $1.5 \times 10^5$ cells/ml (Tables 10 and 13). Biochemical tests revealed differences in the characteristics of the isolated bacteria, with Klebsiella pneumoniae (Tables 12 and 16). In samples (5) and (6) taken from the Al-Azhari neighbourhood (Block 10), the average number of aerobic bacteria was $5.2 \times 10^5$ cells/ml and $1.03 \times 10^8$ cells/ml, respectively (Tables 17 and 21). E. coli was also recorded at an average of $2.1 \times 10^5$ and $3.5 \times 10^5$ cells/ml (Tables 18 and 22).

Biochemical results revealed differences in the characteristics of the bacterial isolates, indicating diversity in bacterial species (Tables 20 and 24). As for samples (7) and (8) taken from grocery shops in the Al-Inqaz neighbourhood (Block 3), the average number of aerobic bacteria was $7.1 \times 10^7$ cells/ml and $4.7 \times 10^5$ cells/ml respectively (Tables 25 and 29). The results also showed a significant increase in the numbers of coliform bacteria, exceeding $1.1 \times 10^6$ cells/ml in both samples (Tables 26 and 30). Biochemical tests revealed that the predominant bacterium was Klebsiella pneumoniae, whilst Bacillus subtilis was the least prevalent (Tables 28 and 32). In samples (9) and (10) taken from grocery shops in the Al-Inqaz neighbourhood (Block 2), the average number of aerobic bacteria was $2.89 \times 10^8$ cells/ml and $8.9 \times 10^7$ cells/ml, respectively (Tables 33 and 37). Coliform bacteria were also recorded at an average of $1.1 \times 10^6$ and $2.1 \times 10^5$ cells/ml, respectively (Tables 34 and 38). The results of the biochemical tests confirmed differences between the isolates, with Klebsiella pneumoniae being the predominant species (Tables 36 and 40).

# RESULTS

The average count of aerobic bacteria in sample 1, taken from Cafeteria A (at Africa International University $10^6 \times$ , was 1,308 cells/ml (Table 1), whilst the average count in sample 2, taken from Cafeteria B) (at the International University of Africa $10^6 \times$ ) was 3,383

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cells/ml (Table 5). The results indicated the presence of E. coli in samples 1 and 2, with averages of 2.8 $\times 10^4$ and 1.5 $\times 10^4$ cells, respectively (Tables 2 and 6). The results of the biochemical tests also showed that there were some differences in the characteristics of the bacteria isolated from samples 1 and 2, with *K. pneumoniae* being the predominant bacterium (Tables 4 and 8).The average count of aerobic bacteria in sample 3, taken from a grocery shop in Al-Azhari neighbourhood, Block 11A ($10^7 \times$ , was 4.923 cells/ml (Table 9), whilst the average count in sample 4, taken from a grocery shop in Al-Azhari neighbourhood, Block 11B ($10^7 \times$ was 2.56 cells/ml (Table 12). The results indicated the presence of E. coli in samples 3 and 4, with averages of 3.5 $\times 10^4$ and 1.5 $\times 10^5$ cells, respectively (Tables 10 and 13). The results of the biochemical tests also showed that there were some differences in the characteristics of the bacteria isolated from samples 3 and 4, with *K. pneumoniae* being the predominant bacterium (Tables 12 and 16). The average count of aerobic bacteria in sample 5, taken from Al-Azhari neighbourhood, Block 10A, was 5.2 $\times 10^5$ (Table 17), whilst the average count in sample 6, taken from Al-Azhari neighbourhood, Block 10B, was 1.03 $\times 10^8 \times$ cells/ml (Table 21). The results indicated the presence of E. coli in samples 5 and 6, with an average of 2.1 $\times 10^5$ and 3.5 $\times 10^5$ cells, respectively (Tables 18 and 22). The results of the biochemical tests also showed that there were some differences in the characteristics of the bacteria isolated from samples 5 and 6, with the predominant bacteria shown in *Tables* 20 and 24. The average count of aerobic bacteria in sample 7, taken from a grocery shop in Al-Inqaz neighbourhood, Block 3A ($10^7 \times$ , was 7.1 cells/ml (Table 25), whilst the average count in sample 8, taken from a grocery shop in Al-Inqaz neighbourhood, Block 3B ($10^5 \times$ , was 4.7 cells/ml (Table (29). The results indicated the presence of E. coli in samples 7 and 8, with an average of more than 1.1 $\times 10^6$ cells in Tables 26 and 30 respectively. The results of the biochemical tests also showed that there were some differences in the characteristics of the bacteria isolated from samples 7 and 8; the predominant bacterium was *K. pneumoniae* and the least common was *B. subtilis* (Tables 28 and 32). The average count of aerobic bacteria in sample 9, taken from a grocery shop in Al-Inqaz neighbourhood, Block 2A ($10^8 \times$ , was 2.89 cells/ml (Table 33), whilst the average count in sample 10, taken from a grocery shop in Al-Inqaz neighbourhood, Block 2B ($10^7 \times$ was 8.9 cells/ml (Table 37). The results indicated the presence of E. coli in samples 9 and 10, with averages of 1.1 $\times 10^6$ and 2.1 $\times 10^5$ cells, respectively (Tables 34 and 38). The results of the biochemical tests also showed that there were some differences in the characteristics of the bacteria isolated from samples 9 and 10, with *K. pneumoniae* being the predominant bacterium (Tables 36 and 40).

**Table 1: Shows the Results of The Aerobic Bacterial Count for Sample No. (1)**

| Dilutions | Number of colonies | Cells/ml |
|---|---|---|
| $10^{-1}$ First dilution | Uncounted | |
| $10^{-2}$ Second dilution | 250 | $10^5 \times$ 0.25 |
| $10^{-3}$ Third reduction | 197 | $10^5 \times$ 1.97 |
| $10^{-4}$ Fourth dilution | 121 | $10^5 \times$ 12.1 |
| $10^{-5}$ Fifth reduction | 38 | $10^5 \times$ 38 |
| $10^{-6}$ Sixth mitigation | 20 | |
| **Average number of cells/ml** | | **$10^6 \times$ 1.308** |

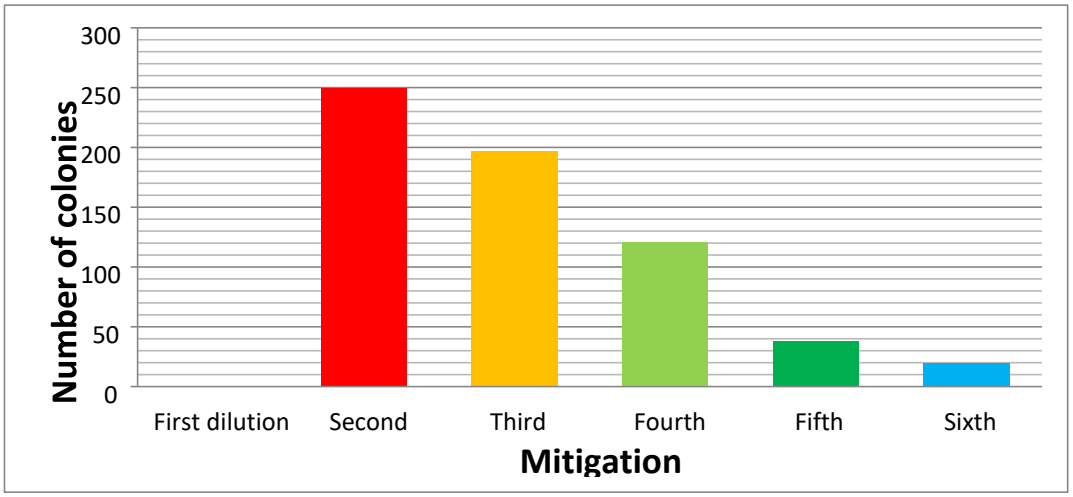


**Figure 1: Shows the colony counts for aerobic bacteria in sample No. (1)**

**Table 2: Shows the results of the probability test for coliform bacteria in sample No. (1)**

| Dilutions | Number of tubes per dilution | Number of tubes in which gas formed |
|---|---|---|
| 10−1 | 3 | 3 |
| 10−2 | 3 | 3 |
| 10−3 | 3 | 2 |
| 10−4 | 3 | 2 |
| 10−5 | 3 | 1 |
| **Most probable number (MPN)** | | **2.8 ×10⁴ cells/ml** |

**Table 3: Shows the results of the confirmatory test for E. coli in sample No. (1)**

| Dilutions | Number of Tubes Per Dilution | Number of Tubes in Which Gas Formed |
|---|---|---|
| 10−1 | 3 | 3 |
| 10−2 | 3 | 3 |
| 10−3 | 2 | 2 |
| 10−4 | 2 | 1 |
| 10−5 | 1 | 1 |
| Total | 11 | 10 |

**Table 4: Shows the Results of The Biochemical Tests for Bacteria in Sample No. (1)**

| Bacteria | SH | GS | I | MR | Gas | KIA | | U | C | SD | No |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Slop | Butt | | | | |
| K. pneumonae | Rod | - | - | - | + | Y | Y | + | + | 10−1 | 01 |
| K. pneumonae | Rod | - | - | - | + | Y | Y | + | + | 10−1 | 02 |
| K. Oxytoca | Rod | - | + | - | + | Y | Y | + | + | 10−1 | 03 |
| K. Oxytoca | Rod | - | + | - | + | Y | Y | + | + | 10−2 | 04 |
| Bacillus alvei | Rod | + | + | - | + | R | Y | + | + | 10−2 | 05 |
| Bacillus cereus | Rod | + | - | - | + | R | Y | + | + | 10−2 | 06 |
| K. Oxytoca | Rod | - | + | - | + | Y | Y | + | + | 10−3 | 07 |
| K. pneumonae | Rod | - | - | - | + | Y | Y | + | + | 10−3 | 08 |
| K. pneumonae | Rod | - | - | - | + | Y | Y | + | + | 10−4 | 09 |
| K. pneumonae | Rod | - | - | - | + | Y | Y | + | + | 10−5 | 10 |

SD = serial dilution. C = citrate. U = urease. KIA = MR = methyl red. I = Indole. GS = Gram stain. SH = shape

**Table 5: Shows the Aerobic Bacterial Counts for Sample No. (2)**

| Dilutions | Number of colonies | Number of cells |
|---|---|---|
| $10^{-1}$ First dilution | Uncount | |
| $10^{-2}$ Second dilution | Uncount | |
| $10^{-3}$Third dilution | 285 | $10^5 \times$ 2.85 |
| $10^{-4}$ Fourth reduction | 193 | $10^5 \times$ 19.3 |
| $10^{-5}$ Fifth reduction | 75 | $10^5 \times$ 75 |
| $10^{-6}$ Sixth reduction | 27 | |
| **Average number of cells** | | **$10^6 \times$ 3.383** |

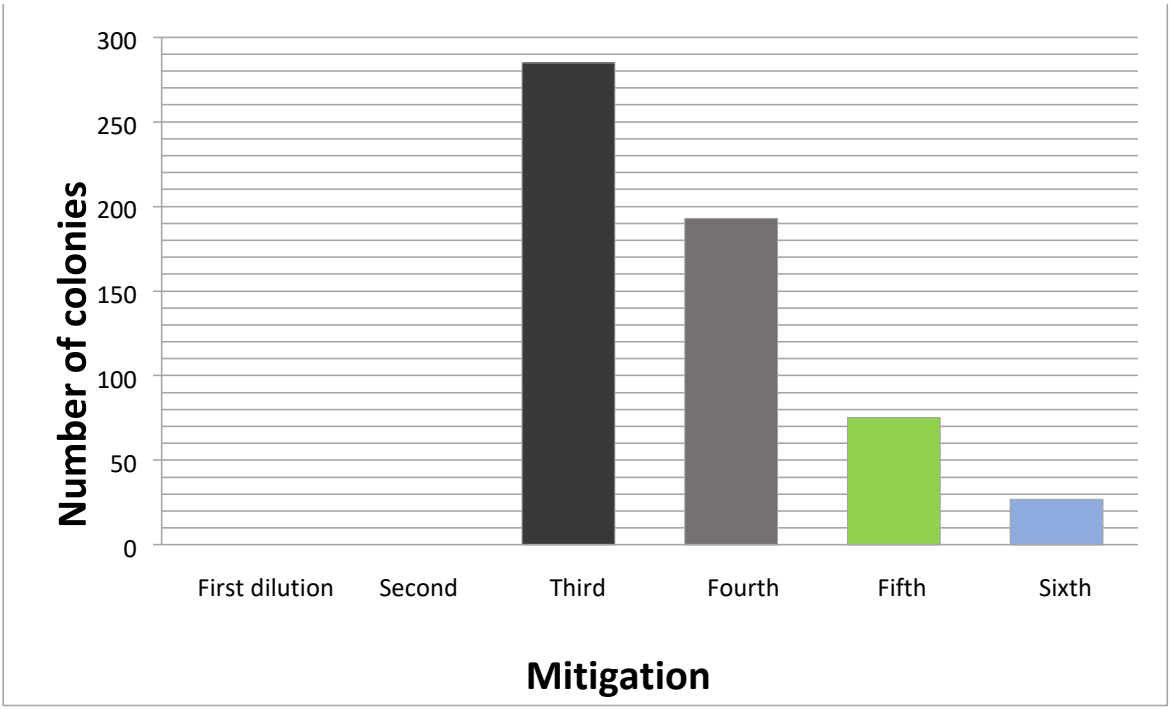


**Figure 2: Shows the Colony Counts for Aerobic Bacteria in Sample No. (2)**

**Table 6: Shows the Results of the Probability Test for Coliform Bacteria in Sample No. (2)**

| Dilutions | Number of tubes per dilution | Number of tubes in which gas formed |
|---|---|---|
| 10−1 | 3 | 3 |
| 10−2 | 3 | 3 |
| 10−3 | 3 | 2 |
| 10−4 | 3 | 1 |
| 10−5 | 3 | 0 |
| **Most probable number (MPN)** | | **1.5 ×10⁴ cells/ml** |

**Table 7: Shows the Results of the Confirmatory Test for E. Coli in Sample No. (2)**

| Dilutions | Number of tubes per dilution | Number of tubes in which gas formed |
|---|---|---|
| 10−1 | 3 | 2 |
| 10−2 | 3 | 2 |
| 10−3 | 2 | 1 |
| 10−4 | 1 | 1 |
| 10−5 | 0 | 0 |
| Total | 9 | 6 |

**Table 8: Shows the Results of The Biochemical Tests for Bacteria in Sample No. (2)**

| Bacteria | SH | GS | I | MR | Gas | KIA | | U | C | SD | No |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Slop | Butt | | | | |
| K. pneumonae | Rod | - | - | - | + | Y | Y | + | + | 10−1 | 01 |
| K. pneumonae | Rod | - | - | - | + | Y | Y | + | + | 10−1 | 02 |
| K. Oxytoca | Rod | - | + | - | + | Y | Y | + | + | 10−2 | 03 |
| Bacillus alvei | Rod | + | + | - | + | R | Y | + | + | 10−2 | 04 |
| K. pneumonae | Rod | - | - | - | + | Y | Y | + | + | 10−3 | 05 |
| K. pneumonae | Rod | - | - | - | + | Y | Y | + | + | 10−4 | 06 |

**Table 9: Shows the Results of The Aerobic Bacterial Count for Sample No. (3)**

| Dilutions | Number of colonies | Cells/ml |
|---|---|---|
| $10^{-1}$ First dilution | Uncounted | |
| $10^{-2}$Second dilution | Uncount | |
| $10^{-3}$ Third dilution | Uncounted | |
| $10^{-4}$ Fourth mitigation | 289 | $10^{6} \times 2.89$ |
| $10^{-5}$ Fifth reduction | 208 | $10^{6} \times$ 20.8 |
| $10^{-6}$Sixth reduction | 124 | $10^{6} \times$ 124 |
| **Average number of cells** | | **$10^{7} \times$ 4.923 cells/ml** |

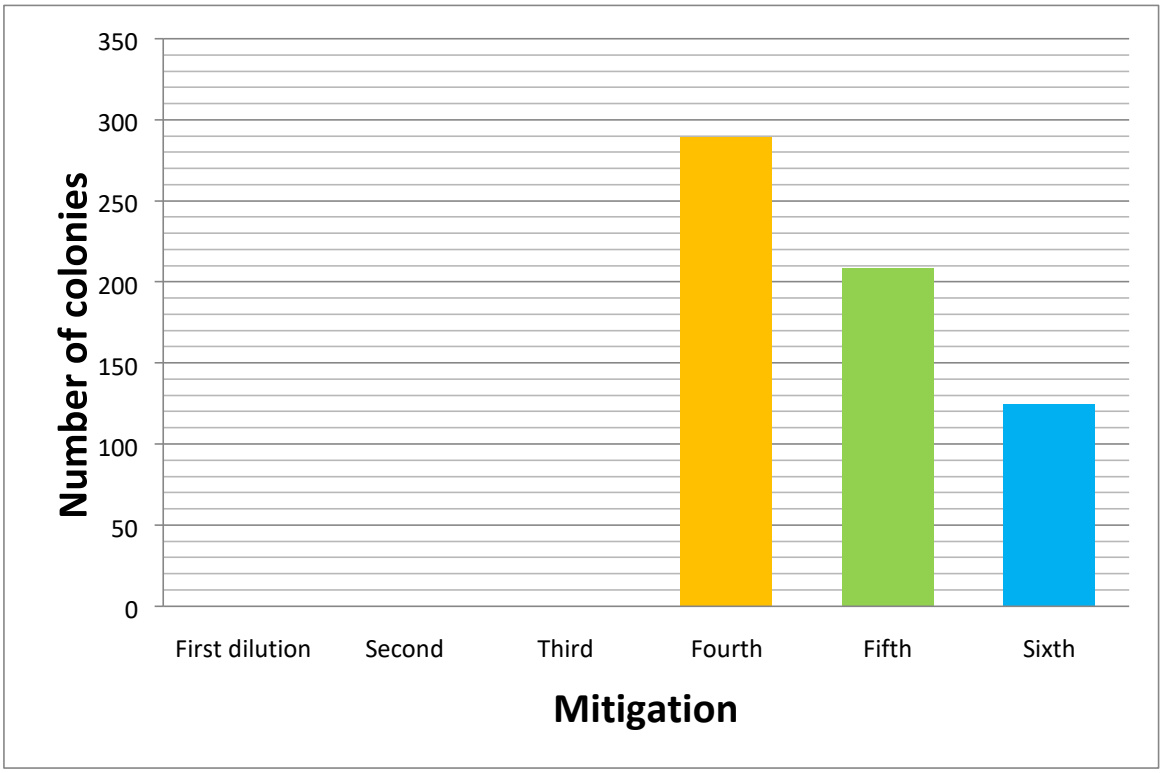


**Figure 3: Shows the Number of Aerobic Bacterial Colonies in Sample No. (3)**

**Table 10: Shows the Results of The Probability Test for Coliform Bacteria in Sample No. (3)**

| Dilutions | Number of tubes per dilution | Number of tubes in which gas formed |
|---|---|---|
| 10−1 | 3 | 3 |
| 10−2 | 3 | 3 |
| 10−3 | 3 | 2 |
| 10−4 | 3 | 2 |
| 10−5 | 3 | 2 |
| **Most probable number (MPN)** | | **3.5 ×$10^{4}$ cells/ml** |

**Table 11: Shows the Results of The Confirmatory Test for E. Coli in Sample No. (3)**

| Dilutions | Number of tubes per dilution | Number of tubes in which gas has formed |
|---|---|---|
| 10−1 | 3 | 3 |
| 10−2 | 3 | 3 |
| 10−3 | 2 | 1 |
| 10−4 | 2 | 0 |
| 10−5 | 2 | 0 |
| Total | 12 | 7 |

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**Table 12: Shows the Results of The Biochemical Tests for Bacteria in Sample No. (3)**

| Bacteria | SH | GS | I | MR | Gas | KIA | | U | C | SD | No |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Slop | Butt | | | | |
| K. Oxytoca | Rod | - | + | - | + | Y | Y | + | + | 10−1 | 01 |
| Bacillus cereus | Rod | + | - | - | + | R | Y | + | + | 10−1 | 02 |
| K. Pneumoniae | Rod | - | - | - | + | Y | Y | + | + | 10−1 | 03 |
| K. Pneumonae | Rod | - | - | - | + | Y | Y | + | + | 10−2 | 04 |
| Bacillus cereus | Rod | + | - | - | + | R | Y | + | + | 10−2 | 05 |
| K. Pneumonae | Rod | - | - | - | + | Y | Y | + | + | 10−2 | 06 |
| K. Pneumonae | Rod | - | - | - | + | Y | Y | + | + | 10−3 | 07 |

**Table 13: Shows the Results of Aerobic Bacterial Counts for Sample No. (4)**

| Dilutions | Number of colonies | Cells/ml |
|---|---|---|
| $10^{-1}$ First dilution | Uncounted | |
| $10^{-2}$Second dilution | Uncount | |
| $10^{-3}$ Third dilution | Uncounted | |
| $10^{-4}$ Fourth mitigation | Uncounted | |
| $10^{-5}$ Fifth attenuation | 256 | $10^{6}\times$ 25.6 |
| $10^{-6}$Sixth dilution | 23 | |
| **Average number of cells** | | **$10^{7}\times$ 2.56 cells/ml** |

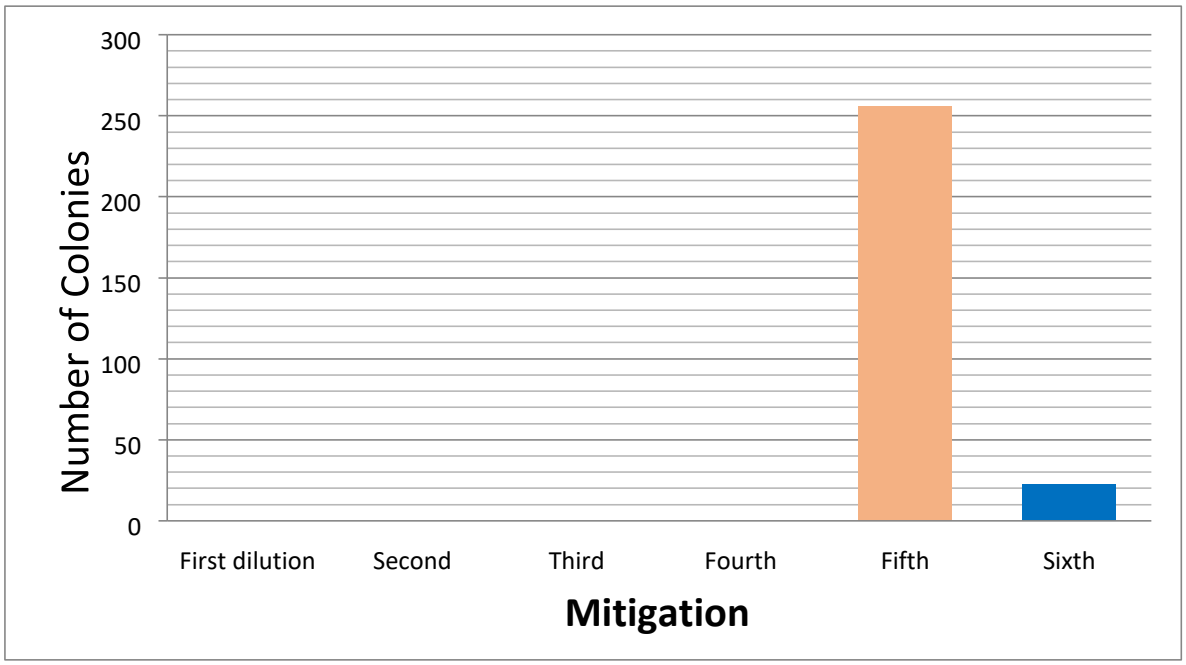


**Figure 4: Shows the Colony Counts for Aerobic Bacteria in Sample No. (4)**

**Table 14: Shows the Results of The Probability Test for Coliform Bacteria in Sample No. (4)**

| Dilutions | Number of tubes per dilution | Number of tubes in which gas formed |
|---|---|---|
| 10−1 | 3 | 3 |
| 10−2 | 3 | 3 |
| 10−3 | 3 | 3 |
| 10−4 | 3 | 2 |
| 10−5 | 3 | 1 |
| **Most probable number (MPN)** | | **1.5 ×$10^{5}$ cells/ml** |

**Table 15: Shows the Results of The Confirmatory Test for E. Coli in Sample No. (4)**

| Dilutions | Number of tubes per dilution | Number of tubes in which gas formed |
|---|---|---|
| 10−1 | 3 | 3 |
| 10−2 | 3 | 2 |
| 10−3 | 3 | 1 |
| 10−4 | 2 | 1 |
| 10−5 | 1 | 1 |
| Total | 12 | 8 |

**Table 16: Shows the Results of The Biochemical Tests for Bacteria in Sample No. (4)**

| Bacteria | SH | GS | I | MR | Gas | KIA | | U | C | SD | No |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Slop | Butt | | | | |
| K. Pneumoniae | Rod | - | - | - | + | Y | Y | + | + | 10−1 | 01 |
| Bacillus cereus | Rod | + | - | - | + | R | Y | + | + | 10−1 | 02 |
| K. Pneumoniae | Rod | - | - | - | + | Y | Y | + | + | 10−1 | 03 |
| Bacillus cereus | Rod | + | - | - | + | R | Y | + | + | 10−2 | 04 |
| K. Pneumonae | Rod | - | - | - | + | Y | Y | + | + | 10−2 | 05 |
| Bacillus cereus | Rod | + | - | - | + | R | Y | + | + | 10−2 | 06 |
| Bacillus cereus | Rod | + | - | - | + | R | Y | + | + | 10−3 | 07 |
| Bacillus cereus | Rod | + | - | - | + | R | Y | + | + | 10−3 | 08 |

**Table 17: Shows the Results of Aerobic Bacterial Counts for Sample No. (5)**

| Dilutions | Number of colonies | Cells/ml |
|---|---|---|
| $10^{-1}$ First dilution | Uncounted | |
| $10^{-2}$Second dilution | Uncount | |
| $10^{-3}$ Third dilution | 300 | $10^5 \times$ 3 |
| $10^{-4}$ Fourth reduction | 74 | $10^5 \times 7.4$ |
| $10^{-5}$ Fifth reduction | 07 | |
| $10^{-6}$Sixth reduction | 00 | |
| **Average number of cells/ml** | | **$10^5 \times$ 5.2** |

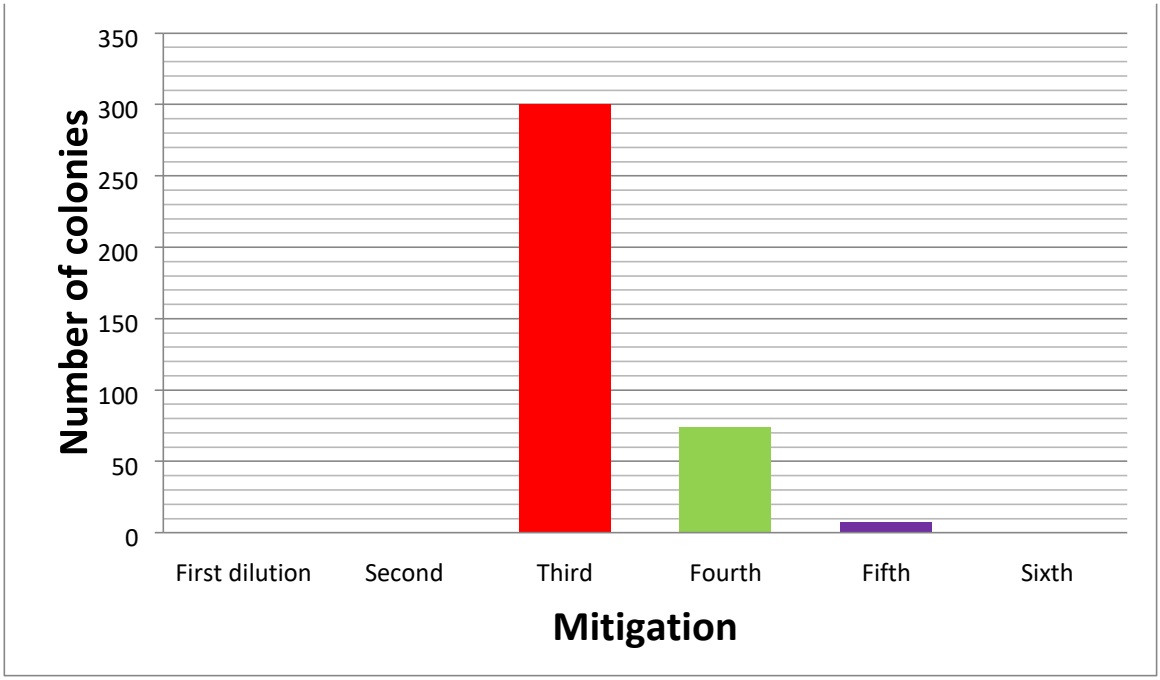


**Figure 5: Shows the Colony Counts for Aerobic Bacteria in Sample No. (5)**

**Table 18: Shows the Results of The Probability Test for Coliform Bacteria in Sample No. (5)**

| Dilutions | Number of tubes per dilution | Number of tubes in which gas formed |
|---|---|---|
| 10−1 | 3 | 3 |
| 10−2 | 3 | 3 |
| 10−3 | 3 | 3 |
| 10−4 | 3 | 2 |
| 10−5 | 3 | 2 |
| **Most probable number (MPN)** | | **More than 2.1 ×$10^5$ cells/ml** |

**Table 19: Shows the Results of The Confirmatory Test for E. Coli in Sample No. (5)**

| Dilutions | Number of tubes per dilution | Number of tubes in which gas formed |
|---|---|---|
| 10−1 | 3 | 3 |
| 10−2 | 3 | 3 |
| 10−3 | 3 | 2 |
| 10−4 | 2 | 2 |
| 10−5 | 2 | 2 |
| Total | 13 | 12 |

**Table 20: Shows the Results of The Biochemical Tests for Bacteria in Sample No. (5)**

| Bacteria | SH | GS | I | MR | Gas | KIA | | U | C | SD | No |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Slop | Butt | | | | |
| K. pneumonae | Rod | - | - | - | + | Y | Y | + | + | 10−1 | 01 |
| K. pneumonae | Rod | - | - | - | + | Y | Y | + | + | 10−1 | 02 |
| K. pneumonae | Rod | - | - | - | + | Y | Y | + | + | 10−1 | 03 |
| K. pneumonae | Rod | - | - | - | + | Y | Y | + | + | 10−2 | 04 |
| K. pneumonae | Rod | - | - | - | + | Y | Y | + | + | 10−2 | 05 |
| K. pneumonae | Rod | - | - | - | + | Y | Y | + | + | 10−2 | 06 |
| K. pneumonae | Rod | - | - | - | + | Y | Y | + | + | 10−3 | 07 |
| K. pneumonae | Rod | - | - | - | + | Y | Y | + | + | 10−3 | 08 |
| K. pneumonae | Rod | - | - | - | + | Y | Y | + | + | 10−4 | 09 |
| K. pneumonae | Rod | - | - | - | + | Y | Y | + | + | 10−4 | 10 |
| K. pneumonae | Rod | - | - | - | + | Y | Y | + | + | 10−5 | 11 |
| K. pneumonae | Rod | - | - | - | + | Y | Y | + | + | 10−5 | 12 |

**Table 21: Shows the Results of The Aerobic Bacterial Count for Sample No. (6)**

| Dilutions | Number of colonies | Cells/ml |
|---|---|---|
| $10^{-1}$ First dilution | Uncounted | |
| $10^{-2}$Second dilution | Uncount | |
| $10^{-3}$ Third dilution | Uncounted | |
| $10^{-4}$ Fourth mitigation | Uncounted | |
| $10^{-5}$ Fifth mitigation | 289 | $10^6 \times$ 28.9 |
| $10^{-6}$Sixth reduction | 177 | $10^6 \times$ 177 |
| **Average number of cells/ml** | | **$10^8 \times$ 1.03** |

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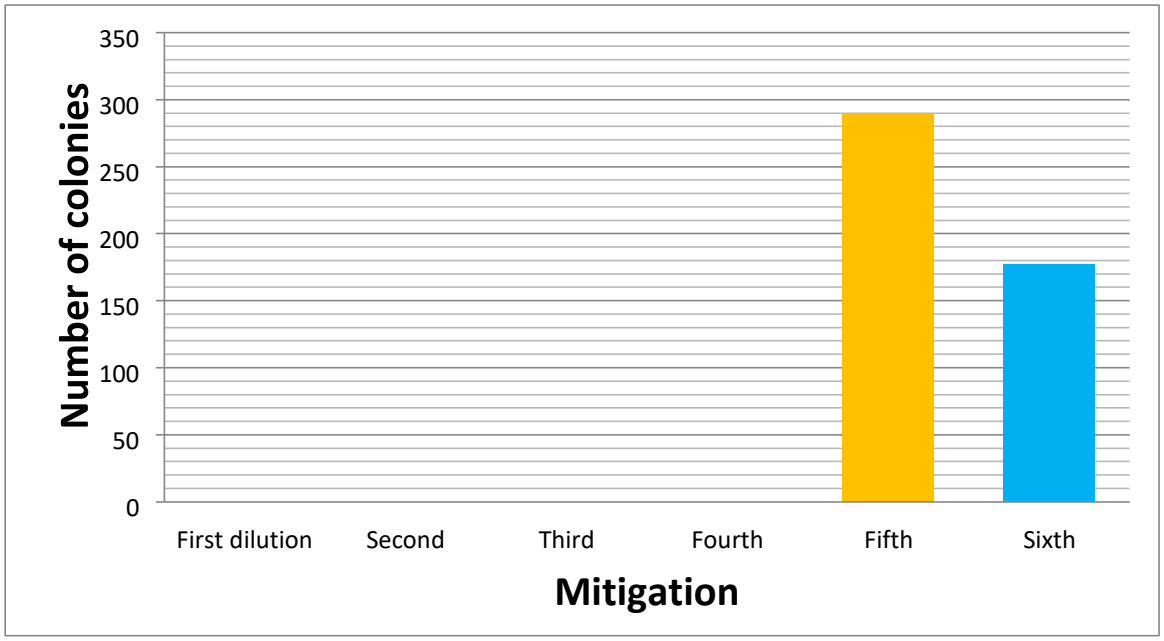


**Figure 6: Shows the Colony Counts for Aerobic Bacteria in Sample No. (6)**

**Table 22: Shows the Results of The Probability Test for Coliform Bacteria in Sample No. (6)**

| Dilutions | Number of tubes per dilution | Number of tubes in which gas formed |
|---|---|---|
| 10−1 | 3 | 3 |
| 10−2 | 3 | 3 |
| 10−3 | 3 | 2 |
| 10−4 | 3 | 2 |
| 10−5 | 3 | 2 |
| **Most probable number (MPN)** | | **3.5 ×10⁵ cells/ml** |

**Table 23: Shows the Results of The Confirmatory Test for E. Coli in Sample No. (6)**

| Dilutions | Number of tubes per dilution | Number of tubes in which gas formed |
|---|---|---|
| 10−1 | 3 | 3 |
| 10−2 | 3 | 2 |
| 10−3 | 2 | 2 |
| 10−4 | 2 | 2 |
| 10−5 | 2 | 2 |
| Total | 12 | 11 |

**Table 24: Shows the Results of The Biochemical Tests for Bacteria in Sample No. (6)**

| Bacteria | SH | GS | I | MR | Gas | KIA | | U | C | SD | No |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Slop | Butt | | | | |
| K. pneumonae | Rod | - | - | - | + | Y | Y | + | + | 10−1 | 01 |
| K. pneumonae | Rod | - | - | - | + | Y | Y | + | + | 10−1 | 02 |
| K. pneumonae | Rod | - | - | - | + | Y | Y | + | + | 10−1 | 03 |
| Bacillus alvei | Rod | + | + | - | + | R | Y | + | + | 10−2 | 04 |
| K. pneumonae | Rod | - | - | - | + | Y | Y | + | + | 10−2 | 05 |
| K. pneumonae | Rod | - | - | - | + | Y | Y | + | + | 10−2 | 06 |
| K. pneumonae | Rod | - | - | - | + | Y | Y | + | + | 10−3 | 07 |
| Bacillus alvei | Rod | + | + | - | + | R | Y | + | + | 10−3 | 08 |
| K. pneumonae | Rod | - | - | - | + | Y | Y | + | + | 10−4 | 09 |
| K. pneumonae | Rod | - | - | - | + | Y | Y | + | + | 10−5 | 10 |
| K. pneumonae | Rod | - | - | - | + | Y | Y | + | + | 10−5 | 11 |

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**Table 25: Shows the Results of Aerobic Bacterial Counts for Sample No. (7)**

| Dilutions | Number of colonies | Cells/ml |
|---|---|---|
| $10^{-1}$ First dilution | Uncounted | |
| $10^{-2}$Second dilution | Uncount | |
| $10^{-3}$ Third dilution | Uncounted | |
| $10^{-4}$ Fourth mitigation | 296 | $10^{6}\times$ 2.96 |
| $10^{-5}$ Fifth reduction | 217 | $10^{6}\times$ 21.7 |
| $10^{-6}$Sixth reduction | 187 | $10^{6}\times$ 187 |
| **Average number cells/ml** | | **$10^{7}\times$ 7.1** |

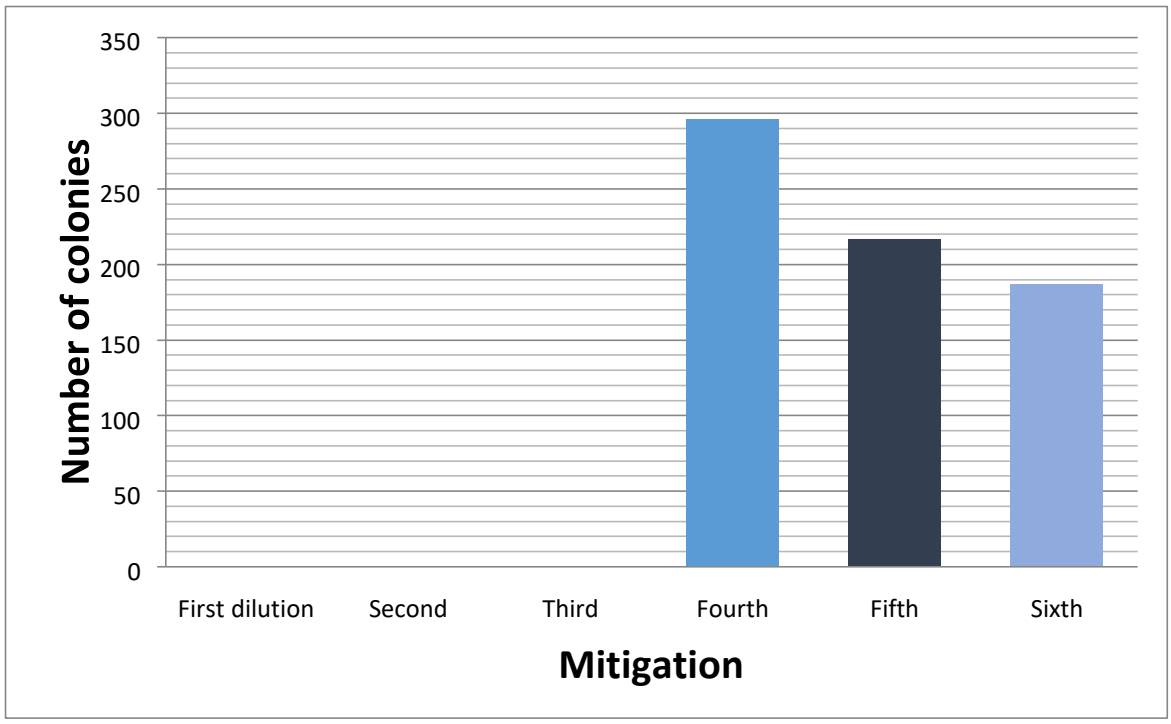


**Figure 7: Shows the Colony Counts for Aerobic Bacteria in Sample No. (7)**

**Table 26: Shows the Results of The Probability Test for Coliform Bacteria in Sample No. 7**

| Dilutions | Number of tubes per dilution | Number of tubes in which gas formed |
|---|---|---|
| **10−1** | **3** | **3** |
| **10−2** | **3** | **3** |
| **10−3** | **3** | **3** |
| **10−4** | **3** | **3** |
| **10−5** | **3** | **3** |
| **Most probable number (MPN)** | | **More than 1.1 ×$10^{6}$ cells/ml** |

**Table 27: Shows the Results of The Confirmatory Test for E. Coli in Sample No. (7)**

| Dilutions | Number of tubes per dilution | Number of tubes in which gas formed |
|---|---|---|
| 10−1 | 3 | 3 |
| 10−2 | 3 | 3 |
| 10−3 | 3 | 3 |
| 10−4 | 3 | 3 |
| 10−5 | 3 | 3 |
| Total | 15 | 15 |

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**Table 28: Shows the Results of The Biochemical Tests for Bacteria in Sample No. (7)**

| Bacteria | SH | GS | I | MR | Gas | KIA | | U | C | SD | No |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Slop | Butt | | | | |
| Bacillus alvei | Rod | + | + | - | + | R | Y | + | + | 10−1 | 01 |
| Bacillus alvei | Rod | + | + | - | + | R | Y | + | + | 10−1 | 02 |
| K. pneumoniae | Rod | - | - | - | + | Y | Y | + | + | 10−1 | 03 |
| Bacillus subtilis | Rod | + | - | - | + | Y | Y | - | + | 10−2 | 04 |
| Klebsiella oxytoca | Rod | - | + | - | + | Y | Y | + | + | 10−2 | 05 |
| Bacillus cereus | Rod | + | - | - | + | R | Y | + | + | 10−2 | 06 |
| Bacillus cereus | Rod | + | - | - | + | R | Y | + | + | 10−3 | 07 |
| K. pneumoniae | Rod | - | - | - | + | Y | Y | + | + | 10−3 | 08 |
| K. pneumoniae | Rod | - | - | - | + | Y | Y | + | + | 10−3 | 09 |
| Klebsiella oxytoca | Rod | - | + | - | + | Y | Y | + | + | 10−4 | 10 |
| K. pneumoniae | Rod | - | - | - | + | Y | Y | + | + | 10−4 | 11 |
| K. pneumoniae | Rod | - | - | - | + | Y | Y | + | + | 10−4 | 12 |
| Bacillus subtilis | Rod | + | - | - | + | Y | Y | - | + | 10−5 | 13 |
| K. pneumoniae | Rod | - | - | - | + | Y | Y | + | + | 10−5 | 14 |
| K. pneumoniae | Rod | - | - | - | + | Y | Y | + | + | 10−5 | 15 |

**Table 29: Shows the Results of Aerobic Bacterial Counts for Sample No. (8)**

| Dilutions | Number of colonies | Cells/ml |
|---|---|---|
| $10^{-1}$ First dilution | Uncounted | |
| $10^{-2}$Second dilution | Uncount | |
| $10^{-3}$ Third dilution | Uncounted | |
| $10^{-4}$ Fourth mitigation | 47 | $10^5 \times$ 4.7 |
| $10^{-5}$ Fifth reduction | 02 | |
| $10^{-6}$Sixth reduction | 00 | |
| **Average number of cells/ml** | | **$10^5 \times$ 4.7** |

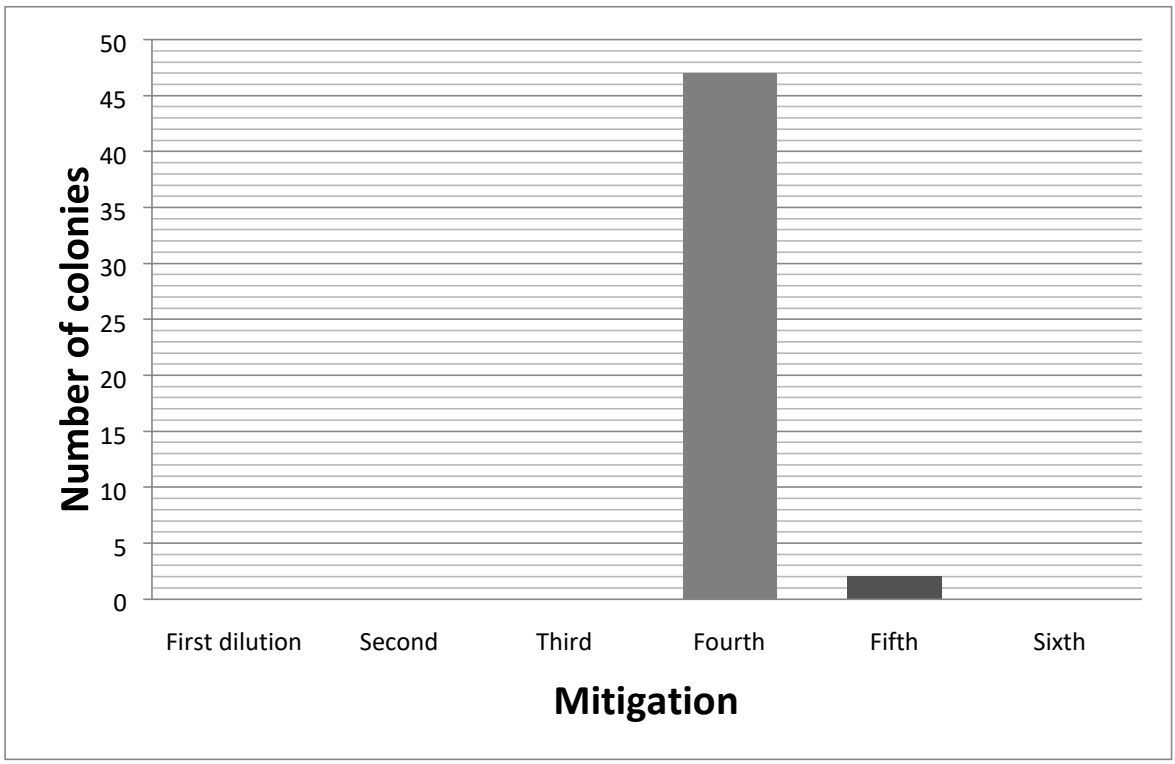


**Figure 8: Shows the Colony Counts for Aerobic Bacteria in Sample No. (8)**

**Table 30: Shows the Results of The Probability Test for Coliform Bacteria in Sample No. 8**

| Dilutions | Number of tubes per dilution | Number of tubes in which gas formed |
|---|---|---|
| 10−1 | 3 | 3 |
| 10−2 | 3 | 3 |
| 10−3 | 3 | 3 |
| 10−4 | 3 | 3 |
| 10−5 | 3 | 3 |
| **Most probable number (MPN)** | | **More than 1.1 ×$10^6$ cells/ml** |

**Table 31: Shows the Results of The Confirmatory Test for E. Coli In Sample No. (8)**

| Dilutions | Number of tubes per dilution | Number of tubes in which gas formed |
|---|---|---|
| 10−1 | 3 | 3 |
| 10−2 | 3 | 3 |
| 10−3 | 3 | 3 |
| 10−4 | 3 | 3 |
| 10−5 | 3 | 3 |
| Total | 15 | 15 |

**Table 32: Shows the Results of The Biochemical Tests for Bacteria in Sample No. (8)**

| Bacteria | SH | GS | I | MR | Gas | KIA | | U | C | SD | No |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Slop | Butt | | | | |
| K. pneumoniae | Rod | - | - | - | + | Y | Y | + | + | 10−1 | 01 |
| Bacillus subtilis | Rod | + | - | - | + | Y | Y | - | + | 10−1 | 02 |
| K. pneumoniae | Rod | - | - | - | + | Y | Y | + | + | 10−1 | 03 |
| Klebsiella oxytoca | Rod | - | + | - | + | Y | Y | + | + | 10−2 | 04 |
| K. pneumoniae | Rod | - | - | - | + | Y | Y | + | + | 10−2 | 05 |
| K. pneumoniae | Rod | - | - | - | + | Y | Y | + | + | 10−2 | 06 |
| K. pneumoniae | Rod | - | - | - | + | Y | Y | + | + | 10−3 | 07 |
| K. pneumoniae | Rod | - | - | - | + | Y | Y | + | + | 10−3 | 08 |
| Bacillus cereus | Rod | + | - | - | + | R | Y | + | + | 10−3 | 09 |
| Klebsiella oxytoca | Rod | - | - | - | + | Y | Y | + | + | 10−4 | 10 |
| K. pneumoniae | Rod | - | - | - | + | Y | Y | + | + | 10−4 | 11 |
| K. pneumoniae | Rod | - | - | - | + | Y | Y | + | + | 10−4 | 12 |
| Bacillus cereus | Rod | + | - | - | + | R | Y | + | + | 10−5 | 13 |
| K. pneumoniae | Rod | - | - | - | + | Y | Y | + | + | 10−5 | 14 |
| K. pneumoniae | Rod | - | - | - | + | Y | Y | + | + | 10−5 | 15 |

**Table 33: Shows the Results of Aerobic Bacterial Counts for Sample No. (9)**

| Dilutions | Number of colonies | Cells/ml |
|---|---|---|
| $10^{-1}$ First dilution | Uncounted | |
| $10^{-2}$Second dilution | Uncount | |
| $10^{-3}$ Third dilution | Uncounted | |
| $10^{-4}$ Fourth mitigation | Uncounted | |
| $10^{-5}$ Fifth mitigation | Uncounted | |
| $10^{-6}$Sixth mitigation | 289 | $10^6 \times 289$ |
| **Average number of cells/ml** | | **$10^8 \times 2.89$** |

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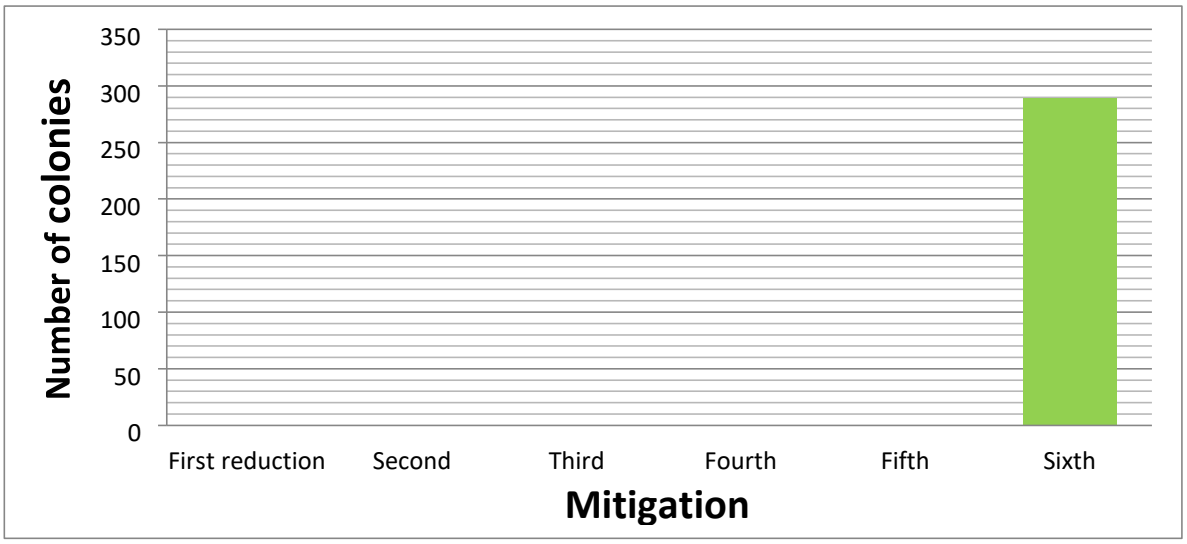


**Figure 9: Shows the Colony Counts for Aerobic Bacteria in Sample No. (9)**

**Table 34: Shows the Results of The Probability Test for Coliform Bacteria in Sample No. 9**

| Dilutions | Number of tubes per dilution | Number of tubes in which gas formed |
|---|---|---|
| 10−1 | 3 | 3 |
| 10−2 | 3 | 3 |
| 10−3 | 3 | 3 |
| 10−4 | 3 | 3 |
| 10−5 | 3 | 2 |
| **Most probable number (MPN)** | | **1.1 ×10⁶ cells/ml** |

**Table 35: Shows the Results of The Confirmatory Test for E. Coli in Sample No. (9)**

| Dilutions | Number of tubes per dilution | Number of tubes in which gas formed |
|---|---|---|
| 10−1 | 3 | 3 |
| 10−2 | 3 | 3 |
| 10−3 | 3 | 3 |
| 10−4 | 3 | 3 |
| 10−5 | 2 | 2 |
| Total | 14 | 14 |

**Table 36: Shows the Results of The Biochemical Tests for Bacteria in Sample No. (9)**

| Bacteria | SH | GS | I | MR | Gas | KIA | | U | C | SD | No |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Slop | Butt | | | | |
| Klebsiella oxytoca | Rod | - | + | - | + | Y | Y | + | + | 10−1 | 01 |
| Klebsiella oxytoca | Rod | - | + | - | + | Y | Y | + | + | 10−1 | 02 |
| K. pneumoniae | Rod | - | - | - | + | Y | Y | + | + | 10−1 | 03 |
| Bacillus cereus | Rod | + | - | - | + | R | Y | + | + | 10−2 | 04 |
| Bacillus alvei | Rod | + | + | - | + | Y | Y | + | + | 10−2 | 05 |
| K. pneumoniae | Rod | - | + | - | + | Y | Y | + | + | 10−2 | 06 |
| K. pneumoniae | Rod | - | - | - | + | Y | Y | + | + | 10−3 | 07 |
| K. pneumoniae | Rod | - | - | - | + | Y | Y | + | + | 10−3 | 08 |
| K. pneumoniae | Rod | - | - | - | + | Y | Y | + | + | 10−3 | 09 |
| K. pneumoniae | Rod | - | - | - | + | Y | Y | + | + | 10−4 | 10 |
| K. pneumoniae | Rod | - | - | - | + | Y | Y | + | + | 10−4 | 11 |
| Bacillus alvei | Rod | + | + | - | + | R | Y | + | + | 10−4 | 12 |
| Klebsiella oxytoca | Rod | - | + | - | + | Y | Y | + | + | 10−5 | 13 |
| Klebsiella oxytoca | Rod | | + | - | + | Y | Y | + | + | 10−5 | 14 |

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**Table 37: Shows the Results of Aerobic Bacterial Counts for Sample No. (10)**

| Dilutions | Number of colonies | Cells/ml |
|---|---|---|
| $10^{-1}$ First dilution | Uncounted | |
| $10^{-2}$Second dilution | Uncount | |
| $10^{-3}$ Third dilution | Uncounted | |
| $10^{-4}$ Fourth mitigation | Uncounted | |
| $10^{-5}$ Fifth reduction | 300 | $10^{6} \times 30$ |
| $10^{-6}$Sixth reduction | 148 | $10^{6} \times 148$ |
| **Average number of cells/ml** | | **$10^{7} \times 8.9$** |

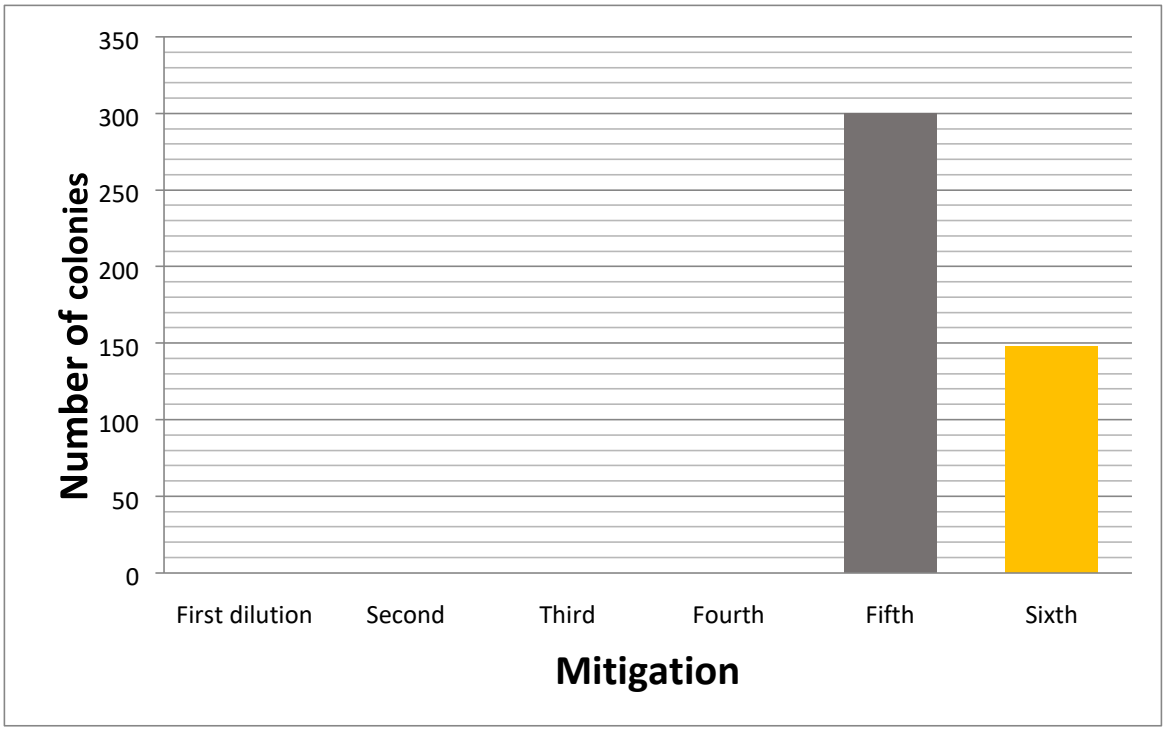


**Figure 10: Shows the Colony Counts for Aerobic Bacteria in Sample No. (10)**

**Table 38: Shows the Results of The Probability Test for Coliform Bacteria in Sample No. (10)**

| Dilutions | Number of tubes per dilution | Number of tubes in which gas formed |
|---|---|---|
| 10−1 | 3 | 3 |
| 10−2 | 3 | 3 |
| 10−3 | 3 | 3 |
| 10−4 | 3 | 2 |
| 10−5 | 3 | 2 |
| **Most probable number (MPN)** | | **$2.1 \times 10^{5}$ cells/ml** |

**Table 39: Shows the Results of The Confirmatory Test for E. Coli in Sample No. (10)**

| Dilutions | Number of tubes per dilution | Number of tubes in which gas formed |
|---|---|---|
| 10−1 | 3 | 3 |
| 10−2 | 3 | 3 |
| 10−3 | 3 | 3 |
| 10−4 | 2 | 2 |
| 10−5 | 2 | 2 |
| Total | 13 | 13 |

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**Table 40: Shows the Results of The Biochemical Tests for Bacteria in Sample No. (10)**

| Bacteria | SH | GS | I | MR | Gas | KIA | | U | C | SD | No |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Slop | Butt | | | | |
| Bacillus cereus | Rod | + | - | - | + | Y | Y | + | + | 10−1 | 01 |
| K. pneumoniae | Rod | - | - | - | + | Y | Y | + | + | 10−1 | 02 |
| Bacillus cereus | Rod | + | - | - | + | Y | Y | + | + | 10−1 | 03 |
| K. pneumoniae | Rod | - | - | - | + | Y | Y | + | + | 10−2 | 04 |
| Bacillus cereus | Rod | + | - | - | + | Y | Y | + | + | 10−2 | 05 |
| K. pneumoniae | Rod | - | - | - | + | Y | Y | + | + | 10−2 | 06 |
| K. pneumoniae | Rod | - | - | - | + | Y | Y | + | + | 10−3 | 07 |
| Bacillus cereus | Rod | + | - | - | + | Y | Y | + | + | 10−3 | 08 |
| Bacillus cereus | Rod | + | - | - | + | Y | Y | + | + | 10−3 | 09 |
| Bacillus alvei | Rod | + | + | - | + | Y | Y | + | + | 10−4 | 10 |
| K. pneumoniae | Rod | - | - | - | + | Y | Y | + | + | 10−4 | 11 |
| K. pneumoniae | Rod | - | - | - | + | Y | Y | + | + | 10−5 | 12 |
| K. pneumoniae | Rod | - | - | - | + | Y | Y | + | + | 10−5 | 13 |

**Table 41: Shows the Results for All Bacterial Species in All Samples, In Terms of Numbers and Percentages**

| Bacteria Sample Site | K.pneumo | K. oxytoca | B. cereus | B.alvei | B. subtilis |
|---|---|---|---|---|---|
| University A | 5 | 3 | 1 | 1 | 0 |
| University A B | 4 | 1 | 0 | 1 | 0 |
| Azhari 11B | 4 | 1 | 2 | 0 | 0 |
| Azhari 11B | 3 | 0 | 5 | 0 | 0 |
| Azhari 10A | 12 | 0 | 0 | 0 | 0 |
| Azhari 10B | 9 | 0 | 0 | 2 | 0 |
| Rescue M 3A | 7 | 2 | 3 | 2 | 1 |
| Rescue M 3B | 10 | 2 | 2 | 0 | 1 |
| Rescue M 2A | 7 | 4 | 1 | 2 | 0 |
| Save M 2B | 7 | 0 | 5 | 1 | 0 |
| Total | **68** | **13** | **19** | **9** | **2** |
| Percentage | **61.26%** | **11.71%** | **17.12%** | **8.11%** | **1.8** |

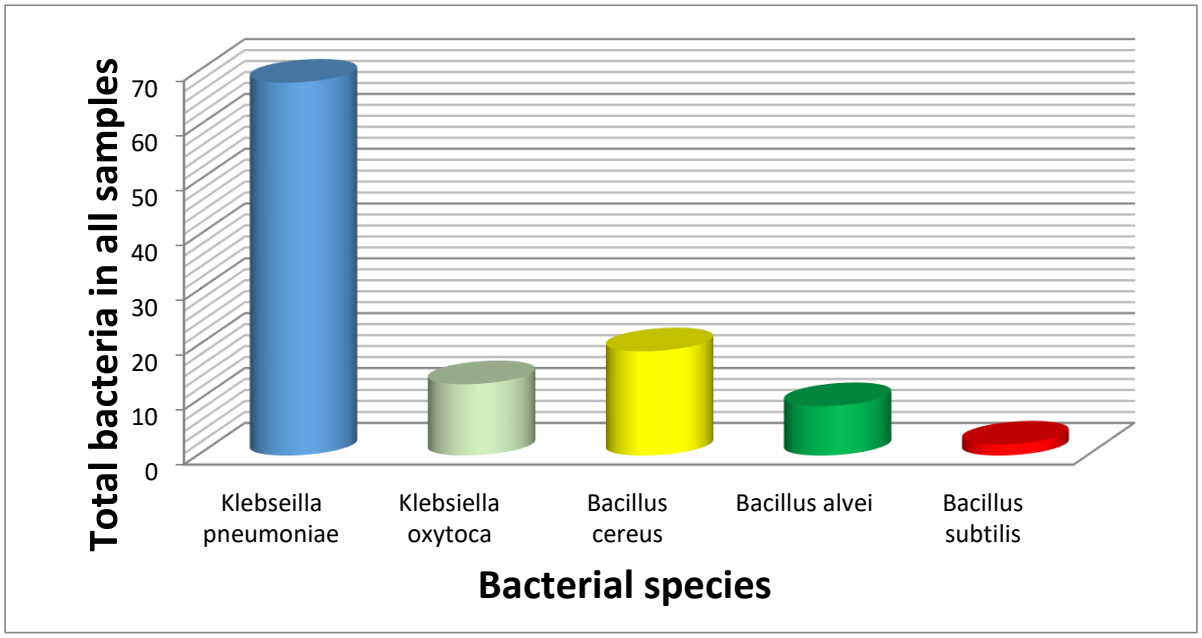


**Figure 11: Shows All Types of Bacteria in All Samples**

# DISCUSSION

**Aerobic bacteria**

According to this study, the contamination of raw milk with bacteria stems from certain environmental factors such as dust and dirt, as well as a lack of awareness of hygiene practices among many workers in milk retail outlets. Consequently, some of these grocers leave milk containers unsealed, which attracts airborne microbes and thus renders the milk susceptible to spoilage.

The results showed that the counts of aerobic bacteria in the study samples ranged from $4.7\times10^5$ to $2.89\times10^8$ cells/ml). It was found that the samples most contaminated with aerobic bacteria were in Al-Inqaz neighbourhood, Block 2 (A), followed by AlAzhari neighbourhood, Block 10 (B), with counts of $2.89\times10^8$ and $1.03\times10^8$ , respectively. The lowest counts were in the Al-Inqaz neighbourhood, Block 3 (B), followed by the Al-Azhari neighbourhood, Block 10 (A), as follows: $4.7\times10^5$ and $5.2\times10^5$ . As for the other samples, they are average, and although there is a variation in bacterial counts between the samples despite the differences in sampling locations, some grocers place milk containers in tightly sealed areas where dust and dirt cannot reach them, and their containers are always closed, while others place dairy containers outside their shops on the street, partially or fully open, to attract customers; this leads to the entry of dust laden with bacteria, which is the reason for the variation in contamination levels.

The results of airborne bacterial counts in Al-Inqaz Block 3(B) and Al-Azhari Block 10(A) showed that they were within normal levels according to the 2007 Sudanese Standards and Specifications, and there were samples very close to these standards, such as those from the University of Africa grocery store. As for the other samples, they exceeded the normal level set by the Sudanese Standards and Specifications.

**Coliform bacteria**

The analysis results showed that the coliform bacterial count ranged between ($1.1\times10^6$ –$1.5\times10^4$ ), i.e. (more than 1,100–28 MPN). The samples with the highest probable count were from Al-Inqaz neighbourhood, Block 3 (A and B), and Al-Inqaz neighbourhood, Block 2 (A). The samples with the lowest probable counts were from Africa University and Al-Azhari Square 11 (A), whilst the remaining samples had intermediate probable counts. The increase is attributed to grocery shop staff not wearing gloves, as well as the presence of open sewers. These results are similar to those obtained by Salman and Hamdan (2011) in Khartoum State in a sample of raw milk; the most probable number (MPN) of coliform bacteria was over 1,100 in both studies, and the reason lies in the sampling area (Khartoum State). The difference, however, is that in the study by Salman and Hamdan, they found that the most probable number of coliform bacteria ranged from zero to over 1,100, whereas in this study, the most probable number ranged from 28 to over 1,100. If this indicates anything, it indicates that the number of samples taken in the studywas very large (644 samples) and that the study period was long and spanned different seasons, whereas this study was conducted over a short period and in a single season.

**Isolation of E. Coli**

The results of the study confirmed that they were consistent with the study conducted by Salman and Hamdan in Khartoum State (2011) in terms of the presence *of Klebseella* in both studies; however, they differ in that this study included the genus *Bacillus sp,* whereas in the study by Salman and Hamdan, E. *coli, Enterobacter, 'Ceratias' and Citrobacter* were found. The reason for this is the difference in seasons; for example, in winter there is a lot of dust and strong winds, which carry bacteria through the air and transport them to places that may appear safe from contamination to the observer, but dust enters and contaminates the milk. There is also a difference in the s of the sampling areas, despite both studies having been conducted in Khartoum State. In the study conducted by Anderson in 2011 in the city of Jemicia (in India), it was found that milk was contaminated with *E. coli* and *Enterobacter*. These results differ from those of this study; this difference stems from the fact that milk contamination in India originates from dust in barns and milk containers, whereas the samples taken from Khartoum State were from grocery shops, which are less prone to contamination. According to a study conducted by Kumur in 2010 in the city of Uttarakhan (India), the bacteria isolated from the milk were *E.* coli and *Staphylococcus.* These results differ from those of this study due to the milk vendors in Indian households, They contaminate the milk with intestinal microbes via contaminated hands, the air and the handling process, all of which is due to a lack of hygiene practices regarding domestic cows in India. According to the study conducted by Slaghuis in 1997 and Salutiano in Brazil (2009), the isolated bacterium was *Bacillus cereus,* and these results correspond to the findings of the present study regarding the presence of this bacterium, which is airborne. However, in this study, in addition to *Bacillus cereus*, *Klebsiella* was found, which is an intestinal bacterium. According to the study conducted by Gogob in Bulgaria in 1977 on raw milk, the bacteria isolated were *Enterobacter cereus, Citrobacter, Klebsiella and E. coli*. These results are similar to those of this study regarding the presence *of Klebsiella,* but differ in the presence of other genera and species of bacteria. This difference is due to the large number of samples (360), which indicates that samples were taken from many locations, thereby increasing the likelihood of the presence of a wide variety of bacterial genera and species.

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