



A Comparative Study On The Antibacterial Property Of Azithromycin And *Ferula Asafoetida* On *Lactobacillus* (Gut Bacteria).

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Abstract: Antibiotics are drugs that are used to treat or prevent certain type of bacterial infections. Despite the fact that antibiotics have considerably increased human health and life expectancy, their alteration of the natural microbiota has been associated with serious negative effects. One such important microbiota is *Lactobacillus*, they are members of the normal microbial ecosystem in the intestinal tract and plays an important role in maintaining stability and diversity of the gut microbiome.

Therefore, a medication that is both anti-pathogenic and shouldn't affect *Lactobacillus* is needed. *Ferula asafoetida* (Hing), an herb which has medicinal properties. The antibacterial property of *F. asafoetida* is tested on pus causing bacteria through Agar well diffusion method. Evaluation was done by observing the Zone of Inhibition. Further, effect of Azithromycin and *F. asafoetida* has been tested on *Lactobacillus* and observed that there was an effect of Azithromycin on *Lactobacillus* whereas, *F. asafoetida* has no adverse effect on *Lactobacillus*.

Index Terms - Antibiotics, Azithromycin, Gut microbiome, *Lactobacillus*, *Ferula asafoetida* (Hing).

I. INTRODUCTION

One of the most commonly utilized pharmacological classes in modern medicine is antibiotics. According to Lu Zhang et al. (2013), antibiotics are often administered orally, intravenously, or by injection. The average human life expectancy rose by eight years between 1945 and 1972 as a result of the use of antibiotics to treat illnesses that were previously likely to result in death for patients.

The amount of antibiotics taken in different countries varies significantly. 2018 saw the publication of the WHO study on antibiotic usage surveillance, which looked at data from 65 countries in 2015 based on daily dosages per 1,000 people. Mongolia had the highest rate of consumption, at 64.4. Burundi scored the lowest, at 4.4. Amoxicillin and clavulanic acid were the most frequently consumed antibiotics (Annie J. Browne, 2021).

Numerous undesirable side effects, ranging from mild to severe, have been connected to several antibiotics (Adam Felman, 2019).

Antibiotic side effects account for one out of every five medication-related emergency room visits, according to the Centers for Disease Control and Prevention (CDC) (Jennie Olopaade, 2021).

Despite having a relatively low per-capita private-sector consumption rate in comparison to many other countries, India uses a substantial amount of broad-spectrum antibiotics, which should ideally be administered sparingly (Shaffi fazaludeen et.al., 2022).

Researchers have shown that certain antibiotics, such as the often administered tetracyclines and macrolides, can kill beneficial gut flora while they are in use (Brian Krans, 2021).

Gut bacteria: According to Hanna Sikorskaa and Wanda Smoragiewicz (2013), the human colon has about 1000 bacterial species and up to 1012 bacteria/g of contents.

Gram-positive, facultative anaerobic or microaerophilic, fermentative organisms are known as lactobacillus. They usually have the shape of straight rods, but in certain situations, helical or coccobacillary forms have been observed. Lactobacillus are crucial for bolstering the immune system and defending the host against dangerous microbes (Alain L. Servin, 2004).

HERBAL MEDICINES:

Like many modern antibiotics, the first antibiotics came from natural sources (IJMM, 2013).

Generally cultural rootedness enduring and widespread use in a Traditional Medical System may indicate safety, but not efficacy of treatments, especially in herbal medicine (Fabio Firenzuoli and Luigi Gori, 2007).

Medicinal plant	Traditional uses	Scientific knowledge
Bergamot (Citrus bergamia)	Fragrances, disinfectant, healer	Photosensitizer, Mutagen-cancerous
Chaste tree (Vitex Agnus castus L.)	Anxiety, convalescence sexual sedative	Premenstrual syndrome
Coltsfoot (Tussilago fanfara L.)	Cough sedative	Hepatotoxic and Mutagenic alkaloids
Garlic (Allium sativum L.)	Influenza and diarrhea, aphrodisiac and abortive. Used against parasites and witches	Platelet antiaggregant. Hypolipidemic and hypotensive herbal remedy
Greater celandine (Chelidonium majus)	Hepatobiliary diseases (yellow latex for yellow bile)	Hepatotoxic
Germander (Teucrium chamaedrys L.)	Depurative, digestive, slimming	Hepatotoxic
Marigold (Calendula officinalis L)	Hemmenagogus, liver depurative gastric ulcer, dysmenorrea	Hemollient and healer (only topic use)
St Jhon's wort (Hypericum perforatum L.)	Burns, gastritis, magical uses	Antidepressant, Induction of CYP3A

(Source: google)

According to experts, those receiving antibiotics should consume probiotic-rich foods like yoghurt and sauerkraut while taking the drugs. On one hand the adverse effects of antibiotics are observed tremendously and on the other hand increase in the herbal medicines has been geared up.

Extracts of *F.asafoetida* shows antibacterial effect on *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Aspergillus niger* (D.Patil et.al., 2015).

Ferula asafoetida: *Ferula asafoetida* is a monoecious perennial herbaceous plant in the UMBELLIFERAE family. *F.asafoetida* is native to Central Asia, ranging from eastern Iran to Afghanistan, and is now primarily grown in Iran and Afghanistan, from which it is exported to the rest of the world. It is not indigenous to India, but it has long been used in Indian medicine and cooking. Typical *asafoetida* contains about 40–64% resin, 25% endogenous gum, 10–17% volatile oil, and 1.5–10% ash.

When *Ferula* plants are 4-5 years old, their huge taproots, which resemble carrots and measure 12.5–15 cm in diameter at the crown, are harvested to make *asafoetida*. In March or April, just before the plants bloom, the stem of the living rhizome root is cut off near to the crown. The exposed area is covered by a dome-shaped structure composed of twigs and dirt. The sliced surface is dripping with a milky fluid. When more latex oozes after a few days, the secretions are scraped off and a fresh slice of the root is cut; occasionally, the resin is also removed with the slice. Repeated resin collection and root cutting are done until secretion stops (about 3 months after the first cut). Sometimes, consecutive incisions made at the intersection of the stem or rhizome and the taproots are used to gather the resin (Augustine Amalraj and Sreeraj Gopi, 2017).

ANTIBACTERIAL EFFECT OF ASAFOETIDA: To treat various fungus and bacteria, *asafoetida* is a spice and herbal remedy. *Asafoetida* crude extracts were tested for their antibacterial efficacy against a variety of bacterial and fungal species.

F.asafoetida have been used as an important source of medicines as they are composed of chemical components such as asaresinotannol, coumarins, phenolics, diterpenes, sesquiterpene etc in their resins, gums, roots, and leaves that are with antibacterial properties. Extracts of *asafoetida* showed significant effect against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Aspergillus niger*. Essential oils extracted from *F. asafoetida* oleo-gum resins (OGRs) at various collection times, designated as OGR1, OGR2, and OGR3, had distinct chemical compositions, including antioxidant, ROS, RNS, H₂O₂, and TBARS scavenging. The essential oil from OGR1 was constituted high levels of bicyclic sesquiterpenes [10-epi- γ -eudesmol] and acyclic sulfur-containing compounds [(E)-1-propenyl sec-butyl disulphide and (Z)-1-propenyl sec-butyl disulphide] showed the highest radical scavenging and the lowest antifungal and antibacterial activities. Essential oil from OGR2 was constituted high levels of acyclic sulphur-containing

compounds [(Z)-1-propenyl sec-butyl disulphide and (E)-1-propenyl sec-butyl disulphide] and bicyclic monoterpenes [β -pinene and α -pinene] and showed moderate radical scavenging, antifungal, and antibacterial activities. Essential oil from OGR3 was constituted high levels of bicyclic monoterpenes [β -pinene and α -pinene] and heterocyclic disulphide [1,2-dithiolane] and showed the lowest radical scavenging and the highest antibacterial and antifungal activities. While the essential oil obtained from the later stages of *F. asafetida* growth could be used in the health industry as a safe and effective source of antibacterial agents, the essential oil obtained from the earlier stages of *F. asafetida* growth could be used as safe and effective natural antioxidants in the food industries to improve oxidative stability of fatty foods during storage. The antibacterial effects of two kinds of *F. asafetida*, Pattani and Irani, whose volatile oils were obtained through hydro-distillation, were investigated against a range of food-borne bacterial and fungal pathogens.

II. MATERIAL AND METHODS:

REQUIRED MATERIALS: Azithromycin, *F. asafetida*, curd, Pus/wound swabs.

i. Isolation of lactic acid bacteria (*Lactobacillus*):

Curd is the best source of lactic acid bacteria. Curd is taken in a sterile beaker. Under the aseptic conditions, curd was serially diluted from 10^{-1} to 10^{-7} from these 7 dilutions, 10^{-3} , is selected. The spread plate technique and streak plate technique are performed on MRS agar medium to isolate pure culture. They are incubated for 24 hours in an incubator at 37°C which is the optimum temperature for *Lactobacillus* bacteria to grow and the isolated colonies were identified by morphological and biochemical characteristics.

ii. Isolation of Pus/wound-causing bacteria:

Pus samples were collected by using sterile cotton swabs which are moistened with sterile saline to prevent drying. For each specimen, 2 swabs were used. They were brought to the laboratory in a sterile container one hour after the collection and processed immediately. One swab was used for gram staining and the other one was inoculated on Mac-Conkey agar and nutrient agar plates for isolating the pathogens. The inoculated plates were incubated at 37°C overnight. After incubation, the plates were observed for growth and the isolated colonies were identified by morphological and biochemical characteristics.

iii. Morphological And Biochemical Characterization Of *Lactobacillus* & Pus/Wound Causing Bacteria:

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

iv. Preparation of Extracts through solvent extraction method.

- a. Methanolic extract of *F.asafetida*: This is an extraction process such as maceration. The drug material (*F.asafetida*) which is grinded into fine powder is taken, and then placed inside a clean container. The extraction solvent(methanol) is then poured on top of the drug material, soaked, and kept for a short period of time. This method is suitable for extraction bioactive constituents that are readily soluble. In addition, it is an appropriate method for preparation of fresh extract before use.
- b. Methanolic extract of Azithromycin:
The solvent extraction method is followed for methanolic extract of Azithromycin.

v. Sensitivity test of methanolic extract of *F.asafetida* & Azithromycin on pus/wound causing bacteria and *Lactobacillus* bacteria.

- a) Agar well diffusion method was followed to test the antibacterial effect of *F.asafetida* on *Lactobacillus* and pus/wound causing bacteria. Aliquots of 20 μl of each sample in 1:1 and 1:5 methanol to methanolic extract of *F.asafetida*[i.e.,10ml of methanol and 10 mg of *Asafetida*,10 ml of methanol and 50 mg of *Asafetida*] were transferred into the wells of nutrient agar plate and MRS agar plate that is inoculated with pus/wound causing bacteria and *Lactobacillus*. respectively. Incubate the agar plates for 24 hours at 37°C , observe the zone of inhibition around the wells.
- b) The powdered form of Azithromycin antibiotic was dissolved in methanol. Aliquots of 20 μl of each sample 1:1 and 1:5 methanol to Azithromycin [i.e.,10ml of methanol and 10 mg of Azithromycin,10 ml of methanol and 50 mg of Azithromycin] were transferred into the wells of the nutrient agar plate and MRS agar plate that

is inoculated with pus/wound causing bacteria and Lactobacillus respectively. Incubate the agar plates at 37 °C for 24 hours, and observe the zone of inhibition around the wells.

III. RESULTS AND DISCUSSION

i. Isolation of Lactic acid bacteria (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 CHARACTERISTICS:

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 Isolated colonies of lactic acid bacteria of dilution factor 10^{-3}



Figure 1.2 Pure culture of LAB by Streaking method

ii. Isolation of Pus/Wound causing bacteria:

Potential strains observed with different colony morphology were streaked on Mac-conkey agar and Nutrient agar 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:2-COLONYPCHARACTERISTICS:

Characteristics	Isolated colony 1	Isolated colony 2
Colour	Yellow	pink
Shape	Cocci	Cocci bunches
Size	Medium	Small
margin	Irregular	irregular

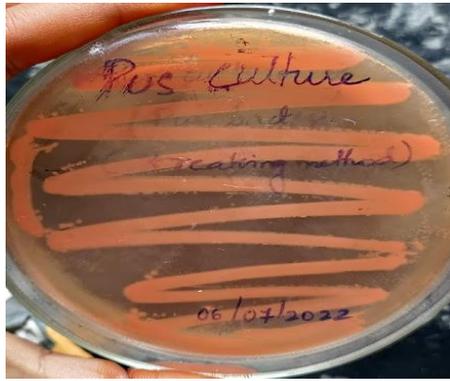


Figure 2 Streaking method of pus/wound bacteria on Mac-conkey agar



Figure 2.1 Streaking method of pus/wound bacteria on Nutrient agar

iii. Morphological And Biochemical Characterization Of Lactobacillus & Pus/Wound Causing Bacteria: MORPHOLOGICAL TESTS OF LACTIC ACID BACTERIA:

1.GRAM'S STAINING:

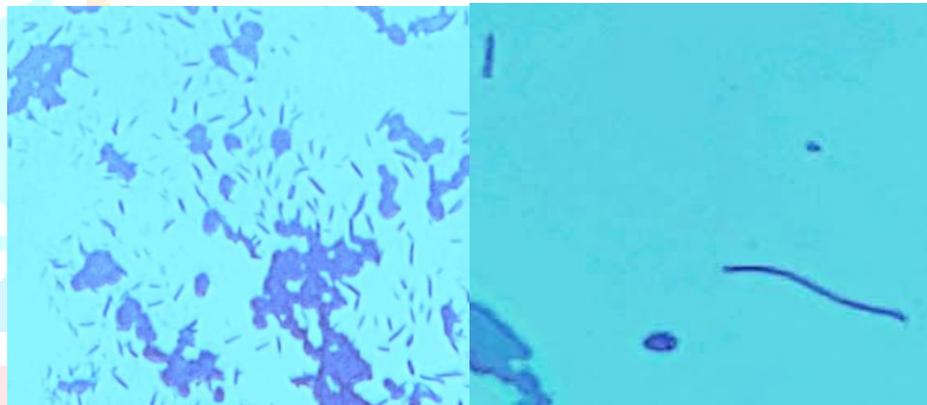


Figure 3 Gram-positive rod-shaped Lactobacillus bacteria.

Observation:

After the process of staining, we observed purple-coloured rod-shaped bacteria.

Result:

From the above observation, the rod-shaped bacteria are identified as Gram- positive Lactobacillus.

2.SPORE STAINING:

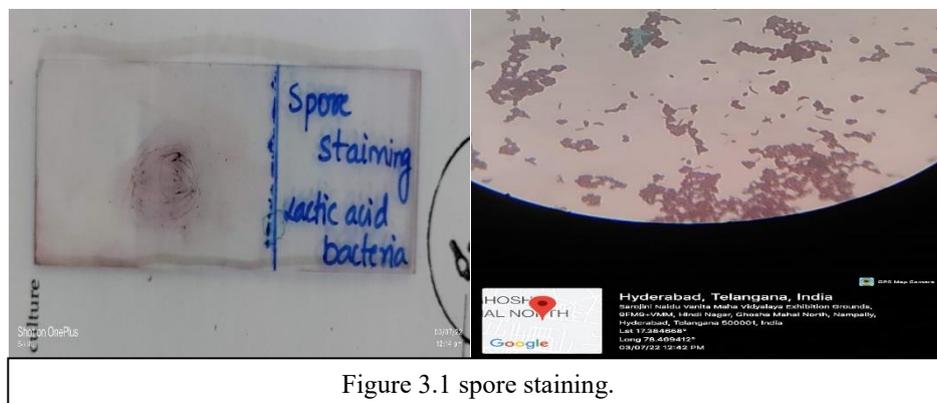


Figure 3.1 spore staining.

Observation:

Endospores appear green and a remainder of the cell or a cell without an endospore i.e., vegetative cells appear light red.

Result:

From the above observation, the bacterial cells of 2 isolates appear to be light red. Hence, they are non-sporulated.

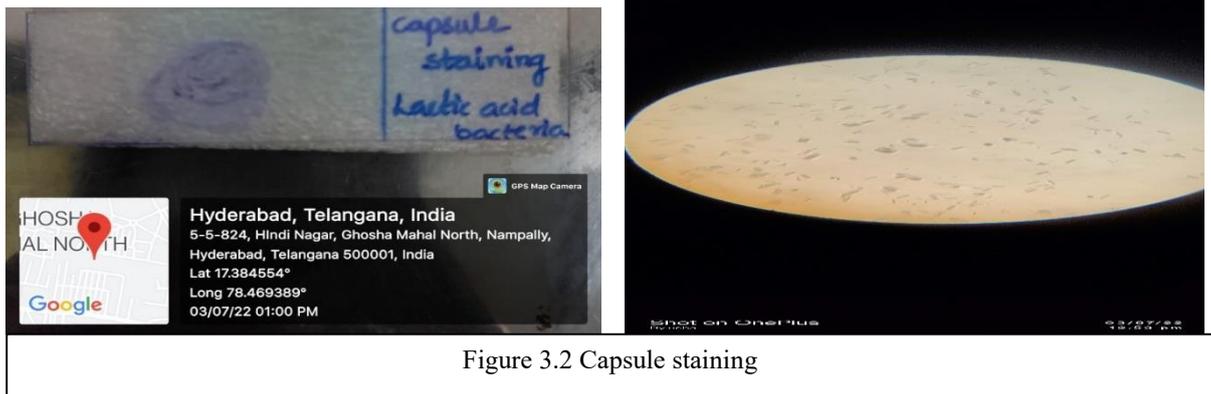
3.CAPSULE STAINING:

Figure 3.2 Capsule staining

Observation:

Capsule appears as light blue layer or ring of varied thickness around the blue violet bacterial cell, but the bacterial cells have no blue layer.

Result:

From the above observation, the bacterial cells of 2 isolates are identified as non-capsulated.

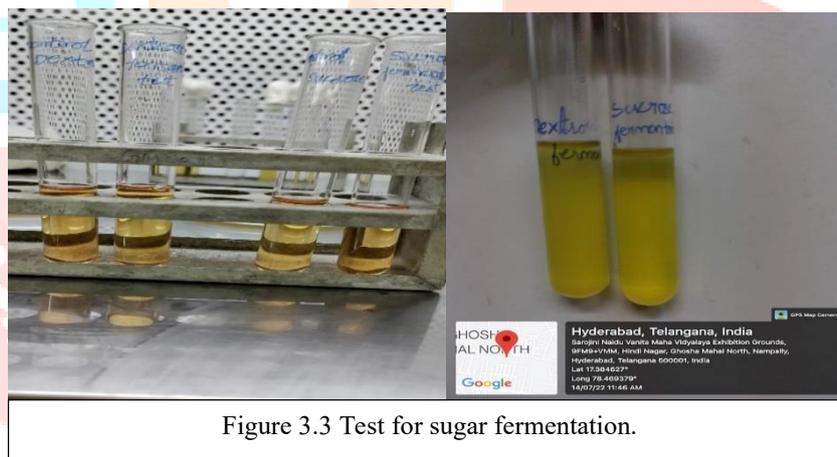
BIOCHEMICAL TESTS OF LACTOBACILLUS:**1.TEST FOR SUGAR FERMENTATION:**

Figure 3.3 Test for sugar fermentation.

Observation:

When 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.

Result:

Colour change indicates that there is an increase in pH as a result of acid formation.

2. METHYL RED TEST:

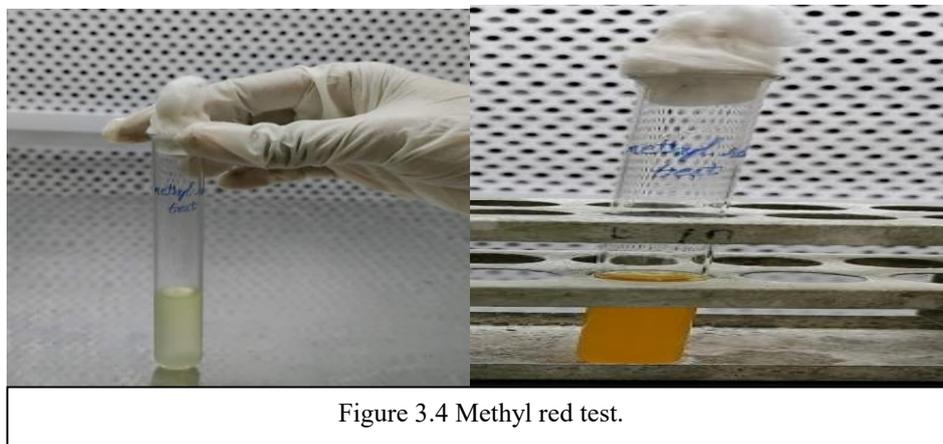


Figure 3.4 Methyl red test.

Observation:

When methyl red is added to the test tubes, no red colour was observed.

Result:

As the colour changes to red orange colour, it shows negative result for the methyl red test.

3. VOGES-PROSKAUER TEST:

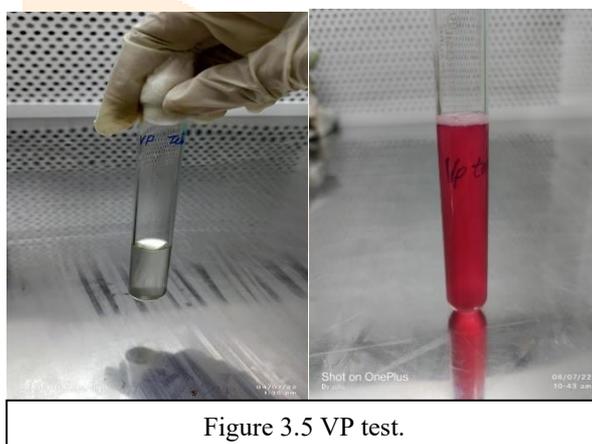


Figure 3.5 VP test.

Observation:

When KOH and α -naphthol is added to the sample, it develops pink colour in 2-5 mins and became crimson in 30 mins.

Result:

As the sample develops crimson colour it shows positive result for the VP test.

4. TEST FOR CATALASE PRODUCTION:

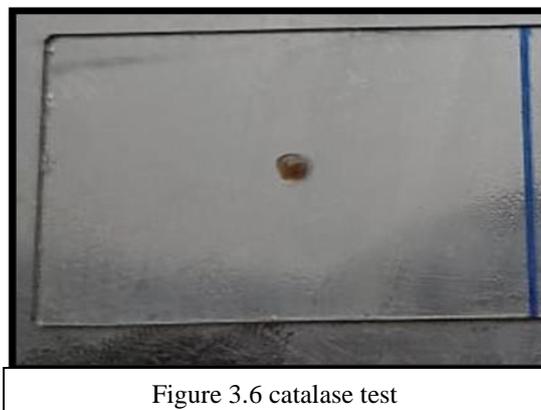


Figure 3.6 catalase test

Observation:

Evolution of bubbles were not observed.

Result:

The given organism is negative for catalase.

5. TEST FOR UREASE ACTIVITY:

Figure 3.7 Urease activity test.

Observation:

Change in the colour of the medium to purple pink.

Result:

Change in the colour is due to urease activity resulting in the formation of NH_3 .

TABLE 3: 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

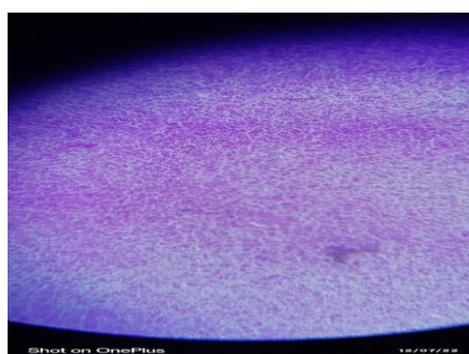
MORPHOLOGICAL TESTS OF PUS/WOUND BACTERIA:**1. GRAM'S STAINING:**

Figure 4 Strain 1- Gram positive bacteria



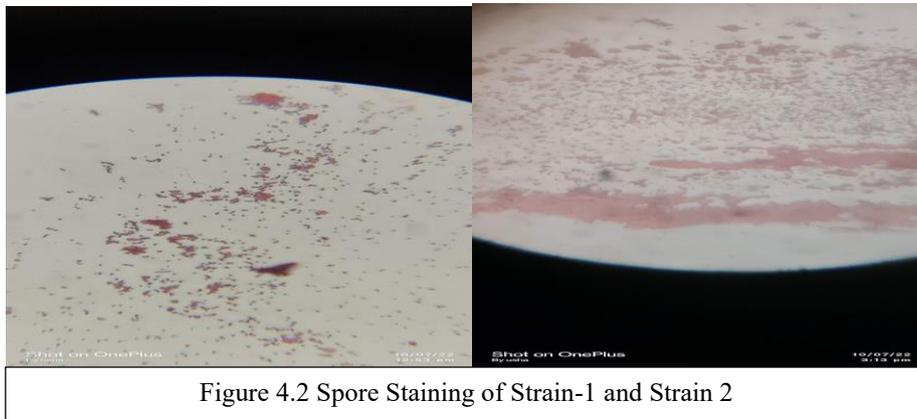
Figure 4.1 Strain 2- Gram negative bacteria.

Observation:

After the process of staining, we observed purple-coloured cocci bacteria and red coloured cocci bunches bacteria.

Result:

From the above observation the strain 1 is identified as Gram positive and strain 2 as Gram negative.

2.SPORE STAINING:**Observation:**

Endospores appear green and remainder of the cell or a cell without endospore i.e., vegetative cells appear light red.

Result:

From the above observation, the bacterial cells of 2 strains appears to be light red. Hence, they are non-sporulated.

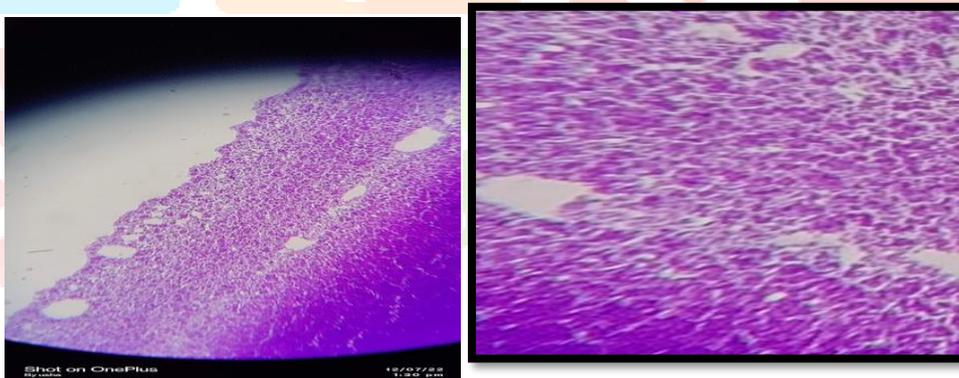
3.CAPSULE STAINING:

Figure 4.3 capsule staining of Strain-1 and strain 2

Observation:

Capsule appears as light blue layer or ring of varied thickness around the blue violet bacterial cell, but the bacterial cells have no blue layer.

Result:

From the above observation, the bacterial cells of 2 strains are identified as non-capsulated.

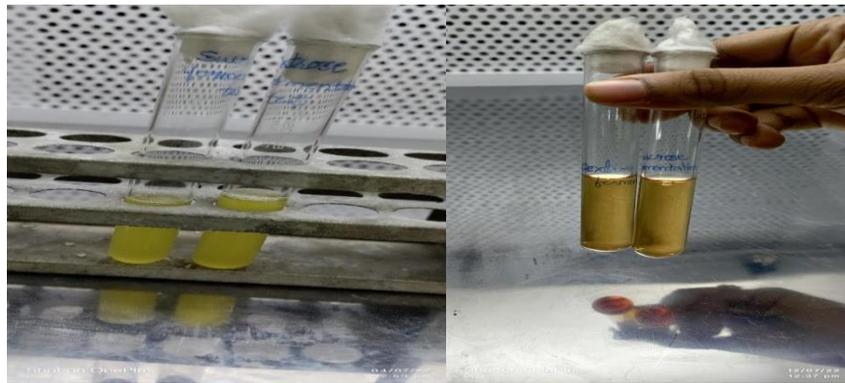
BIOCHEMICAL TESTS OF PUS/WOUND BACTERIA:**1. TEST FOR SUGAR FERMENTATION:**

Figure 4.4 Test for sugar fermentation of Strain 1 and 2

Observation:

When phenol red indicator was added to the test solution, the colour of the strain 1 changed from red to yellow and strain 2 has no change (red in colour).

Result :

Colour change indicates that there is an increase in pH as a result of acid formation. Hence Strain 1 is positive for the sugar test. Strain 2 is negative for the sugar test.

2. METHYL RED TEST:

Figure 4.5 Methyl red test of strains 1 and 2

Observation: When methyl red is added to the test tubes, red colour was observed in strain 1 and orange yellow is observed in strain 2.

Result:

Strain 1- positive for methyl red test, Strain 2- negative for methyl red test.

3. VOGES-PROSKAUER TEST:

Figure 4.6 VP test of Strain 1 and 2

Observation:

When KOH and α -naphthol is added to the sample, it develops pink colour in 2-5 mins and became crimson in 30 mins.

Result:

Strain 1- negative VP test, Strain 2-positive VP test.

4.TEST FOR CATALASE PRODUCTION:

Figure 4.7 Catalase test strain 1



Figure 4.8 Catalase test strain 2

Observation:

Evolution of bubbles were observed due to the formation of oxygen.

Result:

Both the strains 1 and 2 are positive for catalase.

5.TEST FOR UREASE ACTIVITY:

Figure 4.9 Urease activity test of Strain 1 and 2.

Observation:

No Change in the colour of the medium to purple pink.

Result:

strains 1 is negative to urease test and strain 2 is positive.

TABLE 4: Morphological and biochemical tests and their results

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

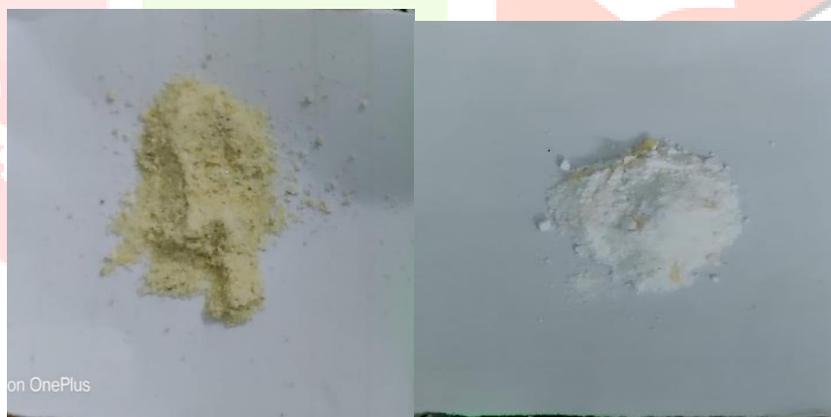
iv. Preparation of Extracts through solvent extraction method.

Figure 5 F.asafoetida powder.

Figure 5.1 Powdered form of Azithromycin

v. Sensitivity test of methanolic extract of *F.asafoetida* & Azithromycin on pus/wound causing bacteria and *Lactobacillus bacteria*.

1) Antibiotic sensitivity test of methanolic extract of Azithromycin on pus/wound causing bacteria.



Figure 6 Zone of inhibition of Azithromycin on strain 1 of pus/wound causing bacteria

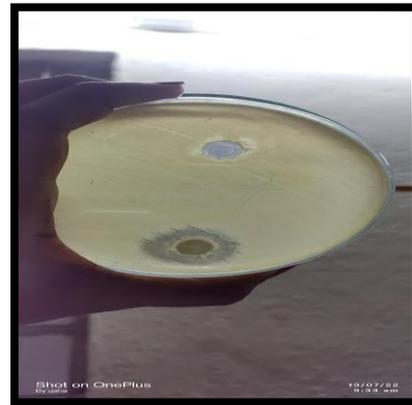


Figure 6.1 Zone of inhibition of Azithromycin on strain 2 of pus/wound causing bacteria.

2) To study the dose dependent effect of *F. asafoetida* on pus/wound causing bacteria.



Figure 7 Zone of inhibition of *F.asafoetida* on stain 1 of pus/ wound causing bacteria.

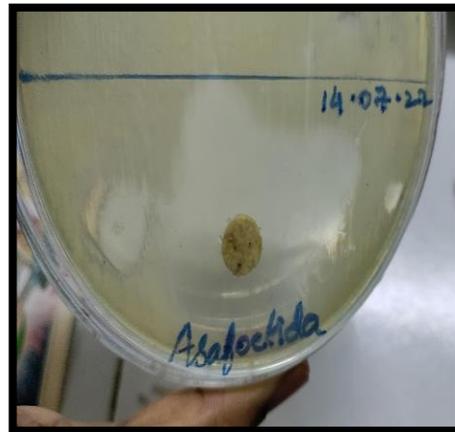


Figure 7.1 Zone of inhibition of *F.asafoetida* on stain 2 of pus/ wound causing bacteria.

3) To study the dose dependent effect of Azithromycin on *Lactobacillus* bacteria.

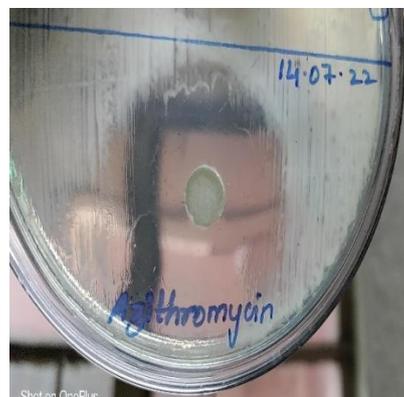


Figure 8 Zone of inhibition of Azithromycin on *Lactobacillus*

4. To study the dose dependent effect of on *F. asafoetida* on *Lactobacillus* bacteria.



Figure 9 Resistivity of *Lactobacillus* to *F. asafoetida*

Observation and result:

- 1) A clear Zone of Inhibition is observed in the Nutrient agar well diffusion method of Azithromycin against pus/wound causing bacteria.
- 2) A clear Zone of Inhibition is observed in the Nutrient agar well diffusion method of *F. asafoetida* against pus/wound causing bacteria.
- 3) A clear Zone of Inhibition is observed in the MRS agar well diffusion method of Azithromycin against *Lactobacillus* bacteria.
- 4) Zone of inhibition is not observed in the MRS agar well diffusion method of *F. asafoetida* against *Lactobacillus* bacteria.

Table 5: Methanolic extracts and their effect on pus/wound bacteria.

Methanolic extracts	dosage	Effect of pus/wound	Zone of inhibition
Methanol	1ml	Resistant	Absent.
Azithromycin	10 mg	Sensitive	Slightly observed.
Azithromycin	50 mg	Sensitive	Distinctly seen
<i>Ferula asafoetida</i>	10 mg	Sensitive	slightly observed.
<i>Ferula asafoetida</i>	50 mg	sensitive	Distinctly seen

Table 6: Methanolic extracts and their effect on *Lactobacillus*.

Methanolic extracts	dosage	Effect of <i>Lactobacillus</i>	Zone of inhibition
Methanol	1ml	Resistant	Absent
Azithromycin	10 mg	Sensitive	Present
Azithromycin	50 mg	Sensitive	Distinctly seen
<i>Ferula asafoetida</i>	10 mg	Sensitive	Present
<i>Ferula asafoetida</i>	50 mg	sensitive	Distinctly seen

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).

A particular class of antimicrobial agent that works against bacteria is an antibiotic. One of the most effective chemotherapies in medical history is undoubtedly antimicrobials. The number of lives they have saved and the impact they have had on the fight against infectious illnesses, which for the majority of human history

were the main causes of sickness and mortality, need not be emphasised here. Research has shown that this is not the case, in contrast to the widely held idea that exposure to antibiotics is limited to the present "antibiotic era." For instance, the presence of tetracycline has been detected in human skeletal remains discovered in ancient Sudanese Nubia between 350 and 550 CE (Bassett et al., 1980; Nelson et al., 2010).

One such antibiotics 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. Azithromycin is effective against chlamydiae, legionella, mycoplasma and mycobacteria.

Because it is effective against *Streptococcus pneumoniae*, *Hemophilus influenzae*, and *Moraxella catarrhalis*, Azithromycin is recommended for treating community-acquired pneumonia and has FDA approval (CAP). Acute otitis media and acute aggravation of chronic obstructive pulmonary disease are two other upper respiratory infection processes for which it has received approval (COPD). In the figure 6 & 6.1, the pus/wound causing bacteria is sensitive to methanolic extract of Azithromycin antibiotic, hence zone of inhibition is observed. Therefore, Azithromycin has approval for the treatment of pharyngitis caused by *Streptococcus pyogenes*, as an alternative to a beta-lactam agent; skin or skin structure infection due to *S. pyogenes*, *Streptococcus agalactiae*, or *Staphylococcus aureus*; *M. avium* complex (MAC) infection treatment and prophylaxis for patients with advanced acquired immunodeficiency syndrome (AIDS); and sexually transmitted infections including chlamydia, gonococcal disease, chancroid (caused by *Hemophilus ducreyi*), and *Mycoplasma genitalium*. Azithromycin is effective against some protozoal species, including *Toxoplasma gondii*, *Plasmodium* sp., and *Babesia* sp., and is sometimes used off-label to treat these parasite disorders in conjunction with antiprotozoal medications (e.g., atovaquone). Lastly, Azithromycin is also used off-label as long-term prophylaxis for bronchiolitis obliterans (BO) in patients who have undergone lung transplantation (Zachary sandman,2021).

Mostly, the pus bacteria includes *Streptococcus aureus*, *Staphylococcus pyogens*, *Enterococcus*, *E.coli*, *Pseudomonas*, *Klebsella*, *Proteus mirabilis*. From the figure 2 and 2.1, the bacteria isolated is pus/wound causing bacteria which are confirmed by the morphological and biochemical tests (Figure 4 to 4.9).

Over the past few decades, dangerous, antibiotic-resistant microorganisms have been found more frequently. The factor fueling antibiotic resistance is the evolution and dissemination of resistance factors within bacterial populations (Richard J. Fair and Yitzhak Tor ,2014).

The mortality rates due to multidrug-resistant bacterial infections are high. Each year, about 25,000 patients in the EU die from an infection with the selected multidrug-resistant bacteria (ECDC/ EMEA Joint Working Group, 2009), and more than 63,000 patients in the United States die every year from hospital-acquired bacterial infections (Rustam I. Aminov,2010).

Recent research has been done on underlying adverse events of long-term Azithromycin(macrolide)therapy for patients with chronic lung diseases, and it has been found that patients treated with long-term macrolides could be at risk of increased infection with nontuberculous mycobacteria. Furthermore, some macrolides may have underlying severe side effects, such as increased cardiovascular events (Hui Li et.al.,2014).

Although azithromycin is often well tolerated, some patients may experience stomach discomfort, headache, or dizziness (1–5% of patients). Also found in 1.5% of patients were transient elevations in transaminases. Azithromycin has also been linked to hearing loss or damage, particularly in patients with COPD and baseline normal hearing. In some cases, these effects looked to be permanent. The US Food and Drug Administration issued a warning in 2012 to consider the risk of fatal heart rhythms (Brendan J McMullan,2022).

Also overuse of antibiotics during the COVID-19 pandemic may lead to increased selection for antimicrobial resistance(Catherine E. Oldenburg et.al.,2021).

Azithromycin effects the *Lactobacillus*, which is one of the important gut bacteria that play vital role in the protection of the host against harmful microorganisms and strengthen the immune system (Alain L. Servin, 2004). Due to its strong effect on the composition of gut microbiota (in figure 8), it keenly shows the adverse effect of Azithromycin on *Lactobacillus* bacteria. A distinct Zone of inhibition is observed indicating that the *Lactobacillus* bacteria is sensitive to the Azithromycin antibiotics. Recent evidence showed that bacteria are important triggers for asthmatic episodes, and that azithromycin reduced the duration of symptoms by half. In the study, 14 days after randomization, children in the AZT group had 23% lower richness and 13% lower

diversity in their fecal samples compared to the placebo group. In particular, the relative abundance of Actinobacteria was reduced (Shaodong Wei et.al.,2018)

New therapies are needed to combat the spread of bacteria resistant to antibiotics. Alternatives to antibiotics must be taken into account in both human and animal medicine due to the drastic fall in the rate of antibiotic development. Products for treating diseases differ from those for preventing them. Vaccines, Probiotics, prebiotics, synbiotics, and competitive exclusion, Disease treatment phage therapy, Endo- or exolysins, Bacteriocins, Predatory bacteria are the alternatives to the antibiotics (Heather K. Allen et.al., 2014).

Since the peak of the antibiotic era in the middle of the 20th century, natural compounds have been effective therapies against pathogenic microorganisms. However, the increasing frequency of antibiotic resistant infections clearly demonstrates that new antibiotics are critical for modern medicine (Sean E. Rossiter et.al., 2017). Herbal medicine is one of the antibiotics that are naturally obtained. The Indian book, Vedas, mentions treatment of diseases with plants. Some of the examples of herbal medicine with antibiotic properties are Black cohosh, Echinacea, Evening primrose, Feverfew, Garlic, Gingko biloba, Ginseng, Hawthorn, St. John's wort, Amalaki, Ashwagandha, Atmagupta, Bilva, Brahmi, Daruharidra, Eranda, Nimba, Shunthi, Yashtimadhu etc (Ashok D.B. Vaidya, 2007).

F.asafoetida is one such herb with medicinal properties. It has been consumed as a spice and a folk medicine for centuries. Recent studies have shown several promising activities particularly relaxant, neuroprotective, memory enhancing, digestive enzyme, antioxidant, antispasmodic, hypotensive, hepatoprotective, antimicrobial, anticarcinogenic, anticancer, anticytotoxicity, antiobesity, anthelmintic and antagonistic effect (Augustine Amalraj, Sreeraj Gopi,2016).

It was revealed that leaf extract of *F.asafoetida* possesses stronger antimicrobial properties against *E. coli*, *S. aureus*, and *S. cerevisiae* than gum extract (Razieh Niazmand and Bibi Marzieh Razavizadeh, 2020). Figure 7 & 7.1, depicts the antibacterial property of *F.asafoetida*. Hence, the stain 1 and stain 2 of pus/wound causing bacteria might be sensitive to the *F.asafoetida* and therefore shows Zone of inhibition around the agar well.

The most common bacteria that causes pus/wound includes *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* (*E. Coli*), *Proteus mirabilis*, *Acinetobacter baumannii/haemolyticus*, *Streptococcus*. According to the studies, *F.asafoetida* is similar to chemical antibiotics such as Azithromycin that controls the bacterial infection.

Though the extracts of *F.asafoetida* has antibacterial property it has no effect on the *Lactobacillus* bacteria (Figure 9). therefore, *Lactobacillus* is resistant to the *F.asafoetida*.

CONCLUSIONS

Antibiotics are the wonder drugs that brought advancements in the medical field.

Antibiotics are used to treat bacterial infections. Azithromycin is an antibiotic medication used for the treatment of a number of bacterial infections. Pus is an accumulation of the dead material. Infections involving the bacteria which are Gram positive and Gram negative bacteria such as *Staphylococcus aureus* and *Streptococcus pyogenes* are especially prone to pus. Both bacteria release toxins that damage tissue, creating pus.

Azithromycin is widely used to cure the infection caused by the pus/wound bacteria. On the other hand due to its antibacterial properties it has adverse effect on the gut microbiome. One such important bacteria that is present in the human gut and getting effected is *Lactobacillus*.

The minimum zone of inhibition was observed when *Lactobacillus* is subjected to 10 mg of Azithromycin. The maximum concentration at which Azithromycin has an effect on *Lactobacillus* is 50 mg which has greater diameter of zone of inhibition when compared to the concentration of 10 mg of Azithromycin.

F.asafoetida also has the antibacterial property which is similar to the Azithromycin. Therefore during the antibiotic sensitivity test of pus/wound causing bacteria, there was a clear appearance of Zone of inhibition around the agar well. Though *F.asafoetida* has antibacterial property, *Lactobacillus* was resistant to it.

Hence, *F.asafoetida* can be used as an antibiotic medicine when infected by the pus/wound causing bacteria without effecting the *Lactobacillus*

REFERENCES

1. Adam Felman: "What to know about antibiotics", January 18, 2019.
2. Ahmed amrajaa abdulraziq. and Sami mohammed salih: "Comparison Effects of Methanolic Extract of Ruta Chalepensis L. with some Antibiotics on Inhibiting Growth of Isolated Bacteria from Wound Inflammation", August.2020.
3. Alain L. Servin: "Antagonistic activities of lactobacilli and bifidobacteria against microbial pathogens". FEMS Microbiology Reviews, Volume 28, Issue 4,2004.
4. Amy Langdon,Nathan Crook Gautam Dantas: "The effects of antibiotics on the microbiome throughout development and alternative approaches for therapeutic modulation" *Genome Medicine* volume 8, Article number: 39, 13 April 2016.
5. Annie J Browne, Dphil,Michael G Chipeta, PhD,Georgina Haines-Woodhouse, MS,Emmanuelle P A Kumaran, MPH,Bahar H Kashef Hamadani, MPH,Sabra Zaraa, MPH,Nathaniel J Henry, BSc,Aniruddha Deshpande, MPH,Robert C Reiner Jr, PhD,Prof Nicholas P J Day, FMedSci,Prof Alan D Lopez, PhD,Prof Susanna Dunachie, PhD,Catrin E Moore, DPhil,Prof Andy Stergachis, PhD,Prof Simon I Hay, DSc,Christiane Dolecek, PhD: "Global antibiotic consumption and usage in humans, 2000–18: a spatial modelling study". November 11, 2021.
6. Ashok D.B. Vaidya and Thomas P.A. Devasagayam: "Current Status of Herbal Drugs in India: An Overview" July 2007.
7. Brendan J McMullan: "Brendan J McMullan" VOLUME 38 : NUMBER 3 : JUNE 2015.
8. Catherine E. Oldenburg, ScD, MPH; Benjamin A. Pinsky, MD, PhD; Jessica Brogdon, MPH, TM; Cindi Chen, MS; Kevin Ruder, BS; Lina Zhong, BS; Fanice Nyatigo, BS; Catherine A. Cook, MPH; Armin Hinterwirth, PhD; Elodie Lebas, RN; Travis Redd, MD, MPH; Travis C. Porco, PhD, MPH; Thomas M. Lietman, MD; Benjamin F. Arnold, PhD, MPH; Thuy Doan, MD, PhD: "Effect of Oral Azithromycin vs Placebo on COVID-19 Symptoms in Outpatients With SARS-CoV-2 Infection. A Randomized Clinical Trial" August 10, 2021 Volume 326, Number 6.
9. Celeste De Monte , Simone Carradori , Arianna Granese , Giovanni Battista Di Pierro, Costantino Leonardo and Cosimo De Nunzio: "Modern extraction techniques and their impact on the pharmacological profile of *Serenoa repens* extracts for the treatment of lower urinary tract symptoms"11 August 2014.
10. Emanuele Rinninella, Pauline Raoul, Marco Cintoni, Francesco Franceschi, Giacinto Abele Donato Miggiano,Antonio Gasbarrini and Maria Cristina Mele:"What is the Healthy Gut Microbiota Composition? A Changing Ecosystem across Age, Environment, Diet, and Diseases". 10 January 2019.
11. Fabio Firenzuoli and Luigi Gori: Herbal Medicine Today: Clinical and Research Issues",2007.
12. Hanna Sikorska , Wanda Smoragiewicz: "Role of probiotics in the prevention and treatment of meticillin-resistant *Staphylococcus aureus* infections",2013.
13. Hanna Sikorska, Wanda Smoragiewicz : Role of probiotics in the prevention and treatment of meticillin-resistant *Staphylococcus aureus* infections". 2013 Dec.
14. Heather K. Allen, Julian Trachsel, Torey Looft, Thomas A. Casey: "Finding alternatives to antibiotics" 20 June 2014.
15. Hui Li, Ding-Hui Liu, Lu-Lu Chen, Qi Zhao, Yan-Zhe Yu, Jing-Jing Ding, Li-Yun Miao, Yong-Long Xiao, Hou-Rong Cai, De-Ping Zhang, Yu-Biao Guo, Can-Mao Xie: "Meta-Analysis of the Adverse Effects of Long-Term Azithromycin Use in Patients with Chronic Lung Diseases" January 2014 Volume 58 Number 1.
16. L. Barth Reller, Melvin Weinstein, James H. Jorgensen, Mary Jane Ferraro. "*Clinical Infectious Diseases*, Volume 49, Issue 11, 1 December 2009, Pages 1749-1755.
17. Lu Zhang, Ying Huang, Yang Zhou, Timothy Buckley, Hua H. Wang: "Antibiotic Administration Routes Significantly Influence the Levels of Antibiotic Resistance in Gut Microbiota". Aug 2013.
18. M. L. Mohammed Kaleem Arshan1 , Ankit Jurla and Sneha Khadse: "Isolation and identification of bacteria from wound infection and their antibiogram", Volume V Issue IV, April 2020.
19. Razieh Niazmand and Bibi Marzieh Razavizadeh: "Ferula asafoetida: chemical composition, thermal behavior, antioxidant and antimicrobial activities of leaf and gum hydroalcoholic extracts" 17 August 2020.
20. Retsema J,Girard A,Schelkly W,Manousos M,Anderson M,Bright G,Borovoy R,Brennan L,Mason R, Spectrum and mode of action of azithromycin (CP-62,993), a new 15-membered-ring macrolide with improved potency against gram-negative organisms. *Antimicrobial agents and chemotherapy*. 1987 Dec;

21. Richard J. Fair and Yitzhak Tor: “Antibiotics and Bacterial Resistance in the 21st Century” June 24, 2014.
22. Rustam I. Aminov: “A brief history of the antibiotic era: lessons learned and challenges for the future” December 2010 | Volume 1 | Article 134.
23. S. D. Pati, S. Shinde, P. Kandpile and A. S. Jain: “evaluation of antimicrobial activity of asafoetida”, 1 February 2015.
24. Sean E. Rossiter, Madison H. Fletcher, and William M. Wuest: “Natural Products as Platforms To Overcome Antibiotic Resistance” 11 October 2018.
25. Shaffi FazaludeenKoya, SenthilGanesh,SakthivelSelvaraj,Veronika J.Wirtz,SandroGalea,Peter C.Rockers: “Consumption of systemic antibiotics in India in 2019”.The Lancet RegionalHealth - Southeast Asia2022;4: 100025Published online volume 4 September,2022.
26. Shaodong Wei, Martin Steen Mortensen, Jakob Stokholm,AskerDaniel Brejnrod,JonathanThorsen,Morten Arendt Rasmussen, Urvish Trivedi,Hans Bisgaard,Søren Johannes Sørensen: “Short- and long-term impacts of azithromycin treatment on the gutmicrobiota in children: A double-blind, randomized,placebo-controlled trial” 16 November 2018.

