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A STUDY ON THE EFFECT OF AZITHROMYCIN ON GUT BACTERIA-LACTOBACILLUS

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Abstract: The antibiotics have considerably increased human health and life expectancy, but due to its over-dosage and frequent consumption the gut microbiome has negative effects. One such important microbiota is Lactobacillus; they are members of the normal microbial ecosystem in the intestinal tract and play an important role in maintaining the stability and diversity of the gut microbiome. The most frequently consumed antibiotic is Azithromycin. The methanolic solution of Azithromycin of 10 mg concentration has been tested on Lactobacillus bacteria, through an Agar well diffusion method and studied its effect on Lactobacillus through evaluating the Zone of inhibition. The test has revealed that the gut bacteria (Lactobacillus) is sensitive to Azithromycin antibiotic.

Index Terms **– Azithromycin, Microbiome, Lactobacillus, Antibiotic sensitivity, Zone of inhibition.**

INTRODUCTION

Antibiotics are one of the most widely used classes of drugs in medicine today. Antibiotics are usually taken by orally, intravenously or by injection (Lu Zhang et, al.,2013). The amount of antibiotics consumed varies greatly between nations. The WHO report on antibiotic usage surveillance, which was published in 2018, examined data from 65 countries from 2015 as determined by daily dosages per 1,000 inhabitants. With a rate of 64.4, Mongolia had the highest consumption. At 4.4, Burundi received the lowest score. The most often ingested antibiotics were amoxicillin/clavulanic acid (Annie J Browne,2021).

Some antibiotics have been linked to a wide range of unfavorable side effects ranging from moderate to severe (Adam Felman, 2019). According to the Centre for Disease Control and Prevention (CDC),1 in 5 medication-related emergency room visits is due to antibiotic side effects (Jennie Olopaade,2021).

India consumes a significant amount of broad-spectrum antibiotics, which should ideally be used sparingly, despite having a relatively low per-capita private-sector consumption rate compared to many other nations (Shaffi fazaludeen *et.al*., 2022).

Azithromycin antibiotic consumption is worldwide. A macrolide antibiotic called Azithromycin prevents bacterial protein synthesis, quorum sensing, and biofilm development. It is transported at high concentrations to infection sites by successfully accumulating in cells, particularly phagocytes, as evidenced by quick plasma clearance and widespread tissue distribution. Azithromycin is prescribed for bacterial infections of the respiratory, urogenital, cutaneous, and other types, and it has immunomodulatory effects on conditions such as diffuse panbronchiolitis, post-transplant bronchiolitis, and rosacea(Michael J. Parnham et.al.,2014). Further, Some antibiotics, including the often-prescribed tetracyclines and macrolides, can destroy good gut flora while being used, according to researchers (Brian krans, 2021).

Gut bacteria: Numerous microorganism species, including bacteria and yeast, make up the gut microbiota. The dominant gut microbial phyla are Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, Fusobacteria, and Verrucomicrobia, with the two phyla Firmicutes and Bacteroidetes representing 90% of gut microbiota. The Firmicutes phylum is composed of more than 200 different genera such as Lactobacillus, Bacillus, Clostridium, Enterococcus, and Ruminicoccus (Emanuele Rinninella *et.al.*, 2019). The human colon contains as many as 10^{12} bacteria/g of contents and >1000 bacterial species (Hanna Sikorskaa and Wanda Smoragiewicz, 2013).

Lactobacillus are Gram-positive, fermentative, facultative anaerobic or microaerophilic organisms. They typically form straight rods, but spiral or coccobacillary forms have been seen under particular circumstances. Lactobacillus play an important role in the protection of the host against harmful microorganisms and strengthens the immune system (Alain L. Servin, 2004). According to experts, those receiving antibiotics should consume probiotic-rich foods like yoghurt and sauerkraut while taking the drugs (D.Patil *et.al*., 2015).

Antibiotic sensitivity tests: The proper treatment of a patient's health against fatal infections requires the use of antibiotic susceptibility testing (AST), which establishes effective antibiotic dosage and creates a profile of empirical therapy (Zeeshan A.

Khan, 2019). The most common AST method in laboratory is disc diffusion since it is inexpensive, simple to use, and applicable to a wide variety of bacterial species and drugs (Ina Gajic, 2022). There are different methods such as the Disc diffusion method, Agar well diffusion method, Micro dilution method etc for antibiotic sensitivity tests. The tests can be evaluated by observing the Zone of Inhibition. The zone of inhibition is a circular area surrounding the antibiotic site where bacteria colonies do not grow. The zone of inhibition can be used to assess the bacteria's sensitivity to the antibiotic (H.S. Bhargav, 2016).

MATERIALS AND METHODS

Materials: Azithromycin, curd, Methanol, MRS agar medium

Methods:

i. Isolation of Lactobacillus: Curd is the best source of lactic acid bacteria particularly Lactobacillus bacteria which is one of the gut bacteria. Curd is taken in a sterile beaker. Under the aseptic conditions, the curd was serially diluted from 10^{-1} to 10^{-7} from these 7 dilutions, the appropriate dilution test tube is selected to isolate the Lactic acid bacteria. Then the desired colony i.e., Lactobacillus colony is identified. The spread plate technique and streak plate technique are performed on MRS agar medium to isolate pure culture of Lactobacillus. They are incubated for 24 hours in an incubator at 37ºC which is the optimum temperature for Lactobacillus bacteria to grow and the bacteria is tested for its morphological and biochemical characteristics.

ii. Tests for morphological and biochemical characteristics of Lactobacillus:

1. Gram staining
2. Spore staining
3. Capsule staining
4. Sugar fermentation test
5. Methyl red test
6. V.P test
7. Catalase test
8. Urease test

iii. Preparation of Azithromycin antibiotic solution: The antibiotic is to be taken in a solution form since the agar well diffusion method is followed for which the powdered form of the Azithromycin antibiotic was dissolved in methanol.

iv. Antibiotic sensitivity tests:

Agar well diffusion method is followed for the Antibiotic sensitivity test. 20 µl of Azithromycin antibiotic solution is taken for the antibiotic sensitivity test and evaluation was done by observing the Zone of inhibition.

RESULTS

i. Isolation of Lactobacillus:

Potential strains observed with different colony morphology were streaked on MRS agar medium to obtain pure cultures and they were taken for further study. In this present study, we selected 2 strains based on the dominating nature.

table:1 colony characteristic:

Characteristics	Isolated colony 1	Isolated colony 2
Colour	white	White
Shape	circular	irregular
Size	small	medium
margin	irregular	irregular

Among the selected 2 strains, isolated colony 2, has rod-shaped bacteria which is the major characteristic of Lactobacillus in curd. Thus, isolated colony 2 is chosen for culturing of Lactobacillus bacteria.

figure 1 Serial dilution of curd

figure 2 Isolated colonies of lactic acid bacteria.

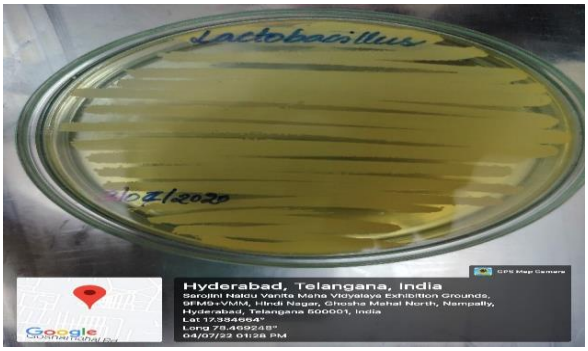

figure 3 Pure culture of Lactobacillus.

ii.Tests for morphological and biochemical characteristics of Lactobacillus:

1. **Gram staining**: After the process of staining, we observed purple-coloured rod-shaped bacteria. Hence, the rod-shaped bacteria are identified as Gram-positive Lactobacillus.

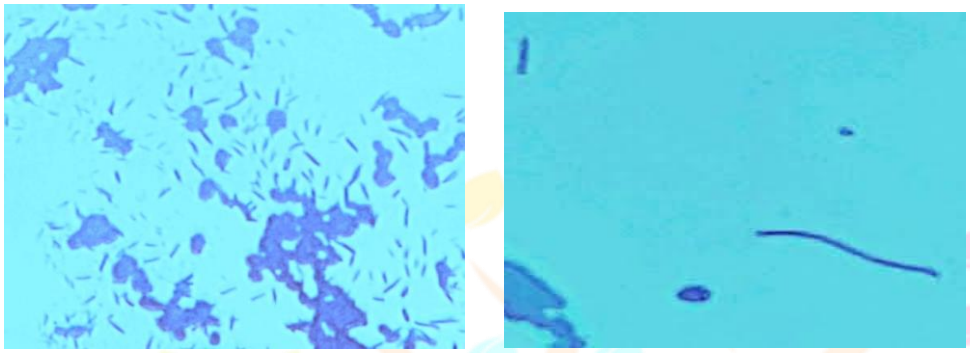

figure 4 Gram-positive rod-shaped Lactobacillus bacteria.

2. **SPORE STAINING:** Endospores appear green and a reminder of the cell or a cell without an endospore i.e., vegetative cells appear light red. The bacterial cells of the 2 isolates appear to be light red. Hence, they are non-sporulated**.**

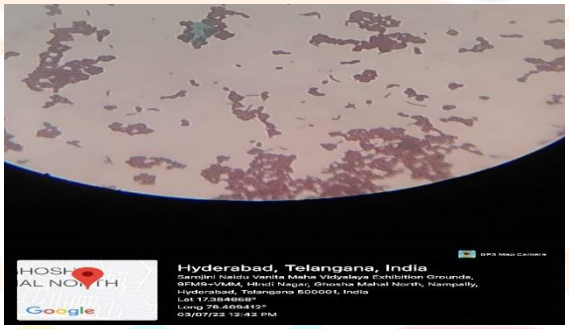

figure 5 spore staining.

3. **CAPSULE STAINING:** Capsule appears as a light blue layer or ring of varied thickness around the blue violet bacterial cell, but the bacterial cells have no blue layer. Hence, the bacterial cells of 2 isolates are identified as non- capsulated.

figure 6 Capsule staining.

4. **SUGAR FERMENTATION TEST:** When the phenol red indicator was added to the test solution, the colour of all the isolates in the test solution changes from red colour to yellow colour indicating the fermentation process and acid formation.

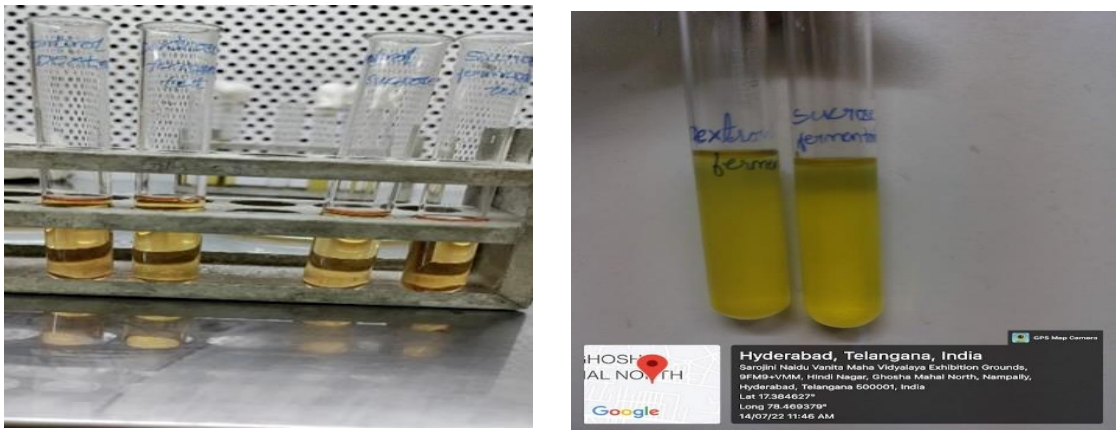

figure 7 Test for sugar fermentation

5. **METHYL RED TEST:** When methyl red is added to the test tubes, no red colour was observed. As the colour changes to red-orange colour, it shows a negative result for the methyl red test.

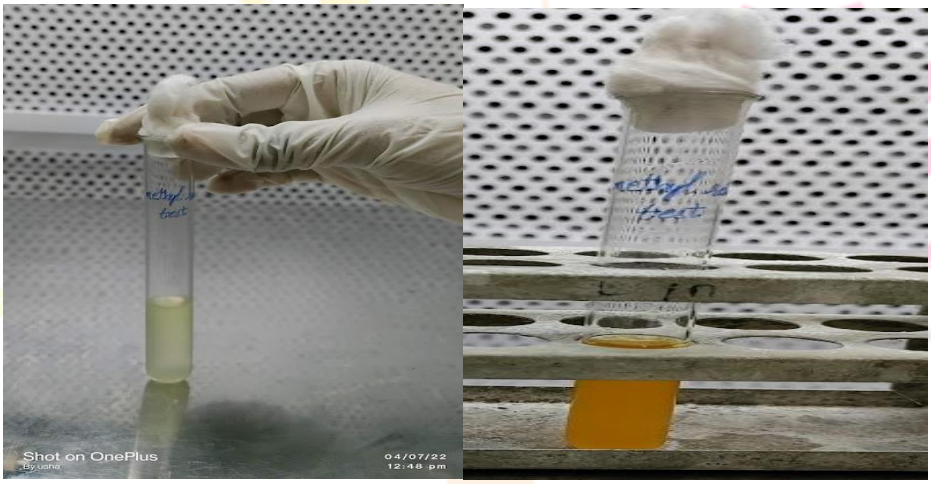

figure 8 Methyl red test.

6. VOGES-PROSKAUER TEST: When KOH and ∝-naphthol is added to the sample, it develops pink colour in 2-5 mins and became crimson in 30 mins. As the sample develops crimson colour it shows a positive result for the VP test.

figure 9 VP test**.**

7. TEST FOR CATALASE PRODUCTION: The evolution of bubbles were not observed. Hence, the given organism is negative for catalase test.

figure 10 catalase test

8. TEST FOR UREASE ACTIVITY: Change in the colour of the medium to purple pink resulting in the formation of NH3. Hence, the test is positive for urease activity.

figure 11 Urease activity test.

table 2: Morphological and biochemical tests and their results.

S.No	Tests	Results
1	Gram's staining	Gram's positive
2	Spore staining	Negative
3	Capsule staining	Negative
4	Sugar fermentation test	Positive
5	Methyl test	Negative
6	VP test	Positive
7	Catalase test	Negative
8	Urease test	positive

iii. Preparation of Azithromycin antibiotic solution:

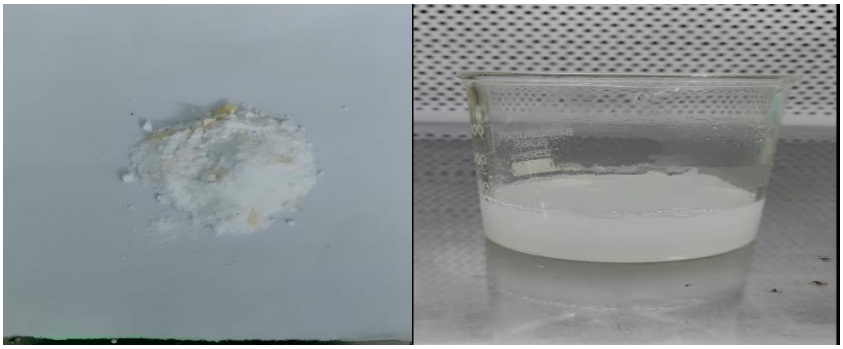

figure 12 Azithromycin powdered form and solution

iv. Antibiotic sensitivity tests:

Agar well diffusion method is followed for the Antibiotic sensitivity test. A clear Zone of inhibition is observed around the circular well of Azithromycin antibiotic.

figure 13 Zone of inhibition

table 3: methanolic extracts of azithromycin and its effect on lactobacillus.

Methanolic extracts	dosage	Effect on Lactobacillus	Zone of inhibition
methanol	10 ml	Resistant	absent
Azithromycin	10 mg	Sensitive	present

DISCUSSION:

Over the past 80 years, the widespread use of antibiotics has prevented millions of deaths, advanced technology, and eliminated untold numbers of harmful and commensal bacteria (Amy Langdon et.al.,2016). One such antibiotic that is widely used is Azithromycin. Azithromycin is an antibiotic that fights bacteria. It is an erythromycin derivative that has significantly increased activity against gram-negative bacteria, such as Enterobacteriaceae, and covers a wide range of gram-positive species (Zachary sandman,2021). Because it is effective against skin or skin structure infection caused due to bacteria such as Streptococcus *pyogenes*, Streptococcus *agalactiae*, Staphylococcus *aureus* etc., Azithromycin is used as an antibiotic (Zachary sandman,2021). Although Azithromycin is often well tolerated, some patients may experience stomach discomfort. It has a severe effect on gut bacteria (Brendan J McMullan,2022). Azithromycin affects the Lactobacillus, which is one of the important gut bacteria that play a vital role in the protection of the host against harmful microorganisms and strengthening the immune system (Alain L. Servin, 2004). In this study, the methanolic solution of Azithromycin of 10 mg concentration has been tested on Lactobacillus bacteria. A distinct Zone of inhibition is observed indicating that the Lactobacillus bacteria is sensitive to the Azithromycin antibiotic. Hence, new therapies are needed to combat the spread of bacterial infection. Alternatives to antibiotics must be considered to prevent the adversities.

CONCLUSION:

Hence, overconsumption of antibiotics especially those which are consumed frequently i.e. Azithromycin shows adverse effects on Lactobacillus bacteria and due to this the other gut microbiota will also be affected which also results in various digestion problems. Therefore, an alternative to the Azithromycin antibiotic is to be considered. Ayurvedic herbal plants such as Ferrula *asafoetida*(Hing), Moringa *oleifera*, Tulasi leaves etc. which have antibacterial properties that can be consumed in a required amount without affecting the gut bacteria.

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