Antibacterial activity of Zinc oxide nanoparticles on *Staphylococcus aureus*

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**ABSTRACT:**

**Introduction:** One of the main health problems is increasing resistance of bacteria to antimicrobial agents world-wide. Nanoparticles can be useful for preventing the distribution of resistant bacteria in the hospital and community. This study was carried out to determine the antimicrobial activity of zinc oxide (ZnO) nanoparticle against isolated *Staphylococcus aureus*.

**Method of study:** This study was conducted on 268 *Staphylococcus aureus* which was isolated from healthy carriers, patients and food materials in Golestan province, in north-east Iran. Antimicrobial activity of ZnO nanoparticles (10-30 nm diameters) against *Staphylococcus aureus* was carried out by Agar dilution method and subsequently by Microdilution method for the determination of its minimum inhibitory concentration (MIC). Distribution of MIC were evaluated and statistically analyzed on the basis of source, antibacterial resistance, the ability of biofilm formation and virulence factors.

**Results:** The minimum concentration of ZnO nanoparticles that could prevent the growth of isolated *Staphylococcus aureus* was 625 µg/ml. MIC₉₀ and MIC₅₀ were 1250 and 5000 µg/ml, respectively. The Mean MIC of ZnO nanoparticle on *S.aureus* which was isolated from food material (2108.6 µ/ml) was more than two other groups; patients: 1614.2 µg/ml and Healthy Carriers: 1503.9 µg/ml. This difference was statistically significant (P = 0.02). There was not any statistically difference in the mean of MIC among *S.aureus* which was isolated from the patients, but the MIC value was lower in the strains which were isolated from blood cultures. The mean MIC value of ZnO in *S.aureus* resistant and sensitive to Gentamicin were 1564.25 and 2343.7, respectively (p = 0.04) and in resistant and sensitive to Erythromycin were 1530.59 and 2103.4, respectively (p = 0.06). There were not any significant differences among the strains according to antibiotic resistance profiles, the ability to produce biofilm, and age typing groups.

**Conclusion:** In addition to the size of nanoparticles and the concentration of bacteria, we found that the efficacy of ZnO nanoparticles dependent mainly on the source of *Staphylococcus aureus* isolation and its resistance to Gentamicin and Erythromycin.

**Keywords:** antimicrobial susceptibility, zinc oxidenanoparticle, *Staphylococcus aureus*

**INTRODUCTION:**

Nowadays in parallel with the progress and change in human lifestyle, the incidence of some infectious diseases has decreased but yet controlling the microorganisms and preventing the causes of antibiotic resistance is one of the most important concerns with the health researchers. Hospital acquired infections (HAI) has been one of the biggest problems around the world and
controlling the distribution of infections, especially in hospitals is a serious challenge (2).
Increase in antibiotic resistance, is one of the most important reason of hospital-acquired infections and its control need to use more expensive drugs combinations with more side effects. That's why the researchers concentrate on using a combination of low-risk and effective control of bacteria resistant to antibiotics more than before. Using nano-material and evaluation of their antimicrobial effects is one of the ways to reduce the HAI (3).
Zinc oxide nanoparticles ZnO are such combinations that their purity is very high. On the other hand, in addition to it doesn't make resistance against antibiotics but also its toxicity and pollution is very rare (4).
Antimicrobial properties of Zn²⁺ were known a long time ago and applied traditionally by people. Some studies have shown that Zn²⁺ and other metal ion via attachment and inactivation of sulfhydryl groups (-SH) in enzymes inhibit the protein and enzyme activation (5).
Staphylococcus aureus is one of the most important pathogens for humans. It can causes a wide variety of diseases in human-beings, from simple and mild infections such as; skin rash, boils, carbuncle, sty and abscesses to some sever and Life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome and septicemia (6).
Staphylococcus aureus harbored a wide range of pathogenic and virulence factors, hemolysins, different toxins, attachment factors and capable of producing biofilm. In the other hand it can acquire drug resistance genes from other bacteria or by mutation. Resistance of this bacterium to methicillin and in lower prevalence to Vancomycin, turning it to superbug bacteria (1). Each year these drug-resistant bacteria infect and kill many people around the world.
In some studies, researcher found that ZnO nanoparticles have inhibitory effect on pathogenic bacteria such as Staphylococcus aureus (7).
This study was carried out to determine the minimum inhibitory concentration (MIC) of ZnO nanoparticles on isolated Staphylococcus aureus according to their source isolation, drug resistance profile and presence or absence some virulence factors.

METHODS:
A: preparation of zinc oxide nanoparticles:
White powder with a diameter 10-30 nm of zinc oxide nanoparticles (Co. US Research Nonmaterial) is commercially obtained. A homogenized suspension was prepared by sonication ZnO nanoparticle in water with ultrasonic devices (Q-Sonica- XL 2000) at frequency HZ20000.
Prepare Mueller-Hinton agar with final concentration of ZnO nanoparticles from 5000 - 2500 - 1250 - 625 -312/5 -156 µ/ml.
B: Staphylococcus aureus isolates preparation:
This study was performed on 268 isolates of Staphylococcus aureus which had been isolated previously in Gorgan, North of Iran (8, 9, 10). Out of 151 isolates from patients admitted to hospitals and private laboratory in Gorgan, 32 isolated from healthy carriers and 84 isolated from different food materials and Col standard strain. The antibiotic susceptibility profile, mecA gene presence and some other virulence factors of these S. aureus isolates were previously determined
Overnight culture of each S. aureus used to make suspensions equivalent to (1.5X10⁸ cfu/ml) 0.5 McFarland turbidity (11).
C: MIC of ZnO nanoparticle:
MIC was determined by Agar dilution method (12). Briefly, 20 µl of bacterial suspension were added to each Muller-Hinton agar media contain different concentration of ZnO nanoparticle and the growth bacteria were evaluated after 24 h incubation culture media in 37°C (figure1).
D: Biofilm formation in S. aureus isolates:
The ability of biofilm production in S. aureus isolates were carried out with crystal violet
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staining in plastic 96 micro titre plates, based on the previously described method (13, 14)

**RESULTS:**
Mean and standard deviation of MIC ZnO nanoparticle on 268 S. aureus isolates was 1445.7 ± 1765µg/ml. The lowest concentration of nanoparticles that prevent the growth of some of the isolates was 625µg/ml and the highest is estimated more than 5,000 µg/ml. MIC<sub>50</sub> and MIC<sub>90</sub> of the isolates was 1250µg/ml, 5000 µg/ml, respectively.

![Figure 1: Determination of MIC ZnO nanoparticles on Staphylococcus aureus by agar dilution method. In this case, MIC is determined 1250 µg/ml.](image)

Comparison of MIC ZnO nanoparticle on S.aureus isolates based on their source of isolation showed that the MIC in subjects which isolates from food material was more than two other group (Table 1) and this difference was statistically significant (P = 0.02)

**Table 1:** The MIC ZnO nanoparticles on S.aureus isolates according to their source, Gorgan, north of Iran

<table>
<thead>
<tr>
<th>Source*</th>
<th>Number</th>
<th>Mean (µg/ml)</th>
<th>Standard deviation(µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient</td>
<td>151</td>
<td>1614.2</td>
<td>1415.6</td>
</tr>
<tr>
<td>Carrier</td>
<td>32</td>
<td>1503.9</td>
<td>950.6</td>
</tr>
<tr>
<td>Food</td>
<td>84</td>
<td>2108.6</td>
<td>1597.5</td>
</tr>
<tr>
<td>COL strain</td>
<td>1</td>
<td>1250</td>
<td>-</td>
</tr>
<tr>
<td>Total†</td>
<td>267</td>
<td>1756.5</td>
<td>1445.7</td>
</tr>
</tbody>
</table>

*Source S.aureus isolates, † one standard isolate (COL)

Among different clinically samples, The MIC value ZnO in Staphylococcus aureus was no different in MIC value were found among various food material, dairy- bakery and meat, which S.aureus isolated source (data not presented).

**Table 2:** The average MIC of ZnO on S. aureus isolated from patients, Gorgan, north of Iran

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Number</th>
<th>MIC µg/ml</th>
<th>Standard deviation µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine</td>
<td>45</td>
<td>1738.8</td>
<td>1501.6</td>
</tr>
<tr>
<td>Wound</td>
<td>33</td>
<td>1734.8</td>
<td>1514.4</td>
</tr>
<tr>
<td>Blood cultures</td>
<td>43</td>
<td>1322.6</td>
<td>946.9</td>
</tr>
<tr>
<td>Hand</td>
<td>3</td>
<td>1666.6</td>
<td>721.6</td>
</tr>
<tr>
<td>Nose</td>
<td>29</td>
<td>1487.0</td>
<td>979.9</td>
</tr>
<tr>
<td>Other</td>
<td>30</td>
<td>1763.8</td>
<td>1802.5</td>
</tr>
<tr>
<td>Total</td>
<td>183</td>
<td>1600.6</td>
<td>1354.8</td>
</tr>
</tbody>
</table>

Comparison of MIC ZnO nanoparticle on S.aureus assessed in 123 isolates. Data showed that among according to their antibiotic resistance profile was nine antibiotic include: Penicillin, Cefazolin,
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Tetracycline, Ampicillin, Cefixime, Nalidixic acid, Erythromycin, Gentamicin, Clindamycin and Vancomycin were tested, only the MIC value in strains which sensitive to gentamicin and erythromycin (Table 3) was statistically lower than resistant strains (P = 0.04). For other tested antibiotics difference MIC between sensitive and resistant strains, weren't meaningful.

**Table 3:** Comparison of MIC value ZnO nanoparticles in *S. aureus* based on drug resistance

<table>
<thead>
<tr>
<th>Drug</th>
<th>Gentamicin</th>
<th>Erythromycin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average MIC</td>
<td>Standard Deviation</td>
</tr>
<tr>
<td>Resistant</td>
<td>2343.75</td>
<td>1658.3</td>
</tr>
<tr>
<td>Sensitive</td>
<td>1564.25</td>
<td>1402.9</td>
</tr>
<tr>
<td>Total</td>
<td>1665.65</td>
<td>1453.558</td>
</tr>
</tbody>
</table>

*MIC of ZnO nanoparticle (µg/ml)

The mean MIC ZnO nanoparticle and standard deviation against MRSA and MSSA strains was 1655.7 ± 1369.7 and 1572.5µ ± 1347.5µg/ml, respectively and this difference was not statistically significant. It wasn’t found a significant relationship in MIC ZnO on *S. aureus* isolates based on their potency for biofilm formation. In *S. aureus* that able to produce biofilm the MIC ZnO was 1658/9 ± 1408µg/ml and for strains which unable to produce biofilm was 1784/1 ± 1397.5 µg/ml. For One hundred sixty four isolates of *Staphylococcus aureus* agr typing were carried out in previous study (15), MIC where mentioned in table 4 showed that in Division 4 agr average was higher than others, but this difference was not statistically significant (p> 0.05).

**Table 4:** Mean and standard deviation MCI of ZnO nanoparticles against *Staphylococcus aureus* on the basis of agr typing

<table>
<thead>
<tr>
<th>Agr</th>
<th>Number</th>
<th>Mean MIC ZnO</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>No typeable</td>
<td>28</td>
<td>2205.3</td>
<td>1946.3</td>
</tr>
<tr>
<td>Agr</td>
<td>62</td>
<td>2084.6</td>
<td>1752.3</td>
</tr>
<tr>
<td>Agr 2</td>
<td>26</td>
<td>1658.6</td>
<td>1117.2</td>
</tr>
<tr>
<td>Agr 4</td>
<td>43</td>
<td>1607.5</td>
<td>1520.8</td>
</tr>
<tr>
<td>Agr 4</td>
<td>5</td>
<td>2500.0</td>
<td>2296.3</td>
</tr>
<tr>
<td>Total</td>
<td>164</td>
<td>1925.3</td>
<td>1662.7</td>
</tr>
</tbody>
</table>

*MIC of ZnO nanoparticle (µg/ml)

**DISCUSSION:**

Due to the beneficial effects of metal nanoparticles on microorganisms and control of many infectious diseases and prevent them from becoming chronic, many scientists believe that nanoparticles could create a great revolution in the field of health. Several studies showed that the antibacterial effect of zinc oxide nanoparticles (ZnO) on *Staphylococcus aureus* is more than other metal oxide nanoparticles such as CuO, CeO2, Al2O3, TiO2, MgO (16). In this study, in order to determine the minimum inhibitory concentration (MIC) of zinc oxide nanoparticles ZnO was performed on 268 on *Staphylococcus aureus* isolates. The results showed that the minimum concentration of ZnO nanoparticles that prevents the growth of *Staphylococcus aureus* in this area was 625µg/ml or 7.5 Micro-molar which is similar to other studies. The MIC ZnO on *S. aureus* in Haris (17), Raghupati and colleagues (18) Jones and colleagues were (16) were 500, 500 and 420 µg / ml, respectively. MIC of ZnO in a study which conducted by Hussein-Zadeh (19) was 1250 µg/ml. In the other hand the MIC ZnO against *S. aureus* in some other studies (20-22) was less...
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than 100 \( \mu g/ml \). This difference may be due to difference in size nanoparticle, differences between strains of bacteria (23) and concentration of bacteria(24). In smaller nanoparticle the surface area ratio to volume increased and therefore reactive groups is increasing as a result the toxicity of nanoparticles increases. In this study we finding that difference in efficacy of ZnO nanoparticles on \textit{S.aureus} related to at least two other character's of this bacteria; its resistance to gentamicin or erythromycin and the source of isolation this bacteria.

We found that the MIC ZNO on \textit{Staphylococcus aureus} which isolated from foods was significantly higher than isolates isolated from human and this difference was statistically significant. Perhaps bacteria which present in foods have more contact with the zinc oxide composition. According to our finding we confirmed the use of this nanoparticle in food material such as orange juice (25).

On the other hand, our study showed that the average MIC isolated nanoparticles of zinc oxide in the carrier is less than isolates from patients. Removal of \textit{Staphylococcus aureus} in the nose employed in health centers (healthy carriers) is one of the main concerns in the official hospitals of infection controls. But our results indicate that probably using the nanoparticles for infection control in hospitals is not of good performance but it has a better therapeutic effect on the isolates of the patients. It should be noted that a similar study in this field was not found; therefore larger studies to prove the validity of these findings, future studies are suggested.

Resistance \textit{S.aureus} isolates to Gentamicin and Erythromycin increased significantly the MIC ZnO or in other words reduces the efficiency of zinc oxide. Since these antibiotics affect on protein synthesis, resistance to them is due to changes in specific receptors in the ribosome or efflux pump. For this reason changes in specific proteins on ribosome may be reduces the ability of zinc oxide nanoparticles to bind to the ribosome and inhibit protein synthesis (26). Our results showed that the mean MIC of ZnO in MRSA strains are more than MSSA isolates, although this difference was not statistically significant but it can be a confirmation of higher resistance of MRSA strains. Haris and colleagues in their study showed that MIC of zinc oxide nanoparticles in the strains of MRSA were achieved to 2000 \( \mu g/ml \) (17), which is similar to our findings. Most of MRSA isolates in our region is belong to SCCmec type3 (under publication) which is the largest in SCCmec types (27), it may carrier the genes responsible for lower sensitivity to nanoparticles.

**CONCLUSIONS:**

Our results showed that the lowest MIC ZnO on \textit{Staphylococcus aureus} isolates is 625\( \mu g/ml \). The average MIC in bacteria which isolated from food material was significantly higher than samples isolated from human. There is a significant relationship between the MIC ZnO and bacterial resistance of erythromycin and gentamicin. It may be related to the change on the structure of the ribosome in these bacteria.

**REFERENCES**

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