**ASSESSMENT OF ANTIBACTERIAL SUSCEPTIBILITY PATTERN OF BACTERIA PRESENT IN DIFFERENT YOGHURT SAMPLES**

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**ABSTRACT**

The primary objective of this study was to assess antibiotic resistance in commercially available *Lactobacilli* probiotics. While these probiotics are generally regarded as safe, their extensive use has raised concerns about the potential reservoir of antibiotic-resistance genes, which could be vertically transmitted. Additionally, external genetic elements may facilitate the horizontal transfer of resistance genes to pathogens and the human gut microbiota, posing risks to the host.

The research focused on evaluating the antibacterial susceptibility of *Lactobacillus sp.* isolated from six yogurt samples, including five commercially available products and one homemade sample. Isolated colonies of *Lactobacillus sp.* were confirmed through various methods, including Gram staining, Endospore staining, and biochemical tests such as IMViC and Catalase tests. Antibacterial susceptibility was examined using 12 antibiotic discs from the A1 Axiom multidisc ring for Gram-positive isolates, employing the disc diffusion method.

Variability in the resistance and susceptibility patterns was observed among *Lactobacillus sp.* from the six yogurt samples. The Milky Mist sample exhibited the highest resistance profile, followed by the Karimnagar yogurt sample. Notably, all *Lactobacillus sp.* from the samples showed resistance to the Ampiclox (ACX20) antibiotic disc. Given the rising concern of antibiotic resistance, probiotic strains susceptible to certain antibiotics may become resistant in the future, underscoring the importance of considering antibiotic susceptibility in probiotic safety assessments.

Statistical analysis using One-way ANOVA on the zones of inhibition revealed a non-significant p-value of 0.920517, indicating that the datasets were not statistically different. The sample variance was determined to be an average of 71.59838, reflecting the spread of values obtained in the study.

**Keywords:** LAB, Human gut microbiota, Antibacterial susceptibility, Zone of inhibition, ANOVA.

1. **INTRODUCTION**
2. **Background Study:**

Milk is an important part of many people’s traditional diets around the world. The vast majority of milk produced is consumed at home and is rarely sold. However, high temperatures and a lack of refrigeration facilities have made it impossible to process and preserve fresh milk. Hence, traditionally leftover liquid milk is converted into partially shelf-stable goods such as yogurt, cheese, acidified milk, butter, and ghee at the home level. Acidification of milk through fermentation is an ancient method of milk preservation. (Ogwaro, 2002)

In different parts of the world, different methods of fermentation are used, which results in a variety of fermented milk products such as kumiss, kefir, acidophilus milk, and yogurt. (Tamime, 1980)

Product quality and consumer satisfaction are critical factors in boosting demand for various types of yogurt. The increase in per capita annual yogurt consumption in the majority of countries has been allocated to improved knowledge about the health advantages of yogurt, the rising availability of fruit or flavoured yogurt, and the variety of product presentations. (Küçüköner)

Yoghurt’s healthy food profile is attributed to the probiotic impact of yogurt microorganisms according to (Guarner) yogurt bacteria are probiotic living microorganisms that give a health advantage to the host when administered in sufficient concentrations the health-promoting properties of live lactic acid bacteria in yogurt including protection against gastrointestinal upsets improved lactose digestion by mal digesters, a lower risk of cancer, lower blood cholesterol, improved immune response and the ability to help the body assimilate protein, calcium, and iron. (Zubeir) (Owiah, 2017)

Yogurt is a popular fermented dairy product that is consumed all over the world. Lactic acid fermentation of milk is achieved through the action of starting culture containing *Streptococcus thermophilus* and *Lactobacillus bulgaricus* (Salama, 2022)

Conjugated linoleic acid has been demonstrated to be a powerful natural and anti-carcinogen that can also lower the risk of cardiovascular disease, fight inflammation, reduce body fat, particularly belly fat, lower cholesterol, and triglycerides, raise metabolism, reduce insulin resistance, and improve the immune system. (Hartigh, 2019)

1. **Yoghurt culture bacteria:**

The proteolytic activity of the two yogurt bacteria is mild yet significant, resulting in the symbiotic development of the two organisms and taste component generation. *Lactobacillus bulgaricus* can hydrolyze caseins, whereas *Streptococcus thermophilus* has low proteinase activity. (Tamime, Yoghurt: Technology and Biochemistry, 1980)

1. **Antibacterial Susceptibility Testing:**

For both bactericidal and bacteriostatic drugs, antibacterial susceptibility testing determines the concentration of antibiotics that inhibit bacterial growth. (Brown, 2016) (Sanguinetti) (AS, 2007) (Belkum, 2019) The importance of accurate antibacterial susceptibility testing in at least guiding antibiotic use in the clinical cannot be underestimated. (Doern, 1994)

Antibacterial susceptibility testing is critical for the development of novel antibacterial since it allows us to (i) determine the preclinical activity of drug candidates and identify lead compounds, (ii) determine the possibility of resistance development and (iii) offer estimations of potential in vivo and more importantly, clinical efficacy when testing drugs in biological matrices reproducing infection sites, such as blood/plasma/ serum, lung bronchiolar lavage fluid/sputum, urine, biofilms, and so on. (Breteler, 2011)

1. **MATERIALS AND METHODS**
2. **Sample Collection:**

Homemade yogurt and five different yogurt samples were collected randomly from the local market under sterilized conditions to check the antibacterial susceptibility pattern in the laboratory.

1. **Culturing on MRS Agar Medium:**

All the glassware and media were autoclaved at 120ºC and 15 lbs pressure, before following any kind of procedures for maintaining sterile conditions and to avoid contamination.

The six different yogurt samples were serially diluted through a series of standard volumes of sterile diluent, to reduce the concentration of cells present in the sample. Small volumes of each dilution were used to make a series of spread plates on the MRS Agar. Following incubation, some of the dispersed cells form isolated colonies. A colony is a big group of bacterial cells on solid media that can be seen with the naked eye as a distinct entity. In this approach, it was assumed that a colony is formed from a single cell and hence represented as a clone of pure culture. After incubation, by looking down at the top of the colony, the general shape of the colony and the shape of the edge or margin were deciphered. The nature of the colony elevation was seen when viewed from the side with the plate held at eye level. After identifying a well-isolated colony, it was picked up and streaked onto a new medium to obtain a pure culture. *Lactobacillus* pure colonies were obtained and validated using gram staining, endospore staining, and biochemical tests such as biochemical assays such as IMViC tests and catalase tests.

1. **Screening of Antibacterial Drug Resistance:**

Loop full of culture from the pure culture of the same morphology type were taken and inoculated into the sterile 2mL of nutrient broth for all the test isolates. Incubated the broth to produce a bacterial suspension of moderate turbidity. The derived suspension of bacterial cultures from the broth were further used in inoculation for further analysis. Sterile Nutrient agar plates were labeled according to the test cultures. The dry surface of the nutrient agar was inoculated using the spread plate technique by adding 0.5mL of Nutrient broth. Dipped the spreader in alcohol and flamed it thoroughly, once cooled, gently spread the entire plate for uniform distribution of the sample. A1 Axiom multi discs were used for Gram-positive bacterial isolates. These impregnated discs were carefully passed down to ensure contact with the agar surface. The disc must not be relocated once it came in contact with the agar surface. Incubated the plates for 24hrs in inverted position within 15mins after the discs were applied.

1. **Reading and Interpretation of Results:**

After incubation the zone of inhibition was calculated using callipers and recorded down for observations. A zone of inhibition interpretation standard chart was used to classify the isolates based on sensitive, moderately sensitive, and resistant respectively.

Table 1Standard chart of A1 Axiom Antibiotic multidrug for Gram-Positive Isolates

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Antimicrobial Agent** | **Code** | **Content** | **Resistant****mm or less** | **Intermediate****mm** | **Susceptible****mm or less** |
| **Amikacin** | **AK** | **30mcg** | **14** | **15-16** | **17** |
| **Ampiclox** | **ACX** | **20mcg** | **22** | **23-27** | **28** |
| **Ciproflaxacin** | **CIP** | **5mcg** | **15** | **16-20** | **21** |
| **Clarithromycin** | **CLR** | **15mcg** | **13** | **14-17** | **18** |
| **Cefotaxame** | **CF** | **30mcg** | **14** | **15-22** | **23** |
| **Sparfloxacin** | **SF** | **5mcg** | **15** | **16-18** | **19** |
| **Cefuroxime** | **CR** | **30mcg** | **14** | **15-17** | **18** |
| **Cefoperazone** | **CFP** | **75mcg** | **14** | **15-18** | **19** |
| **Gentamicin** | **G** | **10mcg** | **12** | **13-14** | **15** |
| **Roxythromycin** | **RX** | **15mcg** | **13** | **14-17** | **18** |
| **Cefadroil** | **CD** | **30mcg** | **14** | **15-17** | **18** |
| **Azithromycin** | **AZ** | **15mcg** | **13** | **14-17** | **18** |

 mcg= micro grams

1. **Statistical Analysis:**

All results were analysed using descriptive statistical techniques such as mean and standard deviation. One way ANOVA was employed to test the significance and p-value <0.05 was considered as significant. All statistical analyses were performed by Microsoft Excel sheet.

1. **RESULTS AND DISCUSSION**
2. **Morphological, cultural and biochemical analysis of isolated *Lactobacillus sp.* of different yoghurt samples:**

Primary identification of all the six different yoghurt samples were done by gram staining and endospore staining procedures. Morphological and various selected biochemical tests had been advocated as per the Bergey’s manual of Systematic Bacteriology. After performing these procedures, it was observed that all the bacterial isolates of six different yogurt samples were gram-positive rods and they were non-endospore producers **(*Table 2).*** By performing biochemical tests like IMViC and Catalase, the bacterial isolates from six different yogurt samples were confirmed as *Lactobacillus sp.* **(*Table 2)***

1. **Screening for Multidrug Resistance for Gram-positive Isolates:**

Antibacterial susceptibility pattern was screened for the resistance and susceptibility of *Lactobacillus sp.* against A1 Axiom multidisc ring for Gram-positive bacterial isolates by using the disc diffusion method. *Lactobacillus sp.* of six different yogurt samples showed the difference in resistance and susceptibility pattern against 12 antibiotics of the A1 Axiom multidisc ring for Gram-positive bacterial isolates. The diameters of the zone of inhibitions around the discs were measured to the nearest milli meters using calipers or rulers accordingly. The isolates were classified as sensitive, moderately sensitive or intermediate, and resistant according to the interpretative standard zone of inhibition chart (***Table 1).*** All the responses of *Lactobacillus sp.* of six different yogurt samples in terms of resistance and susceptibility pattern against antibiotics were recorded in the tabular form (***Table 3) (Figure 1).*** Among all the *Lactobacillus sp.* of six different yogurt samples, the *Lactobacillus sp.* of Milky mist yogurt sample showed the highest resistance profile by showing resistance against six antibiotic discs of A1 Axiom multidisc ring for Gram-positive isolates such as ACX20 (21mm), CF30 (12mm), LE5 (15mm), G10 (10mm), AN30 (11mm) and CFP75 (14mm). This was followed by the Karimnagar yogurt sample, which showed resistance against five antibiotics of A1 Axiom multidisc ring for Gram-positive bacterial isolates such as ACX (10mm), CF30 (0mm), BA25 (15mm), CR30 (0mm) and RX15 (11mm). All the *Lactobacillus sp.* of six different yogurt samples showed resistance against the Ampiclox (ACX20) antibiotic. ***(Table 3).***

Table 2 Morphological, cultural and biochemical characteristics of bacterial isolates from different yoghurt samples

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **S.No** | **Yoghurt Samples** | **Morphological and cultural characteristics** | **Gram’s staining** | **Endospore staining** | **Motility test** | **Biochemical****Characterization of Bacterial Isolates** |
| **Indole** | **MR** | **VP** | **Citrate** | **Catalase** |
| 1. | **Home made** | 1mm, White,shiny smooth, round | **Gram****Positive bacilli** | Non Endosporic | **Non motile** | **-** | **+** | **-** | **-** | **-** |
| 2. | **Heritage** | Small, 0.1-0.5mm, rough dull and round | **Gram****Positive bacilli** | Non Endosporic | **Non motile** | **-** | **+** | **-** | **-** | **-** |
| 3. | **Jersey** | Small, 0.1- 0.5mm, roughdull and round | **Gram Positive****bacilli** | Non Endosporic | **Non motile** | **-** | **+** | **-** | **-** | **-** |
| 4. | **Milky mist** | Small, 0.1- 0.5mm, roughdull and round | **Gram Positive****bacilli** | Non Endosporic | **Non motile** | **-** | **+** | **-** | **-** | **-** |
| 5. | **Nandini** | 1.0 mm white, rough, irregularand round | **Gram****Positive bacilli** | Non Endosporic | **Non motile** | **-** | **+** | **-** | **-** | **-** |
| 6. | **Karimna gar** | 1mm, White,shiny smooth, round | **Gram****Positive bacilli** | Non Endosporic | **Non motile** | **-** | **+** | **-** | **-** | **-** |

Table 3 Determination of diameter of zone of inhibition of Lactobacillus sp. of different yoghurt samples in mm

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| *Antibiotic Code* | *Homemade* | *Heritage* | *Jersey* | *Milky mist* | *Nandini* | *Karimnagar* |
| *ACX20* | ***19(R)*** | ***19(R)*** | ***20(R)*** | ***21(R)*** | ***0(R)*** | ***10(R)*** |
| *CIP5* | ***19(I)*** | ***22(S)*** | ***18(I)*** | ***24(S)*** | ***30(S)*** | ***35(S)*** |
| *CLR15* | ***18(S)*** | ***15(I)*** | ***21(S)*** | ***19(S)*** | ***24(S)*** | ***30(S)*** |
| *CF30* | ***0(R)*** | ***12(R)*** | ***12(R)*** | ***12(R)*** | ***15(I)*** | ***0(R)*** |
| *BA25* | ***0(R)*** | ***22(I)*** | ***19(I)*** | ***21(I)*** | ***11(R)*** | ***15(R)*** |
| *LE5* | ***19(I)*** | ***19(I)*** | ***21(I)*** | ***15(R)*** | ***40(S)*** | ***41(S)*** |
| *CR30* | ***30(S)*** | ***12(R)*** | ***11(R)*** | ***16(I)*** | ***0(R)*** | ***0(R)*** |
| *AZ15* | ***17(I)*** | ***15(I)*** | ***15(I)*** | ***15(I)*** | ***17(I)*** | ***15(I)*** |
| *G10* | ***29(S)*** | ***18(S)*** | ***18(S)*** | ***10(R)*** | ***19(S)*** | ***14(I)*** |
| *AN30* | ***30(S)*** | ***17(I)*** | ***17(I)*** | ***11(R)*** | ***10(R)*** | ***30(S)*** |
| *CFP75* | ***28(S)*** | ***1(R)*** | ***14(R)*** | ***14(R)*** | ***15(I)*** | ***18(I)*** |
| *RX15* | ***17(I)*** | ***15(I)*** | ***16(I)*** | ***16(I)*** | ***18(S)*** | ***11(R)*** |
| *AVG=* | ***18.83*** | ***15.58*** | ***16.83*** | ***16.166*** | ***16.58*** | ***18.25*** |

 (R)= Resistant, (I)= Intermediate, (S)= Susceptible

1. The *Lactobacillus sp*. isolated from the homemade yogurt had found to be resistant against three antibiotics used, **ACX 20** (19mm), **CF 30** (0mm), and **BA 25** (0 mm) While recorded to be moderately sensitive towards **CIP 5** (19mm), **LE 5** (19mm), **AZ 15** (17mm) and **RX 15** (17mm). The most efficient zones and also the maximum were towards **CLR 15** (18mm), **CR 30** (30mm), **G10** (29mm), **CFP 75** (28mm) and **AN30** (30mm). The average inhibitory effect is found to be 18.83mm.
2. The *Lactobacillus sp*. isolated from the Heritage yogurt sample had found to be resistant against four antibiotics used, **ACX 20** (19mm), **CF30** (12mm), **CFP75** (1mm) and **CR30** (12mm) while recorded to be moderately sensitive towards **CLR15** (15mm), **BA25** (22mm), **LE 5** (19mm), **AZ15** (15mm), **AN 30** (17mm) and **RX 15** (15mm). The most efficient zones and also the maximum were towards **CIP 5** (22mm) and **G 10** (18mm). The average inhibitory effect is found to be 15.58mm.
3. The *Lactobacillus sp.* isolated from the Jersey yogurt sample had found to be resistant against four antibiotics used, **ACX 20** (20mm), **CF 30** (12mm), **CR 30** (11mm), and **CFP 75** (14mm). while recorded to be moderately sensitive towards **CIP 5** (18mm), **BA 25** (19mm), **LE 5** (21mm), **AZ 15** (15mm), **AN 30** (17mm) and **RX 15** (16mm). The most efficient zones and also the maximum were towards **CLR 15** (21mm) and **G 10** (18mm). The average inhibitory effect is found to be 16.83mm.
4. The *Lactobacillus sp.* isolated from the Milky Mist yogurt sample had found to be resistant against six antibiotics used, **ACX20** (21mm), **CF30** (12mm), **LE5** (15mm), **G10** (10mm), **AN30** (11mm) and **CFP75** (14mm). While recorded to be moderately sensitive towards **BA 25** (21mm), **CR 30** (16mm), **AZ 15** (15mm) and **RX15** (16mm). The most efficient zones and also the maximum was towards **CIP5** (24mm) and **CLR15** (19mm). The average inhibitory effect is found to be 16.166mm.
5. The *Lactobacillus sp.* isolated from the Nandini yogurt sample had found to be resistant against four antibiotics used, **ACX20** (0mm), **BA25** (11mm), **CR30** (0mm), and **AN30** (10mm). While recorded to be moderately sensitive towards **CF30** (15mm), **AZ15** (17mm) and **CFP75** (15mm). The most efficient zones and also the maximum were towards **CIP5** (30mm), **CLR15** (24mm), **LE5** (40mm), **G10** (19mm), and **RX15** (18mm). The average inhibitory effect is found to be 16.58mm.
6. The *Lactobacillus sp.* isolated from the Karimnagar yogurt sample had found to be resistant against five antibiotics used, **ACX20** (10mm), **BA25** (15mm), **CR30** (0 mm), **CF30** (0 mm) and **RX15** (11mm). While recorded to be moderately sensitive towards **AZ 15** (15mm), **G10** (14mm), and **CFP75** (18mm). The most efficient zones and also the maximum were towards **CIP5** (35mm), **CLR30** (30mm), **LE5** (41mm), and **AN30** (30mm). The average inhibitory effect is found to be 18.25mm.



Figure 1 Determination of diameter of zone of inhibitions of Lactobacillus sp. from six different yogurt samples

Zone of inhibitions of *Lactobacillus sp.* which was measured in mm by comparing with the standard chart of A1 Axiom multidisc ring of gram-positive isolates were graphically represented by using a Microsoft Excel sheet ***(Figure 2).***



Figure 2 Graphical representation of multidrug resistance for Lactobacillus sp. from six different yoghurt samples using Microsoft Excel sheet

1. **Statistical analysis:**

Statistical analysis was carried out by using a Microsoft Excel sheet and the data summary of *Lactobacillus sp.* of six different yogurt samples in the form of mean, standard deviation, standard error, and sample variance were tabulated ***(Table 4)***

Graphical representation of data summary of statistical analysis was depicted in the form of a graph using a Microsoft Excel sheet ***(Figure 3)***

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Yogurt samples | N | Mean | Standard deviation | Standard Error | Sample variance |
| Homemade | **12** | **18.8333333** | **10.2410345** | **2.95633202** | **104.8788** |
| Heritage | **12** | **15.8333333** | **5.664215156** | **1.63411807** | **32.08333** |
| Jersey | **12** | **16.8333333** | **3.32574895** | **0.906006102** | **11.06061** |
| Milky Mist | **12** | **16.1666667** | **4.32399927** | **1.24823107** | **18.69697** |
| Nandini | **12** | **16.5833333** | **11.3654847** | **3.28093282** | **129.1742** |
| Karimnagar | **12** | **18.25** | **13.143439** | **3.7941841** | **172.75** |

N= Number of antibiotic discs in A1 Axiom multidisc for Gram-positive isolates

Figure 3 Graphical representation of data summary of Lactobacillus sp. from six different yoghurt samples

To determine the values of zone of inhibition obtained were statistically analyzed by using One-way ANOVA. ANOVA test was the starting step in analyzing variables that affect a given data set. This test permits a comparison of more than two groups at the same time to check in case a relationship exists between them. The result of the ANOVA equation, the F statistic, permits the analysis of multiple groups of data to determine the variability between samples and within samples. If no real difference exists between the tested groups, which is called the null hypothesis, the result of the ANOVA's F-ratio statistic will be near to 1. The significance was tested using One-way ANOVA, with p-value <0.05 being significant. The experimental data showed a p-value of 0.920517. This means that the datasets were not statistically significant ***(Table 5)***

The standard error of the regression is the average distance that the quantified counts fall from the regression line. In our case, the observed values fall an average of 2.3033007 units from the regression line. These standard errors are useful in precise predictions of the sampling. Sample variance determines the spread of the values of different counts obtained, the sample variance in the study was found to be an average of 71.59838 respectively ***(Table 5)***

A One-way ANOVA is used to compare two means from two independent (unrelated) groups using the F-distribution. The null hypothesis for the test is that the two means are equal. Therefore, a significant result means that the two means are unequal.

Table 4 Statistical analysis- ANOVA summary for bacterial isolates

|  |
| --- |
| SUMMARY |
| Groups | *Count* | *Sum* | *Average* | *Variance* |
| Homemade | 13 | 244.83 | 18.83308 | 96.13889 |
| Heritage | 13 | 202.58 | 15.58308 | 29.40972 |
| Jersey | 13 | 218.83 | 16.83308 | 10.13889 |
| Milky mist | 13 | 210.166 | 16.16662 | 17.13889 |
| Nandini | 13 | 215.58 | 16.58308 | 118.4097 |
| Karimnagar | 13 | 237.25 | 18.25 | 158.3542 |
| ANOVA |
| Source of Variation | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
| Between Groups | 101.611 | 5 | 20.32221 | 0.283836 | 0.920517 | 2.341828 |
| Within Groups | 5155.083 | 72 | 71.59838 |  |  |  |
|  |  |  |  |  |  |  |
| Total | 5256.694 | 77 |   |   |   |   |

ss= sum of squares

ms= mean of squares

**IV. CONCLUSION**

The main aim of this study was to perform the antibacterial susceptibility testing of bacterial isolates from six different yogurt samples. Primary identification of bacterial isolates of all the six different yogurt samples were done by Gram staining and Endospore staining procedures. Morphological and various selected biochemical tests had been advocated as per Bergey’s Manual of Systematic Bacteriology. After performing these procedures, it was confirmed that the bacterial isolates that were isolated from six different yogurt samples were *Lactobacillus sp.* The antibiotic susceptibility was determined by using the disc diffusion method, against 12 antibiotics of the A1 Axiom multidisc ring for Gram-positive bacterial isolates. The resistance and susceptibility of *Lactobacillus sp.* of six different yogurt samples were confirmed by comparing them with a standard chart of A1 Axiom multidisc for Gram-positive isolates. Among all the *Lactobacillus sp.* of different yogurt samples, the *Lactobacillus sp.* of Milky mist yogurt sample showed the highest resistance profile by showing resistance against six antibiotic discs of A1 Axiom multidisc ring for Gram-positive bacterial isolates such as ACX20 (21mm), CF30 (12mm), LE5 (15mm), G10 (10mm), AN30 (11mm) and CFP75 (14mm). This was followed by the Karimnagar yogurt sample, which showed resistance against five antibiotics of A1 Axiom multidisc ring for Gram-positive bacterial isolates such as ACX (10mm), CF30 (0mm), BA25 (15mm), CR30 (0mm) and RX15 (11mm). All the *Lactobacillus sp.* of six different yogurt samples showed resistance against the Ampiclox (ACX20) antibiotic. A graphical representation of the multidrug resistance of *Lactobacillus sp.* of six different yogurt samples was carried out by using an Excel sheet.

Statistical analysis of *Lactobacillus sp.* of six different yoghurt samples were carried out by One-way ANOVA and Excel sheet. To evaluate the values of zones of inhibition of *Lactobacillus sp.* against antibiotics in various yogurt samples, one-way ANOVA was used statistically. ANOVA test was the starting step in analyzing variables that affect a given data set. This test permits a comparison of more than two groups at the same time to check in case a relationship exists between them. The result of the ANOVA equation, the F statistic, permits the analysis of multiple groups of data to determine the variability between samples and within samples. If no real difference exists between the tested groups, which is called the null hypothesis, the result of the ANOVA's F-ratio statistic will be near to 1. The significance was tested using One-way ANOVA, with p-value <0.05 being significant. The experimental data showed a p-value of 0.920517. This means that the datasets were not statistically significant. The spread of the values of different counts obtained was determined by sample variance, which in the study was found to be an average of 71.59838 respectively. A One-way ANOVA was used to compare two means from two independent (unrelated) groups using the F-distribution. The null hypothesis for the test was that the two means were unequal.

Because antibiotic resistance is on the rise, the security of these probiotic strains is becoming increasingly important and it is impossible to ignore their capacity to spread antibiotic resistance genes to pathogenic or commensal bacteria because *Lactobacillus sp.* which are susceptible to specific antibiotics, may develop resistant in the future. As a result, antibiotic susceptibility should be regarded as a critical tool for evaluating the safety of probiotics.

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