Frontier in Medical & Health Research

Volume 3, Issue 1, 2025 ISSN: (e) 3007-1607 (p) 3007-1593

COMPARATIVE ANALYSIS OF ANTIBIOTIC AND CITRUS FRUIT JUICES SENSITIVITY ON STAPHYLOCOCCUS AUREUS GROWTH

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DOI: https://doi.org/10.5281/zenodo.15047310

Keywords
Staphylococcus aureus, MSSA, MRSA, citrus fruits.

Article History
Received on 24 January 2025
Accepted on 24 February 2025
Published on 03 March 2025

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Abstract

Introduction: *Food poisoning is a major problem worldwide. Most of the time main cause is Staphylococcus aureus bacteria. The bacteria itself don't cause disease but toxin produced by bacteria causes food poisoning and can lead to severe condition. Common symptoms during illness are nausea, vomiting, abdominal pain and diarrhea. To kill bacteria different antibiotics are being used. Plants and their extract like fruits, oils can be a good alternate to overcome the situation. We can use organic food as medicine e.g citrus fruits. These fruits not only kill the bacteria but it also helps to fulfill nutritional requirements.*

Methodology: *This study was an experimental and observational study. This experiment was performed at B.J Microlab, Gulzar-e- Quaid Rawalpindi. Muller Hinton agar was used to perform experiment. Three culture plates were taken (C1, C2 & C3) one for antibiotics (Linzolid & Gentamicin) and the others for citrus fruit juices of C. Sinensis (sweet orange) And C. limon species (lemon).*

Results: *The main focus was to find out citrus fruits as an alternative of antibiotics. Quite a good results of citrus fruits against the growth of Staphylococcus aureus were observed, especially lemon juice have amazing bactericidal properties and if any one antibiotic is considered to kill bacteria instead of traditional antibiotics, linezolid can be used. It will not only kill bacteria but also it will try to minimize toxins produced by bacteria and also it will cure the dehydration caused by food poisoning can be recovered by consuming citrus fruit juices. Also the citrus fruits have vitamin C which is helpful in increasing the immunity.*

INTRODUCTION

In this 21st century many illnesses has occurred, diagnosed and treated, as some of the bacterial infections are easily diagnosed and treated with antibiotics nowadays but some bacteria has created antibiotic resistance and cannot be treated in any way using medications. One of the examples is Staph-food poisoning, Staphylococcus aureus (S. aureus) is a cagey human pathogen causing a range of illness from self-limiting to fatal. (Bencardino & Vitali, 2019) Numerous infections, affecting the skin, soft tissues, endovascular locations, and internal organs might be brought on by it. Due to its high rate of morbidity and death S. aureus is still a significant infection in both hospitals and the general population. Toxins are a common way to characterize virulence factors of S.aureus, as poisonous materials are the standard definition of toxins because they are produced by the producing organism and directly affect the host, they differ from other virulence factors, which are chemicals that raise a pathogen's capacity to cause disease in a wider sense. (Otto, 2014) A gastrointestinal illness known as Staph food poisoning is brought on by consuming food contaminated with toxins made by the Staphylococcus aureus (Staph) bacteria. SFP Staphylococcus aureus food poisoning is a prevalent disease, and the number of cases had been increased since 1884, when the first case was documented, to become one of the most common causes of foodborne illness. (Mourenza, Gil, Mateos, & Letek, 2021) One of the most widespread food-borne illnesses in the world is staphylococcal food poisoning which is caused by eating food contaminated with enterotoxin-producing strains of coagulase-positive staphylococci, primarily Staphylococcus aureus. (Argudín, Mendoza, & Rodicio, 2010) S. aureus enterotoxins (SEs) are powerful gastrointestinal exotoxins produced by S. aureus during the logarithmic phase of development or during the transition from the exponential to the stationary phase of growth. They are active at concentrations ranging from nano-grams to micrograms. (Argaw & Addis, 2015) Twenty to thirty percent of people have S. aureus on their skin and mucous membranes all the time.(Fetsch et al., 2014)

Staphylococcus aureus is a immotile, facultative anaerobic, gram-positive coccus. The organism produces catalase and coagulase. Staphylococci survive desiccation and tolerate high levels of salt. The organisms are able to grow in a wide range of temperatures (7°C to 48°C with an optimum of 30°C to 37°C), pH (4.2 to 9.3, with an optimum of 7.0 to 7.5); and sodium chloride concentrations (up to 15% NaCl). These characteristics enable the bacteria to survive in a wide variety of foods, especially fermented food products like cheese. Two type of strains has been used in research i.e, MRSA (Methicillin-resistant Staphylococcus aureus) and MSSA (Methicillin sensitive Staphylococcus aureus). MRSA is distinguished by its resistance to an entire class of antibiotics known as beta-lactams. Among the antibiotics in this class includes methicillin, as well as the more often prescribed penicillin, amoxicillin, and oxacillin. Initially penicillin was able to kill Staphylococcus aureus but by the time bacteria became able to destroy penicillin by producing enzyme known as penicillinase.(Pantosti & Venditti, 2009) Stapylococcus aureus has developed methicillin resistance due to a mutation in a penicillin-binding protein. It is one of the few instances of chromosome-mediated drug resistance via phage transduction that is significant to medicine.MSSA methicillin sensitive Staphylococcus aureus is an organism which can be treated by a group of antibiotics known as beta-lactam. (Siddiqui & Koirala, 2018)

For growth and isolation outside human body Mannitol salt agar (MSA) and Muller Hinton Agar (MHA) is commonly used. Certain species are unable to withstand high osmotic pressure. Except for the salt-tolerant Staphylococci, the growth of these non-tolerant organisms is inhibited by media. Only salt-tolerant Staphylococci can grow on Mannitol Salt Agar due. The medium can support the growth of non-pathogenic Staphylococci, but it doesn't create acid, therefore the medium stays pink. (Nassar, Hazzah, & Bakr, 2019) the agar medium used in this study was Mueller Hinton medium as in 1941, Mueller and Hinton created Mueller Hinton Agar (MHA) for the isolation of pathogenic Neisseria species. The Kirby-Bauer disk diffusion technique is now more routinely employed for regular

susceptibility testing of non-fastidious microorganisms. Mueller-Hinton agar (MHA) is the standard susceptibility testing medium for the Kirby-Bauer method. (Argaw & Addis, 2015)

Food poisoning is not caused by bacteria but it is caused by the enterotoxins produced by bacteria as mentioned earlier. Rapidly developing symptoms are frequently described as intense vomiting and nausea early symptoms include nausea followed by involuntary, distinctive vomiting. Other symptoms that are frequently reported include overall weakness, dizziness, shivering, diarrhea, and abdominal pain that may or may not be accompanied by a mild temperature. (Rajkovic, 2016) The illness is normally self-limiting and resolves within 24 to 48 hours of commencement. It is occasionally severe enough to necessitate hospitalization, particularly in newborns, the elderly, or the disabled. (Argudín et al., 2010)

To treat bacteria different antibiotics are being used for example, Vancomycin, Teicoplanin are commonly used. Instead of these traditional drugs we have used Linezolid and Gentamicin. Initially penicillin was used to treat Stapylococcus aureus but now majority of Staphylococcus aureus strains are penicillin resistant. Vancomycin gram-positive, cell-wall active glycopeptide antibiotic. Vancomycin has antibacterial activity that is mainly concentration-independent. Teicoplanin is a glycopeptide that, in general, has equal action against gram-positive aerobic and anaerobic cocci. Teicoplanin also kills bacteria in a time-dependent manner. Although there are not many clinical trials that compare it to vancomycin. (Rayner & Munckhof, 2005) Linezolid is a synthetic antibacterial drug. (Clemett & Markham, 2000) Linezolid is the first member of the class of antibiotics known as oxazolidinones. Oxazolidinones were initially launched in 1978 for their efficiency in plant disease control. It inhibits bacterial protein synthesis by binding to rRNA on both the 30S and 50S ribosomal subunits. It prevents the creation of the initiation complex, which can shorten the length of the produced peptide chains and slow down the translation reaction. Linezolid may also inhibit the expression of virulence factors, resulting in fewer toxins generated by Gram-positive bacteria. (Hashemian, Farhadi, & Ganjparvar, 2018) Due to less toxin production linezolid would be a good choice of drug as in Staph.aureus poisoning it is all about toxin more than bacteria. In 2012 a salavage therapy was done between Vancomycin and Linezolid and the results were somehow these, the vancomycin MIC did not differ significantly between the linezolid-based salvage therapy group (mean SD, 1.45 0.36 mg/mL) and the glycopeptide-based therapy group (1.54 0.51 mg/mL, P Z 0.49). Furthermore, the vancomycin MIC 1.5 mg/mL (73%, P > 0.99). (Shorr, Kunkel, & Kollef, 2005) Hence, in previous studies it is proved that linezolid can be used as a replacement of vancomycin. Gentamicin is an aminoglycoside antibiotic. It exhibits bactericidal activity against aerobic gram-negative bacteria making gentamicin a good option to treat several common infections. Since, gentamicin has minimal gastrointestinal absorption, its administration is usually by parenteral routes, including systemic, topical, and ophthalmic formulations. (Chaves & Tadi, 2020) Aminoglycosides (e.g., gentamicin) are frequently used in clinical settings along with other antimicrobial drugs such as beta-lactams or glycopeptides to treat serious infections caused by gram-negative and gram-positive organisms. (Tam, Kabbara, Vo, Schilling, & Coyle, 2006) Considering this as an edge we have used Gentamicin susceptibility on Staphylococcus aureus growth. In recent years it has been observed that many resistant strains are breaking out or drug can't treat them due to MDR (Multiple Drug Resistance). The citrus fruit have a unique pleasant and a quality flavor in all over the world, and all the species were spread on different areas on earth. Botanists have suggested that no other equals them in beauty of leaf, delightful odor of flower, or none more beautiful than those we know under the name of citron, lemon, and orange trees or splendor and taste of fruit. (Afroja et al., 2017) In this modern world, the dependency of different medication is increasing day by day to treat any of the disease, although medication have adverse effect. (Liya & Siddique, 2018)T he citrus fruit including Lemon, Orange, Citron, Lime, Shaddock, Bergamot orange, Grape-fruit and many more belong to the family of Rutaceae family, this family consist of 1300 species found on the Earth. These fruit have immense

importance not just because of their flavour or nutritional diet but also beneficial for the maintenance of healthy life and prevention of disease or used as a medicine. (Shakya et al., 2019) Some of the citrus fruit have a broad spectrum of importance in biomedical and pharmaceutical industry, most likely in anti-bacterial, anti-virulent, anti-fungal, or anti-diabetic and anti-cancerous activities because of alkaloids. (Liya & Siddique, 2018)

The plants including vegetable or fruit were used as a first medical treatment or alternative treatment against any inflammation, wound, or any infection for centuries. (Afroja et al., 2017) The citrus fruit have wide range of uses including anti-inflammatory, anti-oxidative, anti-tumor, as well as anti-microbial activity, these properties leads to a healthy life. (Shakya et al., 2019) Skin is the largest organ of the body, which perform multiple important functions. When skin of the human can be effected by any injury or having any wound which can leads to bacterial infection. A citrus fruit known as Lime or (Citrus aurantifolia) peel has the ability to resist the growth of bacteria specially Staphylococcus aureus, it is almost 80% sensitive. (Ekawati, Pradana, & Darmanto, 2019) The essential oils which are extracted from the citrus fruits and different herbs are full of natural properties including antimicrobial effects, anti-inflammatory effect and having those characteristic which inhibit the growth of microbes which are harm full. The Essential Oils are widely used by the food industry and pharmaceutical industry for the treatment of different diseases. (Song et al., 2020) The medicine which are extracted from the plants or vegetable or citrus fruit have no or less harm-full effect on human health therefore, 80% of the world population depends on it. (Afroja et al., 2017) The citrus fruit contain phytochemical property which is very useful in medication. (Shakya et al., 2019) Plants are the natural source of developing any Drug or medicine, in the pharmaceutical industry a large number of medicine were extract from different plant including vegetable and citrus fruit. (Afroja et al., 2017) In the past, different vegetable and fruits were used for medication instead of drugs. (Liya & Siddique, 2018) In the pharmaceutical industry, the product were formulated based on the traditional or past knowledge obtain from the different sources including books or etc. (Afroja et al., 2017)

Due to presence of these resistant strains we have to use alternative drugs but those drugs can be much more toxic and is of very high potency and may cause major damage to human body including the organs like liver, stomach and kidneys etc. To overcome risks instead of using high potency drugs if any organic alternative can be found it will be a revolutionary change in the field of medicine. Human history has deep root of organic food i.e plants, fruits, oil extract etc to treat diseases. It will be much cheaper in cost for patient. In recent studies it is found that citrus fruit can be a good alternative. Citrus fruits reported for enormous number of biological activities such as anti-cancer, anti-diarrheal, antibacterial, antifungal, antiviral, insecticidal and antioxidant. All the citrus types are well known for antimicrobial activities. (Sekar et al., 2013) In one of the study it is observed that lime oil has great antimicrobial activity against Staphylococcus aureus. (Aibinu, Adenipekun, Adelowotan, Ogunsanya, & Odugbemi, 2006)

In addition to its attractive scent, limonene has anti-inflammatory and antibacterial properties and their essential oil shows a inhibition zone against staphylococcus aureus. Antibacterial substances like linalool and citral are present in the peels of grapefruit and tangerines. (Meryem, Mohamed, Nour-Eddine, & Faouzi, 2023) Not only these qualities but citrus fruits also have nutritional capacity as the common symptoms of food poisoning include vomiting which may leads to dehydration. Vitamin C, which is essential for proper functioning of the human body, is said to be present in citrus fruits. (Malik, Najda, Bains, Nurzyńska-Wierdak, & Chawla, 2021) C. limon (lemon) and C. sinensis (sweet orange) will give a good response in sensitivity of Staphylococcus aureus. As citrus fruits are acidic in nature the acidity of fruits will cause damage to cell wall of bacteria which proves its bactericidal activity and these juices will also help to fight against common symptoms of Staph.aureus food poisoning e.g dehydration and electrolyte balance while giving the immune boost through vitamin C in citrus fruits. This study was conducted to find out best alternative

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of Staphylococcus aureus food poisoning instead of antibiotics, as well as to observe best bactericidal citrus fruit among all and to highlight the nutritional value of citrus fruits during the Staph food poisoning infection.

Methodology:
This study was an experimental and observational study, it was conducted at B.J research Micro lab in Gulzar-e-Quaid Rawalpindi, Pakistan. As well as:

• This experimental research was performed on Muller Hinton agar (MHA) plate.

• Three culture plates were taken, labelled as C1, C2 and C3.

• Antibiotics susceptibility was performed by disk diffusion method on C1 while citrus fruit sensitivity was checked by Agar Well diffusion method on C2 and C3.

• Pure colonies of Staphylococcus aureus (MRSA & MSSA) for the purpose of sensitivity colonies and inoculated on all the plates of MHA.

• C1 was used for sensitivity of antibiotics while disk diffusion method has been used and we have used two antibiotics Linezolid and Gentamicin. The MH agar on the C1 plate was divided into two halves and one side was innoculated with MRSA while the other was innoculated with MSSA and each side had both the drugs diffused on it, as shown in fig 1.1.

• C2 and C3 plate was used for resistant sensitivity of citrus fruits by Agar Well diffusion method. The citrus fruits juices used were of C. limon (lemon) and C. sinensis (sweet orange).

• The C2 was innoculated with MSSA (Methicillin sensitive Staphylococcus aureus), and the agar plate was divided into four quarters and four wells were made, one in each quarter, 2 quarters have the well diffused with sweet ornage juice with 50 microliter and 100 microliter concentration each and labeled as "O", while the other 2 quarters were diffused with lemon juice with 50 microliter and 100 microliter concentration each and labelled as "L", as shown in fig 1.2.

• The C3 was innoculated with MRSA (Methicillin resistant Staphylococcus aureus), and the agar plate was divided into four quarters and four wells were made, one in each quarter, 2 quarters have the well diffused with sweet ornage juice with 50 microliter and 100 microliter concentration each and labeled as "O",while the other 2 quarters were diffused with lemon juice with 50 microliter and 100 microliter concentration each and labelled as "L", as shown in fig 1.3.

• The plates were incubated for 24 hours and zone of sensitivity were seen and comapred.
All C1, C2 and C3 culture plates were innoculated with Staphylococcus aureus culture of both types ie. MRSA and MSSA whereas C1 contain only antibiotics e.g Linezolid and Gentamicin and C2 and C3 were used for citrus fruits juices of C. limon (lemon) and C. sinensis (sweet orange) and no other microbe other than Staphylococcus aureus (MRSA & MSSA) was used in our experiment.

RESULTS
Two strains of Staphylococcus aureus were used, one was Methicillin-resistant Staphylococcus aureus (MRSA) and the other one was Methicillin-sensitive staphylococcus aureus (MSSA).

Antibiotic susceptibility:
Visible results of linezolid on Stapylococcus aureus (MRSA & MSSA) growth were observed, as linezolid had more sensitivity against MRSA, as shown in fig 1.1.

Linezolid:
Against MRSA a zone of inhibition of about 30 mm diameter was observed, while on MSSA a 35mm long zone of inhibition was observed.

Gentamicin:
A slightly positive action of gentamicin was seen, but a very small zone of inhibition against Staphylococcus aureus growth was observed. Against MRSA no zone of inhibition was observed but

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against MSSA a 3mm long zone of inhibition was seen.

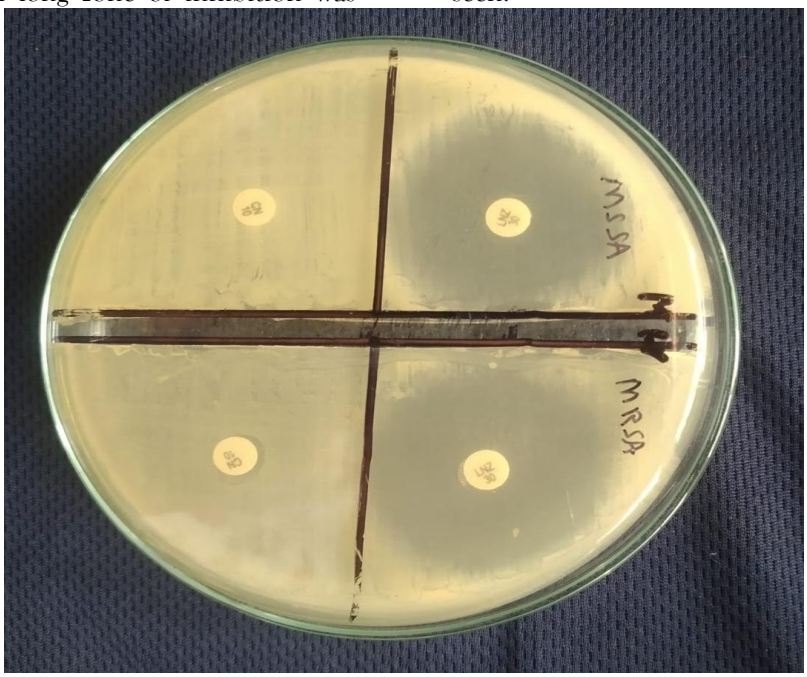

Figure 1.1 : Zone of inhibition of Linezolid and Gentamicin on Staphylococcus aureus (MSSA & MRSA)

Citrus fruits sensitivity:

Citrus fruits juices showed quite a good result on growth of Staphylococcus aureus. Citrus fruits juices used in this research were of C.limon (lemon) and C.sinensis (sweet orange).

Lemon C.limon:

With lemon juice good results were seen. Against MSSA lemon had 8-10 mm long zone of inhibition around the well of 100 microliter concentration, while around 50 microliter concentartion a 4mm long zone of inhibition was also observed on the C2 plate, as shown in fig 1.2. Against MRSA a 3mm long zone of inhibition was observed around 100 microliter concentration well. On the other hand, the well with 50 microliter no zone of inhibition was observed on the C3 plate, as shown in fig 1.3.

Sweet Orange C.sinensis:

As the C.sinensis (sweet orange) does not have the bactericidal properties, although against MRSA a 1.5mm zone of inhibition was observed around the well consiting 100 microliter of orange juice but no zone of inhibition was observed around of 50microliter well on the C3 plate, as shown in fig 1.3. While against MSSA it has a bit good results, as around the well with a concentration of 100 microliter, a 5mm zone of inhibition was observed and around the well of 50 microliter a 2.5mm zone of inhibition was observed on the C2 plate, as shown in fig 1.2.

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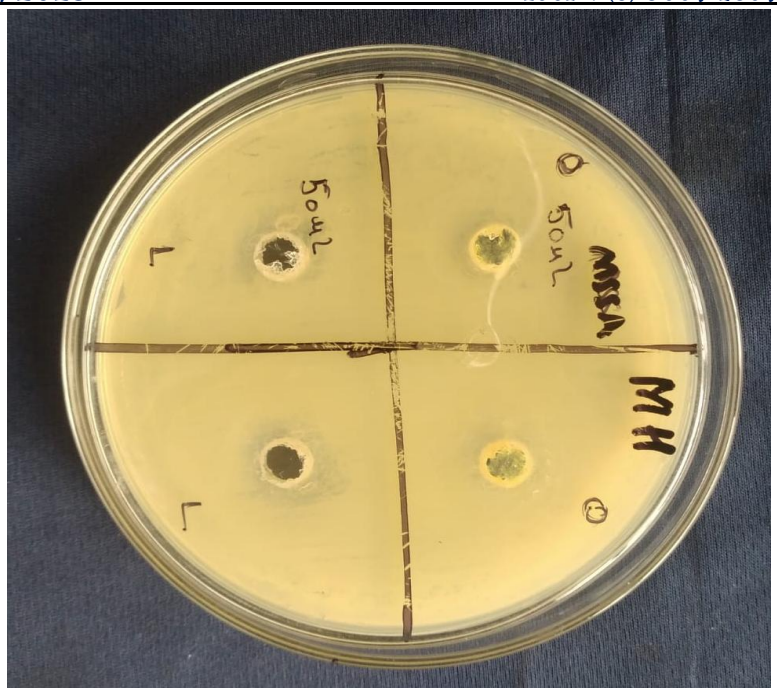

Figure 1.2: The zone of inhibition of Citrus juices (C.limon and C. sinensis) on Staphylococcus aureus (MSSA)

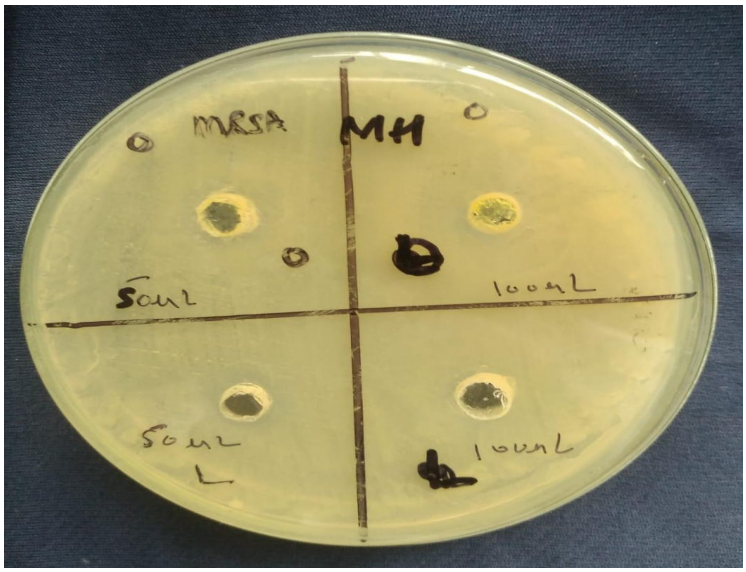

Figure 1.3: The zone of inhibition of Citrus fruit juices (C.limon and C.sinensis) on Staphylococcus aureus (MRSA)

The results can be observed by considering the table 1.1 and 1.2 below:

Table 1.1: Antibiotic sensitivity of Linezolid & Gentamicin on Staphylococcus aureus (MRSA & MSSA).

Antibiotics	MSSA	MRSA
Linezolid	35mm	30mm
Gentamicin	3mm	0mm

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Table 1.2: Antimicrobial activity of citrus fruit juices Lemon & Or5ange on Staphylococcus aureus (MRSA & MSSA).

Citrus Fruits	MSSA (mm)	MRSA(mm)
Lemon (50ml)	4	0
Lemon (100ml)	9	3
Orange (50ml)	2.5	0
Orange (100ml)	5	1.5

DISCUSSION:

Nowadays food poisoning is very common and most of the time cause behind the illness is Staphylococcus aureus bacteria or its toxins. Most of the Staph.aureus strains are now resistant to first line antibiotics. The first case of Staph.aureus was reported in 1884.Common symptom of disease are nausea, vomiting and abdominal pain. In case of bacterial killing different antibiotics are being used.

A research was done by (Rayner & Munckhof, 2005) on antibiotics used in infection caused by Staphylococcus aureus. The main two drugs they described for MRSA were vancomycin and teicoplanin. As most of the Staph.aureus strains are now resistant to traditional antibiotics. But vancomycin has been linked with ototoxicity and nephrotoxicity and teicoplanin has not much clinical trials. (Argaw & Addis, 2015) Keeping this point in mind we used linezolid which is considered as new drug to treat infection. Amazing results of linezolid against MRSA and MSSA both were observed, as against MRSA a zone of inhibition of about 30 mm diameter was observed, while on MSSA a 35mm long zone of inhibition was observed.

In another study done by (Chaves & Tadi, 2020) they found that gentamicin can be used in combination to other drugs to kill gram positive bacteria. In this study gentamicin was used as second drug and a slightly small zone of about 2mm only on MSSA, but no zone was observed against MRSA, as a slightly positive action of gentamicin was seen, but a very small zone of inhibition against Staphylococcus aureus growth was observed. Against MRSA no zone of inhibition was observed but against MSSA a 3mm long zone of inhibition was seen.

In a prior study done by (Aibinu et al., 2006), reported anti-bacterial activity of citrus fruits. Taking this point as a key, this study focused on citrus fruits and check their susceptibility against MRSA and MSSA.

According to this experiment, the results of citrus fruits. C.limon (lemon) and C.sinensis (sweet orange) were used. An amazing results of C.limon 8-10 mm long zone of inhibition against MSSA was observed and MRSA had 3mm zone. Whereas, C.sinensis (sweet orange) had minimal susceptibility in comparison to lemon. Against MSSA a 2.5mm long and on MRSA a 1.5 mm long zone was observed, as from the past studies the citrus fruit have wide range of uses including anti-inflammatory, anti-oxidative, anti-tumor, as well as anti-microbial activity, these properties leads to a healthy life. (Afroja et al., 2017) A citrus fruit known as Lime or (Citrus aurantifolia) peel has the ability to resist the growth of bacteria specially Staphylococcus aureus, it is almost 80% sensitive. (Ekawati et al., 2019) In recent studies it is observed that citrus juices have great result on staphylococcus aureus .The agar diffusion method was used to test the antibacterial effects of juices from three different citrus (C.) fruit species—C. limon (lemon), C. aurantium (bitter orange), and C. paradisi (grapefruit)—against certain bacteria e.g staphylococcus aureus. The diameter of the zone of inhibition around the extracts was also measured. The results confirmed the presence of antibacterial activity of the citrus juice. The highest inhibition zone (18 mm) was observed with a 10% concentration of C. paradises juice on Staphylococcus aureus. (Tawfik, Al-Haliem, & Al-Ani, 2010)

CONCLUSION AND RECOMMENDATIONS

After performing and observing this experiment we can conclude this that instead of vancomycin and teicoplanin we can use linezolid to treat Staph food poisoning as it is a good option. It will not only kill the bacteria but also prevent toxins production.

Beside this when we are talking about organic treatment/alternative citrus fruit is a good option. They not only kill the bacteria but at the same time they are helpful in fulfilling the nutritional requirements which are drained from the body during the diarrheal condition. For the future researchers we recommend that they should look for more alternatives for treating this fatal Staph food poisoning by organic means as it is a major matter of concern but is neglected in our society/ common household. They can also study on citrus fruit juices to cure other conditions and disorderssuch as fungal infections and cancers. Due to unavailability of fruits we can only use only two of the citrus fruit available out in the market in the month of August. Future researchers should try other citrus fruits like grape fruit, bitter orange etc. Previous studies prove that these fruits have more bactericidal properties. Instead of traditional medicine we should go for antibiotics that have minimal resistance still they are not in use.

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