Selenium nanoparticles inhibit Staphylococcus aureus-induced nosocomial infection, cell death and biofilm formation
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ABSTRACT

Background: Staphylococcus aureus that is a major health problem in hospitals, recognized as the greatest important nosocomial pathogen and usually causing postoperative wound infections. Antibiotic resistance by Staphylococcus aureus has developed to be common, and resistance to almost all antibiotics has been found among the most strains of this pathogen. Biofilms are generally more resistant to both host’s defense mechanisms and to antibiotics than planktonic unicellular microbes. Therefore, it is increasingly recognized as important health problem for patients with microbial infections. Moreover, Staphylococcal diseases has found to be connected with programmed cell death in order to cause numerous diseases in human hosts. All that made Staphylococcus aureus infections are hard to treat. Selenium has been examined for numerous medical applications and evidence suggested that Staphylococcus aureus growth can be inhibited in the presence of selenium nanoparticles.

Objectives: The specific objective of this study therefore was to investigate the prevalence, antimicrobial susceptibility patterns and associated risk factors of Staphylococcus aureus in patients with surgical site infections and burns infection. In addition to confirm that selenium nanoparticles may be used to effectively prevent and treat Staphylococcus aureus infections and thus should be further studied for such applications.

Methods: A cross-sectional study was conducted from January 1, 2022 to December 30, 2022 among patients with surgical site infections and burns infection at Al- Najaf city hospitals in Iraq country. A total of 275 Staphylococcus aureus isolates were recovered from hospital patients with postoperative wound infection or burns infection isolation and identification were based on standard bacteriological and biochemical criteria. Biofilms formation in vitro was carried out by microtiter plate spectrophotometric technique. Through using the standard disc diffusion technique, isolated strains of Staphylococcus aureus were tested for antibiotic susceptibility patterns. Host cell killing by Staphylococcus aureus was assayed by using florescent microscopy. Finally, selenium nanoparticles to inhibit bacteria were tested by measuring the sizes of the inhibition zone for bacterial growth.
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Results: Of the 630 surgical patients who resident in Al- Najaf city hospitals and who had developed surgical site infection, Staphylococcus aureus was isolated from 275 (43.6%) cases. The results show that most Staphylococcus isolates were resistant to most antibiotics and have ability for biofilms formation and inducing host cells kill. Results of this study also provided that strongly inhibited growth of Staphylococcus aureus in the presence of selenium nanoparticles after 24 hours at 10, 20, 40 and 80 μg/ml.

Conclusion: The incidence of Staphylococcus aureus infection in surgical and burn wards of Al-Najaf city hospitals in Iraq country was found to be high. In addition, most isolates were extremely resistant to major antimicrobial agents and has ability for inducing biofilms formation as well as host cells kill. Moreover, this study suggests that selenium nanoparticles may be used to effectively prevent and treat Staphylococcus aureus infections and thus should be further studied for such applications.

Keywords: Staphylococcus aureus, Antibiotic susceptibility, Biofilms formation, Host cells kill, Selenium nanoparticles, Al- Najaf city hospitals in Iraq country

INTRODUCTION
Postoperative wound infection characterizes the second most common type of nosocomial infection and remains a relatively common postoperative complication and the most common reason for surgical readmission (Pal et al., 2019).

Staphylococcus aureus has been known to cause deep wound infections for nearly a century and has been implicated as a reason for nosocomial infections and superinfections in patients receiving antimicrobial agents such as surgical cases (Tong et al., 2015). Staphylococcus aureus infections are complicated, as the bacterial strains have become increasingly resistant to numerous commonly used antibiotics. For instance, a methicillin-resistant Staphylococcus aureus infection which is difficult to treat, as it is resistant to a large group of antibiotics (beta-lactams) including oxacillin, penicillin, and amoxicillin (Tran and Webster, 2011).

Methicillin Resistance Staphylococcus aureus (MRSA) is now well understood to be a prominent cause of nosocomial infections and a general health concern globally (Chatterjee and Otto, 2013, Hogea et al., 2014). In addition, MRSA colonizing the anterior nares and skin of humans are the major sources of surgical site infection as well as nosocomial spread. This bacterium inevitably spread through skin contact and potentially can get into the bloodstream leading to sepsis, the primary cause for shock and circulatory collapse (Ito et al., 2014). MRSA can spread further to other tissues, such as kidney, lung, liver, heart and bone marrow, with severe clinical complications caused by endocarditis, osteomyelitis and urethritis (Haim et al., 2010).

Biofilms are aggregates of unicellular microorganisms forming multicellular structures that adhere to inert surfaces. Pathogenic bacteria can form biofilms on inert surfaces of implanted devices such as catheters, prosthetic heart valves and joint replacement (Khatooon et al., 2018). The ability of Staphylococcus aureus to form biofilms in vivo is considered to be a major virulence factor influencing its pathogenesis. High rates of biofilm forming Staphylococcus aureus have been diagnosed in diabetic food and prosthetic hip infection with increased resistance to antimicrobial agents (Mottola et al., 2016). Moreover, Staphylococcal diseases associated with programmed cell death. Thus, it is found that Staphylococcus aureus activities programmed cell death in order to cause numerous diseases in human and animal hosts (Missiakas and Winstel, 2020).

Selenium has been studied for numerous medical applications such as anticancer applications. Selenium as a dietary supplement has been established to decrease the risks of many types of cancers including prostate cancer, lung cancer and esophageal and gastric-cardiac cancers (Foroozandeh and Aziz, 2018). Furthermore, selenium-enriched probiotics have been shown to
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strongly inhibit the growth of pathogenic Escherichia coli in vivo and in vitro (Yang et al 2009). A sequences of organoselenium compounds for example 2,4,6-tri-paramethoxyphenylenenopyrylum chloride, 9-para-chlorophenyl loctahydroselenoxanthene, and perhydroseleleoxanthene have been synthesized and shown to have antibacterial activities in vitro, particularly against Staphylococcus aureus (Ratushnaya et al., 2002).

Because of growing antibacterial resistance, nanotechnology is paving the way for more effective and sensitive methods of detecting and treating bacterial infections. Nanotechnology enables researchers to synthesize nano-sized particles (that is, particles smaller than 100 nanometers in at least one dimension) and to use them in a variety of applications. Moreover, nanoparticles possess increased surface areas and thus have increased interactions with biological targets for instance bacteria compared with conventional, micron particles. Furthermore, nanoparticles enter cells more easily than micro particles (Borchers and Pieler, 2010).

In view of all that has been mentioned so far, one may suppose that nano-antibacterial particles will likely exert stronger effects on bacteria than their micro-counterparts. Therefore, the objective of this study is to determine the frequency, antimicrobial susceptibility patterns and related risk factors of Staphylococcus aureus in patients with surgical site infections and burns infection. In addition to confirm that selenium nanoparticles possibly used to effectively inhibit and treat Staphylococcus aureus infections and perhaps this study will be exposed a new type of antibacterial selenium nanoparticle that capable of reducing Staphylococcus aureus growth which is causes some big problems in our hospitals.

METHODS

Study area and swap sample

The study was conducted at Al-Najaf city hospitals, in surgical and burn wards. This study was conducted from January 1, 2022 to December 30, 2022. Swab samples were collected from admitted patients suspected of surgical site infection or burns infection. Our inclusion criteria were surgical wounds with purulent discharge, serous discharge, signs of sepsis (fever, erythema, induration, and pain), and a medical diagnosis indicating surgical site infection.

Sample collection and handling

Swab samples were collected from inpatients with postoperative infected wounds and infected burns using a sterile cotton swab under an aseptic condition.

Culture and identification

The collected swabs were streaked on blood agar plate (Oxid, UK) as soon as they arrived to the laboratory and then incubated at 37°C for overnight. Suspected colonies on the morphological basis and Gram’s stained smears were further subculture on Mannitol salt agar plates (Oxoid, UK), the plates were incubated at 37°C for overnight. Biochemical reactions performed to confirm the identification of Staphylococcus aureus isolates were including, coagulase (free and bound coagulase), hemolysis production, DNase test, detection of thermostable deoxyribonuclease (TDNase), and acetone production. Staphylococcus aureus was identified based on characteristic yellow colony surrounded by yellow zone on manniol salt agar; β hemolytic colonies with yellowish pigment on blood agar; gram positive cocci singly in pair, in short chain or clusters, catalase positive and coagulase production and mannitol fermentation.

Antimicrobial susceptibility testing

The antimicrobial susceptibility testing of all Staphylococcus aureus isolates were conducted by a disc diffusion method. From a pure culture, 3–5 selected colonies of Staphylococcus aureus had been taken and transferred to a tube containing 5 ml sterile nutrient broth (Oxoid) and were mixed gently. Then a homogenous suspension was formed and incubated at 37°C until the turbidity of the suspension became adjusted to a 0.5 McFarland Standard (Bacterial concentration of 1.5 × 108 CFU/ml).

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A sterile cotton swab was used and the excess suspension was removed by gentle rotation of the swab against the surface of the tube. The swab was then used to distribute the bacteria evenly over the entire surface of Mueller Hinton plate (pH 7.2-7.4) (Oxoid).

The inoculated plates were left at room temperature to dry for 3–5 minutes and a set of 14 antibiotic discs (Oxoid) on the surface of a Muller Hinton plate. The criteria used to select the antimicrobial agents to be tested were based on their availability and frequent prescriptions for the management of wound and burn infections in the hospital.

The plates were then incubated at 37°C for 24 hours. Diameters of zones of inhibition around the discs were measured to the nearest millimeter using a metal caliper, and the isolates were classified as resistant according to the standardized table supplied by the CLSI.

Measure the ability of Staphylococcus aureus for biofilms formation

The ability of Staphylococcus aureus isolates to form biofilms was detected by ELISA technique following the method described by Stewart and Parker in (2019).

Measure the ability of Staphylococcus aureus for inducing cell death

At the desired time point after infections and treatments, cells were fixed onto the coverslips as described below. The media was aspirated off and the cells were washed with PBS. The cells were then fixed by the addition of 3.2% paraformaldehyde (Agar Scientific (R1026)) diluted with PBS for 20 minutes at room temperature. Cells were then rinsed in PBS and stored at 4°C. Images were captured on an epifluorescence upright microscope with an X60 1.40 NA objective lens fitted with appropriate standard filter blocks.

Preparation and detection of selenium nanoparticles by heterochromia

Selenium nanoparticles have been prepared according to (Sasidharan et al., 2015). Orange peel extract was prepared from 3 gm crushed orange peels/100 ml distilled water, placed in a glass flask with a capacity of 200 ml and mixed by continuous stirring for 30 minutes at a temperature of (70 °C ± 5) using a heating device with magnetic. Then the extract was cooled at the laboratory temperature (25 °C ± 2 °C) for half an hour, after which it was filtered using layers of cotton fixed in a funnel fixed at the top of the conical flask to collect the extract. Then the extract was placed in a centrifuge to separate the impurities at 5000 rpm for 5 minutes. Separate the extract from the precipitate and collect the extract in a glass beaker and complete the volume to 100 ml with distilled water. Then add 100 ml of (SeO2) solution at a concentration of (0.5 mM). The final solution was placed on a heating device with continuous stirring for a period of not less than 5 hours and at a temperature of (70 °C ± 5) until the color of the extract changed and the ruby red color stabilized, which is attributed to the phenomenon of surface Plasmon resonance, which is a property possessed by many minerals, including selenium, silver and zinc as a result of to reach the diameter of its particles to the nanoscale. It is an indication of the formation of selenium nanoparticles.

Characterization of selenium nanoparticles

For Characterization of selenium nanoparticles, it is used that: Infrared Spectrometer (FTIR) used to know the active groups present in the sample. In order to confirms the synthesis of nanoparticles the spectroscopy using a UV-Vis spectrophotometer for visible and ultraviolet rays has been used and the absorbance spectrum appeared at 320 nm. On the other hand, X-ray diffraction (XRD) that is a common analytical technique has been used to analyze the molecular and crystal structures of nanoparticles in order to qualitative and identification of different compounds, the quantitative accuracy of chemical species and the measurement of crystallinity, isotypes, and particle sizes. In addition, Scanning Electron Microscope (SEM)
have been used to determine the shape and size of selenium nanoparticles.

Nanoparticles concentrations preparation
Nanoparticles powder was dissolved at a concentration 500 µg/ml using a sterile distilled water then used to prepare a six concentrations of nanoparticles solution (10, 20, 40 and 80 µg/ml).

Wells diffusion method
The well diffusion method was conducted against bacterial species Staphylococcus aureus isolates. Staphylococcus aureus bacteria were plated on agar plates, and then the plates were incubated at a temperature of 37 °C for 18 hours. A single bacterial colony was transferred from the agar plate to a test tube containing 5 ml of the sterile nutrient broth. The bacterial culture tubes were then incubated for 1.5 hrs at 37°C in an orbital shaker incubator at 200 rpm. The density of growth was monitored from time to time and compared with the density of McFarland standards. 100 µl of the bacterial culture was transferred to the center of the Mueller-Hinton agar medium plate and spread using a sterile L-shape spreader then left to dry. The plates were prepared while consistent with four wells with a diameter of 5 mm. The wells then were labelled with four concentrations (10, 20, 40 and 80 µg/ml) of selenium nanoparticles then were used by filling each well with a particular concentration. In addition, two discs of antibiotics (Oxacilin (OX), and Aztreonam (ATM)) were added at the center of the plate using sterile forceps. The plates were incubated at a temperature of 37°C for 24 hours. After the incubation time, the diameters of the inhibition zones were measured using the ruler in millimeters and compared with the standard references.

RESULTS
Sociodemographic Distribution
In the present study, of the 630 surgical patients or burns patients who resident in Al- Najaf city hospitals and who had developed surgical site infection or burns infection, Staphylococcus aureus was isolated from 275 (43.6%) cases. It is found 197 (71.6%) males and 78 (28.4%) females have surgical site or burns infection with Staphylococcus aureus (Table 1).

In addition, Table (2) showed the distribution Staphylococcus aureus isolates from surgical site or burns infection in human participants according to age groups, it seems that people between 18 to 30 years formed the highest rate (52.7%) of participants.

TABLE 1: Distribution of Staphylococcus aureus isolates according to gender

<table>
<thead>
<tr>
<th>Variables</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>197</td>
<td>71.6</td>
</tr>
<tr>
<td>Female</td>
<td>78</td>
<td>28.4</td>
</tr>
<tr>
<td>Total</td>
<td>275</td>
<td>100</td>
</tr>
</tbody>
</table>

TABLE 2: Distribution of Staphylococcus aureus isolates according to age groups.

<table>
<thead>
<tr>
<th>Variables</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ages in years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18-30</td>
<td>145</td>
<td>52.7</td>
</tr>
<tr>
<td>31-40</td>
<td>67</td>
<td>24.4</td>
</tr>
<tr>
<td>41-50</td>
<td>30</td>
<td>10.9</td>
</tr>
<tr>
<td>≥ 51 years</td>
<td>33</td>
<td>12</td>
</tr>
<tr>
<td>Total</td>
<td>275</td>
<td>100</td>
</tr>
</tbody>
</table>
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Table (3) revealed that according to the residence, those reside in urban areas were higher infected with Staphylococcus aureus than those reside in rural areas (63.3% Vs 36.7%) respectively.

**TABLE 3:** Distribution of Staphylococcus aureus isolates according to residence.

<table>
<thead>
<tr>
<th>Variables</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Residence</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rural</td>
<td>101</td>
<td>36.7</td>
</tr>
<tr>
<td>Urban</td>
<td>174</td>
<td>63.3</td>
</tr>
<tr>
<td>Total</td>
<td>275</td>
<td>100</td>
</tr>
</tbody>
</table>

Antimicrobial susceptibility pattern and biofilms formation

A total of 275 Staphylococcus aureus isolated from surgical site infection or burns infection were tested for their susceptibility patterns against 14 antibiotics by disc diffusion method. The results in table 4 show that all isolate were resistant to Cefixime (100%). Moreover, the majority of the isolates were resistant to the following antibiotics in about more than 50%; Piperacillin, Lincomycin, Erythromycin, Colxacillin (63.6, 69.5, 56 and 83.6 respectively). In addition, Staphylococcus aureus isolates showed less than 50% of resistance against Ofloxacin, Gentamicin, Amikacin, Rifampicin, Cefotaxime, Ciprofloxacin, Vancomycin, Clindamycin, and Augmentin (44, 22.5, 5.8, 13.5, 33.1, 5.8, 0.36, 46.9, and 42.2 respectively).

**TABLE 4:** Distribution of antibiotic resistance of human Staphylococcus aureus isolates.

<table>
<thead>
<tr>
<th>Type of antibiotic</th>
<th>Resistant Isolates (No.=275)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td></td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>121</td>
<td>44</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>62</td>
<td>22.5</td>
</tr>
<tr>
<td>Amikacin</td>
<td>16</td>
<td>5.8</td>
</tr>
<tr>
<td>Piperacillin</td>
<td>175</td>
<td>63.6</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>37</td>
<td>13.5</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>91</td>
<td>33.1</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>16</td>
<td>5.8</td>
</tr>
<tr>
<td>Lincomycin</td>
<td>191</td>
<td>69.5</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>1</td>
<td>0.36</td>
</tr>
<tr>
<td>Cefixime</td>
<td>275</td>
<td>100</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>129</td>
<td>46.9</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>154</td>
<td>56</td>
</tr>
<tr>
<td>Colxacillin</td>
<td>230</td>
<td>83.6</td>
</tr>
<tr>
<td>Augmentin</td>
<td>116</td>
<td>42.2</td>
</tr>
</tbody>
</table>

The ability of Staphylococcus aureus for biofilms formation

20 isolates that enrolled from each resistance antibiotic isolate to detect their ability to form biofilms in vitro, table 4 revealed that the all 20 (100%) Staphylococcus aureus isolate that is resistance for Cefixime also has ability for biofilms formation. Moreover, the bacterium which resistance for Ofloxacin, Lincomycin, and Colxacillin has ability to induce biofilms formation 85%. In addition, Staphylococcus aureus that resistance for Clindamycin, Erythromycin, has ability to induce biofilms 55%. On the other hand, bacterial resistance for Gentamicin, Cefotaxime, Augmentin formatted biofilms in about 45%. The results also show that there is no biofilms formation with Staphylococcus aureus which is resistance for
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Amikacin, Rifampicin, Ciprofloxacin, Vancomycin.

**TABLE 5:** The ability of Staphylococcus aureus resistance for antibiotics to induce biofilm formation

<table>
<thead>
<tr>
<th>Type of antibiotic</th>
<th>Biofilm formation (Isolates No.=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>17</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>3</td>
</tr>
<tr>
<td>Amikacin</td>
<td>0</td>
</tr>
<tr>
<td>Piperacillin</td>
<td>9</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>0</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>9</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>0</td>
</tr>
<tr>
<td>Lincomycin</td>
<td>17</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>0</td>
</tr>
<tr>
<td>Cefixime</td>
<td>20</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>11</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>11</td>
</tr>
<tr>
<td>Coloxacin</td>
<td>17</td>
</tr>
<tr>
<td>Augmentin</td>
<td>9</td>
</tr>
</tbody>
</table>

The ability of *Staphylococcus aureus* to killing host cell

After investigated the ability *Staphylococcus aureus* for antibiotic resistance and biofilm formation, we next wished to investigate the potency of these strains in killing host cells. At the end time point, after infections and treatments, cells were fixed onto the coverslips as described in our methods. Images were captured on an epi-fluorescence upright microscope.

We found that *Staphylococcus aureus* were potently cytotoxic for cells. These data indicate the ability of *Staphylococcus aureus* to killing host cells.

**FIGURE 1:** *Staphylococcus aureus* has ability to induce cell death in cells line.

**Assay of selenium nanoparticles for *Staphylococcus aureus* inhibition growth in vitro**

The ability of selenium nanoparticles to inhibit bacteria was tested by measuring the sizes of the inhibition zone for bacterial growth. The results showed that selenium nanoparticles made from orange peels have effective activity against bacteria and that the inhibitory activity of bacteria increases with increasing concentration.
FIGURE 2: Selenium nanoparticles suppresses Staphylococcus aureus.

DISCUSSION

The handling of clinical wound conditions has always been a restricting factor in surgical therapy outcomes. In spite of ongoing advancements that have been accomplished, surgery sites infection persists happen at an unacceptable acceleration, a perennial commanding cause of financial waste (Alexander et al., 2011). Staphylococcus aureus has extended been identified as one of the major causes of nosocomial human infections (Samia et al., 2022, Marshall et al., 2004). This study was accomplished to reveal the prevalence of Staphylococcus aureus in wound clinical samples, 275 (43.6%) Staphylococcus aureus isolates existed out of 630 samples. The outcomes are comparable to those ones recorded in studies of (Mohammed et al., 2021) and (Al-Hamdani, 2012), with positive Staphylococcus aureus growth of (31.6%) and (22.4%) respectively. (31.6%) and (22.4%) respectively. Here, the results also indicate that S. aureus is the main reason for pyogenic cases and an elevated number of Staphylococcus aureus isolates were encountered in children and male patients. This could be due to low immunity in children and the increased possibility to contact with infectious factors among males. These results agree with findings of (Gurung et al., 2020, Obanda et al., 2022, Nwankwo and Nasiru, 2011).

Staphylococcus aureus, in the course of a period, has acquired resistance to diverse traditionally used antibiotics. Most Staphylococcus aureus isolates were multi-drug resistant. A more resistance (above 50%) was observed in the case of Cefixime, Colxacillin, Piperacillin, Lincomycin and erythromycin. Different factors may have provided such an extent of resistance, like the misemploy of antibiotics by clinical experts, unprofessional consultants, and parishioners (Ventola, 2015, Llor and Bjerrum, 2014). Some of these factors may include inappropriate medicine procedures, insufficient patient instruction, restricted diagnostic skills, unofficial sale of antibiotics, and lack of proper working drug regulatory agents. An additional factor that is considered to raise resistance problems is the use of diverse antimicrobials in animals such as cows and poultry (Ayukekbong et al., 2017, Bacanlı and Başaran, 2019).

In Iraq country as in many other countries, it is a usual routine that drugs are illegally bought, without a prescription, and misused antibiotics by individuals, consequently contributing to develop and spread of antimicrobial resistance (M’Aiber et al., 2022, Hussein et al., 2020, Williams, 2008). Other causative aspects might be inadequate hygienic requirements, considering the development of drug-resistant pathogens with insufficient management. In other words, lack of knowledge about routine susceptibility tests of resistant isolates and management of antibiotic resistance (Mello and Oliveira, 2021). As such, following the right hospital rules are critical for an adequate clinical approach toward antibiotic resistance issue (Kabiru Olusegun and Samuel Oluwasegun, 2017, Avershina et al., 2021). It was documented resistance in <50% of Staphylococcus aureus isolates to Ofloxacin, Gentamicin, Amikacin,
Rifampicin, Cefotaxime, Ciprofloxacin, Clindamycin, and Augmentin from both surgical and burns wards, in agreement with (Askary and Malih, 2021, Cong et al., 2020). Vancomycin was the most effective antibiotic for S. aureus isolates of VRSA in this study with 0.36% resistance. It has been reported that the vanA gene, which is expressed on a plasmid, is responsible for resistance in VRSA. In spite of the risen difficulties in the cure for VRAS infections, the total number of human VRSA infections is still little (Rehm and Tice, 2010, McGuinness et al., 2017). In a recent study, 13.8% of S. aureus clinical isolates were found to be VRSA, such this high prevalence of VRSA in clinical samples suggests the high risk of subsequent development in Staphylococcus aureus towards Vancomycin (ElSayed et al., 2018).

In this study, more than half of Staphylococcus aureus have the power to create a biofilm formation in vitro, which is inherently resistant to antibiotics, representing a considerable part of infections, is not a trivial matter, and being especially challenging. These results are in agreement with previous reports affirming the capacity of Staphylococcus aureus to construct biofilm in vitro (Jordan et al., 2022, Trautner and Darouiche, 2004, del Pozo and Patel, 2007, Neopane et al., 2018). However, the ability of Staphylococcus aureus to form biofilms in vivo have also been reported (Oliveira et al., 2007, Lade et al., 2019). So as to gain a finer knowledge of the quantitative microtiter plate spectrophotometric technique, it was of interest to use it as a simple and sensitive method not only for the detection of biofilm formation but also for measuring the removal and killing efficacy of biofilm agents. The results indicate the successful use of microtiter plate spectrophotometric assay to determine biofilm formation (Stewart and Parker, 2019).

Interestingly, our results also show that host cells in culture can be virtually killed following Staphylococcal infections. The results are in keeping with other literature indicating that Staphylococcal infections are associated with programmed cell death (Deplanche et al., 2019, Howden et al., 2023, Missiakas and Winstel, 2020). A study by Soe and coworkers has constructed a direct conclusion that the intracellular adaptation of Staphylococcus aureus is crucial for pathogenicity. Colonizing a special intracellular area allows evasion from host immunity and the antibiotic effects (Soe et al., 2021).

Nanoparticles have been increasingly applied to a broad scope of medicinal applications. The effectiveness of nano-scaled structures is due to the large surface-to-volume ratio. This allows more unique functional locations to link with bioactive molecules, such as genes, antibodies, and drugs (Logothetidis, 2012, Levy, 2008). Nanoparticles can easily penetrate different types of cells including cancer cells, bacteria, and viruses by various pathways. (Foroozandeh and Aziz, 2018). Nanoparticles can convert laser light to heat that able to eliminate cancer cells (Jain et al., 2006 and Ni et al., 2008.). The same principle can be applied to bacterial growth-function inhibition, as employing nanoparticles is believed an appealing approach to preventing pathogens (Hoseini-Alfatemi et al., 2018, Li et al., 2010, Otnola and Afolayan, 2018, Shamaila et al., 2016). However, few researchers have documented using nanoparticles to effectively kill bacteria, in particular antibiotic-resistant Staphylococcus aureus.

Although selenium nanoparticles (SeNPs) have earned a reputation in the scientific community as anticancer agents, their great influence on pathogenic bacteria growth remains largely unexplored (Filipović et al., 2021). The results show that selenium nanoparticles assembled using a simple colloidal synthesis approach extremely inhibited Staphylococcus aureus-induced nosocomial infections. This inhibitory effect of SeNPs might prevent Staphylococcus aureus from forming biofilms and inducing the cell death phenomenon, after that may prevent its infection. Besides, the results indicated that a low concentration of SeNPs (10 µg/ml) was effective to kill Staphylococcus aureus. Consequently, these results suggest that SeNPs may be used to virtually restrain Staphylococcus aureus infections and hence must be investigated using further examinations.
**CONCLUSION**

The prevalence of Staphylococcus aureus in surgical and burns wards of Al-Najaf city hospitals were found to be high. Vancomycin has been a relatively effective drug against Staphylococcus aureus infections. Increased resistance levels were marked to Cefixime, Coxlacinil, Pipercillin, and Lincomycin among Staphylococcus aureus isolates from surgical and burn units. Moreover, the isolation Staphylococcus aureus found has ability for biofilms formation and inducing host kill. Finally, the selenium nanoparticles created here by a simple colloidal synthesis method, strongly inhibited the growth of Staphylococcus aureus.

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