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Therapeutic Alternatives against Multidrug-Resistant *Staphylococcus Aureus*

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Abstract*---Staphylococcus aureus*** is a gram-positive pathogen that can cause peripheral to serious infections in almost all tissues, especially in immunocompromised people, since the introduction of the first antimicrobial agents, it has evolved into one of six "ESKAPE" pathogens that are responsible for the majority of nosocomial infections and able to escape the biocidal action of antibiotics with high resistant rate and pass it into other bacteria. This evolutionary ability of *S. aureus* to gain resistance, has made it important to get insight into resistance mechanisms to be able to develop new antimicrobial agents. Lemon is a flowering medicinal plant belongs to the family Rutaceae, which contains different antimicrobial components. Nanotechnology has enabled the use of nanoparticles for the treatment of antimicrobial-resistant bacteria. Gold nanoparticles have been reported for their most desirable properties as compared to any other noble metal-based nanoparticles, Titanium dioxide NPs have also been explored as metallic NPs and have been successfully applied in MRSA therapy. This study aimed to study the inhibitory effects of fresh sour and sweet lemon juices plus gold and titanium dioxide nanoparticles against multidrug resistant *Staphylococcus aureus.* A total of (110) different clinical samples had been collected from hospitals looking for *Staphylococcus aureus* isolates. After its identification and diagnosis, the inhibitory effects of seven antibiotics, plant extract (sweet and sour lemon juices) and the gold and titanium dioxide nanoparticles effect were applied against *Staph aureus* isolates. *Staphylococcus aureus* was identified as 20.9% from burn, wounds and urine samples, showing a high resistance rate against most used antibiotics; both lemon juice types showed a significant inhibition effect on *S. aureus* growth however it was more with the sour type (P value less than 0.05), also gold and titanium dioxide

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nanoparticles showed a significant inhibitory effect (P value less than 0.05). *Staph. aureus* is a widely distributed multidrug-resistant bacteria and this resistant can be overcome by alternative substances other than chemical antibiotics; whether natural as lemon or metal as Nanoparticles.

Keywords---*Staphylococcus aureus,* Nanoparticles, antibiotic resistance, lemon, MDR.

Introduction

Staph. aureus is the causative agent of a wide range of infections in humans and animals with a significant impact on public health. Host specialization, ability to acquire and loss resistance and virulence genes as well as its zoonotic potential posed a significant public health implication [1]. Its disease can manifest as skin and soft-tissue infections, pneumonia, surgical-site infections, bloodstream infections, endocarditis, septic shock, and many others, as well as causing a numerous of infections; in addition to its ability to acquire antibiotics resistance [2].

Staphylococcus aureus develops resistance to antimicrobial agents by different means, such as horizontal gene transfer of different mobile genetic elements, including bacteriophages, plasmids, Staphylococcus cassette chromosomes, transposons, and pathogenicity islands [3].

Lemon is a flowering medicinal plant belongs to the family Rutaceae. It is a small evergreen tree native to Asia [4]. Lemon fruit is a herbal plant that has the main content of alkaloid compounds which have the function as anticancer, antibacterial, antifungal, antiviral and antidiabetic [5].

Citrus extract form lemon, lime and grape fruit had antibacterial activity against *Pseudomonas aeruginosa, Staphylococcus aureus, proteus, klebsiella, E. coli* and that lime and lemon juice had better antibacterial activity and compared favorably to the commercial antibiotic disc [6].

Nanotechnology has enabled the use of nanoparticles (NPs) for the treatment of antimicrobial-resistant bacteria. The strength antimicrobial NPs' properties results from their large surface area to volume ratio, which also reduces the likelihood of antibiotic tolerance. NPs ranging from 10 to 100 nanometers (nm) in size are thought to possess unique physical and chemical features. Recently, NPs have been used as an alternative method for treatment of various antibiotic-resistant bacterial infections and may solve the problem of multidrug-resistant bacteria [7]. Gold nanoparticles (AuNPs) have been identified as an attractive candidate for delivery into their targets. The acceptance of AuNPs as an excellent candidate for drug delivery was due to its unique properties especially in the transport and release of the therapeutic agents to the target site. The therapeutic agents to be delivered could be small drug molecules or large biomolecules, like proteins, DNA, or RNA and effectiveness of their release is a prerequisite for efficient therapy [8]. Titanium dioxide (TiO2) NPs have also been explored as

metallic NPs and have been successfully applied in MRSA therapy. Their application with different combinations of antibiotics, such as cephalosporins, glycopeptides, and azalides, shown anti-MRSA activity in a disk diffusion assay. Under UV photoactivation, TiO2 NPs form free radicals that lead to their enhanced killing of MRSA [9].

Aim

This study aimed to study the inhibitory effects of fresh sour and sweet lemon juices, gold and titanium dioxide nanoparticles against multidrug resistant *Staphylococcus aureus.*

Materials and Method

Microbial isolation and Identification

A total (110) different clinical samples had been collected from hospitals looking for *Staphylococcus aureus* isolates. Its identification and diagnosis according to [10].

Antibiotic Susceptibility Testing

It was performed by using a pure culture of previously identified bacterial isolate. The most effective antibiotic for each bacterial isolate was determined as recommended by CLSI; seven antibiotic types (Trimethoprime, Clindamycin, Tetracycline, Azithromycin, Amikacin, Trimethoprime sulfamethoxazole, and Gentamicin) determined by CLSI [11].

Plant extract collection

Fresh fruits listed at table (1) used in this study were obtained from the local market at Hilla City, Iraq, 2022. The fresh fruits were washed in running tap water in laboratory, surface sterilized with 70% alcohol, rinsed with sterile distilled water and cut open with a sterile knife and the juice pressed out into a sterile universal container separately and then filtered (using Millipore 0.45 filter paper) into another sterile container to remove the seeds and other tissues and used freshly without refrigeration.

Table (1): Plant extracts used in this study

No	Scientific name	English name	Parts of plant used	Local Arabic name
1	*Citrus limon L*	Sour Lemon	Fruit (juice)	نومي حامض
2	*Citrus limetta*	Sweet Lemon	Fruit (juice)	نومي حلو

Antimicrobial activities

The screening of antimicrobial activities of each crude aqueous lemon extract on the tested bacteria used approximately 20µl of each extract (applied as 100%, 75%, 50% and 25%) was inoculated onto wells were made in the spread plate

culture of each microbial isolates; interpretation was determined according to CLSI[11][12].

Preparation of (Gold and TiO2) Nanoparticles:
Preparation of these nanoparticles as solutions for their later application as antimicrobial agents against tested bacteria; according to [13][14].

The effect of Lemon juices on Bacterial Growth:
The prepared lemon juices (for both sour and sweet) where applied separately in a triplets to bacterial suspension, five concentrations (25, 50, 75 and 100%) of lemons were prepared and applied. 96 well plate was used, incubated for 24 hours, later on ELISA reader at 405 nm was applied to read the results [15].

The effect of AuNPs and Tio2NPs on Bacterial Growth at 24 and 48 hours Incubation time:
The prepared nanoparticles solutions (for both Gold and Titinium dioxixe) where applied separately in a triplets to bacterial suspension, five double serial dilution of nanoparticles' solutions were prepared and applied. 96 well plate was used, incubated for 24 and 48 hours, later on ELISA reader at 405 nm was applied to read the results.

Statistical analysis

Bonferroni test recommended by Danial (1988) was used for statistical analysis ($P \leq 0.05$) to show if there is any significant differences between lemon extracts, effect of different concentrations of gold and titanium nanoparticles.

Ethical approval: The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with patients verbal and analytical approval before sample was taken. The study protocol and the subject information and consent form were reviewed and approved by a local ethics committee according to the document number 217 (including the number and the date in 18/11/2021) to get this approval.

Result

A total of 23 (20.9%) isolates of *S. aureus* were revealed and distributed as following: 5/33 (15.2%) isolates from burns and 8/25 (32.0%) from wounds, while 10/52 was (19.2%) isolates from urine. Present results showed that *S. aureus* isolates that recovered from clinical specimens were resistant to most used antibiotics, and these results in details were as follow: It showed high resistance to Trimethoprim (73.9%) while resistance to Clindamycin and Tetracycline was (65.2%) and (60.9%) respectively. While, it is less resistance to Azithromycin (43.5%) and to Amikacin (39%); resistance to Ciprofloxacin was (34.8%) and for Trimethoprim Sulfamethoxazole was (30.4%), finally, the resistance percentage to Levofloxacin was (26%) and to Gentamicin (21.7%) as shown in figure 1.

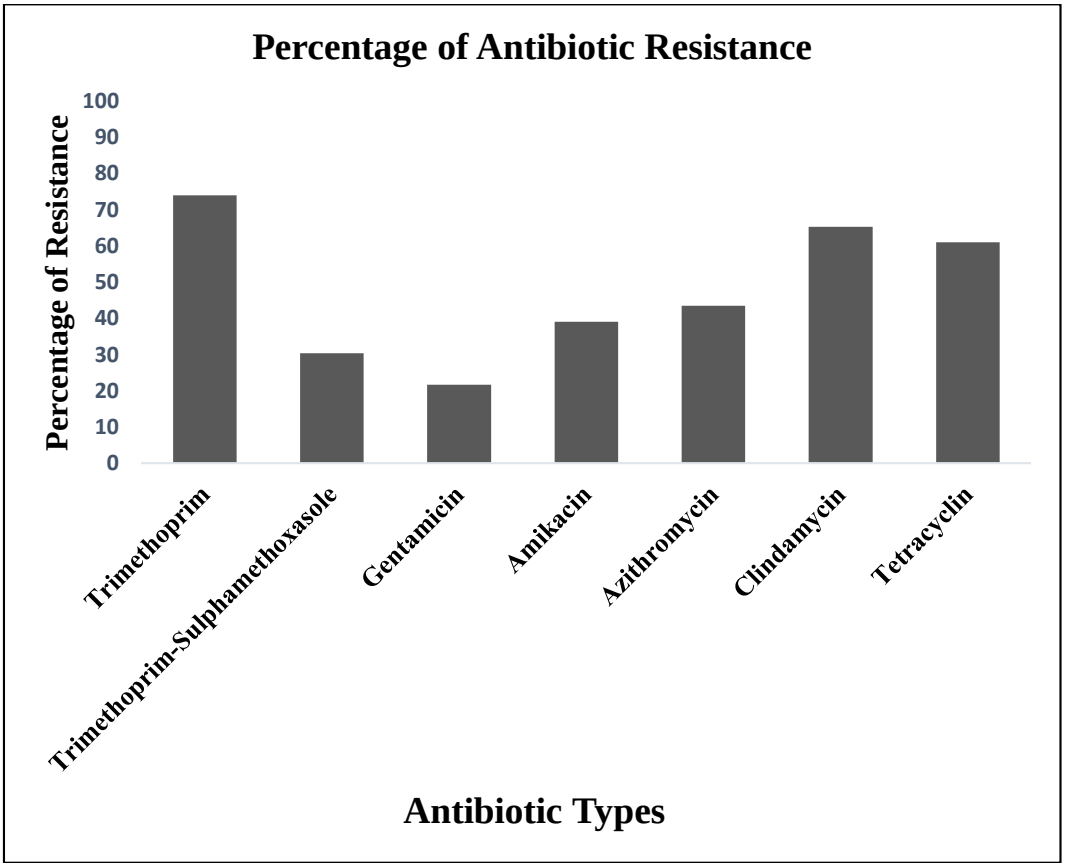

Figure (1): Percentages of antibiotic resistance among *S. aureus* isolates to different antibiotic types by disk diffusion method.

Effect of Sour lemon and Sweet lemon on *Staph aureus* growth

In this study, the effect of lemon juice of both types sour and sweet in different concentrations (100%, 75%, 50% and 25%) for each one on *S. aureus* isolates and their effect was examined and expressed in percentage value; and these results showed that Sour Lemon had higher inhibition effect on *S. aureus* in (100% and 75%) concentrations as it is statistically significant (p value less than 0.05) when compare higher concentrations with lower one; as the sour lemon made inhibition in a percentage (91.3%) for *S. aureus* while (25%) concentration made lowest inhibition effect (52.2%) of *S. aureus* growth. However sweet lemon showed lower effect in its different concentrations in (100%) concentration made (74%) inhibition effect on *S. aureus* while (25%) concentration just inhibit (13.04%) of bacterial growth, as it is statistically not significant (p value more than 0.05) when compare sour with sweet lemon; as shown in figure (2).

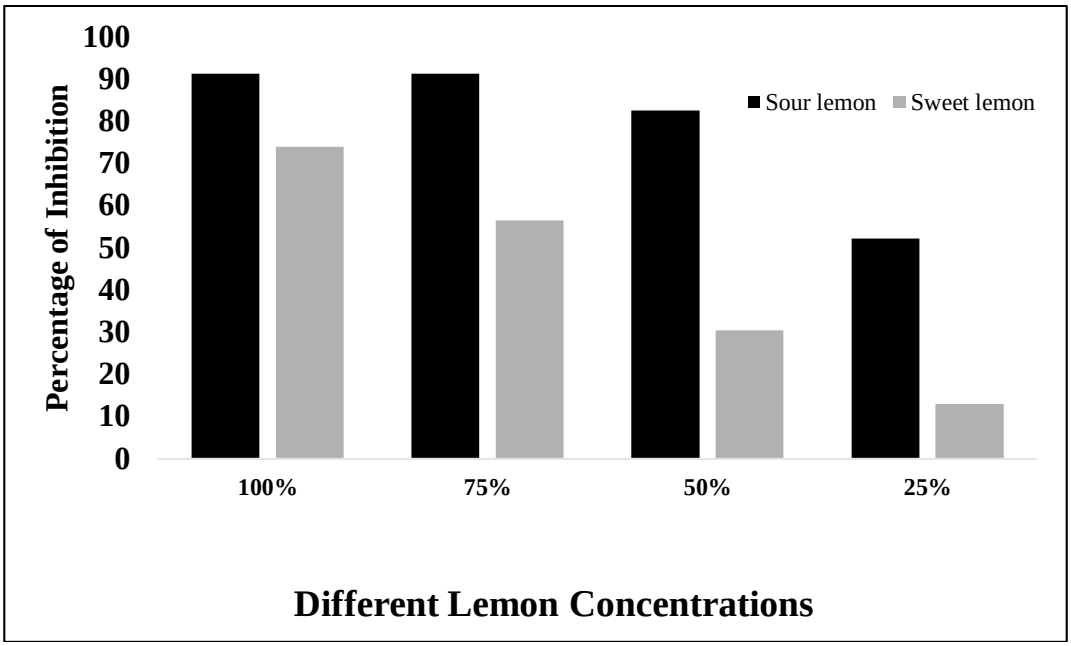

Figure (2): Inhibitory effect of Fresh Lemon (sour and sweet) Juices of the growth of *staph aureus*

Effect of AuNPs and TiO2NPs on *staph aureus* growth

In this study AuNPs and TiO2NPs effects were examined against *S. aureus* growth by making five double serial dilutions for each stock concentrations as (1/2, 1/4, 1/8, 1/16 and 1/32) and their initial stock concentrations were (2000 N) and (400N) for AuNps and TiO2NPs respectively; then after incubation for 24 and 48 hours the bacterial growth had been monitored and checked with spectrophotometer; results were cleared as follow and it revealed that there was statistically significant effect ($P<0.01$) on the growth especially with the first three dilutions, as shown in figure (3).

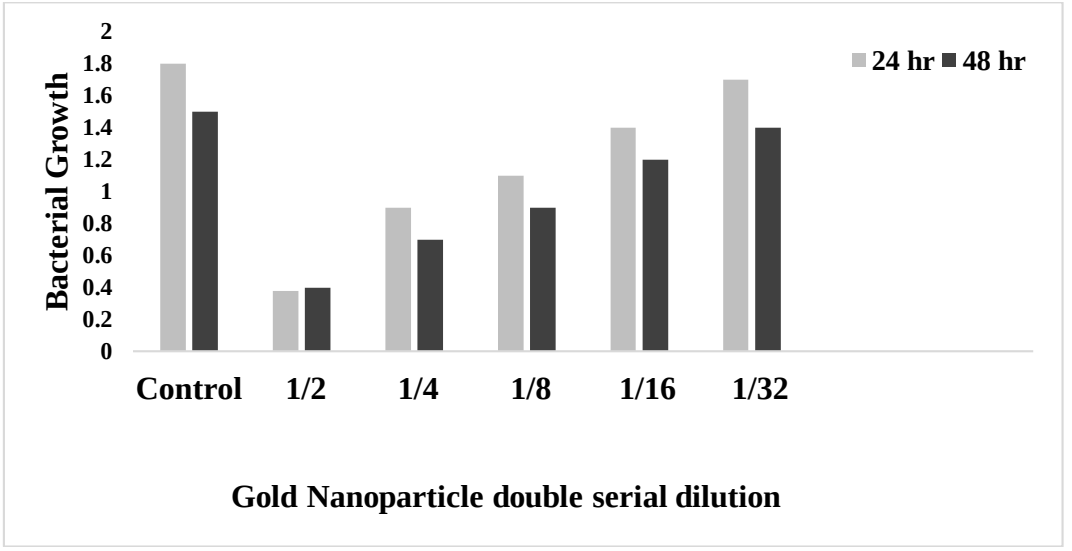

Figure (3): *Staphylococcus aureus* growth inhibition by double serial dilution of Gold (AuNPs) Nanoparticles

This figure showed that the highest concentration of AuNPs after diluted to (1/2) made the highest inhibition effect for bacterial growth and this inhibition minimized the bacterial growth from (1.8) to (0.35) after 24hours of incubation while after 48 hours of incubation bacterial growth minimized from 1.5 to 0.4. While the lowest concentration of AuNPs that made by diluted the nanoparticles for (1/32) showed the lowest inhibition effect on *S. aureus* growth after 24 and 48 hours of incubation.

While regarding TiO2NPs cause significant inhibition with p value less than 0.01 especially with the first two dilutions, and this was relatively a similar inhibition effect to that produced by AuNPs on *S. aureus* growth by the same serial double dilutions and results showed that when TiO2NPs concentration diluted to (1/2) appeared the highest inhibition effect on bacterial growth that minimized the growth of bacterial isolates from (2) to (0.7) after 24 hours of incubation and from (1.5) to (0.4) after 48 hours of incubation. Whereas the lowest concentration of TiO2NPs that prepared by diluting to (1/32) showed the lowest inhibition effect on *S.aureus* growth represented by minimizing the bacterial growth from (2) to (1.9) and (1.5) only after 24 and 48 hours of incubation respectively, as in figure (4)

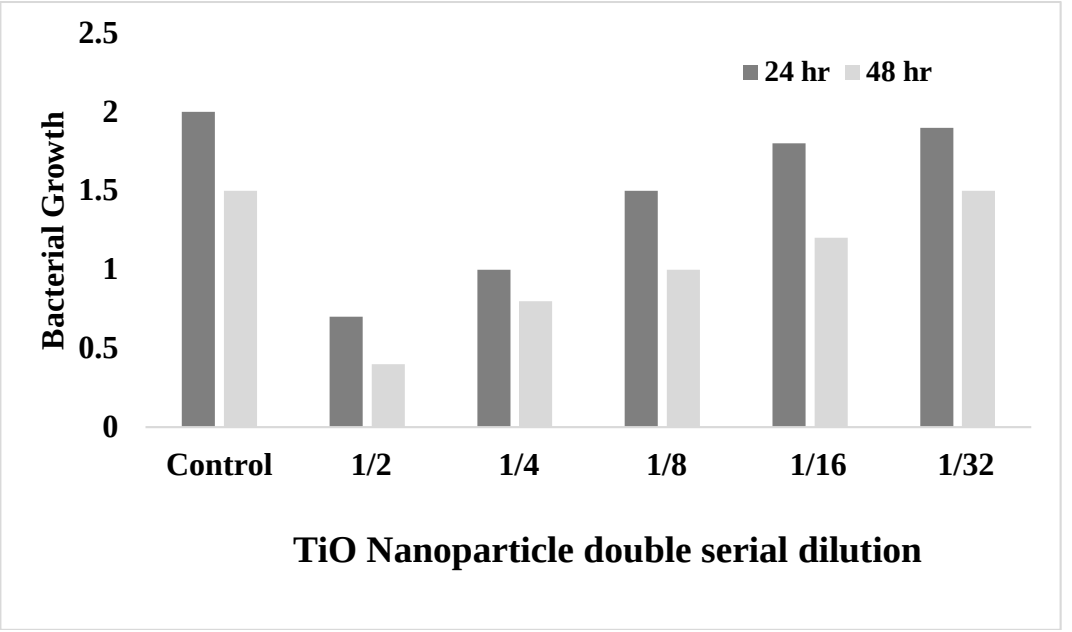

Figure (4): *Staphylococcus aureus* growth inhibition by double serial dilution of TiO2 (TiO2NPs) Nanoparticles

Discussion

The high prevalence of *S. aureus* isolates recovered from urine (19.2%) In present study, all patients were catheterized, this is the justification for high result in present study and this can be explained by its ability to attach to and aggregate onto uroepithelial cells, *S. aureus* is therefore, an important causative pathogen of UTI Kitano *et al*[16]. Also, the obtained prevalence of infections caused by *S. aureus* may be due to this infectious agent is most likely associated with endogenous source as it is a member of the skin and nasal flora, and also with contamination from the environment, surgical instruments, or from hands of healthcare workers.

Susceptibility of *Staphylococcus aureus* to Antibiotics

S. aureus showed a highly resistance values to most of the commonly used antibiotics in hospitals where development of antibiotic resistance is often related to the overuse, and misuse of the antibiotics prescribed. As Iraq is one of the developing countries where all types of antibiotics are sold over the counter, an attitude that encourages self-medication resulting in this antibiotic resistance problem. Also this finding was alarming as infection due to multi-drug resistant *S. aureus* isolates are difficult to treat. Also, resistance in *S. aureus* may be primarily associated with thickening of bacterial cell wall, and the source of highly resistant organisms in the population could be food, water, and/or person-to-person transfer. Lack of proper hand hygiene and overcrowding in health care facilities may enhance the spread of the antibiotic resistant organisms. Uncontrolled consumption of antimicrobial agents is likely to play a major role in the development of antibiotic resistant bacteria as suggested by Ruppe *et al*[17].

The problem in staphylococcal infections is an increasing resistance to many antibiotics because of their extensive usage and due to its ability to rapidly acquire resistance, where the resistance genes could be found on plasmids, transposons, and genomic islands and can be easily transferred horizontally between strains and species; thus it is better to limit their irregular usage to reduce resistance and maintain the beneficial activity of the drug in treating different infectious agents Febler *et al*[18].

Effect of Sour lemon and Sweet lemon on *Staph aureus* growth

The result of this study showed compatibility with Moosavy *et al*[19] result who found the lemon was found to have a significant inhibitory effect against *S. aureus*, The Gram-positive bacteria showed higher susceptibility values than gram-negative bacteria because the cell wall of gram-positive bacteria contains a polysaccharide which is a water-soluble polymer, which acts as incoming positive ions. This soluble nature shows that the gram-positive cell wall is more polar. The flavonoid compounds contained in lemon juice are non-polar, causing disruption of the cell wall function as a giver of cell shape and protects the cells from osmotic lysis. Disruption of the cell wall will cause lysis of the cell Batubara *et al*[20].

Effect of (gold nanoparticles and Titanium dioxide nanoparticles) on *staph aureus* growth

Present result agreed with Zhang *et al*[21] who found that AuNPs only been showed inhibition at a very high concentration of 197 µg/mL. While regarding TiO2, it agreed with Babaei *et al*[22] who showed the decrease in population of *S. aureus* in the presence of titanium dioxide nanoparticles. the TiO2 nanoparticles proved to be very active on the tested Gram-positive strains, this differential sensitivity of Gram-negative and Gram-positive bacteria toward nanoparticles could be explained by the fact that the liquid medium is probably favoring the close interaction between the suspended nanoparticles and the Gram-positive microbial cells, which could better attach and anchor to the surface of the microbial cell, causing structural changes and damages leading to cell death.

Conclusion

Staph aureus is a serious multi-drug resistant pathogen and this resistant causing a large problem, thus application of alternatives is very important to overcome this trouble. These alternatives can include plant extracts as fresh lemon juice especially sour more than sweet; as they are beneficial, effective and not risky. Also can apply nanoparticles that aid in delivery of drugs into their targets via their benefits in transport and release of the therapeutic agents to the target site.

References

1 Luzzago, C., Locatelli, C., Franco, A., Scac- cabarozzi, L., Gualdi, V., Viganò, R., Sironi, G., Besozzi, M., Castiglioni, B., Lanfranchi, P. and Cremonesi, P. 2014. Clonal diversity, virulence-associated genes and antimicrobi- al resistance profile of Staphylococcus aureus isolates from nasal cavities and

soft tissue infec- tions in wild ruminants in Italian Alps. Veteri- nary Microbiology, 170(1):157-61
2 Lu, T., & DeLeo, F. R. (2015). Pathogenesis of Staphylococcus aureus in Humans. Human Emerging and Re-emerging Infections: Viral and Parasitic Infections, 711-748.
3 Bitrus AA, Peter OM, Abbas MA, Goni MD. Staphylococcus aureus: A Review of Antimicrobial Resistance Mechanisms. Veterinary Sciences: Research and Reviews. 2018;4(2).
4 Al-Jabri, N. N., & Hossain, M. A. (2018). Chemical composition and antimicrobial potency of locally grown lemon essential oil against selected bacterial strains. Journal of King Saud University-Science, 30(1), 14-20.
5 Nurfita, D. 2017. Factors Related to Diarrhea in Toddlers at Bulu Lor Health Center, Semarang City. Faculty of Public Health. 11 2 149-154.
6 Tawfik, N. O., Al-Haliem, S. M., & Al-Ani, W. N. (2010). Evaluation of the antibacterial activity of citrus juices: an in vitro study. Al-Rafidain Dental Journal, 10(2), 376-382.
7 Elbehiry, A., Al-Dubaib, M., Marzouk, E., & Moussa, I. (2019). Antibacterial effects and resistance induction of silver and gold nanoparticles against Staphylococcus aureus-induced mastitis and the potential toxicity in rats. MicrobiologyOpen, 8(4), e00698.
8 Shittu, K. O., Bankole, M. T., Abdulkareem, A. S., Abubakre, O. K., & Ubaka, A. U. (2017). Application of gold nanoparticles for improved drug efficiency. Advances in Natural Sciences: Nanoscience and Nanotechnology, 8(3), 035014.
9 Vanamala, K., Tatiparti, K., Bhise, K., Sau, S., Scheetz, M. H., Rybak, M. J., ... & Iyer, A. K. (2021). Novel approaches for the treatment of methicillin-resistant Staphylococcus aureus: Using nanoparticles to overcome multidrug resistance. Drug Discovery Today, 26(1), 31-43.
10 Forbes BA, Sahm DF and Weissfeld AS. 2007. Bailey and Scotts' Diagnostic microbiology 12th. ed. Elsevier China.
11 CLSI (National Committee for Clinical Laboratory Standards). 2002. Methods for dilution antimicrobial susceptibility tests of bacteria that grow aerobically. Approved Standard M100-S12. Wayne. PA, NCCLS.
12 Prescott LM, Harley J and klein DA. 2002. Microbiology 5th. ed, McGraw-Hill New York. pp. 809-811.
13 Al-Jbory, N., Jalal, N. and Ghaleb, R. (2019). Wound Healing Properties and Cytotoxicity of Gold Nanoparticles on MDCK Cell Lines (in Vitro Study). Indian Journal of Public Health Research and Development. 10(7):1412-1414.
14 Mubdir, D. M., Al-Shukri, M. S. and Ghaleb, R. A. (2021). Antimicrobial Activity of Gold Nanoparticles and SWCNT-COOH on Viability of Pseudomonas aeruginosa.annals of RSCB; 25(3): 5507-5513.
15 Hindi, N. K. K., & Chabuck, Z. A. G. (2013). Antimicrobial activity of different aqueous lemon extracts. Journal of Applied Pharmaceutical Science, 3(6), 74.
16 Kitano, H., Shigemoto, N., Koba, Y., Hara, T., Seiya, K., Omori, K., ... & Ohge, H. (2021). Indwelling catheterization, renal stones, and hydronephrosis are risk factors for symptomatic Staphylococcus aureus-related urinary tract infection. World Journal of Urology, 39(2), 511-516.
17 Ruppe, E., Hem, S., Lath, S., Gautier, V., Ariey, F., Sarthou, J., Monchy, D. and Arlet, G. (2019). CTX-M β-lactamases in Escherichia coli from

community-acquired urinary tract infections, Cambodia. Emerg. Infect. Dis., 15(5): 741-748.
18 Febler, A.T.; Wang, Y.; Wu, C.; Schwarz, S. Mobile macrolide resistance genes in staphylococci. Plasmid 2018, 99, 2–10.
19 Moosavy, M. H., Hassanzadeh, P., Mohammadzadeh, E., Mahmoudi, R., Khatibi, S. A., & Mardani, K. (2017). Antioxidant and antimicrobial activities of essential oil of Lemon (Citrus limon) peel in vitro and in a food model. Journal of food quality and hazards control, 4(2), 42-48.
20 Batubara, N. A., & Lindawati, Y. (2019). Effect of lemon on saliva and Staphylococcus aureus. Padjadjaran Journal of Dentistry, 31(2), 85-90.
21 Zhang, Y., Shareena Dasari, T. P., Deng, H., & Yu, H. (2015). Antimicrobial activity of gold nanoparticles and ionic gold. Journal of Environmental Science and Health, Part C, 33(3), 286-327.
22 Babaei, E., Dehnad, A., Hajizadeh, N., Valizadeh, H., & Reihani, S. F. S. (2016). A study on Inhibitory Effects of Titanium Dioxide Nanoparticles and its Photocatalytic Type on Staphylococcus aureus, Escherichia coli and Aspergillus flavus. Applied Food Biotechnology, 3(2), 115-123.